BIVMiB040 BB/L013711/1
1 August – 31 October 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
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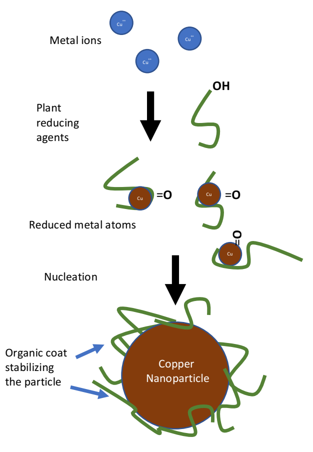
A pilot study to characterize plant-derived compounds that promote the synthesis of copper nanoparticles from contaminating copper ions in waste water

"This study has helped us see the potential future use of plants, plant cell culture or specific plant-produced compounds to remove contaminating copper and other trace metals from, for example, waste water in order to synthesize commercially valuable metal nanoparticles." Andrew Moore, Northumbrian Water Ltd

Ahmed Mohamed, Keith Lindsey and Jennifer Topping, Durham University; Andrew Moore, Northumbrian Water Ltd



Schematic of metal nanoparticle formation in a plant extract (taken from Makarov *et al*., 2014).

RESULTS: Leaf extracts from either coriander or mint were able to facilitate the formation of copper nanoparticles (CuNPs) from copper sulphate solution. Characterization of the CuNPs showed their size ranged from 28-36 nm, they were surrounded by proteins and a proportion of them existed as CuO. The crucial role of plant proteins or protein-containing moieties in CuNP formation was shown by removal of the protein fraction from the plant extracts; CuNPs were not formed in the protein-free fraction. Proteomic analysis revealed that although there was variability between the plant species studied, 105 proteins were associated with the CuNPs formed by both the mint and coriander extracts. Further work, and evidence from the literature, suggested that CuNP formation may be dependent upon protein mixture composition, rather than individual proteins. The second part of our work focused on the potential applications of the CuNPs. The bioactivity of the bio-synthesized CuNPs was compared with commercially available CuNPs in several biological assays. No difference between the two types of CuNPs was observed, confirming that the bio-CuNPs could be used successfully in biotechnological applications.

INITIAL AIMS: Contamination of land and waterways by toxic metals is a serious environmental problem, particularly in areas where mineral mining was once widespread. If the polluting metal can be sequestered into bioactive metal nanoparticles then the nanoparticles could have value in various applications, and the land would be decontaminated using an eco-friendly approach. Plant compounds are thought to be able to precipitate metal ions from dilute solutions to form metal nanoparticles through reduction of the metal ions into metal atoms that coalesce into nanoparticles. There are several possible plant compounds that can act as bio-reductants including flavonoids, terpenoids, sugars and proteins. In this project we studied the formation of copper nanoparticles (CuNPs) from a copper sulphate solution following the addition of plant leaf extract from either mint or coriander, with the aim of gaining a better understanding of how this process occurs, as well as characterising the CuNPs and the bioactive constituents within the plant extracts.

- Plant proteins can be used to reclaim copper in solution in the form of CuNPs
- Bio-CuNPs have the same bioactive properties as commercial (chemically produced) CuNPs
- Further work needed to identify which (if any) of the identified proteins in isolation are sufficient to form CuNPs

BIVMiB018
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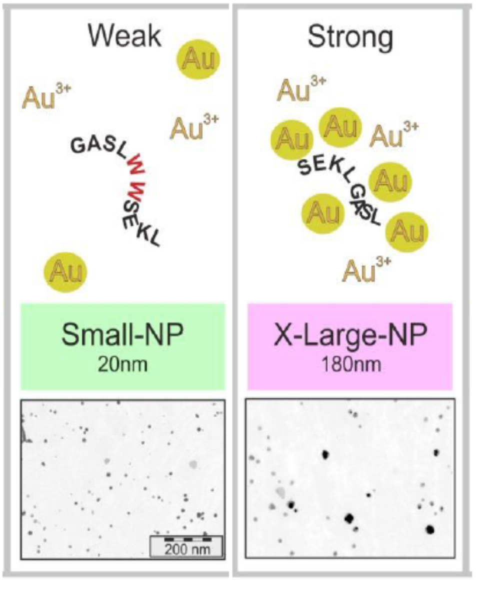
The use of platinum group metal nanoparticles in wastes from roadside verges for the production of high-value catalysts

