LBNet Business Interaction Vouchers (BIV)

Projects funded since 2014

2/1/2018

LBNet is a BBSRC-funded Network in Industrial Biotechnology and Bioenergy

BIV01: Testing the effect of polyethylene glycol as an enzyme enhancer in lignocellulosic hydrolysis

Academic Partner	**Industrial Partner**
Charles J. Banks, University of Southampton	Nicholas Thompson, Fiberight Ltd

Public Summary

Fiberight Ltd will work with the University of Southampton on the production of second generation biofuels from waste lignocellulosic feedstock, with a particular focus on municipal solid waste (MSW). The work will progress the concept of extracting clean fibre from MSW through a washing process, followed by the use of commercial enzyme preparations to prepare a sugar-rich fermentation substrate. The aim of the current work funded by a BIV is to improve the yield of sugar obtained from the use of proprietary enzyme preparations. Specifically, the work will look at the potential for enhancing enzymatic hydrolysis using an additive aimed at enhancing enzyme penetration into the structure of the lignocellulosic fibres. The work will attempt to elucidate the mechanism by which this enhancement occurs, especially when it interacts with other compounds in the complex matrix of MSW. The collaboration will generate new experimental data and allow it to be analysed in the context of Fiberight's business activity. It will contribute towards improving the existing technology used by the company and provide an answer to an important technical problem.

Public Project Outcome

The business of Fiberight Ltd is the production of second generation biofuels from waste lignocellulosic feedstocks, with a particular focus on municipal solid waste (MSW). The company is also interested in developing other value-added commodities based on a sugar platform. Over the past four years Fiberight has progressed the concept of extracting clean fibre from MSW through a washing process, and then using commercial enzyme preparations to prepare a sugar-rich fermentation substrate. Although reasonably content with the yield of sugar obtained from the use of proprietary enzyme preparations, the company believes that even better yields and higher efficiency in enzyme use could be obtained through further research and development. In conjunction with the University of Southampton as the research provider, work was therefore carried out to look at the potential for enhancing enzymatic hydrolysis using surfactant additives. The research showed that use of the chosen additive led to a 40% reduction in the amount of enzyme required for breakdown of the fibre product, and also provided insights into the mechanism by which this enhancement occurs. As the cost of enzymes is one of the major factors affecting process economics the result is important in both scientific and financial terms, and has fully demonstrated the value of this type of collaboration.

BIV02: Structural characterisation of novel GH9 family enzymes from marine polychaetes

Academic Partner	**Industrial Partner**
John McGeehan, University of Portsmouth	Kirk Schnorr, Novozymes A/S

Public Summary

There is a clear need to find alternative energy sources to meet the needs of an expanding global population. Our current reliance on fossil fuels is unsustainable and has damaging effects on the environment. One area that has great potential to make a difference is to use an alternative to fossil fuels for transportation. In short, the aim is to replace the fuel in our cars, trains and aircraft with sustainable biofuels. Second Generation Biofuels avoid the issues of competing with food crops by utilising plant waste, or 'biomass', such as woody material, bagasse from sugar cane and corn stover (the remaining stalks, leaves and husks following a food cereal harvest) rather than growing dedicated fuel crops. Although the technology already exists to do this, the major hurdle remains cost. Unlike sources such as corns and beets, it is difficult to extract the sugars from woody material because of the way the sugars are protected within plant cell walls. Industrial processes currently use digestive enzymes discovered in fungi and bacteria, however, there remains great scope to improve their efficiency to make the process economically viable. We are prospecting novel enzymes from marine organisms and, in partnership with Novozymes, we propose to characterise a range of cellulases from marine worms. Novozymes has the facilities to produce these enzymes in large quantities at their dedicated fermentation facilities in Denmark, while at the University of Portsmouth, we have the expertise to study their properties including solving their 3D structures. This partnership has the potential to provide Novozymes with new templates for protein engineering and produce better, more cost-effective enzymes for the degradation of waste biomass. Long term success in this research area will be the production of transportation fuels that don't rely on dwindling fossil fuel reserves, are sustainable and are kinder to the environment.

