Silvio Silvério da Silva
Anuj Kumar Chandel *Editors*

Biofuels in Brazil

Fundamental Aspects, Recent Developments, and Future Perspectives

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The One who gives life the entire universe,
Is Immortal: He is the One Lord of all.

Guru Nanak Dev

Foreword

Biofuels in Brazil: *Fundamental Aspects, Recent Developments, and Future Perspectives* has been compiled to cater the needs of graduate and post-graduate students, researchers in academia and industries, managerial organizations, biotech business, policy makers, policy analysts and readers at large. This book apprises the technical updates on bioenergy research of Brazil eventually discussing the system biology based approached to manufacture bioenergy crops, biomass pre-treatment, improved microbial strains for the fermentation of sugar solution super enzyme titers, techno-economic analysis of biofuels production, technical aspects of biodiesel and other renewable hydrocarbons production, feedstock availability in Brazil and biofuel policy issues in Brazil.

Among the sustainable biofuels, bioethanol is the most promising and sustainable alternative to gasoline in the context of Brazil. Brazil has played a key role in implementation of bioethanol as an alternative of gasoline. Sugarcane juice is the primary source of ethanol production in Brazil. However, in future definitely the second generation feedstock like biomass residues need to be taken into consideration vigorously. Brazilian Ministry of Science and Technology through the Research and Projects Financing (FINEP) and NIST Bioethanol development via CNPq, FAPESP has developed a network of more than 20 research institutions working on the promotion of biofuels production in Brazil. The success rate of bioethanol policy from Brazil can be followed by other developing nations such as India, China and others for reducing their dependency on oil import thus saving foreign exchange reserves.

This book also disseminates the key information on life cycle assessment of biofuels, arable land and climate changes after implementing bioenergy options. Essentially, this book focuses on the Brazil Government policies for the promotion of biofuels in the country. The success of bioenergy program in Brazil is a learning example for other countries particularly agricultural rich countries for implementing the affordable policies for the commercialization of biofuels. Overall, this book is a special collection of quality chapters written by the peers of field updating research/analysis on bioenergy options in Brazil. I am confident in forwarding this

book to the worldwide readers to learn about the biofuels development in Brazil, technical aspects of biofuels production and other basic ingredients of biofuel research in the Brazilian context.

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Preface

Life is energy. A sustainable supply of energy is required for the overall human development. The Sun is the primary source of energy on Earth. Fossil energy, the major source of energy (80 % of current world power consumption), is the result of energy entrapped by plants through photosynthesis in past eras. Fossil energy is a finite source and is likely to be exhausted sooner rather than later due to the fast pace of urbanization and increased use worldwide. Regular price hikes and environmental damage caused by excessive use of fossil fuels are the major alarming concerns. Owing to these geopolitical factors, the time is now to look out intensively for alternatives to fossil fuels. Renewable or bioenergy is the suitable answer as it can be produced directly from natural resources.

Bioenergy sources are diverse and broad in range. It can be categorized mainly as solar, wind, hydrothermal, and biomass-derived. Amongst all the renewable resources, biomass is one of the most promising answers, particularly for transportation fuels. Brazil is the fourth largest country in the world and is largely blessed by nature for appropriate fertile land, rain, light, and water. Brazil is representative of renewable energy program in the world and ranks second in ethanol production. The government of Brazil has taken appreciable initiatives in order to make the bioenergy program successful. Sugarcane-juice-derived ethanol, so-called first-generation ethanol, is the principle component of bioenergy in Brazil. However, cellulosic ethanol or second-generation ethanol is a prospective energy source in the near future. There are numerous research programs nationwide to make cellulosic ethanol a reality in the near future with financial help from the Ministry of Science and Technology, Government of Brazil. Many countries, particularly the developing world, can learn from the success stories of the Brazilian Bioenergy program and implement the policies for their energy security and betterment of socioeconomic status.

This book aims to disseminate the current advances in the bioenergy program of Brazil starting from feedstock analysis, availability, chemical composition, technical aspects, technoeconomic analysis, and government policies. This book is a fine and unique collection of 19 book chapters written by specialists in the related research area, who afford critical insights into several topics, review of current research, and discuss future progress in this area. Broadly, this book intends to

provide critical insight and background research analysis on raw materials, processing, synthesis, recovery, and application as energy sources, comparative account on major alternative energy producing countries in addition to feedstock variation and analysis. In regard to technical updates, this book highlights the system biology-based approaches for the development of new energy feedstocks, microorganisms, and enzyme titers. Furthermore, recent technical progress made toward pretreatment, enzymatic hydrolysis of biomass, and the fermentation of sugars into ethanol is also mentioned. Similarly, these aspects have also been discussed for the production of other biofules such as biohydrogen, biodiesel, or bio oil. An assessment on technological development for capturing, regeneration, and storage of solar energy, wind energy, and turbines is also made along with future directions. Comparative technoeconomic and life cycle analysis of various biofuels have been discussed in the last section along with the commercialization of cellulosic ethanol and other by-products. Additionally, initiatives taken by the Brazil Government for implementing effective bioenergy policy for the promotion of biofuels through research, commercialization, and private investment support have been apprised to the readers. Apart from researchers and graduate students of microbial biotechnology, and chemical and industrial engineers, this book will assist the business community and policy analysts who deal with geopolitical analysis of bio-based products, bioenergy, and their marketing.

We greatly appreciate the scholarly contribution of authors who added highly informative chapters in this book. We thank Isabel Ullmann, Hanna Hensler, Anette Lindqvist, and the production staff of Springer Verlag, Germany for their timely suggestions and support to publish this book. We would like to thank our colleagues Andre Ferraz, George J. M. Rocha, M. G. A. Felipe, Adriane M. F. Milagres, Walter Carvalho, Rita C. L. B. Rodrigues, Ines C. Roberto, and Messias B. Silva for the completion of this book. We are grateful to FAPESP, CNPq, CAPES, and University of São Paulo, Brazil for the financial support and infrastructure setup. We express our sincere thanks to our families for their unconditional support and cooperation while editing this book. Last but not least, we welcome the reader's suggestions to improve the future editions. We think that Readers' benefits are the best reward for editors, contributing authors, and publisher.

Silvio Silvério da Silva
Anuj Kumar Chandel

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Editors Biography

Prof. Silvio Silvério da Silva is a professor at the Department of Biotechnology, Engineering School of Lorena, University of São Paulo, São Paulo, Brazil. He completed his doctorate in Biochemical and Pharmaceutical Technology from the University of São Paulo (USP) and *Gesellschaft Fuer Biotechnologishe Forshung* (GBF), Germany in 1994. Prof. Silva offers consulting services to various scientific journals, ad hoc institutions, and biotechnological industries. He has published more than 130 papers in peer reviewed international journals and presented more than 455 papers in international conference proceedings. He has also 18 book chapters to his credit. He has recorded two patents on technological processes for xylitol production. Prof. Silva has guided 4 post-doctoral fellows, 11 doctoral students, 26 masters' dissertations, and 58 scientific initiation students in the area of Applied Microbiology, Biochemical Engineering, and Biotechnology. He has successfully completed 19 research projects funded by Brazilian Government and private funding agencies, including International Cooperative Projects. He also received important awards in biotechnology field for his contribution. His research area is Micro-biotechnology harnessing the potential of lignocellulosic feedstock for the production of Xylitol and Bioethanol since the last 25 years. He has visited several international research institutes from various countries for exchange of scientific knowledge on the various aspects of xylitol and bioethanol production.

Dr. Anuj Kumar Chandel completed his doctorate in 2009 from Jawaharlal Nehru Technological University, Hyderabad, India. After his master's from the Indian Institute of Technology Roorkee in 2000, Anuj joined Dalas Biotech Ltd., Bhiwadi, for the large-scale production of penicillin acylase and antibiotic intermediates. Subsequently, he worked at University of Delhi in a research project funded by the Department of Biotechnology (DBT), Government of India. Later he joined Celestial Labs Ltd., Hyderabad as a research associate. After this, he did post-doctoral studies at University of Stellenbosch, South Africa. Anuj worked as a post-doctoral fellow at Engineering School of Lorena, University of São Paulo, Brazil on biofuels development in a thematic project funded by Bioen-FAPESP.

Currently, Anuj is working at the Department of Chemical Engineering, University of Arkansas, Fayetteville, Arkansas, USA. He is the author of 3 books on D-xylitol, lignocellulose degradation and Brazilian Bioenergy development. Anuj has published 51 articles in peer-reviewed journals and 16 book chapters. He has also recorded one Brazilian patent on biomass pretreatment.

Chapter 1
Techno-Economic Analysis of Second-Generation Ethanol in Brazil: Competitive, Complementary Aspects with First-Generation Ethanol

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Abstract Brazil achieved important success in the implementation of ethanol as a reality renewable energy source after the inception of the National Alcohol Program (PROÁLCOOL) in 1970. Today, ethanol produced from sugarcane replaces almost 50 % of gasoline in Brazil. More than 448 bioethanol production (first-generation ethanol) units are functional, which fulfill the 25 % ethanol blending to gasoline that eventually reduces the import of 550 million oil barrels improving the socioeconomic status and saving foreign exchange reserves. Brazil has more than 80 % of its light vehicles running on bioethanol, reducing greenhouse gas emissions. At present, this demand for ethanol is being met through first-generation (1G) ethanol which is directly produced from sugarcane juice and molasses. However, significant research in bioenergy in the last two decades has shown the possibilities of commercialization of second-generation (2G) ethanol, which can be produced from sugarcane bagasse (SB) and straw (SS), complementing 1G ethanol. Nevertheless, both the residues (SB and SS) are an excellent source for cogeneration of heat and power (CHP) in sugarcane processing units. Process simulation studies have provided additional source of information on the overall use of sugarcane for ethanol production and CHP. For the evaluation of the fullest utilization of sugarcane and its by-products, CTBE (Brazilian Bioethanol Science

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and Technology Laboratory) has developed the Virtual Sugarcane Biorefinery (VSB), a comprehensive assessment framework to evaluate a sustainability standpoint (economic, environmental, and social), different biorefinery alternatives. This chapter reviews the important insights made into bioethanol production in Brazil. Technical configuration for 1G and 2G ethanol production and sustainability of ethanol (economic and environmental assessment) have also been discussed.

Keywords Brazilian bioenergy · Fuel ethanol · Sugarcane · Sugarcane residues · Techno-economic analysis · Environmental assessment · Bioelectricity · Second-generation ethanol

1.1 Introduction

Energy encompasses all the important features of the overall growth of human development. As per the human development index (HDI) set by the United Nations, nearly 4 kW per capita power consumption is required (Dale and Ong 2012). Developed countries reach this HDI by heavy usage of fossil energy; China, India, and other developing countries are approaching their increasing energy needs also with fossil fuels, whereas Brazil is the only exception, depending heavily on renewable energy. In the present scenario, fossil energy is the major source of energy (80 % of the world power consumption) in the form of oil (35.03 %), coal (24.54 %), and gas (20.44 %) (Goldemberg 2007). The use of fossil energy is considered as one of the most important man-made factor impacting on global economy and weather (Vertès et al. 2006). It is a widely accepted fact that fossil energy sources are finite and generally exported from politically unstable nations. Moreover, continuous huge demand of gasoline, low and expensive recovery yields, and oil spills are making the situation worse. The limited sources of fossil fuels in the world may not fulfill the increased demand for gasoline in the future. Already, experts have claimed that "peak-oil" (conferring the maximum rate of oil production) has arrived and the oil production rate after this point must decline (Kerr et al. 2011).

Keeping all the aforementioned points in view, the momentum is shifting toward the implementation of renewable energy sources. Biomass derived fuels have the potential to create a transition in the global economy from fossil fuel to a renewable fuel economy, however, it needs intensive technological and multidisciplinary efforts (Vertès et al. 2006; Yuan et al. 2008; Herrera 2006; Ohlrogge et al. 2009). Among the renewable energy sources (constituting around 13.61 %), biomass derived fuels, particularly "bioethanol" is gaining significant importance due to its inherent properties. Ethanol produced from cane juice (in Brazil) and corn starch (in USA) is already an established energy commodity. Brazil and USA are the two major countries that have successfully implemented bioethanol as an alternative

energy source and have shown the signs of global energy commodity making ethanol fully competitive with gasoline and suitable for replication in other countries like India, China, etc. (Goldemberg 2007). Approximately >37.85 billion liters of ethanol (today this figure has more than doubled) is produced globally per year from corn, sugarcane, and sugar beet through fully mature and well-established processes (Rass-Hansen et al. 2007).

1.2 Brazil and Bioethanol

Sugarcane juice derived ethanol has replaced nearly 50 % of gasoline consumption in Brazil. Ethanol production and its implementation have achieved unprecedented success in Brazil as a fuel and gasoline additive. Sugarcane productivity and technical advancements led the ethanol production increase from 0.6 billion liters in 1975/1976 to 24 billion liter in 2012/2013 (Goldemberg 2013; Canilha et al. 2013).

In 2012/2013, it is expected that Brazilian sugar–alcohol mills will process more than 602 million tons of sugarcane, accounting for the production of roughly 39 million tons of sugar and 24 billion liters of ethanol. Experimentally, each ton of processed sugarcane generates approximately 270–280 kg of bagasse and 140 kg of straw (Canilha et al. 2013). Therefore, taking this value into account, it can be estimated that Brazilian mills will produce around 163–169 million tons of sugarcane bagasse and 84 million tons of straw in the 2012/2013 harvest (Canilha et al. 2013). In addition to sugarcane juice derived ethanol (first-generation), the exploration of lignocellulosic residues of sugarcane (bagasse and straw) also has a great potential for ethanol production (Chandel et al. 2012a; Dias et al. 2012a, b). In Brazil, tremendous research efforts are on the way to develop a robust technological setup for the cellulosic ethanol production at commercial scale. Sugarcane is a primary source of renewable energy in Brazil and can be considered as a model feedstock for bioethanol production. The net energy balance (ratio of energy contained in a given volume of ethanol divided by the fossil energy required for its production) for ethanol production from sugarcane is very high (8.2–10) compared with other feedstock sources such as corn (1.3), sugar beet, and wood (approximately 2) (Goldemberg 2008).

The Brazilian National Alcohol Program (PROÁLCOOL) was launched in 1974 to decrease gasoline consumption and thus reduce oil imports. Since then, it has gained significant success and today there is no more government subsidy to the producers (Goldemberg 2008). Nowadays, in the Brazilian automobile sector, more than 90 % of new cars are flex-fueled driven which can run on gasoline as well as on ethanol. Since 1976, ethanol saved 1.51 billion barrels of gasoline correspondingly saving 75 billion US$ (BNDES and CGEE 2008). The successful Brazilian ethanol program can be a learning curve for other countries. Table 1.1 shows the data on ethanol production in various countries and the projected demand per year of ethanol up to 2020/2022.

Table 1.1 Profile of gasoline consumption, ethanol production, and futuristic ethanol demand as per the mandates up to 2020/2022

Country	Gasoline consumption in 2007 (billion liters)[a]	Ethanol production in 2008 (billion liters)[b]	Ethanol demand (on the basis of present mandates up to 2020/2022)
USA	530	34	136
European Union	148	2.3	8.51
China	54	1.9	5.4
Japan	60	0.1	1.8
Canada	39	0.9	1.95
United Kingdom	26	0.03	1.3
Australia	20	0.075	2.0
Brazil	25.2	27	19.6
South Africa	11.3	0.12	0.9
India	13.6	0.3	0.68
Thailand	7.2	0.3	0.7
Argentina	5.0	0.2	0.25
The Philippines	5.1	0.08	0.26
Total	943.2	67.3	178.7

Source [a] OECD/IEA (2010)
[b] REN21 (2009) and Goldemberg (2013)

1.3 Critical Analysis of Technological Routes for Cellulosic Ethanol Production

The production of bioethanol from lignocelullosic materials is assumed to present the largest potential among the possible alternatives to increase bioethanol production in the world without compromising food security, even though it is not yet a reality in an industrial scale (Kazi et al. 2010; Dias et al. 2012a). Lignocellulosic materials are abundant and cheap, do not compete with food crops (Ojeda et al. 2011; Dias et al. 2012a), and consequently, have larger potential to be used as feedstock for the production of sustainable biofuels (Dias et al. 2012a). Ethanol production from lignocellulosic biomass usually contains four major unit operations: (1) pretreatment, (2) enzymatic hydrolysis, (3) fermentation of sugars into ethanol, and (4) ethanol recovery.

The pretreatment is perhaps the single most crucial step as it has a large impact on all the other steps in the process (Galbe and Zacchi 2012). It is responsible for removing lignin or hemicellulose from the lignocellulosic material, and allows cellulose accessibility, enhancing the surface area of substrates for improved sugars recovery after enzymatic hydrolysis. An ideal pretreatment must meet the following requirements: minimum chemical requirement, low residence time, low investment cost, high amount of sugars recovery with less degradation of sugars or the ability to subsequently form sugars by hydrolysis, and minimum formation of inhibitory by-products (Kumar et al. 2009; Rocha et al. 2012). The pretreatment

process can be categorized into four major categories: physical, physico-chemical, chemical, and biological. Each type of pretreatment has inherent specificity in terms of mechanistic application on cell wall components with the applied conditions. Physicochemical and chemical pretreatment is fast, effective, but non-specific, and generates hemicellulosic derived inhibitors. Biological pretreatment methods are used for delignification but hampers by slow reaction rates and nonselectivity. The pretreated material needs to be submitted to enzymatic hydrolysis for the utmost sugars recovery (Agbor et al. 2011). The extent of enzymatic hydrolysis depends on lignin removal and the employed hydrolysis conditions. Hemicellulosic hydrolysates obtained after acid catalyzed reactions generally have cell wall derived inhibitors, i.e., furans, furfurals, phenolics, weak acids, among others. These inhibitors affect the efficiency of microorganisms employed in fermentation process leading to poor ethanol yields. It is essential to eliminate these inhibitors prior to microbial fermentation in order to obtain the desired ethanol yields. Several detoxification methods like evaporation, calcium hydroxide overliming, use of membranes, ion-exchange resins, activated charcoal, and enzymatic detoxification using laccases are in practice to overcome these inhibitors (Chandel et al. 2013a). Simultaneous detoxification of hydolysates and fermentation (SDF) is also possible for ethanol production using two different microorganisms (microorganism eliminating inhibitors + microorganism for ethanol production).

There are four configuration processes to produce ethanol from lignocellulosic biomass: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP) (van Zyl et al. 2011). The most common process is separate (or sequential) hydrolysis and fermentation (SHF), where hydrolysis of pretreated lignocellulosic material is done first and the resultant sugar solution is fermented separately into ethanol in different vessel/reactor. SHF is a lengthy process but has shown optimum sugars production followed by their conversion reaching the desired ethanol yields. Both the processes can be carried out employing the most appropriate conditions. After enzymatic hydrolysis, the solid material can be used for cogeneration of heat and electricity. SSF (simultaneous saccharification and fermentation) or SSCF (simultaneous saccharification and co-fermentation) are other configuration processes where the enzymatic hydrolysis of pretreated lignocellulosic material and the fermentation of released sugars or mixture of sugars (pentose + hexose) is carried out simultaneously. SSF/SSCF has shown great advantages over SHF in the terms of reducing processing time and process complexities. However, the temperature difference in both the reactions is of concern (Olofsson et al. 2008). Enzymatic hydrolysis usually shows the best results at 50 °C, while ethanol fermentation is done at 30 °C. Therefore, pre-hydrolysis at 50 °C can be inducted to initiate the hydrolysis for some time followed by the execution of fermentation reaction. To obtain the maximum ethanol yield, thermotolerant yeast or ethanol producers could be more relevant as they can grow and produce ethanol at the hydrolysis temperature (<50 °C). Furthermore, risk of contamination can be avoided using thermo

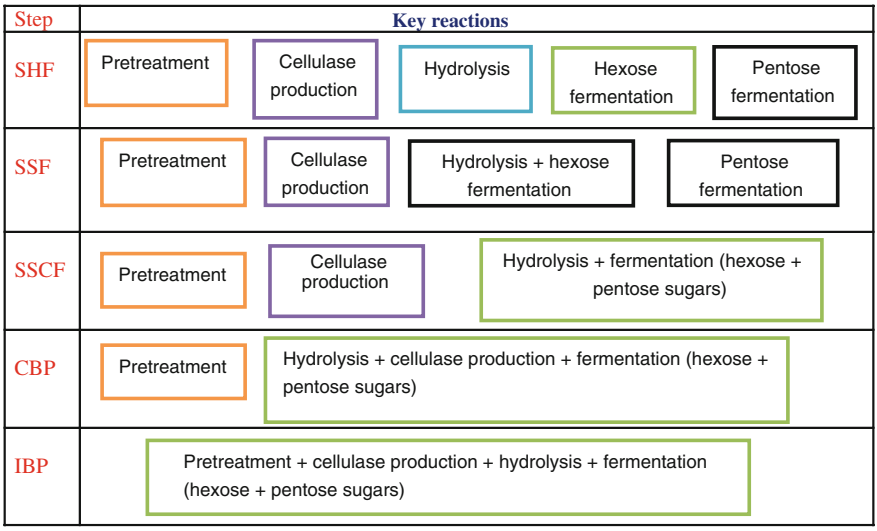

Fig. 1.1 Summary of technical routes for ethanol production from biomass under various process configurations. Each *box* represents the specific reaction performed in sequential order. *SHF* separate hydrolysis and fermentation, *SSF* simultaneous saccharification and fermentation, *SSCF* simultaneous saccharification and co-fermentation, *CBP* consolidated bioprocessing, *IBP* integrated bioprocessing

tolerant microorganism in SSF. Another advantage of SSF/SSCF configuration is to avoid the enzyme inhibition by the released glucose as it is simultaneously converted into ethanol by the microorganism. Moreover, capital cost investment and the processing time could be minimized by employing SSF/SSCF (Olofsson et al. 2008). The latest development in process configuration is CBP (consolidated bioprocessing), wherein the enzyme production and hydrolysis of pretreated lignocellulosic material followed by the fermentation of sugars can be performed in a single reactor (Olson et al. 2012). The key difference between CBP and the other strategies of biomass processing is that only one microbial community is employed both for the production of cellulases and fermentation (Cardona and Sánchez 2007).

Realizing the importance of process integration, IBP (integrated bioprocessing) could provide an important breakthrough in developing an economic and sustainable platform for cellulosic ethanol production. IBP includes the microbial assisted pretreatment of biomass followed by enzyme recovery and delignified biomass saccharification coupled with microbial conversion of released sugars into ethanol simultaneously in a single reactor (Chandel et al. 2013b). Nevertheless, ethanol production through IBP has not been tried as yet. In IBP, there is involvement of at least more than one microorganism (one microorganism for biodelignification and another microorganism for ethanol production). The recovered sugars solution from pretreated lignocellulosic biomass can be used for

ethanol production via modified fermentation strategies like fed-batch, recycling of immobilized cells in continuous fermentation, and semi-continuous processing. Figure 1.1 shows an overview of the technical configurations required in each processing routes for 2G ethanol production.

1.4 Process Simulation and Co-products Utilization

Process simulation using computational tools have been increasingly used to evaluate biorefinery configurations, since it allows the integration of process steps, technologies, and routes, thus providing critical information to assess technical feasibility, detecting process bottlenecks and potential advantages. In this context, CTBE (Brazilian Bioethanol Science and Technology Laboratory), one of the national laboratories of the Brazilian Center of Research in Energy and Materials (CNPEM) developed a comprehensive tool—VSB (Virtual Sugarcane Biorefinery)—based on simulation platforms for the evaluation of different technologies through assessment of their sustainability indicators (economical, environmental, and social) (CTBE 2012).

The integration of 1G (from sugarcane juice) and 2G (from bagasse and straw) was assessed using the VSB. The main process configurations and parameters adopted in the construction of the VSB are described in the following sections.

1.4.1 Process Simulation for 1G Ethanol Production

According to BNDES and CGEE (2008), approximately 70 % of the sugarcane processing units in Brazil are annexed plants. First-generation ethanol production from sugarcane takes place in autonomous distilleries or annexed plants. Autonomous distilleries produce only ethanol. In an annexed plant, a fraction of the sugarcane juice is diverted for sugar production and the remaining fraction along with the molasses (residual solution of sugars that came from sucrose crystallization) is used for ethanol production. Usually, the annexed plant operates using half of sugarcane juice for sugar production. Part of the reason for the success of ethanol production in Brazil is the flexibility of annexed plants to produce more ethanol or more sugar, depending on market demands.

The sugarcane processing facility is self-sufficient on its energy consumption: all the thermal and electric energy required for the production process is produced in combined heat and power generation (CHP) systems using bagasse as a fuel. If sugarcane straw is recovered from the field, it may also be used as a fuel to increase energy generation. A scheme of the sugar, ethanol, and electricity production process from sugarcane is shown in Fig. 1.2. In an autonomous distillery, the unit operations related to the sugar production (left side of Fig. 1.2) is not included in the sugarcane mill.

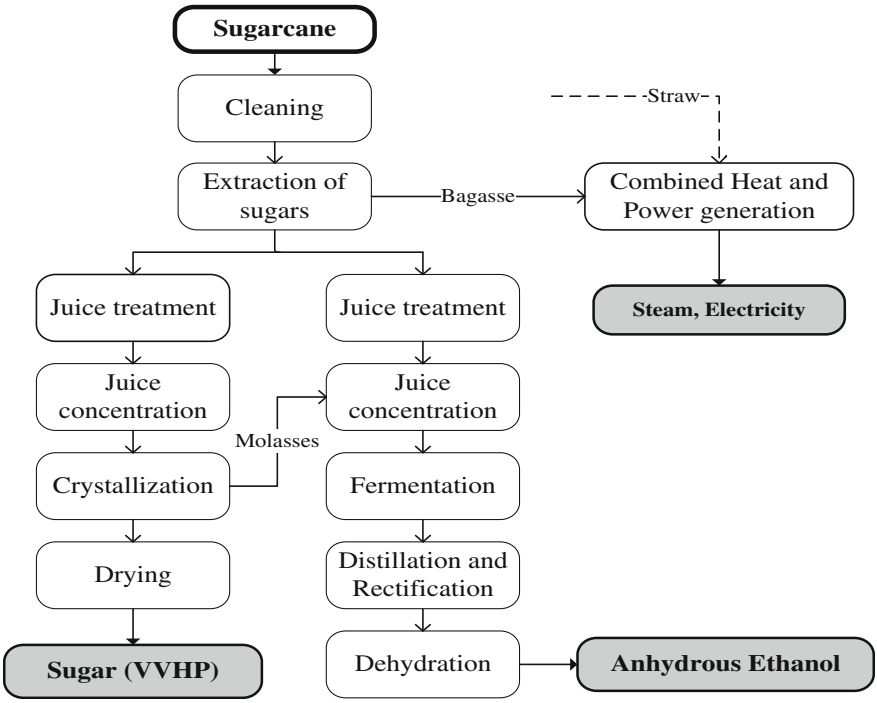

Fig. 1.2 Block-flow diagram of the production of sugar, ethanol, and electricity from sugarcane (CTBE 2012)

1.4.1.1 Process Description and Governing Parameters of the Sugarcane Processing Facility

The basic configuration of an annexed plant (1G) and the related process parameters for ethanol and sugar production have been summarized in this section. The capacity of the sugarcane processing facility has been considered for the processing of 500 metric tons of sugarcane (TC) per hour, during 167 days/year and processing a total of 2 million TC/year.

Sugarcane Reception and Cleaning

The sugarcane arrives at mills with dirt and other impurities dragged in the harvesting process. Therefore, upon reception in the factory, sugarcane must be cleaned. The efficiency of dirt removal in sugarcane washing is 90 % (BNDES and CGEE 2008). Sugarcane cleaning is usually carried out using wash water, which is recycled to the cleaning process after removal of dirt and other impurities.

The amount of sugar lost during the whole sugarcane washing may be calculated as 25 % of the losses for the mechanically harvested sugarcane washing (3.2 kg/TC) as observed by Rein (2007). However, usually no washing is carried out for mechanically harvested (chopped) sugarcane due to the high sugar losses that would occur. The same authors consider that the average amount of water dragged with sugarcane during washing is 7.5 t/100 TC.

Sugarcane Processing and Juice Extraction

After cleaning, sugarcane is fed to the cane preparation system, on which a series of equipment (shredder, hammers, etc.) are used to cut open the sugarcane structure and enhance sugar extraction in the following operation. After preparation, sugarcane passes over a magnet that removes eventual metallic particles dragged along prior to entering the mills.

Juice extraction is usually done using crushing mills, where sugarcane juice and bagasse are separated. Water at a rate of 28 wt% of the sugarcane flow (imbibition water) is used to improve sugars recovery. The imbibition water temperature is 50 °C (Ensinas 2008) and the efficiency of sugar extraction in the mills is 96 % (Walter et al. 2008). Sugarcane juice contains water, sucrose, and reducing sugars, in addition to impurities such as minerals, salts, organic acids, dirt, and fiber particles, which must be removed prior to fermentation. A rotary screen is used to remove solid particles from the juice. The fibers obtained in this screen return to the mills for further recovery of sugars, while the juice is sent to juice treatment. Efficiency of dirt and bagasse removal in the screen is around 65 % (Mantelatto 2010).

Juice Treatment

The aim of the juice treatment process is to separate as much as possible the dissolved and suspended juice impurities without reducing sucrose concentration. It must be done soon after milling to prevent yeasts and enzymes action. Thus, following extraction, the juice undergoes chemical treatment to remove other impurities. This process consists of juice heating from 30 to 70 °C, addition of phosphoric acid or lime followed by a second heating operation, up to 105 °C (Copersucar 1987). Hot juice is flashed to remove dissolved air and after addition of a flocculant polymer, impurities are removed in a settler, where mud and clarified juice are obtained. A filter is used to recover some of the sugars carried along with the mud, and the separated solids are recycled to the process prior to the second heating operation. Bagasse fines, also called bagacillo, and wash water are used in the filter to improve recovery of sugars. The clarified juice is fed to the screens to remove solid particles that were not removed in the clarifier. The clarified juice, at 98 °C, destined for sugar production, contains around 15 wt% solids (Mantelatto 2010) and it is concentrated on a five-stage multiple effect

evaporator (MEE) up to 65 wt% solids. In the annexed distillery, a fraction of the syrup, as well as final molasses, are used to concentrate the clarified juice destined for ethanol production up to around 22 wt% solids, which is cooled and fed into the fermenters.

Sugar Production

The sucrose present in the syrup as sugar crystals is separated from the solution in equipment called vacuum pans and crystallizers, usually operated under vacuum and in fed-batch mode. The syrup is fed into the vacuum pans, where water is removed in a similar way as in the evaporators. The mixture of sugar crystals and molasses (liquid part) inside the equipment is called massecuite. When the amount of material reaches the limit of the vacuum pan (at the end of a batch), the massecuite is transferred to crystallizers and, after an appropriate residence time, it is sent to centrifuges that separate the crystals and the molasses. It is possible to exhaust more the molasses (recuperating more sugar) by repeating the process one or two more times. The washing water temperature (at centrifuges) is 110 °C (Mantelatto 2010).

It is assumed that crystals are separated using the two-boiling system approach, where two types of sugars are produced (Jesus 2004): grade "A" sugar (final product) and grade "B" sugar (intermediate sugar that is produced and recycled inside the process as "B" Magma, a solid–liquid stream rich in sugar crystals). The Brix of the "A" sugar is 99° (Ribeiro 2003) and purity (VVHP—very very high polarization) 99.6 % (Bazico 2010). For the "B" sugar, Brix is 98° (Ribeiro 2003) and purity 88 % (Camargo 1990). The final sugar is dried in a rotary dryer at 100 °C (Camargo 1990) and cooled before shipment.

Fermentation for Ethanol Production

After the juice treatment, concentrated juice is mixed with molasses and sent for ethanol production in the fermenters. A fed-batch fermentation process with cell recycle is assumed. The temperature of fermentation is 33 °C and conversion of sugars into ethanol is about 89.5 % (Mantelatto 2010), which is slightly lower than the conversion in an autonomous distillery, due to the presence of molasses from sugar production. In this process, yeast cells solution is fed to the fermenters prior to sugarcane juice addition. During fermentation, gases released in the fermenters are collected and sent to an absorption column where the entrained ethanol is recovered using water. After the completion of fermentation reaction, the wine is sent to the centrifuges, where cells are separated from the ethanol solution. Cells obtained in the centrifuges are treated in a separate reactor by the addition of sulphuric acid and water, to decrease bacterial contamination. After this treatment, the cells are recycled to be used in another batch. Some part of the yeast cream, also known as alcohol distillery yeast extract, is removed before being recycled.

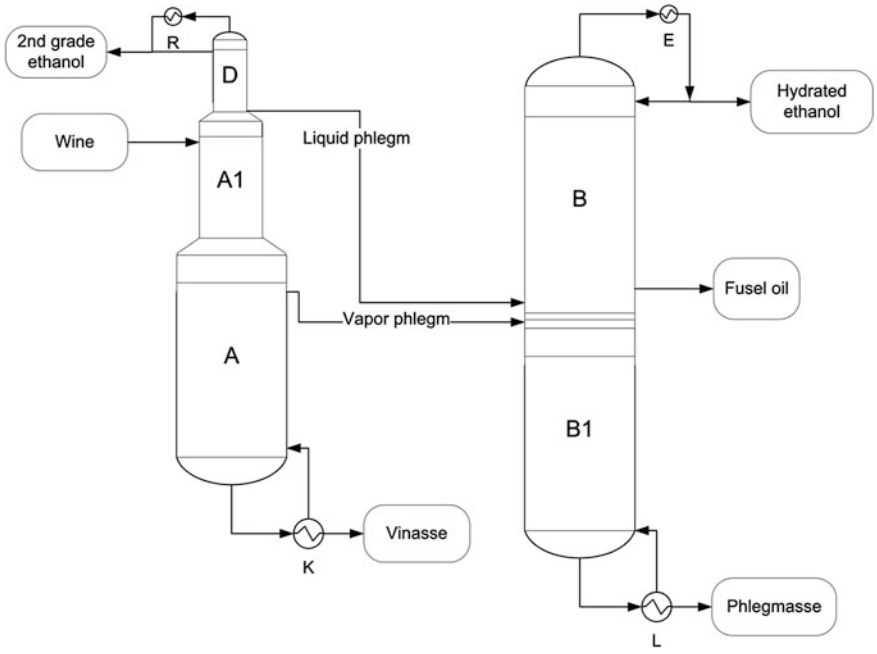

Fig. 1.3 Simplified scheme of the distillation columns (CTBE 2012)

This product is used mostly as protein source for animal feed. The produced wine is mixed with the alcoholic solution obtained in the absorption process (to recover ethanol from the CO_2 stream) and sent for purification. Ethanol content in the wine fed to the distillation columns is 8.5°GL (Mantelatto 2010).

Distillation

The distillation aims at concentrating the wine until alcoholic content is up near the azeotropic point for the hydrated ethanol production, with ethanol content between 92.6 and 93.8 wt% (92.6 and INPM 93.8°) (Dias 2008). Wine is sent to a series of distillation and rectification columns (Fig. 1.3). Distillation columns comprise two set of columns A, A1 and D, and rectification columns B1 and B, each located one above the other. Wine is preheated in the condenser of column B (heat exchanger E) and by exchanging heat with the bottom of column A (heat exchanger K) before being fed into the top of column A1. Ethanol-rich streams (phlegm) are obtained on top of column A and at the bottom of column D, and then fed to column B-B1. Vinasse is produced at the bottom of column A, containing less than 200 ppm of ethanol, while second grade ethanol is obtained from the top of column D. Hydrated ethanol is produced on top of column B and nearly pure

water (phlegmasse) is obtained at the bottom of column B1. Fusel alcohol, containing most of the higher alcohols, is obtained as a side withdrawal in column B.

Dehydration

The hydrated ethanol must be dehydrated to achieve alcohol content over 99.3 % (mass) to be blended with gasoline. The ethanol dehydration cannot be made by conventional distillation due to the azeotropic nature of ethanol solution (95.6 % mass) at atmospheric pressure. Thus, alternative methods of separation must be used to produce anhydrous ethanol (Dias 2008). The dehydration process for anhydrous ethanol production can be carried out considering azeotropic distillation with cyclohexane or adsorption on molecular sieves. The adsorption on molecular sieves is a separation process with reduced energy consumption and without solvent if compared to the azeotropic distillation. In this process, a zeolite bed is used to adsorb water from hydrated ethanol to produce anhydrous ethanol. Usually three beds are used, one of which is always in regeneration, to remove accumulated water.

Combined Heat and Power Generation

Traditionally, cogeneration systems (CHP—combined heat and power generation) used in Brazilian sugarcane mills are based on the Rankine cycle (Fig. 1.4). During sugarcane processing, the juice is separated from the fibers, which produces large amounts of bagasse (approximately 140 kg/TC, dry basis). This bagasse is used as a fuel in the cogeneration system to supply thermal, mechanical, and electrical demand for sugar and ethanol production process. Formerly, low efficient boilers (22 kg f/cm^2) were used to produce steam and electricity for the plant. However, the restructuring of the electricity sector in Brazil and the incentives for energy production from renewable sources have driven to an increase in investments for the production of surplus electricity in the mills. As a result, more efficient boilers and turbines have been employed in order to produce high pressure steams (65 kg f/cm^2), and generate large amounts of electricity. As a consequence, new sugarcane mill projects considering the use of Rankine cycles with steam at higher levels of temperature and pressure, employing extraction-condensing steam turbines and burning all bagasse produced in the mills have been developed. The surplus electricity generated in this new configuration can be sold to the power grid, improving the revenues of the company.

The amount of electricity produced by the sugarcane processing plant may be increased significantly when sugarcane straw is collected from the field and transported to the factory for further processing. Around 140 kg of straw (dry basis) is produced per ton of sugarcane stalks, but part of the straw is usually left in

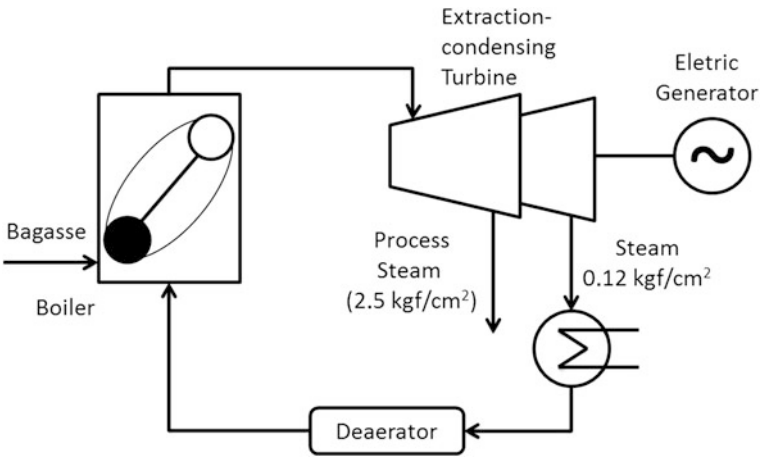

Fig. 1.4 Scheme of back-pressure and extraction-condensing turbines based on Rankine cycle (CTBE 2012)

the field in order to provide for weed and disease control as well as nutrient recycling (Hassuani et al. 2005). However, removal of 50 % of the straw from the fields is considered feasible (Dias et al. 2009; Hassuani et al. 2005; Walter and Ensinas 2010).

Besides being used as a fuel in boilers for the production of steam and electricity, sugarcane lignocellulosic material (bagasse and straw) may also be used as feedstock for second-generation ethanol production. Since it is composed basically of cellulose, hemicellulose, and lignin, it may be converted into fermentable sugars (hexoses and pentoses) through pretreatment and hydrolysis processes. Nevertheless, the amount of surplus lignocellulosic material used as feedstock depends on the energy consumption of the whole production process. In this way, reduction on process steam demand may lead to an increase in the amount of surplus bagasse and straw, which can be employed as feedstock for second-generation ethanol production when lignocellulosic material hydrolysis technologies are available. The residues of the pretreatment and hydrolysis operations (residual cellulose, lignin, and eventually biogas from pentoses biodigestion) may be used as fuels increasing the amount of lignocellulosic material available as feedstock for 2G (Dias et al. 2011, 2012a, b).

Different cogeneration systems were simulated in VSB to represent the integrated 1G and 2G ethanol production processes (Fig. 1.5). The considered alternatives for cogeneration systems as well as the main parameters adopted in the computer simulations are shown in Table 1.2.

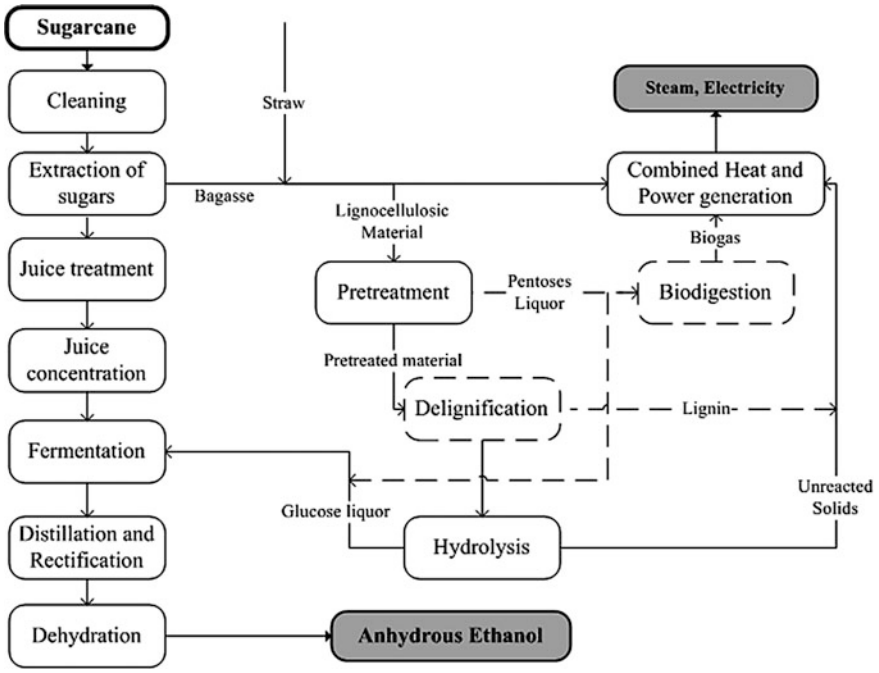

Fig. 1.5 Block-flow diagram of the integrated first- and second-generation ethanol production process from sugarcane (CTBE 2012)

Table 1.2 Main parameters adopted in the cogeneration system (CTBE 2012; Dias et al. 2013a)

Parameters	Boiler pressure (kg f/cm^2)			
	22	42	65	90
Steam pressure (kg f/cm^2)	22	42	65	90
Steam temperature (°C)	300	400	485	520
Steam production (kg steam/kg bagasse)	2.50	2.36	2.23	2.18
Boiler efficiency—LHV basis (%)	85.8	87.0	87.2	87.7
Gases outlet temperature (°C)	180	160	160	160
Electricity demand—direct drives (kW h/TC)	16	16	–	–
Electricity demand—electric drives (kW h/TC)	30	30	30	30
Direct steam drive efficiency (%)	55	55	–	–
Steam turbines efficiency (%)	78	78	85	85
Generator efficiency (%)	98	98	98	98

1.4.2 Process Simulation for 2G Ethanol Production

Currently, one of the greatest concerns worldwide is the large-scale production of alternative forms of energy, such as biofuels, which could reduce greenhouse gases emissions and improve energy security when compared to their fossil counterparts

(Chavez-Rodriguez and Nebra 2010). In this context, bioethanol has received special attention, as it is already produced in large scale and used as automotive fuel (Seabra et al. 2010).

In first-generation plants, sugarcane juice is used for sugar and ethanol production, while sugarcane bagasse is used as a fuel in the boilers, providing heat and power to the plant. However, in the context of expansion of the production and consumption of ethanol, bagasse is considered as a potential feedstock for 2G ethanol, since it does not compete with food crops and is less expensive than conventional agricultural feedstocks (Alvira et al. 2010). In this case, 2G ethanol production processes can be integrated with 1G ethanol plants, sharing part of the infrastructure such as juice concentration, fermentation, distillation, cogeneration, and water cooling systems. Another important residue that may be employed for bioethanol production in the sugarcane industry is sugarcane straw, which includes sugarcane leaves and tops, usually burnt or left in the field (Dias et al. 2011; Macrelli et al. 2012).

For integration of 2G ethanol process with 1G, biomass pretreatment and hydrolysis are usually considered in the processing of lignocellulosic material, since it does not contain monosaccharaides readily available to be fermented. In some cases, fermentation of the pentoses released during the pretreatment step to ethanol can also be carried out; however, conventional microorganisms employed in alcoholic fermentation are not able to ferment pentoses.

Preliminary assessments were carried out using VSB considering two levels of development: current technology—hydrolysis with low yield and low solids loading and biodigestion of C5 liquor—and a second level, potentially available in 2020 (futuristic scenario)—hydrolysis with higher yield and solids loading, C5 fermentation into ethanol, and lower investment and enzyme cost. Process alternatives are shown in Fig. 1.5. Operational conditions and yields are described in subsequent sections.

Due to the high recalcitrance of biomass, pretreatment process is required to increase the accessibility of cellulolytic enzymes toward cellulose (Alvira et al. 2010). Although different types of pretreatments were tested in different conditions over the years, advances are still needed for overall costs to become competitive (Rabelo et al. 2011; Chandel et al. 2010a). In VSB, steam explosion is defined as the pretreatment process where most of the hemicellulose is hydrolyzed into pentoses, with small cellulose loss and no lignin solubilization (Ojeda et al. 2011). The pretreated solids are separated from the pentoses liquor via filtration. In order to allow an increase in hydrolysis yield for the futuristic scenario, an alkaline delignification step of the solid fraction was included after pretreatment, so most of the lignin is removed, decreasing its inhibitory effects on the following enzymatic hydrolysis step (Rocha et al. 2012). Table 1.3 presents main operational conditions and yields for steam explosion pretreatment and alkaline delignification. Cellulose obtained from pretreatment is converted into glucose after saccharification using cellulolytic enzymes. Enzymatic hydrolysis allows the process to be carried out in milder conditions than acid hydrolysis. In addition, enzymes offer the advantage of producing higher yields of sugars with little degradation (Mussatto et al. 2010;

Table 1.3 Parameters adopted in the pretreatment and deliginification processes (CTBE 2012)

Parameter	Value
Pretreatment—hemicellulose conversion	70 %
Pretreatment—cellulose conversion	2 %
Pretreatment—temperature	190 °C
Pretreatment—reaction time	15 min
Alkaline delignification—lignin solubilization	90 %
Alkaline delignification—temperature	100 °C
Alkaline delignification—reaction time	1 h
Alkaline delignification—solids loading	10 %
Alkaline delignification—NaOH content	1 % (m/V)

Table 1.4 Parameters adopted in the enzymatic hydrolysis and sugars recovery (CTBE 2012)

Parameters	Values
Hydrolysis—cellulose conversion (current/future scenarios)	60/70 %
Hydrolysis—hemicellulose conversion (current/future scenarios)	60/70 %
Hydrolysis—solids loading (current/future scenarios)	10/15 %
Hydrolysis—reaction time (current/future scenarios)	72/48 h

Table 1.5 Parameters adopted in C5 biodigestion and fermentation for 2G ethanol production (CTBE 2012)

Parameters	Values
Pentose biodigestion—chemical oxygen demand (COD) removal	70 %
Pentose fermentation to ethanol	80 %

Chandel et al. 2012b). Tables 1.4 and 1.5 show the enzymatic hydrolysis operating conditions and sugars yields.

Saccharomyces cerevisiae is one of the traditionally used microorganisms in 1G ethanol production from corn and sugarcane due to its high efficiency in fermenting hexose to ethanol, and superior tolerance to low pH (Zhang et al. 2010) and high ethanol concentration. However, for use in the 2G ethanol production process, microorganisms that can convert C5 sugars into ethanol are limited and generally have low ethanol and inhibitors tolerance and take longer incubation times (Girio et al. 2010). In order to increase sugar yields, efficient conversion and utilization of hemicellulosic sugars has become an important task and an opportunity to reduce ethanol production cost (Alvira et al. 2010).

Alternatively, C5 liquor may be biodigested, producing biogas for use as fuel, increasing the amount of surplus lignocellulosic material. Pentoses biodigestion

Table 1.6 Some examples of the process simulation for analysis of cost incurred for ethanol production from various feedstock

Raw material	Production technology	Production cost of ethanol (US$/L)	Reference
Sugarcane bagasse	1G + 2G ethanol	0.39	Dias et al. (2012c)
Corn stover	Dilute acid pretreatment, enzymatic hydrolysis and ethanol fermentation	1.36/L of ethanol (gasoline equivalent)	Kazi et al. (2010)
Corn stover	SSCF	6 US$/gallon	Lynd et al. (2005)
Corn stover	CBP	1.11 US cents/L	Lynd et al. (2005)
Sugarcane bagasse	1G + 2G ethanol	0.40	Macrelli et al. (2012)
Corn stover	Ion-liquid pretreatment	6 US$/gallon	Klein-Marcuschamer et al. (2011)
Straw		0.73	Gnansounou and Dauriat (2010)
Eucalyptus		0.56	
Poplar		0.76	
Switchgrass		0.71	
Tall Fescue (*Festuca arundinacea* Schreb)	Dilute acid	0.83	Kumar and Murthy (2011)
	Dilute alkali	0.88	
	Hot water	0.81	
	Steam explosion	0.85	
Empty fruit bunches	Dilute acid hydrolysis, enzymatic hydrolysis, and fermentation with recombinant *Zymomonas mobilis*	0.49 (with cogeneration) 0.58 (without cogeneration)	
Rice husk	Dilute acid hydrolysis, enzymatic hydrolysis, and fermentation with recombinant *Z. mobilis*	0.53	Qunitero et al. (2013)
Coffee cut stems		0.585	
Sugarcane bagasse		0.684	

and fermentation parameters are shown in Table 1.5. Other applications of pentoses include production of xylitol, lactic acid, 2, 3-butanediol, butanol, furfurals, and other valuable products (Chandel et al. 2010b; Girio et al. 2010). However, fermentative production of D-xylitol from hemicellulosic hydrolysate has been considered one of the most beneficial processes to cater to the needs of various commercial sectors (Silva and Chandel 2012) (Table 1.6).

1.4.3 Screening Method Applied to Analysis of Technological Parameters in 1G Ethanol Production Process

Mathematical models are useful tools for development, analysis, and optimization of industrial processes. Models can be defined as a dataset and abstract ideas used to explain a phenomenon of interest and relate the parameters of a given process. A well-adjusted model can predict the parameters behavior so precisely that it becomes a practical and cheap way to obtain information about the process under study. Therefore, if the model is improved, it also improves the description of the reality.

Incorporating mathematical models into computational simulation platforms is not frequently applied to sugarcane-based biorefineries due to its complexity, specificity, variability, interaction with environment, and other inherent characteristics.

In VSB, the simulation for 1G ethanol production is described by variables such as fermentation yield, steam consumption, steam pressure in boiler, among others. The variable values used in this simulation were collected initially from the literature or provided by specialists. In addition, this information was complemented and validated with data from Brazilian sugar and ethanol mills. However, inspite of intense efforts in collecting variable values, this process is a difficult task in the modeling procedure of 1G ethanol production due to its complexity and natural variability.

In this context, screening methods are presented as useful tools to quantify the impact of inputs variations on a given model response (Ruano et al. 2012). Therefore, if a small change in an input variable leads to a large variation in a certain response parameter of the model, this variable is considered important and its determination must be as precise as possible (Cangussu et al. 2003). Assuming that only some input variables contribute significantly to the outcome, screening methods facilitate data collection by limiting the maximal precision to inputs considered most important (Rivera et al. 2013).

Besides being used to obtain information about the degree of importance of each variable, screening methods are frequently used to validate the model itself. This validation reports whether the model follows (or not) an expected behavior. In the simulation for 1G ethanol production in VSB, after screening procedure the ranking of technological parameters can be analyzed to assess if the results agree with what is expected for the current ethanol production in Brazil. Therefore, specialized information (practical knowledge) from professionals is extremely important for analysis and improvements of this model. As a result, screening methods are also mechanisms to detect and adjust model inadequacies (Cangussu et al. 2003). Screening procedure can be performed through design of experiments (DOE) such as central composite design (CCD) (Montgomery 2001) and simulation models.

A study performed in the VSB context illustrates the efficiency and usefulness of CCD as a method to screen the main variables in 1G ethanol production process. Initially, the main input variables of the process were identified as: (i) fermentation yield, (ii) steam consumption, (iii) steam pressure in boiler, (iv) juice extraction yield, (v) residual ethanol concentration in vinasse, and (vi) alcohol content in wine. The influence of these variables on ethanol output and surplus electricity has been studied in the screening procedure. The interpretation of the results was accomplished from the analysis of the model features and the expected behavior of the input variables, bearing in mind the knowledge of the current 1G ethanol production. Therefore, the analysis involved the collaboration among the specialists in the sugarcane sector and CTBE research team.

Variables under study were ranked by CCD with a significance level of 99 %. For Ethanol Output, the input variables juice extraction yield and fermentation yield were shown to be significant. An efficient juice extraction means that a large amount of sugar will be available to the fermentation process without increasing the amount of milled cane. The variable fermentation yield is of significance as it influences directly on the amount of ethanol produced and also in the main dependent variables such as, fermentation time, volume of fermenter, among others.

The variables steam consumption reduction, resulting from energy integration in the production process, and steam pressure in boiler were significant for the surplus electricity parameter. Decreasing the consumption of steam there will be more steam available for the cogeneration process; therefore, more electricity will be produced. The boiler steam pressure is directly related to the electricity cogeneration. More electricity will be produced by the plant with a higher boiler pressure.

The screening procedure was successfully used to identify the relevant variables in 1G ethanol production process. In this procedure, the CCD proved particularly efficient to obtain information about the significance of the input variables. Thus, it was concluded that through screening methods it is possible to understand the behavior of the technological parameters and compare it with the current process.

1.5 Techno-Economic Analysis of Sugarcane-Based Biorefineries

In order to provide a comparison in terms of economic viability, important indicators from Economy Engineering, such as internal rate of return (IRR), production costs of products, beyond others, can be estimated considering a set of scenarios related to different biorefinery alternatives. The principles for the evaluation of economic viability are based on a cash flow projected for each technological scenario to be evaluated, taking into account the investment needed for the project and all expenses and revenues for an expected project lifetime. The main operating expenses (OPEX) and revenues might come from mass and energy

balances obtained from computer process simulation. The basis for the monetary values related to the capital expenses (CAPEX) can be obtained from the literature, consulting with engineering companies, experts, and others.

Several studies were carried out at CTBE following the techno-economic and environmental aspects of first- and/or second-generation ethanol production from sugarcane (Dias et al. 2012a, b, 2013a, b; Cavalett et al. 2012; Junqueira et al. 2012). In this section are summarized the most important findings from the previous studies carried out at CTBE related to the techno-economic analysis and environmental impacts of 1G ethanol and 2G ethanol productions from various biorefinery configurations.

1.5.1 1G Ethanol Production Process

Environmental and economic aspects of autonomous distilleries and annexed plants in Brazil were compared by Cavalett et al. (2012). In addition, optimized technologies for autonomous distilleries and annexed plants were considered in the study and benefits of more flexible scenarios for annexed plants were examined. In such configurations, CAPEX proved to be an important issue, since it increases from autonomous to annexed plants and from fixed to flexible plant, having significant impact on the IRR. Another important observation was that annexed plants present higher IRR for both flexible (favoring sugar production) and fixed plants. Although autonomous distillery also presented good results, it is important to take into account that market oscillations can considerably change and flexibility may be decisive for maintaining and even improving the sugarcane biorefinery profitability.

1.5.2 Integrated and Stand-Alone 2G Ethanol Production Processes

Dias et al. (2012c) evaluated different scenarios for integrated and stand-alone 2G ethanol production from sugarcane bagasse and straw. Five scenarios were selected to demonstrate the economic and environmental impacts of 2G ethanol production in comparison to an optimized autonomous 1G ethanol production plant in Brazil. Results showed that the current integrated 1G and 2G ethanol production scenario, characterized by higher investment cost in 2G (due to the fact it will be one of the first plants), higher enzyme cost, lower yield, and lower solids load in the hydrolysis step presents lower IRR in comparison to the optimized 1G ethanol production. However, the integrated 1G and 2G ethanol production considering future scenarios, where target parameters are used for second-generation processes and ethanol can be also produced from C5 sugars, is more attractive to the investor than the optimized 1G ethanol production.

1.5.3 Different Process Configurations for 2G Ethanol Production Process

Junqueira et al. (2012) assessed economic and environmental impacts of different options for the 2G ethanol production process integrated to the 1G sugarcane biorefinery. The study evaluated two pretreatment options (steam explosion and hydrothermal processing), as well as two alternatives for pentose utilization (biodigestion and fermentation to ethanol). A delignification step was also analyzed after both pretreatments. Results showed that hydrothermal pretreatment based on liquid hot water has higher energy consumption than steam explosion; consequently, larger ethanol production is obtained from steam explosion pretreated bagasse. These results are in accordance with economic and environmental analyses, which shows that the process with steam explosion presents the largest IRR and lower life cycle environmental impacts.

Further, Dias et al. (2012b) evaluated different configurations for the 2G ethanol production process (e.g., pretreatment with steam explosion coupled or not with delignification, pentose biodigestion or fermentation to ethanol, solids loading for hydrolysis) in an integrated 1G and 2G ethanol production biorefinery. The results were used to evaluate which process alternatives provide higher ethanol yield, pointing toward the direction in which research should be oriented. Computer simulations of integrated 1G and 2G ethanol production process from sugarcane showed that high hydrolysis yields (that may be achieved using low solids loading on the hydrolysis reactor) do not lead to the best results in terms of overall ethanol production. Because the lignocellulosic material (sugarcane bagasse and straw) used as feedstock in 2G is also used as fuel, low solids loading requires more steam on the concentration step. Therefore, even though that scenario leads to the highest 2G ethanol production per lignocellulosic material processed (around 200 and 400 L/t dry lignocellulosic material (LM) for the processes with pentose biodigestion and fermentation, respectively), lower yields and higher solids loading lead to larger amounts of ethanol produced per ton of sugarcane (up to 122 L/TC for 20 % solids, as opposed to 116 L/TC for the process with pentose fermentation and 5 % solids loading in hydrolysis). This study confirmed the importance of evaluating the whole process to better understand it and to guide further experiments aiming at the viability of 2G ethanol production process.

Dias et al. (2013a) evaluated different cogeneration systems configurations for integrated 1G and 2G ethanol production, as well as different destinations for the pentose (biodigestion or fermentation to ethanol) obtained after pretreatment of the LM. Economic analyses showed that coupling electricity production with 2G ethanol production in the integrated process improves its economic results, even when electricity is produced in relatively low amounts using low efficiency boilers (22 bar boilers). Another interesting finding of the study is that high pressure boilers (82 bar) consume more bagasse than low pressure boilers, thus decreasing final ethanol output. Nevertheless, revenues obtained with the sale of electricity in the processes employing cogeneration systems with high pressure boilers outweigh

the losses in ethanol yield and the increased investment of these cogeneration systems. Among the evaluated process configurations, the one with 65 bar boilers presents the lowest environmental impacts in most categories including global warming potential. In the context of C5 use, pentose fermentation allows a large increase in the total ethanol production (40–50 % higher than 1G production) compared to the gains of pentose biodigestion (around 30 %).

1.5.4 Improving 2G Ethanol Production Through Optimization of 1G Plant

Dias et al. (2012a) evaluated some improvements in the 1G ethanol production process, aiming at reducing process steam consumption; maximizing surplus LM; or increasing electricity output. The process improvements analyzed in the study decreased the steam consumption in the 1G ethanol production in an autonomous distillery. Results showed that a considerable increase in the amount of surplus LM can be obtained with the use of efficient cogeneration systems (among other process improvements) and the recovery of 50 % of the straw. Significant increase in ethanol production in optimized autonomous sugarcane distilleries integrated with 2G ethanol production, along with electricity production, can be obtained if high pressure boilers are employed. Gains on ethanol production in an integrated 1G and 2G ethanol production process are possible when efficient, low pressure boilers are employed.

1.5.5 Flexibility on 2G Ethanol and Electricity Production

Dias et al. (2013b) evaluated a flexible biorefinery with the ability of diverting a fraction of the lignocellulosic material (sugarcane bagasse and straw) either for electricity production or as feedstock in 2G ethanol production. The flexible sugarcane biorefinery selling surplus electricity in the spot market when prices are favorable presented better economic returns than the conventional biorefinery using all surplus lignocellulosic material as feedstock for 2G ethanol production. The flexible biorefinery and the plant with maximum ethanol production lead to the highest cut-off in carbon dioxide emissions. However, biorefineries producing more ethanol present higher environmental impacts per unit of ethanol produced than the configuration with maximum electricity production due to the high impacts of chemicals used in the 2G process. The study concluded that even though flexible biorefinery has a high IRR, changes in ethanol prices affect the IRR more significantly compared with increases in electricity spot market prices. Thus, if ethanol prices increase, the fixed biorefinery operating with maximum ethanol production will be more advantageous in economic terms.

1.6 Life Cycle Assessment of Sugarcane-Based Biorefineries

Life cycle assessment (LCA) is a recognized method for determining the environmental impact of a product (good or service) during its entire life cycle, from extraction of raw materials through manufacturing, logistics, use, and final disposal or recycling. In LCA, substantially broader environmental aspects can be covered, ranging from GHG emissions and fossil resource depletion to acidification, toxicity, water, and land-use aspects; hence it is an appropriate tool for quantifying environmental impacts of a product system. The ISO 14040 series provides a technically rigorous framework for carrying out LCAs (ISO 2006a, b). The method consists of four main steps: goal and scope definition, inventory analysis, impact assessment, and interpretation. First, the goal and scope provides the context for the assessment and explains to whom and how the results are to be communicated. This step includes detailing of technical information—such as defining the functional unit, system boundaries, assumptions and limitations of the study, impact categories, and methods used to allocate environmental burdens in cases where there is more than one product or function.

Life cycle inventory (LCI) is the methodological step where an overview is given of the environmental interventions (energy use, resource extraction, or emission to an environmental compartment) caused by or required for processes within the boundaries of the studied system. With its translation of the product system's environmental flows from the life cycle inventory phase (LCI) into scores that represent their impacts on environment, life cycle impact assessment (LCIA) is essential for the interpretation of the results in relation to the questions posed in the goal definition (Finnveden et al. 2009). The challenge of LCIA is to evaluate the potential impact of the emitted substances by using a procedure that is ideally simple, applicable consistently to all substances, uses a common unit of measure, and gives results that are comparable between impact categories.

A life cycle interpretation is necessary for identifying, quantifying, checking, and evaluating information from the results of the LCI and/or the LCIA. This interpretation should also raise significant environmental issues, including an evaluation of the study considering completeness, sensitivity, and consistency checks; and limitations.

Regarding the possibilities of using different LCIA methods, Cavalett et al. (2013) used seven different LCIA methods for a comparative assessment of ethanol and gasoline in Brazil. The study provided an updated and comprehensive LCI for sugarcane ethanol in Brazil considering the stages of agricultural production, transport, ethanol production, and its final use. Results showed that the use of different LCIA methods can lead to different comparative environmental impacts of ethanol and gasoline, mainly when single-score indicators are applied. A relative convergence in the results of equivalent environmental impact categories using different midpoint LCIA methods was observed. Results of the comparison of the five midpoint LCIA methods showed that ethanol presents

better environmental performance than gasoline in important categories such as global warming, fossil depletion, eco-toxicities, and ozone layer depletion but worse environmental performance than gasoline in the categories acidification, eutrophication, photochemical oxidation, and agricultural land use.

Calculated environmental impacts using the LCA methodology presented by Cavalett et al. (2012) indicate that sugarcane biorefinery optimization technologies for 1G ethanol production have a great potential for a significant decrease of the environmental impacts in sugarcane biorefineries (for both autonomous and annexed plants). Ethanol production in annexed plants presented slightly lower environmental impacts in comparison to autonomous distilleries mainly due to allocation rules used in the study. Results also showed that flexibility in annexed plants produce little effect on the environmental impacts when the entire ethanol production chain is considered.

Dias et al. (2012c) observed that a current integrated ethanol production plant (1G and 2G) has potential to decrease the environmental impacts in relation to 1G ethanol production process. The study identified that the use of high amount of sodium hydroxide in the alkaline delignification step has strong influence in the increase of the 2G ethanol environmental impacts. Junqueira et al. (2012) also found that alkaline delignification contributed to higher environmental impacts in the 2G ethanol production process. Also, pentose fermentation should be emphasized in experimental studies instead of biodigestion to produce biogas, since fermentation to ethanol leads to the best technical, economic, and environmental results in the 2G ethanol production process.

Galdos et al. (2013) showed the importance of including black carbon emissions for the calculation of global warming and human health environmental indicators. The results quantitatively demonstrated that the technological trends considering past, present, and future scenarios for ethanol production in Brazil is showing lower environmental impacts. Avoiding the preharvesting burning of LM will decrease the emissions of black carbon and greenhouse gases in the sugarcane production phase. In addition, increase in yield of sugarcane per hectare, and of ethanol per ton of sugarcane, will eventually decrease the environmental impacts per unit of biofuel produced. Results showed that 2G ethanol production plays a key role in increasing the amount of ethanol produced per unit of area. Results also indicated that the Brazilian sugarcane sector presents a trend of using more efficiently the resources per unit of ethanol produced, as well as promoting good management practices that reduce its environmental impacts.

1.7 Conclusions and Future Recommendations

Among the renewable energy sources, use of ethanol as transportation fuel has achieved significant success in countries like Brazil and USA. This review shows the potential of computer-aided process modeling and simulation, life cycle

assessment, processing technological routes for biochemical ethanol production (1G + 2G) from sugarcane juice, and lignocellulosic residues of sugarcane.

Brazil is the largest sugarcane producer (625 million tons of sugarcane in 2011) in the world showing the tremendous potential of sugarcane ethanol as sustainable energy source governing the economic, strategic policy, and environmental impacts on the nation. Today, in Brazil, 44 % of energy matrix used is renewable, and 13.5 % of renewable energy is derived only from sugarcane. In the context of the Virtual Sugarcane Biorefinery, from CTBE has extensively worked toward developing rigorous process simulation with the help of in-house derived experimental database to perform mass and energy balances, which is helpful for robust scale-up, and allows a better understanding of economic and environmental impacts. Evaluating several scenarios for 1G and 2 G ethanol production (standalone and integrated plants) in the dynamic context of biorefineries using sugarcane as a main energy driver, it was concluded that integrating 2G to 1G ethanol production improves its economic results. Moreover, in the context of C5 use, pentoses fermentation allows a large increase in the total ethanol production (40–50 % higher than 1G production) compared to the gains of pentoses biodigestion (around 30 %). Another interesting finding of the study is that high pressure boilers (82 bar) consume more bagasse than low pressure boilers, thus final ethanol output is small if very high pressure boilers are used. Furthermore, 2G ethanol may favorably compete with bioelectricity production when sugarcane straw is used in addition to the application of improved technologies using low cost enzymes for biomass hydrolysis. In regard to determining environmental impacts by LCA methodology, optimized cellulosic ethanol production technologies could have a great potential for significant decrease in the environmental impacts of present sugarcane biorefineries (autonomous and annexed plants).

Summarizing all the important features, 2G ethanol production in Brazil seems promising in the present scenario, which is a learning example for many countries in order to harness their natural agro resources. This is a fundamental step toward the development of renewable and sustainable sources of energy.

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Chapter 2
An Assessment of Brazilian Government Initiatives and Policies for the Promotion of Biofuels Through Research, Commercialization, and Private Investment Support

Luís Augusto Barbosa Cortez, Gláucia Mendes Souza, Carlos Henrique de Brito Cruz and Rubens Maciel

Abstract This chapter describes some of the scientific and technological achievements that have contributed to develop sugarcane bioenergy as a major contributor to the Brazilian energy matrix. Today, modern bioenergy plays a key role in the Brazilian economy, with 18 % of Brazilian energy usage coming from sugarcane, with ethanol used as fuel and bagasse to generate electricity. This chapter also discusses the Brazilian biodiesel opportunities and biofuels for aviation, which hold promise for the future. The long-term role played by the Brazilian government in promoting biofuels is considered as a key factor to success, particularly with sugarcane ethanol. Government-funded research agencies have played a strategic role in consolidating knowledge and human capacity to maintain leadership in the bioenergy sector. Brazil presents exceptional conditions to expand bioenergy industry (ethanol, biodiesel and biofuels for aviation) and also bioelectricity and green chemistry. To this end it is necessary to create conditions

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for the increase of the private R&D expenditures, as well as governmental actions to train human resources in the area of bioenergy. With new research centers, graduate programs have the potential to contribute to increasing competence at all stages of bioenergy development.

2.1 Introduction

By the end of the nineteenth century, the Brazilian energy matrix was dominated by traditional bioenergy such as the extraction of firewood. In the early twentieth century, use of hydroelectricity and fossil fuels (coal and oil) had gained prominence, imparting complexity to the Brazilian energy matrix. However, even before the end of the Second World War, oil began to dominate the transportation energy sector and hydroelectricity also gained importance. Nevertheless, both traditional (firewood and charcoal) and modern (ethanol and bagasse) forms of bioenergy have remained important. The importance of wood fuel, however, has diminished in the energy matrix, while the use of sugarcane for energy purposes has gained momentum, especially since 1975 (Guerra and Cortez 1992). Today, modern bioenergy plays a key role in the Brazilian economy, with 18 % of Brazilian energy usage coming from sugarcane, with ethanol used as fuel and bagasse to generate electricity.

In this chapter, the major scientific and technological achievements that have contributed to the rising prominence of sugarcane as a bioenergy in the Brazilian energy matrix are presented. This chapter also presents the Brazilian biodiesel market and biofuels for aviation, which hold promise for the future. In this paper, biofuel is defined as a bio-based liquid fuel, but the definition could be extended to include solid biofuels, such as wood and eucalyptus. However, because Brazil is experiencing an upsurge in the use of modern biofuels such as bioethanol and biodiesel, this chapter will be confined to the limited definition.

2.2 Sugarcane Ethanol in Brazil

Brazilian research on sugarcane ethanol as an automotive fuel began in the 1920s with studies conducted by the National Institute of Technology (INT). The addition of ethanol to imported gasoline was mandated by law in Brasil (1931). In 1933, the Brazilian Institute for Sugar and Alcohol (IAA) was created because of a developing crisis in the sugar sector over the use of sugarcane for ethanol. The IAA's focus was to help sugarcane producers while also reducing the consumption of imported gasoline. In 1938, Law No. 737 was passed, requiring the compulsory addition of sugarcane ethanol to all gasoline in Brazil. This law remains in effect even today, with different percentages of ethanol added depending on economic constraints (Fig. 2.1).

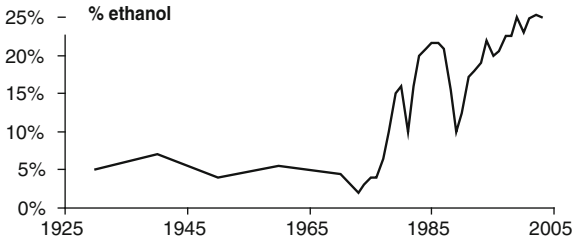

Fig. 2.1 Average ethanol content in the gasoline in Brazil between 1925 and 2005 (Nogueira 2008)

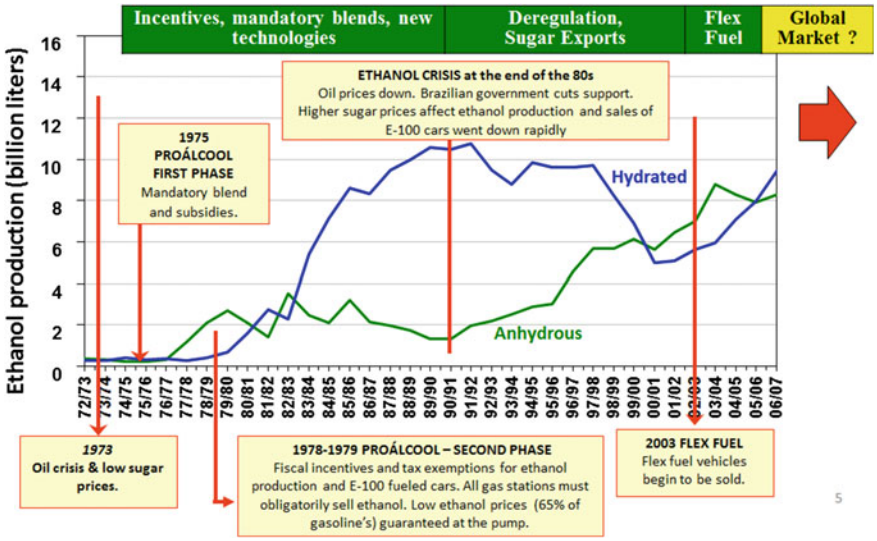

Fig. 2.2 Phases of Proálcool, 1972–2007 (Datagro 2006; elaborated by ICONE and UNICA)

The first oil crisis in 1973 seriously hurt the Brazilian economy. At that time, the country imported nearly 80 % of its oil, which represented approximately 50 % of total imports. An immediate solution was required to reduce Brazil's dependence on oil. The newly elected President, Ernesto Geisel, who was the former president of Petrobras, enacted a number of measures for the energy sector. On November 14th, 1975, by Decree No. 76.593, the Brazilian government created the National Alcohol Program, also known as "Proálcool."

The Proálcool program has had varying levels of success and failure (Fig. 2.2). Financed by subsidies and liberalization policies, the program has survived changes in regimes (from military to democratic), considerable variations in the price of oil and sugar, and economic and political crises over the past 38 years. The most critical period occurred in the second half of the 1980s, when the ethanol car (E100) accounted for 90 % of total sales of new vehicles in the country. In 1989, ethanol

production did not match the domestic demand, primarily because of a lack of planning. Many consumers felt aggrieved and lost confidence in the ethanol car, which dramatically reduced the sales to almost zero in the following years.

The 1990s were marked by a restructuring of the sugar-ethanol sector through gradual deregulation, which allowed Brazil to become a major exporter of sugar. The automotive industry had already begun favoring the sale of cars running on gasoline-ethanol blends (ranging from E20 to E25). With a growing fleet, domestically consumed ethanol was more anhydrous than hydrous, and the demand for both types of ethanol made the total demand for ethanol more or less constant.

By the late 1990s, many consumers had tried using different percentage blends of ethanol and gasoline, popularly known as a "cocktail."

In the twenty-first century, the rising price of oil has given sugarcane ethanol a new impetus in Brazil. The automotive industry realized that consumers want a vehicle with a flexible engine that would work with any proportion of ethanol in the fuel mixture. The consumer does not want to be at the mercy of price fluctuations, which are common today during the sugarcane inter-harvest period, or be held hostage to a fuel that could be depleted and thus devalue their assets (cars). Concurrently, flex-fuel vehicles (FFV) models were being launched in the U.S., and in 2002, the Ford Fiesta was introduced in Brazil. In 2003, Volkswagen launched the GOL as their first Brazilian flex-fuel vehicle.

The "lambda probe," a sensor developed by Bosch and Magneti Marelli (MM) to identify blends of fuel, constituted a considerable qualitative development for Brazilian flex engines operating in bi-fuel vehicles. Today, approximately 90 % of the new cars sold in Brazil are flex-fuel vehicles that allow the consumer to select between fuel types, offering greater protection against the fluctuating prices of ethanol and gasoline.

In Brazil, the successful use of sugarcane ethanol was the result of a learning trajectory based primarily on incremental innovations (Furtado et al. 2011). Copersucar (1989), Leite (1990), Magalhães et al. (1991), Moreira and Goldemberg (1999), Moraes (1999), BNDES (2008), Cortez-Coord (2010) Souza and Macedo (2010) and Rosillo-Calle et al. (1998, 2000) describe in detail the history of Proálcool and how Brazil created this internationally recognized success story.

2.2.1 Sugarcane Agricultural Research

Brazil became the world leader in the production and use of sugarcane-derived fuel ethanol and thanks to a successful combination of long-term actions of the government and private sector, including important agronomic research activities. When the National Alcohol Program was implemented in 1975, Brazil was already a major producer of sugarcane (the second largest after India), milling approximately 100 million TC/year. However, the production of fuel ethanol was modest, at approximately 600 million liters per year.

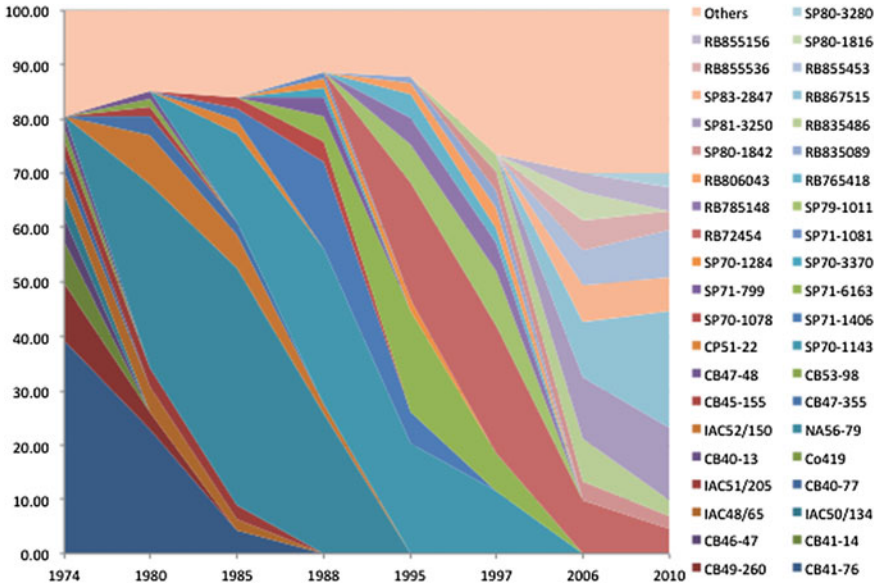

Fig. 2.3 Diversification of commercial sugarcane varieties from 1984 to 2010 in Brazil (*Source* Costa et al. 2011)

Important research centers were also studying sugarcane, including the Agronomic Institute of Campinas (IAC), which had started its breeding program in 1933 and collaborated with the emerging sugar industry in the State of São Paulo to generate important information in the areas of plant nutrition and agricultural practices, forming the basis of existing technologies (IAC and IACSP), and the IAA breeding program of Campos, RJ Station, which was responsible for the CB varieties of sugarcane.

In the early 1970s, the National Program for Genetic Improvement of Sugarcane (Planalsucar) was created by the Brazilian federal government and the Copersucar breeding program (SP varieties) was created with the funds from the private sector.

Until 1975, Brazil had depended on a few sugarcane varieties (Fig. 2.3), including the predominant variety NA5679 from Argentina. However, Planalsucar played an important role in preparing researchers to experiment with sugarcane varieties and creating a sugarcane "genetic bank" in Alagoas, northeast Brazil. New findings emerged in soils, herbicides, diseases, and biological control of sugarcane pests. After the IAA disbanded, Planalsucar resumed its activities in 1990. Its researchers, who were reorganized within federal universities, created the Institutional Network for the Sugar and Alcohol Segment Development (RIDESA) to continue its research and discovery on sugarcane. RIDESA consists of ten federal universities (UFPR, UFSCar, UFV, UFRRJ, UFS, UFAL, UFRPE, UFMT, UFG, and UFPI), has 34 stations, and is responsible for RB varieties of sugarcane.

In the private sector, the State of São Paulo's Sugarcane, Sugar & Alcohol Producers Cooperative—Copersucar was created in 1978, forming the Copersucar

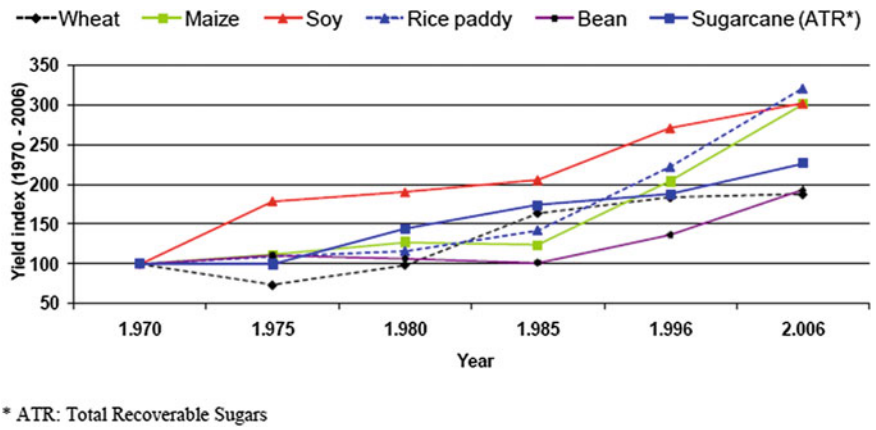

Fig. 2.4 Increased yield per hectare for selected Brazilian crops (1970–2006) (Elaborated by M.P. Cunha (CTBE) using data from Brazilian Agricultural Census (IBGE) and from the Brazilian Agroenergy Yearbook 2009)

Technology Center (CTC). After 2004, the CTC was renamed as the Center for Sugarcane Technology and then the Sugarcane Research Center (http://www.ctcanavieira.com.br/). The CTC has played a key role in technology transfer for both the agricultural and industrial sectors. The CTC produced significant advances in agricultural management and in areas such as agricultural mechanization, microbiology fermentation, energy and water conservation, and application of vinasse and filter cake (Burnquist and Landell 2005).

The CTC was established to conduct research and develop new technologies for application in agricultural activities, logistics and industrial sectors, and to create new varieties of sugarcane, with the technology provisioned to cooperative mills. The CTC is responsible for varieties that make up approximately 60 % of the crops of cooperative units and 45 % of the crops of other producers.

The breeding program of the IAC Sugar Cane Program was reorganized in 1994 by the Cane Center of Ribeirão Preto, which has 128 research units operating in 12 states in Brazil that are committed to the integration of science and technology. Some of the contributions of the Cane Center were highlighted by Dinardo-Miranda et al. 2008. This program has the support of APTA units in the cities of Piracicaba, Jaú, Mococa, Pindorama, Assis and Adamantina, and breeding farms in Goianésia (GO) and Luis Eduardo Magalhães (BA).

There are currently four active sugarcane programs in Brazil: IAC, CTC, RIDESA, and Canavialis. The success of the breeding programs is evident from the evolution of sugarcane agricultural productivity in Brazil (Fig. 2.4). Other crops such as corn have shown a remarkable increase in agricultural productivity in recent decades; however, the agro-industrial yield of sugarcane ethanol is approximately 7,000 L/ha.year in Brazil whereas the yield of ethanol from corn is approximately 3,500 L/ha.year in the U.S. Although important progress has been

made in developing new cane varieties in Brazil, more research is required, particularly in developing areas where sugarcane is expanding, such as Central Brazil.

2.2.1.1 Other Crops for Ethanol Production

When the Proálcool program was first implemented, the possibility of using other raw materials, such as cassava and sorghum, in the production of ethanol was considered. Production of biodiesel is experiencing a similar growth pattern, including encouraging different cultures, decentralizing production, and giving access to small producers. Initiatives of the Brazilian Ministry of Science and Technology/Secretary of Industrial Technology (MIC/STI) (1980) included designing a mini-distillery to produce 10,000 L/day of cassava alcohol and experiments with EMBRAPA to create an SP model using small-scale diffusers instead of mills and operating with sorghum and sugarcane (Figueiredo et al. 1984). Another important project was led by Petrobras in Curvelo, MG, in 1978, who attempted to implement a cassava ethanol distillery that was developed by INT (Motoyama 2004). The project failed primarily because of agronomic difficulties related to large-scale cassava production. Many of the problems associated with alternative feedstocks are related to a lack of long-term research.

2.2.2 Other Agricultural Issues in Ethanol Production

Numerous researchers and Brazilian institutions have played an important role in the development of sustainable sugarcane ethanol, with significant contributions to sugarcane ethanol production originating in Brazilian universities and research centers. The sections below include some of the most important contributions.

2.2.2.1 The End of Sugarcane Burning

Sugarcane burning prior to harvesting is a major environmental problem that pollutes the air and increases respiratory diseases in winter. In September 2002, the legislature of the State of São Paulo passed legislation mandating the gradual reduction of sugarcane burning in São Paulo until 2017: State Law No. 11.241, of 19 September, 2002, shown in Fig. 2.5. "Raw" sugarcane harvesting (without burning) is now practiced in more than 80 % of the harvested areas in São Paulo, which grows over 50 % of Brazil's sugarcane. Raw cane harvesting is expected to reach 100 % of harvested areas in São Paulo State as mandated by the law. However, the harvest of sugarcane without burning introduced a new technological challenge: mechanically and economically harvesting the entire cane (stalks and straw) without compacting the soil, consuming high amounts of fuel, losing sugarcane, damaging the stumps, and introducing too many impurities to the product.

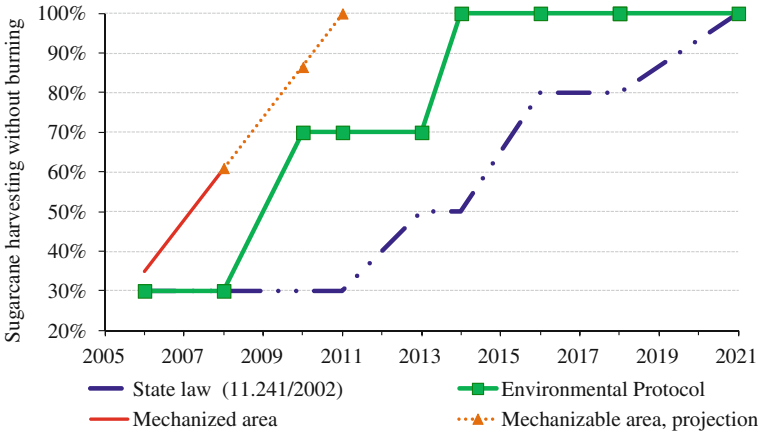

Fig. 2.5 Evolution of the cessation of sugarcane burning and sugarcane harvesting mechanization in the State of São Paulo from 2001 to 2021 (adapted of Macedo 2007)

The CTBE is developing a mechanization approach for sugarcane harvesting (Braunbeck et al. 2005) called a "Controlled Traffic Structure (ETC)" which is specifically designed for low impact mechanization and will introduce no-till farming and precision agriculture for growing sugarcane. (http://www.bioetanol.org.br/interna/index.php?chave=baixoimpacto).

Cane harvesting without burning also introduces the problem of eliminating the cane trash. Burning sugarcane eliminates trash, transforming it into emissions and ash. However, when sugarcane is harvested without burning, left behind trash (composed primarily of leaves and tops) makes the harvesting process less efficient. Significant amounts of sugarcane trash left on the fields confers a degree of agronomical benefit, such as a reduction of soil erosion and moisture loss; however, certain agricultural practices must be changed to manage soil pests that are attracted to the trash. Agronomists recommend that no more than 50 % of the trash be removed from the field, preferably simultaneous with the harvesting process to keep weeds under control. Although the trash material (primarily fibers) may represent an opportunity for generating electricity or second-generation ethanol, there are not enough incentives to make use of the trash economically viable.

2.2.2.2 Recycling Vinasse as Fertilizer (Fertirrigation Technology)

In the early years of Proálcool, vinasse was identified as a major environmental contaminant because it has a high organic load and was commonly disposed of in waterways. An Esalq-USP research group studied vinasse application on the ground, which eventually became a routine practice (Gloria 1975, 1976). Subsequently, the CTC developed more the fertirrigation technology. Today, applying

vinasse to the ground helps the sugarcane industry save significant amounts of potassium (Copersucar 1978, 1980; Freire and Cortez 2000 and Fredo et al. 2008).

In addition, Cetesb regulation controls the inappropriate use of vinasse in fertirrigation to prevent groundwater contamination. This standard establishes criteria for calculating the maximum amount of vinasse to be used depending on soil-type and other parameters (*Vinhaça–Critérios e Procedimentos para Aplicação no Solo Agrícola P4.231* Dez/2006 http://www.cetesb.sp.gov.br/tecnologia/camaras/P4_231.Pdf). Although fertirrigation technology represents a good solution in most cases, a reduction is still required in the volume of produced vinasse and the GHG emissions potential.

2.2.3 The Brazilian Industrial Model for Ethanol Production

2.2.3.1 Scale and Model of Ethanol Production in Brazil

When the Proálcool program was first established, the government proposed that a "standard distillery" (capacity of 120,000 L of alcohol/day) be used for production of ethanol. This plant size, now considered too small, was challenged by many researchers who believed that smaller units would favor small-scale production and increase social benefits. The MIC/STI (1981) proposed production models based on micro-distilleries. A research group from USP—São Carlos proposed a model based on "integrated mini-ethanol plants" that was capable of producing 20,000 L of ethanol/day and could, in theory, become a "more social" version of the ethanol program (Corsini 1992). At the time, there were several companies selling units of up to 1,000 L/day, but the small-scale distilleries never achieved the levels of productivity, quality, and economic viability of the large plants. At present, a standard plant has a production capacity of approximately 1 million liters of ethanol/day, although the largest plants in Brazil can produce 4 times that amount.

2.2.3.2 The Brazilian Model of Simultaneous Production of Sugar and Ethanol

The creation of what is known as the "Brazilian model" of simultaneous production of sugar and ethanol was the result of the combined efforts of several researchers: José Paulo Stupiello, Esalq—USP, who contributed to sugar production technology; Young Park, UNICAMP, who contributed to the microbiology of ethanol and fundamental studies on fermentation using sugarcane and other raw materials; and Carlos Vaz Rossell and Jaime Finguerut, CTC, who contributed to optimizing and building the concept of a "flex plant" (Copersucar 1990). These efforts resulted in significant advancements in the ethanol industry, as evidenced by the reduction in fermentation time.

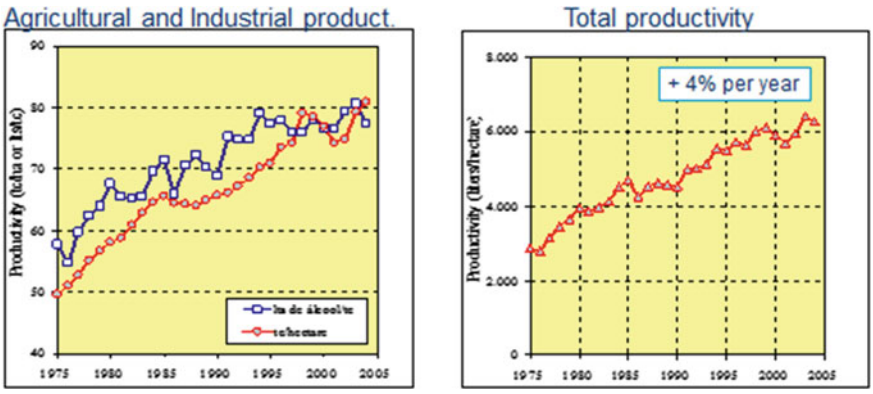

Fig. 2.6 Agro-industrial productivity evolution of sugarcane ethanol through R&D from 1975 to 2005 (*Source* Brito 2012)

These contributions substantially improved ethanol's agro-industrial productivity, which is an indicator based on improvements in agricultural and industrial productivity (Fig. 2.6).

2.3 Development of Ethanol and Flex Fuel Engines in Brazil

One of the first publications on the subject of ethanol and flex fuel engines was the book "Internal combustion engines and ethanol engines," published in 1937 by Eduardo Sabino de Oliveira from the IAA. Much later, Urbano Ernesto Stumpf (CTA) contributed important research on the study of engines fueled by alcohol and received patent #PI8106855-7 for an "alcohol-specific carburetor" on 23/10/1981 (O'Donnell 2009). Another important contribution was made by Romeo Corsini (USP—São Carlos), who received a patent (#PI8402740-1) for the "MAV—pre-evaporized alcohol engine." Additional recognition should be given to the work of Fernando Barata de Paula Pinto of Maxion International Motors, who helped to develop alcohol engines and flex fuel engines. Francisco Nigro (IPT) and Henry Joseph Jr. (ANFAVEA and Volkswagen) helped to develop the alcohol engine and flex-fuel ethanol. Although Brazil has achieved important success in using ethanol fuel, there are still significant challenges to overcome in the design of the engine, which must undergo major changes for use with hybrid engines. Figure 2.7 shows the irregular ethanol vehicle production in Brazil since 1979.

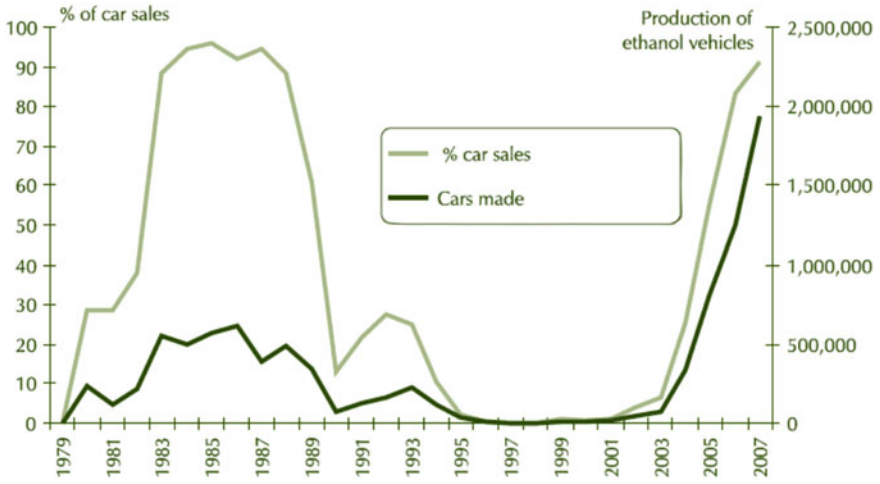

Fig. 2.7 Production and sales of ethanol-based automobiles in Brazil (BNDES 2008)

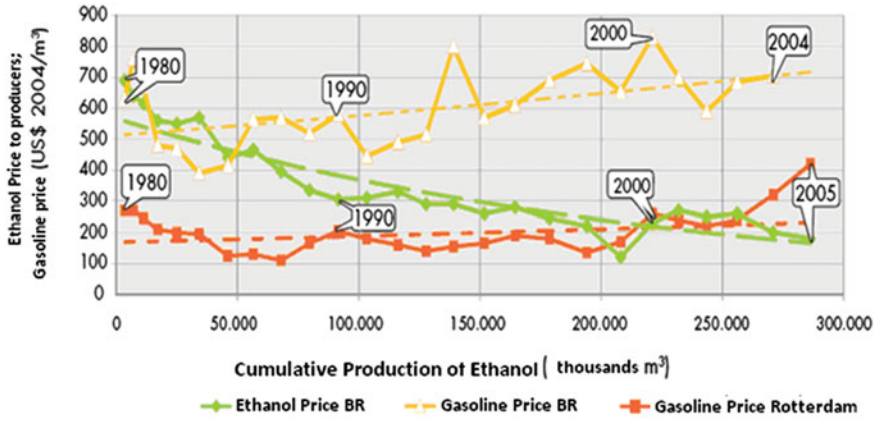

Fig. 2.8 Sugarcane ethanol learning curve (Goldemberg et al. 2008)

2.4 Sustainability of Sugarcane Ethanol

The work of Silva et al. (1978) is also worth mentioning because it is considered as a reference publication on Proálcool and should be recognized as one of the first to address energy balance in the production of ethanol for different crops (Nogueira 1987).

A learning curve proposed by Goldemberg et al. (2004) and collaborators illustrates how production costs have decreased as the quantity of ethanol has increased (Fig. 2.8).

Several research institutions have made significant contributions in the area of sustainability (Camargo 1990), including the IPT (Bonomi 2004), CETESB (Technical Standard P4.231/2006) and INT (STI/INT 1976). Studies have been performed on the digestion of vinasse; however, the digestion of vinasse has not been incorporated in ethanol distilleries, with the exception of Usina São Martinho. UNICA also published an important contribution that summarized 12 major themes related to ethanol's energy and environmental sustainability (Macedo-Coord 2005). Several current ethanol research groups have concentrated their efforts in trying to understand the socioeconomic and environmental issues arising from sugarcane ethanol production and use (Macedo et al. 2008).

2.4.1 Impacts of Ethanol Fuel Use on Population Health

The academic community has made important contributions to public health issues related to vehicle emissions. Saldiva from the Laboratory of Pathology—Medicine (USP) studied the impact of ethanol fuel usage and the emissions generated by more than 5–6 million cars (mostly flex-fuel) in the city of São Paulo. According to Saldiva, replacing gasoline with ethanol fuel is an important public policy measure that improves the health of populations living in large cities because ethanol fuel helps to alleviate air pollution (UNICA 2009 and http://www.worldcat.org/identities/lccn-no00-41804).

2.4.2 Social Science Research Related to Sugarcane Ethanol

Research on the social and economic impacts of sugarcane ethanol production and use in Brazil has received substantial attention from researchers. Presented below is a list of important contributions in ethanol production and use.

Balsadi and Borin (2006) applied a "Quality Index" based on earnings, level of formality, education, and other forms of economic support to analyze the sugarcane sector with regard to improvement of employment (both in quantity and quality) and concluded that each of these indicators had shown significant improvement over the period studied, from 1990 to 2002.

Moraes (2007, 2009, and 2011a , b) analyzed several aspects of the sugarcane, sugar, and ethanol labor markets in Brazil, including the evolution of socioeconomic indicators (number of workers, wages, work formalization, and conditions, etc.), sugarcane worker migration, and income determinants for workers in sugarcane plantations and in the sugar and ethanol industries (influence of education, labor unions, region, etc.).

Moraes (2011a) analyzed the social externalities of fuels and compared indicators between the sugarcane-ethanol and oil industries. They also estimated the socioeconomic impacts of substituting gasoline with ethanol.

Chagas et al. (2011) analyzed the effects of increased sugarcane production on municipal revenues in the State of Sao Paulo. Their results suggested that there is a significant and substantial increase in revenue with increased shares of sugarcane in the municipal agricultural output.

Hofmann (2006) analyzed the effects of increased ethanol production on the reduction of poverty in Brazil. The effects of increased ethanol production on the country's food security is primarily positive. Lack of food security in Brazil is strongly associated with poverty, which should diminish with the increase in employment and income that results from an expansion of sugarcane agribusiness, thus, compensating for the negative effect of eventual increases in food prices.

Martinelli et al. (2011) compared development indicators in municipalities of the State of São Paulo. A series of indices were used, including the following: the human development index (HDI) of the UN, an HDI index based on São Paulo's social responsibility index (SRI) and the Rio de Janeiro municipal development index (MDI). The results showed that the HDI, SRI, and MDI for cattle municipalities were significantly lower than for all the other categories, with the highest results in municipalities with both sugarcane and processing mills, which were higher than nonrural municipalities.

Assato and Moraes (2011) analyzed the socioeconomic impacts of the expansion of the sugarcane sector in two municipalities of Mato Grosso do Sul State and found an increase in aggregate income and improvements in education because of educational programs installed after the expansion of the sugarcane industry.

Satolo and Bacchi (2013) evaluated the effects of the expansion of the sugarcane sector on the municipal per capita GDP in São Paulo State. The results from a dynamic spatial panel indicated a positive impact on per capita GDP.

Moraes (2007) and Oliveira (2009) showed that sugarcane production results in higher wages than other crops, a greater level of formal relations (meaning legal protections) and a lower presence of child labor. Sugarcane is a crop with a greater reliance on the external market and a larger production scale, so labor relations tend to be more formalized and in line with legislation.

Sallum (2007), Moraes and Pessini (2004), and Moraes (2009, 2011a) analyzed the institutional and organizational environment of the labor market in the sugarcane industry and observed that there are clear and specific rules governing the labor market. The authors also demonstrated that employer associations and labor unions within the state of São Paulo were strong and highly active and engaged in wage negotiations for the sugarcane workers at the beginning of each harvest season.

2.4.3 Planning Land Use for Bioenergy in Brazil

The prospects of a rapid expansion of the sugarcane sector for the production of bioenergy intended for export has had a major impact on land-use planning in Brazil, such as the Brazilian Land Use Model (BLUM) (Nassar et al. 2009), the Agro-Ecological Zoning of Sugarcane, which was prepared by the Ministry of

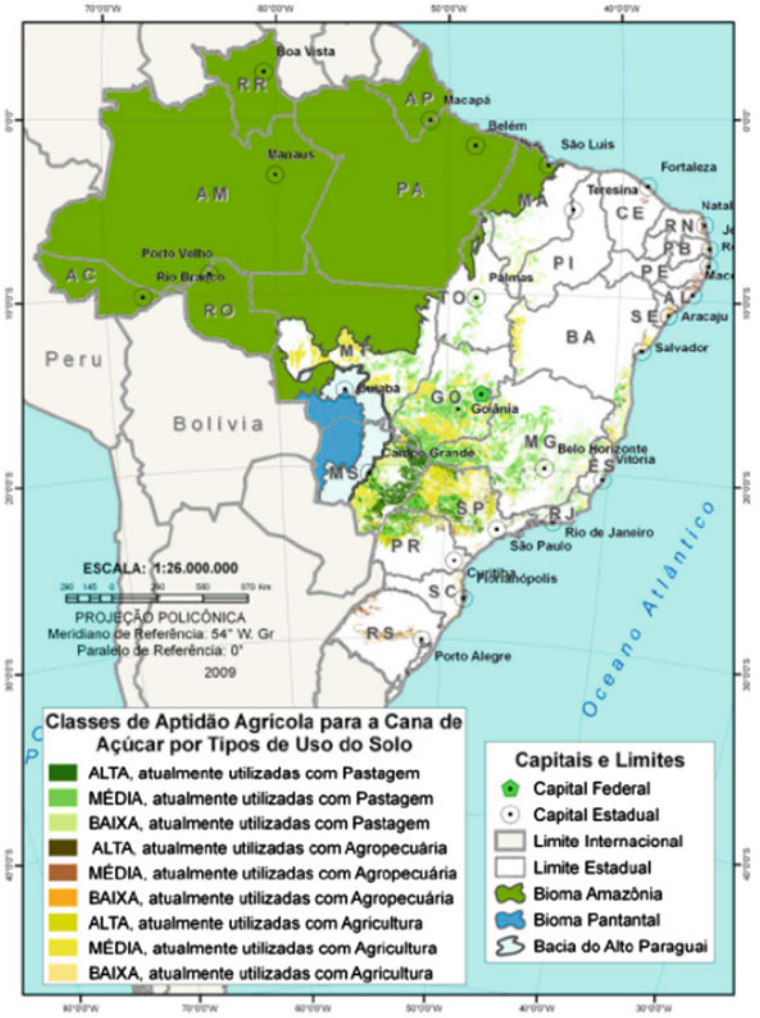

Fig. 2.9 Sugarcane agro-ecological zoning in Brazil (MMA 2009) http://www.cnps.embrapa.br/zoneamento_cana_de_acucar/ZonCana.pdf

Environment (MMA) (Fig. 2.9), the Agro-environmental Zoning for the cultivation of sugarcane, which was released on 18/09/2008 by the government of the State of São Paulo (Fig. 2.10) and based on the work of the BIOTA FAPESP, and the IAC work coordinated by Orivaldo Brunini in 2008, which resulted in the publishing of an agro-climatic suitability map for the state of São Paulo.

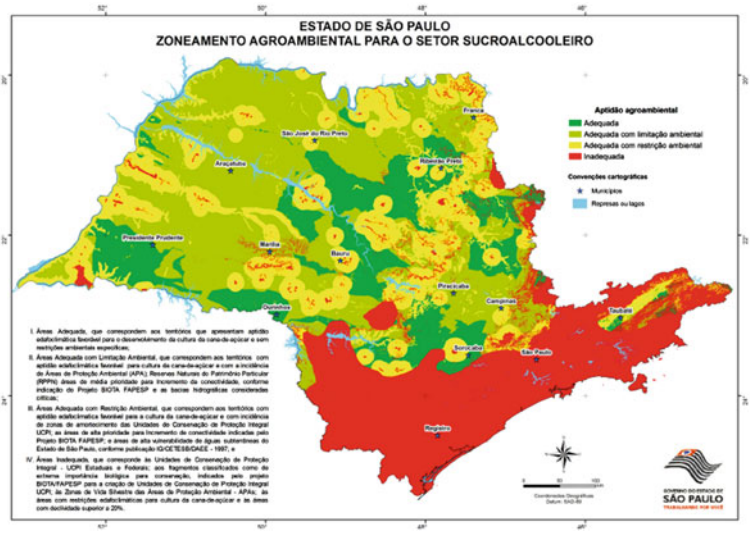

Fig. 2.10 Science-based sugarcane agroenvironmental zoning in São Paulo State http://www.ambiente.sp.gov.br/etanolverde/zoneamento-agroambiental/

2.4.4 The R&D Contribution of the Sugar and Ethanol Private Sector

The sugar and ethanol private sector has contributed substantially to consolidating the industry. In agriculture, contributions have been made to research on new varieties and on planting techniques such as the "plene" planting system, which was developed by Syngenta (http://www.syngenta.com/COUNTRY/BR/PT/PRODUTOSEMARCAS/PLENE/Pages/Tecnologia-plene.aspx). (Several industries have introduced harvesters (e.g., John Deere, Case, Valtra, Santal) and Jacto is developing a new concept (ETC) with CTBE (http://www.bioetanol.org.br/noticias/detalhe.php?ID=NDY2).

The New Holland/CTC partnership on raw sugarcane harvesting has developed a system that simultaneously harvests sugarcane and straw and cleans the straw before industrial use (http://www.bioetanol.org.br/noticias/detalhe.php?ID=NDY2).

Brazilian industry has also conducted important research on sugarcane ethanol. Dedini researched the integration of ethanol and biodiesel production and the organosolv process of hydrolysis of bagasse, known as Dedini Rapid Hydrolysis (DHR) (Dedini 2008). Dedini and Fermentec developed a process for reducing amounts of vinasse (http://www.slideshare.net/tabVlae/dedini-fermentec-vinasse-concentration). Dedini also introduced environmentally sustainable solutions designed to reduce water consumption in the process of ethanol production (BIO-WATER) and recycle solid and liquid waste for use as a fertilizer (BIOFOM).

Additional innovations were developed by Braskem, who produced green plastics (polyethylene) using ethanol as the raw material (http://www.braskem.com.br/site.aspx/plastic-green).

In the recent years, significant efforts in increasing ethanol production have been made by the private sector in conjunction with federal and state agencies, such as the GranBio project in Alagoas, Brazil where an ethanol plant is being built and is expected to start operations in 2014 (http://www.novacana.com/n/etanol/2-geracao-celulose/graalbio-preve-usinas-etanol-2g-210313/%23).

2.4.5 *Fostering Bioenergy Research in Brazil*

Research funding in Brazil is comprised of federal, state, and private initiatives. In building the Brazilian research system, public agencies have been instrumental in improving the quality of research and consolidating graduate programs. Public agencies have made considerable efforts in stimulating businesses to fund research programs, supporting innovative R&D for small businesses, and establishing partnerships for jointly funding research with medium and large companies.

Both the National Research Council—CNPq, which promotes research in Brazil, and the National Fund for Research and Projects—FINEP, which is dedicated to supporting projects with the participation of industry, have been active in financing bioenergy research since the beginning of Proálcool. CNPq played a key role in the early stages of the Proálcool program when it published the book "Rating the technological ethyl alcohol" (Anciães 1978), which was a benchmark in the field of ethanol production. CNPq also acted decisively in offering scholarships to graduates within and outside Brazil at a time when national programs were not yet fully developed.

In the latter half of the 1990s, the federal government created "sectorial funds" (Pacheco 2007). CT-Energ (http://www.mct.gov.br/index.php/content/view/1410/CT___Energ.html) was designed to stimulate energy research and innovation in Brazil and addressed all technical aspects of energy except oil, which had its own specific fund, CT-Petro. Coordinated by ANEEL and ANP together with energy utility companies, funds were raised for R&D and energy efficiency. Bioenergy research to promote innovation that results in better yields and more reliable services has benefited from these resources. However, much more would be gained if there was greater integration of resources, for example, especially in overcoming difficulties related to bioelectricity.

The State of São Paulo Research Funding Agency—FAPESP has contributed considerable resources to bioenergy research (FAPESP 2007). In 1998, the SUCEST project, funded by FAPESP as part of its Genome Program, initiated the sequencing of sugarcane-expressed sequence tags (ESTs). Sequencing the giant genome of sugarcane, where one gene is represented by an average of 10 alleles, is a major challenge. At the time, collections of ESTs represented as a fast alternative

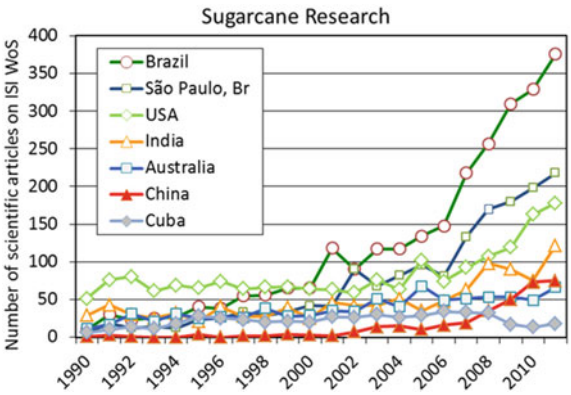

Fig. 2.11 Number of scientific articles on sugarcane cited at ISI WoS for main sugar-producing countries (*Source* Brito 2012)

for the initial characterization of a genome. SUCEST significantly contributed to the identification of genes associated with agronomic traits of interest. Approximately, 43,000 genes were identified (Vettore et al. 2003). This initiative was instrumental in the formation of a network of genome researchers in Brazil and placed the country in the lead of sugarcane-indexed international publications (Fig. 2.11). A survey of research on sugarcane and ethanol funded by FAPESP in recent years has been published in the book "Brazil world leader in knowledge and technology of sugarcane ethanol."

