Sheffield Cell-Mineral Interface Research Programme

Outline of Research Programme

BACKGROUND

The programme is a major strategic development by the University of Sheffield to build a critical mass of research excellence in Cell-Mineral Interface Research. This £1M programme, together with a £2.6M SRIF (Strategic Research Infrastructure Fund) spend in Environmental Engineering and in Nanotechnology, links strengths in Environmental Engineering Science to those in Surface Chemistry, Nanotechnology, Biological Imaging and Molecular Biology. The programme is funded primarily by the Engineering and Physical Sciences Research Council (EPSRC), with additional support from the Biotechnology and Biological Sciences Research Council (BBSRC) and The University of Sheffield.

Natural Attenuation (NA) of organic pollutants in sediments, soils and aquifers is a low-intensity method of remediation that relies on natural biodegradation processes to remove contaminant mass and reduce risk. Reliable prediction of risk and the ability of natural processes to remove contamination demands a much higher scientific base for understanding and quantifying the *in situ* processes than is currently available. Microbiological processes are poised for a major step-change over the coming decade in conceptual understanding and quantitative approaches to interpreting and predicting process rates. Post-genomic science and technology offer tremendous potential for environmental applications, but there are also enormous challenges. These are due primarily to the complexity of natural environments. This includes 1) the complexity of indigenous microbial consortia and ecosystems, 2) unknown microbial species and physiology and 3) the complex physical and chemical matrices that constitute soils, aquifers and sediments. Due to this complexity, these environments are not amenable to idealised system analysis. Although there is extensive knowledge on the biochemical degradability of compounds, it is not possible to predict trends in biodegradation with site characteristics. Conceptual models for site controls on biodegradation are largely restricted to electron acceptor availability, and N and P limitation. However, there are a number of clues about potentially important linkages that may systematically control trends in microbial activity within these spatially heterogeneous mineralogical and aqueous geochemical environments.

- Cell numbers and biodegradation activity are considered to predominate via attached growth on geological solids
- Degrading populations preferentially colonise surfaces that are relatively rich in limiting nutrients
- Biodegradation relies strongly on spatially intimate fermentation and respiration processes within biofilms
- Respiration in the absence of soluble oxidants can remain robust via sparingly soluble mineral oxidants

These lines of evidence point to a dominant, but poorly understood, role of attached microbial activity within the context of biofilm growth in aquifers. We currently have the opportunity to study Natural Attenuation (NA) performance at field scale within a EU project at 6 hydrocarbon-contaminated sites across Europe. The study will quantify average rates of dominant biodegradation processes for a wide range of *in situ* conditions. In the absence of a fundamental understanding of biofilm development and activity, we are limited to an empirical approach: we can monitor groundwater quality, estimate biodegradation rates and quantify a selection of site properties but we cannot predict trends in NA performance. These challenges, coupled with developments in molecular biological, biological imaging and atomic force (AFM) methods for environmental systems, provide a tremendous research opportunity to develop the necessary fundamental biodegradation science to support NA and related passive remediation technologies.

OBJECTIVES OF PROPOSED WORK

We have drawn together a highly committed multidisciplinary team to elucidate key interactions between microbial biofilm activity, aquifer conditions and biodegradation performance. The aim is to establish a critical mass of expertise that will create a step-change in our understanding of the molecular basis for cellular interaction with the environment. The key deliverables are extensive experimental data on model systems down to nanometre observation scale, a rigorous framework linking regulation of the cell-mineral interface with changes in environmental conditions and exceptional interdisciplinary training for researchers. The resulting conceptual and process models will be tested against observed biodegradation rates at field sites in the EU project and with environmental samples from a wide range of sites. The top figure on the following page outlines the scope of the project.