Public Project Outcome

The deconstruction of lignocellulosic biomass into useful products such as soluble sugars is an essential step in the development of the large-scale replacement of current oil-based liquid transportation fuels. Despite a growing amount

of research in recent years, the efficient enzymatic digestion of ligno-cellulose remains a significant economic challenge. Prospecting glycoside hydrolases (GH) that are equipped with novel enzymatic properties has the potential to greatly improve the overall efficiency of a wide range of industrial processes, including the production of bioethanol from sustainable sources.
A detailed sequence comparison of the molecular phylogenetic relationships of GH9 family enzymes revealed an unusual distribution of surface properties across the kingdoms of life. Of particular interest are the marine polychaetes that were shown to have low predicted isolectric point values compared to other animal GH9s. It has been suggested that the unusually acidic surface charge observed in some marine GH enzymes is linked to valuable properties including salt tolerance and resistance to harsh chemical environments.
Bringing together the expertise in recombinant protein expression at Novozymes together with the biophysical and structural expertise at the University of Portsmouth, we have been able to characterise two novel GH9 enzymes from the marine worms *Perinereis brevicirris* (PbCel9) and *Terebella lapidaria.* This work has been successful in solving the detailed 3D atomic structure of one of these enzymes and this data will provide the team at Novozymes with a platform for structure-based protein engineering.
Overall, marine polychaetes present a novel range of previously uncharacterised glycoside hydrolases. GH enzyme efficiency is the rate-limiting step for an economically viable saccharification process. We are therefore engaged in a large prospecting exercise for enhanced or novel enzymatic properties, particularly those that are relevant to industrial processes such as high activity and stability in the presence of enzyme inhibitors found in lignocellulosic biomass. We hope that this new line of investigation lays the foundation for a longer-term partnership on a range of novel enzymes.

BIV03: Valorising poppy straw waste streams

Academic Partner	**Industrial Partner**
Simon McQueen-Mason, University of York	Stephen Poulston, Johnson Matthey Technology Centre

Public Summary
The utilisation of agricultural waste, such as poppy straw produced after extraction of high value chemicals by the pharmaceutical industry, represents one of the most attractive alternatives for biorefining. This project explores the valorisation of poppy biomass waste by developing processing alternatives for conversion of poppy straw into valuable chemicals and fuels. The academic team at the University of York will determine the composition and optimise the pretreatment and hydrolysis of straw, while the industrial partner, Johnson Matthey, will explore catalytic alternatives for the conversion of these biomass fractions into high value products.

Public Project Outcome
We have successfully analysed the sugars content and quantity of polysaccharides, lignin and insoluble material in poppy straw residues following opiate extraction in order to allow Johnson Matthey to evaluate the potential of this material for value generation. We then subject the poppy biomass to hydrolysis with commercially available cellulases following a range of pre-treatments conditions. Johnson Matthey are evaluating these results of our analyses and the potential of the sugars produced for chemo-catalytic up-grading.

BIV04: An innovative way to delivery Ca and Cu to plants safely using lignin

Academic Partner	**Industrial Partner**
David Stainton, University of Lincoln	Apostolos Papadopoulos, Crop Intellect Ltd

Public Summary
Lignocellulosic biomass from forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. Lignin, which represents 15-35% of wood, is the most abundant renewable organic material on the earth, can be used either directly or chemically modified, as a binder, dispersant agent for pesticides/herbicides, emulsifier, and as a heavy metal sequestrate. Lignosulphonates from the paper industry produced during pulping are readily soluble in water and have detergent-like properties. They have the ability to enclose certain metal ions and keep them in solubilised form. Copper has been used in agriculture to reduce fungal attacks and to control certain bacterial diseases. In recent years the use of copper particularly in vegetable production has been banned and only a small amount is allowed to be used. The present projects aims at preparing a formulation that will make copper more readily available to the plant and adhere to the foliage providing a long lasting protection by fungal attacks reducing the need for frequent chemical fungicide treatments.

Public Project Outcome
Crop Intellect ltd and the University of Lincoln have worked collaboratively using an Innovation Voucher provided by the

BBSRC sponsored group "Lignocellulosic Biorefinery Network (LBNet)". The aim of the project was to utilise existing lignin compounds and derivatives in combination with crop nutrition to increase uptake and safety of foliar applications. Existing formulations shown stronger efficacy on model plants when applied on the foliage. Crop Intellect will be utilising the outcome of this research to increase the effectiveness of their existing formulations and produce more based on Ca and Cu elements. The University of Lincoln will support the efforts of research to develop further innovations using lignin and its derivatives.

