

#### Biotransforming Phenylpropanoids derived from Biorefining: a Toolkit Approach

**Prof. Rob Edwards, University of York**

Phenylpropanoids (PPs) are metabolites found in all terrestrial plants which are used in nature to make a range of polyphenolic compounds, with dietary functions as flavourings and antioxidants. In this project, we propose to copy the pathways which exist in nature and take PPs left over as low value by products of biofuel and plant fibre production and biotransform them into high value polyphenolic intermediates of value to the food industry. The biosynthetic process proposed would be assembled in yeast, using enzyme pathways which can be assembled together in a modular manner to generate a wide range of end products from the limited range of PPs available from biorefining. To illustrate the technology, we will generate glycosylated dihydrochalcones which are 100s of time sweeter than sucrose and already extensively marketed as artificial flavour enhancers. While our process will use renewable feedstock and biological processing, currently the synthesis of such compounds requires non-sustainable chemical transformations. In terms of novelty, our programme has the capability of generating end products derived from PPs which have not been described in nature, but which based on chemical precedence would have a strong likelihood of being biologically active as flavourings or nutraceuticals. Therefore in addition to providing an alternative route to existing successful products, through using a modular approach for metabolic engineering we have the potential to generate new market leading products in the future. The proposed process involves a series of individual enzyme steps, each of which serves as a biotransformation module (BM), which can then be linked to the next biosynthetic step to produce the compounds of interest. These BMs will be co-ordinately expressed in different combinations using polyprotein expression technology, essentially enabling the directed reconstruction of plant secondary metabolic pathways in yeast.

#### Fine chemicals from lignocellulosic fermentation residues using heterogeneous catalysis

**Dr Michael Jarvis, University of Glasgow**

A problem in the production of liquid biofuels from lignocellulosic biomass is that polymeric lignin resists degradation and blocks the conversion of polysaccharides to fermentable sugars. In the call documentation's biorefinery diagram the fermentation residue, consisting of lignin and polysaccharides protected by it, is used only to generate heat and power. Obtaining marketable small molecules from this insoluble polymeric residue is a stated aim of the call, and we describe a rational, modular way to achieve this.

Model fermentation residues will be prepared from Miscanthus, willow and spruce biomass. Lignin in pure, polymeric but soluble form will be released from these into ethanol solution by mild acid fragmentation. In this form, uniquely, it will be suitable for hydrogenolytic depolymerisation using heterogeneous catalysis. The monomeric products will be the starting point for catalytic transformations to a potentially wide range of aromatic fine chemicals and industrial feedstocks that are currently made from oil. An example will be selected for proof of concept.

Working with what is effectively a waste (at best, combustible) product from biofuel fermentation permits a flexible approach to lignin utilisation. It places almost no constraints on the other steps in the biofuel production process, which means that these steps can be optimised for profitable yield of the primary fuel product. With raw material costs in the region of £40/tonne and current prices of typical aromatic chemicals around £1000-£1500/tonne, the relatively simple technology required leaves room for profit.

#### Process Intensification for Acceleration of Bio & Chemo Catalysis in Biorefining

**Prof. Adam Kowalski, University of Liverpool**

This proposal addresses "enhancing product value", while providing capability in "integrative bioprocessing". The Ultra Mixing and Processing Facility (UMPF) provides engineering capability to demonstrate that clean bio- and chemocatalysis can be used, as integrated unit operations adjunct to the fuel production stream of the biorefinery, to produce high value aromatics from lignin and monomers from unsaturated oils. The examples chosen to demonstrate the valorisation of by-products of biofuel production are (a) production of monomers from oils in biphasic systems and (b) enzymatic processing of recalcitrant lignin fractions. Both examples rely directly on the use of a novel proprietary Process Intensification technology (UMPF) to overcome mass transfer limitations. Application specific knowledge is provided by University of Bath and Nottingham respectively. The UMPF has been shown in a parallel industry sectors to improve energy efficiency of distributive and dispersive mixing process to produce biphasic systems with large surface area. Scalability is built into the project, as are cleaner processing, reduced waste, energy efficiency and optimised unit operations; concepts central to the environmental, social and economic sustainability of integrated biorefining processes. The basic principles of the UMPF design are a uniform process experience to all molecules and maximisation of the specific area to increase the rate of intra and intermolecular events. We believe that these principles will lead to processes with better selectivity and enable reactions to occur with reduced or even no aids such as phase transfer catalysts, (hazardous) solvents and surfactants. Resulting sustainability gains will be assessed through the use of structured approaches such as Product-Driven Process Synthesis methodologies which are suited for developing conceptual designs and which will support more comprehensive analysis (e.g. life cycle analysis) in follow on proposals.

