FAPESP BIOEN Program

Highlights 2010 - 2016

BIOEN

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Léo Ramos Chaves/Revista Pesquisa FAPESP

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Credits

EDITORS

BIOEN-FAPESP Program Coordinators

Glaucia Mendes Souza (Institute of Chemistry, University of São Paulo)
Marie-Anne Van Sluys (Institute of Biosciences, University of São Paulo)
Rubens Maciel Filho (Faculty of Chemical Engineering, University of Campinas)
Heitor Cantarella (Campinas Agronomic Institute)
André M. Nassar (Agroicone)

FAPESP Scientific Director

Carlos Henrique de Brito Cruz (FAPESP)

PRODUCTION COORDINATOR

Mariana P. Massafera (BIOEN-FAPESP Secretariat)

DIAGRAMATION AND INTERVIEWS

Jornalismo Júnior, ECA-USP

PHOTOGRAPHY

Claudio Arouca (FAPESP)
Eduardo Cesar (FAPESP)
Leandro Negro (FAPESP)
Léo Ramos Chaves (FAPESP)

ACKNOWLEDGEMENTS

Maria da Graça S. Mascarenhas (Gerência de Comunicação, FAPESP)
Tatiane Britto Costa (Gerência de Comunicação, FAPESP)
Mônica Luri Shindo *(Agência FAPESP)*
Alexandra Ozorio de Almeida *(Pesquisa FAPESP)*
Valter Rodrigues *(Pesquisa FAPESP)*

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MISSION

The BIOEN Program aims to integrate comprehensive research on the bioenergy supply chain from field to users, thus assuring Brazil's position among the leaders in the area of Bioenergy. Research includes biomass production from sugarcane and other plants, processing those to biofuel and bioelectricity production as well as evaluating their impacts and sustainability.

BIOEN: impactful results and international leadership in Bioenergy

FAPESP began to plan its BIOEN program in 2005. More than 300 researchers currently participate in the program, which is co-funded by private companies such as Boeing, Peugeot-Citroën, Microsoft, Braskem, Oxiteno, Mahle Metal Leve, and Vale, as well as by overseas agencies such as BE-Basic, BBSRC (United Kingdom) and the European Union.

FAPESP's scientific director, Professor Carlos Henrique de Brito Cruz, says the program creation had three major drivers. Firstly, bioenergy is economically and environmentally crucial for the state of São Paulo and for Brazil as a whole. Secondly, to broaden research to address the research challenges associated to commercially producing second- and third-generation sugarcane bioethanol (which remains a challenge globally, according to Brito Cruz) integrating those processes with the first-generation ethanol production. And thirdly, raise the profile of Brazilian scientists within the international debate on bioenergy, particularly in the field of sustainability: traditionally, American and European scientists led such discussions. As a result of this third objective, BIOEN researchers, jointly with two other FAPESP programs (BIOTA and Climate Change), led a team of 137 researchers from 24 countries and produced the SCOPE Bioenergy & Sustainability Report, which was published in 2015.

As FAPESP was organizing BIOEN, worldwide interest in low carbon fuels was growing, driven by the increasing recognition of global climate change, so that the program was naturally integrated into a global initiative of the utmost importance – the substitution of fossil fuels with renewable fuels to decrease green house gases emissions.

With local motivation alongside world interest in an issue so vital for Brazil, it was necessary to identify academic leaders with the ability to embrace the challenge and bring together the scientific community. The leadership of Professors Glaucia M. Souza and Marie-Anne Van Sluys was essential in the creation of BIOEN, with its first calls for projects announced in 2009. The program was organized around a core of basic science with countless opportunities for connections to the development of applications, in such a way that several companies have signed up to the program. The program's wide range of topics is a distinguishable factor, as it ranges from molecular biology of plants and microorganisms to sustainable agriculture and the application of biofuels in engines and fuel cells.

As next steps, BIOEN intends to intensify research into sustainabil-


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ity and the socio-economic impacts of bioenergy production, as well as seeking to better develop the linkage between first- and second-generation ethanol production: "What we thought in 2007 was: 'We need to get into second-generation production in order to leave the first generation behind.' We now know that we need to get into second-generation production in order to have more first-generation production, and that first-generation production enables second-generation production to be competitive," says Brito Cruz.

"BIOEN has been successful because it has brought together São Paulo's scientific capacity with a technological opportunity that has a great potential for economic and social impacts," Brito Cruz says, and goes on to comment: "Projects carried out in partnership with corporations have boosted São Paulo's scientific and technological development because the scientific capacity we have here is competitive on a world scale."

The BIOEN program is organized into five divisions:

1. Biomass for bioenergy;

2. Biofuels technologies;

3. Biorefining
(alcohol chemistry, sugar chemistry, oil chemistry);

4. Engines
(the application of ethanol in automobile engines: internal combustion engines and fuel cells; aviation applications);

5. Sustainability
(social, environmental and economic impacts).

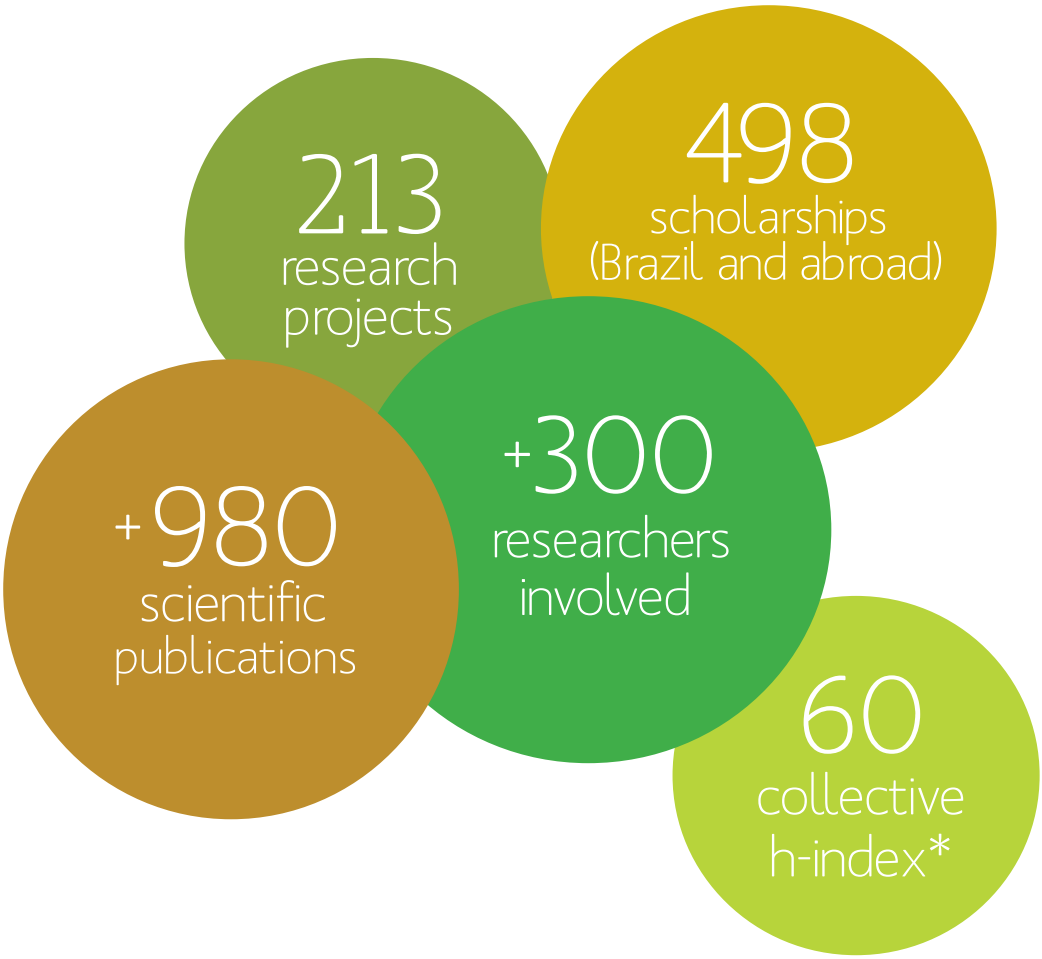
Soundly underpinned by research in topics related to these five areas, the Program aims to generate a large repertoire of knowledge and train scientists and professionals to further develop the ethanol and biomass industries and related technologies.

BIOEN is one of the most important bioenergy research programs in the world because of its combination of joint basic and applied research efforts, the prime quality of its scientific production, and the many examples of strong networks set right from the start. Furthermore, the project is groundbreaking in its ability to transform high-quality scientific production into services, products, jobs and income.

FIGURES

BIOEN has achieved significant advances towards its initial objectives. Around 1,000 scientific publications were published, and the projects encompass 24 fields of knowledge and more than 300 researchers from 30 countries. Over the last six years, the program has organized some 40 scientific events, and its coordinators have given more than 120 lectures on the activities carried out and the scientific results obtained within BIOEN.

At present, 213 research projects and 498 scholarships (in Brazil and overseas) have been granted within BIOEN, and the excellent research carried out by its researchers has been acknowledged in the shape of several awards (around 20), won by faculty and students. Until 2016, around $200 million had been awarded in the form of investment by government (including State Universities and Research Institutes) as well as investment received from seven private companies that are co-funding some of the projects. In total, BIOEN has 14 partners from private enterprise and the government sector, providing institutional support as well as direct participation in the Program's activities.



*according to Google Scholar, on Jan 2017

7
private companies co-funding projects

24
fields of knowledge

BRL 115mi
public investment as research grants

25
patents filed

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PARTNERS

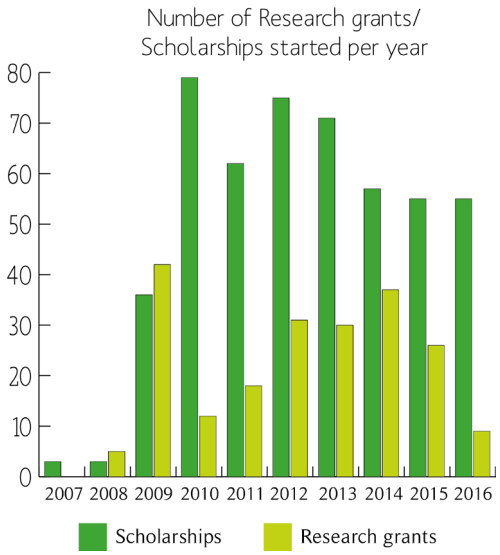
The program seeks to encourage applied research. It does this through partnerships with corporations interested in cooperation between their own laboratories and academic laboratories in order to promote joint investigation. Partnership projects are chosen by means of requests for specific proposals, and are analyzed in line with FAPESP's usual rules.

