Institution: The University of Huddersfield

Unit of Assessment: 5 Biological Sciences

Title of case study: Microbial Production and Metabolism of Polysaccharides

1. Summary of the impact

University of Huddersfield research into the microbial production and metabolism of polysaccharides has had a significant impact in two distinct areas. In the food and health care industry it has driven developments in the use of bacterial starter cultures, leading to the adoption of new techniques to produce fermented products with proven functionality. In the policy arena, in modelling gas production by microorganisms, it has made a major contribution to the safety case for the disposal of nuclear waste, highlighting the economic and environmental benefits of underground storage. In each instance the reach of the research's impact has been international with the biggest beneficiaries residing in Europe and North America.

2. Underpinning research

An understanding of the potential health benefits associated with probiotic bacteria has led to commercial interest in related research. Work by the University of Huddersfield's Department of Biological Sciences has focused on relating the biological activity of the polysaccharides secreted by probiotic organisms (exopolysaccharides – EPSs) to their structure and, working with international collaborators, to correlate variation in EPS structures with changes in the sequence of the EPS synthesis genes. This work is only possible because of our development of methods for production and isolation of pure EPSs for use in characterization studies and in measurement of biological activity **[1, 2, 3].**

Huddersfield's early studies in this area were funded by two large European Framework grants, spanning 1998 to 2003 **[G1, G2]**. Both programmes featured academic partners from Europe and Scandinavia and industrial partners from France (Rhodia Foods) and Poland (Biolacta Texel). Rhodia and Biolacta's focus was on the development of EPS cultures with specific functionality-texture attributes. Initially led by Professor Valerie Marshall (Head of Department, 1998–2005, retired) and Professor Andrew Laws (Director of Research, School of Applied Sciences, 1998–), the Huddersfield team was responsible for coordinating these European projects, for selecting cultures with the ability to produce EPSs **[1]**; establishing methods for the controlled production of functional EPSs from lactic acid bacteria (LAB) **[2]**.

In 2003, Dr Paul Humphreys (Senior Lecturer 2002–2010, Reader in Microbiology 2010–) joined the research team and work was extended to include probiotic strains of bifidobacteria. New characterization techniques were developed including real-time measurement of polysaccharide chain length. At the same time the team also started working closely with Dr Ruas-Madiedo and colleagues at the Instituto de Productos Lacteos de Austurias (IPLA), part of the Spanish Research Institute, CSIC. The probiotic team at IPLA–CSIC is recognized as one of the leading government funded research groups studying the biological activity and genetics of bifidobacteria. IPLA–CSIC works closely with the Spanish dairy sector. With IPLA, the Huddersfield microbiology team has jointly published work describing both the gene sequence and the structure of an EPS from a bifidobacterium **[3].** This work was funded through a grant from CSIC **[G3].**

In related research, the team has also used its expertise to study microbial polysaccharide metabolism – particularly gas and small molecule generation during microbial-catalysed cellulose decomposition. This work, initiated by Humphreys in 2002 and carried out by both Humphreys and Laws since 2009, has helped provide a comprehensive understanding of the fate of cellulosic materials in waste facilities, which is widely regarded as a key factor in the safety case for proposed underground nuclear waste repositories. **[4, 5, 6].** This work was funded by a large EPSRC grant **[G4]** and small grants from the UK-Nuclear Decommissioning Authority (NDA) and Ontario Nuclear Waste Management Organisation (NWMO) **[G5, G6].**

The research investigated the microbial production of highly mobile gases such as methane, carbon dioxide and hydrogen. Being radioactive and highly flammable, these gases pose a threat to the safe storage of intermediate and low-level radioactive waste if directly released into the environment **[4]** The team also studied the production and subsequent fate of small organic molecules, generated during cellulose hydrolysis, that can complex with radionuclides and potentially escape into the geosphere through water courses. The principal outcome was a computational model, developed by Humphreys **[6a, b],** for estimating gas production during the microbial decomposition of cellulosic waste.

3. References to the research

[1] **Marshall, VM**, **Laws, AP**, Gu, Y, Levander, F, Radstrom, P, De Vuyst, L, Degeest, B, Vaningelgem, F, Dunn, H, and Elvin, M: 'Exopolysaccharide-producing strains of thermophilic lactic acid bacteria cluster into groups according to their EPS structure' (2001), *Letters in Applied Microbiology*, 32(6), 433-437. DOI**:** 10.1046/j.1472-765X.2001.00937.x

[2] Harding, LP, **Marshall, VM**, Hernandez, Y, Gu, Y, Maqsood, M, and **Laws, AP**, 'Structural characterisation of a highly branched exopolysaccharide produced by Lactobacillus delbrueckii subsp bulgaricus NCFB2074' (2005), *Carbohydrate Research*, 340(6) 1107-1111. DOI: 10.1016/j.carres.2005.01.038

