SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

Grant Agreement no. 251132

Collaborative Project
EU 7th Framework Programme
ENERGY

Project duration: 1st October 2010 – 30th September 2014

Deliverable 9.15
"Industrial Showcase Event"

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Workpackage: 9
Workpackage Leader: Simon McQueen-Mason

Due date: Month 36
Actual submission date: Month 45

Dissemination Level: PU

SUNLIBB Project (Grant number 251132)

Del No: 9.15	**Deliverable Name:** Industrial Showcase Event			
WP: 9	**Lead partner:** P1	**Dissemination level:** PU	**Delivery due date (project month):** Month 36	**Actual delivery date:** Month 45

Objective: To represent SUNLIBB at an international industrial showcase event and disseminate information about SUNLIBB's activities and achievements.

Results: SUNLIBB was represented with a stand at the 22nd European Biomass Conference and Exhibition in Hamburg, Germany, from 23rd to 26th June 2014.

Elke Theeuwes from Ecover (SUNLIBB partner 5) and Ioanna Dimitriou from the University of Sheffield (SUNLIBB partner 11) displayed 4 posters, delivered a talk and distributed leaflets explaining the aims and objectives of SUNLIBB and the progress made in the Project so far. Posters were provided by Anne Readshaw, Leo Gomez and Tom Attard from the University of York (SUNLIBB partner 1) and Elke Theeuwes.

Post-event statistics (retrieved on 26th September 2014 from the following website: http://www.conference-biomass.com/Previous-Event.70.0.html) showed that the conference had been attended by 1340 participants from 66 countries. Participants by country were listed as: Germany 24%, Italy 10%, UK 6%, China 5%, Netherlands 5%, USA 4%, Denmark 4%, Spain 4%, France 3% and Belgium 3%.

At the SUNLIBB stand, approximately 20 interested parties asked questions and exchanged business cards, with a view to establishing further professional contact in future.

Discussion /Conclusion: Posters and leaflet are attached to this report, and are available for download from the SUNLIBB website, www.sunlibb.eu.

SUNLIBB Objectives

Improve feedstock quality, in order to reduce the high economic costs associated with converting biomass to second generation bioethanol.

Add value to the overall process of conversion in biomass biorefining, by upgrading residues and by-products and producing other value streams from the feedstock, in addition to bioethanol.

Enhance the economic sustainability of second generation biomass by bringing together in model (or pilot) biorefineries our improved feedstock, enhanced conversion processes and added-value product extractions.

Improve the conversion process by which we produce sugars for fermentation.

Ensure that the new processes developed fulfil sustainability requirements by reducing GHG emissions, cutting other forms of air pollution, having minimal impacts on local environments and biodiversity, building sustainable rural industries and not impacting on food production or prices.

European Consortium Partners

- University of York, CNAP, UK.
- University of York, Green Chemistry, UK.
- Borregaard, Norway.
- Biogemma, France.
- Ecover, Belgium.
- INRA, France.
- North Energy Associates Ltd, UK.
- Processum, Sweden.
- University of Cambridge, UK.
- University of Leeds, UK.
- University of Sheffield, UK.
- VIB, Belgium.
- Wageningen University, The Netherlands.
- Biotech Consultants Ltd, UK.

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SUNLIBB

Sustainable Liquid Biofuels
from
Biomass Biorefining

www.sunlibb.eu

What is SUNLIBB?

SUNLIBB is an EU-funded consortium project, working to overcome technical barriers to second-generation bioethanol production.

SUNLIBB brings together key researchers and industrial innovators working in areas such as feedstock improvement, pre-treatment and saccharification, generation of added-value products (especially from lignin) and fermentation.

The work is focused on 3 closely-related grasses; maize, miscanthus and sugarcane, which are major bioenergy crops in Europe and Brazil.

SUNLIBB collaborates closely with a sister consortium in Brazil (CeProBIO). This cooperation provides an opportunity for some of the best researchers in our respective regions to work together on a globally important issue.

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SUNLIBB/CeProBIO Publications

"Side by side comparison of chemical compounds generated by aqueous pretreatments of maize stover, miscanthus and sugarcane bagasse." Gomez, L. D., Vanholme, R., Bird, S., Goeminne, G., Trindade, L.M., Polikarpov, I., Simister, R., Morreel, K., Boerjan, W. & McQueen-Mason, S. J. (2014) Bioenerg. Res. Epub ahead of print.

"The pattern of xylan acetylation suggests xylan may interact with cellulose microfibrils as a two-fold helical screw in the secondary plant cell wall of *Arabidopsis thaliana*." Busse-wicher, M., Gomes, T.C.F., Tryfona, T., Nikolovski, N., Stott, K., Grantham, N. J., Bolam, D.N., Skaf, M.S. & Dupree, P. (2014) The Plant Journal. Epub ahead of print.

"The potential of C4 grasses for cellulosic biofuel production." Van der Weijde, T., Alvim-Kamei, C.L., Torres, A.F., Vermerris, W., Dolstra, O., Visser, R.G.F. & Trindade, L.M. (2013) Front Plant Sci. 4:107

"Microwave-enhanced formation of glucose from cellulosic waste." Fan, J., De Bruyn, M., Zhu, Z., Budarin, V., Gronnow, M.J., Gomez, L.D., Macquarrie, D.J. & Clark, J.H. (2013) Chemical Engineering & Processing. 71:37-42

SUNLIBB/CeProBIO Publications

"Lignification in sugarcane: biochemical characterization, gene discovery and expression analysis in two genotypes contrasting for lignin content." Bottcher, A., Cesarino, I., Santos, A.B., Vicentini, R., Mayer, J.L., Vanholme, R., Morreel, K., Goeminne, G., Moura, J.C., Nobile, P.M., Carmello-Guerreiro, S.M., Anjos, I.A., Crese, S., Boerjan, W., Landell, M.G. & Mazzafera, P. (2013) Plant Physiol. 163 (4): 1539-57

"Evaluating the composition and processing potential of novel sources of Brazilian biomass for sustainable biorenewables production." Lima, M.A., Gomez, L.D., Steele-King, C.G., Simister, R., Bernardinelli, O.D., Carvalho, M.A., Rezende, C.A., Labate, C.A., de Azevedo, E.R., McQueen-Mason, S.J. & Polikarpov, I. (2014) Biotechnology for Biofuels 7: 10

"Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production – part1." Lima, M.A., Lavorente, G.B., da Silva, H.K.P., Bragatto, J., Rezende, C.A., Bernardinelli, O.D., de Azevedo, E.R., Gomez, L.D., McQueen-Mason, S.J., Labate, C.A. & Polikarpov, I. (2013) Biotechnology for Biofuels 6:75

SUNLIBB/CeProBIO Publications

" European Union Leadership in biofuels regulation: Europe as a normative power?" Afionis, S. & Stringer, L. C. (2012) Journal of Cleaner Production. 32: 114-123

"Investigating laccase and titanium dioxide for lignin degradation." Kamwilaisak, K. & Wright, P.C. (2012) Energy & fuels. 26 (4): 2400-2406

"Developing sustainable biofuels." McQueen-Mason, S.J. (2013) Pan European Networks. 7: 244-245

"Composition and structure of sugarcane cell wall polysaccharides; implications for second-generation bioethanol production." DeSouza, A.P., Leite, D.C.C., Pattathil,S., Hahan, M.G. & Buckeridge, M.S. (2013) Bioenergy Res. 6: 564-579

"Sugarcane as a bioenergy source: History, performance and perspectives for second-generation bioethanol." De Souza, A.P., Grandis, A., Leite, D.C.C. & Buckeridge, M.S. (2014) Bioenergy Res. 7:24-35

SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

What is SUNLIBB?

SUNLIBB is an EU-funded consortium project, working to overcome technical barriers to second-generation bioethanol production. SUNLIBB brings together key researchers and industrial innovators working in areas such as feedstock improvement, pre-treatment and saccharification, generation of added-value products (especially from lignin) and fermentation. The work is focused on 3 closely-related grasses; maize, miscanthus and sugarcane, which are major bioenergy crops in Europe and Brazil. The project collaborates closely with a sister consortium in Brazil (CeProBIO). This cooperation provides an opportunity for some of the best researchers in our respective regions to work together on a globally important issue.

Background

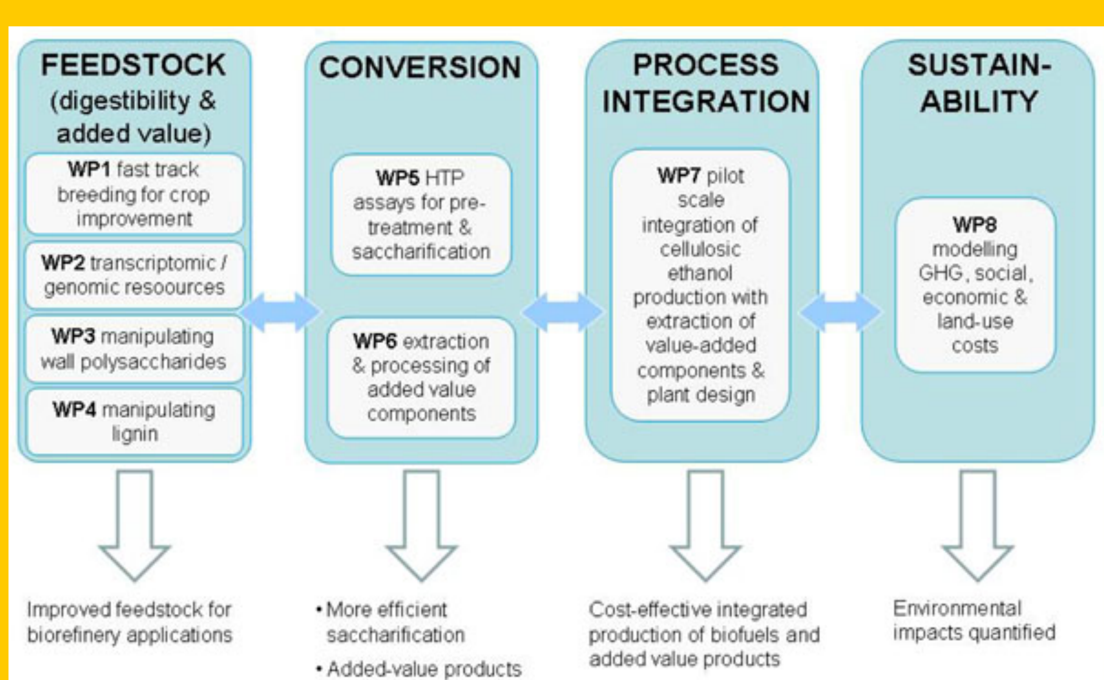
Humanity faces a dilemma: how to escape our dependence on fossil fuels and reduce greenhouse gas (GHG) emissions, without further exacerbating the environmental impacts of agriculture. This issue faces all of us, because GHG emissions that are produced locally have global impacts. Also, we operate in a global economy, where commodities such as biofuels are traded on the world stage.

First-generation or conventional biofuels are made from sugar, starch or vegetable oil and have attracted criticism because their production is energy intensive and may increase food prices. In comparison, second-generation biofuels can be made from agricultural residues or waste, which otherwise may be costly to dispose of. Second-generation biofuels clearly have the potential to deliver considerable benefits, by increasing the environmental sustainability of bioenergy production.

Lignocellulosic residues from temperate EU crops such as maize, tropical Brazilian crops such as sugarcane and dedicated biomass crops such as miscanthus are all potential feedstocks for second-generation bioethanol production. However, it is hard to extract useful sugars from this woody or fibrous biomass, because of the presence of complex carbohydrates such as lignin, hemicellulose and cellulose, which lock the useful sugars in. The sugars have to be released using enzymes or pre-treatments before they can be fermented to make bioethanol. As a result, several technical challenges need to be overcome before second-generation biofuels could be produced in an economically competitive manner.

The aim of the SUNLIBB-CeProBIO project is to combine European and Brazilian research strengths to open the way for cost-competitive lignocellulosic bioethanol production, whilst ensuring that this also brings about the anticipated environmental benefits. The project integrates Brazilian expertise in sugarcane breeding and bioethanol process engineering with EU expertise in genomics, plant science and green chemistry.

Project Overview



Maize, miscanthus and sugarcane

Closely-related grasses, all with C4 metabolism. Knowledge generated in one species can be transferred to the others.

