ChemEngDayUK 2015
Biological Engineering Abstracts

BE-01

A multivariate model analysis of a freshwater lake community under differing nutrient regimes

D.A.Russo[1], A.Beckerman[2], J.Pandhal[1]
1. Department of Chemical and Biological Engineering, University of Sheffield
2. Department of Animal and Plant Science, University of Sheffield

Eutrophication has caused widespread problems in freshwater lakes. Simple pairwise relationships between biotic and abiotic variables have proven to be insufficient to predict the dynamics of its environmental impact. We applied a multivariate statistical approach (structural equation modelling) to examine the role of water quality and nutrients on the structure and composition of functional microbial groups (phytoplankton, bacteria) in freshwater lake ecosystems under differing nutrient regimes. We found evidence of the decoupling of the phytoplankton biomass–nutrient paradigm. When nutrients are in sufficiently high quantities, water quality has a stronger influence on biological variation. We also found that nutrients and water quality act independently in the ecosystem but can create a synergistic effect. In addition, a direct link between temperature and cyanobacteria was found. Our methodological framework can assist researchers and decision-makers in lake management.

BE-02

A Proteomic Investigation of the Molecular Basis of Tau Pathology in Alzheimer's Disease

Z. Da, J. Heng, P. DiMaggio
Department of Chemical Engineering, Imperial College London

Recent studies have proposed a model for AD progression based on the endocytosis of tau filaments, which result in further tau aggregation events in healthy cells [Guo JL and Lee VM., 2011]. Our work proposes to investigate whether phosphorylation of tau is involved in this spreading mechanism. Specifically, we have expressed and purified recombinant protein tau from E. coli to generate in vitro tau filaments, which will be used to treat HEK-293 cells that express protein tau. Metabolic labelling and liquid chromatography tandem mass spectrometry (LC-MS/MS) will then be used to distinguish recombinant from cellular tau and identify any changes in the phosphorylation marks. An added advantage of our methodology for studying tau pathology is that we are able to purify untagged protein tau from the complex cellular background, so this approach could be extended to the study of animal models and post-mortem tissues.

BE-03

Appraisal of the mixing performance of a shaken bioreactor with conical bottom.

G. Rodriguez[1], T. Anderlei[2], M. Micheletti[1], A. Ducci[3]
1. Department of Biochemical Engineering, University College London
2. Adolf Kühner AG, Dinkelbergstrasse 1, CH-4127 Birsfelden, Switzerland
3. Department of Mechanical Engineering, University College London

Mammalian cell based cultures are typically developed in shaken bioreactors (OSRs). Once the process is optimised at small scale, it is implemented in stirred tank reactors (STRs), which is bioreactor type most commonly used at production level. Process transfer from shaken to stirred cultures often becomes a bottleneck, hence the industry's recent interest in large OSRs. The aim of the work is to characterize the mixing and flow dynamics in a cylindrical orbitally shaken bioreactor with conical bottoms of different heights. The rationale for a conical bottom is to ease the suspension of micro-carriers, which are used for the cultivation of cells not yet adapted for suspend culture, such as legacy cell lines and stem cells. Particle Image Velocimetry, PIV, and Dual Indicator System for Mixing Time, DISMT, were employed to assess the mixing performances in the bioreactor with a conical bottom.

BE-04

Biobased polymer production

A. Ferre-Guell, J. Winterburn
School of Chemical Engineering and Analytical Science, University of Manchester

Oil-based plastics are widely used in everyday life. However, oil reserves are limited, hazardous materials are employed for production and plastics become recalcitrant waste once used. Thus there is a need to find alternative materials produced using renewable resources with low environmental impact. Polyhydroxyalkanoates (PHA) are a family of microbially produced, biodegradable plastics with similar properties to conventional plastics. PHA have potential to be used in everyday products, but industrial production is hindered by high production costs. Thus, novel fermentation and downstream processes are required. Alternatives investigated in the past few years include the use of low cost, renewable feedstocks as a carbon source. In this work, an experimental and computational approach is used to study the effect of nutrient limitation requirements to trigger PHA production in batch and fed-batch fermentations leading to the design of improved fermentation protocols.

BE-05

Biochemical production of succinic acid from bio-refinery glycerol through continuous fermentation

A. Rigaki, C. Webb, K. Theodoropoulos
School of Chemical Engineering and Analytical Science, University of Manchester

Continuous process with cell recycle was assessed computationally and confirmed experimentally to be the optimal operational mode for the production of succinic acid from crude glycerol, which is the major by-product of the bio-diesel production process. The system's behaviour has been examined based on a double substrate limitation model in batch mode and modifications were made to incorporate streams for fed-batch and continuous bench scale systems. Sensitivity analysis was performed initially to investigate the order of significance of the decision parameters and process optimisation followed. In all cases, the maximisation of yield and productivity was defined as the objective function and the initial conditions along with the feeding and the recycling strategy where applicable, consisted the degrees of freedom. The model was validated against experimental results and the superiority of the continuous process with cell recycle was verified in all the performance indicators.