Specific objectives are:

1. Quantify rates of biodegradation with the numbers and activity of attached microbial colonies on minerals and rock
2. Characterise the physical and community structure of biofilms with molecular biology methods and fluorescence microscopy
3. Elucidate the biological physical chemistry of the cell surface that controls microbial attachment on minerals
4. Develop a fundamental understanding of how biofilms and related biodegradation activity respond to environmental conditions
5. Develop a framework to predict trends in attached growth and biodegradation activity and test against field-scale observations.

EXPERIMENTAL PROGRAMME

Laboratory Microcosm Experiments

In addition to the Post-Doctoral Research Associate, the programme initially includes 7 PhD studentships (6 are advertised with this position) and technician support. The project will be organised around a common set of microcosm experiments with a wide range of observation and analysis techniques directed towards these. The scope of the biodegradation experiments and the related projects are outlined below.

Microcosms will be designed as batch reactors and flow-through micro-reactors reactors containing mineral phases, aqueous solutions and microbial inocula. The Groundwater Group at Sheffield have established a variety of batch and flow reactor techniques to study biodegradation processes for a wide range of environmental conditions and contaminants. Conditions to be varied in the proposed experiments are

1. Microbial populations; pure and mixed cultures of aerobes, anaerobes and fermentors
2. Mineral phase present ($Fe(OH)_3$(s), Al_2O_3(s), SiO_2(s)),
3. Relative concentration of respiratory electron acceptors (O_2, NO_3^-, Mn(III,IV), Fe(III), SO_4^{2-}, HCO_3^-),
4. Relative concentration of nutrients (organic carbon compounds, N and P solute species),
5. Growth phase of the microbial populations and
6. Ionic composition of the groundwater (pH, ionic strength).

Fine-grained silica sand and glass plates (cm size) will be used as a solid substrate for attached microbial growth. Alumina and iron oxide surfaces will be prepared separately by metal ion hydrolysis and solid phase precipitation onto silica grain suspensions and the glass plates. Work will be initiated with well-characterised individual strains of bacteria. Parallel investigations will develop microbial ecosystems using inocula from field sites. Enrichment cultures with growth media of selected nutrient and electron acceptor composition will be used to select for populations with significant syntrophic activity. The dominant strains of the enriched populations will be isolated, characterised and used further as mixed inocula in order to study the organisational structure and interaction within biofilms containing fermentors, acetogens, hydrogen-utilising and methanogenic populations.

Scope and Design of Multi-Factorial Experiments. We propose to use Toluene as the main source of organic carbon because of it's well characterised biochemical degradation pathways. Comparison under a limited number of experimental conditions will be extended to other constituent compounds of hydrocarbon pollution (benzene, ethylbenzene, xylene. Consideration will be given at the start of the project to statistical design of multi-factorial experiments in order to reduce the number of combinations required to cover a suitable range of conditions and combinations.

PhD 1: Cell and Mineral Surface Chemistry

High precision potentiometric titrations will be carried out on aqueous suspensions of the silica, alumina and iron oxide grains in order to determine surface charge dependence on pH and ionic composition. Analogous titrations will be carried out on cells harvested from the microcosms. These will compare the chemistry of the cell surface and associated extracellular polymeric substances with changes in nutrient status and growth phase. Extracellular macromolecules will be analysed for TOC, Total proteins, Pyrolysis-GC-MS, DNA (to check on cell lysis) and analysis by the potentiometric and spectroscopic methods to assess the charge, polymer speciation and macromolecular conformation as a function of solution conditions. Non-invasive methods to directly examine cell surfaces and the biological matrix of biofilms include fluorescence microscopy with specific fluorophores (for example calcofluor white and lectins) for use with confocal laser scanning microscopy. In addition to the biological imaging studies described below, we propose to apply FTIR microspectroscopy to visualise and chemically characterise biofilm samples.

