

A review of the Integrated Biorefining Research and Technology Club (IBTI Club)



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A report for the Biotechnology and Biological Sciences Research Council (BBSRC)

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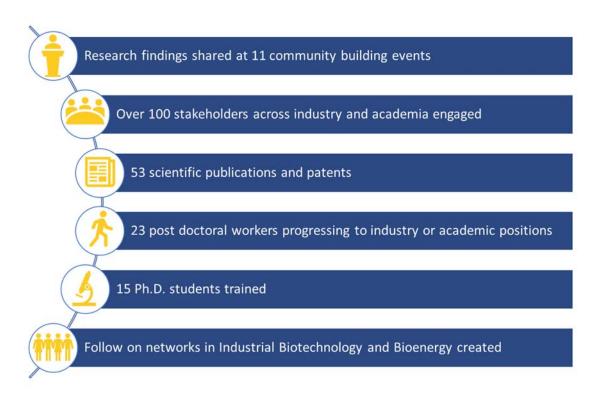
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1 Executive Summary

The aim of the IBTI club was to establish a programme of strategic biorefining research covering diverse raw materials and the essential conversion technologies necessary to realise their economic potential. The Club was to provide a mechanism to combine academic expertise in the biosciences with a relevant group of companies that would guide the research towards industrial development.

In response to 3 calls for proposals, the club invested £6.25Mn across 12 research projects. The research portfolio reflected the broad interests of the Club, with projects providing new insights into crop breeding, novel approaches to biomass pre-treatment and projects aimed at valorising existing biorefinery by-products.

The Club created an active biorefining research community, funded research activity contributing to the development of 38 post-doctoral researchers and Ph.D. researchers. The research outputs generated by the project team have been published in over 50 scientific papers and has contributed to a patent publication. Commercial activities related to the work of the club includes the creation of a technology spin out company and the formation of a consultancy business.



Several of the research themes initiated through IBTI funding have moved onto industrial research programmes:

- The work of Prof. Bugg at the University of Warwick has led to a number of ongoing projects to evaluate the industrial potential of lignin decomposition and production of added value chemicals and polymers. This has led to a significant collaboration with Biome Bioplastics in developing a process for producing aromatic chemicals from agricultural residues such as straw. These chemicals are being processed to produce new sustainable plastics, with improved properties over existing bioplastics.
- Prof. Bancroft developed a novel genetic tool for identifying variation in crops for marker-assisted breeding programmes and understanding disease susceptibility for Ash Dieback.
- Prof. Edwards developed synthetic biology tools in yeast which enable the transformation of low value bioproducts from biorefining into high-value intermediates for use in the food industry. His research has also evolved, with the knowledge gained through the IBTI project, to develop rapid screening systems for herbicide toxicity underpinning a collaboration with Syngenta.

The Club provided a community forum enabling a discussion on the scientific and business needs of the biorefining industry. These discussion groups became the basis of wider networks which ultimately evolved into several of the BBSRC Networks in Industrial Biotechnology and Bioenergy including FoodWasteNet, Plants to Products and the lignocellulosic Biorefinery Network LBNet. Collectively these networks have a total of 1,441 members with over 930 academics and 450 industrialists. LBNet, led by Simon McQueen Mason and Tim Bugg, commissioned a landscape study on the potential for integrated lignocellulosic biorefineries in the UK based on a range of feedstock types. This document assesses the indicative feasibility of various biorefinery scenarios that might be appropriate to the UK and therefore strategically influence relevant research project developments from the community and inform policy of infrastructure needs.

For the first time in the UK, the IBTI Club successfully brought together a group of industrial and academic researchers with an interest in biorefining. Several academic members were new to the biorefining area, demonstrating the ability of the club to attract research knowledge and capabilities, aligned but not previously utilised on biorefining projects. The research initiated in the Club continues through industrial collaborations clearly meeting the objectives of the Club to undertake fundamental research of strategic commercial interest.

In conclusion the IBTI Club represents a successful investment in strategic research, providing a stimulus for biorefining research activity, creating a platform for community building and providing a basis for ongoing technology development and networking activities.

2 IBTI Club Background

The UK's bioeconomy directly provides £36.1 billion in gross value added (2 per cent of the national total), provides 600,000 jobs and represents an important part of the overall economy¹.

The current bioeconomy is dominated by activity in the agricultural and food value chains but as the power of industrial biotechnology grows, the ability to convert renewable raw materials into added value pharmaceuticals, chemicals, polymers, increases in commercial attractiveness.

Around 2006-2007 the Biotechnology and Biological Sciences Research Council (BBSRC) recognised that an increased investment in research was required to underpin the needs of industry in developing biological processes to produce chemicals, polymers and pharmaceuticals.

As part of its strategy to meet this need, BBSRC invited the Biosciences KTN to identify industrially relevant research challenges and companies interested in forming the basis of a Research and Technology Club. Through industrial engagement and discussion, ten companies ranging from start-ups to multinationals agreed to support the formation of the club. From the outset, the importance of physical and engineering sciences to the biorefining process was recognised and therefore the need for the Engineering and Physical Sciences Research Council (EPSRC) to take an active role in the club.

In August of 2008, the BBSRC in partnership with EPSRC launched the Integrated Biorefining Research and Technology Club (IBTI Club) A total investment of approximately £6M was available to fund underpinning basic research in biorefining technologies. In addition to the BBSRC and EPSRC investments (£4MIn and £1.2MIn respectively) the industrial members of club invested £660,000.

An important feature of the club was the building of an active biorefining research community. An objective was that the club would bring together academic groups and industrial stakeholders and provide a platform for the dissemination of grant funded research outcomes to club members.

To enable knowledge exchange between club members and the wider biorefining community a fund was created to support, through bursaries, visits by academic members of the club to learn new scientific methods and techniques, and build new collaborations.

¹ The British Bioeconomy: An assessment of the impact of the bioeconomy on the United Kingdom economy, Capital Economics 2015

Funding was also made available to support PhD students through the provision of IBTI Club training grants and hence support the development of a future generation of scientists.

3 Club Governance and Operation

The IBTI Club was joint managed by BBSRC and EPSRC who also managed the peer review of research proposals submitted to the Club. The assessment of applications was performed by the IBTI Club Steering group, comprised of Club Industry Member representatives and selected academics, who ranked applications based on science quality and industrial relevance and ultimately provided funding recommendations to BBSRC and EPSRC.

To enable the effective co-ordination of club activities and the flow of information between grant holders and club members, BBSRC appointed an Academic Coordinator (Chris Knowles, Oxford Innovation) and an Industrial Coordinator (Adrian Higson, NNFCC). The academic coordinator worked with the academic community to develop project proposals and monitor the progress of funded projects and studentships. The industrial coordinator worked with industry members to canvass views, promote club activities and keep industry members informed on developments.

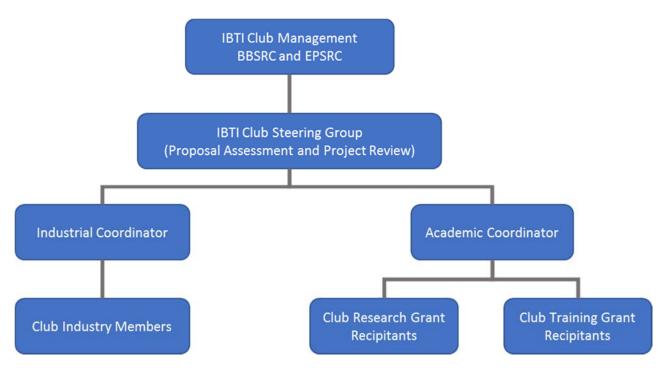


Figure 1. IBTI Club Structure

Working together, the club coordinators ensured the success of the Club through facilitating networking between funded research groups and industry.

4 IBTI's Industrial Members

Biorefining not only encompasses a wide range of technologies but also requires the participation of various stakeholders from crop breeders, renewable feedstock processors, technology developers and product manufacturers.

The participating industrial members of the IBTI club reflected this diversity and together provided a biorefining value chain perspective, from raw materials to processed products. The club members are listed below;

4.1 KWS UK

As part of the KWS Group, KWS UK is one of the UK's leading supplier of agricultural seeds (cereals, oil-seeds, sugar beet and maize). The KWS business is focused on providing UK growers with innovative new crop varieties capable of meeting demanding end market needs. For more information see <u>www.kws-uk.com</u>

4.2 Syngenta

Syngenta is world leading agribusiness, it has two core business areas, seeds and crop protection. Syngenta has 4,000 employees in R&D globally, working to find new and improved ways of raising the quality and yield of crops worldwide. For more information see <u>www.syngenta.com</u>.

4.3 Home Grown Cereals Authority

Home Grown Cereals Authority (HGCA is now part of The Agriculture and Horticulture Development Board (AHDB)) was a statutory levy board, funded by farmers, growers and others in the cereals and oilseeds supply chain. HGCA generated and disseminated information to support a competitive and sustainable arable industry and will continue to perform this function as AHDB oilseeds and cereals. For more information see https://cereals.ahdb.org.uk/.

4.4 InCrops Enterprise Hub

The InCrops Enterprise Hub was a not-for-profit technology transfer company based in the East of England. The InCrops project centred on providing business support to regional companies and entrepreneurs developing products and technologies based on alternative and non-food crops. InCrops is no longer operating.

4.5 Biocaldol

Biocaldol specialised in the development of new business systems for the agro industry. They offered proprietary, environmentally friendly micro-organisms, process design and engineering to produce second generation biofuels, animal feed and other biomass-derived products. Biocaldol is no longer operating but company expertise is available through Biotech Consultants Limited.