BE-06

Biosorption of certain selected toxic heavy metals and methylene blue by use of anionised microalgal loaded luffa cylindrica.

A. Emene, R. Edyvean
Department of Chemical and Biological Engineering, University of Sheffield

Heavy metals and organic pollutants are commonly released into the water environment and there is an urgent need for better treatment methods to eliminate these toxic substances. Although many conventional techniques have been utilised, adsorption is of considerable interest. Adsorption has been found to be most effective method in the removal process due to its low cost, high efficiency, regeneration and recovery; and a minimized production of sludge. It involves the use of biosorbents to remove heavy metal ions and organic pollutants from aqueous solutions. A new approach is proposed in this research based on the high percentage composition of cellulose in LFC. This study will alter the LFC surface by chemically modifying the LFC by an anionic monomer. A new strategy with the use of this anionized treated LFC immobilised with algae and fungi respectively, will be established for the simultaneous removal of selected heavy metal ions and a model organic pollutant.

BE-07

Chaperonin-Inspired Enzyme Protection by Mesoporous Silica

M.M.Lynch, M.M.Nigra, M.-O.Coppens
Department of Chemical Engineering, University College London

Enzymes catalyse organic chemical reactions in a rapid, selective, and environmentally-friendly manner. They have myriad applications, especially in pharmaceutical manufacturing, but their stabilities are constrained to physiological environments. Attaching enzymes to nanostructured materials can increase their operational stability by providing them with tailored scaffolds that protect against denaturing conditions. Mesoporous silica SBA-15 boasts an inert, stable matrix with tunable pore size and surface chemistry. To investigate SBA-15's suitability for enzyme immobilisation, we measure the adsorption of model enzymes onto the material. We also quantify the catalytic activity of these enzymes both before and after exposure to unfavourable pH, ionic, or temperature conditions. Our studies indicate that SBA-15 is a valuable candidate for enzyme immobilisation, and its tunability is key to its development in biocatalytic applications.

BE-08

Cyanobacterial biofuels: Investigating hydrogen production in Synechocystis PCC 6803 using proteomics

A. Landels[1], N. Couto[1], C. Evans[1], J. Noirel[2], P.C. Wright[1]
1. Department of Chemical & Biological Engineering, University of Sheffield
2. Chaire de bioinformatique, Laboratory 'genomics, bioinformatics and applications', Conservatoire national des arts et métiers, Paris

Solar radiation is the most prominent source of energy on earth, however our ability to store this energy is currently limited. Cyanobacteria are uniquely placed to address this challenge. They have the ability to harvest and store sunlight highly efficiently in a stable molecular form that can be used later. Hydrogen is an ideal energy storage molecule as it burns cleanly in air, releasing the energy and producing water with no carbon-based side reactions. There are a number of different cyanobacteria and algae currently under investigation as solar bio-hydrogen candidates, however the existing body of data is insufficient to successfully engineer past the biological complexity. Here we show proteomic data from hydrogen-producing conditions in Synechocystis PCC 6803, the cyanobacterium with the most comprehensive body of existing data supporting it. This data will be used in conjunction with other systems-level data to generate a comprehensive model for hydrogen production.

BE-09

Deformation of a tissue phantom by an oscillating bubble for drug delivery

M. Tinguely, M. G. Hennessy, A. Pommella, O.K. Matar, V. Garbin
Chemical Engineering, Imperial College London

Microbubbles are used as ultrasound contrast agent for biomedical imaging, as well as in drug delivery. The ultrasound-driven oscillations of the microbubbles can promote the uptake of drugs by cells or tissues through local deformation of the cell surface. From a drug delivery perspective, it is crucial to understand the mechanical effect of microbubbles on tissues. To this end, we use high-speed video microscopy to observe the deformation of a tissue phantom, agarose gel, by an oscillating bubble. The deformation is measured by tracking particles embedded within the surface of the gel. The bubble generates Rayleigh waves that propagate along the surface of the gel. The characteristics of the deformation are strongly dependent on the rheological properties of the gel. We develop a Kelvin-Voigt viscoelastic model to explore the dependence of the deformation on the gel properties.

BE-10

Effect of C12-homoserine lactone on the protein expression of Pseudomonas aeruginosa

J.Mukherjee, N. Couto, P.C. Wright, C. Biggs
Department of Chemical and Biological Engineering, University of Sheffield.

Most gram-negative bacterial cells communicate with each other via acyl-homoserine lactone (HSL) quorum sensing molecules. The Pseudomonas aeruginosa quorum sensing autoinducer C12-HSL is an important bacterial virulence factor that plays an important role in the regulation of virulence genes expression and their effect of changes in the cellular system. The overall aim of this project is to understand the effect of autoinducers C12-HSL on the changes in the cytosolic protein expression of P. aeruginosa PAO1 wild type (WT) and various quorum sensing mutant strains (Lec:Lux, ΔLasI and ΔLasR). Conducting a high throughput quantitative proteomic analysis (iTRAQ) using an Electrospray ionization quadrupole time of flight mass spectrometer, we found significant differential expression of proteins of the mutant strains as compared to the WT. The Fatty Acid metabolism and the TCA cycle pathway was up- whereas the Pentose Phosphate pathway was down-regulated.

