The importance of oxygen in large-scale fermentations.

Dr TW Overton

t.w.overton@bham.ac.uk
Biochemical Engineering, School of Chemical Engineering, The University of Birmingham.

Oxygen can be utilised by many species of bacteria as a terminal electron acceptor. The industrially important bacterium *Escherichia coli* respires oxygen; this mode of growth yields more energy than anaerobic fermentation. The switch from aerobic to anaerobic growth is regulated by a number of transcription regulators, including FNR and ArcA, which activate transcription of genes required for growth in oxygen-limited conditions.

In large-scale industrial bioreactors, oxygen transfer and dispersion throughout the culture is often suboptimal, leading to pockets of oxygen limitation. Bacteria passing through these pockets will sense and respond to oxygen depletion, leading to activation of anaerobic metabolism. Aerobic energy generation pathways will be down-regulated in favour of anaerobic fermentative pathways. Thus, suboptimal growth will occur, due to interruptions in aerobic respiration and expenditure of energy during the adaptation to anaerobic fermentation.

This project aims to characterise the nature of the gene regulatory response to heterogeneities in oxygen concentration in large bioreactors. Scale-down model systems will be used to replicate conditions in a large bioreactor, allowing oxygen concentration gradients and oscillations to be simulated. Reporter gene fusions will be used to measure intercellular oxygen concentrations, redox state and the stress state of the bacteria. Flow cytometry will be used to characterise heterogeneities in bacterial physiology within a mixed population (using differentially permeable dyes) and heterogeneities in gene regulation (using promoter - fluorescent reporter gene fusions). The links between oxygen sensing and responses to stress will also be studied.

This project will utilise a wide variety of molecular biology techniques such as PCR-based cloning, site-directed mutagenesis, analysis of cellular proteins using electrophoresis, biochemical assays, fluorescence measurement and flow cytometry. It will also involve microbial fermentations in bioreactors and analysis of cultures using online measurement techniques (GC-MS). Students will have access to a newly-refurbished pilot plant facility and a well-equipped molecular microbiology laboratory. There are opportunities to develop this project in a number of directions, either towards more applied areas or into ‘pure’ molecular microbiology.

References

Constantinidou C, Hobman JL, Griffiths L, Patel MD, Penn CW, Cole JA, Overton TW. (2006) A reassessment of the FNR regulon and transcriptomic analysis of the effects of nitrate, nitrite, NarXL, and NarQP as *Escherichia coli* K12 adapts from aerobic to anaerobic growth. *J Biol Chem* **281:** 4802-15.

Hewitt CJ, Nebe-Von Caron G, Axelsson B, McFarlane CM, Nienow AW. (2000) Studies related to the scale-up of high-cell-density *E. coli* fed-batch fermentations using multiparameter flow cytometry: effect of a changing microenvironment with respect to glucose and dissolved oxygen concentration. *Biotechnol Bioeng*. **70:** 381-90.