4.6 TMO Renewables

TMO Renewables was a biotechnology development company offering a second-generation ethanol from distiller's grains process as a bolt-on to existing corn ethanol plants. The company now trades as ReBio Technologies Ltd, an industrial biotechnology company specialising in the development of proprietary routes to key commodity platform biochemicals from renewable resources. For more information see <u>www.rebio.co.uk</u>

4.7 Green Biologics

Green Biologics is a renewable chemicals company producing butanol, acetone and related derivatives from renewable resources through a Clostridial ABE (Acetone-Butanol-Ethanol) fermentation. The company operates a production facility in Minnesota, USA. The company's R&D function is based in Oxfordshire. For more information see www.greenbiologics.com/

4.8 British Sugar Group

British Sugar Group (now AB Sugar) is one of the largest sugar producers in the world. In addition to sugar, the company also suppliers a range of co-products including animal feed, ethanol and, soil conditioning and landscaping products. For more information see www.britishsugar.co.uk

4.9 Croda International

Croda International is a world leader in natural based speciality chemicals. The company's activities fall into two areas; Consumer Care, consisting of personal, health, home and crop care and Industrial Specialities such as lubricants and lubricants additives and, polymers and polymer additives. For more information see <u>www.croda.com</u>

4.10 BP Biofuels UK

BP Biofuels UK is part of BP, one of the world's largest energy companies. BP had several interests in the development and production of biofuels including the development of

cellulosic ethanol processes the production of bio-isobutanol. For more information see <u>www.bp.com.</u>

5 The Research Agenda

The IBTI Club was launched to support underpinning basic research in biorefining technologies. Biorefining was defined as the fractionation and processing of renewable biomass feedstock for industrial applications. To ensure cost efficiency and environmental sustainability, biorefineries need to be highly efficient, producing multiple products with minimal waste.

What is a Biorefinery?

IEA Bioenergy Task 42 defines a biorefinery as 'the sustainable processing of biomass into a spectrum of marketable products and energy'. It can be a facility or cluster of facilities that aims to utilise as much of a biomass feedstock's components as possible, minimising waste.

Using a range of different types of biomass (e.g. lignocellulose, grasses) to produce a range of different product and commodities through several conversion pathways is described as 'Integrated Biorefining'. It is analogous to a modern-day petroleum refinery, where a range of chemical and energy products are produced from crude oil, optimising for both the feedstock and the market status of the products refined. Biorefining therefore is not just concerned with the physical conversion of biomass to products, but represents the upstream, midstream and downstream processing of biomass.

The key drivers for the development of biorefining as part of the wider biobased economy are sustainability, security and economics; all of which are inexplicably linked. The dangers of climate change, diminishing fossil reserves and fluctuating oil prices has focussed attention on generating energy and products from renewable resources, with the biobased economy having an integral part in global economic and sustainable development. The development of biorefining into commercially viable and sustainable processes is a major undertaking which requires multidisciplinary research at the biology, chemistry and engineering interface. With this is mind the IBTI club operated around three science themes;

- Optimisation of feedstock composition
- Integrative bioprocessing, and
- Enhancing product value.

Although biorefining is often associated with biofuels production, a diverse array of products can be produced either as an adjunct to biofuels production or as standalone processes. With this in mind, the Club took a broad view on the types of desirable outputs obtainable from a biorefinery. Examples of relevant products included;

- Speciality oils varying in carbon chain length, desaturation and substitution
- Surfactants
- Flavours and nutraceuticals
- Terpenoids
- Phytopharmaceuticals
- Polymers and monomeric precursors

5.1 Optimisation of feedstock composition

Sustainable biorefining requires that value be obtained from as much of the biomass as possible. This can be achieved through efficient extraction and conversion technologies and through the optimisation of feedstock quality.

The IBTI Club targeted the development of traits to increase the quantity of valuable components and the ease with which these components could be extracted.

The club placed a requirement for bulk production of chemicals and polymers to be based on biomass crops and agricultural residues while also recognising the potential of crops and algae to provide speciality and fine chemicals including bioactive molecules. The club sought to extend the range and quantity of both bulk and specialty products through feedstock improvements based on marker-assisted breeding, genetic engineering or a combination of both.

5.2 Integrative bioprocessing

The development of a biorefining process requires a multidisciplinary effort. From the development of physical and chemical treatment of biomass to allow the component parts

to be processed, through biotechnological transformation with or without chemical transformation, to the final recovery and purification of the end production, requires research at the interface of chemistry, engineering and biotechnology.

With biotechnology at the heart of club activities an important research goal was to achieve high levels of substrate utilisation using either real or artificially constituted biorefinery feedstock along with high consumption rates.

5.3 Enhancing product value

Alongside the production of a primary product, biorefining processes generally produce one or more co or by-products, for example the production of distillers dried grains and soluble (DDGS) alongside wheat or corn derived bioethanol.

Adding value to by-products through further processing can significantly improve the economics of the overall biorefinery process. The IBTI club targeted the use of novel biotransformation and fermentation processes to convert large volume co-products such as DDGS and lignin into new polymers or chemicals or new extraction processes to recover valuable components from by-product streams.

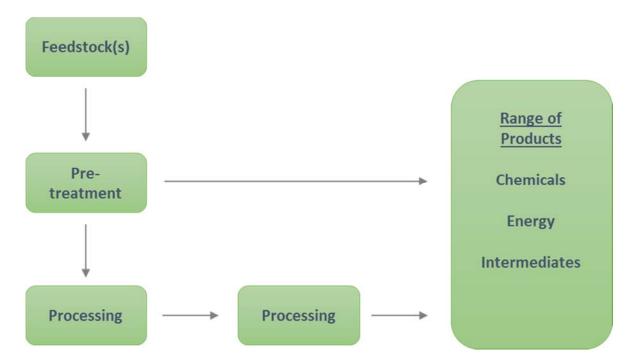


Figure 2. Example schematic of a biorefinery (simplified)

6 Community and Legacy

The IBTI club built, for the first time in the UK, a community focused on the various aspects of biorefining.

Through a combination of research grants, Ph.D. training grants and long term active business engagement, the club brought together a community of over 100 stakeholders covering agriculture and biorefining.

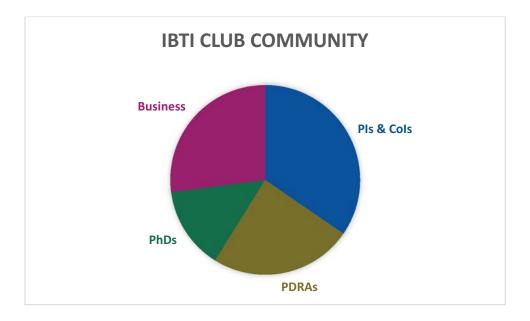


Figure 3. IBTI club stakeholder group

The relationships and community developed by the club were instrumental in formation of several of the BBSRC's Networks in Industrial Biotechnology and Bioenergy (NIBB). The NIBB represent the £18 million investment in 13 unique Networks fostering collaborations between academia, industry, policy makers and NGOs. As with the IBTI Club the networks have a central aim to identify novel approaches to tackle research challenges, translate research and deliver key benefits from industrial biotechnology.

The research and discussion stimulated by the IBTI club ultimately led the concept and formation of three networks, the lignocellulosic Biorefinery Network, the Food Waste Network and the Plants to Products Network and these networks and their members represent a legacy of the community initially built through IBTI with over 930 academic and 450 industrial members.

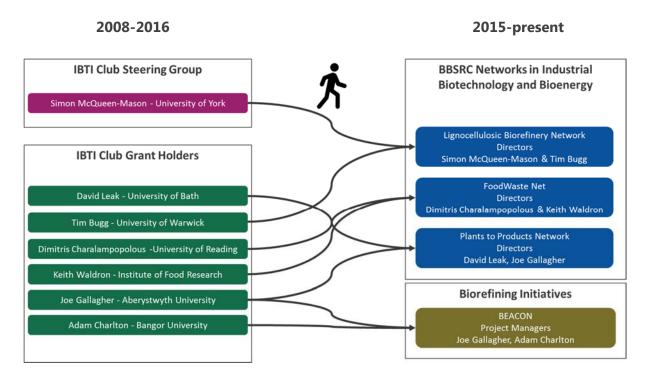


Figure 4. IBTI club as a catalyst for ongoing networks and initiatives

Beyond the NIBB, Club members are active in other biorefining initiatives such as the BEACON project delivered by the Universities of Aberystwyth, Bangor and Swansea.

7 The Projects

The IBTI Club issued three calls for research proposals. Calls one and two were deliberately broad to allow applicants to propose research projects to address a range of challenges whereas the third call specifically addressed the potential to add value to Distillers Dried Grains with Solubles (DDGS) as a biorefinery co-product. Across the 3 calls the club invested £6.25Mn across 12 research projects running from 2009 to 2015.

The following section provides a description of each projects, the published outputs from each project are listed in section 11.

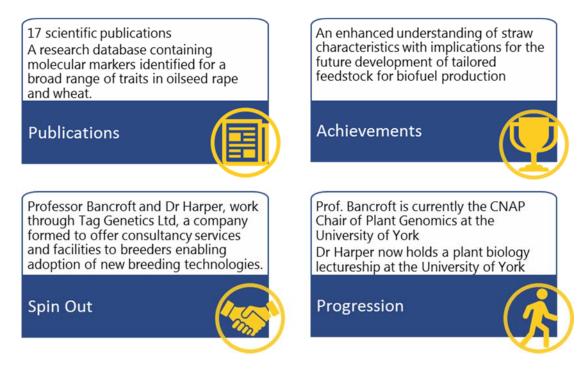
7.1 Funded research projects - Round 1

7.1.1 Optimisation of Wheat and Oilseed Rape Straw co-products for bio-alcohol production

| Principle Investigators | Ian Bancroft (John Innes Centre) |
|-----------------------------------|---|
| | Keith Waldron (Institute of Food Research) |
| Co Investigators | N/A |
| Post-Doctoral Research Associates | Andrea Harper (John Innes Centre) |
| | Samuel Collins (Institute of Food Research) |
| Associated PhD students | Charlotte Miller (John Innes Centre) |
| | Ian Wood (Institute of Food Research) |

Various forms of biomass represent potential feedstocks for degradation and fermentation to produce alcohols as liquid biofuels. Residual lignin and protein-containing materials may be suitable for further exploitation, such as processing for animal feed or production of platform chemicals. This provides the potential to substantially substitute for fossil fuels, with the associated sustainability and environmental benefits.

Despite the obvious benefits, concerns are raised in the eventuality that energy crops compete directly with UK food crops for land use. With the growing recognition of the vulnerabilities of world (and UK) food security, and associated food price volatility, the use of 'waste' biomass associated with production and processing of food crops represents an ideal feedstock for conversion to biofuel. By utilising the non-edible residues of wheat and OSR production instead of the edible grain and seed, this eliminates the 'food vs. fuel' debate while increasing the value of the straw and enhancing the financial viability of food production. Some characteristics of straw can make biofuel production particularly challenging however, such as the recalcitrant nature of lignocellulose meaning that straw based biofuel production is associated with high processing costs.