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BE-11

Engineering a biosensor for detection of quorum sensing molecules in drinking water

E. K. Court[1], S. Hunt[2], C.A. Biggs[1]
1. Department of Chemical & Biological Engineering, University of Sheffield
2. Department of Clinical Dentistry, University of Sheffield

Vibrio cholerae is a waterborne pathogen which is prevalent in the developing world with 100-120,000 deaths per year. Current detection methods are lab based, with a timescale of hours to days. We are engineering a fast, sensitive, mobile surveillance system that can be used in the field. The basis of the biosensor design is to detect chemicals involved in 'quorum sensing' (QS). Our biosensor consists of a reporter component, and a sensor component made up of the pathogens own intracellular QS signalling pathway genes whose proteins will detect the chemical molecule CAI-1 and drive expression of reporter genes. We aim to present the development of a biosensor chassis that can be easily adapted to detect different pathogens, whilst keeping in mind the need to scale up in the future by designing a system that can be manufactured cheaply and easily.

BE-12

Engineering oxidative stress resistance in CHO cell factories

D. L. Fairbrass[1], D. C. James[1], Bo Kara[2]
1. Department of Chemical and Biological Engineering, University of Sheffield
2. Fujifilm Diosynth Biotechnologies

Oxidative stress is a term given to an imbalance in the amount of Reactive Oxygen Species (ROS) created within a cell, and the ability of its defence mechanisms to effectively deal with ROS. Oxidative stress is known to cause damage to DNA, proteins and lipids (Turrens, 2003). Mitochondria are the cell's predominant producer of ROS (Murphy, 2009); it has also been shown that increased protein folding in the Endoplasmic Reticulum results in an increase in ROS levels (Malhotra, 2008), an issue particularly pertinent for hard-to-express proteins. As well as many enzymes dedicated to ROS reduction, the cell maintains a glutathione pool to buffer the increase of ROS (Lu, 2009). In addition, there are several anti-oxidant compounds that can mitigate ROS production. Commercially available anti- and pro-oxidants were screened for impact on cell growth and productivity. The results of this screen will inform engineering strategies for increasing cell line resistance to oxidative stress.

BE-13

Engineering Ralstonia eutropha for biological carbon dioxide capture and utilization (synbioCCU).

A.O. Johnson, A.M. Othusitse, T.S. Wong

ChELSI Institute and Advanced Biomanufacturing Centre, Department of Chemical and Biological Engineering, University of Sheffield

With increasing focus on biological CO2 capture and utilization approaches, emphasis has shifted towards engineering CO2-fixing organisms such as Ralstonia eutropha. Vital to this is the development of synthetic biology toolbox. We present here a comprehensive database (REDatabase) of this mixotroph, detailing relevant experimental techniques for the benefits of researchers in the field. We also report a Mathematica programme for designing oligonucleotides to target and edit specific gene(s) in the organism's genome using CRISPR-Cas technique. We hope to leverage on these resources to optimise R. eutropha for enhanced CO2 fixation by deleting the can gene encoding β-carbonic anhydrase to elicit a high carbon requirement phenotype, overexpressing RuBisCO and α-carbonic anhydrase – its key CO2-fixation hubs, deleting its phaCAB operon for bioplastics biosynthesis, and engineering sustainable production of formate as energy source to transform R. eutropha into an industrial strain.

BE-14

Engineering the bacterial flagellum: conversion into a high efficiency protein secretion machine

C.A. Green[1], M. Hicks[1], F. Ying[1], P.C. Wright2, G.P. Stafford[1]
1 Integrated BioSciences, School of Clinical Dentistry, University of Sheffield
2 ChELSI, Chemical and Biological Engineering, University of Sheffield

When manufacturing proteins in bacteria, secretion into culture media is desirable -product is free of cytoplasmic contaminants, proteolysis reduced, and downstream processing simpler. We have focussed on re-engineering the flagellar type III secretion system (FT3SS) -a one-step high capacity secretion machine, which assembles the flagellum by extruding thousands of proteins through the flagella lumen. Characterisation of the FT3SS shows a structure that is amenable to engineering into an efficient protein secretion device. We have constructed E. coli with truncated flagella and demonstrated directed secretion of a range of proteins in a modular secretion construct (harbouring a secretion signal, purification tag and cleavage sites to aid further processing). We aim to improve secretion of a number of proteins by altering regulatory circuits to increase the capacity of the FT3SS. We also demonstrate use of metabolic manipulations to aid production of proline rich eukaryotic proteins.

