BIVMiB032 BB/L013711/1
16 January – 14 July 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Embedding technical expertise in the optimisation of trace metal supplementation strategies for successful biomethane production

"The work has been an eye-opener for the company in terms of the potential for optimisation of the plant, and a huge benefit in terms of skills transfer to our staff" Michael Mason, Tropical Power Ltd

Yue Zhang, University of Southampton and Michael Mason, Tropical Power Ltd

RESULTS: The project developed methods that are helping a UK company, which has built and is currently operating Africa's first grid-connected anaerobic digester, to determine more precisely the trace element requirements for optimum digestion of their novel agricultural waste feedstocks. We developed a method suitable for use in Africa that uses simple multi-purpose apparatus to test which trace elements are actually required. The methodology was made available to the industrial partner in the form of a training video and a detailed description of the procedures. In addition we helped our partner company interpret historical data from the digestion plant and provided them with a simple spreadsheet-based calculator to allow them to maintain steady state concentrations of essential elements in the digester in proportion to the feed added. The work also added tantalisingly to growing evidence that minor trace elements such as tungsten may play a critical role in the function of these microbial systems.

Tropical Power's grid-connected anaerobic digester in Kenya

INITIAL AIMS: Transformation of waste biomass into bioenergy is a key component in 21st century industrial biotechnology. It is increasingly clear that successful biomethanisation of mixed biomass requires complex enzyme systems that are produced by both natural and engineered synthetic microbial communities. Trace quantities of metals, which are required by certain essential metallo-enzymes, are needed to ensure that these microbial systems function in the most effective and productive way. There is a growing commercial market in trace metal supplements, but formulations of these are often generic rather than based on specific requirements. The current project will transfer knowledge and expertise in determining trace metal requirements to a UK company that uses novel waste feedstocks in Africa for renewable biomethane production. This will enable the company to formulate specific trace metal mixtures for optimum plant performance, and the scientific knowledge gained will contribute to the creation of future markets for UK suppliers of tailored supplements.

- **Collaboration broadened into new scientific areas help meet bioenergy needs of lower income countries**
- **Academic and industrial collaborators partnering on stage 1 GCRF application**

Biotechnology and Biological Sciences Research Council

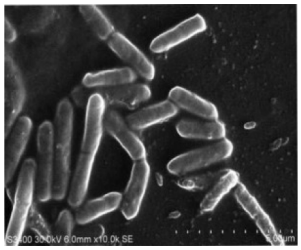
BIVMiB043 BB/L013711/1
1 November 2017 – 4 January 2018
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

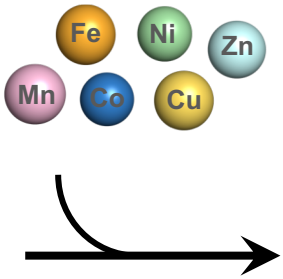
Investigating the link between metal homeostasis, sporulation, and solvent production in the *Clostridial* ABE fermentation process

"This study advanced understanding of metal requirements for commercial and research and development processes."

Peter Chivers, Durham University; Liz Jenkinson, GBL

OUTCOMES: Biomass samples were collected by GBL at 3-h intervals for metal content analysis and RNA isolation. An asporogenic mutant that did not show changes in metal content was sampled in parallel to understand the genetic requirements for changes in metal homeostasis. The project also provided key knowledge transfer – the metal content and RNA sample analysis were carried out at Durham by a GBL scientist. The informal communications during the 3-week visit will enhance future work at both sites.





AIMS: Solventogenic Clostridia were first used for fermentative acetone-butanol-ethanol (ABE) production roughly 100 years ago. The project focused on establishing a detailed picture of the changes in metal utilisation and homeostasis at the onset of sporulation using a combination of RNASeq and ICP-MS to correlate initiation of the sporulation genetic program and the role of metalloenzymes and proteins.

- **Understanding metal utilisation during sporulation could lead to improved control of fermentation processes**

Biotechnology and Biological Sciences Research Council

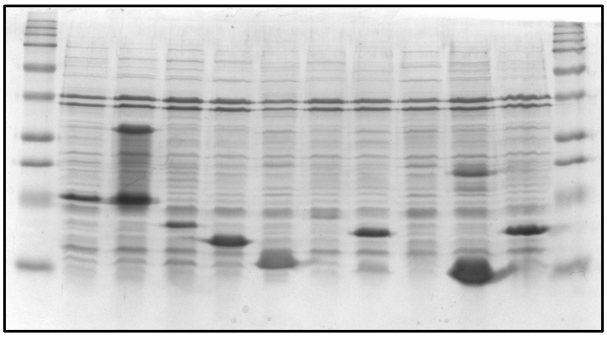
BIVMiB035 BB/L013711/1
1 April – 30 September 2017
Funded by a Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Metal utilisation in *Clostridium* microbial biocatalysts

"For GBL, this study highlighted the importance of metal requirements for commercial and research and development processes."