BIV05: Testing bacterial lignin degrading enzymes for delignification of municipal solid waste

Academic Partner	Industrial Partner
Tim Bugg, University of Warwick	Nicholas Thompson, Fiberight Ltd

Public Summary

Fiberight Ltd are developing a process for conversion of municipal solid waste into sugar for biofuel production. Lignin is a recalcitrant aromatic polymer found in lignocellulose, which must be removed as part of this process. The project will investigate whether bacterial enzymes discovered recently in Prof Bugg's research group would be useful biocatalysts for removal of lignin as part of this process.

Public Project Outcome

Samples of recombinant bacterial lignin-oxidising enzymes Dyp1B from *Pseudomonas fluorescens* and manganese superoxide dismutase from *Sphingobacterium* sp, T2 identified in Professor Bugg's research group were tested for their effects on the delignification of samples provided by Fiberight Ltd.

BIV06:Sonochemical pretreatment of biomass

Academic Partner	Industrial Partner
Leonardo Gomez, University of York	Narinder Bains, SERE-Tech Innovation Ltd

Public Summary

By-products from agriculture and forestry such as straw and trimmings have potential as sustainable feedstocks for production of fuel and chemicals. However, these materials are not easy to deconstruct and digest to release sugars from the constituent cellulose and hemicellulose polymers. Usually a harsh pre-treatment is needed to enable enzymes to access these polymers and this can produce inhibitors of fermentation such as furfurals as well as adding expense and energy to the process.

Application of ultrasonic irradiation has been shown to have potential as a benign pre-treatment, enhancing ethanol yield from lignocellulosic feedstocks such as wheat straw.

SERE-Tech Innovation Ltd, is a company with specialist expertise in use of ultrasonics in the food and drinks industry. SERE-Tech will work with the University of York (UoY), supported by the Biorenewables Development Centre (BDC), to establish whether pretreatment of lignocellulosic material with SERE-Tech's specialist equipment can replace or enhance existing deconstruction methods for pretreatment of straw for ethanol production.

Public Project Outcome

This successful BIV between SERE-Tech and the University of York demonstrated that pretreatment of lignocellulosic material with SERE-Tech's specialist equipment could improve existing deconstruction methods for pretreatment of straw for ethanol production. Results from this study support the potential that this technology could be an effective pretreatment of wheat straw for biofuels and high-value chemicals, and will form the basis of future significant collaborative work in this area. UoY and Seretech are exploring possible funding opportunities at UK and European level to scale up and integrate this work.

BIV07: Softwood Waste as a Source of Renewable Platform Chemicals

Academic Partner	Industrial Partner
Leonardo Gomez, University of York	Gary Wheatley, Advances Extraction Technology Ltd

Public Summary

Softwood waste represents a valuable plant derived biological resource, currently an under-utilised co-product stream resulting from forestry & related operations. The aim of the project is to demonstrate the feasibility of converting this biomass, using biotechnological processes, into feedstocks suitable for the production of key "platform chemicals" capable of replacing the petroleum derived materials, chemicals and energy currently used in the chemical industry.

Public Project Outcome

Advanced extraction technology (AET) Ltd is currently developing a method to use subcritical water for the treatment of lignocellulosic waste materials. This technology has the potential to create a fermentation substrate that is high in sugars and low in inhibitors. To aid in the development of this technology, LBNet funded a business interaction voucher allowing the Centre for Novel Agricultural Products (CNAP) and AET Ltd to complete a short and fruitful project, supported by the Biorenewables Development Centre (BDC), to carry out lignocellulosic analysis on soft wood treated with subcritical water. The project used chromatography to look for the presence of furfural and 5-hydroxymethylfurfural (HMF), inhibitors to fermentation that are often created by the pre-treatment of lignocellulosic waste. This project shows that the technology created by AET Ltd has the potential to remove the hemicellulosic and lignin fractions during pretreatment with low formation of inhibitors of fermentation such as 5-HMF and furfural. We would recommend further studies to develop this work, including fermentation studies and exploring fully the effects of this treatment on the lignocellulosic fractions at each step of treatment.