"The results from this collaboration have enabled us to develop techniques and gain experience which will help towards the development of alternative plant-based remediation practices for sweeper wastes." Yorwaste Ltd

Neil Bruce and Elizabeth Rylott, University of York;
Richard Bate, Yorwaste Ltd

OUTCOMES: We have achieved our objectives to investigate nanoparticle formation using synthetic peptides and analyse catalytic activity in the subsequently pyrolysed nanoparticle-containing plant biomass. Our promising results demonstrate that the expression of synthetic peptides in plants can be used to alter nanoparticle size and subsequent catalytic activity in plants. As part of our third objective, to determine if plants could be used to selectively take up platinum group metals from sweeper wastes, we have shown that sweeper wastes contain detectable levels of valuable metals. However, our studies show that further work is needed to understand the phytotoxicity behind these wastes so that they can be optimised to allow plant growth. Our wider studies indicate that synthetic biology could be used to develop plants that can selectively take up platinum group metals from metal-rich wastes.



Peptide sequences control the size of nanoparticles (NP)

INITIAL AIMS: **Platinum group metals are rare elements that are particularly used in catalytic converters on road traffic vehicles. Over time, palladium and other valuable metals are lost via exhaust fumes and deposited onto roads and verges. Waste collected from road sweepers contains detectable levels of palladium. Plants can take up platinum group metals as nanoparticles in their tissues. With the ultimate goal of recycling these rare metals, the aims of this project were to:**

1. Investigate if the expression of synthetic peptides in plants can control nanoparticle size
2. Analyse catalytic activity in plant biomass that contains pyrolysed nanoparticles
3. Determine if plants can be used to selectively take up platinum group metals from sweeper wastes

- **Results from this project are being investigated further as part of funding from the New Zealand Ministry of Business, Innovation and Employment Global Strategic Partnership**

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18 May – 17 November 2016
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Metals IN BIOLOGY

Studies into the uptake and distribution of metal oxide nanoparticles in plants

"The collaboration has allowed us to apply expertise in mass spectrometry to an industrially relevant area in seed enhancement, springboarding further research into the area of nutrient delivery." Croda Europe

Sheffield Hallam University   CRODA

Neil Bricklebank, Malcolm Clench & Catherine Duckett, Sheffield Hallam University
Kathryn Knight & Marta Dobrowolska, Croda Europe

OUTCOMES: Two samples of barley seeds coated with zinc oxide particles and different seed treatments were prepared by Croda. Seeds from each sample were germinated at Sheffield Hallam University and harvested at different growth points. The selected germinated seeds were embedded in gelatin, cryosectioned and then analysed by LA-ICP-MS. Untreated, germinated seeds were used as the control. The results provide a two-dimensional 'map' (figure) showing the location of the zinc within the seed at different time points throughout its germination. The results clearly show that as the seed is germinated the zinc is transported from the coating applied to the surface and into the shoot of the seedling. The results are being used by Croda to assess the effectiveness of their products that are used in commercial seed coatings, including surfactants, adjuvants and formulation aids, on the uptake of metals by plants

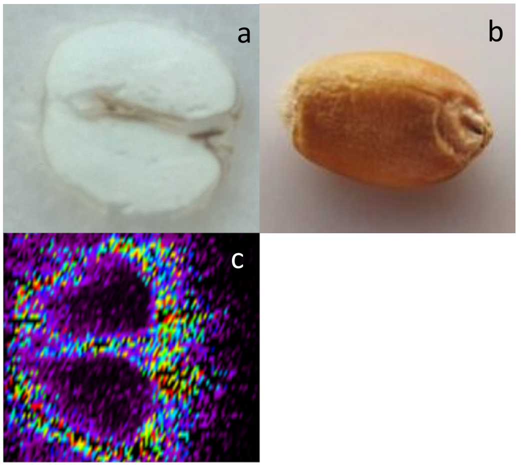

Image of (a) un-germinated seed, (b) section of treated seed tissue, and (c) LA-ICP-MS image showing distribution of zinc in seed tissue.