Peter Chivers, Durham University; Liz Jenkinson, Green Biologics

OUTCOMES: Metal contents were measured at different times during a GBL fermentation protocol used for Research and Development. The ICP-MS analysis revealed a large increase in metal content in *Clostridium,* coincident with the onset of butanol production. To complement the experimental data, bioinformatics analyses identified ten candidate metal sensor genes. These genes encode regulator proteins responsible for sensing and responding to changes in metal content. In several cases, their regulatory targets have also been identified. The proteins encoded by these genes have been overexpressed in *E. coli* to facilitate future studies of their metal selective transcriptional responses. These results will enable more detailed analysis of metal homeostasis networks in *Clostridium* to understand their link with butanol production or competing processes.



SDS-PAGE analysis of overexpression of *Clostridium* metal sensor proteins in *E. coli*

INITIAL AIMS: Little is known about the metal-demands of *Clostridium* strains during solvent production. This project will explore the metal requirements of solventogenic *Clostridium* during commercial and research and development processes used by Green Biologics. Metal content will be analysed by ICP-MS. A complementary aim is to identify the metal sensor genes responsible for maintaining metal homeostasis so that they may be overexpressed in *E. coli* to define the metal selective response of each protein.

- **Understanding metal demands could lead to improved control of fermentation processes**

PoCMiB028 BB/L013711/1
1 July – 31 August 2017
Funded by Metals in Biology BBSRC NIBB Proof of Concept grant
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

LPMOs: a new face in biomass breakdown?

"The on-going collaboration with Novozymes into the chemistry and activities of biomass-degrading enzymes continues to provide very fruitful research, not only in the discovery of the fundamental chemical processes exhibited by these enzymes but also their potential in biomass processing." Paul Walton, University of York

Paul Walton and Gideon Davies, University of York;
Jens Erik Nielsen Novozymes, Denmark

OUTCOMES: This project examined the structure and reactivity of a new class of *lytic polysaccharide monooxyegenase* (LPMO) enzyme, which were expressed in eukaryotic systems to high yield by Novozymes. The objectives of the work were to establish whether the enzyme required metal ions for maximal activity, and if so, how those metal ions interacted with the enzyme. We also aimed to establish whether the enzyme was active on lignocellulosic substrates with an oxidoreductase type action. A series of metal-binding studies was performed using isothermal calorimetry experiments, from which it was determined that metal binding was weak and non-specific, unlike the canonical class of other LMPOs. Electron paramagnetic resonance studies showed that the enzyme bound copper. Structures of the enzyme showed that the new class of LPMOs forms interactions with lignocellulosic-type substrates near the active site. This is now an area of active investigation.

INITIAL AIMS: The efficient conversion of abundant biomass into liquid biofuel is of vital importance in meeting the world's energy demands. Despite the unrivalled calorific potential of biomass, which is composed mostly of lignocellulose, it has not been possible until recently to convert it through to bioethanol. The reason for this is the chemical recalcitrance of the cellulosic biomass. One promising way to breakdown lignocellulose involves the use of enzymes, especially lytic polysaccharide monooxygenases (LPMOs). LPMOs have overturned our understanding of biomass conversion as they boost significantly the conversion of biomass to ethanol. This project aims to study a new exciting class of metal-containing LPMOs which do not contain the usual active-site amino acids, thereby offering new insight into how biology performs the conversion of biomass and consequently our ability to use biomass as a sustainable fuel source.

- This work is continuing with BBSRC NIBB follow-on funding to examine the reaction of lignin components with the enzyme

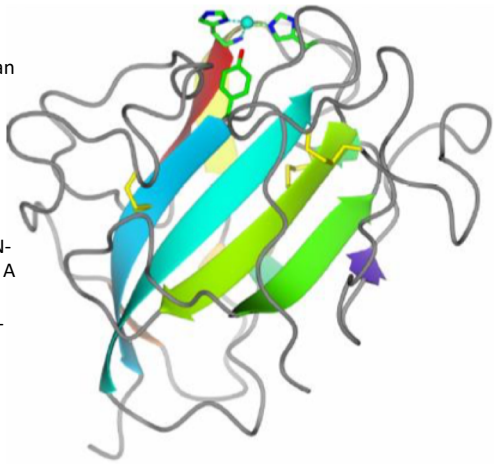
Biotechnology and Biological Sciences Research Council

ISCFPOCMiB038 BB/L013711/1 BB/SCA/MetalsinBiology/17
1 December 2018 – 31 January 2018
Funded by Metals in Biology BBSRC NIBB
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Arginine-terminated LPMOs: a new face in biomass breakdown? Follow-on studies

"The new form of LPMO enzymes is intriguing as it does not contain the usual amino acids at its active site, suggesting that it could be active on a new range of biomass components." **Paul Walton, University of York**

Paul Walton and Gideon Davies, University of York;
Jens Erik Nielsen, Novozymes, Denmark



The overall structure of a typical LPMO has an extended flat face in the middle of which lies the enzyme's active site, containing copper ion coordinated by a N-terminal histidine. A new type of LPMO has a conserved N-terminal arginine instead of an N-terminal histidine.

