

**INTEGRATED BIOREFINING RESEARCH AND TECHNOLOGY CLUB (IBTI CLUB)
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Optimization of Wheat and Oilseed Rape Straw Co-products for Bio-alcohol Production	
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<p>Various forms of biomass represent potential feedstocks for degradation and fermentation to produce alcohols as liquid biofuels, with residual protein-rich materials being suitable for further exploitation, such as processing for animal feed. This provides the potential to substantially substitute for fossil fuels, with the associated sustainability and environmental benefits. However, some of the sources evaluated in recent years could 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, it is becoming increasingly clear that the displacement of food crops by crops grown solely for biofuel is inappropriate. There are, however, several sources of "waste" biomass associated with the UK production and processing of food crops. These provide potential feedstocks for "biorefining" to recover biofuels and animal feed from residues after grain/seed recovery. This approach does not compete with food crops; indeed the increased value of co-products such as straw should enhance the financial viability of food crop production in the UK.</p> <p>Major UK food crops such as wheat and oilseed rape produce more straw co-product than harvested grain or seeds. This straw constitutes a plentiful potential feedstock. However, these crops have been bred for the quality and yield of grain or seeds, not for the composition of the straw they produce. Past studies have revealed variation for the major chemical constituents of importance for bioalcohol production.</p> <p>We are already involved in an ongoing research programme, funded by Defra, which is developing the methodology for the efficient exploitation of biomass residues from the food chain. The proposed research dovetails with this project, providing the means to optimise the principal feedstocks: wheat and oilseed rape straw. Combined, these two projects will establish the supply chain (from breeders and farmers to bio-alcohol producers and the motor industry) needed to deliver the financial and societal benefits of the science.</p> <p>We aim to thoroughly analyse the composition of straw from current wheat and oilseed rape varieties, and of genetic material in use by breeders, for compositional characteristics of importance for biofuel production. We will use recently developed technologies to explore variation of the sequences and expression of tens of thousands of genes in each of wheat and oilseed rape, and relate these genetic characteristics to the compositional characteristics of the lines in order to develop markers for use in subsequent breeding programmes. Using the information we have gained, we will hypothesise the processability characteristics of differing potential feedstocks and test these using a pilot plant system.</p>	

Aromatic Feedstock Chemicals from Degradation of Lignin	
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<p>One of the biggest problems facing society today is our dependence on dwindling fossil fuels, and the contribution to global warming of power stations, car transport and domestic heating that make use of fossil fuels. Not only is oil used to prepare fuel, but also an essential by-product of oil refining is the production of raw materials for chemical and pharmaceutical synthesis. In 50-100 years time, all of these chemicals will need to be produced from renewable, nonpetroleum sources, but at present we have very limited methods to do this, so a lot of new technology needs to be developed in a relatively short space of time.</p> <p>The carbon content of plant lignocellulose, found in plant cell walls, represents an abundant source of renewable carbon. One component of lignocellulose is an aromatic polymer called lignin that binds the cellulose cell walls together. Lignin is very hard to break down, so at present lignocellulose is broken down via a "pre-treatment" step, usually involving heating with acid and steam to 200 oC, which consumes a lot of energy. Therefore, if we could use Nature to break down the lignin, we would improve the efficiency of lignocellulose breakdown, and liberate useful aromatic by-products.</p> <p>We have recently developed a new method for identifying strains of bacteria that are able to break down lignin, and we have already found several strains of bacteria that are able to do this. We will isolate the lignin-degrading enzymes from these strains, and use molecular genetics to produce large quantities of these enzymes for further studies. We will then use these strains to try to produce useful aromatic chemicals from breakdown of lignocellulose. One example is vanillin, which is used for food flavouring in products such as vanilla ice cream; other examples are phenols that are used in the manufacture of plastics.</p>	

In Silico Study of Lignocellulosic Biofuel Processes

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In theory, a genome sequence provides all of the information necessary to define the structure of the biological system of interest. For example, knowing all of the enzymes in a cell and the substrates that each one accepts and all of the products that each one can make, it is possible to formulate a bioreaction master global network that represents the complete repertoire of possible biochemical reaction systems within that cell. In this study, we plan to use a "genomescale" metabolic network (gsmn), reconstructed from the sequence data for a number of species with applications in biofuel production. Gsmns have already been published for a number of medically- and industrially-important species, including *Streptomyces coelicolor* and *Mycobacterium tuberculosis* (these 2 by us) facilitating novel approaches to process design and identification of antibiotic targets, respectively.