PhD 2: Molecular Microbiology of Biofilms

An essential component of this study is to determine the structure of microbial communities in natural aquifers and compare with mixed populations in laboratory microcosms. We will apply rapid DNA profiling methods[40] that provide molecular fingerprints for the different member species of a prokaryotic community represented within extracted DNA. Group, species-specific and functional gene primers will be used to monitor the diversity of microbial populations at plume fringes, where contaminant and redox gradients are manifested at the EU field sites.

PhD 3: Biological Imaging of Microbial Biofilms

Crucial deliverables will be visualisation and quantification of physical structure including pore and throat distributions that control diffusion mass transfer and the spatial distribution and physiology of microbial populations. Work will initially focus on pure cultures of aerobes but will progress to anaerobes and the mixed cultures described above. Fluorescence *in situ* hybridisation (FISH) and confocal scanning laser microscopy (CFLSM) will be used to identify, quantify and localise the different microbial species within the mixed culture biofilms and examine associations between different species. The application of species-specific probes will allow the identification, quantification and spatial relationship of species to be determined.

PhD 4: Development of Reporter Gene Biosensors

We currently hold a BBSRC grant (50/E15832) devoted extensively to developing fluorescent chemical sensors of the physical environment. We will expand this approach using biological sensors of the environment and microbial function. Our aim will be to target pivotal genes associated with key activities (e.g. xyl genes for aerobic toluene degradation), as well as genes associated with major geochemical processes (hence groups of bacteria; e.g. methanogens, methyl coenzyme M reductase genes, mcrA; acetogens, formalytetrahydrofolate synthetase genes). Combinations of organisms, promoters and FPs will be used to visualise these activities within biofilms. In addition, sensitive detection methods (e.g. quantitative Real Time-PCR) using primers directed against active genes will be used in this project as a first step only, towards a genomic approach to eventually develop arrays carrying a range of activity assays for multiple degrative processes.

PhD 5: The Chemistry of Microbial Cell Wall Polymers

Simple fluorescence intensity and lifetime measurements will be used to establish adsorption isotherms for the binding of specific polymeric species to the surface of mineral particles as a complement to the potentiometric and FTIR studies (PhD 1). Emission anisotropy experiments, in particular, time-resolved anisotropy measurements (TRAMS) will be used to study the changes in conformational behaviour of polymers as they bind to mineral surfaces and the way in which the conformational freedom of bound species is affected by varying amounts of surface coverage and the solution composition. These observations will be systematically carried out under a range of aqueous- and mineral surface- chemical conditions and compared with changes in measured polymer- and cell- mineral interaction forces (PhD 7). This constitutes fundamental physical-chemical studies of cell-mineral interfaces that are likely to produce novel results as a study in its own right.

PhD 6: The Physical Chemistry of Cell-Mineral Interactions

We propose to use a molecular force probe (MFP) from Asylum Research, which is designed to measure molecular forces from the pN scale and greater. Cell-surface interactions can be measured by replacing the MFP tip with a larger surface (e.g. a micro-machined glass bead), to which the cell can be attached. The deflection of the cantilever as the surface is approached provides the necessary information on the cell-surface interaction as a function of distance from the surface. Extra cellular polymers such as alginates can also be attached to MFP tips, allowing a qualitative understanding of the polymer mineral interactions in comparison with the cell as a whole. The final stage of these investigations will involve **direct** measurement of cell-mineral adhesion by replacing the MFP tip with a cell wall fragment where the extra cellular polymer matrix remains associated with the cell wall structure. The essential scope of the investigations will be sufficient data on model systems to contribute significantly to the mathematical modelling, and to demonstrate the viability of direct interfacial measurements between mineral and cell surfaces.

