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Sustainable Liquid Biofuels from
Biomass Biorefining (SUNLIBB)

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SUMMARY

The SUNLIBB project was funded under the European Union's Seventh Framework Programme (FP7) within the Energy theme: Second Generation Biofuels – EU Brazil Coordinated Call. SUNLIBB (GA 251132) started on 1st October 2010 for 4 years and collaborated with CeProBIO, a parallel project in Brazil. The project officially concluded on 30th September 2014.

Key Messages

- 1. First generation (1G) biofuels have triggered a highly contentious debate on the international sustainability agenda, due to their links to energy security, transport, trade, food security and climate change concerns.**
- 2. Second generation (2G) biofuels rely on inedible sources, thereby increasing their sustainability. Also, 2G biofuels are more energy efficient, less water and land intensive, and are expected to achieve greater GHG emissions reductions.**
- 3. The aim of SUNLIBB, an EU FP7-funded project was to combine European and Brazilian research strengths in order to open the way for environmentally, as well as socially and economically sustainable 2G bioethanol production.**

Background

First generation biofuels are mainly produced from food crops, such as grains, sugarcane and vegetable oils. However, using food crops to make biofuels has been widely criticized, because of potential adverse effects on food security. It also increases competition for land-use and may exacerbate climate change. Developing second generation (2G) biofuels has emerged as a more attractive option. It is intended that these 2G biofuels will be manufactured from inedible sources, such as woody crops, energy grasses, or agricultural and forestry residues. Such sources have little or no market value, and often are disposed of by burning or burial. Apart from mitigating social concerns regarding food production, 2G biofuels are also more energy efficient, less water and land intensive, and are expected to achieve greater reductions in emissions of Greenhouse Gases.

Residues from sugarcane and leaves and stems from maize, as well as 'whole-crop' miscanthus, are all potential raw materials (called feedstock) for 2G ethanol production. This feedstock, known

as lignocellulosic (woody) biomass, is mainly composed of the cell walls of plants. Sugarcane, maize and miscanthus are closely related, so developing them as biomass crops raises common technical challenges. This means that there is the opportunity for breakthroughs in discoveries in cell wall biochemistry and genetics in one species to be rapidly exploited in the others.

Despite the potential sustainability benefits of 2G bioethanol, the current inefficiency of conversion from biomass to biofuels makes it economically uncompetitive. Taking up this challenge, the SUNLIBB consortium's multi-disciplinary team of scientists – in cooperation with those of CeProBIO, our sister project in Brazil – has combined European and Brazilian research strengths, so as to open the way for environmentally, socially and economically sustainable 2G bioethanol production.





Photo 1: *SUNLIBB Miscanthus field in Wageningen University*

Objectives

To create biofuels, feedstock is first broken down into sugars (saccharification). The sugars are then fermented (converted) to produce bioethanol. Unlike easily-processed first generation biofuels, complex substances like lignin which form the woody, inedible tissues of plants are much harder to break down and are more complicated to convert to fuels and/or chemicals with currently available methods. This greatly impedes their commercialisation. The plant cell walls that make up lignocellulosic biomass are composed of various polysaccharides (sugar polymers). Indeed, almost all crops have more sugar potentially available in the cell walls of their stems and leaves than in their grains.

Consequently, our first objective was to identify the genes that affect the cell wall composition of our target plants. Exploiting this knowledge may allow us to improve feedstock quality and hence reduce the high economic costs associated with converting biomass to 2G bioethanol. Towards this end, SUNLIBB's first four Work Packages (WPs) have taken different approaches. WP1 has sought to identify specific regions of the chromosome (i.e. Quantitative Trait Loci, QTL) that contain genes which influence the

saccharification potential of our chosen crops. WP2 has focused on generating data that could further our understanding of the genes that are switched on when secondary cell walls are formed. WP3 has advanced understanding about the composition of biomass polysaccharides and the genes that are involved in their formation, while WP4 has investigated the genes involved in lignin formation.

Our second objective was to improve the conversion process by which we produce sugars for fermentation from woody materials. Having worked on improving feedstock quality, SUNLIBB's first WPs then sent to WP5 genetic lines with contrasting saccharification potential. The task of the latter WP was to test various new methods (pre-treatments and new enzyme combinations) to extract sugars in a more cost and energy efficient manner.

Our third objective was to develop value streams, other than ethanol, for which potential markets already exist, in order to enhance the profitability of 2G biofuels. WP6 focused on extracting valuable products from plant material, before it was used as a biofuel feedstock. A wide range of useful molecules, including waxes, alkanes, polycosanols and sterols can be obtained using specially developed techniques, including supercritical CO₂ extraction. Lignin itself can be a source of valuable building blocks for chemical synthesis, and ways to obtain these were also investigated as part of WP6. WP7 sought to come up with the most efficient processes to maximise the actual value obtained from the biomass. Data generated via computers, lab-scale experiments and pilot-scale trials in biorefineries have been employed for this purpose.