BIV08: Investigating the potential for integrating large scale biofuel and bioenergy production

Academic Partner	**Industrial Partner**
Simon McQueen-Mason, University of York	Mark Flower, Drax Group plc**:**

Public Summary

Reducing carbon emissions is important if we are to avoid the worst scenarios of predicted climate change due to global warming. It is generally accepted to that replacing fossil fuels with non-food plant biomass is an important component for reaching the UK's ambitious carbon reduction targets. Replacing petroleum for liquid fuel production requires large quantities of sustainable plant biomass being processed in centralised biorefineries. The economics of establishing such refineries is challenging on a number of fronts, especially feedstock supply and capital investment. Drax power has undertaken an ambitious programme to replace coal with woody plant biomass for electricity production, and is now uniquely placed in the UK with a well-developed and sustainable biomass supply chain. Producing bioalcohols (e.g. ethanol or butanol) from plant biomass uses roughly 30% of the biomass feedstock, such that the remainder can be used for bioenergy production, primarily through combustion. We propose to carry out a desk-based study to consider potential scenarios for the integration of biofuel and bioenergy production based on the Drax site. The project will consider the technologies currently available to achieve this aim, examine the general techno-economic considerations, and identify potential technology providers.

Public Project Outcome: to follow

BIV09: Investigation of a route to bio-based polymers using an ionic liquid

Academic Partner	**Industrial Partner**
Jason Hallett, Imperial College London	Paul Mines, Biome Technologies plc

Public Summary

This is the first step in a combined chemoenzymatic conversion of Hydroxymethylfurfural (HMF) to Furandicarboxylic acid (FDCA), by taking advantage of the high conversion yields and biocompatibility of ionic liquids as solvents for carbohydrates. FDCA can then be used to manufacture novel bioplastic copolymers with interesting properties.

Public Project Outcome

The market for bioplastics is constrained by the functional capability and the cost of production of the current generation of materials. Biome's strategy is to bring to market novel, highly functional polymers derived from lignocellulose in a sustainable way. One of the key, near market bioplastic material precursors is FDCA. This molecule has the potential to replace terephthalic acid in various polyesters (e.g. PET) and to be an intermediate to other polymers, fine chemicals, pharmaceuticals and agrochemicals. Biome, working with the University of Liverpool, Chemistry Department has already patented an enzymatic route to make FDCA from HMF and has further interests to improve the stability of applied enzymes and their conversion rate in other than aqueous solutions. This BIV project provided Biome with the excellent opportunity to work with Imperial College, Chemical Engineering Department on anhydrous ionic liquids in which the key enzyme was successfully stabilised in a nanoconjugate from preserving its thermal stability and activity.

BIV10: Assessing the potential of cocoa crop residues for biorefining and bioenergy applications

Academic Partner	**Industrial Partner**
Simon McQueen-Mason, University of York	Isabella Van Damme, Mars Chocolate UK Ltd

Public Summary

Several millions of tonnes of cocoa beans are harvested every year, primarily for the production of chocolate. Cocoa beans are produced by the tree Theobroma cacao in large fleshy pods, which are usually discarded after harvest and currently have very limited value. These abundant crop residues could potentially serve as a feedstock for biorefineries for producing a range of chemicals and fuels but until now there has been little investigation into their composition, which is a prerequisite in determining routes to potential value creation. Mars sources cocoa from Western Africa and is keen to help develop new ways to improve the sustainability of the production processes as well as stimulate new economic activities for cocoa growing communities. In this project, we will carry out analyses of the composition of the lignocellulosic biomass of cocoa pods and determine the amount and composition of the major polysaccharides, phenolics and lipids found in these materials in order to provide baseline data from which to develop strategies for generating value from these materials.

Public Project Outcome

Cocoa beans, the raw material for the production of chocolate, are produced in large, fleshy pods which are usually discarded. At present, very little is known about the composition of the pod husks, which is crucial to develop methods to process them and create economic value. Mars Chocolate sources cocoa from SE Asia, Western Africa and South America and is keen to help develop ways to improve the sustainability of the production processes as well as stimulate new economic activities for cocoa growing communities. In this study, we provide a detailed analysis of the composition of the biomass of cocoa pod husks from selected Mars Chocolate providers in Ecuador, Indonesia and Ivory Coast to obtain baseline data which to develop stratergies to increase value from this crop residue. We determined the amount and composition of major lipids, matrix polysaccharides, cellulose, hemicellulose and pectin, as well as the content of water solubles, solid residues (ash) starch, protein and lignin. We found that cocoa pods from three regions analysed have similar biomass components profiles, with high lignin (>20%) and protein (>7%), and low lipids and starch contents. The saccharification potential is not very high; up to 45% of the total biomass are available sugars, of which less than 30% were released after hydrolysis. The treatment conditions assayed, however, were relatively mild; harsher treatments might help improve the yield to potentially serve as feedstock for biorefineries. The high protein content might represent an untapped resource that could merit further analysis.