INITIAL AIMS: The uptake of metals is essential for the growth and development of healthy plants. Plants obtain the metals they need from soil or from fertilizers applied to the growing plant. One of the most important metals is zinc, which is found in many metalloenzymes. Zinc is also essential for humans who gain it from dietary grains and vegetables. In this project we will study the effect of zinc, in the form of a formulation containing zinc oxide, on the growth of plants and use a new analytical tool — known as laser ablation-Inductively coupled plasma-mass spectrometry (LA-ICP-MS) — to study the uptake and distribution of zinc in plants.

- **Results used by Croda to assess the effectiveness of products used in commercial seed coatings on the uptake of metals by plants**
- **Innovate UK funding won to advance the work**
- **Croda awarded a BBSRC iCase studentship to continue the work**

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01 February 2016 – 31 July 2016
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Microbial recovery of metals from contaminated *Miscanthus* used in the industrial remediation of degraded landscapes

"With this funding, we were able to kick start a new collaboration, bringing technologies together that wouldn't have been possible from any other funding source."

Chris Chuck, University of Bath; **Michal Mos**, Terravesta Ltd

OUTCOMES: The project demonstrated that the most suitable technique for metal recovery from *Miscanthus* grown on contaminated land was hydrothermal liquefaction. The hydrothermal liquefaction of the *Miscanthus* produced a reasonable bio-oil yield, and in addition the majority of metals from the *MIscanthus* partitioned in the aqueous phase or the solid residue and could be recovered and/or recycled easily. Further work to increase the bio-oil content needs to be conducted, as well as further optimisation to partition the metals into the solid residue while decreasing the carbon content.

Chris and Michal discuss their collaborative work

INITIAL AIMS: Metal leaching from mining and other industrial activity has the potential to degrade landscapes across the globe. However, several techniques have recently been trialled and brought to market to restore the natural capital of such areas. One of the most promising is growing *Miscanthus x giganteus*, an energy crop that can remove metal contamination while being used as a biofuel feedstock. However, the processing of the contaminated *M. x giganteus* remains an issue. In this study we explored the use of the oleaginous yeast *M. pulcherrima* — which produces metal chelators such as pulcherriminic acid — as a method of valorising the *Miscanthus* biomass into a range of products including a palm oil substitute, and removing the metal waste into a smaller containable volume. This method was compared to hydrothermal processing of the *Miscanthus* waste.

- **Hydrothermal liquefaction was the most suitable technique for metal recovery**
- **Further work is underway to assess the applicability of the technique**

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Metals IN BIOLOGY

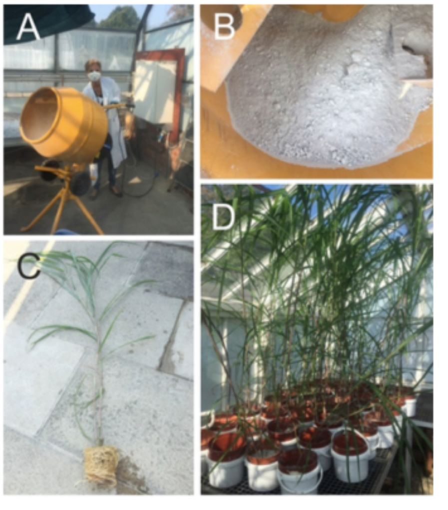
Investigating uptake and catalytic potential of *Miscanthus* grown on palladium mine wastes

"*We are very pleased that this BBSRC NIBB-funded Business Interaction Voucher has made it possible for us to establish contact with the University of York and support their research towards the development of additional uses for UK-grown Miscanthus.*" Mike Cooper, Miscanthus Nursery Ltd