OUTCOMES: In this project we examined the activity of this new LPMO using ultraviolet–visible spectroscopy and electron paramagnetic resonance. We also determined the structure of the enzyme, gaining particular insight into how it interacts with its substrates. This finding opens up the possibility of tailoring the enzyme to carry out new types of reactions which are of importance to biomass degradation. While we cannot report the details of the findings due to IP reasons, the project was successful in that we developed a new type of assay for this class of enzymes and we also demonstrated that the LMPOs had a new type of enzymatic activity on a range of substrates.

INITIAL AIMS: The generation of fuels and commodity chemicals from sustainable biomass hinges on a single key issue: that biomass (e.g. wood, plant matter) is very hard to break down in a controlled manner. The use of copper-containing lytic polysaccharide monooxygenases (LPMOs) — natural enzymes that are highly efficient at breaking down cellulose — could help circumvent this. In this project, we seek to maximise the ability of a new type of LPMO to break down woody biomass. Our previous Metals in Biology-funded studies showed that this new LPMO can bind metal ions, and we have also obtained a full molecular structure of the enzyme using X-ray diffraction. The structure of this new class of LPMOs suggests that they could be active on lignin and/or lignin components and that, indeed, these LPMOs use metal ions as part of their catalytic cycle. If true, this would represent a wholly new activity for LPMOs and be an exciting addition to the field of biomass degradation.

- **The partners are in discussions on how to take the project forward**

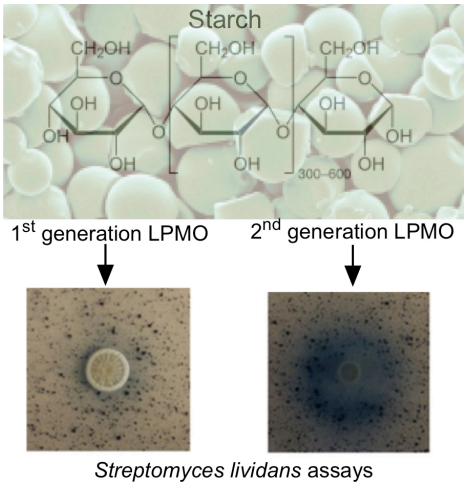
BIVMiB042 BB/L013711/1
23 October 2017 – 4 January 2018
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Creating new starch-active copper LPMOs through the generation of loop libraries

"We are pleased to be part of this research project as we believe that LPMOs will have significant role in starch hydrolysis and starch modification. So far the development of the LPMOs has progressed very swiftly and we are excited to see the next steps in this project." Johannes de Bie, WeissBioTech

Jonathan Worrall, University of Essex; Johannes de Bie, WeissBioTech

OUTCOMES: To create second-generation starch-active lytic polysaccharide monooxygenases (LPMOs), loops that form the active surface surrounding the catalytic copper ion were targeted. In total, there are five active surface loops that may be considered important for interaction and specificity with starch. Active surface loop libraries have been designed *in silico* with saturating mutations in selected amino acid positions in each loop based on sequence variability within the starch-active LPMO family. This business interaction voucher allowed for the synthesis — by combining site evaluation library and combinatorial library technology — of two out of the five loop libraries in a starch-active LPMO. These two loop libraries will be screened in *Streptomyces lividans* for enhanced activity relative to the wild-type LPMO. Following this, we will conduct *in vitro* characterisation of recombinantly produced proteins and then test the activity of the selected variants under industrial conditions.



Improving the degradation efficiency of insoluble starch granules through the creation of 2nd generation LPMOs.

INITIAL AIMS: The efficient deconstruction of plant biomass into biofuels and other chemicals is a key challenge to secure a low carbon economy. In nature, many microorganisms secrete enzymes that can break down recalcitrant biomass that is composed mostly of lignocellulose into soluble substrates. Harnessing the catalytic power of these enzymes to treat biomass outside of their natural habitats is challenging and a major goal of industrial biotechnology. Recently, a new class of enzyme that drastically increases the efficiency of biomass conversion has been identified. These enzymes contain a copper ion and are called lytic polysaccharide monooxygenases (LPMOs). The aim of this project is to assess whether second-generation LPMOs with enhanced substrate activities can be created. As a proof of principle, we will use a starch-degrading LPMO as a template to design and synthesize DNA libraries that will then be screened for substrate activity.

- Two loop libraries from a starch-active LPMO were synthesised
- Further project work between the academic and industrial partners is ongoing