To date BIOEN includes projects co-funded by companies such as: Braskem, ETH Bioenergia, Mahle Metal Leve, Microsoft Research, Oxiteno, PSA Peugeot Citroën do Brasil, and Vale. Among other important partners of the program are: BBSRC, EU, BE-Basic Consortium, Boeing, Dedini, Oak Ridge National Laboratory, and Research Councils UK. FAPESP also supports research in smaller companies whose output is linked to the scientific fields within BIOEN. Approximately 15% of the funds allocated for the program go into partnerships with small entrepreneurs (PIPEs).

HUMAN RESOURCES TRAINING

In order to train highly-qualified human resources, the State of São Paulo Bioenergy Research Center (Centro de Pesquisa em Bioenergia do Estado de São Paulo — SPBioenRC), a joint initiative bringing together FAPESP, USP, UNICAMP, UNESP and the State of São Paulo Government, was created and hired 20 new faculties for bioenergy activities; this has led these universities to build laboratories dedicated to research in this field. In August 2015, a Workshop entitled "New Researchers and the Expansion of Bioenergy Research" was held at FAPESP São Paulo, where research projects were presented by the newly hired faculties, and SPBioenRC was officially launched. The São Paulo State Universities set up a Joint International Bioenergy Graduate Program to welcome students from around the world and provide them with training at Ph.D. level.

Workshop "New Researchers and the Expansion of Bioenergy Research" — Launch of the SPBioenRC. FAPESP, August 4th, 2015



DISSEMINATING THE KNOWLEDGE

Over the last six years the program has organized countless events such as conferences, meetings, and workshops, as well as postgraduate disciplines. One highlight is the Brazilian Bioenergy Science and Technology Conference (BBEST), which has become a Brazilian benchmark in the field of bioenergy, and has been staged twice in the town of Campos do Jordão. The first edition took place in August 2011, and brought together such renowned local and international researchers as José Goldemberg, Chris Somerville, Richard B. Flavell, and Bruce Dale. The second edition of BBEST was held in October 2014. Scientific and technological breakthroughs in bioenergy were discussed, as well as the potential of global biomass production, its impacts, and also strategies for disseminating bioenergy production and use sustainably throughout the world. On the occasion, four parallel sessions dedicated to a selection of BIOEN projects were held, enabling members of BIOEN's International Advisory Board to carry out the annual assessment of the Program. The next BBEST will be held in 2017.

BIOEN also organizes a Cycle of Seminars, which takes the form of a graduate course, taught by lecturers and specialists in bioenergy and broadcast live on the internet. The Seminars entitled Topics in Bioenergy, Biofuels, and Renewables Chemistry were offered in 2016 for the third time, now as part of the activities of the International Ph.D. Program in Bioenergy (PIPG-Bioenergia).

BBEST 2011

BIOEN RESEARCH HIGHLIGHTS

>> How to join BIOEN

There are two ways of requesting to join the BIOEN Program: one is for ongoing projects, while the other is exclusively for new projects.

Detailed instructions can be found at:

bioenfapesp.org/joinbioen

The foundation to achieve sustainable expansion of bioenergy production is biomass yield efficiency. Whatever the final application — whether for liquid biofuels, bio-electricity, plastics or other compounds — high-yield plants are essential in order to bring down costs and minimize the amount of land required. Additionally, biomass for bioenergy should ideally be produced with a limited use of inputs or water in order to keep environmental impacts to a minimum. Research in biomass ranges from agronomy and plant breeding, development of biotechnology and the use of systems and synthetic biology approaches to boost yields.

In agronomy, BIOEN supports research into soil fertility and plant nutrition, soil handling, and pest and disease control. Although advances in these fields are incremental, they are no less important. For example, they enable adjustments in recommendations for fertilization in order to improve yields. Recommendations for micronutrients in sugarcane have therefore been revised as a result of studies developed within BIOEN that demonstrated a need for the use of larger amounts of zinc. However, results

BBEST 2014

can sometimes be unexpected. Research has shown that mineral fertilization cannot yet be replaced by the inoculation of nitrogen-fixing bacteria, and this suggests that there is still some way to go before supplying sugarcane with nitrogen fixing bacteria becomes fully feasible.

In the field of plant breeding, BIOEN has set up a far-reaching research network in conjunction with the State of São Paulo breeding programs IAC and RIDESA. The infrastructure needed for crossbreeding ancestral lineages of sugarcane, using controlled photoperiod greenhouses that allow progenies carrying characteristics of interest to be obtained and germplasm to be characterized, was set up within IAC. RIDESA has characterized progenies that differ in terms of sugar content, and has created a panel of varieties that is being mapped. Both genetic improvement programs seek to broaden the genetic foundation used in their sugarcane improvement programs, and are working on molecular tools with colleagues from USP and UNICAMP for molecular marker assisted breeding. .

In biotechnology, BIOEN experts have sequenced the genome of a commercial cultivar and developed a new assembly algorithm in partnership with Microsoft Research. A research network for sequencing the sugarcane genome has been organized that involves groups from Brazil, USA, France, Australia and China, and runs an annual workshop during the Plant & Animal Genome Conference in San Diego to discuss the results achieved. Today the sugarcane genome is held to be the most challenging genome in terms of sequencing and assembly because of its multiple copies and chromosomes. Obtaining a reference genome paves the way for the development of tools for sugarcane biotechnology, despite the use of tools developed for other plants, even if such tools are not always suitable. Again within the field of biotechnology, BIOEN has groups working on the development of genetically modified sugarcane plants, achieving results in the increase of sugar content, increased saccharification and drought tolerance. These developments are important not only because they enhance yields, but also because they help develop second-generation ethanol. The community has also geared major efforts to introducing systems biology approaches, producing results in the analysis of the transcriptome, proteome and metabolome, and has worked to integrate these results.

In the development of biofuel production, what stands out is research enabling the improvement of first generation processes (those processes using sugarcane juice) in all their stages, which brings significant yield gains (increased ethanol, energy and sugar production for example) with reduced production costs, promoting lower needs of energy to the process, and considerably reducing water consumption. Critical steps such as fermentation and obtaining hydrous and anhydrous ethanol have been extensively investigated and improvements have been proposed. Meaningful contributions have been made for the development and assessment of second-generation processes which uses lignocelullosic materials such as sugarcane bagasse and the residues of agricultural and forestry activities to produce ethanol. These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Members of the Scientific Advisory Committee — SCOPE Bioenergy & Sustainability volume. FAPESP, February 28th, 2013

processes have been shown to have the potential to increase ethanol production without increasing the sugarcane planted area, thus enabling more sustainable production of the biofuel. Surveys have shown that it is feasible to integrate first- and second-generation processes, thus forming the ideal environment, even worldwide, for developing processes and enhancing them in order to reduce production costs (using first generation as a learning curve for second generation). Pre-treatment stages involving hydrothermal, acid catalyzed hydrothermal, steam explosion, organosolv and AFEX processes have been researched, which were the first five methods tested at the laboratory and pilot scales. Pioneering results in the kinetics and conversion of these processes, as well as the definition of operating conditions so as to maximize efficiency, have been obtained by the projects funded within BIOEN. Research into acid hydrolysis and above all enzyme hydrolysis has been developed and has shown which next steps are necessary, as well as critical points to be considered at this stage of the advance of the second-generation ethanol production process. Another standout has been research looking into different ways of integrating first- and second-generation processes with an emphasis on the production of electricity to be exported to the energy grid, which is actually a competitive advantage within the Brazilian process of second-generation ethanol production. A range of conceptual projects have been put forward and assessed using laboratory data, pilot plants and prediction methods based on mathematical models and process simulators, enabling decisions to be made that take economic, environmental and sustainability data into consideration.

A network of scientists jointly with private sector collaborators has made significant contributions to the field of biorefining. Processes to obtain products ranging from commodities to high value-added products (including medical, cosmetic and foodstuff applications) have been put forward and assessed. The bio-refinery concept presented and widely discussed in studies funded by BIOEN encompasses a vast range of options to add value to agribusiness by providing data, information and solutions with the potential for real development of the so-called bioeconomy. Solutions to obtain chemicals from ethanol have been put forward (such as polymers and solvents) based on the fermentation of first-generation sugars (the biochemical route) as well as through the thermochemical route. Several concepts and proposals for conceptual projects have been worked out and assessed showing the potential of biorefineries to bring economic and social benefits to the State of São Paulo and Brazil as a whole.

In the field of sustainability, BIOEN initiatives have considerably extended knowledge on the environmental impact of sugarcane growing, demonstrating that the production of ethanol and the cultivation of sugarcane have beneficial effects on the reduction of greenhouse gases (GHGs) and on carbon stocks in the soil. It is known that nitrous oxide emissions caused by nitrogenous fertilizers in the field have a great impact on ethanol's GHC balance. BIOEN studies have shown that nitrous oxide emissions in the São Paulo sugarcane crop are well below the reference figures used by the IPCC. However, with the application of vinasse, nitrous oxide emissions from nitrogen fertilizers rise considerably. Additional studies are ongoing to reduce this unwanted effect. The importance

Launch of the SCOPE Bioenergy & Sustainability volume. FAPESP, April 14th, 2015

of not burning the straw that is left on the field after harvesting, thus increasing the stock of carbon in the soil, is better understood today. This not only improves the quality of the soil and thus enables sustainable biomass production, but also is one way of mitigating global warming by removing CO_2 from the atmosphere. Proper soil management, reducing the amount of tillage, also helps keep carbon stocked in the soil.

In addition to studies of environmental impacts, the BIOEN Program has enabled greater knowledge of the international ethanol market. Analyses of the growth of sugarcane crops, the modeling of changes in the area under other crops, and changes in land use in Brazil have played an important role in influencing international dialog and demonstrating that Brazilian ethanol is an advanced biofuel. These studies have contributed also to the discussion of the public policies necessary for the expansion of bioenergy worldwide.

