Chapter 1
Routes to Second-Generation Bioethanol in Brazil: Foundation of the National Institute of Science and Technology of Bioethanol

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Abstract Along the last 8 years, the National Institute of Science and Technology of Bioethanol (INCT-Bioetanol) reached several important milestones towards the main objective of producing second-generation bioethanol from sugarcane bagasse. The sugarcane cell wall structure and architecture is now better understood, dozens of new cell wall hydrolases had their genes and enzymes fully characterized, sugargane physiology and its responses to climate change were investigated, and novel yeast strains capable to metabolize pentoses have been discovered. At the same time, history and politics involved in sugarcane as a bioenergy source have been studied and revised. Thus, simultaneously with other large bioenergy programs in the USA and Europe that were set up to improve renewable energy production, the INCT-Bioetanol in Brazil managed to produce important data in basic research. All this data is expected to help industry to produce not only more ethanol, but also new biomaterials to foster bioeconomy. This book reports some of these findings.

Keywords Bioenergy • Bioethanol • Second generation • FAPESP • BioEn • INCT

The National Institute of Science and Technology of Bioethanol (INCT-Bioethanol) was founded in 2008, at the same time that other international initiatives such as the Energy Biosciences Institute (EBI) in the United States and the European Bioenergy Research Institute (EBRI) in England. The main goal of all those

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institutes was to contribute to increasing the knowledge and the development of new routes related to bioenergy.

The motivation or the creation of the INCT-Bioethanol was closely related to the launching of the fourth International Panel on Climate Change report (AR4) in 2007, which was strongly highlighted in Brazil. At the same time, the São Paulo Research Foundation (FAPESP) started the Bioenergy Research Program of São Paulo (BIOEN) and a few months latter, the Brazilian Government set up a series of strategic science and technology institutes (the INCT Program) to try to solve problems that could improve the industrial performance in the country. Just after the foundation of BIOEN, a group of researchers gathered in a workshop to discuss the scientific challenges to be faced in order to lead sugarcane bioethanol to a new technological status. After this workshop, 33 laboratories across six different Brazilian states became part of the INCT-Bioethanol proposal.

It was clear that the production of renewable energy could significantly contribute to mitigating CO_2 emissions in the planet, and that Brazil was already contributing to such mitigation due to previous development of consistent technologies to produce bioethanol from sugarcane. At that moment, however, the available technologies to produce bioethanol relied only on the use of sucrose (first-generation bioethanol), whereas the bagasse was being used exclusively to produce electricity and the leaves had no or little use. So, the presentations and discussions during the workshop that originated the INCT-Bioethanol proposal involved questions related to the possibility of use of those residues to significantly increase the production of bioethanol in Brazil and to the knowledge that would be necessary to achieve this goal. There were formidable problems to be solved and the group of researchers present at the meeting quickly realized that it was necessary to start a research project focused on basic science in order to produce the knowledge that could be used for technological development.

Some points highlighted during the 2008 workshop became milestones of INCT-Bioethanol for the decade to come. These milestones were the following:

1. *Understand the structure and architecture of the sugarcane cell walls*: Although the main components of sugarcane cell walls were already known, there was lack of knowledge related to the structure of the polymers (polysaccharides and lignin), and to the glycosidic linkages and interactions among polymers that needed to be explored to help understand how to hydrolyze sugarcane cell walls.
2. *Find ways to hydrolyze sugarcane cell walls*: In 2008, both sulfuric acid and enzymatic hydrolysis were available options to hydrolyze sugarcane bagasse. However, local initiatives such as the Dedini pilot plant that were using sulfuric acid ended up discontinuing its unit, and the acid route was abandoned. Thus, the focus started on the enzymatic routes. But, very little was known about enzymes capable of hydrolyzing biomass. More than that, it was clear that at the same time as the cell wall of sugarcane would become known, it would be possible to engineer enzymes to improve their catalytic properties. In addition, it was realized that modern molecular biology tools could be used to engineer the sugarcane, so that endogenous hydrolytic mechanisms could be controlled and used to help the hydrolysis as a biological pretreatment.

3. *Understand sugarcane photosynthesis and growth, especially under climate change conditions*: We were aware that the biomass-producing capacity of some of the sugarcane varieties was much higher than it was seen in the field. Thus, it was possible to turn sugarcane even more productive than thought at that time. For that, physiological, metabolic, and gene expression sugarcane responses to different stresses would have to be deeply studied, so that we would be able to give support to the production of sugarcane in a changing climate.
4. *Understand sugarcane genome and genetics*: We knew that it would be quite complicated since sugarcane is a polyploid and we needed to develop new tools to manipulate sugarcane genome and genetics. The traditional breeding coupled with the development of those new tools would bring really great advances in this area. Another challenge was to genetically transform sugarcane. As we intended to learn more about gene expression related to several processes, it would be natural to transform plants either for purely scientific purposes (proofs of concepts) or to produce new varieties that could actually be used in the field.
5. *Find ways to improve extant yeast strains or discover novel species of them that could ferment pentoses efficiently and produce ethanol*: Although the Brazilian 1G technology was highly efficient for ethanol production from sucrose, the strains used did not process pentoses with the same efficiency. Thus, genetic manipulation of known strains and screening of microorganisms capable of fermenting pentose were necessary.
6. *Develop new pretreatments and associate them with hydrolysis and fermentation*: Sugarcane biomass, both bagasse and trash, were hard to hydrolyze and new methods to pretreat biomass had to be tested in order to suggest possible procedures to be used in industry.