BE-15

Engineering the sustainable production of biofuels and value added products from microalgae

M. Huete-Ortega, R. Kapoore, G. Padmaperuma, D. Pandey, K. Okurowska, S. Vaidyanathan
Department of Chemical and Biological Engineering, The University of Sheffield

Depleting fossil fuels and danger of increasing CO2 emissions, has spurred interest in searching for renewable and sustainable energy sources, such as biofuels. Photosynthetic microalgae have great potential for biofuel production as they can achieve higher biomass than plants and store high quantities of energy-rich compounds, such as triacylglycerol, which can be converted into biofuels. Furthermore, their photoautotrophic growth only requires naturally available sunlight and CO2. In our group the ultimate goal is to engineer sustainable production of biofuels and bioproducts from microalgae. The research objectives are to i) understand their biochemistry and physiology through a systems approach ii) develop an optimized workflow for conducting metabolomics analyses iii) evaluate microalgae consortia in production capabilities and iv) engineer atmospheric CO2 fixation and transformation of inorganic carbon to valuable organic (bio)chemicals, including biofuels such as bio-diesel.

BE-16

Expanding the molecular toolbox for synthetic biology.

P. Jajesniak, T.S. Wong
ChELSI Institute and Advanced Biomanufacturing Centre, Department of Chemical and Biological Engineering, The University of Sheffield.

We describe three unique molecular tools that allow for efficient, less resource-intensive and more advanced manipulation of biological systems and can greatly expedite the development of synthetic biology. QuickStep-Cloning is a novel gene cloning method that overcomes major drawbacks of existing, sequence-independent cloning methods. It allows for seamless integration of DNA fragments into any plasmid of choice at any position, in a time-efficient and cost-effective manner. QuikChange HT Protein Engineering System, launched by Agilent Technologies and further developed by us, is a powerful tool for creating high-quality mutant libraries for directed evolution. Its wide range of potential applications include the highly sought-after protein stabilisation. Finally, we describe a new method for simultaneous alteration of multiple genes that should enable identification of genes encoding for complex phenotypes and help to improve properties of industrially-relevant bacterial strains.

BE-17

Exploring the molecular mechanisms of aggregation in cyanobacteria

Esther Karunakaran[1], Joop de Vries[2], Henny van der Mei[2] and Catherine A. Biggs[1]
1 ChELSI Institute, Department of Chemical and Biological Engineering, University of Sheffield
2 The University Medical Centre Groningen, University of Groningen, Netherlands

Cyanobacteria are increasingly being used in various biotechnological processes from biofuel generation to water treatment. Profitable access to the biomass produced depends heavily on the processes in place to harvest the cells from the liquid environment. Sedimentation i.e. gravitational settling of bacterial cell clusters, is a traditional cell/liquid separation technique. Aggregation of cells occurs either naturally with the help of surface adhesins and extracellular polymeric substances or can be induced by the addition of coagulants and flocculants. A common cause of reduced biomass recovery is either an over dosage of coagulants or an inappropriate choice of coagulants. In this work, we demonstrate that by combining XDLVO modelling and force spectroscopy using an atomic force microscope (AFM), a deeper understanding of the molecular mechanisms underlying aggregation can be obtained in two cyanobacterial strains; Synechococcus PCC 7002 (a marine strain) and Synechocystis PCC 6803 (a freshwater strain). AFM and the knowledge of cell surface properties can be used, in the future, to identify novel biocoagulants, estimate effective dosage ranges for biocoagulants and design smart, effective cell/liquid separation strategies.

BE-18

Foam fractionation for the recovery of trehalolipid biosurfactants produced by a marine bacterium, Rhodococcus sp. PML026.

S. Bages [1], D.A. White [2], P.J. Martin [1]
1. School of Chemical Engineering and Analytical Science, The Mill, The University of Manchester
2. Plymouth Marine Laboratory, Plymouth

Trehalolipids are glycolipid biosurfactants with interesting applications in the environmental, petroleum and biomedical industries. Despite the properties biosurfactants possess such as low toxicity, biodegradability and stability at extreme conditions, their commercialisation has been limited due to the high production costs, especially associated to downstream processing. Foam fractionation is a separation method that uses foam to remove surface active compounds from a solution. Foam fractionation has been applied to recover a trehalolipid biosurfactant produced by a marine bacterium, Rhodococcus sp. strain PML026, when grown on hydrophobic substrates. Different operating conditions have been studied to recover the biosurfactant from a fermentation broth. This study demonstrates the successful recovery of a trehalolipid biosurfactant using foam fractionation, which aims to reduce biosurfactant production costs and going a step forward towards its industrial commercialisation.

BE-19

Identification of Novel Factors for Improving Escherichia coli for Microbial Fuel Cell Applications

S. Hall, S. Jaffé, G. Fowler, J. Posada, A. Rennie, P.J. Hall, P.C. Wright
Department of Chemical and Biological Engineering, University of Sheffield

Microbial Fuel Cell (MFC) technology, among other applications, aims to recover energy from wastewater by harnessing bacteria capable of donating electrons to an extracellular acceptor as part of their respiration. These electrogenic bacteria break down waste molecules, generating current directly by donating the electrons to an electrode. Three mechanisms have been identified for achieving extracellular electron donation: direct electron transfer from cell membrane proteins to the external acceptor; transfer along extracellular appendages, or “nanowires”; and transfer through secreted mediators, such as flavins. Few specific pathways have been identified and characterised in electrogenic strains, however. This work presents a synthetic biology approach to creating the ideal electrogen, through the creation and screening of genomic libraries of electrogenic bacteria in Escherichia coli, with an aim to identify and characterise novel pathways and factors affecting electrogenicity.