PhD 7: Physical - Chemical Modelling of Cell-Mineral Interactions

Mathematical expressions for the polymer enthalpic and entropic contribution to the interaction potentials at the surfaces of “soft spheres” and bacterial polymer adhesion to solid surfaces have been developed. Soft spheres are defined as particles surrounded by a bound polymer and therefore bacteria may be considered as soft spheres due to the presence of EPS. These expressions will be used as a first approximation for the respective contributions to interaction potential between cells and mineral surfaces in close proximity. Significant advancement on previous approaches will be the independent experimental validation of the conformation of polymers using TRAMS and overall interaction potentials using scanning force measurements as described above. Model predictions of interaction potentials between the cell-mineral surfaces and polymer-mineral surfaces will therefore be tested against such independent measurements. In addition, the model will be tested at macroscopic scale by comparing the extent of measured and calculated cell-mineral adhesion/repulsion forces for a range of environmental conditions with the associated attached cell numbers determined by biological imaging methods.

INTEGRATION OF RESULTS ACROSS SPATIAL SCALES

Development of conceptual models to predict trends in biodegradation activity with aquifer conditions is based on 4 hypotheses.

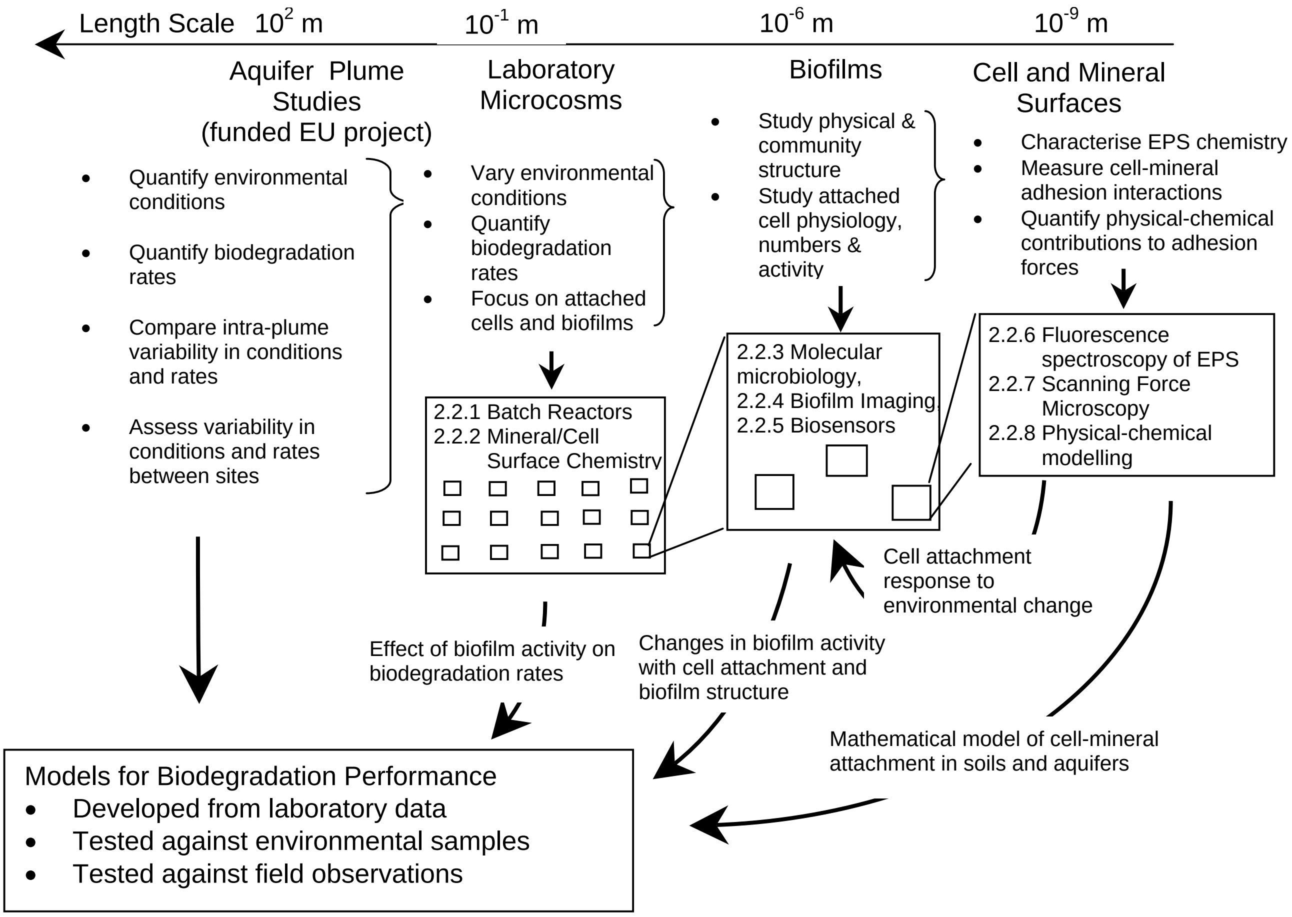
1) Biodegradation activity at field scale depends on geochemical and mineralogical conditions affecting biofilm formation.
2) Attached growth activity in turn depends on the numbers of attached cells and the physical and community structure of the biofilm.
3) Biofilm structure varies with environmental conditions that affect cell-mineral adhesion.
4) Cell regulation of adhesion occurs by variation of cell wall and extracellular chemistry in response to environmental conditions.