The objective of this project was to evaluate wheat and oilseed rape (OSR) residues from a wide range of varieties to elucidate key characteristics of the cell walls that determine the potential yield of bio-alcohol and investigate further the genetic bases and their variation and sequencing for these compositional traits to develop markers for use in breeding programmes.

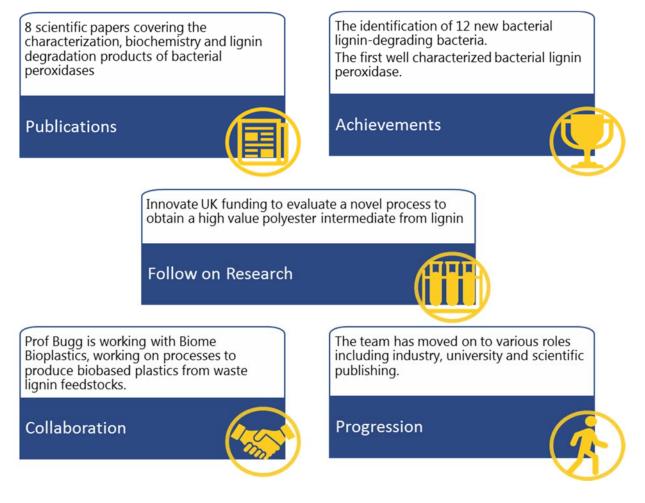
The project demonstrated that different varieties of wheat and oilseed rape produce straw with differing processability characteristics with implications for the future development of tailored feedstock for biofuel production.

| Principle Investigators | Timothy Bugg (University of Warwick) |
|-----------------------------------|---|
| Co Investigators | N/A |
| Post-Doctoral Research Associates | Elizabeth Hardiman (University of Warwick) Charles Taylor (University of Warwick) Zoe Mycroft (University of Warwick) |
| Associated PhD students | Paul Sainsbury (University of Warwick) Mark Ahmad (University of Warwick) |

7.1.2 Aromatic feedstock chemicals from degradation of Lignin

Diminishing fossil fuel resources and the unsustainable impact these fuels have on the environment when used for heat, power and transport are two of biggest challenges facing modern society today. It is not just in the energy sector, however, that these challenges pose a threat: the by-products of oil refining are used in the production raw materials to produce plastic, chemicals and pharmaceuticals. Soon, these products will need to be synthesised from 100% renewable feedstocks with carbon-based lignocellulose, available in abundance,

the key alternative to petro-feedstocks. As lignin is the only component of lignocellulose based on aromatic units, it is a key raw material in the production of high-value aromatic chemicals and polymers.

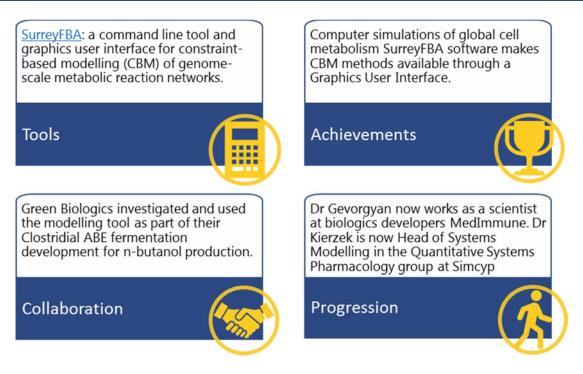


Producing chemicals and intermediates from lignin can be challenging however as its chemical structure means it is naturally very difficult to break down, often resulting in the need for intense pre-treatment steps (acid or high temperatures) to liberate the lignin. The objective of this project therefore was to identify bacterial strains that are capable of breaking lignin down and developing new methods using these bacteria to convert lignin to high-value aromatic chemicals.

The project produced important outputs and resulted in a number of significant results. A new colorimetric assay method was developed for monitoring lignin breakdown. Using this assay method, 12 new bacterial lignin-degrading bacteria were identified from soil samples, and their lignin degradation capability assessed. A bacterial lignin-degrading peroxidase enzyme DypB was identified in *Rhodococcus jostii* RHA1. This is the first bacterial lignin peroxidase to be well characterised. Using gene deletion strains of *Rhodococcus jostii*, high levels of metabolites from lignin degradation were produced by fermentation of lignocellulose, including vanillin, a valuable chemical for the food/flavour industry.

| Principle Investigators | Mike Bushell (University of Surrey) |
|-----------------------------------|---|
| Co Investigators | Norman Kirby (University of Surrey) |
| | Claudio Avignone-Rossa (University of Surrey) |
| | Andrzej Kierzek (University of Surrey) |
| Post-Doctoral Research Associates | Albert Gevorgyan (University of Surrey) |
| Associated PhD students | Kaylee Herbert (University of Surrey) |

7.1.3 In Silico study of Lignocellulosic biofuel processes

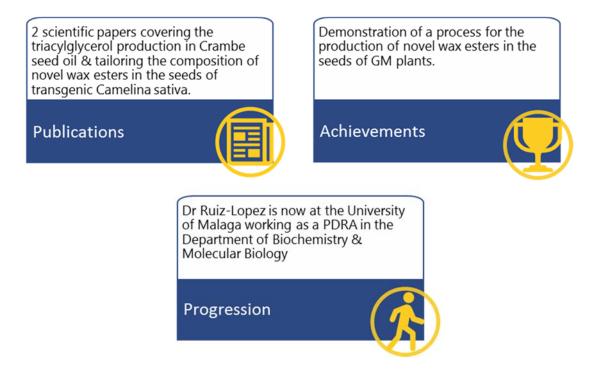


A genome sequence, theoretically, provides all the necessary information required to define a biological system of interest. Knowledge of all the enzymes in a cell and all its substrate pairs, for example, and the products each can make, makes it possible to formulate a bioreaction master global network that represents a complete set of possible biochemical reactions within a given cell.

The objective of this study was to link the genome sequences of 'model' organisms capable of degrading biomass to the genomes of organisms capable of producing ethanol from biomass to produce a computer simulation platform that could predict optimal characteristics of manufacturing processes involving pairs of woody biomass degrading and bio-alcohol producing microbial cultures.

7.1.4 Engineering oilseeds to synthesise designer wax esters

| Principle Investigators | Jonathan Napier (Rothamsted Research) |
|-----------------------------------|---|
| Co Investigators | Frederic Beaudoin (Rothamsted Research) |
| Post-Doctoral Research Associates | Noemi Ruiz-Lopez (Rothamsted Research) |
| Associated PhD students | Royah Vaezi (Rothamsted Research) |



Waxes are a type of lipid that have several useful properties, especially as lubricants. The modern industrial world is dependent on the smooth running of millions of machines and processes, all of which require lubricants to reduce friction and wear & tear. Currently, most lubricants are made from crude oil by chemical transformations, a process which consumes a diminishing natural resource both as a feedstock and as a source of energy to drive the reaction. It is therefore important that waxes can be efficiently produced from renewable resources.

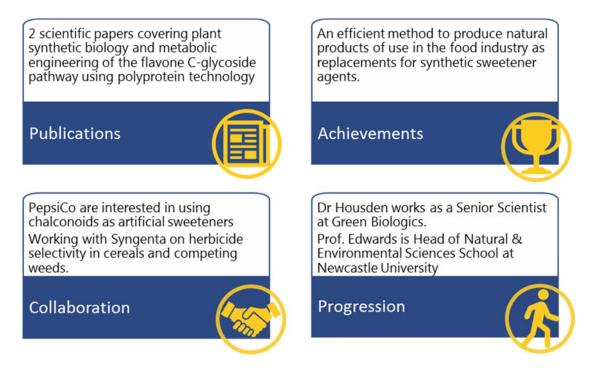
Although plants can produce oils from their seeds, they are not very effective as lubricants as they are prone to oxidation which makes them sticky. The jojoba plant is unique however, as it makes a different class of oil to that found in all other plant seeds, through the accumulation of wax esters which have superior lubricating properties compared with the normal seed oils which accumulate triacylglycerols. Jobaba plants grow in arid conditions however, which precludes their wider commercial use in European agriculture. To circumvent this problem, this project proposed to transfer the genes for wax biosynthesis from jojoba (and other organisms which make wax esters) to suitable temperate plant species in order to synthesise and accumulate wax esters in the seeds of a dedicated non-food oilseed crop, providing a novel and sustainable source of these lipids for use as bio-lubricants. The project demonstrated how to make novel wax esters in the seeds of GM plants.

7.2 Funded research projects - Round 2

7.2.1 Bio transforming Phenylpropanoids derived from Biorefining: A Toolkit Approach

| Principle Investigators | Rob Edwards (University of York) |
|-----------------------------------|--|
| Co Investigators | N/A |
| Post-Doctoral Research Associates | Hazel Housden (University of York) Federico Sabbadin (University of York) |
| Associated PhD students | Keir Bailey (University of York) |

Phenylpropanoids (PPs) are metabolites found in all terrestrial plants which are used in nature to make a range of polyphenolic compounds which have dietary properties as flavourings and antioxidants. The objective of this project was to derive a flexible process for biotransforming simple PPs left over as low value by-products (lignin) from biofuel and plant fibre production and bio-transforming them in to high-value polyphenolic intermediates of value to the food industry. This was achieved using a novel polyprotein technology to assemble a multienzyme biosynthetic pathway in the yeast species *Saccharomyces cerevisiae*, then using the engineered yeast to ferment PPs to high value products.

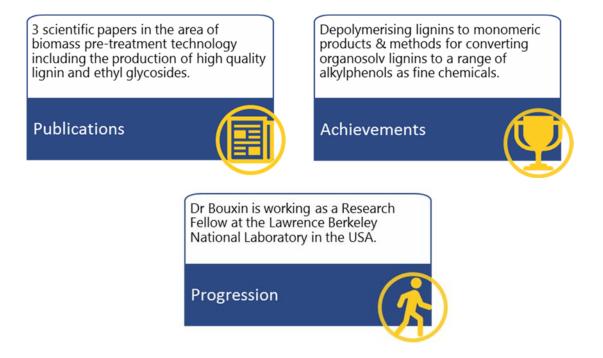


The project demonstrated an efficient method to produce natural products of use in the food industry as replacements for synthetic sweetener agents. The technology is both of commercial interest and gives new insights into the metabolic regulation of multi-enzyme pathways.