BIV11: High-throughput industrial enzyme analysis and optimisation of DASH for commercial research service

Academic Partner	Industrial Partner
Paul Dupree, University of Cambridge	Kirk Schnorr and Kristian Krogh, Novozymes AS

Public Summary

The development of a sustainable bioeconomy will require the discovery and development of a suite of glycan-degrading enzymes with novel activities and substrate specificities. To achieve this end, there is a substantial need for analytical techniques which can yield detailed information about enzyme substrate specificity and site-of-attack in a high-throughput manner. A method for the analysis of the products of glycan-degrading enzymes, which is both high throughput and yields detailed information about enzyme activities, has been developed in the Biochemistry Department, University of Cambridge. Cambridge wishes to develop the process for large-scale glycan analysis, for the eventual aim of establishing an analytical service company. Cambridge and Novozymes will partner to provide a proof-of-principle analysis of a subset of Novozymes enzymes. Such work will: give Novozymes deep and rapid insight into the activity of a chosen range of its enzymes and allow Cambridge to optimise protocol, workflow and costings for the analytical service. Finally, this will enable the establishment of commercial relationships, which we will explore continuing following the completion of the project.

Public Project Outcome

The aim of the project was to analyse a range of mixed-linkage (MLG)-active glucanases against MLG using high-throughput capillary electrophoresis (CE), and to thereby elucidate site attack and substrate specificities for the enzymes used. The project also served as a proof-of-concept for a service to be offerd by a spin-out company form the University of Cambridge, Cambridge Glycoscience Ltd. We produced a final report documenting enzyme product profiles, and deducing likely sites of attack has been assembled, against MLG. We optimised our CE protocol and workflow for rapid analysis of large batches of glycans was successfully achieved. Precise costings and sample turnaround times for CE were determined, to help develop analytical service business proposition. Finally, our library of known oligosaccharide structure retention times was extended to include mixed-linkage glucan-derived oligosaccharides.

BIV12: Feasibility of extracting value from lignocellulose held in complex matrix waste materials

Academic Partner	Industrial Partner
Stuart Wagland, Cranfield University	Tom Everitt, Ecoganix

Public Summary
Options for recycling waste materials are in a constant state of review and they are intrinsically linked to, inter alia, the market value of the recyclate and/or the cost of conversion. Paper materials, which comprise lignocellulose structures, are subjected to repeated reprocessing cycles which reduce their integrity and forms lignocellulose-containing wastes which cannot be readily used in conventional paper products. This project explores the potential for the conversion of lignocellulosic materials which are currently recycled at negative or low values to be manufactured into higher value products, therefore the upscaling of lignocellulosic by-products.

Public Project Outcome
De-inking sludges arise during the re-pulping of recycled paper products. These by-products contain lignin, short-chain celluloses, inorganic matter (clay, fillers etc) and are currently seen as negative or low value materials. In the UK, many thousands of tonnes of de-inking sludge are produced annually and the project industrial partner Ecoganix manages over 200 ktpa. This project demonstrated it is feasible to use low-cost commercially available cellulase to produce significant quantity of glucose from de-inked paper pulp. The highest specific glucose yield archived in the study was 3 g g-1 VS. Higher enzyme concentration promotes glucose yields, however it is anticipated a higher yield can be achieved under low enzyme concentration if a longer incubation time can be adopted.

BIV13: Testing of bacterial lignin degraders for delignification of distillery spent lees for anaerobic digestion

Academic Partner	**Industrial Partner**
Tim Bugg, University of Warwick	Derek Rodman, Clearfleau Ltd

Public Summary
The project will examine whether bacterial lignin-degrading strains from Prof Bugg's research group can be used to as a delignification pretreatment for distillery spent lees prior to anaerobic digestion in Clearfleau's anaerobic digestion facilities.