[3] Leivers S, Hidalgo-Cantabrana C, Robinson G, Margolles A, Ruas-Madiedo P, **Laws AP**: 'Structure of the high molecular weight exopolysaccharide produced by *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 and sequence analysis of its putative EPS cluster' (2011). *Carbohydrate Research*, 346(17), 2710-7. DOI: 10.1016/j.carres.2011.09.010

[4] Metcalfe, R, Watson SP, Rees, JH, **Humphreys, P**, and King, F: 'Gas generation and migration from a deep geological repository for higher activity radioactive waste', Nuclear Waste Assessment Team Report (2008) NWAT/NDA/RWMD/2008/002, http://a0768b4a8a31e106d8b0-50dc802554eb38a24458b98ff72d550b.r19.cf3.rackcdn.com/geho1108bozn-e-e.pdf

[5] **Humphreys, PN**, West JM, and Metcalfe, R: 'Microbial effects on repository performance', Quintessa Report to Radioactive Waste Management Directorate', (2009) QRS-1378Q-1, Version 2.0, http://eprints.hud.ac.uk/7613/1/Microbial_Effects_on_Repository_Performance.pdf

[6a] Avis, J., West, A., Walsh, R., Calder, R., Suckling, P., **Humphreys, P**. and King, F. 'Detailed Modelling for the Postclosure Safety Assessment of OPG's DGR.', *In: 'Waste Management, Decommissioning and Environmental Restoration for Canada's Nuclear Activities', Sep 11 - Sep 14 2011'*, (2011) Ontario, Canada, p. 27

[6b] Suckling, P., Avis, J., **Humphreys, P.** and King, F. *T2GGM Version 2: Gas Generation and Transport Code* (2011) Canada: NWMO, Ontario, Canada

Additional indicators of quality:

The work on exopolysaccharide production was supported by either the European Community or directly by European Research institutes with Huddersfield coordinating the work of Internationally recognised research teams [Grants to Marshall and Laws **G1-G3**]. The work on microbial gas production has been supported by government funding agencies in Canada and Europe, again, demonstrating the international excellence of our research outputs **G4-G6**.

Grants[G1-G6]:

[G1] Awarded to Marshall (PI); INCO Copernicus IC15-CT98-0905; European Grant (1998–2002) 225,000 euros.

[G2] Awarded to Marshall (PI) & Laws (CoI); FAIR CT-98-4267; European Grant (1999–2002)

1,088,355 euros.

[G3] Awarded to Laws (PI); Spanish Plan Nacional de I+ D through project AGL2009–09445, (2009–2011) 20,000 euros.

[G4] Awarded to Humphreys (PI); The Post-Disposal Behaviour of C-14 and Irradiated Graphite; EPSRC EP/I036354/1 (2011–2015) £728,414.

[G5] Awarded to Humphreys and Laws; UK funding for Nuclear work – Nuclear Decommissioning Authority contracts; sponsors include-SERCO, Quintessa and AMEC; (2008–2013) £50,413.

[G6] Awarded to Humphreys (PI); International funding for Nuclear work; sponsors include DGR-Canada, NAGRA-Switzerland, VTT-Finland**;** (2008–2013) £34,020.

4. Details of the impact

Huddersfield's research into the microbial production and metabolism of polysaccharides has had a significant impact in two distinct areas. Firstly, research into EPS production has impacted on manufacturing industry, both the dairy industry and manufacturers of personal care products, where it has driven developments in the use of bacterial starter cultures. Secondly, work on microbial gas production has impacted the policy arena surrounding the disposal of nuclear waste, where it has informed the debate on safety and helped deliver economic and environmental benefits.

Throughout the impact period the methods established for screening LAB cultures for EPS production have been adopted by industry. Initially applied to fermented milks, the techniques are now used for various fermented products with functional properties. For example the personal care industry has recruited EPSs as agents for modifying the viscosity and performance of personal care products. The methods developed at Huddersfield for characterizing EPSs have been adopted by Croda (Europe) who have acknowledged that our work has provided *'valuable input to development of our strategy for characterizing product candidates'* **[A]**.

The work has also guided government research organisations outside the UK. For instance, the Spanish Dairy Institute (Instituto de Productos Lacteos de Astruias – IPLA) is currently using the results of Huddersfield's research into EPSs from bifidobacteria to identify specific mutant cultures that are good EPS producers and have proven health benefits. IPLA scientists have described Huddersfield's contribution to the Institute's ongoing work in this field as having *'pivotal importance'* in strengthening the research direction of the Probiotic group **[B]**.