7.2.2 Fine chemicals from lignocellulosic fermentation residues using heterogeneous catalysis

| Principle Investigators | Michael Jarvis (University of Glasgow) |
|-----------------------------------|--|
| Co Investigators | David Jackson (University of Glasgow) |
| Post-Doctoral Research Associates | Florent Bouxin (University of Glasgow) |
| Associated PhD students | Ashley McVeigh (University of Glasgow) |

During liquid biofuel production from lignocellulosic biomass, the polysaccharides must first be liberated from the lignocellulose matrix via pre-treatment processes. Following this, they are broken down to their constituent oligomers and monomers before undergoing conversion to biofuels.



Challenges associated with using lignocellulose as a feedstock for biofuel however include the fact that lignin, as a recalcitrant material, can resist degradation which blocks the conversion of polysaccharides embedded within its structure to fermentable sugars. In addition, lignin is not often utilised in an effective manner and is mostly collected as a residue which, at best, is combusted to provide some process heat demand in the biofuel production process. As lignin is the most abundant aromatic feedstock on the planet, it is an important feedstock from which to produce renewable aromatic chemicals.

The aim of this project was to exploit the lignin fraction of lignocellulose (following biofuel production) to produce high-value marketable molecules. Specifically, the project aimed to extract soluble model lignin residues then using heterogenous catalysis, depolymerise the lignin to produce a group of molecules called alkylphenols.

The project developed optimised or partially optimised pre-treatment conditions for softwoods, hardwoods and cereal straw and investigated methods for depolymerising lignins, in lignocellulosic biomass fermentation residues, to monomeric products and methods for converting organosolv lignins to a range of alkylphenols with potential as fine or performance chemicals.

7.2.3 Process Intensification for Acceleration of Bio and Chemo Catalysis in Biorefining

| Principle Investigators | Adam Kowalski (University of Liverpool) Mike Egan (University of Liverpool) |
|-----------------------------------|--|
| Co Investigators | Janet Scott (University of Bath) Gill Stephens (University of Nottingham) |
| Post-Doctoral Research Associates | Sivaram Pradhan (University of Liverpool) |
| Associated PhD students | Alison Woodward (University of Nottingham) |

Controlled Deformation Dynamic Mixing (CDDM) technology is the result of more than 2 decades of R&D within Unilever Research Labs and with the engineering involvement of TecExec Ltd. Since 2007, ongoing development of the CDDM technology has been carried out at the Ultra Mixing and Processing Facility at the University of Liverpool. Using this highly controllable and scalable approach, extremely intensive processing conditions can be used to increase reaction rates, reduce processing times, shrink processes and significantly reduce CAPEX/OPEX, as well as to develop novel materials and application areas.



This project focussed on 'enhancing product value' of biofuel by-products while at the same time providing capability in integrative bioprocessing, using the University of Liverpool's Ultra Mixing and Processing Facility (UMPF). The facility provides the engineering capability to show that bio and chemocatalysis can be used as integrated unit operations adjunct to the fuel production stream of a biorefinery, to produce high value aromatics from lignin and monomers from unsaturated oils. The project relied on a novel proprietary 'Process Intensification' technology which helps overcome mass transfer problems.

Although the project allowed the mixing technology to be used in a new industrial area i.e. biocatalysis, the project encountered a familiar challenge in lignin processing. Under process

conditions (enzymatic or thermochemical depolymerisation) chemical intermediates tend to repolymerise into an intractable material.

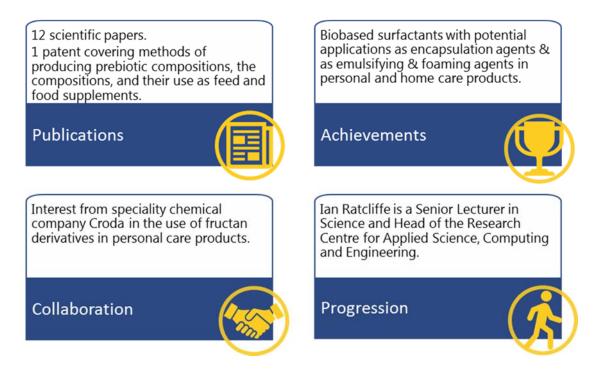
The IBTI project represented one aspect of the UMPFs potential. In 2012 CDDM Technology (CDDMtec) was formed as joint venture between Unilever Ventures Ltd and TecExec Ltd. The company aims is to build businesses around CDDM technology for applications beyond Unilever's fields of interest.

| 7.2.4 | Isolation fractionation and modification of fructans from rye-grass to produce |
|-------|--|
| | novel biosurfactants and polymers as part of a rye-grass biorefinery |

| Principle Investigators | Peter Williams (Glyndwr University) Adam Charlton (Bangor University) Joe Gallagher (Aberystwyth University) |
|-----------------------------------|--|
| Co Investigators | Steve Fish (Aberystwyth University) |
| Post-Doctoral Research Associates | Ian Ratcliff (Glyndwr University) Gwynn Lloyd Jones (Bangor University) Andrew Cairns (Aberystwyth University) Lesley Turner (Aberystwyth University) |
| Associated PhD students | Matthew Evans (Glyndwr University) Rakesh Bhatia (Aberystwyth University) |

The economic and sustainable development of a biorefinery is dependent on the valorisation of all co-products and on the maximisation of feedstock molecules. This project was concerned with the establishment of a sustainable biorefinery based on rye-grass; a feedstock that can yield a wide range of molecules called fructans. Fructans are molecules of interest as they can be chemically modified to produce novel polymers and surfactants which have application in a range of commercial formulations, especially in the cosmetic and personal care industries.

The objective of this project was to exploit perennial rye-grass, through producing and isolating fructans from high-sugar strains and fractionating these fructans into a range of sizes, using separation techniques, that can be chemically modified to produce novel polymers and surfactants.



The project demonstrated that fructan could be isolated and purified at pilot scale and that the fructans could be converted to molecules with reducing groups for Maillard reactions using an endo-hydrolase.

Using a combination of physical pre-treatment (ultrasound) and an environmentally friendly bleaching agent (sodium percarbonate) the grass fibre co-product could be converted to a colour free, odourless fibre for a range of applications including speciality paper and packaging, acoustic insulation and, porosity and vapour composite boards.

The project led to the production of a range of biobased surfactants that have potential application for the encapsulation and delivery of active hydrophobic compounds as well as for application as emulsifying and foaming agents in personal care and home care products.

Although the fructans have the potential to be useful surfactants the production economics are challenging, and any development will depend on the utilisation of all biorefinery outputs.

7.2.5 Evaluation of consolidated bioprocessing as a strategy for production of fuels and chemicals from lignocellulose

| Principle Investigators | David Leak (Imperial College London) |
|-----------------------------------|---|
| Co Investigators | Nilay Shah (Imperial College London) |
| | Cleo Kontorvadi (Imperial College London) |
| | Xioa Xu (Imperial College London) |
| Post-Doctoral Research Associates | Jeremy Bartosiak-Jentys (Imperial College London) |
| Associated PhD students | N/A |

4 scientific papers covering consolidated bioprocessing of lignocellulosic biomass including metabolic modelling, and assessment of glycosyl hydrolase secretion. The project demonstrated that that consolidated bioprocessing could be achieved on a small scale but bioreactor designs are required for process scale up.

Publications



Achievements

Dr Bartosiak-Jentys went on to hold positions at CPI, Zuvasyntha and the Univeristy of Bath. He is currently Research & Technology Manager, Biotechnology at Croda Europe Ltd.

Progression

As already highlighted, to produce chemicals and fuels from lignocellulosic biomass, it must first undergo pre-treatment. A typical pre-treatment involves the physico-chemical disruption of the biomass followed by enzymatic hydrolysis of the polysaccharides to its monomer units. Pre-treatment processes as well as the conversion of sugar monomers to fuels and chemicals (e.g. via fermentation) take place as distinct processes, requiring different types of enzymes and therefore increased costs. These processing costs could potentially be lowered through consolidating the necessary and desirable functions in to a single microorganism that can convert lignocellulose to a useful product through replacing much or all the externally sourced enzymes with enzymes produced by the fermenting organisms for example. This type of processing is an example of a one-step consolidated bioprocess (CBP).

The objective of this project was to evaluate the feasibility of using CBP as a method for lignocellulosic biomass conversion into products using *Miscanthus x giganteus* as a substrate and a genetically engineered *Geobacillus thermoglucosidasius* bacterium; a thermophile with some existing capabilities that make it suitable for lignocellulosic feedstock processing. By

creating suitable recombinant strains, the objective was to enable researchers to establish where process bottlenecks arose through looking at fluid dynamic behaviour of mixtures with high solids loadings and modelling the conversion process.

The project demonstrated that that consolidated bioprocessing could be achieved on a small scale. However, the project also showed that it would be impossible to put the required amount of undigested lignocellulosic material into a bioreactor and mix it effectively based on a simple batch process and current bioreactor designs. Therefore, for this to become a realistic process, different operating strategies and bioreactor designs are required.

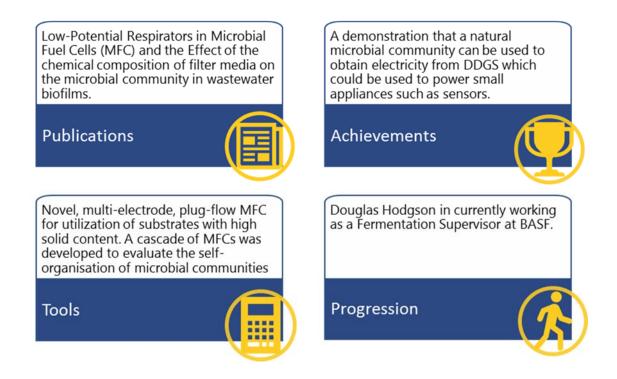
7.3 Funded research projects - Round 3

7.3.1 A study of metagenomics-informed biochemical functionality of microbial fuel cells using DDGS as a substrate

| Principle Investigators | Mike Bushell (University of Surrey) | |
|-----------------------------------|---|--|
| | Claudio Avignone-Rossa (University of Surrey) | |
| Co Investigators | Julian Marchesi (Cardiff University) | |
| | Bob Slade (University of Surrey) | |
| | John Varcoe (University of Surrey) | |
| Post-Doctoral Research Associates | Douglas Hodgson (University of Surrey) | |
| | Ann Smith (Cardiff University) | |
| | Sonal Dahale (University of Surrey) | |
| Associated PhD students | N/A | |

During grain-to-bioethanol and whisky production, a by-product called 'dried distillers grains with solubles' (DDGS) is produced. This by-product has traditionally been used to make animal feed however there has been growing interest amongst stakeholders in enhancing its value as an industrial feedstock.

