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A study of metagenomics-informed biochemical functionality of microbial fuel cells using DDGS as a substrate

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This proposal addresses a BBSRC initiative that aims to enhance the value of Dried Distillers Grains with Solubles (DDGS), a byproduct of grain-to-bioethanol and whisky production. DDGS will become increasingly abundant in the UK as bioethanol production develops. It is currently mainly used as a cattle feed but there is also interest in developing it as an industrial feedstock

A microbial fuel cell (MFC) is a device that contains an anerobic culture of microorngnaisms, capable of directly converting chemical energy to electrical energy. A typical microbial fuel cell consists of anode and cathode compartments separated by a cation (positively charged ion) specific membrane. In the anode compartment, nutrients are oxidized by microorganisms, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, while protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

We will develop a microbial fuel cell that will process DDGS prior to drying and use as an animal feed. The MFC will generate electricity (to reduce consumption by the biorefinery) and enhance the protein content of the animal feed product.

The species of micro-organisms added to the MFC will be determined by analysing all of the genes present in whole populations of micro-organisms (metagenomics) under a range of conditions and using a computer simulation which highlights the most important genes to carry out the desired functions of the MFC. The population composition will be further fine-tuned by feeding the microbes with nutrients as rewards for achieving the desired characteristics, forcing it to evolve to the most effective distribution of species.

Development of a process scheme for the production of high value functional products from DDGS

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DDGS is the major co-product of bioethanol fermentation and is produced at very large quantities annually worldwide. Currently, DDGS is a low value agro-industrial product produced by distillers or bioethanol factories, and is primarily used as a protein-rich animal feed. A major issue with this application, which reduces its utilisation compared to soybean and canola meals, is its compositional variability, which consequently affects its nutritional quality and digestibility.

The aim of the proposed work is to develop a novel, scalable and economically viable process that will transform DDGS into several medium to high value products, namely a prebiotic food ingredient, gluten protein for film packaging, betaine and choline for use as nutritional supplements, and crude dietary fibre. The proposed process is based on the biorefinery concept in which the agricultural raw material is transformed into several value-added streams, which are either end-products or starting materials for secondary processing. Developing such a multi-stream process using DDGS as the raw material would be pioneering for the biorefinery industry as it would add considerable value to DDGS.

Prebiotics are non-digestible food ingredients that have a beneficial effect on health through their selective metabolism by bacteria in the intestinal tract, and are attractive prospects in the digestive health market. The objective will be to transform arabinoxylan (AX), which consists 30-50% of DDGS, into arabinooligosaccharides (AXOS); these have been shown to have prebiotic activities and over the last five years have attracted considerable commercial interest. A commercially attractive prospect is to target the production of AXOS with relatively high molecular weights (MW) in an effort to increase the persistence of the prebiotics in the colon and target delivery into the distal region. This would increase the beneficial effects of prebiotics as most of the colonic diseases, principally ulcerative colitis and bowel cancer, predominantly originate in the distal region. Gluten on the other hand consists 30-40% of DDGS and will be used to produce biodegradable film packaging material. The research will focus on extracting and characterising the gluten and evaluating the properties of the films. This will open up new applications for DDGS gluten with high market potential and economic benefits. Finally, betaine and choline have important biological functions for human health and as such they have received a lot of commercial interest as nutritional supplements. They are present in wheat and consequently in DDGS at much higher concentrations than in other natural food sources, and therefore extraction of these compounds from DDGS has considerable economic and market potential.

The proposed process consists of several scalable unit operations including the separation of DDGS into a soluble and non soluble stream, the fractionation of the soluble stream into gluten, AX, betaine and choline, the controlled hydrolysis of AX to AXOS, and the purification of AXOS. Key factors influencing the efficiency, scalability and economic feasibility of the process are (i) the development of efficient processing steps for the separation of the raw material into the target compounds, with high yields and purities, (ii) the utilisation of highly active enzymes that lead to the controlled synthesis of AXOS with specific MW and prebiotic activities and (iii) the production of gluten films with suitable morphological and functional properties for commercial use. The work will be carried by a multidisciplinary team of researchers from the University of Reading and Rothamsted Research and will bring together unique expertise in wheat biochemistry, bioprocessing, protein science, food ingredient functionality and gut microbiology.

Fractionation and exploitation of the component value of DDGS

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Some fermentation processes, eg brewing, use cereal starch as their source of carbohydrate. Typically, the residue of the cereal grain is not separated until the end of the fermentation process. In distilleries or in processes designed to produce alcohol for fuel, the liquid stream then goes through distillation which leaves a liquid residue ("thin stillage"). In large scale operations, the liquid and grain residues are dried to produce "distillers dried grain and solubles" (DDGS) which can be used as animal feed. In the UK, at least 2 large-scale wheat to alcohol plants will soon be operating. These will convert low-grade feed-wheat (of which the UK typically has a surplus) to alcohol for use as an automotive fuel, co-producing large quantities of DDGS, of a fairly consistent composition. The industrial members of IBTI have set a challenge of adding value to this DDGS, which this project addresses.

Apart from the starch, cereal grain is composed mainly of protein, fibre and other non-starch carbohydrate and fats. The main animal feed value is contained in the protein, but the high fibre content means that DDGS is only useful for ruminant animals. In this project we intend to separate some of the protein, carbohydrate and fats and use them to produce higher

value products, while still retaining the option to use the protein component as an animal feed, possibly for poultry. The latter is important as using DDGS as animal feed replaces imported soybean and thus, can reduce the greenhouse gas (GHG) emissions associated with soybean production and importation. Therefore, the challenge breaks down into 2 parts: 1) devising methods to remove the non-starch carbohydrate and fat from the DDGS without destroying the feed value, and 2) finding ways to gain added value from the extracted components.

For the first part we have assembled a multidisciplinary team who are experts in addressing the engineering, biological and animal nutrition components of this project. From a process engineering perspective it would actually make sense to use the separated distiller's grain, before addition of the "solubles", as our starting material. This would be difficult to obtain, so we will recover the grain component from the DDGS. Removal of the fats could be done with an organic solvent, but this might leave undesirable residues in the animal feed. As an alternative we will investigate the use of super-critical carbon dioxide (SCCO₂) extraction; a gentle, residue free method used for making decaffeinated coffee. The fibre and other carbohydrates will be removed mainly with enzymes, but we will need to find gentle physical pre-treatments (hot water or a short steam treatment) to facilitate enzyme access to the carbohydrates.

In (2), we will focus on upgrading the carbohydrate and protein components. The carbohydrate could be used in a second fermentation process, if an organism was available that could convert the carbohydrates to useful products. To reduce the cost of this process we would need to find/create an organism that could use most of the carbohydrate polymers directly, rather than adding separate enzymes, so this part of the programme will focus on identifying suitable enzymes and the genes that encode them to put into established process organisms. Producing additional fuel or other chemicals by a secondary fermentation will not only improve the economics but also the GHG balance of the process. The proteins contained in wheat grain are rather specialised in their make-up, having a high frequency of certain amino acids. Availability in large volumes offers a unique opportunity to make specific chemicals, and the feasibility of exploiting this renewable chemicals approach will comprise a second strand of activity. If successful, this will also have a GHG benefit.

Together with projected uses of the fatty fraction we will combine data from the whole exercise into an economic model for independent evaluation by potential users.