A number of patents have also cited the research, including United States patent number US 73232 B2, which was granted in January 2008 to Luc De Vuyst and Bart Degeest, of Vrije Universiteit Brussel, for a method for preparing yoghurt and other fermented milk products using a starter culture with an EPS-producing micro-organism **[C]**.

A number of agencies with responsibility for developing the safety case for proposed underground storage facilities for nuclear waste have drawn on Huddersfield's expertise in modelling the microbial decomposition of cellulose and gas generation. This work was funded through several successful bids for research contracts from the UK's Nuclear Decommissioning Authority (£65,000 during the impact period **[G5, G6]**) and Ontario Power Generation (OPG), which is responsible for Canada's nuclear waste disposal facilities. Acknowledging their significance, researchers from SERCO, the National Nuclear Laboratories and other UK universities have contributed experimental data for use in Humphreys' computational models. As a result, Huddersfield's work has helped shaped UK and Canadian government policy in this increasingly important area. This is evidenced by Huddersfield's contributions to a series of key position papers and technical documents, chief among them 2010's *Near Field Evolution Status Report, Radionuclide Behaviour Status Report* and *Gas Status Report* for the NDA **[D–F]** and 2011's Environmental Impact Statement Summary and Postclosure Safety Assessment for OPG **[G–H]**.

The latter relate to OPG's proposed Deep Geologic Repository (DGR) Project for Low and Intermediate Level Waste at the Bruce nuclear facility in the Municipality of Kincardine.

Huddersfield's work has been crucial to what OPG has described as *"a thorough, traceable, step-wise assessment process"* to identify the potential environmental repercussions of all phases of the project. Humphreys' modelling has helped to demonstrate the likely effects of microbial decomposition in the years following closure, leading to the conclusion that the repository will take *"hundreds of thousands or even millions of years"* to re-saturate. A public hearing into the project, which was the first of its kind in Canada, took place in September 2013. Dr Humphreys contributed as a 'microbiology expert' and his comments are cited in the transcript of the meeting **[I].** If approved, the DGR is expected to be operational by 2018.

In the UK the NDA has suggested the building of such facilities will bring about not just major environmental benefits but considerable economic impacts. It has been estimated that the initial work to investigate the potential geology at a selected candidate site would require an average of around 440 staff and take around 10 years to complete; construction would require 840 staff; and operation would require 560 staff. Huddersfield's work in this field is therefore helping to lay the foundations for what experts believe will be a major new industry.

5. Sources to corroborate the impact

[**A**] Croda Biotechnology, Croda Europe Ltd. **Contact 1**.

[**B**] Instituto de Productos Lacteos de Asturias, Consejo Superior DE Investigaciones Cientificas, Carretera de Infiesto, Spain. **Contact 2.**

[**C**] United States patent number US 7323199 B2, De Vuyst, L, and Degeest, B: Method for the preparation of yoghurt and other fermented milk products
http://www.google.co.uk/patents/US7323199

[**D**] NDA REPORT: NDA/RWMD/033 – Near Field Evolution Status Report
http://www.nda.gov.uk/documents/upload/Geological-Disposal-Near-field-evolution-status-report-December-2010.pdf (see page 150)

[E] NDA REPORT: NDA/RWMD/034 – Radionuclide Behaviour Status Report
http://www.nda.gov.uk/documents/upload/Geological-Disposal-Radionuclide-behaviour-status-report-December-2010.pdf (see pages 115 and 117)

[F] NDA REPORT: NDA/RWMD/O37 – Gas Status Report
http://www.nda.gov.uk/documents/upload/Geological-Disposal-Gas-status-report-December-2010.pdf (see pages 112 and 113)

[**G**] Ontario Power Generation Deep Geologic Repository Project for Low and Intermediate Waste report: Environmental Impact Statement Summary (2011)
http://www.opg.com/power/nuclear/waste/pdf/NWMO%20216%20-%20EIS%20Summary.pdf (see page 44 for use of Huddersfield model for gas generation)

[**H**] Ontario Power Generation Deep Geologic Repository Project for Low and Intermediate Waste report: Postclosure Safety Assessment (NWMO DGR-TR-2011-25, 2011)
http://www.nwmo.ca/uploads/DGR%20PDF/Licensing/Postclosure-Safety-Assessment.pdf (see page 76, inter alia, for use of Huddersfield model for gas generation)

[**I**] Canadian Nuclear Safety Commission Microbial Effects Review (CNSC IR-EIS-21)
http://www.ceaa-acee.gc.ca/050/documents/p17520/94516E.pdf (see page 23 Line 21)