BE-20

Improving the efficiency of prokaryotic glycosylation

B.Strutton, S.Jaffe, J.Pandhal, P.C.Wright
Department of Chemical and Biological Engineering, The University of Sheffield.

Glycosylation is a post translation modification preserved throughout the three domains of life. It was most recently discovered in prokaryotes with the identification of N-linked glycosylation machinery in C. jejuni which was successfully transferred into E. coli opening up the idea of producing glycoproteins in the model bacterial host. The glycans producible in E. coli can mimic the first 5 sugar residues seen in humans but the production and transfer of these glycans to the target protein is poor. Through various metabolic engineering techniques, bacterial host genes have been identified that can aid in glycoprotein production. We aim to over express these in combination with varying target proteins and glycosylation machinery, to see if these genes can improve the efficiency in a combinatorial way; potentially proving that engineering strategies can be applied universally and not on a protein to protein, or glycan to glycan basis.

BE-21

Mathematical modelling and experimental studies of chlamydomonas reinhardtii growth and lipid accumulation

M. Bekirogullari[1], J. Pittman[2], C. Theodoropoulos[1]
1. School of Chemical Engineering and Analytical Science, University of Manchester
2. Faculty of Life Sciences, University of Manchester

The objective this work is to establish links between algal strains grown in large raceway open ponds and innovative bioproduct generation technologies including fuels and chemicals in order to achieve positive energy balance and environmental sustainability. Specifically, multi-parameter quantification and predictive modelling will be investigated to describe biomass growth and lipid production in bench-scale batch systems. The purpose of modelling of such closed batch systems is to identify kinetic parameters which lead to the algae growth and productivity. Subsequently, the kinetic parameters defined in the batch system through modelling and optimisation studies will be used to further develop novel harvesting techniques and oil extraction methods for industrial scale pond use. The validated predictive closed batch system model will be used to construct open raceway pond models for maximization of the biomass production and as well as maximization of oil production in such systems.

BE-22

Mechanical Properties of Neutrophils, Bone Marrow-Derived Hematopoietic and Mesenchymal Stem Cells before and after being Treated with Cytokines

M. Du[1], N. Kalia[2], Z. Zhang[1]
1 School of Chemical Engineering, University of Birmingham
2 School of Clinical and Experimental Medicine, University of Birmingham

Stem cells injected for therapeutic purposes encounter complex mechanical obstacles in circulation, leading to low adhesion efficiency. Previuously studies have shown that pretreating with cytokines could enhance their recruitment within injured sites, but the mechanism is not clear. Neutrophils, Hematopoietic and Mesenchymal Stem cells before and after being treated with SDF-1α or H2O2 were mechanically tested. The Young's modulus values of the tested cells were determined. The results suggest that neutrophils were smaller in size and mechanically stiffer than stem cells, and HSCs showed similar deformability to MSCs though they possessed different sizes. Pre-treating the cells with cytokine significantly increased the deformability of HSCs, but did not induce any significant change in stiffness of MSCs. This study directly demonstrates the physical influence of cytokines on stem cells, and may also be exploited for sorting stem cells based on their difference in mechanics.

BE-23

Metabolic Modelling of Cupriavidus necator DSM545 for biosynthesis of poly(3-hydroxybutyric acid) from glycerol.

C. Sun, C. Webb, K. Theodoropoulos
School of Chemical Engineering and Analytical Science, The University of Manchester.

In a novel fermentation process, bacterial strain Cupriavidus necator DSM 545 is able to utilise glycerol, an abundant by-product from biodiesel industry, to synthesis poly(3-hydroxybutyric acid), a biodegradable plastics favoured by industrial applications. Metabolic engineering proves to be a useful practice to optimise the performance of cells, thereby improving the overall performance of the process. Therefore, a genome-scale metabolic model of C. necator has been constructed to gain in-depth insight into cellular metabolism, and predict specific enzymes whose manipulation would result in desirable change in both PHB synthesis rate and yield. Flux balance analysis (FBA) and flux control analysis (FCA) were used to develop the model based on flask- and bioreactor-scale fermentations. To validate the model, the strain is to be metabolically engineered as suggested by the model, subsequently compared with the original strain in terms of process performance.

BE-24

Microbial Oil production through yeast fermentation of biorefinery waste glycerol.

E.Karamerou, C. Theodoropoulos, C. Webb
School of Chemical Engineering and Analytical Science, The University of Manchester

Oil and its derivatives are materials of high importance as they fire the fuel, pharmaceutical and food industry. More oil is needed in order to meet the increasing demand, preferably from alternative oil sources free from competition with land and food issues. Oleaginous yeast have the edge over traditional oil sources since they offer short life cycle, high oil yield with similar compositions to plant oils and can consume a variety of substrates, including waste. Process improvements are necessary in order to reduce the high cost of the large scale production of microbial oil. This work focuses on the application of crude glycerol, a biorefinery waste, as fermentation substrate for oil production from the yeast Rhodotorula glutinis with overall aim the design of a cheaper process and use of it in integrated biorefineries. The bioprocessing conditions effect in oil yield are examined experimentally and computationally with the help of a kinetic model.