2.4.5.1 Scientific Publications in Sugarcane in Brazil

In Brazil, the Institute of Sugar and Alcohol, created in the 1930s, provided a major contribution to the study of bioenergy by publishing the magazine *Brasil Açucareiro* and *Anuário Açucareiro*, which was a yearbook-type of publication that was started in 1935 and discontinued in 1975. The Society of Technical and Sugar and Ethanol Producers of Brazil—STAB currently represents the only scientific magazine in the country in the sugar-ethanol sector. An inventory of publications held by Vian and Corrente (2007) allows a better understanding of how the industry disseminates its knowledge.

2.4.5.2 Scientific Publications in Sugarcane in Indexed Journals Highlighting the Evolution of Research in Brazil and the State of São Paulo

The SUCEST project was succeeded by two initiatives led by researchers at USP and UNICAMP and a "spin-off" biotechnology company. The SUCEST-FUN project (http://sucest-fun.org), which was started in 2003, focused on the identification of genes associated with agronomic traits of interest (such as yield,

tolerance to biotic and abiotic stresses, mineral nutrition, sugar content, and responses to climate change). The project was based at the Institute of Chemistry, USP, and was a collaboration of groups from USP, UNICAMP, UFSCar, CTC, IAC, UFRJ, UFPE, UFRPE, UFAL, and RIDESA. Genes associated with sucrose content and drought resistance have been patented, and transgenic plants were developed that should contribute to the improvement of sugarcane. Concurrently, a project based at CBMEG, UNICAMP, developed molecular markers, genetic-statistical tools, and a functional genetic map for breeding progenies of the CTC, IAC, and RIDESA programs. These initiatives allowed the development of molecular tools that promise to accelerate the release of new cultivars by classical breeding programs (Cantarella et al. 2012).

Alellyx was found in February 2002, by a group of molecular biologists and bioinformaticians involved in the SUCEST project or the FAPESP Genome Program. Set in Campinas, Alellyx operated in partnership with CanaVialis, which was found in 2003, and whose focus was sugarcane breeding. Together, Allelyx and CanaVialis represented one of the most modern sugarcane breeding programs in the world and had an important influence on the FAPESP Genome Program. After the initial phase of venture capital investments by Votorantim New Business, both companies were acquired by Monsanto in what was one of the biggest purchases of a start-up company in Brazil at the time.

In 2008, FAPESP created the FAPESP Bioenergy Research Program BIOEN (http://bioenfapesp.org). BIOEN is organized in five divisions: Biomass, Biofuel Technologies, Biorefineries, Engines, and Sustainability and Impacts. FAPESP establishes partnerships with national and international funding agencies and businesses in its efforts to articulate and integrate research initiatives. CNPq resources, for instance, were mobilized under Pronex and INCT joint grants. The private sector is also represented with BIOEN agreements that involve public research institutions and companies such as Braskem, Dedini, Oxiteno, Microsoft Research, ETH, Boeing, BP, PSA, BE-Basic, and Vale, all of which share human, material, and financial resources. In 2013, the program had grown to more than 400 researchers in over 20 countries. Considering its size, broad research spectrum and the depth of its goals, BIOEN can be considered as one of the most important bioenergy research programs in the world. BIOEN was built on a solid base of exploratory academic research that is generating new knowledge and highly qualified experts, which are essential for enhancing the industry's ability to run on ethanol technologies and increasing internal and external competitiveness.

A strategically important FAPESP project was the Project for Public Policy Research on Ethanol (PPP Ethanol), developed in partnership with APTA (www.apta.sp.gov.br/cana). PPP Ethanol promoted a broad discussion of the entire ethanol production chain from sugarcane, with researchers from academia and the private sector. This research produced a technology roadmap for the sector and resulted in the publication of the book "Bioethanol from Sugarcane: research & development productivity and sustainability" (Cortez-Coord. 2010).

2.4.6 New Frontier Research on Sugarcane Bioenergy

2.4.6.1 Breeding, Molecular Biology, Genomics, and GMOs

In 2009, BIOEN catalyzed a series of discussions on biotechnological paths for the improvement of sugarcane, including the development of new sugarcane varieties with increased yield, tolerance to stress (especially drought), and adaptations to the soil and climate conditions of areas where sugarcane expansion is taking place (mid-west, south, and north east) (Hotta et al. 2010). Defined research priorities included (1) obtaining the sequence of a reference genome of sugarcane (2) developing molecular markers and genetic maps to assist breeding programs in the choice of parents and progenies (3) understanding the physiological processes underlining the partition of carbon, photosynthesis, and use of water (4) discovering genes and functional genomics for identifying genes of interest, and (5) researching the stable production of transgenic plants. Challenges and bottlenecks in breeding were recognized as well as the role of high-performance technologies in overcoming breeding issues.

Improving yield and resilience of sugarcane varieties can be achieved by integrating agronomic practices, adequate management practices, traditional breeding and molecular-assisted breeding, and development of transgenic plants. The discovery of genes associated with agronomical and physiological traits will provide the necessary knowledge for the development of cultivars dedicated to the production of bioenergy (energy cane) or to be used as biofactories.

It is expected that the amount of information regarding sugarcane will increase exponentially in the coming years in response to research incentives. The development of integrated databases, such as the SUCEST-FUN Database (http://sucest-fun.org) is essential for the optimal use of research results. In particular, collecting large amounts of sequence data will require the implementation of bioinformatics and databases for information management. This demand will be similar in systems biology projects designed for an integrated understanding of the various aspects of plant growth and their adaptive development to different environments. Project data management will be absolutely crucial for the integration of heterogeneous data coming from different methodologies and groups.

2.4.6.2 Second-Generation Technologies

Lignocellulosic Ethanol (hydrolysis)

Hydrolysis research in Brazil began with José Carlos Campana Gerez, Institute of Chemistry, UNICAMP, in the late 1970s with studies on acid hydrolysis. These works led to the installation of a pilot plant on the UNICAMP campus, but the project was discontinued in the early 1980s because of a lack of resources. However, the process resulted in patent #PI8203026-0 (1982).

A 1979 initiative of the federal government saw the passage of Law No. 6.768 that established the company COALBRA—Coke and Alcohol Wood S/A, which was based on technology of Russian origin and designed to convert wood into methanol by acid hydrolysis. This project was coordinated by Sérgio Motta, who acquired a full-scale industrial unit that was installed in Minas Gerais, near Uberlândia, but was discontinued because of technical difficulties and a lack of funding. At the beginning of the 1980s, CESP (Companhia Energética do Estado de São Paulo) installed a pilot plant to gasify wood and produce methanol from synthesis gas, which was also discontinued.

Research on cellulosic ethanol production started again in the mid-1990s with studies by Dedini in partnership with the CTC and FAPESP and coordinated by Carlos Eduardo Vaz Rossell. The research, based on the organosolv pretreatment of bagasse, was used in a demonstration plant installed at São Luiz Mill in Pirassununga, SP. The plant had the capacity to produce 5000 L of ethanol/day, taking advantage of the synergy of an integrated process in the first-generation. Because of technical problems regarding the sugarcane bagasse supply, the production of inhibitory compounds for fermentation and a viable use for the fraction of lignin, it became clear that additional research was required at a smaller scale. However, the studies indicated important technical challenges that helped to drive future studies in the area.

In 2005, the Ministry of Science, Technology & Innovation- MCTI, created the Bioethanol Network, which was coordinated by Rogério Cezar de Cerqueira Leite, UNICAMP. The network's activities involved several universities and research institutions (such as CTC) and attempted to identify necessary skills and contribute to the development of a technology to produce cellulosic ethanol in Brazil (http://cenbio.iee.usp.br/projetos/bioetanol.htm). After 3 years of intense work, the Bioethanol Network program was able to identify skills as well as scientific and technological barriers and served as a foundation for the creation of the hydrolysis program at CTBE (National Laboratory of Science and Technology of Bioethanol). The hydrolysis program at CTBE is coordinated by Carlos Eduardo Vaz Rossell and aims to make improvements in the four basic areas of enzymatic hydrolysis: pretreatment, enzymes, hydrolysis, and fermentation. Facilities were installed at the CTBE for conducting experiments at laboratory scale and pilot plant scale (up to 500 L). The pilot plant is a flexible unit, and the cellulosic ethanol conceptual process is designed to make use of the biorefinery concept, which may introduce many potential product options in addition to bioethanol (http://www.bioetanol.org.br/).

CENPES/Petrobras is also developing a program to research cellulosic ethanol, especially in the area of enzyme production, in association with universities and domestic and foreign companies. Dedini has also worked with several companies in search of robust and economically competitive enzymatic hydrolysis processes. Other groups have also emphasized research work in the area of enzymatic hydrolysis, such as the CTC and Luiz Ramos at UFPR.

Considering the worldwide efforts in hydrolysis, it is now understood that the problem is quite complex and offers an opportunity to bring together more basic research, such as understanding the deconstruction of the cane fiber, and technological challenges, such as creating efficient processes for the production of enzymes and obtaining enzymes robust enough to operate in an industrial environment at lower prices. Given the highly favorable characteristics of fiber and the availability of utilities, the 1G plant environment is considered very suitable for coupling a 2G plant. In addition, ethanol fuel hydrolysis technology will allow the plants to make use of the biorefinery concept to produce high-value molecules, such as polymers, and develop innovative products.

The BNDES PAISS Program to Promote Second-Generation Ethanol

The PAISS is a joint initiative of the BNDES and FINEP, which are a selection of business plans and development projects that include the development, production, and commercialization of new technologies intended for the industrial processing of biomass derived from sugarcane. The purpose of PAISS is to organize requests for financial assistance under the two institutions to allow greater coordination of actions for development and better integration of available financial support instruments http://www.bndes.gov.br/SiteBNDES/bndes/bndes_pt/Areas_de_Atuacao/Inovacao/paiss/.

The PAISS program is prepared to invest R$1 billion (nearly US$400 million) to install pilot plants and demonstrate innovative technologies in this area. The company GraanBio (GranBio) is planning a demonstration unit for second-generation bioethanol from sugarcane bagasse that should be operational by the first semester of 2014. The mill will have the capacity to produce 82 million liters/year, and it will use innovative solvent-free processes in the pretreatment step. The initiative will offer an opportunity to evaluate the technology and obtain process data and information for the design of large-scale units.

Thermoconversion Technologies: Torrefaction, Gasification, Pyrolysis, and Combustion

Experimental work on the thermoconversion of biomass (torrefaction, pyrolysis, gasification, and combustion) was started at the Institute for Technological Research (IPT) in São Paulo in the 1970s when the combustion laboratory was created.

Two groups stand out in this area: Carlos Luengo at IFGW-UNICAMP, who conducted basic research into the processes of thermal conversion of biomass pyrolysis and roasting, and Saul D'Avila at FEQ-UNICAMP, who researched and formed many frames in gasification and pyrolysis of biomass. Concurrently, CIETEC of Rio Grande do Sul was conducting research in the area of thermal biomass conversion. The CTC in the 1990s led first phase of the GEF project with the support from the World Bank to conduct research and gather information about

the gasification of straw and bagasse. This project was designed for the eventual construction of an advanced gasification pilot plant, but was halted because of funding problems. CHESF, with the participation of Shell and support from GEF (Global Environmental Facility), attempted to complete the second phase with a focus on gasification of eucalyptus wood, but because of administrative and financial difficulties, the second phase of the project was never completed. The IPT currently continues to work in this area in collaboration with Swedish industries that research pyrolysis charcoal. The work performed by the UFMG (Maria Emilia Rezende) resulted in the creation of the company Biocarbo, although it is not involved in managing sugarcane biomass. As a result of the efforts of Saul D'Avila, José Cláudio Moura and Themistocles Rocha, the Termoquip company was started in the region of Campinas and produces biomass gasifiers, including Petrobras, and has been instrumental in the creation of various reactors used for research at UNICAMP (FEQ, FEM and AEC) and UNIFEI. A spin-off company called Bioware produces thermal conversion technology for sugarcane and produces products such as bio-oil, acids and pyrolytic carbon. A lab-scale pyrolysis and gasification unit was designed and constructed specifically to process sugarcane bagasse and straw and is currently in operation with the focus of obtaining kinetic data for the reaction and gathering useful information for process scale-up. The project is coordinated by Rubens Maciel Filho at FEQ/UNICAMP.

2.4.7 New Bioenergy Research Centers and a Graduate Program in Bioenergy in Brazil

The new generation of bioenergy research centers in Brazil linked to the resurgence of ethanol have been motivated by events of this century such as the electricity "blackout" in 2002, production of flex-fuel cars, and interest by the United States in second-generation ethanol research, which is considered more sustainable than the first-generation when compared to ethanol from corn and other cereals.

In the early 1990s, he National Reference Center for Biomass-CENBIO was created to develop research activities in conjunction with universities and companies in the area of bioenergy. CENBIO has made important contributions to government policies at the state level, such as studies conducted by the State Committee for Bioenergy and coordinated by José Goldemberg (Goldemberg et al. 2008).

In 2005, a project coordinated by Rogério Cerqueira Leite was begun that performs a series of studies with the Center for Strategic Studies and Management in Science, Technology, and Innovation—CGEE to study issues involved with the possibility of replacing 10 % of all gasoline consumed worldwide with ethanol from sugar cane by 2025, which would constitute an increase of approximately 10 times the ethanol currently produced in a season (Leite 2009 and Leite et al. 2009). This study allowed Brazil to better understand the importance of producing high-level

research toward the sustainable use of whole-sugarcane resources. Created officially in 2008, the National Laboratory of Science and Technology of Bioethanol—CTBE at the CNPEM in Campinas, SP began 5 research programs covering basic research, mechanization, minimum impact agriculture, hydrolysis, and virtual biorefinery sustainability.

The federal government created the Agroenergy Embrapa Center in 2006 in Brasilia research issues related to biodiesel, ethanol, and the energetic use of agricultural and forest residues.

Petrobras Biofuels has promoted the engagement of CENPES in the research of biofuels and is primarily studying second-generation ethanol.

The reorganization of IAC sugarcane research that was started in the 1990s led to the creation of the Centro Cana IAC/APTA in Ribeirão Preto, where laboratories were installed to research plant breeding, agricultural entomology, biotechnology, agribusiness technology, and management of sugarcane varieties. The research center currently houses a public collection of germplasm of sugarcane.

The creation of the State of São Paulo Bioenergy Research Center was also important. This center is a consortium of three state universities in São Paulo (USP, UNICAMP and UNESP) and FAPESP and will have a research budget of $75 million. The infrastructure is funded by the government of the State of São Paulo, and the universities are already hiring professors. FAPESP's role is to fund high-quality research in bioenergy.

A new Ph.D. program in bioenergy is being jointly implemented by USP, UNICAMP, and UNESP in an attempt to create highly qualified human resources. This initiative demonstrates the commitment of these universities in promoting long-term research and innovation in the field of bioenergy in Brazil. The new program will have a strong international orientation and will collaborate with the best universities and research centers around the world http://agencia.fapesp.br/en/17301.

2.5 The Use of Vegetable Oils for Biodiesel Production

2.5.1 Brazilian Biodiesel Research from Vegetable Oils

When Proálcool was first begun, the federal government also proposed "Proóleo," a national program based on the production of vegetable oil fuel to replace diesel.

Unlike the sugar and alcohol sector, which had already organized, Proóleo did not have a sector or culture to lean on in 1970s. At the time, soy was a nascent culture in Brazil, and other oilseeds, including palm, were not produced commercially or at scale. Fernando Homem de Melo and Eduardo Giannetti da Fonseca from USP, writing in "Proálcool, Energy and Transports," calculated land requirements for different cultures of oil crops (Melo and Fonseca 1981).

In 1969, Leopold Hartman at FEA—UNICAMP published an important article on the transesterification of vegetable oils and created the Laboratory of Oils and

Fats with the support of GTZ (Informativo SBCTA 2005). The first patent for biodiesel and aviation jet fuel in Brazil (PI8007957) is credited to Expedito Parente in 1980. Ulf Schuchardt from IQ—UNICAMP patented the use of vegetable oils for fuel purposes (#PI 8302366-6) and published "Continuous reactor with organic heterogenized catalysts for transesterification of vegetable oils" in 1982 and "Process for the preparation of esters with organic catalysts and method of rapid determination of the composition of oils and fats" in 1983.

Although there was available technology to produce biodiesel in Brazil, problems remained concerning the raw material. Replacing diesel fuel would require the cultivation of an energy crop for oil, as sugarcane was for ethanol. Although the cultivation of palm is considered equivalent to sugarcane, an agricultural sector did not exist that was sufficiently organized to realize this potential. However, recent information indicates that the palm crop is expanding in the State of Pará.

Biodiesel is popular in Europe, where it is produced with rapeseed, and was initially considered as an option in Brazil that could meet social goals and alleviate dependence on diesel. On January 13, 2005, the federal government enacted Law No. 11.097, which created the National Program for Production and Use of Biodiesel—PNPB. Despite initially suggesting the production of biodiesel in family units and making use of transesterification of ethanol, more than 80 % of biodiesel in Brazil is currently produced using soybeans and methanol because of the absence of other oil crops on the scale of soybeans and the technical difficulties in the transesterification of ethanol.

If Brazil invests in oil crops with energy potential such as oil palm and produces the equivalent of the biodiesel yield in Malaysia and Indonesia, which is approximately 5,000 L of oil/ha year, then a sustainable program to replace diesel with biodiesel can be developed, an example of which has been developed in Colombia.

2.5.2 Other Routes for Biodiesel Production

The sector of renewable chemicals is also considering of producing new compounds and biofuels via biological routes, which is called synthetic biology. Synthetic biology is engaged in the construction of new components and biological systems or redesigning natural systems using evolutionary processes. These artificial systems can perform new tasks, such as the production of plastics, biokerosene and bio-gasoline. A major initiative is underway by Amyris, a California company that opened a subsidiary in Brazil for the production of new biofuels such as biodiesel and aviation fuel from sugarcane sucrose (Amyris 2008). Amyris's initial goal was to develop technology that allowed the production of an antimalarial drug, artemisinin, by microorganisms. The platform is being applied industrially to the development of yeast with the ability to produce gasoline and

kerosene and to the scheduling of production processes for farnesenes. This approach must be improved to match production requirements and market prices, especially with respect to bio-kerosene.

The production of biodiesel from algae should also be mentioned, particularly for its ability to minimize land use for biofuels. This technology aims to produce third generation biofuels, which are produced from the use of CO_2.

A group led by Franco and Maciel at FEQ-UNICAMP and Ana Teresa Lombardi (UFSCar) have been researching the use of algae to produce biodiesel; however, production of biodiesel from algae is still somewhat advanced in Brazil, although it deserves attention, especially because it is so innovative in that it does not require land and makes use of CO_2. Numerous lab-scale studies are evaluating the use of microalgae as a lipids feedstock for biodiesel, and many interesting options are under consideration, especially those that integrate CO_2 from ethanol mill fermentation to increase microalgae growth (research project funding by FAPESP 2008/57873-8).

2.6 Future Perspectives of Biofuels for Aviation in Brazil

In October 2011, Boeing, Embraer and FAPESP formally agreed to investigate how Brazil could contribute to the production of sustainable biofuels for aviation in an attempt to reduce CO_2 emissions. The result was Sustainable Aviation Biofuels Brazil, a national assessment of the technological, economic and sustainability challenges, and opportunities associated with the development and commercialization of sustainable aviation biofuel in Brazil.

Multiple possible pathways to produce biofuels for aviation were identified during the project. Certification requirements for use in commercial aviation are established internationally according to ASTM D7566, which contains one special annex for each approved alternative jet fuel production process. Figure 2.12 presents an overview of all identified pathways pertinent to Brazil, including the denomination and status of the ASTM approval process. As depicted, two of the final jet fuel production processes are already approved (green boxes in Fig. 2.12), and several others are still under analysis in ASTM's Emerging Fuels Committee.

The project concludes that Brazil has exceptional conditions to develop and produce sustainable biofuels for aviation. However, additional research is required to develop the identified pathways and additional effort is required to improve the transportation infrastructure to lower raw material costs. More information can be found here: http://www.fapesp.br/publicacoes/flightpath-to-aviation-biofuels-in-brazil-action-plan.pdf.

Recently, the Brazilian National Agency for Oil, Natural Gas and Biofuels-ANP approved resolutions for biofuels for aviation in Brazil (http://www.petronoticias.com.br/archives/31531).

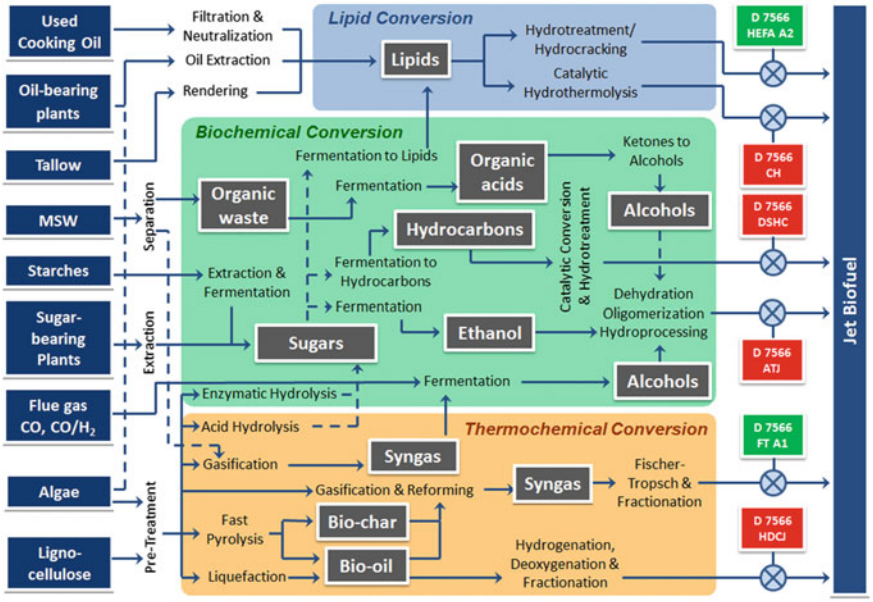

Fig. 2.12 Identified pathways for the production of sustainable jet biofuel in Brazil [*Note HEFA* Hydroprocessed Esters and Fatty Acids; *CH* Catalytic Hydrothermolysis; *DSHC* Direct fermentation of Sugars to Hydrocarbons; *ATJ* Alcohol to Jet; *FT* Fischer-Tropsch hydroprocessed-synthesized paraffinic kerosene; *HDCJ* Hydrotreated Depolymerized Cellulosic to Jet] (Boeing et al. 2013)

2.7 Conclusions

Brazil has a prosperous future in the field of bioenergy. The participation of sugarcane in the Brazilian energy matrix has grown 1 % per year since 2002, reaching 19 % in 2010. Although the potential is much larger, not only for sugarcane but for eucalyptus, palm tree, and innumerous other crops, the country has already built a significant history in the bioenergy area. However, much more can be done, particularly in adding combined value to products and utilizing concepts that produce sustainable solutions to improve the industry in the new agricultural frontiers of Brazil.

The long-term role played by the Brazilian government in promoting biofuels is considered as a key factor to success, particularly with sugarcane ethanol. Government-funded research agencies have played a strategic role in consolidating knowledge and human capacity to maintain leadership in the bioenergy sector.

However, investments in research and development of human resources in the area of bioenergy must also grow proportionately, particularly in the private sector. With new research centers, graduate programs have the potential to contribute to increasing competence at all stages of bioenergy development.

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Chapter 3
Renewable Liquid Transportation Fuels: The Cornerstone of the Success of Brazilian Bioenergy Program

Veronica de Araujo Bruno and Adilson Roberto Gonçalves

Abstract This chapter presents the historical evolution, together with social and economical aspects concerning biofuels in Brazil. The country plays an important role due to its large land area and the tropical climate, favoring sugarcane and soybean cultures. In respect to ethanol, Brazil has reached process and technology maturity, the production is rising and the market will grow in the coming decades, drived especially by flex-fuels engines. Currently, attention is also focused on second-generation ethanol, obtained from lignocellulosic materials. Networks for ethanol production are much more structured, integrated, and developed than those for the production of biodiesel. Addition of 2 % biodiesel from soybean to regular diesel, contributed significantly to increase domestic production of this biofuel, pushing Brazil to a global context. In 2010, this percentage increased to 5 % and is forecasted to reach 20 % in 2020. When anhydrous ethanol from sugarcane is mixed with gasoline at a 25 % ratio, 1900 kg CO_2 eq/m^3 of bioethanol is avoided. The use of biodiesel to replace diesel fuel reduces 90 % emissions of burning gas and 78 % of smoke emissions.

3.1 Introduction

During the 1970s OPEC decided to raise the oil price by 70 %. Countries depending on this fuel were forced to develop new sources of energy. As one of those countries, Brazil began the intensification of programs supporting the energy matrix diversification, oil crisis being a driver for the biomass fuel's development.

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However, the national interest in biofuels dates back much earlier. Since 1920, Brazil produces vegetable oils and the first official push for the production of renewable fuels was in 1938: law 737 ruling the blending of ethanol in gasoline nationwide (Goldemberg et al. 2004).

Currently, research in renewable fuels is focused on reducing greenhouse gas emissions and on energy security supply (Masieiro and Lopes 2008). Brazil plays an important role due to its large land area and the tropical climate, favoring sugarcane and soybean cultures. The energy agriculture could be incorporated in the Brazilian economy without competing with agriculture for food, as well as be possible to perform multiple cultures within the year.

In respect to ethanol, Brazil has reached process and technology maturity, the production is rising and the market will grow in the coming decades (Brazilian Government 2011). Brazilian ethanol production can be divided into four stages. The first was from the beginning to the 1970s, when production was intended mainly to manufacturing beverages, considering ethanol as a by-product of sugar production. The second stage began with the creation of Proalcool in 1975 which determined that ethanol be mixed with gasoline in cars. Already in 1979 the second phase of the program was initiated, the production reached 3.4 million m^3, and the goal of the program became the incentive for the production of cars that moved entirely on ethanol. At that time, there was great expansion in the sector due to the large number of autonomic distilleries. In 1985, the production of Brazilian ethanol accounted for about 80 % of world production. The program, however, lived the end of its apogee with the drastic drop in oil prices, reducing the attractiveness. Recently, after almost 20 years, ethanol production has entered a new stage with automotive *flex fuel* engines that operate with fractions of gasoline and ethanol in any proportion (Milanez et al. 2008).

Currently, attention is also focused on second-generation ethanol, obtained from lignocellulosic materials. Ethanol from straw and sugarcane bagasse is promising and a sustainable alternative to the by-products from the sugarcane industry and does not imply the need for an additional plantation area. However, technical difficulties are at play for the extraction of sugars from lignocellulosic matrix for subsequent fermentation. Hydrolytic enzymes together with chemical and physical treatments are necessary to break the biomass structure, allowing the cellulose be accessible (Masiero and Lopes 2008).

While studies of renewable fuels started in Brazil in the 1920s, only in 1975 the use of vegetable oils for energy purposes was actually proposed. This occurred through the Pro-Óleo program, which aimed to sell surplus of vegetable oil, including a blend of 30 % vegetable oil in diesel and increase up to 100 % in long-term use. Decrease in oil price in 1986 decreased research incentives. In 2004, biodiesel production was resumed with the National Program for Production and Use of Biodiesel (PNPB), which included the addition of 2 % biodiesel to regular diesel, contributing significantly to increase domestic production, pushing Brazil to a global context. In 2010, this percentage increased to 5 % and is forecasted to reach 20 % in 2020 (ANP 2011).

In Brazil, ethanol is responsible for the majority percentage (16 %) of energy from biomass, while less than 3 % is derived from biodiesel. According to the Ministry of Mines and Energy in 2010–2011, about 28 million m^3 of ethanol, and 2.4 million m^3 biodiesel (MME 2011) were produced.

3.2 Sugarcane and Soybeans: The Foundations of the Brazilian Biofuel

Brazil has 152.5 million ha of total available arable land (17.9 % of the territory), and 62.5 million ha (7.3 %) are already under use (Safras e Mercado 2012).

Currently in Brazil, 90 % of vegetable oil is produced from soy and 80 % of industries produce biodiesel using soybean oil as feedstock. The remaining corresponds to animal fat (15 %) and other oilseeds (5 %) (Castellanelli 2008).

According to Embrapa (2011), the 2010/2011 national harvest produced 75 million tons of soybean, making Brazil the second largest producer in the world, standing just behind the United States. Soybean emerges as the main raw material for the production of domestic biodiesel (Embrapa 2011).

Sugarcane occupies the first position in agricultural production (675 million tons in 2011), making Brazil the world's largest sugarcane producer, but the second ethanol producer, United States being the first.

In Brazil there are two crops of sugarcane, depending on rainfall patterns: one occurs in the South-Central region (April to December) and the other in the North-Northeast (October to March). Thus an integration of crops occurs, allowing ethanol supply throughout the year (Gorren 2009).

The ideal climate provides a long, hot season with high solar irradiation and moisture from rainfall, and another season reasonably dry but sunny and cool, frost-free for the ripening and cultivation. The total amount of water decreases in the cane growing, going from 83 % in young up to 71 % in mature plants. However, the sucrose content varies from 10 to 45 % (Embrapa 2009).

Besides, the natural conditions of the Brazilian territory are favorable to growing sugarcane for obtaining ethanol; other advantages are also found compared to alcohol from corn. Planting corn uses high amounts of pesticides from fossil fuels; processing the alcohol from corn emits more CO_2 and the productivity is low compared to ethanol from sugarcane (Schaeffer 2007).

Although Brazilian ethanol still finds obstacles for its export, the country configures worldwide as a protagonist in the production of clean, renewable energy, and recognized due to its large potential in the field (Rached 2011).

3.2.1 Ethanol

There are two types of ethanol, hydrated and anhydrous. The alcohol resulting from biological fermentation of sucrose is hydrated, a colorless liquid whose composition is approximately 5 % water. To obtain anhydrous ethanol, a further dehydration step is needed to decrease the percentage of water to 0.5 % (Gorren 2009).

Distilleries are those capable of producing ethanol and sugar in varied proportions, while independent distilleries are those that are dedicated exclusively to the production of alcohol.

Production of alcohol is a series of interconnected unit operations, with the main objective of converting sugar into alcohol, consisting basically of two main steps, fermentation and distillation.

After grinding, the sugar syrup (molasses) is adjusted regarding sugar concentration, acidity, nutrients, and antiseptics. Yeast is added and, after fermentation time, the wine is separated from the yeast that is recovered for a new use cycle. The wine is transferred to decanters and after other cleaning processes distilled to obtain ethanol (Castro 2011). The most widely used process in the ethanol industry is *Melle-Boinot-Almeida,* consisting of a batch process with cell recycling.

3.2.2 Biodiesel

Biodiesel is a synthetic fuel made from vegetable oils, animal fats, algae, or fungi. The most common way of producing this fuel is through transesterification and esterification of vegetable or animal fats and oils (Krawczyk 1996). Brazilian biodiesel is derived mostly from soy. In 2010, they produced in Brazil approximately 680,000 tons of soybean oil (CONAB 2012).

The first step in the biodiesel production process is the preparation of raw materials. The oil received is stored in a tank and later pumped to a decanter which separates the materials in suspension and then limed. Oil and alcohol are then pumped into the reactor, where the transesterification occurs.

The next step is the separation of coproducts. The mixture is transferred to a separator, where the esters are glycerin and stored in separate storage tanks. Finally, purification of the esters is performed, and distillation of biodiesel and glycerin.

A major problem in obtaining biodiesel is the large quantities of glycerin formed as a by-product. The production of 90 m^3 of biodiesel generates 10 m^3 of glycerin (SBQ 2009). New uses for glycerin have been investigated such as a composite for reducing friction in oil rigs.

Table 3.1 Adjustments percentage of anhydrous ethanol added to gasoline (MAPA 2011)

Regulation (Brazilian nomenclature)	Scope	Percentage (%) of ethanol added to gasoline
Decreto 19.717—Feb, 20 1931	Brazil	5
Decreto 59.190—Sept, 8 1966	Brazil	25
Portaria CNP 94—July, 1 1976	Pernambuco (NE)	10
Portaria CNP 88—May, 19 1977	São Paulo	20
Portaria CNP 245—June, 30 1981	Center–South	12
Portaria CNP 142—Nov, 16 1989	Brazil	13
Portaria MAPA 278—Nov, 10 2006	Brazil	23
Portaria MAPA 7—Jan, 11 2010	Brazil	25
Portaria MAPA 678—Aug, 31 2011	Brazil	20
Portaria MAPA 105—Feb, 28 2013	Brazil	25

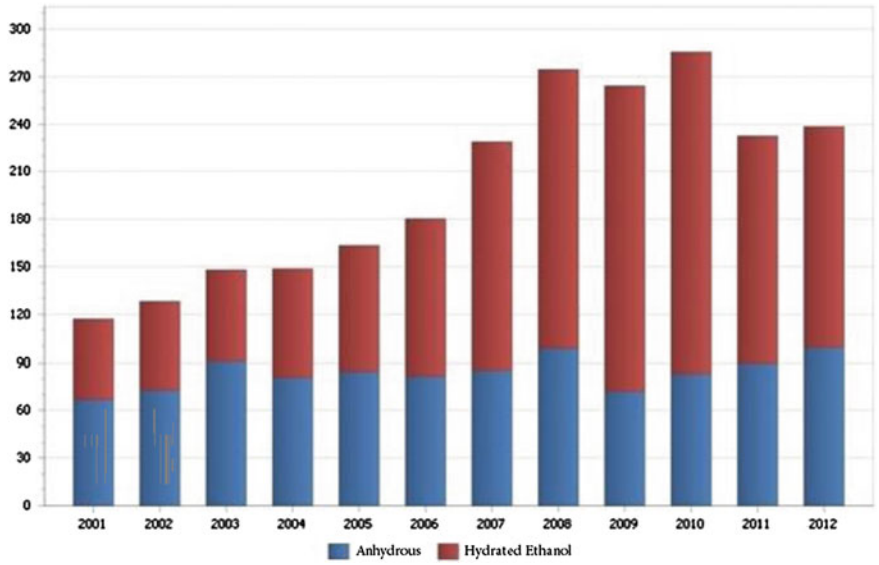

Fig. 3.1 Production of anhydrous and hydrated ethanol million liters over the years (IBP 2012)

3.3 Brazilian Strategies

In Brazil, the use of the mixture of anhydrous ethanol in gasoline, dating back to the 1930s, has been subject to adjustments, discussions, and energy policies. Percentages of anhydrous ethanol range depending on the region and the economic policy of the time. Different regulations have been applied over the years, as observed in Table 3.1.

Figure 3.1 shows the production of anhydrous and hydrated ethanol in Brazil.

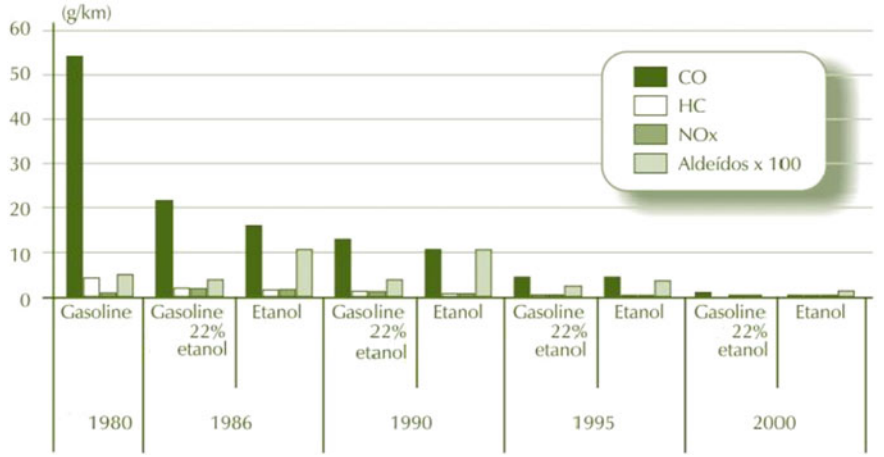

Fig. 3.2 Evolution of car emissions in Brazil (IBAMA 2006)

The exhaust gases produced by combustion reactions inside motors released into the atmosphere became one of the major reasons for concern about the pollution of the environment, specifically the atmospheric air. A well-known fact is that burning ethanol, or their mixture with gasoline, releases smaller amounts of carbon monoxide, sulfur oxides, hydrocarbons, and other polluting compounds. Figure 3.2, compiled from data from IBAMA, presents the reduced greenhouse gas emissions in Brazil over the years.

More recently, in 2003, official rule determined the evaluation of Brazilian biodiesel production to understand its current situation, availability, as well as advantages and disadvantages. With satisfactory results in hand, the Federal Government decided to immediately take the necessary measures to ensure that biodiesel became representative in the Brazilian energy matrix and thus created the National Program for Production and Use of Biodiesel (NPPB) in December 2004. The main objectives of the program were the stimulus to the formation of a national biodiesel market, a definition of tax model, creating lines of funding and development of farmers organizations for both counting on the collaboration of the main industries involved in producing this biofuel chain.

Biodiesel can replace diesel oil, obtained in the fractionation of oil in diesel cycle engines, for example, present in trucks, buses, airplanes, and tractors or can be added to it in high proportions. The Act 11.097/2005 made compulsory the addition of 2 % biodiesel to diesel from 2008 throughout the national territory. A new adjustment increased this percentage to 5 % in 2010 and, currently, a regulatory mark was sent to the National Congress to increase this percentage to 7 % by 2014 and to 10 % by 2020, which would mean 7.5 billion liters of biodiesel produced and consumed in the country. Studies show that biodiesel can be added up to 20 % without compromising the efficiency and without being necessary adjustments to the engine.

3.4 Biofuels and the Environment

Modern environmental concepts rule every industrial process, sustainability being inserted and prioritized in order to use from nature and return to it in the same proportion. The development of biofuels has confirmed to be the most viable solution to the energy problems, especially when biofuels replace petroleum, mitigating greenhouse gas emissions. Any CO_2 generated by burning biofuel is incorporated again to the carbon cycle through photosynthesis. However, biofuel production, from cultivation to the final consumer, uses fossil fuels.

Currently, the production of anhydrous ethanol from sugarcane generates 440 kg CO_2 eq/m^3 of bioethanol, while ethanol from corn generates 1700 kg CO_2 eq/m^3. This is mainly because other fractions of maize as a source of energy are not used. When anhydrous ethanol from sugarcane is mixed with gasoline at a 25 % ratio, 1900 kg CO_2 eq/m^3 of bioethanol is avoided (Macedo et al. 2008).

Like ethanol, biodiesel has smaller net greenhouse gas launched in the atmosphere than burning different fossil fuels. The use of biodiesel to replace diesel fuel reduces 90 % emissions of burning gas and 78 % of smoke emissions.

These rates are measured from the entire lifecycle of biofuels production, soil preparation, use of pesticides and fertilizers, harvesting, manufacturing, storage, distribution, and use as fuel.

3.5 Perspectives and Conclusions

Brazil not only sets up as one of the most developed countries regarding the use of renewable energy sources, but also because this sector is constantly expanding. Climate and territorial conditions were always favorable to agriculture in the country, and as a result the production of biofuels from plant biomass became especially extremely viable in a global scenario which seeks to mitigate the use of fossil fuel origin.

Ethanol from sugarcane and biodiesel produced by soybeans are the most significant liquid biofuels, a scenario constructed over the years as a combination of a number of factors that favored such production forms. The current Brazilian's moment shows that networks for ethanol production are much more structured, integrated, and developed than those for the production of biodiesel. However, despite its more recent history, soybean biodiesel has grown quickly and studies aimed to its development prove to be promising.

By analyzing some aspects regarding biofuels, we conclude that they have undoubtedly great environmental advantages over fossil fuels. But there is a notable difference between these two biofuels. While sugarcane is planted mostly to meet the fuel market, to be the most advantageous among the crops for this purpose, soybean is planted with the aim of meeting the food market. Biodiesel production using soybean becomes a consequence of its large crop in the country, but among other oilseeds soybean does not have greater benefits in all aspects.

Biofuels question has long ceased to be purely energetic and has achieved social, political, and economic sphere, becoming a government policy.

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Chapter 4
Socio-Economic and Ambient Impacts of Sugarcane Expansion in Brazil: Effects of the Second Generation Ethanol Production

André Luis Squarize Chagas

Abstract The growing demand for clean energy sources to replace petroleum has substantially expanded the use of biofuels—fuels produced from agricultural products. For Brazil, instead of representing a hindrance to growth because of the need for changes in the country's energy mix, this represents a great opportunity to generate value and income, since the country has clear comparative advantages in producing these fuels from renewable sources. The main biofuel in the country is ethanol, made from sugarcane. The country's cane growing sector has been undergoing intense transformations, with the attraction of foreign capital, opening of new distilleries and intensification of mergers and acquisitions. However, doubts have been raised about the socioeconomic effects of the spread of sugarcane growing, such as the effects on the environment, labor market, social conditions and food prices, among others. This work reviews the papers that discuss these impacts. The results suggest that the expansion in recent years helps to improve the capital-labor relationship; the sugarcane growing is not the cause of increased land and food prices; the environmental indicators in sector is better than fossil fuel sector, or other relevant concurrent; the sector has no significant effects (positive or negative) on social conditions in cane growing regions, and that the sector can contribute positively by increasing local tax revenue.

Keywords Sugarcane · Social impacts · Environmental impacts · Second generation ethanol production

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4.1 Introduction

The growing demand for clean energy sources to replace fossil fuels has caused great expansion of bioenergy, fuels produced from agricultural products, mainly ethanol and biodiesel. These products can be obtained from different sources of raw materials. In the case of ethanol, we highlight sugarcane and corn, and in the case of biodiesel, soybeans, canola, palm oil, castor oil, among other various sources. The new needs provide increasing prominence to bioenergy, and represents greatest potential to generate income for countries that have comparative advantage in the production of these goods.

Ethanol production from renewable sources places Brazil at the forefront of the search for clean energy, along with the strong presence of hydroelectric energy matrix of the country. Brazil is the only country that has a large-scale program of vehicles with engines that use clean and renewable energy sources. The competitiveness of ethanol produced from sugarcane in Brazil is significantly higher than that of other producers, especially in relation to U.S. corn ethanol, both in the matter of production costs, such as energy balance (BNDES and CGEE 2008).

In recent years, the sector invests in second generation of ethanol, to increase the production. The production of second generation ethanol, or cellulosic ethanol, consists of a first stage of pre-treatment, hydrolysis for liberating the molecules, a second steps in degradation of sugars enzymatic or chemical means, and the last fermentation stage, obtaining as ethanol product (Lee 1997; Chandrakant and Bisaria 1998; Lin and Tanaka 2006; Cardona and Sánchz 2007; Soccol et al. 2010). In contrary than other places, in Brazil, the second generation of ethanol will make deepening the current mode of production (Chagas 2013).

The main raw material to cellulosic ethanol, in Brazil, is the sugarcane bagasse obtained as waste in the process of juice extraction plant. Bagasse is typically fibrous, with high content of lignocellulosic material. Currently, the bagasse is used for cogeneration of electricity, but in future, it can be used to ethanol production (Pandey et al. 2000; Macrelli et al. 2012).

The ethanol production based on sugarcane bagasse is favored, in Brazil, because the production process can be attached to the already existent plants, requiring lower investments, infrastructure, logistics and energy supply. Besides, the bagasse is generated at the industrial units, and with lowest transportation costs. This is a promising scenario because from each 10 million tons of dry biomass, 600 million gallons of ethanol could be produced, considering the use of its cellulosic part only (Soccol et al. 2010). Including the bagasse in production, the ethanol production can increase at least 30 % reaching to 100 % (Santos et al. 2012).

For Brazil, the sugarcane expansion represents a great opportunity to increase the value and income, since the country has clear comparative advantage in the production of renewable fuels (Hoffmann 2006). Nevertheless, there are controversies about the indirect effects of ethanol from sugarcane on the environment, market labor, and social conditions in producer regions (Chagas et al. 2008, 2011; Chagas 2009).

The purpose of this article is review recent studies that analyze the impact of the production of sugarcane, especially in producers regions. In Sect. 4.2, I comment the work that analyses the sugarcane market labor in recent years. The Sect. 4.3 reviews the food versus bioenergy discussion, and Sect. 4.4 discusses the climate impacts of sugarcane production. In the next section (Sect. 4.5), I discuss the social impact of sugarcane production and other impacts, I report in Sect. 4.6. The last section presents the conclusion.

4.2 Labor Market

The most part of the studies about the sugarcane market labor relates to health problems associated to the harvest manual, accidents in job, and energy expenditure and repetitive activity. The main idea associated to the sector workforce is the strong labor-unskilled presence and the temporary labor for manual harvest. This characteristic results in intense migratory flows to producer regions in harvest periods. Additionally, the work is unhealthy and requires high physical exertion, resulting in a number of severe accidents and mortality (Alessi and Navarro 1997; Scopinho 2000; Alves 2006, 2007; Baccarin et al. 2008).

Alves (2006) calls attention to the extreme physical exertion required of workers in the sector, especially those engaged in manual harvesting. Although this aspect is still a problem at present, legislation in the most relevant producing areas has changed to make mechanical harvesting mandatory in the next few years.

Other chapters study the economic relationship between the cane cutter and the sugar mill (Basaldi 2007; Silva 2005). There are also some studies that show the evolution and profile of labor in sugarcane, evidencing the changes in labor relations (Goza 1997; Moraes 2007), and the implications of the process of mechanization in sugarcane sector (Ramos 2007; Staduto et al. 2004).

Some recent studies show, however, that the wage level in sugarcane cultivation is higher than in other cultures. Of course, the highest wage in sector may be consequence of the effort of work. But, other indicators are also better in sugarcane sector than others sector, when is higher the degree of formalization of labor relations (formal signed contract), the presence of child labor is lower. Possibly because it is a culture whose product has greater integration in foreign markets, and larger scale production, its working relationships are more formalized and in accordance with the law (Toneto-Jr and Liboni 2008).

Moraes (2007) analyzed the impact of the end of the burn during the harvest process. The conclusion is that there was an increase in mechanization and changes in the profile of agricultural labor. Additionally, the new planting areas tend to be mainly mechanized (Toneto-Jr and Liboni 2008). The introduction of machines in harvest process is not damaging to employment since it occurs while the sector is expanding. Thus, there is not job destruction. The recent transformation still helps to deepen a feature of the sector. The wages in sector tends to increase with the mechanization.

The situation of labor in the sector tends to improve with increasing mechanization, which will tend to eliminate the aspect in which the sector indicators are worse: the low-skill of work force and the high effort. In addition, mechanization reduce the weight of the primary employment, which are the most common complaints related to fatigue and the intensity of work (Toneto-Jr and Liboni 2008; Hoffmann and Oliveira 2008). Thus, it appears to be unfounded the concerns about the deterioration of working conditions, due to a significant expansion of the sector, due the second generation ethanol, mainly when considering that the expansion of the sector will be with increased mechanization.

4.3 Food Versus Bioenergy

The capacity of expansion of production is a concern that follows the discussions on the sugarcane sector in Brazil, repeatedly. The introduction of 5 % ethanol in gasoline, in developed countries, should demand about 90 billion l/year of ethanol. Given the magnitude of these numbers is possible to think of failure of land, which would generate pressure on land and food prices (FAO 2008). With some changes, this is a recurrent debate on economic since Malthus (Abramovay 2010).

There are significant trade-offs, however, involved in the massive expansion of the production of sugarcane and other crops for fuel. Chief among these would be a shift of major amounts of the world's food supply to fuel use when significant elements of the human population remains ill-fed (Avery 2006).

The main criticism argues that the increase in sugarcane production would lead to increased competition for land use, with an increase in land rent. With higher land prices, increase agricultural production costs, impacting food prices (Chagas et al. 2008). If this argument is true, should be a long-term relationship between the sugarcane production, land rent and food prices series.

Chagas et al. (2008) tests the existence in long-term relationship. The results for Granger causality test showed that there is no temporal precedence of sugarcane production on the land rent, but rather the opposite, that is, the price of land which causes (in the Granger sense) the production of sugarcane. The long-term relationship identified establishes a common trend between these two variables, but not statistically significant. Since the coefficient of short-term adjustment to the price of land is not statistically different from zero, with the result of Granger causality test, it is concluded that the price of land is exogenous with respect to the production of sugarcane and the price of food. In other words, the order of causality identified did not show that an increase in the production of sugarcane positively impact the price of land, although the variables walk in the same direction.

With respect to the price of food, the long-term relationship with production of sugarcane follows opposite direction to what would be expected. This result is robust if the change range used for measuring the cost of food to consumers.[1]

Chagas et al. (2008) concludes that the growth in agricultural demand explains the incorrect association between increase production of sugarcane and food price. In fact, the effect of China's demand tends to pressure the international commodities price while pressing the production too.

4.4 Ethanol and Environmental Impacts

Considering the environment impact of sugarcane production, the main concern refers to the risk of contamination of soil, water use, shifting other crops to forest regions, fires, use of protected areas (springs, riverbanks, mountain tops, etc.), among others. Several studies were undertaken in order to estimate the amount of fossil energy expended in the production of sugarcane in the Brazilian conditions. Among which may be mentioned: Macedo (1998), Macedo et al. (2008), Urquiaga et al. (2005), Pimentel and Patzek (2008), Oliveira et al. (2005), Oliveira (2008).

The conclusion of these studies is that the energy balance of sugarcane (ratio of total energy contained in the fuel produced and fossil energy invested in its production) is quite varied. Studies undertaken by researchers in Brazil estimate this relationship between 8 and 9, and can reach to 12, under certain conditions. However, studies performed abroad indicate far less expressive numbers, around 3.7 and 1.1. The main reason for this divergence of findings refers to the assumptions adopted in the calculation.

The studies carried out abroad (Pimentel and Patzek 2008; Oliveira et al. 2005; Oliveira 2008) assume very outdated technology in field operations, resulting in a consumption of fossil energy much higher than what would be reasonable. Pimentel and Patzek (2008) estimated consumption of 2,596 Mcal (approximately 10,640 MJ) per a thousand liters of ethanol, due to energy use in the preparation stages of cleaning and crushing of sugarcane in conveyor belts, filters and centrifuges and heating the juice for fermentation. These values of power consumption account for about a half of all the energy contained in the ethanol. However, Brazilian mills produce all the energy they consume these processes from burning bagasse in high pressure boilers, whose steam generated drives turbines that produce electricity cogeneration unit. So it is not correct to assume that these energy costs come from fossil source (Chagas 2013).

Soares et al. (2009) presents a comprehensive review of available data and factors of fossil energy consumption in the production of sugarcane in the

[1] The argument, however, seems valid with respect to the price of food at wholesale. This difference between the consumer price and wholesale price, is possibly due to the fact that wholesale prices closely follow production decisions, while consumer prices also depend on industrial dynamics and technological innovations, that can dampen the effects of any reallocation of land use.

Brazilian conditions.[2] Whereas a liter of ethanol produces 21.45 MJ of energy, a hectare of sugarcane can produce 6,510 l of ethanol per year, generating 139,639 MJ of energy, approximately nine times the fossil energy invested in agricultural operations.

Thus, from an environmental point of view, the sugarcane sector has very positive results, due to use of a renewable feedstock for the production of cleaner fuels, enabling both the use of by-products and generate a less polluting energy to country. Additionally, the sector complements the hydroelectric power supply, because the harvest of sugarcane in Midwest and South occurs in the dry season and low in the reservoirs (Chagas 2013).

Nevertheless, there are other environmental concerns related to the production of ethanol and sugarcane, like environmental impacts due new investments, legal reserves, harvest's burning, by-products generated in the production process.

To control the environmental impacts due new investments, all business plans should obtain an environmental license and submit the Environmental Impact Assessment and Environmental Impact Report (EIA-RIMA, in Portuguese acronym) requires study prior to any activity that may potentially cause environmental degradation. Actually, three types of licenses are required: preliminary permit approving the location and design of the project and establishes the requirements for obtaining licenses following; installation permit and operating license. The latter is 3 years for the production of sugar and 2 years for ethanol and should be requested renewal before expiration. Licensing is the responsibility of the state environmental authority, except in cases where venture beyond the limits of the state.

In relation to land use, it is stipulated the requirement for a legal reserve of approximately 20 % of the total area that cannot be used beyond the preservation of permanent protection areas (riparian forests, river springs, etc.). This is a problem in the sector, since historically the sugarcane producers with advanced plantations in all areas, including the areas of permanent protection.[3] The intensification of monitoring has led the implementation of significant programs of riparian forests restoration and protection to the sources. It is noticed that there was a significant progress in the areas of sugarcane plantation on protected areas, mainly in the past. In many regions these problems are being repaired. Increased supervision and greater control of compliance have led to recovery of protected areas.[4]

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[2] The estimate of total fossil energy used in field operations, including the transportation of cane to the mill and the supply of inputs, is 12329.7 MJ/ha/year. Already tickets fossil energy associated with the material used in the construction and equipment of plants representing 2,611 MJ/ha/year, totaling 14940.8 MJ/ha/year.

[3] In São Paulo state, 8.1 % of the cane area refers to riparian forests. Of this area, 3.4 % have natural forest and 0.8 % was reforested (Chagas 2013).

[4] There is a discussion regarding the responsibility for the preservation of protected areas and legal reserves. The plants produce using own cane, sugarcane produced on land owned and leased areas, and third-party sugarcane, obtained from the suppliers. In relation to land owned and third-party suppliers, there is no doubt about the responsibility, but in relation to leased areas is doubtful.

In the past, many rivers in Brazil were contaminated by stillage discharged by the mills. Currently, the water withdrawn for industrial process is almost entirely treated and reused in the plant itself, causing low water uptake; industrial systems are virtually closed. The sugarcane produced in the traditional regions of Midwest and Southwest regions uses virtually no irrigation, depending basically of rains. The capture of water in surface and/or groundwater is controlled by the state and depends on the granting of grants by the environmental agency (Department of Water and Power, in the case of São Paulo). In several watersheds of the São Paulo state the capture, consumption and disposal of effluents are charged, which should induce a reduction in funding and better treatment of wastewater.

Currently, almost all by-products are utilized in the production process of the sugarcane mills. The stillage (vinasse) is used for fertilization in the field in a process called fertirrigation[5]. Another by-product used as fertilizer is the filter cake.

Also in relation to the consumption of fungicides (practically zero) and pesticides, these are lower than the other crops. The borer control (major pest) and leafhopper is made by biological means. Only the control of ants, beetles, and termites are made by chemical means. The use of pesticides (fungicides, herbicides, and insecticides) is also regulated by federal law and controlled by state or federal agency depending on the state.[6]

The burning of harvest is a public health problem, as will comment below, because it causes respiratory problems. This practice tends to occur with greater intensity during the dry season, which intensified their negative effects. Burning is a practice designed to facilitate manual harvesting of sugarcane. The law prohibits certain types of fires in certain areas and times. The controlled burning of sugarcane is regulated by specific federal law (Decree 2661/98), and in the State of São Paulo has a specific law more restrictive (State Law 11.241/02). The trend is that this practice be ended in a few years, both by regulatory pressures to reduce the emission of pollutants and their harmful effects, as by the economic stimulus resulting from full use of the cane (sugarcane juice, straw, leaves and bagasse). The issues related to the labor market, formalization of hand labor and workforce enhancement contribute too.[7]

The advance in mechanization could contain the burning process. Harvest mechanization of sugarcane reached 65.2 % of the harvested area in the state of São Paulo, in 2011/2012 season. Data from the Environmental Protocol Sugarcane Industry shows that sugarcane production in the state has been fulfilling the targets

[5] As the vinasse is a valuable organic fertilizer and a source of replacement water into the soil, your use reduces the need for fertilizers and water. There exist rigorous restrictions on the amount of vinasse used by area, so there is no problem of contamination of the soil, and the plants.

[6] The limits of use are determined and monitored by specific department, and the producers are required to return the packaging used.

[7] In São Paulo, the Environmental Protocol signed between the plants, sugarcane producers and the government establishes the order of the burned areas for mechanization in 2014 and in all areas in 2017.

set for reducing burned. Since 2007, when the proposal was signed between the sugarcane industry and the Government of São Paulo State, mechanization increased from 34.2 to 65.2 % of the harvested area.

4.5 Social Conditions and Sugarcane Production

Piketty et al. (2008) have shown that the sugarcane culture has not played a significant role in reducing poverty and inequality in the country. Indeed, for the state of São Paulo (Brazil's main cane producing state), the authors concluded that the sector contributed to the concentration of income.

Camargo-Jr and Toneto-Jr (2008) have found a positive association between sugarcane growing and sugar and alcohol production and socioeconomic indicators. In general, municipalities with strong involvement in the sugar-alcohol sector perform better on socioeconomic indicators, and in some cases even outperform the greater São Paulo Metropolitan Region (SPMR), the state's main region in economic terms. Silva (2008) also found the same positive impact when no cross-effects on other variables are considered.

However, when consideration is made for the fact that the sector's presence can affect local human development through its impact on other variables, he found that the situation is reversed and the sector's presence has net negative impacts.

The above studies do not take into consideration the full heterogeneity of producing regions, treating regions with different aptitudes for distinct crops as the same. A more reasonable assessment must compare similar places with and without sugarcane, which is clearly impossible to do. To overcome this difficulty, Chagas et al. (2011) apply matching methods to estimate the impact of determined treatments on treated subjects, as explained in the following section.

Chagas et al. (2011) implements a spatial propensity score matching test, an original contribution to this type of study. This methodology is useful because it deals with the fact that one cannot immediately compare average indicators of cane producing regions with those of nonproducing ones, since the probability of production is not a random variable. Thus, spatial factors need to be considered to control for the probability of producing or not.[8]

Although there are arguments in favor and against the sector's impacts on local social conditions in growing regions, Chagas et al. (2011) indicates that the

[8] To calculate the spatial propensity score were considered neighborhood effects, as the proximity of county to sugar mill and a dummy variable for those located in states dense sugarcane production. The spatial effects capture both the fact that in a region whose neighbors are producers, the probability of producing sugarcane is higher (dependence or spatial autocorrelation), as well as the specific soil and climate of each region. The second part seeks to control the probability of production take place in regions near the plants (potential plaintiffs production). The last one captures state effects specific, such as legislation, ease of flow of production, access to tax incentives, etc.

presence of sugarcane is not relevant to determine their social conditions, whether for better or worse. It is thus likely that public policies, especially those focused directly on improving education, health and income generation/distribution, have much more noticeable effects on the municipal HDI than sugarcane production. Possibly public policies, especially those aimed directly at improving education, health, production and income distribution, have greater impacts on the HDI-M.

However, the same is not true when consider the healthy impact of sugarcane production, mainly to respiratory problems that it associate to burning of sugarcane. The burning is meant to increase the productivity of workers. It facilitates the harvest, easing access to the plants and reducing work hazards (dry leaves are harmful, there might be poisonous insects and snakes). It takes place in the beginning of harvest, which coincides with the dry season. Many studies relate sugarcane burning to increases in fine particulate matter, coarse particulate matter and black carbon concentration, especially in burning hours (Lara et al. 2005). Allen et al. (2004) observe increases in the concentration of substances as nitrite, sulfite, oxide of carbon, and others. The sugarcane is harvested by unskilled workers, mostly manually. The literature also indicates that exposition to classical pollutants (matter, sulfite, nitrite, oxide carbon, etc.) can affect negatively human health (Sicard et al. 2010), especially for young, elderly and woman people (Braga et al. 1999; Roseiro 2002; Gonçalves et al. 2005).

The burning of sugarcane generates a massive quantity of smoke that spreads in the region, reaching cities and becoming a potential threat to the human health. Few studies have linked the burning of sugarcane straws with respiratory diseases in the producing regions. Although the pollution from sugarcane burning may be as harmful as the pollution from traffic and manufacturing Mazzoli-Rocha et al. 2008, many studies relate its impact on health, for specific municipalities or for larger regions: Arbex et al. (2000, 2004), Cançado et al. (2006), Arbex et al. (2007), Ribeiro (2008), Uriarte et al. (2009), Carneseca et al. (2012). These studies consider only the local, or short-distance, effects of burning to respiratory health, ignoring the effects on neighboring municipalities.

Chagas et al. (2013) proposes to measure the impact of burning on respiratory problems of children, teenagers, and elderly people, working with a balanced panel of 644 municipalities, from 2002 to 2011, and using a spatial difference-in-difference technique, to control for the effect of sugarcane burning on non-producing regions in the vicinity of producing regions. The paper concludes that sugarcane burning significantly increases the incidence of respiratory problems in producing regions. The use of a spatial diff-in-diff model allowed the authors to find out that the effect on nonproducing nearby regions is also significant and quantitatively relevant, at least 66 % of the effect on producing regions. The results suggest that this method makes it possible to better identify the impact, not only in the treated regions, but on the nontreated too.

4.6 Other Impacts

The expansion of sugarcane growing has speeded up substantially in recent years in the state of São Paulo, because of increasing demand for both sugar and alcohol. This expansion has been accompanied by debate over the costs and benefits of this expansion. Chagas et al. (2010) verify the effects of the increased production of sugarcane specifically on municipal revenues.

To do this, the authors gathered a panel data, to control for possible specific effects of each municipality. Given the time persistence of revenue, the traditional fixed- and random-effects models can generate biased estimators, so, they consider a dynamic panel model to be better. Moreover, in view of the geographic dimension of the phenomenon studies, they also introduced spatial controls.

The results of the tests suggest that for all the revenue categories studied there is a significant and substantial increase in revenue with the expanded participation of sugarcane in the municipal agricultural output. This effect can be from the direct and indirect effects of the sector on the local economy and its relation with neighboring municipalities.

The value of agricultural production of sugarcane is greater per hectare than for most other crops, indicating a greater value of agricultural income. Cane also employs more workers per hectare (considering both agricultural and industrial workers), once again showing greater generation of income. The greater geographic integration between the agricultural and industrial phases and the demand for services at plants should cause greater impacts on generation of urban income, meaning a greater multiplying effect on local economic activity. Therefore, even though it brings some negative effects, the growing and processing of sugarcane appears to have significant impacts on generation of income, which is partly captured by municipal tax revenues, as suggested by the results of this study. In other words, if on the one hand the expansion of sugarcane creates pressures on municipal spending on public services for migratory workers and greater health expenditures because of the deleterious effects of burn-off, among others, these municipalities appear to have higher revenues that offset the higher spending pressures.

4.7 Final Remarks

The need to reduce the use of fossil fuels in global energy poses risks and opportunities. The discussion of alternatives is urgent and necessary. Biofuels—fuels produced from agricultural products—are on a possible alternative. Their environmental, social, and economic impacts need to be scaled. In general, the studies suggest that the net effects are positive when compared with fossil fuels. For Brazil, this process appears to have more beneficial consequences than negatives. The country has very large share of renewable energy as inputs, such as hydropower and ethanol from sugarcane.

The expansion of the production of sugarcane for ethanol production seems inevitable and desirable. Brazil has indisputable comparative advantage in the production of ethanol: sector productivity per land use is increased and costs are significantly lower. Nevertheless, doubts prevail about the impacts of the sector in the economy as a whole, the social welfare and on the other sectors. This consider a relevant literature about these issues.

The expansion in recent years has helped to improve the capital-labor relationship, due the change in production technic (mechanization). This change will tend to eliminate the aspect in which the sector indicators are worse: the low-skill of work force and the high effort. In addition, mechanization reduce the weight of the primary employment, which are the most common complaints related to fatigue and the intensity of work.

Regarding the conflict between land uses for energy production versus food production, the results do not support their existence. The long-term relationship identified establishes common path between the production of sugarcane and the price of land, but this relationship is not statistically significant. Indeed, there are indications that the price of land is not determined by the production of sugarcane, although this result may be compromised by sample period and for the errors of measurement of the variable price of land available.

With regard to the price of food, there are apparently long-term relationship between this variable and the production of sugarcane, but in the opposite direction to what would be expected if it were valid the argument that the production of sugarcane increases the price of food.

This result is robust to number of price change used to measure the price of food to consumers. The conflict, however, seems valid with respect to the price of food at wholesale. The eventual realization that the increased production of sugarcane can push the price of land is, possibly, a result of a combination of several factors that contribute to increase agricultural production in general, with the increase of demand for these products, due to the increase in world income (especially poor and populous countries, like China and India). If this is true, it is possible to get in the short term, expanding the production of sugarcane and increase the price of food. Clearly, future work will test the robustness of this hypothesis.

In terms of strategic option for the country, it must be examined whether the increased production of other commodities, with lower value added and with much weaker links with other productive sectors, it is more advantageous to invest in the production of commodity that can provide advantages comparative energy to the country.

The use of ethanol as fuel generates positive environmental impact when compared to oil, which has made many countries to adopt measures to encourage its production and use. Moreover, there has been a massive investment to discovery of new energy sources and development of new technologies for the production of ethanol from different materials. The main focus, in developed countries, is the development of the process of hydrolysis, which will enable the production of ethanol from cellulosic materials.

The utilization of all by-products generated in the production process increase the environmental benefits of sector. The recent regulatory acts contribute to make more transparent the actions of the sector on the carbon savings. Additionally, the sector complements the hydroelectric power supply, because the harvest of sugarcane in Midwest and South occurs in the dry season and low in the reservoirs.

Another aspect discussed refers to the impacts of the industry on the social conditions of the locations which concentrate the production of sugarcane. Aside from the potential benefits that the industry can bring to the country (such as employment generation, income, and revenue), it may be that in the regions producing the burden is greater than the bonuses, considering the pressure for local public services, urban infrastructure, etc.

The results, however, suggest that the presence of the industry in one location is not relevant to determine their social conditions, for better or for worse. Possibly, there are public policies which should be much more obvious impacts on the HDI, particularly those related directly to the improvement of the conditions of education and health as well as to improve production and income distribution. Future works could be done by testing this hypothesis.

With respect to health impact, however, it seems that the burning of harvest has a negative impact over the respiratory cases in producers region, impacting non-producers neighbors regions too. The deepening of technical change, with greater adoption of mechanization, can have a positive impact in this regard.

Finally, it seems that low agricultural sectors generate direct revenue, especially for city government, due to the low incidence of taxes on such activity in Brazilian federative system. But, it is also true that agricultural production is inevitable, much more municipalities. In this case, it may be that the multiplier effects of the production of sugarcane are larger than that of competing products, so that their expansion will increase the revenue of municipalities.

The results corroborate this hypothesis. For all categories of income is considered significant and substantial increase in revenue with the expansion of the production of sugarcane. This effect may possibly be outdated. An increase in sugarcane production this year will have impact on the share from transfer from the next period. Thus, the revenue gains obtained in the counties producing sugarcane may offset any spending pressures that arise due to the characteristics of the sugarcane industry. The expansion of sugarcane in the counties, replacing other agricultural activities, appears to result in increased tax revenues.

In summary, the results suggest that (i) the sugarcane expansion seems help to improve the relationship between sugarcane mill and workers, (ii) is not the expansion of the production sector the main reason to push the price of food, (iii) the environmental indicators in sector is better than fossil fuel sector, or other relevant competitor, (iv) the sector has no significant effects (positive or negative) on social conditions in cane growing regions, (v) the mechanization may contribute to reduce the sector impact in people's health, and (vi) the sector can contributes to public policies, to the extent that generates indirect effects on the rest of the economy, increasing the local revenue.

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Chapter 5
Integrated Production of 1G–2G Bioethanol and Bioelectricity from Sugarcane: Impact of Bagasse Pretreatment Processes

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Abstract The industrial plant for production of second-generation (2G) ethanol from sugarcane bagasse will most probably be integrated to the already existing facilities for first-generation (1G) ethanol from sugarcane juice. This will allow lower investment costs, since the former would be able to take advantage of the existing infrastructure, setup for the later. Nevertheless, the exploitation of sugarcane bagasse as raw material must take into account that this biomass is also used as boiler fuel in order to produce steam to meet process demands. Additionally, steam demand is highly dependent on the pretreatment used. In this context, five pretreatments were chosen and an ethanol production process was proposed for each of them. Steam demand was calculated and used to determine the maximal bagasse that could be diverted from steam production. Among the pretreatments considered, the alkaline one presented the higher increase in ethanol production (5.7 L/tonne of sugarcane). This was due the almost complete cellulose hydrolysis and the lower steam demand of this process. On the other hand, pretreatment and hydrolysis reactor volumes were first and second higher, respectively, for this pretreatment. This suggests that, from an economic perspective, steam explosion (with a 2G ethanol production of 2.8 L/tonne of sugarcane) might be a better option.

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5.1 Introduction

The main liquid biofuel, nowadays and in the foreseeable future, is bioethanol, and the fermentation of carbohydrates coming from different crops is the dominant technology for its production. In Brazil, sugarcane is the raw material for this biofuel and the consolidated production process uses sugarcane juice (essentially sucrose) as substrate for ethanolic fermentation by *Saccharomyces cerevisiae*. This production process, known as first generation (1G), utilizes sugarcane bagasse as fuel to the boiler, which is important, in the cogeneration system, both to produce steam to the process and to cogenerate electric energy, in the turbines coupled to electric generators. The electric energy produced is used in-house and, when integration with the electricity companies' lines is possible, it is sold to the grid.

The production of bioethanol can be increased if the carbohydrates present in sugarcane bagasse are used in the fermentation steps as well, giving rise to the so-called second-generation (2G) ethanol. Since the main constituents of bagasse are polymers of carbohydrates (around 45 % of cellulose and 27 % of hemicellulose, dry basis), they can, in principle, be separated from the other components of bagasse (lignin, ashes), and then hydrolyzed in order to make their sugars accessible to be fermented. Since bagasse is already at the 1G plant site, it is natural to think of a 2G plant integrated to the 1G one, with common pieces of equipment, like the cogeneration system (combined heat and power system) and distillation columns.

The production of 2G ethanol from bagasse, nevertheless, has two points of concern. First of all, not all bagasse can be used to produce 2G ethanol, since bagasse is necessary as fuel to the boiler, to produce steam and electric energy both to 1G and to the new 2G process. Second, bagasse must be pretreated to make cellulose more accessible to enzymes in the enzymatic hydrolysis (the dominant technology for industrial application), turning this step more efficient. Several alternatives of pretreatment of bagasse have been studied and, from the process point of view, they differ in many aspects: in the ratio of water used, in which component (cellulose, hemicellulose, lignin) is mostly attacked, in the recovery of their different constituents, in the crystallinity of obtained cellulose, etc. Of course, since these different options of pretreatment use different ratios of water and produce different qualities of constituents, they impact differently in the integrated production of 1G–2G bioethanol (and bioelectricity). It is important to bear in mind that many 1G plants have long-term contracts to sell their surplus of electric energy. And the diversion of bagasse for production of 2G ethanol could change the surplus of generated electric energy by the industry, due to the less amount of bagasse burnt and to the higher steam consumption of the integrated 1G–2G bioethanol and bioelectricity production plant. Of course, part of the so-called crop trash (leaves, straw, tips, etc.) might be used for bioelectricity production. This surplus feed for the boiler would certainly be important for an economic assessment, but it does not change the relative performances of the different pretreatment processes.

Here, different pretreatment options reported in the literature are revised and some of them are chosen for a study of their impact on bioethanol and bioelectricity production in 1G–2G autonomous distilleries (i.e., no sugar production is considered). Since it is not the aim of this chapter to assess the economic feasibility of different pretreatments, the use of sugarcane trash was not considered in our analysis, for the sake of simplicity.

5.2 Bagasse Pretreatment

A number of different pretreatments for biomass, including bagasse, have been investigated over the past decades. These include milling, steam explosion, ammonia fiber expansion (AFEX), organosolv extraction, the use of microwaves, of acid, alkaline, or oxidative mediums, of liquid hot water, or the use of ionic liquids. Many studies consider just one pretreatment for bagasse and some of them use two or more pretreatments in sequence in order to evaluate their combined effects, but an evaluation of the overall integrated process is still lacking.

Milling is a purely physical pretreatment, used to enhance the access of the enzymes cocktail to the fibers. Furthermore, it has the advantage of not producing fermentation inhibitors (Silva et al. 2010).

Among the physicochemical options of pretreatment, steam explosion of bagasse (Chen et al. 2010; Dias et al. 2011b; Kaar et al. 1998) applies a sudden decrease in pressure in order to expose the material fibers. Hemicellulose is autohydrolyzed at high temperatures prior to the decompression, while lignin remains almost intact, retained with the exposed cellulose (yielding the so-called cellulignin). AFEX uses anhydrous ammonia to break down lignin–carbohydrate linkages. In opposition to steam explosion, hemicellulose is not removed in AFEX as a separate liquid stream (Krishnan et al. 2010). Liquid hot water pretreatment (Hernandez et al. 2012; Wang et al. 2012; Yu et al. 2013) uses liquid water at high pressure and temperature (150–230 °C) in order to hydrolyze mainly the hemicellulose fraction, which is removed as a liquid stream.

Chemical pretreatments make use of acid, alkali, oxidant, or solvent in order to attack different constituents of lignocellulosic material. Weak acid has been investigated by many authors (Pietrobon et al. 2011; Rocha et al. 2011; Vasconcelos et al. 2013; Zhao et al. 2007) in order to make hemicellulose soluble, and to facilitate the enzymatic hydrolysis of cellulignin (Canilha et al. 2012). Very different process conditions are reported in the literature (with respect to temperature, space time, the acid that is used and its concentration). These conditions will influence the transformation of the formed monosaccharides, through undesired reactions, into other compounds, that are inhibitors to fermentation process. Alkaline pretreatment aims to remove lignin from the biomass and has also been studied by many researchers lately (Fuentes et al. 2011; Hernandez et al. 2012; Rabelo et al. 2009; Wu et al. 2011). It requires lower temperatures than other chemical pretreatments, but, on the other hand, is a slower pretreatment, requiring

longer space times (Canilha et al. 2012). Addition of surfactant to alkaline medium has been also investigated (Cao and Aita 2013) in order to promote a better removal of hydrophobic compounds (lignin) due to the decrease in surface tension. Oxidation pretreatment (Cheng et al. 2008; Martín et al. 2007; Martín et al. 2008; Rabelo et al. 2011) uses pure oxygen or air combined with water or alkaline solutions (alkaline-oxidative pretreatment) to attack lignin structure. According to Martín et al. (2007), toxic furaldehydes and phenol aldehydes have their formation diminished with the use of the alkaline-oxidative alternative. The use of solvents to solubilize lignin in the pretreatment characterizes the so-called organosolv pretreatment (Mesa et al. 2010; Wolf 2011). The action of the solvent solution on the biomass is often catalyzed by acid or alkali. Compared to alkaline pretreatments, organosolv is supposed to have the advantage of solvent recovery and less use of water.

The combination of established pretreatments is commonly found in the literature. For example, Giese et al. (2012) studied the effect of acid pretreatment followed by alkaline delignification on the enzymatic hydrolysis of sugarcane bagasse, and showed that a decrease in enzyme loading costs could be achieved. A combination of steam explosion and alkaline pretreatments is also found in the literature, in order to remove lignin from the solid fraction after steam explosion, leading to a fourfold increase of ethanol production (Wanderley et al. 2013). Zhao et al. (2011) combined alkaline and peracetic acid pretreatments and their results show a better digestibility by cellulases. The combination of weak acid with organosolv pretreatment with NaOH was the focus of Mesa et al. (2011), who concluded that this combination was very efficient, increasing the glucose concentration of the enzymatic hydrolyzed stream.

Other types of pretreatments, not so extensively investigated in the literature, are the ones that make use of microwaves or ionic liquids. The former is used as an alternative source of heat, aiming to achieve the temperatures required by different pretreatments, to which the microwave is combined (Binod et al. 2012). Ionic liquids, by their turn, may dissolve cellulose. Zhu et al. (2012) applied them to promote the performance of the enzymatic hydrolysis.

5.3 Selected Conditions for the Different Pretreatment Analysis

As it can be seen in Sect. 5.2, the effect of several pretreatments in the composition and hydrolysis susceptibility of sugarcane bagasse has been reported. Since not all options of pretreatment are in equal state of development, only five of them were chosen for an analysis of their influence on the performance of the integrated 1G–2G bioethanol-bioelectricity production process: steam explosion, organosolv, liquid hot water, weak acid, and alkaline ones.

Table 5.1 Pretreatment conditions, according to each selected literature article

Pretreatment	Pretreatment conditions
Steam explosion (Warderley et al. 2013)	Temperature: 200 °C Reaction time: 7 min
Organosolv (Wolf 2011)	Temperature: 190 °C Reaction time: 90 min Solid/liquid ratio: 9.1 % (m/v) Reagent: ethanol/water solution (50 %, m/v)
Liquid hot water (Yu et al. 2013)	Temperature: 180 °C Reaction time: 20 min Solid/liquid ratio: 5 % (m/v)
Weak acid (Rocha et al. 2011)	Temperature: 190 °C Reaction time: 10 min Solid/liquid ratio: 9.1 % (m/v) Reagent: 1 % (m/v) of H_2SO_4 and 1 % (m/v) of acetic acid
Alkaline (Yu et al. 2013)	Temperature: 110 °C Reaction time: 1 h Solid/liquid ratio: 14.3 % (m/v) Reagent: 0.18 % (m/v) of NaOH

In the literature, it is possible to find a great number of papers for each one of these selected pretreatments. Nevertheless, only a few provide enough information for a complete process analysis. The main criterion for our selection of pretreatment conditions was the existence of complete reported information in the literature. For complete reported information, one can cite sugarcane bagasse composition (both before and after the pretreatment is applied), mass yield, pretreatment conditions (temperature, pressure, reactants concentration, reaction time, solid/liquid ratio), and enzymatic hydrolysis conditions (reaction duration and attained conversion, load of solids, enzyme concentration and temperature). Cellulose recovery and sugar yields were also considered, since pretreatment conditions that led to higher recovery and yields were preferred.

Table 5.1 shows the articles selected from the literature and, based on them, the pretreatment conditions that were considered.

In order to standardize the results and facilitate their comparison, a unique composition for sugarcane bagasse was used. This value was calculated as the average of the compositions reported in the literature. For the sake of simplicity, the references for natural bagasse composition were suppressed, but the values are shown in Table 5.2. The compositions of bagasse after each pretreatment, as reported in the selected articles, were adapted for this specific bagasse. It was assumed that the percentage solubilized of each component remained the same as described in the article, despite the differences in bagasse composition. The composition of the pretreated biomass, after each pretreatment, is shown in Table 5.2.

Table 5.2 Bagasse mass composition before and after pretreatments

Pretreatment	In natura bagasse	Steam explosion	Organosolv	Liquid hot water	Weak acid	Alkaline
Reference		Warderley et al. (2013)	Wolf (2011)	Yu et al. (2013)	Rocha et al. (2011)	Yu et al. (2013)
Cellulose	44.9	50.2	64.2	59.6	59.6	60.7
Hemicellulose	27.7	9.5	17.6	4.5	2.7	29.2
Lignin	23.6	34.9	11.8	29.8	31.9	3.6
Ash	3.8	5.4	6.3	6.0	5.8	6.5
Solid mass yield		68.0	60.1	63.5	65.1	58.5

The yield of enzymatic cellulose hydrolysis is exceedingly dependent on the pretreatment used, and cannot be inferred by composition of the biomass after the procedure. As stated beforehand, hydrolysis conditions and the resulting conversion of cellulose were explicitly reported on all articles that were used as base for our analysis of the processes, i.e., all these articles also reported experimental results of enzymatic hydrolysis of the material. It is worth mentioning that none of these publications optimized the hydrolysis conditions. Since the pretreated bagasse has different digestibility after each pretreatment, it is expected that process conditions will be distinct. The conditions used in each study are shown in Table 5.3.

Neither ethanol nor biogas production from the hemicellulose fraction was considered in this analysis. Except for the steam explosion pretreatment, the pentoses concentration in the liquid fractions obtained was too low for direct use as raw material for these processes and a concentration step would increase steam demand and decrease the bagasse available for 2G ethanol production. This might be circumvented by a heat integration of the process, which is outside the scope of this chapter.

An integrated 1G–2G bioethanol and bioelectricity production plant was considered in the process analysis. In this scenario, bagasse is diverted from the combined heat and power system to the 2G sector (set of unit operations necessary for production of 2G bioethanol). Since process steam demand (1G and 2G) is met by the bagasse combustion, there is a limit to the amount of bagasse that can be diverted to 2G sector. A typical 1G process was considered, based on the simulations performed by Dias et al. (2012).

Based on the data provided by the selected literature articles, a process was proposed for each of the pretreatments chosen. Solvent recovery was only implemented for the organosolv pretreatment, as shown in Fig. 5.1. The other four pretreatments shared the same basic process, shown in Fig. 5.2. It is worth mentioning that the processes proposed do not take full advantage of the possible heat and mass integration for the combined 1G and 2G process.

Table 5.3 Cellulose hydrolysis conditions, according to each selected literature article

Pretreatment	Hydrolysis condition
Steam explosion (Warderley et al. 2013)	Cellulose to glucose conversion: 32 % Reaction time: 24 h Solid/liquid ratio: 8 % (m/v) Enzyme load: 10 FPU/g of cellulose
Organosolv (Wolf 2011)	Cellulose to glucose conversion: 52 % Reaction time: 42 h Solid/liquid ratio: 9.1 % (m/v) Enzyme load: 20 FPU/g of lignocellulosic material
Liquid hot water (Yu et al. 2013)	Cellulose to glucose conversion: 52.3 % Reaction time: 72 h Solid/liquid ratio: 5 % (m/v) Enzyme load: 15 FPU/g of lignocellulosic material
Weak acid (Rocha et al. 2011)	Cellulose to glucose conversion: 75 % Reaction time: 48 h Solid/liquid ratio: 2 % (m/v) Enzyme load: 15 FPU/g of lignocellulosic material
Alkaline (Yu et al. 2013)	Cellulose to glucose conversion: 99 % Reaction time: 72 h Solid/liquid ratio: 5 % (m/v) Enzyme load: 15 FPU/g of lignocellulosic material

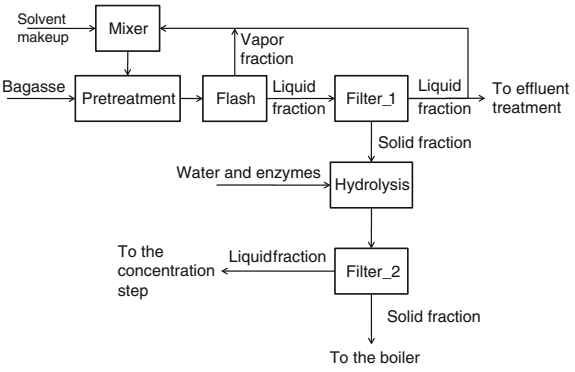

Fig. 5.1 Organosolv pretreatment simplified block diagram

5.4 Results

The results for the organosolv pretreatment show that the proposed solvent recovery step was not adequate and more ethanol was lost in this step than produced by the fermentation of the glucose produced in the hydrolysis of cellulose. Therefore, a more complex system must be implemented, including a distillation column for the liquid fraction of Filter_1 (Fig. 5.1). Since it is not the scope of this chapter to make such a detailed simulation, the organosolv pretreatment was not further considered.

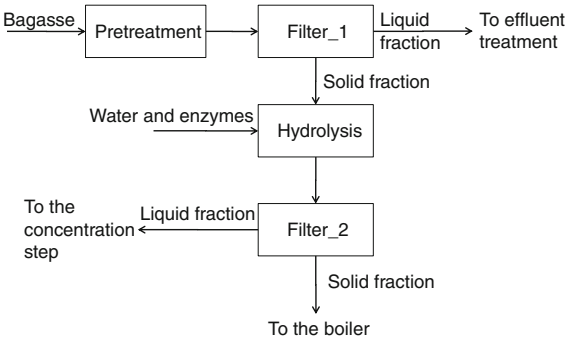

Fig. 5.2 Steam explosion, liquid hot water, weak acid, and alkaline pretreatments simplified block diagram

Table 5.4 Main results for the process simulations

Pretreatment	Steam explosion	Liquid hot water	Weak acid	Alkaline
Specific production of 2G bioethanol(L/TdB)	104.2	94.0	198.0	239.0
Specific steam consumption of 2G sector (kg of steam/kg of dry bagasse)	3.9	13.3	22.4	6.9
Maximal amount of bagasse diverted to 2G production (kg of dry bagasse/TC)	27.3	15.0	10.0	23.7
Decrease in electric energy production (kW/TC)	34.5	19.0	12.6	29.9
Increase in ethanol production (L/TC)	2.8	1.4	2.0	5.7

TdB ton of dry bagasse, *TC* tonne of sugarcane

Table 5.4 shows the main results of the simulations for each pretreatment. The specific ethanol production was estimated considering the cellulose hydrolysis yields provided by the references and assuming 90 % of the theoretical fermentation yield. No inhibition effect by the byproducts of the hydrolysis was considered, since it was supposed that glucose would be concentrated and fermented together with the sugarcane juice from first-generation process, in the same bioreactor, thus diluting the inhibitors. Specific steam consumption, as shown in Table 5.4, takes into account the steam produced by burning the residual solids from Filter_2. Since the steam demand for both first- and second-generation ethanol must be provided by the combustion of these residual solids and bagasse, the steam consumption determines the amount of bagasse available for 2G ethanol production. Considering a production of dry bagasse of 140 kg per ton of sugarcane (Dias et al. 2011b), a steam production of 4.5 kg per ton of dry bagasse (based on information from Dias et al. 2011a) and a steam consumption of 360 kg per tonne of sugarcane in the 1G process (Seabra and Macedo 2011), it is concluded that 1G process is responsible for the consumption of 57.3 % of the

Table 5.5 Volumes of the main pieces of equipment as a function of the amount of bagasse processed

Pretreatment	Pretreatment reactor volume (m^3/TdB)	Hydrolysis reactor volume (m^3/TdB)
Steam explosion	0.35	243
Liquid hot water	7.01	569
Weak acid	1.84	1665
Alkaline	7.01	917

TdB tonne of dry bagasse

bagasse. Therefore, approximately 60 kg of bagasse per ton of sugarcane would be available for 2G ethanol production. Table 5.4 shows the maximal amount of bagasse that could be diverted to 2G ethanol production considering each pretreatment.

From Table 5.4, it is possible to determine that it is necessary, only for the 2G sector, 37.4 kg of steam per each liter of 2G bioethanol produced for the steam explosion pretreatment. For the other pretreatments these values are 141.5, 113.1, and 28.9 for, respectively, liquid hot water, weak acid, and alkaline. The 1G plant has a consumption around 4.0 kg of steam per each liter of 1G bioethanol produced (Seabra and Macedo 2011). This suggests that the requirement of thermal energy for producing 2G bioethanol is still high. Furthermore, liquid hot water and weak acid pretreatments have intense steam consumption and would certainly benefit from process heat integration.

The decrease in electric energy production shown in Table 5.4 considers only the bagasse diverted to 2G ethanol. It does not consider the decrease related to steam extractions at high pressures to meet the 2G steam demand (between 12 and 16 bar). If the selling of electric energy to the grid were considered, its market price could be used to estimate the minimal ethanol selling price for 2G ethanol.

Reactor volumes were also considered, as an approximation of the investment costs for the comparison among the pretreatments. Table 5.5 shows the volume of both the pretreatment reactor and the hydrolysis reactor as a function of the bagasse flow into the system. Weak acid presented the highest total volume, mainly due to its low solid loads in the hydrolysis reactor. This is also the reason for the large volume of the hydrolysis reactor for the alkaline pretreatment. This suggests that it is important to improve solid loads in the enzymatic hydrolysis of cellulose.

Among the considered pretreatments, alkaline obtained the higher increase in ethanol production. This was due both to its higher conversion of cellulose to glucose in the hydrolysis reactor and to its low steam consumption. On the other hand, its reactor volumes were the second higher, which suggests that steam explosion might be a better option when the overall investment costs are considered, despite its lower ethanol production.

5.5 Conclusion

The production of 2G ethanol from sugarcane bagasse, integrated to the 1G process, for five different pretreatments were analyzed from a process perspective. Considering the experimental results reported in the literature and used in this study, ethanol production could be increased by 5.7 L/TC for the best pretreatment option (alkaline). For the same process, electric energy production is decreased by 29.9 kW/TC. Its market value could be used to determine ethanol minimum selling price in an economic analysis. If investment costs are considered, the results for the demanded volumes for the pretreatment and hydrolysis reactors suggest that steam explosion might be a better option, despite its lower 2G ethanol production. As a general case, an increase in solid loads both in the pretreatment and in the hydrolysis reactor could greatly benefit the 2G ethanol process feasibility.

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Chapter 6
Potential Biomass Resources for Cellulosic Ethanol Production in Brazil: Availability, Feedstock Analysis, Feedstock Composition, and Conversion Yields

Boutros F. Sarrouh, Júlio C. dos Santos, Mário Antônio A. Cunha and Ricardo F. Branco

Abstract Due to economic, geopolitical, and environmental issues, the world's attention turns to alternative energy sources, especially for second-generation ethanol. In addition to economic considerations, other factors such as energy security, greenhouse gas emissions, and global climate change are boosting scientific researches concerning alternative bioenergy. According to the literature projections, more than 10 % of all gasoline used in the world can be replaced by biofuels over the next 15–20 years. Currently, Brazil is faced with the prospect of a significant increase in demand for ethanol. This prediction holds up in some market realities, as increasing domestic consumption of hydrous ethanol by the successful introduction of the alternative flex fuel vehicle market in automotive lightweight and expansion of Brazilian ethanol exports due to the increasing global interest in mixing alcohol with gasoline. In this context, all the potential sources for ethanol production must be considered wherein cellulosics stands out as an important alternative. In this chapter, we discuss about the potential to produce ethanol from lignocellulosic materials, a renewable source largely available in the

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world. Particularly, some aspects of potential sources in Brazil are described. First, however, brief comments on the composition of these materials and some analytical methods used to characterize them are exposed.

6.1 Introduction

The agro-industrial waste can contain many substances of high value. If appropriate technology is used, this material can be converted into commercial products or raw materials for secondary processes using biotechnological processes. It is therefore important to develop new techniques aimed at using these residues to obtain products useful to mankind, and with this goal, the use of fermentation processes have been extensively studied (Baudel et al. 2005; Delgenes et al. 1998).

In Brazil there is a great potential to exploit renewable sources with competitive costs in international terms. When searching in Brazil for production of low cost energy, the choice falls on a renewable alternative. Among the larger countries, it is a unique case. The analysis of Brazilian resources to be exploited commercially by merit of maintaining lower cost indicates a power supply with a large share of renewables, with the same mix adopted in recent decades: use of energy derived from sugarcane and exploitation of the hydroelectric potential of the country.

Brazil is the world's largest producer of ethanol derived from sugarcane and as a consequence there is generation of large amounts of lignocellulosic biomass from this industrial sector which can be converted into second-generation ethanol. Additionally, as the country has a robust agro-industrial park, with industries using different vegetal feedstocks, several residues with potential for alcohol production are generated. There are however some bottlenecks that need to be overcome to make the production of second-generation ethanol competitive and viable economically compared to the first generation.

The use of the full potential of any cellulosic feedstock is associated to a deep knowledge of its composition. This, before any discussion on potential feedstocks to second-generation production in Brazil, some comments will be carried out on analytical methods used for the compositional characterization of the material. Particularly, some brief comments will be put on the new approaches related to analytical strategies used for lignocellulosics.

6.2 Feedstock: Composition-Advanced Analytical Techniques for Characterization

Wastes from vegetable biomass are composed mainly of macromolecular fractions from plant cell wall: cellulose, hemicellulose, and lignin.

Cellulose is a polysaccharide composed of glucose molecules linked through intermolecular interactions, which result in a crystalline structure which gives high

strength and insolubility of the molecule to the action of chemicals. Nevertheless, the pulp is prone to chemical or enzymatic hydrolysis.

The hemicellulose, xylan, unlike cellulose, comprises various types of sugars being considered, therefore, a heteropolymer. It consists of pentoses (D-xilose, β-and α-L-arabinose), hexoses (β-D-manose, β-D-glucose and α-D-galactose) and/or uronic acids (α-D-glucuronic acid, α-D-4-O-α-metilgalacturonicoacid, and D-galacturonic acid). This polysaccharide is more prone to hydrolysis. Xylan is the second most polysaccharide found in nature.

Lignin is formed by a complex structure which gives the plant a higher mechanical strength. This is a compound highly resistant to chemical and enzymatic degradation and as overlying other polysaccharides, is a problem when trying to use the crushed sugarcane to produce ethanol because it prevents access to the other tissues. However, there are microorganisms that can carry out their degradation.

Other substances that can be extracted from the plant material are waxes, alcohols, lipids, steroids, fatty acids, hydrocarbons, and flavonoids. Some of these compounds can be toxic extractives. These molecules may vary according to the species examined and the type of processing to which the material is subjected.

The remaining materials are considered nonextractive substances, mainly ashes. These inorganic compounds are known as salts or minerals such as potassium, silicon, manganese, sodium, calcium, among others. The composition of nonextractive substances depends on soil conditions, climate, and environment.

Due to biomass heterogeneity, robust analytical methods are needed to support and enable biomass conversion processes. The chemical composition of a biomass feedstock varies as a function of many factors including plant genetics, growth environment, harvesting method, and storage (Hames et al. 2003).

Detailed analysis of the chemical composition of herbaceous and woody biomass feedstocks is labor-intensive, expensive, and time-consuming. Rapid methods of analysis would enable timely estimates of biomass quality, and may provide a tool for evaluating the biomass composition as it enters the conversion plant (Sanderson et al. 1996).

Different techniques are used to quantify these compounds, including High Performance Liquid Chromatography (HPLC) and Gas Chromatography-mass Spectrometry (GC/MS). However, these techniques, although largely used due to its high accuracy, require processing samples in the laboratory, which are time and cost consuming. In this sense, currently, the use of other techniques in qualitative and quantitative analysis of solid, liquid, and gas is already widely recognized. The most widespread ones are absorption infrared (NIR–NIR and FTIR-absorption infrared Fourier transform), Raman scattering, fluorescence, and photoacoustic. In particular, infrared spectroscopy methods combined with statistical analysis of spectra have been successfully used in various areas such as food, environment, and pharmaceuticals. Recently, the use of Near Infrared (NIR) official method was accepted as recognized by agencies such as the American Society for Testing and Materials (ASTM) and the United States Pharmacopoeia (Haack 2010).

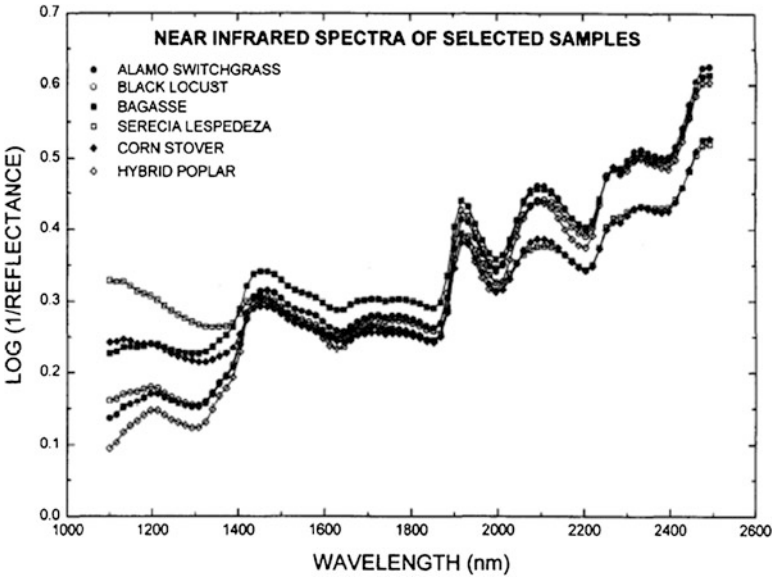

Fig. 6.1 Representative near infrared spectra of various biomass feedstocks. Reproduced from Sanderson et al. (1996)

The near infrared spectroscopy has been used to determine the quality parameters as soluble solids, polarizable sugars, and reducing sugars present in sugarcane. Likewise, infrared spectroscopy Fourier Transform is also used in the investigation of solid bagasse from sugarcane (Thermo Nicolete Corp 2001). Near infrared reflectance spectroscopy (NIRS) has been used commercially as a rapid and effective analysis tool to estimate lignocellulosic composition (Sanderson et al. 1996).

NIRS is characterized as a nondestructive technique, with an easy sample preparation and management (no reagents are required), rapid (1 min per spectrum) and inexpensive. Near infrared radiation (700–2,500 nm) is absorbed by various bonds, such as C–H, C–C, C = C, C–N, and O–H, characteristics of organic matter, as shown in Fig. 6.1 (Ludwig and Khanna 2001).

Similarly, Raman scattering is extremely efficient and of high sensitivity widely used in the identification of organic compounds, including those of interest in lignocellulosic: aliphatic acids, furans, and phenolic compounds. The advantage of Raman spectroscopy with respect to the infrared absorption lies in its greater sensitivity. Moreover, one should consider the complementary character of the techniques, since no active compounds, Raman and infrared active (Lupoi and Smith 2012).

Another promising technique used for lignin structure analysis is known as pyrolysis Gas Chromatography–Mass Spectrometry (Py–GC–MS). This analytical method utilizes a microscale quartz reactor inserted into a platinum wire probe

capable of heating to high temperatures at extremely fast rates. This pyroprobe is directly coupled to a GC–MS instrument through a transfer line, allowing rapid analysis. Py–GC–MS revealed a variety of pyrolytic products, including methoxyphenols and other aromatic compounds derived from the monomeric units coumaryl, coniferyl, and sinapyl alcohols within the lignin structure (Mendu et al. 2011).

6.3 Potential Feedstocks

6.3.1 Sugarcane Bagasse

In Brazil, studies have been conducted systematically for biofuel production (high environmental value) and biomolecules (high added value) for organic conversion, mainly using sugarcane bagasse as feedstock. Deployment of ethanol technology from sugarcane bagasse in Brazil is favored because the production process can be attached to existing units of sugar and alcohol industries, requiring lower investment, infrastructure, logistics, and energy supply. Furthermore, bagasse is generated in industrial plants, and as such, free transportation costs.

Brazil is the largest producer of sugar in the world. Therefore, it is one of the countries that generates waste bagasse from sugarcane for which new techniques of exploitation are always needed. For the season 2013/2014, the culture of sugarcane is expected to continue to expand. The prediction is that Brazil will attain an increase of 408 ha of planted area, which is equivalent to 4.8 % compared to the 2012/2013 crop. São Paulo, Minas Gerais, Goiás, and MatoGrosso do Sul should be the states with the largest increase in planted areas with 141.400, 106.100, 101.100, and 43.500 ha, respectively. This growth is due to the expansion of new planting areas for the sugar-alcohol industries already in operation. The total cultivated area with sugarcane in 2013/2014 is estimated at 8.933.00 ha, distributed in all producing states according to their characteristics.

The forecast for total sugarcane to be ground is 653.81 million tons, an increase of 11.0 % compared to the 2012/2013 crop, which was 588.92 million tons, meaning that the amount to be ground to be 64.89 million tons more than in the previous harvest.

The forecast for sugarcane production in 2012/2013 was approximately 196 millions of tons, according to the proportion indicated by Procknor (2000).

Sugarcane bagasse is composed of the fibrous material obtained after sugarcane is crushed to extract the juice. Much of the bagasse is used by the industry itself as an energy source, and the plants themselves use up to 80 % of bagasse as an energy source to replace fuel oil in the heating process of the boilers and for the generation and sale of electricity (Teixeira et al. 2007). There are, however, non-energy uses for sugarcane bagasse, some of them already made viable commercially. Bagasse plays an important role as a raw material in the paper industry and cardboard manufacturing clusters, as alternative materials in construction, animal

feed, and microbial biomass production, acoustical, fodder for agriculture, xylitol, ethanol, hydroxy methyl furfural, alkaloids, and enzymes (Sarrouh and Silva 2008; Carvalho et al. 2008; Neureiter et al. 2004; Howard et al. 2003; Pandey et al. 2000). However, there is still a surplus (10–20 %) of this waste which is not used, causing serious environmental pollution and storage (Teixeira et al. 2007).

6.3.2 Sweet Sorghum Straw and Bagasse

Sorghum is able to produce more than 2,500 L of ethanol per hectare during off-season of sugarcane. The development of this culture will generate an improvement in the productivity of sugarcane by providing a favorable period for its harvest (Monsanto 2013).

Sweet sorghum can also be produced between May and December, being economically more competitive than sugarcane in marginal areas or potentially in rotation for ethanol production and cogeneration for electricity production (Ceres 2013).

The sorghum plant's culture became popular in many parts of the world due to its ability to adapt to a wide range of environments, especially under water stress conditions (Williams et al. 1999). Table 6.1 shows the chemical composition of sweet sorghum plant.

As sorghum plants mature more quickly than canesugar and achieve an optimum level of sugar in different periods of the year, sugar obtaining by the ethanol industries can extend the operating season for up to 60 days or more. Figure 6.2 shows that by extending the operational season of the plant the price of ethanol becomes more competitive.

In the 1980s, sweet sorghum plant (Sorghum bicolor) was introduced in Brazil as an alternative to ethanol production during the off-season of canesugar. The initiative was promoted by Pro-alcohol program developed by the Federal Government to encourage the use of alcohol and other energy sources as an alternative to gasoline in a period of global oil crisis. However, at the beginning, the large-scale production of this raw material was not successful because there were no adequate hybrids suitable for the Brazilian regional planting peculiarities. Today, 30 years later, sweet sorghum has become a commercial reality due to technological advances in the industry, especially breeding technologies for sugarcane and sweet sorghum. Figure 6.3 shows the main differences between sugarcane and sweet sorghum, especially in terms of productivity and cultivation cycle.

Sorghum has great potential as an annual energy crop. While primarily grown for its grain, sorghum can also be grown for animal feed and sugar. Sorghum is morphologically diverse, with sorghum grain being of relatively short stature grown for the grain, while forage and sweet sorghums are tall and grown primarily for their biomass.

The main product obtained from sweet sorghums is the fermentable sugar-rich juice that is produced and accumulated in the stalks in a similar fashion as

Table 6.1 Chemical characterization of the sweet sorghum grains, juice, and bagasse

Grains (% wet base)		Juice (% wet base)		Bagasse (% dry base)	
Starch	70.1	Soluble solids	18	Cellulose	38.5
Proteins	11.2	Sucrose	8.5–12.4	Hemicellulose	21.4
Humidity	11.6	Glucose	2.1	Lignin	17.6
Fibers	1.82	Fructose	1.2	Protein	1.1
Lipids	3.54	Starch	0.5	Extractives	13.7
Ashes	1.8	Water	84	Ashes	3.7

Barcelos (2012), Rossell (2011), Panagiotopoulos et al. (2010), Wu et al. (2007)

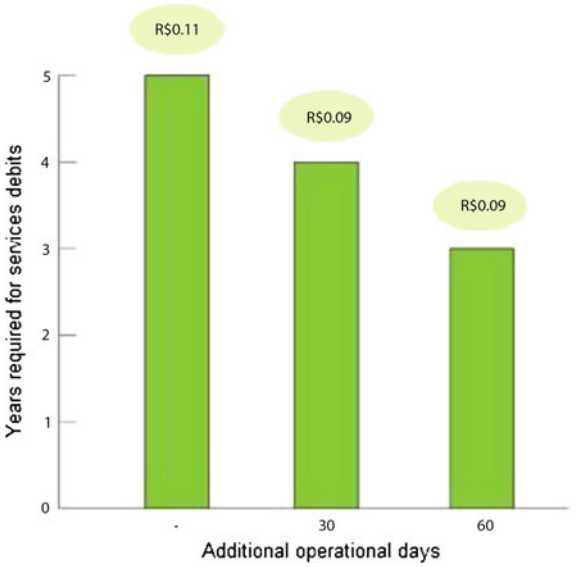

Fig. 6.2 Relation between additional operational days by using sweet sorghum and the price of 1 L of ethanol expressed in real (R$1 = US$0.5). Reproduced from Ceres (2013)

sugarcane. The extracted sweet juice is mainly composed of sucrose, glucose, and fructose, and thus can be directly fermented into ethanol with efficiencies of more than 90 % (Wu et al. 2010).

Considering two crop harvests a year, sorghum can yield about 15.62 ton/ha of biomass which can be exploited to produce second-generation ethanol. In addition, 15.6 ton/ha of panicles with a high value for silage or as direct feed is produced (Cardoso et al. 2013).

Current interest in bioethanol research is focusing on how to efficiently liberate sugar molecules from lignocellulosic feedstock for increased bioethanol production. Therefore, integration of the sugars from sweet sorghum bagasse (cellulosic residue after cane extraction) with sugar derived from the stem/cane will further increase ethanol yield and also make bioethanol affordable (Aleke 2011).

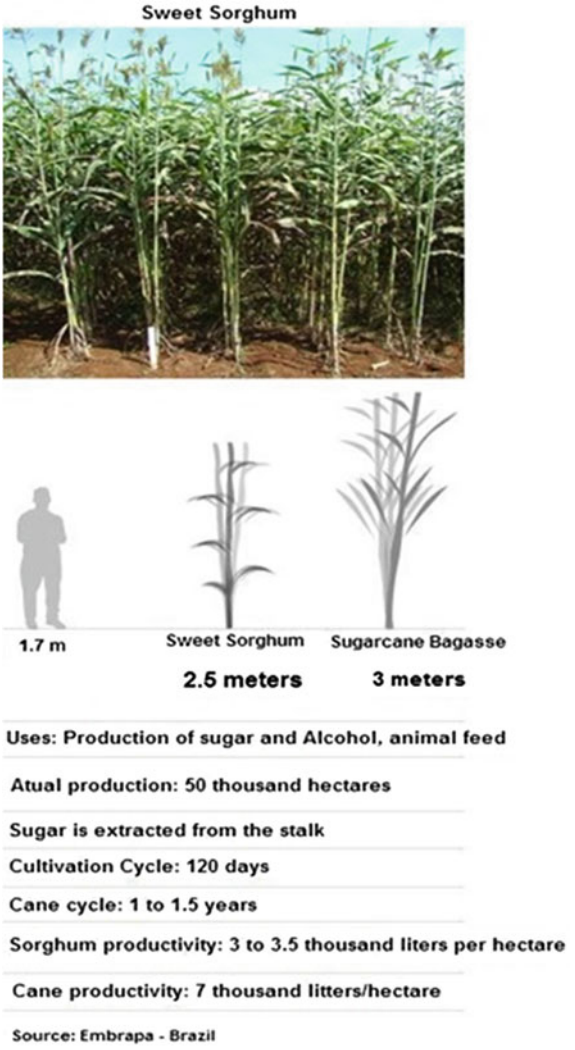

Fig. 6.3 Comparison between sugarcane and sweet sorghum. Reproduced from Globo (2013)

6.3.3 Corn Stover

Corn is a grain that is produced and known worldwide due to its rich starch content. In Brazil, the production this year (2013) was estimated as 79 million tons, which is approximately 10 % more than the last harvest 2012, this increase was a reflect of an increase in the planted area and culture efficiency due to demand and governmental incentives (CONAB 2013). The main production regions are the Center-West and South, representing, respectively 42 and 33 % of

the national production, being the estates of MatoGrosso (Center-West) and Paraná (South), the top two producers (CONAB 2013).

However, corn culture is one of the agricultural activities that most generate residues, because the corncob represents little considering the whole plant. Therefore, after the harvest the stem, leaf (straw), and cob are left in the field; these residues are known as corn stover (Gil et al. 2013). Figure 6.4 shows the residues of corn culture after the harvest. According to Lindstrom et al. (1981) (quoted in many scientific articles) the ratio between corn harvested and stover above ground is 1:1 (16 % moisture), much greater than sugar bagasse, mentioned before). Brazil alone in (2013) produced around 66 million tons (dry weight) of corn stover. Nevertheless, only part of this amount can be removed and used, an average of 40 % can, on a sustainable basis, be removed (Kadam and McMillan 2003; Walsh et al. 2000). It is advised to leave some stover on the field in order to prevent soil erosion. The removal optimization of corn stover from the field has been studied by Gil et al. 2013. Therefore, considering these data, in 2013 Brazil generated 26.4 million tons (dry weight) of corn stover available as raw material.

Corn stover can be used for many purposes as listed below:

(1) Farm/animal uses: It is a potential feed for dairy cattle (Adams 1998). However, it is not a high-quality feed, its biggest disadvantage is its physical character.
(2) Fuels: It can be used as a fuel, after milling, in a boiler furnace, as most lignocellulosic material.
(3) Biobased materials: Particleboard has been produced from bagasse and wheat straw as well as other types of fibers (Karr and Sun 2000; Karr et al. 2000). Building panels have been made from several crop residues including wheat straw, rice straw, and bagasse, and can also be made from corn stover. A drawback to manufacturing particleboard using these fibers, like corn stover, is the need for expensive resin binders (Kadam and McMillan 2003). Pulp and paper: Corn stover possesses cellulose, as other agricultural residues, therefore corn stover-based pulp and paper production is a viable alternative (Wagner et al. 2000). Using corn stover has advantages as environmental benefit (dioxins are less generated), and according to Kadam and McMillan (2003) the lower lignin content requires less bleach than that needed for wood pulp.
(4) Chemicals: Corncobs can be feedstock for producing furfural (Foley and Vander Hooven 1981; Riera et al. 1991).
(5) Miscellaneous: Hog manure and corn stover can compose potting soil (Adams 1998). Corn stover can also be put along the roadsides to prevent soil erosion and can be similarly used for slope stability (Zinkand 2000).

Although about 26 dry tons per year of corn stover is available in Brazil, based on the above recent estimates and many uses without much transformation, if one looks at corn stover chemical composition its real potential will be noted.