In recent years bioenergy has been extensively analyzed in regard to sustainability issues including environmental, social and economic impacts of land-use, industrial practices and possible benefits resulting from its use and production. SCOPE (Scientific Committee on the Problems of the Environment) is a body set up in 1969 to scientifically analyze environmental issues worldwide. Jointly with SCOPE, FAPESP engaged three of its research programs, given the current world context, to undertake in-depth studies on the sustainability of bioenergy: BIOEN (Bioenergy Research), BIOTA (Studies of Characterization, Conservation, Restoration and Sustainable Use of Biodiversity) and RPGCC (Research Project in Global Climate Change). Glaucia Souza, BIOEN coordinator and leader of the FAPESP-SCOPE Bioenergy & Sustainability Project, points out the importance of this multidisciplinary approach: "It is an outstanding work in part because we have involved three of FAPESP's major programs to analyze the problems of mankind and the environment through different approaches."

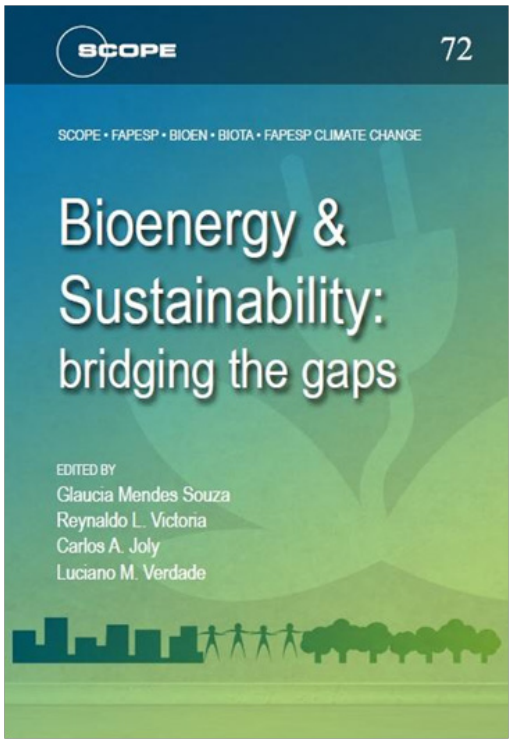
SCOPE Bioenergy & Sustainability mobilized 137 researchers from 24 countries and 83 institutions that met at UNESCO in Paris to discuss how bioenergy performs in the energy security, food security, environmental and climate security, sustainable development and innovation nexus. The results led to a 700-page book, described today as the "bioenergy Bible." It brings together a synthesis of scientific knowledge to inform policy-making, and has been downloaded more than 36,000 times. Controversies associated with the production and use of bioenergy are also presented: (i) the feasibility of producing it in different countries, the conflict between the production of food and the production of energy, (ii) social benefits and environmental issues per se, such as the impact of changes in land use on biodiversity and ecosystems, all of which are hotly debated throughout the world. The issue of public policies that are necessary to expand sustainable bioenergy pervades all of these discussions precisely because it is the core of the work in SCOPE, an agency that produces science-based recommendations in order to help create policy.

The conclusions of the study show that there is enough land to

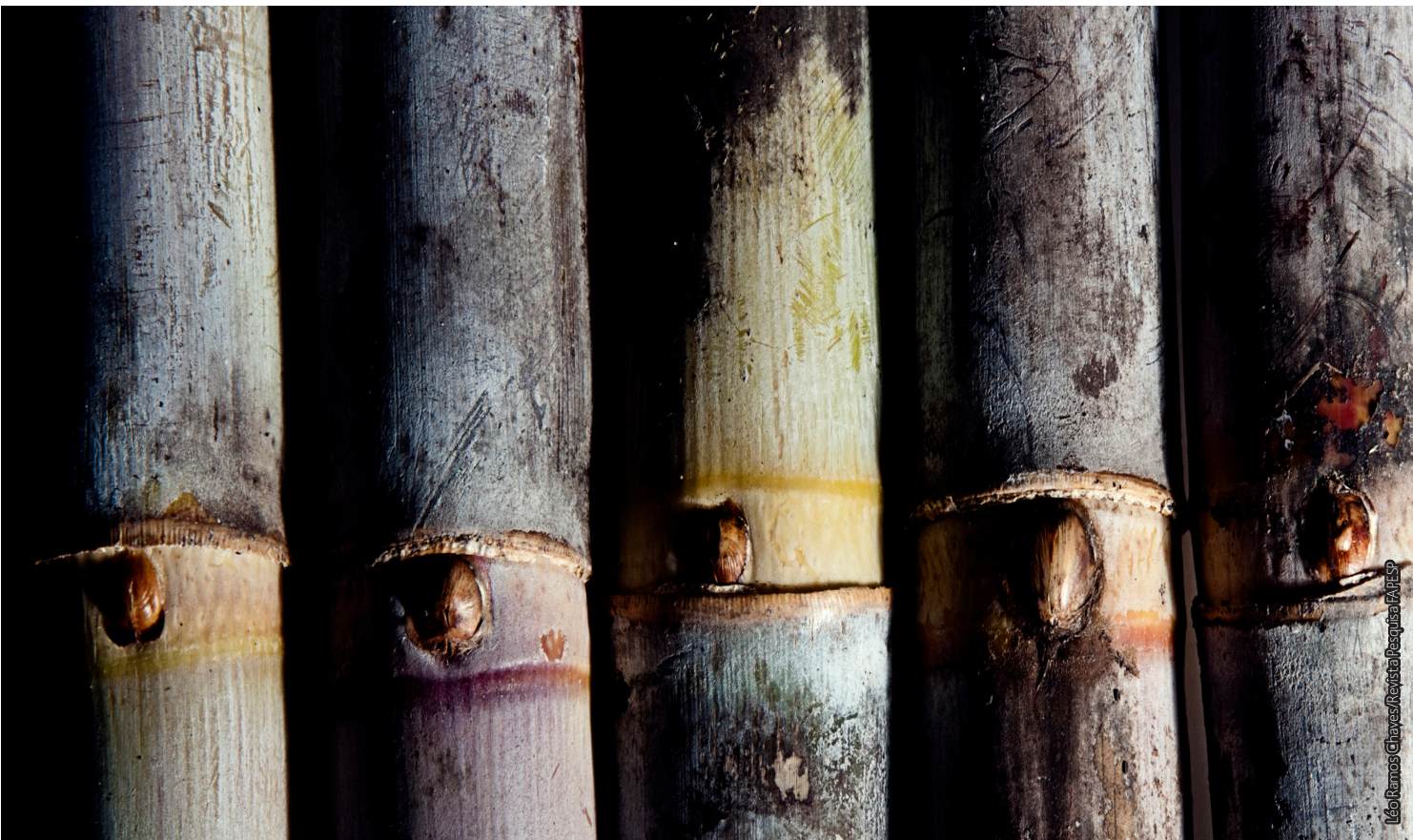
enable the expansion of bioenergy without competing with the production of foods, and that if managed sustainably, bioenergy can make a significant contribution to the solution of environmental, social and economic problems. Also, it became clear that there is no single solution.

The group involved in the project also produced eight scientific articles and an editorial footnote in the Environmental Development Journal as well as a policy brief, which is essentially a summary of the material in the work with objective recommendations to inform policy makers. The summary aims to guide and inform politicians in many countries about these issues so that their decisions can be technically and scientifically sound. After all, sustainable policies do not only affect the economy: they also influence social development. The policy brief is available in Portuguese and in English.

The project proved very important in a context where dependence on fossil fuels is less and less feasible and bioenergy is proving crucial for climate change mitigation efforts. Building a diversified energy matrix is essential and alternative, less-polluting technologies that consume less energy and do not contribute to the greenhouse effect are increasingly necessary; a scientific basis is a vital part of the process for delivering such options.



Cover - SCOPE Bioenergy & Sustainability volume



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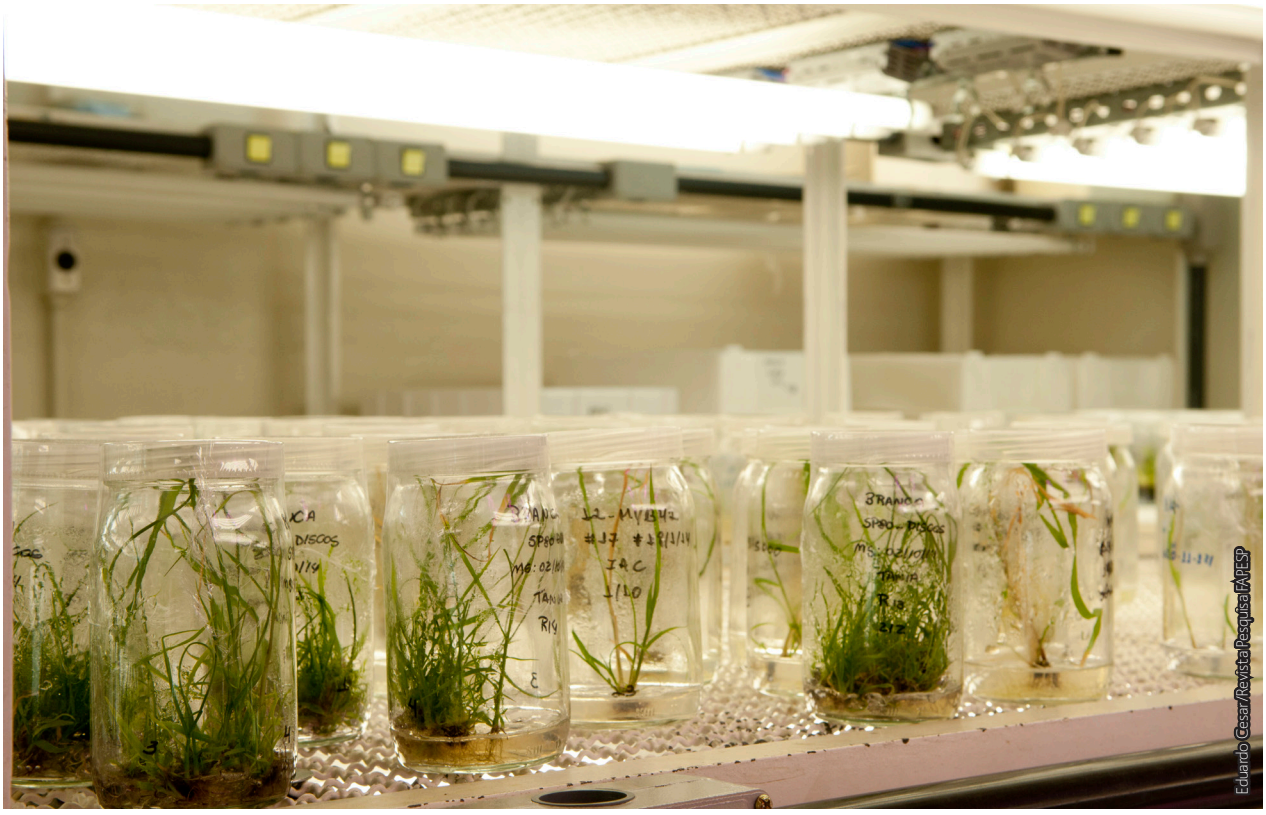
BIOMASS DIVISION