BE-25

Mixing Simulations for the Scaling-up of Biochemical Processes.

I.S. Fragkopoulos, A. Rigaki, C. Webb, C. Theodoropoulos
School of Chemical Engineering and Analytical Science, The University of Manchester

The main objective of this work is to investigate the hydrodynamics in a biochemical process at different scales to improve its performance. Vlysidis et al. (2011) have developed a batch reactor model for the prediction of succinic acid production from glycerol. Although the ODE based model was deemed to accurately predict the concentration transients in small-scale reactors, differences are observed between the model predictions and the experimental observations in scaled-up reactors. CFD simulations are performed here to investigate the mixing importance in such systems. The proposed CFD model couples the k-ε model with convection/diffusion as well as the reaction processes taking place in the fermentation system. The resulting set of PDEs is solved simultaneously leading to efficient prediction of the concentration transients in the volume of the agitated fed-batch bioreactor.
References: Vlysidis A., M.Binns, C.Webb and C.Theodoropoulos, Biochem.Eng.J., 1-11, 2011.

BE-26

Model based design of a gene metabolic system using model predictive control

A. H. Bhatti[1], G. Thomas[2], V. Dua[1]
1. Department of Chemical Engineering, University College London
2. Department of Cell and Developmental Biology, University College London

Design of dynamic biological systems can be quite difficult as there are many complex processes that can govern the system outcome. One method for designing such systems is by using nonlinear model predictive control (NLMPC). In general NLMPC problems are formulated as solving on-line a finite horizon optimisation problem. In this work we use NLMPC to help control a genetic network of Acetyl Co-enzyme A (Acetyl-CoA) and its co-factors, termed the metabolator (Fung et al, 2005). The methodology is based upon a simultaneous solution of the model equations and the optimisation problem. The dynamic model equations are transformed into algebraic equations through a neural network transformation, and are then embedded within the overall optimal design problem. The modelling results are verified against the experimental results from the literature. The design results provide valuable insight into the metabolator system and future work will be to test these design results experimentally.

BE-27

Modelling and optimal control of non-viral siRNA delivery

E. Jamili, V. Dua, M. Stamatakis
Department of Chemical Engineering, University College London

Gene therapy can be defined as the insertion of genetic materials into selected cells in the body for obtaining a therapeutic effect. In this project, mathematical and computational modeling is used for analysis, optimisation and control of siRNA delivery. Compartmental models were developed using the experimental data reported in the literature to describe the transport of siRNA between the various compartments. The model development involves estimating the rate constants of siRNA translocation over the cellular barriers. Then, the developed models are used to obtain optimal gene infusion rates. This work includes providing a balance between two conflicting objectives: high efficacy and low toxicity. One of the important constraints is on the time to take into account the cell multiplication so that the effect is manifested before the cell division takes place. Thus, a mathematical modeling and control framework to incorporate the time constraints for cell division has been developed.

BE-28

Online Glucose Detection with Highly Porous Gold Electrodes

D.W.Ferdani[1], H. du Toit[2], M. Di Lorenzo[2]
1. Centre for Doctoral Training in Sustainable Chemical Technologies, University of Bath.
2. Department of Chemical Engineering, University of Bath

Diabetes affects approximately 347 million worldwide. In order to live longer and healthier lives, people who suffer from diabetes need to carefully regulate their blood glucose levels. This in turn requires regular blood glucose monitoring that generally involves disposable elements and invasive procedures. Cost-effective and non-invasive sensors are crucial for the good management of this disease. We have recently reported a methodology for the rapid and cost-effective deposition of highly porous gold (hPG) onto metal electrodes. The resulting electrodes have a foam-like open structure, which is the ideal support for enzyme immobilisation at high loadings. We have successfully developed a rapid and efficient methodology for the immobilization of glucose oxidase (GOx) onto hPG. The resulting electrode is extremely selective to glucose, even at concentrations as low as 1 µM. This sensitivity makes hPG/GOx an ideal candidate material for the monitoring of glucose in transdermal fluids.

BE-29

Pore Curvature Effects on Protein Confinement in Mesoporous Silica

J. Siefker[1], M. Krutyeva[2], M. Nigra[1], M.-O. Coppens[1]
1 Department of Chemical Engineering, University College London
2 JCNS-1/ICS-1, Jülich Centre for Neutron Science, Julich, Germany

Confinement of biomolecules in structured nanoporous materials can offer many desirable advantages in both biological and synthetic systems. Our previous studies on confinement effects in nanoporous particles have shown that geometric properties such as surface curvature play a significant role in addition to classic properties such as charge and hydrophobicity. Previous results using SBA-15 indicate that high concave surface curvature can stabilize the native protein structure and protect enzymes from extreme environmental conditions. Here, we expand our investigation of curvature beyond SBA-15 to include additional confinement geometries, including the Ia3d (KIT-6) and Im3m (SBA-16) cubic space groups, and complete further analysis using small angle neutron scattering to further elucidate protein structure. These results allow for further insight into the relationship between protein structure and confinement geometry.