We plan to use genome scale modelling to demonstrate the utility of in silico experimentation to Biorefining. The approach will link genomes, capable of carrying out lignocellulose degradation to genomes able to produce biofuels, with a view to predicting processes that will form the basis for an in vivo study. As far as we are aware, this will be the first project to link genome scale models in this way, and will therefore represent a scientific advance in addition to providing pragmatic information. This 2-stage (biomass degradation followed by a separate bioethanol production stage) is potentially more efficient than a single microbial processing step.

Biomass Degradation

The genomes of two "model organisms" (the fungus *Trichoderma reesei* and the bacterium *Clostridium thermocellum*) have recently been sequenced and these species will, therefore, be included in the study. However, considering the current dependence on acid and heat pre-treatment in lignocellulose degradation, enzymes that are stable and active at low pH values and at high temperatures are of particular value. Thus, enzymes derived from thermophilic and acidophilic organisms known to degrade lignocellulose hold significant promise for industrial processes, and, for this reason *Caldicellulosiruptor saccharolyticus* and *Acidothermus cellulolyticus* (both of which have been sequenced) will be included in the biomass degradation stage.

Biofuel production

A significant yield limiting factor is the toxicity of ethanol to the fermenting host. Most fermenting organisms such as *S. cerevisiae* cannot tolerate high ethanol concentrations resulting in a product that must then be concentrated through an expensive and energy-intensive distillation step. *Pichia stipitis* represents one yeast species of relevance to biofuel research based on its natural ability to ferment xylose. Its recently sequenced genome revealed insights into the metabolic pathways responsible for this process and this species will be included in our second stage modelling. *Zymomonas mobilis*, which has been described as having considerable potential for biofuel production has also been sequenced recently and will be included. Finally, the use of *E. coli*, which has been engineered to produce isobutanol and other alcohols, using non-fermentative pathways, will be included. The feed-stocks to be examined for biomass degradation will include lignocellulose (with and without chemical pre-treatment) and co-substrates, which may enhance bioenergetic efficiency of metabolism. Each of the four species will have gsmns constructed. In each case, a "draft" gsmn will be prepared (using the sequence annotations) which will be refined by the addition of further reactions in order to "close" the model. Each gsmn includes numerous (often hundreds of) "input gates", ie potential substrates, predicted by the gene sequence but never tried in the laboratory. Using in silico models, we will be able to examine the effect of combinations of substrates that would require many years of experimentation in vivo.

Engineering Oilseeds to Synthesise Designer Wax Esters

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Waxes are a type of lipid which have useful properties, specially as lubricants. The modern industrial world is dependent (literally) 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 this deminishing natural resource both as a feedstock and as a source of energy to drive the reaction. In other words, petrochemically-derived lubricants are unsustainable. Whilst plant seeds produce oils, these are not very effective as lubricants as they are prone to oxidising which makes them sticky. However, one particular plant, jojoba, makes a different class of oil to that found in all other plant seeds. Jojoba seeds accumulate wax esters, rather than triacylglycerols, and waxes have far superior lubricating properties compared with the normal seed oils. Unfortunately, jobaba plants only grow in American deserts, which preclude their use in European agriculture. To circumvent this problem, we propose to transfer the genes for wax biosynthesis from jojoba (and other organisms which make wax esters) to suitable plant species for evaluation and testing. It is proposed to use *Arabidopsis* as a model system to identify the best combination of genes to make waxes with the most useful properties, and having determined these, introduce them into a dedicated industrial oilseed crop, *Brassica carinata* (Ethiopian mustard). Importantly, by using a crop species that is not used to produce food, this will ensure that our waxes (to serve as biolubricants) will not enter the human food chain. Overall, this project aims to demonstrate the feasibility of substituting plant-derived wax esters for petrochemically-derived lubricants, providing a new green, sustainable source of these important compounds.