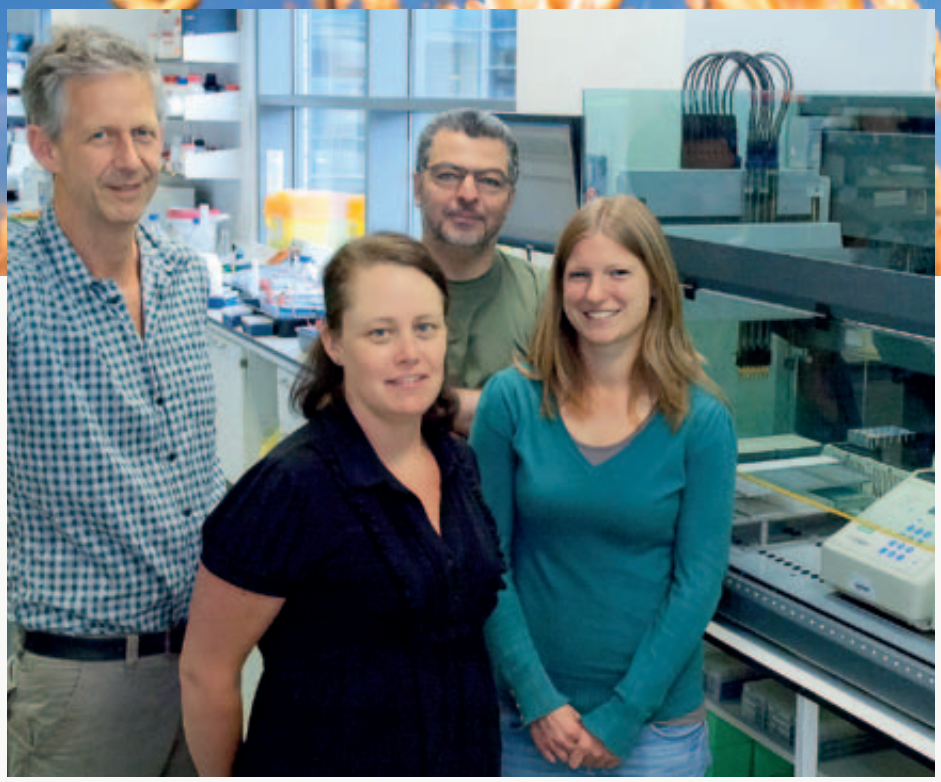
Second generation biofuels – making energy from waste



Left to right: Simon McQueen-Mason, Caragh Whitehead, Leonardo Gomez and Poppy Marriott

The Centre for Novel Agricultural Products (CNAP), a specialized research center within the Biology Department at the University of York, UK, is using Freedom EVO® workstations in its research into the potential of plant-and microbial-based renewable resources.

Plant biomass is one of the greatest reserves of fixed carbon on the planet and, as such, has huge potential as a renewable energy source to replace fossil fuels. The main structural material in plants, lignocellulose, is composed of cellulose, hemicellulose, and lignin and has a long history as a source of energy. A drawback, however, is that the carbon required is largely present in cell walls, and is not easily accessible. A 20-strong group based at the University of York and led by Professor Simon McQueen-Mason is looking closely at plant cell wall biology, and investigating ways of successfully and cost-effectively releasing and processing the lignocellulose biomass from cell walls. In contrast to first generation biofuels which are produced from food crops, the York Group is concentrating on saccharification as a method of breaking this complex carbohydrate into its monosaccharide components, opening doors into a second generation of biofuels potentially produced from food crop waste such as leaves and stems.

In order to screen thousands of plant samples, both from classical breeding experiments as well as specifically created plant mutants and different enzymes, the Group needed to develop ways of miniaturizing and automating the saccharification assay and turned to Tecan for a solution. Dr Leonardo Gomez, who developed the assay, said: “We approached Tecan because of its reputation for developing flexible, reliable and easy-to-use automated systems, and we have certainly found that to be true. We first chose a Freedom EVO 200 workstation that is very flexible and we have used it for other applications besides the standard saccharification analysis, including the determination of the kinetics of enzyme preparations, the activity of cellulases and in comparing a matrix of different enzymes and substrates.”

In the automated process, ground and aliquoted plant samples of a defined particle size first undergo a mild pre-treatment by heating the samples in the presence of alkaline or acid solution, followed by hydrolysis and finally a colorimetric detection assay for the free sugars based on MTBH (3-methyl-2benzothiazolinonehydrozone). Pre-treatment, hydrolysis and the assay steps of sugar determination are all performed on the Freedom EVO system, equipped with a Liquid Handling arm with eight fixed tips, a sample loading area, a heated shaker, a Monitored Incubator Option (MIO™) shaking incubator and three thermocyclers. The thermocyclers achieve even and fast heat distribution across the microplates which has proven to be crucial to the MBTH assay. Figure 1 shows an overview of the saccharification process and the employed hardware and labware.

Saccharification assays of plant material are prone to variation due to the heterogeneous and largely insoluble plant samples and the use of crude enzyme mixtures. Automation of the assay removes this problem and has improved the overall assay by standardizing conditions, like incubation timings. This was clearly seen in a comparison of the manual method with the automated protocol which showed a decrease in the coefficient of variation from 9 % to 5.5 %, dramatically increasing the repeatability and comparability of the test results. Depending on the conditions, 80 samples in quadruplicates on four microplates are typically analyzed per day on York's first platform. However, as the interest in second generation biofuels grew, the Group acquired a second Freedom EVO system to increase the number of analyses it can perform in its laboratory.

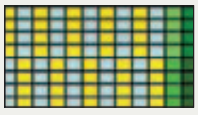
Leo concluded: "We are very pleased with our choice of automated platform; we have successfully miniaturized this process and created an efficient and reliable workstation that handles high throughputs with ease."

See the Group's Freedom EVO workstation in action and described clearly in the video entitled 'High throughput saccharification assay for lignocellulosic materials' at **www.jove.com/details.php?id=3240**

To find out more about Tecan's Freedom EVO liquid handling platform visit **www.tecan.com/freedomevo**

To find out more about the Centre for Novel Agricultural Products (CNAP), visit **www.york.ac.uk/org/cnap/**

Figure 1: Labware, hardware and incubation conditions used in the saccharification analysis

Process	→ Pre-treatment	→ Hydrolysis	→ Sugar determination
Labware			 
Plate layout	standard 1 standard 2 standard 3 standard 4 sample sample 		 Determination in triplicate
Hardware	 Heating block	 Oven/shaker	 Thermocyclers
Temperature	90 °C	50 °C	60 °C
Duration	30 min	8 h	15 min