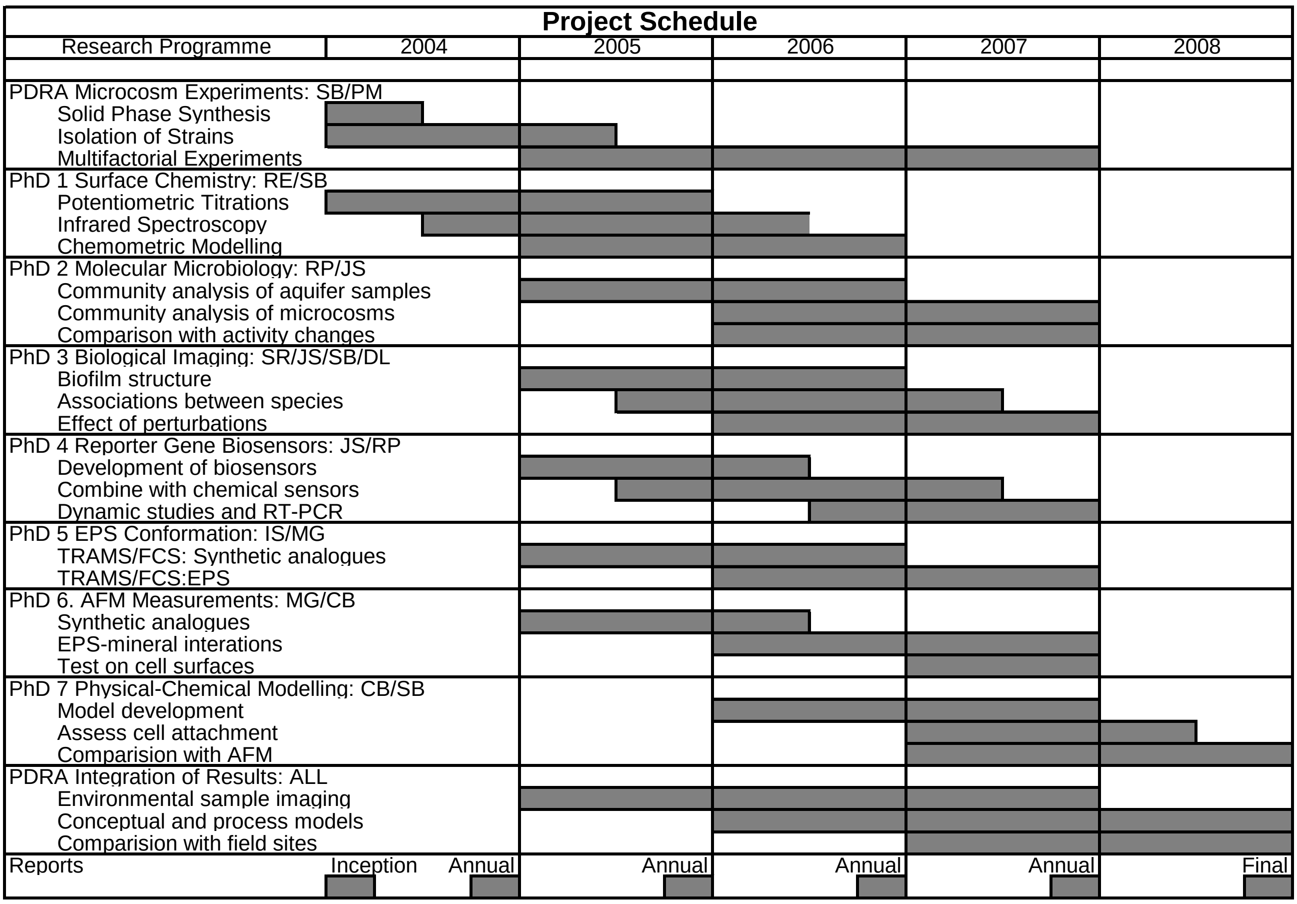
<u>Integration with Field Observations.</u> Integration will take place by hierarchical testing of these hypotheses, progressing in descending order of physical scale from 1) to 4). This will be led by the Post-Doctoral Researcher (PDRA) with a major responsibility to draw together the wide range of results from the above PhD investigations. Hypothesis 1) will be tested by predicting trends in biodegradation activity with field conditions based on the outcome of the microcosm experiments, and testing the prediction against a ranking of observed biodegradation rates at the sites. Hypothesis 2) will be tested by comparing biodegradation rates for pure cultures in microcosms with the numbers of attached cells