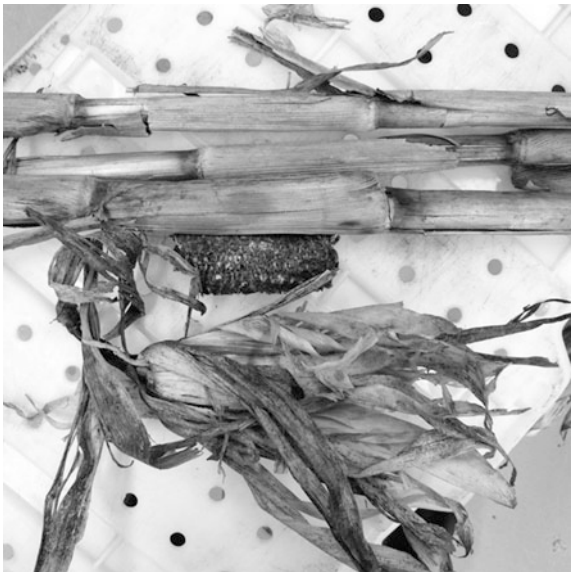

Fig. 6.4 Residue of corn cultures, known as corn stover (stem, leaf and cob). Reproduced from Stephanie Chen Design Lab (2013)

Corn stover is a lignocellulosic material, hence there are three parts (cellulose, hemicellulose, and lignin) as mentioned before. In 6.2 is presented the composition of corn stover (Kim and Holtzapple 2005).

It must be pointed out the amount of glucan (cellulose), xylan, and arabinan (hemicellulose) in corn stover, according to Table 6.2 sums up 60 % of the material. Considering that these polymers are composed of fermentable sugar (glucose, xylose, and arabinose), one can affirm that most of the corn stover is a sugar source, which makes this material ideal for sustainable processes.

6.3.4 Soybean Waste

The Global production of soybeans in 2011/2012 was 236.38 million tons (USDA 2013) and in this scenario, Brazil is the second largest producer, surpassed only by the United States. The Brazilian soybean production in 2012 was 65.7 million tons (almost 30 % of the global production) and its estimated production is 81.1 million tons for 2013 (IBGE 2013).

Soybean (Fig. 6.5) has a wide field of industrial applications including the production of vegetable oil, meal for animal feed, margarine, protein extracts, polymers, pharmaceuticals, soaps, printing inks, fertilizers, and others. The oil and meal are the main derived products from soybeans and also in the beneficiation process of the grain are generated wastes such as shells and soy molasses.

Table 6.2 Corn stover chemical composition. Kim and Holtzapple (2005)

Composition	% mm^{-1} (dry weight)
Glucan	36.1
Xylan	21.6
Arabinan	3.6
Klason lignin	17.2
Acid-soluble lignin	3.6
Protein	3.5
Acetyl	3.2
Ash	6.9
Others (*mannan, galactan, uronic acid and non-structural sugar*)	6.1

6.3.4.1 Soybean Hulls

Soybean hulls are a residue obtained from the rupture of the grains in the early stages of the process and also is the main by-product of this oilseed processing industry. This biomass represents 8–10 % of the total weight of the grain (Lia et al. 2011; Gnanasambandan and Proctor 1999). Considering the Brazilian soybean crop in 2012, around 5.9 million tons of bark were generated in the country and, in the same period, produced 21.3 million tons in the world. The high production volume of this residue can become attractive for Biorefining and production of high-value products in countries such as Brazil and the United States.

The chemical composition of soybean hulls may vary depending on growing conditions, grain growth, and efficiency of the extraction process. The method of removing the bark is done with greater intensity when it is desired a soybean meal with higher protein content that can interfere with the chemical composition of the bark. In Table 6.3 is described the chemical composition of this biomass according to Cassales et al. (2011), Yoo et al. (2011) and Mielenz et al. (2009).

Glucose is the main component of the polysaccharide fraction (39.7 %), followed by xylose (19.6 %) and arabinose (5.9 %), respectively. Other components are present as acetic and glucuronic acid (2.6 %), cellobiose (1.6 %), lignin (9.1 %), protein (1.13 %), ash (0.6 %), and extractives (3.2 %) (Cassales et al. 2011). The authors suggested this residue as a potential biomass for bioethanol production due to the presence of fermentable sugars associated with low lignin content. The lignin present in soybean hulls is lower than found in other wastes like sugarcane bagasse (22.8 %), corn stover (16.3 %), wheat straw (18–20 %), corncob, among others (Merali et al. 2013; Siqueira et al. 2013; Cybulska et al. 2012). The degradation of lignin produces compounds toxic to microorganisms and the smaller content of this macromolecule in the lignocellulosic wastes are desirable for use in processes of bioconversion. Note that soybean hulls have lower amounts of lignin hydrolyzate and contain minor amounts of toxic phenolic derivatives than hydrolysates of other waste. Soy hulls also contain appreciable quantities of minerals (P, K, Mn, Ca, Fe, Cu, S, and Zn), which can facilitate fermentative processes, since some may participate as enzymatic cofactors and the auxiliary microbial metabolic activity.

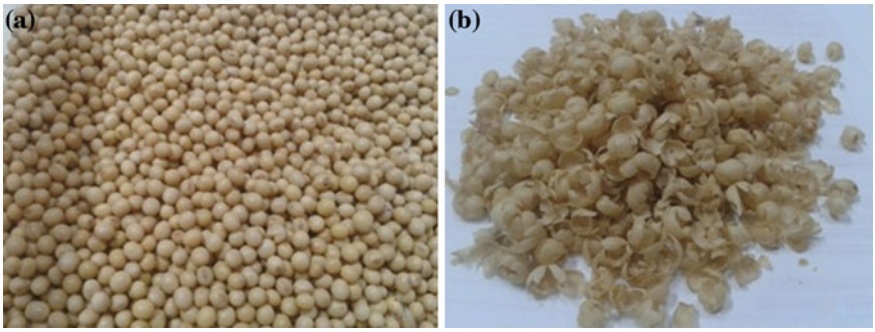

Fig. 6.5 Soybean grain (**a**) and soybean hulls (**b**)

Table 6.3 Chemical composition (mass fraction % on a dry basis) of soybean hull

Composition	Mielenz et al. (2009) (%)	Cassales et al. (2011) (%)	Yoo et al. (2011) (%)
Cellulose	29–51	39.7	35.4
Hemicellulose	10–20	25.5	17.2
Lignin	1–4	9.1	2.3
Pectin	6–15	–	–
Protein	9–14	13.1	–
Ash	1–4	0.6	–

6.3.4.2 Soybean Molasses

Soy molasses is a by-product of soy protein concentrate production. After the oil is removed from the crushed soybean, the defatted flakes (white flakes) or soybean meal is washed with 70–90 % aqueous ethanol to remove the carbohydrates and concentrate the protein. The washed solids have a protein content of at least 65 % (dry matter basis) and are known as soybean protein concentrate (Long and Gibbons 2013). The sugars present in the soybeans are extracted by ethanol–water mixture, and after recovery of ethanol is sourced molasses with a moisture content of around 50 % (Fig. 6.6).

Soybean molasses is composed mainly of carbohydrates, lipids, protein, fiber, and ash. The most abundant carbohydrates are sucrose, raffinose, and stachyose, present at percentages of 28.4, 18.6, and 9.7, respectively, based on dry weight (Silva et al. 2012). Reducing sugars and sucrose are obtained by enzymatic hydrolysis of the stachyose and raffinose oligosaccharides using α-galactosidase and invertase (Silva et al. 2012). In Table 6.4 is described the chemical composition of this biomass according to Long and Gibbons (2013), Silva et al. (2012), Siqueira et al. (2008) and Knudsen et al. (2007).

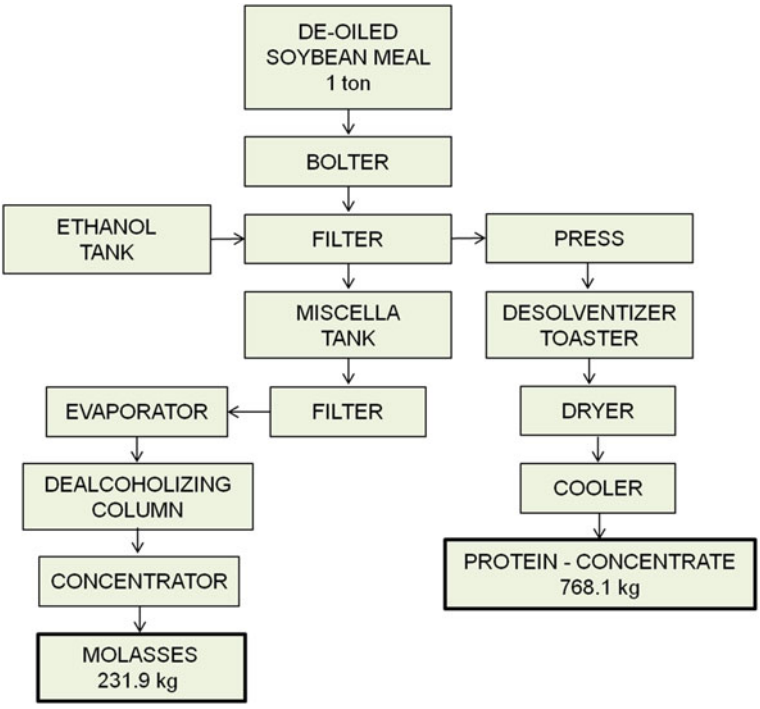

Fig. 6.6 Production process of the soybean protein-concentrate. Reproduced from Siqueira et al. (2008)

6.3.5 Eucalyptus and Forest Residues

The world forest area totals over 4 billion hectares in the world, covering 31 % of the total land area of the earth. The five most forest-rich countries are the Russian Federation, Brazil, Canada, the United States of America, and China, which account for more than half of the total forest area (53 %) (FAO 2010).

Forests are fundamental from an ecological viewpoint, but are also very important economically, with the world trade in 2007 reaching about US$10 trillion (BSS 2008). Products from forest resources are among the top ten main internationally traded ones, corresponding to 3 % of global trade (about US$300 million). In this context, silvicuture is an important tool that allows the economical use of these resources preventing further deforestation of native vegetation and contributing to reforestation or afforestation (i.e., planting of trees on land that was not previously forested) (FAO 2010; BSS 2008).

Since 2000, Brazilian silvicuture has surpassed forest extraction in production value. The sector's gross production value of forest-based sector associated with forestry in Brazil corresponded to about 30 billion US dollars in 2012, representing an important social activity, with the producing chain generating about 4.4

Table 6.4 Chemical composition (mass fraction % on a dry basis) of soybean molasses

Composition	Knudsen et al. (2007)	Siqueira et al. (2008)	Silva et al. (2012)	Long and Gibbons (2013)
Total carbohydrate	–	57.3	–	–
Glucose	–	0.243	–	4.67
Fructose	–	0.127	–	2.96
Galactose	–	0.254	–	
Sucrose	35.3	21.3	25.99	18.5
Raffinose	3.7	9.7	11.74	25.5
Stachyose	18.9	18.6	15.50	34.2
Proteins	8.4	9.4	6.44	11.7
Lipids	15.5	21.2	15.60	4.91
Fibers		5.7	–	–
Ash	6.9	6.4	7.88	21.9

million jobs and resulting in an investment of about 75 millions US dollars in social improvement, education, and environmental programs, benefiting 1.3 million people and approximately one thousand communities located around the companies. The largest forest plantations in Brazil are found in the southern and south-eastern regions, especially in the states of Minas Gerais, São Paulo, and Parana. The sector involves a number of industrial segments such as Pulp and Paper, Industrialized Wooden Panels, Mechanically Processed Wood, Charcoal-fired Steelworks, and Biomass, among others (ABRAF 2013; IBGE 2012; Gonçalves et al. 2008).

The main species of Brazilian forestry correspond to Eucalyptus. Indigenous to the Australasian region, this angiosperm from the genus *Eucalyptus* includes about 800 species, with mainly the following cultured in Brazil: *Eucalyptus grandis, Eucalyptus citriodora, Eucalyptus camaldulensis, Eucalyptus saligna, Eucalyptus urophilla*, among others, including hybrids as *Eucalyptus urograndis, obtained from E. urophilla* and *E. grandis* (Ministério da Agricultura, Pecuária e Abastecimento 2010; Coppen 2002). The last is the mostly clonally propagated and the most planted, owing to its excellent adaptation to regions with low to moderate water deficiency and low fertility soils (Gonçalves et al. 2008).

According to ABRAF (2013), the Eucalyptus planted area in Brazil in 2012 reached 5.10 ha, 53 % of which is located in southeast Brazil, with the northern region of the Brazilian Amazon representing only 6.2 % of this area. Eucalyptus corresponds to 70.8 % of total area of forest plantations. Plantations are harvested at short rotation periods of about 7 years before coppice and the productivity is in the range of 35–55 m^3 per hectare per year (BSS 2008). The main use of this genus of tree in Brazil is in the industrial segment of Pulp and Paper.

Another important group of species is pine, which corresponded to 22 % of the total area of forest plantations in Brazil in 2012. The remaining 7.8 % of planted area is used with species of Acacia, Araucaria, Populus, Teak, Rubber Tree, and Paricá.

Forest harvest practices or forest product processing results in a large quantity of residues, a part of which remains in land and contributes to fertilize the soil, but much of it is wasted and can pile up, resulting in environmental problems and representing a loss of valorous raw material. For instance, besides previously discussed for agricultural sources, wood residues are suitable lignocellulosic raw material to be used in second-generation ethanol production.

According to Ferreira-Leitão et al. (2010), forestry wastes correspond to parts of trees not profited for cellulose production, such as tips and branches, which contribute to soil fertility upon degradation. Also, according to these authors, these wastes are by nature heterogeneous in size, composition, and structure, including mainly small pieces of wood, as tree bark, corresponding to about 71 % of total waste, and sawdust about 22 % of the total.

Industrial waste wood can be grouped into sawdust, wood shavings, solids from wood, bark, and others and are generated from the transportation of the roundwood industry, to its handling and processing. The residues can be from sawmills and plywood manufacturers, or from industries like pulp and cellulose, wood panel, or furniture. Besides, there are urban residues from civil construction, municipal and urban landscaping, or wood packaging (Wiecheteck 2009).

In an early work, Ferreira-Leitão et al. (2010) estimated the total potential of generation of forest residues in Brazil in 52.8×10^6 tons (dry mass). Concerning the waste available in Brazil to energy production, another simple estimative of the magnitude of total mass can be carried out by considering only the residue generated after processing of timber logs and taking into account that, in this case, about 50 % of the wood mass is wasted (Coelho et al. 2012). According to IBGE (2012), 125.9×10^6 m^3 of timber logs were produced in Brazil in 2011. Using the correspondence of 0.68 t to each m^3 (BSS 2008; Coelho et al. 2012), 75.54×10^6 tons of timber logs were produced, generating about 37.77×10^6 tons of residues in the country in 2011. As this mass was generated only during timber processing, residues that were left in the field were not taken into consideration.

Besides the total biomass production, the composition of the material is fundamental information to be considered, because ethanol production is obtained by carbohydrate fraction, but lignin content is an important parameter in initial pathways of the process of production of second-generation ethanol. The composition is variable and depends on the wood species of origin of waste. Trees are generally classified into two broad categories known as "softwoods" (gymnosperms) and "hardwoods" (angiosperms or dicotyledonous angiosperms) (Álen 2000). Softwoods are also referred to as coniferous wood, and include species of pines, e.g., Hardwoods, in turn, include species of eucalyptus, oak, and poplar, among others. Table 6.5 shows the range of variation of main components for hardwood and softwood species.

As shown in Table 6.5, although the cellulose content is not so different for hardwood and softwood, in general, hardwoods have comparatively higher tenor of hemicellulose and lower tenor of lignin. However, there are exceptions to this rule: tropical hardwood has higher lignin content compared to softwood.

Table 6.5 Percentage of the main components of woods (Álen 2000)

Composition	Percentage of content (% dry mass)	
	Softwood	Hardwood
Cellulose	40–45	40–45
Hemicellulose	25–30	30–35
Lignin	25–30	20–25
Extractives	2–5	2–5

Specifically in relation to Eucalyptus, Emmel et al. (2003) related the following composition for industrial chips of *E. grandis*: 44.65 % of cellulose, 25.77 % of lignin, 15.33 % of hemicellulose (xylan), 3.25 % of extractives in benzene:ethanol (2:1, v/v) and 11 % of unidentified components which included 4-O-methyl-glucuronic acid and acetyl groups present in heteroxylans.

6.3.6 Other Potential Raw Materials

There are a number of other potential raw materials to be used to produce ethanol in Brazil. The use of a specific one is dependent on factors as regional availability and logistic aspects. Following, two examples of important possibilities are commented.

6.3.6.1 Cassava Residues

Another important crop in Brazil with great potential for ethanol production is Cassava (*Manihotesculenta*, *Crantz*), a woody widely cultured in the tropical regions of Africa, Asia, and Latin America (Wanapat and Khampa 2007; Boonnop et al. 2009; Ferreira-Leitão et al. 2010). An annual crop, this plant of the family *Euphorbiacea* represents the third most important source of calories in the tropics, after rice and maize (FAO 2013; Silva et al. 2001). The root is composed almost entirely of carbohydrate which can be used as an important food source.

The annual world production of cassava root in 2011 was about 252 millions tons, with Brazil producing about 25 million tons (FAO 2013). Its tuber contains 70 % starch by dry weight and has been used as a promising feedstock for fuel ethanol (Huang et al. 2010; Sanchez and Cardona 2008). It is possible to develop a system to produce ethanol from the whole plant using both starch and cellulose available. This is a true mainly considering residues of the cassava crop, estimated to weigh 144–257 % of the root weight, and residues from processing, such as bran from the root peeling, cassava waste liquor from pressing and waste fibers generated in industrial production of starch and cassava flour (Ferreira-Leitão et al. 2010). If an ethanol producing process from starch cassava is taken into account, massive amount of residues will be produced as by-products, nearly half a ton of cassava residues for producing 1 ton of ethanol, cellulose accounts for nearly one-quarter of the dry residue weight (Zhang et al. 2011, 2013).

6.3.6.2 Peanut Hulls

In Brazil, peanut hulls represent another vegetable biomass that can be used for second-generation ethanol production. The world peanut production is approximately 29 million metric tons per year, with the U.S. being the world's third largest producer, after China and India. Brazil occupies the 17th rank of peanut producers (Soyatech 2013). Brazil produced 226.5 million metric tons of peanuts in the 2010/2011 agricultural season in a land area of 84.100 ha. Several value-added products have been obtained from peanut as peanut oil and butter, peanut flour, and roasted peanuts.

Peanut shells are abundant lignocellulosic residues that could be considered as raw materials for ethanol production in China, India, and United States. Although Brazil is the 17th peanut producer, its production is relatively significant and therefore large quantities of shells are generated per year. This biomass has high contents of cellulose as can be seen in Table 6.6.

Cellulose from peanut shells can be chemically or enzymatically hydrolyzed to glucose and subsequently converted into ethanol. A potential barrier for the hydrolysis of this biomass could be its high content of lignin. Boonmee (2012) reports lower total sugar yield (22.8 g/100 g dry weight) after acid hydrolysis compared to other biomass such as bagasse, rice hull, leaf, and stalk of sugarcane (43.8–49.6 g/100 g dry weight). The high lignin content could contribute to obtaining hydrolyzate rich in compounds toxic to microbial cell. However, the employment of appropriate detoxification systems associated with the use of adapted cells could overcome this problem.

6.4 Potential for Second-Generation Ethanol Production

6.4.1 Sugarcane Bagasse

Sugarcane bagasse is a fibrous by-product resulting from grinding of the cane for sucrose extraction and can have many uses, from producing energy by combustion in boilers to soil incorporation or as part of the bovine diet. Even after extraction of sucrose and other nutrients, bagasse still contains a lot of organic matter, thus being a possible source of more energy and other fine chemicals. The alcohol obtained from bagasse is known as second-generation ethanol (lignocellulosic ethanol). However, due to the complexity of its fibrous components, many studies are still needed to improve the efficiency of ethanol production. The crushed vegetal material is rich in polysaccharides (complex sugars) such as cellulose and hemicellulose, compounds commonly found in the cell walls of plant cells. Lignin is also contained in this organic mass. These three materials together constitute more than 75 % of the biomass and confer mechanical strength to the plant. The remaining biomass is composed of substances such as proteins, vegetable, and mineral oils.

Table 6.6 Chemical composition (mass fraction % on a dry basis) of peanut shell

Composition	Al-Masria and Guenther (1999) (%)	Boonmee (2012) (%)	Riville et al. (2012) (%)	Kuprianov and Arromdee (2013) (%)
Cellulose	42.1	22.1	40.5	51.3
Hemicellulose	11.5	12.1	14.7	10.7
Lignin	37.4	35.2	26.4	45.5
Protein	5.7	–	–	–
Ash	2.8	2.2	–	6.3

The deployment of the technology of second-generation ethanol from sugarcane bagasse in Brazil is favored because the production process can be attached to existing units of sugar and alcohol industries, requiring lower investment, infrastructure, logistics, and energy supply. Furthermore, the bagasse is generated in industrial plants, and as such, free transportation costs. This is promising because from every 10 million tons of dry biomass, 600 million gallons of ethanol can be produced considering the use of only a part of cellulosic fibers (Soccol et al. 2010).

6.4.2 Sweet Sorghum Straw and Bagasse

During the processing of sweet sorghum by industries, only stems are used in the manufacture of alcohol. The pulp and seeds are discarded and in some cases are used for animal production. Sorghum bagasse is normally employed in furnaces at power plants as energy source. Nowadays and after the development of innovative technologies, sorghum bagasse is used for the production of ethanol as a source renewable energy (Oliveira et al. 2009).

According to Barcelos (2012) the composition of sweet sorghum's juice and bagasse is similar to sugarcane, as well as the efficiency of bagasse for cogeneration (2.150 kcal kg^{-1} for sugarcane bagasse and 2.200 kcal kg^{-1} for sorghum bagasse).

In comparison with other feedstocks, the characteristics of sorghum cellulosic fiber are similar to that of other nonwoody sources, such as cotton stalks and corn stover (Reddy and Yang 2005), and sorghum has lower lignin levels than many woody and nonwoody fiber sources (Godin et al. 2010).

The sweet sorghum bagasse presents a Brix of 16.5 %, differing from sugarcane bagasse. According to Silva et al. (2007) sugarcane bagasse presents a Brix of 20 %. This difference between sweet sorghum bagasse and sugarcane bagasse is probably due to the method of juice extraction. Since, sugarcane has thicker stems than sorghum, the grinder can better extract the juice from the cane. The same authors confirm that, the density of sorghum bagasse is lower than that of sugarcane bagasse, thus requiring reactors with larger capacity for the development of the hydrolysis processes.

Sorghum fiber hydrolyzates are liquors rich in both hexoses and pentoses, therefore production of bioethanol from these matrixes is possible only with the use of osmotolerant and pentose fermenting yeast or bacterial strains. Ballesteros et al. (2004) obtained 16.2 g ethanol/L when hydrolyzates obtained from sweet sorghum bagasse were fermented with *Kluyveromyces marxianus*.

According to Saldívar-Serna et al. (2012) experimental data obtained from sweet sorghums cultivated in Central Mexico indicated that these materials are capable of yielding 6.38 tons of sugar/ha/cut. Consequently, when adequately bioconverted, they have the potential of producing 4.1 L ethanol. Regarding lignocellulosic fraction, if 15.3 ton of bagasse/ha is obtained containing 29 % cellulose and hemicellulose and 5.4 % of remaining unextracted soluble sugars, up to 2,400 L of ethanol can be obtained.

According to Hess et al. (2007) the logistic cost, including the harvesting, collecting, preprocessing, transporting, and handing of the raw materials, has an important share in the whole cost of ethanol production. Thus, if sweet sorghum bagasse (SSB) could be effectively utilized for ethanol production integrating with juice fermentation, the overall cost of refining ethanol from sweet sorghum would be reduced by sharing the co-logistic cost.

6.4.3 Corn Stover

As abovementioned, corn stover is a potential sugar source that can be used for many purposes, therefore this material can also be used as sugar source for second-generation ethanol. Since it was already discussed before, in this part it will not be described pretreatments and treatments, and fermentation details, however studies have been conducted in this area in order to optimize these processes (Kim and Holtzapple 2005; Gáspár et al. 2007).

In order to calculate more accurately the corn stover potential to produce ethanol one must make some consideration: (a) Both cellulose and hemicellulose will be converted to ethanol; (b) Considering sugar extraction and purification efficiency of 50–70 %; (c) Fermentation for ethanol of both cellulose and hemicellulose of 40–45 % and; (d) 10 % of the corn stover will be used for energy (furnaces) and animal feed. These estimates are based on experimental observation and on the literature (Mosier et al. 2005; Kaar and Holtzapple 2000; Kadam and McMillan 2003). After these considerations, it is possible to calculate ethanol production. In Brazil, in 2013 alone it was possible to generate, from 19.80,000 million tons of corn stover 3.96,000–6.23,700 million tons of ethanol (4.96,000–7.8,200 millions of L), which is 250–394 L of ethanol per ton of corn stover (dry weight). Other countries, for example the U.S.A., which has a large corn plantation area, could produce 14.00,000–20.00,000 millions of L of ethanol per year (Sokhansanj et al. 2002).

6.4.4 Soybean Residues

6.4.4.1 Soybean Hulls

Pretreatment processes are are necessary for the use of soybean hulls in the production process of bioethanol. The objective of pretreatment is to break the protective barrier of lignin and disrupt the crystalline structure of cellulose, thus making more accessible carbohydrate enzymes to increase the yield of fermentable sugars. There have been few reports of studies on processes for pretreatment and hydrolysis of soybean hulls in the literature.

Thermo-mechanical extrusion pretreatment of soybean hulls followed by enzymatic hydrolysis was described by Yoo et al. (2011) as a feasible way for cellulose to glucose conversion. Thermo-mechanical extrusion was shown to be a feasible pretreatment method for lignocellulosic ethanol production. Values of cellulose to glucose conversion of until 95 % were obtained. Cellulose conversion from extrusion pretreatment of soybean hulls was comparable or better than that obtained from traditional chemical pretreatments utilizing acid and alkali.

Soybean hulls were evaluated as a substrate for production of ethanol by fermentation with *Saccharomyces cerevisiae* D_5A and simultaneous enzymatic saccharification by Mielenz et al. (2009). The authors obtained ethanol concentrations of 25–30 g/L, while under these conditions corn stover, wheat straw, and switch grass produced 3–4 times lower ethanol yields.

Since there is a valuable market for soybean hulls as animal feed, little attention has been given to this biomass for the production of second-generation ethanol. In Brazil this biomass is not competitive for the production of ethanol fuel yet, especially when compared with the sugarcane bagasse due to the large quantities of bagasse generated by numerous ethanol plants in the country. However, considering that ethanol is used in industrial plants producing soy protein in the form of alcohol solution with a concentration of 70 %, the deployment unit producing ethanol from soybean hulls associated with producing plant protein concentrate can be feasible and economically attractive.

Similarly, ethanol is also used in the production of biodiesel from soybean oil obtained by the transesterification method. In the transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of strong acid or base to produce a mixture of fatty acids alkyl esters and glycerol. For a complete stoichiometrically transesterification a 3:1 molar ratio of alcohol per triglyceride is required.

Soy is the crop most used in Brazil for the production of biodiesel and in this sense, industrial complexes consisting of units associated with the production of ethanol and biodiesel derived from soybean residues may become a promising strategy in the future.

6.4.4.2 Soybean Molasses

Conversion of the carbohydrates from soybean molasses into ethanol through simultaneous saccharification and fermentation using commercial enzymes (cellulase, β-glucosidase, and pectinase) and *S. cerevisiae* NRRL Y-2034 or *Scheffersomyces stipitis* NRRL Y-7124 was examined by Long and Gibbons (2013). *S. cerevisiae* and *S. stipitis* produced about 12.5 and 6.00 g/L ethanol, respectively, on molasses.

Letti et al. (2012) reports 78.3 % maximum theoretical yields and 24.2 g/L of ethanol in flasks fermentation using *Zymomonas mobilis* NRRL 806 and 96.0 % maximum theoretical yields, with productions of 29.3 g/L of ethanol in bioreactor fermentation. Bioreactor fermentation using *S. cerevisiae* LPB1 was also studied and it was reached 89.3 % of the theoretical maximum value.

Silva et al. (2012) studied the production of ethanol from natural soybean molasses by fermentation with *S. cerevisiae*, the enzymatic hydrolysis of soybean molasses by α-galactosidase and the subsequent fermentation with *S. cerevisiae* (HF) and ethanol production via simultaneous hydrolysis and fermentation (SHF) of soybean molasses. The results showed that, although the fermentation of natural soybean molasses provided a fermentation yield of 72.9 % under optimized conditions, hydrolyzed soybean molasses provided a fermentation yield 7.6 % higher using the HF process and 8.2 % higher using SHF. Both fermentation processes resulted in lower concentrations of residual sugar. Comparing the processes of hydrolysis and subsequent fermentation (HF) and SHF, it appears that the production of ethanol (54.0 g/L) were higher in HF when compared to the values for the simultaneous process (49.2 g/L ethanol).

In Araucária Town (Paraná state) is located the world's first company to produce ethanol from soybean molasses. The company can produce up to 10,000 L of hydrous ethanol per day and it has license from Brazilian National Agency of Petroleum to produce and commercialize ethanol fuel for cars.

Ethanol production from soybean molasses on industrial scale for use as fuel still is not economically advantageous compared to other biomasses such as sugarcane bagasse. However, the transformation of biomass into ethanol by the company generating the waste can become an attractive prospect, especially when alcohol is used as solvent for the industry itself or used in power generation or such fuel for the company's fleet.

6.4.5 Forest Residues

With relation to forest residues, the main drawback to produce ethanol can be related to the high degree of difficulty to liberate fermentable sugars from lignin seal that composes the macromolecular net of the material. This is true mainly for eucalyptus or hardwoods (Mcintosh et al. 2012).

Different conversion yields for the process have been related, in dependence of evaluated conditions and use of hexoses or pentoses in fermentation. Mcintosh et al. (2012), evaluated conditions for dilute acid pretreatment of eucalypt (*Eucalyptus dunnii*) and spotted gum (*Corymbiacitriodora*) forestry thinning residues for bioethanol. In their work, the authors observe that in the optimized conditions, an enzyme cellulose hydrolysis yield of 74 % is theoretical. *S. cerevisiae* efficiently fermented hexoses from crude *E. dunnii* cellulosic hydrolysate within 30 h, yielding 18 g/L ethanol, representing a glucose to ethanol conversion rate of 0.475 g/g (92 %). In another work, Silva et al. (2011), besides fermentation of hexoses from cellulose with *S. cerevisiae*, have also evaluated the pentoses fermentation from hemicellulosic fraction of residual wood chips of cellulose industry. Dilute acid pretreatment was used to produce hemicellulosic hydrolysate; its fermentation was carried out using a flocculating strain of *Pichia stipitis*. The process resulted in 15.3 g/L of ethanol in 40 h of fermentation, corresponding to a yield of 0.32 g/g. Still considering that work, the solid fraction generated after pretreatment was subjected to enzymatic hydrolysis, which was carried out simultaneously with glucose fermentation (SSF: Simultaneous Saccharification and Fermentation Process), using a strain of *S. cerevisiae* and resulting in 28.7 g/L of ethanol in 55 h. According to the authors, the global yield of the ethanol production process was 100 L of ethanol/ton of eucalyptus wood chips.

The global yield of ethanol production from Eucalyptus biomass was considered by Gonzales et al. (2011) as equivalent to those in corn stover. In that work, software aided simulation was carried out for technical and financial performance of high yield Eucalyptus biomass in a cocurrent dilute acid pretreatment followed by enzymatic hydrolysis process. The authors have considered an ethanol yield per ton of dry Eucalyptus biomass of 347.6 L of ethanol, with average carbohydrate content in the biomass of 66.1 %.

6.5 Future Trends

According to Saldívar-Serna et al. (2012) the genetic improvement of crops is one of the most promising research priorities in agricultural production with high economic relevance. In the case of sorghum and sugarcane for fuels there are important advances in the development of biomass, sweet and high yielding grain varieties and hybrids, but is yet one of the most important and critical research topics. New cultivars should be adapted to marginal lands and they must be resistant to pests, other phytopathogens, and stable facing water stress (Saldívar-Serna et al. 2012).

According to Reddy and Yang (2005) the main obstacle in creating new hybrids for ethanol production is the “non additive” character of their relevant traits, such as plant height, total soluble solids, juice production, and lignin:cellulose:hemicellulose ratio. On the other hand according to Turhollow et al. (2010), the genetic mapping combined with its relatively fast hybridization and field tests, can facilitate the design and development of dedicated bioenergy cultivars.

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Chapter 7
Advances in Methods to Improve the Sugarcane Crop as "Energy Cane" for Biorefinery: An Appraisal

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Abstract Plant biomass is a source of renewable energy and biomolecules amenable to feed environmentally sustainable biorefineries. Chemistry, biotechnology, and process engineering advances will make biorefineries feasible in technical and cost aspects. Efforts have been concentrated in assessing plant biodiversity and crop potentialities for manipulation of physiological responses such as carbon fluxes toward soluble, storage, and structural sugars, waxes, oils, phenolics, and many other products. Thanks to advances in the "omics" field by the use of model plants, these issues have been addressed, allowing for a better comprehension of the general plant metabolism with concomitant inferences to important crops, like sugarcane. Plant cell walls are one of the most abundant, renewable, and useful biomaterial on the earth. However, wall polymers are entrapped in an imbricated structural organization. Thus, the viability of using such feedstock in a bio-based economy will greatly depend on the integration of "green" and "white" technologies in the production processes to efficiently extract and use molecules and energy stored in biomass. In this chapter, we discuss some principles underlying biorefination and bottlenecks under the crop physiology aspects—including *Saccharum*. Correlations between biomass yield and properties with environmental factors are revisited.

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7.1 Photosynthesis and the Potential Biomass Yield of Primary Production

Photosynthesis is responsible for all plant biomass entering the biosphere. After incorporated into the first and simple trioses-phosphate sugars, carbon is then partitioned toward different classes of substances, such as structural and soluble carbohydrates, lignin, protein, and many other biomolecules.

The primary plant productivity (P_n) is under influence of many environmental factors such as competition with weeds, diseases, nutrient scarcity, mineral toxicity, temperature, and water availability. The primary productivity is usually taken to describe the physiological yield potential (Y_p) parameter,

$$Y_p = \eta \cdot P_n$$

where Y_p represents the product of the harvest index (η) by the primary productivity (P_n). The harvest index (η) means the partition of the biomass produced by photosynthesis toward effectively harvested products, while the primary productivity (P_n) denotes the total biomass accumulated during the growing season as a result of photosynthetic activity (Evans and Fischer 1999).

The primary productivity (Monteith and Moss 1977) for a culture is a parameter associated with climatic conditions, photosynthesis efficiency, and allocation of biomass. Therefore, the occurrence of stresses greatly impacts the primary productivity, since it affects intrinsic photochemical and biochemical performances of photosynthesis. The primary productivity (P_n) is expressed as

$$P_n = S_t \cdot \varepsilon_i \cdot \varepsilon_c \cdot K^{-1}$$

where:

S_t = Annual Integral of the Incident Sun Light Energy (MJ m^{-2})
ε_i = Light harvesting efficiency
ε_c = Efficiency conversion of absorbed light into biomass
K = Energy content of the total biomass (17.5 MJ Kg^{-1}) or total carbohydrates (15.9 MJ Kg^{-1}).

Countries located in the intertropical regions receive a great amount of solar energy during the entire year. The annual mean daily horizontal global solar radiation in any region of Brazil during a decade of study (1995–2005) was much higher (1500–2500 kWh m^{-2}) than that for the majority of countries located in Europe (Pereira et al. 2006): Germany (900–1250 kWh m^{-2}), France (900–1650 kWh m^{-2}), or Spain (1200–1850 kWh m^{-2}). In Brazil, the Northeast and Central regions receive the largest amount of daily global solar radiation (about 5.6 kWh m^{-2} or 20.16 MJ m^{-2}) in the fall and winter seasons (between June and September), when clear sky days are usual.

The primary productivity depends on the photosynthetically active radiation (PAR), since it represents the range of the visible spectrum (approximately 40 %)

effectively available for photosynthesis. Taking into consideration the Northern and Central regions of Brazil, the annual mean for the daily PAR is estimated as 2.4 kWh m^{-2}. This is the same as 8.64 MJ m^{-2} or 8.64 × 10^4 MJ ha^{-1} day^{-1}. This energy is in part converted by plants into chemical potential energy (C–C bounds) used for respiration or storage. It is known that a C_4 plant, like sugarcane, will convert, the best as possible, only 6 % of the total incident radiation into carbohydrates, due to losses such as reflection, transmission, heat convection, and metabolic costs (Zhu et al. 2008). This is equivalent to 1.21 × 10^4 MJ ha^{-1} day^{-1} being converted into structural or storage carbohydrates. For each Kg of C_4 produced biomass, 15–17.5 MJ of energy equivalent is required (Lorimer et al. 2010). In this sense, 1.21 × 10^4 MJ h^{-1} day^{-1} may potentially render 690 kg of biomass ha^{-1} day^{-1} or 251.8 ton ha^{-1} $year^{-1}$. This represents an estimate for the annual mean of the sugarcane primary productivity (total biomass).

On the basis of ecophysiological and agronomic studies (Larcher 2003), the sugarcane harvest index (economic yield/biomass production yield) ranges from 0.6 to 0.85, which allow us to estimate the yield potential (Y_p) for sugarcane in the Northeast and Central Brazilian regions, which is about 182 ton ha^{-1} $year^{-1}$ (or 18 kg m^{-2} $year^{-1}$). However, in practice sugarcane aboveground harvestable biomass is reported (Larcher 2003) to be around 6–8 kg m^{-2} $year^{-1}$ (60–80 ton ha^{-1} $year^{-1}$). Hence, the exploitation of lignocellulosic residues for second-generation biofuels has the potential to increase Y_p as a function of the increment of η.

Despite high yield potential estimated for cane in Brazil, the average productivity was 69.4 ton ha^{-1} in 2012. In 2011 and 2012, the Northeast region experienced a severe drought that greatly affected the ratoon crop, that did not sprout. A great variation in sugarcane productivity in the different regions was registered in this period. The North/Northeast region averaged 49.7 ton ha^{-1}, whereas the Midwest/South region harvested 72.4 ton ha^{-1}. Acre, a hot and humid north state, registered 95 ton ha^{-1} in 2012, one of the best records for the period of 2012 (da Silva 2013). Since 2011, climatic instabilities have been causing huge productivity losses. In 2011, about 571 million tons of cane were harvested, 10 % less than the year before.

For many crops abnormal seasons may cause atypical flowering and more propensity to diseases. In this sense, biotic and abiotic stresses are the main causes of great losses in productivity every year in the world. In nematode infested areas, yield loss up to 50 % may occur, and the costs for managing weed infested areas are 15–30 % higher (Barela and Christoffoleti 2006).

7.2 Basic Concepts on Biorefinery

Biorefineries are a set of processes to produce energy and products from renewable feedstocks, such as plant, algae, animal, or bio-based wastes, with minimal environmental impact. Biorefineries will potentially mitigate the indiscriminate

use of ancient carbon reserves that produce greenhouse effects. Biorefination will also allow the development of new products and processes that will aggregate value to biomass. On the social and political scenarios, it could also open opportunities for adoption of policies to allow the participation of a global market involving land use, technology transfer, and employments. Conceptually, it needs to operate with minimal cost and time, produce low or zero environmental impacts, to be large-scale operated, and must generate social benefits.

The US Department of Energy defined that a biorefinery is an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products (DOE 2014). Also, the American National Renewable Energy Laboratory (NREL 2009) stated that: "A biorefinery is a facility that integrates biomass conversion processes and equipments to produce fuels, power and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum. Industrial biorefineries have been identified as the most promising route to the creation of a new domestic biobased industry" (NREL 2009). A more general definition is: "Biorefining is the transfer of the efficiency and logic of fossil-based chemistry and substantial converting industry as well as energy production onto the biomass industry" (Kamm et al. 2012).

The great development recently experienced in biotechnology, chemistry, and process engineering points out limitless possibilities for biorefination. For a long time, microorganisms have been used to produce or transform many biomolecules of interest (drugs, food, textile, recombinant enzymes), much like a micro-biorefinery plant. The biological systems may be considered as high-end biofactories (Fig. 7.1) that one scarcely knows how to operate, but keep huge capabilities to be exploited through genetic engineering.

Cell wall polymers, which represent a rich resource of important biomolecules for biorefination, are entrapped in an imbricated structural organization in the wall. Therefore, the viability of using such feedstock in the bio-based economy will greatly depend on development and proper integration of "green" and "white" technologies in the production processes. At the "green side" there is a need to improve crop performance and biomass traits and at the "white side" industry has been challenged to deal with efficient conversion of biomass into products and energy (Vanholme et al. 2013). In the recent years, many forestry (Liu 2010), agriculture (Mariano et al. 2013), aquaculture (Demirbas and Demirbas 2011), and waste feedstocks (Vanholme et al. 2013; Weiland 2010) have been assessed for their potential use as biomass resources (Vanholme et al. 2013).

Three stages of the biorefineries development can be identified (Kamm et al. 2012): (a) Generation I biorefinery: Still limited biomass/feedstock utilization, basically, a dry milling ethanol plant using grains as raw material. (b) Generation II biorefinery: A more flexible mill that uses wet technology to produce different types of end products from grains (oil, syrup, ethanol, starch). (c) Generation III biorefinery: Uses agricultural or forest biomass to produce ethanol, chemical, and plastics. Reviews on possible industrial processes and products can be found at (Jong et al. 2008). Concerning biomass feedstock, two generations can be

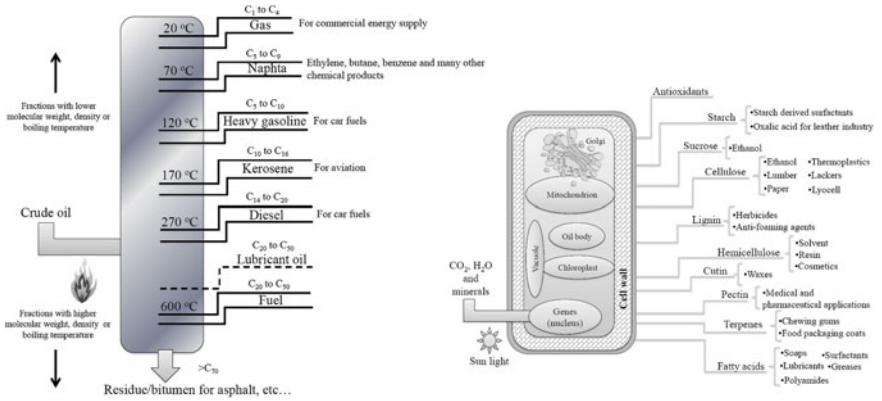

Fig. 7.1 Parallel between a traditional refination and the biorefination

identified: The first-generation feedstocks consist of crops with potential use as food or fuel, such as crop for bio-oils, sucrose, and starch, and therefore raise many debates about impacts on food prices and plantation area, mainly in countries with limited agricultural land. The second-generation feedstocks refer to the possible use of any part of the plant, such as the plant cell wall fibers, increasing the potential utilization of the whole harvestable biomass (Kamm et al. 2012).

Concerning biorefinery implementation, two main approaches are objects of study (Jong et al. 2008): (a) A value chain approach, by which interesting compounds are identified and isolated directly from biomass or through (bio)conversion steps. By this approach, a great and concomitant development in separation and bioconversion of products is required, and (b) Integrated process chain approach, by which universal biomass substrates are first transformed into common building blocks, also used by the petrochemical refinery. This facilitates the integration with the current feasible and economically viable petrochemical facilities. Here, the challenge is to improve the transformation of biomass into these common universal building blocks.

7.2.1 The Plant Biomass and Its Potential Use as Biorefinery Feedstock

7.2.1.1 Plant Cell Wall

The plant cell wall is a rich resource of biopolymers and monomers for biorefineries. It is classified as primary or secondary, according to its composition and structural organization.

Primary walls contain cellulose, structural proteins, and a hydrated polysaccharide matrix consisting of hemicelluloses and pectin. The primary walls are

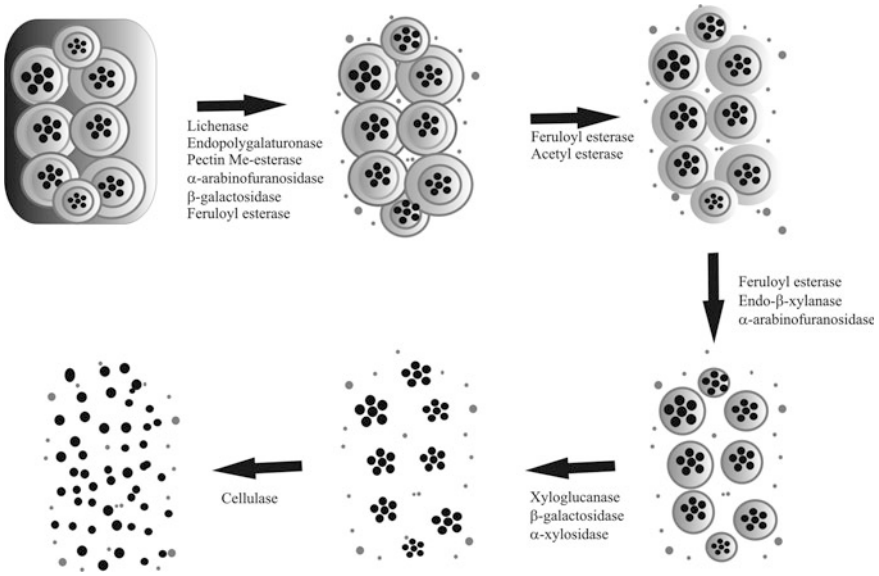

Fig. 7.2 The stepwise use of different cell wall degrading enzymes to fractionate the sugarcane biomass (*Source* Adapted from Souza et al. 2012)

usually classified as type I or type II. Type I walls are present in dicots and noncommelinoid monocots. Xyloglucan is the major hemicellulose found in type I walls, which also contains abundant amounts of pectic polysaccharides. Type II walls, found in commelinoid monocots, are abundant in cellulose and only negligible amounts of pectin and proteins are found (Carpita 1996; Pauly and Keegstra 2008). In Poales, such as sugarcane, arabinoxylan is the predominant hemicellulose (Souza et al. 2012).

Secondary walls are thicker than primary walls and may be deposited in different layers (S_1–S_3) according to the microfibrils orientation (Higuchi 1996). Bamboo has cell walls with much more layers (Parameswaran and Liese 1976). The deposition of secondary wall ceases cell enlargement. The secondary walls contain cellulose and arabinoxylan and/or glucomannans as hemicellulose (Pauly and Keegstra 2010). In secondary walls, pectin is replaced by lignin, which makes them very impenetrable to solutes and enzymes (the so-called recalcitrance).

In order to use lignocellulosics as feedstock in biorefineries, the plant fibers need to be first fractionated. This involves chemical, physical, and/or enzymatic processes to disrupt the native fibers configuration. The cell wall fractionation through enzymatic methods is clean and preserves the chemical identity of the original polymer in the fragments released (Fig. 7.2).

The main derivatives of C5 and C6 sugars with great economic potential were reported by the US-DOE to be organic acids such as lactic and succinic acids, sugar alcohols (sorbitol), and ethanol (Bozell and Petersen 2010). In addition, fine

chemicals (enzymes, vaccines) are examples of products that will be facilitated by biorefineries.

Next, we briefly discuss the potential use of plant biomolecules as substrates for biorefination, with reviews and original papers indicated for each topic.

Cellulose: Cellulose, when hydrolyzed, releases glucose, which can be readily fermented to produce second-generation ethanol (2G). Second-generation ethanol will be commercially produced in Brazil from 2014. The use of lignocellulosics from different crops could enter the process chain to increase ethanol yields. Second-generation ethanol from lignocellulosics on its maturity could increase the current Brazilian production by 50 % without expanding the current sugarcane agricultural frontiers. The first commercial Brazilian plant for the 2G ethanol estimates the initial production of 82 million liters ethanol per year. Today, about 22 billion liters of ethanol is consumed in Brazil, but until 2020 a demand of 47–68 billion litters is expected (Viana 2013).

Hemicelluloses: Hemicellulose from type II primary walls or secondary walls releases mainly pentoses when hydrolyzed. It is a source of C5 sugars, such as xylose. Xylose can be converted into xylitol and furfural. Xylitol is a sweetener with antimicrobial, remineralization, and teeth hardening activities and therefore enters the formulation of chewing gums and toothpaste (Roberto et al. 1999; Sarrouh and da Silva 2013). The first report of furfural production was in 1831, by Dübereiner, who reported the distillation of bran with diluted sulfuric acid. Industrial technology for furfural production from pentose was developed by Quaker Oats in the 1900s. DuPont has established the production of nylon-6.6 since 1960 from furfural, despite the plant furfural has been replaced by fossil-based substrates since then. Furfural is also used in the manufacture of phenol plastics, varnishes, and pesticides (Montané et al. 2002).

Pectin: Pectin is a complex polysaccharides composed of four main pectic compounds: homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), and xylogalacturonan (XGA). XGA exhibits the basic HG core with xylosil substituents attached to it, while RG-II, the more complex pectic component, is the modification of HG with four different side chains exhibiting a great diversity of sugar linkages (Atmodjo et al. 2013; Carpita and McCann 2009). Pectin is synthesized in Golgi apparatus and recently, it has been reported that many glicosyltransferases physically interact to form large supramolecular complexes responsible for the synthesis of pectic compounds (Atmodjo et al. 2011). Pectin enters the secretory pathway and is deposited in the apoplast in a highly methyl-esterified form (Driouich et al. 2012) that is subsequently modified (de-methyl-esterified) by the action of Pectin Methyl Esterases (PME), cross-linking of divalent cations, usually Ca^{2+} or RG-II dimerization through borate-diester linkages between two RG-II (Carpita and McCann 2009). These listed modifications are thought to increase the cell wall stiffness, although the modification of the cell wall status by pectin seems to depend on the organ type and situation, thus complex effects on plant development and growth might be expected (Derbyshire et al. 2007; Peaucelle et al. 2012). The overexpression of a PME inhibitor in Arabidopsis provoked biomass and saccharification efficiency increases, indicating

the potential of the biotechnological modification of pectin metabolism for 2G ethanol (Lionetti et al. 2010). A pectin-based biorefinery includes its use as gelling agent, thickener, stabilizer in jams and drinks, and gelatin. The complex pectin structure reflects its many potential functions and applications. Pectin may act as a signaling molecule in plant defense responses (Davis and Hahlbrock 1987), may serve for many medical and pharmaceutical applications such as in drug delivery or gene delivery systems and tissue engineering. The multitude of ways by which the complex pectic structure can be further chemically modified offers opportunities to discover novel anti-cancer and anti-metastatic drugs (Jackson et al. 2007; Munarin et al. 2011; Munarin et al. 2012).

Lignin: The presence of lignin in plant biomass has been mainly associated with the recalcitrance of biomass to saccharification. Lignin impacts the yield of fermentable sugars and fermentation efficiency of lignocellulosics in different manners. It hinders the access of carbohydrate degrading enzymes to C6 and C5 sugars in the cell wall. Lignin inhibits cellulase activity by adsorbing them, increasing the need for higher enzyme loads during saccharification (Jørgensen and Olsson 2006; Berlin et al. 2006). Concerning lignin applications, many aromatic products might be produced from it. Vanillin and gallic acid are examples of building blocks that have attracted great interest (Walton et al. 2003). Vanillin can be used as basic monomeric unit for the production of herbicides, anti-foaming agents or drugs, such as papaverine, L-dopa, and trimethoprim (Loureiro et al. 2011).

7.2.1.2 Plant Oils

Plant oils and animal fats are sustainable alternatives to mineral oils in the production of lubricants, fuel (biodiesel), surfactants, cosmetics, and emulsifiers. Many hydrophobic substances depend today basically on the petroleum. The market potential for the bio-based oils is immense and the development of oils with different properties will depend on the assessment of different biomass resources.

Edible and nonedible plants like soybean, rapeseed, sunflower or castor beans oils, and animal fats like fish oil and tallow are sources of lipids. The richness of oils with still unknown properties is huge, reaching up to a thousand types if plant and animal lipids are combined. The lipid source depends on the region, season, and knowledge of plants (Chou 2011).

The Brazilian Agroenergy Plan (Oliveira and Ramalho 2006) recommends the search for new raw materials with higher energy content. It also advises for the cultivation of oleaginous plants according to the particularities of each State or Region, preferably where they are already introduced and consumed. Species with high potential for oil-based biorefineries are *Jatropha curcas*, L. (pinhão-manso), *Acrocomia aculeata*, Jacq (macaúba), *Astrocaryumm urumuru*, Mart (tucumã), *Orbignya phalerata*, Mart. (babaçu), and *Maximiliana maripa* (inajá).

The assessment of natural diversity of native plants has much to offer due to the infinity of bio-oils with different characteristics that can be found. However, the domestication of native plant species is still in its infancy, concerning the exploitation of their genetic potentials. Biotechnology will be helpful to manipulate quantitatively and qualitatively the lipid profile in plants or algae biomass. The biotechnological advances on lipid metabolism will greatly depend on the knowledge of how fatty acid biosynthesis is regulated in the oleaginous crops. Equally important is the definition of new routes to the desired products and public policies for the regional development. Technical support for local producers will be important for the implementation of standard production methods to keep the quality control of the raw material, associated with sustainable production practices.

7.2.1.3 Waxes and Suberin

Another class of plant substances with potential application in biorefineries are waxes and suberin, which are produced and deposited outside the epidermis, forming a water repellent barrier to protect the plants against biotic and abiotic stresses.

Waxes are composed of very long fatty acids produced by the endoplasmic reticulum of epidermal cells. Many years of genetic studies on molecular aspects of waxes biosynthesis in Arabidopsis have led to the characterization of fatty acid elongating enzymes and waxes transporters (Bernard and Joubès 2013). In agreement with their protective function against exacerbated water loss, the wax biosynthesis is upregulated at the transcription level by water deficit. In addition, abscisic acid (ABA)—a plant hormone that plays a major role in water stress signaling, increases wax synthesis and decreases cutin permeability (Kosma et al. 2009). The MYB96 transcription factor has been implicated in the regulation of a set of genes in an ABA dependent manner in Arabidopsis plants undergoing water stress, including those related with wax metabolism (Seo et al. 2011).

In addition, waxes protect external plant tissues avoiding the establishment of a prolonged humid environment that could propitiate pathogens colonization. Indeed, anti-feeding function has also been attributed to the inner layer of the waxes (intracuticular compartment) that contains aromatic and triterpenoid compounds that could counteract herbivory (Eigenbrode and Espelie 1995). The aromatic compounds present in the wax are also known to absorb UV-B and UV-C radiation, and the cuticle concentrations of these constituents were shown to provide moderate UV protection in some plant species (Krauss and Markstädter 1997).

In this scenario, plant waxes have potential applications as raw material for production of inseticides, cosmetics, sealing agents, and much more, based on their natural properties already identified.

Suberin constitutes the periderm layer that is deposited during secondary growth of many plant species—the best known is *Quercus suber*, the cork oak tree. The apoplastic deposition of suberin protects polysaccharide cell walls from

decomposition, as evidenced by the slow chemical decay of cork bark in soil (Vane et al. 2006). Suberin incrustation in the apoplastic space makes the cell walls a highly selective barrier against water, solutes, and gases. Although still a topic of debate (Naseer et al. 2012), plants use suberin along lignin to build the Casparian band in root endodermis and exodermis to restrict the transport of substances and microorganism through the apoplastic pathway, conferring additional absorption selectivity for the roots in soil.

The fine structure of suberin is still uncertain due to difficulties on the substance isolation. Nonetheless, some compositional analyses reveal that suberin can be chemically described as a bio-polyester, mainly comprising ω-hydroxy acids; α,ω-dicarboxylic acids (diacids) with low amounts of fatty acids and alcohols. The carbon chains length ranges from C16 to C32. Glycerol and minor amounts of aromatic phenylpropanoids may also be part of the aliphatic suberin polyester (Franke et al. 2012).

Suberin has the potential to be source of new hydrophobic plant-derived polyesters. Industrial cork by-products may also render antioxidants, triterpenes, and other lipophilic compounds (Santos et al. 2013; Sousa et al. 2006). The cork stopper and agglomerate industries generate considerable amounts of suberin-rich residues. The exploitation of such biomass resource is therefore in agreement with the biorefineries concepts (Sousa et al. 2011).

The state of the art on suberin and cuticular waxes biosynthesis, deposition, and regulation is reported by (Buschhaus and Jetter 2011; Franke et al. 2012; Samuels et al. 2008) and (Bernard and Joubès 2013).

7.2.1.4 Rubber

Rubbers are natural polymers with unique properties and are mainly obtained from "Seringueira" (*Hevea brasiliensis*). Nonetheless, guayule (*Parthenium argentatum Gray*), a xerophytic shrub growing mainly in the arid regions of Mexico, has been pointed as a good source of natural rubber. To make exploitation of guayule rubber feasible, domestication programs must be conducted, especially because this species can grow in arid and semiarid areas around the world, bringing economic importance to these regions (Thompson and Ray 1989).

For medical purposes, rubber must be hypoallergenic and the research field of rubber-associated proteins is of great importance. The rubber genetic breeding program has been conducted in Brazil since the 1930s, for yield and resistance against *Microcyclus ulei* P. Henn (Filho and de Resende 2000). Interspecific crosses among *Hevea brasiliensis*, *H. benthamiana* and *H. pauciflora* are the basis of the "seringueira" genetic breeding program. However, little is known about the genes participating in the biosynthesis and traits of rubber, and how they are regulated at molecular level.

7.2.1.5 Cogeneration of Energy

Cogeneration is the autonomous production of energy in a mill by burning of crop residues. The thermal energy generated this way can supply the plant demand for energy and generate a surplus that can be exported, benefiting other industrial, home, or commercial installations nearby. Combustion processes using high efficiency, multi-pass, steam turbines to produce electricity can currently achieve an overall efficiency of 35–40 % (McKendry 2002). Cogeneration makes the processing of biomass independent of other energy sources, eliminating the need for electric substations, and does not increase the carbon budget in the atmosphere since it displaces the use of fossil fuels to generate energy.

In the past, the burning of exceeding sugarcane trashes was a common practice, but today much of the trash is used as soil cover and the remaining for cogeneration. With the foreseen second-generation ethanol from lignocellulosics, viability studies will be needed to decide how much trash will be partitioned to cogeneration, fermentation, and soil cover.

Any biomass crop is expected to give the same calorific power when burnt ($\sim$17–21 MJ kg^{-1}) (McKendry 2002). Thus, the possibility of decreasing the calorific energy output in benefit of increasing the yield of fermentable sugars by traditional genetics or molecular breeding aggregates more value to the plant material than increasing calorific energy, since ethanol is used by vehicles and can also be an exportation product. Considering the integration of lignocellulosics into the process chain, fiber content and composition in bagasse and crop trashes (tops and leaves) should receive attention by breeders in order to decrease biomass recalcitrance. Hence, it would be expected that new varieties improved for biomass yield and fiber composition for better saccharification are preferred over cogeneration purpose.

7.3 A Few Considerations on Plant Molecular Physiology Aspects Influencing Biomass Yield and Quality

It is well documented that biomass yield and quality begins to be regulated since the cell cycle (Francis 2011; Ng et al. 2013) until the last steps accomplished by enzymes in the metabolic pathways leading to plant products of interest (Gray et al. 2012). Subtle changes introduced in the final raw material might affect the feasibility to obtain the monomers or polymer of interest. The best known example is how lignin imposes recalcitrance to the cell wall enzymatic degradation (Jung et al. 2013).

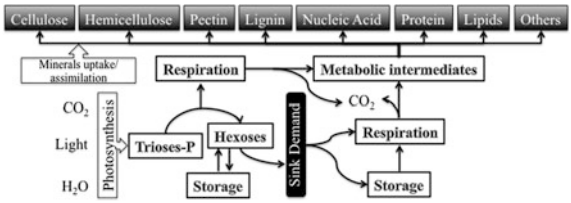

Fig. 7.3 Carbon flux under demand

7.3.1 Carbon Partition and Allocation

7.3.1.1 Sink and Source Relations

The carbon fixed through photosynthesis may flow through primary and secondary plant metabolism. Respiration is responsible for production of energy and metabolic precursors at the expense of high losses of carbon fixed by photosynthesis. The rates by which carbon flows toward the synthesis of different classes of compounds is highly dictated by the sink and source relations (Fig. 7.3).

It is accepted that the increase in sink strength leads to a higher demand for photoassimilates, upregulating the functioning of the photosynthetic machinery (McCormick et al. 2006). For instance, if plants are attacked by herbivores, the remaining leaves seems to compensate for the loss of photosynthetic area by improving the functional efficiency of photosynthesis in the remaining leaves (Thomson et al. 2003). Under normal developmental conditions, the cell proliferation and expansion in active growing regions must be accompanied with higher C inputs provided by source leaves through phloem. Apical regions of root and stems, sprouts, sugar loading on storage tissues, seeds, and grains are some examples of high C demanding organs.

In sugarcane, invertase activity is highly required by the young developing internodes, which exhibits a high C turnover for the synthesis of metabolic intermediates (Rae et al. 2005; Rose and Botha 2000; Whittaker and Botha 1997). During the internode elongation, a high sink strength mediated by invertase, which can convert sucrose into glucose and fructose, is established in these tissues, favoring for sucrose delivery in the young expanding internode cells (Rose and Botha 2000). On this agreement, metabolomic studies have shown that as the internodes elongate (become older), invertase activity and hexose accumulation decreases, favoring the increase in sucrose content (storage) in culm parenchyma, which assumes a sucrose storage function (de carli Poelking 2012). Sugarcane can store up to 25 % of its fresh weight as sucrose in parenchyma (Moore and Maretzki 1996).

In sugarcane, the mechanism of sucrose phloem unloading to the vascular parenchyma is not well understood. In the sugarcane stem, the vascular bundles are surrounded by a layer of cells that become lignified as the internodes mature (de carli Poelking 2012). This lignification forms a barrier that difficults the apoplastic mechanism for the phloem unloading (Jacobsen et al. 1992), if only the apoplastic pathway is used. A sucrose transporter in sugarcane (ShSUT1) with homology to

the SUT/SUC family of plant sucrose transporters was identified (Rae et al. 2004). *ShSUT1* was expressed predominantly in mature leaves that were exporting sucrose and in stem internodes actively accumulating sucrose. They also found that a simplastic tracer dye can move from phloem into the vascular parenchyma cells and then, through the first lignified cell layer of the parenchyma cells which surround the vascular bundle. This suggests that sucrose may be able to enter the storage parenchyma through symplastic connections.

The downregulation of pyrophosphate: fructose 6-phosphate 1-phosphotransferase (PFP), a key enzyme in the primary C metabolism operating at the glycolysis level, led to increased sucrose content in immature internodes (Groenewald and Botha 2007). These data confirm that respiratory activity is intense in immature internodes, and that the impairment in the conversion of the fructose 6-phosphate and pyrophosphate (PPi) into fructose 1,6-bisphosphate plus inorganic phosphate (Pi) bottlenecks the C flow through respiration, promoting sucrose accumulation. Interestingly, these authors also reported that some field grown transgenic lines had high fiber content. This is an evidence that it is possible to manipulate the C metabolism toward high sucrose and fiber content, creating mixed-purpose sugarcanes that could produce juice for sugar and 1G ethanol and fiber for 2G ethanol production. However, these authors did not report whether the transgenic lines were more susceptible to diseases, since a higher sucrose content would also mean more resources for plant pathogens.

7.3.1.2 Tillering Response

When sugarcane is cultivated for high sucrose yield purposes, the number of culms per cultivated area is often associated to cane yield and used as a criteria for the sugarcane payment. In a work accessing the inheritance of yield-related traits in sugarcane, 227 individuals from a cross between the Australian variety Q165 and a *Saccharum officinarum* accession were evaluated during three years for stalk weight, stalk diameter, stalk number, stalk length, and total biomass (Aitken et al. 2008). In this work, the authors mapped two alleles of a candidate gene showing homology with the *teosinte branched 1* (*tb1*) gene from maize. This gene in maize has a prominent role in regulating branching, although the authors reported a minor effect in sugarcane (Aitken et al. 2008).

The *teosinte branched 1* (*tb1*) gene belongs to the TCP gene family. The members of this family encode putative basic helix-loop-helix DNA-binding proteins that may play a role in organ growth. The *tb1* related genes may encode negative regulators of branching and their function has been recently investigated in rice tillering. The overexpression or RNAi suppression of a maize *tb1* gene in rice plants reduced or increased, respectively, the number of tillers and panicles of transgenic rice (Choi et al. 2012). This effect was less pronounced for plants growing in paddy fields than for those growing in greenhouse, suggesting that environment also plays a role in controlling this trait.

Tillering involves perception and transduction of environmental clues by plant hormones. Strigolactone is a class of carotenoid derived plant hormones participating in the control of tillering in monocots and dicots (Shinohara et al. 2013). Besides controlling branching, strigolactones are also involved in processes like root branching, hyphae branching in arbuscular mycorrhiza, and seed germination of parasitic weed by the exudates of the host plant roots (Matusova et al. 2005).

Tillering may change the sink/source relation in plants. An evidence that tillering imposes a high sink strength in plant development is that in barley, cyclic crossing and selections led to high head numbers and tiller mortality, low kernels numbers per head, low kernel weight, and high susceptibility to lodging (Benbelkacem et al. 1984). Thus, tillering may exert negative impacts in grains productivity (reproductive development) due to sinks competition, mainly if branching occurs during seed filling. In this circumstances, neither reproductive nor vegetative development can be supported by adequate water and nutrient supply. In the case of sugarcane, the number of culms (vegetative development) against low flowering and grain production (reproductive development) is a desirable trait to be selected by breeding or produced by genetic engineering.

The tillering intensity in sugarcane is variable. In order to increase the number of culms in sugarcane, induction of sprouting in the initial phases of the plant development has been recommended (Silva et al. 2007). The highest number of tillers (10–20) may occur after 4 months of cultivation and then decrease as a result of probably tillers competition for resources, such as water, light, and nutrients (Castro and Christoffoleti 2005). Limited resources may cause some tillers to abort with possible increase of the diameter of the remaining ones. Increased stalk diameter suggests resources availability for the crop. Therefore, increased tillering may also need to be supported by better crop management practices.

In the ratoon crop, sprouting is highly dependent on water availability and drought stress greatly impacts the success of the culture in ratoons. Design of canes tolerating large variations in water soil potential, for regions experiencing remarkably drought in defined seasons is an important strategy to increase cane yields.

Despite tillering being an important component of yield, high plant densities may inhibit tillering by the initiation of a shading avoidance response, mediated by phytochromes, particularly the PHY B type, which senses the decrease of red:far-red light ratio in the transmitted light under shading condition. Under shading, branching is inhibited, plants grow taller, produce less tillers and reproductive structures, leaf expansion is inhibited and higher mortality of young vegetative tillers may also be found (Casal et al. 1986). The signaling pathway by which strigolactones operates in tillering or shading avoidance responses seems to be down stream of the PHY B light quality perception and probably crosstalks with auxin signaling pathways to control the developmental responses (Brewer et al. 2013).

7.3.1.3 Lignin Accumulation

Ligning is a complex polymer from the plant secondary metabolism and plays an important role in the plant growth and development. It is the second most abundant polymer on the earth, after celulose. About 30 % of atmospheric CO_2 is fixed as lignin (Boerjan et al. 2003) which constitutes 10–40 % of the total plant dry matter (Sederoff et al. 1999).

Lignin biosynthesis is a considerable sink of carbon fixed by plants. Since plants cannot degrade it to recover the carbon invested in its synthesis, there might be a fine control of spatial and temporal lignin deposition in the cell wall (Rogers et al. 2005).

Lignin biosynthesis is influenced either by internal (genetics and physiology) or environmental factors. Environmental stimuli and developmental cues regulate the carbon flux toward lignin, and this is also accomplished by a complex network of tissue-specific transcriptional factors—TFs (Rogers and Campbel 2004; Bonawitz and Chapple 2010). The identification of regulatory cis-elements targeted by different TF's families in the promoter region of many genes from the monolignol biosynthesis pathways is helping to understand the transcriptional regulation of lignin genes (Zhao and Dixon 2011).

Conserved motifs, such as AC elements, which are targeted by MYB TF's are ubiquitous to regulatory region of genes encoding enzymes participating in the phenylpropanoid and monolignol biosynthesis (Bugos et al. 1991; Sablowski et al. 1994). MYB recognized elements are present in the promoter of *PAL, 4CL, C3H, CCoAMT, CCR, C4H, COMT,* and *CAD* genes (Zhou et. al. 2009; Zhao and Dixon 2011) and can regulate the lignin genes in a positive or negative manner. Lignification must also be controlled in a tissue-specific manner in order to avoid improper lignin deposition. Repressors of lignin biosynthesis genes should work in this occasions. For instance, AtMYB32 and KNOX are well known TFs repressing lignin accumulation. AtMYB32 is highly expressed in flowers but not in lignified tissues, whereas KNOX keeps the meristematic cells in shoot apical meristem in an undifferentiated and unlignified state (Tsiantis et al. 1999; Zhao and Dixon 2011). Conversely, the lignin deposition may occur through the inactivation of repressors instead the induction of activators, as exemplified for PAL, CCR, and F5H, which are not usually expressed in epidermis, but under attack of pathogens, their transcripts substantially increases (Bhuiyan et al. 2009).

TFs have a central role in the transduction of plant intrinsic signals leading to alterations in the metabolism of lignin. However, the post-translational regulation of these proteins and their possible interacting partners are still not well known. Moreover, lignin biosynthesis related TF's might also crosstalk with hormonal pathways, as exemplified by *AtMYB*32, which is strongly activated after auxin treatment (Preston et al. 2004).

The potential of general regulators of lignin biosynthesis might be exploited in order to reduce biomass recalcitrance, but selective downregulation of lignin biosynthesis genes should be preferred since many developmental traits may be affected by general regulators due to lignin biosynthesis network crosstalks with

hormonal signaling, which controls many aspects of plant development. For instance, the down-regulation of the enzyme hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase (HCT) in *Arabidopsis thaliana* and *Medicago sativa* significantly reduced lignin levels and cell wall recalcitrance to saccharification, but also impacted plant growth due to enhanced accumulation of salicilic acid (Gallego-Giraldo et al. 2011). Salicilic acid content increased conversely to lignin content in *HCT*-down-regulated *M. sativa.* Also, other lignin metabolism related genes were down-regulated, exemplifying that a single gene alteration can exert a great impact in the metabolism due to the interconnected signaling pathways (Lee et al. 2011).

Many genes involved in lignin biosynthesis are under light, circadian cycle, and sugar levels control. It has been shown that *C4H*, *COMT*, *CCoAOMT* and *CCR* expression oscillates according to the circadian clock (Rogers et al. 2005). Since carbon fluxes also exhibit oscillation—starch is usually accumulated during the day and hydrolyzed at night—there might be a control through sugar signaling and circadian rhythm to optimize the metabolic fluxes of C toward different pathways (Rogers et al. 2005; Zhao and Dixon 2011). Then, a question arises: Which environment diverts C toward phenolics or carbohydrates? Stresses are a driving force guiding the plant metabolism to surveillance. Light, temperature, and water stresses are a few examples of conditions that can saturate the photosynthetic electron transfer chain leading to oxidative stress. Plants are usually adapted to a range of environmental conditions. Under acclimated condition, the metabolism can make its shifts properly. However, plants usually dispose of more energy than they, in fact, can use in the environment and the synthesis of aromatic compounds such as lignin and flavonoids may serve as a protective mechanism to channel the excess of reducing power in the photosynthetic electron transport chains toward the high energy-consuming phenylpropanoid biosynthesis. Aromatic phenolics exhibit a remarkably abundance of Pi electrons involved in aromaticity, conferring them a high reductional state and thus, a remarkably antioxidant characteristic. Stoichiometry analysis of mass-energy equivalence confirms that in order to produce 1 g of lignin, 2.7–3.0 g of glucose may be required, since lignin has 30 % more energy than carbohydrates, in average (Novaes et al. 2010).

The accumulation of phenolics is a typical response to elevated visible irradiance or UV-A/B in the environment (Guo et al. 2008; Matus et al. 2009; Shin et al. 2007). Under clear skies, the abundance of visible and UV light is high and plants need to protect themselves. UV-B perception has always been a puzzle in the field of plant photomorphogenesis until the identification of a plant UV-B receptor in Arabidopsis (Rizzini et al. 2011). The characterization of UVR8 impaired plants has shown that UVR8 is involved in the regulation of a set of photomorphogenic responses, including: inhibition of hypocotyl elongation, leaf expansion regulation, stomatal differentiation, and accumulation of phenolics (Morales et al. 2013; Wargent et al. 2009). UVR8 has been implicated in the regulation of a set of genes involved in protection against oxidative stress and hormone signaling. Interestingly, PAL—the key enzyme of the phenylpropanoid pathway entrance, was positively regulated by UV-B light in an UVR8-dependent manner. Thus, the

regulation of PAL encoding genes is an evidence that lignin and other phenylpropanoids synthesis are much influenced by environmental factors such as light and injuries caused by mechanical stress and pathogens (Rogers and Campbel 2004). Not surprisingly, sites of pathogen penetration may exhibit accumulation of phytoalexins.

Since PAL is the first enzyme of the phenylpropanoid pathway, it drives the C metabolism to the synthesis of these compounds (Sewalt et al. 1997). Concomitantly, C must also be diverted toward cellulose and hemicellulose synthesis. However, the precise control levels by which these shifts operate are not thoroughly understood. At least in Arabidopsis, the biogenesis of secondary wall shares a common TF network that also regulate lignin biosynthesis genes. The NAC, SND1, with their homologs—NST1, NST2, VND6, and VND7 belong to this network and may regulate cellulose, xylan, and lignin biosynthesis related genes (Zhong and Ye 2009). SND1 and NST1 double knockout lines exhibited a complete loss of secondary wall thickening, suggesting their relation with multiple cell wall components biosynthesis (Zhong and Ye 2009; Boejan et al. 2010). Moreover, it has been proposed that SND1 and MYB46 are on top of the signaling cascade controlling lignin, cellulose, and xylan deposition (Zhong and Ye 2009; Zhao and Dixon 2011).

There are evidences that the flux toward secondary metabolism might be limited by the primary metabolism precursors. An evidence is that the Arabidopsis mutant *sex*1 is defective in starch degradation and as a consequence, many genes involved in the monolignol biosynthesis exhibit low transcript levels and the plants accumulate less lignin (Rogers et al. 2005). When grown in dark with supplemented sucrose, lignin accumulation is restored in *sex*1. This finding supports the idea that sucrose availability through starch degradation may stimulate lignin accumulation.

When starch is normally degraded but cellulose synthesis is impaired, then lignification can be invoked, including defense responses, as reported by (Delgado et al. 2003). On this agreement, defense responses seem to be activated in detriment of energy invested in vegetative growth. The competition of C partitioning toward lignin or polysaccharides in plants have been confirmed by direct and forward genetics. A *Pinus taeda* CAD mutant exhibited negative correlation between biomass yield and lignin content. Down-regulated 4CL plants also exhibited lignin reduction, which was counterbalanced by increase in cellulose and hemicellulose contents, on agreement with a reduced C flux toward lignin might be compensated by cellulose and hemicellulose accumulation (Hu et al. 1999). Therefore, the carbon flux toward cellulose and hemicellulose often correlates with growth and yield while lignin deposition marks the cessation of cell growth and proliferation.

The lignin profile has been associated with its reactivity. In the Kraft process, the noncondensed ether bonds (β-O-4-) are more amenable to the delignification, whereas C–C bonds (β-β, β-1, β-5 e 5-5) are more resistant to chemical degradation. Modifications of key steps of the lignin metabolism may render plants less recalcitrant for pulping and saccharification purposes (Baucher et al. 1998,

Lapierre et al. 2000, Baucher et al. 2003). The Shikimate pathway is the beginning of phenylpropanoid biosynthesis (Schmid and Amrhein 1995). This pathway provides prephenate, which is formed by the combination of phosphoenolpiruvate (PEP), produced by glycolysis, and erytrose-4-phosphate, a precursor derived from the Calvin-Benson cycle or pentose phosphate oxidative pathway—PPOP (Amthor 2003). The synthesis of monolignols takes place after the phenylalanine deamination, successive hydroxylations, and o-methylations, that modify the aromatic ring of the cinnamic acids produced. The latter steps reduce the cinnamic acids into the monolignols, which are then incorporated into lignin. The *p*-coumaril, coniferil, and synapil alcohols are referred as H, G, and S type lignin, respectively. The relative amount of H, G, and S units is temporally and tissue-specific controlled and may also receive influence of the environment (Boerjan et al. 2003; Bonawitz and Chapple 2010). However, only minor qualitative differences in H, G, and S composition were detected in bagasse from different varieties of sugarcane bagasse (Lopes et al. 2011).

Lignin polymer is formed by the action of many enzymes leading to the sequential deposition of *p*-hydroxyphenil (H), guayacil (G), and then syringil (S) (Boerjan et al. 2003; Donaldson 2001). Transgenic plants down-regulated for single genes of the lignin biosynthetic pathway exhibited phenotypical alterations that allowed for the functional characterization of the particular lignin related genes in the plant growth and development (Table 7.1).

As an example of the potential manipulation of these enzymes, in a recent study, transgenic sugarcane expressing low levels of COMT exhibited increased yield of fermentable sugars with much less enzyme loads (Jung et al. 2013). In the same work, authors reported that reduction of lignin content by 6 % improved saccharification efficiency by 19–23 % without significant changes in agronomic traits, such as plant height, tillering, brix, or stalk diameter. Nevertheless, lignin decreases of above 8 % increased saccharification in more than 28 %, but impacted biomass yield. Combining metabolism manipulations could alleviate the negative side effects due to drastic lignin reductions through other compensatory effects.

7.3.2 The Water Status

Stomatal responses to root water status is triggered by the transmission of a chemical signal in xylem sap, suggesting that root water status directly affects CO_2 assimilation. In sugarcane, pots, and field grown crops exhibited stomatal and root hydraulic conductance correlation result in homeostatic regulation of leaf water potential (Smith et al. 2005).

Water deficit negatively affects the cell expansion and, therefore, cell proliferation and biomass yield. In addition, all the basic physiological processes are affected. The turgor pressure governs the cell expansion process as expressed by the growth rate equation:

Table 7.1 Effects of downregulation of phenylpropanoid genes on lignin content and profile

Enzyme	Impact on lignin profile	References
PAL—phenylalanine ammonia-lyase	The low expression of PAL decreases the G unit content and the overall lignin content	(Boerjan et al. 2003)
C4H—cinnamate 4-hydroxylase	The low expression of C4H decreases the S unit content in lignin and the overall lignin content	(Boerjan et al. 2003)
COMT—caffeic acid/5-hydroxyferulic acid 3/5-O-methyltransferase	The down-regulation of COMT decreases the lignin content up to 30 % in some plants. S lignin type is reduced and occurs incorporation of 5-hydroxyconiferyl alcohol into lignin	(Zhong et al. 2008)
CCoAOMT—caffeoyl CoA O-methyltransferase	Reduced CCoAOMT activity results in lower content of lignin and increased S/G ratio due to reduction of G units	(Parvathi et al. 2001)
F5H (FAH-1)—coniferaldehyde/ferulic acid 5-hydroxylase	Arabidopsis F5H mutants have depletion of S lignin, with concomitant presence of dibenzodioxins and phenylcoumaran. The overexpression of F5H in Arabidopsis markedly increases S lignin content	(Franke et al. 2000)
4CL—4-coumarate: CoA ligase	The down-regulation of the 4CL in some plants reduces lignin content and increases the cell wall-bound hydroxycinnamic acids	(Boerjan et al. 2003)
CCR—cinnamoyl-CoA reductase	CCR down-regulated plants exhibit increase in the S/G ratio due to a decrease in G units. However, the lignin becomes more condensed. In Arabidopsis, CCR1 mutants are dwarf and exhibit collapsed xylem due to reduced lignin content in the cell wall	(Boerjan et al. 2003; Ruel et al. 2009)
CAD—Cinnamyl alcohol dehydrogenase	CAD deficient plants exhibit minor changes in lignin content. In tobacco and poplar, the down-regulation of CAD leads to the incorporation of sinapaldehyde and coniferaldehyde into the lignin polymer	(Boerjan et al. 2003; Ralph et al. 2001)

$$GR = m(\psi_P - \Upsilon).$$

GR is the growth rate, *m* is the cell wall extensibility, ψ_P is the turgor pressure (the pressure that the wall exerts upon the symplast) and Υ is the yield threshold (the pressure value in which the cell wall resists to plastic deformation). If $\psi_P = \Upsilon$, then $GR = 0$. Under normal hydration conditions, ψ_P is slightly higher than Υ (0.1–0.2 MPa), that means the cell expansion may be affected by even low decreases in water content. Besides affecting the ψ_P, the soil water deficit also causes structural changes in the wall, since the xylem sap becomes more alkaline. Wall extensibility coefficient (*m*) decreases due to the alkalinity of the apoplastic

fluid, which inhibits the activation of the expansions, whose activity is required for the acidic growth mechanism mediated by auxins. Another possible explanation for the decrease of expansibility, and thus growth rate (*GR*) under long-term water deficit condition is that Υ may also be affected due to biochemical changes in the cell wall that may not be easily reverted if (Radin et al. 2010).

7.3.3 The Important Role of Roots

Much attention is given to the improvement in harvestable aerial plant organs. The ability to better explore the soil for water and minerals, properly responding to stress signals, will make the whole plant perform better in the environment. Roots are strong carbohydrate sinks in the plants, which respond to a variety of stresses. In drought stressed roots of sunflower, decrease in respiration rates were detected (Burton et al. 1998; Hall et al. 1990). On the other hand, during grain filling, root respiration rates increase significantly (Hall et al. 1990) in order to support the aerial parts with water, minerals, and hormones. Therefore, the impact that drought causes in root respiration rates also down-regulate photosynthesis. On the other hand, it has been recently reported that salt stressed rice roots increased the cyanide-resistant respiration, through alternative oxidase (AOX), which probably mediated cell death in the tissues (Feng et al. 2013).

In sugarcane, the root system is continuously renewed, since the older roots lose their absorptive function and die by a mechanism still unknown. The absorptive function is assumed by the new roots produced. The ratoon is known to be sensible to water deficit, probably due to inhibition of aerobic respiration, responsible for the turnover of carbon stored in culms for the new sprouts. In such situation the root system may also play an important role for the success of the ratooning.

Root performance should be addressed to improve the use of soil available resources and increase aboveground biomass. It is possible that improvements in yield through breeding have come at the expense of roots performance for water and nutrient uptake (Smith et al. 2005). To investigate this, old and new cultivars should be revisited in order to address this question.

In the biorefinary scenario, a potential use of roots as bio-factory is discussed by (Skarjinskaia et al. 2013). Recombinant proteins, such as vaccines, could be produced in the hairy root system of edible plants for biomedical and pharmaceutical applications. If therapeutical molecules are produced this way, there will be no cost with expensive protein extractions and purifications and the biomolecule could be kept active improving its recovery. The plant cell wall would retain and gradually release the bioactive substances as the plant tissues goes through the gastrointestinal tract.

7.4 Final Considerations

Biorefinery is a broad and recent concept that complies the appeal for a cleaner and sustainable way to produce energy and commodities from biomass, although it still must find its ways to be cost competitive.

The transition of the current oil refineries to the total "green" refineries is challenging and may not be implemented in the short term. It is expected that integration of biomass and petrochemical platforms could allow for the use of basic building blocks, more flexibility, and initial cost reduction. In Brazil, the flex fuel car is an example of adaptation of platforms to a transition market demanding alternative fuels.

The 2G ethanol technology still faces the challenging task to be efficient, cheap, and clean and the hardest mission is to efficiently deconstruct the lignocellulosic biomass. It is accepted that the achievement of higher yields of fermentable sugars might occur through the route of reduction of biomass recalcitrance by genetic breeding and transgenics, allied to the use of recombinant microbial enzymes mix cheaply produced. Associated with less aggressive biomass pretreatments, these approaches would allow for higher recovery of fermentable sugars and enzymes.

In spite of being a prominent energy crop, faster advances in sugarcane genetic breeding is hampered by a high ploidy and complex genome structure. Molecular breeding is thus expected to speed up the generation and release of new lines. How then should the future cane be? There are speculations that breeding for sucrose decreases fiber content and vice versa. However, recent experiments have shown that either directions are possible but not mutually exclusive. Regarding the still little exploited sugarcane genetics and molecular physiology, it is not risky to say that *Saccharum* genus still reserves a huge breeding potential. An exciting time for biorefineries is emerging as new findings and concepts in the field of "green" and "white" technologies arise.

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Chapter 8
The Essential Role of Plant Cell Wall Degrading Enzymes in the Success of Biorefineries: Current Status and Future Challenges

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Abstract The viability of cellulosic ethanol depends on the optimal use of biomass component through the biorefinery concept and this requires the integration of unit operations that are involved in the production of fuel and chemicals. In this regard, enzymes are important tools to improve the efficiency and sustainability of a biorefinery process. Therefore, a comprehensive approach and full understanding of the structure and function relationships that are involved in the enzymatic hydrolysis of lignocellulosic materials is a fundamental step toward the optimization of these bioconversion processes.

8.1 Introduction

The viability of cellulosic ethanol depends on the optimal use of biomass component through the biorefinery concept and this requires the integration of unit operations that are involved in the production of fuel and chemicals. In this regard, enzymes are important tools to improve the efficiency and sustainability of a biorefinery process. Therefore, a comprehensive approach and full understanding of the structure and function relationships that are involved in the enzymatic hydrolysis of lignocellulosic materials is a fundamental step toward the optimization of these bioconversion processes.

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Biomass conversion may be performed by chemical or biochemical routes. For many reasons ranging from process efficiency to environmental issues, most of these pathways are ideally performed by biochemical catalysts (enzymes) such as polysaccharide hydrolases (Mussatto et al. 2010). However, the relatively high cost of enzymes and the complexity of carrying out enzymatic hydrolysis in large scale are still limiting the implementation of biorefineries for fuels and chemicals based on lignocellulosic materials. Besides glycoside hydrolases, it is also widely known that other enzymes have an important role in the deconstruction of the plant cell wall. These include oxidases that are involved not only in lignin degradation but also in the chemical modification of carbohydrates. Hence, a full spectrum of enzymes is required to deal with the wide diversity of chemical linkages and chemical environments that are found in the plant cell wall. This chapter attempts to describe the essential role of plant cell wall degrading enzymes in the success of biorefineries, particularly with regard to the use of lignocellulosic materials for fuels and chemicals.

8.2 Plant Cell Wall

The physical and chemical association of the three main components of the plant cell wall, cellulose, hemicelluloses, and lignin, has been the subject of many reviews that are found in the specialized literature (Higuchi 1985; Matthews et al. 2006; Coughlan and Hazlewood 1993). In short, linear chains of β-(1 → 4)-glucans (cellulose) interact with one another by hydrogen bonding to produce well-organized crystalline regions that are regularly interrupted by less-organized or "amorphous" regions in which these chains are more randomly oriented. These ribbons of polysaccharide chains are embedded in a matrix of hemicelluloses and lignin, whose distribution and close association defines the outstanding physical and chemical properties of this natural composite (Fig. 8.1).

A short review of the chemical and structural properties of the main plant cell wall macromolecular components is presented below. However, important but minor components such as pectic materials were not included in this chapter. Details about this class of compounds may be found in reviews that are already available in the literature (Jayani et al. 2005).

8.3 Cellulose

Cellulose is a linear homopolysaccharide composed of anhydro-D-glucopyranose units joined together by β-(1 → 4) glycosidic linkages (Fig. 8.2). The equatorial orientation of the anomeric hydroxyl of the β-D-glucopyranosyl units confers linearity to the cellulose chains, which interact with one another to produce aggregates of great molecular order whose supramolecular structure is

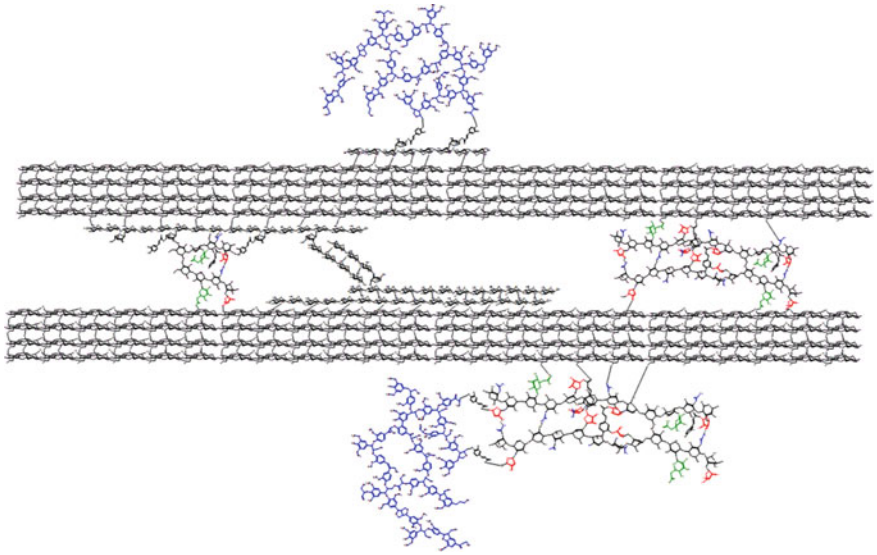

Fig. 8.1 Structural representation of a lignin-carbohydrate complex, in which cellulose and lignin are interconnected by their chemical interaction with arabinoferuloyl xylan units

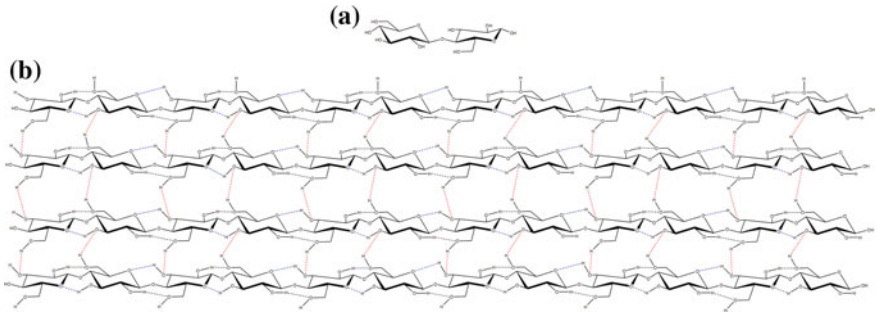

Fig. 8.2 Cellulose structure. **a** Cellobiose unit. **b** Inter- and intramolecular interactions among cellulose chains, with hydrogen bonds (HB) shown in different colors: *red* for intermolecular HB involving C6 and C3; *black* for intermolecular HB between C2 and C6; *blue* for intramolecular HB involving C3 and the hemiacetalic oxygen atom

architecturally stabilized by a network of intra- and intermolecular hydrogen bonding (Zhang and Lynd 2004). As a result, adjacent cellulose chains are held together as flat layers, allowing the more hydrophobic faces of the ribbons to stack (Matthews et al. 2006). However, there is a thermodynamic limit beyond which this molecular order is gradually lost, characterizing a transition to less-organized regions in which the cellulose chains are more randomly oriented (amorphous regions). Hence, depending on its "amorphous character," the whole structure

presents cavities or pores that are able to hold relatively large amounts of water by capillarity (Mihranyan et al. 2004).

Cellulose chains may organize themselves in different ways, forming allomorphs that are known as cellulose I, II, III, or IV. The natural cellulose form is the metastable cellulose I, which contains two coexisting phases, I_α (triclinic) and I_β (monoclinic), and the ratio between them varies depending of its origin, being the type I_α commonly found on algae and bacteria, while type I_β is primarily found in higher plants. The main difference between celluloses I_α and I_β lies on the displacements of the sheets relative to one another and cellulose I_α can be converted to I_β by bending during microfibril formation (Jarvis 2000). For cellulose I_α, the chains are regularly displaced from each other in the same direction, whereas for cellulose I_β, this displacement is found in alternating directions. This difference leads to different water adsorption profiles as well as different chemical accessibilities for conversion (Matthews et al. 2006).

The other cellulose allomorphs are not natural. Cellulose II is generally obtained either by mercerization or by regeneration of cellulose in organic solvents and ionic liquids (Jhonson 1969; Okano and Sarko 1985). Cellulose III can be produced by treatment with liquid ammonia or in the presence of some amines (e.g., ethylene diamine). This way, cellulose III_I derived from cellulose I while cellulose II leads to cellulose III_{II}. Finally, cellulose III can be treated with glycerol at high temperature to produce cellulose IV and, by doing so, cellulose IV_I and IV_{II} can be obtained from cellulose III_I and III_{II}, respectively (Loeb and Segal 1954; Tsuda and Mukoyama 1957).

For many years, cellulosic materials have been extensively studied as a source for ethanol production. In this case, conversion of lignocellulose to fermentable sugars (mostly glucose and xylose) may be carried out by acid or enzymatic hydrolysis (Caes et al. 2013; Yabushita et al. 2013). However, the use of acid hydrolysis may lead to lower sugar yields due to the use of more drastic reaction conditions, in which an array of both hydrolysis and fermentation inhibitors are usually produced (Ramos 2003). Due to its higher specificity and lower environmental impact the enzymatic hydrolysis of cellulose has received much more attention from the international scientific community as well as from the industry.

The enzymatic conversion of cellulose to glucose is primarily performed by the synergic/concerted action of three main classes of hydrolases, which are usually referred to as the cellulolytic complex or cellulases: endo-β-(1 → 4)-glucanases (EC 3.2.1.4) (EnG), exo-β-(1 → 4)-glucanases (EC 3.2.1.91) (ExG), and β-(1 → 4)-glucosidases (EC 3.2.1.21) (βG). Many EnG and ExG enzymes are able to adsorb on the substrate surface through a carbohydrate-binding module (CBM), which is connected to the catalytic domain by a linker peptide (Notenboom et al. 2001). Several researches have shown that CBMs increase the performance of cellulases and other hydrolases. The role of CBM in hydrolysis was recently shown by Várnai et al. (2013). These authors were able to show that more than 60 % of cellulase genes do not have a CBM or any alternative protein structure linked to them (dockerins) (Várnai et al. 2013). Furthermore, the effect of CBM was more pronounced at low total solids (1 wt%, dry basis), being more important

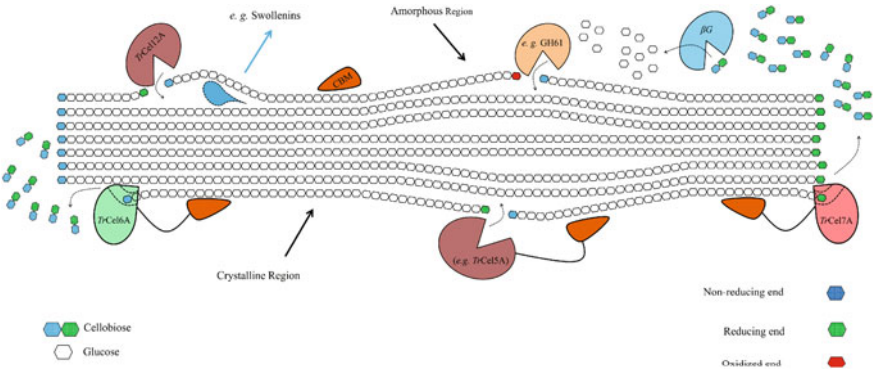

Fig. 8.3 Hydrolytic and nonhydrolytic enzymes on the synergic action of the cellulose conversion

for ExGs than EnGs of *Trichoderma reesei*. The results suggest that CBMs would not be required at high total solids because these conditions would already promote enough enzyme-to-substrate interactions for hydrolysis to occur.

EnG enzymes have a catalytic domain with a cleft shape active site that is able to break down glycosidic bonds along the cellulose chain, acting mainly at the less-organized "amorphous" regions (Rabinovich et al. 2002). This reaction leads to the formation of two new chain ends triggering off the so-called endo-exo synergism. ExG enzymes have a tunnel-shaped catalytic site through which the cellulose chains must penetrate prior to eliciting its catalytic activity, releasing mostly cellobiose. These enzymes need to adsorb on to the cellulose surface in order to facilitate this process (Beckham et al. 2010). Once captured by cellulase enzymes, the cellulose chain is forced to unglue/unbind from the surface and its gradual solubilization starts processively by ExG enzymes. Finally, cellobiose and other low molecular mass oligomers are converted to glucose by the action of βG enzymes. Figure 8.3 shows a pictorial representation of the enzymatic hydrolysis of cellulose.

T. reesei is the most widely studied organism for the production of cellulases. Wild-type *T. reesei* strains are able to secret at least four EnGs (*Tr*Cel5A, *Tr*Cel12A, *Tr*Cel7B *Tr*Cel45A), two ExGs (*Tr*Cel7A, *Tr*Cel6A), at least one xyloglucanase (*Tr*Cel74A, with EnG activity), and several βGs (Foreman et al. 2013). However, it is known that *Tr*Cel7A, *Tr*Cel6A, and *Tr*Cel5A are the predominant enzymes in the enzymatic pools of *T. reesei* (Nidetzky and Claeyssens 1994). Therefore, considering that the expression levels of βG by *T. reesei* are enough for the growing cells but insufficient for industrial applications, enzymes from other fungi such as *Aspergillus* spp. must be used to supplement this enzyme component. In addition, besides being more tolerant to end-product inhibition, the βG enzymes from *Aspergillus* spp. are able to act not only on cellooligosaccharides (COS) but also on insoluble COS with an average degree of polymerization of 20 (Sakamoto et al. 1985).

TrCel7B is the major endo-acting enzyme from *T. reesei*, showing 6–10 % of its total cellulase production (Ståhlberg 1991; Nidetzky and Claeyssens 1994). *Tr*Cel7B has been reported as catalytically active on both soluble (modified cellulose such as CMC) and insoluble cellulosic substrates as well as on xylans and glucomannans (Shoemaker et al. 1983). On the other hand, the TrCel5A is not able to act on xylans but it is also active on soluble and insoluble cellulosic substrates including mannans (Henrissat et al. 1985; Macarron et al. 1996; Karlsson et al. 2002). Unlikely the major EGs from *T. reesei*, minor enzyme components, such as *Tr*Cel12A and *Tr*Cel45A, can also act on both soluble and insoluble substrates including glucomannans.

Other cellulolytic enzyme systems have been investigated in their performance to hydrolyze cellulosic substrates, such as the proteome of *Neurospora crassa* (Phillips et al. 2011), *Penicillium* cellulases (Marjamaa et al. 2013), Cel7A proteins from different thermophilic fungi (Voutilainen et al. 2008) and several EnG enzymes from GH families 5, 6, 7, 9, 12, and 45 (Vlasenko et al. 2010), among others. A thorough description about fungal enzymes that are able to degrade lignocellulosic materials can be found elsewhere (Dashtban et al. 2009).

Cellulolytic enzymes represent one of the most important enzymes for the development of biorefineries. However, ancillary proteins have also been identified as important auxiliary tools to achieve high conversion rates in cellulose saccharification (Arantes and Saddler 2010; Ekwe et al. 2013) such as expansins, swollenins, and lytic polysaccharide monooxygenases (LPMO). Cellulose binding proteins can promote the deagglomeration of the cellulose chains at crystalline regions causing amorphogenesis and this seems to be a critical step toward the development of high accessibilities (Din et al. 1991; Chen et al. 2010). Interestingly, Reese et al. (1950) suggested about 60 years ago that cellulolytic enzymes may require the action of nonhydrolytic proteins in order to promote the disruption of the substrate polymer packing.

The presence of expansins in plant tissues have been originally described by Cosgrove and co-workers (Cosgrove 1999; Cosgrove 2000a, b). Expansins are proteins of 25–27 kDa of molecular mass and their mechanism of action consists on break the noncovalent bonds between cell wall polysaccharides, thereby inducing the plant cell wall extension and swelling (Cosgrove 2000a; Lee et al. 2001). Also, Yuan et al. (2001) proposed that some cellulases such as TrCel12 may have expansin-like properties in addition to its hydrolytic activity.

Likewise expansins, swollenins can also break down the physical interactions among cellulose chains. Jäger et al. (2011) expressed the *T. reesei* swollenin protein in a recombinant *Kluyveromyces lactis* strain and studied the effect of this recombinant swollenin on cellulosic substrates. In general, treatment with swollenin led to a decrease in both substrate particle size and crystallinity while increasing the extent of cellulase adsorption on cellulose. As a result, high cellulose hydrolysis rates were obtained. Gourlay et al. (2013) showed that *T. reesei* swollenin affected especially xylan of pretreated corn stover substrate, enhancing the production of sugars in hydrolysis. More recently, Kang et al. (2013) characterized a novel recombinant swollenin from *Penicillium oxalicum* with regard to

its ability to facilitate cellulose hydrolysis. This new swollenin consists of a family 1 CBM connected to a family 45 endoglucanase-like domain by a linker.

In 2005, Vaaje-Kolstad et al. (2005) identified a novel bacterium able to secret a chitin binding domain (CBP21) that is able to break down chitin while increasing the substrate accessibility to chitin hydrolases. Based in this, CBP21 was classified as a family 33 carbohydrate-binding module (Cantarel et al. 2009). This study revealed that CBP21 cleaves glycosidic bonds in chitin by oxidation, leading to the generation of a terminal gluconic acid residue and a normal nonreducing chain end. These and other authors have also demonstrated that CBP21 is able to increase the accessibility of cellulose to cellulolytic enzymes (Harris et al. 2010; Eijsink et al. 2008; Vaaje-Kolstad et al. 2005) but the mechanism of CBP21 action was only clarified by Vaaje-Kolstad et al. (2010).

New studies with CelS2, a CBM33 protein from *Streptomyces coelicolor*, showed that it produces aldonic acids on the cellulose surface. Like other oxidative enzymes, CelS2 also depends on the presence of divalent metal ions. Westereng et al. (2011) revealed that these enzymes are copper-dependent monooxygenases. Interestingly, CBM33 was also characterized as a copper-dependent lytic enzyme (Vaaje-Kolstad et al. 2012).

Recently, a new type of fungal protein was discovered and classified as family 61 Glycoside Hydrolases (GH61, LPMO) (Harris et al. 2010; Quinlan et al. 2011; Beeson et al. 2012). Likewise CBP21, this enzyme catalyses the oxidative cleavage of polysaccharides, generating new chain ends while modifying the charge distribution of the cellulosic substrate surface. The activity of these oxidative enzymes depends upon the presence of a divalent metal ions and an electron donor. Also, unlike ExG enzymes, their activity on crystalline cellulose does not require the pull-out of a cellulose chain from the surface of the crystalline matrix (Vaaje-Kolstad et al. 2010). These and other authors have shown that oxidative enzymes such as those belonging to LPMO and which are abundant in fungal genomes increase the rate of conversion of cellulosic materials by enzymatic hydrolysis. Figure 8.4 shows one hypothesis for the action of LPMO. Oxidized cellulose chain ends are partially converted to aldonic acid and this highly solvated-opened structure forces these chains to pull out from the surface, leading to a gradual disaggregation of the cellulose structure and to an increase in the availability of new reaction sites for both ExG and EnG.

Anaerobic microorganisms are also able to produce multi-enzymatic complexes called cellulosomes that are able to deconstruct the structural organization of plant polysaccharides (Fontes and Gilbert 2010). In this system, several types of cellulolytic and hemicellulolytic enzymes are assembled in scaffolding subunits that are connected to the whole cell by protein-to-protein noncovalent interactions involving docking and anchoring protein models that are referred to as docherins and cohesins, respectively (Bayer et al. 1994). Like most fungal cellulases, the cellulosome systems have a CBM in mainly their anchoring protein in order to bind to the cellulose surface (Bayer et al. 1994). Furthermore, recently the cellulosomal enzymes had showed synergistic action on the presence of cellulases (Resch et al. 2013).

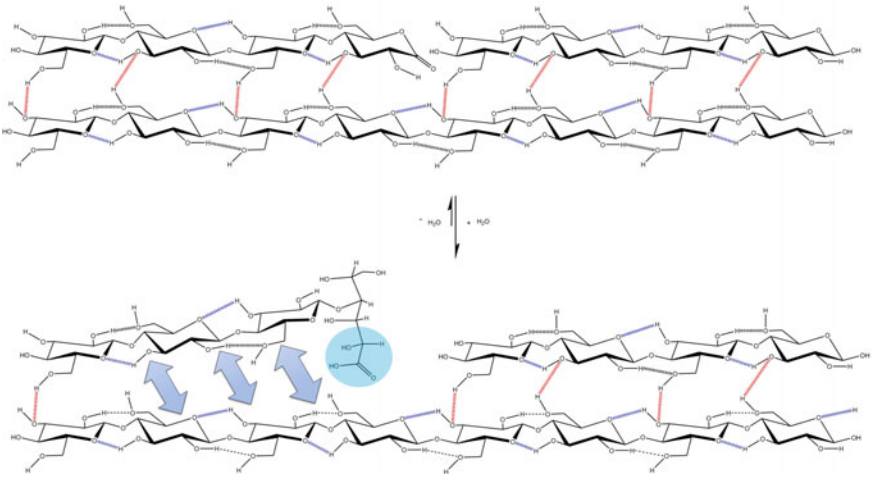

Fig. 8.4 Release of a single chain from the crystalline region after the enzyme-mediated oxidation of cellulose

8.4 Hemicelluloses

Hemicelluloses are heteropolysaccharides that are strongly associated with cellulose by hydrogen bonds as well as van der Waals forces. Their primary structure ranges from linear to highly branched polymeric chains with varying degrees of substitution which, upon acid hydrolysis, may release different types of monosaccharides such as D-mannose, D-galactose, D-xylose, D-glucose, glucuronic acid, 4-*O*-methyl-D-glucuronic acid, and L-arabinose addition of L-rhamnose and D-galacturonic acid present in rhamnogalacturonans of pectin materials (Bon et al. 2008). Compared to cellulose, these heteropolysaccharides have lower thermal and chemical stabilities probably due to their lower crystallinity index and lower degree of polymerization, reasons for what they are much more susceptible to both acid and alkaline hydrolysis (Ramos 2003).

The main hemicellulose components of dicotyledonous angiosperms are xylans and these usually correspond to about 20 wt% of plant dry mass (Singh et al. 2003). However, in monocotyledonous plants, xylans are no more than 2 wt% of plant dry mass. The main backbone of these polysaccharides is composed of anhydro-D-xylopyranosyl residues that are linked together by β-(1 $\rightarrow$ 4) glycosidic bonds in which substituents are usually found such as α-L-arabinofuranosyl, α-D-4-*O*-methylglucuronosyl and *O*-acetyl groups (Sunna and Antranikian 1997). In angiosperms, 10 % of the D-xylopyranosyl residues are substituted on C-2 position by *O*-acetyl (Coughlan and Hazlewood 1993). Figure 8.5a shows a theoretical model of the xylan structure as well as the enzymes involved on its degradation.

Most xylanases are classified in the hydrolase families 10 and 11 (Biely et al. 1997). The main difference between these two families is addressed to their

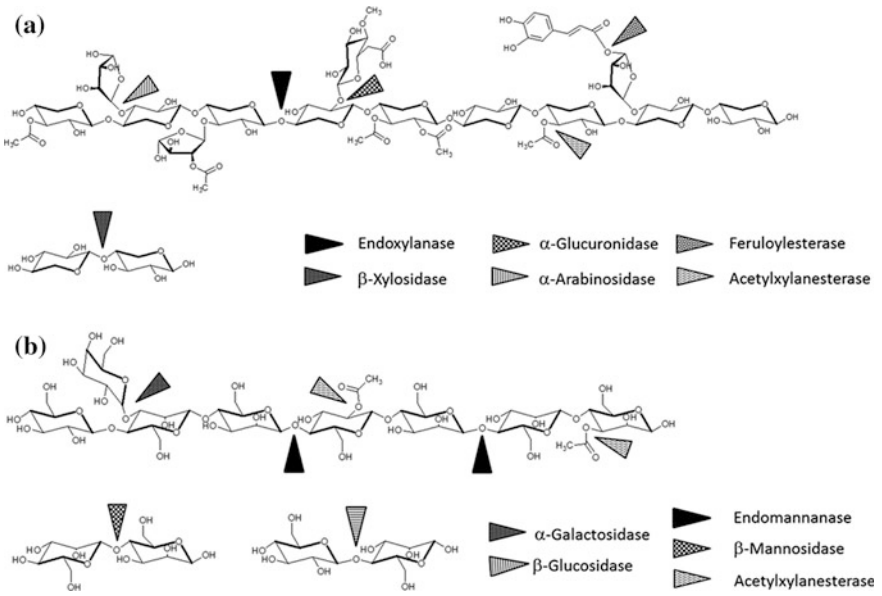

Fig. 8.5 **a** Xylan and **b** glucomannan structures and the main enzymes involved in their enzymatic hydrolysis

catalytic properties. Therefore, they usually display a greater catalytic versatility, particularly in the hydrolysis of highly substituted xylans. Likewise cellulases, some xylanases have a CBM in their structure, bring either a xylan- or a cellulose binding module (Shareck et al. 1991; Sakka et al. 1993).

Generally, endoxylanases act on xylans releasing mainly xylobiose, xylotriose, and branched xylooligomers up until xylopentaose. In addition, most endoxylanases hydrolyze nonsubstituted xylans more efficiently and their tolerance to the presence of side chain varies from one enzyme to another.

Considering the high degree of substitution of xylans, endo-acting enzymes are dominant to the exo-mode. However, although xylans are mainly composed of β-(1 → 4) linkage, some xylanases are able to hydrolyze β-(1 → 3) linkages. Furthermore, exo-acting enzymes show great affinity for polymeric xylan, however, β-xylosidase rather to act on the xylooligosaccharides. As described earlier, glucomannans may be present as one hemicellulose component of the plant cell wall. They have a primary backbone composed of anhydro-D-mannose and anhydro-D-glucose linked together by β-(1 → 4) glycosidic bonds and this may be furnished by side chain groups such as acetyl and anhydro-D-galactosyl groups. Therefore, like other polysaccharides, different enzymes are required for their total hydrolysis. Figure 8.5b shows the theoretical model of a (galacto)glucomannan fragment as well as the enzymes involved on its degradation.