Our final objective was to develop life cycle assessment (LCA) models suited to biorefineries, so as to ensure that our products are sustainable at all stages of the process. In addition, WP8 was tasked with reviewing all pertinent guidelines, policy and

regulatory frameworks for sustainable biofuels in both the EU and Brazil, in order to take into account any kind of influential developments that could affect the future potential to harness benefits from this work.

Outcomes

Apart from keeping the programme of work on course to achieve our research aims, our progress has been possible through highly successful co-operation between European and Brazilian groups. A strong sense of community has evolved through the sustained integration of our efforts, and many joint publications have been produced (see list below). In particular, the following key breakthroughs have taken place:

a.) To be able to identify QTL, detailed genetic maps are required, which are in turn compiled from large collections of genetic markers (DNA sequences with a known physical location on a chromosome). WP1 has successfully identified QTL for digestibility and saccharification in maize, while possible QTL for saccharification have also been identified in miscanthus. Our Brazilian partners have discovered 7 saccharification QTL, and a genetic map for sugarcane is not far away.

b.) WP2 has identified candidate genes involved in cell wall biosynthesis, by using biological databases (bioinformatics) to find genes that have the same function in different species (orthologs). An orthology web-interface database has been developed, and is designed to become a tool that can be used by the wider scientific community to study gene expression in any crop, even when its genome has not been fully sequenced.

c.) In WP3, the polysaccharides making up the cell wall matrix have been characterised in maize and miscanthus. A new model has been published, suggesting how hemicellulose and cellulose (2 components of the cell wall) interact. In particular, 88 gene candidates involved in matrix polysaccharide synthesis have been identified across sugarcane, maize

and miscanthus. Several new genes have been discovered that are important in the synthesis of xylan (a type of hemicellulose).



Photo 2: *SUNLIBB maize fields in France (INRA, 2014)*

d.) WP4 has used a procedure known as CSPP (Candidate Substrate Product Pairs) to discover the complex metabolic pathways involved in lignin production in maize and sugarcane. This knowledge has been used to identify a set of novel target lignin genes. Some of these genes have been shown to be involved in lignin biosynthesis in maize, using techniques that switch off the candidate genes so that information about their function can be obtained.

e.) WP5 has developed a method (High-Throughput saccharification assay) that allows the speedy and efficient testing of a great number of samples. This method has been used for maize and miscanthus, and the expertise has been shared with our CeProBio partners in Brazil, where a similar assay for sugarcane has been established.

f.) WP6 has extracted and fractionated waxes from maize and sugarcane bagasse using supercritical CO₂. Washing machine tests developed by one of our industrial partners, Ecover, have shown that these waxes are promising antifoaming agents.

g.) WP7 has carried out pilot scale fermentations of sugars from maize and miscanthus. Feasibility studies of maize and miscanthus biorefinery concepts have been

carried out to examine their techno-economic potential. It was shown that improved feedstock resulted in better overall ethanol yields and lower production costs of the biorefinery for both crops. Ethanol yields were higher for the maize biorefinery due to the increased sugar content of the pretreatment products, as well as the higher cellulosic and hemicellulosic content of the maize genotypes.

h.) WP8 has developed various MS Excel workbooks for calculating primary energy inputs and prominent greenhouse gas emissions associated with the production in biorefineries of biofuels and biochemicals, such as acetone, butanol and ethanol. Apart from biorefinery operation, workbooks cover the provision of all three feedstocks, comprising cultivation, harvesting and transportation. Finally, in addition to reviewing all pertinent guidelines, policy and regulatory frameworks for sustainable biofuels in both the EU and Brazil, WP8 has also studied EU-Brazil trilateral biofuel cooperation initiatives with African countries. It was found that environmental sustainability concerns have been an important barrier to international policy cooperation, but that Brazil remains an internationally important source of knowledge and expertise for other countries to turn to in developing their own biofuels policies.

Conclusion

The SUNLIBB consortium has made significant progress towards the objectives established at the beginning of the project. It has successfully addressed several issues that were identified as significant gaps in the technical knowledge necessary to develop lignocellulosic biomass as a viable feedstock for biofuel production. In some WPs, achievements have even extended beyond those for which we originally aimed. These results are largely thanks to excellent cooperation between different WP members and between the SUNLIBB and CeProBIO projects. Interactions have been reinforced

by exchange visits that have taken place between European and Brazilian partners during the course of the Project. For instance, SUNLIBB researchers from York, Leeds and Cambridge were frequently hosted by various CeProBIO partners and vice versa. Several of these constructive relationships are likely to continue long into the future.



Photo 3: *SUNLIBB Miscanthus sinensis* (Wageningen University – Assoc Prof Luisa Trindade)

Project public website address

For further public information on the SUNLIBB project, please refer to our website: www.sunlibb.eu.

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EMBRAPA
Universidade Estadual de Campinas
Universidade Federal de Viçosa (UFV)

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