University of York AgriKinetics

Neil Bruce & Elizabeth Rylott, University of York
David Stone, AgriKinetics Ltd
Mike Cooper, Miscanthus Nursery Ltd

OUTCOMES: To simulate mine waste, *Miscanthus* plants were grown on synthetic mine tailings (containing kaolinite, gravel and palladium). The plants were dosed fortnightly with potassium cyanide (KCN) to solubilise palladium in the tailings. We achieved our objective to test the effect of multiple KCN treatments on palladium uptake by *Miscanthus.* Although the application of KCN significantly increased the concentration of palladium in the aerial tissues, additional KCN applications did not enhance palladium uptake. There was also an increase in the appearance of necrotic tissues with increasing number of KCN applications. These results suggest that palladium, or other metals in the tailings, were accumulating in the plants to phytotoxic levels. Our studies indicate that achieving palladium levels required for use as a commercially-comparable catalyst is difficult. Our further studies are investigating if lower levels of palladium in plant biomass can be used in alternative catalysis methods (controlled, low-energy pyrolysis), as well as whether synthetic biology methods can be used as an improved alternative to KCN solubilising treatments.



Mixing (A) and appearance (B) of synthetic mine tailings. Four-month old *Miscanthus* plants (C) and plants one week after planting (D) in synthetic mine tailings.

INITIAL AIMS: Following palladium mining and extraction, mined areas and waste tailings need to be re-vegetated. Tailings still contain significant levels of palladium but recovery using conventional methods is currently uneconomical. Plants can be used to re-green mined areas and have the potential to 'phytoextract' residual levels of precious metals, which could be used as catalysts. Because the insolubility of palladium in the waste is a major limitation to uptake, this project will determine the effects of solubilising treatments on palladium uptake and accumulation in *Miscanthus*.

- Sequential KCN applications did not enhance *Miscanthus* palladium uptake
- Lower palladium levels and other solubilising methods are under investigation

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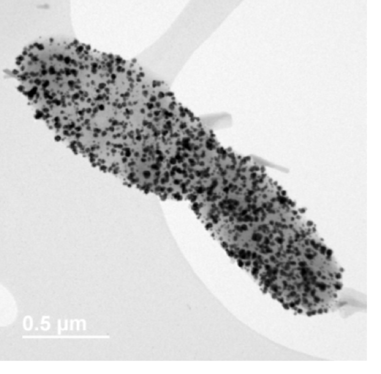
Exploiting the commercial potential of novel biometallic catalysts

"The BIV provided a quick, convenient and effective route for us to bring together the Manchester group's expertise in bioproduction of metal particles with Johnson Matthey's catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts." Nigel Powell, Johnson Matthey.

Jon Lloyd, Nick Turner and Richard Kimber, University of Manchester; Nigel Powell, Johnson Matthey

OUTCOMES: Metal-reducing bacteria can accumulate metals from process environments in the form of catalytically active nanoparticles, offering a simple and green method for high-value nanoparticle production. These nanoparticles have many applications, including in the production of fine and speciality chemicals such as pharmaceutical intermediates, fats and oils, and upgrading of fuels and biorenewables. Bimetallic nanoparticles offer a number of advantages over their monometallic counterparts due to the combined properties of the two metals present, and through new properties created from the synergy between these metals.

We investigated the potential for a metal-reducing bacterium to produce bimetallic nanoparticles from metal solutions containing a range of metals supplied in combination. Metallic nanoparticles were biosynthesised at the University of Manchester and then Johnson Matthey's scanning transmission electron microscopy facilities were used to characterise the products. We found that the pattern of distribution of the metallic nanoparticles was highly dependent on the combination of metals supplied to the cells. Evidence was provided for the formation of bimetallic nanoparticles for some examples of metal combinations, and these nanomaterials are the focus of future work.