Public Project Outcome
Bacterial lignin-degrading strains discovered in Prof. Bugg's group have been tested for delignification of distillery spent lees as an aid to anaerobic digestion. One bacterial isolate has shown enhanced gas production, indicating that it may be a useful microbial biotransformation.

BIV14: Levoglucosan and cellobiosan utilisation for bioethanol production

Academic Partner	**Industrial Partner**:
David Leak, University of Bath	John Nicholas, Nova Pangaea

Public Summary
Nova Pangaea have a patented novel lignocellulose pretreatment technology which is aimed towards a renewable and sustainable zero carbon, fossil-fuel-free future. The Nova Pangaea Technology (NPT), lignocellulosic pretreatment and fractionation technology fully fractionates biomass in order to produce **C-5 and C-6 sugars** of industrial purity, in large scale volumes at competitive prices, so that the fuel and chemical sectors have a realistic replacement for crude oil and gas based intermediates. The NPT process delivers a **C-5 stream** of sugars containing above 90% of the available hemicellulose sugars as monomers, which can be converted to bioethanol. The second stream of **C-6 sugars** are **levoglucosans,** which are **anhydro-sugars** (primarily anhydro-glucose) formed by elimination reactions occurring during the pyrolysis of the cellulose rich biomass, post-hemicellulose removal. Some **cellobiosans** are also formed, as well as compounds which may be toxic in fermentation processes. To valorise the pre-treatment process it will be necessary to efficient ferment these molecules to produce bioethanol or other high-value biochemicals. However, the streams of sugars (C-5 and C-6) also contain quantities of **toxic compounds** emanating from sugar degradation during the initial thermochemical and pyrolysis pretreatment process, which makes its fermentation by microorganisms problematic. During any form of pretreatment, it is well known that various compounds are produced which can inhibit downstream fermentation and need to be removed prior to fermentation. These compounds include organic acids (formic acid, **acetic acid**, levulinic acid etc.), **furfural** and hydroxymethyl furfural **(HMF)** (Klinke *et al*., 2004; Olsson and Hahn-Hagerdal, 1996; Palmqvist and Hahn-Hagerdal, 2000; Palmqvist *et al*., 2000; Larsson *et al*., 1999; Helle *et al*., 2003). The team in Bath University recently isolated a yeast strain (awaiting patent) which can rapidly **detoxify biomass toxins without reducing the sugar content**. Additionally we have **2 industrial ethanologenic lignocellulosic fermenting organisms** (yeast and thermophilic bacteria) that can fully ferment xylose and glucose sugars to bioethanol. The thermophilic bacteria

(*Geobacillus thermoglucosidiasus* TM242) have the added advantage of fermenting oligomeric sugars and all biomass sugar types (**xylose, arabinose, fructose, galactose, mannose, glucose, etc). This bio-detoxification is more efficient and greener than the chemical detoxifications currently being used in the industry.** In this BIV we propose to collaborate with Nova Pangaea in **determining the fermentability of their levoglucosans and cellobiosans produced in the cellulose pyrolysis sugars stream**. There is limited information on the direct fermentability of these anhydro-sugars; acid hydrolysis has been applied in some reports to hydrolyse them to glucose prior to chemical detoxification and fermentation. Another option is the recombinant expression of Levoglucosan Kinase (Layton et al 2011; Dai et al 2009), an enzyme normally found in a few organisms including *Lipomyces starkeyi* (Ning et al 2008) *Aspergillus* species (Xie et al 2005) and *Sporobolomyces salmonicolor* (Kitamura & Yasui 1991) that allows the direct utilisation of levoglucosans. But, information on other organisms with this capability is rare. Evidence of success in these preliminary investigations will form the basis of a more extensive project on the 2 different sugar streams conversion to bioethanol.