The main mission of the INCT-Bioethanol was the production of knowledge that could help industry, rather than the development of specific technologies. Other organizations were set up at the same time to take care of the technological development, such as the CTBE (National Laboratory of Science and Technology of Bioethanol) in Campinas, São Paulo. This center was designed at that time to bridge the gap between basic science and technological development of the sector, making the links with industry.

After 8 years of research in this field, Brazil has developed a couple of initiatives to produce second-generation (2G) ethanol from sugarcane, but the integration of the basic knowledge into industrial technology has proven to be rather slow. It has to be noted, though, that the forecasts made by the INCT-Bioethanol were that the basic research needed at least one decade to produce enough knowledge and provide an efficient connection with industry. Therefore, we expect that the knowledge produced by the INCT-Bioethanol will start to be incorporated within the middle of the second decade of the twenty-first century, depending on the economic return that such incorporation signalizes to the companies.

The INCT-Bioethanol has not yet completely solved yet the main problems stated in the six milestones listed above, but it has significantly advanced the science necessary for sugarcane bioethanol technology development. Some of the achievements made and their implications to society are shown in Fig. 1.1. The

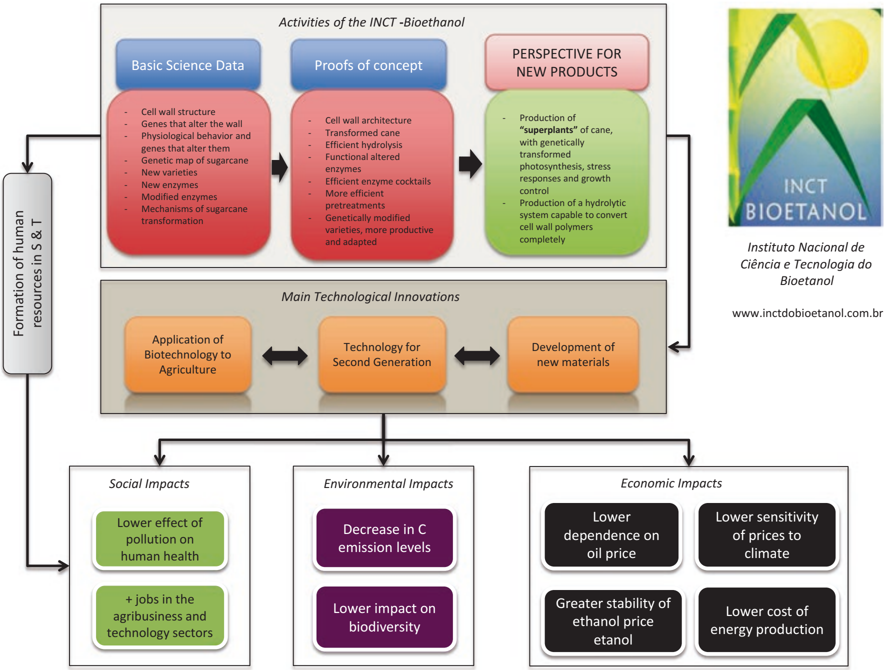

Fig. 1.1 Scheme with the main activities of the INCT-Bioethanol and its consequences for innovation and sectors of the society

science produced by the institute has been reported in hundreds of papers, book chapters, and theses released by the research groups along the last 8 years (http://www.inctdobioetanol.com.br). The first book was published in 2011, reporting the advances reached at that time (Buckeridge and Goldman 2011). We now present a second book, which brings chapters that update aspects related to the sugarcane cell wall (Chaps. 2, 3, and 4) and genetics (Chap. 9), enzymes (Chaps. 5, 6, and 7), pentose fermentation (Chap. 8), sustainability (Chap. 10), and international policies (Chap. 11).

Although this book does not cover all the science produced by the INCT-Bioethanol, it brings an important sample of some of the main achievements reached by the group after almost one decade of hard work. We expect that the scientific knowledge compiled in this book will help students, researchers, and the industry to improve even more the production of 2G bioethanol, influencing industrial and environmental sectors and helping to use the benefits of renewable energy for mitigation and adaptation to the global climate changes.

Reference

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