Objectives

The SUNLIBB programme of work aims to:

- **Improve the feedstock quality of lignocellulose in biofuels crops in order to allow truly cost-effective ethanol production.** This will be achieved using modern crop breeding approaches and cutting-edge plant cell wall research, to introduce improved digestibility traits to elite varieties, thus reducing the high costs associated with biomass conversion. To do this, we are incorporating dedicated gene identification programs that will discover genes for better quality polysaccharide content, improved digestibility and lignin that is easier to break down.
- **Add value to the overall process of conversion in biomass biorefining, by upgrading residues and by-products and producing other value streams from the feedstock, in addition to bioethanol.** We are developing technologies for the extraction of valuable materials such as waxes and oligosaccharides, as well as carrying out novel underpinning science to allow valuable materials to be generated from the breakdown products of lignin. These processes have been developed at laboratory scale and are being tested in pilot processing plants.
- **Improve the conversion process by which we produce sugars for fermentation.** We aim to do this using our improved feedstock with unique combinations of pre-treatments and new enzymes that are being produced in Europe and Brazil for the biochemical conversion of lignocellulosic material.
- **Develop integrated processes that capture maximum value from lignocellulosic biomass, by integrating a range of product streams in addition to bioethanol.** We are bringing improved feedstock, added value product extractions and improved saccharification and fermentation combinations together in pilot biorefineries in Europe and Brazil. This includes in-silico optimisation of energy balances, design of the plant, cost evaluation, exploration of efficient co-generation and reduced energy use for steam and cooling operations, as well as development of new thermo-chemical pre-treatments.
- **Ensure that the new processes developed fulfil sustainability requirements by reducing GHG emissions, cutting other forms of air pollution, having minimal impacts on local environments and biodiversity, building sustainable rural industries and not impacting on food production or prices.** We are developing and applying quantitative Life Cycle Assessment (LCA) models and qualitative approaches for other sustainability criteria suited to biorefineries. We are assessing the full spectrum of relevant impacts, using data generated by SUNLIBB and data available from ongoing work in partner organisations and public sources.

Consortium partners

SUNLIBB

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- Borregaard, Norway.
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- Ecover, Belgium.
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- University of Cambridge, UK.
- University of Leeds, UK.
- University of Sheffield, UK.
- VIB, Belgium.
- Wageningen University, The Netherlands
- Biotech Consultants Ltd, UK.

CeProBIO

- Universidade de São Paulo
- Universidade Federal de São Carlos
- Laboratório Nacional de Ciência e Tecnologia do Bioetanol
- Empresa Brasileira de Pesquisa Agropecuária
- Universidade Estadual de Campinas
- Universidade Federal de Viçosa

Publications

Please visit our website: www.sunlibb.eu

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SUNLIBB Work Packages – Aims and achievements

The work of the SUNLIBB Project is divided into Work Packages, each with a leader responsible for carrying out particular tasks according to their area of expertise.

1 Genetic approaches to improve biomass quality for cellulosic biofuel production – Work Package 1

Institut National de Recherche Agronomique (INRA), Biogemma, Wageningen University (WU).

WP1 aims to develop robust methods for improvement of biomass quality in maize and to transfer this knowledge to miscanthus and sugarcane. Mapping populations have been developed to find Quantitative Trait Loci (QTL) involved in biomass composition, digestibility and saccharification potential. In maize (INRA), QTL for saccharification and digestibility have been found (they are not co-localised). In miscanthus (WU), screening for saccharification QTL using BIOMIS and SUNLIBB mapping populations is on-going. Similar work on sugar cane is progressing in Brazil.

Lines of maize and miscanthus with contrasting saccharification characteristics have been sent for pilot experiments.

The cell walls of mutants (from Biogemma) with knock-out mutations in key candidate genes are being characterised.

2 Transcriptomic and genomic resources for biomass improvement – Work Package 2

WU, INRA, Biogemma, University of York (UoY).

The aim of WP2 is to generate transcriptomic data for genes involved in secondary cell wall biosynthesis in maize, miscanthus and sugarcane. A better understanding of cell wall biosynthesis will allow the tailoring of lignocellulosic biomass for more efficient conversion into Biobased products. For miscanthus, this requires the identification of miscanthus ortholog genes from model species and other C4 plants, and the extraction of high quality RNA during different stages of miscanthus development.

To uncover key genes involved in the synthesis and modification of the different components of the cell wall an **orthology web-interface database** has been developed.

Recently, deep transcriptome sequencing data (Illumina and 454 pyrosequencing) of *Miscanthus sinensis* have been released, which redirected our work to a bioinformatics focus using these datasets. OrthoMCL was used to create a multi-species ortholog database, using these RNA-seq data and the predicted proteome of twelve other species. Putative protein function for *M. sinensis* was inferred through orthology relationships. With this strategy, our aim is to directly identify candidate genes involved in secondary cell wall biosynthesis, via the annotation of transcripts and identification of gene orthologs. At the moment, we are using cell wall genes from maize, differentially expressed in an internode transcriptome dataset, to find candidate miscanthus orthologs and paralogs present in the database. Comparative expression analyses will then be performed using contrasting miscanthus genotypes. Tests with designed primers for ortholog lignin genes in miscanthus are currently underway, to be followed by a set of hemicellulose genes. A web-interface of the database is currently being updated to increase its usability. Our ultimate goal is to develop a tool that enables biologists, without extensive bioinformatics knowledge, to efficiently study *de novo* transcriptome data from any orphan crop. The tool is currently being designed to facilitate the identification of candidate transcripts through phylogenetic relationships and to enable design of specific and degenerate primers for analysis of known and novel transcripts.

3 Understanding cell wall polysaccharides to underpin biomass improvement – Work Package 3

University of Cambridge, Biogemma, UoY.

WP 3 aims to understand more about biomass polysaccharide composition and structure in maize and miscanthus, and investigate genes involved in their biosynthesis. So far, the main oligosaccharides composing the miscanthus GAX (glucuronoarabinoxylan) have been identified. This knowledge is transferrable to most grasses, and will help to create grasses with improved hemicellulose for digestibility and/or saccharification.

4 Understanding and obtaining value from lignin – Work Package 4

VIB Department of Plant Systems Biology, University of Ghent, WU, Biogemma.