NEW PRODUCTION POSSIBILITIES FOR GM SUGARCANE

Study of lignin metabolism in plants promises production of cheaper, more efficient ethanol

This study, coordinated by Professor Paulo Mazzafera from UNICAMP (State University of Campinas, Institute of Biology), begun in 2008 and it focuses on the cell-wall polymer lignin. Lignin is a component of the cell wall, where cellulose — a polymer of glucose — is also found, and which, when unfolded, may undergo fermentation to produce second-generation ethanol, also known as lignocellulosic ethanol. The project had four main goals. 1) to analyze lignin, sucrose and cellulose in sugarcane varieties, differing in lignin content, grown in six different locations in São Paulo State. These locations had temperature, water availability, and irradiation characteristics. Based on the results, a study of genetic expression of lignin related genes and a more detailed analysis of lignin composition was planned. 2) to search SUCEST (a database of Expressed Sequence Tags) for ESTs coding for transcription factors involved in the lignin metabolism and use the information in studies with plants grown under greenhouse controlled conditions (water supply, nitrogen fertilization, light intensity, low temperature) and field conditions, in order to establish correlations between transcription factors gene expression and lignin composition. 3) to search for ESTs coding for orthologs of peroxidases and laccases, and to use the information in controlled studies to assess the involvement of these enzymes in lignin biosynthesis. 4) to carry out a system biology study using the data emerging from biochemical and gene expression studies.

The interest in identifying the genes involved in lignin biosynthesis in commercial sugarcane varieties lies in the possibility of using these genes to create genetically modified plants with reduced lignin content with altered composition. Lignin in the cell wall interweaves with cellulose fibers and restricts



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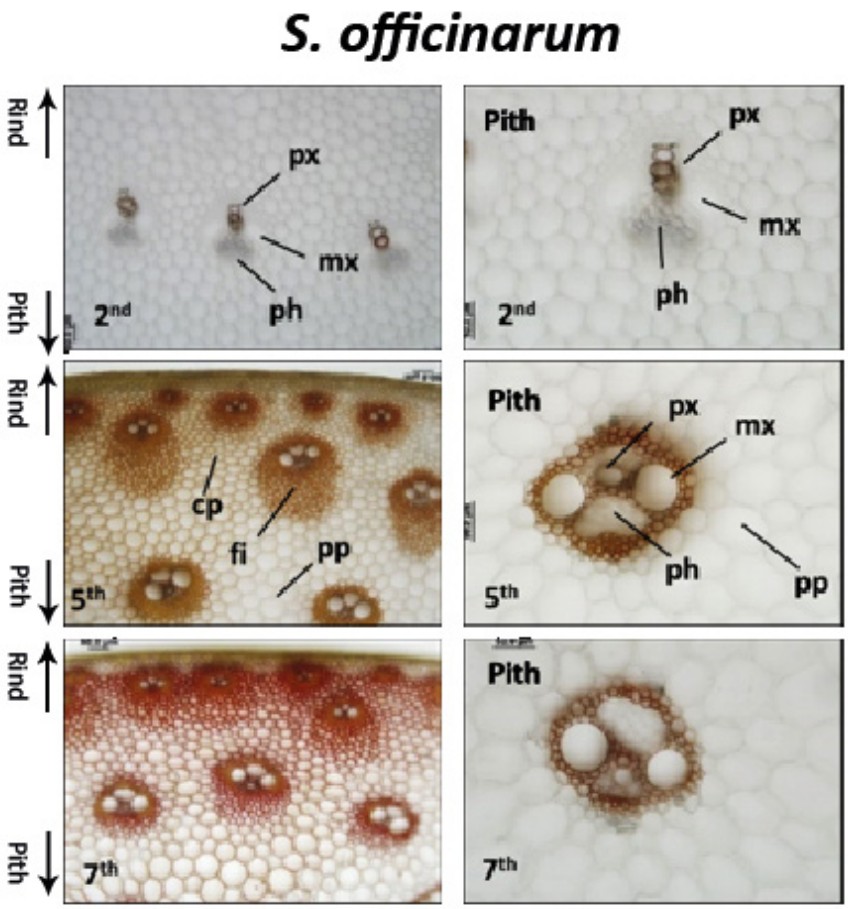
the access of hydrolytic enzymes that can break down cellulose into glucose units, which in turn can be fermented and give rise to the second-generation ethanol. Additionally, lignin can inhibit the growth of the yeast used to ferment glucose. Cutting down the quantity of lignin can therefore enhance the fermentation process. The lignin composition can also be changed so as to increase the efficiency of fermentation, since the nature of its components can lead to greater or lesser recalcitrance.

SUCEST was used in order to isolate the genes identified and linked to lignin biosynthesis, and their expression was evaluated in nursery and field assays. The results led some genes to be selected as candidates for further trials to change the quantity or composition of lignin in sugarcane through the production of genetically modified plants.

The research enabled post-doctoral student Dr. Paula Nobile, who took part in the initial study, to carry out the actual sugarcane genetic transformation with the selected genes. Dr. Nobile is currently heading a Young Researcher (Jovem Pesquisador — JP) project funded by FAPESP that is ongoing at the IAC Sugarcane Center in Ribeirão Preto. Another post-doctoral student engaged in the project, Dr. Michael dos Santos Brito, likewise a JP coordinator at IAC, is studying the identified transcription factors.

The main outcome of the project was the selection of candidate genes to be used in the genetic transformation of sugarcane and thus to enhance the efficiency of second-generation ethanol production, making the fuel more affordable and less expensive to produce. Additionally, the extended use of bioethanol rather than fossil fuels can help reduce CO_2 emissions, mitigating the effects of global warming.

- Project Title: "Control of lignin biosynthesis in sugarcane: many gaps still to be filled" (2008/58035-6)
- Coordinator: Paulo Mazzafera (Instituto de Biologia, UNICAMP)



Histological sections showing lignin deposits in new (top), intermediate (middle) and mature culms (bottom) of S. officinarum (credits to Juan Pablo Portilla Llerena, M.Sc. student, FAPESP scholarship holder)

SUGARCANE GENOME SEQUENCING PROJECT AIMS TO BOOST BIOENERGY PRODUCTION

Complexity of sugarcane genome is a major hurdle to increasing yields

Sugarcane has proven to be an excellent fuel producer: for this reason it is being used as an alternative to fossil fuels. Professor Glaucia Mendes Souza is a Full Professor at the University of São Paulo's Institute of Chemistry, and coordinates the FAPESP BIOEN Research Program; she leads a project entitled "Development of an algorithm for the assembly of the sugarcane polyploid genome", which aims to boost energy production from sugarcane.

It is urgent in today's concerning world climate scenario to replace energy obtained by burning fossil fuels. However, it is no simple task to create renewable fuels: in the case of bioenergy it depends on using high-yield plants that adapt to environments with minimal use of inputs and water, and thus mitigate environmental impacts. The major hurdle to improving energy output from sugarcane lies in the complexity of the plant's genome. Unlike human beings, who possess two copies of 23 chromosomes (one inherited from the mother and the other from the father), sugarcane possesses 8 to 12 copies, owing to many years of improvement guided by means of the cross breeding from several species towards increased sugar content and disease resistance. With a huge polyploid genome, it is hard to assemble the complete genome, thereby moving forward in the development of biotechnology for the crop.

Léo Ramos Chaves/Revista Pesquisa FAPESP

The project led by Professor Glaucia Souza, along with researchers from the Institute of Chemistry, the Biosciences Institute and the "Luiz de Queiroz" School of Agriculture, in collaboration with researchers from Microsoft Research (USA), aims to sequence the sugarcane genome and develop an assembly that separate the several copies of each gene of the plant.

Dr. Souza is also coordinating a project analyzing networks of genes that have to do with agronomic traits of interest. Plants can be improved when it is known how their genetics operates and which genes are responsible for certain characteristics such as stress tolerance. Dr. Souza highlights the generation in the laboratory of drought resistant GMO plants. The expression of genes associated with drought resistance was analyzed and by generating GMOs it was possible to raise the growing potential of sugarcane in formerly unfeasible areas.

In addition to the genome difficulty, sugarcane adoption faces challenges in the dispute for market share with oil companies. Because oil happens currently to be more economically advantageous, it is receiving far more investment. The oil industry — far more consolidated and promising greater financial returns — has traditionally been more attractive to investors. And fluctuations in the price of a barrel of oil are reflected in the degree of interest in renewable energy. Despite all the benefits shown by ethanol, when the price of a barrel of oil is low and therefore profitable to produce, renewable forms of energy are left aside. The opposite also happens.

However, at the United Nations Climate Change Conference COP21 in December 2015, it was agreed that bioenergy is an immediate necessity. "COP showed once and for all that every country will have to take effective steps. It is still early days, but at least agreement has been reached that we should all seek to reduce emissions, and this means phasing out oil, period. That's where bioenergy comes in. If we can manage to develop drought-resistant higher-yield sugarcane, possessing a fiber or sugar content that adapts to a range of applications, as many economically advantageous agricultural characteristics as possible, then the lower the cost, the lower is the environmental impact, and the greater will be the profit," says Souza.

>> Projects Titles: "Development of an algorithm for the assembly of the sugarcane polyploid genome" (2012/51062-3); "Sugarcane signaling and regulatory networks" (2008/52146-0)

>> Coordinator: Glaucia Mendes Souza (Instituto de Química, USP)

BIOFUELS TECHNOLOGIES DIVISION

NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY (INCT) IN BIOETHANOL - A NEW PROSPECT FOR BIOENERGY

An overview of the INCT for Bioethanol and its importance for advances in bioenergy production in Brazil

The National Institute of Bioethanol Science and Technology (INCT), one of Brazil's leading bioenergy research projects, involves an array of institutes and researchers dedicated to the study of several processes involved in bioethanol production in order to enhance these products and make them more attractive to the market. Professor Marcos S. Buckeridge, the coordinator of the project, was also one of its founders.