The objective of this project was to develop a microbial fuel cell (MFC) capable of processing DDGS prior to drying and use as animal feed to generate electricity and reduce consumption of the biorefinery. (An MFC being a device that contains an anaerobic culture of micro-organisms, capable of directly converting chemical energy to electrical energy.)



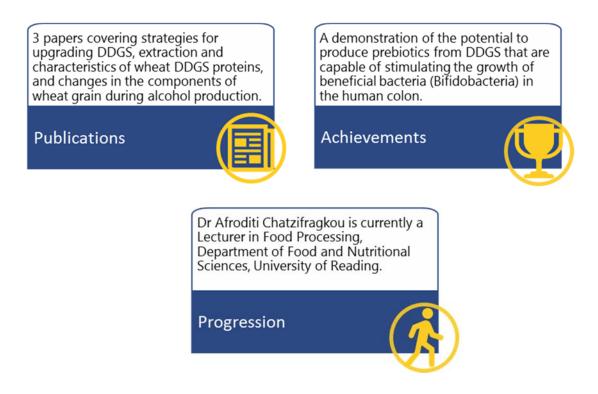
To select the correct micro-organisms for use in the MFC, all genes present in whole populations of microorganisms were analysed under a range of conditions, then a computer simulation was used to highlight the most important genes required to carry out the desired function of the MFC.

The project demonstrated that a natural microbial community can be used to obtain electricity from DDGS (a by-product of the bioethanol industry) which could be used to power small appliances such as sensors. After passing through the MFC the DDGS would exist as an improved animal feed, as the MFC process results in the removal of many indigestible components.

The learning from project has enabled the group to engage in new projects looking at waste water treatment and assess the potential for the evolved microbial communities to produce solvent molecules.

7.3.2 Development of a process scheme for the production of high value functional products from DDGS

| Principle Investigators | Dimitrious Charalampopoulos (University of Reading) Peter Shewry (Rothamstead Research) |
|-----------------------------------|--|
| Co Investigators | Glenn Gibson (University of Reading) Bob Rastall (University of Reading) Richard Frazier (University of Reading) Alsion Lovegrove (Rothamstead Research) |
| Post-Doctoral Research Associates | Dr Afroditi Chatzifragkou (University of Reading) Dr Parvathy Prabhakumari (University of Reading) Dr Andrea Monteagudo (University of Reading) Dr Ondrej Kosik (Rothamsted Research) |
| Associated PhD students | N/A |



The DDGS produced as co-product of bioethanol fermentation is currently produced in large quantities annually worldwide. Although its current primary use is as a protein-rich animal feed, an issue with this application (which hinders its utilisation compared to soybean and canola meals) is its compositional variability, which consequently affects its nutritional quality and digestibility.

The objective of this work was to develop a novel, scalable and economically viable process that will transform DDGS into several medium to high value products, namely a prebiotic food ingredient, gluten protein for film packaging, betaine and choline for use as nutritional supplements, and crude dietary fibre. The process was based on the biorefinery concept in which the agricultural raw material is transformed into several value-added streams, which are either end-products or starting materials for secondary processing.

The project demonstrated the potential to produce prebiotics from distiller's grains. The extraction of proteins from wet solids or DDGS results in a solid residue with high carbohydrate content (~50%) which through the application of selective enzymes can be converted to xylo-oligosaccharides capable of stimulating the growth of beneficial bacteria (Bifidobacteria) in the human colon.

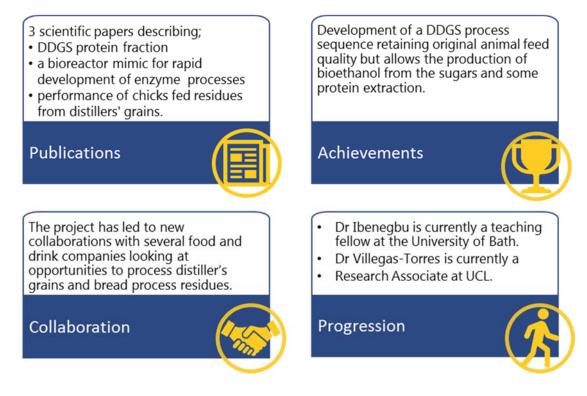
| Principle Investigators | David Leak (University of Bath) | |
|-----------------------------------|--|--|
| Co Investigators | Gary Lye (University College London) Caroline Rymer (University of Reading) | |
| | | |
| | Santos (University of Birmingham) | |
| Post-Doctoral Research Associates | Chris Ibenegbu | |
| | Maria Francisca-Villegas-Torres | |
| | Ricardo Roque | |
| Associated PhD students | N/A | |

7.3.3 Fractionation and exploitation of the component value of DDGS

As outlined in the previous two project descriptions, increasing the value of the DDGS byproduct of bioethanol production is of currently of interest to stakeholders. Presently, DDGS is used as a high protein animal feed however its high fibre content limits its use to ruminant animals who can internally process the fibrous material prior to digestion. If the non-starch carbohydrate and fat could be removed from the DDGS effectively and used as a feedstock for high value chemicals, it would leave residual protein to be used as feed, enabling processed DDGS to be utilised across several markets.

This application also carries with it potential GHG benefits, as separating the DDGS protein for feed and associated non-starch carbohydrates, and fat for high value products could reduce the need to cultivate and grow soybean (with associated GHG emissions) in comparison to scenarios where all the DDGS was used solely to make high value products.

This project therefore focussed on developing methods to remove non-starch carbohydrates and fat and from DDGS without destroying the feed value and finding ways to gain added value from the extracted components.



Various combinations of extraction were tested to determine the most suitable process flow. A sequence that started with supercritical CO₂ extraction of oil, followed by alkaline ethanol based extraction of some protein followed by the production of bioethanol from the high sugar fraction post steam explosion and hydrolysis, with the remaining solids retaining their original animal feed quality was found to be the preferred combination.

Techno-economic analysis of a stand-alone extraction process showed that the most cost sensitive part of the process was the initial supercritical CO_2 extraction. However, in an ethanol biorefinery, CO_2 and ethanol are readily available and recoverable, which would reduce the costs of the oil extraction step considerably, making the process designed, economic.

The understanding and techniques developed in the project have led to new collaborations with several food and drink companies looking at opportunities to process distiller's grains and bread process residues.

8 **Results dissemination and community building**

A key aim for the IBTI Club was to build of a community of researchers working across biorefinery development, ultimately capable of building multi-disciplinary consortia to address the complex challenges of biorefinery research.

Community building centred on biannual research dissemination events which brought IBTI funded researchers together with industrial and academic club members. The events provided an opportunity for IBTI funded researchers to present project progress and outline future plans, while receiving scientific and commercial feedback from club members.

The IBTI community was further enhanced through the award of 15 IBTI Club Ph.D. training grants. Students were encouraged to attend club events and had the opportunity to present posters and give both flash and short presentations to the club and hence develop a range of soft transferable skills.

| Supervisor | Institution | Student |
|----------------------|----------------------------|------------------|
| Prof. Ian Bancroft | John Innes Centre | Charlotte Miller |
| Prof Keith Waldron | Institute of Food Research | Ian Wood |
| Prof Tim Bugg | University of Warwick | Paul Sainsbury |
| Prof Mike Bushell | University of Surrey | Kaylee Herbert |
| Prof Jonathan Napier | Rothansted Research | Royah Vaezi |
| Prof David Jackson | University of Glasgow | Ashley McVeigh |
| Prof David Leak | University of Bath | Ali Hussein |
| Prof Peter Williams | Glyndwr University | Matthew Evans |
| Prof Rob Edwards | University of York | Keir Bailey |
| Paul Fraser | RHUL | Amanda Kozlo |
| Dr Joe Gallagher | Aberystwyth University | Rakesh Bhatia |
| Prof Gill Stephens | University of Nottingham | Alison Woodward |
| Rowan Mitchell | Rothamsted Research | Lucy Hide |
| Prof Nilay Shah | Imperial College London | Kristian McCaul |
| Greg Tucker | University of Nottingham | Paul Waldron |

9 Case Studies

As described previously a key goal for the IBTI Club was the development of the UK's research capacity in biorefining. This involved encouraging academics with research interests applicable to biorefining to undertake biorefining research, creating an environment for academics and industrialists to develop research relationships and form collaborations for future research and provide a mechanism to train the next generation of academic and industrial, scientists and engineers in the field of biorefining.

This section highlights three IBTI projects that have had particular success and describes the impact of IBTI funding on the development of biorefining research and collaborations that have led to economic and societal benefits.

9.1 Using enzymes to convert the lignin found in biomass to useful aromatic chemicals - Professor Tim Bugg

The science;

Principle Investigator Professor Tim Bugg's background is in mechanistic enzymology of bacterial aromatic degradation: how microbes metabolise aromatic chemicals in the environment.

Through discussions with colleagues and other research centres at the University of Warwick, Professor Bugg and his peers recognised the need and opportunity to apply his research expertise to the biorefining sector and that he was ideally placed to investigate the use of lignin as a key aromatic chemical feedstock.

He was awarded a PhD studentship which resulted in the development of a biochemical assay to follow the bacterial degradation of lignin. At this time, and by fortunate coincidence, the IBTI call for funding was announced and Professor Bugg was successful in his application allowing his continued research into bacterial lignin decomposition.

A new colorimetric assay method for monitoring lignin breakdown was developed, and using this method, 12 new lignin-degrading bacteria were identified from soil samples and their lignin degradation capability assessed. From this, the first ever detailed characterisation of the bacterial lignin-degrading enzyme: peroxidase DypB from *Rhodococcus jostii* RHA1 was produced. Furthermore, using gene deletion strains of *Rhodococcus jostii*, metabolic pathways were elucidated and the potential to control lignin decomposition towards chemicals such as vanillin, a valuable chemical for the food/flavour industry, was demonstrated.