WP4 aims to mine transcriptomic data for lignin biosynthesis genes, to make gene silencing or over-expression constructs for maize and miscanthus. Integrated metabolic maps have been made for maize. A set of novel target lignin genes has been identified in C4 grasses, using a systems biology approach.

5 Biomass deconstruction – Work package 5

UoY, INRA, University of Sheffield, VIB. U. Ghent.

WP5 aims to understand the deconstruction of lignocellulose at the biochemical level, to optimise cost and energy efficiency. A High-Throughput saccharification assay has been developed at The University of York for maize and miscanthus. This expertise has been shared with Brazil, where a similar assay has been established for sugarcane. Experiments have been carried out to optimise pre-treatments, and trials of new saccharification enzymes have been conducted. (See adjacent poster).

6 Generating Added Value from Biomass – Work Package 6

UoY , Borregaard, Ecover, Processum, U. of Sheffield.

WP6 aims to identify the extraction and processing steps required to generate added-value products from biomass. Hydrophobic molecules have been characterised and extracted using supercritical CO_2. Extraction of waxes has been optimised. (See adjacent poster).

7 Integrated process engineering to obtain full value from biomass processing – Work Package 7

U.of Sheffield, Processum, Biotech Consultants Ltd. (BTCL), Ecover, Borregaard.

WP7 aims to trial new processes that extract added-value products from partial pre-treatments (before enzymic saccharification) and to develop integrated process systems that obtain maximal value from lignocellulose, using data from WP5 and WP6.

Lab-scale and *in-silico* fermentations have been optimized. Pilot-scale fermentation trials are on-going. Cost modelling is still to be done.

8 Sustainability Assessment – Work Package 8

North Energy Associates, University of Leeds, Processum.

The purpose of WP8 has been to determine and compare the impacts of biofuel production in integrated biorefineries with other sources of biofuels and conventional fuels. The context of this work is the existing and emerging policies for biofuels, the frameworks for calculating their associated greenhouse gas emissions, and the application of sustainability criteria in the European Union (EU) and elsewhere. Investigation of this essential context has been undertaken by the Sustainability Research Institute at the University of Leeds, which has documented findings in the "Sustainability and Policy and Regulatory Review Report". This addresses policy and regulatory frameworks at EU and individual Member State level, assessing the effectiveness of sustainability criteria in relation to relevant governance.

Supporting activities included, among others; preparation of two Policy Briefs for the project's website (www.sunlibb.eu); an exchange visit to Brazil for dialogue with partners in the sister CeProBio Project at the University of Sao Paulo; interviews with stakeholders on bilateral (EU-Brazil), as well as trilateral (Brazil-Africa-EU) co-operation, with regard to second generation biofuels; presentations at conferences in Verona (2011) and The Hague (2013); publication of papers in the Journal of Cleaner Production, as well as in International Environmental Agreements: Politics, Law and Economics.

Both quantitative and qualitative sustainability assessments have been performed in this Work Package, co-ordinated by North Energy Associates Ltd with support from Processum Biorefinery Initiative AB and other partners, including the Department of Chemical and Biological Engineering in the University of Sheffield, and the Green Chemistry Centre at the University of York. Quantitative assessment involved developing 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 relevant feedstocks, consisting of cultivation, harvesting and transportation. Workbooks are based on the principles of life cycle assessment and accommodate different calculation methodologies including the European Commission's Renewable Energy Directive. The initial workbook models biorefinery operation and a feedstock supply chain based on sugar cane and bagasse. Subsequent workbooks represent biorefineries based on whole maize and stover, and miscanthus.

Qualitative assessment has been undertaken for biorefinery pathways based on maize and miscanthus feedstocks. Reports were produced on supporting sustainability criteria for these potential biorefinery feedstocks in the EU, addressing land use, soil erosion, fertility and carbon, water use, emissions to water and air, biodiversity and other impacts. The activities of this Work Package will conclude with a "Sensitivity and Comparative Analysis Report". This will document the effects on quantitative results of varying the values of key parameters in the workbooks, which will reflect the final feedstock specifications and biorefinery concepts of other relevant partners. Qualitative aspects of sustainability criteria will also be incorporated by ranking and grading relevant impacts.

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Screening of sugars and phenolics released during pretreatment of miscanthus, maize and sugar cane bagasse for potential added value products from c4 crops