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

**Dr David Leak, Imperial College London**

To improve the greenhouse gas balance associated with using renewables for fuels and chemicals and avoid the food versus fuel conflict, we must move to using lignocellulose (LC) from crop wastes or purpose grown feedstocks. Although the economics have improved in recent years, the costs associated with recovering fermentable carbohydrate from LC need to be improved. A typical pretreatment involves physico-chemical disruption followed by enzymatic hydrolysis of the carbohydrate polymers. Conceptually, a more economic process would replace much or all of the externally sourced enzymes with enzymes produced by the fermenting organism. This is the basis of "Consolidated Bioprocessing" (CBP). Currently CBP is little more than a concept. In this project we intend to use the thermophilic facultative anaerobe *Geobacillus thermoglucosidasius* to build a CBP platform, using miscanthus as a substrate, in order to evaluate the benefits/potential problems associated with CBP. *G. thermoglucosidasius* is a good platform for CBP as it already metabolises cellobiose and short chain xylans. We have isolated other *Geobacillus* spp which are cellulolytic, xylanolytic and pectinolytic and have the genetic tools to add the relevant catabolic capabilities to this host. Miscanthus is a high yielding grass which is under consideration as an "energy crop" in Europe and the USA. We will pre-treat miscanthus with 2 different ammonia based pre-treatments which largely attack the lignin and use these to gain process data on the effectiveness of different fermentation scenarios, measuring a range of different process parameters to enable effective modelling. These will include biological factors such as growth, metabolite and enzyme productivity (linked to transcription, translation, secretion and sequestration), rates and nature (progression) of utilisation of complex substrates. Different processing regimes (batch and fed batch) will be considered and studies of fluid dynamics made throughout.

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

**Prof. Peter Williams, Glyndwr University**

This proposal is concerned with the establishment of a sustainable biorefinery based on rye-grass and brings together a team of academics with complementary expertise ranging from plant biology, to biochemistry, chemistry and colloid science. The project exploits perennial ryegrass which produces a diverse range of fructan molecules (branched/linear) varying in composition from 3-90 fructose units with different bond types (beta 2,1 and beta 2,6) that will be chemically modified to produce novel polymers and surfactants. These molecules will be produced either directly or through the action of fructan hydrolysing enzymes (endo and exo). Isolation will include the use of novel ultrasound technologies to aid the release of plant sugars from the rye-grass and removal of coloured impurities. Membrane filtration technologies will be used to fractionate the fructans according to their molecular size. The fructans will then be modified to produce a range of polymers and surfactants that can be used in the formulation of a broad range of products including cosmetics, personal care products, pharmaceuticals, surface coatings etc. Chemical modification will be achieved by reacting the fructans with alkenyl succinic anhydrides and alkyl acyl chlorides under relatively mild aqueous conditions. Maillard -type complexes will be produced with a series of proteins and protein hydrolysates using the enzyme treated-fructans. By choosing fructans of varying chain length it will be possible to produce materials which can perform as wetting agents, emulsifiers and rheological modifiers. The performance of the surfactants will be assessed by measurement of the contact angle and dynamic surface tension while the performance of the polymers will be investigated by measurement of the steady shear viscosity.