Bimetallic nanoparticles produced during the project

INITIAL AIMS: This project brings together biotechnologists from the University of Manchester and experts in industrial catalysis at Johnson Matthey, a leading multinational specialty chemicals and sustainable technologies company headquartered in the UK. This project will facilitate collaborative discussions required to underpin the development and exploitation of a new generation of 'biometallic' industrial catalysts. These are based on naturally occurring metal-reducing bacteria that are able to accumulate metals from process environments (as catalytically active nanoparticles), while also expressing enzymes that are able to extend the range and complexity of industrial reactions that can be produced from these novel microorganisms. This novel extension of synthetic biology has the potential to transform several sectors of UK industry, including those of industrial biotechnology and makers and users of catalysts, simplifying current processes, underpinning novel reactions and extending the range of available products.

- Obtained further funding through a BBSRC NIBB Proof of Concept award
- Industrial partner supported successful BBSRC Responsive Mode grant awarded to academic partner

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Metals IN BIOLOGY

Biosynthesis of bimetallic nanoparticles for fine and specialty chemical production

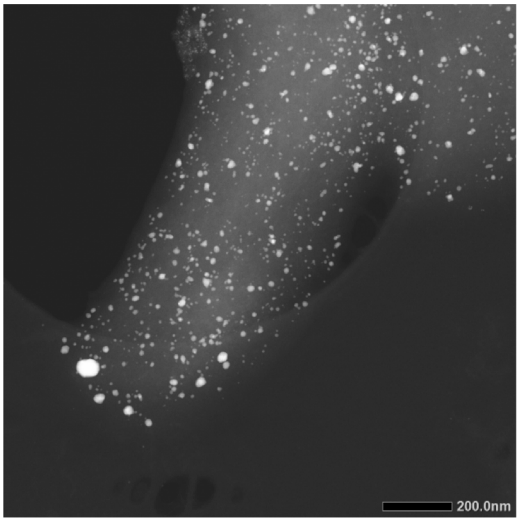
"This award provided a quick, convenient and effective route to bring together the Manchester group's expertise in bioproduction of metal particles with Johnson Matthey's catalysis know-how. We have begun to determine the potential of this technology for the production of novel catalysts." Nigel Powell, Johnson Matthey

MANCHESTER 1824 The University of Manchester

JM Johnson Matthey Inspiring science, enhancing life

Jon Lloyd and Richard Kimber, University of Manchester; Nigel Powell, Johnson Matthey plc

RESULTS: Building on from a successful Business Interaction Voucher project with Johnson Matthey, we continued to investigate the biosynthesis of novel bimetallic nanoparticles for fine and specialty chemical production. Electron microscopy revealed that different metals have varying affinities for forming bimetallic nanoparticles and that the bimetallic nature is also affected by the order the metals are supplied to the bacteria. In addition, we found that the pH buffer used during synthesis can exert some control over the formation of the bimetallic nanoparticles. This knowledge will help us tailor these products going forward. Several of the biosynthesised nanoparticles showed promising catalytic activity. Although they did not perform to the same level as a commercial catalyst, this project has provided us with valuable insights into the optimisation of these bionanocatalysts which we are continuing to explore.



Electron microscope image of bacteria that contain bimetallic nanoparticles. Image provided by G. Goodlet, Johnson Matthey Technology Centre.

INITIAL AIMS: Metal-reducing bacteria are able to recover a wide range of metals from process environments as catalytically active nanoparticles. This project will produce bimetallic nanocatalysts for use in fine and speciality chemical production. Bimetallic nanoparticles offer advantages over monometallic catalysts due to the properties that arise from the presence and synergy of the two metals, offering increased efficiency and specificity for speciality chemical production. This novel biotechnological process offers a simple, cost-effective, environmentally friendly synthesis route for bimetallic catalyst production.