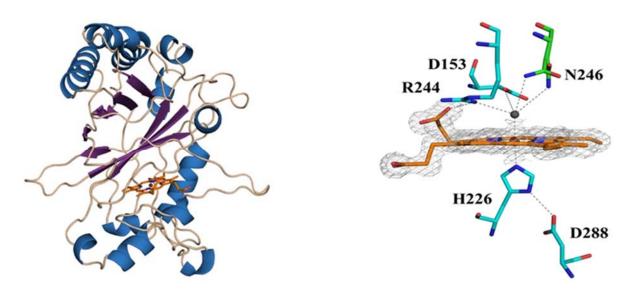


Figure 5. Crystal structure of peroxidase DypB and peroxidase DypB active site

According to Professor Bugg, one of the main challenges using bacterial enzymes for lignin degradation is the fact the genetic pathway for this type of degradation is still poorly understood; meaning that gathering more genome sequence data is essential to enhance understanding. Another challenge, which is particularly important when considering scaling-up, is the time taken to for these biological processes to produce chemicals and polymers. At the laboratory scale, this is in the order of a few days, which is too slow for industrial production, meaning that more research will be required to investigate how to speed up the production processes.

In the field of biological lignin degradation, the traditional focus of research has been largely on fungi as principle degraders. Research into the potential of using bacteria as process tools for the conversion of lignin to chemicals and polymers has undergone significant development since Professor Bugg and his team's ground-breaking work. These include the work of Greg Beckham and his team at the National Renewable Energy Laboratory (NREL) in Colorado, USA, who are actively designing and developing chemical and biological catalysts to deconstruct and upgrade lignin to value-added molecules.

The project team;

Prof Bugg is a Professor of Biological Chemistry at the University of Warwick. Members of his research team who worked with him as part of this IBTI grant included Mark Ahmad (PhD), Liz Hardiman (PDRA), Paul Sainsbury (PhD), Zoe Mycroft (PDRA) and Charles Taylor (PDRA).

Mark Ahmad is currently teaching abroad while Liz Hardiman is now working in an administrative role in a New Zealand University. Paul Sainsbury is currently head of membership, marketing and communications at the Society for Microbiology and editor of the microbiologist magazine, Zoe Mycroft is working for a biotechnology company in Birmingham and Charles Taylor is working with Southern Water.

The project team has gone on to new employment in a variety of roles, demonstrating the flexibility and transferable skills that training and exposure to the IBTI Club provided.

To be continued;

The Bugg laboratory has continued its research and formed a close collaboration with Biome Bioplastics, one of the UK's leading developers of biobased plastics. As part of their R&D portfolio, Biome were looking to develop novel aromatic based polymers as alternatives to the more widely developed aliphatic biobased polymers. While aliphatic molecules are relatively easy to access from renewable resources, the aromatic chemicals widely used in plastics such PET or polystyrene are hard to produce. However, the processing of lignin presents a potential route to aromatic chemicals and after a wide and international search for potential collaborators Biome recognised the value of Prof. Bugg's work within IBTI and began discussions on collaboration. If a new route to novel aromatic chemicals could be developed it may be possible to produce new plastics with improved performance and lower environmental impacts.

The collaboration between Biome and Prof. Bugg began with a 2013 feasibility study, funded through Innovate UK. The study evaluated the feasibility of production and commercialisation of a substituted phthalic acid from lignin using pathway engineering and through scale-up of a novel fermentation processes. A further ERA-IB funded project, involving INRA and CSIC, is investigating lignin degradation in an alternative host, *Pseudomonas putida*. The project takes the knowledge gained from studying *Rhodococcus jostii*, to re-programme the relatively well know metabolism of *Pseudomonas putida*, and use high activity lignin-degrading enzymes, to produce two pyridine dicarboxylic acids for use in the production of novel plastics.

"The foundational work performed by Prof. Bugg as part of IBTI, gave us the confidence to begin a programme of industrial development around biobased aromatic polymers from lignin. Through working with Prof. Bugg and other UK leading academics we are making considerable progress to realising our vision, with over a hundred different polymers combinations prepared for performance testing and an initial kg sample produced" Paul Mines, CEO, Biome Bioplastics

This initial work has led to two further projects funded through the Industrial Biotechnology Catalyst Programme. The first project, involving researchers at the Universities of Warwick and Leeds, seeks to demonstrate that lignin metabolites previously extracted at laboratory scale can be produced in a commercially viable manner. The project is investigating the selective decomposition of lignin using whole cell or isolated enzymes under fed batch or continuous reactor conditions. Ultimately the developed processes will be trialled at larger scales using the facilities available at the Centre for Process Innovation, CPI. The second project, involving the Universities of York and Liverpool, is focused on the technologies required to produce polymers from the lignin derived monomers. The project is developing biocatalytic routes to highly functional polyesters and assessing their properties and lifecycle benefits.

Although there is further development and scale-up work to do; the initial research performed within IBTI has evolved to a point where real commercial opportunities for the production of lignin derived polymers can be envisaged

9.2 New plant breeding techniques maximising resource efficiency and biorefining productivity - Professor Ian Bancroft

The science;

Before the IBTI project, Professor Bancroft's research focussed on *Brassica napus* (Oilseed Rape (OSR)). The crop is used as a model system for studying the evolution of polyploid genomes under breeding selection, providing insight into how genome changes affect crops in areas such as vegetable oil composition and the uptake and use of micronutrients. The IBTI funding had a significant impact on Professor Bancroft's research allowing him to extend his crop portfolio to include wheat in addition to Brassica species.

In the IBTI project more than 50 characteristics, relevant to the use of wheat and rapeseed straw for bio-alcohol production, were analysed. From this, molecular markers for these characteristics were identified, based on gene sequence variation and/or gene expression variation, and made available for use in marker-assisted breeding programs. The project also resulted in the development of the integrated methodology termed 'Associative Transcriptomics', which identifies both gene sequence variation and gene expression variation correlated with trait variation. The contribution of the IBTI grant towards the development of the Associative Transcriptomics methodology is noteworthy as the funding enabled this methodology to develop from a theoretical concept at the start of the project to a practical technique at the end and is now a widely used genetic tool.

The work on Associative Transcriptomics by Bancroft and team led to further research on Ash (tree) Dieback caused by the fungal pathogen *Hymenoscyphus fraxineus*. The disease known as Ash dieback was identified in the UK in 2012, ash is the UKs third most abundant species of broadleaf tree, covering 129,000 hectares, there are concerns that if the disease was to establish in the UK large numbers of trees would have to be destroyed.

This work, published in Nature Scientific Reports, successfully identified markers for low susceptibility to ash dieback via Associative Transcriptomics, the first case of using this approach to identify markers associated with disease susceptibility in plants. The work on Associative Transcriptomics also led to funding from Defra to investigate the characteristics of OSR crops such as establishment success, nitrogen use efficiency and susceptibility to diseases such as stem canker.

The use of Associative Transcriptomics in plant breeding is in its infancy but the development and training provided through the IBTI project is building the UKs capacity and capability in the method.

The project team;

Prof. Bancroft is currently the CNAP Chair of Plant Genomics at the University of York. The PDRA Dr Andrea Harper now has a plant biology lectureship at the University of York and works with Bancroft providing consultancy services via Tag Genetics Ltd. PhD student Charlotte Miller, who worked on the project, took up a post-doctoral research place at the John Innes Centre and has accepted a postdoctoral position at the Salk Institute.

To be continued;

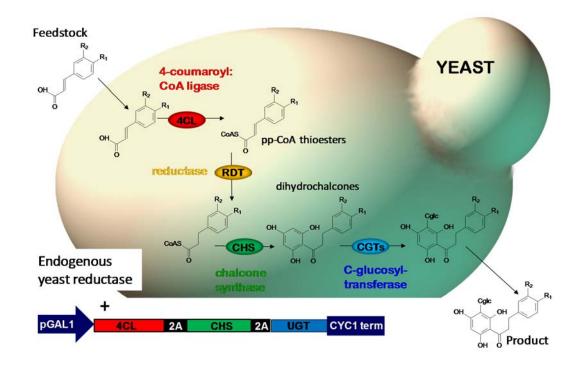
Although development of the Associative Transcriptomics methodology was a key outcome of this project, the techniques are regarded as complicated. Encouraging crop breeders to employ it as common-practice in real-world applications was highlighted as a key challenge by Professor Bancroft. Also, intellectual property can be a barrier to university – plant breeder partnerships. To ameliorate these issues, Tag Genetics Ltd. was formed. The company offers consultancy services and facilitates the adoption of these innovative technologies by crop breeders.

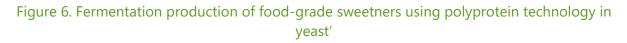
9.3 Synthetic biology for transforming low value by products into high value intermediates for the food industry - Professor Robert Edwards

The science;

Professor Robert Edwards current research interests are focussed around the biotransformation of synthetic compounds and natural products in plants and the manipulation of these pathways for applications in crop protection and biorefining using technologies including synthetic biology. He became involved in the IBTI Club through his role as science lead at the Bioscience for Business Knowledge Transfer Network (KTN) but had not undertaken any specific research directly related to biorefining before this. Adding value to the by-products of biofuel and biobased products production is important for the economic viability of a biorefinery operation. For example, the non-fermented residue from bioethanol production contains significant quantities of extractable chemicals including phenylpropanoids such as ferulic acid. Through the IBTI project the Edwards group demonstrated how they could be transformed into high-value polyphenolic intermediates of interest to the food industry as new flavour enhancing products. This was achieved by assembling a multienzyme biosynthetic pathway in the yeast species *Saccharomyces cerevisiae*, then using the engineered yeast to ferment phenyl propanoids to produce interesting food ingredients including dihydrochalcones, widely used as artificial sweeteners and flavour masking agents.

The IBTI club has enabled Edwards to dedicate part of his research to a work programme in the field of biotechnology and helped create several academic and industrial contacts within the field.





The IBTI club has also influenced the direction of the Edwards research group, notably through the use of polyprotein technology developed in the IBTI project in further yeast engineering programmes. In particular, polyproteins have been used to characterise herbicide detoxification pathway enzymes from crops and weeds using yeast expression systems. This has resulted in further major funding from Syngenta to study herbicide selectivity mechanisms (£350k) and is part of The Black-GRass Herbicide Resistance Initiative (BGRI) funded by BBSRC and AHDB (£3.4m).