The Bioethanol INCT sprang out of worldwide concern for the production of renewable energy on the planet, and the need to make new alternatives feasible. Researchers from São Paulo state universities such as USP, UNESP and UFSCar turned their attention to Brazil's bioethanol production which, although well-advanced and renowned worldwide, still required improvement. "Our first-generation ethanol is so highly efficient that we are already thinking of how to improve it. We hope to take a genetics route to increase the amount of sugar in the plant, making the sugarcane grow faster," says Buckeridge. "At this moment we are working on the possibility of setting up a second generation project — that is to say instead of only using sugarcane sugar (sucrose), we would begin to try to degrade the cell walls, and produce ethanol from what is left over in the straw and the bagasse," he says. Studies have made calculations that pointed out that, with second generation, it would be possible to boost bioethanol production by 40%. Buckeridge explained the structure of the team that achieved the desired result: "In the group working with the physiology and cell biology center, we adopted a strategy of understanding the physiology of sugarcane and how it responds to the global climate change, by growing it in environments with high concentration of carbon dioxide and also by submitting it to drought conditions." For cell biology, "we used chemical and biochemical tools to shed light on a basic point: to seek to understand how the polymers in the cell wall associate. Without this basic knowledge, we would have been unable to move forward with the project, because our idea was to use enzymes

Léo Ramos Chaves/Revista Pesquisa FAPESP

to degrade the cell wall, produce sugars from this degradation, and thus lead to fermentation."

The project was split into two phases. In the first phase there were four research fronts, and a relatively long period of study was needed before any practical applications, in order to get to know the plant and its functioning in depth. Using highly accurate genetic techniques and powerful equipment, the group of researchers tries to understand the genotype complexity of sugarcane, the feature that makes the assembly of its genome such a challenging task.

Another group within the Bioethanol INCT aimed to understand how this genome could be exploited so as to generate an adapted plant. In order to enhance the production of second-generation bioethanol, they needed to import, from Australia and South Africa, those plants that gave rise to sugarcane, because the Brazilian varieties had been developed for the production of sucrose (first generation) and would not be usable for the second generation. These genetic techniques were applied over the last six years, since INCT was set up.

A third group was tasked with prospecting for fungi and enzyme engineering, and dedicated itself to find new organisms and detecting genes and enzymes that could hydrolyze the cell wall by using several other techniques — above all molecular biology, biochemistry and chemistry — in order to develop enzymes cocktails as desired. "With the knowledge about the genetics of sugarcane now available to the group, we can realistically think of a sugarcane plant capable to degrade itself before entering the process, making much easier the hydrolysis of its cell walls", says Buckeridge.

The fourth and final group was tasked with building upon the advances achieved by the previous groups, and testing ways of enhancing sugar production. That was the structure of the first phase of the project, from 2009 until December 2016.

The second phase of the project — ongoing at the moment — is marked by application of the concepts learned during the first phase. "We are now focusing our efforts on how to hydrolyse the cell wall of sugarcane efficiently, using all the knowledge acquired in the first phase", says Buckeridge. "What we have today in terms of knowledge of the cell wall and the enzymes capable to hydrolyze it, may give rise to a made-in-Brazil enzyme cocktail. That is what we expect to reach in the second phase."

The project's legacy will be extremely important: unique know-how that may go beyond sugarcane and be applied to other plants such as maize and rice. Researchers in the field of bioenergy will also have access to the INCT laboratories and equipment. As Buckeridge stresses, "from the outset, our role has been very clear: we are scientists and we produce knowledge."


Eduardo Cesar/Revista Pesquisa FAPESP

» Project Title: "National Biotechnology Institute for Bioethanol" (2008/57908-6)

» Coordinator: Marcos Silveira Buckeridge (Instituto de Biociências, USP)

BIOFUELS: MUCH YET TO EXPLORE

Dozens of students mobilized to enhance biofuel output


Eduardo Cesar/Revista Pesquisa FAPESP

Part of Professor Antonio Meirelles's research project was completed in 2015. The study, carried out at UNICAMP and USP, seeks to develop technologies for the production of biofuels, oilseed and sugarcane by-products, and add value to foods produced from these raw materials. "In ethyl biodiesel, our intention is to develop a production technology using ethanol all the way from the vegetable oil extraction stage to the final biodiesel production stage," says Meirelles.

He explains that he has chosen to investigate this subject because he has been working in the field since his M.Sc. When the FAPESP BIOEN Program call for proposals was published, his personal connections to other researchers in the field facilitated the studies.

Meirelles says that the goals of the research can be summed up as: developing an ethyl biodiesel production route using ethanol from the oil extraction stage all the way until purification of the biofuel itself; enhancing and perfecting alcohol distillation technologies; and developing technologies for recovering, adding value to, and formulating oilseed and sugarcane plant products.

The conclusions reached in the study were highly satisfying, according to Meirelles. In addition to the participation of approximately 100 students (post-doctoral students, masters students, and so on), 100 scientific articles were published. He also points out that "in terms of technologies, we can list three main outcomes: we developed technologies for ethyl biodiesel production, which led to two patent requests at INPI (Brazil's National Institute for Industrial Property); enhancement and optimization of alcohol distillation technologies, further leading to two patent requests at INPI; and the development of technologies to exploit and/or formulate some bioproducts."

The project stands out in Brazilian science and its social importance is also noteworthy. The development of a technology to allow ethyl biodiesel to be produced along with edible vegetable oils is extremely beneficial for Brazil's vegetable oil industry (along the same lines as how the successful sugar and alcohol industry has benefited from the simultaneous production of ethanol and sugar).

Meirelles goes on to explain that an enhanced technology to produce ethyl biodiesel would bring costs down and boost the chances of investment in the industry, while enhancing byproducts that have an added value potential: "These are aspects possibly benefiting the population at large, in addition to tying in with the goals of BIOEN to improve production based on renewable resources."

Meirelles added that although oils and fats are well-developed industries in Brazil, research into biofuels, assisted by more than 25 investigators in Brazil and the rest of the world, still has a lot to contribute.

>> Project Title: "Phase equilibrium and purification processes in the production of biofuels and biocompounds" (2008/56258-8)

>> Coordinator: Antonio José de Almeida Meirelles (Faculdade de Engenharia de Alimentos, UNICAMP)

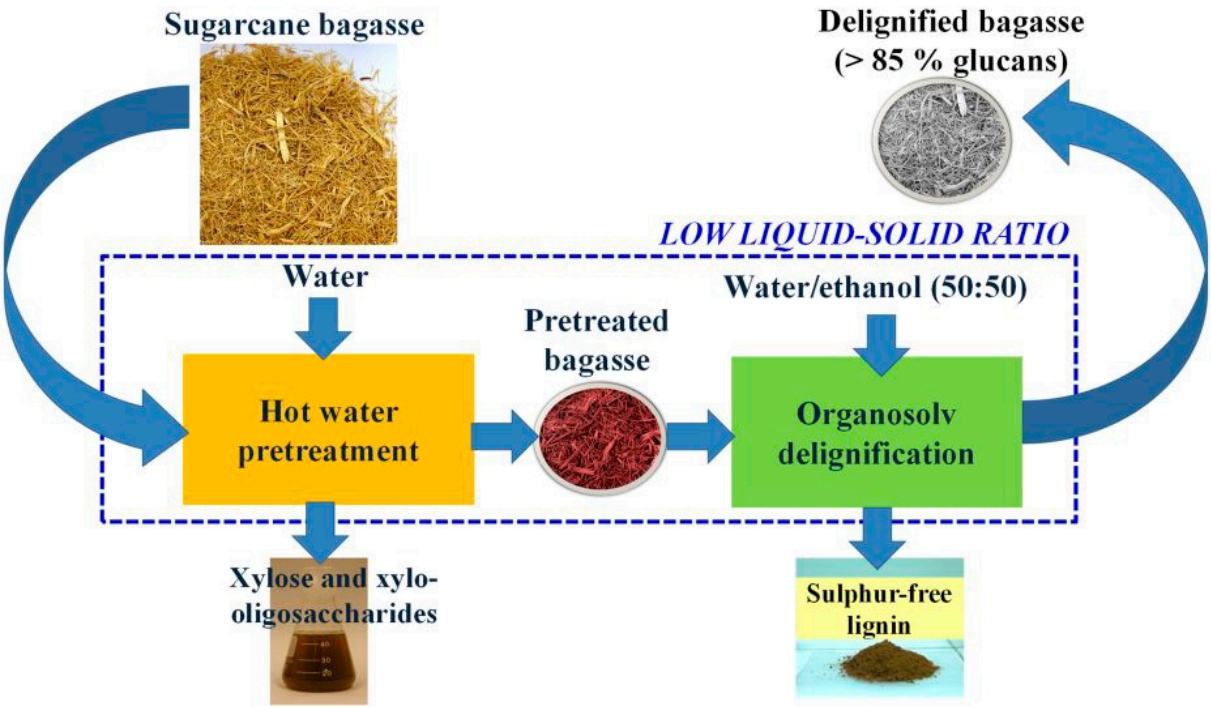
STUDY HELPS BRIDGING THE GAPS IN SUGARCANE PROCESSING

Significant results in the study of sugarcane delignification

The project was carried out entirely at the São Carlos Institute of Chemistry. The team is comprised of researchers from the Institute itself and two other Faculties of the University of São Paulo (the Institute of Physics and the Engineering School, both on São Carlos campus). The group responsible for the research has more than 20 years of experience in this field and it is coordinated by Dr. Antonio Aprigio da Silva Curvelo.

The project has two principal lines of investigation. Firstly, the solvency of lignin in delignification reactions, and secondly, the development of analytical methods to study the composition of lignocelullosic materials. The core objective of the study was to generate processes to transform sugarcane bagasse and straw into commercial products.

Five further goals came to light as a result of laboratory studies. They are: (I) to develop a quantitative analytical methodology to determine the chemical make-up of sugarcane bagasse and straw, which are essential steps towards direct use or transformation into other byproducts; (II) to obtain lignin patterns in order to guide more efficient delignification methods and generate commercial products; (III) to create a delignification process through organosolv, thus enabling a complete and cost effective recovery of cellulose, hemicelluloses and lignins; (IV) acid hydrolysis of cellulose and hemicelluloses—substrates of ethanol and byproduct production; and (V) the development of an analytical method to control delignification and acid



Isolation of macromolecules in sugarcane bagasse

hydrolysis with a view to future application in industrial processes.