The main backbone of both glucomannans and galactoglucomannans is hydrolyzed primarily by β-(1 → 4)-endomannanases (EC 3.2.1.78). One of the

problems relies on the fact that some mannanases are able to hydrolyze not only the β-(1 → 4) linkage between two mannose residues but also the β-(1 → 4) linkage between glucose and mannose residues (Kusakabe et al. 1988; Tenkanen et al. 1997). Glucomannans are also efficiently hydrolyzed by endoglucanases (Mikkelson et al. 2013).

As the concentration of oligomers builds up as a result of hydrolysis, other enzymes such as β-mannosidase (EC 3.1.1.25) and β-glucosidase assume their role in converting these substrates in the monomeric constituents. These enzymes are able to remove mannose or glucose from the nonreducing end of mannooligomers. Furthermore, *T. reesei* β-xylosidases and *Aspergillus niger* β-mannosidases may also catalyze the removal of xylose and mannose units from the chain ends of xylans and mannans, respectively (Margolles-Clark et al. 1996; Ademark et al. 1999). Also, some endoglucanases are able to hydrolyze not only the internal glycosidic linkages of cellulose but also those found in other polysaccharides such as xyloglucans due to the cleft shape of their catalytic domain. In addition, these enzymes are also able to act on mixed β-(1 → 3, 1 → 4)-glucans.

Endo-(1 → 3)-β-D-glucanases are able to catalyze the hydrolysis of β-(1 → 3) linkages; however, these enzymes show limited activity on the mixed glucans. On the other hand, endo-(1 → 3, 4)-β-D-glucanases are able to hydrolyze both (1 → 3) and (1 → 4) β-linkages. Furthermore, some exo-glycosyl hydrolases are able to cleave β-(1 → 3) linkages in glucans by a processive action from the nonreducing end, releasing glucose as its main end-product.

Figure 8.5 shows the average side groups that have been already found in xylans and glucomannans. Therefore, the enzymes required to unfurnish these polysaccharides are clearly different from those involved in the hydrolysis of the main chain. The main enzymes involved in the removal of these side chains are α-glucuronosidases, α-D-galactosidases, α-arabinofuranosidases, acetyl xylan esterases, and ferulic acid esterases. For instance, α-glucuronosidases (EC 3.2.1.139) carry out the partial hydrolysis of heteroxylans releasing both of glucuronic and 4-*O*-methylglucuronic acid residues.

α-D-Galactosidases has not been as thoroughly studied as other enzymes but their specific activity is critical for the complete hydrolysis of softwood mannans. The role of this enzyme is to catalyze the hydrolysis of α-D-galactosyl side groups that are covalently linked to the O-6 position of the anhydro-D-mannose backbone residues (Puls 1997).

In the case of α-arabinofuranosidases, besides being active on the removal of side chains from xylans, some of these enzymes have been reported as catalytically active in the hydrolysis of pectins, arabinans, and arabinoxylans (Hata et al. 1992; Saha 2000; Ximenes et al. 1996). Besides, these enzymes are particularly important for the deconstruction of the plant cell wall because arabinose units are connected to ferulic acid residues in lignin carbohydrate complexes.

As mentioned earlier, acetyl groups are present in several types of hemicelluloses such as xylans and galactoglucomannans. In hardwood and herbaceous xylans, the level of acetyl groups is much higher than in the case of softwoods. However, acetyl groups can be removed from these polysaccharides by the action

of acetyl xylan esterases (AXEs) and, like other enzymes already described in this work, AXEs' specificity depends on the nature of the substrate and its degree of polymerization. Furthermore, AXEs can also show synergism with other enzymes such as xylanases (Poutanen et al. 1990; Bartolome et al. 1997). For biorefinery processes, the use of AXEs must be carefully planned because the release of acetyl groups from the hemicellulose structure decreases the pH and this may be not favorable to some fermenting microorganisms (de Mancilha and Karim 2003; Martin and Jonsson 2003; Lima et al. 2004).

Non-saccharide side chains can also be found in hemicelluloses, such as in the case of ferulic acid in herbaceous and hardwood xylans. Ferulic acid is normally esterified at the C-2 position of an arabinosyl residue (Fig. 8.5a) and its role is apparently associated to the three-dimensional stability of the polymer network (Mathew and Abraham, 2004). Basically, ferulic acid units may be involved in the crosslinking of adjacent xylan backbones by ether linkages forming diferulate bridges, and may also play an important role in linking hemicelluloses directly to the lignin component (Bartolome et al. 1997) (Fig. 8.1). Ferulic acid esterases (FAEs) are responsible for removing ferulic acid decorations from xylans and some of these enzymes are also effective in releasing coumaric acid from similar chemical environments (Donaghy and McKay 1997). Likewise, some FAEs may differ from each other by the affinity to the substrates that they act upon, either polysaccharides (xylans and pectins) or substituted xylan oligomers (de Vries and Visser 1999). Furthermore, new studies have demonstrated the presence of synergism between xylanases and FAEs, and also an enhanced catalytic activity in FAE/xylanase fusion proteins (Faulds et al. 1995; de Vries et al. 2000; de Vries and Visser 2001; Yu et al. 2003). However, likewise AXEs, the activity of FAEs may lead to the release of aromatic compounds that are inhibitory to fermentation microorganism.

The use of a specific ratio of hollocellulose degrading enzymes, including EnGs, ExGs, βGs, xylanases, β-xylosidases, mannases, and β-mannases, is a critical step toward the completed hydrolysis of lignocellulosic materials and this ratio must be in agreement with the pretreatment technology applied in the process (Várnai et al. 2011). In the case of hemicelluloses, both debranching and depolymeration enzymes are required to improve the extent by which these polysaccharides are hydrolyzed. However, different criteria may apply when the desired products are oligosaccharides with special properties for special uses.

The synergistic action among debranching and depolymeration enzymes with different specificities has already been extensively reported. For instance, the synergism between α-glucuronosidases and endoxylanases in the hydrolysis of wheat xylans led to the highest release of 4-*O*-methylglucuronic acid (de Vries et al. 2000). Therefore, α-arabinofuranosidases can act synergistically with many different enzymes such as xylanases, acetyl xylan esterases, and ferulic acid esterases (Kroon and Williamson 1996; Coutinho and Henrissat 1999; de Vries et al. 2000; Puls 1997; Bachmann and McCarthy 1991).

The factors affecting the performance of hydrolytic enzymes in biorefinery processes are diverse and originate from enzyme characteristic, process conditions,

and substrates. The high catalytic efficiency of individual proteins and optimal ratio of mixture components are the first prerequisites for efficiency. Thermal stability has been shown to be beneficial for enzymes, due to better stability, higher conversion rates, and flexibility in terms of process design (Viikari et al. 2007). Nonproductive adsorption on biomass, especially on lignin reduces the availability of enzymes for hydrolysis, and results in enzyme inactivation especially in high temperature (Rahikainen et al. 2011). Enzyme inhibition has been extensively studied, and it can be caused by several compounds, such as sugars and oligosaccharides, various chemical compounds being often degradation products of biomass, and also by each other on biomass surfaces. The inhibitory environment can be improved by milder pretreatment conditions and by intelligent design of the process. The behavior of enzymes in high dry matter conditions, applied in industrial conditions differ clearly from that in laboratory conditions which are in most cases used for screening and evaluation studies. High dry matter has consequences in the performance of enzymes (e.g., Jørgensen et al. 2007) as well as to fundamental features such as the effect of CBMs in hydrolysis (Várnai et al. 2013).

8.5 Lignin

Lignin is the most abundant polyphenolic compound in nature, reaching 20–30 % of the lignocellulosic biomass produced worldwide (Fengel and Wegener 1989). Its hydrophobic and complex structure is mainly formed by the following units: 4-(3-hydroxyprop-1-enyl)-phenol, 4-(3-hydroxyprop-1-enyl)-2-methoxyphenol and 4-(3-hydroxyprop-1-enyl)-2, 6-dimethoxyphenol. Like hemicelluloses, the lignin type and distribution depends on the plant species and varies from one tissue to another. Besides, the chemical characteristics of isolated lignin depend largely on the method used for extraction.

Unlike cellulose and hemicelluloses, the lignin building blocks or monomeric units are not disposed in order and their crosslink includes ether linkages between aromatic rings and aliphatic chains (β-*O*-4′ and *O*-α 4′) and different carbon-to-carbon bonds involving aliphatic chains (β-β', α-α', and α-β'), aliphatic chains and aromatic rings (β-5′, β-1′, α-1′, and β-6′), and aromatic rings (5-5′) (Higuchi, 1985). According to Lee (1997), the most important linkages in the lignin structure are the β-1 and β-*O*-4 types, the latter of which corresponding to more than 50 % of its polyphenolic structure. Figure 8.6 shows the model structure of a lignin fragment derived from *P. albis* (Higuchi 1985) in close association with a feruloylated arabinoxylan, forming a lignin-carbohydrate complex.

Some microorganisms are able to produce lignin-degrading enzymes such as lignin peroxidase (LiP) and manganese peroxidase (MnP), which are extracellular heme proteins (Shin et al. 2005; Sharma et al. 2011). In a general, LiP catalyses the conversion of aromatic compounds in the presence of H_2O_2 to their corresponding aldehydes or ketones, and the hydroxylation of benzylic methylene groups. On the other hand, MnP may behave as an oxidase or a peroxidase (Singh et al. 2011).

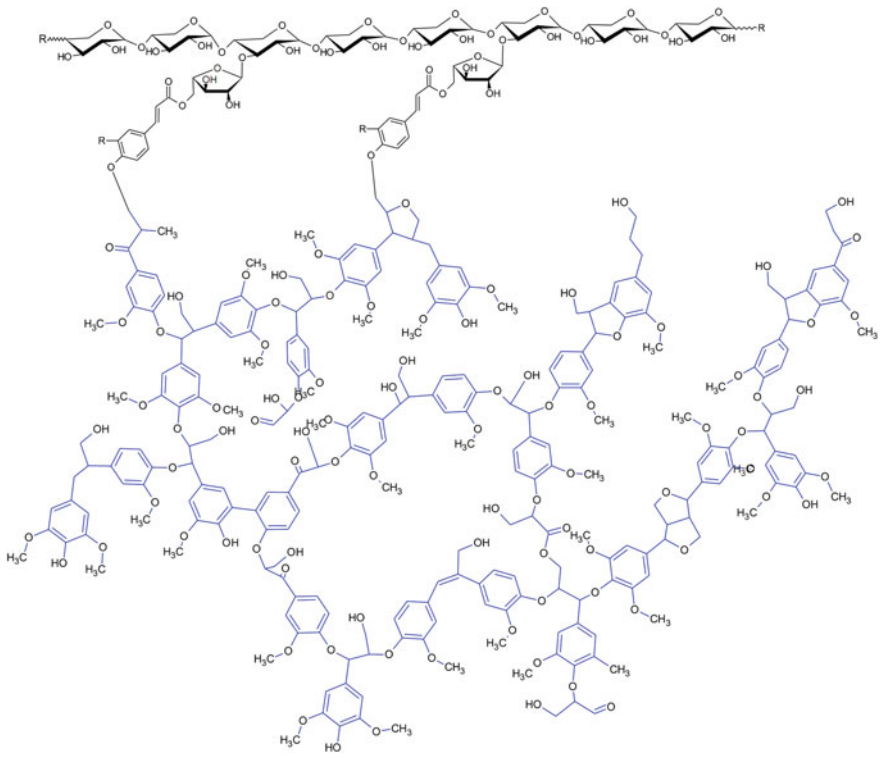

Fig. 8.6 Lignin fragments connected to an arabinoferuloylxylan residue

MnP acts by oxidating Mn^{2+} to Mn^{3+} with H_2O_2 in order to convert aromatic compounds to polycyclic aromatic hydrocarbons (Steffen et al. 2002; Shin et al. 2005). Therefore, LiP and MnP are known as primary enzymes for degradation of lignin. Besides the LiP and MnP, laccases are also known to degrade lignin to a certain extent (Youn et al. 1995; Eggert et al. 1997). Several studies have demonstrated the use of laccases in the detoxification of aromatic compounds but its role in lignin degradation has not been well established as yet. Furthermore, it is known that mushrooms can grow on lignocellulosic materials using plant carbohydrates as the carbon source while secreting lignin-degrading enzymes.

In general, oxidative enzymes require the presence of cofactors such as metallic ions and H_2O_2 and for this reason it is very difficult to carry out a bioprocess with simultaneous use of lignin-degrading enzymes and carbohydrate-degrading enzymes. Therefore, for the biorefinery processes development based on the use of the lignocellulosic materials, these enzymes are mainly useful for the biological pretreatment of the substrate such as in the case of biopulping (Aguiar and Ferraz 2012).

8.6 The Role of Enzymes in the Biorefinery

Apart from improvements in the development viable enzyme technologies for converting biomass to fuels and chemicals, insights on the pretreatment technologies of the lignocellulosic materials also represent a key factor for the industrial biorefinery based on agroindustrial wastes. In other words, pretreatment is crucial for the technical and economic viability of the overall process.

There are several pretreatment technologies already available for separating the main plant cell wall components in different streams: steam explosion with and without the use of an exogenous catalyst (Ramos 2003), dilute acid hydrolysis (Larsson et al. 1999), liquid hot water (Laser et al. 2002; Mosier et al. 2005), wet oxidation (Martin et al. 2007), ammonia fiber expansion (Balan et al. 2009; Chundawat et al. 2010), alkali extraction (Gupta and Lee 2010), alkaline hydrogen peroxide (Xiang and Lee 2000), organosolv extraction (Araque et al. 2008; Obama et al. 2012), and treatment with ionic liquids (Li et al. 2010), among others. When removed, hemicelluloses and lignin can be utilized in direct applications or as precursors for a wide range of industrial chemicals and materials. For instance, lignin can be directly used as a fuel (Menon and Rao 2012) or be converted to many value-added products including activated carbon (Demirbas 2004), binders (Dizhbite et al. 1999), dispersants, emulsifiers, and sequestrants (Suhas and Ribeiro 2007; Adler, 1997), vanillin and polyurethanes (Borges da Silva et al. 2009). Lignin can also be used in blends with polyhydroxyalkanoates (Ghosh et al. 2000) and polylactides and polyglycolides (Doherty et al. 2011), in epoxy resins (Wang et al. 1992) and as antioxidant in asphalts (Pan 2012). By contrast, hemicelluloses such as xylans can be converted to furfural (Montané et al. 2002), hydrogen (Caye et al. 2008), succinic acid (Nghiem, 2005), xylitol (Felipe et al. 1997), and xylooligosaccharides (Vazquez et al. 2000). Finally, apart from its more classical uses, cellulose can be converted to glucose to produce ethanol (Wyman 1994; Sun and Cheng 2002), lactic acid (Hofvendahl and Hahn-Hägerdahl 2000), succinic acid (Wang et al. 2011), and acetic acid (Wang et al. 2013) by fermentation, or used in pharmaceutical applications (Cherian et al. 2011) and as reinforcing agent in nanocomposites (Alves et al. 2013).

Figure 8.7 shows a simplified scheme for a biorefinery based on lignocellulosic materials. This biorefinery involves a multistep process in which the first step is the pretreatment of the biomass to render its macromolecular components amenable for further processing. The outputs of this process could be used as it is or be converted into chemical building blocks for further processing into polymers, chemicals, fuels, energy, and composite materials.

According to the applied pretreatment technology, different substrates are produced and their chemical composition would require a different enzyme composition for optimal enzymatic hydrolysis. In fact, this is a major challenge for commercial enzymes because none of them can be claimed as universal in their application to substrates with different compositional analysis and physical

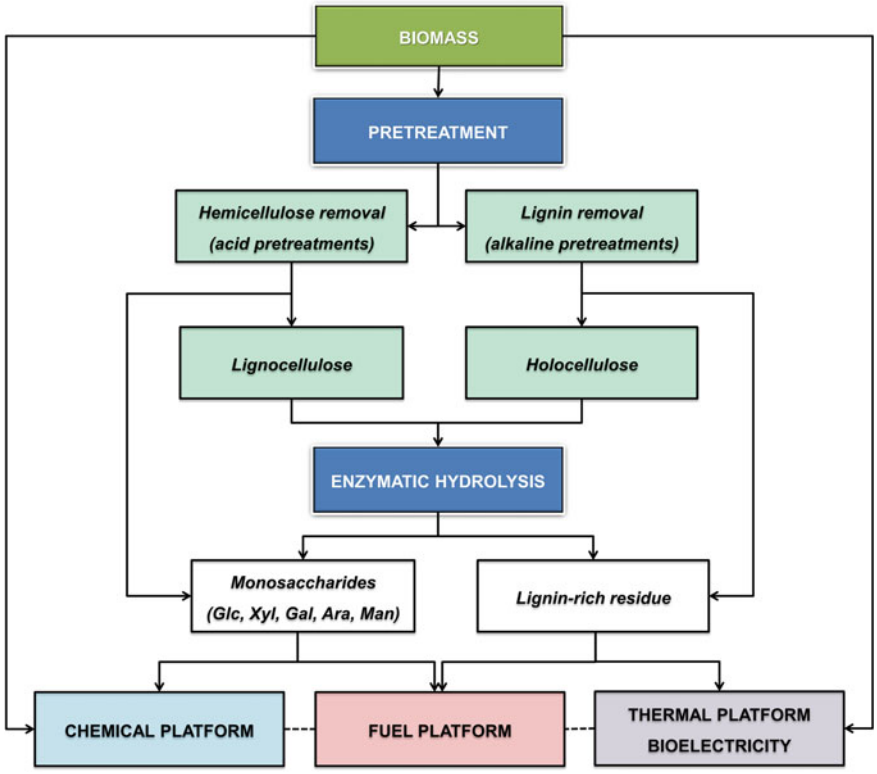

Fig. 8.7 Conceptual schematic biorefinery to the technology of system integration energy

properties such as degree of crystallinity, degree of polymerization, particle size, available surface area, and pore volume distribution.

Acid pretreatments tend to remove most of the hemicelluloses as water-soluble mono- and oligosaccharides (the so-called hemicellulose hydrolysate), leaving a lignocellulosic material whose hydrolysis would require an enzyme cocktail that is less susceptible to hydrophobic interactions and to the inhibitory effects of aromatic compounds such as phenolic acids derived from lignin. The immediate consequence of this pretreatment option is the possibility of using the hemicellulose hydrolysate for a variety of applications including ethanol production after partial detoxification. Also, by enzymatic hydrolysis of acid-pretreated materials, glucose is obtained as the main product and this could be one important issue for the desired integration of cellulosic ethanol into the currently existing first-generation ethanol producing technologies. Finally, the lignin-rich residue obtained after enzymatic hydrolysis could be used for co-generation or bioelectricity and also for other applications in the fuel and chemical platforms.

Alkaline pretreatments are able to extract the lignin component of plant biomass and depending on the extent of lignin extraction, the resulting fibrous

material may be classified as holocellulose. In this case, higher hemicellulase activities would be required in the enzyme cocktail to achieve complete hydrolysis of the delignified cellulosic material. Alternatively, the hemicellulose component could be extracted from these substrates in its poly- or oligomeric form, allowing its use as a polyelectrolyte, sizing agents, food additives, thickeners, films, and as a component of natural composites. Also, the lignin component can be obtained in higher molecular mass and with a lower degree of condensation, opening a venue of possible industrial applications in resins, emulsions, adsorbents, carbon fiber, films, polymers, adhesives, and composites.

With the abundance of biomass wastes, the development of new technologies that will make use of biomass for materials production beyond biofuels represents an important opportunity to fully utilize the resources. Development of efficient techniques to fractionate lignocellulosic biomass into its core components will facilitate research on the production of specific biomass-derived sugars, building block chemicals, and ultimately value-added commodity chemicals while preserving the concept of the biorefinery approach by promoting effective utilization of all feedstock fractions.

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Chapter 9
Mapping of Cell Wall Components in Lignified Biomass as a Tool to Understand Recalcitrance

André Ferraz, Thales H. F. Costa, Germano Siqueira and Adriane M. F. Milagres

Abstract Lignocelulosic biomass is recalcitrant to enzymatic digestion because terrestrial plants develop an efficient manner to grow upward and resist the microbial degradation of the polysaccharides contained in their cell walls. The complex cell ultrastructure, varied tissues, and the composite characteristic of the cell walls are among the several factors explaining the recalcitrance of lignified plants. Mapping the macromolecular components in the cell walls has proved to be useful to understand the varied recalcitrance of different biomass tissues. Available data indicate that lignin and hemicellulose greatly affect the final digestibility of the lignocellulosic materials. Removal of these components from the cell walls with varied pretreatments or even using lignin- and/or hemicellulose-depleted plants indicate that a critical characteristic of the cell wall to be digestible is to present most as possible available cellulose. This chapter revises some basic information on cell wall structure and advance in the knowledge compiling information on the mapping of cell wall components by several techniques and showing that the removal of cellulose encapsulating components is a key factor to increase cell wall porosity and digestibility by hydrolytic enzymes.

9.1 Introduction

Lignocelulosic biomass is recalcitrant to the enzymatic digestion because terrestrial plants develop an efficient manner to grow upward and resist the microbial degradation of the polysaccharides contained into their cell walls. Indeed, only a small group of organisms is able to digest the lignified cell walls in natural environments. They comprise soft-, brown-, and white-rot fungi, which use an intricate extracellular system to decompose the lignified cell wall macromolecules into small

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compounds that can pass across the cell membrane integrating the intracellular metabolism (Sanchez 2009; Nilson 2009). The complex cell ultrastructure and the composite characteristic of the cell walls are among the several factors explaining the recalcitrance of lignified plants. In this chapter, an overview of lignocellulosic biomass ultrastructure and some key characteristics of the plant cell walls are revised to show the origins of the recalcitrance in lignified plants. Understanding the origins of the recalcitrance is fundamental for the development of the future industry involved with the biomass conversion to monomeric sugars. Mapping the macromolecular components in the cell walls, determining how the chemithermomechanical pretreatments affect these components distribution and how the pretreated materials respond to the enzymatic digestion was revisited. The aim was to delineate the correlations between the changes occurred during the removal of cell wall components with the subsequent efficiency in the enzymatic hydrolysis of the polysaccharides. Emphasis is given on the recalcitrance of sugarcane, which is the main lignocellulosic substrate for the polysaccharides conversion into monomeric sugars for use in the second-generation biofuel industries in Brazil.

9.2 Fundamental Aspects of Biomass Recalcitrance

9.2.1 Ultrastructure and Cell Types in Lignified Biomass Revisited

The ultrastructure of wood and nonwood biomass is well known based on microscopic studies of these materials. Complete revisions on this subject have been published in wood chemistry textbooks (Fengel and Wegener 1989; Thomas 1991; Wiedenhoeft and Miller 2005; Daniel 2009) and in book chapters dedicated to the study of grass monocotyledons such as sugarcane (Moore 1987). In this chapter, a brief review on key aspects of the ultrastructure and cell types in lignified biomass was revisited to show the cell diversity in these plants and the importance of secondary walls for the processes involved in biomass conversion.

Wood biomass contains axial and radial cell systems. The axial cells are oriented along the longitudinal direction of the trunk whereas radial cells are orientated perpendicularly to the axial cells. The amount of each cell system varies according to the wood classification and among wood species. The gymnosperms (conifers, also referred as softwoods) and the angiosperms (deciduous or broadleaf trees, also referred as hardwoods) produce the solid material known as wood biomass. The gymnosperms present a relatively simple ultrastructure where the cells named tracheids dominate the secondary xylem (Fig. 9.1). The tracheids are part of the axial cell system of gymnosperms, presenting conduction and mechanical support functions. The tracheids are long cells with approximate dimensions of 20–65 μm wide and 1.4–4.6 mm long (Fengel and Wegener 1989). In gymnosperms, the reserve materials of the plant, such as starch, are stored mainly in ray parenchyma cells that are part of the radial cell system.

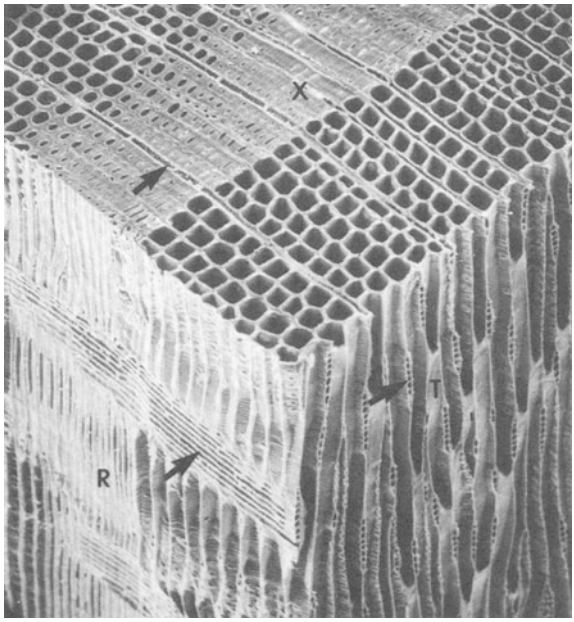

Fig. 9.1 Scanning electron microscopy of a gymnosperm showing all available faces. *X* cross-section (transversal) showing tracheids in early wood (*wide*, *thin-walled cells*) and late wood (*narrow*, *thick-walled cells*); *T* tangential surface with ray parenchyma cells (indicated by *arrows*) viewed from the tangential cut; and *R* Radial surface with the ray parenchyma (indicated by *arrows*) viewed from the radical cut (Reproduced with modifications from N.C. Brown Center for Ultrastructure Studies at SUNY-ESF, Syracuse, NY, http://www.esf.edu/scme, previously published in Thomas 1991)

The angiosperms present a more complex anatomy where the fibers have the support function similar to that of the tracheids in gymnosperms. However, together with the fibers, the angiosperms present vessels as an integral part of the axial cell system (Fig. 9.2). The presence of vessels represents the main difference between angiosperms and gymnosperms. The vessel elements are cells connected each other along the longitudinal axes of the tree forming a cell system (vessels) with the main function of water conduction. The fibers are narrower and shorter cells than the tracheids of gymnosperms with approximate dimensions of 15–40 μm wide and 0.6–1.6 mm long (Fengel and Wegener 1989). In addition to fibers and vessels, the angiosperms present abundant parenchyma cells that can occur in the ray tissue of the radial cell system, but also as longitudinal parenchyma, making part of the axial cell system. As in gymnosperms, the main function of the parenchyma cells is the storage of reserve materials for the tree.

In monocotyledons such as sugarcane, the cell distribution along the axial axes of the plant is different from that observed in gymnosperms and angiosperms. The internodes of monocotyledons present vascular bundles surrounded by parenchyma cells as illustrated in Fig. 9.3. Each vascular bundle contains a small phloem (sieve

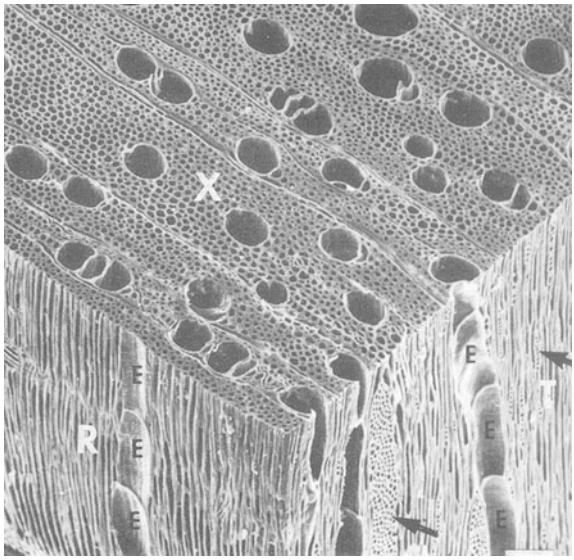

Fig. 9.2 Scanning electron microscopy of an angiosperm showing all available faces. *X* cross-section (transversal) showing fibers (*narrow cells*) and vessels (*wide cells*); *T* tangential surface with multi-cell ray parenchyma (indicated by arrows); and *R* Radial surface showing vessel elements in details *E* (Reproduced with modifications from N.C. Brown Center for Ultrastructure Studies at SUNY-ESF, Syracuse, NY, http://www.esf.edu/scme, previously published in Thomas 1991)

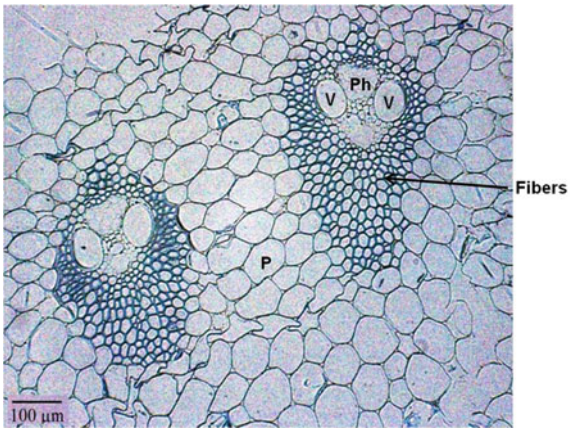

Fig. 9.3 Transversal cut of a monocotyledon (sugarcane hybrid, *Saccharum* sp.) visualized through optical microscopy illustrating mature vascular bundles surrounded by parenchyma cells. *V* Vessels; *Ph* Phloem cells; *P* Thin-walled parenchyma cells; and (Fibers) *Thick-walled fibers* in the vascular bundle (Micrograph provided by the authors)

elements and companion cells), whereas most of the bundle area is composed of vessels and fiber cells that present similar functions as described for angiosperms. The fibers are similar to angiosperms fibers with approximate dimensions of 15–25 μm wide and 0.6–1.7 mm long (SanJuan et al. 2001). The parenchyma cells surrounding the vascular bundles have the main function of storage of reserve materials, which in the case of sugarcane is composed mainly of sucrose.

Table 9.1 Abundance of different cell types in biomass from gymnosperms, angiosperms, and monocotyledons (Chum et al. 1985; Fengel and Wegener 1989)

Biomass origin (%)	Cell type		
	Fibers	Vessels	Ray parenchyma
Gymnosperms			
Volume	90–95	absent	5–10
Dry mass	95–98		
Angiosperms			
Volume	45–65	10–40	10–30
Dry mass	70–85	10–15	4–8
Monocotyledons			
Volume	20–60	1–10	30–70

In monocotyledons such as sugarcane, the thickness of the cell walls in different cell types is relevant when biomass conversion is under scrutiny because the parenchyma cells can represent up to 70 % of the internodes' volume. However, most of the parenchyma cells present very thin cell walls that contrast with vessels and especially fibers, which develop an extensive deposition of thick secondary walls. Therefore, the secondary walls of fibers are the most important process material in biomass conversion, since the secondary wall represents most of the dry matter of the mature plant. Table 9.1 illustrates the volume and mass proportion of different cell types in gymnosperms, angiosperms, and monocotyledons. There is a great variation inside each plant group, but a general trend is that the gymnosperms tracheids are responsible for more than 95 % of the secondary xylem dry mass. In angiosperms, the fibers account for 70–85 % of the dry matter and the vessels represent 10–15 %. In both cases, the parenchyma cells present only a minor participation in the secondary xylem dry mass (Chum et al. 1985; Fengel and Wegener 1989).

The average thickness of the different wall layers is variable according to the wood classification and the cell type. In general, the primary wall and the middle lamella are very thin and difficult to be distinguished each other by microscopic techniques. The thickness for the pair primary wall plus middle lamella (also referred as compound middle lamella) is in the range of 0.05–0.1 μm, independently on the origin of the lignified biomass. In contrast, the three layers of the secondary walls together (S1, S2, and S3, when present) comprise approximately 2 and 4.2 μm in early and late wood tracheids of gymnosperms, respectively (Fengel and Wegener 1989). In angiosperms, the secondary wall of fibers presents the approximate thickness of 3 to 5 μm, whereas in longitudinal and radial parenchyma it can reach 0.7 and 1.3 μm, respectively (Daniel 2009). In the sugarcane monocotyledon, the fiber cell walls were reported to present an average thickness of 4 μm, whereas vessel and parenchyma cell walls present average values of 2.7 and 1.7 μm, respectively (SanJuan et al. 2001). The average value of 1.7 μm for the parenchyma cell walls suggests that secondary wall deposition occurs at least in some parenchyma cells of sugarcane.

Table 9.2 Dry mass contents of four different regions dissected from sugarcane internodes (Costa et al. 2013)

Internode region	Dry biomass content (%)		
	Sugarcane cultivars		
	A	B	C
Pith	7	6	6
Interface pith-rind	18	19	20
Rind	50	63	58
Outermost fraction	25	12	16

In monocotyledons, the volume occupied by parenchyma cells can vary from 30–70 % of the internode, but it should represent less than 10–20 % of the internodes' dry mass. An example of the dry mass significance of each cell type in sugarcane can be obtained by dissection of the internodes into several regions. In a detailed study of three different cultivars of sugarcane, Costa et al. (2013) dissected the sugarcane internodes into four different fractions between the pith and the outermost part of the stalk. These fractions corresponded to the pith, the pith-rind interface, the rind, and the outermost fraction, which composed approximately 10, 29, 49, and 12 % of the internodes' volume, respectively. Micrographs of transverse cuts from each internode region showed that wide parenchyma cells predominated in the pith, while a small number of vascular bundles containing thin-walled-large-lumen fibers were also observed. In contrast, vascular bundles containing numerous thick-walled fibers were observed in the rind fraction. The pith–rind interface was comparable to the pith, whereas the outermost fraction contained epidermis cells, a ring of thick-walled cortical cells and small parts of the peripheral vascular bundles. These observations confronted with the mass proportion of the regions (Table 9.2) indicated that the central region of the internode (pith plus the interface pith-rind), in which the parenchyma cells predominated, accounted to a maximum of 25 % of the dry material. In contrast, the rind and outermost fractions, in which the vascular bundles predominated, contained the remaining 75 % of the dry matter of the internode.

9.2.2 Composite Characteristics of Secondary Walls Limiting the Enzyme Access to the Cellulose Chains

Some characteristics of the lignocellulosic substrates are critical to limit the enzymatic hydrolysis of cellulose and hemicellulose present in the lignified cell wall. Besides the anatomical tissue complexity of lignified biomass, the secondary cell walls of lignocellulosic materials are true composites since the strands of cellulose microfibrils are embedded with an amorphous matrix composed of lignin and hemicelluloses (Fengel and Wegener 1989; Daniel 2009). In some grass monocotyledons, usually consumed by ruminants, hydroxycinnamic acids make bridges between hemicellulose chains or between lignin and hemicelluloses and

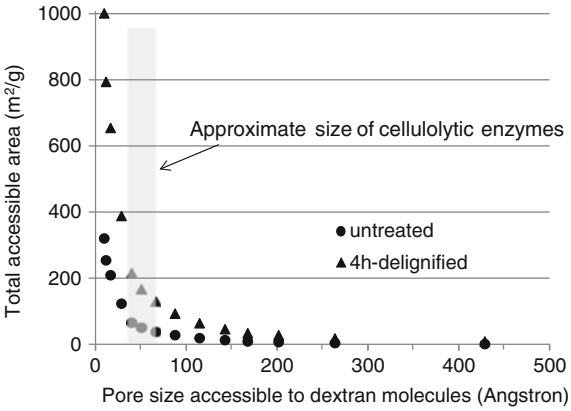

Fig. 9.4 Surface area accessible to dextran molecules of different sizes in untreated and 4-h delignified sugarcane bagasse. The lignin content in each material was 21 and 6 %, respectively (Reproduced with modifications from Santi Jr. 2011)

are also important for the formation of this type of embedding matrix (Grabber et al. 2002; Lam et al. 1994). In all cases, the cell walls of these materials exhibit porosity of molecular scale dimensions, which limits the permeability of the enzymes through the cell wall composite. The low porosity of the cell walls is, therefore, one of the main causes of lignocellulose recalcitrance. For example, in untreated and 4-delignified sugarcane bagasse, the area accessible to molecules of approximately 50 Angstrons, which is the approximate size of cellulolytic enzymes (Rollin et al. 2011), increases from 56 m^2/g to 172 m^2/g (Fig. 9.4), indicating that lignin removal let voids in the cell wall matrix. This change in the material porosity caused by lignin removal presented a remarkable effect in both, cellulose and xylan conversion to monomeric sugars by commercial cellulases, since they were limited to 20 and 9 % in untreated material, and increased to 96 and 85 %, respectively, in the 4-h delignified material (Santi Jr. 2011).

The effect of the matrix composed by hemicellulose and lignin on enzymatic hydrolysis of cellulose has been investigated for several lignocellulosic materials (Liao et al. 2005; Mussatto et al. 2008; Lee et al. 2009; Mendes et al. 2011; Siqueira et al. 2011). All these works indicate that lignin and hemicellulose removal from each material enhances the hydrolysis ability of the lignocellulosic material. However, it is noteworthy that the complete removal of these components is not necessary to achieve cellulose hydrolysis levels higher than 80 %. Thus, a comprehensive study of the structural features that cause the major effects on enzymatic hydrolysis of lignocellulose is still needed to finally determine a correlation between lignin and/or hemicellulose removal and digestibility.

Pan et al. (2005) suggested that lignin reduces the cellulose hydrolysis by two distinct mechanisms: by forming a physical barrier that impedes or prevents enzyme access to the cellulose and by unproductively binding cellulolytic enzymes. In fact, lignin irreversibly adsorbs the cellulase enzymes, preventing their action on cellulose. A consequence of both phenomena is that biomass samples with high lignin content are poorly hydrolyzed by celulases. Otherwise,

Table 9.3 Extracted components from sugarcane bagasse treated with sodium chlorite and the corresponding initial rates of enzymatic hydrolysis of cellulose in the treated samples (measured after 4 h of reaction) (Siqueira et al. 2013)

Treatment time with sodium chlorite (h)	Extracted lignin (%)	Extracted hemicellulose (%)	Extracted cellulose (%)	Initial hydrolysis rate (% h^{-1})
0	0	0	0	2.8
1	41.0	0	0	7.8
2	63.2	0	0	10.2
3	72.2	9.2	0	10.4
4	76.2	10.3	0	12.3

for samples with low lignin content, many enzymes can adsorb onto cellulose, which is effectively and rapidly digested (Chang and Holtzapple 2000).

Many studies have been conducted to access the effects of the lignin removal in the enzymatic hydrolysis of cellulose. Some chemical treatments are able to selectively remove this component, providing information about the role of lignin in limiting the enzymes action. The challenge on the selective removal of cell wall components is to remove one of them without changing the structure or chemical properties of the others. However, all the treatments, even the selective ones, affect the remaining components, making it difficult to study a single effect. Lignin can be removed using specific ionic liquids, such as 1-ethyl-3-methylimidazolium acetate, which solubilizes mainly lignin, preserving the other cell wall components (Lee et al. 2009). Oxidative methods, such as chlorite treatment, are widely studied and have given some information about restriction of enzymatic hydrolysis by lignin (Siqueira et al. 2013; Várnai et al. 2010; Kumar et al. 2013). The chlorite treatment consists of the hourly addition of sodium chlorite and acetic acid to the biomass at a reaction temperature of 70 °C. During the reaction, chlorine dioxide is formed as the main product, which can efficiently oxidize lignin (Browning 1968; Gellerstedt 2009).

Siqueira et al. (2013) studied the effect of lignin removal with sodium chlorite in the subsequent enzymatic hydrolysis of sugarcane bagasse, one of the typical grass monocotyledons used in biomass conversion processes. The bagasse was treated for 4 h and biomass samples were collected after each hour of treatment, generating four substrates with different lignin contents. The chlorite delignification was selective up to 2 h of treatment, removing 60 % of the initial lignin from the sugarcane bagasse. After that reaction time, part of the hemicellulose was also extracted from the samples (approximately 10 %). However, even up to 4 h of treatment, the cellulosic fraction was not solubilized (Table 9.3). Considering the difficulty to selectively remove a single component, the chlorite treatment has been considered one of the best treatments to study the effect of lignin in the enzymatic hydrolysis. The data presented in Table 9.3 corroborate that the enzymatic hydrolysis rates are determined by the accessibility to the substrate. Removing lignin from the substrate provided more accessible cellulose, resulting in higher hydrolysis rates. However, during the enzymatic hydrolysis, the reaction rate

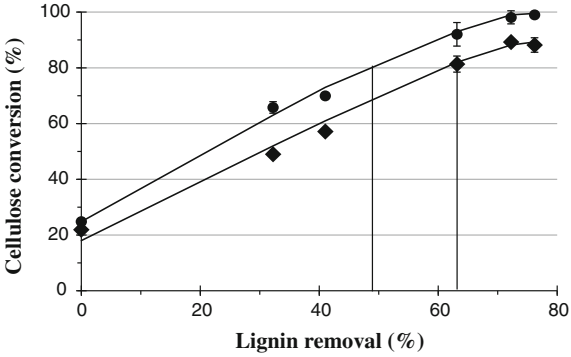

Fig. 9.5 Cellulose conversion after 72 h of enzymatic hydrolysis as a function of extracted lignin, cellulases with β-glucosidase (*filled circle*) and cellulases without β-glucosidase (*filled diamond*) (Reproduced from Siqueira et al. 2013)

decreases mainly because the more accessible cellulose is hydrolyzed first, whereas the residual material is enriched in recalcitrant substrate (Arantes and Saddler 2011).

One of the goals of selective removal of lignin is to access how much of lignin has to be removed to achieve good hydrolysis levels (above 80 %), either to design better pretreatments or to develop plants with lower lignin contents. Studies have shown that removing between 40 and 60 % is enough to reach good hydrolysis (Lee el al. 2009; Siqueira et al. 2013). For sugarcane bagasse, Siqueira et al. (2013) showed that removing 63 % of the initial lignin, more than 90 % of the cellulose was converted into glucose, while all cellulose was hydrolyzed after removing 72 % of the lignin (Fig. 9.5). The authors also combined the effect of lignin removal and the addition of β-glucosidases to the reaction medium. With the addition of β-glucosidase, less lignin needs to be removed to achieve similar hydrolysis levels. In the example of the Fig. 9.5, setting 80 % hydrolysis as a goal, 63 % of the lignin has to be removed if β-glucosidase is not added to the system. However, supplementing the enzyme mixture with β-glucosidase, the same hydrolysis level is achieved if 48 % of the lignin is removed.

Hemicellulose also exerts a great influence on the enzyme attack to the cellulose chains because this component is closely related to cellulose into the cell walls, covering part of the microfibrils. In the same manner, as illustrated for selective lignin removal from the lignocellulose matrix, some published work successfully removed hemicellulose from brewer's spent grain (86.5 % removal of hemicellulose and only 14 % removal of lignin) and evaluated the digestibility of the resulting solid material (Mussatto et al. 2008). In this case, the enzymatic conversion of cellulose to glucose was 3.5 times increased compared to the untreated material, attaining a value of 78 %. However, the real effect caused by the presence of hemicellulose in the enzymatic cellulose hydrolysis is not conclusive since the acidic removal of hemicellulose also change the content of crystalline cellulose in the sample as well as can diminish the cellulose degree of polymerization. In addition, some recent reports indicate that the presence of xylan in the substrate can facilitate its swelling creating more accessible surface area for the interaction of the enzymes with the cellulose present in the substrate (Ju et al. 2013).

An example of multivariate effects occurring during hemicellulose removal can be assessed in some reports indicating that despite the hemicellulose removal facilitate cellulose hydrolysis by increasing the porosity of the lignocellulosic ultrastructure, the content of crystalline cellulose increases after the acid pretreatments owing to the coupled removal of amorphous cellulose from the lignocellulosic material (Kim and Holtzapple 2006). The lignocellulose crystallinity (also referred as crystallinity index, CrI) has been considered as a significant parameter that affects negatively the biomass digestibility despite the different cell wall composition of samples (Lee et al. 2009; Xu et al. 2012). However, some contrary data have indicated that CrI did not affect the efficiency of the hydrolysis. This controversy remains, mainly if complex substrates, instead of pure cellulose such as cotton fibers, are under scrutiny. Several factors, substrate and enzyme related, may confound the isolated effects caused by CrI. Residual lignin and hemicellulose are among the materials that can interfere in the data interpretation related to the enzymatic hydrolysis of complex lignocellulosic substrates, since the action of cellulases is also affected by irreversible adsorption of enzymes onto these components as discussed below (Palonen et al. 2004; Rahikainen et al. 2011). Mixtures of pure cellulose substrates and purified enzymes indicate that the specific enzymes can attack both amorphous and crystalline cellulose simultaneously, with no accumulation of crystalline cellulose (Hall et al. 2010). Consequently, CrI alone may not adequately explain differences in observed hydrolysis rates and should be considered just one of several parameters that affect the enzymatic hydrolysis of cellulose in a complex biomass sample (Pu et al. 2013).

9.2.3 Unproductive Binding of Cellulolytic Enzymes on Lignin

As mentioned before, lignin has an important negative effect on the enzymatic hydrolysis of cellulose, mainly because its presence in the cell wall matrix limits the accessibility of the enzymes to the cellulose polymer. However, this is not the single inhibitory role that lignin plays in the conversion of cellulose to glucose. The enzymatic hydrolysis of cellulose is a heterogeneous reaction, which requires the adsorption of the enzymes to the cellulosic chains. This adsorption depends on the pairing of aromatic amino acid residues from the enzyme with the glycoside-exposed surface of the cellulose. This enzyme-substrate interaction occurs mainly due to the presence of tyrosine residues in the enzyme helped by hydrogen bonds between glutamine residues and cellulose hydroxyls (Linder et al. 1995). The coupling is not specific and the celluloytic enzymes can bind to other molecules such as lignin, which reduces the amount of available enzymes to act on cellulose. The adsorption of cellulolytic enzymes on molecules that differs from cellulose is usually named as unproductive binding. The phenomenon was observed in a variety of different biomasses subjected to a range of different pretreatments, and is considered an important inhibitory effect of lignin during the enzymatic

hydrolysis of cellulose (Chernoglazov et al. 1988; Palonen et al. 2004; Berlin et al. 2005; Nakagame et al. 2010; Rahikainen et al. 2011).

The extent of enzymes binding depends on the lignin structural features (Linder et al. 1995; Palonen et al. 2004). This aspect is relevant, because during the pretreatment employed to increase the efficiency of the enzymatic hydrolysis of biomass, chemical modifications in lignin can change its affinity for the cellulolytic enzymes. For example, some pretreatments that increased the phenolic hydroxyl contents of residual lignin also increased the capacity of the lignin to bind to proteins (Sewalt et al. 1997; Rahikainen et al. 2013). In contrast, pretreatments that turned lignin more hydrophilic (mostly by generation of acid groups) were effective to diminish the unproductive binding of cellulases on the pretreated material (Nakagame et al. 2011a; Lou et al. 2013). The main effect of introducing acid groups in lignin is that, at the pH of the enzymatic hydrolysis (usually from 4.8–5.0), at least part of these acid groups are ionized, giving to lignin a negative charge. The cellulases with isoelectric point below 4.8 (most of them present isoeletric poins between 3.6 and 8.5) are also negatively charged at pH 4.8–5.0, which causes repulsion of the enzyme to the lignin moieties, diminishing the unproductive binding (Nakagame et al. 2011b). Because of this, some authors suggest the use of higher pH values for enzymatic hydrolysis (Lou et al. 2013). In addition to the pretreatment effect on the lignin capacity to bind cellulases, different biomasses also differ on unproductive binding properties. For example, Nakagame et al. (2010) demonstrated that the lignin from wood biomass binds more to cellulases than lignin from monocotyledon agricultural residues.

The cellulolytic complex is a mixture of several enzymes that differ on molar mass, isoelectric point, and hydrophobicity. Because of this, unproductive binding studies have been conducted with the whole cellulase mixture and also with some purified enzymes. For example, comparing the major enzymes involved in the cellulose breakdown, it is apparent that the cellobiohydrolase Cel7A from *T. reesei* binds more strongly to lignin than endoglucanase Cel5A produced by the same fungus. The binding affinity of Cel7A was also 3 times higher than Cel5B, despite the lignin tested (Palonen et al. 2004).

Another interesting aspect of unproductive binding is the role of cellulose binding modules (CBMs). Cel7A, which contains a CBM, binds faster and to a larger extent to lignin films compared to Cel7A lacking the CBM (Rahikainen et al. 2013). It is suggested that the three aligned tyrosines (Y5, Y31, and Y32) present in the CBM are important for the hydrophobic interactions that drive the CBM-cellulose binding (Linder et al. 1995). However, it is probable that these amino acid residues are also important for the unproductive binding of the protein to hydrophobic lignin surfaces.

Unproductive binding is a problem for the enzymatic hydrolysis of cellulose not only because it decreases the amount of available protein, but also because of the thermal inactivation of the enzymes. Most of the adsorption studies are conducted at low temperature (4 °C) to avoid structural changes in the substrates. At this temperature, the bound enzymes can be recovered with almost the same activity. However, at hydrolysis temperatures of 45 °C or more, the protein–lignin

interactions are intensified and the proteins lose their native structure, becoming denatured and irreversibly bound to lignin (Rahikainen et al. 2011).

Adsorption is a concentration-dependent phenomenon, with the available surfaces becoming saturated as the protein concentration increases. Because of this, the unproductive binding can be overcome if the enzyme loading is relatively high (Nakagame et al. 2010; Kumar et al. 2012). Making the cellulose more accessible is another way to avoid unproductive binding because the cellulases bind faster to cellulose than to lignin (Tu et al. 2009; Kumar et al. 2012). However, overcoming unproductive binding at low enzyme loadings is still a challenge.

As the unproductive binding is not a specific interaction, the addition of other proteins prior to the addition of cellulases can reduce the amount of cellulases bound to the lignin. For example, Yang and Wyman (2006) added bovine serum albumin (BSA) to the reaction mixture and measured the cellulase activity in the supernatant during the course of hydrolysis. After 72 h, the cellulose activity in the supernatant was 20 % of the initial activity if BSA was not added to the reaction. In contrast, when BSA was added 1.5 h before cellulases addition, 50 % of the initial activity remained in the liquid fraction after 72 h. Another way to decrease the unproductive binding is to add surfactants to the reaction mixture. The presence of surfactants can increase the desorption rates, reducing the amount of lignin-bound enzymes (Eriksson et al. 2002).

9.3 Topochemical Distribution of Cell Wall Components and Its Correlation with Varied Recalcitrance in Different Cell Types

9.3.1 Lignin and Hydroxycinnamic Acids

Several microscopic techniques have been used to detect lignin and other aromatic compounds directly into the cell layers of lignified plants. The most traditional technique involves the UV absorption of lignin moieties that enables the direct assessment of lignin contents in each cell layer (Fergus et al. 1969; Koch and Kleist 2001). Based on this technique, fine details on lignin deposition into cell walls, middle lamella, and cell corner have been revealed. Textbooks on wood chemistry present classical data for some wood species indicating that most of the lignin contained in the lignocellulosic materials is located in the secondary walls simply because the lignified secondary walls are the thickest layers and represent most of the dry matter in wood biomass. An overall view of lignin distribution in the cell wall layers of wood biomass is summarized in Table 9.4. The highest concentration of lignin is always found in the cell corners, followed by the middle lamella and then secondary walls. However, 65–75 % of the total lignin available in the gymnosperms is located into de tracheid secondary walls. In angiosperms, approximately 60 % of the lignin is in the secondary walls of fibers, 20 % is in the

Table 9.4 Approximate lignin distribution and concentration in the several cell layers of gymnosperms and angyosperms (adapted with modifications from Fengel and Wegener 1989; Henriksson 2009)

Wood type	Cell type	Cell layer	Contribution to the total lignin content (%)	Lignin concentration in the layer (%)
Gymnosperms early wood				
	Tracheid	Secondary wall (S1–S3)	65	24
		Middle lamella plus primary wall	21	49
		Cell corner	14	64
Gymnosperms late wood				
	Tracheid	Secondary wall (S1–S3)	75	22
		Middle lamella plus primary wall	14	51
		Cell corner	11	78
Angiosperms wood				
	Fiber	Secondary wall (S1–S2)	60	19
		Middle lamella plus primary wall	9	40
		Cell corner	9	85
	Vessel	Secondary wall	9	25
		Middle lamella plus primary wall	2	40
	Ray cells	Secondary wall	11	25

cell walls of other cell types, and the rest is distributed in the middle lamella and the cell corners (Table 9.4).

In the last decades, the UV microspectrophotometry evolved to 0.25 μm^2 of geometrical resolution and appropriate softwares translate the absorption intensities of the spots in the cell layers into multicolored pixels to illustrate the lignin distribution in the biomass tissues. An example of this mapping technique is shown in Fig. 9.6 for the early wood tracheids from the gymnosperm *Pinus taeda*.

In monocotyledons, there are fewer studies related to the lignin distribution in the cell layers and cell types. However, the general trend of the major proportion of lignin in the secondary walls is valid, with the highest concentrations also observed in the cell corners and middle lamella. In addition to lignin, the cell walls of monocotyledons can also present UV absorption assigned to the presence of hydroxycinnamic acids. For example, the distribution of lignin and hydroxycinnamic acids in different cell types of sugarcane was formerly studied by He and Terashima (1990, 1991) using microautoradiography and UV microspectrophotometry. These authors demonstrated that the lignification of vessels occurred in the early cell maturation stage followed by lignification of fibers. In contrast, the UV absorption spectra of the parenchyma cell walls suggested the predominance of hydroxycinnamic acids instead of lignin. More recently, Siqueira et al. (2011) mapped the lignin occurrence in different cell types of mature sugarcane samples.

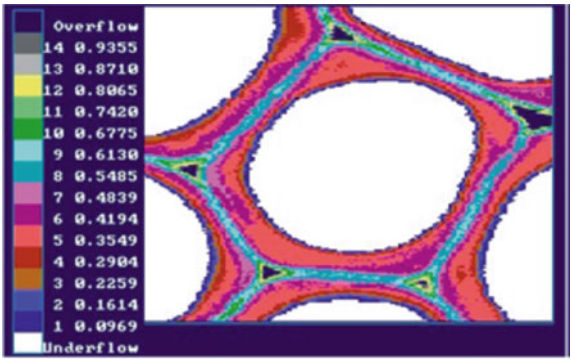

Fig. 9.6 UV micrograph of and early wood tracheid from the angiosperm *Pinus taeda* with 0.25 μm^2 of geometrical resolution. Appropriate software translates the absorptions intensities at 278 nm (shown in the left of the image) into multiple colors to illustrate the lignin distribution in the biomass tissues. The image clearly indicates the cell corners as the region with the highest absorption (colored with *black*), followed by the middle lamella (colored with *gray* to *light blue*) and by the secondary cell wall (colored with *pink* to *dark blue*) (Micrography provided by the authors and previously published in Mendonça et al. 2004)

The highest UV absorbance was detected in the cell walls of vessels followed by fibers and then parenchyma. UV spectra of fiber and vessel cell walls presented bands near to 278 nm and 315 nm (Fig. 9.7). The band at 278 nm was assigned to the aromatic rings of lignin, whereas the strong band at 315 nm was assigned to hydroxycinnamic acids linked to the lignin and/or arabino-methylglucurono-xylan backbones often found in grasses (He and Terashima 1991; Lybeer et al. 2006). The spectra from the parenchyma cell walls revealed the lowest absorbance values, the band at 278 nm was not resolved, and the most intense absorption appeared at 315 nm, which is consistent with the predominance of hydroxycinnamic acids as the main UV absorbing compounds in these cell walls. The parenchyma cells found in the central part of the internode (pith) presented even lower absorbance values as seen in Fig. 9.7.

Selected areas of the sugarcane fibers scanned at the geometrical resolution of 0.25 μm^2 corroborated previous studies with wood tissues. The most intense absorbance values were observed in the cell corners and the middle lamella followed by the cell walls (Fig. 9.8). It is noteworthy that the absorption intensities at 278 nm (proportional to the lignin concentrations) in the cell corners and middle lamella of the sugarcane fibers are lower than those observed in the fibers of the gymnosperm *Pinus taeda* (Fig. 9.7).

Confocal Raman microscopy also has been used to map the lignocellulose components into the cell layers (Agarwal 2006; Gierlinger and Schwanninger 2006). In this case, an especially set Raman spectrometer is attached to the microscope enabling the spectrum record in defined areas of the cell layers similarly to the described before for UV microspectrophotometry. Usually the Raman band intensities at the regions of 1,519–1,712 and 978–1,178 cm^{-1} are used to

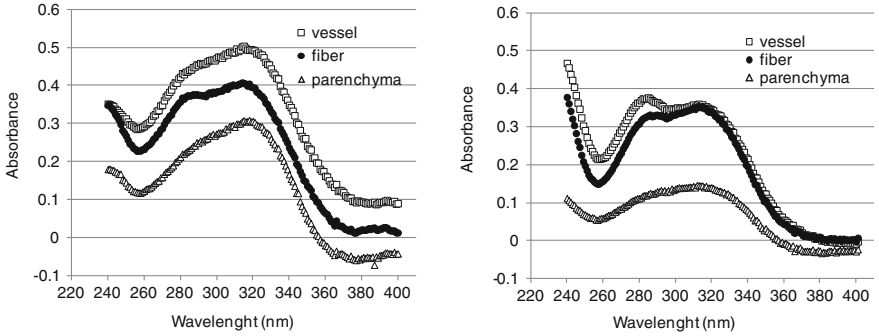

Fig. 9.7 UV spectra recorded from 1 μm^2 areas selected in the cell walls of different cell types excised from the rind (*left*) and the pith (*right*) region of mature sugarcane (UV spectra provided by the authors and previously published in Siqueira et al. 2011)

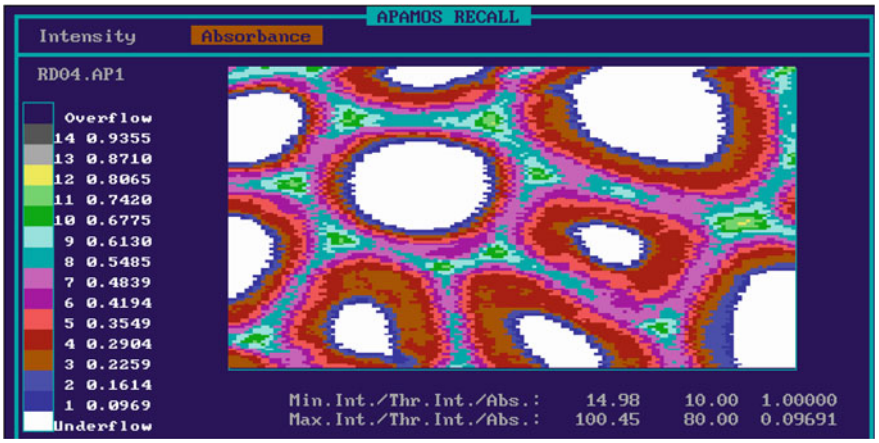

Fig. 9.8 UV micrograph of fiber cells from the monocotyledon *Saccharum sp* with 0.25 μm^2 of geometrical resolution. Appropriate software translates the absorptions intensities at 278 nm (shown in the *left* of the image) into multiple colors to illustrate the lignin distribution in the biomass tissues. The image clearly indicates the cell corners as the region with the highest absorption (colored with *light*- and *dark green*), followed by the middle lamella (colored with *light green* to *pink*) and by the secondary cell wall (colored with *pink* to *dark blue*) (Micrography provided by the authors and previously published in Siqueira et al. 2011)

map lignin and cellulose distribution, respectively. Stimulated Raman scattering (SRS) microscopy was recently used to map the cell wall components in maize (Ding et al. 2013). With this promising technique, the signal intensity at 1600 cm^{-1} (aromatic breathing modes, primary assigned to lignin moieties) was used to reveal the lignin localization and abundance into the cell walls, similarly to the discussed before for the UV microspectrophotometry. In addition, the Raman signal intensity at 2900 cm^{-1} (C–H stretch, primarily assigned to polysaccharides)

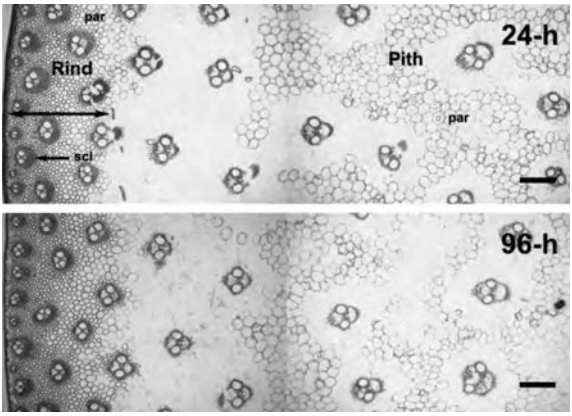

Fig. 9.9 Transversal cuts of maize internodes treated with the rumen biota for 24 and 96 h of digestion. Note that a group of parenchyma cells was completely removed (digested) from the samples, whereas some other parenchyma and all vascular bundles resisted to the digestion even after 96 h of treatment (Reproduced with modifications from Jung and Cassler 2006)

was used to reveal the localization and abundance of polysaccharides into the cell walls. The technique has been applied as a two-color SRS microscopy of fresh samples where the polysaccharides and lignin absorptions were related to two different colors in the produced image.

With the advance in microscopic techniques for mapping the lignocellulosic components into the cell walls, some attempts to correlate the topochemical distribution of lignin and hydroxycinnamic acids with the in vitro recalcitrance of the biomass material to hydrolytic enzymes have been reported. In the case of untreated grass monocotyledons, the recalcitrance varies according to the cell type and maturation stage (Siqueira et al. 2011; Zeng et al. 2012; Jung and Casle 2006). Some reports indicate that the parenchyma cells from the maize internode are promptly hydrolyzed by commercial cellulases or by the rumen biota (Fig. 9.9). This occurs because these cells are not extensively lignified and contain a limited amount of hydroxycinnamic acids as compared to other cells in the biomass material (Costa et al. 2013; Zeng et al. 2012; Jung and Casler 2006; Ding et al. 2013; Siqueira et al. 2011). In contrast, the rind region of the monocotyledon internodes contains highly lignified vessels and fibers arranged in the vascular bundles that are very recalcitrant to enzymatic hydrolysis (Fig. 9.9). Similar results were obtained with sugarcane samples as described by Costa et al. (2013). The authors evaluated the digestibility of the same sugarcane cultivars and internode regions previously described in the Table 9.2 of this chapter. As illustrated in the Fig. 9.10 (for the cultivar C from Table 9.2), the outermost fraction and the rind regions from the sugarcane internodes were very recalcitrant, whereas the pith–rind interface and the pith were significantly less recalcitrant.

As already discussed, several publications related to the enzymatic digestion of wood and nonwood substrates indicate that the selective lignin removal from the

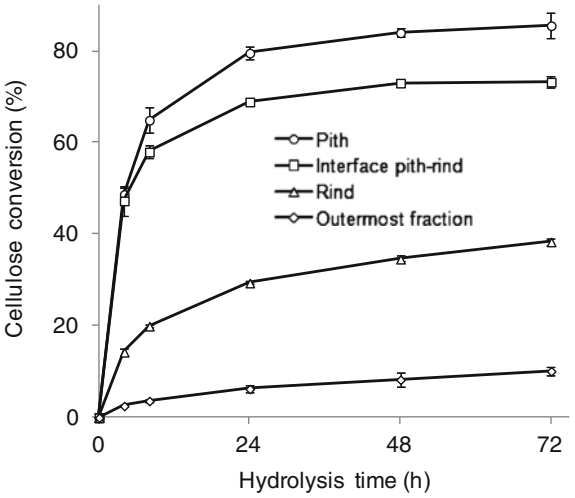

Fig. 9.10 Cellulose conversion to glucose catalyzed by commercial cellulases acting on different regions of untreated sugarcane internodes. (Reproduced with modifications from Costa et al. 2013)

lignocellulosic material promptly diminishes its recalcitrance (Lee et al. 2009; Siqueira et al. 2011; Siqueira et al. 2013; Ding et al. 2013). In the case of sugarcane, a detailed UV microspectrophotometric evaluation of the lignin removal from fiber cell walls corroborated that the highly lignified fibers from the rind region of the internode become less recalcitrant as a function of the lignin and hydroxycinnamic acids removal (Siqueira et al. 2011). In contrast, even untreated parenchyma cells from the pith region (characterized by low UV absorbance of the cell walls) were promptly hydrolyzed by commercial cellulases (Fig. 9.10). The data indicated that the action of the cellulolytic enzymes was not restrained by the aromatics occurring in the pith parenchyma, but it was strongly controlled by the high lignin content present in the fiber cell walls from the rind region of the internode. The chlorite treatment (delignification) of the pith region, rich in parenchyma cells, did not enhance cellulose conversion, whereas the application of the same treatment to rind cells, rich in vascular bundles, led to significant removal of hydroxycinnamic acids and lignin, resulting in a significant enhancement of the cellulose conversion by commercial cellulases.

9.3.2 Hemicellulose

As stated before, there is a clear correlation between lignin removal from the fiber cell walls and the increased efficiency in the subsequent cellulose hydrolysis of the delignified residue induced by enzymes. However, the important role of hemicellulose on the recalcitrance should not be ruled out. For example, several pretreatment processes are developed under acidic conditions resulting in hemicellulose removal. In most cases, the pretreated residue with reduced hemicellulose content is

less recalcitrant to the enzymatic digestion by commercial cellulases (see previous chapters in this book). Therefore, the hemicellulose encapsulating the cellulose microfibrils can also help to explain part of the recalcitrance of the cell walls of lignified biomass.

Hemicelluloses are synthesized from varied monosaccharides including D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid. Most of the hemicelluloses are branched and present varied levels of acetyl groups substituting free hydroxyls in the monomers of the polysaccharide backbone (Fengel and Wegener 1989; Teleman 2009). The hemicelluloses are found in both, primary and secondary cell walls, as well as in the middle lamella at low concentrations. In primary cell walls, pectin is also of great importance because it is a key component of immature cells and parenchyma cell walls. Xyloglucan, arabinoxylan, and pectic polysaccharides are the major polysaccharides present in plant primary cell walls besides cellulose. In grass monocotyledons, the primary cell walls may also present high levels of hydroxycinnamates and structural proteins (Carpita 1996; Vogel 2008). Xyloglucan is in close association with cellulose microfibrils and may be the main barrier to cellulose access in primary cell walls (Hayashi and Kaida 2011). In secondary cell walls, the hemicelluloses generally represent 25–35 % of the dry matter and are composed mainly by galactoglucomannans and arabinoglucuronoxylans in gymnosperms. 4-O-methyl-glucuronoxylans and glucomannans are the most frequent hemicelluloses in the secondary cell walls of angiosperms. In grass monocotyledons, 4-O-methyl-glucuronoarabinoxylan is the main hemicellulose in the secondary cell walls (Teleman 2009; Masarin et al. 2011).

Once the hemicelluloses may cooperate with recalcitrance of lignocellulosic materials, and its contents vary in different tissues and cell types, the evaluation of the topochemical distribution of this component becomes relevant in the biomass conversion studies. A singular past work on mapping hemicelluloses in the tracheids of gymnosperms was presented by Hoffmann and Parameswaran (1976). The authors carefully delignified spruce tissues with sodium chlorite and then oxidized the reducing ends of the hemicelluloses. The carboxyl end groups were then contrasted with colloidal iron, silver, or lead. Imaging ultrathin cuts of these samples by electron transmission microscopy revealed that the highest concentration of hemicelluloses was found in the S1 layers with the concentration decreasing toward the secondary wall zones composed by the S2. The authors also assigned the dense contrast observed in the cell corners and the compound middle lamella (middle lamella plus the primary cell walls) to the presence of hemicelluloses and pectin, which naturally present acid groups.

One of the recent strategies used to map the hemicelluloses into the cell walls involves the immunolabeling of the polysaccharide chains of interest with monoclonal antibodies. The detection is performed by adding a secondary antibody labeled with gold particles or fluorescent compounds followed by the analyses of the cell walls under electron or fluorescent microscopy, respectively (Kim and Daniel 2012a, b; Petersen et al. 2012). Figure 9.11 illustrates fiber and vessel cell walls of mature xylen from aspen wood based on the fluorescence detection of

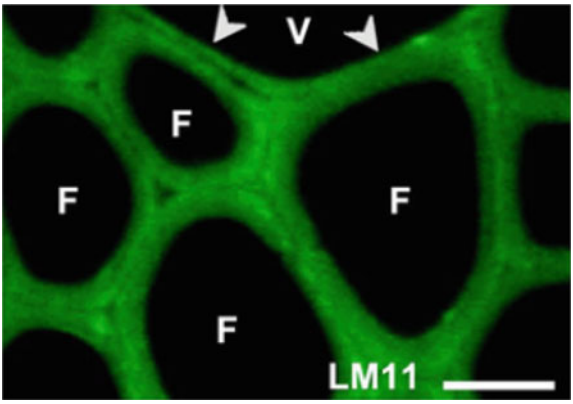

Fig. 9.11 Example o immunofluorescence localization of substitute xylans in fiber *F* and vessel *V* cell walls of mature xylen from apen wood. LM11 denotes the antibody used to bind to substituted xylan structures. The bar size corresponds to 10 μm. Note that the cell corners and middle lamella were poorly labeled whereas secondary walls were strongly labeled with the LM 11 antibody indicating the regions where substitute xylan predominates in the mature xylem (Reproduced from Kim et al. 2012)

antibodies selected to bind to substituted xylan structures (Kim et al. 2012). The differentiation between low- and highly substituted xylans is also possible by using two different specific antibodies (McCartney et al. 2005). Using these techniques, Kim et al. (2010) mapped the xylan distribution in the gymnosperm *Cryptomeria japonica*. During the tracheid maturation, the xylan deposition started in the corner of the S1 layers of tracheids. Using two different antibodies (LM10 that binds to low-substituted xylans and LM11 that binds highly substituted xylans) the authors suggested that structurally different types of xylans may be deposited in the tracheid cell layers according to the development stage.

The topochemical distribution of xylan was also assessed in an angiosperm hybrid (*Populus tremula* L. and *P. tremuloides* Michx) (Kim et al. 2012). Xylan immunolocalization in differentiating xylem cells indicated that the xylan deposition begins in the fibers (at the cell corner of the S1 layer as in the tracheids of gymnosperms), followed by vessels and ray cells. Xylan was not immunodetected in the cambial and radial zones because these tissues present cells with mostly primary cell walls and then low xylan content. However, in mature xylem, xylan was strongly detected in all cell types and layers, including the middle lamella.

In bamboo, an important fast grow grass monocotyledon, the immunofluorescence technique has revealed that the xylan deposition in the cell walls increased along maturation of a growing plant. The authors confirmed that in mature tissues, there was a higher xylan content in the vascular bundles, especially in thickened secondary cell walls of the fibers (Chang et al. 2013). In sugarcane, the immunolocalization of xylan has not been attempted up-to-date.

Another method to map hemicellulose in lignocellulosic materials involve synthetic or natural special peptides called “carbohydrate binding modules” that

may bind to specific carbohydrates of plant tissues, such as xylan. These binding modules may be labeled with fluorescent compounds or immunotargeted with modified antibodies in order to detect the signals by microscopy. Through this technique xylan chains were detected in pulp fibers, wood sections, and tobacco (Hervé et al. 2009; Filonova et al. 2007).

As reported for lignin, the heterogeneous distribution of hemicelluloses also has been associated with the varied recalcitrance of different tissues of lignocellulosic materials. For example, xyloglucan topochemistry indicated that this polysaccharide is in close association with cellulose microfibrils and may be the main barrier to cellulose access in primary cell walls (Hayashi and Kaida 2011). Nonetheless, xyloglucan immunofluorescence is generally associated with that of pectin and xylan and it is difficult to differentiate these polysaccharides in the thin primary cell walls.

The spatial distribution of hemicelluloses in different internode regions and tissues of some grasses such as sugarcane and maize may also differ significantly. Such fact permits to evaluate how the hemicelluloses may affect recalcitrance in each case. For example, sugarcane and maize present different hemicelluloses contents (mostly assigned to 4-O-methylglucuronoarabinoxylans with some cross-link with hydroxycinnamates bridges) in different anatomical regions of their internodes. Recent studies showed that the total hemicellulose content in these grasses increases from pith toward rind (Costa et al. 2013; Bairros-Rios et al. 2012; Zeng et al. 2012; Siqueira et al. 2011). The same studies showed that untreated rind tissues are very recalcitrant whereas the pith region can be easily hydrolyzed by commercial cellulases. These reports suggest that the different hemicellulose contents in the each region are in some way correlated with the recalcitrance of the material. In fact, the study of Costa et al. (2013) indicated that the sum of hemicellulose and lignin (the cellulose embedding components) in each sugarcane region was a key factor to explain the varied recalcitrance of the different tissues.

9.4 Pretreatments Affecting the Cell Wall Components Distribution and the Effects on Enzymatic Hydrolysis of Polysaccharides

As discussed in the previous sessions, lignin and/or hemicellulose removal from the cell walls can improve the access of the cellulolytic enzymes to the cellulose chains. In fact, the partial removal of these components is the basis for the pretreatment processes. Mechanical disruption of the cell wall ultrastructure, including cell wall rupture, can also enhance the enzyme access to the cellulose chains; however, pure mechanical pretreatments are extremely energy consuming and will not be considered in this chapter.

The technological basis of several biomass pretreatments were considered in other chapters of this book. Briefly, the pretreatments are necessary to increase the porosity of the cell wall, through the removal of physical barriers caused by lignin

and/or hemicellulose, and allow the enzymatic hydrolysis of lignocellulosics. The focus in this chapter is on the mapping of the lignin and/or hemicellulose removal or redistribution in some technical pretreatments with the aim to show how the cell wall become more accessible to the enzymes after the pretreatment.

To remove part of the lignin in technological processes various chemicals such as alkaline salts, sulfites, ammonia, and organic solvents have been exploited (Mosier et al. 2005; Hendriks and Zeeman 2009). The alkaline pretreatments also affect the cellulose degradation by enzymes because, in some reaction conditions, cellulose I arrangement is transformed in cellulose II after neutralization (Hall et al. 2011; Hendriks and Zeeman 2009).

An example of lignin removal from spruce wood tracheids during the alkaline/sulfite treatment can be observed in Fig. 9.12 (Koch et al. 2003). In alkaline reaction media, the lignin is progressively removed from the lumen toward the middle lamella. Therefore, lignin is first removed from the secondary walls and only at advanced delignification stages it is removed from the middle lamella (Jayme and Torgersen 1967; Procter et al.1967; Goring 1981; Koch et al. 2003). This is a relevant observation when delignification pretreatments are involved because only the internal surfaces of the cell walls become accessible to the enzymes owing to the increased porosity. The external surfaces of the cell walls continue capped by a middle lamella layer and are not accessible to the enzymes. A simple demonstration of this phenomenon was showed during the evaluation of the alkaline and alkaline/sulfite pretreatment of sugarcane bagasse (Mendes et al. 2011). Fibers released from the vascular bundles after the pretreatment were observed by light microscopy before and after 96 h of enzymatic digestion by commercial cellulases (Fig. 9.13). The action of the enzymes on the internal surfaces of the fibers can be observed as cavities caused in the secondary cell walls. In the untreated fibers only small cavities are observed and the cellulose conversion to glucose was limited to 20 % (Fig. 9.13a and d). The alkaline pretreated material becomes less recalcitrant and the cavities were more dispersed along the internal surfaces of the fibers (Fig. 9.13b, e). In this case, the cellulose conversion reached 50 %. Most of the alkaline/sulfite pretreated material was susceptible to the enzymatic hydrolysis, since 85 % of cellulose conversion was obtained. The few resistant fibers, after 96 h of enzymatic digestion, still presented the cavities in the cell walls starting from the internal surfaces of the fibers (Fig. 9.13c, f). In all cases, a middle lamella layer outside of the nondigested fibers can be visualized as a dark contrasting layer that have restrained the permeation of the enzymes from the external surface of the fibers.

Besides lignin removal, the residual lignin in the alkaline/sulfite pretreated materials is modified by the incorporation of sulfonic groups (Gellerstedt 2009). The sulfonic acid groups turn the residual lignin less hydrophobic allowing an increased capacity of water retention by the fibers (Mendes et al. 2013). This characteristic is critical because the swollen fibers become more porous, facilitating the enzyme permeation toward the secondary walls of the pretreated material. The cellulotytic enzymes apparently do not adsorb irreversibly to the lignin in this type of pretreated material, which diminishes the enzyme load

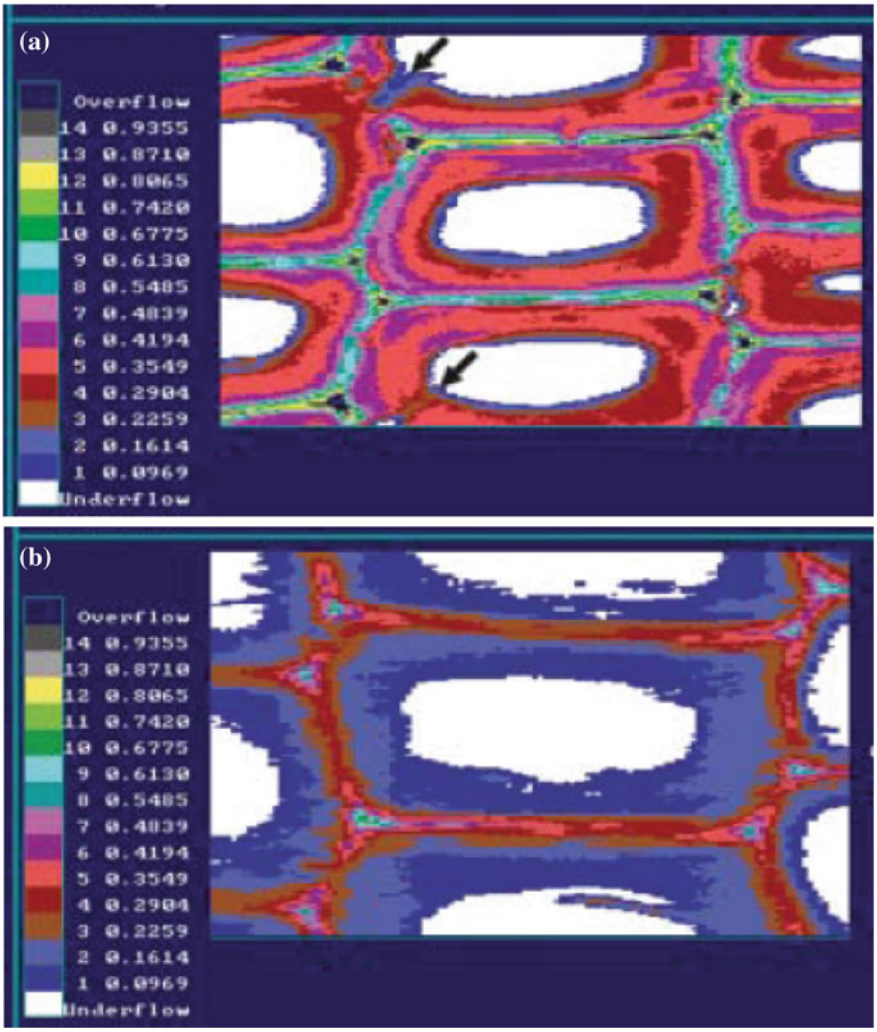

Fig. 9.12 UV micrographs of late wood tracheids from spruce wood treated under alkaline/sulfite processes after incipient delignification, 30 min (**a**), and advanced delignification, 120 min (**b**). The absorption intensities at 278 nm (shown in the *left* of the image) are translated into multiple colors to illustrate the lignin distribution in the biomass tissues. *Pink*, *light blue*, and *green*, as well as *yellow* and *black* correspond to mid to strong UV absorptions indicating high lignin concentrations. *Blue* to *brown* colors indicate low UV absorption intensities and low lignin concentrations. The images clearly indicate that the cell corners and the middle lamella remain present even after a long treatment time when the lignin originally present in the secondary walls were significantly removed (Reproduced with modifications from Koch et al. 2003)

required for efficient hydrolysis as well as turn the enzymes recycling more feasible (Zhu et al. 2009; Liu and Zhu 2010). These combined effects have been claimed to bring low enzyme consumption and costs in this type of process.

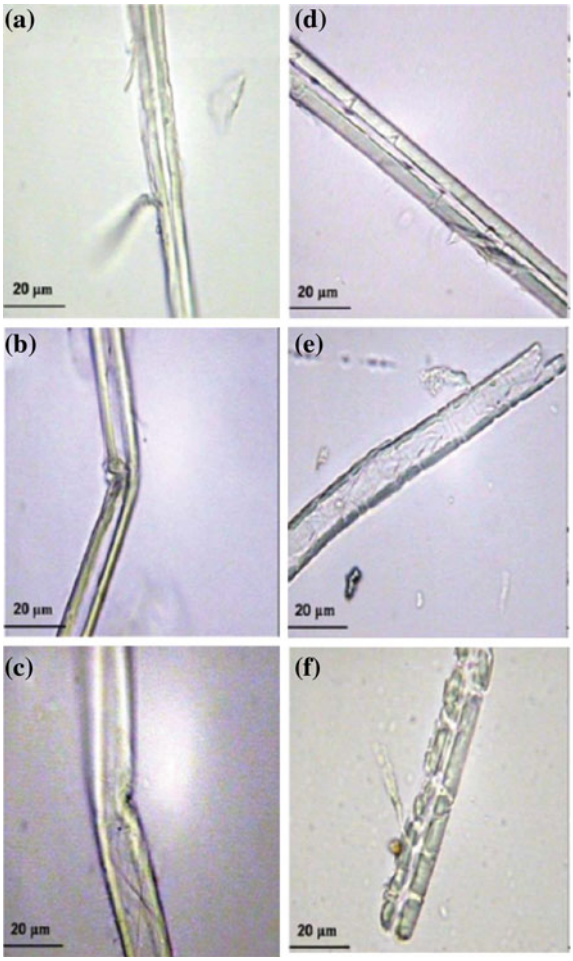

Fig. 9.13 Light micrographs of selected fibers from sugarcane bagasse treated under alkaline and alkaline/sulfite processes, before (**a**–**c**) and after enzymatic digestion with commercial cellulases (**d**–**f**). **a** and **d** Untreated fibers; **b** and **e** Fibers pretreated with the alkaline process; **c** and **f** Fibers pretreated with the alkaline/sulfite process. (Micrographs provided by the authors, previously published in Mendes et al. 2011)

Some pretreatments performed under acidic conditions, such as steam explosion and hydrothermal processing, cause partial removal of hemicelluloses (Ramos 2003). In these treatments, acetyl groups linked to the hemicelluloses are cleaved and, therefore, act as hydrolysis catalysts. The removal of the hemicelluloses increases the porosity and the internal surface area of the pretreated material, facilitating the accessibility of the enzymes for the subsequent cellulose hydrolysis (Himmel et al. 2007). Lignin, on the other hand, is removed to a limited extent from the material but it is rather redistributed on the internal cell surfaces owing to the softening occurred at temperatures above 130 °C. A former work presenting this phenomenon was published by Michalowicz et al. (1991), which studied the ultrastructural changes in poplar cell wall during steam explosion treatment by transmission electron microscopy of ultrathin cross-sections of the xylem. The authors described the "melting" of the lignin, which agglomerated as droplets

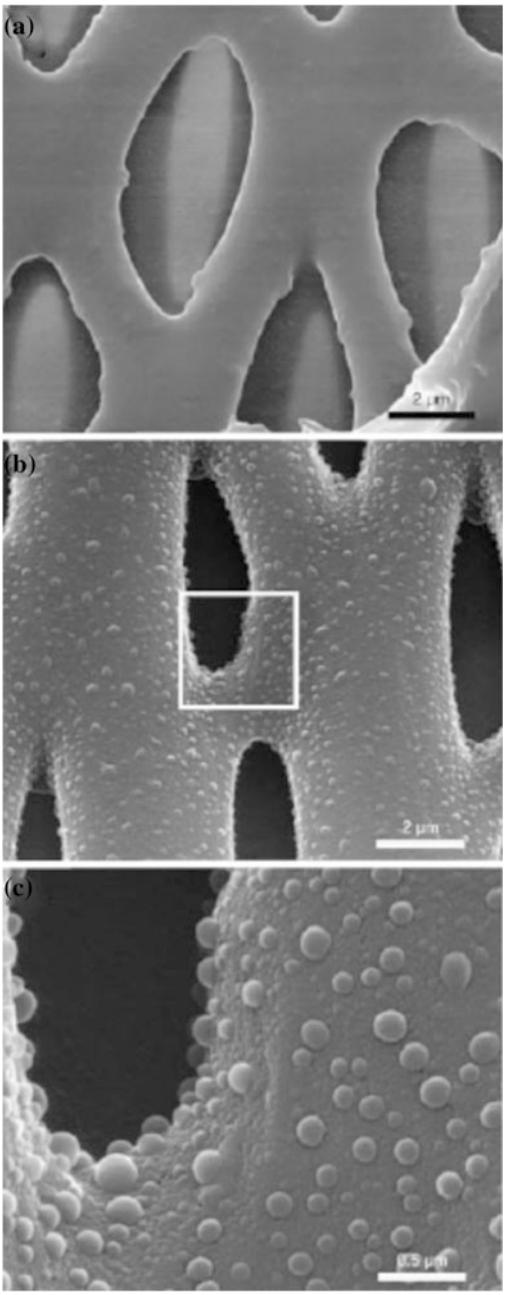

Fig. 9.14 Scanning electron micrographs (SEM) of corn stover fiber surfaces treated with 0.8 % sulfuric acid at 150 °C for 20 min. **a** Control sample and **b** and **c** treated samples at different magnifications. The bars represents 2 µm (**a** and **b**), and 0.5 µm (**c**). Note that the lignin droplets diffuse from the secondary cell walls toward the internal surfaces of the lumen (Reproduced from Donohoe et al. 2008)

inside the secondary wall and diffused toward the lumen. Donohoe et al. (2008) demonstrated a similar redistribution of lignin in the fibers of corn stover treated under acidic conditions as illustrated in Fig. 9.14.

Similar to that reported for lignin, part of hemicelluloses present in secondary cell walls of lignocellulosic materials can also migrate during dilute acid pretreatment. This phenomenon was demonstrated using fluorescence microscopy and scanning electron microscopy based on semiquantitative analysis of xylan antibody signals detected in the cell walls (Brunecky et al. 2009). An interesting aspect of these techniques was that the pixel intensities were quantified along the cell wall to identify the distribution and the removal of xylan occurring during the dilute acid pretreatment. The authors observed that a decrease in the average signal intensity detected in the inner cell walls of fibers from corn stover closely matched the progressive loss of xylan determined by chemical analysis.

9.5 Concluding Remarks

The complex cell ultrastructure and the composite characteristic of the cell walls are among the several factors explaining the recalcitrance of lignified plants. Understanding the origins of this recalcitrance is fundamental for the development of the future industry involved with the biomass conversion to monomeric sugars. Mapping the macromolecular components in the cell walls has proved to be useful to understand the varied recalcitrance of different biomass tissues, as well as how the removal of individual components can affect the final digestibility of the pretreated material. Data available to date indicate that parenchyma cells of monocotyledons are significantly less recalcitrant than fibers and vessels. However, even in parenchyma rich materials such as sugarcane bagasse, this cell type represents a minor fraction of the dry biomass. Consequently, pretreatments are necessary to remove some of the cellulose embedding components (lignin and hemicellulose) in order to enhance the cell wall digestibility. The information revised in this chapter indicates that lignin removal from the cell walls significantly enhance the digestibility of the material by commercial enzymes. Hemicellulose removal can also help on some extent and the general trend is that the diminished recalcitrance in pretreated materials or in different tissues of the biomass is obtained when the cellulose become more accessible to the enzymes. In this subject, some recent data support that the available cellulose can be estimated in the biomass materials as the content of cellulose divided by the sum of the embedding components, hemicellulose, and lignin.

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Chapter 10
Dilute Acid Pretreatment and Enzymatic Hydrolysis of Sugarcane Bagasse for Ethanol Production

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Abstract Many efforts have been dedicated to understanding and refining different technologies to promote the conversion of the sugars contained in the sugarcane bagasse into ethanol. One of the promising strategies include the pretreatment of the bagasse with dilute sulfuric acid followed by the saccharification of the remaining polysaccharides with enzymes, and by the fermentation of the generated monosaccharides (both hexoses and pentoses) with yeasts. In the present chapter, data regarding the characterization and conditioning of the raw material as well as its pretreatment, saccharification, and fermentation are disclosed to illustrate that the sugarcane bagasse, like many other agroindustrial residues, consists of a heterogeneous material and that its different constituent sugars, not necessarily only plant cell wall polysaccharides, may need to be recovered under different experimental conditions if high conversion yields are to be achieved using the proposed technology.

10.1 Introduction

10.1.1 Sugarcane and Sugarcane Bagasse

Brazil is one of the large producers of sugarcane in the world. Sugarcane represents one of the most important agroindustrial cultures in the country, with plantations concentrated in the center-south and northeast regions; mainly in the state of São Paulo (Conab 2013).

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Each metric ton of sugarcane processed in the mills for extraction of the juice, used in the production of sugar and/or ethanol, generates about 280 kg of moist bagasse; a lignocellulosic material, usually burned for power generation (Cenbio 2013).

The sugarcane bagasse, as any other lignocellulosic material, is composed of three major constituents: cellulose, hemicellulose, and lignin. Cellulose, a polysaccharide formed exclusively by glucose units, represents the main component. In the plant cell wall, the elementary fibrils of cellulose are formed by the association of linear cellulose chains that are maintained by intra and intermolecular hydrogen bonds; which explains, at least in part, its resistance to microbial degradation (Bragatto et al. 2012). Hemicellulose is a general name given to a family of heteropolysaccharides (Ebringerová 2006), whose structures may include hexoses (D-mannose, D-glucose, and D-galactose) and pentoses (D-xylose and L-arabinose) as well as small amounts of deoxihexoses (L-fucose and L-rhamnose) and uronic acids (glucuronic acid, galacturonic acid, and methylglucuronic acid). Properties such as low degree of polymerization and amorphous structure make the hemicelluloses less stable to biological degradation than cellulose (Ek et al. 2009). Lignin, on the other hand, is a non-carbohydrate macromolecule that confers stability to the plant cell wall due to the formation of a composite, together with the polysaccharides (cellulose and hemicellulose), highly resistant to microbial degradation. Its structure derives from three primary precursors (coniferyl, sinapyl, and coumaryl alcohols), but the biological synthesis generates complex structures with different types of linkages (mainly β-O-4, α-O-4, β-1, β-5, 5-5, and β-β) oriented towards all the spatial directions (Fengel and Wegener 1989).

In addition to the major constituents, lignocellulosic materials exhibit varying amounts of other substances, sometimes referred to as minor constituents. Such constituents include organic and inorganic compounds, and are divided into two classes: the first comprise materials known as extractives, being extractable with water or neutral organic solvents, or volatilized in the presence of steam; the second refer to non-extractable materials, including certain inorganic salts and proteins (Browning 1967). The qualitative and quantitative compositions of these so-called minor constituents, however, depend on several factors, including the origin and the age of the material (Kai 1991).

10.1.2 Production of Ethanol from Sugarcane Bagasse

The structural sugars contained in the sugarcane bagasse, including cellulose and hemicellulose, represent potential substrates that could be used for increasing the ethanol production by the sugarcane processing mills (Carvalho et al. 2007). The challenge consists of quantitatively recovering the constituent sugars, ideally in their monomeric forms, and in efficiently converting them into ethanol; in an economical way (Wyman 1994).

In order to overcome the bottlenecks associated with the conversion of the sugars contained in the sugarcane bagasse into ethanol, many efforts have been dedicated to the understanding and refinement of different technologies able to achieve the above mentioned targets. One of the promising strategies include the pretreatment of the bagasse with dilute sulfuric acid, followed by the saccharification of the remaining polysaccharides with enzymes and by the fermentation of the generated monosaccharides with yeasts (Canilha et al. 2009).

10.1.2.1 Pretreatment with Dilute Sulfuric Acid

Pretreatment of lignocellulosic materials with dilute sulfuric acid is one of the most effective methods for solubilizing hemicelluloses and generating solids highly reactive to enzymatic saccharification (Canilha et al. 2011).

Depending of the pretreatment severity, the solubilized sugars can undergo degradation reactions and give rise to compounds like furfural and hydroxymethylfurfural (HMF) which inhibit the bioconversion (Palmqvist and Hahn-Hagerdal 2000; Carvalho et al. 2004a). Similar behavior is observed for the small fraction of lignin soluble in acid medium, which may reveal itself as composed by strong inhibitors of both the enzyme saccharification of polysaccharides (Ximenes et al. 2010) and the yeast fermentation of monosaccharides (Carvalho et al. 2004b); not to mention the inhibitory effect of acetic acid, released from certain hemicelluloses (Han et al. 2006).

As the pretreatment conditions that maximize the recovery of xylose in the hemicellulosic hydrolysate are generally different from the pretreatment conditions that maximize the recovery of glucose during the enzymatic hydrolysis of the pretreated solids, a two-step pretreatment can be employed (Nguyen et al. 2000; Söderstrom et al. 2003). The approach consists of hydrolyzing the hemicellulose under milder conditions and, then, in conditioning the resulting pretreated solids under more drastic conditions.

10.1.2.2 Saccharification with Enzymes

The enzymatic hydrolysis of pretreated lignocellulosic materials is specific and carried out under mild conditions of pH and temperature, which avoids the generation of inhibitors and leads to high sugar yields (Taherzadeh and Karimi 2007).

Cellulose is hydrolyzed into glucose by the coordinated action of endoglucanases, exoglucanases, and β-glucosidases (Alvira et al. 2010). Depending on the pretreatment conditions, residual hemicellulose may remain in the pretreated solids and hinder the action of cellulases; in these cases, the use of hemicellulases, in association with cellulases, improves the efficiency of saccharification (Öhgren et al. 2007a).

Besides compounds such as furans, phenolics, and organic acids, originated or released during the pretreatment, the sugars released during the enzymatic

saccharification of the pretreated solids can themselves act as inhibitors of the enzymes: glucose inhibits the action of β-glucosidases; cellobiose, the activity of exo and endoglucanases (Andric et al. 2010). Such inhibition, however, can be overcome by integrating the saccharification and fermentation operations in a process configuration like the simultaneous saccharification and fermentation (SSF), in which the glucose released during the enzymatic saccharification of the cellulose is simultaneously converted into ethanol by an appropriate microorganism; which prevents the inhibition of the enzymes (Öhgren et al. 2007b).

10.1.2.3 Fermentation with Yeasts

The sugars generated in the pretreatment of the lignocellulosic material with dilute sulfuric acid and/or in the saccharification of the pretreated solids with enzymes must be quantitatively converted into ethanol (Kuhad et al. 2011); for process economy and environmental sustainability.

Although *Saccharomyces cerevisiae*, the yeast long used by humankind for ethanol production in hexoses-based media, can be genetically engineered to produce ethanol from pentoses like xylose and arabinose (Bettiga et al. 2009), there are also a number of yeast species able to naturally produce ethanol from such substrates; among them: *Pachysolen tannophilus*, *Candida shehatae*, and *Scheffersomyces* (formerly *Pichia*) *stipitis* (Jeffries and Kurtzman 1994).

Among the yeasts that naturally produce ethanol from pentoses, *S. stipitis* stands out, due to its high substrate-to-product conversion efficiency and versatility towards the consumption of different substrates; even though it may present stringent oxygen requirements for the production of ethanol from pentoses, and in spite of its susceptibility towards inhibiting compounds (Agbogbo and Coward-Kelly 2008).

10.2 Case Study

In the following, a case study that reports results of an ongoing project that deals with the conversion of the sugars contained in the sugarcane bagasse into ethanol by pretreatment with dilute sulfuric acid followed by enzyme saccharification and yeast fermentation is presented.

Data regarding the characterization and conditioning of the raw material as well as its pretreatment, saccharification, and fermentation are disclosed to demonstrate that the sugarcane bagasse is a heterogeneous material and that its different constituent sugars may need to be recovered under different experimental conditions if high conversion yields are to be achieved during the conversion as a whole.

10.2.1 Materials and Methods

Figure 10.1 presents a schematic representation of the ongoing project; the main results of which, achieved up to now, are hereafter presented.

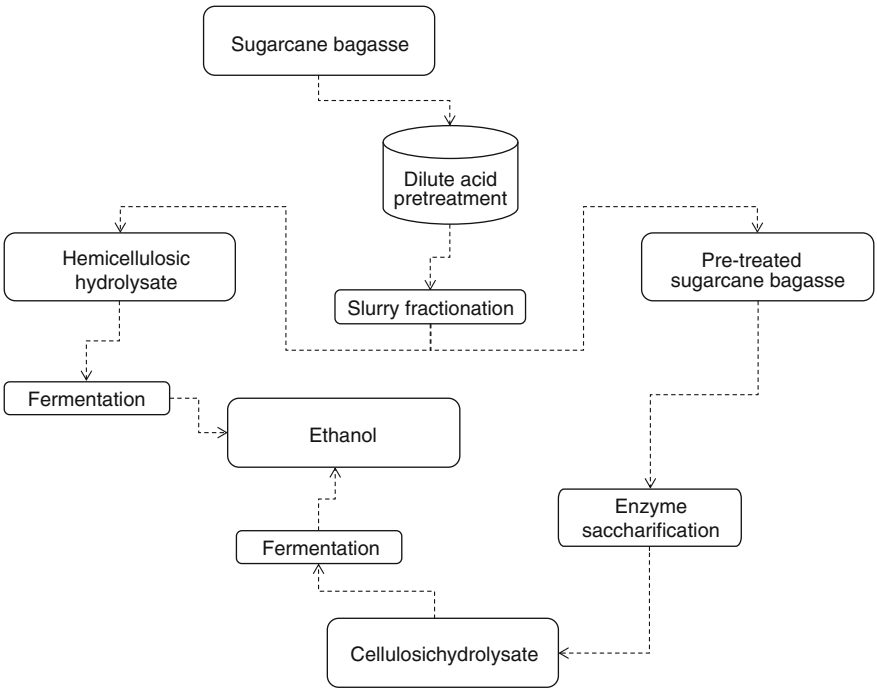

Fig. 10.1 Schematic representation of the study

10.2.1.1 Raw Material, Characterization, and Conditioning

Two samples of sugarcane bagasse, acquired from different mills located in São Paulo state, hereafter nominated as sugarcane bagasses A and B, were used in the study. Bagasse A was milled in a hammer mill and the fines were removed, with the help of compressed air, by using a device consisting of a plastic box in which the bottom was replaced by two intertwined 20 mesh sieves (Fig. 10.2). For the fractionation, 150 g of sample were transferred into the box; next, through a lateral opening, compressed air was injected during 40 s. The fractions of bagasse retained (bagasse without fines) and not retained (fines) by the device were characterized regarding the distributions of particle sizes and chemical compositions. In addition, samples of the raw bagasses A and B were knife-milled to pass through a 20 mesh sieve. The milled bagasses were packed into cellulose thimbles (4.5 g) and submitted to solid–liquid extraction in a Soxhlet extraction system.

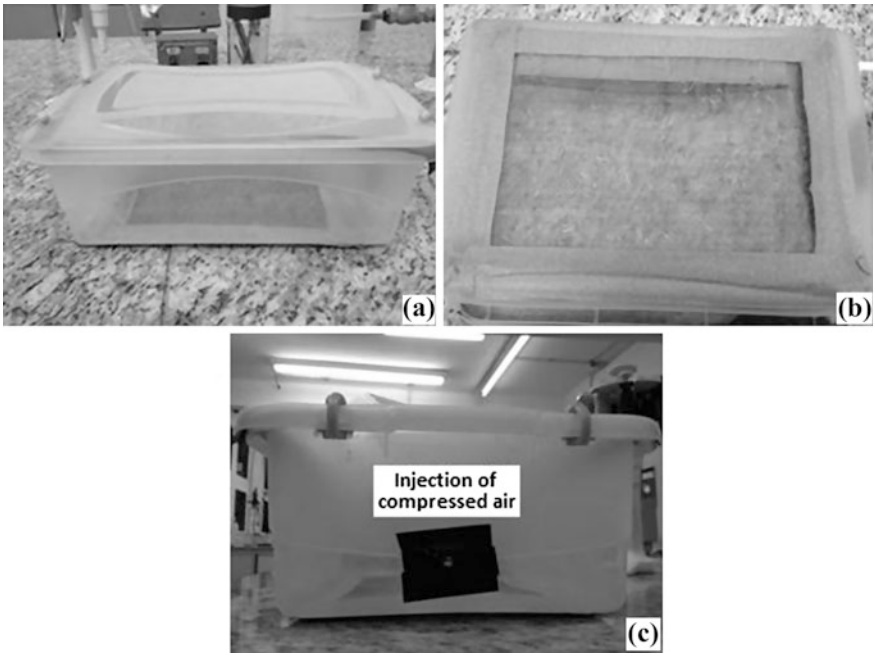

Fig. 10.2 Photos of the device used to fractionate the sugarcane bagasse with the help of compressed air: **a** (*top* view); **b** (*bottom* view); **c** (*front* view, with the entrance for injection of compressed air)

Water, ethanol or water followed by ethanol were used as extracting solvents. After adding 800 mL of the solvent into the receiving flask, the Soxhlet apparatus was assembled and the heating mantle was turned on. Two extractions in each solvent were performed, each one lasting 12 h. At the end of the extractions, the solids were quantitatively transferred into weighing bottles which were then oven-dried at 105 °C until constant weight. The chemical compositions of both extracted and non-extracted samples were determined in the sequence.

The distributions of particle sizes were analyzed by determining the mass fractions selectively retained on a set of standard sieves with openings of 3.35, 1.70, 0.85, 0.42, and 0.21 mm, respectively. For each analysis, 20 g of sample was transferred into a magnetic stirrer in which the sieves were stacked in order of decreasing apertures; afterwards, the system was set to vibrate for 1 h. The chemical composition of solid materials were determined according to the traditional two-step acid hydrolysis procedure of Klason, employing one of two methods (Sluiter et al. 2010; Gouveia et al. 2009) previously shown to lead to similar results (Canilha et al. 2011). Sugars were quantified by HPLC using a refraction index detector and a Biorad Aminex HPX-87H column at 45 °C. Sulfuric acid 0.01 N at a flow rate of 0.6 mL/min was used as eluent, and the injection volume was 20 μL. Furfural and hydroxymethylfurfural concentrations were also

determined by HPLC, using a UV–VIS detector at 280 nm and a Hewlett-Packard RP18 column at 25 °C. Acetonitrile: water (1:8) supplemented with 1 % acetic acid was used as eluent at flow rate of 0.8 mL/min. The injection volume was 20 μL. The profiles of UV light absorption of the aqueous and alcoholic extracts were determined in an UV–VIS spectrophotometer, after appropriate dilution. The results were expressed in terms of relative absorbances, which were calculated by multiplying the absorbance values at the different wavelengths by the dilution factors used in each determination (Carvalho et al. 2008). The content of sugars in the extracts were determined by the phenol–sulfuric acid method (Dubois et al. 1956), using glucose as standard.

10.2.1.2 Pretreatment of the Sugarcane Bagasse with Dilute Sulfuric Acid

Sugarcane bagasse A, as received from the mill, was used in the pretreatments, which were performed in a pilot reactor (total capacity of 100 L) heated by direct steam. In each experiment, an initial mass of 20 kg, including bagasse (15 % dry mass basis), sulfuric acid, and water was used. The temperature, the acid concentration, and the time of pretreatment at the target temperature (heating and cooling were fast and, consequently, not considered) were varied according to the experimental design shown in Table 10.1. After the pretreatments, the *slurries* were separated into liquid (hemicellulosic hydrolysates) and solid (pretreated solids; pretreated sugarcane bagasse in Fig. 10.1) fractions; the pretreated solids were exhaustively washed with hot water and dried at room temperature, while the hemicellulosic hydrolysates were maintained frozen. The chemical compositions of the pretreated solids and of the hemicellulosic hydrolysates were determined as described previously.

10.2.1.3 Enzymatic Saccharification of the Pretreated Solids

The 20 pretreated solids obtained under the different pretreatment conditions were subjected to enzymatic hydrolysis using a mixture of enzymes characterized previously (Santos et al. 2011). The assays were performed in 125 mL Erlenmeyer flasks containing 12.5 mL of sodium citrate buffer (100 mM, pH 4.8) supplemented with sodium azide (0.02 % w/v), 10 % of solids, and 0.025 g of Tween 20 and 10 FPU of cellulases per gram of bagasse; the final volume of each assay was 25 mL, completed with distilled water. The experiments were performed at 45 °C in a rotatory shaker at 150 rpm. Samples were withdrawn in 24 h intervals, boiled for 5 min and centrifuged for 30 min at 12,000 g. The supernatants were analyzed by HPLC.

Table 10.1 Real and coded values of the independent variables according to the 2^3 central composite full factorial design with 6 central points

Exp	Temperature (°C)	Acid concentration (% w/w)	Time (min)	Temperature (coded)	Acid concentration (coded)	Time (coded)
1	140	1	20	−1	−1	−1
2	160	1	20	1	−1	−1
3	140	3	20	−1	1	−1
4	160	3	20	1	1	−1
5	140	1	40	−1	−1	1
6	160	1	40	1	−1	1
7	140	3	40	−1	1	1
8	160	3	40	1	1	1
9	150	2	30	0	0	0
10	150	2	30	0	0	0
11	150	2	30	0	0	0
12	131.91	2	30	−1.81	0	0
13	168.09	2	30	1.81	0	0
14	150	0.19	30	0	−1.81	0
15	150	3.81	30	0	1.81	0
16	150	2	11.90	0	0	−1.81
17	150	2	48.09	0	0	1.81
18	150	2	30	0	0	0
19	150	2	30	0	0	0
20	150	2	30	0	0	0

10.2.1.4 Fermentation of the Hemicellulosic Hydrolysates

Scheffersomyces stipitis DSM 3651, previously used by Canilha et al. (2010), was employed in the fermentation assays. The inoculum was grown by transferring cells from a malt extract agar slant into a 500-mL Erlenmeyer flask containing 200 mL of synthetic medium consisting of xylose (30 g/L), yeast extract (3 g/L), malt extract (3 g/L), and peptone (5 g/L). The flasks were incubated in a rotatory shaker at 30 °C and 200 rpm for 24 h, and the cells were collected by a 30 min centrifugation at 2,000 *g*; followed by suspension in sterile distilled water.

The 20 hemicellulosic hydrolysates obtained under the different pretreatment conditions had their pHs adjusted to 6 with NaOH and, after removal of the precipitates, were sterilized by autoclaving at 111 °C for 15 min.

The fermentations were carried out in 125-mL Erlenmeyer flasks containing 50 mL of medium and inoculated with 3 g/L cells (dry weight). The fermentation media were composed by the autoclaved hydrolysates supplemented with yeast extract (3 g/L), malt extract (3 g/L), and peptone (5 g/L). The flasks were maintained in a rotatory shaker at 30 °C and 200 rpm for 120 h. Samples were periodically collected to determine the concentrations of sugars and ethanol, by HPLC.

10.2.1.5 Statistical Analysis of the Results

In order to evaluate the effects of the pretreatment conditions on the composition and enzymatic saccharification of the pretreated solids, as well as on the composition and fermentation of the hemicellulosic hydrolysates, empirical models (Eq. 10.1) were adjusted to the experimental data generated according to the experimental design.

$$Y_i = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \qquad (10.1)$$

where Y_i represents the dependent variable, b_0, b_i, b_{ii} and b_{ij} represent the regression coefficients, and x_i and x_j represent the independent variables. The significance of the regression coefficients kept in the models was evaluated considering, as statistically significant coefficients, those that, in general, exceeded the confidence level of 95 %.

10.2.2 Results and Discussion

10.2.2.1 Raw Material, Characterization, and Conditioning

Figure 10.3 presents the particle size distributions determined for bagasses A and B.

The profiles observed for the two bagasses showed that they consist of heterogeneous materials that present polydisperse distributions of particle sizes. Considering this heterogeneity, reduction, and/or fractionation of the raw material before conversion is a strategy described in the literature (Gámez et al. 2006; Hernández-Salas et al. 2009; Pietrobon et al. 2011). Small particle sizes have increased surface areas (Driemeier et al. 2011); moreover, the diffusion of chemical reagents such as dilute H_2SO_4 has been shown to be optimized for smaller particles (Kim and Lee 2002).

Table 10.2 presents the chemical compositions determined for bagasses A and B, before and after the extractions with water followed by ethanol.

Considerable differences were observed in the compositions of the two materials, with bagasse A being poorer in cellulose and richer in ash. Moreover, the extraction with solvents removed a considerable portion of "pseudo-lignin", due to the fact that some extractives can condense and precipitate during the compositional analysis (Hatfield and Fukushima 2005). A similar behavior was already observed for other lignocellulosic materials (Grohmann et al. 1986; Nguyen et al. 2000); and, to remove such interfering compounds, the National Renewable Energy Laboratory (NREL/USA) recommends successive extractions with water and ethanol (Sluiter et al. 2008).

Further analysis of the effects promoted by extraction of the raw material with solvents, performed with bagasse B, showed that the water extraction solubilized

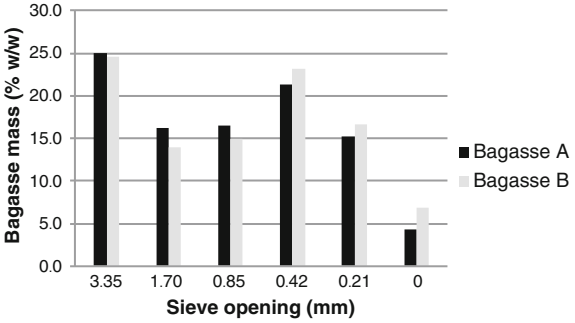

Fig. 10.3 Distributions of particle sizes for bagasses A and B, determined by sieving

Table 10.2 Chemical compositions determined for bagasses A and B, before and after the extractions with water followed by ethanol

Component (% w/w)	Before extraction		After extraction	
	Bagasse A	Bagasse B	Bagasse A	Bagasse B
Cellulose	38.8 ± 0.1	46.4 ± 0.3	38.3 ± 0.3	45.0 ± 0.1
Hemicellulose	26.6 ± 0.0	27.2 ± 0.2	27.8 ± 0.0	25.8 ± 0.1
Lignin	27.9 ± 0.1	24.8 ± 1.0	22.5 ± 2.6	19.1 ± 0.2
Ash	6.7 ± 0.0	1.6 ± 0.2	5.3 ± 0.2	1.0 ± 0.0
Extractives			6.1	9.1

6.0 % of solids, while ethanol extraction led to a similar content of extractives (5.7 %). The sequential extraction with both solvents, however, reduced the dry weight of the raw material in 9.1 %, thus showing that the solvents dissolved, at least in part, structurally different compounds (Table 10.3).