BE-30

Production of poly (3-hydroxybutyrate) (PHB) by Cupriavidus necator ATCC 17699.

R.Yousuf, J. Winterburn
School of Chemical Engineering and Analytical Science, the University of Manchester

Biopolymers are a group of polymers which occur naturally, being produced by a range of living microorganisms. The raw material for production of these polymers can be derived from either renewable or synthetic resources. Current industrial interest is moving towards producing environmentally friendly biopolymers with a high degree of biodegradability using modern biotechnology. Polyhydroxybutyrate (PHB) is a polyester, produced by a range of microorganisms, commonly found in soil bacteria with a biological role similar to starch in plants, which is accumulated in the cell cytoplasm as crystalline granules.The main aspect of this project is to investigate ways to reduce the cost of PHB produced by Cupriavidus necator, through utilisation of low cost carbon sources and development of improved fermentation processes. C.necator has been chosen as it capable of degrading a large number of chemical compounds and it is a well understood bacteria that produces PHB from simple carbon sources.

BE-31

Proteopolymersome: A Tool for the Study of Microsomal Monooxygenases.

H. E. M. Omar Ali [1,2,] M. Nallani [2], T. S. Wong [1]
1. ChELSI Institute and Advanced Biomanufacturing Centre, Department of Chemical & Biological Engineering, The University of Sheffield
2. School of Materials Science and Engineering, Nanyang Technological University, Singapore.

The microsomal monooxygenase system contains different protein components, including cytochrome P450 enzyme and its redox partners. These membrane-bound enzymes play a very important role in drug metabolism and detoxification. Their study is vital for understanding protein-drug interactions, drug design . The requirement of membrane environment hinders research into these enzymes. My work aims to tackle this problem by utilising polymersomes as a platform for functional protein expression and enzyme study. Polymersomes are self-assembled polymer vesicles with their architecture mimicking cellular membranes. We aim to achieve direct protein insertion, expressed using cell-free expression systems, into polymersome thereby creating proteopolymersomes. Proteopolymersome with correctly folded and active enzymes can be used as a model to answer important questions of the microsomal monooxygenase system, high-throughput drug screening and in vitro production of metabolites.

BE-32

Pushing the Limits of Detection in Mass Spectrometry: A ChIP-MS Workflow for the Characterisation of Histone Post-translational Modifications

A.J.Borg[1], R.Grimley[2], P.A.DiMaggio[1]
1 Department of Chemical Engineering, Imperial College London
2 Pfizer/Neusentis, Granta Park, Cambridge

Histone post translational modifications (PTMs) are required for the recruitment of proteins involved in many important nuclear processes. Technological advancements in chromatin immunoprecipitation sequencing (ChIP-seq) and liquid chromatography tandem mass spectrometry (LC-MS/MS) have advanced our understanding of histone PTMs but require at least 10^6 cells for a single replicate, making the study of animal models or purification of histone subsets by ChIP problematic. We propose utilising mixed-integer linear optimisation tools from the Chemical Engineering scheduling community to develop a LC-MS/MS retention time based targeted scan programme for characterising histone PTMs from limited amounts of starting material. This is demonstrated by analysis of histone PTM changes from rat tissue containing 10^5 cells where a 50-fold improvement in sensitivity is observed, reducing the number of animal sacrifices by ten-fold and minimising problems associated with animal heterogeneity.

BE-33

Quantitative phosphoproteomics study of Sulfolobus solfataricus P2 in responding to carbon source change

W. Qiu, T.K.Pham, P.C.Wright
Department of Chemical and Biological Engineering, The University of Sheffield

The quantitative phosphoproteome network of Sulfolobus solfataricus P2 in responding to carbon source change (glucose vs tryptone) was investigated using a combination of the optimised P-peptide (phosphopeptide) enrichment and iTRAQ techniques by LC-MS/MS. A combined use of modified SIMAC and 4 cycles of continuous incubation of TiO2 beads were optimal for P-peptide enrichment in this study. A total of 109 P-peptides corresponding to 100 P-proteins (phosphoproteins) were detected. Only a few of these P-proteins were differentially regulated (glucose vs tryptone). Most of the quantified P-proteins involved in CCM were unaffected. Furthermore, the detection of regulated P-proteins provide interesting targets for future work, such as the universal stress proteins (SSO2778); critical enzyme (SSO0248) and molecular switch (SSO0625). Here is the 1st quantitative study of P-proteins in S. solfataricus, offering potential application for other achaeal studies.

BE-34

Simulation and calculation of periodic steady-state conditions in a sequencing batch reactor for biological wastewater treatment.

A.A.Rasheed, D.Dionisi
Materials and Chemical Engineering Research Group, School of Engineering, The University of Aberdeen.