The project team;

Prof. Edwards is currently the Head of the School of Natural and Environmental Sciences at Newcastle University. Several researchers who worked on the IBTI project with Prof. Edwards have remained in the field of biotechnology. One PDRA, Dr Housden currently works as a Senior Scientist and IP Manager at biobased butanol producer Green Biologics, one of the UKs leading industrial biotechnology companies and member of the IBTI Club. The other PDRA, Dr Sabbadin currently holds a Post-Doctoral Research position at the University of York, and the PhD student, Dr Bailey is currently working as a Post-Doctoral Research Assistant at the University of Leeds.

To be continued;

The project has resulted in an interesting potential collaboration with food and drink giants PepsiCo, who are interested in Edward's work on chalconoids which can be used as artificial sweeteners in the food manufacturing industry.

As part of learning gained from the project, for the Edwards group, one of the biggest challenges in the field of synthetic biology is the general ambivalence towards genetic modification, especially its application in the food chain. Other challenges highlighted by Edwards includes a reluctance of flavour and fragrance SMEs to explore biotechnology as method of production, as much of their backgrounds are firmly grounded in traditional synthetic chemistry. Their limited experience or knowledge of bioscience inhibits their uptake of industrial biotechnology as a manufacturing method. Related to biotechnology in the wider context is the challenge of developing products from an expensive technology with enough added value to make it economical. In other words, suitable applications need to be sought that provide sufficient payback through biorefining approaches.

10 Delivery against IBTI objectives

The aim of the IBTI club was to establish a programme of strategic biorefining research utilising a diverse range raw material feedstock and focussed on developing the conversion technologies necessary to realise their economic potential.

The Club was to provide a mechanism to combine the UK's academic expertise in the biosciences with a relevant group of companies that will help ensure the research funded through the Club was taken forward to industrial development. Through industrial collaboration the bioscience and associated technologies generated during the research programme could be applied efficiently to establish a competitive UK bio-economy.

In respect to these aims the IBTI club was successful in developing both a broad research portfolio covering feedstock optimisation and process development. The club's portfolio covered the processing of distillers' grains and solubles, as a co-product of ethanol production, the production of fructans from rye-grass as novel surfactants, lignin processing to produce fine chemicals, *in silico* modelling, microbial fuel cells, a synthetic biology approach to phenylpropanoid synthesis as synthetic sweeteners and the development of GM seeds for wax ester production.

Inevitably not all research is adopted by industry however several projects generated industrial interest and have moved forward to industrial development, and leading to product development. Notable examples include the interest of food and drink giants PepsiCo in the work of Prof Robert Edwards and the ongoing collaboration between Prof Tim Bugg and Biome Bioplastics.

The Club also provided platform for discussion on research and business needs in the field of biorefining. These discussions were the catalyst for the creation of informal special interest groups; ultimately leading to the formation of several BBSRC Networks in Industrial Biotechnology and Bioenergy, research grants and policy activities supporting UK biorefining.

In conclusion the Club was successful in meeting its objectives with it aims continuing to be realised through ongoing collaborative industrial R&D projects and through the networking activities of the Networks in Industrial Biotechnology and Bioenergy.

11 Publications

11.1 Optimisation of Wheat and Oilseed Rape Straw co-products for bioalcohol production

Steam Explosion of oilseed rape straw: establishing key determinants of saccharification efficiency. Wood, I. P., Wellner, N., Elliston, A., Wilson, D. R., Bancroft, I., Waldron, K. W., Bioresource technology, 162, 175-83 (2014)

Variation in the chemical composition of wheat straw: the role of tissue ratio and composition. Collins, S. R., Wellner, N., Martinzez Bordonado, I., Harper, A, L., Miller, C. N., Bancroft, I., Waldron, K., Biotechnology for biofuels, 7 (1), 212 (2014)

Dissection of the control of anion homeostasis by associative transcriptomics in Brassica napus. Koprivova, A., Harper, A. L., Trick, M., Bancroft, I., Kopriva, S., Plant physiology, 166 (1), 442-450 (2014)

Quantification of reducing sugars in biomass hydrolysates: Improving the speed and precision of the dinitrosalicyclic acid assay. Wood, I. P., Elliston, A., Ryden, P., Bancroft, I., Roberts, I.M., Waldron, K. W., Biomass and bioenergy. 44, 117-121 (2012)

Associative transcriptomics study dissects the genetic architecture of seed glucosinolate content in *Brassica napus*. Lu, G., Harper, A., Trick, M., Morgan, C., Fraser, F., O'Neill, C., Bancroft, I., DNA Research. 21 (6), 613-625 (2014)

A novel and fast approach for population structure interference using kernel-PCA and optimisation. Genetics. Popescu A, A., Harper, A. L., Trick, M., Bancroft, I., 198 (4), 1421-1431 (2014)

Methodology for enabling high-throughput simultaneous saccharification and fermentation screening of year using solid biomass as a substrate. Elliston, A., Wood, I. P., Soucouri, M. J., Tantale, R, J., Dicks, J., Roberts, I, N., Waldron, K. W., Biotechnology for biofuels, 8 (1), 2 (2015)

Effect of *Brassica napus* **cultivar on cellulosic ethanol yield.** Biotechnology for biofuels. Wood, I, P., Wellner, N., Elliston, A., Wilson, D., Bancroft, I., Waldron, K. W., 8, 99 (2015)

Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat. Harper, A., Trick, M., He, Z., Clissold, L., Fellgett, A., Griffiths, S., Bancroft, I., Plant Biotechnology Journal, 14, 1207-1214 (2015)

Associative transcriptomics of traits in the polyploid crop species *Brassica napus*. Harper, A, L., Trick, M., Higgins, J., Fraser, F., Clissold, L., Wells, R., Hattori, C., Werner, P., Bancroft, I., Nature Biotechnology, 30 (8), 798-802 (2012)

Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). Feng, L., Chen, B., Zu, K., Wu, J., Song, W., Bancroft, I., Harper, A. L., Trick, M., Liu, S., Gao, G., Wang, N., Yan, G., Qiao, J., Li, J., Li, H., Xiao, X., Zhang, T., Wu, X., DNA research, 21 (4), 355-367 (2014) **Extensive homoeologous genome exchanges in allopolyploid crops revealed by mRNAseqbased visualization.** He, Z., Wang, L., Harper, A.L., Havlickova, L., Pradhan, A.K., Parkin, I.A.P, Bancroft, I., Plant Biotechnology Journal, 1-11 (2016). DOI: 10.1111/pbi.12657

Elucidation of the genetic basis of variation for stem strength characteristics in bread wheat by Associative Transcriptomics. Miller, C. N., Harper, A.L., Trick, M., Werner, P., Waldron, K., Bancroft, I., BMC Genomics 17, 500 (2016)

Carbohydrate microarrays and their use for the identification of molecular markers for plant cell wall composition. Wood, I. P., Pearson, B. M., Gutierrez, E. G., Havlickova, L., He, Z., Harper, A. L., Bancroft, I., Waldron, K., PNAS 114:6860-6865, (2017)

Variation across a wheat genetic diversity panel for saccharification of hydrothermally pretreated straw. Collins, S. R. A., Wilson, D. R., Moates, G, K., Harper, A. L., Bancroft, I., Waldron, K., Biotechnology for Biofuels 10:227, (2017).

Variation in susceptibility to microbial lignin oxidation in a set of wheat straw cultivars: influence of genetic, seasonal and environmental factors. Konstantopoulou, M., Slator, P. J., Taylor, C. R., Wellington, E. M., Allison, G., Harper, A. L., Ian Bancroft, I., and Timothy D.H. Bugg, T. D. H., Nordic Pulp & Paper Research Journal 32:493-507, (2017)

Dissecting the complex regulation of lodging resistance in *Brassica napus.* Charlotte N. Miller, Andrea L. Harper, Martin Trick, Klaus Wellner, Peter Werner, Keith Waldron, Ian Bancroft, Molecular Breeding Accepted

11.2 Aromatic feedstock chemicals from degradation of Lignin (University of Warwick)

The emerging role for bacteria in lignin degradation and bio-product formation. Bugg T. D. H., Ahmad, M., Hardiman, E. M., Singh, R., Current opinion in biotechnology, 22 (3), 394-400 (2011). DOI: 10.1016/j.copbio.2010.10.009

Breaking down lignin to high-value chemicals: the conversion of lignocellulose to vanillin in a gene deletion mutant of Rhodococcus jostii RHA1. Sainsbury P. D., Hardiman, E. M., Ahmad, M., Otani, H., Seghezzi, N., Eltis, L D., Bugg, T. D. H., ACS chemical biology, 8 (10), 2151-2156 (2013)

Characterization of dye-decolorizing peroxidases from Rhodococcus jostii RHA1. Roberts J, N., Singh, R., Grigg, J. C., Murphy, M. E. P., Bugg, T. D. H., Eltis, L. D., Biochemistry 50 (23), 5108-5119 (2011). DOI: 10.1021/bi200427h.

Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Ahmad M., Taylor, C. R., Pink, D., Burton, K., Eastwood, D., Bending G. D., Bugg, T. D., Molecular bioSystems, 6 (5) 815-821 (2010). DOI: 10.1039/b908966g **Identification of DypB from Rhodococcus jostii RHA1 as a lignin peroxidase.** Ahmad M, Roberts, J. N., Hardiman, E. M., Singh, R., Eltis, L. D., Bugg, T. D., Biochemistry, 50 (23), 5096-5107 (2011). DOI: 10.1021/bi101892z

Isolation of bacterial strains able to metabolize lignin from screening of environmental samples. Taylor C. R., Hardiman, E. M., Ahmad, M., Sainsbury, P. D., Norris, P. R., Bugg, T. D., Journal of applied microbiology, 113 (3), 521-530 (2012). DOI: 10.1111/j.1365-2672.2012.05352.x

Pathways for degradation of lignin in bacteria and fungi. Bugg T. D., Ahmad, M., Hardiman, E. M., Rahmanpour, R., Natural product reports, 28 (12), 1883-1896 (2011). DOI: 10.1039/C1NP00042J

Variation in susceptibility to microbial lignin oxidation in a set of wheat straw cultivars: influence of genetic, seasonal and environmental factors. Konstantopoulou, M., Slator, P. J., Taylor, C. R., Wellington, E. M., Allison, G., Harper, A. L., Ian Bancroft, I., and Timothy D.H. Bugg, T. D. H., Nordic Pulp & Paper Research Journal 32:493-507, (2017)

11.3 In Silico study of Lignocellulosic biofuel processes (University of Surrey)

SurreyFBA: a command line tool and graphics user interface for constraint-based modeling of genome-scale metabolic reaction networks. Gevorgyan A., Bushell M.E, Avignone-Rossa C., Kierzek A. M., (2011)., Bioinformatics 27 (3): 433-434 (2011).