Public Project Outcome:

We sought to produce ethanol from thermolysis anhydrosugar media produced by Nova Pangaea Technologies' from their recently patently NPT Process, which aims to produce fermentable sugars from biomass without the need for enzymes. Using Birchwood as feed stock, NovaPangaea performs an initial acid hydrolysis that separates a hemicellulose liquid stream from residual cellulose solids. The cellulose is then further pyrolysed (thermolysis process) to char and a bio-oil stream that contains anhydrosugars (mainly levoglucosan). This later stream contains several toxic compounds including phenolics, acids, furans and alcohols and was used in our experiments. Our results showed that direct fermentation of pure cellobiosan and levoglucosan (ie material bought commercially) was impossible by the microbial strains we tested (*Saccharomyces* sp, *Bacillus* strain, *Anoxybacillus* and *Geobacillus* species) without prior acid hydrolysis. However, after acid hydrolysis, the pure sugar streams were fermented by all microbial strains with yields of over 95% theoretical ethanol production. The NovaPangeaea anhydro-sugar stream was also hydrolysed with acid (using the same conditions used for acid hydrolysis of pure anhydrosugars), and neutralised prior to fermentation by *Saccharomyces* sp. An in-house yeast strain was also used to detoxify some of the sugar stream prior to fermentation. Fermentation studies indicate that bio-detoxification enabled complete fermentation of the sugar stream, when diluted 1:1 with water, in 24hrs with yields of more than 95% based on total sugar content. However, without bio-detoxification, fermentation of this sample was much slower and produced lower ethanol yields. Without dilution, the bio-detoxified samples were only 61% fermented based on total sugar content, but this is significant compared to only 15% for non-detoxified samples. This indicates that the detoxification regime will need to be optimised and other means of toxicity reduction exploited. Thus we conclude that thermolysis media by NovaPangaea is made of mainly levoglucosan which can be hydrolysed with low concentrations of sulphuric acid to produce fermentable glucose. However, the media contains toxins which can be partially removed by bio-detoxification; fermentation of undiluted media after bio-detoxification remained problematic and remains to be optimised.

BIV15: Cannabinoids extracts from hemp by-products

Academic Partner	Industrial Partner
Simon McQueen-Mason, University of York	Damian Bove and Dominic Bartle, Adact Medical Ltd

Public Summary

Hemp provides raw materials, seeds and fibres for many industrial applications. The processing of the fibres leads to the production of large amount of 'dust' (up to 30%) which, at the moment, is considered a waste. This dust is largely composed of cell wall and epidermis. Hemp is well-known to accumulate cannabinoids and certified hemp varieties containing less than 0.2% Tetrahydrocannabinol, the main psychoactive compound, are legally grown under licence. Recently, extracts containing non-psychoactive cannabidiol (CBD) have been strongly associated with alleviating symptoms of pain and reducing seizure in epilepsy patients, leading the market for this product to grow rapidly. At the University of York, we have demonstrated that specific dust samples, obtained from processing hemp fibres, contain significant levels of cannabinoids. This led to the award of a POC with BBSRC NIBB P2P where, as collaboration between the Centre of Novel Agricultural product and the Green Chemistry Centre of Excellence, we demonstrated the economic viability of using hemp dust by-products for cannabinoid extraction. In this Business Interaction Voucher, we will aim at developing two additional steps to the extraction set up in the POC to improve this commercial value: a purification step to obtain cannabinoid samples with higher concentration and an esterification step to broaden the way CBD could be administrated. This will build on the relationship that has been established within the NIBB P2P POC between the UK hemp processing actors and facilitate research technology transfer by improving the commercial potential of the innovation. Furthermore, if successful, it will also enhance the Industrial partner proposition and may lead to commercialisation.

Public Project Outcome: to follow

BIV16: Continuous bio-production of pyridine 2,5-dicarboxylate using lignin-derived monomers, vanillin and guaiacol, towards sustainable biopolymer manufacture

Academic Partner	Industrial Partner
Sam Bryan, University of Nottingham	Paul Mines, Biome Technologies Plc

Public Summary

Lignin represents a renewable carbon feedstock that has transformative potential as a replacement for petroleum carbon feedstocks. However, approximately 50 million tonnes of lignin is produced worldwide as a poorly valorised waste product. Lignin is comprised of aromatic monomer building blocks. Enabling production of economically viable lignin-derived biochemicals, these monomers must be selectively released from the lignin and utilised as a carbon source by microorganism cell factories. Cupriavidus necator is a robust, metabolically diverse microorganism cell factory, capable of utilising aromatic monomers as carbon sources; however, C. necator is unable to degrade lignin. This makes C. necator an ideal candidate to introduce selective and targeted lignin degradation via metabolic engineering. This research is positioned at the forefront of generating a metabolically engineered strain capable of breaking down lignin and utilising the monomers to make industrially relevant products, which will have a major impact on next generation biopolymers.

Public Project Outcome: to follow