Figure 10.4 shows that the aqueous extracts presented peaks of absorbance at 200 and 280 nm (A), while the alcoholic extracts exhibited peaks at 220 and 315 nm, with a shoulder at 290 nm (B). Although, for the same solvent, 2nd extractions removed much lower amounts of soluble compounds than 1st extractions (A and B), previous extractions of the raw bagasse with water did not seem to have affected the qualitative and quantitative profiles of light absorption by consecutive ethanolic extracts (C).

Considering that peaks of maximum absorbance (λ_{MAX}) near 280 nm are typical of lignin-derived aromatics and that phenolic acids, originally ester-linked to carbohydrates, show a bathochromic shift from 280 nm to a shoulder near 290 nm with $\lambda_{MÁX}$ near 320 nm (Akin 2007), it is supposed that the aqueous and alcoholic extracts were rich in aromatics and phenolic acids, respectively.

As measured by the phenol–sulfuric acid method, the amount of sugars recovered in the aqueous extracts (73.3 mg total sugars/g bagasse) was higher than that recovered in the alcoholic extracts (49.4 mg total sugars/g bagasse), which can be explained by the comparatively higher solubility of sugars in water (Alves et al. 2007). Moreover, the sequential extraction with both solvents increased the recovery of sugars to 99.2 mg total sugars/g bagasse, an unusually high value

Table 10.3 Chemical compositions determined for bagasse B, before and after the extractions with water, ethanol, and water followed by ethanol

Component (% w/w)	Before extraction	After extraction		
		Water	Ethanol	Water + Ethanol
Cellulose	46.4 ± 0.3	44.6 ± 2.0	46.2 ± 0.4	45.0 ± 0.1
Hemicellulose	27.2 ± 0.2	26.2 ± 1.5	27.8 ± 0.3	25.8 ± 0.1
Lignin	24.8 ± 1.0	22.0 ± 0.6	19.3 ± 0.2	19.1 ± 0.2
Ash	1.6 ± 0.2	1.2 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
Extractives		6.0	5.7	9.1

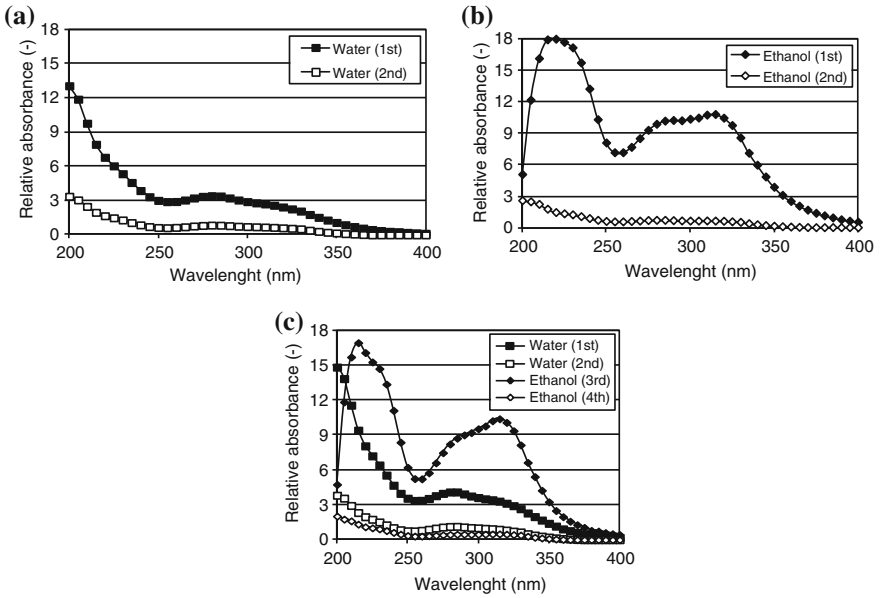

Fig. 10.4 UV-light absorption spectra of the extracts prepared from sugarcane bagasse B. Extracting solvents: **a** (water); **b** (ethanol); **c** (water followed by ethanol)

compared to the content of sucrose expected to be found in this industrial byproduct, 0.2–5.0 % (Tewari and Malik 2007).

Figure 10.5 shows the distributions of particle sizes determined for bagasse A before and after hammer-milling.

As can be seen, the strategy of milling was effective in reducing the proportion of particles with larger sizes, thereby contributing to homogenization by comminuting the material. More than grinding their sugarcane bagasse sample, Gámez et al. (2006) selected particles smaller than 0.5 mm before the pretreatment with dilute H_3PO_4; Hernández-Salas et al. (2009), on the other hand, selected the fraction which passed through a 1.68 mm sieve but was retained by a 0.149 mm sieve.

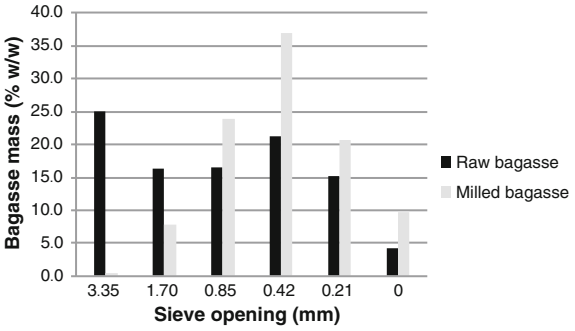

Fig. 10.5 Distributions of particle sizes for bagasse A before and after hammer-milling, determined by sieving

As already mentioned, after milling, bagasse A was fractionated by using a device designed to accomplish the removal of fines with the help of compressed air. As illustrated in Fig. 10.6, the fraction of fines, in fact, exhibited smaller particles in comparison to the milled bagasse. In spite of this, the fractionation was not selective; the major part of the whole mass consisted in particles retained by sieves with openings bigger than or equal to 0.21 mm.

Samples of both fractions were extracted with water followed by ethanol, and, subsequently, submitted to compositional analysis. The relative contents of some constituents are presented in Table 10.4.

Significant differences ($p < 0.05$) were observed for the contents of water extractives, xylan, insoluble lignin, acetyl, and ash. While the fines presented higher contents of water extractives and ash, the bagasse without fines exhibited higher contents of xylan, insoluble lignin, and acetyl groups. In turn, the total content of sugars determined in the extract prepared from the fraction of fines was higher than that determined in the extract prepared from the fraction without fines, of 2.5 % (dry mass basis). Sanjuán et al. (2001) also demonstrated that, when the sugarcane bagasse was extracted with hot water, the amount of extractives depended on the nature of the sample in terms of constituent cell types. While 10.3 % of solids were solubilized from pith cells, the amount of compounds extracted from vascular bundles did not exceed 1.1 %. When ethanol was used as solvent, the contents of extractives were of 3.4 and 1.1 %, respectively.

Considering the aforementioned, it is important to point out that the production of goods from lignocellulosic feedstocks by biochemical means can be strongly influenced by the nature and content of both structural and nonstructural constituents. For example: the extractives can be either toxic compounds (Venalainen et al. 2004) or fermentable carbohydrates that can undergo degradation reactions under high temperatures and low pHs (Bower et al. 2008); a high content of ash imply in higher consumption of acid during the pretreatment (Linde et al. 2006); a reduced content of lignin can make the structural carbohydrates more accessible to

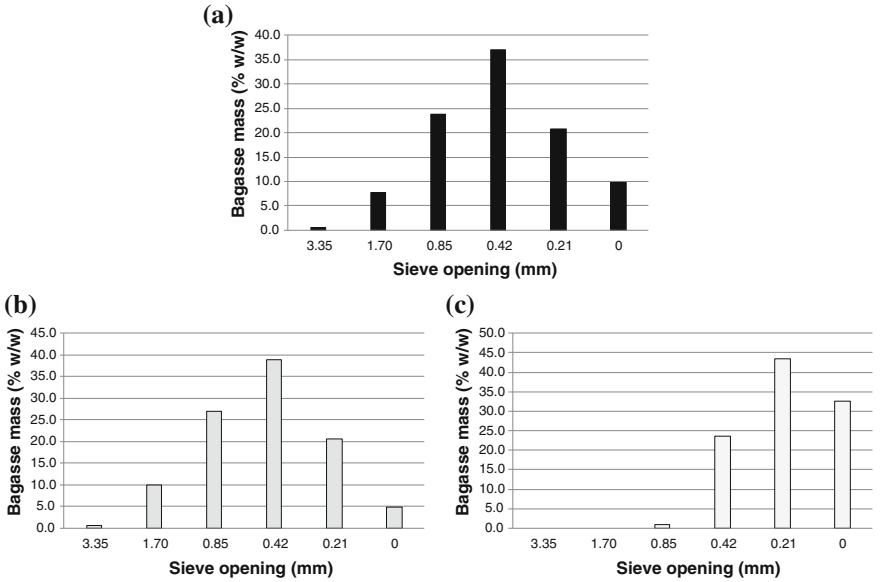

Fig. 10.6 Distributions of particle sizes for bagasse A before and after fractionation, determined by sieving: **a** (milled bagasse); **b** (milled bagasse, fraction without fines); **c** (milled bagasse, fraction of fines)

Table 10.4 Chemical compositions determined for the fractions of bagasse A, generated by using the device shown in Fig. 10.2

Component (% w/w)	Fraction without fines	Fraction of fines
Extractives (water)	–	12.2 ± 1.0
Extractives (ethanol)	–	2.6 ± 1.6
Glucan	–	–
Xylan	–	14.3 ± 0.1
Arabinan	1.6 ± 0.1	–
Soluble lignin	3.7 ± 0.1	–
Insoluble lignin	–	11.8 ± 1.2
Acetyl	3.5 ± 0.3	–
Ash	1.7 ± 0.4	–

enzymatic hydrolysis (Yang et al. 2009; Siqueira et al., 2011); and so on. Therefore, appropriate selection of the material that will be used as raw material during the conversion may be advantageous.

10.2.2.2 Effects of the Pretreatment Conditions on the Composition and Enzymatic Saccharification of the Pretreated Solids, and on the Composition and Fermentation of the Hemicellulosic Hydrolysates

The compositions of the different pretreated solids and the corresponding cellulose-to-glucose conversions with enzymes, as well as the concentrations of xylose and furfural in the respective hemicellulosic hydrolysates and the corresponding sugars-to-ethanol conversions with yeasts, are presented in Table 10.5.

Regarding the compositions of the pretreated solids, the hemicellulose content showed the greatest variation in function of the experimental conditions employed during the pretreatment, with relative contents varying from 0.14 to 17.62 % (dry mass basis). Cellulose and lignin were much less solubilized, and their relative contents varied from 52.38 to 65.81 % and from 21.25 to 35.48 %, respectively.

The effects of the pretreatment conditions on the content of hemicellulose and on the efficiency of cellulose saccharification after 24 h of hydrolysis were analyzed statistically (Table 10.6).

Acid concentration was the variable that affected the hemicellulose content the most, followed by temperature and time. Hsu et al. (2010) also observed that temperature and H_2SO_4 concentration were the major variables influencing the hemicellulose content in rice straw pretreated with dilute H_2SO_4.

In turn, cellulose conversion into glucose was influenced mostly by temperature, followed by acid concentration. A similar behavior was observed by Cai et al. (2012) when studying the pretreatment of corncobs with dilute H_2SO_4.

The empirical model proposed to explain the effects of the pretreatment variables on the hemicellulose content (coefficients shown in Table 10.6) was reduced by setting the time of pretreatment at maximum, giving rise to Eq. 10.2. For predicting the cellulose conversion after 24 h of hydrolysis as a function of the pretreatment conditions, the values of the regression coefficients shown in Table 10.6 were used directly (Eq. 10.3).

$$\mathrm{Hc} = 2.28 - 2{,}95\,A - 3{,}06\,B + 1{,}51\,B^2 \tag{10.2}$$

$$\mathrm{C_c} = 49.09 + 5.97\,A + 4.08\,B - 3.23\,B^2 \tag{10.3}$$

where Hc is the hemicellulose content and C_C is the efficiency of cellulose conversion after 24 h of hydrolysis, both obtained as functions of the coded values of temperature (A) and acid concentration (B).

The response surfaces corresponding to the above mentioned models are shown in Fig. 10.7.

As can be seen, the increase in the acid concentration and in the temperature used during the pretreatment with dilute H_2SO_4 led to pretreated solids with lower hemicellulose contents and, thus, exhibited improved cellulose saccharification efficiencies. In practice, the maximal efficiency of cellulose saccharification (63.76 % after 24 h; 87.72 % after 72 h) was achieved in experiment 8 (pretreated

Table 10.5 Chemical compositions and yields of cellulose saccharification after 24 h of hydrolysis (pretreated solids) as well as xylose and furfural concentrations and yields of sugars conversion into ethanol (hemicellulosic hydrolysates) determined in the experiments of the statistical design (Table 10.1)

Std	Pretreated solids				Hemicellulosic hydrolysates		
	Cellulose (% w/w)	Hemicellulose (% w/w)	Lignin (% w/w)	C_C (%)	Xylose (g/L)	Furfural (g/L)	$Y_{P/S}$ (g/g)
1	53.56	14.51	23.51	30.99	13.04	0.08	0.48
2	57.97	5.93	26.96	55.81	17.29	1.28	0.36
3	62.35	4.61	28.92	49.20	19.53	1.09	0.38
4	63.94	0.79	31.77	54.82	13.52	3.73	0
5	59.41	9.05	29.18	44.37	16.59	0.48	0.39
6	64.01	1.82	29.85	53.28	14.08	3.53	0
7	61.64	5.38	30.21	44.90	20.53	1.66	0
8	59.76	0.14	35.48	63.76	7.02	4.40	0
9	62.02	5.47	29.55	49.76	11.71	1.02	0.40
10	61.62	5.88	29.28	42.69	19.61	1.40	0.36
11	60.86	5.48	29.46	52.05	19.71	1.32	0.36
12	55.31	11.13	23.43	41.45	18.27	0.34	0.36
13	64.04	1.23	30.05	57.30	1.43	4.68	0
14	52.38	17.62	21.25	25.35	8.61	0.11	0.47
15	61.31	4.28	26.83	42.69	20.13	1.20	0.25
16	58.11	8.78	29.41	39.55	17.94	0.59	0.33
17	65.81	3.04	30.47	45.11	17.07	1.68	0
18	60.28	1.56	30.21	50.30	21.05	1.68	0.26
19	60.34	5.18	30.04	46.34	20.50	2.04	0.30
20	62.13	5.38	28.27	46.17	20.08	1.74	0.32

Table 10.6 Values of the regression coefficients maintained in the models proposed to predict the content of hemicelulose and the efficiency of cellulose saccharification within 24 h of hydrolysis (pretreated solids)

Variable	Hemicellulose content			Cellulose saccharification		
	Coefficient	S. Error	*p*	Coefficient	S. Error	*p*
Constant	4.76	0.53	–	49.09	1.59	–
A	−2.95	0.50	<0.0001	5.97	1.49	0.0010
B	−3.06	0.50	<0.0001	4.08	1.49	0.0146
C	−1.37	0.50	0.0153	–	–	–
B^2	1.51	0.44	0.0035	−3.23	1.31	0.0252
Model			<0.0001			0.0006
Lack of fit			0.3133			0.0894
R^2	0.85			0.65		

A Temperature; *B* Acid concentration; *C* Time

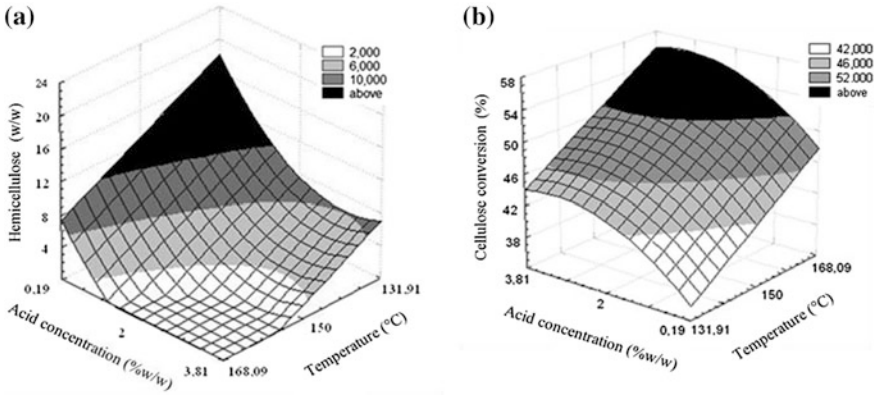

Fig. 10.7 Response surfaces showing the effects of acid concentration and temperature on the content of hemicellulose (**a**) and on the efficiency of cellulose conversion into glucose after 24 h hydrolysis (**b**); pretreated solids

solids exhibiting a hemicellulose content of only 0.14 % w/w), which confirms that the enzymatic conversion of cellulose into glucose depends on the chemical composition of the pretreated solids, which, in turn, depends on the conditions employed during the pretreatment with dilute sulfuric acid.

Back into the data presented in Table 10.5, regarding the compositions of the hemicellulosic hydrolysates, it can be seen that the concentration of xylose ranged from 1.43 to 21.05 g/L; the concentration of furfural, from 0.08 to 4.68 g/L. In turn, the yield of conversion of the major sugars (xylose + glucose) into ethanol varied from 0 to 0.48 g/g.

Table 10.7 shows the values of the regression coefficients of the models proposed to predict the concentrations of xylose and furfural as functions of the levels of the independent variables used during the pretreatment with dilute sulfuric acid.

Regarding carbohydrates, the concentration of xylose was heavily dependent of the temperature used during the pretreatment; the acid concentration was kept in the model because the interaction between temperature and acid concentration was also significant. Regarding the sugar dehydration product, all the three independent variables influenced the furfural concentration significantly ($p < 0.05$); temperature was the most influential variable, followed by acid concentration and time of pretreatment, respectively.

Neureiter et al. (2002) found that the acid concentration, and not the temperature, was the most important variable impacting the xylose yield from sugarcane bagasse, although temperature had a strong influence on furfural generation. Aguilar et al. (2002), on the other hand, observed that both temperature and acid concentration influenced the kinetics of xylose generation from xylan and of xylose degradation into furfural.

The model proposed to explain the effects of the pretreatment conditions on the concentration of xylose in the hemicellulosic hydrolysate is described by Eq. 10.4.

Table 10.7 Values of the regression coefficients maintained in the models proposed to predict the concentrations of xylose and furfural (hemicellulosic hydrolysates)

Variable	Xylose concentration			Furfural concentration		
	Coefficient	S. Error	*p*	Coefficient	S. Error	*p*
Constant	18.61	1.18	–	1.40	0.15	–
A	−3.32	0.91	0.0030	1.20	0.14	< 0.0001
B	1.41	0.91	0.1467	0.51	0.14	0.0026
C	–	–	–	0.40	0.14	0.0128
A^2	−2.55	0.81	0.0076	0.42	0.13	0.0044
B^2	−1.17	0.81	0.1723	–	–	–
AB	−2.66	1.23	0.0496	–	–	–
Model			0.0036			< 0.0001
Lack of fit			0.4664			0.1200
R^2	0.70			0.87		

A Temperature; *B* Acid concentration; *C* Time

For predicting the concentration of furfural, the respective model (coefficients shown in Table 10.7) was reduced by setting the time of pretreatment at maximum (Eq. 10.5).

$$C_X = 18.61 - 3.32A + 1,41B - 2.55A^2 - 1.17B^2 - 2.66AB \quad (10.4)$$

$$C_F = 2.12 + 1.20A + 0.51B + 0.42A^2 \quad (10.5)$$

where C_X and C_F are the concentrations of xylose and furfural in the hemicellulosic hydrolysate, both obtained as functions of the coded values of temperature (*A*) and acid concentration (*B*).

The response surfaces corresponding to the abovementioned models are shown in Fig. 10.8.

As can be seen, the use of high acid concentration in association with low temperature during the pretreatment with dilute sulfuric acid increased the xylose concentration in the hemicellulosic hydrolysate; high temperatures, however, caused degradation of xylose into furfural.

Table 10.8 shows different combinations of the pretreatment variables, encoded at their maximum and minimum levels, and the respective predictions for the content of hemicellulose and the enzymatic conversion of cellulose into glucose after 24 h of saccharification, as well as for the xylose and furfural concentrations in the correspondent hemicellulosic hydrolysates.

As illustrated, the conditions of pretreatment of the sugarcane bagasse with dilute H_2SO_4 that maximize the concentration of xylose in the hemicellulosic hydrolysate and the enzymatic saccharification of cellulose in the pretreated solids are different. To maximize the xylose concentration (23.70 g/L), the pretreatment needs to be carried out with maximum acid concentration associated with minimal temperature, regardless of time of pretreatment. On the other hand, to achieve maximum cellulose saccharification (56.79 %), the pretreatment needs to be

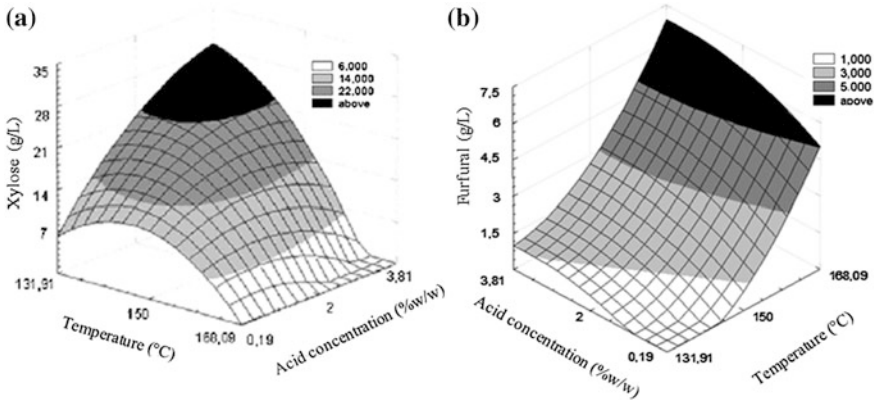

Fig. 10.8 Response surfaces showing the effects of acid concentration and temperature on the concentrations of xylose (**a**) and furfural (**b**); hemicellulosic hydrolysates

Table 10.8 Predictions of the levels of the dependent variables (C_X, C_C, H_C and C_F) as functions of the levels of the independent variables (A, B and C)

A (°C)	B (% w/w)	C (min)	C_X (g/L)	C_C (%)	H_C (%)	C_F (g/L)
−1.81	−1.81	−1.81	1.20	20.34	23.12	0
1.81			6.61	41.96	12.47	3.27
−1.81	1.81		23.70	35.17	12.04	0.79
1.81			0	56.79	1.39	5.14
−1.81	−1.81	1.81	1.20	20.34	18.18	0.38
1.81			6.61	41.96	7.54	4.73
−1.81	1.81		23.70	35.17	7.10	2.24
1.81			0	56.79	0	6.59

A Temperature; B Acid concentration; C Time; C_X and C_F xylose and furfural concentrations in the hemicellulosic hydrolysate; H_C Hemicellulose content in the pretreated solids; C_C Enzymatic conversion of cellulose into glucose within 24 h of hydrolysis

carried out with maximum acid concentration associated with maximum temperature, also regardless of time. This behavior may be due to the fact that, when the pretreatment is conducted at high temperature, the extent of hemicellulose removal is high, which improves the cellulose saccharification in the pretreated solids. Under such severe conditions of pretreatment, however, xylose is dehydrated to furfural, diminishing the xylose concentration in the hemicellulosic hydrolysate. A similar behavior was observed for rice straw (Hsu et al. 2010) and switchgrass (Shi et al. 2011).

As a real example obtained in the present study: a high concentration of furfural (4.40 g/L) and a low concentration of xylose (7.02 g/L) were observed in experiment 8; which, however, as already pointed out, led to solids highly digestible.

Table 10.9 Values of the regression coefficients maintained in the model proposed to predict the yield of sugars (xylose + glucose) conversion into ethanol (hemicellulosic hydrolysates)

Variable	Coefficient	S. Error	*p*
Constant	0.280	0.03	–
A	−0.110	0.03	0.0010
B	−0.086	0.03	0.0049
C	−0.098	0.03	0.0019
A^2	−0.046	0.02	0.0636
Model			0.0002
Lack of fit			0.0384
R^2	0.75		

A Temperature; *B* Acid concentration; *C* Time

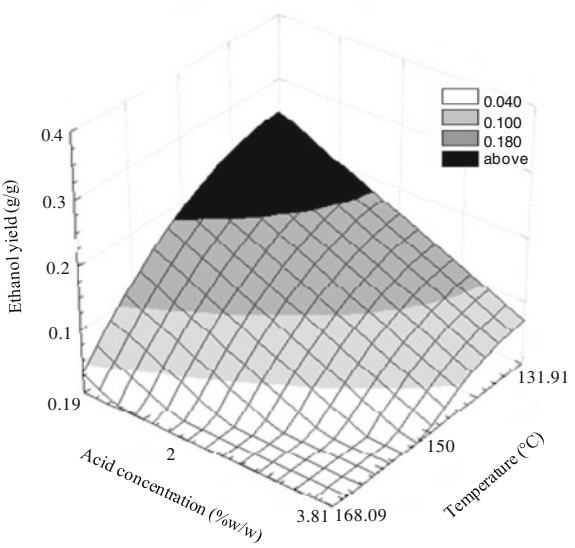

Fig. 10.9 Response surface showing the effects of acid concentration and temperature on the yield of sugars (xylose + glucose) conversion into ethanol; hemicellulosic hydrolysates

Last, but not least, Table 10.9 shows the values of the regression coefficients of the model proposed to predict the yield of sugars (xylose + glucose) conversion into ethanol as a function of the levels of the independent variables used during the pretreatment with dilute sulfuric acid.

As can be seen, all the three independent variables influenced significantly ($p < 0.05$) the bioconversion of the major sugars contained in the hemicellulosic hydrolysate into ethanol; temperature, again, was the most influential variable.

In order to elaborate the response surface shown in Fig. 10.9, the complete model that correlates the level of the dependent variable with the levels of the independent variables was simplified by setting the time of pretreatment at maximum (Eq. 10.6).

$$Y_{P/S} = 0.10 - 0.11A - 0.09B - 0.05A^2 \quad (10.6)$$

where $Y_{P/S}$ is the yield of sugars (xylose + glucose) conversion into ethanol, obtained as function of the coded values of temperature (A) and acid concentration (B).

The data show that the efficiency of converting sugars into ethanol exhibited by the yeast *Scheffersomyces stipitis* in the hemicellulosic hydrolysate is optimized when the pretreatment is carried out under conditions of low severity, due to the low content of inhibitory compounds. This conclusion is supported by the manuscript written by Scordia et al. (2010), which reports data obtained when pretreating *Saccharum spontaneum* with oxalic acid and fermenting a selected hemicellulosic hydrolysate with *Scheffersomyces stipitis* CBS 6054.

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Chapter 11
Scale-up Pretreatment Studies on Sugarcane Bagasse and Straw for Second-Generation Ethanol Production

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Abstract Sugarcane juice-derived ethanol (1G ethanol) has been the major renewable energy source in Brazil after the inception of National Alcohol Program in 1970. The remaining part, after the processing of sugarcane and extraction of juice (sugarcane bagasse-SB and straw-SS), are the promising sugar feedstock for cellulosic ethanol (2G ethanol) due to their abundant availability round the year and high energy content. However, sugar recovery from lignocellulosic biomass is not easy and needs intensive processing. Pretreatment to overcome the recalcitrance of these feedstocks and sugar recovery constitute almost 30 % cost of 2G ethanol production. Several pretreatment methods have been studied recently aiming to either lignin removal or hemicellulose from SB/SS for the subsequent enzymatic hydrolysis for fermentable sugar production. However, steam explosion and dilute sulfuric acid have been emerged out as two successful options for the pretreatment of SB/SS. Pilot level studies at our institute (Laboratório Nacional de Ciência e Tecnologia do Bioetanol—CTBE, Campinas, Brazil), for the pretreatment of SB/SS considering steam explosion and dilute acid pretreatment, have shown the promising results. Both the pretreatment strategies are scalable and reproducible at the commercial level. This chapter deals with the experiments made on SB/SS for the steam explosion and dilute acid hydrolysis and the sugar recovery after enzymatic hydrolysis. Furthermore, process configurations for saccharification of pretreated biomass and the conversion of released sugars into ethanol have also been discussed.

Keywords Sugarcane bagasse · Sugarcane straw · Scale-up pretreatment · Enzymatic hydrolysis · Ethanol production

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11.1 Introduction

Contemporary industrial developments increased energy demands, and rapid pace of urbanization have necessitated for the search of economic and environmentally sustainable energy sources. Ethanol made from either sugar from sugarcane, corn, sugar beet (1G ethanol) is a well-established process in countries like USA, Brazil, China, India, and others have shown a promising option for transportation fuel. However, the food versus fuel concerns have alarmed the researchers and policy makers to implement the ethanol production from the second-generation feedstocks like sugarcane bagasse, corn stover, grasses, and dedicated energy crops. Biomass-derived cellulosic ethanol may provide unique environmental, economic strategic benefits, and can be considered as a safe and cleanest liquid fuel alternative to fossil fuels (Goldemberg 2008). Brazil and USA are the major ethanol producing countries in the world from sugarcane juice and corn grains, respectively. Brazilian mills is likely to produce around 163–169 million tons of sugarcane bagasse and 84 million tons of straw in the 2012/13 harvest (Canilha et al. 2012). Undoubtedly, SB is a preferred source of co-generation of heat and power in sugarcane processing industries for the sugars and first-generation ethanol production. However, the remaining amount of SB or SS can be used for the 2G ethanol production for the fullest valorization of biomass (Chandel et al. 2012a). For the effective conversion of lignocellulosic material into ethanol, there are three major steps involved first, thermochemical pretreatment—a preprocessing step that improves enzyme access to the cellulose; second enzymatic saccharification—use of cellulases and hemicellulases; and third, fermentation of released sugars by specialized organisms.

Pretreatment is an important tool for practical cellulose conversion processes, and has a strong impact on ethanol production from lignocellulosics following the biorefinery concept (Galbe and Zacchi 2002). The goal of pretreatment is either to break the lignin seal or hemicellulose removal and disrupt the crystalline structure of cellulose for the maximum sugar recovery after enzymatic action (Taherzadeh and Karimi 2007; Yang and Wyman 2008). Several pretreatment technologies have been investigated for the pretreatment of SB/SS (Chandel et al. 2012b). However, steam explosion and dilute acid hydrolysis of SS/SB have presented the most promising results in terms of improved sugar recovery from the substrates upon cellulase-mediated action.

During steam explosion pretreatment, biomass is heated with saturated steam, followed by a sudden decompression of the pressurized system. Thus, steam penetrates into the lignocellulosic matrix and condensates to form liquid water inside the fibers which is rapidly evaporated causing the explosion of fibers. Acetyl groups are hydrolyzed and the released acetic acid mechanistically acts on hemicellulose into monomeric products (Hu and Ragauskas 2012). Additionally, partial degradation of lignin or delocalization of lignin moities is also possible increasing the accessibility of cellulases enzymes toward the substrate for the hydrolysis of cellulose into glucose (Chen et al. 2007).

Dilute acid hydrolysis is another method which is particularly well suited for the pretreatment of SB/SS. Dilute mineral acids such as sulfuric acid effectively solubilize the hemicellulose fraction of the cell wall at high temperature eventually increasing the accessibility of cellulolytic enzymes action to the carbohydrates present in the pretreated substrate (Santos et al. 2011). The remaining cell wall fraction after dilute acid hydrolysis is called cellulignin which have shown 60 % sugars recovery after enzymatic hydrolysis. The hemicellulose fraction of SB/SL after steam explosion or dilute acid hydrolysis is depolymerized primarily into pentose sugars (xylose and arabinose) and hexose sugars (glucose, galactose, mannose, etc.) along with inhibitory compounds (Canilha et al. 2012). The effectiveness of steam explosion and dilute acid hydrolysis as pretreatment for SB and SS has been shown in laboratory- and pilot scale experiments (Rocha et al. 2011, 2012a, b).

The enzymatic hydrolysis is a promising and environmentally feasible method for saccharification of lignocellulosics to sugars. Further, in order to develop integrated process configurations, enzymatic hydrolysis and fermentation of released sugars may be combined in a single vessel, the so-called simultaneous saccharification and fermentation (SSF), enzymatic hydrolysis, and co-fermentation of pentose and hexose sugars by single or mixed microorganisms (SSCF). In the line to advance the process intensification, process like consolidated bioprocessing (CBP) have been emerged wherein the enzyme production, enzymatic hydrolysis, and co-fermentation of released sugars into ethanol is possible in single vessel (Lynd et al. 2005). Microbial delignification has given the new idea in order to develop highly integrated process for ethanol production. It is possible for pretreatment of biomass, enzyme production, enzyme hydrolysis, and co-fermentation of released sugars into ethanol in a single vessel. This process may be called as integrated bioprocessing (IBP). The idea of IBP is in nascent stage and is subjected to multidisciplinary research efforts for its realization.

This chapter entails about the experimental outcome of pilot scale studies concerned with steam explosion and dilute acid hydrolysis of sugarcane bagasse and straw. Various process configurations for second-generation ethanol production have also been discussed.

11.2 Pretreatment by Steam Explosion

The steam explosion pretreatment of SB or SS is probably one of the most commonly applied methods among the physical–chemical methods. This technology can achieve high reaction rates has a high potential in many industrial fields: the paper and textile industry, extraction and fermentation biotechnology, fine chemicals, and biodegradable polymer production (Focher et al. 1988; Hendriks and Zeeman 2009; Mosier et al. 2005; Hu and Ragauskas 2012).

Pretreatment by steam explosion has been proposed as one of the most promising methods in the separation of the main components of lignocellulosic materials: cellulose and hemicellulose. When subjected to high steam pressures for

certain period of time, this material suffers a process known as self-hydrolysis whereas when it is subjected to high temperatures the links owned by the biomass become weak and brittle in some parts. This process allows, after the decompression, the defibration, and reduction of the material to smaller particles, meaning that the hemicelluloses are hydrolyzed in soluble sugars and the lignin is partially modified, increasing its susceptibility for enzymes and chemical reagents (Martín et al. 2002, 2008; Rocha et al. 2011).

Several works have been reported in the literature showing the technological advances of this technique of pretreatment for plants biomass, aiming a wide range of applications of the main components of this material.

11.2.1 Reaction in a 200L Pilot Reactor

Rocha et al. (2013) studied the pretreatment by steam explosion with sugarcane bagasse for 20 reactions in a 200L pilot reactor with 1.3 MPa pressure, equivalent to 13 kgf·cm^{-2} at 190 °C, and a period of 15 min. The results showed an excellent reproducibility, resulting in an average yield in mass of 66.1 % and a standard deviation of 0.8 %. The average results of the main components of these reactions were 57.5 ± 1.6 % in cellulose, 6.6 ± 1.5 % in hemicellulose, and 32.5 ± 2.4 % in lignins. The solubilization of hemicellulose was an average of 82.7 % with a standard deviation of 4.3 %.

The cellulignin fractions obtained in pretreatment was submitted to an alkaline delignification in a pilot scale. The steam explosion pretreated bagasse was reacted with a NaOH solution 1.0 % (w/v). The delignification reactions were made in a stainless steel 316L reactor with a 350L capacity, fitted with mixing and heating systems, using a solid–liquid ratio 1:10 (w/v). The operation was carried out at 100 °C for 1 h.

The amount of cellulose increased to an average of approximately 87 % and the removal of hemicellulose and lignin exceeded 90 %, showing an excellent removal of lignin from the biomass. It solubilized a 92.7 % average with a standard deviation of 3.9 %. The hemicellulose hydrolysis was 94.7 % with standard deviation of 0.9 %. The process hydrolyzed 31.1 % of cellulose with a deviation of 3.5 %. It evidences that even in milder conditions, the steam explosion pretreatment and alkaline delignification processes causes a substantial cellulose loss. Figure 11.1 shows a flowchart of the separation processes for the main components of SB, with the pretreatment of steam explosion followed by alkaline delignification.

The micrographs of the SB pretreated by steam explosion and delignification are shown in Fig. 11.2.

The micrographs with magnification of 200 times reveals aggregated fibers, due to the complex cellulose-hemicelluloses-lignin-extractives. A high content of marrow flakes is observed which evidences an element ringed at the top that probably came from a xylem vase during the grinding of sugarcane.

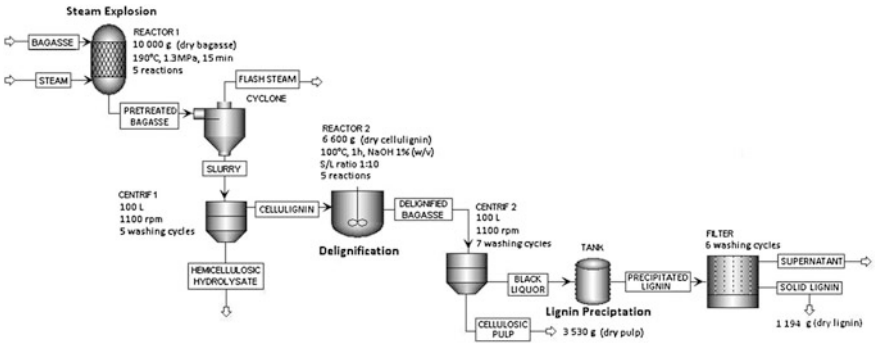

Fig. 11.1 Flowchart of the separation processes for the main components of sugarcane bagasse (Rocha et al. 2013)

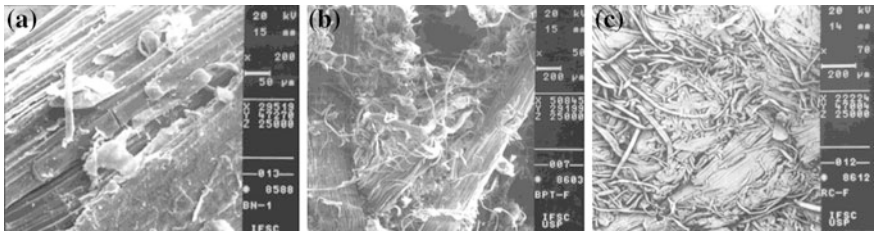

Fig. 11.2 Micrographs of bagasse in natura (**a**), pretreated by steam explosion (**b**), and bagasse pretreated by steam explosion and delignified (**c**)

The fiber characteristic of the SB pretreated by steam explosion showed a structural disintegration and disaggregation, resulting in fiber damage. The surface structure of fibers is apparent from the highest enlargement.

After the removal of main components of vegetable biomass by the processes of delignification of SB pretreated by steam explosion, is observed a total disaggregation of the fibers of bagasse rich in cellulose, evidencing a largest surface of contact and accessibility to chemical or biological attacks, such as enzymes cellulolytic in processes of saccharification.

11.2.2 Steam Explosion Reaction in a 2.5 m^3 Reactor

Industrial-scale steam explosion pretreatment of SS for enzymatic hydrolysis of cellulose for production of second-generation ethanol was studied by Oliveira et al. (2013a). Pretreatment was conducted in a 2.5 m^3 reactor for 15 min at 180, 190, and 200 °C, respectively. The flowchart process is shown in Fig. 11.3.

The chemical composition of raw straw, after the pretreatment and the pretreatment and delignification process, respectively, are shown in Table 11.1.

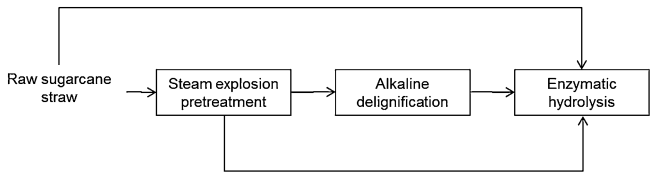

Fig. 11.3 Schematic representation of the processing of sugarcane straw by steam explosion pretreatment, alkaline delignification, and enzymatic hydrolysis

11.2.3 Steam Explosion Reaction in a 5 m^3 Industrial Reactor

Oliveira et al. (2013b) studied the pretreatment of bagasse carried out in the industrial reactors of the Mill. This company has three 5 m^3 reactors of Caldema® for steam explosion pretreatment of SB or SS, as shown in Fig. 11.4. The pretreatment was performed under the 15.5 $kgf \cdot cm^{-2}$ pressure at temperature near a 200 °C for 7 min.

The cellulignin fractions obtained in the pretreatment applied a pilot scale alkaline delignification. The steam explosion pretreated bagasse was reacted with a NaOH solution 1.0 % (w/v). The delignification reactions were made in a stainless steel 316 L reactor with a 350L capacity, fitted with mixing and heating systems, using a solid–liquid ratio 1:10 (w/v). The operation was carried out at 100 °C for 1 h.

The chemical compositions of the pretreated and pretreated and delignified SB are shown in Table 11.2.

Pretreatment indicated a mass yield of 68 %, approximately 78 % hydrolyses of hemicelluloses and solubilizing 20 % of the cellulose without a significant reduction in lignin content. The alkaline delignification had a mass yield of 59.1 %, while lignin content was reduced by 90 %, and hemicelluloses decreased by 95 %.

Currently, major advances have been achieved as the pretreatment by steam explosion, especially as new equipment, such as continuous reactors, which allows to obtain kinetic and thermodynamic pilot scale seeking an extension to an industrial scale (Fig. 11.5).

That justifies the technique as one of the most promising for obtaining second-generation ethanol.

11.3 Hydrothermal Pretreatment

Hydrothermal pretreatment is gaining attention as an environmentally friendly solvent and an attractive reaction media for a variety of applications. In this process, at 150–230 °C range temperatures, lignocellulosic materials undergo hydrolysis reactions in the presence of the hydronium ions generated by water auto-ionization, which act as catalysts.

Table 11.1 Chemical composition of in natura, pretreated, and delignified sugarcane straw samples

Steam explosion pretreatment							
Components (%)	Raw sugarcane straw	PTS 180 °C	PTSD	PTS 190 °C	PTSD	PTS 200 °C	PTSD
Mass yield	100	58	59	57	55	56	54
Cellulose	39.8 ± 0.3	47.8 ± 0.2	73.0 ± 0.2	48.6 ± 0.7	74.8 ± 0.3	48.7 ± 0.5	74.6 ± 0.5
Hemicellulose	28.6 ± 0.2	16.2 ± 0.2	9.5 ± 0.7	7.3 ± 0.2	6.1 ± 0.6	3.7 ± 0.1	5.9 ± 0.4
Lignin	22.5 ± 0.1	32.5 ± 0.1	8.8 ± 0.4	38.1 ± 0.2	10.3 ± 0.3	41.8 ± 0.2	14.0 ± 0.4
Ash	2.4 ± 0.3	3.5 ± 0.1	7.1 ± 0.8	5.2 ± 0.2	8.5 ± 0.8	5.2 ± 0.1	7.0 ± 0.7
Extractives	6.2 ± 0.3	NA	NA	NA	NA	NA	NA
Total	99.6 ± 1.2	100.0 ± 0.7	99.3 ± 2.2	99.1 ± 1.3	99.7 ± 2.0	99.33 ± 1.0	101.3 ± 0.7

PTS Pretreated material with steam explosion
PTSD Pretreated and delignified material
NA Not available
Percentage Mean of three replicate analyses (Oliveira 2012)

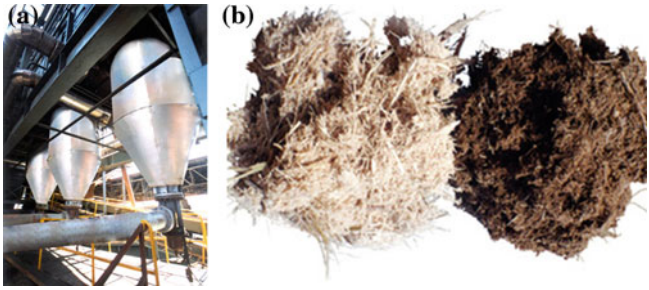

Fig. 11.4 Set reactors of pretreatment by steam explosion with 5 m^3 capacity to treat 3400 kg of bagasse per hour (**a**), and sugarcane bagasse before and after the pretreatment by steam explosion (**b**)

Table 11.2 Chemical compositions of the raw, pretreated, and delignified sugarcane bagasse samples

Steam explosion pretreatment			
Components (%)	Raw sugarcane	PTS	PTSD
Mass yield	100.0	68.0	59.1
Cellulose	43.8 ± 1.1	51.7 ± 0.6	90 ± 2
Polyoses	25.8 ± 0.8	8.9 ± 0.1	3.4 ± 0.3
Lignins	22.1 ± 0.8	34.3 ± 0.3	5.5 ± 0.2
Ash	1.4 ± 0.2	5.5 ± 0.2	1.4 ± 0.1
Extractives	6.1 ± 0.3	NA	NA
Total	99.2 ± 0.8	100.3 ± 0.4	99.9 ± 0.5

PTS Pretreated material with steam explosion
PTSD Pretreated and delignfied material
NA Not available

Fig. 11.5 Continuous reactor of steam explosion AdvanceBio LLC-USA with a capacity of 75 $kg.h^{-1}$

Fig. 11.6 Rotatory reactor, REGMED AU/E-20

11.3.1 Reaction in 20L Reactor

Hydrothermal pretreatment of sugarcane bagasse for production of second-generation ethanol was studied by Silva (2009). The pretreatment was conducted in a 20L reactor (REGMED AU/E-20) indicated on Fig. 11.6, for 10 min at 180, 190, and 195 °C, respectively.

The chemical composition of raw sugarcane, after the hydrothermal pretreatment and after the pretreatment and delignification process, are shown in Table 11.3.

This pretreatment indicated a mass yield of 62.1, 51.7, and 49.6 % for hydrothermal pretreatment performed at 180, 190, and 195 °C, respectively. Approximately, 89 % of hemicellulose was hydrolyzed during this pretreatment at 195 °C.

The heterocyclic ether bonds of hemicelluloses are the most susceptible to this type of reaction, leading both to generation of oligosaccharides and to splitting of the acetyl groups from the hemicellulosic fraction of the raw materials. In further reaction stages, the hydronium ions generated from acetic acid auto-ionization also act as catalysts in the degradation of polysaccharides.

The hydrothermal pretreatment solubilizes almost 25 % of the cellulose content, without a significant reduction in lignin content, however, the cellulose could be largely preserved during hydrothermal pretreatment and the dissolution is low, occurs an enhancement in the cellulose digestibility in the enzymatic hydrolysis, due to the solubilization of the hemicellulose.

The alkaline delignification step hydrolyses almost 80 % of the lignin content, for the cellulignin pretreated in 195 °C, with a mass yield of 59.7 %. Lignin is not significantly solubilized during autohydrolysis, but during the pretreatment a

Table 11.3 Chemical composition of *in natura*, hydrothermal pretreated and delignified sugarcane bagasse samples

Hydrothermal pretreatment							
Components (%)	Raw sugarcane bagasse	PTH 180 °C	PTHD	PTH 190 °C	PTHD	PTH 195 °C	PTHD
Mass yield	100.0	62.1	75.1	51.7	68.6	49.6	59.7
Cellulose	42.8 ± 0.3	54.3 ± 0.3	65.3 ± 0.6	60.8 ± 0.9	73.1 ± 0.6	63.4 ± 1.1	79.2 ± 0.6
Hemicellulose	25.9 ± 0.3	15.4 ± 0.2	12.3 ± 0.1	8.9 ± 0.4	7.1 ± 0.1	5.9 ± 0.1	3.7 ± 0.2
Lignin	22.1 ± 0.2	26.2 ± 0.1	19.8 ± 0.7	24.9 ± 0.7	17.3 ± 0.9	28.5 ± 1.2	14.2 ± 0.3
Ash	1.4 ± 0.1	4.1 ± 0.6	2.9 ± 0.9	5.4 ± 0.1	2.6 ± 0.0	2.1 ± 0.1	3.6 ± 0.4
Extractives	6.1 ± 0.1	NA	NA	NA	NA	NA	NA
Total	98.3 ± 1.0	100.0 ± 1.2	100.3 ± 2.3	100.0 ± 2.1	100.1 ± 1.6	99.9 ± 1.5	100.7 ± 1.5

PTH Pretreated material with hydrothermal pretreatment
PTHD Hydrothermic pretreated and delignfied material (NaOH 1.0 % (m/v), 100 °C for 1 h)
NA Not available
Percentage Mean of three replicate analyses (Silva 2009)

redistribution of the lignin occurs, which causes the lignin to cease acting as a steric barrier to enzymatic hydrolysis (Rohowsky et al. 2013).

The hydrothermal pretreatment is known to form only minor amount of fermentation inhibitor products as long as the pH is kept between 4 and 7. Other fractions of lignocellulosic materials different from hemicelluloses can also react in the presence of water; for example, cellulose and lignin can be partially depolymerized by similar hydrolysis reactions (Garrote et al. 1999; Silva et al. 2011).

11.4 Dilute Acid Pretreatment

Among different types of pretreatment on lignocellulosic materials, the hydrolysis using diluted acid is the most used technical on the removal of hemicellulose and has been applied to various agricultural wastes, using a wide range of catalysts such as: sulfuric, hydrochloric, phosphoric, and nitric acid (Ramos 2003; Seabra 2008; Mussatto and Teixeira 2010). This pretreatment process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing.

11.4.1 Dilute Acid Pretreatment in a 350L Reactor

Silva (2009) performed a diluted sulfuric acid pretreatment of sugarcane straw for production of second-generation ethanol in 350L reactor for 10 min at 120 °C (Fig. 11.7). The chemical composition of raw straw, after the diluted sulfuric acid and after the pretreatment and delignification process are shown in Table 11.4.

The objective of this process is the hydrolysis of sugars present in hemicelluloses: xylose, arabinose, and others, which are water soluble, rendering the cellulose fraction more amenable for a further enzymatic treatment (Hendriks and Zeeman 2009; Gírio et al. 2010), once that the presence of hemicellulose and lignin in lignocellulosic biomass are responsible for reduction of enzymatic saccharification efficiency (Mussatto et al. 2008).

According to Table 11.4, the diluted acid pretreatment performed with sugarcane straw showed almost 22.7, 67.0, and 32 % of cellulose, hemicellulose, and lignin solubilization, respectively. The delignification step improves the lignin solubilization to 67.8 %, summarizing almost 78 % of solubilization in both processes (pretreatment and delignification). The micrographs of the raw sugarcane straw, pretreated by diluted sulfuric acid and the pretreated and delignified are shown in Fig. 11.8.

Micrographs of sugarcane straw prior to pretreatment and delignification process exhibited the recalcitrant external surface. It is necessary to treat the material before the enzymatic or acid hydrolysis to breakdown the cell and tissues, resulting

Fig. 11.7 Picture of the 350L reactor, were a diluted sulfuric acid pretreatment of sugarcane straw were performed for production of second-generation ethanol

Table 11.4 Chemical composition of raw sugarcane straw pretreated by diluted sulfuric acid and pretreated and delignified

Diluted sulfuric acid Pretreatment			
Components (%) Mass yield	Raw sugarcane straw 100.0 %	PTA 120 °C 56.8 %	PTAD 63.1 %
Cellulose	38.1 ± 0.2	51.9 ± 0.1	74.2 ± 0.2
Hemicellulose	29.2 ± 0.3	17.0 ± 0.1	9.1 ± 0.2
Lignin	24.2 ± 0.2	29.0 ± 0.1	14.9 ± 0.2
Ashes	2.4 ± 0.1	1.9 ± 0.0	1.0 ± 0.0
Extractives	5.9 ± 0.2	–	–
Total	99.8 ± 1.0	99.8 ± 0.3	99.2 ± 0.6

PTA Pretreated material with diluted sulfuric acid
PTAD Material pretreated by diluted sulfuric acid and delignfied with NaOH 1.0 % (m/v), 100 °C for 1 h

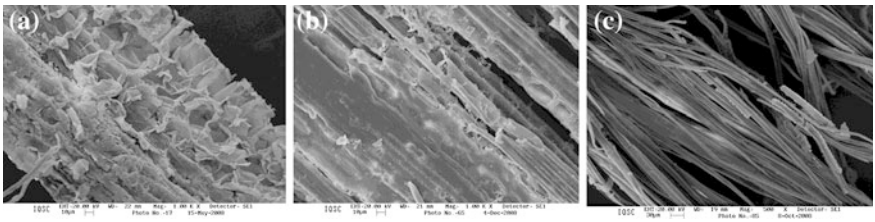

Fig. 11.8 Micrographs of raw (**a**), pretreated by diluted sulfuric acid (**b**) and pretreated and delignified (**c**) sugarcane straw

in "free" cellulose fibers (C). The micrographs also reveal the remotion of parenchyma cells of sugarcane straw after the acid pretreatment.

The acid pretreatment can also be conducted with concentrated acid to increase the solubility of hemicellulose, however, requires intensive care because these

reagents are toxic, corrosive, favor higher solubilized lignin precipitation, increased formation of hydroxymethylfurfural and furfural (degradation products of cellulose and hemicellulose, respectively), and increased release of acetic acid by acetylated hemicellulose, and these compounds act as inhibitors of the fermentation process for ethanol production (Fengel and Wegener 1989; Shevchenko et al. 1999; Liu and Wyman 2003; Ramos 2003). Of all chemical pretreatments, historically dilute sulfuric acid (0.5–1.5 %, T above 160 °C) has been most favored for industrial application, because it achieves reasonably high sugar yields from hemicellulose: at least xylose yields of 75–90 % (Hamelinck et al. 2005; Seabra 2008), and also improves the subsequent process which is the enzymatic hydrolysis of cellulose (Yu et al. 2008).

After the processes of removing or altering the recalcitrant lignocellulosic biomass structures, via pretreatment, the substrate dramatically changes and become more susceptible to acid and/or enzymatic hydrolysis. Many pretreatments act differently on the cell wall structure, with varied results as cellulosic microfibrils exposure and lignin removal (Joshi et al. 2011; Iranmahbooba et al. 2002).

Usually, defined by the cleavage of chemical bonds by the addition of water (Eq. 11.1), the main step of the hydrolysis process is to generate fermentable monomeric sugars from biomass cellulose content by the following reaction (Wyman et al. 2005).

$$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6 \tag{11.1}$$

Thus, each glucose unit in the long chain combines with a water molecule, and 180 mass units of glucose are released from 162 mass units of glucan and 18 mass units of water, an 11.1 % mass gain (Wyman et al. 2005).

The cellulose hydrolysis is carried out by cellulase enzymes which are composed mainly by (1) endoglucanase, (2) exoglucanase or cellobiohydrolase, and (3) β-glucosidase. The endoglucanases cleaves the "middle" β-1, 4-glycosidic bonds on cellulose chains to form glucose, attacking randomly; the exoglucanases or cellobiohydrolases attack the nonreducing end of cellulose chain to form the cellobiose units, cellobiose are the unit formed by 2 glucose linked by a glycosidic linkage, and the β-glucosidases or cellobiase converts cellobiose into D-glucose (Joshi et al. 2011; Zhang and Lynd 2004; Liu et al. 2009). In general, the enzymatic hydrolysis consists of three steps: adsorption of enzymes onto the surface of the substrate, the biodegradation of cellulose to fermentable sugars, and desorption of the enzymes (Fig. 11.9) (Sun and Cheng 2002).

Table 11.5 shows the values of enzymatic conversion, for pretreated and pretreated and delignified samples of sugarcane straw and sugarcane bagasse.

The biomass recalcitrance, due to the presence of hemicellulose and/or lignin in the sample, affects directly the cellulose conversion. The enzymatic conversion of sugarcane straw pretreated by steam explosion showed at 200 °C the high cellulose yield conversion. As noticed for all pretreatments the enzymatic conversion proportionally increases with the raise of pretreatment temperature, due to the presence of different contents of hemicellulose and/or lignin in the sample which

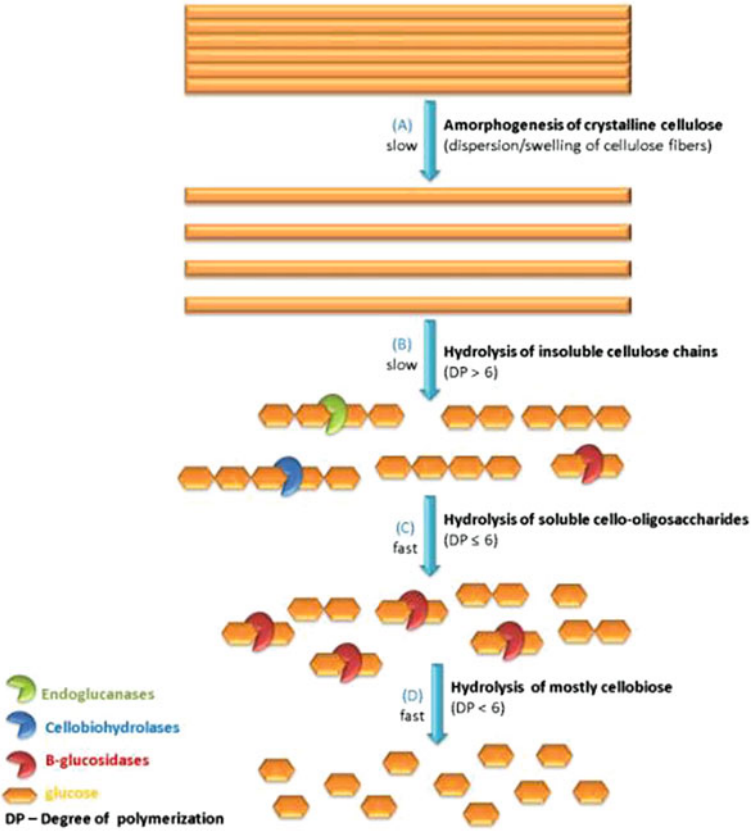

Fig. 11.9 Proposed mechanism for cellulose depolymerization by cellulases (Arantes and Saddler 2010)

affects directly the cellulose conversion. The sugarcane bagasse and straw cellulignin obtained from steam explosion pretreatment performed at 200 °C showed 52 and 80 % of cellulose conversion, respectively.

According to Santos et al. (2012), the enzymatic hydrolysis efficiency, from hardwoods, was correlated to the wood chemical composition and lignin characteristics, with lignin content, enzyme adsorption on substrate and, the ratio of syringyl/guaiacyl of the substrate as the most important key features. The lignin content cannot explain a correlation with enzymatic hydrolysis, but several studies showed several changes in cellulose conversion, resulted from lignin removal (Kooa et al. 2012).

The delignification step of the sugarcane straw pretreated by steam explosion and diluted sulfuric acid load to a significant enhancement of the enzymatic hydrolysis of the material pretreated at 180 °C. However, the effect of delignification was not similar for all the pretreatment conditions. Differently for the cellulignin yield conversion, the improvement of the enzymatic convertibility after delignification decreased with the delignification process temperature.

Table 11.5 Cellulose conversion by enzymatic hydrolysis, for pretreated, and delignified samples of sugarcane straw and sugarcane bagasse

Enzymatic conversion								
Hydrolysis conditions	15 FPU/g of celluclast and 10 UI/g of β-glicosidase							
Pretreatment	Steam explosion				Diluted sulfuric acid	Hydrothermal		
Samples	Sugarcane straw[a]			Sugarcane bagasse[b]	Sugarcane straw[c]	Sugarcane bagasse[c]		
PT conditions	1	2	3	4	5	6	7	8
Raw material	16.0 ± 1			15.0 ± 0.3	7.7 ± 1.3	6.0 ± 0.3		
PT	58.8 ± 1	69.7 ± 2	80.0 ± 2	52 ± 2	51.4 ± 3	37.4 ± 0.5	56.9 ± 0.7	69.2 ± 2.6
PTD	85.1 ± 4	73.0 ± 1	71.5 ± 4	76.0 ± 0.7	85.0 ± 0.5	73.0 ± 0.7	82.3 ± 0.6	89.2 ± 2.2

1 PTS in a 2.5 m^3 reactor for 15 min at 180 °C; *2* PTS in a 2.5 m^3 reactor for 15 min at 190 °C; *3* PTS in a 2.5 m^3 reactor for 15 min at 200 °C; *4* PTS in 5 m^3 industrial reactor at 200 °C for 7 min, *5* PTA in H_2SO_4 1 % (m/v), 120 °C, 10 min, *6* PTH 180 °C in a 20L reactor for 10 min; *7* 190 °C in a 20L reactor for 10 min; *8* 195 °C in a 20L reactor for 10 min

[a] Oliveira et al . 2012

[b] Oliveira et al. 2012, 2013a

[c] Silva et al. 2009

Concerning the low enzymatic rate, many hypotheses have been proposed, such as lignin content, enzymatic activity, temperature, pH, enzyme source and enzyme concentration, the linkage between lignin and carbohydrates, the hydrophobic interaction between lignin and enzyme, enzyme inactivation, substrate accessibility and reactivity cellulose crystallinity enzyme synergism, surface obstacles, low porosity, and fractal nature of the substrate (Caminal et al. 1985; Monney et al. 1998; Chen et al. 2007; Xu and Ding 2007; Kumar and Wyman 2009; Park et al. 2010; Jalak and Väljamäe 2010; Wada et al. 2010; Kurasin and Väljamäe 2011; Paul and Teli 2011; Bansal et al. 2012). The important key enzymatic hydrolysis development is to identify the main causes for the slowdown enzymatic rate: key factors that have remained challenging (Bansal et al. 2012).

According to Santos et al. (2012), the enzymatic hydrolysis efficiency, from hardwoods, was correlated to the wood chemical composition and lignin characteristics, with lignin content, enzyme adsorption on substrate, and the ratio of syringyl/guaiacyl of the substrate as the most important key features (Santos et al. 2012). The lignin content cannot explain a correlation with enzymatic hydrolysis, but several studies showed several changes in cellulose conversion, resulted from lignin removal (Kooa et al. 2012).

The Crystallinity index of cellulose has been reported as one of the most important structural parameter and the major limiting factor during enzymatic hydrolysis (Chang and Holtzapple 2000; Park et al. 2010), although the overall carbohydrate conversion of enzymatic hydrolysis did not showed a correlation with lignin removal and sample crystallinity index (Yu et al. 2011; Ioelovich and Morag 2011).

Another important affecting factor of the enzymatic hydrolysis is drying of the wet sample. The difference between the enzymatic conversion of the nondried and dried lignocellulosic (cellulose) samples is due to the fact that drying process can cause an irreversible collapse of the pore structure, decreasing the hydrolysability and the cellulose access (Ioelovich and Morag 2011).

The major limitations in the commercialization of second-generation ethanol biofuel, by breakdown of cellulose with enzymes, are their high cost, low-specific activity, and slow rates of hydrolysis (Bansal et al. 2012). The enzymes contribute in about 25 % of the biomass conversion process, excluding feedstock cost, to obtain biofuels (NREL 2012).

Because of its specificity, a lot of research groups and biotechnology companies are focused on the improvement of the enzymatic hydrolysis process, by protein engineering (Himmel et al. 2007) and substrate engineering, to make the lignocellulosic substrate less recalcitrant to enzymatic action, and provide enzymes with more hydrolyzation capability (Ragauskas et al. 2006; Bansal et al. 2012). It is shown in Table 11.6 the enzymatic hydrolysis of different biomass as the respective yields of conversion.

Table 11.6 Enzymatic hydrolysis of vegetal biomass

Hydrolysis of biomass cellulose				
Lignocellulosic subtrate	Type of hydrolysis and conditions	Catalyser dosage	Sugar conversion (%)	References
Sugarcane bagasse	Enzymatic hydrolysis substrate condition: sugarcane bagasse pretreated by acid followed by the step with NaOH 1 %	25 FPU Accellerase 1500 and 50 UI of Beta-Glucanase per gram of biomass	The total cellulose conversion increases significantly from 22.0 % (value for the untreated bagasse) to 72.4 %	Rezende et al. (2011)
Sunflower stalks	Enzymatic hydrolysis substrate condition: sunflower stalks pretreated by hydrothermal pretreatment	23–31 FPU celuclast, 15 IU β-glucosidase per g of pretreated substrate	90 % glucose conversion can be obtained after 72 h enzyme action on pretreated sunflower stalks at 220 °C	Diaz et al. (2011)
Corn stover	Enzymatic hydrolysis substrate condition: pretreated corn sttover	15 FPU cellulase per g cellulose	One-stage hydrolysis reach a yield of 62.8 % (72 h), 70.2 % with enzyme recycling and 76.1 % with the supplement of fresh enzyme to eliminate enzyme recovery procedure, were obtained in 24 h	Yang et al. (2010)
Bagasse pulp	Enzymatic hydrolysis substrate condition: bagasse pulp prepared from the treatment process with active oxygen and MgO-based solid alkali	15 IU/g of Fusarium oxysporum enzyme extract	82.38 % sugar yield	Xiea et al. (2013)
Wood	Enzymatic hydrolysis substrate condition: cellulose fiber from wood pulp	25 FPU celluclast and 123 CBU Novozym 188 continuous additions every 2 h	First: continuous addition of substrate during hydrolysis—increase by 50 % the concentration hydrolyzed products second: continuous and simultaneous addition of enzyme and substrate during hydrolysis step led to very high concentrated hydrolysates (170 g/l)	Jacquet et al. (2012)
Sugarcane straw	Enzymatic hydrolysis substrate condition: sugarcane straw pretreated by steam explosion at 180, 190 and 200 °C for 15 min and pretreated and delignified straw by sodium hydroxide	15 FPU.mL celluclast, 10 IU g Novozym 188	Delignification increase the enzymatic conversion (from 58.8 % in the cellulignin to 85.1 % in the delignificated pulp)	Oliveira et al. (2013b)

11.5 Microorganism for Cellulosic Ethanol Production: An Asset in Biorefinery

The sugar syrup obtained after thermochemical or enzymatic hydrolysis of lignocellulosic materials is used for ethanol fermentation. Lignocellulose hydrolysates contain a variety of sugars, i.e., glucose, xylose, cellobiose, xylose, mannose, and others, however, glucose and xylose are the principle sugars in hydrolysates comprising more than 90 % (Hahn-Hägerdal et al. 2007). In order to obtain the desired ethanol yields and productivities, it is necessary to convert maximum amount of sugars into ethanol. Therefore, the ideal organism for the production of ethanol from lignocellulosic hydrolysate would be the one, which can utilize various forms of sugars generated by lignocellulose hydrolysis. The ability to ferment pentoses along with hexoses is not wide spread among microorganisms (Chandel et al. 2011).

Conventional ethanol producers in industries such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* are capable of converting only hexose sugars to ethanol. Yeasts such as *Candida shehatae, Pichia stipitis*, and *Pachysolen tanophillus* have shown abilities for the conversion of xylose and glucose into ethanol (Hahn-Hägerdal and Pamment 2004). However, their low ethanol and substrate tolerance and poor ethanol productivities make them a limited choice for cellulosic ethanol production at industrial scale. Of the various xylose-fermenting yeasts, *P. stipitis* has shown greater ethanol production than *C. shehatae*. This was due to the increased uptake of xylose, glucose, mannose, cellobiose, and galactose (Chandel et al. 2011). Commercial exploitation of these yeasts for ethanol production from xylose is restricted mainly by their low ethanol tolerance, slow rates of fermentation, difficulty in controlling the rate of oxygen supply at the optimal level plus sensitivity to inhibitors generated during pretreatment, and hydrolysis of lignocellulosic substrates (Hahn-Hägerdal et al. 2007).

Nevertheless, xylose can be converted to xylulose using the enzyme xylose isomerase and traditional yeasts can ferment xylulose to ethanol although the process is not cost-effective. Arabinose and other pentose sugars are often present in hemicellulosic hydrolysates depending on the source, but only a few yeast strains can barely ferment arabinose to ethanol, thus no naturally occurring yeast can ferment all these sugars to ethanol.

The Table 11.7 shows the substrate range of various yeasts. Genetically engineered organisms are now being employed for ethanol fermentation, these can greatly improve ethanol production efficiency and reduce the cost of operation (Dien et al. 2000). The recombinant strains of *Escherichia coli* with the genes from *Zymomonas mobilis* for the conversion of pyruvate to ethanol have been constructed. The recombinant plasmids with xylose reductase and xylitol dehydrogenase genes from *P. stipitis* and xylulokinase gene from *S. cerevisiae* have been transformed into *Saccharomyces* sp. for the co-fermentation of glucose and xylose (Hahn-Hägerdal and Pamment 2004). Though new technologies have greatly improved bioethanol production, yet there are still a lot of problems that have to be solved. The major problems include maintaining a stable performance of

Table 11.7 Native yeast and fungal species capable of fermenting xylose to ethanol

Microorganism	Sugar utilization pattern	References
C. shehatae	Has both active and positive transport system for xylose uptake: produces moderate amount of xylitol does not grow anaerobically requires biotin and thiamine	Jeffries and Shin (1999)
C. boidinii	Produces large amount of xylitol: oxidizes methanol	Ko et al. (2008)
P. stiptis	Ferment all sugars found in wood some strains ferment xylan	Nigam (2002)
F. oxysporum	Ferments 20 different carbon sources including xylitol; does not use xylan or cellulose: converts xylose to ethanol, acetic acid, carbon dioxide	Suihko et al. (1983)
Mucor species	Ferment pentoses	Sharifia et al. (2008)
P. tannophilus	Ferment xylose glucose and glycerol metabolize xylose anaerobically produce large amount of xylitol	Zhao et al. (2008)

genetically engineered yeast in commercial scale fermentation operations (Dien et al. 2000), developing more efficient pretreatment technologies for lignocellulosic biomass, and integrating optimal components into an economic ethanol production system (Hahn-Hägerdal et al. 2007).

11.5.1 Process Routes for Cellulosic Ethanol Production

In the past, all the three major fermentation process (Batch, Fed-batch, and Continuous) have been employed for biomass conversion into ethanol. Nevertheless batch fermentation has been the preferred choice for cellulosic ethanol production due to its simplicity and fast conversion rates. The desired choice of fermentation strategies usually depend upon the kinetics of fermenting microorganism, type of hydrolysate, and process economics. Generally, batch fermentation has some limitations like the capacity which in turn reflects into low productivity and labor intensive (Dien et al. 2000). In general, fed-batch fermentation is not successful for biomass to ethanol production. In reality, fed batch fermentation is more suitable production process where the product is biomass associated. In such cases for getting the higher concentration of required product, more biomass is needed. But ethanol production does not relate directly to cell mass as it is not intracellular or even periplasmic originated metabolite. Fed-batch operation can be more useful where lignocellulose hydrolysate contains a high concentration of inhibitors so by feeding the hydrolysate with slow rate, the effect of inhibitors can be minimized to microorganism which can give a high concentration of ethanol with a considerable good yield but at the helm of more time consumption, which will result in low productivities (Olsson and Hahn-Hägerdal 1996).

There are two methods to increase cell density—Immobilization and recycling of cell mass which leads to higher productivity and ultimately the requirement of fermenter size and therefore the capital cost becomes lower.

By supplying fresh lignocellulose hydrolysate to fermenting medium and simultaneously withdrawing spent broth containing cells and ethanol, it becomes continuous mode of cultivation. Both types of continuous systems—closed (where cells are retained) and open (cell withdrawal) were applied for bioethanol fermentation. The main problem in continuous cultivation is that it takes a long start up time to establish steady state. The continuous process eliminates much of the unproductive time associated with cleaning, recharging adjustment of media and sterilization (Chandel et al. 2009). Prolonged continuous operation with the same yeast can result in generation of a culture that becomes well adapted to the particular feed and processing conditions.

11.5.2 Separate Hydrolysis and Fermentation

In Separate hydrolysis and fermentation (SHF) process, biomass hydrolysis and fermentation is performed separately. The recovered sugar solution from lignocellulosic biomass either by thermochemical methods or enzymatic action contains a variety of sugars: glucose, xylose, arabinose, mannose, cellobiose, and others. This sugar solution can be fermented into ethanol by a suitable ethanol producer. The fermentation of sugars into ethanol have several options to be adopted such as batch, fed-batch, and continuous. The SHF process has been studied extensively in the laboratories. During the SHF process, both yeast and enzymes can work at their optimal temperature, but an accumulation of end products can reduce the efficiency of hydrolysis (Margeot et al. 2009). Table 11.8 summarizes the examples of ethanol production from lignocellulosic biomass employing SHF.

The SHF is a lengthy, cumbersome process which requires more process steps and equipment/vessels making process overall more costly. The recovered sugar solution can be concentrated by vacuum evaporation and subsequently can be employed for fermentation process to obtain high ethanol concentration. Furthermore, the yeast cell mass can also be recovered and conditioned for the use in next fermentation reaction. The solid lignocellulosic biomass recovered after thermochemical hydrolysis or enzymatic hydrolysis can be used in co-generation for the heat and electricity production. These features may be very useful to economize the cellulosic ethanol production process following SHF process configuration.

11.5.3 Simultaneous Saccharification and Fermentation

The SSF have been investigated as a method of lignocellulosic conversion to economic ethanol production (Takagi et al. 1977; Olofsson et al. 2008). Hydrolysis and fermentation of released hydrolysate with ethanol producing microorganisms can be performed simultaneously, which is referred to as SSF (Ohgren et al. 2006; Rudolf et al. 2005).

Table 11.8 Recent reports on the cellulosic ethanol production from lignocellulosic raw materials adopting various process configurations

Raw material	Type of pretreatment	Process configuration	Microorganism used	Ethanol production (g/l) or ethanol yield (g/g or %)	References
Mixture of glucose and xylose	NA	Fed-batch	*Scheffersomyces* (*Pichia*) *stipitis*	40.7 g/L	Unrean and Nguyen (2013)
Saccharum spontaneum	Soaking in aqueous ammonia	Recycling of cells	*Saccharomyces cerevisiae* VS_3	21.66 ± 0.62 g/L (yield, 0.434 ± 0.021 g/g)	Chandel et al. (2009)
Cassava mash	NA	Continuous	*S. cerevisiae* CHFY0321	86.1 g/L, and 91 % ethanol yield	Moon et al. (2012)
Triploid poplar	Biodegradation (Fungal pretreatment with *Trametes velutina* D10149)	SSF	*S. cerevisiae*	5.16 g/L	Wang et al. (2013)
Wheat straw	Dilute acid	SHF	Recombinant *Escherichia coli* strain FBR5	41.1 ± 1.1 g ethanol/L	Saha et al. (2011)
Sugarcane bagasse	Ammonia fiber expansion (AFEX)	SHF	Recombinant *S. cerevisiae* (424A LNH-ST)	34–36 g/L of ethanol with 92 % theoretical yield	Krishnan et al. (2010)
Switch grass	AFEX	SSCF	Recombinant *S. cerevisiae* (424A LNH-ST)		Jin et al. (2010)
Spruce	Steam pretreatment	Fed-batch SSCF	*S. cerevisiae*	68.9 %	Hoyer et al. (2010)
Rice straw	Dilute acid	SHF	*Pichia stipitis*	0.44 ± 0.02	Lin et al. (2012)
Miscanthus giganteus	Liquid hot water	SSF	Active dry yeast *S. cerevisiae*	98.27 %	Li et al. (2013)
Sugarcane straw	Ball milling	SHF	*S. cerevisiae*	91.8 %	da Silva et al. (2010)

(continued)

Table 11.8 (continued)

Raw material	Type of pretreatment	Process configuration	Microorganism used	Ethanol production (g/l) or ethanol yield (g/g or %)	References
Water hyacinth	Microbial pretreatment with white rot fungus, *Echinodontium taxodii*	SHF	*S. cerevisiae*	0.192 g/g of dry matter	Ma et al. (2010)
Sorghum straw	NA	Deep-bed solid state fermentation	Thermotolerant *Issatchenkia orientalis* IPE 100	0.25 g-ethanol/g-dry stalk	Kwon et al. (2011)
Jerusalem artichoke tuber		CBP	*S. cerevisiae* DQ1	128.7 g/L	Guo et al. (2013)
Corn stover	AFEX	Continuous SSCF	Recombinant *S. cerevisiae* (424A LNH-ST)	80 % glucose-to-ethanol conversion and 47 % xylose-to-ethanol conversion	Jin et al. (2013)

NA Not available

For the maximum conversion of released sugars (pentoses and hexoses) into ethanol, mixture of yeasts can be used in the fermentation reaction, so-called simultaneous saccharification and co-fermentation (SSCF). In general, SSF has been pivotal to achieve fast hydrolysis reaction rates with low enzyme loadings and high ethanol yields in addition to saving manifold processing time and minimizing the equipment usage and capital cost (Chandel et al. 2010).

In contrast to a set-up where hydrolysis and fermentation are separated (SHF), glucose released during enzymatic cellulose hydrolysis, is simultaneously fermented in an SSF set-up (Olofsson et al. 2008). Thus, glucose or any other released sugars will not accumulate during SSF and enzyme inhibition due to glucose can be avoided. This was confirmed in SSF of steam pretreated corn stover with *S. cerevisiae* TMB3400 (Ohgren et al. 2006). If SSF or SSCF process performed efficiently, ethanol could be produced at prices competitive with that of petroleum fuel.

The decrease in capital investment has been estimated to be more than 20 % (Wingren et al. 2003). SHF and SSCF economics was also analyzed using cellulase enzymes in both configurations with SSF being less expensive by about 10 %; and estimated the ethanol production cost of 0.56–0.67 $/L (Wingren et al. 2003). According to the National Renewable Energy Laboratory (NREL, Colorado, USA) estimations, ethanol production cost of 20 cents per liter is possible in another 15 years from lignocellulose biomass employing designer cellulases and SSF (Wingren et al. 2003). However, there are also disadvantages of SSF such as the optimum temperature (45–50 °C) for enzymatic hydrolysis of cellulosics is usually higher than microbial fermentation of hydrolysates into ethanol (30 °C). Therefore, the thermotolerant ethanol producing microorganisms will be a desired choice to be incorporated in SSF or SSCF to avoid the ethanol yield loss.

11.5.4 Consolidated Bioprocessing

The CBP is a consolidated technological platform summarizing all the critical steps of bioethanol production, i.e., cellulase production, substrate hydrolysis, and fermentation of released sugars into ethanol in one step. CBP can effectively save the processing time, processing costs, energy while reducing the number of involved steps (Lynd et al. 2005). However, it is very difficult to find such microorganism that can perform all these reactions. CBP can save the bioethanol production cost drastically due to elimination of requirement of enzymes addition from outside and separate hydrolysis (Olson et al. 2012). The need of hour is to develop such microorganism which can perform all these steps simultaneously. Several technological developments have been attempted aiming to develop the microbial traits for the incorporation in CBP platform (van Zyl et al. 2011; Olson et al. 2012).

Goyal et al. (2011) developed a yeast consortium showing endoglucanase, exoglucanase, and β-glucosidase enzyme titers aiming to utilize cellulose for growth coupled with hydrolysis and ethanol production in one vessel (1.25 $g \cdot L^{-1}$, 87 % of theoretical value). Jin et al. (2012) studied the ethanol production from

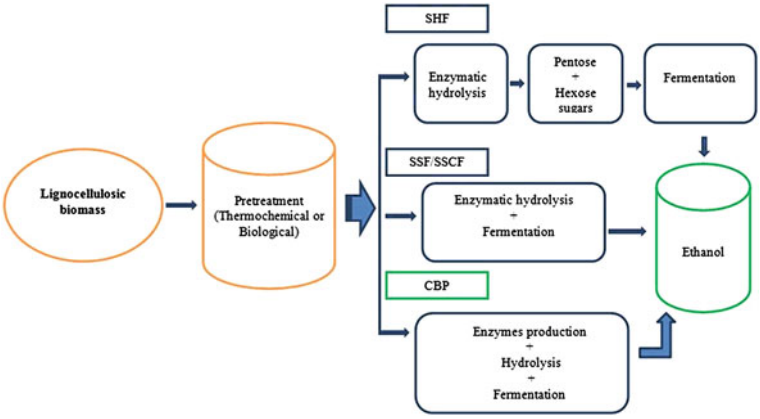

Fig. 11.10 Schematic paradigm of major process configurations for cellulosic ethanol production from lignocellulosic biomass

delignified corn stover (ammonia pretreated) using CBP approach. They found 48.9 % glucan conversion and 77.9 % xylan conversion after 264 h with 7 $g{\cdot}L^{-1}$ ethanol production by *Clostridium phytofermentans* ATCC 700394.

11.5.5 Integrated Bioprocessing

All the four essential steps of biomass conversion into ethanol could be performed in a single vessel sequentially first time termed as IBP, which is distinguished from other less highly integrated configurations in that it does not involve a dedicated process step for pretreatment. All necessary steps can be done in the same vessel in order to combine the overall process steps. During the microbial pretreatment of LB, microorganisms secrete a cocktails of plant cell wall degrading enzymes which can be recovered (on-site enzyme production) and subsequently can be used for the saccharification of pretreated biomass alone or with the supplementation of necessary enzymes from outside. There is involvement of at least two microorganisms in IBP (first for delignification and second for ethanol production from released sugars from pretreated cellulosic biomass). IBP may provide a unique breakthrough for cheap cellulosic ethanol production due to economic advantages and time savings. However, there is no practical report using IBP for ethanol production as yet (Fig. 11.10).

11.6 Conclusions

As can be drawn from the above discussion, the success of next-generation biofuels, such as cellulosic ethanol will depend on the efforts in reducing capital costs, financial support during scale up, establishing feedstock supply arrangements, and

overcoming blend wall constraints (Coyle 2010). There are a number of components that affect the cellulosic ethanol production cost, estimating 14.5 % for enzymes, 36.4 % for feedstock, 20 % of capital, and 29.1 of other components such as pretreatment and fermentation steps (Coyle 2010).

The survey collected showed that in 2012, the cost of cellulosic ethanol production was $0.94 per liter, around 40 % higher than the $0.67 per liter (L) cost of producing ethanol from corn (Isola 2013). According to the world's leading producer of enzymes, the cost of enzymes for cellulosic ethanol had been reduced significantly in the last 2 years to about 50 cents per gallon, reducing total production costs in the near term to about $2 per gallon (Novozymes 2010). The costs of cellulosic ethanol, that have fallen significantly, are expected to decline more as companies scale-up production, but further advance in these technologies are required to turn this process into a competitive fuel with first-generation ethanol and gasoline (Ziolkowska et al. 2011; Isola 2013). Dilute acid hydrolysis and steam explosion are the successful pretreatment technologies used for the sugarcane bagasse and straw which can be applicable in industrial-scale operations.

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Chapter 12
Novel Yeast Strains from Brazilian Biodiversity: Biotechnological Applications in Lignocellulose Conversion into Biofuels

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Abstract The bioprospection of novel biochemical traits from world biodiversity is far underexploited. Brazil is one of the richest megadiverse countries, and a source of new species and strains with potential application to biotechnological processes. Among the organisms of interest, yeasts capable of fermenting sugars from lignocellulosic biomass have particular interest for the development of efficient fermentative technologies in the production of biofuels, like second-generation ethanol, and other chemicals, like xylitol. In this chapter, recent studies performed with novel Brazilian D-xylose- and/or cellobiose-fermenting yeasts are highlighted. The new isolates from the genus *Scheffersomyces* and *Spathaspora* represent an important contribution of new species and strains to yeast taxonomy and ecology, and their characterization a first screening for potential biotechnological applications. These yeasts species and strains represent a new set of biological material that can be used directly in the conversion of lignocellulosic biomass into value-added bioproducts, or a source of genetic material for the improvement of the fermentative capacity of industrial microorganisms, like the yeast *Saccharomyces cerevisiae*, toward the production of second-generation biofuels.

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12.1 Introduction

Yeast biotechnology embraces a variety of processes involving these microorganisms, their enzymes, and metabolites for the production of fermented foods and beverages, fuels, chemicals, and pharmaceuticals. Traditional attributes of yeasts include their primary roles in fermentation processes, particularly in alcoholic fermentation. The use of *Saccharomyces cerevisiae* in ethanol production processes is well documented and has been exploited for centuries, namely in the production of alcoholic beverages. The application of ethanol from fermentative origin as a partial or total substitute of gasoline in spark-ignition internal combustion engines is continuously increasing and has been significantly used for decades in Brazil and USA—first-generation (1G) bioethanol. The interest in bioethanol as a renewable energy source, especially as fuel for transportation, has been fluctuating substantially during most of the last century mainly in response to oil crises.

The growing concern about the environmental consequences of the intensive use of fossil fuels have been promoting innumerous policy actions toward the reduction of greenhouse gas (GHG) emissions, including incentives for the use of renewable sources with low impact in environment for the production of fuels and chemicals. Lignocellulosic materials from forestry, agriculture, agro-industry, or municipal solid waste constitute the most abundant renewable raw feedstock in the world. The composition of lignocellulosic materials varies among plant species but generally consists of ~25 % lignin and ~75 % carbohydrate polymers (cellulose and hemicellulose) (Zaldivar et al. 2001). The cellulosic and hemicellulosic fractions can be separated from the lignin and depolymerized by physical, chemical, and biochemical processes (pretreatment and enzymatic hydrolysis) to obtain their constituent sugars, mainly hexoses (glucose) from cellulose and pentoses (usually D-xylose) from hemicellulose, which, in turn, can be converted to ethanol by biological processes (fermentation). The economic viability of ethanol produced from lignocellulosic materials—second-generation (2G) bioethanol—is dependent on the complete utilization of the carbohydrate fraction, including hemicellulose (van Vleet and Jeffries 2009; Gírio et al. 2010). However, *S. cerevisiae* lacks the ability to ferment a number of saccharides derived from cellulose and hemicellulose. It cannot readily utilize cellulose and hemicellulose, nor the disaccharides cellobiose and xylobiose (Lynd et al. 2002), neither can ferment the pentose sugars D-xylose and L-arabinose (Hahn-Hägerdal et al. 2007; van Vleet and Jeffries 2009; Gírio et al. 2010). On the other hand, among yeasts other than *Saccharomyces* spp., referred to as nonconventional yeast (NCY) (Boekhout and Kurtzman 1996) are those that consume the pentose sugars D-xylose (Skoog and Hahn-Hägerdal 1988), L-arabinose (Dien et al. 1996; Fonseca et al. 2007) and lignocellulose-derived di- and trisaccharides (Freer and Detrov 1983; Parekh and Wayman 1986; Golias et al. 2002; Ryabova et al. 2003). The so-called NCYs provide alternative biocatalysts for metabolic engineering of *S. cerevisiae* toward the utilization of all the sugars present in lignocellulosic materials (Hahn-Hägerdal et al. 2007).

The increased exploration of NCYs including detailed characterization of their physiology, metabolism, and genomics are inevitably leading to a wide range of useful biotechnological and industrial applications (Wolf et al. 2003; Buzzini and Vaughan-Martini 2006).

12.2 Yeast Biotechnology and Biodiversity

Yeasts are unicellular fungi characterized by a widespread distribution throughout all biomes in the world in association with sugary substrates like flowers, fruits, tree exudates, leaves, and mushrooms. These organisms also occupy a diverse variety of micro-ecosystems and are well adapted to a wide range of weathers, altitudes, substrates, and geographical locations, being found in glaciers, high salinity lakes, water, soil, air, and gut of a variety of vertebrates and invertebrates (Rosa and Péter 2006; Starmer and Lachance 2011).

Yeasts have benefitted humankind for millennia. These microorganisms have wide-ranging fundamental and industrial importance in scientific, food, medical, and agricultural disciplines. Traditional industrial attributes of yeasts include their primary roles in many food fermentations and other long-standing industrial processes, like the production of fuel ethanol, single cell protein (SCP), feeds and fodder, industrial enzymes and small molecular weight metabolites, and as host for the heterologous expression of proteins of interest. Several of these processes and products have reached commercial scale, while others are still under development (Johnson and Echavarri-Erasun 2011).

Once that microbial biodiversity is the source of innovation in biotechnology (Bull et al. 1992), the exploration and isolation of yeasts from various habitats is a growing field where novel species and its physiological abilities are potentially useful in the search of new products by means of direct application in industrial processes or metabolic engineering approaches of industrial microorganisms, like the yeast *S. cerevisiae* (Barriga et al. 2011). The number of fungi present on Earth is estimated between 1.5 and 3.3 million species (Hawksworth 1991, 2012) and about 100,000 species have already been described (Hibbett et al. 2011). Currently, nearly 1,500 yeast species among 149 genera are recognized (Kurtzman et al. 2011). Therefore, it is estimated that the vast majority of the potential biodiversity of yeasts is still unknown, which supports a need for increasing efforts to study the biological diversity of these microorganisms, especially in mega diverse countries from the tropical regions of the planet. To date, most of yeast species cataloged has been discovered in countries from the Northern hemisphere. Relatively few studies dedicated to yeast biodiversity have been done in tropical zones of the planet and in Southern hemisphere countries that embrace abundant and diverse ecosystems. Particularly, South America is a region that offers great potential in terms of biodiversity (Barriga et al. 2011), being Brazil, the largest country located in this continent, considered one of the world's richest megadiversity countries (Mittermeier et al. 2005).

12.2.1 Brazilian Biodiversity as a Potential Source of New Yeasts

Brazil spans 8.5 million km^2. Its geographic space presents a great diversity of climate types, physiognomy, soils, and vegetation. These great ecological variations led to the formation of distinct biogeographical zones or biomes within the country: Amazon, the world's biggest rainforest (which spans 49 % of the Brazilian territory); Pantanal (1.7 %), the biggest flood plain; Cerrado (23.9 %), with savannahs and woods; Caatinga (9.9 %), with semiarid forests; Pampas' meadows (2 %); and the Atlantic rainforest (13 %) (IBGE 2010). Two of these biomes—the Atlantic rainforest and the Cerrado—are classified as *hotspot* regions, areas with high biodiversity, elevated levels of endemism, and great anthropic pressure. Moreover, the Atlantic rainforest is considered as one of the five leading biodiverse *hotspots* of the planet (Myers et al. 2000). These relevant ecosystems, together with the Amazonian forest, also embraces ecoregions, defined as a relatively large unit of land or water containing a characteristic set of natural communities that share a large majority of their species, dynamics, and environmental conditions (Dinerstein et al. 1995; Olson and Dinerstein 1998). Ecoregions function effectively as conservation units at regional scales because they hold similar biological communities and because their boundaries roughly coincide with the area over which key ecological processes most strongly interact (Orians 1993; Noss 1996).

Forest ecosystems are an attractive site for the collection of yeasts (Morais et al. 2006). Approximately 2.3 million km^2 of Brazil—27 % of its total area and almost 17 % of the world's global stock—comprises tropical moist forests, making it the third highest ranked country in terms of remaining frontier forest and the first in plant biodiversity among frontier forest nations. Its tropical forest endowment and its importance to global biodiversity are unparalleled in the world (Lele et al. 2000; Morais et al. 2006). Ecosystems such as forests are considered a mosaic of patchy habitats for organisms, consisting of soil, litter, tree stems, trunks, canopy, flowers, and fruits, a feature that supports a huge biodiversity of microorganisms and represents different niches for colonization of yeasts (Morais et al. 2006). Until now, the studies on yeast from Brazil's ecosystems have focused mainly on Atlantic rainforest (Morais et al. 1992, 1995a, 1996; Prada and Pagnocca 1997; Abranches et al. 1998; Araújo et al. 1998; Ruivo et al. 2004, 2005, 2006; Rosa et al. 2007a; Barbosa et al. 2009; Cadete et al. 2009; Pimenta et al. 2009; Santos et al. 2011; Morais et al. 2013a, b). Few studies have been conducted on Cerrado ecosystem (Morais et al. 2004; Rosa et al. 2007b, 2009, Canelhas et al. 2011; Barbosa et al. 2012; Safar et al. 2013) and Amazonian forest sites (Mok et al. 1984; Morais et al. 1994, 1995b; Vital et al. 2002, Cadete et al. 2012a, b, 2013), which, in association with the frequent discovery of new yeast species regardless of the sampling area, increases the impact of the rarity of studies on yeast from

Brazilian biomes. In this chapter, the recognition of these ecosystems as potential locals for research on yeast biodiversity toward its biotechnological application for biomass conversion to biofuels (2G bioethanol) and other bioproducts is shown.

12.3 Fermentation of Lignocellulosic Sugars

Lignocellulose is a complex and chemically rich material represented by the physical–chemical interaction of cellulose, a linear glucose polymer, with hemicellulose, a highly branched sugar heteropolymer, and lignin, a high molecular weight and cross-linked aromatic macromolecule (Ferreira-Leitão et al. 2010). The pretreatment of lignocellulosic materials by hydrothermal or other acidic methods generates a liquid fraction also named hemicellulosic hydrolysate. The conversion of the hemicellulosic hydrolysate is a challenge on ethanol production from lignocellulose, due to the presence of inhibitors of microbial metabolism (Almeida et al. 2007) and to the heterogeneity of sugars usually found in this fraction—oligosaccharides like xylooligosaccharides (XOS), and monosaccharides like D-xylose L-arabinose, D-galactose, D-glucose and D-mannose. The identification or development of microbial strains able to efficiently ferment these sugars is mandatory for the successful industrial production of ethanol (Hahn-Hägerdal et al. 2007; Fukuda et al. 2009; van Vleet and Jeffries 2009; Gírio et al. 2010; Ferreira et al. 2011).

The desired properties of strains required for fermenting lignocellulosic hydrolysates are: the efficient utilization of hexoses and pentoses; high ethanol titers, yields and productivities; high tolerance to ethanol, fermentation inhibitors, low pH and high temperature; high viability and vitality; and others process-specific characteristics like sugar co-consumption and appropriate flocculation properties (Hahn-Hägerdal et al. 2007; Pasha et al. 2007). The yeast *S. cerevisiae* is the most commonly used microorganism in traditional industrial fermentations, including current sucrose-, starch-, and cellulose-based bioethanol production. *Saccharomyces cerevisiae* is also generally recognized as safe (GRAS) and can ferment efficiently simple hexose sugars, such as D-glucose, D-mannose and D-galactose, and disaccharides like sucrose and maltose, reaching ethanol concentrations as high as 20 % (v/v) (Gírio et al. 2010). Moreover, this species has a relatively good tolerance to lignocellulose-derived inhibitors and to high osmotic pressure (Almeida et al. 2007). The major inconvenience to the use of *S. cerevisiae* for lignocellulosic fermentation is its lack of natural ability to utilize the pentose sugars D-xylose and L-arabinose (Hahn-Hägerdal et al. 2007; van Vleet and Jeffries 2009; Gírio et al. 2010). Contrary to *S. cerevisiae*, other yeast species, and also bacteria and filamentous fungi can ferment pentoses. However, despite the existence of pentose-fermenting microorganisms and the innumerous efforts on metabolic engineering of *S. cerevisiae*, it is still challenging to reach high ethanol productivities from pentose sugars while simultaneously withstanding fermentation inhibitors (Hahn-Hägerdal et al. 2007; Chandel et al. 2011). The identification

and/or development of new yeast strains which ferment hemicellulosic sugars will improve prospects for lignocellulosic ethanol production (Jeffries and Kurtzman 1994; Hahn-Hägerdal et al. 2007; van Vleet and Jeffries 2009). The exploitation of biodiversity through the identification of novel microorganisms and their unique traits and the use of adaptation strategies and/or metabolic and evolutionary engineering approaches are contributing to the development of novel cell factories for the production of bioethanol, other biofuels, and biochemicals from lignocellulosic materials (van Maris et al. 2006; Sanchez et al. 2010; Fonseca et al. 2011; Nielsen et al. 2013).

12.3.1 D-xylose-Fermenting Yeasts

Pentose (C5) sugars can constitute up to 70 % of the fermentable sugars in hydrolysates (Gírio et al. 2010). Once that high ethanol yields are required for the development of economically feasible second-generation ethanol processes, the conversion of C5 sugars is a prerequisite for a cost-effective lignocellulosic ethanol production (van Vleet and Jeffries 2009; Gírio et al. 2010). As the major pentose in hemicelluloses from hardwood, cereals, and other herbaceous crops, D-xylose is the second most abundant sugar component in lignocelluloses and the second most abundant carbohydrate in nature after glucose (Jeffries 2006; Watanabe et al. 2007). This feature makes D-xylose the C5 sugar most studied in lignocellulose fermentation processes. Several bacteria, yeasts, and filamentous fungi naturally ferment D-xylose to ethanol (Jeffries 1983). Yeasts have advantages over bacteria for commercial fermentations due to larger size, thicker cell wall, better growth at low pH, less stringent nutritional requirement, higher tolerance to fermentation products, and greater resistance to contamination (Jeffries 2006), whereas presenting higher rates of sugar consumption and product formation than filamentous fungi (Skoog and Hahn-Hägerdal 1988).

12.3.1.1 D-xylose Metabolism

The pentose phosphate pathway (PPP) is the biochemical route for D-xylose metabolism. This pathway is found in virtually all cellular organisms providing D-ribose for nucleic acid biosynthesis, D-erythrose-4-phosphate for the synthesis of aromatic amino acids, and NADPH for anabolic reactions. The PPP consist of two parts. The oxidative part converts the hexose D-glucose-6-phosphate into the pentose D-ribulose-5-phosphate, plus CO_2, and NADPH. The nonoxidative part converts D-ribulose-5-phosphate into D-ribose-5-phosphate, D-xylulose-5-phosphate, D-sedoheptulose-7-phosphate, D-erythrose-4-phosphate, D-fructose-6-phosphate, and D-glyceraldehyde-3-phosphate. D-glyceraldehyde-3-phosphate and D-fructose-6-phosphate can be converted to pyruvate in the Embden-Meyerhof-Parnas pathway (glycolysis). Pyruvate can either be decarboxylated and

reduced to ethanol or can enter the tricarboxylic acid cycle. To enter the central carbon metabolism, D-xylose must first be converted to the intermediate compound of the PPP, D-xylulose-5-phosphate, and, essentially, two different pathways are available in nature for the conversion of D-xylose into D-xylulose: reduction/oxidation-based pathways and isomerization-based pathways (Bettiga et al. 2008). In D-xylose-utilizing yeasts, aerobic fungi, and other eukaryotes, this proceeds via a two-step reduction and oxidation mediated by xylose reductase (*XYL1*, Xyl1p, XR) and xylitol dehydrogenase (*XYL2*, Xyl2p, XDH), respectively (Kötter et al. 1990; Jeffries 2006). D-xylose is first reduced by XR to xylitol which is then oxidized to D-xylulose through XDH. In bacteria and some anaerobic filamentous fungi, the D-xylose is directly converted into D-xylulose by a xylose isomerase (*xylA*, XI) (Walfridsson et al. 1996; Kuyper et al. 2003; Jeffries 2006). After D-xylose conversion to D-xylulose through XR/XDH or XI, the metabolism proceeds via phosphorylation of D-xylulose, a reaction catalyzed by xylulokinase (*XKS1* or *XYL3*, Xks1p or Xyl3p, XK) (Jeffries 2006).

The D-xylose-oxido-reductase pathway found in yeasts faces cofactor requirements—NAD(P)(H)—by XR and XDH, which has great impact in xylitol and ethanol yields from D-xylose fermentation under oxygen-limited conditions (Bruinenberg et al. 1983). The relevance of this topic led to an extensive characterization of these enzymes with respect to enzymatic activity, specificity, and cofactor requirement in yeasts grown under different experimental conditions. The existence of XRs strictly NADPH-dependent or showing dual cofactor specificity, with preference for NADH or NADPH has been shown (Bruinenberg et al. 1983, 1984a; Yablochkova et al. 2003; Hou 2012). XDH activities are virtually NAD^+-dependent. Indeed, no correlation was observed between the ability to ferment D-xylose and the activity of $NADP^+$-linked XDH (Bruinenberg et al. 1984a). Although the existence of NAD(P)H-utilizing XRs, NADPH is still the preferred cofactor in most of known D-xylose-fermenting yeasts. Therefore, the different cofactor requirement of XR and XDH (NADPH and NAD^+, respectively) leads to the accumulation of $NADP^+$ and NADH. In addition, the absence of transhydrogenase in yeast prevents cofactor interconversion (Bruinenberg et al. 1985; Dellomonaco et al. 2010). Whereas $NADP^+$ can be reduced through recycling D-fructose-6-phosphate (via D-glucose-6-phosphate) in the oxidative PPP during pentose metabolism (Bruinenberg et al. 1983; Fonseca et al. 2008), NADH is mainly oxidized to NAD^+ through oxygen in the respiratory chain. Under oxygen limitation, NAD^+ is not efficiently regenerated, and xylitol is accumulated (Gírio et al. 2010). Thus, yeasts harboring strictly NADPH-dependent XR produce xylitol as the major product of D-xylose fermentation under oxygen-limited conditions (Bruinenberg et al. 1984b; Gírio et al. 1994; Silva et al. 1996; Fonseca et al. 2007). Yeasts producing a XR with dual cofactor specificity can oxidize NADH to NAD^+ in this step, thereby reducing xylitol formation and allowing D-xylose fermentation to proceed under oxygen-limited conditions (Bruinenberg et al. 1983). A direct relationship between the dual cofactor dependence of XR with regard to NADH-linked activities and the ability to ferment D-xylose to ethanol with high

efficiency by naturally or mutated D-xylose-fermenting yeasts has already been demonstrated (Bruinenberg et al. 1984a; Watanabe et al. 2007; Bengtsson et al. 2009; Runquist et al. 2010; Hou 2012).

12.3.1.2 Ecology, Taxonomy, and Phylogenetic Relationships of D-xylose-Fermenting Yeasts

The first researches focused on D-xylose conversion to ethanol by yeasts emerged in the 1980s. These studies attempt to isolate and screen D-xylose-fermenting yeasts (Nigam et al. 1985; du Preez and Prior 1985), to demonstrate the fermentation of this pentose by several strains (Gong et al. 1983; Toivola et al. 1984), or by particular species (Jeffries 1981; Schneider et al. 1981; du Preez and van der Walt 1983; Dellweg et al. 1984), and to verify the impact of several parameters in this process, like the oxygen supply (Delgenes et al. 1986; Skoog and Hahn-Hägerdal 1990), mixtures of hemicellulosic sugars (du Preez et al. 1986; Jeffries and Sreenath 1988), high D-xylose concentrations (Slininger et al. 1985), the nitrogen sources, and the pH (Jeffries 1985). Studies addressing the transport of D-xylose were also initiated in this decade (Kilian and Uden 1988). In common, these reports revealed as best D-xylose-fermenting yeasts producing ethanol the species *Pachysolen tannophilus, Scheffersomyces* (*Pichia) stipitis*, and *Scheffersomyces (Candida) shehatae*, although the recognition of other D-xylose-fermenting species. Indeed, until a few years ago, the majority of studies concerning D-xylose conversion to ethanol have been conducted with these three yeast species, being *Sc. stipitis* considered the best D-xylose-fermenting yeast and the source of genes for metabolic engineering of *S. cerevisiae* for D-xylose fermentation (Kötter et al. 1990). More recently, the isolation of new D-xylose-fermenting species and strains especially from the *Spathaspora* clade in particular habitats, among which Brazilian ecosystems stands out for its important contribution, came to cause upheavals in this scenario (Nguyen et al. 2006; Barbosa et al. 2009; Cadete et al. 2009, 2012a, b, 2013; Morais et al. 2013b).

D-xylose-fermenting yeasts exhibit as a common feature the association with vegetal biomass sources (Fig. 12.1). These microorganisms have been isolated from tree exudates (Nigam et al. 1985), wood-boring insects (Toivola et al. 1984; Suh et al. 2003; Nguyen et al. 2006; Urbina et al. 2012, 2013), decaying wood (Toivola et al. 1984), rotten fruit, and tree bark (Rao et al. 2008). This behavior can be explained by the role of yeast ecology in these substrates and by the fact that biomass materials contain D-xylose in their structures. Typically, yeasts are considered as primarily decomposers among the earlier colonizers of nutrient rich substrates, where they are followed by a succession of organisms that degrade dead organic matter. Land plant tissues (stems, flowers, and fruits) are rich in organic compounds and moisture, and consequently provide a favorable environment for yeast growth. Likewise, exudates of leaves, roots, flowers, and tree trunks are good habitats in which yeasts flourish. Many yeast species that are found in live or decaying plant parts are associated with insects that also use these habitats as

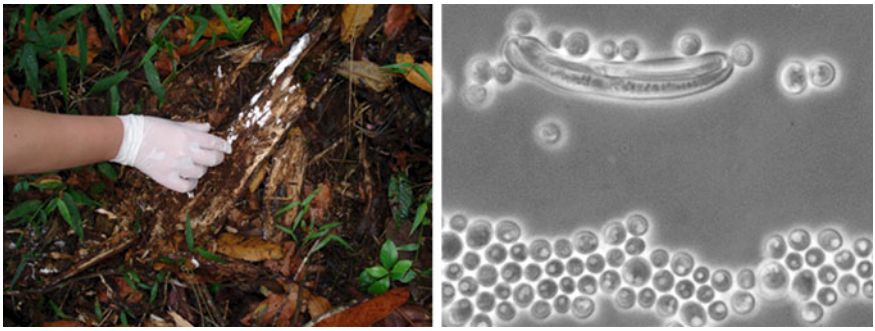

Fig. 12.1 Sampling of rotting wood in an Atlantic rainforest site (Nova Friburgo, RJ, Brazil) to isolate D-xylose-fermenting yeasts (on *left*) and budding yeast cell and asci of *Spathaspora passalidarum* NRRL Y-27907 (type strain) cultured on diluted V8 agar after 5 days at 20 °C (on *right*)

feeding or breeding sites. In general, these three-part associations (insect-yeast-plant) are maintained by reliance on reciprocal benefits exchanged by the insect-yeast partners. Often the yeast supplies essential nutrients or beneficial supplements to the insect while the insect provides transportation of the yeast to new habitats (Starmer and Lachance 2011).

Besides being linked to common substrates, D-xylose-fermenting yeasts display close phylogenetic relationships. Although these microorganisms appear scattered throughout the subphylum Saccharomycotina (Kurtzman et al. 2011; Urbina et al. 2012), the species that have been reported to exhibit the highest rates of D-xylose fermentation and ethanol production are members of the clades *Scheffersomyces* (Suh et al. 2006; Jeffries et al. 2007; Kurtzman and Suzuki 2010; Urbina et al. 2012), and *Spathaspora* (Nguyen et al. 2006; Wohlbach et al. 2011; Hou 2012; Long et al. 2012; Cadete et al. 2013). Both clades belong to the same family, Debaromycetaceae, and are phylogenetically closed to each other in relation to other clades of this family (Kurtzman et al. 2011). The ascosporic species assigned to the genus *Scheffersomyces* were initially identified within a polyphyletic genus, *Pichia*, whose classification based on phenotypic similarities, such as formation of hat-shaped ascospores and inability to assimilate nitrate as a sole source of nitrogen resulted in the placement of phylogenetically distant species within the same genus (Kurtzman and Suzuki 2010; Kurtzman 2011). The combined analyses of sequences of the D1/D2 domains of the large subunit and the nearly complete small subunit rRNA genes showed that the species described as *Pichia stipitis*, *P. segobiensis*, and *P. spartinae* were distantly related to *Pichia membranifaciens*, the type species of the genus *Pichia* (Kurtzman and Robnett 1998), but phylogenetically close to each other, leading to the propose of the genus *Scheffersomyces* (Kurtzman and Suzuki 2010) to the reclassification of these species. Currently, the genus *Scheffersomyces* is represented by 16 species (Cadete et al. 2012b; Urbina et al. 2012, 2013) including the asexual species previously

described as *Candida* (*C. amazonensis, C. coipomoensis, C. ergatensis, C. gosingica, C. insectosa, C. lignicola, C. lignosa, C. queiroziae*, and *C. shehatae*) and now identified as *Scheffersomyces* (*Sc. amazonensis*, *Sc. coipomoensis* and so on) and by the species *Sc. stipitis* (type species) *Sc. segobiensis*, *Sc. spartinae*, *Sc. quercinus, Sc. illinoinensism, Sc. virginianus,* and *Sc. cryptocercus.* In addition to the update of the genus, the *Scheffersomyces* clade was divided into three subclades:

(1) the early diverging *S. spartinae* and *S. gosingicus (C. gosingica)* subclade, being this last species able to ferment cellobiose;
(2) the cellobiose-fermenting *Sc. ergatensis* subclade, comprising the species *Sc. amazonensis*, *Sc. coipomoensis*, *Sc. ergatensis*, *Sc. lignicola,* and *Sc. queiroziae*;
(3) the largest, D-xylose-fermenting *Sc. stipitis* subclade presenting the remaining species. Within the *Scheffersomyces* clade, Brazilian isolates have so far contributed with new *Sc. stipitis* and *Sc. shehatae* strains (Ferreira et al. 2011; Cadete et al. 2012a; Martiniano et al. 2013a, b) and mainly to the description of the new species *Sc. amazonensis* (Cadete et al. 2012b) and *Sc. queiroziae* (Santos et al. 2011).

The *Spathaspora* clade was first described to harbor the species *Sp. passalidarum*, the first teleomorphic species of the genus (Fig. 12.1), and the anamorphic species *C. jeffriesii*. Both species were isolated from wood-boring beetles collected, respectively, in Louisiana (USA) and Chiriqui (Panama) (Nguyen et al. 2006). Today, the *Spathaspora* clade is represented by the teleomorphic species *Sp. passalidarum, Sp. arborariae, Sp. brasiliensis, Sp. roraimanensis*, and *Sp. suhii*, and by the anamorphic species previously described as belonging to the genus *Candida, Sp. jeffriesii, Sp. lyxosophila, Sp. materiae, Sp. insectamans, Sp. subhashii*, and *Sp. xylofermentans* (Nguyen et al. 2006; Barbosa et al. 2009; Cadete et al. 2009, 2012a, 2013). Among these species, six were described from isolates associated to rotting wood sampled in Brazilian biomes: *Sp. materiae* (Barbosa et al. 2009), *Sp. arborariae* (Cadete et al. 2009), and *Sp. brasiliensis, Sp. roraimanensis*, *Sp. suhii* and *Sp. xylofermentans* (Cadete et al. 2013). Also, six new *Sp. passalidarum* strains, a species described from a single isolate and recently shown as the best ethanol from D-xylose-producing yeast ever reported (Hou 2012; Long et al. 2012) were obtained from a forest reserve located in the Brazilian Amazonian forest (Cadete et al. 2012a).