Over the four years' duration of the project, the group achieved important academic results, carrying out several other studies and publishing papers: nine in indexed international periodicals, and two which came out as complete works in Congress proceedings.

From a practical point of view, the results obtained during the study of the solvency of lignin are an important contribution in the conversion process of sugarcane bagasse and straw into ethanol. Three particular results stand out: the first led to significant improvements in the treatment of the operation owing to the use of lower liquid/solid ratios (solution/sugarcane bagasse); the second was a pioneering use of carbon dioxide in operations to remove hemicelluloses and lignin and the third was the use of solubility parameters in order to choose the best organic solvent to be used in organosolv delignification processes.

The study bridges the gap perceived by the academic and industrial fields. This means that the project helps consolidate Brazil's leadership in the sugar-alcohol industry enabling it to valorize the integral plant biomass of sugarcane, which is crucial to ensure the sustainability of future conversion processes.

The development of new professionals to work in this field is another positive outcome of the project. It trained graduate students (M.Sc.s and Ph.D.s) with know-how in plant biomass, allowing them to pursue careers in Universities and/or Industry. The importance of this study is borne out by the scientific and technological production achieved as a result of the project, attesting to its success.


Léo Ramos Chaves/Revista Pesquisa FAPESP

>> Project Title: "Development of analytical methodologies and organosolv delignification applied to straw and bagasse from sugarcane" (2007/51755-0)

>> Coordinator: Antonio Aprigio da Silva Curvelo (Instituto de Química de São Carlos, USP)

NEW ROUTES FOR PLANT BIOMASS BIOCONVERSION

Novel enzyme biocatalysts to push forward fuel and bioproducts production from plant biomass

The study of enzymes involved in the bioconversion of plant biomass is a BIOEN-FAPESP project coordinated by Professor Fabio Squina, a graduate of the University of São Paulo's Pharmaceutical Sciences School. The study aims to develop a collection of enzymes for a wide range of biotechnological applications.

Combining metagenomics, proteomics and high-throughput screening approaches, researchers routinely prospect and clone recombinant proteins, to produce them by heterologous expression system. These proteins are enzymes that act in the bioconversion of plant biomass, such as: endo- and exo-cellulases and β-glucosidases, endo- and exo-xylanases, arabinofuranosidases, feruloyl esterases, mannanases, arabinanases, laminarases, lichenases, xyloglucanases and other auxiliary activities.

By characterizing the enzymes mined in this study, Squina's team added scientific value to the project. "We carried out comprehensive biochemical and functional studies, which enabled us to assign enzymes to specific biotechnological applications, as well as correlated function with structure in order to better understand the molecular features of biomass and enzyme interactions", he says.

The project also addresses the development of protein hyper-secretion technology using filamentous fungi. Filamentous fungi are an exceptional cell machinery for the production and secretion of enzymes, and can produce raised titers of target proteins in agricultural byproduct or even industrial waste substrates.

Thousands of enzymes were identified in the research, more than 50 enzyme activities were produced and studied, derived from filamentous fungi, hyperthermophilic bacteria, soil and termite metagenomes, and artificially synthesized genes.

In addition to the applications foreseen in the conversion of biomass to biofuels, the collection is also driving the development of biotechnological routes to produce nutraceutical compounds, animal feed ingredients or cosmetics.

The study was carried out at Brazilian Bioethanol Science and Technology Laboratory (CTBE) within the Ministry of Science, Technology and Innovation (MCIT)'s National Center for Research in Energy and Materials (CNPEM).

» Project Title: "Library generation for biomass-conversion enzymes from soil metagenome" (2008/58037-9)

» Coordinator Fabio Marcio Squina (CTBE)

Léo Ramos Chaves/Revista Pesquisa FAPESP

BIOREFINERIES DIVISION

A PROCESS FOR THE TOTAL PRODUCTION OF BIOETHANOL AND ZERO CO_2 EMISSIONS

The project aims to develop alternative routes for the production of ethanol and to optimize sugar and alcohol production while avoiding emissions of polluting gases

The Thematic Project entitled "An integrated process for the total production of bioethanol and zero CO_2 emissions" was carried out from 2010 to 2015 with the support of FAPESP and CNPq, aiming to propose a pioneer bioethanol production process integrating biochemical and thermochemical routes to use sugarcane bagasse and straw as raw material, and providing an innovative concept with zero emissions of undesired products - including the CO_2 and vinasse, the later a feedstock to biogas. The proposed process is considered an environmental-friendly approach suitable to be applied in the Brazilian first generation bioethanol plants.

Coordinated by Rubens Maciel Filho, the project was carried out at the State University of Campinas (UNICAMP) and was assisted by a number of undergraduate, M.Sc., Ph.D., and postdoctoral students and fellows. Francisco Maugeri Filho, Maria Isabel Rodrigues, Aline Carvalho da Costa, Maria Regina Wolf Maciel, Dejanira de Franceschi de Angelis and Carlos Eduardo Vaz Rossell were principal investigators of the project.

The overall objective of the project is to improve existing ethanol production (fermentation of sugarcane molasses, known as first-generation bioethanol), and to suggest a range of options to produce second-generation ethanol output (based on biomass), through biochemical and the thermochemical routes. Among the possible process approaches is bioethanol generation from biological or catalytic fermentation of synthesis gas, as well as from algal biomass, a study in which the researchers were pioneers.

One of the major drivers of the thematic project is the possibility of reducing CO_2 emissions, a major contributor to global warming. The CO_2 released in first- and second--generation processes is absorbed and recycled either to boost the growth of microalgae or as a base molecule to produce other chemicals. These microalgae are used to produce lipids that can be conver-

Fermentation pilot plant

ted into biodiesel, or to accumulate sugars that will be fermented into ethanol (the biochemical route).

The experimental part of the project was carried out in the laboratories of UNICAMP, especially at Laboratory of Design, Optimization and Advanced Process Control (LOCA) and Laboratory of Separation Processes (LDPS). Simulation of the process to assess scalability, and investigation of the effects of operational and project variables for the process performance evaluation were made in Aspen Plus®. Rigorous and specific simulations were carried out using Fortran programming, using proprietary softwares, which enabled more detailed simulation of, for example, supercritical processes.

The study enabled promising processes to be developed for every stage in the generation of ethanol (as well as that of other chemicals, such as butanol and other alcohols) and — in the case of the thermochemical routes — determined which catalysts were the most suitable. The project helped identify and quantify the organic acids produced from process conditions and types, investigate routes using currents formerly rejected in industrial processes, such as vinasse and CO_2. The work allowed the identification of new lines of research and technologies and first steps to be taken in them.

Professor Rubens Maciel Filho believes the project succeeded in showcasing alternatives with the potential to create new business models for Brazil's bioethanol, which will help grow domestic technology, generate new jobs, make ethanol more competitive in the energy field, and boost the country's sustainable development. As a consequence of this research, a number of patents are available in the market, which may provide alternative and new approaches for the second-generation ethanol, electricity and chemical products from renewable sources.

In his opinion, the possibility of linking a project to BIOEN-FAPESP is essential to draw attention to alternative sustainable processes proposed in this research, seeking to come up with new ideas to help Brazil's bioenergy production, because innovation is necessary in every field. He stated that it is extremely important that professionals in this field become aware of the research and studies that have being carried out, as well as of their potential, in order to help the decision-making activities.

>> Project Title: "An Integrated Process for the Total Production of Bioethanol and Zero CO_2 Emissions" (2008/57873-8)

>> Coordinator: Rubens Maciel Filho (Faculdade de Engenharia Química, UNICAMP)

NEW PLATFORM ENABLES VIRTUAL BIOREFINERY TO BE BUILT

Project aims to enhance output of first-generation mill on the basis of mathematical models

Professor Antonio Bonomi, a researcher at CTBE (Brazil's National Bioethanol Science and Technology Laboratory), has been coordinating a project to develop a library of mathematical models capable of representing computationally the production process of sugar, first-generation ethanol and bioelectricity, reproducing what happens in the sugarcane processing factories.

The project is funded by FAPESP and has involved partnership with researchers from the University of São Paulo (USP), the State University of Campinas (UNICAMP), the Federal University of ABC (UFABC — with operations in several municipalities in the ABC region) and the Federal University of São Carlos (UFSCar).

The software platform used for the development is the free process simulation tool called EMSO (Environment for Modeling, Simulation and Optimization) and simulates every step of the production process: reception and preparation of the sugarcane, extraction and concentration of the juice, crystallization — in the production of sugar, fermentation and distillation — in the production of ethanol, cogeneration of electricity and biodigestion of vinasse. With these simulations, it is possible to define the operating conditions at which the biorefinery should work in order to maximize products outputs and profits.

EMSO thus operates as a tool to create mathematical models. These models allow simulation of procedures and calculation of how much sugar, ethanol, lignocellulosic material and electricity is being produced by the mill, as well as showing what amount of such products could be expected if the mill was running in the most efficient manner possible. Process variables can thus be manipulated to reach the desired objectives, such as maximum profitability, or prioritizing ethanol production, without ignoring sugar and electricity, up to the optimum situation.

Léo Ramos Chaves/Revista Pesquisa FAPESP

Bonomi states that the importance of the EMSO project goes beyond enhancing the process of obtaining first-generation ethanol. "The more optimized the production of first-generation ethanol, the more lignocellulosic material will be available for use in producing second-generation ethanol, and the more efficient the process will become."

Researcher Vera Lucia Reis, also working at CTBE, believes the major benefit of the platform is its ability to predict all of these changes without a need for constant testing of the variables in the actual mill. The

digital biorefinery will therefore help to reduce the cost of these studies.

Another aspect being studied within the platform is to assess different ways of using the methane in the biogas produced in the vinasse biodigestion step. On average, ordinary reactors produce biogas with 60% methane content and it is used to generate electricity or as direct heating source. But if the desired end product is biomethane, a purification step must be added enabling 96.5% methane to be obtained in the gas. In addition to replacing diesel in agricultural machinery and trucks, the biomethane could also be used in the public natural gas distribution grid, leading to an economically and environmentally favorable alternative.