SUNLIBB

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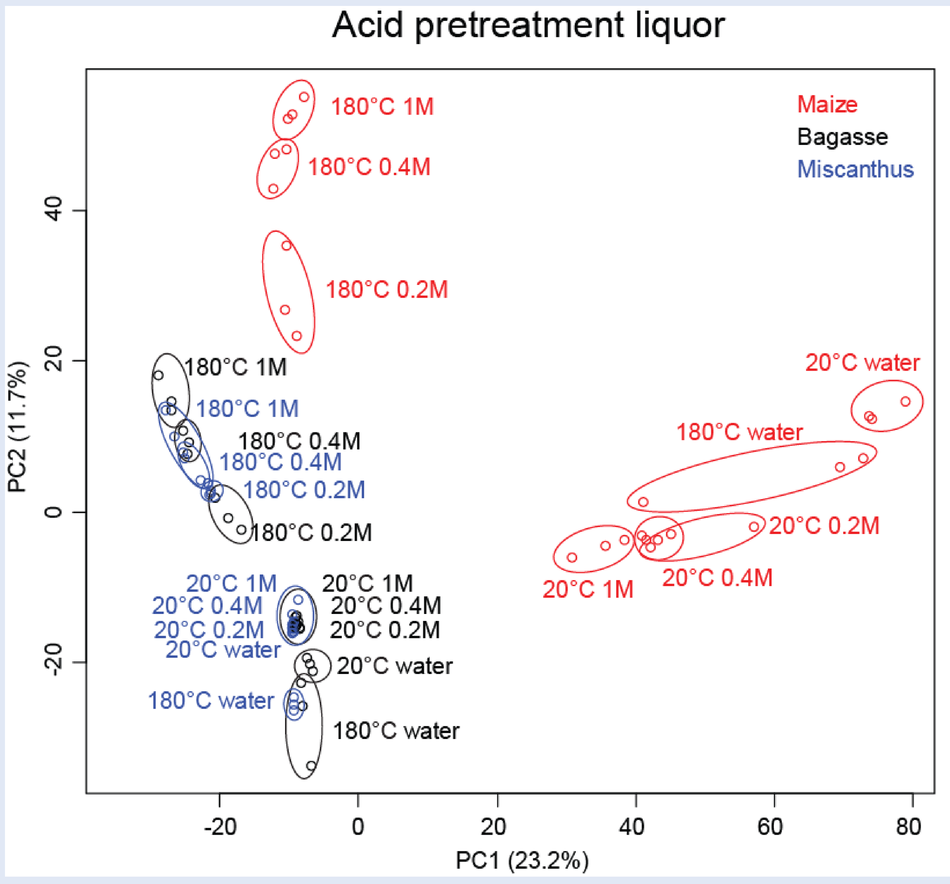
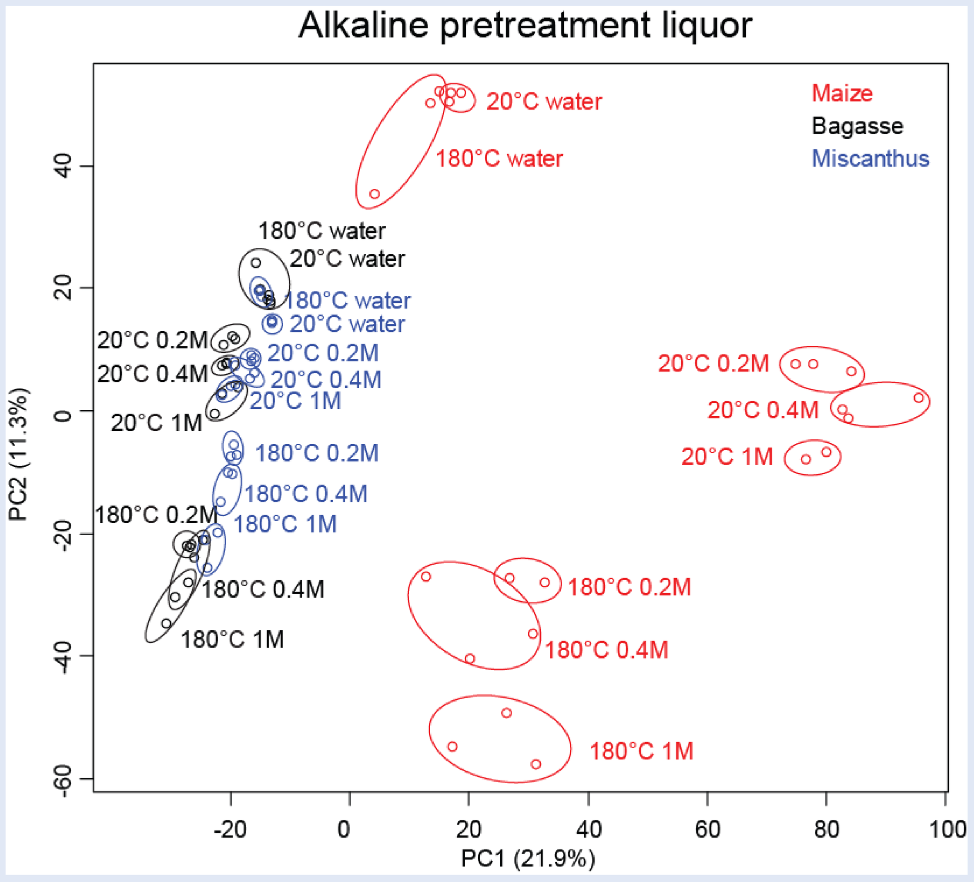
INTRODUCTION

The viability of cellulosic fuels hangs on the reduction of the costs involved in the conversion processes. An approach to reach a realistic cost for conversion of biomass into large volumes/low cost products is **by identifying added value products** that can be obtained during the production of biomass derived fuels. This biorefinery concept involves the benefits of reducing the cost of the overall process**, replace petroleum derived materials and chemicals, and reduce waste streams**. Pretreatments have been considered for a long time as the key to increase the efficiency of pretreatments in terms of increased fuel production after fermentation. An interesting new approach to evaluate pretreatments is the preservation of the valuable chemicals in the pretreatment liquors in order to add value to the process from products obtained by fractionation of the biomass. In the present work we characterise the sugars and lignin derivatives present in the **pretreatment liquor of maize, miscanthus and sugar cane bagasse under a range of acid and alkaline pretreatments**.

EFFECT OF PRETREATMENTS





To reveal the general similarities and differences between the pretreatment conditions, all samples were integrated and aligned after UHPLC-MS analysis and PCA was performed. The composition of the pretreatment liquor with acid 20 °C is not very different from water pretreatment. However, the pretreatment liquor with acid 180 °C clearly separated from the 20 °C, indicating that the **acid needs higher temperatures to be effective**. In terms of biomass, the PC1- and PC2-based PCA plots showed a **high similarity between miscanthus and bagasse**, while maize samples were clearly different.

MONOSACCHARIDES

The monosaccharide composition of these liquors was analysed using high-performance anion-exchange chromatography (HPAEC). In most conditions used, **glucose** and **xylose** were the most abundant monosaccharides, these are followed by **arabinose** (up to 39%), **galactose** (up to 10%) and **mannose** (up to 6%). Alkaline conditions release a complex mixture of monosaccharides with a large representation of C5 sugars. Acid conditions, on the other hand, produce liquors with higher proportion of glucose, particularly at low temperatures.