and with respiration activity (from imaging of electron acceptor flux and biosensor activity). Hypothesis 3) will be tested by comparing changes in biofilm structure with environmental conditions, and ranking the extent of attached cell numbers and activity against rankings of favourable/adverse conditions for cell attachment based on the chemical speciation of cell and mineral surfaces and the physical-chemical modelling of adhesion interaction potentials. Hypothesis 4) will be tested by comparing changes in cell and mineral surface chemistry, and the resulting calculated interaction potentials and AFM measurements, with changes in environmental conditions (particularly growth phase and nutrient status).

Integration with Process Models. We have the capability with computational methods developed from that grant to implement the results of this study into detailed process models of biodegradation within biofilms. Such process models would explicitly address microbial attachment and associated biodegradation activity'. First, together with the modellers on the Platform Grant, the appropriate mathematical descriptions and parameter values will be developed, as part of the above integration. Second, these formulations and parameter values will be used directly in numerical models.

Project Organisation Diagram





Investigators Profile: The Sheffield Cell-Mineral Interface Research Programme

Groundwater Protection and Restoration Group (GPRG)

The Groundwater Protection and Restoration Group (GPRG: http://www.shef.ac.uk/gprg/) is led by Prof. Steven Banwart and Prof. David Lerner FREng, with important support from Visiting Professors Phil Morgan and Roger Pickup. It is a multi-disciplinary grouping of ~20 research workers, with a current research programme of about £4M with funding from EPSRC, NERC, BBSRC, EU, Environment Agency and industry. GPRG have a national and international reputation for research on groundwater pollution, reflected in the 2000 award of a Queen's Anniversary Prize for Higher Education awarded to Sheffield University for *Environmental Outreach*, and the Department's recent 5B RAE rating.