- Project Title: "Simulation of a first-generation sugarcane biorefinery using the EMSO platform" (2011/51902-9)
- Coordinator: Antonio Maria Francisco Luiz Jose Bonomi (CTBE)

MATHEMATICAL MODELS FACILITATE PRODUCTION OF BIOPRODUCTS

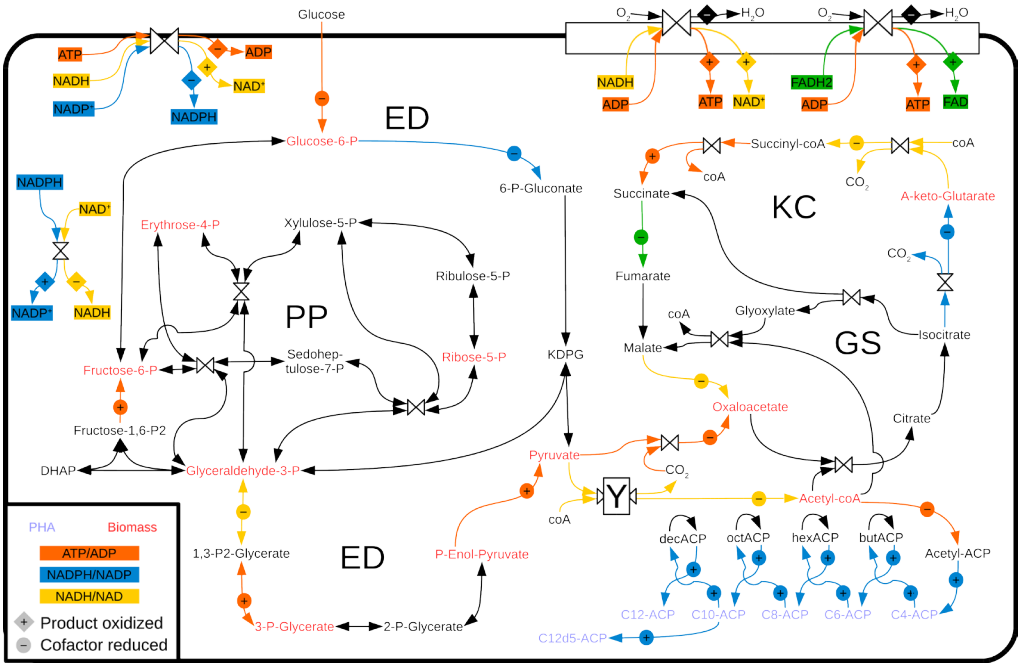
Mathematical models based on the metabolic characteristics of bacteria make lab tests more rational

Professor Marilda Keico Taciro, Ph.D., a chemical engineering graduated from University of São Paulo Polytechnic School (EPUSP), has coordinated a Young Researcher Project supported by FAPESP to introduce a line of research in metabolic engineering, a field that has been neglected in Brazil.

The study, engaging engineers, mathematicians and biologists from different units of the University of São Paulo (Polytechnic School, Institute of Mathematics and Statistics & Institute of Biomedical Sciences) and engineers from the National University of Colombia, develops mathematical models to simulate the behavior of certain bacteria so as to reduce work on laboratory bench scale, by predicting the efficiency of bioproduct formation.

"The study was a great challenge: providing a different focus for a group accustomed to molecular biology studies," Taciro says. Due to this pioneering study it is now possible to bring together research lines from microbial metabolism and chemical engineering to increase the production of biomolecules.

Taciro emphasizes that instead of utilizing industrial microorganism at sub-optimal conditions, biotechnological processes could first evaluate them, applying the mathematical models developed, to achieve their full potential. To boost efficacy, the team has analyzed some bacteria and built mathematical models simulating their behavior, using data from the bacterial genome sequence. These equations are used to simulate many hypothetical situations, and in many cases reducing the number of laboratory experiments.



Core model on genomic scale: ~400 reactions, ~250 metabolites. Simplified model for flow analysis using elemental modes: 46 reactions, 50 metabolites.

In February 2016, the project reached its five-year milestone: it introduced new technologies that are now being used in other projects of the Laboratory of Bioproducts. It is now possible to improve biotechnological processes based on metabolic fluxes analysis using engineering tools and mathematical models, even on a genomic scale. Professor Taciro says that apart from the innovation aspect of this research in Brazil, it can bring together knowledge from a range of fields in the University.

However, this is only one of the researchers' tasks: they also analyze the trajectories of labeled carbon in the metabolic pathways of the bacteria in order to know how its metabolism works and to discover which metabolic pathways are used in specific experimental conditions.

Now that this line of research has been introduced, the team aims to extend study cases and the microbial platforms used, because the microorganisms can use different metabolic network, resulting in different behaviors. For the time being, the genomes of three bacteria have been sequenced and their metabolic network analyzed.

Finally, they aim to discover the best way of producing molecules by rational work, improving the process, and obtaining enhanced results through the choice of the best microorganism/platform to obtain a given product, whether by manipulating the process operating conditions or by specific genetic modifications in the microorganism used in production.

>> Project Title: "Metabolic engineering applied to the production of biopolymers" (2010/51692-1)

>> Coordinator: Marilda Keico Taciro (Instituto de Ciências Biomédicas, USP)

ENGINES DIVISION

ENGINE DEDICATED TO ETHANOL MAY INFLUENCE THE SCENARIO OF THE SUGAR AND ALCOHOL INDUSTRY

In addition to providing a new alternative to existing engines, the project aims to cut down particulate emissions in urban areas

Aiming to exploit ethanol as a fuel and to come up with new possibilities for the automobile industry, researchers from UNICAMP Mechanical Engineering School, the USP Polytechnic School, Instituto Tecnológico de Aeronáutica (ITA) and Instituto Mauá de Tecnologia (IMT), supported by FAPESP and Peugeot-Citroën, are studying an advanced engine designed to run exclusively on ethanol. The main advantages of the model under analysis are greater compression rates, greater burning speed, and greater latent heat of vaporization.

According to Professor Waldyr L. R. Gallo, coordinator of the project entitled "A conceptual study for an advanced ethanol engine", carmakers have not carried out studies based on the exclusive use of ethanol with new technologies: they have chosen to rely on the flex-fuel model. This technology does not allow the technical characteristics of ethanol to be fully exploited, although such features would provide the flexibility demanded by consumers.

The study is exploring existing technologies that have not yet been tested in engines of this type. Technologies to control compression rates and the engines' volumetric displacement (for which a patent has been requested by Professor Janito Vaqueiro Ferreira, also involved in the study), as well as controlling engine load by means of variable camshafts, are considered promising. The project will also test other technological features, such as turbocharging, exhaust gas recirculation, and direct fuel injection.

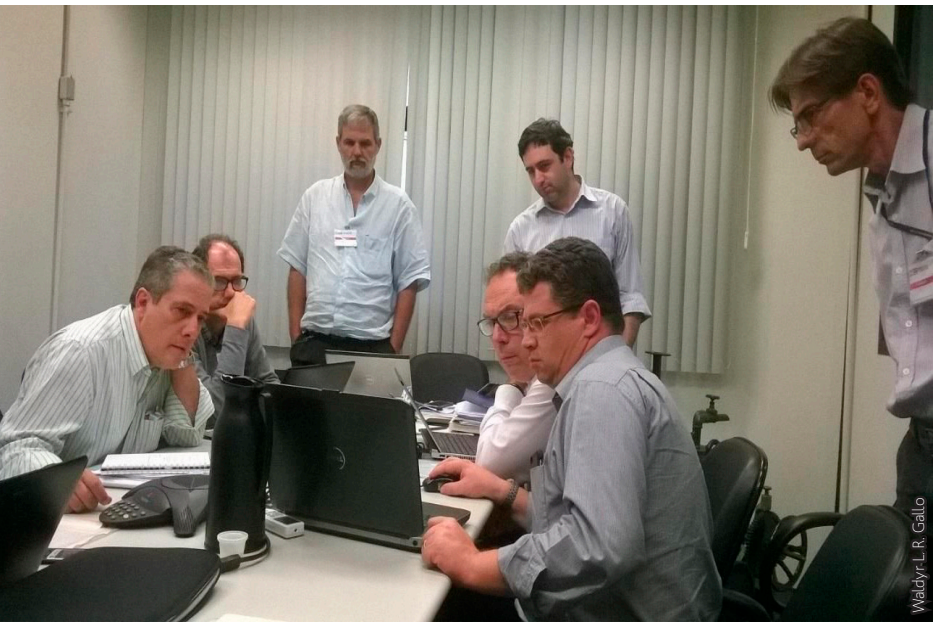
Carried out at the "Prof. Urbano E. Stumpf" Engineering Research Center (Centro de Pesquisa em Engenharia — CPE), the study encompasses a range of topics from the basic phenomenology of phy-

Eduardo Cesar/Revista Pesquisa FAPESP

sical processes in engines, to the relationship among the engines and the vehicles in which they are adopted. It comprises six subthemes: "Exploratory experimental tests — ethanol engines"; "Comprehensive studies on the preparation of the mixture and turbulent combustion in ethanol engines"; "Ethanol spray combustion for multipoint fuel injection [MPFI] engines"; "Thermodynamic simulation of the indicated performance for ethanol engines"; "Innovative solutions for ethanol engines"; and "Simulation of dynamics, tension analyses, design and testing of low weight components".

These six subthemes led the study to employ a range of highly specific methodologies involving computing packages to study the mechanical stresses on engine components or the formation of fuel spray; home-made programs for the thermodynamic performance simulation; several types of laser anemometry measurement techniques; a very particular test engine with optical access to the cylinder; as well as fully equipped test bench for real size engine testing.

The project entered its third year in November 2016, with certain scientific and technological challenges still to overcome. However, according to plans, the next two years point to an engine type that may increase the demand for ethanol, thus directly influencing the sugar and alcohol industry. "If successful, the project will pioneer a market niche for ethanol engines," says the coordinator, "whose efficiency will be very close to that of diesel engines. [They] will be able to replace small urban-use diesel engines (vans, SUVs, smaller trucks), thus reducing emissions of particulates in cities."



Work meeting at which some of the principal researchers are present to discuss the results of an engine test performed in the Mauá Institute of Technology. From left to right: Prof. Gallo (UNICAMP), Prof. Pedro Lacava (ITA), Prof. Janito (UNICAMP, standing), Eng. Rafael Serralvo (Peugeot, standing), Eng. Franck Turkovics (Peugeot, seated), Prof. Clayton (Mauá Institute of Technology, seated), and Prof. Guenther (USP, standing).