- **The partners will continue to work together to optimise and tailor the catalytic activity of these materials**
- **We will then seek to identify potential avenues for further funding**

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Metals IN BIOLOGY

Bioaccumulation of platinum from waste

"Finding an easier and cheaper way to reclaim of platinum which would otherwise be lost to the environment is important for global resource management." Helen Carney, Teesside University

Teesside University · University of York · TeeGene Biotech

Helen Carney and Caroline Orr, Teesside University; Frans Maathuis, University of York; Pattanathu Rahman, TeeGene Biotech

OUTCOMES: We tested several microbial species — *Shewanella algae, Pseudomonas aeruginosa, Bacillus megatherium*— that were suggested by the literature to have biosorption properties and so have the ability remove platinum from solution. Our studies showed that under the conditions tested, *S. algae* removed more platinum from a solution (hexachloroplatinic (IV) acid, see figure) than *P. aeruginosa* or *B. megaterium*. The optical density of the platinum solution was not reduced by *B. megaterium* and a reduction of only between 5.7% – 6.5% was observed with *P. aeruginosa.* However, a a 55% reduction was shown with *S. algae*. This work confirmed the findings of other studies that showed that *S.algae* could take-up platinum ions from solution. The results add to knowledge in an important area for waste management, since finding an easier and cheaper way to reclaim platinum that would otherwise be lost to the environment is important for global resource management. A logical next step to this work would be to determine the optimal conditions (salt concentrations, temperature, time) required by biosorbants to remove platinum from the two broad categories of waste – high volume, low concentration (e.g. sewage waste or mining waters) and low volume, high concentration (e.g. electroplating discharge). Additional work could consider the role of contaminants and whether uptake is passive or involves hydrogenase enzymes.

Dilutions of hexachloroplatinic (IV) acid prior to analysis. Darker solutions contain more platinum.

INITIAL AIMS: Platinum is a scarce metal, being one of the least abundant elements in the earth's crust and as such has a high material value. This research will focus on the recovery of platinum from wastewaters, where it is present as a soluble, ionic form. Platinum enters wastewater from a range of sources such as metal refining and chemical industries as well as hospital waste, where it can be found as a component of chemotherapy drugs. Bacteria can take-up and accumulate platinum using both active and passive methods, often referred to as biosorption and bioaccumulation respectively. This project, which is a collaboration between TeeGene Biotech, Teesside University and University of York, will investigate the potential of microbes to recover platinum from solutions, with the aim of recycling the recovered metal. The project aims to identify a suitable microbe that can be used in a waste refining process and identify any physicochemical factors that influence platinum recovery.

- **Ongoing business–academia relationship sustained via dissertation research**

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Assessing the bioavailability of metal ions accumulated by DRAM® filters

"Through the BIV, the network has allowed us to better evaluate the readiness level of our collaborator's developing technology. It may be possible to scale up this technology for commercial metal recovery." Epona Technologies Ltd

Louise Horsfall, University of Edinburgh
Leigh Cassidy, Epona Technologies Ltd

A DRAM filter

OUTCOMES: This project assessed the potential to recover Cu(II) from DRAM® media filters and convert it to metallic copper nanoparticles using *M.psychrotolerans*. Using a $CuSO_4$ solution, Cu(II) was accumulated on DRAM filters. Approximately 20% of this bound Cu(II) could be made available for biotransformation into metallic copper nanoparticles. These particles were visualised and positively identified by electron microscopy. Thus *M.psychrotolerans* can form nanoparticles from Cu(II) accumulated on DRAM® filters. Approximately 12% of Cu(II) was reduced by *M.psychrotolerans* to metallic copper nanoparticles. Although the proof of principle study was successful with model solutions and controlled copper solutions, the industrially used DRAM® media — with its unknown contaminants —is currently beyond our methods of nanoparticle isolation and analysis, so further work is needed to identify nanoparticles from this source.

INITIAL AIMS: There is increasing concern over environmental copper levels, their toxicity and the adverse effects on humans and wildlife. Epona Technologies Ltd has developed DRAM® (device for the remediation and attenuation of multiple pollutants) filters, which can accumulate polluting copper ions from industry and agriculture. We would like to determine whether the copper ions accumulated by DRAM® filters can be transformed into copper nanoparticles by *Morganella* sp., so offering a way to recycle copper.

- *M.psychrotolerans* can form copper nanoparticles from Cu(II) accumulated on DRAM® filters
- Paper: *Cueva & Horsfall (2017) Microb Biotechnol. 10: 1212-1215*