Steve Banwart is the Principal Investigator and has a leading international track record in reactive processes in groundwater. Major achievements include demonstrating the importance of the biodegradation of natural organic matter to the performance of spent nuclear fuel repositories. The work is summarised in a review article featured on the cover of *Environmental Science and Technology (ES&T)*[1]. He has an exceptional track record in the aqueous coordination chemistry of mineral surfaces, including speciation at the mineral-water phase interface[2,3] the chemical kinetics of mineral dissolution[4,5] and heterogeneous electron transfer[6,7]. He has a strong track record in fundamental descriptions of reactive processes, and methods to scale theoretically rigorous process descriptions from model laboratory systems to the physical scale and complexity of field sites. Key results that successfully predicted field mineral dissolution rates from lab data for the first time are published in *ES&T* as a Research Communication[8] warranting rapid publication. He leads lab studies in an EPSRC/NERC project on predicting adsorption of organic compounds in soil, and leads the GPRG participation in the EPSRC Faraday Partnership on Remediation of Polluted Environments. He recently co-authored a major new book on Mine Water Pollution[9].

David Lerner has a long-standing record of successful field research projects, starting with the first UK survey of organic pollution under a city, showing how ubiquitous chlorinated solvent pollution is. The EU-funded Coventry Groundwater Investigation followed which considered the sources and movement of chlorinated solvents in a multi-layered, fractured rock environment and resulted in 14 journal publications. The multi-institution '4 Ashes' project on natural attenuation successfully linked laboratory process studies, field investigation and numerical modelling[10], and has lead to a new hypothesis for the size of naturally attenuating plumes which is being tested in CORONA, an EU project which he leads. He is a Fellow of the Royal Academy of Engineering, co-editor of the leading journal in his field, Journal of Contaminant Hydrology, and vice-chair of the Technology and Research Group of CL:AIRE.

Roger Pickup is Visiting Professor of Molecular Microbial Ecology at the University of Sheffield and is a microbial ecologist/environmental microbiologist located at the Centre for Ecology and Hydrology, Windermere. He has approximately 90 publications and £4.5M in research funding since 1984. His research interests are in the application of molecular techniques to problems in microbial ecology with

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emphasis on detection of specific bacteria, relating community structure to function, non-culturable but ecologically significant bacteria and evolution of genetic elements in bacteria isolated from deep sediments. Prof. Pickup has experience in managing large contracts and research projects within the field of microbial ecology and collaborates extensively with a number of UK universities as well as being a partner on EU projects.

Phil Morgan is Visiting Professor of Environmental Microbiology at the University of Sheffield. He is Technical Director of Environmental Simulations International Ltd and has over 17 years professional experience in bioremediation R&D (ICI, Shell and supervising PhD students in collaboration with a number of UK universities) with over 35 publications in peer-reviewed journals, book contributions and conference proceedings. He is recognised as a leading EU expert in the assessment, fate and biological impact of contaminants in soil and groundwater and in the practical application of Monitored Natural Attenuation (MNA) and bioremediation. His core research experience is in microbial physiology, and he possesses a unique breadth of experience with MNA, bioremediation technologies and chlorinated solvent contamination. His expertise in bioremediation is highlighted by his role as Chairman of the Programme Management Committee of the DTI Bioremediation LINK programme.

Department of Chemical and Process Engineering

Dr Robert Edyvean has some 25 years experience in studying the interaction of microorganisms with materials, at surfaces and in the planktonic phase. This has included microbial mineralisation and the influence of microbial metabolism on the mineral structure, the bio-degradation of materials and the biosorption of metals from solution. Originally involved with microbiological corrosion aspects of slimes and hydrogen sulphide and the interactions within microbial consortia and with dissolved metal ions, more recent work has included studies on the microbiological colonisation and biomineralisation in sandstone, *ex situ* soil decontamination and the development of biofilms in low nutrient environments. Funding has been by EPSRC, BBSRC, European Commission, British Council, water companies and industry. Robert Edyvean is a Chartered Engineer and Chartered Professional Biologist, past President of the Institute of Corrosion and has over 150 publications on biodeterioration, biodegradation and biosorption. He leads Environmental Engineering research in the RAE 4-Rated department.