Sequencing batch reactor (SBR) is one of the biological treatment systems used for domestic and industrial effluents. It is an activated sludge process designed to operate under non-steady state conditions and its operation is characterised by five consecutive periods (fill, react, settle, draw and idle). This study is the first of its kind to develop a mathematical model that calculates the periodic steady-state conditions (microbial cell and substrate concentrations) during SBR treatment of both readily and slowly biodegradable substrates. Monod equation was used to describe microbial growth kinetics and the numerical method used to solve the model comprised of a set of differential equations that represents the biological reactions taking place in the reactor. Excel-Solver was used for the simulation to obtain the steady state conditions without calculating the transient (unsteady state). The results highlighted the effects of various process variables on the performance of the SBR.

BE-35

The Biological Conversion of Methane to Methanol Using Fine Bubble Aeration

C.Bjorck, W.B.Zimmerman, J.Pandhal, P.D.Dobson
Department of Chemical and Biological Engineering, The University of Sheffield

There are multiple drivers for interest in the biocatalytic conversion of methane to methanol: the energy intensive commercial synthesis of methanol, the vast quantity of methane wasted globally, and greenhouse gas mitigation strategies. A class of bacteria known as methanotrophs catalyse the reaction of methane to methanol under ambient conditions using the methane monooxygenase (MMO) enzyme. This reaction is followed by the sequential oxidation of methanol to formaldehyde, formate and carbon dioxide. The key challenges are to stop cellular reactions after the first step and overcome the inhibitory effects of methanol accumulation. This project will investigate use of an airlift bioreactor with fine bubble aeration to maximise mass transfer of methane to the aqueous phase and also in the reverse direction in use of the sparging gas for stripping of the volatile methanol product. Product removal will prevent reaction inhibition while also avoiding over oxidation of methanol.

BE-36

Theoretical investigation of different population coexistence in bioreactors with mixed microbial cultures.

I.M. de Oliveira e Silva, D.Dionisi.
School of Engineering, The University of Aberdeen

Open-mixed culture fermentations have gained increasing attention to produce renewable chemicals, since costly sterilisation is not required and more complex substrates can be used to produce a wide range of products. An example of this technology is the possible use of mixed culture fermentation in biorefineries to produce value added chemicals from organic wastes or lignocellulosic biomass. This work investigates the coexistence of different microbial populations at particular operational conditions in a continuous anaerobic bioreactor. By using a mathematical model with glucose as only carbon source in the feed stream, two different strains are inoculated into a bioreactor to produce specific products: ethanol and acetic acid. The study has demonstrated that, although only one population can occur at steady state condition, coexistence of different populations may become possible with dilution rate fluctuations in the bioreactor, thereby producing more than one product.

BE-37

Use of Microcalorimetry to Study Inorganic and Organic Molecules in Aqueous Solutions: Implications for Catalysis, Purification and Formulation Unit Operations.

J.W. Bye, J. McGregor, R.J. Falconer
Department of Chemical and Biological Engineering, The University of Sheffield.

Pressure perturbation calorimetry is an under-utilised technique for studying aqueous solutions but it provides a unique insight into the relationship between water and solutes. In this technique the pressure is increased from 1 to 5 bar and the heat required to maintain the temperature (ΔQ) is measured and compared to a pure water reference. Results from recent research demonstrate its application to studying inorganic molecules in solution and water-alcohol mixtures. The interpretation of the data will be discussed along with its application to development of catalysis, purification and formulation unit operations.

BE-38

Using foam fractionation to intensify downstream processing of biosurfactant

C. Kaisermann, P.J. Martin
School of Chemical Engineering and Analytical Science, The University of Manchester

In recent years, a renewed interest for foam fractionation has emerged as a single economic solution to two bioprocess engineering issues: foaming of surface active molecules in fermentation broth and high cost of downstream processing. The downstream processing of surface active molecules such as biosurfactants can account for 60% of the total cost of production. Foam fractionation can be applied to enrich a low concentrated biosurfactant from high volume broth by the absorptive bubble separation method. It is also inexpensive and has the potential to be integrated into a continuous production system. In this study, the operating parameters and their influence on the efficiency of continuous foam fractionation for the recovery of a lipopeptide biosurfactant were studied. The optimal operating airflow, feed flow and temperature were determined in stripping mode. Further work will be required to establish the global optima in production when the column is integrated to the bioreactor.

BE-39

Utilizing alternative carbon sources for the production of polyhydroxyalkanoates

F. Guzman-Lagunes, J.B. Winterburn
School of Chemical Engineering and Analytical Sciences, The University of Manchester

Polyhydroxylkanoates (PHA) have been extensively studied as a biodegradable and biocompatible alternative to petroleum based plastics. Although a range of PHAs have been successfully produced, industrial production costs are an obstacle to wider use. Cupriavidus necator is a model biopolymer producer, being able to accumulate more than 90% of its weight as polyhydroxybutyrate (PHB).

To explore the possibility of utilising low value food waste streams as a substrate for PHB production different carbon sources were tested, using C. necator ATCC17699 flask cultures. A synthetic medium with an initial concentration of rapeseed oil, as the sole carbon source, of 25 g/L led to a final PHB concentration of 2.48 g/L. An improvement in yield was achieved using fructose as carbon source, initial concentration of 10 g/L, obtaining a PHB concentration of 2.41 g/L. It was found that glycerol-based media give rise to lower growth rates when compared to oil and fructose based media.