12.3.1.3 Studies with Brazilian Yeasts

In the past few years, the Brazilian biodiversity has contributed with new D-xylose-fermenting yeast species and strains, and studies conducted with these organisms regarding physiology, biochemistry, molecular biology, and genomics have already been published or are still in progress. All these research efforts show potential results concerning the bioconversion of D-xylose to ethanol or xylitol under different culture conditions (Table 12.1).

Table 12.1 Main fermentation product (ethanol or xylitol) yield [$Y_{p/s}$ ($g.g^{-1}$)] and productivity [Qp ($g.l^{-1}.h^{-1}$)] achieved in defined or hydrolysate fermentation media by Brazilian D-xylose-fermenting strains

Clade	Species	Strain	Fermentation medium	Main fermentation product	$Y_{p/s}$ ($g.g^{-1}$)	Qp ($g.l^{-1}.h^{-1}$)	Reference
Scheffersomyces	*Sc. stipitis*	UFMG-XMD-15.2	YPX[a]	Ethanol	0.28	0.51	Cadete et al. (2012a)
		UFMG-HMD-32.1			0.22	0.23	
		UFMG-XMD-15.2	ScBHH[b]		0.34	0.20	
		UFMG-IMH-43.2			0.19	0.13	Ferreira et al. (2011)
	Sc. shehatae	BR6-2AI	YPX		0.45	0.35	Martiniano et al. (2013a)
		CG8-8BY			0.47	0.37	
		PTI-1BASP			0.44	0.36	
		BR6-2AY			0.48	0.37	
		CG8-8BY	ScBHH		0.3	0.15	
		BR6-2AY			0.21	0.11	
		CG8-8BY	ScBHH		0.33	0.21	Martiniano et al. (2013b)
		CG8-8BY	ScBHH		0.2	0.12	
		UFMG-HM-52.2	ScBHH		0.35	0.13	Chandel et al. (2013)
		UFMG-HM-52.2	ScBCH[c]		0.28	0.20	
Scheffersomyces	*Sc. amazonensis*	UFMG-XMD-24.1	YPX	Xylitol	0.59	–	Cadete et al. (2012a)
		UFMG-XMD-26.2			0.58	–	
		UFMG-HMD-26.3			0.57	–	
		UFMG-XMD-40.2			0.55	–	
		UFMG-XMD-40.3			0.56	–	
Spathaspora	*Sp. passalidarum*	UFMG-HMD-1.1		Ethanol	0.36	0.75	
		UFMG-HMD-1.3			0.35	0.72	
		UFMG-HMD-2.1			0.31	0.62	
		UFMG-HMD-10.2			0.33	0.69	
		UFMG-HMD-14.1			0.37	0.68	
		UFMG-HMD-16.2			0.33	0.64	
		UFMG-HMD-1.1	ScBHH		0.2	0.09	
		UFMG-HMD-14.1			0.18	0.10	

(continued)

Table 12.1 (continued)

Clade	Species	Strain	Fermentation medium	Main fermentation product	$Y_{p/s}$ ($g.g^{-1}$)	Qp ($g.l^{-1}.h^{-1}$)	Reference
Spathaspora	*Sp. arborariae*	UMFG-HM-19.1A	YPX	Ethanol	0.50	–	Cadete et al. (2009)
			$G_{20}X_{20}A_{10}^{d}$		0.46	0.21	Cunha-Pereira et al. (2011)
			RHH[e]		0.45	0.16	
			ScBHH		0.14	0.04	Martiniano et al. (2013b)
	Sp. brasiliensis	UMFG-HMD-19.3	YPX	Xylitol	0.16	–	Cadete et al. (2012a)
	Sp. roraimanensis	UFMG-XMD-23.2		Ethanol	0.26	0.21	
	Sp.suhii	UFMG-XMD-16.2			0.33	0.27	
		UFMG-HMD-16.3			0.27	0.22	
	Sp. xylofermentans	UFMG-HMD-23.3			0.18	0.10	
		UFMG-HMD-25.5		Xylitol	0.22	–	
	Sp. roraimanensis	UFMG-XMD-23.2	ScBHH		0.61	–	
	Sp. suhii	UFMG-XMD-16.2			0.57	–	

[a] YPX = D-xylose, peptone and yeast extract
[b] ScBHH = sugarcane bagasse hemicellulosic hydrolysate
[c] ScBCH = sugarcane bagasse cellulosic hydrolysate
[d] $G_{20}X_{20}A_{10}$ = glucose, D-xylose and arabinose
[e] RHH = rice hull hydrolysate

The species *Sc. stipitis* and *Sc. shehatae* have been the D-xylose-fermenting yeasts better described in the past decades and the source of genes for metabolic engineering of *S. cerevisiae* (Bruinenberg et al. 1984a; Verduyn et al. 1985; du Preez et al. 1986; Ligthelm et al. 1988; Prior et al. 1989; Skoog and Hahn-Hägerdal 1990; Kötter et al. 1990). It is expected that the screening, identification, and characterization of new and unrelated species from biomes harboring high biodiversity would even be more beneficial for yeast biotechnology, once the access to that genetic diversity will certainly conduce to the identification of new traits (Lachance 2006). Also, it is well known that there are variations within strains from the same species, and some metabolic abilities/disabilities are not necessarily linked to the species but result rather from strain variability (Barriga et al. 2011). Thus, the bioprospection toward the identification of new yeasts able to convert lignocellulosic sugars would generate a portfolio of species, strains, and varieties suitable for exploitation purposes to the conversion of lignocellulose into value-added products, like 2G bioethanol, other advanced fuels, and chemicals.

In the past years, a strong effort has been made in Brazil for the identification of novel yeasts able to ferment lignocellulosic sugars (e.g., D-xylose and cellobiose). *Scheffersomyces stipitis* strains have been isolated from the gut of wood-boring insects collected in a natural reserve of Atlantic rainforest (Cadete 2009; Ferreira et al. 2011) and from rotting wood sampled in forest reserves of Amazonian forest (Cadete et al. 2012a). *Scheffersomyces shehatae* strains have been isolated from rotting wood of Atlantic rainforest (Cadete 2009; Chandel et al. 2013) and different natural habitats within Brazilian forests, like bromeliads, mushroom, and palm tree (Martiniano et al. 2013a, b).

The new D-xylose-fermenting strains have been recently tested for D-xylose fermentation under different conditions, including define medium and hemicellulosic hydrolysates. The production of ethanol from D-xylose by *Sc. stipitis* UFMG-IMH-43.2 was evaluated in a hemicellulosic hydrolysate obtained by dilute-acid hydrolysis of sugarcane bagasse (Ferreira et al. 2011). The supplementation of the fermentation medium (with $MgSO_4 \cdot 7H_2O$, yeast extract and/or urea) was required, and yeast extract was reported as favoring ethanol production (Ferreira et al. 2011). Also, initial D-xylose concentration and inoculum load showed significant ($p < 0.05$) influence on ethanol production. The best results (ethanol yield and productivity of 0.19 $g.g^{-1}$ and 0.13 $g.l^{-1}.h^{-1}$, respectively) were obtained using the hydrolysate containing an initial D-xylose concentration of 30 $g.l^{-1}$, supplemented with 5.0 $g.l^{-1}$ yeast extract and inoculated with an initial cell concentration of 2.0 $g.l^{-1}$ (Ferreira et al. 2011).

Two *Sc. stipitis* strains isolated from the Brazilian Amazonian forest were tested in complex medium (YPX) with D-xylose as sole carbon source and peptone and yeast extract as nitrogen sources and in detoxified sugarcane bagasse hydrolysate (Cadete et al. 2012a). Great differences were observed in the behavior of each strain during the fermentation assay. In the complex medium, strain UFMG-XMD-15.2 showed the best ethanol production results, yielding 0.28 $g.g^{-1}$ ethanol, with productivity equal to 0.51 $g.l^{-1}.h^{-1}$, whereas strain UFMG-HMD-32.1 presented yield and productivity of 0.22 $g.g^{-1}$ and 0.23 $g.l^{-1}.h^{-1}$,

respectively. Due to its good ethanol production achieved in the complex medium, *Sc. stipitis* UFMG-XMD-15.2 was evaluated together with several NCY strains and species using hemicellulosic hydrolysate. Among the microorganisms tested, this strain was the best D-xylose-fermenting, reaching ethanol yield of 0.34 $g.g^{-1}$ and productivity equal to 0.20 $g.l^{-1}.h^{-1}$.

Scheffersomyces shehatae UFMG-HM-52.2 was assayed in batch fermentations of hemicellulosic and cellulosic hydrolysates prepared from sugarcane bagasse pretreated with oxalic acid (OA) and detoxified using calcium hydroxide *over-liming* or subjected to enzymatic hydrolysis after OAFEX pretreatment (Chandel et al. 2013). In detoxified hemicellulosic acid hydrolysate, this strain reached an ethanol yield and productivity of 0.35 $g.g^{-1}$ and 0.13 $g.l^{-1}.h^{-1}$, respectively. When the cellulosic fraction was fermented after enzymatic hydrolysis, an ethanol yield of 0.28 $g.g^{-1}$ and productivity equal to 0.20 $g.l^{-1}.h^{-1}$ were obtained. Additionally, *Sc. shehatae* UFMG-HM-52.2 showed a similar growth pattern in both hydrolysates, being more than 80 % of the sugars utilized within 24 h of incubation. To compare the behavior of different strains from the same species, four *Sc. shehatae* strains (BR6-2AI, CG8-8BY, PT1-1BASP, BR6-2AY) were evaluated under the same D-xylose fermentation conditions (Martiniano et al. 2013a). These strains were grown in YPX medium and detoxified hemicellulosic hydrolysate from dilute-acid pretreatment of sugarcane bagasse. All the strains showed high ethanol yields when cultured in complex medium. *Scheffersomyces shehatae* BR6-2AY presented the maximum ethanol yield (0.48 $g.g^{-1}$) followed by the strains CG8-8BY (0.47 $g.g^{-1}$), BR6-2AI (0.45 $g.g^{-1}$), and PT1-1BASP (0.44 $g.g^{-1}$). The productivities ranged from 0.35 to 0.37 $g.l^{-1}.h^{-1}$. Among all these four strains, CG8-8BY and BR6-2AY were selected for ethanol production from hemicellulosic hydrolysate due to their high ethanol production yields in defined media. The fermentation performances of both strains were lower using the hydrolysate as culture medium, due to the presence of undesired toxic compounds (e.g., acetic acid) in this substrate even after detoxification. *Scheffersomyces shehatae* CG8-8BY and BR6-2AY showed ethanol yields and productivities of 0.30 $g.g^{-1}$, 0.15 $g.l^{-1}.h^{-1}$, 0.21 $g.g^{-1}$, and 0.11 $g.l^{-1}.h^{-1}$, respectively. As the best *Sc. shehatae* isolate selected in this study, the strain CG8-8BY was further characterized (Martiniano et al. 2013b). Two different media formulations were used for inoculum preparation and fermentation medium, using yeast extract and rice bran extract (RBE) as nitrogen sources supplementing a detoxified hemicellulosic hydrolysate from dilute-acid pretreatment of sugarcane bagasse. This strain showed an ethanol yield of 0.33 $g.g^{-1}$ and productivity equal to 0.21 $g.l^{-1}.h^{-1}$ using a fermentation medium supplemented with RBE. On the contrary, the same strain, when grown in hydrolysate supplemented with yeast extract, exhibited an ethanol yield and productivity of 0.20 $g.g^{-1}$ and 0.12 $g.l^{-1}.h^{-1}$, respectively. All these results demonstrate the influence of several fermentation conditions and the intraspecific variability among strains from the same species in the performance of D-xylose conversion to ethanol.

Apart from the importance of the isolation and identification of the new D-xylose-fermenting strains of the *Scheffersomyces* clade from natural habitats in

Brazil, the new six isolates belonging to the species *Sp. passalidarum* reported as associated with rotting wood in the Brazilian Amazonian forest are the most relevant finding (Cadete et al. 2012a). This occurrence is relevant not only because before the isolation of these strains, this species was represented by a single isolate, the type strain, but also due to the recent reports highlighting *Sp. passalidarum* as the major naturally ethanol producer from D-xylose (Hou 2012; Long et al. 2012). The first demonstration of D-xylose fermentation under "anaerobic" conditions by *Sp. passalidarum* (NRRL Y-27907, type strain) resulted in high ethanol production yield, fast cell growth, and rapid sugar consumption with D-xylose being consumed after glucose depletion (Hou 2012). In this work, it was further demonstrated that for this species, D-xylose conversion takes place by means of NADH-preferred xylose reductase and NAD^+-dependent xylitol dehydrogenase. Thus, the capacity of *Sp. passalidarum* to utilize D-xylose under "anaerobic" conditions was proved to be possible due to the balance between the cofactor's supply and demand through its XR–XDH pathway. It has also been shown that this species simultaneously assimilate glucose and D-xylose aerobically and simultaneously co-ferment glucose, cellobiose, and D-xylose with an ethanol yield of 0.42 $g.g^{-1}$ and productivity of 0.53 $g.l^{-1}.h^{-1}$, exhibiting a specific ethanol production rate on D-xylose more than three times that of the corresponding rate on glucose (Long et al. 2012). Moreover, in this work, an adapted strain of *Sp. passalidarum* produced ethanol from a nondetoxified hardwood hydrolysate with yield of 0.34 $g.g^{-1}$. Metabolome analysis of *Sp. passalidarum* before onset and during the fermentations of glucose and D-xylose showed that the flux of glycolytic intermediates is significantly higher on D-xylose than on glucose. High affinity of its xylose reductase activities for NADH and D-xylose combined with allosteric activation of glycolysis probably account in part for its unusual capacities (Long et al. 2012). So far, the performance of the Brazilian *Sp. passalidarum* strains was evaluated in YPX medium and in detoxified hemicellulosic hydrolysate from dilute-acid pretreatment of sugarcane bagasse (Cadete et al. 2012a). In this study, all the strains were responsible for the highest ethanol production in complex medium, yielding from 0.31 to 0.37 $g.g^{-1}$ ethanol, with productivities of 0.62 to 0.75 $g.l^{-1}.h^{-1}$, which are far above those found in *Sc. stipitis* and *Sc. shehatae*. When the hemicellulosic hydrolysate was used as fermentation medium, the production of ethanol by the strains *Sp. passalidarum* UFMG-HMD-1.1 and UFMG-HMD-14.1 was detected, but with lower yields (0.20 and 0.18 $g.g^{-1}$, respectively) when compared to the results in complex medium. However, this production can be enhanced through evolutionary engineering (Long et al. 2012) or protoplast fusion (Hou and Yao 2012).

New D-xylose-fermenting yeast species are important contributions to a better understanding about the evolution process and the metabolism of this pentose among such microorganisms. Although described and characterized as a cellobiose-fermenting yeast (Cadete et al. 2012b, Urbina et al. 2012), *Sc. amazonensis* is also able to ferment D-xylose, but with a remarkably xylitol yield (0.55 to 0.59 $g.g^{-1}$) and, consequently, low ethanol yields (0.07 to 0.08 $g.g^{-1}$) in YPX medium (Cadete et al. 2012a). The recently discovered species from the *Spathaspora* clade,

Sp. brasiliensis, Sp. roraimanensis, *Sp. suhii*, and *Sp. xylofermentans*, are capable of producing ethanol and xylitol from D-xylose at different concentrations (Cadete et al. 2012a, 2013). Under aerobic conditions in YP medium with 2 % of D-xylose (Cadete et al. 2013), *Sp. xylofermentans* UFMG-HMD-25.1 reached the maximum ethanol yield (0.34 $g.g^{-1}$) followed by *Sp. roraimanensis* UFMG-XMD-23.2 (0.29 $g.g^{-1}$), *Sp. suhii* UFMG-XMD-16.2 (0.14 $g.g^{-1}$) and *Sp. brasiliensis* UFMG-HMD-19.3 (0.12 $g.g^{-1}$). When the fermentation process was shift to a less oxygenated condition conducted in complex medium with D-xylose (Cadete et al. 2012a), *Sp. suhii* UFMG-XMD-16.2 and UFMG-HMD-16.3 produced more ethanol (0.33 and 0.27 $g.g^{-1}$) than xylitol (0.21 and 0.17 $g.g^{-1}$). The production of ethanol by *Sp. roraimanensis* UFMG-XMD-23.2 was also higher (0.26 $g.g^{-1}$) than xylitol (0.19 $g.g^{-1}$). Inversely, *Sp. brasiliensis* UFMG-HMD-19.3 produced similar amounts of both products (0.13 $g.g^{-1}$ and 0.16 $g.g^{-1}$ of ethanol and xylitol yields, respectively). The strains *Sp. xylofermentans* UFMG-HMD-25.1 and UFMG-HMD-23.3 exhibit different behaviors in this assay. Whereas UFMG-HMD-25.1 showed a higher yield of xylitol (0.22 $g.g^{-1}$ against 0.14 $g.g^{-1}$ ethanol yield), the reverse was observed for UFMG-HMD-23.3 (0.18 $g.g^{-1}$ ethanol yield and 0.13 $g.g^{-1}$ xylitol yield). In this study, two of these four new species were cultured in detoxified hemicellulosic hydrolysate from dilute-acid pretreatment of sugarcane bagasse. *Spathaspora suhii* UFMG-XMD-16.2 and *Sp. roraimanensis* UFMG-XMD-23.2 achieved the highest xylitol yields (0.57 and 0.61 $g.g^{-1}$) and the lowest ethanol yields (0.23 and 0.22 $g.g^{-1}$), respectively.

Spathaspora arborariae has been the most studied new D-xylose-fermenting yeast from Brazilian ecosystems, as denoted by the significant number of studies published with this species (Cadete et al. 2009; Cunha-Pereira et al. 2011; Hickert et al. 2013; Martiniano et al. 2013b). The type strain, UFMG-HMD-19.1A, is capable of producing ethanol and xylitol from D-xylose, being ethanol the main fermentation product. This yeast showed ethanol yields equal to 0.50 $g.g^{-1}$ in batch D-xylose fermentation (Cadete et al. 2009), 0.45 $g.g^{-1}$ in nondetoxified rice hull hydrolysate (Cunha-Pereira et al. 2011) and 0.14 $g.g^{-1}$ in detoxified sugarcane bagasse hemicellulosic hydrolysate supplemented with RBE (Martiniano et al. 2013b). When co-cultured with *S. cerevisiae* ICV D254 in nondetoxified rice hull hydrolysate in bioreactor cultures under oxygen limitation (Hickert et al. 2013), hexoses and pentoses from the hydrolysate were converted to ethanol and xylitol, with yields of 0.48 and 0.39 $g.g^{-1}$, respectively. Regarding the ability of this yeast to produce ethanol from D-xylose, a major influence of the fermentation media was revealed.

12.3.2 Cellobiose-Fermenting Yeasts

Cellulose is the most abundant biopolymer on Earth and has great potential as a renewable energy source. The enzymatic hydrolysis of cellulose, followed by fermentation to ethanol, is a promising green alternative for the production of

transportation fuels (Lynd et al. 2002). However, its crystalline structure makes this polymer insoluble and inaccessible to cellulolytic enzymes, and therefore a pretreatment step is required for its biochemical conversion processing (Gray et al. 2006; Olofsson et al. 2008; Chauve et al. 2010).

In nature, cellulose is degraded mostly by fungi and bacteria, which excrete a number of hydrolytic and oxidative enzymes (Lynd et al. 2002; Horn et al. 2012), including cellulases, hemicellulases, and enzymes involved in lignin breakdown. Cellulases are divided into endoglucanases (EGs), cellobiohydrolases (CBHs), and β-Glucosidases (BGs). Endoglucanases (EGs) attack cellulose chains at random positions generating cello-oligosaccharides. CBHs are exo-acting enzymes that release cellobiose units from cellulose chain ends. The hydrolysis of cellulose is completed by β-Glucosidases (BGs), which hydrolyze cellobiose and soluble cello-oligosaccharides to glucose (Singhania et al. 2013). Cello-oligosaccharides and cellobiose are potent inhibitors of endoglucanases and cellobiohydrolases. The catalytic activity of the BGs is rate limiting in the saccharification of cellulose. β-Glucosidases not only determine the rate but also the extent of cellulose hydrolysis by relieving end product inhibition of CBHs and EGs (Lynd et al. 2002; Olofsson et al. 2008). In addition, the produced glucose also inhibits β-Glucosidase and exerts feedback inhibition (Krogh et al. 2010).

To be economically feasible, the hydrolysis of cellulose must be conducted at a high dry matter concentration, which inevitably results in a high concentration of hydrolysis endproducts and makes the product inhibition of enzymes a major challenge in rate limiting for lignocelluloses hydrolysis in high-solid conditions and enzyme engineering (Kristensen et al. 2009; Olofsson et al. 2008; Teugjas and Väljamäe 2013). To minimize the end product inhibition, the most often applied setup development is a process called simultaneous saccharification and fermentation (SSF), whereby glucose is constitutively removed by fermentation to ethanol due to the addition of a fermenting organism in parallel with hydrolytic enzymes (Olofsson et al. 2008). However, the rate of ethanol production during SSF can be limited by degradation of cellobiose to glucose because *Saccharomyces cerevisiae* cannot directly use cellobiose and cello-oligosaccharides (Lee et al. 2013). Cellulases preparations with sufficient β-Glucosidase activity are expensive to produce. To bypass the use of BGs and lessen the need of these hydrolytic enzymes, researchers have investigated the use of cellobiose itself as a fermentable sugar (van Rooyen et al. 2005). Using a yeast capable of fermenting both glucose and cellobiose in a coupled system may have several advantages, like circumventing the endproducts inhibition of the cellulase complex and increasing the effective activity of the cellulolytic enzymes (Freer and Detrov 1983), thereby enhancing the ethanol production.

Following the same reasoning adopted for studies with D-xylose-fermenting yeasts, studies toward the use of native cellobiose-fermenting yeast strains for SSF can be direct either as an alternative or co-culture usage with *S. cerevisiae* or to generate yeast strains capable of fermenting cellobiose and cello-oligosaccharides. However, studies on bioprospecting yeasts capable of fermenting cellobiose are scarce. Most works in this area are focused on screening the ability to ferment

cellobiose within species from culture collections (Maleszka et al. 1982; Freer and Detrov 1983; Gondé et al. 1982; Toivola et al. 1984; Morikawa et al. 1985) or to demonstrate the property of one or few yeast strains to convert cellobiose to ethanol (Blondin et al. 1983; Parekh and Wayman, 1986; Spindler et al. 1992; Golias et al. 2002; Ryabova et al. 2003). Therefore, studies of bioprospecting facing such microorganisms are innovative and of interest.

12.3.2.1 Studies with Brazilian Yeasts

Although limited to a few studies, the discovery of new Brazilian cellobiose-fermenting yeast species and strains open a range for future researches in this field. Both *Sc. queiroziae*, a new species described from six isolates related to rotting wood and wood-boring insects collected in areas of Atlantic rainforest (Santos et al. 2011; Morais et al. 2013b), and *Sc. amazonensis*, a new species described from five isolates related to rotting wood from the Brazilian Amazonian forest (Cadete et al. 2012a, b) are able to ferment cellobiose. An ethanol yield from cellobiose (0.32 $g.g^{-1}$) obtained with two *Sc. queiroziae* strains, UFMG-CLM-5.1 and UFMG-IMX-6.1, is in the same range as those obtained during glucose fermentation by these yeasts in YP medium with 2 % of cellobiose or glucose (Santos et al. 2011). Moreover, both UFMG-IMX 6.1 and UFMG-CLM 5.1 lack (or have very low) periplasmic β-Glucosidase activity, with rates of cellobiose hydrolysis of less than ~ 5 $U.g^{-1}$ dry yeast cells at pH 5.0 or 7.0. When the yeast cells were permeabilized, a significant BG activity could be verified at pH 5.0 (29–47 $U.g^{-1}$ dry yeast cells), and especially at pH 7.0 (167–230 $U.g^{-1}$ dry yeast cells), which is consistent with an intracellular β-Glucosidase as being responsible for cellobiose hydrolysis by this species (Santos et al. 2011). Recently, the conversion of D-xylose to ethanol was also demonstrated by new *Sc. queiroziae* strains (Morais et al. 2013b), a feature that enhances the potential of this species in fermentative process of biomass conversion.

Scheffersomyces amazonensis has also the ability of ferment D-xylose, with a remarkable production of xylitol (Cadete et al. 2012a). In a fermentation assay performed in complex medium with D-xylose as sole carbon source, all the strains belonging to this species achieved the highest xylitol yields (0.55–0.59 $g.g^{-1}$) accomplished with a low ethanol production (0.07–0.08 $g.g^{-1}$) when compared to the other strains tested (Cadete et al. 2012a). Once that xylitol is one of the most expensive polyol sweeteners in the world market and has been the subject of specific health claims (Saha 2003), the biotechnological production of xylitol using microorganisms is of economic interest, and yeasts demonstrating this capacity can be used in processes of bioconversion of hemicellulosic hydrolysates to this high-added value product.

12.4 Final Remarks and Future Perspectives

Brazilian biodiversity has allowed the isolation, identification and characterization of novel yeasts species and strains able to ferment lignocellulosic sugars. In view of its efficient D-xylose/cellobiose fermentation, these yeasts are a new source of genes coding enzymes (including sugar transporters) to engineer industrial strains for the production of 2G bioethanol and other advanced biofuels and chemicals. Several studies performed with *Sp. passalidarum* in the past years makes this species the most attractive microorganism within this field. The recent genome sequencing of *Sp. passalidarum* type strain (Wohlbach et al. 2011) is an important tool to elucidate the remarkable conversion of D-xylose to ethanol attained by this yeast and to develop unprecedented D-xylose-fermenting *S. cerevisiae* strains. Under this scenario, Brazilian *Sp. passalidarum* strains arise as a great set of "tools" to be explored and exploited for industrial production of lignocellulosic ethanol and other advanced biofuels.

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Chapter 13
Trends in Biodiesel Production: Present Status and Future Directions

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Abstract The use of renewable fuels, an alternative that reduces the generation of greenhouse gases, is one proposal to mitigate the effects that contribute to global warming. Brazil is the fourth largest producer of biodiesel in the world, and growth expectations of the productive capacities have generated huge technological and environmental challenges. In this context, this chapter discusses some of the relevant aspects of biodiesel production in Brazil, including sustainability of raw materials, conventional technology limitations, and further presents technological alternatives as strategies that will guide the future directions which can result in processes with greater environmental and economic returns.

13.1 Introduction

Global warming is a serious environmental problem these days (Kerr 2013). Consequently, substantial climate changes have been observed, causing major socioeconomic and environmental impacts to society and biodiversity. The emission

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of greenhouse gases from the use of fossil fuels on a large scale after the industrial revolution is seen as the main cause of this phenomenon (Beck 2013).

Most of the energy consumed in the world comes from fossil oil, natural gas, and coal. Fossil fuels are widely used as a transportation and machinery energy source due to their high heating power, availability, and quality combustion characteristics (Hassan and Kalam 2013). However, as foreseen, fossil fuel resources will inevitably be depleted, while demand for energy is increasing due to population growth, technological progress, and urbanization. Thus, estimates for 2100 suggest that worldwide energy demand will be five times greater than today (Hossain and Davies 2013). At the same time, a report of the Intergovernmental Panel on Climate Change (IPCC) pointed out the use of renewable fuels as an alternative to mitigate the emission of greenhouse gases (IPCC 2007).

Several countries have investigated, developed, or are considering the introduction of biofuels in their national energy programs. Particularly, Brazil has developed programs that use biofuels and other renewable energy sources, for both transport and power generation. In 2002, the Incentive Program for Alternative Sources of Energy (PROINFA) was implemented with the purpose of developing alternative and renewable sources of energy for electricity production, taking into account the characteristics and potential region, aimed at reducing emissions of greenhouse gases. More recently, the National Program for Production and Use of Biodiesel (PNPB) was launched in 2004 to regulate the production and distribution of Brazilian biodiesel from various sources of raw materials, in a sustainable way, both technically and economically, with a focus on social inclusion and regional development. Thus, small farms can cultivate oilseeds according to regional characteristics in order to produce biodiesel. However, although soybean is the main raw material marketed by the program, other crops such as sunflower, peanut, sesame, castor, and soybean oil itself have a higher market value. Therefore, some of the oil can be sold to the program, but can also be used for manufacturing products with higher commercial value.

Biodiesel production in Brazil is an overcoming history when compared with the ethanol production, since ethanol has a consolidated technology in relation to biodiesel, which is still incipient (Sallet and Alvim 2011). The methods for biodiesel production are well known (Basha et al. 2009). However, the chemical transesterification using methyl alcohol or ethyl alcohol, in some cases by one or two reaction steps, has been adopted as a conventional route for its production at an industrial scale. In general, biodiesel production can be considered as a simple process. However, production on a large scale presents challenging technological and production cost problems. In addition, as the raw materials used in the production of biodiesel fuel are sometimes the source of food for humans and/or animal consumption, controversy, and competition between biofuels and food, sustainability and limited land for use, and deforestation have been generated (Elbehri et al. 2013).

This chapter discusses some of the aspects of biodiesel production in Brazil, including feedstock used, technological routes established, and alternative

processes such as unconventional methods of characterization as well as the challenges that must be met to make this process more competitive and attractive in terms of industrial and environmental concerns.

13.2 Raw Materials for Biodiesel Production

For biodiesel production, the raw materials frequently used around the world, according Pahl (2008), are rapeseed oil (59 %), soybean oil (25 %), palm oil (10 %), sunflower oil (5 %), and other sources (1 %) which include: coconut, jatropha sp, camelina, peanut, safflower, mustard, hemp, corn (maize), waste frying oil, animal fat, and algae. For Brazil, Fig. 13.1 shows the profile of use of raw materials in the production of biodiesel based on the average values of the first half of 2013, according to data reported by the National Agency of Petroleum, Natural Gas and Biofuels (ANP). Brazil is the second largest soybean producer in world, and as can be seen, soybean biodiesel represents more than 72 % compared to other raw materials, followed by beef tallow and cotton which are approximately 20 and 3 %, respectively. The explanation for this national scene is due to investments in the production chain over decades, which has resulted in the development of new varieties of species, genetic improvement, and plague control and, consequently, a higher rate of productivity compared to other oilseeds, resulting in a relatively lower cost of soybean production.

However, other crops have been gaining ground as sources of raw materials (Fig. 13.2). Furthermore, it is predicted that over the years, this growth will become more significant insofar as technological advances in agriculture are reached, especially for those oilseeds with higher energy density than soybeans (Table 13.1), i.e., the higher oil content of seeds. In fact, this may be possible because of the geographic characteristics of Brazil, which is basically a tropical country that has a large territory, important water resources, regular rainfall, high biodiversity, and well-developed agricultural technologies, therefore, having great potential for bioenergy production (Sthel et al. 2009).

Many studies have demonstrated the potential of some of these oilseeds in biodiesel production. Macedo et al. (Macedo et al. 2011a) studied the thermal properties of biodiesel obtained from oiticica oil, while Andrade et al. (2012a) produced biodiesel through moriche palm oil (Buriti oil) to evaluate thermal behavior in blends with diesel. Other studies investigated the potential of using different raw materials such as macaw palm oil (Ferrari and de Azevedo Filho 2012), babassu oil (Freitas et al. 2009; Nascimento et al. 2009), Pequi oil (Macedo et al. 2011b), in the production of biodiesel.

Although there is still much to be done for its implementation on an industrial scale, microalgae has been identified as third-generation biodiesel and presents several benefits over other raw material resources, such as land use, potential cultivation in nonfertile locations, and especially its faster growth and high lipid-to-biodiesel yield (Torres et al. 2013). According to Demirbas and Demirbas

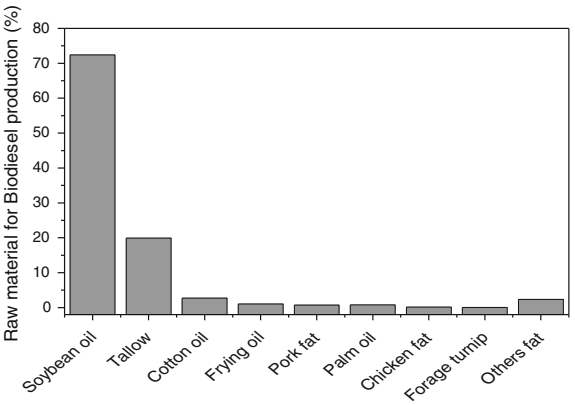

Fig. 13.1 Raw materials used for Brazilian biodiesel production during the first 6 month of 2013 (ANP 2013)

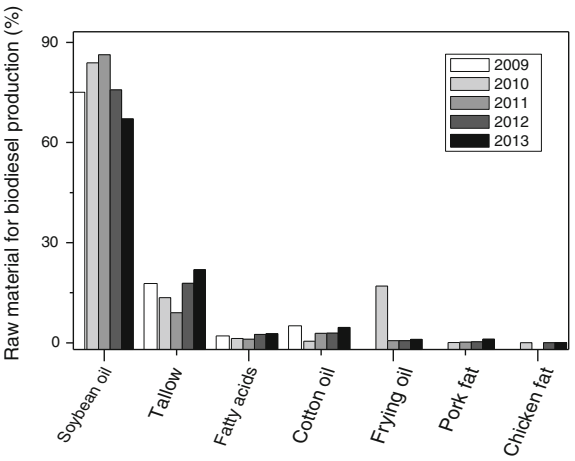

Fig. 13.2 Profile of the use of raw materials used in biodiesel production in Brazil between 2008 and 2013 (ANP 2013)

(Demirbas and Demirbas 2011), high oil species of microalgae cultured in optimized growth conditions in photobioreactors have the potential to yield 19.000–57.000 L of microalgal oil per acre per year; consequently, the yield of oil from algae is over 200 times the yield from the best-performing plant vegetable oils. More than three thousand species of algae have been identified;thus the choice of highly producing strains of oils for industrial application will probably depend on genetic improvement. The oil content of some microalgae is very attractive for biodiesel production, since it can be higher than 70 %. For example, the oil content of *Schizochytrium sp*. and *Botryococcus braunii* are 50–77 and 25–75 %, respectively (Chisti 2007). Similarly, single cell oils (SCOs) accumulated by oleaginous microorganisms have recently emerged as a potential feedstock for production of biodiesel. This is a very interesting raw material because the biomass can be produced in conventional fermenters using low-cost substrates as carbon sources, such as sugar cane bagasse (Kamat et al. 2013), glycerol generated from biodiesel

Table 13.1 Several oilseeds with potential to produce biodiesel from vegetable oil in Brazil

Oilseed type	Oil content (%)	Oil yield (ton/ha)	Brazilian region[a]	Refs.
Babassu palm (*Orbignya phalerata*)	60–68	0.12[b]	Largest producer is the State of Maranhão	(Freitas et al. 2009)
Canudo-de-pito (*Mabea fistulifera*)	40	–	Minas Gerais, Rio de Janeiro, and São Paulo	(CENBIO 2013)
Crambe (*Crambe abyssinica Hochst*)	35	0.45–2.5[b]	Plantations extend through central warmer regions of Brazil	(Vargas-Lopez et al. 1999)
Cream nut or monkey pot (*Lecythis pisonis Camb.*)	54.80	–	Ceará to Rio de Janeiro, South Bahia, and North Espírito Santo	(CENBIO 2013)
Linseed (*Linum usitatissimun L.*)	33–43	0.4–1.45	Brazil South Region, especially Rio Grande do Sul	(CENBIO 2013)
Macaw palm (*Acronomia aculeata*)	20–25	1.5–5.0	Midwest and North, Minas and São Paulo	(do Amaral et al. 2011)
Maraja (*Bactris tomentosa Mart.*)	28	–	Maranhão and Pará	(CENBIO 2013)
Monguba (*Pachira aquática Aubl*)	56–58	–	Entire Amazon region to Maranhão	(CENBIO 2013)
Moriche palm (*Mauritia flexuosa*)	29	–	Acre, Amazonas, Bahia, Ceará, Goiás, Tocantins, Maranhão, Pará, Piauí, São Paulo	(CENBIO 2013)
Palm (*Opuntia cochenillifera*)	22	2.0–8.0[b]	Amazonas and North Region	(Queiroz et al. 2012)
Jatropha (*Jatropha curcas L*)	50	1.2–1.5[b]	Goiás, Minas Gerais and in the Northeast.	(CENBIO 2013)
Rapeseed (*Brassica napus L. var. oleifera*)	34–40	0.8	Goiás, Mato Grosso do Sul, Paraná, Rio Grande do Sul	(CENBIO 2013)
Safflower (*Carthamus tinctorius*)	30–45	0.7	A very promising planting in the semiarid region in Brazil	(CENBIO 2013)
Sessame (*Sesamum indicum L.*)	50–60	0.24	Goiás, Mato Grosso and Southeast (mainly in São Paulo)	(CENBIO 2013)
Sunflower (*Helianthus annus L.*)	40–47	0.774[b]	Alagoas, Ceará, Goiás, Mato Grosso, Mato Grosso do Sul, Paraná, Rio Grande do Norte, Rio Grande do Sul, Sergipe	(Bergmann et al. 2013)

(continued)

Table 13.1 (continued)

Oilseed type	Oil content (%)	Oil yield (ton/ha)	Brazilian region[a]	Refs.
Tucuma palm (*Astrocaryum aculeatum*)	30–50	–	Acre, Amapá, Amazonas, Pará, and Rondônia	(CENBIO 2013)
Tung-oil tree (*Aleurites moluccanus*)	35–40	0.790	Rio Grande do Sul	(CENBIO 2013)
Turnip forage (*Raphanus sativus L.*)	30	2.2	Mato Grosso, Mato Grosso do Sul, Minas Gerais, and São Paulo	(CENBIO 2013)
Ucuúba (*Virola surinamensis*)	58–60	–	Amazon region to Maranhão and Pernambuco	(CENBIO 2013)
Western soapberry (*Sapindus saponaria L.*)	30	–	Amazon region to Goiás and Mato Grosso	(CENBIO 2013)

[a] IBGE-Brazilian Institute of Geography and Statistic (http://www.ibge.gov.br/english/)
[b] Yield oil (Bergmann et al. 2013)

production (Xu et al. 2012), biomass pyrolytic sugars (levoglucosan) (Lian et al. 2013), and aqueous fractions rich in organic short chain (C1–C4) obtained in thermochemical conversion processes of biomass (Lian et al. 2012).

The choice of suitable feedstocks must also answer technical questions. Thus, parameters such as flash point, viscosity, density, acid value, cetane number, and oxidative stability, among others, must be observed for both biodiesel and diesel/biodiesel blends. Depending on the chemical composition of the raw material, some properties of the produced biodiesel may be undesirable. In these cases, the use of additives may be required to attenuate these effects (Focke et al. 2012; Ali et al. 2013). Thus, the production of biodiesel from oils with high iodine value, for example, can result in a product susceptible to oxidation. Similarly, raw materials with high content of saturated fatty acids result in biodiesel which tends to have solidification problems with temperature variations (Knothe et al. 2005).

13.3 Process of Biodiesel Production: Trends and Alternatives

In 2010, Brazil became the second world producer of biodiesel with a production of 2.4 billion of liters, approximately, second only to Germany. However, in 2011, both the United States and Argentina increased production, and now Brazil is the fourth world producer of biodiesel, as shown in Fig. 13.3.

At present, 69 Brazilian plants are authorized for biodiesel production, corresponding to a total capacity of 22,334.06 m^3/day. Among these, 11 plants are allowed to expand their production capacity, while 3 other new plants should be built, providing an increase of 9 % in the current production capacity of biodiesel in Brazil (ANP 2013).

Figure 13.4 shows the values of biodiesel production in Brazil in relation to the overall production. The global average annual growth rate over the period from the end of 2005 through 2011 was approximately 37 %. Compared with global production, the Brazilian profiles were similar; biodiesel production increased from 70 million liters in 2006 to 2.7 billion liters in 2011. Thus, the Brazilian average annual growth rate over the period from 2008 to 2011 was 33 %. In 2012, biodiesel production continued to expand, but at much lower rate, nearly 1.7 %, while the global production was just 0.4 %. However, the Brazilian biodiesel market is not open, and the ANP itself, which currently regulates sales through public auctions, gives preference to companies with the "Social Fuel Label."

Industrially, biodiesel production is achieved by chemical transesterification of oils/fats using methanol or ethanol in some cases, by one or two reaction steps in the presence of a homogeneous alkaline catalyst such as KOH or NaOH or its corresponding alkoxide ($Na^+CH_3O^-$or $K^+CH_3O^-$). These alkoxides can be produced in a very simple way, dissolving sodium hydroxide in alcohol, before its addition to the reaction medium. Basically, in this process one mole of oil reacts with three moles of alcohol, but in industrial practice alcohol is used in excess to

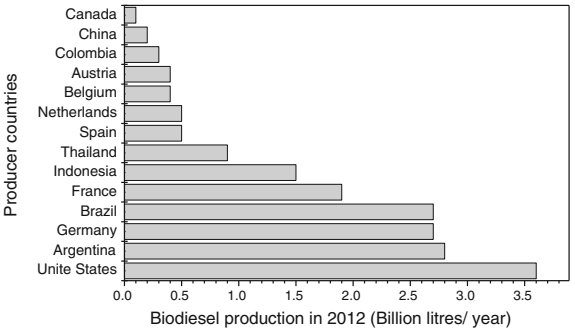

Fig. 13.3 Global ranking of biodiesel production for 2012 (REN21 2013)

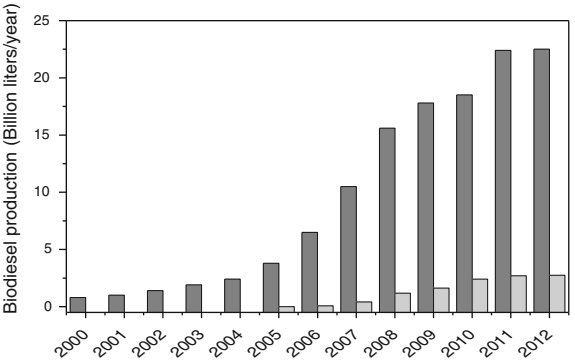

Fig. 13.4 Biodiesel production record by years. Symbols: ■ Global production (REN21 2013); ■ Brazilian production (ANP 2013)

displace the equilibrium of the reaction and promote the formation of the product. As a result, a mixture of alkyl esters of fatty acids of a long chain and glycerin as byproduct is obtained (Fig. 13.5).

From a technological point of view, this is a relatively simple process which can be conducted at atmospheric pressure and under moderate conditions of temperature (50–60 °C), resulting in high conversion rates at relatively low reaction times (Meher et al. 2006). When low-cost oils and fats (waste frying oil, etc.) are used as raw material, they cannot be converted to biodiesel using alkaline catalyst because of their large amounts of free fatty acids. Therefore, two-step processes are required. First, the free fatty acids are converted to fatty acid methyl esters by an acid catalyst; in the second step, transesterification is completed using an alkaline catalyst.

A general description for a conventional process is synthesized in Fig. 13.6, in which a mixture of oil, methanol, and catalyst is fed to a system of two stirred tanks reactors; after reaction in the first reactor, the glycerol is removed from the first reactor before being fed into the second reactor. The subsequent sections allow separation of biodiesel as the light phase from glycerol, and it is purified by washing, neutralization, and then vacuum dried. Meanwhile, the glycerol is separated from the residual fraction, neutralized and the excess of methanol is removed by evaporation and recycled in the process (Knothe et al. 2005).

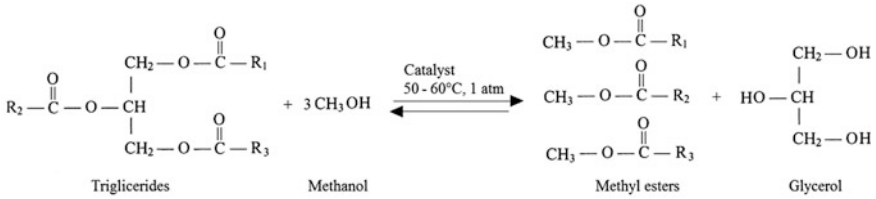

Fig. 13.5 Simplified scheme for biodiesel production by chemical transesterification

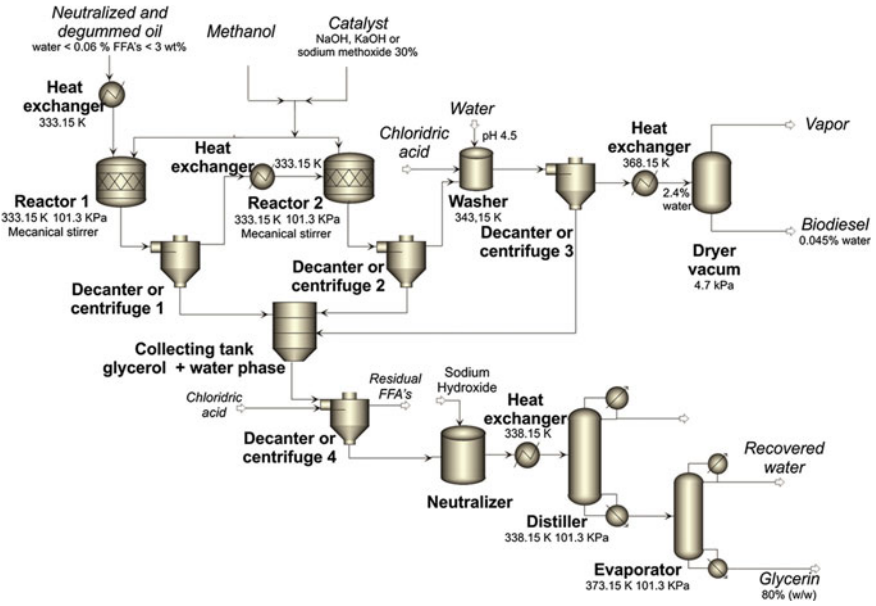

Fig. 13.6 Flow sheet for conventional biodiesel production

The presence of free fatty acids and water in the reaction facilitates soap formation; the need to neutralize the catalyst after the reaction; the impossibility of catalyst retrieval; as well as requirements for treatment of effluents generated during the washing steps are some drawbacks that currently require attention in the process. Furthermore, when free fatty acid content is very high, this process can also be carried out using homogeneous acid catalysts despite the alkali, but reaction times are longer, requiring much higher oil molar ratio oil: alcohol and reaction temperatures.

On the other hand, transesterification processes in situ have been reported for a diversity of oils using methanol (Abo El-Enin et al. 2013). In these processes, the ground oilseed is mixed directly, instead of purified oils, with alcohol and catalyst, to produce alkyl fatty acid esters, as shown in the generic scheme (Fig. 13.7). The molar ratio oil: alcohol higher than the value calculated according to stoichiometry has been tested alkaline and acid catalyst. The simplicity of the process will pave

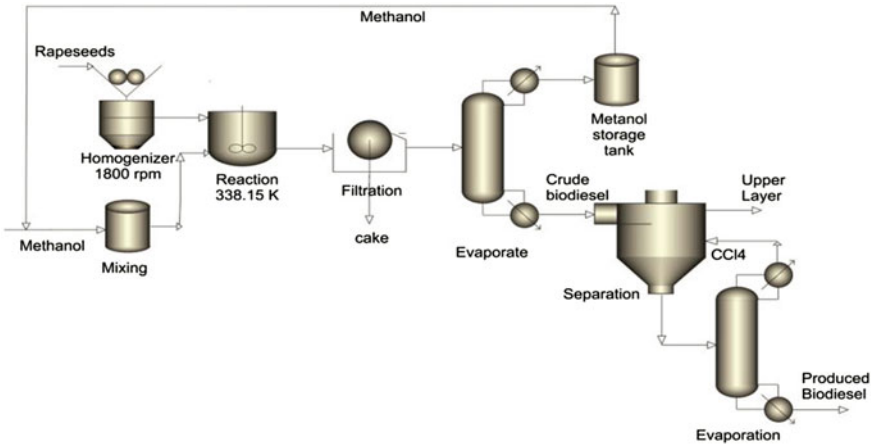

Fig. 13.7 Pilot scale for in situ transesterification from rapeseeds (Modified from Abo El-Enin et al. 2013)

the way for oil seed growers to move away from overdependence on crushing and solvent extraction plants.

Emphasis can be also placed on the technology developed by PETROBRAS (Brazil) for the biodiesel production known as Grain route. In the process, biodiesel is obtained directly from oilseed plant grain using ethanol, which works not only as an acyl acceptor, but also as a solvent in the oil extraction. This process eliminates steps such as oil extraction and refining; its feasibility on an industrial scale was assessed in a small plant in Rio Grande do Norte (Brazil) (Sauer et al. 2006).

The production of biodiesel from algae is also a process that is under discussion. A variety of high value-added byproducts can be produced from algae; therefore, it is likely that in the future production of biodiesel from algae will be the least important application. Basically, bioreactors for algal culture consist of open ponds, photobioreactors, and closed systems. Open pond systems are shallow ponds in which algae are cultivated, while photobioreactors include different types of tanks or closed systems. The biodiesel from algal oil is similar to biodiesel produced from vegetable oils. The biggest challenges of this process are the choice or development of bioreactors, the definition of the best choice to formulate nutrients, and methods for biomass processing to extract the oil, which involves costly steps such as concentration, separation, drying, and oil extraction. After obtaining the oil, the following steps involve transesterification and purification of biodiesel, alcohol recovery and separation of glycerin, as in the conventional process. Economic evaluation studies have been reported comparing different alternatives (Gallagher 2011; Nagarajan et al. 2012). Closed systems are relatively expensive compared to open ponds due to the required infrastructure costs. However, open systems are more vulnerable to bacterial contamination. Thus, the tubular photo bioreactor seems to be the most satisfactory choice for producing algal biomass on the scale needed for biodiesel production (Demirbas and Demirbas 2011).

Another common issue in this process that must be addressed relates to the use of methanol for biodiesel production. Ethyl esters proved to be a viable alternative to diesel fuel being more sustainable than methyl esters (Brunschwig et al. 2012). Methanol is a product known to be toxic and although it can be obtained from synthesis gas (syngas) of biomass, it is usually produced from fossil materials, such as natural gas. In anyway, in Brazil lacks self-sufficiency in the CH_3OH production. In this sense, the use of ethanol instead of methanol fossil is a very attractive alternative. A technological condition in Brazil that is quite atypical with respect to most countries engaged in biofuels production, especially for being the second largest ethanol producer in the world, reaching in 2012 a production of 21.6 million liters of ethanol. But either way it would be necessary to increase the production levels to meet demand for biodiesel in the next years.

Technological advances have made possible the construction of industries which are able to process raw materials from various sources, using both methylic and/or ethylic routes, in batch or continuous processes (Table 13.2). Stand out Barralcool plant, designed with Dedini-Balestra technology and built-in integrated way with a sugarcane industry. In addition, processes that use centrifuges instead of decanters to separate biodiesel from glycerol, such as Westfalia technology, improve the separation phases, but production cost can be increased as a consequence of high energy consumption. On the other hand, some Brazilian research technologies that can modify the conventional reaction step are summarized in Table 13.3. Several of these emerging technologies seem to be cost-effective and environmentally friendly operations in comparison with conventional biodiesel production technologies. In some cases, the conventional processes without great modification can be adapted, e.g., ultrasound reactors when the mechanical stirrer is replaced with ultrasound equipment. Furthermore, the use of packed-bead reactors, which are well known in other industrial processes, are limited by the lack of optimization studies applied to biodiesel production.

On the other hand, extensive research activity has been observed to use heterogeneous catalysts as alternative to the use of conventional homogeneous catalysts (Table 13.4). In these systems are required typical reaction temperatures and molar ratios of oil: alcohol more higher, however, probably one of the most important advantages is that these catalysts do not produce soap, can be recovered and consequently, their use results in processes with lower environmental impact. Heterogeneous catalysts may be chemical or enzymatic, the latter consisting of enzymes and cells (whole cells), either free or immobilized. Particularly, in the case of chemical heterogeneous catalysts, many studies have focused on both alkaline and acid catalysts, as well as the reaction mechanisms and their physicochemical properties that influence biodiesel yields (Islam et al. 2013; Semwal et al. 2011; Endalew et al. 2011). There is still no consensus, however, as to whether alkaline catalysts are a better choice than acid in terms of reaction rate and biodiesel productivity. One disadvantage of the use of a solid catalyst is the formation multiphasic system, which leads to diffusion limitations that decrease the reaction rate (Semwal et al. 2011).

Table 13.2 Some examples of Brazilian industries designed to produce biodiesel production by methylic and/or ethylic routes (BiodieselBR 2013)

Industry	Feedstock	Process	Technological route	Production capacity (10^6 L/year)
Araguassu	Soybeans (80 %), sunflower, cotton, castor beans	Own technology/continuous	Methylic/ethylic	36
Barralcool	Soybeans	Dedini-Balestra batch/ continuous	Methylic/ethylic	60
Bigfrango	Animal fat, recycled oil	Own technology/batch	Methylic/ethylic	2
Bio Petro	Soybeans	Own technology/continuous	Methylic/ethylic	70
Biopar Parecis	Animal fat		Methylic/ethylic	36
Bioverde	Soybeans (40 %), cotton (50 %), recycled oil (10 %)	Own technology/batch	Methylic/ethylic	181
Cooperbio	Soybeans, sunflower, cotton, animal fat, recycled oil	Own technology/continuous	Methylic/ethylic	166
Delta Biocombustíveis	Soybeans, cotton, crambe, and beef tallow		Methylic/ethylic	108
Fertibom	Soy, Sunflower, jatropha, animal fat, recycled oil, peanut	Own technology/batch	Ethylic	120
Granol (Anapolis city, GO)	Soybeans (90 %), cotton (90 %) Forage turnip, animal fat, recycled oil	Dedini-Balestra continuous	Methylic/ethylic	372
Granol	Soybeans (90 %), cotton, forage turnip, animal fat, recycled oil	Westfalia/continuous	Methylic/ethylic	336
SP Bio	Soybeans (80 %), animal fat, recycled oil	Own technology/batch	Methylic/ethylic	25

Table 13.3 Unconventional technologies for biodiesel production

Type of reactor	Characteristics	Refs.
Microwave irradiation	Palm oil using ethyl alcohol with *Pseudomonas fluorescens* immobilized. Conversion 97.56 %, 12 h, 43 °C, productivity of 64.2 mg ethyl esters $g^{-1}h^{-1}$	(Da Rós et al. 2013)
	Macaw acid oil, ethanol (1:9), and commercial enzymes, use of microwave increased about one order the biocatalyst activity	(Nogueira et al. 2010)
	Adapted domestic microwave oven with babassu coconut oil, methanol and KOH, 70 s, conversion >90 %	(Nascimento et al. 2009)
Heterogeneous fixed bed	Soybean oil ethanol 3:1, lipase from *B. cepacia,* conversion of 95 %, 46 h, 50 °C, alcohol added in two steps (0 and 7 h) and 1 % (m/m) of water. Column of 17 mm (ID) and 100 mm high, recirculation (1.5 mL/min) bottom– top	(Salum et al. 2010)
	Pellets of mixed oxides in a reactor 30 cm long column flowed with soybean oil (168 g/h) and methanol or ethanol (89 g/h) at 100 °C reached 80 % yield (methanol) and 40 % (ethanol). At 180 °C, ethanolysis reach yields up to 90 %	(Suarez and da Silva 2012)
Ultrasonic cavitation	Using commercial immobilized enzymes, mild irradiation power supply (100 W), 60 °C in 4 h, 90 wt% of conversion	(Batistella et al. 2012)
	Soybean oil with ethanol (1:24) and potassium hydroxide (1.5 %) using low-frequency ultrasound (20 kHz), reaction mixtures between 39 and 52 °C. With three-step reaction, the yield was of approximately 98 % after 6 min	(Brito et al. 2012)
	Soybean oil, ethanol and NaOH at room temperature, for 30 min produce yield of 91.8 %	(Rodrigues et al. 2009)
	Using a frequency of 24 kHz beef tallow, methanol (6:1), NaOH (0.5 %), 70 seg, 60 °C, conversion 92 %	(Teixeira et al. 2009)
Reactive distillation column	Simulation and experimental reaction of soybean oil, ethanol, catalyzed with NaOH, attained a conversion of 99.84 wt% after 6 min	(Da Silva et al. 2012)
Reactive extraction	Simulation of batch and continuous processes demonstrated the possibility of applying biocatalyst system (*C. rugosa* immobilized in tetraethyl orthosilicate with magnetite) in the reactive zone using external magnetic fields	(Dussan et al. 2010)
2 step reaction by transesterification	Two step transesterification procedure which starts with a basic catalysis, followed by an acidic catalysis. 97 % conversion for waste cooking oil and soybean oil and 98 % for linseed oil were achieved	(Guzatto et al. 2011)

(continued)

Table 13.3 (continued)

Type of reactor	Characteristics	Refs.
Supercritical microtube reactor	Soybean oil, supercritical ethanol (1:20), catalyst-free, carbon dioxide as co-solvent (0.2:1 mass). 598 K, 20 MPa, oil to ethanol molar ratio of 1:20 and using a CO_2 to substrate mass ratio of 0.2:1	(Trentin et al. 2011)
	Soybean oil and macaw oil with supercritical methyl acetate (1:5), without catalyst, at 20 MPa, with 45 min at 350 °C obtain yield of 44 % for soybean oil and with macaw oil 83 % of yield	(Doná et al. 2013)
Ultra-shear reactor	Equipment with rotor–stator mixing with high speed and intense shear frequency. Soybean, ethanol, NaOH, 1:6:1.35, conversion of 99.26 wt%, with 12 min, 78 °C and constant agitation of 7900 rpm	(Da Silva de et al. 2011)

The use of a co-solvent can help to solve this problem, but in industrial practice this method should not be used to avoid increasing cost production. In addition, another important aspect that must be observed concerns the particle size of these catalyst systems, which are usually synthesized as very small particles or fine powder. Conceptually, high reaction rates should be expected when catalysts with high surface area are used (Levenspiel 1999). However, this may result in the formation of clusters due to the physiochemical properties of the reaction medium oil: alcohol. Consequently, on one hand it affects the performance of the catalysts and on the other side, more complex and expensive downstream steps are required.

Bifunctional heterogeneous catalysts have also been studied as a potential alternative means to simultaneously develop biodiesel production by esterification and transesterification reactions (Borges and Díaz 2012; Farooq et al. 2013), but to attain a catalyst bifunctional with adequate surface area, size, and porous volume and high activity, as well as being inexpensive, more investigations are required. However, Axens has commercialized a process for the production of biodiesel via heterogeneous catalysis at elevated temperatures (180–220 °C) and consequently higher pressures, known as Esterfip-H process. The transesterification reaction makes use of rapeseed oil and methanol and as catalyst a spinel, e.g., one co-mixes the alumina support material with zinc (Bournay et al. 2005).

In addition, Albemarle Corporation (www.albermarle.com), a leader in the market of heterogeneous catalysts, has a pilot plant demonstration (BECON Pilot Plant) in Iowa (USA) with a capacity of 300,000.00 gal/year for the production of biodiesel via heterogeneous catalysis, using the catalyst known as GoBio T300. This process uses vegetable and algae oils and operates at pressures and temperatures similar to conventional homogeneous catalysis process.

In a similar way, large efforts have been made to investigate enzymatic transesterification (Tan et al. 2011; Gog et al. 2012). Lipases are the most studied enzymes and they show great potential for enzyme immobilized on organic or inorganic supports. Basically, the high biochemical specificity of lipases, which allows conducting the reactions under mild conditions of temperature, as well as the ease of biocatalyst reuse in several reaction cycles, are some of the major

Table 13.4 Some study cases of Biodiesel production using heterogeneous catalysts

Catalysts	Oil, alcohol (molar ratio % cat)/Temperature/Reaction time/ Conversion	Refs.
HUSY and Ce/HUSY zeolites	Soybean oil, ethanol (30:1: 0.001 mol)/200 °C under constant stirring (1000 rpm) and autogenous pressure (20 bar)/ 24 h/ >97 % for barium	(Borges et al. 2013)
Alkaline compounds of strontium $SrCO_3 + SrO + Sr(OH)_2$	Babassu, methanol (1:6:1.0 %)/65 °C/ 1 h/ >95 %/reusability 6 times	(de Carvalho et al. 2013)
$Ni_{0.5}Zn_{0.5}Fe_2O_4$ ferrites doped with Cu	Soybean oil, methanol (1:20:4 %)/160 °C/ 2 h/ >42 %	(Dantas et al. 2013)
Sn(IV) complexes: Butyl stannoic acid, di-*n*-butyl-oxo-stannane and dibutyl tin dilaurate	Soybean FFAs, methanol (simultaneous transesterification/ esterification) (4:1:0.01)/160 °C/ 1 h/ >90 %	(Brito et al. 2012)
Alumina impregnated with potassium iodide	Bran oil, methanol (15:1: 5 %)/–/92 h/95.2 %	(Evangelista et al. 2012)
Iodide potassium incorporated on mesoporous molecular sieves (SBA-15 and MCM-41)	Sunflower oil, methanol (1:15:1 e 2 %)/60 °C/4 h/∼85 %	(de Galvão et al. 2012)
Lewis acid/surfactant rare earth trisdodecylsulfate	Waste cooking soybean oil with 8.8 wt.% of free fatty acids, ethanol (1:6:10 %)/100 °C/1 h/76–86 %	(de Mattos et al. 2012)
Mesoporous silica active phase (La50SBA-15)	Soybean oil, ethanol (20:1:1 %), at inert atmosphere (N_2)/ 343 K/6 h/>80 %	(Quintella et al. 2012)
Prepared from the waste material, Amazon flint kaolin and activated with 4 M sulfuric acid	Esterification of distillate produced by deodorization of palm oil and methanol (1:60)/160 °C/4-h/92.8 %	(do Nascimento et al. 2011)
$H_3PW_{12}O_{40}$ (HPA)	Oleic acid, methanol (esterification) (1:1:0,1 g)/25 °C/10 h/ 80 %	(Sepulveda et al. 2011)
Mixed oxides $(Al_2O_3)_{0.8}(SnO)_{0.2-x}(ZnO)_x$ $(0.2 \leq x \leq 0)$	Soybean oil, methanol/100 °C/3 h/>80 %/200 h	(Suarez and da Silva 2012)

attractions of this alternative. Novozym 435 has been reported to be an effective biocatalyst for biodiesel production (Da Rós et al. 2012a). Also, some successful strategies combine the use of enzymes with unconventional reactors, resulting in considerable improvements in the productivity of biodiesel production, as in the case of a microwave reactor used for the transesterification of beef tallow by *Burkholderia cepacia* lipase immobilized on silica-PVA, where full conversion was achieved only at 8 h reaction (Da Rós et al. 2012b, 2013). More recently, the preparation of biocatalysts with magnetic properties has motivated the scientific community, and some work has been applied to the production of biodiesel (Wang et al. 2011; Ngo et al. 2013; Liu et al. 2012). These systems allow the separation of the magnetic biocatalyst by applying an external magnetic field at the end of the reaction. However, the main obstacles to the use of immobilized enzymes are the high cost and low conversion rates as a function of the reaction time (Jang et al. 2012). Thus, whole cells are an attractive alternative because they can be obtained at much lower cost than purified enzymes (Gog et al. 2012; Andrade et al. 2012b), but the long reaction times required and low conversion rate are still undesirable. In these cases, diffusional limitations imposed by cellular walls certainly contribute to lower conversion rates. Therefore, to further reduce the cost of biocatalysts in biodiesel production, new immobilization procedures with higher activity and stability still need to be explored.

13.4 Characterization of Biodiesel Through Unconventional Techniques

The growth of biodiesel production simultaneously demands research for the development and implementation of analytical techniques to evaluate the quality of biodiesel, as well as of diesel/biodiesel blends. Basically, biodiesel quality is determined through the analysis of chemical composition and several physical properties, which have been extensively reviewed (Knothe et al. 2005; Knothe 2006; Monteiro et al. 2008).

Nevertheless, there is demand for new procedures and, in this scenario, photothermal techniques arise as alternatives and unconventional methodologies for the characterization of biodiesel. Photothermal methods include various techniques and phenomena based on the absorbed optical energy into heat conversion. Basically, these techniques consist of detecting very small variations in temperature resulting from the absorption of the given modulated radiation, and therefore it is possible to determine physical properties such as conductivity and thermal diffusivity of the raw material, biodiesel, and blends with diesel. Among the different types of phothermical techniques, two have been shown to be sensitive to the study and characterization of the thermal biodiesel properties: (a) photopyroelectric, and (b) thermal lens. Lima et al. (2009) conducted a study with soybean samples to show the influence of waste and antioxidants in the thermal properties of the samples. Castro et al. (2011) reported a study which

observed a correlation between the iodine number and the thermal diffusivity for biodiesel samples attained from oils of several sources and consequently with different fatty acid composition. Another study developed by Guimarães et al. (2009) also presents measures of diffusivity, conductivity and effusivity for mixtures of diesel/biodiesel. Furthermore, Ventura et al. (2012) conducted a study in which the thermal lens technique was applied to biofuel samples to test their potential to distinguish diesel from biodiesel in binary mixtures. More recently, Crespo (2013) presented a study on the correlation between the NOx emissions of biodiesel with the thermal and rheological properties. This study may serve as a new methodology for the characterization of NOx emissions from the combustion of this type of biofuel.

13.5 Application of Glycerin

Since the beginning of the program of biodiesel production in Brazil there has always been concern with respect to the glycerol accumulation. Over the years, increase in biodiesel production has created a scenario that is indeed alarming. Glycerol, a by-product of the transesterification, represents about 10 % of the total biodiesel, e.g., just in 2012 approximately 274 million liters were produced in Brazil. Research focusing on new applications for glycerol is being developed to improve the economic viability of biodiesel production and its environmental impact (Quispe et al. 2013). Some of the main uses of refined glycerin include food, personal care products, and oral hygiene products, which make up approximately 64 % of total consumption (Stelmachowski 2011). Other applications include its use in the manufacture of pharmaceuticals and cosmetics (Tan et al. 2013). But the range of applications is now much broader, including its use in the production of chemicals, fuel additives, production of hydrogen, development of fuel cells, ethanol production, animal feed, co-digestion, and co-gasification (Leoneti et al. 2012) as well as single cell oil (Xu et al. 2012). In addition, an economic evaluation study found that attaining acrolein, hydrogen, and 1,2-propanediol from glycerol was feasible from a technological standpoint, with good profitability. At the same time, the conversion of glycerol into value-added products such as 1,3-propanediol, PHB, and ethanol (da Silva et al. 2009) also proved cost-effective and a high margin of difference between the cost of production and sale using glycerol was observed (Cardona et al. 2010). As can be observed, there are many applications, but the current panorama of glycerol accumulation will be changed only if the technologies to transform it in chemical products of high value are used on a large scale. A strategy that seems particularly promising would be to look for applications inside the biodiesel process, as addictive or as raw material for methanol production, eliminating the need of acquisition from methanol from natural gas, transforming the biodiesel completely in a renewable process. However, in some way, glycerin will probably be available in the market, at low cost, in the next few decades.

13.6 Concluding Remarks

Brazil has a large potential for bioenergy production derived from plants and several biomass, as well as other residual sources. The question in debate between energy versus food may be increasingly attenuated as new raw materials not competing with the food chain begin to gain more attention in biodiesel production. Thus, the expectation of using algae continues to be of great relevance, although more research is required to reduce processing costs. In general, further efforts should be made to reduce the cost of biodiesel production. Intense research activity is occurring with the search for new chemical and biological catalysts, proposing improvements and alternative processes, but on the industrial scale, development of new technologies including the imminent use of glycerol to add value to the process, such that they become more efficient and friendly with the environment, is imperative. Other aspects no less important that were not addressed here, but which should be observed, include problems in the stock, stability, and formation of sediments after the manufacturing process of biodiesel and even increasing the level of NOx emission with increasing future demands of blends of diesel/biodiesel B10, B20, etc. Hence, major challenges in scientific and technological development in this branch of biofuels, and particularly biodiesel, will be specifically required to achieve these goals in a sustainable way.

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Chapter 14
Critical Technological Analysis for Enzymatic Biodiesel Production: An Appraisal and Future Directions

Marcelle Alves Farias and Maria Alice Z. Coelho

Abstract Biodiesel has attracted considerable interest in recent years as an alternative energy source, once the world petroleum and gas resources will soon be exhausted. Additionally, the biodiesel is a biodegradable and renewable fuel. The conventional alkaline process for biodiesel production generates undesirable by-products such as soaps, which make difficult biofuel separation and purification. This technology becomes less interesting in Brazilian industry, once the significant amount of raw material, available in Brazil, has as characteristic high acidity value. In this scenario, to find an alternative technology that could eliminate these problems is desired by Brazilian biodiesel market. Designed to overcome these drawbacks, the enzymatic biodiesel production has been studied due to some relevant advantages over conventional process, such as: glycerol can be easily recovered without any complex process, free fatty acids contained in the oils can be completely converted to esters and subsequent wastewater treatment is not required. Nevertheless, despite the advantages of using enzymatic biodiesel production, the enzymatic route is not an industrial-scale reality yet. There are some challenges that should be overcome before biocatalysts can be made feasible for biodiesel production, like their higher cost, biodiesel productivity, and enzyme inhibition.

14.1 Introduction

The global interest in biofuels is growing in Europe, North America, Asia, and Brazil and its production is expanding faster than conventional oil supply (Nogueira et al. 2011). Chemical catalysis is a well-established process for

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biodiesel production. The homogeneous alkali-catalyzed transesterification process has been extensively applied to the large-scale synthesis of alkyl esters, especially due to the low cost of base catalysts and their efficiency even at low concentrations. The chemical reaction, however, has some disadvantages as it is energy-intensive, requires several separation/purification steps and generates significant amounts of wastewater to be treated (Feltes et al. 2012). The drawbacks in the homogeneous alkaline transesterification process have encouraged researchers and biodiesel industry to look into different biodiesel production methods.

In this sense, particular attention has been dedicated to the use of lipases as biocatalysts for biodiesel production due to compatibility with quality variations of the raw material (especially triglycerides with high free fatty acid content), their favorable conversion rate obtained in gentle conditions and, relatively simple downstream processing steps for the purification of biodiesel and by-products (Gog et al. 2012). Additionally, the use of lipase as a biocatalyst can minimize wastewater treatment and improve the glycerol recovery (Vyas et al. 2010). Other advantage of enzymatic route is a more favorable life cycle assessment when compared to chemical catalysis (Harding et al. 2008). Nevertheless, comparatively to conventional chemical processes, the major obstacles for enzymatic biodiesel production remain to be the cost of lipases (Yaakob et al. 2013), the relatively slower reaction rate and lipases inactivation caused by methanol and glycerol and, in this way, this technology already presents economic disadvantage (Marchetti et al. 2007). Then, in order to overcome these challenges, new technologies have been developed aiming at finding alternatives for enzymatic biodiesel production at industrial scale. These technologies should take into account the enzyme choice (source or if it is free or immobilized), medium condition that can improve the productivity, beyond the process design related to efficiency and operational cost.

Undoubtedly, Brazil is a promising country for biotechnological products development, such as enzymes for the bioenergy industry, once its biodiversity and environmental characteristics can contribute to this opportunity.

14.2 Lipase as Catalyst

Lipases (triacylglycerol ester hydrolases E.C.3.1.1.3.) are enzymes classified as hydrolases that can catalyze both the hydrolysis and the synthesis of esters from glycerol and long-chain fatty acids. The last reaction occurs only in the presence of water traces (Paiva et al. 2000). These enzymes, under specific conditions, are also capable to catalyze reversible reactions: interesterification, aminolysis, and transesterification reactions. The microbial lipases are glycoprotein and its molecular weight can vary between 19 and 60 kDa, presenting around from 258 to 544 amino acids residues and the majority of them have hydrophobic characteristics (Jaerger and Reetz 1998).

Figure 14.1 shows the main reactions catalyzed by lipases. The lypolitic reactions occur in the lipid-water interface. Jaeger et al. (1999) reported two

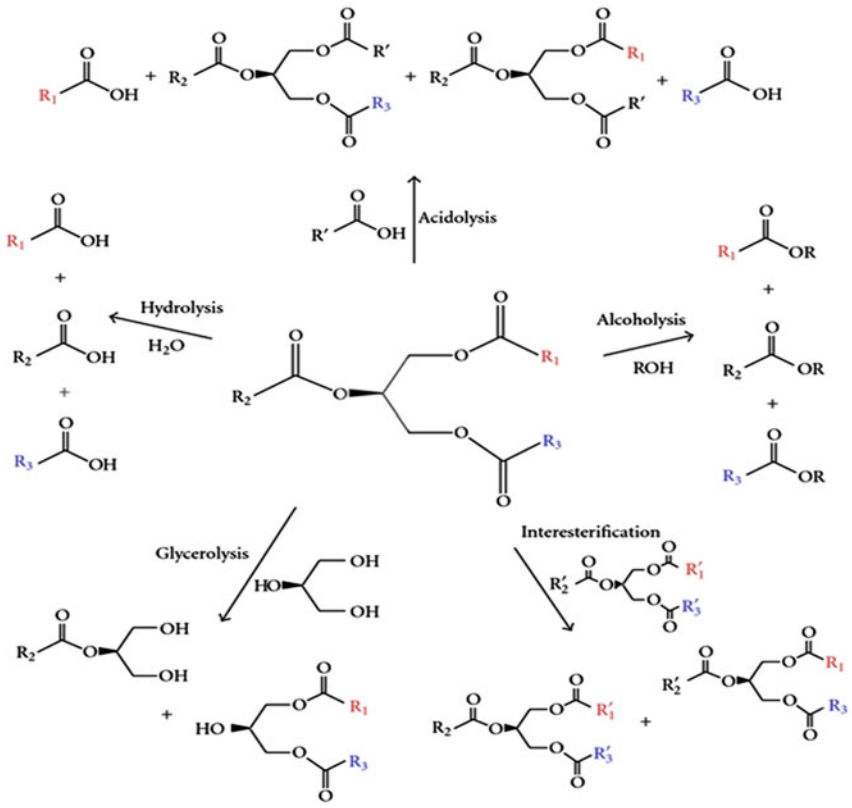

Fig. 14.1 Reactions catalyzed by lipases (Ribeiro et al. 2011)

different classification criterias to distinguish between a lypolytic enzyme from "true lipases"(E.C. 3.1.1.3): (a) It should be activated by the presence of an interface, i.e., its activity should sharply increase as soon as the triglyceride substrate forms an emulsion. This phenomenon was termed "interfacial activation" (b) It should contain a "lid," which is a surface loop of the protein covering the active site of the enzyme and moving away on contact with the interface. Therefore, these hypotheses seem to be unsuitable for classification, mainly because some enzymes have a lid but not exhibit the interfacial activation (Verger 1977). Although there is no strict definition available for the term "long-chain," glycerol esters with an acyl chain length of <10 carbon atoms, usually indicates the presence of an esterase. It should be emphasized that most lipases are perfectly capable of hydrolyzing these esterase substrates.

The lipases belong to α/β hydrolases family, considering the structural aspect and, its activity depends on the catalytic triad. In most lipases, the access to catalytic site is controlled by helicoidal structure called lip, that under specific conditions, is responsible for covering the catalytic site. Its role is to block the

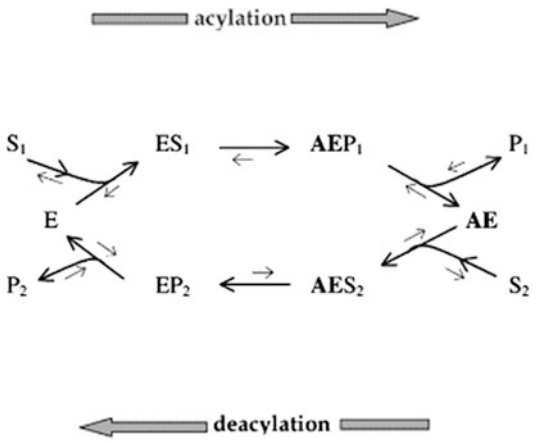

Fig. 14.2 Classically proposed mechanisms of hydrolysis, esterification, and alcoholysis reactions catalyzed by lipases *ME* methyl ester, *EE* ethyl ester, *MeOH* methanol, *EtOH* ethanol, *AG* Fat acid (Vaysse et al. 2002)

Reaction	S_1	P_1	S_2	P_2
Hydrolysis	ME	MeOH	H_2O	AG
	EE	EtOH	H_2O	AG
Esterification	AG	H_2O	MeOH	ME
Alcoholysis	EE	EtOH	MeOH	ME

active site in the absence of substrate (closed conformation) while an hydrophobic interface induces a conformational modification of the lid, rendering the active site of the lipase accessible to the substrate (open conformation). Knowledge of their three-dimensional structures and the factors that determine their regiospecificity and enantiospecificity are traditionally essential to tailor lipases for specific applications (Jaeger and Reetz 1998).

As mentioned, the reactions involving lipases occur in lipid-water interface and, as consequence, the kinetics cannot be described by Michaelis–Menten equation, once this model is only valid to homogenous phase catalysis (Jaeger and Reetz 1998). The reactions catalyzed by lipases usually follow the "ping-pong bi–bi" mechanism (Haffner et al. 1999).

Vaysse et al. (2002) proposed a general resumed scheme showed in Fig. 14.2. From this scheme, it is possible to find the main differences between substrates (S_1 and S_2) and products (P_1 and P_2), considering hydrolysis, esterification, and transesterification reactions catalyzed by lipases, using methanol (MeOH) and ethanol (EtOH) as acyl acceptors. Regarding the substrates, it is important to note that S_1 is the acyl donor and S_2 is the acyl acceptor.

The transesterification reaction is a term that is widely used to describe an important class of organic reactions, where one ester is converted into another. The transfer of an acyl group can happen between an ester and an acid (acidolysis), one ester and another ester (interesterification) or between an ester and an alcohol

(alcoholysis). The alcoholysis reaction catalyzed by lipases, involves two-step mechanism for each ester bond of the triglyceride molecule. In the first step, the ester bond is hydrolyzed and the alcohol moiety is released, followed by an esterification with the second substrate (Kaieda et al. 1999). Some basic differences have been identified between the transesterification of triacylglycerols (TAGs) and the esterification of free fat acids (FFAs). According to Freire et al. (2011), the transesterification is a sequence of three reaction (diacylglycerol and monoacylglycerol are formed as intermediates), and the esterification involves parallel reactions of FFAs in order to produce biodiesel. The last reaction is quicker than the former one. The higher polarity of FFAs, compared to TAGs, makes the short-chain alcohols more soluble in the reaction medium. Moreover, water is one of the esterification products and shifts the equilibrium toward hydrolysis when the concentration exceeds optimal level.

14.3 Process Variable for Enzymatic Biodiesel Production

Production of biodiesel by lipase is critically influenced by various process variables such as the choice of enzyme (source and kind), temperature, water content, water activity, enzyme amount, oil to alcohol ratio, addition of organic solvents, among others. In this sense, the most relevant variables whose affect the enzymatic biodiesel production will be discussed in this item.

14.3.1 The Choice of Enzyme

The lipases present many advantages when compared to others catalysts. In this context, it is important to point out the biocompatibility, biodegradability, and environmental benefits (Marchetti et al. 2007).

Lipases from bacteria and fungi are the most commonly used for transesterification process and, the choice of lipase will depend on the origin as well as the formulation of the enzyme. In general, the best enzymes are able to reach conversions above 90 %, while reaction temperatures vary between 30 and 50 °C. Reaction time also vary greatly from a low of 8 h for immobilized *Pseudomonas cepacia* lipases transesterifying jatropha oil with ethanol, to a high of 90 h for the same free enzyme transesterifying soybean oil with methanol (Fjerbaek et al. 2009). Thus, besides the source, it becomes necessary to evaluate different raw material (oil and fat), acyl acceptors, free or immobilized enzymes, among others variables.

Free enzymes are far cheaper than immobilized ones. They can be purchased in an aqueous solution composed by enzymes plus nothing more than a stabilizer to prevent enzyme denaturation (glycerol or sorbitol) and a preservative to inhibit microbial growth (Freire et al. 2011). However, in some cases, these enzymes can

loss the activity in the presence of some compounds. Improved immobilization technology has provided an enhanced level of reusability, operational stability, and optimum temperature, resulting in higher conversion rates and shorter reaction time, respectively. Until present, most thoroughly investigated commercial immobilized lipases are Novozym 435, Lipozyme TL IM, Lipozyme RM IM, and Lipase PS-C. Different immobilization methods can be applied for lipases used in biodiesel production: adsorption, cross-linkage, entrapment, encapsulation, and covalent bonding (Gog et al. 2012).

To overcome the higher costs of enzymes, compared to homogeneous chemical catalysts, its reutilization is essential (Li et al. 2012; Tan et al. 2010; Yan et al. 2011). The longer the reuse of the same enzyme, higher productivity will be obtained for a given batch of enzyme and, as consequence, the biodiesel production price will decrease. Efficient reuse is dependent upon whether the enzymes can obtain and maintain a high initial activity without inactivation or inhibition (Fjerbaek et al. 2009). Moreover, the size of immobilized enzyme helps its separation from reaction medium when it is compared to free enzyme.

14.3.2 Water and Acidy Impact

The water concentration in enzymatic transesterification has an important hole for enzyme conformation, protecting the protein against structural deformation (Fjerbaek et al. 2009). For the lipase-catalyzed biodiesel production in predominantly nonaqueous media, in fact, water plays multiple roles and it has strong influence on the catalytic activity and stability of the lipase (Gog et al. 2012). Lipases have a unique feature that consists in catalyze the reaction at interface between water and organic phase. Generally, the enzyme activity depends on the interface and, the increasing of interfacial surface is well inclined to enzyme activity maintenance. However, the excess of water can conduct undesirable reactions, like hydrolysis during transesterification process. Thus, the water quantity required to maximize the lipolytic activity will depend on raw-material, immobilization support, and the solvent used (Tan et al. 2010).

The effect of water concentration in enzymatic biodiesel catalysis by *R. oryzae, C. rugosa, P. fluorescens*, Novozym 435, and *B. cepacia* showed that lipase activity was lower in water absence. This result demonstrates that a minimum water concentration would be required for enzyme activation. With increasing amount of water added to the medium, there was also an increased formation of ester, confirming the rise of lipase activity (Al-Zuhair and Emirates 2007). On the other hand, Shimada et al. (1999) emphasized that the higher water addition in the reaction, the lower would be the ester formation. Corroborating Shimada et al. (1999), Azócar et al. (2011) investigated biodiesel production, in anhydrous medium, by lipase from Novozym 435, using frying oil as raw-material and methanol as acyl acceptor. The low concentration of water allowed the esterification of fatty

acids (already presented in raw material) at the beginning of reaction and, after, the transesterification reaction producing methyl esters.

Considering the acidity of raw material, comparing the transesterification process by chemical catalysis to the enzymatic one, besides being a cleaner technology, the last presents advantages over the alkaline chemical catalysis. In chemical catalysis, the raw material needs to be previously treated when it has high concentration of fat acids, in order to diminish the acidity (Freedman et al. 1984; Kaieda et al. 1999; Zhang et al. 2003). The pretreatment is also important to reduce the saponification of free fat acids, caused by alkaline neutralization process that promotes difficulties in the separation of biodiesel and glycerol. Additionally, it can generate alkaline residual water (Meher et al. 2006; Mittelbach 1990) and can cause environmental impact and higher energy consumption.

Different from alkaline transesterification, the enzymatic technology for biodiesel production do not form "soap" (when raw materials with significant amount of fatty acids are used) and, it can esterify the FFA in one unique step, without need of subsequent washing process. Thus, enzymes are potential catalysts for biodiesel production in industrial scale, decreasing the costs with regard to raw material treatment. The enzymatic technology becomes very attractive because it will not be necessary to have a raw material with strict specification (acid and water concentration) being its commercial value lower than the raw material used for alkaline chemical transesterification.

14.3.3 Organic Solvent

The use of organic solvents in enzymatic biodiesel synthesis improves mutual solubility of hydrophobic triglycerides and hydrophilic alcohols and also protects enzymes from denaturation by high concentrations of alcohols (Gog et al. 2012). Additionally, it reduces the viscosity of the reaction mixture increasing the diffusion rate reducing mass transfer problems around the enzyme. For immobilized enzymes, nonpolar solvents might maintain the residual water around the enzyme increasing the water activity locally and solvents might help stabilizing enzymes (Fjerbaek et al. 2009).

Tert-butanol has been shown as an excellent solvent for maintaining the enzyme activity (Liu et al. 2011; Chattopadhyay et al. 2011) due to its ramification, which is more miscible in triacylglycerol when compared with linear alcohols with the same carbon number (Azócar et al. 2011). Royon et al. (2007) investigated the biodiesel production using cotton seed oil as raw material, lipase as biocatalyst from immobilized *Candida antartica* and *tert*-butanol as a solvent. The results showed some advantages when *tert*-butanol was used: (a) In the presence of this solvent, high reaction rates and yield were obtained. (b) The quantity of enzyme needed to catalyze the reaction within a reasonable time periods was lower than that of other systems. (c) A very simple, one step continuous reactor was used

for biodiesel production. (d) No catalyst regeneration steps were needed for lipase reuse. (e) The operational stability of the catalyst was high even at 50 °C.

However, according to Kumari et al. (2007) and Soumanou and Bornscheuer (2003), the use of solvents can greatly reduce the enzyme activity, increasing investment with reagents and fixed assets, and can increase the reactor volumes to fit the additional volume of solvent. Then, the enzymatic technology using solvent-free system becomes very interesting feature for future industrial scale. Nevertheless, additional efforts are necessary to conquer the reaction time reduction.

14.3.4 Temperature

The enzymatic transesterification is generally performed at lower temperature, when it is compared to chemical reaction, in order to prevent loss of lipase activity. Optimum temperature determined by various lipases, used for biodiesel synthesis, ranges between 30 and 55 °C (Gog et al. 2012). Strategically, this range of temperature results from interaction between operational stability and high conversion rate.

Generally, immobilized lipases present greater temperature resistance when compared to free ones. According to Fjerbaek et al. (2009), the binding to the carrier material gives stability to the enzyme and promote a decrease of the thermal effect, avoiding deactivation when compared to the free enzyme. Table 14.1 presents examples of lipases from different microorganisms and their respective temperatures.

14.3.5 Acyl Acceptor

Different acyl acceptors have been studied for enzymatic biodiesel production, and the alcohol is the main chemical molecule chosen. Many alcohols as methanol, ethanol, 2-propanol, and 2-butanol have been studied as acyl acceptors for enzymatic transesterification of triacylglycerols; however, they can affect lipase activity through different mechanisms. Jech et al. (2003) used different alcohols aiming at evaluating the lipase inhibition level. Linear alcohols as methanol, ethanol, propanol, and butanol, and also ramified ones as isopropanol and isobutanol were investigated. All linear alcohols tested were toxic to enzyme. The inhibition level was inversely proportional to the carbon number presented in alcohol chain. When the linear chain is compared to the ramified one, the latter is less impactable to lipase activity (Jech et al. 2003).

Shimada et al. (1999) and Watanabe et al. (1999) proposed a different solution for this drawback. They reported the gradual addition of alcohol into reaction medium in order to minimize the enzyme activity loss. Shimada et al. (2002) recommended methanol addition in steps, once the methanol is more soluble in

Table 14.1 Temperatures used for enzymatic biodiesel production (Fjerbaek et al. 2009)

Temperature (°C)	Lipase, fatty acid/oil/tallow and alcohol
50–60	*P. fluorescens*, oleic acid, propanol, and butanol
70	*P. fluorescens*, oleic acid, propanol, and butanol
20–60	Novozym 435, soybean and rapeseed oils mixture, methanol
25–60	Novozym 435/Lipozyme TL IM/Lipozyme RM IM, soybean oil, methanol

acyl ester than in triacylglycerol. Rodrigues et al. (2010) reported the addition of ethanol in two steps in order to promote the ethanolysis of soybean oil by immobilized enzyme of *Thermomyces lanuginosus* and the best result reached 100 % of conversion.

Depending on acyl acceptor used for biodiesel production, it will influence the fuel proprieties, it means, the behavior of fluidity (Lee et al. 1995; Wang et al. 2005) and lubricity (Drown et al. 2001) when in contact with different temperature levels. The alcohols used for enzymatic or chemical process need to have a low commercial value, in order to reduce the total manufacturing cost. In this sense, methanol and ethanol appear as good options because of their lower price, when compared to secondary and tertiary alcohols. Despite the higher price, these last alcohols are also appropriate to biodiesel production, since these compounds are responsible to form an ester with low fluidity point. However, according to Stamenković et al. (2011), the complexity of alcoholysis conditions carry on economically unviable for secondary and tertiary alcohols.

In an industrial process, the acyl acceptors, besides having a low cost, need to be commercially available in large scale. Taking into account these considerations, methanol and ethanol continue to be very interesting alternatives for biodiesel production, once they present competitive prices and market availability.

14.3.6 Phospholipids

The enzymes showed considerable inhibition by phospholipids presented in the crude oil, during the biodiesel production (Lai et al. 2005; Wei et al. 2004). The phospholipids are the main components removed by degumming process and, because of this limitation, the use of refined oil is relevant for enzymatic biodiesel production. The best alternative for phospholipids removal would be the use of phospholipases, followed by enzymatic transesterification reaction. This strategy was investigated by Jang et al. (2012) and reached conversion up to 89 %. According to Séverac et al. (2011), the inhibition caused by the presence of phospholipids, in crude high-oleic sunflower oil, was eliminated by using *tert*-butanol. This solvent was chosen, because of its medium polarity. Additionally, *tert*-butanol helps to preserve the activity of Novozym 435 as well as improve its stability in the medium.

14.3.7 Glycerol Formation

Although glycerol being a by-product formed during the enzymatic transesterification, the influence of this compound on enzyme activity is relevant. During biodiesel production, Soumanou and Bornscheuer (2003) reported that glycerol could inhibit transesterification reaction by limiting the mass transfer due to its insolubility in the oil. According to Lee et al. (2011), the effect of glycerol on enzyme activity was tested using Novozym 435 and Lipozyme RM IM. Both enzymes were incubated in a mixture of canola oil with glycerol (0–15 % w/w) for 2.5 h prior to activity assay (the immobilized lipases were washed with isopropyl alcohol, in order to avoid the effect of glycerol adsorbed onto support). The results obtained by this experiment showed that high glycerol contents did not influence the enzyme performance and, the lowered performance of lipases by glycerol during biodiesel synthesis in mainly due to mass transfer limitation rather than direct inhibition of the enzyme. However, some authors corroborated that the enzyme deactivation could be caused by glycerol, especially under a solvent-free system (Robles-Medina et al. 2009; Talukder et al. 2009) and can inhibit the enzyme activity by forming a hydrophilic coat on its surface that exclude TAG from the active site (Véras et al. 2011). According to Xu et al. (2011), glycerol formed in biodiesel synthesis by immobilized lipases can severely reduce the reaction rate by surrounding the catalyst in a hydrophilic layer, thereby limiting the mass transfer of substrate to the enzyme.

14.4 Process Design: Technological Trends

The process setup is very important needing to consider the above discussed technical issues like, reaction/product mixture, solubility of alcohol, enzyme stability and recovery, among others (Nielsen et al. 2008). Regarding the enzyme characteristics, it is relevant to include the reuse of this biocatalyst. For free enzymes, this can be achieved using an ultrafiltration or centrifugation unit and, for immobilized ones, different techniques and matrix (support) are available for immobilization.

Considering such features, a reactor configuration for industrial applications has an important role to make the enzymatic biodiesel production economically feasible. Then, there are several different processes to be considered, in order to develop a process design: batch, continuous stirred-tank reactors and packed-bed reactors. Other possible solutions were described in the literature, according to Fjerdaek et al. (2009): fluid beds, expanding bed, recirculation membrane reactors, or reactors with static mixers.

According to Nielsen et al. (2008), the batch design is a typical process used in laboratory scale due to the simple setup. All reagents used in the reaction are introduced from the start, whereas stepwise addition of alcohol (mainly methanol)

is recommended. On the other hand, this process setup in large scale promotes a long reaction time and the gradual decline of enzyme activity according to the number of reuses. As time goes by, the plant capacity will decrease and, eventually, becomes unacceptably low.

The continuous stirred-tank reactor (CSTR) consists of a continuous supply of substrate feed and product withdrawal. The design requires multiples tanks in series to assure the same degree of conversion for the same reaction. It is important to note that this process has interesting advantages like: (a) the reaction can hold enzymes of different age/activities; (b) possibility of introducing separation steps between the tanks in order to eliminate the glycerol formed as byproduct. In contrast, Tan et al. (2010) pointed out the stress caused by stirring, once it would disrupt the enzyme carrier by physical agitation. So, the immobilized enzymes sometimes might not be reused for a long period.

Nielsen et al. (2011) reported an enzymatic large-scale production of biodiesel in two different steps using free enzymes and immobilized ones. First, a liquid formulated lipase is used (Callera^{TM} Trans) for transesterification and the second step is the esterification of FFA with the immobilized enzyme (Callera Ultra). They tested a setup with three CSTRs in series. The FAME content out of the reactors was 67, 85, and 89 % in reactor 1, 2, and 3, respectively, when the system was in steady y state. The remaining FFA, inside the tank 3, was converted to FAME and, in addition, transesterifies the remaining glycerol esters.

Considering the industrial scale of enzymatic biodiesel production, Tan et al. (2010) reported that Lvming Co. Ltd., in 2007, established an enzymatic production line with capacity of 10,000 tons in Shanghai, China. The process is carried in stirred-tank reactor (STR) system and the technique used comes from Beijing University of Chemical Technology, with immobilized lipase *Candida sp.* 99–125 as catalyst. A waste cooking oil have been used as raw material. A centrifuge is used to separate out the glycerol and the water produced during the reaction, and the yield of FAME has reached 90 % of conversion under optimal conditions. Another plant that conducts enzymatic catalysis in China is Hainabaichuan Co. Ltd., Hunan Province. The factory has used the technology of Tsinghua University and commercial Novozym 435 as catalyst.

Together with STR, the packed-bed reactors (PBR) are the most widely used reactors for enzymatic biodiesel production. This system consists of a continuous operation without separation of the catalyst from the reaction product. The PBRs generally use immobilized enzyme packed in column that allows an easy implementation of continuous process. In this way, as biodiesel is a chemical commodity, its production in continuous-flow systems would certainly reduce the operational costs of its production (Freire et al. 2011). However, the main disadvantage is that the resulted glycerol remains at the bottom of the reactor and might deposit on the surface of the immobilized lipase, thus decreasing the catalytic efficiency (Gog et al. 2012). So, it is relevant to know that the glycerol produced in the reaction can be removed between the columns and the inactivation of the enzyme by addition of methanol/ethanol can be solved by stepwise addition before each column. In this sense, immobilizing enzymes for this application

generally has: a positive effect on the operational stability of the catalyst (compared to free enzymes), an easier handling (compared to free enzyme powder), and allows operation under low-water conditions (compared to liquid formulated enzymes) (Nielsen 2008).

Fjerdaek et al. (2009) concluded that for continuous production, it is possible to achieve longtime enzyme stability in PBRs, with or without solvents. The use of solvents in itself only increases production costs as they have to be removed and purified for recycling. On the other hand, the pressure drop caused by the high viscosity of solvent-free systems could become a problem. For large-scale production, PBRs should operate at low flow rates or using larger biocatalyst particle sizes to minimize such a drop in pressure, once with increasing particle diameter, the pressure drop decreases.

In Brazil, most of the process design for enzymatic biodiesel production is based on STR reactors. However, in a recent study from Federal University of Santa Catarina, Dors et al. (2012) demonstrated the potential of lipase as biocatalyst in continuous PBR for biodiesel production using a great variety of raw material. The best result and conditions for transeterification of this study can be found in Table 14.2. According to Table 14.2, PBR is a potential technology for enzymatic biodiesel production. However, a suitable process technology has yet to be established.

14.5 Enzymatic Biodiesel Production: Brazilian Experience

14.5.1 Why Brazil is a Potential Country for Enzymatic Biodiesel Production

There are two interesting arguments that should stimulate technology developments for enzymatic biodiesel production in Brazil. One of them is the possibility of using a raw material with low price, that presents high acidity value. Another reason is the use of agroindustrial residues as substrate, for microbial lipase production.

Brazil has large diversity of oleaginous cultures which have high potential of producing biodiesel. Almost all vegetable oils can be used as raw matter for biodiesel production, which is a promising activity in Brazil due to the potential growth of sunflower, soybean, castor bean, African palm, babassu, cotton, peanut, linseed, macauba, pequi, buriti, sesame, canola, and others (Lopes et al. 2011). Even the great variety of vegetable oils available for biodiesel production, when these oils have high concentration of fatty acids, it is necessary to previously treat this raw material, in order to reduce the saponification of free fatty acids. The pretreatment is indispensable for biodiesel production using alkaline chemical catalysis technology.

Table 14.2 Enzymatic biodiesel production by different design processes

Reactor	Lipase source	Oil/fat alcohol	Condition	Conversion (%)	Solvent	Operation time (h)	References
Packed-bed reactors	*C. antartica* (Novozym 435)	Soybean oil and isopropanol	1 bioreactor continuously operated (τ_R = 1 h, T = 51.5 °C). 1:4 oil/alcohol ratio	75	solvent free	168	Chang et al. (2009)
Packed-bed reactors	*Burkholderia cepacia* (lyophilized and delipidated fermented solid)	Soybean oil and ethanol	1 bioreactor operated in batch mode (τ_R = 46 h, T = 50 °C) with 2 stepwise additions of alcohol and 3:1 alcohol/oil molar ratio	95	solvent free	190	Salum et al. (2010)
Batch system	The recombinant *Rhizopus oryzae* immobilized on macroporous resin and anion exchange resin	Pistaciachinensisbge seed oil (PCO) with methanol	1 bioreactor (T = 37 °C) and methanol to oil molar ratio 5:1, water content 20 % by weight of oil	94	solvent free	60	Li et al. (2012)

(continued)

Table 14.2 (continued)

Reactor	Lipase source	Oil/fat alcohol	Condition	Conversion (%)	Solvent	Operation time (h)	References
Packed-bed reactors	*C. antarctica* (Novozym 435)	Sunflower oil and isopropanol	1 bioreactor continuously operated (T = 50 °C) with oil/ alcohol/ isopropyl ester weight ratioof 35:35:30	90	solvent free	210	Jachmanián et al. (2009)
Packed-bed reactors	*C. antarctica* (Novozym 435)	Cottonseed oil and methanol	1 bioreactor continuously operated (T = 50 °C) with oil/ alcohol/*tert*-butanol weight ratio of 1:2, 4:4	95	t-butanol	24	Royon et al. (2007)
Four-packed-bed reactors	*Pseudomonas cepacia* (commerciallipase Fe3O4 nanoparticlebiocompositecatalyst)	Soybean oil and methanol	4 bioreactor continuously operated (T = 40 °C). The ratio of the volume of soybean oil:distilled water: methanol:n-hexane was 6:3:1:0, 2	over 88	n-hexane	192	Wang et al. (2011)

(continued)

Table 14.2 (continued)

Reactor	Lipase source	Oil/fat alcohol	Condition	Conversion (%)	Solvent	Operation time (h)	References
Batch system	Crude pancreatic lipase	Cottonseed oil and methanol	1 bioreactor (T = 37 °C) and methanol/oil molar ratio was 15:1 and water concentration of 5 % (wt of oil)	75–80	t-butanol	4	Chattopadhy et al. (2011)
Packed-bed reactors	*C. antartica* (Novozym 435)	Crude high-oleic sunflower oil and butanol	1 bioreactor (T = 60 °C) and butanol/oil molar ratio was 5:1	96	t-butanol	48	Séverac et al. (2011)
Batch system	Self-developed *Burkholderia* immobilized onto hydrophobic magnetic particles	Olive oil	1 bioreactor (room temperature) and methanol/oil molar ratio was 4:1 and water concentration of 10 % (wt of oil)	70	solvent free	12	Liu et al.(2012)
Batch system	*Thermomycesl anuginosus* was immobilized by covalent binding onto olive pomace	Pomace oil and methanol	1 bioreactor (T = 25 °C) and methanol/oil molar rate was 6:1, using three-step addition of alcohol	93	solvent free	24	Yücel et al. (2011)

(continued)

Table 14.2 (continued)

Reactor	Lipase source	Oil/fat alcohol	Condition	Conversion (%)	Solvent	Operation time (h)	References
Continuous packed-bed reactor	*P. fluorescens* lipase immobilized on epoxy-polysiloxane–polyvinyl alcohol composite	Palm oil and ethanol	1 bioreactor (T = 50 °C) and methanol/oil molar rate was 9:1, 70 % of the biocatalyst activity was retained even after continuous operation for almost 48 days	87.6	t-butanol	4.6	Dors et al. (2012)
Packed-bed reactors integeated with glycerol separation	Commercial *C. antartica* lipase B immobilized on macroporous acrylic resin	Rapeseed blended with soybean oil and methanol	1 bioreactor (T = 30 °C) and methanol/oil molar ratio was 1:2 and 10 stepwise additions of alcohol	98.6	solvent free	After 8th pass (time not informed)	Hama et al. (2011)
Batch system	Lipozyme TL IM	Castor and jatropha oil blended (1:5) and methanol	1 bioreactor (T = 45 °C) and methanol/oil molar ratio was 1:1, using single stepwise of alcohol	78.3	solvent free	24	Maleki et al. (2013)

Another potential raw material for enzymatic biodiesel production could be tallow and fried oils, once they present low cost and, in many cases, reduction of environmental problems associated to the final deposition of these materials. Brazil has the second largest herd in the world, with a cattle herd of 207.2 million heads in 2007 and is one of the greatest producers and first exporter of beef over the world. The current production in Brazil is around 700,000 tons of tallow per year. As regard used fried oil, there is also a good potential of supply estimated in about 300,000 tons per year (Nogueira 2011).

Take in advance the fact that Brazil generates, annually, thousands of tons of agricultural and agroindustrial residues, the bioconversion of these residues for lipase production, as well as, other value-added products would point out Brazil a prominent position in the future biotechnology developments. Oil cakes of various residues obtained from extraction of oils have been utilized for fermentative production of lipases (solid state fermentation). This is because their residual oil contents serve as inducers for lipase production. Several agricultural residues have been reported to be effective for lipase production and these include brans (wheat, rice, soybean, barley), oil cakes (soy, olive, gingelly, babassu), and bagasse (sugarcane) (Salihu et al. 2012). Additionally, these residues have attracted increasing attention as abundant and cheap renewable feedstock and can diminish the environmental impact of biodiesel supply chain. Nowadays, these residues can be used as animal feed (when it is on specification) or can be discarded in landfill.

Some of the most reported microbial genera that produce lipases using solid state fermentation (SSF) technique are *Aspergillus, Candida, Humicola, Penicillium, Rhizopus, Geotrichum, Mucor, Pseudomonas,* and *Rhizomucor*. In the last decade, lipases have been increasingly studied as biocatalysts for biodiesel production either lyophilized or immobilized (Castro and Castro 2012; Gunasekaran and Das 2005). Additionally, Castilho et al. (2000) compared the economic viability of lipases production by *Penicillium restrictum* using SSF and submerged fermentation strategies. After scale up, authors concluded the returned unitary production cost was 47 % lower than the practiced selling price in the period the study was carried out, and the major reason was the use of low-cost agroindustrial raw materials.

The Brazilian biodiversity and environmental characteristics create an opportunity to be a major producer of biotechnological products, such as enzymes for the bioenergy industry (Castro and Castro 2012). It is also important to notice the great potential of this country to integrate processes, it means, the biocatalyst and raw material for biodiesel production could come from the unique one oleaginous.

14.5.2 Enzymatic Transesterification for Biodiesel Production in Brazil

The transesterification is the most studied process in enzymatic biodiesel production and this reaction consists in one unique step. In fact, the Brazilian academic community is looking for a better conversions and economic viability, considering diversity of raw materials, by-products, and sources of lipases. In this sense, different strategies for transesterification have been tested to reach better conversions.

Freitas et al. (2009) studied different commercial lipases for integrated production of biodiesel and monoacylglycerol, such as: *Candida antartica B* (CAL B), *Pseudomonas fluorescens* (Lipase AK), *Burkholderia cepacia* (Lipase PS), and *Penicillum camemberti* (Lipase G). All lipases were immobilized on silica–PVA composite by covalent immobilization. The assays were performed using babassu oil and ethanol for biodiesel production and glycerol for monoacylglycerol production, in solvent free system. For both substrates, lipase from *B. cepacia* (lipase PS) was found to be the most suitable enzyme to attain satisfactory yields. For biodiesel production, the highest transesterification yield was >98 % in 48 h of reaction at 39 °C using an oil-to-ethanol molar ratio of 1:7. For monoacylglycerol production, the better conditions were oil-to-glycerol molar ratio of 1:15 at 55 °C. This investigation showed the potential integrated process in order to produce one more product and, as consequence, to increase the revenue of possible future technologies.

Rodrigues et al. (2008) studied immobilized lipases (Novozym 435, Lipozyme TL-IM and Lipozyme RM-IM) in enzymatic alcoholysis of three vegetable oils, soybean, sunflower, and rice bran, using different acyl acceptors (ethanol, propanol and butanol). The results showed that each lipase displayed the alcoholysis reactions using the three different alcohols. Novozym 435 presented higher activity in methanolysis, at a 5:1 methanol:oil molar ratio; Lipozyme TL-IM presented higher activity in ethanolysis, at a 7:1 ethanol:oil molar ratio; and Lipozyme RM-IM presented higher activity in butanolysis, at a 9:1 butanol:oil molar ratio. The conversion reached values around 50 % of FAME. The optimal temperature was in the range of 30–35 °C for all lipases and when commercial ones were washed with n-hexane, approximately 90 % of the enzyme activity remained after seven synthesis cycles.

Sangaletti et al. (2012) investigated other strategy to produce biodiesel, used to positively contribute to the development of an integrated and environmentally friendly technology. The authors studied the replacement of hexane by ethanol in soybean oil extraction process and, in this sense the production of biodiesel from oil and ethanol micelle (oil + solvent) by direct transesterification using Novozym 435 as catalyst and ethanol as acyl acceptor was investigated. The best experimental conditions were found using 40 °C of temperature, oil:ethanol molar ratio 1:4.5 and catalyst concentration 9.5 % for 24 h, reaching 85.4 % of fat acid ethyl ester (FAEE) yield. *Tert*-butanol was used as co-solvent and increased the ethyl

esters yield at 18 %, keeping a high FAEE yield (over 70 %) for more than three cycles of enzyme reuse.

Another way, to minimize the costs, consists in using a non-commercial immobilized lipase, in other words, to developed an indigenous technology that provides the production and immobilization of lipases. Moreira et al. (2007) reported the transesterification of palm oil with ethanol in a solvent free system using lipase from different sources (*T. lanuginosus, P. fluorescens, B. cepacia, Penicillium camembertii, and Candida antarctica*, porcine pancreatic), immobilized on hybrid support polysiloxane–poly-(vinyl alcohol). The best performance was attained with the lipase from *P. fluorescens* that reached almost full conversion (99.4 %) in less than 24 h of reaction, under established operational conditions, using 18:1 as molar ratio of ethanol:oil at 58 °C. This is an exceptional option for the Brazilian biodiesel production, because both palm oil and ethanol are readily available in this country. In this sense, Rodrigues et al. (2010) investigated the immobilization and stabilization of lipase from *T. lanuginosus* (TLL) on aldehyde-Lewatit (Lew-TLL). Lew-TLL was 10-fold more thermo stable than the commercial TLL preparation (Lipozyme TL-IM). The stabilized Lew-TLL was used for the enzymatic transesterification of ethanol and soybean oil. When n-hexane was used as co-solvent, the transesterification reached 100 % of conversion after 10 h, while in solvent-free system the yield was 75 %. The ethanol was added in two steps, using 7.5:1 as ethanol:soybean oil molar ratio.

Aiming at finding new alternatives for conversion improvement, Brazilian researches have been investigated different mechanisms/processes to increase enzymatic transesterification yields. Considering this issue, the use of supercritical CO_2 in the production of biodiesel appears to be a very interesting process to be analyzed. Rodrigues et al. (2011) reported a continuous process for biodiesel production in supercritical carbon dioxide. This apparatus consisted of two main sections: a reaction section comprising a high pressure packed-bed enzymatic tubular stainless steel reactor, and a separation section. The transesterification of virgin sunflower oil with methanol was carried using Lipozyme TL IM as a biocatalyst. Fatty acid methyl esters yield exceeded 98 % at 20 MPa and 40 °C for a residence time of 20 s and an oil to methanol molar ratio of 1:24. The authors also informed that Lipozyme TL IM was less efficient using waste cooking sunflower oil as raw material. In this case, a combination of Lipozyme TL IM and Novozym 435 afforded FAME yield nearing 99 %. The use of supercritical carbon dioxide increases both mass and thermal transfer and leads higher reaction rates (Lee et al. 2009; Lozano et al. 2011).

In order to minimize the enzyme inhibition and maximize the conversion of triacylglycerol to ethyl ester, Gamba et al. (2008) investigated the biodiesel production using lipase from *P. cepacia* supported in ionic liquid, 1-n-butyl-3-methylimidazolium bis (trifluorome-thylsulfonyl)imide, as an alternative of "green" method for biodiesel production from the alcoholysis of soybean oil. The transesterification reaction, catalyzed by this ionic liquid-supported enzyme, was able to be performed at room temperature, in the presence of water and without the use of organic solvents. The biodiesel was separated by simple decantation and the recovered ionic liquid/enzyme catalytic system could be reused at least four times

without loss of catalytic activity and selectivity. According to this investigation, 96 % of conversion in 48 h was obtained using 8.2 mmol of ionic liquid (as a support), methanol/water rate of 70:30 at 30 °C. In this studied ionic liquid provides the ideal medium for the stabilization of the enzyme and, additionally, removes glycerol from the reaction medium, avoiding enzyme inhibition.