CHARACTERISED COMPOUNDS

bagasse

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	295	7217	67652	114765	76175	109028	75669	104939
2 *p*-coumaric acid	6668	20777	70675	169709	119810	222879	245659	369714
3 ferulic acid	72	581	19252	37541	42517	43488	63507	64413
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	10	38	80	580	92	1381	100	2656
5 G(8-O-4)Gglycerol 2	29	98	167	1668	217	3175	269	5510
6 G(8-O-4)Sglycerol 1	47	103	799	2419	1043	5720	1154	14754
7 G(8-O-4)Sglycerol 2	48	110	594	2444	709	6467	801	14947
8 S(8-O-4)Sglycerol 1	9	33	190	935	319	3017	315	9469
9 S(8-O-4)Sglycerol 2	4	30	60	804	91	2524	137	7727
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	188	208	10941	13445	13834	12398	15361	15378
11 G(8-O-4)ferulic acid 2	94	126	7733	10033	10931	12316	13641	9476
12 S(8-O-4)ferulic acid 1	6	45	861	8737	1554	8774	1970	7134
13 S(8-O-4)ferulic acid 2	11	59	1111	7532	1781	8296	2096	9286
14 G(8-5)ferulic acid	3	29	1743	4570	2132	4003	2968	3177
15 S(8-5)ferulic acid	6	79	898	12988	1395	13044	3171	13415
16 ferulic acid(8-5)G	44	821	1325	120892	1472	86698	1337	60799
17 ferulic acid(8-5)ferulic acid 1	2	15	818	3538	1144	4388	2489	5130
18 ferulic acid(8-5)ferulic acid 2	9	238	3410	22644	8283	29181	14562	42472

miscanthus

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	39	5529	33318	71394	45682	72479	48139	70987
2 *p*-coumaric acid	6	9446	60403	86250	98954	121878	209581	173383
3 ferulic acid	33	335	21671	21058	31556	28290	44645	43874
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	17	92	153	522	131	1589	173	4167
5 G(8-O-4)Gglycerol 2	41	297	386	2174	435	4528	542	11432
6 G(8-O-4)Sglycerol 1	92	169	705	974	935	3514	1211	10172
7 G(8-O-4)Sglycerol 2	54	144	453	2515	593	6909	831	19912
8 S(8-O-4)Sglycerol 1	39	108	196	755	242	2137	338	8485
9 S(8-O-4)Sglycerol 2	18	74	33	1237	58	2805	149	12244
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	361	159	19369	8215	21451	9190	25050	9032
11 G(8-O-4)ferulic acid 2	207	111	19436	9619	24514	11429	30389	5478
12 S(8-O-4)ferulic acid 1	181	146	1322	6940	1560	6684	2294	6222
13 S(8-O-4)ferulic acid 2	175	159	1106	9062	1457	7589	2086	8065
14 G(8-5)ferulic acid	80	49	13837	13749	12409	12752	11903	12403
15 S(8-5)ferulic acid	118	93	2011	26398	2521	21996	3080	25700
16 ferulic acid(8-5)G	19	37	740	67863	783	38336	630	28023
17 ferulic acid(8-5)ferulic acid 1	2	2	904	2734	1798	3420	2855	4033
18 ferulic acid(8-5)ferulic acid 2	18	45	5686	11220	8007	18032	13989	26838

maize

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	232	2486	6676	18368	12043	20825	13100	17315
2 *p*-coumaric acid	1865	6200	20541	39664	41984	55743	69022	78481
3 ferulic acid	3037	1939	23819	26599	36647	34566	48658	46241
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	5	14	22	382	35	829	34	1638
5 G(8-O-4)Gglycerol 2	5	44	50	816	66	1604	52	3052
6 G(8-O-4)Sglycerol 1	117	108	493	918	571	1938	636	5647
7 G(8-O-4)Sglycerol 2	100	81	375	1511	409	2881	463	6618
8 S(8-O-4)Sglycerol 1	59	84	160	490	209	1022	273	3805
9 S(8-O-4)Sglycerol 2	58	101	188	1253	114	1723	224	4181
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	136	137	6541	6559	7587	6461	9024	9494
11 G(8-O-4)ferulic acid 2	151	110	7038	6243	9587	6003	10568	5153
12 S(8-O-4)ferulic acid 1	87	42	961	6000	1342	4737	1545	5192
13 S(8-O-4)ferulic acid 2	61	76	993	4829	1041	4522	1268	4935
14 G(8-5)ferulic acid	2	37	137	388	128	3356	123	1139
15 S(8-5)ferulic acid	16	5	6897	11540	9550	8099	6893	7437
16 ferulic acid(8-5)G	21	78	2051	54000	2352	28328	874	19045
17 ferulic acid(8-5)ferulic acid 1	3	8	1556	6050	3087	6984	5483	8355
18 ferulic acid(8-5)ferulic acid 2	20	392	7488	17099	12006	22215	17651	30718

Eighteen **phenolic compounds** were structurally characterized based on their MSMS fragmentation and their respective ion traces. Benzoic **1**, *p*-coumaric **2** and ferulic acid **3** are among the highest accumulating compounds (based on MS traces).

Conclusions:

- Miscanthus and Bagasse pretreatment product profile are most similar to each other, while Maize presents a different profile of chemicals released.
- Xylose predominates liquor monosaccharides of Miscanthus and bagasse, while glucose predominates in maize.
- The alkaline preatreatment liquors were substantially more enriched in phenolic compounds as compared to acid. Grasses contain high levels of benzoate, *p*-coumarate and ferulate esters and these compounds are released in alkaline conditions.

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C_4 Waxes as defoamers

Thomas M. Attard, Andrew J. Hunt and Elke Theeuwes

Since the 1990's, decreasing fossil reserves, rising oil prices and concerns over security of supply and sustainability have led to a global policy shift back towards the use of biomass as a local, renewable and low carbon feedstock.

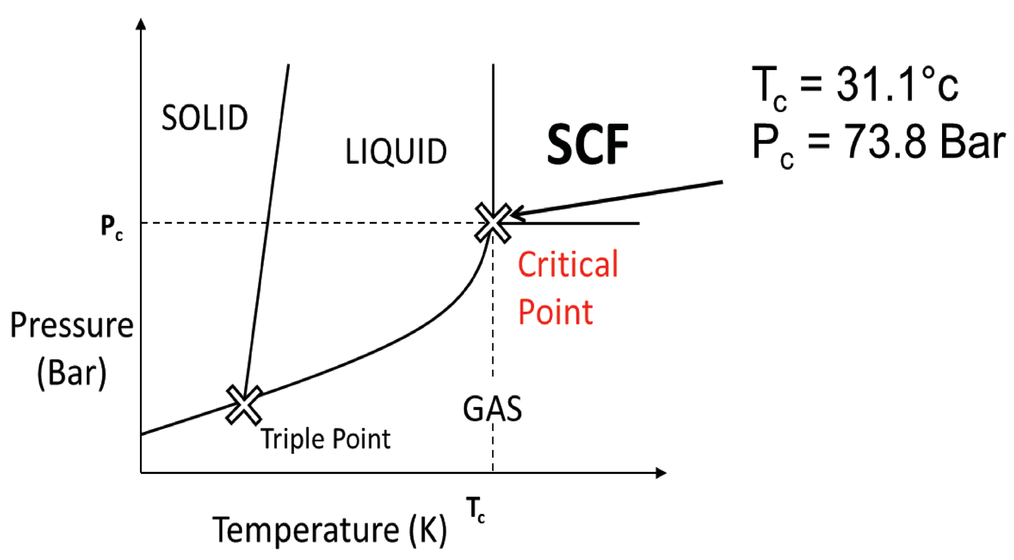
The biorefinery concept that has emerged is analogous to today's petroleum refineries that convert the biomass into multiple value-added products including energy, chemical and materials.[1]