Dr Catherine Biggs is a Lecturer in the Department of Chemical and Process Engineering. Her research into experimental and quantitative modelling of flocculation of biological systems is internationally known with publications in several refereed journals[11,12] and presentation at major international conferences (International Water Association July 2000 and April 2002). Her experience in flocculation modelling also includes 2-D population balance models for inorganic systems[11]. The multidisciplinary nature of current and previous projects have enabled Dr Biggs to develop skills and expertise in process engineering, wastewater technology, particulate processing, environmental microbiology and population balance modelling. Dr Biggs is continuing to work at the interface between biology and engineering systems with current projects in the area of activated sludge flocculation, colloidal characterisation of *E.Coli*, biochemical processes in sewers and remediation of contaminated soils.

Nanotechnology Group

Dr. Mark Geoghegan is Lecturer in Physics in the RAE 5-Rated Department of Physics and Astronomy. He is the research project coordinator for the nanoscale science and technology MSc joint programme with the U. of Leeds. Within this framework he has co-supervised two major (nine month) research projects on polymer - surface interactions using a molecular force probe in a standard geometry[12]. Current research involves the response of polymers, tethered to surfaces, to their aqueous environment, primarily the effect of pH on conformation (EPSRC Fast Track Grant No. GR/R74383/01). Geoghegan has already explored issues whereby polymer conformational changes are important, such as those when tethered polymers enter into complex matrices[13]. Conformational issues are also important in projects on film and polymer stability of topologically rough substrates, studied using scanning force microscopy[14]. He has co-written a major review outlining many issues involving polymers at surfaces[15]. Additional work involves quantifying the forces required to remove adsorbed polymers from solid surfaces; here the adsorbed polymers interact with the tip of the molecular force

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[14] N. Rehse, C. Wang, M. Hund, M. Geoghegan, R. Magerle, and G. Krausch *Eur. Phys. J. E* **4** 69-76 (2001)
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probe as it approaches the surface. Stretching curves are obtained which show force as a function of interfacial separation.

Dr. Linda Swanson is a Senior Lecturer at Sheffield, heading the Photophysics group with an international track record . She is an experienced polymer chemist specialising in the application of photophysical techniques, a field in which she has a world-wide reputation. in promoting the use of photophysical techniques, particularly anisotropy measurements (TRAMS) and energy transfer (FRET) in studies of macromolecular mobility, aggregation, adsorption and complexation. The photophysics group is recognised internationally for its pioneering work on water-soluble polymers and led the fundamental research enabling development of the phosphorescent ink used for postal coding and mail sorting in the UK.

Department of Animal and Plant Sciences

Dr. Julie Scholes is Reader in Animal and Plants Sciences in the RAE 5*-Rated department and has an international reputation in the field of molecular microbe – plant interactions. A key aspect of her research is the development and application of novel, *in vivo*, imaging techniques to study the regulation of plant and microbial metabolism and gene expression. Drs Scholes and Rolfe were recently awarded a grant from the BBSRC Bioimaging Initiative to develop a high resolution, microscope-based imaging system to allow simultaneous imaging of plant chlorophyll fluorescence and reporter genes such as Green Fluorescent Protein (GFP) in microbes or plants (see: http://www.sheffield.ac.uk/~ifpm/index.shtml). More recently she has been developing promoter-reporter gene fusions in microbes to image dynamic changes in the location of microbes within tissues and responses to environmental perturbations. Dr Scholes is currently a member of the BBSRC Plant and Microbial Sciences Committee.

Dr. Steve Rolfe has extensive experience in developing biological imaging systems funded, most recently, under a BBSRC Special Initiative 'Biological Imaging'. This experience ranges from the development of novel equipment and imaging algorithms through to the genetic modification of biological organisms with fluorescent reporter genes. This has been applied most extensively in studying plant-pathogen interactions integrating imaging techniques with molecular, biochemical and physiological analyses.