Considering the conversion optimization, Batistella and Lindomar (2012) reported the use of ultrasonication, once this technique reduces the processing and phase separation time of the transesterification reaction. This work investigated soybean oil transesterification with ethanol using two commercial immobilized lipases (Novozym 435 and Lipozyme RM IM) under the influence of ultrasound irradiation (ultrasonic water bath). Results showed that ultrasound-assisted lipase-catalysis might be a potential alternative route to conventional alkali-catalyzed method, once this experiment reached 90 wt.% of FAME. This yield was obtained at mild irradiation power supply (100 W), and temperature (60 °C) in a relatively short reaction time, around 4 h, using Lipozyme RM IM as catalyst and oil/ethanol molar rate of 1:3. However, catalyst repeated use under the optimum experimental condition resulted in decay in both enzyme activity and product conversion after two cycles. Nogueiraet al. (2010) investigated the biodiesel synthesis from macauba oil (*Acrocomia aculeata*) and ethanol using Novozym 435 and Lipozyme IM, under microwave irradiation. The results showed that the activity of the enzyme had increased about one order of magnitude due to microwave, however the conversion of FAME remained low.

14.5.3 Other Strategies for Enzymatic Biodiesel Production in Brazil

In Brazil, beyond the transesterification process, others strategies have been developed for enzymatic biodiesel production. In this way, enzymatic hydroesterification reactions (hydrolysis followed by esterification step); concomitant esterification and transesterification reactions; and esterification of high acid raw materials have been studied as enzymatic biodiesel production alternatives.

Aiming at using high acid oils as raw material for biodiesel production, Corrêa et al. (2011) studied an alternative route based on esterification of free fatty acids present in by-products obtained from vegetable oil refining, such as palm oil fatty acid distillate (PFAD). PFAD is a byproduct of the production of edible palm oil, which contains 96 wt.% of free fatty acids, becoming quite impossible its use in conventional alkaline transesterification. In this way, the authors investigated the biodiesel synthesis via esterification of PFAD, using methanol and ethanol as acyl acceptors, catalyzed by commercial immobilized lipases (Novozym 435, Lipozyme RM-IM, and Lipozyme TL-IM), in a solvent-free system. The best result was reached when Novozym 435 was used as biocatalyst and ethanol as acyl acceptor (two stepwise additions). The conversion was 93 % after 2.5 h of esterification reaction using 1.0 wt.% of enzyme at 60 °C.

A different strategy to reduce the costs of enzymatic biodiesel production was proposed by Salum et al. (2010). This group demonstrated a possible strategy regarding the use of lipase from *B. cepacia* LTEB11 by solid-state fermentation and added the lyophilized fermented solid (LFS) directly to the reaction medium to catalyze esterification and transesterification reactions. Not only is the solid substrate relatively cheap, but also the steps of lipase extraction, purification, and immobilization are avoided. This fermented solid was packed into a column and used to catalyze the synthesis of biodiesel through the ethanolysis of soybean oil in a medium free of co-solvents. The best conversion was 95 % after 46 h, which was obtained at 50 °C, with an alcohol:oil molar ratio of 3:1, alcohol addition in two steps and the addition of 1 % of (m/m) water to the reaction medium. Fernandes et al. (2007) also found important results using the same strategy. The main result of this research was found after 18 h, reaching 94 % of esterification and after 120 h, up to 95 % for transesterification reaction.

With the advent of LFS as an immobilized system for biodiesel production, the relevance of technology protection becomes to be a very important issue, because of industrial interest. In this way, the patent PI0704791-6 was deposited in order to protect the process of esters production, using oleic acid, ethanol, and LFS (from *B. cepacia*). This technology has a great potential, once this lipase is immobilized in the raw material, avoiding subsequent steps for separation and could be reused for several times.

Beyond these strategies mentioned in this chapter, some researchers have been directed to hybrid catalysis using enzymatic and chemical reactions. Sousa et al. (2010) studied the biodiesel in two steps. The first step consisted in *Jatropha curcas* oil hydrolysis by vegetable lipase from the own plant and, as result, 98 % of triacylglycerols were converted into fat acids after 2 h. The second step of the process converted the fat acids into esters, using methanol as acyl acceptor and niobic acid as catalyst (heterogeneous acid catalysis). The results showed that 97.1 % of methyl esters (high quality biodiesel) were produced. Cavalcanti-Oliveira et al. (2010) also investigated the hybrid catalysis for biodiesel production. First, the hydrolysis was conducted by lipase from *T. lanuginosus*, using soybean oil as triacylglycerol. In this study, the conversion rate was 89 % after 48 h of reaction. Following the process, the fatty acids were transformed into ester, using methanol and ethanol as acyl acceptors. The results were 92 and 83 % using, respectively, ethanol and methanol as acyl acceptors. It is important to note that these results were reached after 1 h of reaction.

14.6 Economic Aspects

The scientific community has published high values of FAME conversion by enzymatic route (Shah et al. 2007; Royon et al. 2007; Salis et al. 2005). Undoubtedly, the main challenge of enzymatic biodiesel production is to prove the

economic feasibility of this process when it is compared to alkaline catalysis. In both process, the capital and operating costs will depend highly on the chosen process design and its implications on purification steps, etc. However, in general terms, processing costs will be a function of factors such as: cost of enzyme, cost of oil (usage of lower-cost high-FFA); cost of alcohol; cost of preprocessing steps; process yield; cost of waste product handling; value of glycerol stream and; posttreatment stages (Nielsen et al. 2008).

Analyzing the costs with raw materials, it constitutes the significant component of overall production costs, and the soy oil feedstock, for instance, is the biggest contributing factor, itself constitute 88 % of the overall production cost. These values are consistent with the results of others costs analysis of biodiesel production from refined soy oil (Haas et al. 2006). Zhang et al. (2003) reported that approximately 70–95 % of the total biodiesel production cost arises from the cost of raw material; that is, vegetable oil or animal fats. Virgin oil costs approximately 2–3 times more than waste cooking oil indicating that use of virgin oil leads to a substantial increase in total manufacturing cost. When cooking oils are used as raw material, the viability of a continuous transesterification process and recovery of high quality glycerol as a biodiesel by-product is an interesting option to be considered to lower the cost of biodiesel (Demirbas 2009).

Since there is no detailed data for biodiesel production (alkaline catalysis) costs in Brazil, Giersdorf (2012) reported that the production costs of this biofuel can only be estimated by using a process model (Haas et al. 2006). The study consists mainly of three different steps: the pretreatment of the feedstock, the alkaline catalysis transesterification, and the purification of biodiesel and co-products. The total biodiesel cost was estimated to be 0.53 USD/L. Since the vegetable oil alone represents 87 % of the subtotal costs, it is obvious that a change in the feedstock and/or feedstock costs/prices will significantly impact the overall production costs. In this way, the possibility of using an enzyme, that accepts high acidity raw materials, appears to be very attractive for economic aspects.

Besides raw material cost, the price of enzymes should be brought down if this biocatalyst intend to compete with the chemical catalyst. Besides the price of enzyme, it is relevant to choose between free and immobilized ones. The immobilized products have a significantly higher price per “activity unit” compared to liquid products. It is difficult to make general comparisons between costs of liquid formulated versus immobilized enzymes, once it will depend very much on the cost of immobilization process. The immobilized lipase that has been extensively used (Novozym 435) has a high price per kilogram, meaning that a very high productivity is required for the process to be cost-effective. One the other hand, new immobilization technology resulted in a much lower selling price for the immobilized lipase and was recently successfully introduced for interesterification (Nielsen et al. 2008). Additionally, immobilized enzymes are required in biodiesel production due to the easier handling and reuse.

In order to compare the economic potential of enzymatic to chemical process for biodiesel production, few studies pointed out the productivity (kg biodiesel/kg enzyme) that can be easily used for cost comparisons. This calculation will also

depend on yield, number of reuse, and enzyme concentration. Nielsen et al. (2008) reported that the maximum price of the enzyme should be the same as when using chemical catalysis, 25 USD/ton biodiesel, thus the enzyme prices can vary from 12 to 185 USD/kg, depending on the productivity in the application. Fjerbaek et al. (2009) also analyzed the comparison between Novozym 435, a price of approximately 1,000 USD per kg, and NaOH as a chemical catalyst, approx. 0.62 USD (Haas et al. 2006). The authors reported that lipases have up to 74 times higher productivity, then considering the productivity, an enzyme cost of 0.14 USD per kg ester compared to 0.006 USD per kg ester for NaOH. If the enzyme purchase cost dropped to 44 USD per kg or the enzyme could be reused around 6 years, the enzymatic route could be competitive based on productivity alone. However, not only enzyme, but the overall process needs to be considered in the economic feasibility for enzymatic biodiesel production.

Sotoft et al. (2010) investigated the economic feasibility using current lipase prices. The authors considered in its study the size and capacity of the plant and the solvent use to make a comparison between different scenarios. One of these comparisons, considered the product price of 8 million tons of biodiesel/year. The literature showed that it can produce biodiesel for 0.55–0.62 €/kg with high quality raw materials and traditional catalysts, while this study showed that it can be produced at 0.75–1.49 €/kg, using solvent free system and lipase as catalyst. In this sense, enzymes showed to be more expensive to use, but if the shelf life and yield of the lipases could be improved, considering also the improvement in environmental impacts, the enzymatic production of biodiesel will sure to become very attractive for industrial prospect.

14.7 Conclusions

The choice between the chemical and enzymatic catalysis is an important decision to make before to invest in biodiesel production. The enzymatic catalysis can offer many advantages, however the acquisition cost involved with this technology is still economically not feasible, mainly due to enzyme cost. Du et al. (2008) elucidated there are two ways to be considered in order to reduce the costs of lipase. One of them would be to reduce the production costs through new lipase development, fermentation optimization, and downstream processing improvement. Another way is to improve/extend the operational life of the lipase, and this can be achieved through enzyme immobilization, alcoholysis reaction optimization, among others. This chapter also demonstrated that the raw material is an important variable to be considered, once it can impact from 70 to 90 % of the total cost involved in conventional biodiesel production, depending on the raw material specification. In summary, the major issues to be investigated, for lipase-mediated alcoholys aiming at biodiesel industrialization, are to reduce the lipase production cost and to develop new technologies that allow the use of poor quality raw

material. In this scenario, enzymatic route is a very promising technology to be developed in Brazil, once it is possible to integrate the variety of raw materials available and the use of agroindustrial residues to produce low-cost lipase.

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Chapter 15
Critical Analysis of Feedstock Availability and Composition, and New Potential Resources for Biodiesel Production in Brazil

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Abstract The worldwide demand for renewable energy has increased considerably in the recent years, and the need for biofuels should increase even more, especially in developing countries. Brazil has 43 % of its energy matrix based on renewable resources and is a leading country in the production of biofuels. The Brazilian National Program for Biodiesel Production and Use (PNPB) that started in 2005 encouraged biodiesel production, leading Brazil to become one of the world's top producers with a production of 2,718.48 thousand m^3 of biodiesel in 2012. Currently, soybean is the main feedstock used for biodiesel production in Brazil. However, as the demand for this fuel is constantly increasing, and soybean has low oil yield and productivity, alternative feedstocks for biodiesel production have been evaluated. In this review, we discuss the feedstocks that are currently most used for biodiesel production in Brazil (i.e., soybean, tallow, and cotton), as well as the more important feedstock alternatives (i.e., oil palm, physic nut, and microalgae) for the future. In addition, an analysis of oil physical–chemical properties and their effects on biodiesel production and quality is presented. Finally, different scenarios for the biodiesel industry in Brazil for the short-, medium-, and long-terms are discussed.

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15.1 Introduction

Environmental issues and energy prices have prompted worldwide increase in biofuels' production in the recent years (Almeida et al. 2012). Biodiesel is a biofuel made of fatty acid monoalkyl esters derived from biologically produced oils or fats, including vegetable oils, animal fats, and microalgal oils. It is possible to produce biodiesel from different vegetable feedstocks and the most appropriate choice will depend on technical, economical, and socioenvironmental competitiveness. Agronomic aspects of the plant feedstock are also important, and the following characteristics should be taken into consideration: (a) oil content and type, (b) productivity (i.e., production per area unit), (c) production systems, (d) crop cycle (i.e., seasonality), (e) regional adaptation (i.e., preferably broad to meet different environmental conditions), (f) socio-environmental development, and (g) oil quality. Given the mentioned factors, different crops have been used as the main source for biodiesel production in different countries. In the United States, for example, soybean oil is considered an essential feedstock, however, in tropical countries such as Malaysia, palm oil is more often used for biodiesel production. In Germany, rapeseed oil is used in the production of biodiesel, and it is distributed in a pure form, free of any additives or blending (Singh and Singh 2010; Atabani et al. 2012). Brazil is currently one of the largest biodiesel producers in the world. The feedstock of choice is soybeans. Using this feedstock, the production of biodiesel in Brazil increased from 0.74 thousand m^3 in 2005 to 2,718.48 thousand m^3 in 2012 (ANP 2013). Indeed, 80 % of the biodiesel produced in Brazil over the years is derived from soybean, a commodity that has a well-established production chain (Fig. 15.1).

The successful establishment of a biodiesel industry in Brazil was only possible due to investments in biodiesel research and strong public policies mainly established by program PNPB (The Brazilian National Program for Biodiesel Production and Use) (MME 2013a, b). PNPB was created in December 2004 with the aim to encourage biodiesel production and its use to replace, partially or completely, petrodiesel. This would reduce Brazil's dependence on petrodiesel imports and promote new sources of renewable energy which are increasingly important in the country's energy matrix. Indeed, renewables contributed with 43 % to the energy matrix in 2012 (EPE 2013). To accomplish this goal, the program brought incentives to family farmers (small farmers) and to the biodiesel producers. Producers buying oil from family farmers benefit from a social seal, which guarantees fiscal incentives, including federal tax reductions of up to 68 %, and special credit lines with favorable rates from governmental banks. The family farmers also benefit from special credit lines. Contracts that established selling oil prices for the family farmers and delivery dates to the producers brought security to the system and encouraged structuring in the sector. With this, Brazilian biodiesel production plants were installed in all five geopolitical regions of the country (Fig. 15.2). Today, the biodiesel production capacity of Brazil is 7,343 thousand m^3/year, but only 37 % of this capacity is being used. Another important characteristic of the

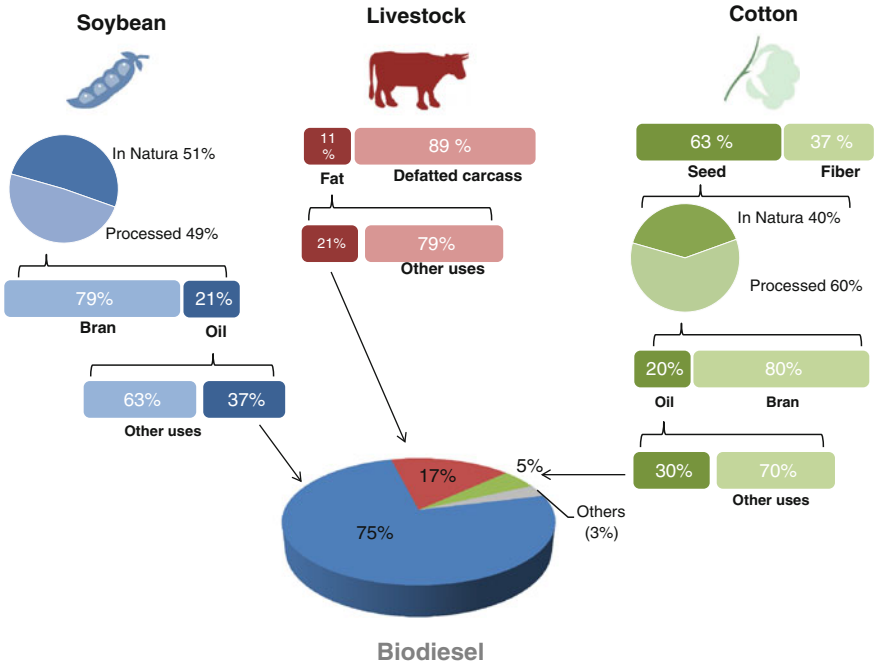

Fig. 15.1 Main feedstocks and their relative contribution to Brazilian biodiesel production (pie). Percentage of each feedstock biomass that is processed in Brazil and their respective oil (or fat for livestock) content are also presented. For 2012, 100 % biodiesel, soybean, and cotton are equivalent to 2,718.48 thousand m^3, 66.4 and 3.1 million tons, respectively. 11 % fat is equivalent to 1,560,000 tons/year

PNPB is the incentive to the diversification of feedstocks for biodiesel production, which focuses on the development and use of oil crops other than soybean to produce biodiesel in specific regions of the country. This would promote regional development and allow a more stable supply of oil throughout the year while decreasing dependence on soybean oil. Brazil holds a large territory and there is a wide variety of feedstocks that could be exploited for biodiesel production, thus diversification of the biodiesel feedstock matrix is not only feasible, but very attractive.

Blending of 2 % of biodiesel into petrodiesel (B2) became mandatory in Brazil since 2008. The same law (N.11.097/2005) stated that the percentage of biodiesel blended into petrodiesel should increase to 5 % in 2013. However, considering the expansion of biodiesel participation in the Brazilian energy matrix, in addition to the economical, social, and environmental benefits of biodiesel and National Energy Policies, the government decided to implement the mandatory B5 blend 3 years ahead of schedule, i.e., in 2010 (Resolução CNPE N° 6, de 16.9.2009—DOU 26.10.2009). Currently, B5 is still in use, but oil and biodiesel producers expect this percentage to increase soon, since the country's biodiesel plants are

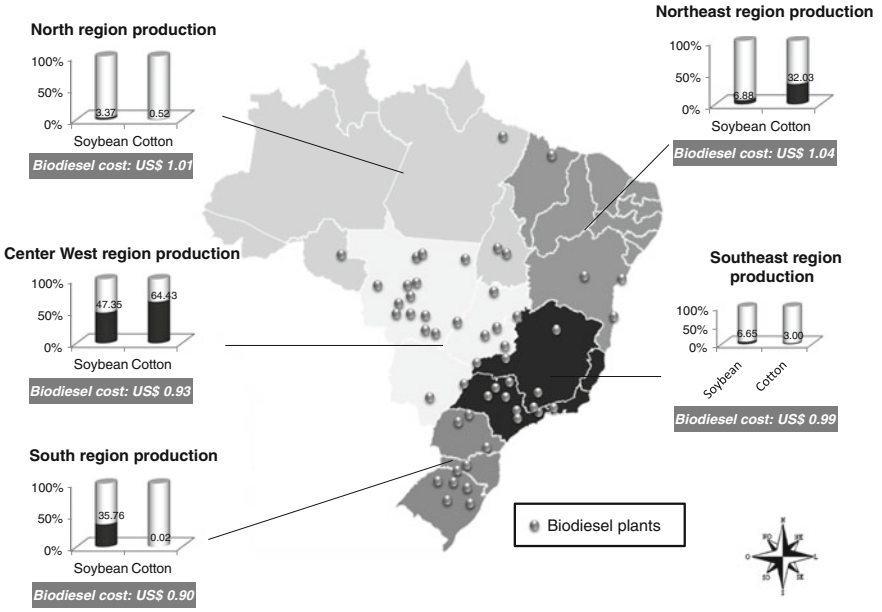

Fig. 15.2 Soybean and cotton production and cost of biodiesel by region in Brazil. Biodiesel production plants are indicated by circles in the map

working well below production capacity. The current production of biodiesel from only soybeans in Brazil would allow mixtures up to B10 levels (Fig. 15.1 and Table 15.1).

Brazil is a tropical country of continental dimensions with approximately 90 million hectares of available arable land. There are also 210 million hectares of pasture land (pasture fields) that could be employed in agriculture after mild recovering. In addition, there are several species able to grow and produce oils in high amounts and with high productivity in the different regions of the country (Bergmann et al. 2013). Characteristics of these crops and their relative technical advantages and disadvantages for biodiesel production were recently reviewed by Bergmann et al. (2013). Altogether, Brazil has a unique opportunity to increase oil production, valorize diversification of feedstocks, and regionalize its production, without the need to expand the production of biodiesel feedstocks into the remaining areas with native vegetation. However, to further develop the biodiesel industry, depending on the crop considered, as many as three, of the following technical-scientific challenges need to be overcome: (i) technological know-how about the feedstock—techniques to achieve high yields and productivity, solving agronomic issues such as seeding, growing, and harvesting problems are needed; (ii) production scale—despite technical know-how, some crops may not be produced yet at a large enough scale to support biodiesel production for a 5 % blend;

Table 15.1 Technical–economical characteristics of feedstocks for biodiesel production in Brazil

Feedstock	Participation in Brazil's biodiesel matrix (%)[a]	Oil cost (US$/ton)[b]	Brazilian annual oil production (t)[c]	Oil destined to Biodiesel production (t)	Oil (%)	Biomass productivity (kg/ha/year)	Oil productivity (kg/ha/year)
Soybean	75	972.00	5,450,000	1,993,082	18–21	2,938[e]	540
Animal fat	17	845.00	1,560,000	329,274	–	–	–
Cotton	5	1,098.00	300,000	90,175	20	2,168[e]	360
Oil palm	<1	858.00	110,000	<40,000	22	20,000	4,000
Physic not	–	–	–	–	38	4,500	1,500
Algae	–	>1,500.00 [c]	–	–	40–50[d]	75,000–230,000[f]	46,000–110,000[f]

[a] (MME 2013a, b); [b] (CONAB 2013a, b); [c] (Nunes 2007) and http://www.agencia.cnptia.embrapa.br; [d] (Stephenson et al. 2011); [e] (CONAB 2013a, b); [f] (Stephens et al. 2010)

(iii) production chain logistics—involves the spatial distribution of feedstock and biodiesel production plants, transportation costs, and all possible diverse uses of the feedstock. Critical analysis of these three challenges is essential to allow the identification of the best candidate crops for the biodiesel industry.

Soybean oil, cotton oil, and beef tallow (Fig. 15.1) are the main feedstocks currently used for biodiesel production in Brazil. In this chapter, we present how the production chains for these feedstocks fit with the biodiesel industry in Brazil. In addition, oil palm (*Elaeis guineensis* Jacq.), physic nut (*Jatropha curcas*), and microalgae are shown as the most important alternative feedstocks for biodiesel production in Brazil. The main challenges to employ them in the industry are discussed. The potential of other feedstocks, such as sunflower (*Helianthus annuus*), coconut (*Cocos nucifera*), babassu (*Attalea speciosa*), castor bean (*Ricinus communis* L.), rapeseed (*Brassica napus*), and other exotic oil producing species found in Brazil have been recently reviewed (Bergmann et al. 2013) and are not discussed here. An analysis of oil physical–chemical properties and their effects on biodiesel production and quality are presented. Finally, different scenarios on the short-, medium-, and long-term perspectives for the biodiesel industry in Brazil are discussed.

15.2 Feedstocks for Biodiesel Production in Brazil

Soybean oil, animal fat, and cotton oil are the three major feedstocks for biodiesel production in Brazil (Fig. 15.1, Table 15.1). Despite their importance in the biodiesel feedstock matrix, biodiesel can be considered a secondary product obtained from these feedstocks. It is important to note that the amount of oil and fat used for biodiesel production represents only a small fraction of the products obtained from these feedstocks (Fig. 15.1). Indeed, the production of soybeans, cotton, and animal beef have been developed for many years in Brazil for other uses and only recently, especially after the PNPB, biodiesel production started to integrate the value chain of these commodities. The well-established production and distribution chains of soybean and cotton in the country is one of the main reasons to use these crop species for biodiesel production, as their oil yield is low, i.e., below 600 kg/ha. Low cost of residual fat from beef production explains its use as a feedstock for biodiesel production. It is important, however, to further develop the biodiesel industry, so that feedstock crops have higher yields of oil, increasing the current yields of only 350–600 kg/ha of cotton and soybean to 5,000 kg/ha, and potentially contributing to regional development. Here, we summarize the advantages and disadvantages of the current substrates used for biodiesel production in Brazil (soybean, cotton, and animal fat) as well as some alternative feedstocks with great potential for biodiesel production (oil palm, physic nut, and microalgae).

15.2.1 Vegetable Oils

Soybean (*Glycine max*): Brazil is one of the top soybean producers in the world. In 2012 it produced 66.4 million tons of grain. This number should be surpassed in 2013, when soybean production may reach 81.5 million tons. National productivity reached 2,938 kg/ha and the planted area 27,721.5 thousand hectares, which represents increments of 10.8 and 10.7 %, respectively, compared to the 2012 numbers (MME 2013a, b).

The Brazilian success in soybean production, processing, and export in the recent years has made its oleaginous seed the main feedstock for biodiesel production in the country. The well-established production chain and distribution logistics assure the soybean market and value, and allow its inclusion as a reliable source of oil in the biodiesel feedstock matrix. It should be noted that only a relative small amount of soybean is used for biodiesel production. Brazil exports approximately 50 % of its production *in natura,* while the other half is processed internally to produce protein bran for animal feed (23.5 million tons in 2012) and edible oil (5.8 million tons in 2012). From the oil produced, only 25–30 % is used in the biodiesel industry (Table 15.1). Inspite of the low oil productivity and the relative small amount of feedstock designated to produce biodiesel, soybean is still the major feedstock for production of this biofuel, representing at least 75 % of the feedstock used (Table 15.1, Fig. 15.1).

Cotton (*Gosssypiuym hirsutum latifolium* Hutch LR): Cotton oil is the third most important feedstock for biodiesel production in Brazil, with 5 % of participation in biodiesel feedstock matrix, only behind soybean oil and beef tallow (Fig. 15.1, Table 15.1) (MME 2013a, b). However, in the Northeast region of the country, in some months of the year, cotton oil becomes the second main feedstock for biodiesel production. In Brazil, cotton is produced mainly in the Center-west and Northeast regions of the country, the states of Mato Grosso and Bahia being the largest producers. In 2012, 3.1 million tons of cotton seeds were produced. Like for soybean, the cotton seed oil content is low, approximately 20 %. This fact together with the relative small area of cotton production (1.4 million hectares) limits the use of cotton oil for biodiesel production. In addition, the cake obtained after oil extraction cannot be used for animal feed, because of the presence of toxic gossypol.

Oil Palm (*E. guineensis* Jacq.) Oil palm is especially suitable for biodiesel production due to high yields of biomass and oil, which are approximately 7 times higher in oil palm than in soybean (Table 15.1). Despite these impressive numbers, palm oil contributes with less than 1 % of the biodiesel production in Brazil. Relatively small-scale cultivation of this palm, estimated at 180,000 hectares, and uses of palm oil in other industries explain its modest contribution to biodiesel production. In Brazil, oil palm cultivation is restricted to specific soil and climate conditions mainly found in the North and Northeast regions of the country. Oil palm has the greatest potential to become the main feedstock for biodiesel production in the future. The Brazilian Federal government launched the National

Program for Sustainable Production of Oil Palm to stimulate cultivation of this crop and also to regulate its expansion and establish its agroecological distribution. In addition, there are oil palm breeding programs aiming to improve oil production and crop resistance to pathogens. In this context, a genetic hybrid of the African oil palm, *E. guineensis*, and the American oil palm, *Elaeis oleifera*, was released in 2010 by EMBRAPA (Cunha and Lopes 2010). This hybrid is resistant to fatal yellowing, which has decimated thousands of plants in the North region of the country. Ongoing R&D programs for the expansion, characterization, and conservation of a germplasm bank, genetic improvement of oil palm, and seed production programs are occurring. Oil acidity and time-costly and time-consuming manual harvest of fruit bunches are two important problems to be addressed to improve industrial performance of this crop. Strategies to solve the former problem will be discussed in detail in the following section. The latter problem is expected to be solved with improvements in mechanization of harvesting.

Physic nut (*J. curcas* L.) is a perennial plant of the Euphorbiaceae family, probably native of Central America. *Jatropha* shows potential for high yield of seeds, which are high in oil content (Table 15.1). Despite the advantages of using *Jatropha* for biodiesel production, this species is still under domestication and there are many challenges to be overcome by research. The *Jatropha* varieties currently available in Brazil are not genetically characterized and there is little information about production levels in the different regions of the country. Agronomical production systems are not yet completely validated and more information about propagation, plant density, maintenance, nutrition, and pest management are necessary to allow industrial scale production. Currently, there are many research and developmental efforts to enable biodiesel production with *Jatropha* oil in Brazil, from implementation and analysis of germplasm banks to agronomical studies (Rosado et al. 2010). For instance, population evaluation allowed the identification of 5-year-old plants able to produce 4,500 kg/ha of seeds, which result in approximately 1,500 kg/ha of oil (Sotolongo et al. 2007; Laviola and Alves 2011). Importantly, researchers have identified *Jatropha* cultivars that do not produce phorbol ester in the seeds. This may allow the development of nontoxic commercial cultivars whose cake (i.e., left over from oil extraction) can be used for animal nutrition.

15.2.2 Animal Fats

Animal fat is currently the second most used feedstock for biodiesel production in Brazil (Fig. 15.1, Table 15.1). Although poultry fat and lard are also used to make biodiesel, the vast majority of the fat used to produce biodiesel in Brazil is tallow (ANP 2012a, b).

Brazil is one of the world's top meat producers. In 2012, 7.4 million tons of beef (IBGE 2013), 3.5 million tons of pork, and 11.5 million tons of poultry were produced (IBGE 2013). Tallow is a by-product of the meat and rendering industry

and it is estimated that 10.9 % of the live slaughter animal weight corresponds to tallow (Nelson and Schrock 2006). It is produced in a centralized manner in slaughter/processing facilities and historically it has low-market value (Teixeira et al. 2010). If not directed to other uses, animal fat can be an environmental pollutant.

15.2.3 Microalgae

Microalgae are photosynthetic microorganisms that grow in water and convert CO_2 into carbohydrates, protein, and natural oils. They are recognized as one of the most productive organisms in terms of biomass. In tropical areas, marine phytoplankton biomass can be produced at a rate of 100 tons/ha/year (Ben-Amotz and Jinjiikhashvily 2008). In addition, for some microalgae species, as much as 80 % of their mass is composed of lipids, which can be used to produce biodiesel. Indeed microalgae have the potential to produce oils at quantities up to 110,000 L/ha/year (Table 15.1) (Stephenson et al. 2011). Furthermore, microalgae which are not generally used as human food, can be grown in nonarable land using seawater, brackish water, or even wastewater, and can capture carbon emissions from industrial plants (Carioca et al. 2009; Stephenson et al. 2011). These characteristics render microalgae biomass a promising alternative source for biofuels with minimal problems with direct and indirect land use.

Brazil has great potential for large-scale microalgae production given that the country possesses a large tropical coastal area, with 10,959 km, has approximately 12 % of the world's freshwater supply, and receives average insolation levels of 8–22 MJ/m^2 day (IBGE 2013). Nonetheless, there are significant technological challenges to produce economically competitive, algal-derived biofuel (Stephens et al. 2010). Aiming to reduce production costs (Table 15.1), research efforts on the isolation, characterization, and domestication of highly productive algal strains from Brazil's biodiversity are currently underway. For example, it was shown that a freshwater strain of *Choricystis* sp. can provide 115 % more fatty acids per gram of biomass than soybean grain (Menezes et al. 2013). In another study, Nascimento and coworkers (Nascimento et al. 2013) screened microalgae strains isolated from freshwater lagoons from the Northeast region of Brazil based on their lipid productivity and fatty acid profiles. The highest values for lipid productivity were observed for a *Chlorella vulgaris* strain (i.e., 204.91 mg/L/day) and two *Botryococcus* strains (i.e., 112.43 and 98.00 mg/L/day for *Botryococcus braunii* and *Botryococcus terribilis*, respectively). Comparable levels have been reported for the most promising microalgae species isolated from other parts of the world such as *Nannochloropsis gadinata* (i.e., 310 mg/L/day), *Nannochloropsis salina* (i.e., 170 mg/L/day) and *Phaeodactylum tricornutum* (i.e., 50 mg/L/day) (Radakovits et al. 2012).

Table 15.2 Physical properties of vegetable oils and its biodiesel

Feedstock	Kinematic Viscosity at 38 °C (mm^2/s)		Cetane		Cloud point (°C)		Flash point (°C)		Density (g/cm^3)	
	Oil	Biodiesel	Oil	Biodiesel	Oil	Biodiesel	Oil	Biodiesel	Oil	Biodiesel
Cottonseed	33.5	3.8–4.0	41.8	46–52	1.7		234	182	0.914	0.871
Soybean	32.6	4.1–4.5	37.9	45–53	−3.9	1	254	178	0.913	0.865–0.885
Rapeseed	37.0	4.4–4.6[a]	37.6	51–59	−3.9	−3 to 4	246	127	0.911	0.857–0.882
Palm	39.6	5.7	42.0	62	31.0	13	267	164	0.918	0.867–0.880
Diesel[b]	2–4.5		49 (min)		–		55(min)		0.820–0.860	

Source (Srivastava and Prasad 2000; Singh and Singh 2010); [a] (Ramos et al. 2009) at 40 °C;. [b] EN590:1999

15.3 Biodiesel Composition and Quality

In principle, any vegetable oil can be used directly in diesel engines. In the last two decades in Brazil, several oils have been directly tested in motors (e.g., babassu, castor bean, palm oil, *Jatropha*, macaw palm, and others). However, research has shown that direct use causes adverse effects on engines, such as problems in pumping, atomization, gumming, and piston ring sticking. These problems are due to the high viscosity, density, iodine value, and poor/nonvolatility of oils. Hence, it is essential to modify these characteristics for better combustion of the vegetable oils by, for example, a transesterification reaction for biodiesel production (Kumar et al. 2013). As shown in Table 15.2, this reaction dramatically changes some physical properties of oil.

The most significant components of the oils and fats (conceptually, the difference is that oils are in liquid state at room temperature, whereas grease and fats are in solid state at room temperature; and also that the former are from plant source) are triglycerides and their physical properties depend on the structure and distribution of fatty acids. The majority of feedstocks for biodiesel production have triglycerides composed of 10 different types of fatty acids. These fatty acids have between 12 and 22 carbons in the chain, with 90 % or more having 16 and 18 carbons. Table 15.3 shows the fatty acid composition of oils/fats currently used for biodiesel production in Brazil and also of the most promising feedstocks for future use.

Biodiesel is characterized by physical–chemical properties. Some of these properties include density (g/cm^3), viscosity (mm^2/s), cloud and pour points (°C), flash point (°C), cetane number, oxidation stability, and distillation range, which basically depend on the type of feedstock and their fatty acid composition. Other properties, like acid value (mg KOH/g-oil), ash content (%), copper corrosion, phosphorus (mg/kg), sulfur content, carbon residue, water content and sediment, and glycerin (% m/m) are more affected by processing (Atabani et al. 2012). In Box 1, at the end of this section, explanations of some general properties of biodiesel are presented. Currently, the properties of biodiesel must comply with

Table 15.3 Fatty acid profile of selected oils and fat used in biodiesel production

Fatty acids	Soybean	Cotton	Palm	Physic nut	Tallow
C14:0	0	1	1	0	3
C16:0	12	21	43	15	23
C18:0	3	3	5	6	19
C18:1	23	19	41	35	43
C18:2	56	55	10	44	3
C18:3	6	1	0	0	1
C20:0	0	0	0	0	0
C22:1	0	0	0	0	0
Saturated	15	24	48	21	45
Iodine value	130	105	37	101	35–48

Source (Ma and Hanna 1999; Singh and Singh 2010); Mendonça S. (unpublished)

international biodiesel standard specifications established by one of the various organizations that set fuel standards. Particularly important specifications for biodiesel fuel (B100) include the ASTM 6751 from the American Standards for Testing Materials (ASTM 2012) and the EN 14214 from the European Committee for Standardization (ECN 2008). However, there are other standards available globally such as those from Germany (DIN 51606), considered to be even stricter than the European norms, and the Brazilian resolution (ANP 04/2010) (ANP 2012a, b), which is based on ASTM 6751 and EN 14214.

Some parameters for the quality of biodiesel in different countries and a summary of physical–chemical properties of diesel and biodiesel produced from different feedstocks are shown in Table 15.4. Biodiesel standards in Brazil and in the U.S. are applicable for both fatty acid methyl esters (FAME) and fatty acid ethyl esters (FAEE), whereas the current European biodiesel standard is only applicable for fatty acid methyl esters (FAME). Also, the standards for biodiesel in Brazil and in the U.S. are used to describe a product that is a blending component in conventional hydrocarbon-based diesel fuel, whereas the European biodiesel standard describes a product that can be used either as a stand-alone diesel fuel or as a blending component. These differences in technical specifications are primarily related to the origin of the feedstock and the characteristics of the local markets. Though this currently translates into some significant divergence in specifications and properties of the derived fuels—which could be perceived as an impediment to trade—in most cases it is possible to meet the various regional specifications by blending the various types of biodiesel to the desired quality and specifications (Tripartite Task Force 2007).

The Cold Filter Plugging Point (CFPP) is very important in colder regions, where a high CFPP indicates a high likelihood that the fuel will clog up the vehicle engine. Biodiesel from palm oil and tallow show the poorest performance (highest temperature points) in terms of CFPP, while biodiesel from rapeseed generally

Table 15.4 Biodiesel standards in different countries and comparison with biodiesel characteristics from diverse feedstocks

Characteristic	Brazil (ANP14/2012)	European Union (EN 14.214)	USA (ASTM D6751)	Soybean biodiesel	Rapeseed biodiesel	Cottonseed biodiesel	Palm biodiesel	Jatropha biodiesel
Density at 15 °C (g/cm^3)	0.850–0.900[a]	0.860–0.900	–	0.880–0.884	0.879–0.882	0.875	0.864–0.880	0.864–0.880
Kinematic viscosity at 40 °C (mm^2/s)	3.0–6.0	3.5–5.0	1.9–6.0	4.0–4.2	4.4	4.1	4.5–5.7	4.2–4.8
Cetane number (min.[b])	Report	51	47	45–58.1	54–59	54	62	51–57
Oxidation stability at 110 °C: h (min.)	6	6	3	1.3–3.8	6.4–7.6	1.8	11–13	2.3–3.2
Iodine value -g I_2/100 g (max.[c])	Report	120	115	128	109		57	104
CFPP (°C) (max.[c])	19	0 summer[d]–20 winter	–	−5	−20 to 10	1	10–12	0
Sulfur (mg/kg) (max.[c])	50	10	15	0.2–0.8	0.2	0.01	0.01	0.3
Flash point (min.) °C (min.[b])	100	120	93	160–254	170	150	135–176	163–191

[a] at 20 °C; [b] minimum limit; [c] maximum limit; depends on country and season, the given example is for Germany

Source (Foidl et al. 1996; Demirbas 2008; Ramos et al. 2009; Atabani et al. 2012)

shows the best performance (lowest temperature points). Because of large geographic and seasonal temperature variations, neither the U.S. nor European biodiesel standards have strict specifications for these low temperature properties, though they are among the most important properties in determining the suitability of biodiesel fuels in-use. In Europe, CFPP values must be established for each country according to its climate. In the United States, the value of CP (Cloud Point) is used instead of CFPP, and it is also dependent on the season of the year. According to the Brazilian Resolution, except for castor bean biodiesel, a maximum of 19 °C for CFPP is applicable for the South, Southeast, Midwest, and the state of Bahia. For the other Brazilian regions with tropical climate, there is no recommended value for the cloud point, although it needs to be reported.

The density and viscosity increase with the number of carbons of the fatty acid chain and are reduced by the presence of double bonds. Triglycerides with saturated fatty acids (SUFA) have higher density and viscosity (more solid at room temperature) causing problems to SUFA-derived fuels in cold regions, i.e., they have higher CFPP. Triglycerides rich in polyunsaturated fatty acids present low oxidative stability and low CN, which may lead to oxidation and thermal polymerization, whereas they present better CFPP (low values for CFPP). More saturated triglycerides such as tallow are solid at room temperature. Thus, they are difficult to use as fuel because of the higher values of CFPP, whereas excessive carbon deposits in engine are reported when polyunsaturated triglycerides like rapeseed oil are used as fuel. Vegetable oils are mostly unsaturated and thus more susceptible to oxidation and thermal polymerization reaction (Kumar et al. 2013). To achieve a balance between CFPP and oxidative stability, a biodiesel feedstock should have as high as possible monounsaturated fatty acid content.

The degree of unsaturation may be expressed as iodine value (i.e., amount in grams of iodine which reacts with the double bonds present in 100 g of the sample) and can be used to classify oils into three categories: drying (iodine value greater than 170; e.g., linseed oil), semidrying (iodine value between 100 and 170; e.g., soybean and sunflower); nondrying (iodine value less than 100; e.g., palm oil). Drying oils tend to form films, becoming solid due to polymerization of the chains in consequence of oxidation. On the other hand, nondrying oils are resistant to oxidation and will remain liquid for a long time (Meier et al. 2007). This does not mean that those oils cannot be used for biodiesel production; however, they should be mixed with biodiesel from other sources to achieve the recommended quality standard. For example, adding 5 % of tallow biodiesel to rapeseed biodiesel would increase the cetane number, without significant interference in CFPP. Soybean biodiesel presents an iodine value above the acceptable range established by the European Norm (EN) (i.e., 125–140, where the EN14214 limit is 120) and lower cetane number. The mixture with 20 % of tallow biodiesel could lower the iodine value allowing export to Europe and at the same time increase the cetane number improving fuel characteristics. Similarly, 70 % of palm oil biodiesel could be blended to 30 % Jatropha biodiesel to optimize CFPP of the former and oxidative stability of the latter.

Biodiesel can be produced from beef tallow using the traditional route of NaOH catalysis and methanol with high yields (i.e., 96.26 %) (Araujo et al. 2010). Although biodiesel from vegetable oils and tallow have comparable properties, tallow biodiesel has more saturated fatty esters because beef tallow has more saturated fatty acids (Table 15.3). Among these saturated fatty acids, stearic (C18:0) and palmitic (C16:0) acids are the most abundant. This in turn has consequences to fuel quality. Viscosity of tallow biodiesel at 40 °C is 4.89 mm^2s^{-1}, compared to 4.20 and 3.47 for soybean biodiesel and petrodiesel, respectively (Table 15.4) (Teixeira et al. 2010). Another fuel property is the cold filter plugging point, which can be interpreted as the lowest temperature at which the fuel will flow without problems such as clogging a fuel system. The cold filter plugging point for tallow biodiesel is 15 °C, while for soybean biodiesel it is 4 °C and for petrodiesel it is 10 °C (Teixeira et al. 2010). Therefore, the properties of tallow biodiesel are not the most advantageous, particularly for cold weather climates. However, tallow biodiesel can still be used successfully if blended to soybean biodiesel or petrodiesel (Teixeira et al. 2010). The use of tallow biodiesel only becomes a concern in a scenario of B100, where tallow biodiesel is in high proportion compared to soybean or other vegetable-oils biodiesel. Given that tallow is a by-product of the meat and rendering industries, it is unlikely that the amount of tallow to make biodiesel will increase disproportionately and that this scenario will become a reality.

Microalgae represent a very diverse group and their fatty acid profile varies drastically depending on the species (Nascimento et al. 2013). In addition, cultivation parameters will also affect fatty acid composition (Cabanelas et al. 2013; Xu et al. 2006). Generally, solar incidence, nitrogen, and carbon limitations/source will lead to increase/decrease in saturated/insatured fatty acids. Xu et al. (2006) demonstrated that oil derived from a heterotrofically cultivated strain of *Chlorella protothecoide*s could be used to produce biodiesel that meets the Brazilian National Agency of Oil (ANP) standards (Franco et al. 2013). Furthermore, good quality microalgae biodiesel may also be obtained by using a mixture of oils from different microalgae species, from other biodiesel feedstocks or by optimizing microalgae culture conditions.

BOX 1: Parameters for biodiesel quality

Kinematic Viscosity: The viscosity of biodiesel increases with carbon chain length and degree of saturation and influences the process of fuel burning in the combustion chamber of the engine. Due to decreased efficiency of atomization in the combustion chamber, high viscosity causes heterogeneity in the combustion of biodiesel and residues are deposited in the internal parts of the engine.

Cloud and Pour Points, Cold Filter Plugging Point (CFPP): At low temperatures, biodiesel tends to partially lose fluidity or solidify, leading to fuel flow disruption, clogging of the filtration system, and engine damage due to inadequate lubrication. This causes problems in starting the engine.

Cloud point refers to the temperature at which the liquid begins to become turbid, and the pour point is the temperature at which the liquid no longer flows freely. Both are influenced by feedstock characteristics, and also the alcohol used in the transesterification reaction. Usually, international standard specifications are expressed in CFPP, which is correlated to both cited cloud and pour points and refers to the temperature at which the test filter starts to plug due to fuel components that have started to gel or crystallize. Thus, the higher the CFFP point, the higher the chances that a small decrease in weather temperature will cause problems to the fuel. The CFPP from biodiesel derived from rapeseed oil is between −7 and −12 °C, whereas from animal fat it is between 15 and −1 °C.

Iodine value: It is an indicator of the number of double bonds present in biodiesel. It does not distinguish double bond location (i.e., the fuels' oxidation depends not only on the number of double bonds but also on their proximity to each other), so the iodine value is a weak predictor of biodiesel's oxidation stability or its tendency to form deposits in the engine.

Oxidation and Thermal Stability: There are two types of stability to be considered: stability during long-term storage (oxidation stability) and at high temperatures and/or pressure in the engine (thermal stability). The available data indicate that biodiesel has a good thermal stability, even producing less coke residues in the engines injectors than conventional diesel. Biodiesel's aging or oxidation may lead to high acidity, high viscosity, and formation of gum and sediment that plugs filters. If these latter properties exceed the limits permitted by ASTM D6751, B100, it is considered out of specification and should not be used as fuel. The higher the unsaturation level of the original feedstock, the higher the probability of fuel oxidation. As a rule, saturated fatty acids (e.g., C14:0 or C16:0) are more stable than unsaturated fatty acids (e.g., C18:2 or C18:3). For every double bond added, the fuel's stability decreases 10 times. Other factors, such as exposition to oxygen, light, and high temperatures, and also to contaminants accelerate oxidation. Brazilian and European legislation specify an accelerated test for biodiesel's oxidation stability, called the Rancimat test, recently also adopted as part of the United States standards.

Cetane Number/Cetane Index: Cetane number is an indicator of the ignition quality of a fuel for a diesel engine, and it has a direct influence on motor starting and operation under load. A high cetane number of a fuel indicates good combustion in a diesel engine. Furthermore, in conventional diesel engines, high cetane numbers are correlated with lower nitrogen oxide (NO_x) emission. The larger the number of unsaturations and the shorter the chain of fatty acids that compose the biodiesel, the lower the cetane number is corresponding to a greater the emission of NO_x from the fuel (Kumar et al. 2013). The average cetane number for biodiesel (B100) is 55. For petrodiesel this index is between 48 and 52 (minimum of 40). This is the reason that biodiesel burns much better in a diesel engine than petrodiesel. In terms of legal

requirements, the minimum cetane number for biodiesel (B100) is 51 in Europe and 47 in the United States; the Brazilian legislation does not establish a minimum, but the cetane number should be reported.

15.4 Critical Analysis

Despite the low oil yield of soybeans, this crop has been the major feedstock for biodiesel production in Brazil for a number of reasons. First, technological know-how for soybean cultivation is well established. Brazilian crop breeders made a huge effort to obtain soybean varieties that were viable in the Cerrado (the Brazilian Savanna) region of the country. Cerrado is a vast area in the Center-West region of Brazil with poor and acidic soils that were thought to be inappropriate for agriculture. Research led to the improvement in soil quality. Furthermore, when new tropical soybean varieties were introduced, the flat Cerrado topography proved to be ideal for mechanization and the adoption of an agribusiness model based on technology. The large-scale production of soybeans is the second reason that it has been used as a feedstock for biodiesel production. By definition, the demand for energy is constantly increasing and therefore any feedstock used to produce energy needs to be available in large amounts. Today in Brazil no other feedstock is produced in a large enough scale to reliably supply the biodiesel production chain. The third reason for Brazil's current dependence on soybeans for biodiesel production is that soybeans are planted in all states of the country. This makes it widely available and contributes to lower prices of biodiesel in the regions that concentrate the production (Fig. 15.2).

Currently, tallow and cotton oil play important roles as feedstocks for biodiesel production in Brazil. Tallow is used in the biodiesel industry because it is a low-cost by-product of the meat and rendering industries (Fig. 15.1). However, its offer is not related to the biofuel market. In the long term, it is not expected that tallow will increase its contribution to biodiesel production by more than 20 %, even if one considers that all tallow generated in Brazil is used to produce biodiesel. This is due to the increasing production of biodiesel from vegetable oils and to the fact, as previously discussed, that tallow biodiesel presents quality obstacles that hinder long-term storage or use in higher levels of blending (Table 15.3), as tallow biodiesel has high CFPP around 12 °C. Thus, tallow should always be considered as an additional feedstock to be mixed with biodiesel produced from other oils. Participation of cotton oil in the biodiesel feedstock matrix in Brazil is not expected to increase considerably in the future and should remain around the current 5 % (Fig. 15.1). Like for soybean, cotton seed oil content and yield are low. In addition, as previously mentioned, the cake obtained after oil extraction cannot be used as animal feed because of the toxic compound gossypol.

Palm oil will probably start to play a more important role as a feedstock for biodiesel production over the years in Brazil. As with soybeans, the technological

know-how for oil palm cultivation is available. Given that oil production per hectare is much higher than that of soybeans (Table 15.1), large-scale production of palm oil should not be a problem. The Brazilian government wants to encourage a greater participation of oil palm as a biodiesel feedstock, and it is implementing policies to support this growth in a controlled and organized manner. For instance, there are governmental incentives for oil palm planting in specific areas. This takes into consideration not only which areas have the appropriate soil and climatic conditions, but also restricts planting to areas with a history of anthropic intervention (i.e., agroecological zoning). One of the major problems with oil palm, as well as other perennial crops, is that the farmer needs to invest money over many years before any profits are made. To make this viable, there is a need for specific lines of credit during this period. Another problem that needs to be addressed is that there are many small farmers producing oil palm and the most advanced technology is not always available to them to increase productivity.

In the long term, *Jatropha* may be a good candidate crop for further development of the Brazilian biodiesel program. It has high productivity of fruits and good quality oil for biodiesel production (Tables 15.1, 15.3 and 15.4). However, there are still many challenges to be overcome before large plantings of *Jatropha* are viable, since no commercial cultivars, and consequently no production system, are available. In addition, currently the *Jatropha* cake cannot be used as animal feed, and thus is a negative economic impact in the *Jatropha*-biodiesel production chain.

Biofuel companies are also seeking to achieve commercial production of microalgae and to design economically viable systems for growing microalgae in Brazil. An example is Austria's See Algae Technology (SAT) and the Brazilian JB Group partnership that aim to produce biodiesel from microalgae in a plant in Brazil's Northeast region (http://www.seealgae.com/article32.htm). The US$ 5 million pilot project is based on SAT-designed solar prisms that concentrate sunlight, through optical fibers, on microalgae grown in tanks. Another strategy is being used by the U. S. company Solazyme, which is in partnership with the Brazilian company Bunge in a US$120 million investment (http://solazyme.com/media/2013-01-16). Solazyme uses genetically modified algae that produce oil for renewable chemicals and biofuels cultivated in a closed heterotrophic system. In the microalgae fermentation facility, which is annexed to Bunge's Moema unit in São Paulo (Brazil), the feedstock for the plants will be sugarcane juice. The Brazilian startup company, Algae (http://www.algae.com.br), is building a pilot plant in São Paulo in partnership with the Federal University of São Carlos (UFSCAR). In this model, the carbon source is vinasse, a by-product of sugarcane processing and an environmental pollutant. Also in the Northeast region—taking advantage of its favorable climate with a high number of sunny days—in 2012, Petrobras, the main Brazilian petroleum company, began operating its first microalgae cultivation tanks for the production of biodiesel (http://www.petrobras.com.br/pt/energia-e-tecnologia/tecnologia-e-pesquisa/diversificando-os-produtos/). In the laboratories of the Federal University of Rio Grande do Norte (UFRN), the company selected microalgae species that can be grown in water used for petroleum production.

Regardless of feedstock, the Brazilian National Program for Biodiesel Production and Use needs to set new goals for higher blends of biodiesel into petrodiesel. The biodiesel industry is currently at half its maximum capacity, which means it can easily produce enough biodiesel to supply a B10 demand. However, until the government sets these new goals, the only increase in demand for biodiesel will come from an increase in diesel B5 use. Government planning, even if only for the long term, will be very important so that the biodiesel industry can prepare itself to meet demands, thus avoiding a future shortage of biodiesel. Clearly established goals will be equally important to ensure the availability of feedstocks for biodiesel production in the future.

15.5 Conclusion

The Brazilian biodiesel industry was developed based on the soybean, cotton, and beef production chains. These feedstocks allowed the industry to be established and made Brazil one of the largest biodiesel producers in the world. However, considering the increasing demand for renewable fuels, other potential oil crops for biodiesel production have been evaluated for usage in the short-, medium- and long-term perspectives. Soybean produced in Brazil is enough to easily increase biodiesel mixtures from the current B5 to up to B10 and it should continue to be the main feedstock for biodiesel production in the short term. Alternative feedstocks like oil palm, which have much higher productivity and yields of oil, should increase its participation in the energetic matrix in the medium term. This will help with diversification and regionalization of feedstocks, especially in the North and Northeast regions of the country. Nevertheless, substantial financial investments need to be made in the coming years to support the cultivation of this perennial crop. Finally, *Jatropha* and microalgae may become significant components of the biodiesel production chain in the long term, after technical challenges are surpassed. Clear governmental demands for biodiesel usage and establishment of specific goals for the diversification of biodiesel feedstocks are essential to guarantee the long-term success of the Brazilian National Program for Biodiesel Production and Use.

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Chapter 16
Techno-Economic and Life Cycle Analysis of Biodiesel Production: Perception of Land Use, Climate Change, and Sustainability Measurements

Donato A. G. Aranda, Cecilia M. Soares and Neyda Om Tapanes

Abstract Motivation of this study is the strategic importance of bioenergy and biofuels for sustainable development of the global economy. Brazilian Bioenergy Program has enabled the consolidation of Brazil within the leading countries in the production of energy and renewable fuels. Within this program, biodiesel occupies a prominent position, influenced by significant technological, economic, environmental, and social advantages. This chapter covers issues like the life cycle analysis for the biodiesel production, allowing the mapping of resources, impacts of this economic activity, and the premises of sustainability. It also provides market information by analyzing the demand—production relationship, prices, and product quality supervision. Finally, it presents technical and economic parameters of the main technological routes of biodiesel production in Brazil (hydroesterification and transesterification) using current data and allowing the growing demand for new approaches and technological advances.

16.1 The Biodiesel Market

The next decade biofuel demand is increasing in Europe. However, non-European countries must represent more than 60 % of the world demand by 2030 and about 70 % by 2050. China, India, and Latin America will probably be the leaders in this market (Fig. 16.1).

Brazil holds an important position in the biofuel world scenario. Biodiesel has the advantage to be used pure or with diesel blends in internal combustion compression engines. In addition to the advantages of being produced locally, biodiesel has several environmental advantages compared to regular fossil diésel. Moreover,

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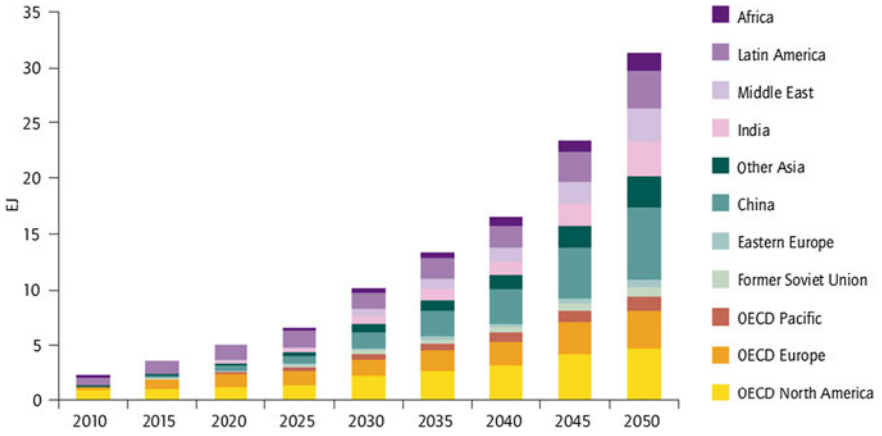

Fig. 16.1 Biofuel demand by region, 2010–2050 (IEA 2010)

social benefits and regional development with significant amount of new jobs and income can be obtained once its production and consumption are promoted in a non-centralized way with multiple feedstock (Ferreira and Oliveira 2010).

There are many factors that contribute to the increase in investments in biodiesel in Brazil. It is possible to mention the environmental pressures, world political instabilities, and uncertainty about the future of oil exploration, the social stimulus to agriculture, and the dependence on foreign diesel oil, where about 18 % of this fuel comes from.

Diesel oil is the principal fuel used in Brazil, because of the extensive use of road logistics all over the country that has been estimulated by the Federal Government since the decade of the 1950s (Fig. 16.2).

Provisional Bill n° 214, from 13 September 2004, the Petroleum National Agency Agência Nacional do Petróleo (ANP) defines technically biodiesel as a fuel for combustion engines with internal compression ignition, renewable and biodegradable, derived from vegetable oils or animal fats which can replace partially or totally fossil diesel (Soares 2008).

In the Brazilian Biodiesel Standard, B100 is defined as a fuel consisting of alkyl esters of long chain fatty acids, derived from vegetable oils or animal fats. The B2 is a commercial fuel composed of 98 % by volume of diesel fuel, as ANP specification, and 2 % of biodiesel. The other compositions follow the models of B2 and B100 (Soares 2008).

The Brazilian law 11,097 of 2005 provides for the introduction of biodiesel into the Brazilian energy matrix, and sets to 5 % in volume its mandatory minimum starting at 2013.

However, since 1 January 2010, diesel fuel sold in Brazil contains 5 % biodiesel. This rule was established by Resolution No. 6/2009 of the National Energy Policy (CNPE), which increased from 4 to 5 % the mandatory percentage blending of biodiesel to diesel oil. The continued rise in the percentage of biodiesel added to

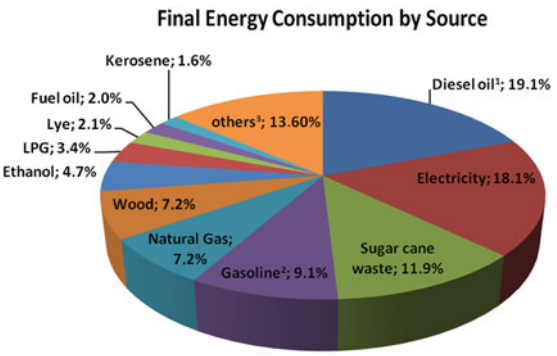

Fig. 16.2 Final energy consumption by source in Brazil, 2011 (Balanço Energético Nacional 2012)

diesel demonstrates the success of the National Program for Production and Use of Biodiesel and the experience accumulated by Brazil in the production and use of biofuels on a large scale (ANP 2013).

It is possible to highlight three groups that have been involved in biofuel production: those who already have the necessary resources (including agribusiness entrepreneurs, oil companies, plant operators, and small farmers); suppliers of products and services (including seed companies, engineering and equipment, and biotechnology), and the market participants (such as farmers, agricultural equipment companies, fertilizer suppliers, and logistics providers) (Caesar 2007 in Soares 2008).

In 2011, the amount of B100 produced in Brazil reached 2,672,760 m^3, against 2,386,399 m^3 in the previous year. Thus, there was an increase of 12 % in biodiesel available in the national market. In 2011, the percentage of B100 compulsorily added to mineral diesel remained constant at 5 %. The main raw material was soybean oil (81.2 %) followed by tallow (13.1 %) (Balanço Energético Nacional 2012).

Since 2005, the Petroleum National Agency performs biodiesel auctions where refineries buy biodiesel to be blended with fossil diesel. The initial purpose of the auction was to generate a permanent market and thereby stimulate the production of biodiesel to meet the national law (ANP 2013).

These auctions were structured for the period between 2005 and 2007, but to preserve the participation of family agriculture in the supply of raw materials, the government preferred to keep the systematic purchase through auctions after that period, rather than direct negotiations between producers and distributors or refineries, as occurs in the ethanol market (Amaral Mendes and Da Costa 2009).

The evolution of the biodiesel auctions can be evidenced in Fig. 16.3, from the first auction, which started with 70,000 m^3 in November 2005, until the thirtieth auction in April 2013 that fetched 488,532 m^3 (auction for 2 months delivery).

Figures 16.4, 16.5, and 16.6 summarize average prices in each biodiesel auction as organized by ANP. After several changes in the auction manner, currently, it involves a direct offering between biodiesel producers and fuel distribution companies.

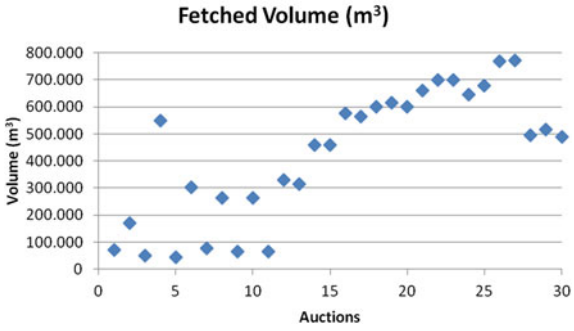

Fig. 16.3 Biodiesel auctioned volume—ANP auctions (ANP 2013)

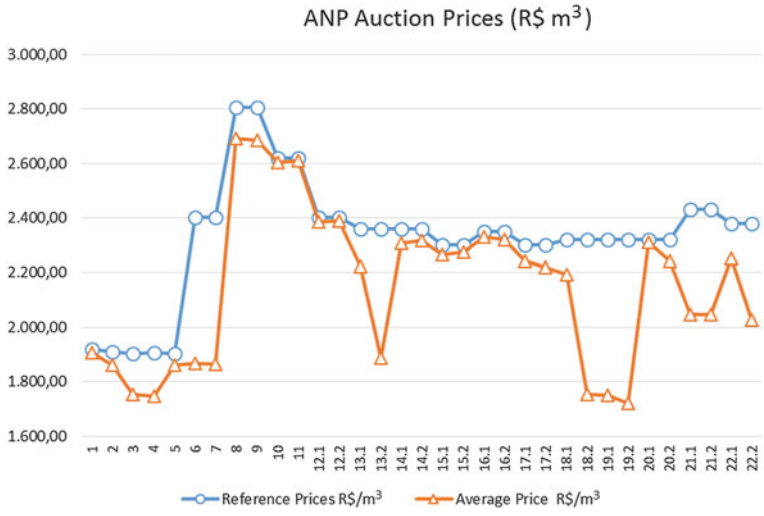

Fig. 16.4 Auction average prices 1–22 (ANP)

Despite being a new industry, the potential biodiesel offer is much higher than the mandatory demand. This means a dangerous industrial idling (Fig. 16.6). Until January, 2013, 64 biodiesel plants obtained operating licenses with a total capacity of 20,207.76 m^3/day. In the next few years, more plants are scheduled to work in addition to some extended size ones.

Biodiesel Program promotes social inclusion through deals involving biodiesel producers and small farmers. Agriculture Development Ministry published the "Instrução Normativa n° 1 de 20/06/2011," which currently regulates the "Social Fuel Seal." Basically, the biodiesel industries with purchase contracts with small farmers are included in the "Programa Nacional de Fortalecimento da Agricultura Familiar (Pronaf)." Once the producers have obtained this seal, they are able to obtain some tax reductions and special fundings. Producers have to purchase at least

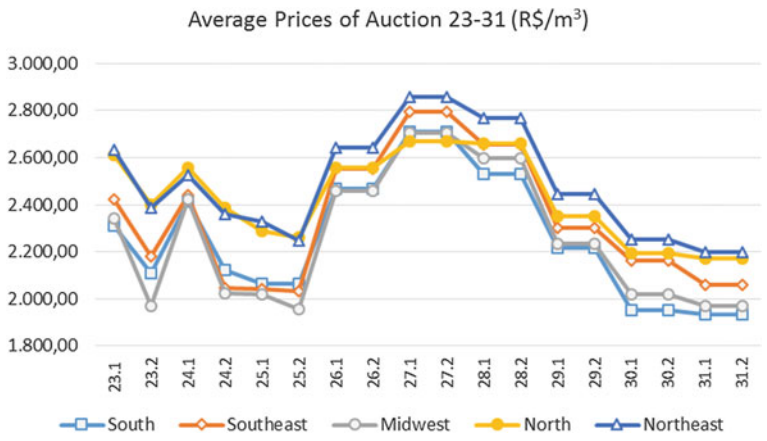

Fig. 16.5 Average prices in biodiesel auctions (ANP auction 23–31)

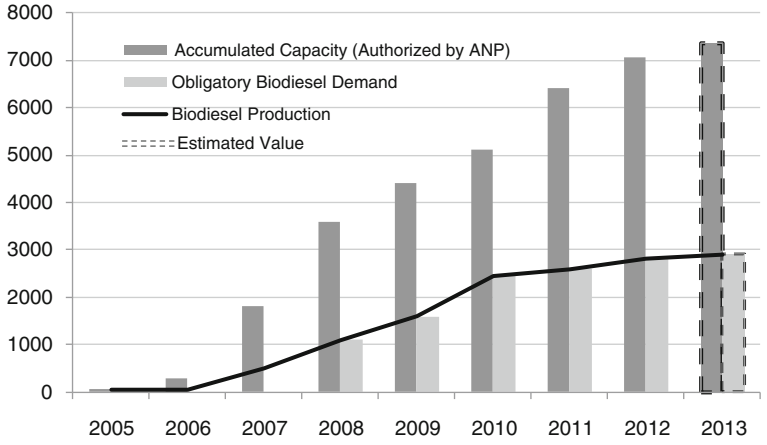

Fig. 16.6 Mandatory demand and operating licensed capacity for biodiesel plants in Brazil, 1000 m^3 (ANP 2013)

30 % of their feedstocks from Northeast, Southeast, and South region small farmers, or 15 % in the case of North or West-Central region (BRASIL/MDA 2011).

A part of biodiesel producers are asking for an open market phase instead of the regulated auctions as is currently done. In this case, ANP activity would be restricted to quality control as well as the blended biodiesel-diesel regulation.

Biodiesel development occurrs in Brazil due to the mandatory process once its price becomes historically higher than mineral diesel. Average Price for B5 in April/2013 was R$2.33/L, 14 % higher than April/2012. This higher price is basically due to the higher prices of mineral diesel and not due to a more expensive biodiesel (Fig. 16.7).

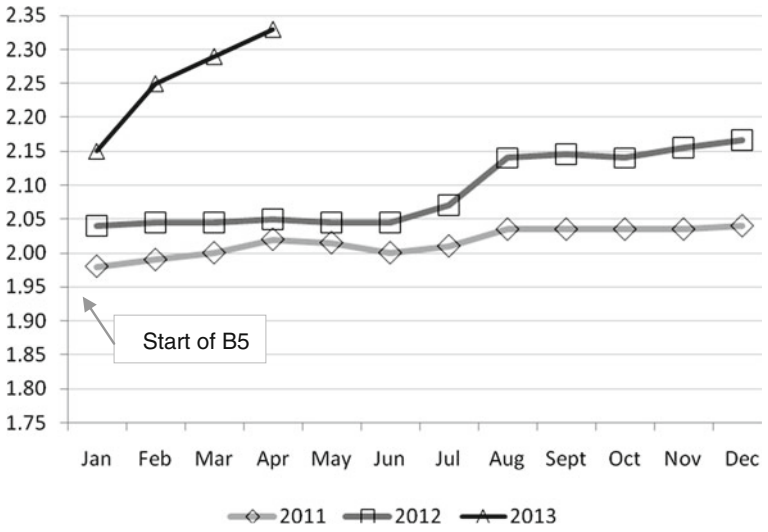

Fig. 16.7 B5 prices (R$/liter) (ANP 2013)

Table 16.1 Brazilian prices for biodiesel and mineral diesel (R$/m^3)

Fuel prices (R$/m^3)	2012	2013*	Δ %
Biodiesel—Auctions—ANP	2,187.91	2,249.42	2.8
Diesel in refinery	1,372.13	1,538.28	12.1
Diesel to distribution companies	1,816.50	2,002.75	10.3
Diesel in fuel station	2,041.25	2,259.50	10.7
Imported diesel	1,448.32	1,604.67	10.8

Source ANP (2013). Dólar/Brazilian Real Exchange, R$2.00/US$1.00
* 1st quarter of 2013

Average prices for biodiesel purchased in the auctions were between R$2,553.46/m^3 and R$2,213.57/m^3 in the first quarter of 2013 (Table 16.1). After that the price dropped to R$1,981.22/m^3. Comparing with the same period in 2012, prices were 2.8 % higher. At the same time, mineral diesel prices increased twice (ABIOVE 2013a, b).

It is important to stress that biodiesel price is ascribed to vegetable oil prices. Actually, biodiesel production cost is about 85 % of vegetable oil. In the Brazilian case, soybean oil price is relevant. It represents 75 % of the feedstocks to biodiesel. Another important factor in the biodiesel historical prices is the large amount of companies offering biodiesel in the auctions. In the ANP auctions, only the maximum prices are fixed; final prices are based on the competitive edge (Amaral Mendes and Da Costa 2010).

16.2 The Environmental Issue on Biodiesel

Once there is oxygen in its structure, biodiesel is able to promote a more complete combustion, reducing emissions of carbon monoxide (CO) and particulate matter, and increasing lubricity, guaranteed by sulfur in diesel, hence improving the life of engine components. There are also reductions in emissions of sulfur oxides because biodiesel does not contain sulfur. Furthermore, biodiesel provides a small increase in emissions of NOx for more than 20 % B20+ (Soares 2008). However, urea solution additive significantly reduces problems ascribed to NOx emissions in diesel engines.

The impact of NOx emissions by replacing diesel with biodiesel is not significant, but the reductions in CO, hydrocarbons, particulate matter, and poly-aromatics imply significant benefits (Monteiro 2005 in Soares 2008).

In general, it is considered that biodiesel is able to reduce the total greenhouse gases (GHG) emissions compared to diesel fuel. However, for a more comprehensive study of emissions from biofuels, it is necessary to consider some variables for the production of biodiesel, such as the production technology route, oilseed, and alcohol used in the process (Soares 2008).

16.3 Life Cycle Analysis of Biodiesel: An Overview of the Brazilian Case

Life Cycle Assessment (LCA) of a product or process is a management technique to quantify the mass flow, energy, and emissions assessing the environmental aspects and potential impacts from its production chain (Soares 2008).

ISO 14040, in the same group of ISO 14000, which establishes guidelines for corporate environmental management, indicates a methodology to life cycle assessment of products and services.

In order to estimate the environmental impact of the production and use of biodiesel, it is proposed a simplified life cycle analysis of its production.

The scope of this life cycle assessment includes two basic steps:

- an initial mapping of the most used vegetable oils in Brazil and potentially usable main raw materials for the biodiesel production;
- a mapping of biodiesel production key data in Brazil.

Another important issue is the land use. Most countries have a conflict between the land used for food plantations and the land dedicated to bioenergetic crops. In the case of Brazil, there are about 850 million ha including forests, cattle pasture, lakes, cities, etc. If one takes into account just the available land for new crops, the official number is about 90 million ha. One million hectares with palm plantation could produce about 5 million mton/year of palm biodiesel. This would mean a

Table 16.2 Biodiesel production by feedstock (m^3 biodiesel)

Feedstock	2008	2009	2010	2011	2012	2013
Soybean oil	801,320	1,250,577	1,960,822	2,152,298	2,042,730	466,588
Beef tallow	206,966	258,035	327,074	357,664	469,215	141,260
Cottonseed oil	18,353	59,631	57,458	84,711	123,325	26,797
Others	140,489	40,206	41,086	78,088	83,683	37,215
Total	*1,167,128*	*1,608,448*	*2,386,438*	*2,672,760*	*2,718,954*	*671,859*

Source/Preparation ANP (2013), ABIOVE 2013a, b—Coordination of economics and statistics

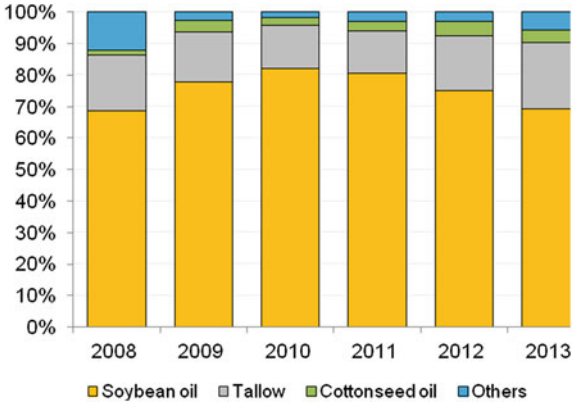

Fig. 16.8 Oilseeds market share for biodiesel production (ANP 2013; ABIOVE 2013a, b)

B10 program just with 1/90 of the available land. Thus, land use in Brazil is not a big issue. On the other hand, most of the Brazilian soybean producers are part of the Round Table on Responsible Soy Association (RTRS), which tracks the soybean origin. Soybean produced in a deforested area or using slavery labor conditions is not allowed.

16.3.1 The Oilseed Producing Biodiesel in Brazil

ABIOVE (Brazilian Association of Vegetable Oil Industries) considers that the biodiesel market is represented by the following oilseeds (Table 16.2).

These data contribute to the formation of the following market share of oilseeds for biodiesel production (Fig. 16.8).

It may be noted that soybean oil, beef tallow, and cottonseed oil contribute, on average, by about 90 % market share for feedstocks producing biodiesel.

It should also be noted that soybean oil is the main feedstock responsible for the production of fuel, representing more than 60 % of the total oilseed. The explanation for this lies in the fact that soybeans have a role as one of the main items of Brazilian agricultural production, due to its development observed more sharply

since the 1970s, which enabled the crop to meet the biodiesel national market in an easy way. Brazil holds the second position in the world ranking for soybean production, behind only to USA.

Based on the soybean oil produced in Mato Grosso state, the farthermost soybean production center from the main consumption biodiesel places (São Paulo, Santos port, Paulínia fuel bases) Delta CO_2 company made an LCA of the pure soybean biodiesel (Delta CO_2 2013). Results indicated a reduction in the greenhouse gases to about 70 % compared to fossil diesel. If one considers the total amount of biodiesel being produced and consumed so far in Brazil, it means about 21 million m ton of avoided CO_2 due to the Brazilian biodiesel experience. It is important to emphasize that animal fat, the second more used feedstock, was not considered in this evaluation. Usually, animal fat biodiesel has an even better environmental performance than soybean.

Beef tallow is the second most widely used feedstock for biodiesel production and has contributed to almost 500,000 m^3 of fuel in 2012. This feedstock is also justified for the production of biodiesel because livestock is one of the main economic activities in Brazil. Brazil has the second largest herd in the world, behind India, currently occupying an area of almost 200 million ha—which is about three-fourths of the occupied area by the entire agricultural industry in the country.

Cottonseed oil contributed in recent years by 2–5 % of oilseeds for biodiesel production in Brazil. Cotton production in the country in 2011/2012 was over 1.8 million tons. Brazil is the fifth largest producer, behind China, India, Pakistan, and the United States, and is the third largest exporter of this oilseed (Abrapa 2013).

Among the other possible oilseeds that produce biodiesel, those that stand out as market reality are palm oil, babassu oil, castor bean oil, and sunflower oil. However, it is expected that the feedstocks used for this fuel would be diversified with advances in research and development in the Brazilian agricultural sector in order to reduce competition with food and land use, and optimize production and implementation costs, providing integration with the use of manpower to the agricultural industry in a socially dignified way.

The main trend for the future of biodiesel production in the country is the use of algae because of a significantly higher biomass productivity at current oil, which reduces the demand for extensive lands, besides the fact that they have carbon dioxide and light as their main inputs.

16.3.2 Biodiesel Production in Brazil

In general, the biodiesel market in the country still lacks important studies and research, such as life cycle assessment for the production and use of fuel associated with the market reality. This was observed through a simplified market research performed with some of the leading producers of biodiesel in Brazil.

These data reflect the difficulty to relate the relevant and reliable data on the life cycle assessment for the production of biodiesel in Brazil. Companies such as Petrobras, which conducts similar ongoing studies reflects a trend of concern about the alignment of fuel data to the company's need. There is also a series of academic studies focused on life cycle assessment of biodiesel, but each with its specific limitations and considerations that do not necessarily refer to a reality in the market.

The factors to be considered and inventoried for a life cycle assessment of the production and use of biodiesel reflecting the reality of Brazil are:

- Origin and indicators of production processes for the oilseeds employed;
- Distance, type of transport, and logistics, possible loss estimates associated with the origin of oilseed and its production process;
- Indicators of the production process for biodiesel;
- Distance, type of transport, and logistics possible losses estimate associated with the oilseed to the biodiesel production process;
- Origin and production process indicators of feedstock, supplies, and utilities for the biodiesel production processes;
- Distance, type of transport, and logistics possible loss estimates associated with the feedstocks, supplies, and utilities to the biodiesel production; and
- Indicators of the biodiesel use to the end consumer.

The indicators of the production of biodiesel should also consider the wastewater generation, solid waste, and gaseous emissions of its productive chain.

Amaral Mendes and Da Costa (2009) define the biodiesel industry as structured by companies with three distinct classifications in relation to its main feedstock: integrated, partially integrated, and nonintegrated.

The integrated companies have the cultivation or marketing of oilseeds step in its supply chain. These companies typically have greater competitiveness in the market due to greater flexibility of marketing products in accordance with the stages of its production.

The partially integrated company has the ability to produce, in addition to biodiesel, vegetable oil, although they do not sell or plant the crop plants.

The nonintegrated companies produce only biodiesel and are vulnerable to the oilseed price market fluctuations.

In relation to feedstocks, it is observed that besides oilseeds, the market mainly uses methanol as the reagent alcohol for the transesterification of the oil, despite the large supply of ethanol in the country. This is also explained by the large use of ethanol as an automotive fuel, as well as sugar and ethanol feedstock, an important item in the food market.

The use of ethanol as a feedstock potentially emits less greenhouse gases, since its production is made from sugarcane, which is renewable and widely exploited in various regions of the country. Methanol, in turn, has its origin in the petrochemical industry and is produced domestically only in the city of Rio de Janeiro, which is a negative fact for the logistics of the reagent to the producers.

The main supply input for biodiesel production is sodium methylate, which is used as a catalyst in the process.

The necessary utilities in this production are basically electricity and steam.

The National Program for Production and Use of Biodiesel was the major regulatory milestone for the compulsory and progressive use of the fuel in the country. However, as already mentioned, it was established by Law 11,097 in 2005, i.e., the growth of this market is still very recent. Therefore, it is still expected to be built a learning curve for the use of biodiesel in order to exploit natural resources in a better way to meet the same standards.

The Brazilian biodiesel market is fragmented. There are several producers with none having a market share larger than 16 %. Petrobras is the largest buyer but Shell, Exxon Mobil, Repsol, and Ipiranga are also important players. An important alternative to Brazilian producers could be export. However, the big international markets, like the European one, have technical restrictions to a pure soybean biodiesel. The main advantages of biodiesel production are ascribed to social and environmental issues. First, because it is labor intensive and can be produced from different types of raw materials and in several regions in the country. Second, it replaces a very important transportation fuel reducing local polutants like soot, carbon monoxide, and SOx. In addition, it significantly reduces greenhouse gas emissions (Amaral Mendes and Da Costa 2010).

The main risks are ascribed to new technology trends both in the case of different types of feedstocks and the so-called second and third generations, as microalgae, for instance. A good opportunity is to try to explore high valued coproducts in this chain.

16.4 Biodiesel Production Technology

As mentioned elsewhere, the price of raw materials has a strong influence on the final cost of production. In general, more than 80 % of the cost is based on this price (Shi and Bao 2008). In order to reduce this cost, efforts are based on cheaper feedstocks and process optimization.

16.4.1 Transesterification

Transesterification is a reaction between a vegetable oil and a short chain alcohol like methanol or ethanol to produce monoesters and glycerol. This is a reversible reaction and an excess of alcohol is used to shift the equilibria. Stoichiometrically, this reaction involves 3 mols of alcohol to each molecule of triglyceride, producing 3 mols of esters and 1 molecule of glicerol. Industrially, at least 6 mols of alcohol is used for each triglyceride molecule in order to obtain a more complete conversion (Fukuda et al. 2001).

From the most studied transesterification catalysts, Brønsted bases and acids are the main ones, with alcoxides and alkaline hydroxides the prefered ones (Suarez et al. 2007).

It is clear in the literature that basic catalysis have operating problems when high amounts of free fatty acids are found in vegetable oil. In this case, soap is produced reducing the yields with associated emulsions. Similar behavior occurs when moisture is in the reaction media. Hydrolysis of esters produce fatty acids, which react with the base catalyst leading to soap and emulsions (Ma and Hanna 1999).

16.4.2 Hydroesterification

Hydroesterification has been presented as a new alternative to biodiesel production. Several studies are conducted on kinetics, catalysts, multiple feedstocks, production costs, and hydroesterification plant instalation (Lima Leão 2007; Gonçalves 2007; Encarnação 2008; Gomes 2009; Leão 2009).

Hydroesterification means a first reaction of triglyceride hydrolysis producing fatty acids and glycerol. Secondly, an esterification reaction, where fatty acids plus methanol or ethanol produce biodiesel and water (Kuss 2012).

Hydrolysis reaction increases the feedstock acidity, thus a fatty acid remotion is not necessary. Thus, any fatty material (vegetable oil, animal fat, used fried oil, brown grease, etc.) can be used in this process with any acidity or moisture content. The ability to use those types of crude feedstocks is the main difference compared to regular transesterification, which always produces soap and reduces yields due to a difficult glicerol/biodiesel separation (Encarnação 2008).

Acid hydrolysis promotes a complete transformation of fatty materials in fatty acids which are converted into biodiesel in the second step. Glycerol does not suffer any contamination due to alcohol or biodiesel contact since it is removed during hydrolysis. Esterification produces biodiesel and water which can be reused in the hydrolysis step (Arceo 2012).

Based on the above mentioned, hydroesterification (hydrolysis plus esterification) is a promising alternative to conventional biodiesel production (Arceo 2012).

16.4.3 Transesterification and Hydroesterification Costs

A large transesterification biodiesel plant usually presents operating costs of about US$70/ton (electricity, steam, chemicals, and labor) (Encarnação et al. 2009). In the hydroesterification process with no homogeneous catalysts and no inorganic acids in the washing step, total operating cost is about US$35/ton. In a medium to large size biodiesel plant (100,000 mton/year), this process costs about US$3.5 million/year in operating costs.

Table 16.3 Transesterification versus hydroesterification comparison

(₵/L)	Transesterification	Hydrolysis + Esterification
Chemicals	4	1
Energy	1	2
Other operating costs	5	3

Source Cruz and Aranda (2011)

Currently, there are several feedstocks that can be used and transformed into an international standard biodiesel with high yields (about 98 %). The transesterification process cannot be applied efficiently to crude feedstocks. Few hydroesterification studies are found in the literature. Lima Leão (2007), who studied hydroesterification of soybean and castor oils obtained high conversions for castor fatty acids esterification (87.24 %) and soybean fatty acids (92.24 %), with niobium-based catalysts (20 %), temperature (200 °C). Chenard et al. (2009) studied the same process using jatropha oil obtaining conversions from 86.60 to 88.35 %.

As about 80 % of biodiesel operating cost is ascribed to feedstock price, hydroesterification allows a significantly better performance in the feasebility of a biodiesel project. In Table 16.3, a comparison between operating costs for hydroesterification and transesterification (in a 50,000 mton/year) is given.

In order to obtain important advances in this growing biofuel demand, new approaches are necessary. Currently, algae in biodiesel research is considered a new frontier in this sector presenting superior yield compared to other conventional plantation. Biodiesel expectations are huge because: (i) algae absorb CO_2; (ii) growing rate is fast; (iii) high lipid content; (iv) can be cultivated in pools, lakes, raceways, and photobioreactors; (v) high yield by acre; and (vi) not considered as a food item. Nevertheless, Brazil should seek an alternative to soybean with higher efficiency, not necessarily ascribed to edible oil prices (Amaral Mendes and Da Costa 2010).

16.5 Conclusions

The approaches in this chapter allow the understanding of the development strategies of biodiesel production and consumption in Brazil, based on technological, economical, social, and environment sustainability assumptions.

It is relevant to pay attention to the rapidly changing industrial capacity to produce biodiesel. Until September 2013, 63 units were authorized to produce this biofuel, with a total nominal capacity of approximately 8 billion L/year. Over 80 % of this capacity is ascribed to social seal companies that are involved with small farmers providing their feedstocks. From 2005 to September 2013, Brazil produced 13 billion L of biodiesel, reducing diesel imports of US$11 billion and contributing positively to the Brazilian Trade Balance.

Finally, it should be emphasized that biodiesel relevance in the Brazilian industry is influenced by a large amount of R&D funding throughout the production chain, ranging from the agricultural stage to the industrial production processes, including coproducts and storage. In this sense, the current tax model gives Brazilian biodiesel a unique feature in the world supported by a biofuel policy with social orientation.

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Chapter 17
Microalgal Feedstock for Bioenergy: Opportunities and Challenges

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Abstract The utilization of algal feedstock for bioenergy can be considered as one of the greatest challenges for biosystems engineering in the near future. Some species of microalgae show high potential for oil accumulation and further utilization of its biomass for biogas generation, pyrolysis, ethanol production, and even as fertilizer. Microalgae can utilize CO_2 as carbon source and can also be grown on nonagricultural environments, such as wastewater facilities, industrial effluents, freshwater, and marine water habitats. The vast research field on microalgae engineering is due to the facts that it can be a source of energy and act as an air and water pollutants removal. There have been considerable advances in engineering its growth, in bioreactor designs, and on lipid accumulation due to chemical, biochemical, and genetic studies. Despite that, there are still some fundamental processing aspects that are considered challenges, either economical, ecological, or technical, such as biomass harvesting and the competition with the higher value products produced from algae, as proteins.

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17.1 Introduction

The pursue of alternative sources for energy in the new century is due to the scarcity of fossil fuels in the near future, i.e., energy security reasons, and also the concern with the environment. According to the Intergovernmental Panel on Climate Change (IPCC), the accelerated production of carbon dioxide as a result of human activity is a major factor which contributes to the greenhouse effect (Houghton et al. 2001). The history of biofuels, which have been considered a green alternative for fossil fuels, has been changing much for the past 40 years. There were an ethanol boom with Brazil and the United States (Ribeiro and Younes-Ibrahim 2001; Goldemberg et al. 2004) and a huge interest in producing biodiesel from oleaginous plants in the last decades (Demirbas 2008; Pousa et al. 2007). Despite these being designed as green alternatives, recent studies imply that ethanol and biodiesel produced from plant feedstocks do not match several criteria for sustainability (Hoekman 2009). The large acreage of corn for ethanol production in the United States, for example, has raised concerns among specialists regarding pollution from pesticides and fertilizers, reduction of biodiversity, soil erosion, and a shift on the equilibrium on the food supply chain (Fargione et al. 2008, 2010; Hill et al. 2009).

An alternative showing promising results are known as second-generation biofuels, i.e., biofuels produced from lignocellulosic residues (Sun and Cheng 2002). These are still being developed and are based on the utilization of sugar monomers released from agro-residual biomass hydrolysis and on the production of biogas from biomass controlled combustion (Hendriks and Zeeman 2009). Despite these efforts are considerable and important for supplying clean energy to human society, microbes have been considered as one of the new potential sources of energy harvesting (Xia et al. 2011; Huang et al. 2009; Li et al. 2008; Millati et al. 2005; Illman et al. 2000; Ratledge and Wilkinson 1988). In the group of microbes, microalgae have earned much attention from the academic society for a vast number of reasons. Some of which are: the tendency of producing more biomass than terrestrial plants per unit of area, they can be produced in marginal lands, in fresh water, and in salt water ecosystems (Chisti 2007) and their non-competition with food systems, since they can be produced in areas where there is no agricultural productivity (Hill et al. 2006). Another characteristic of microalgae that can make its production more feasible and sustainable is its capacity to uptake human produced CO_2 (Benemann and Oswald 1996) as well as removing certain water pollutants (Powell et al. 2008; Munoz and Guieysse 2006).

The interest in microalgae is not something new though from 1978 to 1996, the U.S. Department of Energy funded a program to develop renewable transportation fuels from algae (Sheehan et al. 1998). Their goal was the production of biodiesel from high lipid content algae utilizing waste CO_2 from coal plants, and throughout these almost 20 years of research, there was a considerable advance in metabolism manipulation and bioprocessing engineering for algae growth. In the recent years, there was a development in the field of investigation on genetic modification for

enhancing lipid production (Rosenberg et al. 2008; Radakovits et al. 2010), as well as the studies on biochemical engineering regarding optimization of growth. Factors such as reactor configuration (Vergara-Fernández et al. 2008; Wu and Merchuk 2002, 2004), nutrient loads (Fabregas et al. 2000; Heredia-Arroyo et al. 2010; 2011), light fluxes, and others are some present in the literature.

Another key aspect regarding algae for bioenergy is the utilization of its dry biomass for biogas generation (Vergara-Fernández et al. 2008; Bohutskyi and Bouwer 2013; Mussgnug et al. 2010), for production of other fuels and even as feedstock for char as potential fertilizer (Johnson et al. 2013). Therefore, it can be seen that the trend of microalgae research nowadays is mainly focused on the conversion of algal biomass to fuels and the engineering toward optimization of cultivation methods and oil and lipid enhancing.

The purpose of this chapter is to present some technologies available in the field of growing, harvesting, and utilizing microalgae biomass, the chemical and biochemical nature of microalgae biomass, and the basic concepts of biodiesel, biogas, biohydrogen, bioethanol, and other fuels production from microalgae biomass and lipids. Alongside the technologies, the current challenges and some opportunities are presented.

17.2 Cultivation of Microalgae

As well as any other microorganism, microalgae grow in environments with its basic nutritional needs. In lab scale, there have been studies on formulating specific medias for their growth since the nineteenth century (Lourenço 2006). Basically, all the culture media for microalgae cultivation should be composed of basic macronutrients (C, H, O, N, P, S, K, Mg, Si, and Fe) and micronutrients (Mn, Mo, Co, B, V, Zn, Cu, Se, Br, and I) (Lobban 1994), as well as light and water. Grobbelaar (2004) presented the following ratio for some nutrients: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. There are several classifications, from which two are more useful in this chapter: into marine species, which have affinity for high concentration of salt, and freshwater species (Bilanovic et al. 2009); and the division into autotrophic, heterotrophic, and mixotrophic species, noticing that some species can grow under two or three of these regimes (Heredia-Arroyo et al. 2010).

Light administration is a key factor in both indoor and outdoor systems, affecting especially those microalgae that grow on photoautotrophic regime. For outdoor systems the most common light source is sunlight while in indoor cultivation, artificial light sources are required. Chen et al. (2011) summarized several artificial light sources, from the conventional one (with a high electricity consumption) to more engineered options, such as LED, Optical fiber excited by metal-halide lamp (OF-MH), Optical fiber excited by solar energy (OF-solar), and an option with zero electricity consumption and high operation stability, which is the LED/OF-solar combined with wind power/solar panel.

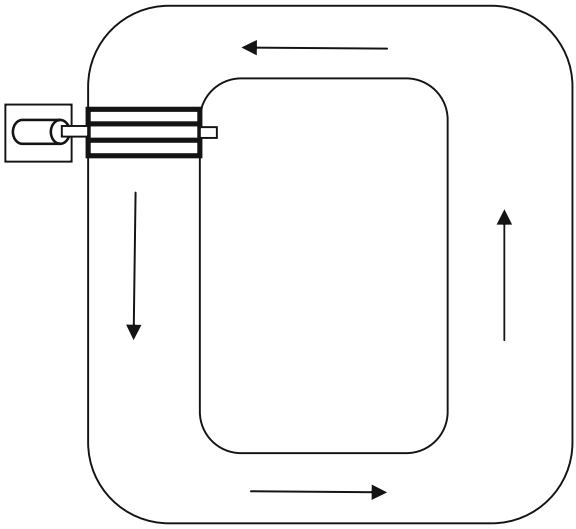

Fig. 17.1 Scheme of a Raceway pond

Producing microalgal biomass nowadays is generally more expensive than crops, although the culture media are inexpensive (Acién Fernández et al. 1999). There should also be a temperature control within 20–30 °C in most cases (Chisti 2007).

17.2.1 Large Scale Production of Photoautotrophic Microalgae

According to Chisti (2007), the only practicable ways to produce microalgae in large scale are in raceway ponds and in photobioreactors.

A raceway pond is a simple and continuous system of microalgae growth. It is consisted on a closed loop recirculation channel, with an average depth of 0.3 m, built generally with PVC, concrete, or compacted earth covered with a plastic film (Chisti 2007; Terry and Raymond 1985). The continuous flow is guided around bends and it is mixed and circulated by a paddlewheel, used also to prevent sedimentation. After its retention growth, culture broth is harvested on the completion of the circulation loop. A simple scheme of a raceway pond can be seen in Fig. 17.1.

There are also circular ponds and unstirred ponds. The main advantage of a raceway pond, when compared to a photobioreactor, is its price, having operational and building costs lower than the other does.

Photobioreactors (PBRs) are closed systems, which have the differential characteristic of enhancing light availability for each microalgae cell in the reactor (Suali and Sarbatly 2012). These are generally built with transparent materials,

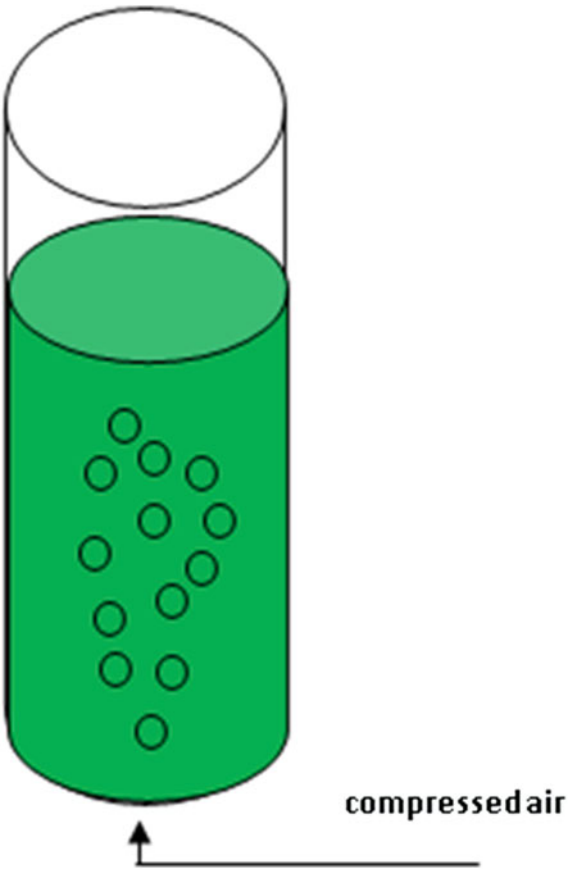

Fig. 17.2 Scheme of a cylinder photobioreactor

allowing light penetration into the culture. These are usually more expensive than raceway ponds, however, they show higher productivity (Lourenço 2006). In the literature, three types of geometry are most cited: the flat-plate, the cylinder, and the tubular.

Tubular and PBRs are constructed with transparent glass or plastic, and these sort of PBRs have been gaining attention from the academic community in the past decades. Geometrically, they can be horizontal, vertical, conical, and even inclined. Mixing can be done either by air lift or by air pumps (Chen et al. 2010). A simple scheme of a tubular PBR can be seen in Fig. 17.2.

The flat-plate bioreactors consist of airlift driven columns or rectangular tanks with a recirculation loop. In this kind of reactor, illumination is provided by an external light source or a bank of lights (Silva et al. 1987).

17.2.2 Cultivation on Heterotrophic Conditions

The so-called heterotrophic cultivation is that one utilizes organic carbon under dark conditions, as purpose for carbon sources and energy. There are a large number of organic substrates that microalgae can assimilate, such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose, which can be derived from residue biomass, such as corn stover, wheat straw, and sugarcane bagasse hydrolysates (Chojnacka 2004).

Heredia-Arroyo et al. (2010) confirmed that heterotrophic growth could result in a higher biomass concentration, when compared to autotrophic conditions for *Chlorella protothecoides* under the conditions they studied; the oil content was similar between these two conditions though. A drawback of using heterotrophic cultivation is the possibility of bacterial contamination.

17.2.3 Cultivation of Mixotrophic and Photoheterotrophic Microalgae

Mixotrophic cultivation is the one when microalgae undergo photosynthesis and use both organic compounds and CO_2 as carbon source (Chen et al. 2011). Cultivating a microorganism under mixotrophic conditions means that this organism would be able to grow under phototrophic, heterotrophic, or both conditions.

There is also a cultivation condition known as photoheterotrophic, also known as photoorganitrophic, photoassimilation, and photometabolism. In this sort of metabolism, light is a requirement to utilize organic compounds as carbon source.

Utilizing mixotrophic and photoheterotrophic conditions to grow microalgae, with a supply of organic compounds from waste resources with zero or even negative carbon and economic costs may be a good opportunity. Some mixotrophic conditions may enhance lipid accumulation, achieving yields significantly higher than those in solely autotrophic conditions (Heredia-Arroyo et al. 2011).

A brief comparison of the characteristics of different cultivation conditions is shown (Chen et al. 2011) in Table 17.1.

17.2.4 Biomass Harvesting Techniques

The recovery of algal biomass consists on a solid–liquid separation process and may account with costs up to 30 % of the total costs in producing microalgal derived fuels (Gudin and Thepenier 1986). The traditional biomass harvesting processes are: flocculation, filtration, flotation, and centrifugal sedimentation. The most appropriate harvesting process depends on a series of factors, such as culture density, size, value of the products, and available technology (Brennan and Owende 2010).

Table 17.1 Comparison among cultivation conditions

Cultivation condition	Energy source	Carbon source	Achieved cell density	Reactor scale-up	Cost	References
Phototrophic	Light	Inorganic	Low	Open pond or PBR	Low	Chen et al. (2011)
Heterotrophic	Organic	Organic	High	Conventional fermentor	Medium	Chen et al. (2011)
Mixotrophic	Light and organic	Inorganic and organic	Medium	PBR	High	Chen et al. (2011)
Photoheterotrophic	Light	Organic	Medium	PBR	High	Chen et al. (2011)

Cell flocculation is a process in which cells are aggregated together, in order to form larger particles for settling, i.e., decanting. There are some process well established, such as chemical autoflocculation (precipitation of carbonate salts with algal cells at an alkaline pH) (Chen et al. 2011), chemical coagulation (with organic and inorganic coagulants) (Grima et al. 1994), and even combined flocculation techniques.

Filtration is known as the unit operation of separating solid particles from a suspension using a screen with a particular pore size, accumulating solids at one side, and decreasing biomass concentration on the other (Grima et al. 1994). This technique shows several advantages toward other methods, such as simplicity in cost and operation, and with some adaptations, it can achieve recovery rates of up to 89 % (Petrusevski et al. 1995). There are some operation limitations, e.g., the reduction of effectiveness when biomass is too concentrated and clogging issues (Chen et al. 2011).

Flotation is based on gravity separation in which air or gas bubbles attach to solid particles and then carry them to the liquid surface (Chen et al. 2011). Flotation can be divided in two major categories: Dissolved air flotation (DAF) and Dispersed air flotation. The main difference between these two is the bubble size; while in the first, the air bubbles are within the diameter range of 10–100 μm, the second one is based on bubbles of 700–1500 μm formed by a high speed agitator (Rubio et al. 2002).

Centrifugation is a traditional harvesting method, even though its operational costs can be high enough to make it unfeasible. It is a quick and effective method, achieving recovery rates of up to 90 % within 2–5 min (Chen et al. 2011). Depending on the situation, it may not be adequate, since high shear forces could damage cell wall.

17.2.5 Oil Extraction Techniques

Oil extraction from microalgal biomass is usually an energy demanding process, due to, among other factors, the requirement of biomass dewatering. The costs of harvesting and dewatering can achieve costs up to 30 % of the total algal biodiesel

Table 17.2 Comparison among biomass drying processes

Method	Advantages	Disadvantages	References
Drum-drying	Fast, efficient	High costs	Chen et al. (2010); Becker (1994)
Spray-drying	Fast, efficient	High costs	Chen et al. (2010); Becker (1994)
Sun-drying	Cheap	Slow, weather dependent	Chen et al. (2010); Becker (1994)
Solar-drying	Cheap	Weather dependent	Chen et al. (2010); Becker (1994)
Cross-flow-drying	Moderate rate and costs	Electricity costs	Chen et al. (2010); Becker (1994)
Vacuum-shelf-drying	Gentle process	High costs	Chen et al. (2010); Becker (1994)
Freeze-drying	Gentle process	Slow, high costs	Chen et al. (2010); Becker (1994)

production (Tampier et al. 2009). The most economically viable way would be natural drying, with solar and wind energy, however this would be a weather dependent process, which could make oil production seasonal. There are a few common drying methods, as well presented by Chen et al. (2010) and Becker (1994) in Table 17.2.

A widely used method for extracting oil from microalgal biomass is using organic solvents. It is a common pathway for extracting oil from oleaginous plants and it has been used for microalgae in most cited cases as well (Grima et al. 1994). The ideal organic solvent should have some characteristics: it has to match the lipid polarity in the cells, it should be cheap, easy to remove, present low to zero toxicity, insoluble in water, recyclable, and efficient in dissolving some targeted components (Chen et al. 2010).

Even though chloroform has high risks of toxicity and flammability, it is a very common solvent used in lipid extraction. Chloroform is able to extract hydrocarbons, carotenoids, chlorophylls (source of the green color of algal oil), sterols, triacylglycerols, wax esters, fatty alcohols, aldehydes, and free fatty acids (Chen et al. 2010). A traditional method which combines chloroform with methanol at a 2:1 v/v ratio is one of the most used in the published studies (Folch et al. 1957). Attempts in utilizing other solvents, such as ethanol, 1-butanol, hexane, isopropanol, and hexane have also been studied (Grima et al. 1994; Medina et al. 1998; Cartens et al. 1996; Nagle and Lemke 1990).

In order to optimize solvent extraction, some mechanical and physic-chemical approaches have been studied to disrupt microalgal cell wall. These include: autoclaving, microwave, sonication, bead-beating, osmotic shock, and cell grinding, i.e., "blending," freeze-press and enzymatic and chemical lysis. Such methods will not be discussed in this chapter, but they are very well presented by Chisti and Moo-Young (1986).

Two new techniques in extracting lipids are the supercritical fluid extraction (SFE) and the subcritical water extraction (SWE). The idea behind SFE is the utilization of supercritical thermodynamic properties, such as better diffusivity and decreased viscosity, which improve diffusion rates through solid materials, aiming higher extraction efficiencies (Anklam et al. 1998). On the other hand, the principle of SWE is the utilization of water under subcritical conditions, which decreases its polarity, improving solubility of nonpolar organic compounds (Chen et al. 2010).

17.3 Microalgal Biomass Utilization

The composition of microalgal biomass varies according to several factors: according to the species, to the growth conditions, temperature, Carbon to Nitrogen ratio, and others (Volkman et al. 1989). This section will describe some technologies used in harvesting bioenergy from microalgal biomass.

There are a few most studied species used in the bioenergy field, such as *Chlorella* sp. (Illman et al. 2000; Heredia-Arroyo et al. 2011; Wang et al. 2010; Liang et al. 2009), *Dunaliella* sp. (Tang et al. 2011; Shuping et al. 2010; Zou et al. 2009; Minowa et al. 1995), *Nannochloris* sp. (Takagi et al. 2000; Demirbas and Fatih Demirbas 2011; Hsieh and Wu 2009), *Parietochloris incisa* (Bigogno et al. 2002), and a few others. Among these, *Nannochloris* and *Dunaliella* are marine microalgae and some *Chlorella* sp. are as well.

17.3.1 Biodiesel from Microalgal Oils

One of the key points in investing technology to achieve a sustainable biodiesel production pathway from nonedible microalgal oil is its high productivity, achieving numbers as high as 5000–100,000 L ha^{-1} a^{-1} (Levine et al. 2010).

As well described by Qiul et al. (2011), production of biodiesel from microalgae consists of a series of steps: lipid extraction, removal of solvent, catalyzed transesterification, and purification. There are also a few other processes, such as hydrolysis followed by esterification (also known as hydroesterification), in situ transesterification, and supercritical transesterification.

The well-known reaction of lipids transesterification consists of a reaction of a fatty ester with an alcohol, in order to form fatty acid alkyl esters and glycerol (Ma and Hanna 1999). This reaction under normal conditions of pressure and moderate temperatures and reaction times usually requires a catalyst, which can be alkaline, acid, or enzymatic (Meng et al. 2009).

Microalgal oil has some characteristics that are not desirable in the biodiesel production: it usually has a high free fatty acid value (Miao and Wu 2006) and it also contains a high degree of polyunsaturated fatty acids (PUFA) when compared to vegetable oils (Chisti 2007). A major implication on having a high free fatty

acid value is making the alkali catalysis, which is the cheapest one, unviable (Lotero et al. 2005). Having a high degree of PUFA makes it more susceptible to oxidation, thus limiting conditions of storage (Chisti 2007).

There are a few publications about the so-called in situ transesterification. This process consists of simultaneously extracting and transesterifing the lipids to produce biodiesel. The biomass has to be dewatered, since water can act as an inhibitor in this process (Chen et al. 2010). This can be a promising strategy, since costs are lowered due to the removal of a step in the whole production.

Due to the fact of having high free fatty acid indexes, the alternative of producing biodiesel from microalgal oils through hydroesterification has also been considered. Hydroesterification is consisted of a hydrolysis followed by an esterification (Diaz et al. 2013; Reyes et al. 2012). Very little has been done yet utilizing microalgal lipids, even though it may be a good alternative for research. Reyes et al. (2012) utilized an autoclave reactor at 250 °C for the hydrolysis reaction and niobium powder for the esterification, achieving conversion rates, i.e., formation of methyl esters up to 91.7 %.

There has been a trend in this field of study using supercritical conditions. Patil et al. (2011) studied the optimization of a single-step supercritical process for simultaneous process for simultaneous extraction and transesterification of wet algal biomass, using methanol as alcohol. They present some advantages of using supercritical conditions, such as that they use modest temperatures, the high rate of production, and the final product price, which, according to the authors, is even lower than the biodiesel produced from traditional transesterification.

Up-scale processes have been studied as well. Li et al. (2007) presented the results of utilizing bioreactors with up to 11,000 L at a biodiesel production rate of 6.24 g L^{-1} and conversions up to 98.15 %. They used immobilized lipase from *Candidia* sp. and the microalgal species was *C. protothecoides.*

Biodiesel from microalgal oils has some advantages when compared to petroleum diesel: it can be a totally renewable and biodegradable fuel, a low carbon footprint, it has low levels of toxicity, and it contains reduced levels of particulates, carbon monoxide, hydrocarbons, and SO_x (Brennan and Owende 2010). Another key point that may lead biodiesel from microalgal oils to a commercial process is its low freezing point and its high energy densities, making it an interesting alternative for the aviation industry (Chisti 2010).

17.3.2 Biogas from Microalgal Biomass

In order to make microalgae a more sustainable source for bioenergy, there must be a use for its residual biomass, i.e., the biomass in which higher value products were removed, such as lipids and proteins. The high productivities of microalgae may release high amount of nitrogen and phosphate into the environment, which would shift the bioenergy harvesting from microalgae toward an unsustainable position. A process that could solve this issue is the anaerobic digestion, converting biomass to

biogas, recovering more usable energy from cell walls. Anaerobic digestion is the conversion of organic wastes into biogas, which consists of methane and CO_2, with traces of other compounds, such as H_2S (Bridgwater 2008).

Theoretically, there is more energy to be harvested through anaerobic digestion, producing a mixture rich in methane, than from lipid extraction (Sialve et al. 2009). So far, it is still a research field with very little work done and published. Only small-scale experiments have been reported achieving efficiencies in the range of 20–80 % (Zamalloa et al. 2011).

Sialve et al. (2009) identified a few challenges in digesting microalgae: the biodegrability can be low depending on the biochemical composition and on the nature of the cell wal,; which may result in ammonia release leading to toxicity in cases with high cellular protein content and in inhibition of the process by sodium, when marine species are considered.

There are a few key points that make microalgal biomass an interesting opportunity for investing in anaerobic digestion. Besides carbon, nitrogen, and phosphorus, there are nutrients in lower concentration, such as iron, cobalt, and zinc, which are able to stimulate methanogenesis (Speece 1996). The theoretical methane production increases with higher lipid content, since lipids are energy condensed structures (Angelidaki and Sanders 2004).

Sialve et al. (2009) calculated the methane potential and ammonia release from anaerobic digestion of several different species of microalgae using data from (Becker 2004). The results of these researchers are presented in Table 17.3.

The quantity and the quality of biogas generated are dependent upon the biomass composition, pH, temperature, solid retention time, hydraulic retention time, and loading rate (Singh and Olsen 2011).

17.3.3 Ethanol from Microalgal Biomass

There are three possible pathways for producing ethanol from microalgae. Algae can assimilate considerable amounts of starch and cellulose, which can be convertible to fermentable sugars. These can be fermented to produce ethanol using a yeast strain, for example. Some species can also produce ethanol during the dark fermentation methabolic pathway; the third possible process is to generate genetic engineering microalgae to produce ethanol directly (John et al. 2011).