11.4 Engineering oilseeds to synthesise designer wax esters (Rothamstead

Research)

The utilization of the acyl-CoA and the involvement PDAT and DGAT in the biosynthesis of erucic acid-rich triacylglycerols in Crambe seed oil. Furmanek, T., Demski, L., Banaś, W., Haslam, R., Napier, J., Stymne, S., Banaś, A., Lipids, 49 (4), 327-333 (2014). DOI: 10.1007/s11745-014-3886-7

Tailoring the composition of novel wax esters in the seeds of transgenic Camelina sativa through systematic metabolic engineering. Ruiz-Lopez, N., Broughton, R., Usher, S., Salas, J., J., Haslam, R., P., Napier, J., A., Beaudoin, F., Plant Biotechnology Journal (2017) 15, 837–849. DOI: 10.1111/pbi.12679

11.5 Bio transforming Phenylpropanoids derived from Biorefining: A Toolkit Approach

Metabolic engineering of the flavone C-glycoside pathway using polyprotein technology. Brazier-Hicks, M and Edwards, R. Metabolic Engineering. 16, 11-20. (2013). DOI: 10.1016/j.ymben.2012.11.004

Plant synthetic biology: a new platform for industrial biotechnology. Fesenko, E., Edwards, R., Journal of experimental botany, 65 (8). 1927-1937 (2014). DOI: 10.1093/jxb/eru070

11.6 Fine chemicals from lignocellulosic fermentation residues using heterogeneous catalysis

Organosolv pretreatment of Sitka spruce wood: conversion of hemicelluloses to ethyl glycosides. Bouxin F, P., Jackson, S. D., Jarcvis, M.C., Bioresource technology, 151, 441-444 (2014). DOI: 10.1016/j.biortech.2013.10.105

Catalytic depolymerisation of isolated lignins to fine chemicals using a Pt/alumina catalyst: part 1-impact of the lignin structure. Bouxin F. P., McVeigh, A., Tran, F., Westwood, N. J., Jarvis, M. C., Jackson, S. D., Green Chem. 17 (2), 1235-1242 (2015). DOI: 10.1039/C4GC01678E

Isolation of high quality lignin as a by-product from ammonia percolation pretreatment of poplar wood. Bouxin F, P., Jackson, D. Jarvis, M.C., Bioresource Technology, 162, 236-242 (2014). DOI: 10.1016/j.biortech.2014.03.082

11.7 Evaluation of consolidated bioprocessing as a strategy for production of fuels and chemicals from lignocellulose

Physical characterisation and yield stress of a concentrated Miscanthus suspension. Botto L, Preuss, K., Robertson, L, X., Xiao, Y, X., Rheologica Acta, 53 (10-11), 805-815 (2014). DOI: 10.1007/s00397-014-0794-y

Metabolic characterization and modelling of fermentation process of an engineered Geobacillus thermoglucosidasius strain for bioethanol production with gas stripping. Niu H, Leak, D. J., Shah, N., Kontoravdi, C., Chemical Engineering Science, 122, 138-149 (2015). DOI: 10.1016/j.ces.2014.09.004.

Enabling Qualitative Colony Screening and Quantitative Analysis of Promoter Strength. Bartosiak-Jentys, J., Eley, K., Leak, D. J., Application of pheB as a Reporter Gene for Geobacillus spp., Applied and Environmental Microbiology, 78 (16), 5945-5947 (2012). DOI: 10.1128/AEM.07944-11

Modular system for assessment of glycosyl hydrolase secretion in Geobacillus thermoglucosidasius. Bartosiak-Jentys J., Hussein, A, H., Lewis, C, J., Leak, D, J., Microbiology, 159 (Part 7), 1267-1275 (2013). DOI: 10.1099/mic.0.066332-0

11.8 Isolation fractionation and modification of fructans from rye-grass to produce novel biosurfactants and polymers as part of a rye-grass biorefinery

Prebiotic composition 2015. PCT Application - (PCT/GB2014/053414)

The emulsification properties of octenyl- and dodecenyl- succinylated inulins. Kokubun, S., Ratcliffe, I., Williams, P. A. Food Hydrocolloids, 50, 145-149 (2015)

Functional properties of modified inulin, Functional properties of modified inulin, Special Publication. Kokubun, S., Ratcliffe, I., Williams, P. A. - Royal Society of Chemistry, 346, 245-251 (2014)

Physicochemical characterisation of inulin and ryegrass fructans. Evans, M., Gallagher, J. A., Ratcliffe, I., Williams, P. A., Gums and Stabilisers for the Food Industry: The changing Face of Food Manufacture: The Role of Hydrocolloids, 17, 73-78 (2014). DOI: 10.1039/9781782621300

Fructan synthesis, accumulation, and polymer traits. I. Festulolium chromosome substitution lines. Gallagher, Cairns, A, J., Thomas, D., Charlton, A., Williams, P., Turner, L, B., Frontiers in Plant Science, 6, 486 (2015). DOI: 10.3389/fpls.2015.00486

Fructan synthesis, accumulation and polymer traits. II. Fructan pools in populations of perennial ryegrass (*Lolium perenne* L.) with variation for water-soluble carbohydrate and candidate genes were not correlated with biosynthetic activity and demonstrated constraints to polymer chain extension. Gallagher, Cairns, A, J., Thomas, D., Charlton, A., Williams, P., Turner, L, B., Frontiers in Plant Science, 6 (2015). DOI: 10.3389/fpls.2015.00864

Genetic Differentiation in Response to Selection for Water-Soluble Carbohydrate Content in Perennial Ryegrass (*Lolium perenne* L.). Gallagher, J, A., Turner, L. B., Cairns, A. J., Farrell, M., Lovatt, J. A., Skøt, K., Armstead, I. P., Humphreys, M. O., Roldan-Ruiz, I., BioEnergy Research, 8 (1), 77-90 (2014), DOI: 10.1007/s12155-014-9491-z

Determination of the degree of polymerisation of fructans from ryegrass and chicory using MALDI-TOF Mass Spectrometry and Gel Permeation Chromatography coupled to multiangle laser light scattering. Evans M, Gallagher, J. A., Ratcliffe, I., Williams, P. A., Food Hydrocolloids, 53, 155-162, 2016. DOI: 10.1016/j.foodhyd.2015.01.015

'Emulsion stabilization using polysaccharide-protein complexes'. Evans, M., Ratcliffe, I. and Williams, P. A Current Opinion in Colloid and Interface Science, 18, 272-282 (2013)

'Synthesis, characterisation and self assembly of biosurfactants based on hydrophobicallymodified inulins'. Kokubun, S., Ratcliffe, I. and Williams, P.A Biomacromolecules 14 (8) 2830-6 (2013). DOI: 10.1021/bm4006529

Components responsible for the emulsification properties of corn fibre. Kokubun, S., Yadhav, M.P., Moreau, R.A. and Williams, P.A. Food Hydrocolloids 41 164-168 (2014)

Self-assembly and emulsification properties of hydrophobically modified inulin. Han, Lingyu, Ratcliffe, I., Williams, P.A. J Agricultural and Food Chemistry 63 3709-3715 (2015)

The emulsification properties of octenyl- and dodecenyl- succinylated inulins. Kokubun, S., Ratcliffe, I. and Williams, P.A., Food Hydrocolloids 50 145-149 (2015)

11.9 A study of metagenomics-informed biochemical functionality of microbial fuel cells using DDGS as a substrate (University of Surrey and University of Cardiff)

Low-Potential Respirators Support Electricity Production in Microbial Fuel Cells. Grüning A, Beecroft, N. J., Avignone-Rossa, C., Microbial Ecology, 70 (1), 266-273 (2014). DOI: 10.1007/s00248-014-0518-y

Effect of the chemical composition of filter media on the microbial community in wastewater biofilms at different temperatures. Naz I, Hodgson D, Ann Smith, Marchesi J, Ahmed S, Avignone-Rossa C, and Saroj D. P., RSC Adv., 6, 104345-104353, (2016). DOI: 10.1039/C6RA21040F

11.10 Development of a process scheme for the production of high value functional products from DDGS (University of Reading and Rothamstead Research)

Extractability and characteristics of proteins deriving from wheat DDGS. Chatzifragkou, A., Prabhakumari, P. C., Kosik, O., Lovegrove, A., Shewry, P. R. and Charalampopoulos, D., Food Chemistry, 198, 12-19 (2016). DOI: 10.1016/j.foodchem.2015.11.036

Biorefinery strategies for upgrading Distillers' Dried Grains with Solubles (DDGS). Chatzifragkou A, Kosik, O., Prabhakumari, P. C., Lovegrove, A., Frazier, R., Shewry, P. R. and Charalampopoulos, D., Process Biochemistry, 50 (12), 2194-2207 (2015). DOI: 10.1016/j.procbio.2015.09.005

Changes in the arabinoxylan fraction of wheat grain during 1 alcohol production. Kosik, O., Powers, S. J., Chatzifragkou, A., Prabhakumari, P. C., Charalampopoulos, D., Hess, L., Brosnan, J., Shewry, P. R. and Lovegrove, A., Food Chemistry, 221. pp. 1754-1762. ISSN 0308-8146 (2017). DOI: 10.1016/j.foodchem.2016.10.109

11.11 Fractionation and exploitation of the component value of DDGDS

The protein fraction from wheat-based dried distiller's grain with solubles (DDGS): extraction and valorization. Villegas-Torres M. F., Ward, J. M., Lye, G. J., New biotechnology 32 (6) 606-611 (2015)

Creation of an ultra scale-down bioreactor mimic for rapid development of lignocellulosic enzymatic hydrolysis processes. Conroy, N., Tebble, I., Lye, G. J., Journal of Chemical Technology & Biotechnology, 90 (11), 1983-1990 (2015). DOI: 10.1002/jctb.4801

Chemical composition and performance of chicks fed residues from the extraction of chemicals from distillers' grains. Rymer., C., Advances in Animal Biosciences 7



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