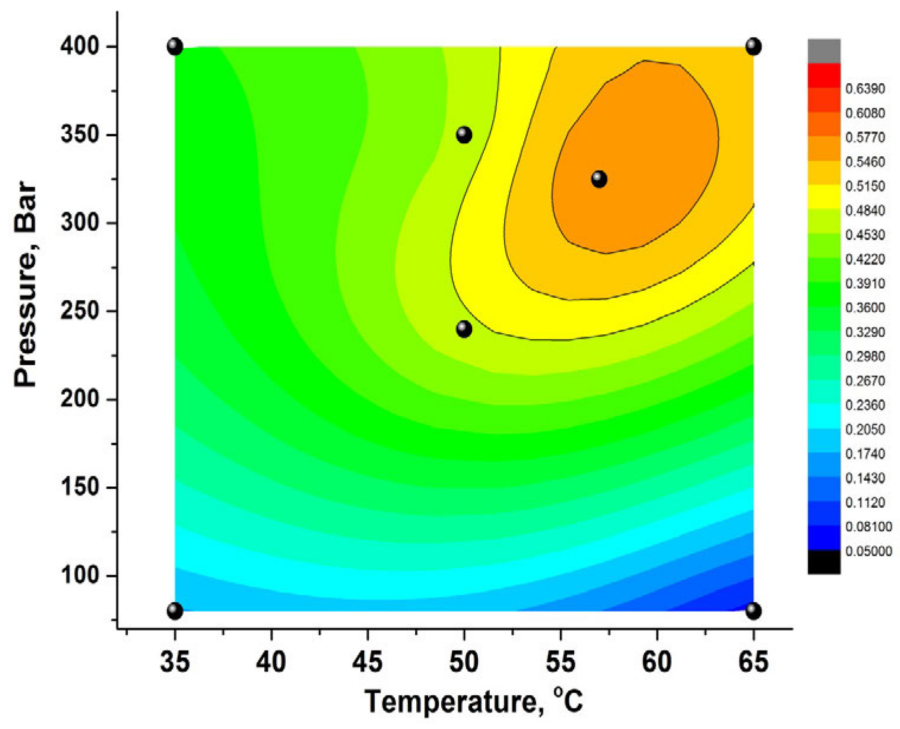
Extraction of valuable phytochemicals, prior to more destructive processes, can significantly increase the range of products and potentially improve the overall financial returns.[2]

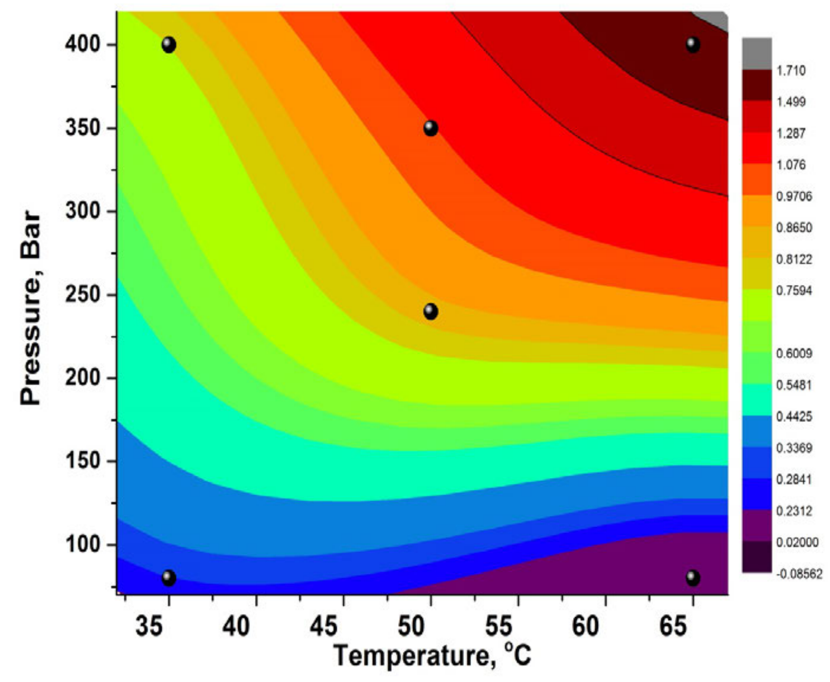
Supercritical Fluid Extraction/Fractionation of Waxes

The conventional techniques for extracting waxes involve the use of volatile organic solvents such as dichloromethane, chloroform and hexane which, apart from having environmental and toxicological effects, are also unselective and extract a number of unwanted compounds.[3] Supercritical CO_2 has several distinct advantages over conventional organic solvents in extractions.

- Selective
- Low surface tension
- High mass transfer rates
- Simple product recovery
- No solvent residues
- Cheap and non-toxic[3]