Starch is stored in microalgal cells and can be extracted from biomass at regular intervals from photobioreactors or open ponds through mechanical processes or by dissolution of cell walls through enzymatic reactions. This starch goes through solvent extraction and then used for microbial fermentation (John et al. 2011). Once again, the biomass composition is a key point to achieve high yield on this sort of fuel. It has been reported that *C. vulgaris* is a good source for ethanol fermentation, due to the high starch content, of around 37 % dry weight, achieving conversion efficiencies of up to 65 % (Hirano et al. 1997). Following well-known procedures, Harun et al. (2010) investigated the feasibility of producing ethanol

Table 17.3 Biomass composition of several different species of microalgae with CH_4 and $N–NH_3$ productivity (VS = Volatile solids)

Species	Protein (%)	Lipid (%)	Carbohydrate (%)	CH_4 (L g^{-1} VS)	$N–NH_3$ (mg g^{-1} VS)	References
Euglena gracilis	39–61	14–20	14–18	0.53–0.8	54.3–84.9	Sialve et al. (2009)
Chlamydomonas Reinhardtii	48	21	17	0.69	44.7	Sialve et al. (2009)
Chlorella pyrenoidosa	57	2	26	0.8	53.1	Sialve et al. (2009)
Chlorella vulgaris	51–58	14–22	12–17	0.63–0.79	47.5–54.0	Sialve et al. (2009)
Dunaliella salina	57	6	32	0.68	53.1	Sialve et al. (2009)
Spirulina maxima	60–71	6–7	13–16	0.63–0.74	55.9–66.1	Sialve et al. (2009)
Spirulina platensis	46–63	4–9	8–14	0.47–0.69	42.8–58.7	Sialve et al. (2009)
Scenedesmus obliquus	50–56	12–14	10–17	0.59–0.69	46.6–42.2	Sialve et al. (2009)

from *Chlorococum* sp. biomass and achieved yields as high as 3.8 g/L using a 10 g/L substrate solution, through fermentation by *Saccharomyces bayanus*.

Besides starch, microalgae can also accumulate cellulose in their cell walls, as a structural polysaccharide. This is a common characteristic among green algae (John et al. 2011). Cellulose can be hydrolyzed into its monomers, i.e., glucose monosugars, and further fermented to ethanol. A huge advantage when comparing biomass residues from algae when to plant materials is the inexistence of lignin in algae, therefore, reducing energy costs and making ethanol production from cellulose a more feasible process.

A second possible pathway is through the metabolic pathway called dark fermentation. In absence of light and in presence of oxygen, microalgae usually maintain their life by consuming starch or glycogen; however, if oxygen is also not available, the oxidative reaction of starch is incomplete, and several other products are formed, such as hydrogen gas, carbon dioxide, ethanol, lactic acid, formic acid, etc. (John et al. 2011). A patented process is based on this sort of fermentation (Ueda et al. 1996), in which microalgal cells contained a large amount of polysaccharides, which were catabolized under dark and anaerobic conditions to ethanol. This process does not apply to all species of microalgae, but according to (Ueda et al. 1996), classes *Chlorophyceae*, *Prasinophyceae*, *Cryptophyceae*, and *Cyanophyceae* are the ones able to be induced to produce ethanol.

As well explained by John et al. (2011), the algal photosynthesis is based on Calvin cycle in which ribulose-1,5-bisphosphate (RuBO) combines with CO_2 to produce two 3-phosphoglyceric acid (3-PGA), which is used to produce glucose and other several metabolites. There is a current attempt trying to redirect the 3-PGA produced to ethanol transformation. This is mainly done by introducing ethanol producing genes, such as pyruvate decarboxylase and alcohol dehydrogenase (John et al. 2011). Deng and Coleman (1999) published a work using modified cyanobacterium (*Synechococcus* sp.) in order to utilize light, CO_2 and inorganic nutrients to produce ethanol and have it diffused from the cell into the culture medium.

Ethanol producing from microalgae is, thus, a challenge for biotech companies. There are a few bottlenecks in these three processes; such as the high cost of starch/cellulose depolymerizing enzymes for pretreatment of algal biomass and the competition with higher value fuels.

17.3.4 Biohydrogen from Microalgal Biomass

Microalgae generally have the necessary genetic, metabolic, and enzymatic characteristics to photoproduce H_2 gas. There are two possible pathways for hydrogen production under anaerobic conditions from eukaryotic microalgae: either as an electron donor in the process of fixating CO_2 or evolved in both light and dark (Ghirardi et al. 2000; Melis and Happe 2001).

During the process of photosynthesis, microalgae convert water into H^+ and oxygen. H^+ can be subsequently converted to H_2 through hydrogenase catalyzed reactions under anaerobic conditions (Melis and Happe 2001; Cantrell et al. 2008). The key for producing hydrogen in this situation is the utilization of anaerobic environments, since oxygen is a key inhibitor to hydrogenases (Akkerman et al. 2002). This reaction is reversible, therefore, hydrogen is either produced or consumed by the conversion of protons into hydrogen gas.

Brennan and Owende (2010) cited two fundamental approaches for photosynthetic H_2 production from water. The first one is a two-stage photosynthesis process in which photosynthetic oxygen production and generation of hydrogen gas are spatially separated. The first stage of this process consists of microalgae growing in normal conditions; the second one consists of privation of sulfur, which induces anaerobic conditions, stimulating hydrogen production (Melis and Happe 2001). This is a time-limited process, and hydrogen yields achieve a maximum after 60 h of production.

The second approach consists of simultaneously producing oxygen and hydrogen gases. In this process, the hydrogenase reaction is fed directly with electrons that are released upon oxidation of water (Ghirardi et al. 2000). There is a considerable higher productivity in this process, when compared to the first, however, hydrogenases are inhibited after a short time by the oxygen produced. Melis and Happe (2001) calculated the theoretical maximum yield of hydrogen using the two-step process and found numbers as high as 198 kg H_2 ha^{-1} day^{-1}.

There is little research yet on biohydrogen production from microalgal derived routes. The main challenge is to achieve high yields, in order to make this process feasible, since the theoretical photochemical efficiency of the photoheterotrophic process is low, of around 10 %. Even achieving high yields, with very high light intensities, still a large surface would be needed to reach a reasonable hydrogen production (Melis and Happe 2001). A summary provided by Melis and Happe (2001) is shown, comparing efficiencies in Photossynthetically active radiation (PAR) and H_2 production, in Table 17.4.

17.3.5 Pyrolysis of Microalgal Biomass

Microalgal biomass can be converted to bio-oil, syngas, and charcoal through pyrolysis. Pyrolysis processes happen at medium range temperatures (350–700 °C) in the absence of air (Goyal et al. 2008). There are a few operating modes of pyrolysis, as well described by Bridgwater (2012), which are called: Flash, Fast, and Slow pyrolysis.

Flash pyrolysis works at moderate temperatures of 500 °C, it has a short hot vapor residence time, of about 1 s, and is deemed to be a viable technique for future replacement of fossil fuels with biomass derived liquid fuels, since there is a high biomass-to-liquid conversion ratio. This ratio achieves numbers as high as 95.5 % (Clark and Deswarte 2011; Demirbaş 2006). Fast pyrolysis also works at

Table 17.4 Energy conversion efficiencies of green algae for H_2 production

Species	Absorbed light ($\mu W/cm^2$)	H_2 (nmol h^{-1})	Efficiency (PAR) %	References
C. reinhardtii (sup)	2.2	44–61	13–18	Melis and Happe 2001
C. reinhardtii (UTEX 90)	8.4	78–104	6–8	Melis and Happe 2001
Chlorella moewusii	9.1	253–337	18–24	Melis and Happe 2001

The results are based on several different light periods and with a heating value of H_2 of 0.23 MJ/mol

the vapor residence time, of around 10–20 s. Slow pyrolysis, on the other hand, is processed at lower temperatures, of around 400 °C and has very long solids residence time. The liquid percentage in these three processes are, respectively, 75, 50, 30 %; the char is known to be around 2, 20, and 35 % and the gas percentage of 13, 30, and 35 % for flash, fast, and slow pyrolysis, respectively. These numbers are based on Bridgwater (2012).

Bio-oil from microalgal biomass has higher quality than the one extracted from lignocellulosic materials (Demirbaş 2006), making it a promising area of studies. Bio-oils have been preferred over the other products of pyrolysis because they have the potential for being upgraded to liquid transportation fuels (Chen et al. 2010).

Pyrolysis of microalgal biomass converts lipids, starch, protein, and cellulose into bio-oil, combustible gas, and charcoal (Chen et al. 2010; Ginzburg 1993). It is interesting to note that the products from heterotrophic and from autotrophic grown microalgae can be very different; these effects are believed to be due to different methabolic pathways during their growth (Miao and Wu 2004).

Some current challenges in making pyrolysis from microalgal biomass feasible, presented by Chen et al. (2010), are: the dewatering process prior to the pyrolysis itself which is a very high energy requiring step, and the fractioning of the resulting bio-oil. Bio-oil can achieve high levels of component complexity and acidity as well. There is a field of studies in testing different conditions and catalysts to improve bio-oil quality (Wan et al. 2009).

A new approach in microalgal biomass pyrolysis is the utilization of microwaves. This technology, developed at the University of Minnesota, provides a few important advantages toward the conventional processes (Du et al. 2011), such as easier to control heating, fewer requirements on the feedstock grinding, cleaner conversion products, produced syngas with a higher heating value, and low cost.

17.3.6 Other Energy Products from Microalgal Biomass

Algal biomass can also be converted to a combustible gas mixture called "synthesis gas," or simply syngas. These reactions consist of partial oxidation of biomass in the range of temperatures from 700 to 1100 °C (Chen et al. 2010). As

well as pyrolysis, gasification products vary according to the temperature, moisture content, and other factors. The major applications of syngas are based as source of thermal energy in gas engines or gas turbines and feedstock for catalytic reforming and fermentation, in order to produce other chemicals.

Gasification of microalgal biomass has had little interest over the past years, thus making a broad field of study available for researchers and research groups with available technology to work on this. Demirbas (2009) aimed at producing H_2 from microalgal biomass, having CO_2, CO, and CH_4 as byproducts through a gasification process. Although there are some companies working on syngas production, such as Ensyn Corp and Plascoenergy Group, both Canadian industries, few research has been developed using microalgal biomass. Gasification and catalytic reforming of residue biomass might be an answer for achieving higher sustainability index from microalgal derived fuels. A new approach is to include algal charcoal, after biomass utilization, as a new product in the portfolio of algal products (Johnson et al. 2013).

There is also the energetic route known as thermochemical liquefaction, which aims to produce liquid fuel from wet algal biomass (Patil et al. 2008). The so-called bio-oil derived from liquefaction is produced at a range of low temperatures, usually from 300 to 350 °C, at high pressures, from 5 to 20 MPa, with the presence of a catalyst and in the presence of hydrogen. The mechanism of bio-oil production through this process is the high water activity in subcritical thermodynamic conditions in order to decompose, i.e., break down biomass to smaller molecules with a higher energy density (Patil et al. 2008). Dote et al. (1994) produced bio-oil with a heating value of 45.9 MJ kg^{-1}, with an yield of 64 % at dry weight of biomass and a positive energy balance of 6.67:1 (output/input). These numbers, especially the last one, may make thermochemical liquefaction of algal biomass a promising alternative for further energetic studies.

Hydrothermal processes have several technical and engineering challenges, such as controlling the ideal heating rate, the residence time, and up-scaling processes. Most studied processes are still in batch conditions, which may show a wide range of temperatures and a long time to have the reactor cooled down before analysis of products. Continuous processes would theoretically show better results, since these problems are minimized (Chen et al. 2010). Therefore, some of the engineering challenges would be improving heating rate, making a homogeneous flow (in order to prevent clogging) and higher-pressure pumps.

A relatively less promising option, which could be used as one of the last steps in the algal biomass lifecycle, is the direct combustion of biomass. Combustion is the oxidation reaction at high rates in presence of air and high temperatures, of 800 °C or more, at furnaces, boilers, or steam turbines. Not only algal, but any biomass should have a maximum moisture content of 50 % (McKendry 2002). A drawback for growing algal biomass solely for combustion is the need for drying, chopping, and grinding, which raises costs, producing a relatively cheap product. Therefore, it may not be feasible to burn directly biomass without extracting higher value products, such as lipids and proteins.

17.4 Challenges in Optimizing Sustainability

Prior to any discussions regarding biomass purification and extraction of products, it is important to discuss what would be the best way to cultivate microalgae. As previously shown in this chapter, the utilization of photobioreactors may yield higher productivity yields; on the other hand, it shows higher costs than other cultivation methods. The cheapest way to produce microalgae in large scale would be utilizing lagoons, lakes, or open ponds. In these, however, without any light administration, there would be a daily loss of biomass of around 25 % due to overnight respiration (Ratledge and Cohen 2008), not even mentioning the possibility of contamination from protozoa, bacteria, other algae, and fungi. Yields in lagoon systems would require up to 2 months for the culture to reach an optimum biomass density, in order to harvest.

As previously described, collecting and concentration algal biomass are cost intensive processes and have been subject of study in order to enhance sustainability from algal biofuels. A study from the 1960s was made comparing several harvesting techniques, including filtration, flocculation, precipitation, ion exchange, and ultrasonic vibration (Golueke and Oswald 1965). The authors concluded that only centrifugation and chemical flocculation were economically viable at the time. With environmental concerns nowadays, chemical flocculation may have some drawbacks, since the usage of chemicals in these processes is not incentivized; centrifugation may not be the most feasible option as well, because it is an energy intensive process and may damage cell wall, which can represent yield loss in some extraction steps. Alternatives, such as the usage of low energy ultrasound waves, are gaining room in microalgae production. These sort of waves allow cell to aggregate and settle down once the ultrasonic field is turned off. The main disadvantage, common among some new techniques, is the high power consumption and low concentration factors (Bosma et al. 2003).

Up to the date, it is proven that oil extraction from microalgae is an expensive and difficult process. There is no well-defined and ready to scale-up method available on the market and most of extractions face challenges with chemical waste and/or high costs of operation. There are some new technologies, such as nano-dispersion (promotes dispersion of nano-sized particles), electroporation, and the usage of co-solvent systems (Chen et al. 2010). Once again, engineering challenges are faced in the oil extraction step, and whichever method makes itself more sustainable and economically feasible will definitely attain market interest. Dewatering is also another key issue previously discussed. Thus, an extensive engineering work must be done in order to make oil production feasible and reduce the minimum cost of today production of US$5600–7000/ton (Ratledge and Cohen 2008).

Although with all the required processing steps in order to achieve the oil extraction step, its characteristics may limit biodiesel production. First of all, even though some microalgae species are able to accumulate up to 70 wt% of lipids

(*Botryococcus braunii*) (Chen et al. 2010) under starvation of N, P, and Si, the overall yield may not be high enough to make it economically feasible. The development of genomic engineering to map all the pathways in the algal cell, especially using *Chlamydomonas reinharttii*, has been done in order to fully understand lipids production and, obviously, address its optimization afterwards.

Algal oil characteristics are also challenges toward commercialization of fungal biodiesel, for example. The high free fatty acid value and the presence of unsaturated bonds are two drawbacks in the biodiesel industry, since series of pretreatments, higher costs with catalysts (since the cheapest and most traditional in biodiesel plants, NaOH and KOH, cannot be used), and lack of oxidation stability are faced.

In the 1960s, Japan started to produce *Chlorella* as a food additive, and since then, the potential of using microalgae in the food industry has grown enormously. Today, the most used species in human nutrition are primarily from *Chlorella*, *Spirulina*, and *Dunaliella* classes (Brennan and Owende 2010). The high content of beta-carotene in *D. salina* (up to 14 %) (Moore 2008) and the usage of *Chlorella* sp. in the pharmaceutical industry make the biofuels industry less advantageous, comparing economically values. Going further, microalgae can also be source of high value PUFA, such as docosahexaenoic acid (*Crypthecodinium* and *Schizochytrium* spp.), eicosapentanenoic acid (*Nannochloropsis*, *Phaedactylum*, *Nitzchia*, and *Pavlova* spp.), γ-linolenic acid (*Spirulina* sp.), and arachidonic acid (*Porphyridium* sp.) (Spolaore et al. 2006). In addition, microalgae can be source for pigments, aquaculture feed, high value fertilizer, and biochemical isotope chemicals, that have higher value than biofuels (Spolaore et al. 2006). The challenge of facing these industries could be deviated using a combined platform, producing these higher value products and also further processing algal biomass, producing biodiesel, methane, bio-oil, etc.

17.5 Opportunities and Other Applications of Microalgae

17.5.1 Growth in Municipal Leachate

There are a few publications about the usage of microalgae toward landfill leachate purification. They could utilize organic compounds present in there as carbon and nitrogen sources (Lin et al. 2007; Cheung et al. 1993). A recent project at the University of São Paulo, Brazil, aims at the utilization of leachate as culture media for *Chlorella* sp. growth. The current stage of this project is the chemical, physical, and nutritional factor screening toward cell growth.

Table 17.5 Reported values of heavy metals uptake by *C. vulgaris*

Metal	mg g^{-1} (metal/biomass)	References
Au	25.02	Ting et al. (1995)
Cd	12.48	Sandau et al. (1996)
Cu	190.62	Mehta and Gaur (2001)
Ni	205.48	Mehta and Gaur (2001)
Pb	17.2	Sandau et al. (1996)
Zn	6.6	Sandau et al. (1996)

17.5.2 Cocultivation with Pelletized Fungus

A recent study at the University of Minnesota (Hu et al. 2013) is based on a novel approach of utilizing microalgae and pelletized fungus for a series of advantages. The coculture enables filamentous fungi, under pelletized morphology, to have microalgae attached on the pellets; which may drastically decrease harvesting costs, avoid second pollution from flocculants and the researchers claim that it also stimulates the algae production (Zhang and Hu 2012).

The fungal pellets (*Aspergilus niger*), with an average diameter of 2–5 mm, act as nuclei for microalgal cells to attach. The proposed mechanism for this phenomenon is due to the production of hydrophobins, which are hydrophobic proteins, a family of low molecular weight amphipathic proteins (Linder 2009) detected hydrophobin on the fungal hyphae, and one of the functions of these proteins is to coordinate the adherence of hyphae to solid substrates. This study still needs inputs for larger scale purposes, and may be one of the answers for enhancing higher sustainability from microalgae-derived fuels.

17.5.3 Metal Sorption

The so-called biosorption is the capability of passive removal of toxic heavy metals such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, and Hg^{2+} by inexpensive biomaterials (Davis et al. 2003). Some green algae, such as *Chlorella* spp., *Cladophora* spp., *Scenedesmus* spp., *Chlamydomonas reinhardttii,* have been studied due to their capability of some metals sorption, and the affinity of sorbing a particular ion is particular to a species and to the physical conditions the cells are grown, since the cell wall composition may change (Mehta and Gaur 2005).

Chlorella vulgaris, for instance, has a broad spectrum of metal sorption, such as those reported in Table 17.5. These numbers are based on (Mehta and Gaur 2001; Sandau et al. 1996; Ting et al. 1995).

17.5.4 Microalgal Biorefinery

The concept of a microalgal-based refinery is based on a traditional petroleum refinery. Since many products are possible to be produced from microalgal biomass, as well as their utilization as cleaning and depolluting agents, microalgae would be able to be source of this type of industry. If adequate project and systems studies are applied, one can achieve maximization on revenues from these organisms, achieving high economic and environmental benefits.

Unlikely a petroleum refinery, a biorefinery utilizes biomass as feedstock for its operation, producing a wide range of products from one or more biological resources (Chen et al. 2010). An integration approach can be applied, making it possible to produce multiple products from a single biomass feedstock, and this system can also be self-sufficient in energy, in case of using biomass residues as energy source.

17.6 Conclusion

Although microalgae have been an interesting field of study in the most diverse areas of engineering, microbiology, and biochemistry, it still needs further technical development to make an algal biorefinery something feasible, making products sufficiently cheap, sustainable, and profitable. As argued by Ratledge (2008), oil contents of algal cells should be at least 40 % or above to be a starting material for biodiesel, for example. According to them, producing methane through anaerobic digestion would yield very little revenue, as well as burning the residual biomass.

Chen et al. (2010) cited a few key economic concerns of the mass algal production systems; which are basically: the cost of the resources for producing microalgae, the cost of construction and maintenances of the culture system, the operational costs of harvesting systems, downstream processing and refining. It is clear that costs vary according to location, solar energy availability, species, etc.

Microalgae does not show any direct competition with the food supply system, which is an attractive point toward its production. However, the well-established method for producing *Spirulina* for consumption is very simple (usage of lakes and natural lagoons, without mechanical stirring and simple methods of harvesting and sun drying) and produces a higher value products than, for instance, fuels. Its biomass is also source of beta-carotene and PUFA, which have a great interest from food and pharmaceutical industries.

A very favorable point when growing microalgae is the carbon capture issue. Also according to Chen et al. (2010), for every ton of algal biomass produced, approximately one ton of carbon dioxide is fixed (assuming 40 wt% of dry algal biomass as carbon). While most plants capture very dilute CO_2 from the

atmosphere, most algae are able to use very concentrated CO_2 as carbon source, allowing it to be part of industrial effluent cleaning processes for example.

Therefore, it is clear that an extensive work must yet be done in order to make microalgae a major source for energy production a feasible option. The dream of making a microalgae-derived refinery is far in the near future reality, but it shows potential of becoming an alternative of supplementing and even replacing non-renewable fuels. The possibilities of growing microalgae under autotrophic conditions and of utilizing its properties to clean air and wastewater are some unique advantages that are counting toward its feasibility. Economic studies have shown also that commodity oils, such as soybean, have doubled and even trebled within one year (Ratledge and Cohen 2008). Following this trend, there will be an equivalent point in which commodity oil and algal oil will match in price and from this point onward, algal oil should be cheaper. Within 10–20 years, there should be innumerable research advances which will probably derive microalgae as a potential energy source in the world.

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Chapter 18
Technological Advancements in Biohydrogen Production and Bagasse Gasification Process in the Sugarcane Industry with Regard to Brazilian Conditions

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Abstract Global warming is caused mainly by the excessive use of fossil fuels (coal, oil, diesel, gasoline, etc.) that emit millions of tons of pollutants into the environment. Besides, the fact that these fossil fuels are nonrenewable resources promotes the research in cleaner energy sources. In this chapter are presented two different technologies that could be introduced in the sugarcane industry to generate electricity and other kinds of clean fuel (producer gas and hydrogen); the case of hydrogen production by ethanol steam reforming and biomass gasification, which appear like promising technologies for energy generation in the sugarcane

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industry. Currently, most hydrogen is obtained from natural gas through a process known as reforming. Other technologic alternatives that may improve the supply of energy to the sugarcane industry is the use of biomass gasifiers in association with cogeneration system utilizing combined cycles to produce simultaneously electricity and heat, a technology known as Biomass Integrated Gasification/Gas Turbine Combined Cycle (BIG/GTCC). Cogeneration, has been accepted by different industries and has gained great application in the sugarcane industry, where the thermic and electric demands are favorable to use this type of energy system. The main fuel used in the process is sugarcane bagasse which is a by-product of sugar and ethanol production processes; the obtained energy is used in the form of mechanical power, electric power, and saturated steam in the processes. The surplus electricity can be sold. Technical, economical, and ecological analyses were performed for introduction of hydrogen production and BIG/GTCC in the sugarcane industry, using bagasse as fuel, in order to identify the better scenarios for electricity and heat generation. The introduction of these technologies will engender innovations in the sugarcane industry and will promote the sector development and as main results will increase electricity production with an economic and ecologic sustainable approach.

18.1 Introduction

The gradual increase in energy demand and environmental pollution caused by combustion processes of fossil fuels, has made necessary the development of new technologies employing alternative fuels, thus facilitating the reduction of dependence on fossil fuels oil, natural gas, and coal. Generally, the combustion process of fossil fuels produce greenhouse gases such as CO_2, NO_x, and others that increases the temperature. Energy consumption, mainly in developed countries, has reached incredible limits, which has led, combined with other factors, the increase in global warming, with large worldwide implications. Biomass is a renewable resource that plays a substantial role in the sustainable energy future. Currently, the sensitivities to environmental issues and energy security have led to the promotion of the use of endogenous renewable energy sources. Biomass as an energy source covers 10 % (50 EJ) of the global primary energy source (IEA 2009). Sugarcane is cultivated in more than 80 countries and the by-products obtained from the sugar production process represent a great biomass potential. The harvest of sugarcane in the producing countries is about 1.2 Gt and potentially its residue can be used for electric power production of 300 TW h y^{-1} (Filippis et al. 2004). Besides, nearly 95 % of hydrogen is produced from fossil-based materials, with steam reforming of methane being the most used and usually the most economical option. However, in this process, carbon is transformed into CO_2 and released into the atmosphere, leading

to global climate change (Navarro et al. 2005); thus the main interest is focusing on alternative methods for the production of hydrogen from renewable energy sources. These processes are being investigated as long-term solutions, while generation of hydrogen from biomass has been recognized as a more feasible option for the near-term solution due to its renewable and carbon-neutral nature (Yang et al. 2006). In this chapter are presented two different technologies that could be introduced in the sugarcane industry to generate electricity and other kind of clean fuel (producer gas and hydrogen); specifically hydrogen production by ethanol steam reforming and biomass gasification, which are promising technologies for energy generation in the sugarcane industry.

18.2 Incorporation of Biohydrogen Production in Sugarcane Industry

Fuel Cell (FC) appears like a promising alternative technology for energy generation, since it is any efficient system that consists of an electrochemistry process. In this process, water, electricity, and heat are generated through the combination of hydrogen and oxygen (Silveira et al. 2009). Hydrogen can be produced from a variety of sources including water and biomass (Silveira et al. 2008). Currently, most hydrogen is made from natural gas through a process known as reforming.

18.2.1 The Steam Reforming Process

For hydrogen production, several technologies can be used. Steam reforming is one of the most common installed in chemical industries. The reforming process efficiency is a function of physical–chemical properties of feedstock, thermodynamic conditions (temperature and pressure of reaction), technical configurations of reformer (dimensions and catalysts), and feedstock and water flows. The reformer to be used depends on the fuel cell, which will use the reforming products. The fuel cell technology determines the hydrogen purity required. Steam reforming occurs in the presence of a catalyst, the syngas produced includes hydrogen (H_2), carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4), among others. Some arrangements to minimize various reactions that can contribute to decrease the hydrogen production are necessary. Since this reaction is endothermic, heat from external sources is necessary. To minimize losses, several products of steam reforming, like the nonreacted fraction of reactants, might be utilized to heat up the reactants (Souza et al. 2006).

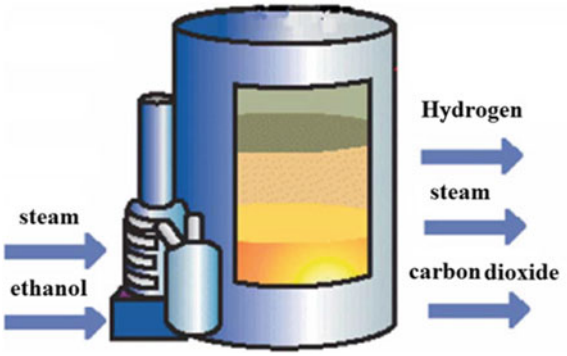

Fig. 18.1 Inlet and outlet flows of ethanol steam reforming process (Silveira et al. 2009)

18.2.1.1 Ethanol Reforming Reactions

Souza et al. (2006) indicated that this way of reforming can be described through the following main reactions:

- *Global reaction*. Ethanol reacts with steam in an endothermic reaction, taking place in the production of carbon dioxide and hydrogen, as shown in Eq. 18.1:

$$C_2H_5OH + 3\,H_2O \rightarrow 2\,CO_2 + 6\,H_2 \tag{18.1}$$

Figure 18.1 shows inlet and outlet flows of ethanol steam reforming process, and Fig. 18.2 shows the prototype developed in São Paulo State University, by Energetic Systems Optimization Group (www.feg.unesp.br/gose).

- *Ethanol steam reforming reaction*. Equation 18.2 shows the reaction where the production of carbon monoxide and hydrogen occurs:

$$C_2H_5OH + H_2O \rightarrow 2\,CO + 4\,H_2 \tag{18.2}$$

- *Water Gas Shift Reaction*. Since carbon monoxide damages fuel cell catalyst, an additional process is necessary to remove it. The Water Gas Shift Reaction (Eq. 18.3), is exothermic, reversible, and occurs at lower temperatures than the forming reaction:

$$CO + H_2O \rightarrow CO_2 + H_2 \tag{18.3}$$

- *Methanation*. Several chemical reactions occur simultaneously. Equation 18.4 shows methane production from carbon monoxide:

$$CO + 3\,H_2 \rightarrow CH_4 + H_2O \tag{18.4}$$

- *Bouduard Reaction*. This reaction (Eq. 18.5) describes carbon production from carbon monoxide decomposition:

$$2\,CO \rightarrow CO_2 + C \tag{18.5}$$

Fig. 18.2 Prototype developed in São Paulo State University

18.2.1.2 Hydrogen Production in Sugarcane Industry

Brazil has the largest and most successful biofuel programs in the world, involving production of ethanol from sugarcane and has the world's first sustainable biofuel economy. Together, Brazil and the United States lead the industrial world in global ethanol production, accounting for 70 % of the world's production (Silveira et al. 2009). The Brazilian sugarcane-based industry is far more efficient than the corn-based industry of USA. In the near future, the sugarcane industry of Brazil could be modified according to our purpose, as shown in Fig. 18.3. In this case, in addition to the production of sugar and ethanol, the Brazilian sugarcane industry will be able to produce biohydrogen.

The goal is innovation in the sugarcane industry production chain through incorporation of hydrogen production process by steam reforming of ethanol.

It is proposed to incorporate ethanol steam reforming to the traditional sugarcane industry process which is composed of extraction, juice treatment, evaporation, cooking, fermentation, and distillation to produce ethanol and sugar as well as electricity generation through cogeneration system, as shown on Fig. 18.3. These processes are described below:

- *Extraction.* In this step the cane is cleaned and milled. The milling consists in breaking the hard structure of the cane and grinding it. To increase the amount of juice, water is added. The bagasse obtained from extraction is used in the boiler of the cogeneration system, as shown in Fig. 18.4 (Pellegrine 2009).

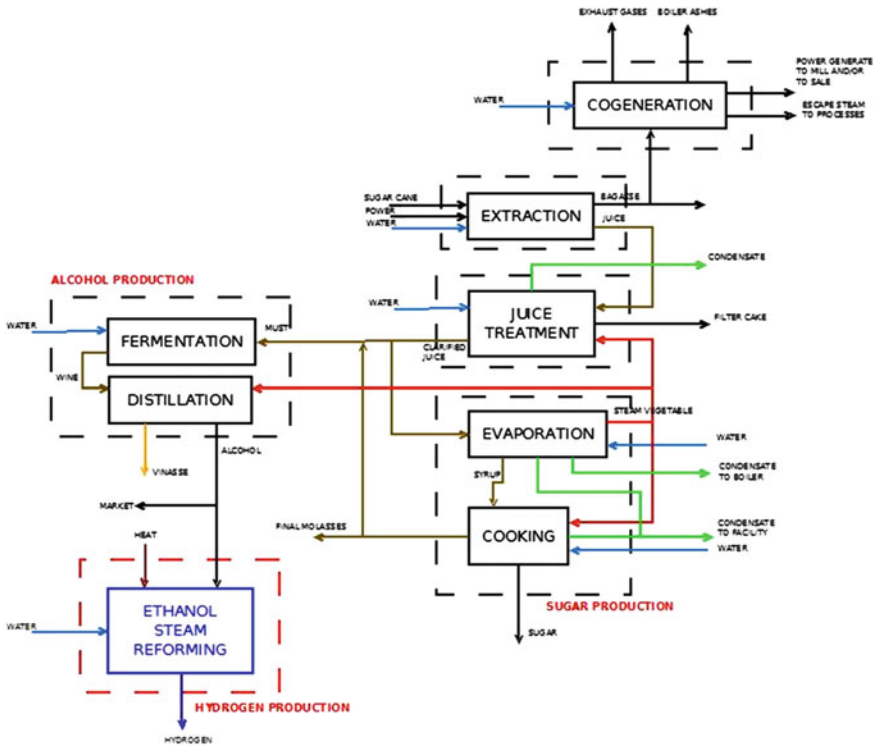

Fig. 18.3 Incorporation of steam reforming process to sugarcane industry

- *Juice treatment*. The juice is first strained to remove large particles. Then it is treated with chemical substances to modify the pH, coagulate the colloidal material (greases, proteins, etc.), and precipitate certain impurities (organic acids, sulfates, etc.). The purification process is chosen according to the sugar type that is desired to produce. After the addition of chemical substances, the mixture is heated with water vapor in high pressure. The insoluble particulate mass (mud) is separated by decantation (Silva 2010). Clarified juice goes to the evaporators without additional treatment. The mud is filtered and the filter cake is washed with water.
- *Sugar production.* According to Castro (2001) the sugar production involves two steps: evaporation and cooking, as described below.
- *Evaporation*. In this step the clarified juice is concentrated. First the juice is passed through heat exchangers to preheat and then to the evaporator stations, typically a series of five evaporators called multiple-effect evaporators. The concentrated juice (syrup) follows to the cooking step (Silva 2010).
- *Cooking*. The syrup goes through the second phase of concentration until it takes the consistency of honey and begins to form sugar crystals. Once crystallization is complete, the massecuite is centrifuged and the crystallized sugar and honey

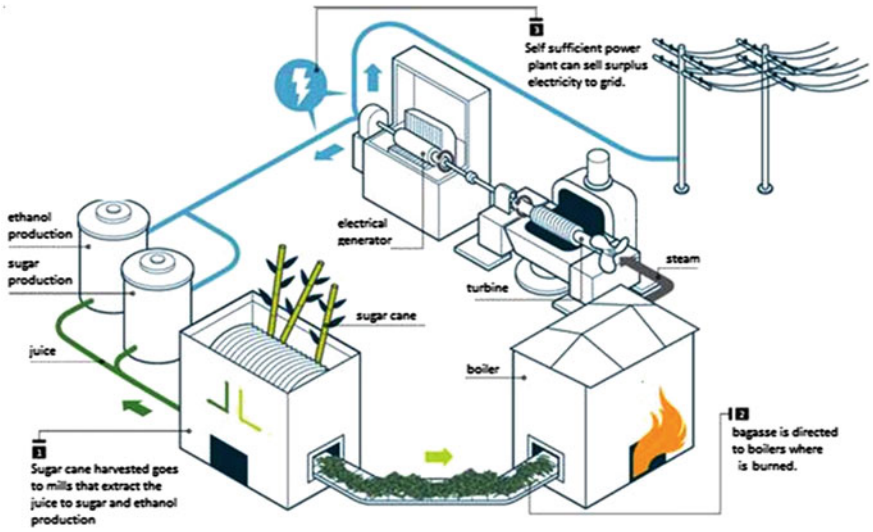

Fig. 18.4 Cogeneration system (Bernardo 2013)

are separated. The crystals obtained are of good quality and the syrup returns to the crystallization process. The end honey, or molasses, can be used as raw material for the fermentation of ethanol. The crystal sugar obtained goes through the refining process, where it is transformed into amorphous sugar, aiming to improve the purification and composition (Silva 2010).

Pellegrine (2009) advocates two stages for alcohol obtaining: fermentation and distillation.

- *Fermentation*. The mud is diluted to correct the concentration and transferred to vats where the fermentation process takes place. In this stage are added nutrients, antiseptic, and yeast, mainly responsible for fermentation. After that, wine is obtained, which goes to the distillation process (Pellegrine 2009).
- *Distillation*. The wine is directed to a decanter and after that to centrifuge where the yeast wine is obtained. It is transferred to a wine reservoir where the alcohol is separated through distillation processes (Silva 2010).

The cogeneration system is shown in Fig. 18.4.

In 18.4, after juice extraction, bagasse is directed to the boilers where it is burned. The steam from boiler goes to the steam turbine, which is connected to the electricity generator. In self-sufficient power plants, the surplus electricity can be sold to grid. As a result of the incorporation and the new configuration of the sugarcane industry will be produced hydrogen in addition to sugar, ethanol, and electricity.

18.3 Incorporation of Biomass Gasification in Sugarcane Industry

Traditionally, sugar mills use bagasse and cane trash with high moisture content as fuel for low pressure boilers to generate steam, using a conventional condensing–extraction steam-turbine (CEST) technology to provide the plant with heat, electricity, and mechanical power. The recent years have seen more modern systems for burning bagasse in suspension that allow to raise the steam pressure and temperature for the purpose of obtaining a higher electric power cycle cogeneration. The plant thermal efficiency is usually in the 15–30 % range, consequently the size of conventional combined heat and power generation plants from bagasse have been limited by these low efficiencies and the amount of fuel within an economical transportation radius.

The BIG/GTCC technology has been identified by several authors (Babu 1995; Larson et al. 2001) as an advanced technology with the potential to be cost-competitive with CEST technology using the biomass by-products of sugarcane processing as fuel, while dramatically increasing the electricity generated per unit of sugarcane processed. This type of technology does not require a large investment demand and can be inserted into the production process of ethanol (Sánchez Prieto and Nebra 2001).

18.3.1 Biomass Gasification Process

Gasification is a thermochemical process in which a carbonaceous substrate is transformed into a fuel gas, through a number of reactions that take place at high temperature in the presence of a gasifying agent (air, oxygen, and/or water vapor). The gasification process includes the following steps:

- *Drying* is an endothermic process and to achieve acceptable efficiencies the maximum amount of moisture in the solid is limited between 20 and 30 % by weight. The drying process begins at temperatures below 100 °C and can be expressed by the following reaction:

$$\text{Biomass}_{\text{wet}} + \text{Heat} \leftrightarrow \text{Biomass}_{\text{dry}} + \text{H}_2\text{O}_{(g)} \tag{18.6}$$

- *Pyrolysis* is an endothermic process, consists of biomass thermal degradation, and is developed at temperatures between 200 and 600 °C. The pyrolysis products are carbon, condensable gases (light and heavy hydrocarbons), and noncondensable gases (methane, water vapor, carbon monoxide, hydrogen, and carbon dioxide). The reaction can be represented as

$$\text{Biomass}_{\text{dry}} + \text{Heat} \leftrightarrow \text{Char} + \text{CO} + \text{CO}_2 + \text{H}_2 + \text{C}_2\text{H}_4 + \text{Tar} \tag{18.7}$$

- *Oxidation*. The oxidation step is important from the energy point of view, since it is the exothermic reaction that releases the energy required to develop the gasification process. The reaction represented by this phase would be:

$$\text{Pyrolisis products} + O_2 \leftrightarrow CO_2 + CO + H_2O_{(g)} + \text{Heat} \tag{18.8}$$

- *Reduction*. The reduction step begins to develop significantly when the solid reaches a temperature around 700 °C. Thus, the char reacts with water vapor, carbon dioxide, and hydrogen, and gases react together to produce the final gas mixture, obtained as a result of the following reactions:

$$\text{Char} + H_2O + O_2 \leftrightarrow CO_2 + \text{Heat} + \text{Ash} \tag{18.9}$$

$$\text{Char} + \text{Heat} + CO_2 \leftrightarrow 2CO + \text{Ash} \tag{18.10}$$

$$\text{Char} + H_2O + \text{Heat} \leftrightarrow CO + H_2 + \text{Ash} \tag{18.11}$$

The producer gas is the principal product of gasification, and its lower heating value (LHV) varies depending on the composition of biomass and the gasifying agent employee. Using air as the gasifying agent, the LHV of the producer gas is in the range between 4 and 6 MJ/Nm^3 and using water vapor or oxygen the LHV is between 8 and 20 MJ/Nm^3 (Reed et al. 2005).

18.3.2 Gasification in Fluidized Bed Reactors

Fluidized bed reactors are those in which the gasifying agent circulates inside them at a rate such that a bed is in a state of fluidization, existing inside the gasifier several conditions that intensify the transfer of energy and material between the fuel and gas. There are two main categories within these types of gasifiers: bubbling and circulating. In the bubbling fluidized beds, the fluidizing velocity–gasifying agent is sufficiently low as there is no significant movement of solid. By contrast, in the circulating fluidized bed, the velocity of the agent is much higher resulting in a solids circulation. This solid is recirculated to the reactor by the use of a cyclone return system to the gasifier. The main advantages of fluidized beds include better control of temperature and reaction rates, high specific capacity, potential scaling to larger sizes, and adaptation to changes of biomass. On the contrary, show moderate–high tars and particulates levels in the exhaust gas and the fuel conversion are not as high as in the fixed bed gasifiers. A comparison between bubbling and circulating fluidized bed gasifiers is shown in Table 18.1 (Williams et al. 1995).

Table 18.1 Comparison between bubbling and circulating fluidized bed gasifiers

Fluidized bed reactor	Temperature (°C)		Biomass	Feed	Gasification agent	Tar content
	Reaction	Exit				
Bubbling	700–1000	700–800	Wood chips, leftover corn cobs, rice husks	Directly in the area of the bed	The bottom of the gasifier	Medium–High
Circulating	700–1000	600–800	Sugarcane bagasse, wood chips, sawdust, rice hulls	Directly in the area of the bed	The bottom of the gasifier	Low

18.3.3 Biomass Gasification Technology for Cogeneration of Heat and Power

Cogeneration study can be divided into three different types of cycles. The conventional cycle in which steam is used at low pressure and temperature and the steam and power generated is just enough for own consumption of the plant. A second cycle is the advanced cycle with similar configuration to conventional cycle but operating with a higher pressure and temperature, which results in significantly greater generation of electricity than the needs of the sugar factory; the excess energy can be sold to external consumers. The third cycle is the BIG/GTCC and also generates an excess of electricity. The combined cycle system is the simplest scheme used for cogeneration, shown in Fig. 18.5. It employs a gas turbine, a heat recovery steam generator without supplementary firing, and steam-turbine (Silveira et al. 2006).

18.3.4 Sugarcane Bagasse as Biomass

There are several studies on the use of sugarcane bagasse as fuel in gasification processes. Olivares (1995) studied different types of bagasse with the objective of introducing it as fuel in a fluidized bed gasifier. Bagasse is a material with high fiber content and low density and has an extensive range of sizes. It exits the sugar production process with a moisture content of approximately 50 % (wet basis); for this reason, a pre-treatment process is necessary that includes drying, crushing, and others in order to improve their properties and facilitate the feeding process to fluidized bed reactors.

One of the principal parameters to evaluate the quality of bagasse is the moisture content because the more humid bagasse will decrease its lower calorific value (LHV), and therefore it will has less available energy for the same amount of fuel.

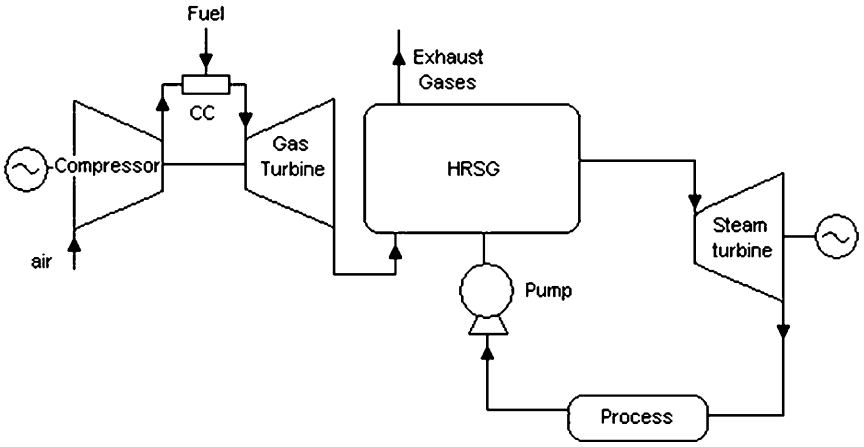

Fig. 18.5 Gas turbine associated with the steam turbine (Combined Cycle)

Table 18.2 shows the main physical and chemical properties of sugarcane bagasse reported by Jenkins et al. (1998). Bagasse is classified as a fuel with high reactivity due to its high content of volatiles and low ash content, making it a good feedstock for gasification.

18.3.5 Combined Cycle Associated with a Fluidized Bed Gasifier

In a combined cycle, the fuel combustion provides the mechanical energy to the electric generator and the exit gases from the combustion are directed to a heat recovery steam generator to produce steam; this steam will drive a steam turbine that will be linked the other electricity generator. It is generally employed in this type of cycle-to-cycle Brayton combination with a Rankine cycle type (Diniz et al. 2013). To have the possibility of entering the gasifier in this cycle, there must be previous drying since the sugarcane bagasse has relative humidity around 50 % *in natura* (Olivares et al. 1995). It is also necessary to clean the producer gas generated, since it contains a load of particulate and tar, as shown in Fig. 18.6.

Research has shown the potential of BIG/GTCC-based systems to be competitive with, if not superior to, conventional combustion power plants because of their higher efficiency, superior environmental performance, and competitive cost (Reed et al. 2005). However, much of the advancements are still under research and development. BIG/GTCC is a combination of two leading technologies: gasification and gas turbine combined cycle. The gasification portion of the BIG/GTCC plant produces a clean gas which fuels the gas turbine. For this system, the gasification stage is carried out in a fluidized bed. Typical operating temperature of

Table 18.2 Main physical and chemical properties of sugarcane bagasse (Jenkins et al. 1998)

Proximate analysis	
Volatile matter (wt%, dry basis)	85.61
Fixed carbon (wt%, dry basis)	11.95
Ash (wt%, dry basis)	2.44
Higher heating value (MJ. kg^{-1}, dry basis)	18.99
Ultimate analysis (wt%, dry basis)	
C	48.65
H	5.87
O (by difference)	42.82
N	0.16
S	0.04
Cl	0.03

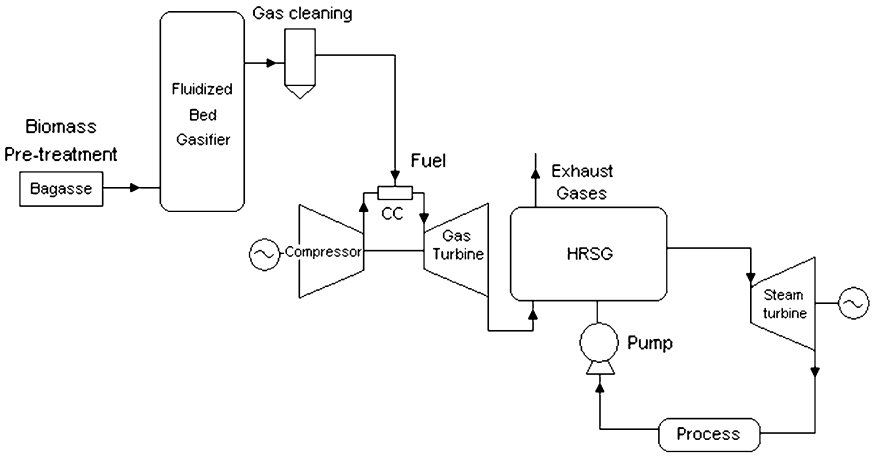

Fig. 18.6 Cogeneration combined cycle associated with the fluidized bed gasifier

a fluidized bed is 800–850 °C. Air is blown through the bed at a sufficient velocity to keep the bed materials in a state of suspension. The fuel particles are introduced at the bottom of the reactor, very quickly mixed with the bed material, and almost instantaneously heated up to the bed temperature and hence the subsequent producer gas generation. After the producer gas has left the fluidized bed chamber, it goes through a cleaning unit. The gas after the cleaner unit is then led to a boost compressor that compresses it to the gas turbine combustion chamber pressure conditions. The exhaust heat from the combustion turbine is recovered in the heat recovery steam generator to produce steam. This steam then passes through a steam turbine to power another generator, which produces more electricity. The combined cycle is more efficient than conventional power generating systems because it reuses waste heat to produce more electricity (Okure et al. 2006).

18.3.5.1 Energy Analysis of the Integration of Gasification of Bagasse in the Sugar and Alcohol Sector

The energy analysis comprises the study based on the first law of thermodynamics, the law of conservation of energy. This type of study is universally valid and is used in the mass and energy balances in the gasifier and other components of the BIG/GTCC system. For realization of this energy, analysis is determined of the operating parameters and efficiencies of the process and its components. However, only the use of the first law of thermodynamics proves to be insufficient in subsequent economic evaluation of an energy system, because it does not estimate the amount of energy available for conversion into work or power. Given this, the study based on only the first law of thermodynamics gives us an incomplete analysis of the potential energy of a system.

18.3.5.2 Exergetic Analysis of Integration of Gasification of Bagasse in the Sugar and Alcohol Sector

Given the limitations of the first law in formulating the quality and quantity of useful energy in a system, the concept of Exergy was created from the second law of thermodynamics. According to Tuna (1999), Exergy is that portion of noble energy that can be completely converted into work reversibly. However, the exergy can be defined as the maximum useful work that can be obtained by an energy carrier (Tsatsaronis 1993). The exergy inefficiency of a system consists in a destruction of exergy associated with irreversibilities. The irreversibility in a system can be decomposed into internal irreversibility, known as the Second Law of Thermodynamics as destruction of energy and external irreversibility, which is the exergy loss to the environment, developing out of the control volume selected for thermodynamic analysis (Valero et al. 2011). The maximum improvement in exergy efficiency for a process or system is obviously determined when the exergy loss or irreversibility is minimized, the latter being determined by the following equation (Sozen et al. 2002; Utlu et al. 2006):

$$I = \sum E_{x_{\text{in}}} - \sum E_{x_{\text{out}}} \tag{18.12}$$

$$e_{x1} = (h_1 - h_0) - T_0(S_1 - S_0) \tag{18.13}$$

$$E_{x1} = m \cdot e_{x1} \tag{18.14}$$

Exergy analysis or even availability analysis is then drawn in this way to achieve the goal of a more effective use of energy resource as it enables the location, cause, and true magnitude of waste and loss. Such information can be used in the design of efficient energy systems and to increase the performance of existing systems. Exergy analysis also provides a broader view of the problem under consideration, avoiding conclusions based purely on the application of the

first law of thermodynamics. Tuna (1999) emphasizes that the analysis of first and second law are not competing, but complementary, and together contribute to a consistent assessment of the thermal system.

18.4 Economic Analysis

In both technologies the methodology to make the economic analysis is similar and is based on engineering economics calculations developed by Silva (2010), who considered the sugarcane industry producing hydrogen using ethanol and gasification of electricity generated in the gas turbine and steam turbine by use of producer gas from bagasse gasification. In order to reach this proposal, an economic analysis based on the investment of the hydrogen production system and BIG/GTCC system were developed considering the input costs, operating cost, maintenance cost, operation period, interest rate, and annuity factor.

The global equation for hydrogen cost is shown in the following equation:

$$C_{H_2} = \frac{I_{\text{nvref}} \cdot f}{H \cdot E_{H_2}} + C_{\text{OP}} + C_{\text{man}} \tag{18.15}$$

The annual cost of obtaining electricity (Cel), US$/kWh, for each selected system is given as

$$C_{\text{el}} = \frac{(I_{\text{pl}} - I_{\text{vcr}}) * f}{H * E_p} + \frac{C_{\text{comb}} * \left(E_{\text{comb}} - E_{\text{cr}} - \frac{\text{Per}}{2}\right)}{E_p} + \text{CM}_{\text{stg}} \tag{18.16}$$

$$f = \frac{q^k * (q-1)}{q^k} \tag{18.17}$$

where:

k is the amortization period or pay-back, given in years. C_{H_2}—Hydrogen production cost (US$/kWh); C_{el}—Electricity production cost (US$/kWh); Inv_{ref}—Reference investment for hydrogen production (×104 US$); f—Annuity factor (1/year); H—Equivalent period of operation (h/year); E_{H_2}—Energy provided by Hydrogen (kW); C_{op}—Operational cost (US$/kWh); C_{man}—Maintenance cost (US$/kWh).

Operational cost using bagasse as fuel is shown in Eq. 18.18, and the operational cost using electricity is shown in Eq. 18.19. According to Kothari et al. (2008), the maintenance cost of steam reformer was estimated as 3 % of investment.

$$C_{\text{OP}} = \frac{E_{\text{fuel}} \cdot C_{\text{fuel}}}{E_{H_2}} + \frac{E_{\text{EtOH}} \cdot C_{\text{EtOH}}}{E_{H_2}} \tag{18.18}$$

$$C_{\text{OP}} = \frac{E_{\text{Elet}} \cdot C_{\text{Elet}}}{E_{H_2}} + \frac{E_{\text{EtOH}} \cdot C_{\text{EtOH}}}{E_{H_2}} \tag{18.19}$$

where:

E_{fuel} Energy provided by sugarcane bagasse (kW);
C_{fuel} Fuel cost (sugarcane bagasse) (US$/kWh);
E_{EtOH} Energy provided by ethanol (kW);
C_{EtOH} Ethanol cost (US$/kWh);
E_{Elet} Electricity consumed by reformer (kW);
C_{Elet} Electricity cost (US$/kWh).

The investment cost (acquisition cost of equipment, installation cost) to produce steam covers the cost of system gas turbine (compressor, combustion chamber, gas turbine electric generator, and other accessories), the heat recovery steam generator is considered as separate module. Thus, the following equation is used to calculate the investment to be made:

$$I_{pl} = I_{stg} + I_{vcr} \tag{18.20}$$

For investment cost of heat recovery steam generator (I_{vcr}) without supplemental fuel burning is used Eq. 18.21, defined as the technique of Boehn (1987) according to the steam production in (kg/h), with a multiplicative factor of 10 % related to the cost of installation of the boiler recovery and valid for production values higher than 800 kg/h and less than 4000 kg/h.

$$I_{vcr} = 1.1 * 160000 * \left(\frac{m_v}{1500}\right)^{0.81} \tag{18.21}$$

The equation final investment cost becomes:

$$I_{pl} = \left(I_{stg} + I_{vcr}\right) * 1.3 \tag{18.22}$$

$$C = C_r\left(S/S_r\right)^m \tag{18.23}$$

where:

C—Equipment cost for an interest capacity *S*; *m*—Incidence factor indicating the economics scale (0.5–1.0); *Cr*—Equipment cost for a reference capacity *Sr*.

Silva (2010) has adapted the reference investment for steam reforming process with hydrogen production range of 1 up to 1500 (Nm^3/h), resulting in Eq. 18.24

$$\text{Inv}_{ref} = \left(\frac{m_{H_2}}{750}\right)^{0.5304} \tag{18.24}$$

The expected annual revenue is calculated as the sum of earnings or annual benefits due to the installation of a system (Silveira and Tuna 2003, 2004).

18.5 Ecological Analysis

At present, practically all known forms of energy production have some kind of interference in the environment. Due to this fact using biomass gasification combined with a cogeneration system is a set of recommended alternative energy, from the environmental point of view.

18.5.1 Ecological Efficiency

The ecological efficiency analysis is based on calculations of equivalent carbon dioxide [$(CO_2)_e$], and pollution indicator (Π_g) for determining the ecological efficiency of the process of hydrogen production by ethanol steam reforming, and for the BIG/GTCC system.

18.5.2 Determination of Equivalent Carbon Dioxide (CO_{2e})

The equivalent carbon dioxide depends on the emission of SO_2, NO_x, and PM, and can be determined using Eq. 18.25.

$$CO_{2e} = CO_2 + 80 * SO_2 + 50 * NO_x + 67 * MP \tag{18.25}$$

18.5.3 Determination of Pollution Indicator (Π_P)

The pollution indicator (Π_P) is the ratio between the amount of CO_{2e} in kg and the power supplied by the producer gas and for the hydrogen production it is shown in Eq. 18.26 (Silveira et al. 2012).

$$\Pi_p = \frac{CO_{2e}}{PCI} \tag{18.26}$$

18.5.4 Determination of Ecological Efficiency (ε)

Ecological efficiency is defined as an indicator for evaluating the performance of a particular system, considering the emissions of pollutants burning 1 kg of fuel. Their values vary between 0 and 1, where the higher the vicinity of 0 means the greater the environmental impact, and if it is proximate to 1, indicates that it is

nonpolluting (zero environmental impact). The ecological efficiency can be determined using Eq. 18.27 (Silveira et al. 2012).

$$\varepsilon = \left[\frac{0.204 * \eta_{\text{system}} * \ln\left(135 - \Pi_p\right)}{\eta_{\text{system}} + \Pi_p}\right]^{0.5} \tag{18.27}$$

18.5.5 Calculation of CO_2 Emissions from Combustion Process of Sugarcane Bagasse

The CO_2 emissions from 1 kg of fuel can be calculated according to Eq. (18.28).

$$M = \frac{(w_1 \times 44 \times 1)\text{CO}_2}{N} \tag{18.28}$$

where:
M_{CO_2}—CO_2 emissions (kg_{CO_2}/kg_{fuel}); Molar mass of fuel (bagasse) (kg/kg_{mol}).

The molar mass of bagasse can be determined based on the elemental composition (Table 18.2). Therefore, the molar mass of bagasse can be calculated through Eq. 18.27.

$$N = (a_1 12) + (b_1 1) + (c_1 16) + (d_1 14) + (e_1 32) \tag{18.29}$$

18.6 Conclusions

Hydrogen, the principal energy carrier to fuel cells, can be produced through various ways, but ethanol steam reforming is an alternative to guarantee the volume of production necessary in the Brazilian case. The integration or association of hydrogen production with sugar industry, can certainly put Brazil in a good classification in the "Hydrogen Era," in the near future. Similarly in terms of ecological efficiency, the fluidized bed gasifier operating with bagasse is an environmentally friendly way, with high ecological efficiency to produce energy in the sugarcane industry. This technology proves that this type of combine cycle is an excellent alternative to the traditional electric power generation technology, based on the Rankine cycle, used in this industry for electricity and heat generation. Thus, these technologies can be inserted with energy and environmental gains in the sugarcane industry.

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Chapter 19
Nonconventional Renewable Sources in Brazil and Their Impact on the Success of Bioenergy

Luís Cláudio Oliveira-Lopes and Cláudio H. Ferreira da Silva

Abstract Brazil has abundant natural sources of renewable energy. Existing renewable sources of energy are discussed and an overview of the energy options in Brazil is assessed with their current situation and future potential. Given the great deal of opportunities in terms of energy from wind, solar, and biomass and their impact in Brazil energy matrix, a diverse and sustainable framework for producing energy needs to be consolidated. The effectiveness of success of the Brazilian renewable energy market strongly depends on legislation and country policies. The growth will be as fast as the country implements energy policy to support the renewable sources of energy by breaking economic, regulatory, or institutional barriers. Furthermore, the impact of the nonconventional renewable energy in the success of bioenergy highly depends of the policymaker initiative on seeking a variety of renewable energy sources and their incorporation into the energy matrix of the country.

19.1 Introduction

Brazil is the fifth largest country in the world with an abundant potential for hydropower, based on several important rivers (Paraná, Tocantins, São Francisco, Iguaçu, Paranaíba, among others). Hydroelectric capacity is complemented by conventional thermal and nuclear plants, totaling 107 GW of installed power capacity (EPE 2009b), of which more than 79 GW is hydropower, 24 GW thermoelectric, 2 GW nuclear, and 602 MW wind. The country posses a great variety

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of natural resources, but their exploitation may cause significant environmental impacts. In the case of hydroelectric power, Brazil exploits only 30 % of its potential, but the remainder is mainly located in environmentally sensitive regions, like Amazon.

Besides the richness in hydropower potential, Brazil is a large country and presents different climate zones that open other potential for renewable source of energy (Meisen and Hubert 2010). While in the central region of the country there is a dry and sunny climate, which gives a great opportunity for solar energy production, a large and windy coast allows a wind power production and perhaps, wave power. In addition to those aspects, Brazil has a large potential of biomass generation, and with that sources for bioenergy production (bioethanol, biobutanol, and biohydrogen). Thanks to an ideal climate for sugarcane, Brazil is the second largest ethanol producer in the world (after the United States). The ethanol from sugarcane in Brazil is a very competitive and mature industry (Pereira et al. 2012).

In this scenario, Brazil has indicated that it is committed to maintaining a large share of renewable source in its energy matrix (Fig. 19.1), through Law no 10.438 of 2002, of the Program for Incentive of Alternative Electric Energy Sources (PROINFA), whose main objectives are to promote the diversification of electric power generation sources, in order to increase supply security, prioritize action that exploit regional and local characteristics and potentialities, and the reduction of greenhouse gas emissions (Pereira et al. 2011). Besides, to promote the use of renewable technologies (wind, biomass cogeneration, and from small hydroelectric plants) through incentives and subsidies, PROINFA planned the installation of 144 power plants (with the installed capacity of 3,299.40 MW), distributed among 63 small hydroelectric plants (1,191.24 MW), 54 wind power plants (1,422.92 MW), and 27 biomass-based power plants (685.24 MW) (MME 2013), with an increase in the Brazilian renewable share of annual energy consumption to 10 %.

Brazil has an electricity generation matrix based mainly on renewable sources (44.1 % in 2011). Figure 19.2 shows the internal supply of electricity in Brazil in 2011 (BNE 2012). A total of 466.8 TW h of was produced in 2011.

With an electricity matrix based mostly on renewable sources (88.8 % in 2011), the country still have many challenges in the future. In fact, a wide variety of renewable source technologies will be needed to meet the challenges of sustainable energy development, considering that biomass, biogas, and small hydropower plants are already competitive compared to traditional generation sources (Ren-21 2013).

Brazil is under strong development and with that it is expected a significant increase in energy consumption (EPE 2007a, b, 2009a, 2013). The availability of the energy system should be increased by over 100 % in the next decades. This scenario represents a large environmental, economical, and social effort for the country. However, it is a great opportunity for implementing the country vocation in incorporating renewable energy to the energy generation matrix (Pottmaier et al. 2013).

In this sense, this chapter addresses nonconventional renewable sources of energy, their impact, and potentialities in the consumption energy profile in Brazil.

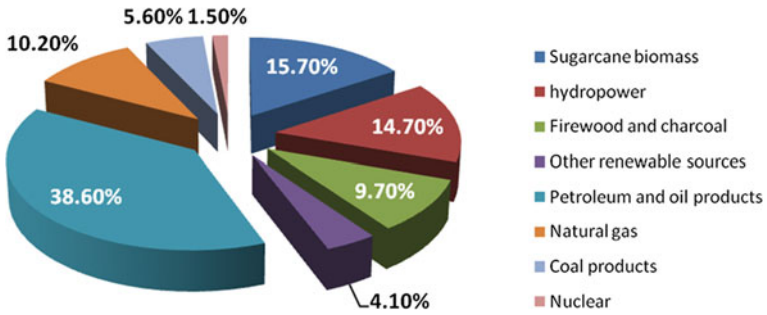

Fig. 19.1 Brazilian energy matrix for 2011 (EPE 2012)

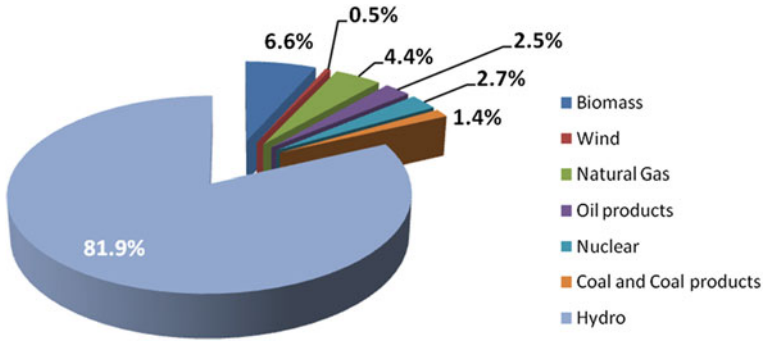

Fig. 19.2 Brazilian internal electricity supply by source in 2011 (EPE 2012)

19.2 Brazilian Potential for Nonconventional Renewable Sources

Considering the environmental situation, the depletion of natural resources, the greenhouse gases issue, and also the challenge of energy planning that allows reconciling supply and demand; it is necessary to move toward a set of solutions where many options or alternatives need to be used, constituting a contribution to the construction of a energy system toward low tariffs, high sustainability, and the use of the local potential thought the country. After all, there exist an important relationship between society and energy, which may be strongly impacted economically and even drive a country perspective of future.

Due to many possibilities of increasing the use of energy renewable sources, this section will not cover the conventional sources. Nevertheless, a quick overview is presented next:

- Hydropower—it is mostly dependent on precipitation and elevation changes; high precipitation levels and large elevation changes are necessary to generate

Table 19.1 Summary of the largest hydroelectric plants in Brazil (*Source* ANEEL 2013)

Dam	River	MW	Reservoir (km^2)	Status	Location
Itaipú	Paraná	14000	1350	OP	Paraná(BR)/Paraguay
Belo Monte	Xingú	11233	516	UC	Pará
São Luiz do Tapajós	Tapajós	8381	722	PL	Pará
Tucuruí	Tocantins	8370	2850	OP	Pará
Santo Antonio	Madeira	3665	271	UC	Rondônia
Ilha Solteira	Paraná	3444	1195	OP	São Paulo
Jirau	Madeira	3300	108	UC	Rondônia
Xingó	São Francisco	3162	60		Alagoas/Sergipe
Paulo Afonso IV	São Francisco	2462	12.9	OP	Bahia
Jatobá	Tapajós	2338	646.3	PL	Pará

significant quantities of electricity and irregularity in the wet/dry seasons may affect the energy production.

In Brazil hydropower is characterized by large reservoirs, located in several hydrographical basins, which are mostly far from the main consumption centers. The National Interconnected System is operated by the National System Operator that optimizes the use of all hydroelectric sources. Brazil has over a hundred hydroelectric plants. This number almost gets doubled when one includes small hydroelectric plants (from 1 to 30 MW and a reservoir area up to 3 km^2). Table 19.1 presents the major hydroelectric plants in Brazil (OP = operating, UC = under construction, and PL = planned).

- Ethanol—is a liquid fuel that can be produced from any primary matter which contains sugar or material that can be transformed into sugars (i.e., starches or cellulose). Together with the U.S. Brazil produce 87 % of the world's ethanol. The vast majority of U.S. produce ethanol from corn, while in Brazil, it is produced by fermentation of sugars from sugarcane using *Saccharomyces cerevisiae*. Approximately 90 % of Brazilian sugarcane production takes place in South-Central Brazil with the remainder grown in Northeastern Brazil. Both production regions are located around 2,500 km (1,550 miles) away from the Amazon region. In addition to an ideal climate for sugarcane, available land to produce sugarcane without deforestation, Brazil has the world's most competitive program of development and ethanol production. Nowadays, the use of *flex fuel* vehicles (that can run on either gasoline or hydrous alcohol) account for 90 % of new car sales in Brazil, the reduction of greenhouse gas emission when using ethanol as a substitute fuel, and the mandatory addition of up to 25 % (volume) of hydrated alcohol to the all gasoline used in Brazil make ethanol a highly competitive substitute fuel in Brazil. Table 19.2 presents the global ethanol production.

Given the vast possibilities and alternatives, the next section will focus on some options in terms of energy from wind, solar, and biomass and their potential and impacts on Brazil energy matrix.

Table 19.2 Global ethanol production in billions of gallons

Country	2007	2008	2009	2010	2011	2012
USA	6.521	9.309	10.938	13.298	13.948	13.300
Brazil	*5.019*	*6.472*	*6.578*	*6.922*	*5.573*	*5.577*
Europe	570	734	1.040	1.209	1.168	1.179
China	486	502	542	542	555	555
Canada	211	238	291	357	462	449
Asia[a]	132	156	527	244	335	397
South America[b]	75	79	83	200	199	223
Mexico and Central America	Na[c]	Na	Na	364	39	19
Australia	26	26	57	66	87	71
Africa	Na	Na	Na	44	38	42
Other	82	128	247	66	Na	Na
WORLD	13.123	17.644	20.303	23.311	22.356	21.812

[a] Excluding China; [b] Excluding Brazil; [c] *Na* not available
Source F.O. Licht, cited in Renewable Fuels Association, Ethanol Industry Outlook 2008–2013 reports

19.2.1 Wind Energy

Air moves due to difference in pressure. In the atmosphere it is simply called as wind, which has kinetic energy (due to its motion). Therefore, wind energy production is related to capturing the wind kinetic energy with turbines, which are designed with a vertical (VAWTs) or a more commonly found horizontal-axis (HAWTs), to spin a shaft linked to a generator that transfers the rotational energy to electricity.

Wind energy has been used for 1000 years, it begins with sailing boats, grinding grains in windmills, water pumping systems, and in the last century has started being used for electricity generation with turbines operating based on large aerodynamic turbine systems in a wind farm, which consists of a group of wind turbines distributed over an area that may be used for agricultural or other purposes or even be located offshore.

Wind energy has several positive aspects: it does not burn fossil fuels, wind turbines are scalable and space-saving when compared to other alternatives for electricity production, and wind energy is an unlimited source for energy production. On the other hand, wind energy uses turbines that are quite noisy, requires several units to produce enough energy compared to other alternatives, has low reliability and consistency due to the wind nature, brings visual pollution, and it is still costly to install.

Table 19.3 presents the worldwide current installed wind power capacity, (282,482 MW), for which less than 2 % are offshore installation.

In Brazil, the use of wind power for electricity started in 1992 with the installation of a small wind turbine in Fernando de Noronha (PE), followed 2 years

Table 19.3 Installed wind power capacity (MW) in 2012 (GWEC 2013)

Rank	Country	Capacity (MW)
1	China	75,564
2	U.S.	60,007
3	Germany	31,332
4	Spain	22,796
5	India	19,564
6	U.K.	8,445
7	Italy	8,144
8	France	7,196
9	Canada	6,200
10	Portugal	4,525
11	Denmark	4,162
12	Sweden	3,745
13	Japan	2,614
14	Australia	2,584
15	*Brazil*	*2,508*
16	Poland	2,497
17	Netherlands	2,391
18	Turkey	2,312
19	Romania	1,905
	World total	282,482

later by Morro do Camelinho, installed in 1994 in the city of Gouveia—MG. Brazil wind energy potential is shown in Fig. 19.3, where it can be seen that the estimated potential for wind energy in Brazil based on the annual mean wind speed at 70 m above ground level is 143.5 GW (272.2 TW h/yr). However, it was only with the PROINFA that the wind energy production presented an important growth. Figure 19.4 shows the historical flowchart for wind energy in Brazil (MME 2012).

Currently wind energy in Brazil accounts for 2 % of national electricity consumption. In 2012 alone, 40 new wind farms came online, adding more than 1 GW of new capacity to the Brazilian electricity grid.

Table 19.4 presents some of the most important wind power plant in Brazil. Most of the plants are located in Rio Grande do Sul, Bahia, Rio Grande do Norte, and Ceará. Last year, the Alto Sertão-I Wind Complex (with 294 MW installed wind capacity) and 14 wind farms, was inaugurated, and the Alto sertão-II Wind Complex (with 386 MW) is currently under construction.

Despite the wind energy growth, Brazil lacks a great deal of investment in infrastructure to connect all those planned/under construction wind farms to the Brazilian network of energy. According the Brazil's National Electric Energy Agency (ANEEL) there are 197 granted wind-based enterprise (5,253,425 kW), 93 under construction (2,346,866 kW) and 96 operating units (2,109,341 kW). Those numbers do not contain the information of those units that were constructed, but still not integrated in the Brazilian distribution system.

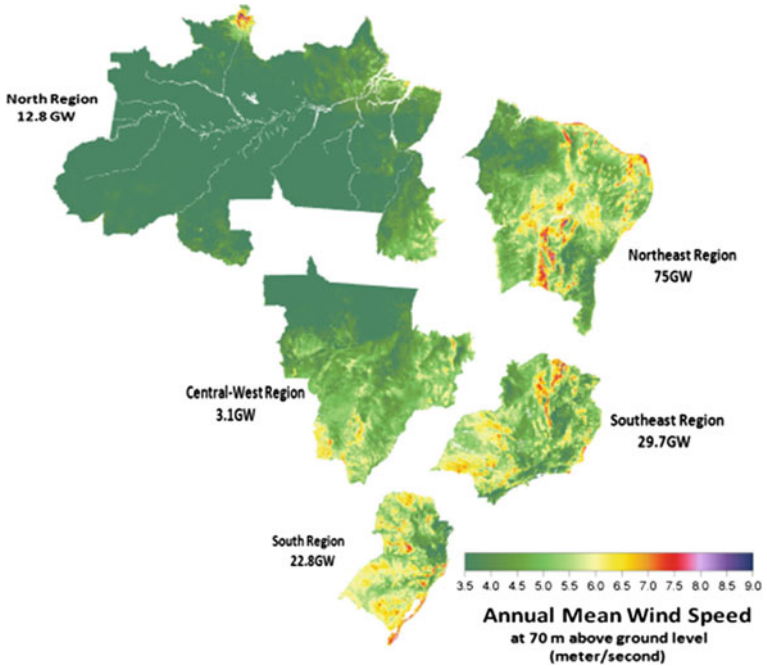

Fig. 19.3 Potential for wind energy in Brazil based on the annual mean wind speed at 70 m above ground level (MME 2012)

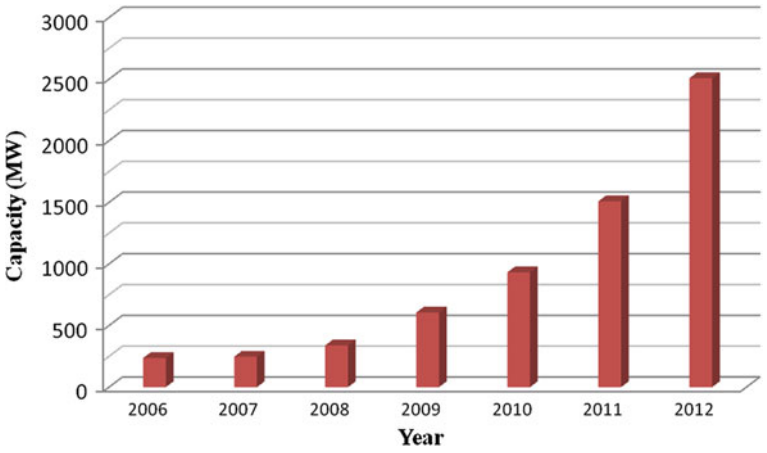

Fig. 19.4 Brazil installed wind power capacity (2006–2012) (MME 2012)

Table 19.4 Largest wind power plants in Brazil by installed capacity

Wind power complex	Installed capacity (kW)	Local	Status
Alto Sertão-I	294,000	Southwest—BA	OPI-2012
Alto Sertão-II	386,000	Southwest—BA	OPC
Alegria complex	151,650	Guamaré—RN	OPP-2011
Praia formosa	105,000	Camocim—CE	OPF-2009
Aracati complex	138,500	Aracati—CE	OPF-2008
Osório complex	150,000	Osório—RS	OPF-2007
Icaraízinho	65,100	Amontada—CE	OPC
Elebrás Cidreira 1	70,000	Tramandaí—RS	OPF-2011
Vale dos Ventos	48,000	Mataraca—PB	OPF-2009

OPI lacks infrastructure for full operation, *OPC* planned or under construction, *OPP* partially operating, *OPF* fully operational (ANEEL 2013)

19.2.2 Solar Energy

Solar energy refers to the use of the energy from the sun for practical use. It is known that the Earth receives 174 petawatts of solar radiation at the upper atmospheres (IPHE 2011), reflecting back around 30 % of it to space. Solar radiation is spread around the world, but strongly depends on the distance from the equator.

Solar power is the conversion of sunlight into electricity either directly using photovoltaics (PV) or indirectly using concentrated solar power (CSP). CSP systems use lenses or mirrors and tracking systems to focus a large area of sunlight into a small beam. PV converts light into electric current using the photoelectric effect.

Brazil has a large majority of its land in the tropics, therefore, the estimated solar incidence for Brazil ranks among the highest in the world. Solar insolation levels are relatively high and promising across the country.

The need for high quality information on solar data for Brazil was addressed by SWERA (Solar and Wind Energy Resource Assessment) Project. SWERA Project was supported by The United Nations Environment Program (UNEP) and the Global Environmental Facility (GEF) (Martins et al. 2008) and in Brazil the project was coordinated by Centre for Weather Forecast and Climate Studies of the Brazilian Institute for Space Research (CPTEC/INPE). The solar irradiation data provided by SWERA was based on the model BRASIL-SR developed by CPTEC/INPE and LABSOLAR/UFSC (Pereira et al. 2000).

According to the SWERA data, (prepared by using BRASIL-SR radiative transfer model and satellite database acquired from 1995 to 2005 with a spatial resolution of 10 km × 10 km) the maximum daily global solar irradiation value (6.5 kW h/m^2) occurs in the semi-arid climate area of Brazilian Northeastern region. The lowest daily global solar irradiation (4.25 kW h/m^2) occurs on the shore of Southern region of Brazil. Those values are much greater than those for the majority of the European countries. BRASIL-SR model was validated through

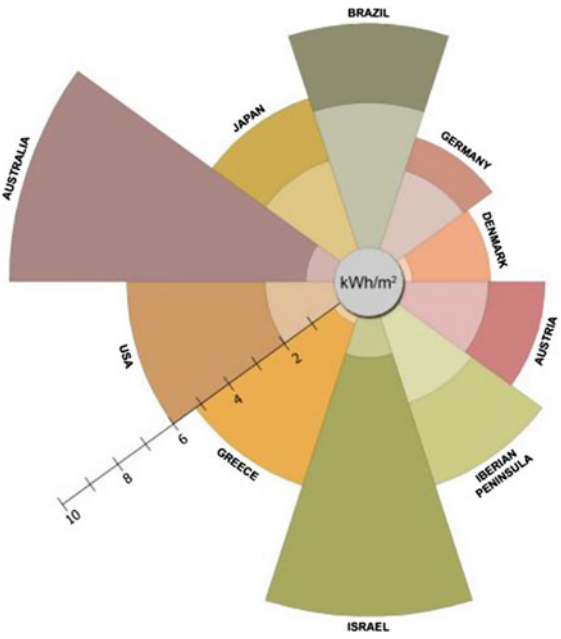

Fig. 19.5 Annual mean daily solar irradiation (kW h/m^2) (IPHE 2011)

a comparison with measured values at the ground stations spread throughout the country with ground data collected by the SONDA (National Organization of Environmental Data System) network stations and by automatic weather stations (AWS). Figure 19.5 shows the annual daily solar irradiation for several countries and Figs. 19.6 and 19.7 show the annual average of daily total global solar irradiation and the daily solar irradiation per region of the country, respectively.

PROCEL (National Program for Electricity Conservation) estimates that there are more than 30 million electric showers installed in Brazil, consuming about 6 % of all electricity produced in the country, accounting for approximately 18 % of the peak demand of the national electric system. This means that to heat water, Brazil is consuming too much resource. This fact indicates that solar thermal can be a smart move for Brazil's demand of water heating systems (Martins and Pereira 2011).

Solar heating is a case of success in all sectors of the Brazilian economy: residential, commercial, and industrial. Belo Horizonte, capital of the state of Minas Gerais in 2010 had 1.87 million square meters of solar panels (Companhia Energética de Minas Gerais 2012), from 1991 to 2010, saving the total of 861,000 TEP (*Ton Equivalent Petrol*).

Besides using solar energy for water heating, there are a few power plants in Brazil for electricity production. According to ANEEL, the installed capacity of photovoltaic power plants is about 7.5 MW (ANEEL). The SER (Sistema de Energia Renovável) announced plans to build a total of 600 MW of solar power capacity in Brazil by 2020. Its first project, a 5 MW capacity solar power

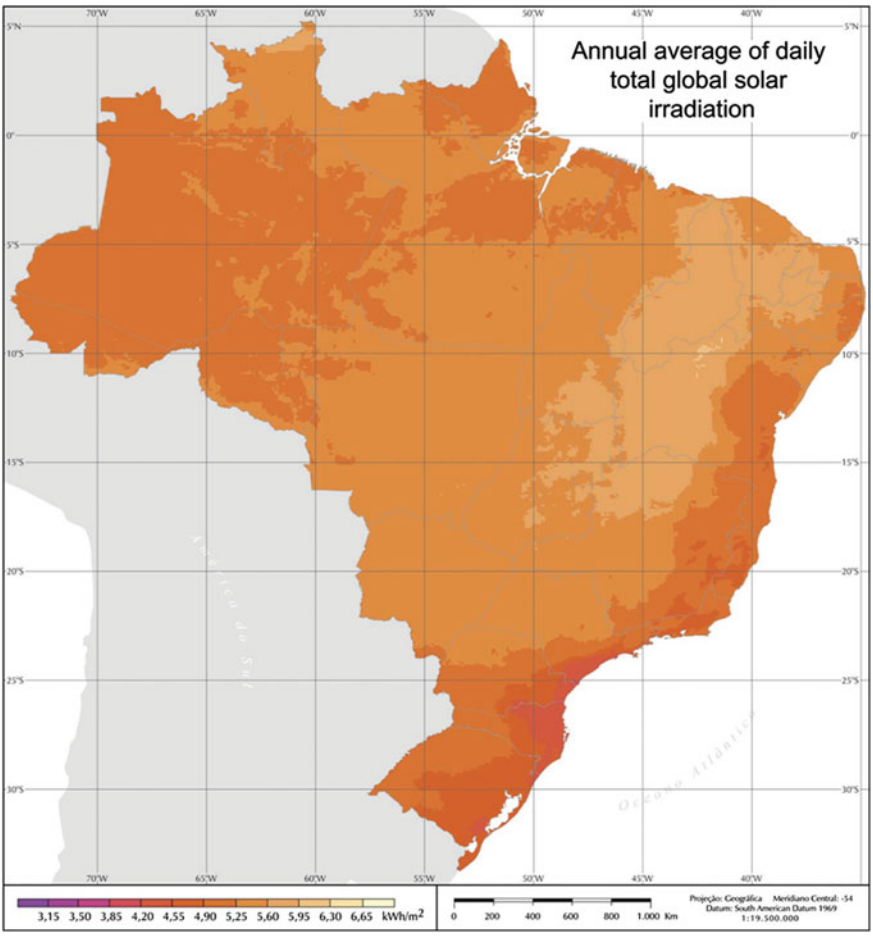

Fig. 19.6 Annual average of daily total global solar irradiation (Pereira et al. 2006)

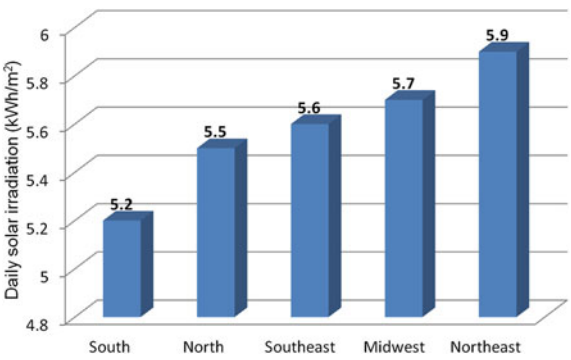

Fig. 19.7 Daily solar irradiation in Brazil for each region (data from Pereira et al. 2006)

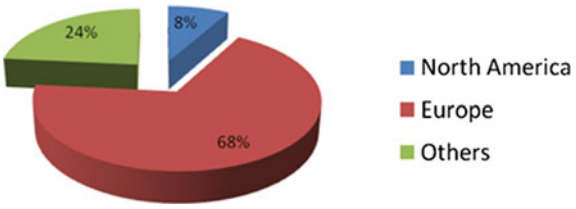

Fig. 19.8 Worldwide cumulative installed photovoltaic (PV) Power 2012 (IEA 2012b)

installation, is currently being built. Because domestic solar PV manufacturing units are in its infancy, the need to import all the components of solar PV systems adds to the solar energy cost in Brazil. That is likely to change and it is expected that the new solar power capacity will be competitive as compared to other electricity sources within 6 years. Figure 19.8 shows the share of all electricity production from photovoltaic power plants in the world.

The Brazilian's most important solar power plants (operating or under construction) are: Tauá power plant (SP), with 1 MWp of installed capacity, Tanquinho (SP) with 1 MWp, Alto do Rodrigues (RN), with 1.1 MW, Itajobi (SP), with 3 MWp, several sport arena (i.e., Mineirão (MG) with 1.5 MWp, Marcanã (RJ), with 3.3 MWp), and Pituaçu (BA) with 404,80 kW. According the Brazil's ANEEL, there are just 19 granted solar-based enterprise (6,637 kW) already in operation. There are many planned CSP plants planned or under construction: Araçuai (MG) (20 MW), Barra (BA) I and II (60 MW), Belém do São Francisco (PE) I and II (60 MW), Ceará (CE) I and II (60 MW), Jaguaretama (CE) I and II (60 MW), Dr. Miguel Arraes (PE) I and II (60 MW), Piauí (PI) I and II (60 MW), Remanso (BA) I to VIII (240 MW), Sousa (PB) I and II (60 MW), and Xique–Xique (BA) I to VIII (240 MW). According to the Empresa de Pesquisa Energética (EPE), the energy plan 2013-2022 plans a solar energy production of 1.400 MW by 2022.

19.2.3 Biohydrogen

About 45 billion kg hydrogen is produced annually (IPHE 2011), but most of it is used for industrial purposes. The dominant technology for hydrogen production is steam reforming from hydrocarbons (Fig. 19.9). However, there are many other production routes including electrolysis, thermolysis, and renewable energy technologies, such as wind, solar, geothermal, and hydroelectric power. Hydrogen production is an industry over a hundred billion dollars. Most of the produced hydrogen is used in oil refining, ammonia, and methanol production.

Biological hydrogen, known as biohydrogen, can be produced through direct biophotolysis, indirect biophotolysis, photofermentation, and dark fermentation (considered the rote with greatest potential by many). Table 19.5 presents the most promising methods and technologies for hydrogen production from renewable sources.

In Brazil, biohydrogen production has attracted great interest mainly due to the possibility of using renewable energy sources, as well as the reuse of waste

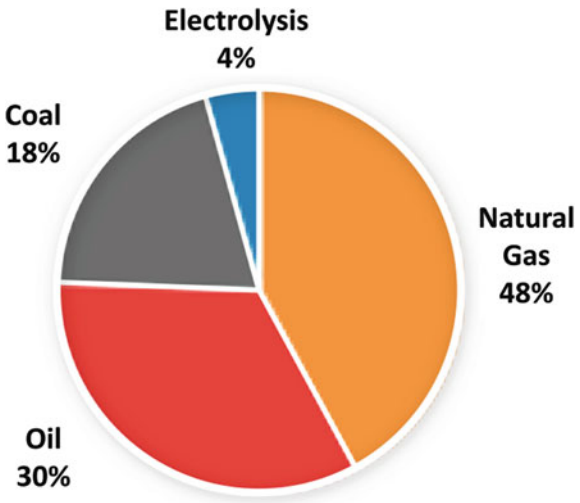

Fig. 19.9 Sources of current worldwide hydrogen production (IPHE 2011)

Table 19.5 Methods for hydrogen production from renewable sources (IPHE 2011)

Energy source	Technology	Process	Prospect
Wind, solar, geothermal, hydro	Renewable electrolysis	Electrolytic	Near-, Mid- and Long-term
Biomass	Gaseification	Thermal	Mid-term
Biomass: ethanol, bio-oil	Reforming		Mid- to Long-term
CSP	High temperature water splitting		Long-term
Microorganisms: algae, cyanobacteria	Photobiological water splitting	Photolytic	Long-term
Semiconductors	Photochemical water splitting		Long-term

materials (lignocelluloses or starch materials, glycerol, palm oil mill effluent, food and dairy wastes, paper mill wastes, among others). In a complementary production unit, one can exploit residual streams from first-generation ethanol, second-generation ethanol, and biodiesel production. A wide range of feedstock could be utilized to produce glycerol via transesterification of oils and greases. The Brazilian annual potential of electricity generation utilizing residues is 318.58 GWh with overall installed potential of 63.72 MW. The potential of electricity generation of other feedstock (castor bean, peanut, and soybean) is 1587 GWh/year with installed potential of 317.49 MW (Souza and Silveira 2011). Additionally, utilizing hydrogen produced through steam reforming of glycerol, high amounts of H-BIO, and biopropane could be produced. Electricity can be produced in a fuel cell which combines hydrogen and oxygen to produce electricity, heat, and water.

An intense research effort has been carried out in the entire world to improve the use of hydrogen as an energy source and also as a carrier of energy. The Brazilian Ministry of Science, Technology, and Innovation has promoted the development of initiatives for the hydrogen economy through programs such as ProCaC—Brazilian Program for Fuel Cells (Centro de Gestão e Estudos Estratégicos 2002) and *ProH*$_2$—Brazilian Program of Science, Technology, and Innovation for the Hydrogen Economy (Centro de Gestão e Estudos Estratégicos 2005, 2010). The main reasons for the country to develop hydrogen technology are: the availability of biomass, biogas, and ethanol can put the country on strategic condition for producing renewable hydrogen and participate in markets for capital goods and services associated with hydrogen.

The Brazilian path to the hydrogen economy is still undefined and many actions for their development (Centro de Gestão e Estudos Estratégicos 2005, 2010) are required: (a) creating a market for hydrogen energy from production to consumption; (b) definition of logistics and required associated developments; (c) implementation of pilot projects, with research and development integrated with collaborative action for exploiting hydrogen energy; and (d) dissemination of hydrogen technology.

19.2.4 Biogas

Sometimes referred to as biomethane, waste gas, or renewable gas, biogas refers to a gas produced by anaerobic digestion with bacteria or fermentation of biodegradable materials and it comprises primarily methane (CH_4) (50–75 %), carbon dioxide (CO_2) (30–40 %), nitrogen (N_2), and may have small amounts of hydrogen sulfide (H_2S), moisture, and siloxanes. Biogas technology (biomethanation), the generation of a combustible gas from anaerobic biomass digestion is a well-known technology.

A biogas plant yield depends not only on the type of feedstock, but also on the plant design, fermentation temperature, and retention time. The most common form of biogas use is to produce combined heat and power (CHP), including internal combustion engines, combustion gas turbines, microturbines, fuel cells, and steam turbines. The largest and most widespread barriers to biogas use are because they are economic, related to higher priority demands on limited capital resources or to perceptions that the economics do not justify the investment. There is also other uses of biogas, non-CHP applications, like injection of purified biogas into natural gas pipelines and as a vehicle fuel.

Germany is the largest biogas producer in the world. German biogas electricity production represented 14 % of total renewable electricity production in 2011. Germany biogas power generation is forecast to increase from 18,244 Gigawatt hours (GWh) in 2012 to 28,265 GWh in 2025. By comparison, the US, the second most productive biogas power producer, is expected to increase generation from a more modest 2012 figure of 9,072–20,936 GWh in 2025. The impressive growth

and current scale of the German biogas industry provides many important insights for other countries that are looking to expand biogas resources (Bilek 2011).

There are small- and medium-sized biogas power plants in Brazil, mostly installed in agroindustrial settings. There also exists biogas production in Brazil from landfills. The main purpose of these plants (based on waste of animal production facility or municipal solid waste) is sanitation and environmental protection, but also producing gas and electricity. In Brazil, in an energetic context only, electricity price is not high enough to guarantee a biogas power plant profitable operation without government incentives.

The growing demand for waste treatment processes and an increased focus on greenhouse gas mitigation are generating demand on a worldwide basis. According to the Atlas of GHG Emission and Energy Potential (2013), Brazil produced approximately 198,000 tons of municipal solid waste (MSW) per day in 2011, of which 90 % of the total waste produced was collected and of this, 58 % was disposed of in sanitary landfills, 24 % went to controlled landfills, and 17 % to dumpsites. Disposal sites have potential to develop greenhouse gas (GHG) mitigation projects, as the final product of decomposition of solid waste under confined oxygen-free conditions is biogas. Of the mitigation projects in Brazil, 50 % consider of capture/flaring of recovered biogas, and the other half consider the energy utilization of biogas. The overall installed capacity stated for electricity generation in the verified Project Design Documents (PDDs) of these projects is 254 MW. The potential of electricity production from biogas from MSW in Brazil is of 282 MW, with the regional share shown in Fig. 19.10.

From an industrial point of view, a great opportunity in Brazil for Biogas production from waste refers to the digestion of vinasse, a byproduct of the ethanol industry, once for each liter of ethanol produced it is also produced 13 liters of vinasse, there is a significant potential energy to be exploited. The stillage is currently used in fertigation, but its transformation into biogas constitutes an economic and environmental benefit. Furthermore, the current implementation requires appropriate monitoring, since the indiscriminate use of vinasse in fertigation can lead to acidification and leaching processes, with impact on soil productivity and contamination of groundwater.

From a commercial livestock industry point of view, Brazil has the potential for exploiting biogas from animal manure. Today there is a growing interest in biogas production and utilization, mainly in the states of Mato Grosso, Minas Gerais, Goiás, Paraná, Santa Catarina, and Rio Grande do Sul, where livestock breeding is predominant. Biogas is most commonly used on the farm where it is produced, mostly for electricity.

The Brazilian National Policy on Solid Waste (known by its acronym in Portuguese, PNRS), which was passed on August 2, 2010, provides the key regulatory framework for the waste sector in the country, and shall hopefully have a positive impact on mitigation and use of produced biogas.

The use of microalgae has gained attention in Brazil and is presented as a promising technology for the production of biomass for energy purposes, and may

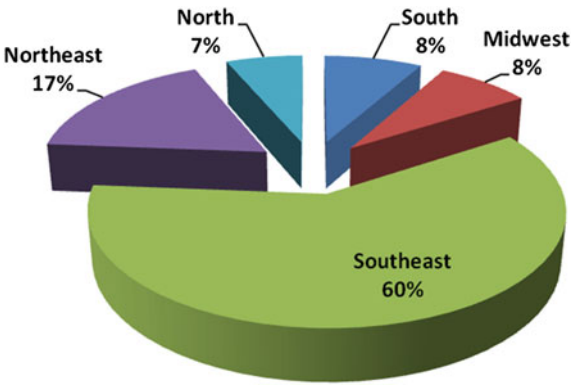

Fig. 19.10 Brazil biogas-to-electricity generation potential (APRELPE 2013)

have application in digestion and consequent production of biogas. Moreover, such systems may be implemented using exhaust gas heat systems allowing the capture of carbon dioxide, which in this case happens to be an input to the process of producing microalgae. The use of microalgae associated with genetics also opens up the possibility of development of liquid fuel in the form of alcohols and oils.

19.2.5 Biodiesel

The Federal Law No. 11,907 of 2005 defines biodiesel as a new fuel in Brazil's energy mix, and since 2008 2 % biodiesel component blended to 98 % diesel oil, known as B2. In the beginning of 2010, the mix requirement increased to 5 % (B5), 3 years ahead of the target established by law. There is the possibility for higher blend percentages up to pure biodiesel (B100) by authorization of the Brazilian Petroleum, Gas and Biofuels Regulator (ANP), which has regulatory and fiscal control.

In 2011, the amount of B100 produced in Brazil reached 2,672,760 m^3, against 2,386,399 m^3 in the previous year. Thus, there was an increase of 12.0 % in biodiesel available in the national market. Table 19.6 presents the installed capacity for B100 production in Brazil.

Additionally, in 2011, the percentage of B100 compulsorily added to mineral diesel remained constant at 5 %. The main raw material was the soybean oil (81.2 %), followed by tallow (13.1 %). Since the launch of the National Biodiesel Production and Use Program in December 2004, up to the end of 2011, Brazil avoided importing 7.9 billion liters of diesel, equivalent to a gain of about US$5.2 billion in the Brazilian trade balance. Nowadays, biodiesel blend is sold in more than 30,000 service stations around the country. Figure 19.11 shows the effect of the government action on the biodiesel production in Brazil. According to the (EPE), if the use of biodiesel in Brazil is kept at the 5 % (B5), the total production capacity in the country is guaranteed until 2019.

Table 19.6 Installed capacity of B100 production in 2012 (ANP 2013)

Producer	Location/State	Installed capacity (m^3/day)
ADM	Rondonópolis/MT	1,352.0
Agrenco	Alto Araguaia/MT	660.0
Bianchini	Canoas/RS	900.0
Bionasa	Porangatu/GO	653.0
Camera	Ijuí/RS	650.0
Caramuru	São Simão/GO	625.0
Caramuru	Ipameri/GO	625.0
Cargill	Três Lagoas/MS	700.0
Granol	Cachoeira do Sul/RS	933.3
Granol	Anápolis/GO	1,033.0
Olfar	Erechim/RS	600.0
Oleoplan	Veranópolis/RS	1,050.0
Petrobras	Candeias/BA	603.4
Others		10,183.1
Total		*20,567.8*

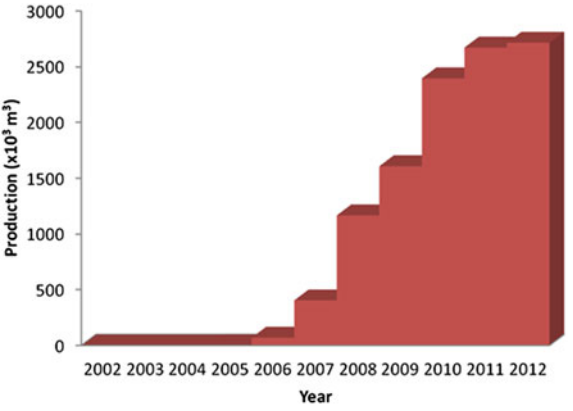

Fig. 19.11 Production of biodiesel (B100) in Brazil (data from ANP 2013)

There is also biodiesel produced from algae. However, although it allows a high yield per acre, current technology and the scale of production make the average cost per liter of biodiesel from algae of 5–10 times greater than the biodiesel plants such as soy, peanut or sunflower. Nevertheless, Brazil is planning to start the world's first algae-based biodiesel plant. The planned capacity is 1.2 million liters each year. The unit is yet to be approved by Brazil's National Petroleum Agency (ANP), but if approved it will be located in the Northeastern Brazilian state of Pernambuco (PE) and the facility will utilize carbon emissions from an ethanol producer unit from sugarcane to speed up the photosynthesis process in the seaweeds and reduce emissions of pollutant gases.

Figure 19.12 shows an outlook for B100 production in Brazil kept the same legislation and addition level in the country.

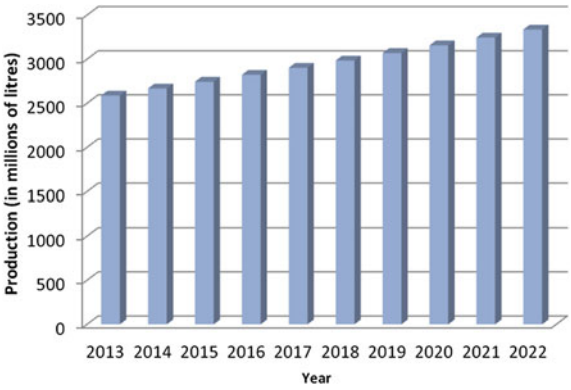

Fig. 19.12 Agricultural outlook 2013–2022 for biodiesel production in Brazil (OECD-FAO 2013)

19.2.6 Other Sources (Biobutanol, Geothermal and Ocean Energy)

19.2.6.1 Biobutanol

Biobutanol, or biogasoline, is an alcohol that is produced from biomass feedstock, by a thermochemical route with biomass gaseification or by fast pyrolysis with a bio-oil steam reform. Biobutanol can be utilized in internal combustion engines both as a gasoline additive and or a fuel blend with gasoline.

As an automotive fuel, butanol is recognized by its "drop-in" characteristics and is a superior blend stock that can be blended with gasoline, diesel, biodiesel, and ethanol (Mariano et al. 2013).

The energy content of biobutanol is 10 % less than that of regular gasoline, but it can be integrated into regular internal combustion engines easier than ethanol. Biobutanol has the potential to reduce the carbon emissions by 85 % when compared to gasoline (Dürre 2007).

Biobutanol is made via fermentation of biomasses and the difference from ethanol production is primarily in the fermentation of the feedstock and minor changes in the separation (Kumar and Gayen 2011). Perhaps the main bottlenecks of biobutanol production are: (a) low final biobutanol concentration caused by butanol toxicity; (b) many by-products, and (c) low biobutanol productivity. However, efforts are currently underway to improve the existing microorganisms used for fermentation and the separation costs of biobutanol from fermentation broth. Through a mixture of genetic engineering and new separation technology (based on membranes for instance), leads to thinks that biobutanol has a promising future in the biofuels market.

A promising development in biobutanol production technology has been recently discovered, and this can impact the biobutanol production in the next years.

A possible trend is the purchases of ethanol fermentation plants by biobutanol companies. These ethanol plants can be retrofitted with advanced separation systems to allow them to produce biobutanol; since biobutanol has inherently higher value than bioethanol this trend can change the biobutanol market in a small period of time. In this context, it is possible to see the formation of biorefinery (Vaz Jr 2011) that is able to integrate biomass conversion processes and equipment to produce fuels, power, heat, and chemicals with greater value from biomass. In Brazil, *HC Sucroquímica* is an example of a sugarcane biorefinery that is currently producing biobutanol (Mariano et al. 2013).

19.2.6.2 Geothermal

Geothermal energy is thermal energy generated and stored in the Earth. The main sources for geothermal energy are the heat flow from the earth's core and mantle (~40 %), and that generated by the gradual decay of radioactive isotopes in the earth's continental crust (~60 %) (IEA 2012a). Bertan (2012) presents the total installed capacity from worldwide geothermal power plant.

In Brazil, the total capacity of low temperature geothermal systems in use is estimated at 362 MWt and the annual energy use at 6536 TJ. About a dozen of the spring systems account for the bulk of this capacity. It is known that regions of very high geothermal gradients are absent in Brazil. This is a natural consequence of the fact that there are no areas of young volcanism or active tectonics. The best sites for extraction of geothermal energy in Brazil are the younger sedimentary basins, which makes the Paraná Basin a suitable place to tap geothermal energy. Furthermore, most of the major springs are located in central Brazil (in the state of Goiás) and in the south (in the state of Santa Catarina). The potential for large-scale exploitation of low temperature geothermal water for industrial use and space heating may be considered in southern and southeastern parts of Brazil, but at this date the geothermal exploitation is quite small and investments needed to convert this energy into electricity would appear to be too expensive at this time. However, other applications can be found like residential and commercial systems utilizing hot water.

19.2.6.3 Ocean Energy

Ocean energy can be defined as energy derived from technologies that utilize seawater as their motive power or harness the water's chemical or heat potential. The sources are: (a) wave; (b) tidal range; (c) tidal current; (d) ocean current; (e) thermal gradient; and (f) salinity gradient. The worldwide resource of wave energy has been estimated to be greater than 2 TW (Cruz et al. 2008).

According to a workshop leaded by Segen Estefen from Alberto Luiz Coimbra Institute and published by IEA, the total wave energy potential for Brazil is around 122 GW. The southeast of the country possesses circa of 50 GW of wave energy potential capacity along its coast, equivalent to three times the capacity of Itaipu

Dam. In Brazil, except for the project in the Port of Pecém (CE), with an installed capacity of 500 kW, the ocean energy potential has not been exploited.

The technologies for the exploitation of the energy potential of the oceans are the least mature. Due to the small number of prototypes in operation, there is still no relevant data on the costs, environmental impacts, strengths, and difficulties of integration with the distribution systems. This is because, with the exception of tidal technology with the use of dams, which has some commercial units operating in the world, all other developments are in precommercial prototype stage.

19.3 Nonconventional Renewable Energy Assessment

Certain aspects need to be considered to assess nonconventional renewable energy production and their potential use in Brazil. The very first point is the fact that the country has indicated that it is committed to maintaining a large share of renewable source in its energy matrix, as shown by the Alternative Sources Incentive Program (PROINFA). Second, Brazil posses a great variety of natural resources and much of this potential are still to be better exploited. Additionally, as the economy growth affects the demand for energy. Furthermore, to examine the competitiveness of renewable sources advantages such as: GHG emission avoidance, energy cost, job creation, required public investment, technology know-how, and the country potential for using it in a sustainable way should be considered (Estefen 2012).

Brazil has an electricity source mostly based on hydroelectric power, however, a mere 30 % of its potential are exploited, but the remainder potential is mainly located in the environmentally sensitive Amazon region, far from where energy it required the most. Additionally, drought greatly affects the country capacity for producing energy.

There are isolated villages and are difficult to access in the national grid, where small hydropower through simple domestic applications would be very promising to develop. According to the Electricity Regulatory Agency (ANEEL) data of 2011, there were 397 SHPs (Small hydroelectric plants) in operation in Brazil (3500 MW), and a growth rate of 10 % per annum with the total potential of around 25.9 GW. Therefore, SHPs are one of the main priorities of the ANEEL and they are ideally suited to meeting the energy demands of small urban centers and rural areas.

Ethanol production is expected to grow in the next decade (RFA 2013). However, the main issue with using sugarcane as a biofuel is the rise in price, which can directly affect consumers. That is why the country must also focus on second-generation biofuel (from non-food staples) so as to avoid this issue.

The use of wind energy will likely be consolidated to achieve as much success as ethanol and hydroelectricity for energy production in Brazil. Wind power requires high initial investments but less important operational costs. The strategy for structuring the wind energy market through public auctions ensured the

participation of the local content, encouraged domestic industry, and drove a reduction by 60 % in the price of wind energy after the PROINFA auction in 2011.

Nowadays, there are at least a dozen manufacturers producing wind turbines in Brazil making Brazil self-sufficient regarding wind turbines and wind farms equipments. Nevertheless, there exist certain difficulties in the consolidation process of wind energy (Silva et al. 2013). To answer to those points, ANEEL proposed a Strategic R&D Call No. 17/2013 (ANEEL 2013), whose main objective is to foster the development of domestic technology in the area of wind energy, seeking to reduce its dependence on technology of the country and to stimulate the production of innovative, high value-added technology adapted to the Brazilian reality.

Solar energy for water heating is by far the most widespread application of solar energy in Brazil, where solar heating industry is well developed and able to supply the country market for this resource. An interesting factor impacting the use of solar energy is that it allows a greater production when there is greater energy demand (Summer time and midday air-conditioning energy requirements). In addition, solar power also allows the implementation of mini-grids local operated. Unfortunately, unlike wind energy, solar energy is not a case of success in PROINFA results. The photovoltaic market in Brazil represents a promising market and is expected to grow quite fast in the coming years. Rural electrification is one of the underserved markets that can benefit from this technology.

Solar PV has also been the subject of intense discussions. In 2011, ANEEL through the R&D Strategic Call No. 13/2011 released the hiring of 18 projects, which aims at the development of technical arrangements and commercial power generation through solar photovoltaic technology, in an integrated and sustainable way. The action seeks to create conditions for the development of technological and technical infrastructure and technology for insertion of solar photovoltaic generation in the national energy matrix (Viana et al. 2011). Hopefully, the results of these pilot projects contribute to demonstrate the technical and economic viability of solar photovoltaic generation of electricity in the country. As the implementation period is 36 months, the results of the implementation of these projects will be available in the next couple of years.

The development of technologies for the production and use of biohydrogen has limitations mainly due to the low price competing energy. The great contribution of these programs was to structure research groups and laboratories with human resources to deal with the technologies associated with biohydrogen in Brazil and the next decade. Some important initiatives are linked to demo projects of city bus powered by biohydrogen, which are being developed in the cities of Rio de Janeiro and São Paulo. Another important step was the normalization by a Special Committee of Studies (EEC) for biohydrogen.

Biohydrogen production is subject to limitations of low conversion efficiencies. Furthermore, there are certain technological bottlenecks associated with the biohydrogen economy: (a) the price for hydrogen is established for its use as a chemical feedstock and this fact does not provide economic viability for energy use in the economy of today; (b) there are several ways to produce hydrogen, but with exception of reforming or water electrolysis, all other approaches require

further developments for a wide and advantageous use as a energy source; and (c) hydrogen use in fuel cells, still requires the development for durability, robustness and prices compatibles with competing technologies. Therefore, it is still necessary to compensate many aspects in order to get biohydrogen production economically feasible. In this scenario, the feasibility of the widespread use of hydrogen is restricted to certain market niches, such as backup power systems.

In the biogas context, the ANEEL has launched a R&D Call focusing on biogas (National Agency of Electric Energy 2012). The goal is to define a model of biogas system applicable to the sanitation sector, producing biogas in sewage treatment plants and the organic fraction of solid waste. Hopefully, the results of this call for strategic project contribute to demonstration and improvement of technical and economic feasibility of generating electricity from biogas derived from waste/wastewater in the country (MMA 2011). As the implementation period of this project is 36 months, the expectation of concrete results will occur around 2016.

The National Program for Production and Use of Biodiesel (PNPB) completed 8 years (Pousa et al. 2007). In this short period of time, the program was able to induce the formation of an industrial park able to meet a demand of about two and a half billion gallons of biodiesel. Nowadays, the dependence of soybeans and the difficulties in promoting the social inclusion of family farmers represent major PNPB challenges.

In all other energy sources covered in this text, there is not a structural framework and legislation in order to produce a competitive energy. Most of the initiative are from private groups (as in the case of biobutanol) or research prototype based mostly on universities.

Finally, regional organizations are fostering development of regional energy trade. Latin America countries could have great mutual benefits in collaborating in energy production and distribution, but many actions will need to be performed to make true that scenario.

19.4 Conclusions

Over the last two decades, energy policy in Brazil has sought to reduce the country's dependence on foreign energy supply and stimulate the development of domestic energy sources. However, it had left behind a huge unexploited potential of renewable energy sources. The effectiveness of success of the Brazilian renewable energy market strongly depends on legislation and country policies. The growth will be as fast as the country implement energy policy to support the renewable sources of energy by breaking economic, regulatory, or institutional barriers. Therefore, the impact of the nonconventional renewable energy in the success of bioenergy depends highly on the policy maker initiatives on seeking a variety of renewable energy sources incorporated into the energy matrix of the country.

The renewable energy future looks very promising in Brazil. However, it is necessary to develop national technology that requires skilled manpower and

scientific development, which depends on education and government policies. Many technologies are already a reality in other parts of the world. However, the national development of a given technology favors sustainable solutions and local application.

Country leadership has to be quite alert in order to guarantee the national development of energy routes that might not be a competitive one in today's energy market, but can be promising under different scenarios or with improved technological solution.

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