» Project Title: "Conceptual study for an advanced ethanol engine" (2013/50238-3)

» Coordinator: Waldyr Luiz Ribeiro Gallo (Faculdade de Engenharia Mecânica, UNICAMP)

IMPACTS AND SUSTAINABILITY DIVISION

STUDIES ASSESS THE CONTRIBUTION OF BIOENERGY IN LATIN AMERICA, THE CARIBBEAN AND AFRICA

Entitled LACAf-Cana-I, the project seeks to increase the electricity supply of these regions through growing sugarcane

Bioenergy is well-established in Brazil, USA and Europe, and has a real potential for production in the Caribbean, Southern Africa and Latin America, says researcher Luís Augusto Barbosa Cortez. The abundance of fertile land and the suitable tropical climate, along with agricultural trends and experience in these regions, have — in recent years — led scientists such as Cortez to consider how to expand sugarcane-based bioenergy production. The goal is to map out ways in which these regions can — over the next few decades — depend less on traditional sources and fossil fuels, and thus guarantee their energy self-sufficiency. The initiative is entitled LACAf-Cane: involving international collaboration, it is being developed at the Interdisciplinary Energy Planning Center (Núcleo Interdisciplinar de Planejamento Energético — NIPE) of the State University of Campinas (UNICAMP) where Cortez works. The LACAf project is a contribution to the Global Sustainable Bioenergy (GSB) project set up in 2009 to bring together professionals from around the world to boost sustainability and study new energy alternatives.

To estimate potential bioenergy production in the regions under study, the project will expand the sugarcane crop over 1% of the grazing area in each territory, calculated at 646.9 million hectares (Mha). Cortez reports that preliminary results are promising: in Latin America and the Caribbean, the change would produce some 20 million cubic meters of ethanol and 15 terawatt-hours (TWh) of electricity, which could replace 15% of the gasoline consumed and provide the equivalent of 2.2% of the electricity in these regions. In southern Africa, with a surplus of 180 million tonnes of sugarcane, the yield would be 15 million cubic meters of ethanol and 13 TWh of electricity, equivalent to 5% of average local consumption. Given the scarcity of electricity supply in southern Africa, where 60% of the population is still without energy access, researchers see the sugarcane project as an important element in future electrification projects.

Alongside other advantages, sugarcane growing promotes renewable energy on a large scale, with smaller production costs, and boosts technological know-how wherever it is used. The project still has a long way to go. LACAf-Cane intends to gather data on the feasibility of execution, then consult with local people in order to receive their input on the new energy scenario. The goal is to adjust the guidance that will be given so that it is consistent with future public policies. Furthermore, despite the fact that the studies did not focus exclusively on Brazil, the country's advanced bioenergy development leads Cortez to affirm that impacts


Léo Ramos Chaves/Revista Pesquisa FAPESP

may be positive on the domestic scenario as well: "Alongside other research, studies such as ours may not only help Brazil contribute to the reduction of greenhouse gases, but may also boost our sustainable expansion through the use of biofuels and enhanced land-use planning." He has been leading specific detailed surveys focusing on each country in southern Africa and Latin America.

>> Project Title: "Contribution of Latin American, Caribbean and Southern African bioenergy production to the GSB-LACAf-Cana-I project" (2012/00282-3)

>> Coordinator: Luís Augusto Barbosa Cortez (Núcleo Interdisciplinar de Planejamento Energético, UNICAMP)

GREENHOUSE GAS EMISSIONS ARE STUDIED IN THE SUGARCANE CROP

Study evaluates greenhouse gas emission rates in the ethanol production process

Most people link greenhouse gas (GHG) emissions to the use of fossil fuels such as gasoline and diesel. Biofuels such as ethanol, for example, are considered to be renewable because they enable rapid carbon (C) cycling: the CO_2 released during the combustion of biofuels has been previously removed by photosynthesis from the air by sugarcane or other plants. Thus, concentrations of atmospheric CO_2, the most important gas involved in intensifying the greenhouse effect, are not supposed to increase with the use of biofuels. However, other greenhouse gases that are even more potent than CO_2 are emitted during agricultural activities associated with the production of biofuel feedstocks such as sugarcane. For example, small quantities of N_2O, which has a global warming potential about 300 times greater than CO_2, are released in sugarcane due to the use of nitrogen fertilizer.

The rate of atmospheric N_2O emission is directly linked to the amount of nitrogen fertilizers applied to crops. Accordingly, Paul Crutzen and colleagues estimated that 3% to 5% of fertilizer nitrogen applied to biofuel crops is released to the atmosphere as N_2O, elevating the emission factors predicted by the IPCC for biofuels far above the reference value (1% of N released as N_2O). In terms of mitigating global warming, this figure would make biofuel produced from less nitrogen use-efficient crops such as maize unfeasible for climate change mitigation, and would significantly compromise the benefits of using sugarcane ethanol.

Taking this into consideration, UFSCAR Sorocaba researcher Janaina B. do Carmo, assisted by a group of researchers from a pool of institutions (Leonardo Machado Pitombo, Johnny R. Soares, Hei-

Hétor Cantarella and Johnny R. Soares

tor Cantarella, Raffaella Rossetto, Cristiano Alberto de Andrade, Luiz Antonio Martinelli, Solange Filoso, and Eiko Kuramae), has been quantifying GHG emissions of agro-ecosystems with the support of FAPESP since 2009.

The research team uses a bottom-up methodology to quantify GHG emissions *in situ*. Results of several field studies conducted by this group in São Paulo indicate that the N_2O emission factor for N fertilizer used in sugarcane is generally lower than 1%, confirming the good sustainability standards of ethanol produced in Brazil. However, when nitrogen fertilizer is applied to sugarcane fields covered by crop trash as opposed to bare soils, N_2O emissions increase. As we know, sugarcane generates large amounts of residual material (straw), which is either used for energy production or left in the field as crop trash to protect the soil or provide other benefits.

Mitigation practices are also being investigated. For instance, the work of Soares et al. (2016) showed that it is possible to decrease the N_2O emissions by up to 95% with the use of nitrification inhibitors along with N fertilizers.

Future work include the identification of processes and the microorganisms driving the biogeochemical processes that cause emissions, so that effective management practices can be developed to reduce emissions and increase the benefits of sugarcane biofuel.

>> Projects Titles: (1) "N_2O, CO_2 and CH_4 emissions from agro-biofuel production in São Paulo State, Brazil" (2008/55989-9); (2) "Nitrogen nutrition of sugarcane with fertilizers or diazotrophic bacteria" (2008/56147-1)

>> Coordinators: (1) Janaina Braga do Carmo (Departamento de Ciências Ambientais, UFSCar); (2) Heitor Cantarella (Instituto Agronômico de Campinas)

UNDERSTANDING THE MICROORGANISMS RESPONSIBLE FOR THE PRODUCTION AND CONSUMPTION OF N_2O

The study aims to investigate organisms and processes contributing to N_2O production in sugarcane fields

Sugarcane is considered one of the most nitrogen-efficient crops for biofuel production. However, this efficiency might be jeopardized by relatively high emissions of potent greenhouse gases such as N_2O during the sugarcane production phase. Much of the emissions are caused by the application of vinasse to sugarcane fields, which is a means to recycle a by-product of the ethanol distillation process and also recycle back to the field nutrients which are essential for sugarcane growth. Practices to prevent or at least reduce greenhouse gas emissions must therefore be adopted.

Not too long ago, N_2O loss during nitrogen cycling was predicted using model microorganisms isolated in the nineteenth century from soils of the temperate zones. Since then, molecular technology has helped determine that different taxa ("species") predominate according to conditions and settings, as shown by a study published in the journal GCB Bioenergy by Professor Carmo's group. Furthermore, the technology has revolutionized our understanding of the microorganisms involved in the reduction of N_2O to N_2 thanks to field studies of this function (N_2O flow) and the abundance of microorganisms in the soil. Consequently, it is now possible to develop research to understand how microorganisms can be used to minimize N_2O emissions and improve the N_2O balance in agricultural systems. Some of these microorganisms are found in vinasse because of contamination in the fermentation process. By controlling them, it may be possible to increase the efficiency of the ethanol production process while lowering N_2O emissions.

The work of Pitombo suggests that the adoption of new model organisms may enhance the sustainability of biofuel production, especially from sugarcane. Biogeochemical processes such as nitrogen fixation, nitrification and the consumption of N_2O by non-denitrifying organisms are highlighted in the study. In addition to new models, a new approach is suggested for microorganisms that have already been studied. For example, groups of microorganisms proposed as nitrogen fixers and obligate endophytes were abundant in the soil, showing that they have the potential to fix nitrogen in free life.

Now, efforts are focused on the study of processes at the laboratory scale, based on observations from field studies. Specific techniques that would not be feasible in the field - such as the use of stable isotopes and transcriptome - are used in the laboratory in order to study microbial activity. With support from FAPESP in partnership with BE-Basic, the laboratory studies have been successful and preliminary results have shown that the microorganisms found in vinasse actually produce N_2O. When vinasse was sterilized or simply stored in a freezer before applied in the soil, emissions were similar to those in treatments without vinasse. Emissions observed from the sterile soil after the application of vinasse confirmed this finding.

Along with Janaina Braga do Carmo, the researchers Eiko Kuramae, Leonardo Machado Pitombo, Elisabete Alves, Juliana Cristina Ramos and Heitor Cantarella also participate in this project.

>> Projects Titles: (1) "Unravelling the sources and sinks of nitrous oxide (N_2O) in sustainable bio-based agriculture" (2013/50940-0); (2) "Microbial networks in control of greenhouse gases emissions in bio based agriculture - MiniBag" (2013/50365-5)

>> Coordinators: (1) Janaina Braga do Carmo (Departamento de Ciências Ambientais, UFSCar); (2) Heitor Cantarella (Instituto Agronômico de Campinas)

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Faculty of Chemical Engineering
University of Campinas

André M. Nassar
Agroicone

PROGRAM MANAGER

Mariana P. Massafera
Institute of Chemistry
University of São Paulo

CONTACT: BIOEN-FAPESP Secretariat
Instituto de Química - Universidade de São Paulo
Av. Professor Lineu Prestes, 748, Bloco 3 Superior, Sala 364, Butantã - CEP 05508-000 - São Paulo/SP - Brazil
Phone: +55 11 3091-8939

For further information:

The FAPESP Program on Bioenergy Research (BIOEN)
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