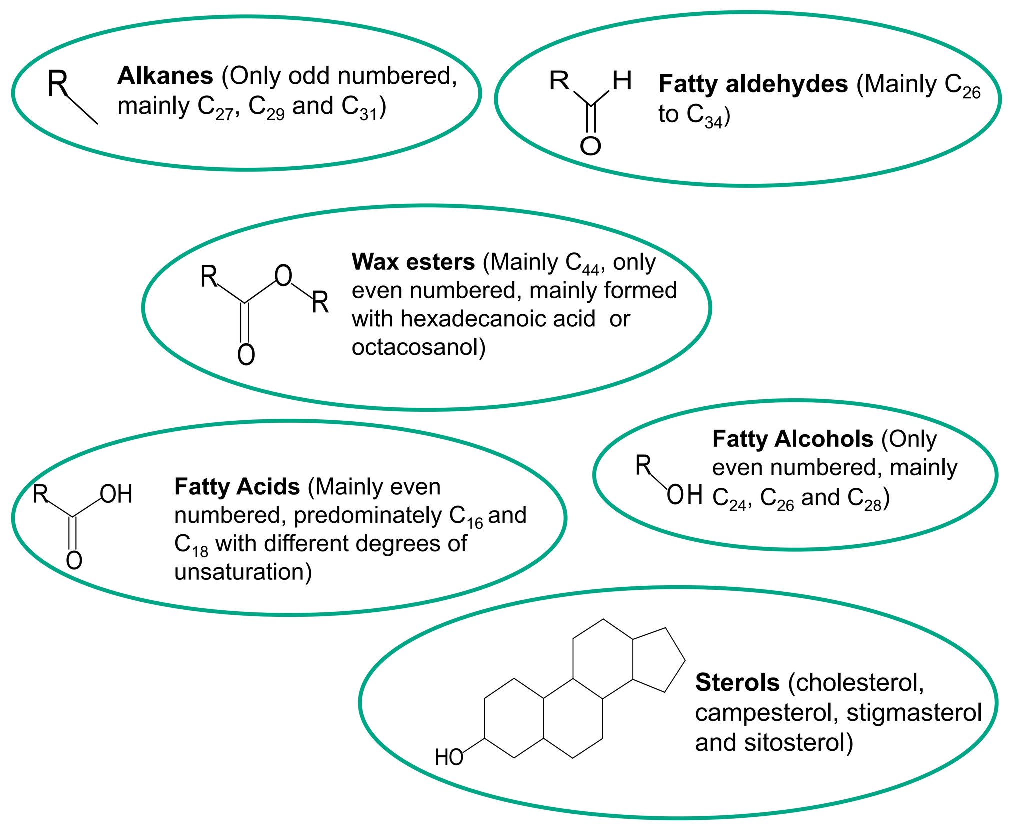


Waxes from maize and sugarcane bagasse contain a wide range of hydrophobic molecules ranging from long-chain hydrocarbons to wax esters.

Extractions of waxes from maize and sugarcane bagasse (SCB) were carried out using optimised conditions obtained using the factorial experimental design. Fractionation of crude waxes isolates different groups of hydrophobic molecules, resulting in wax fractions having distinct properties and melting points.

ATM/50 °C – Maize wax is a liquid at 50 °C

80 bar/35 °C – Maize wax is a yellow powder



Defoaming Properties of waxes

Foam control in laundry applications

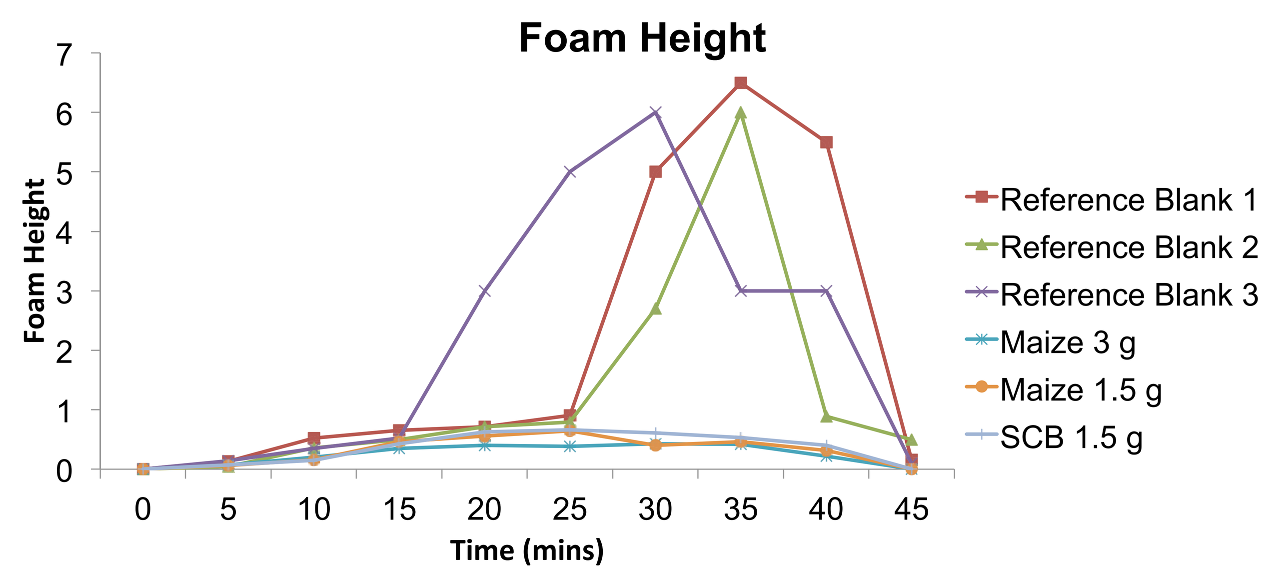
Foam control in horizontal axis washing machines is an important issue. Due to the mechanical agitation, elevated temperature and high surfactant concentration, an excess of foam can be generated resulting in adverse effects on washing performance related to impaired movement of the laundry itself and inefficient rinsing and drainage of the machine. Besides that, the electronic parts of the washing machine may be damaged. Several types of antifoam substances are used for foam control, although they have a negative impact on the environment[4,5]: phosphates (eutrophication), nitrogen-containing compounds (possible carcinogenetic by-products nitrosamines), organic silicon compounds (persistent) and fluoro compounds. At the moment, carboxylates are used in ecological laundry detergents. Another option is renewable hydrocarbons, like waxes, as presented here. The waxes should have a melting point range between 30-50°C and low saponification values[5].

Washing Machine Tests (real-life situation)

The wax samples were tested in the washing machine formulations. In the reference blank test no defoaming agent was added while in the wax washing machine test wax was added to investigate its defoaming properties in a washing machine run.

Reference Blank **Sugarcane** **Maize**

The height of the foam was measured every 5 minutes in order to investigate the efficiency of the wax as a defoaming agent.



Conclusions

Waxes from maize and sugarcane bagasse have been successfully extracted and fractionated using supercritical carbon dioxide. Washing machine tests have shown that the waxes are promising antifoaming agents. Tests on the performance of the surfactants in the presence of the waxes will be carried out.

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