FED-BATCH *E. COLI* CULTURES IN A SHAKEN, SINGLE-USE 24-WELL MINIATURE BIOREACTOR

Mary A. Lunson, Department of Biochemical Engineering, University College London, UK
mary.lunson.12@ucl.ac.uk
Steve R.C. Warr, GlaxoSmithKline, Stevenage, UK
Gary B. Finka, GlaxoSmithKline, Stevenage, UK
Gary J. Lye, Department of Biochemical Engineering, University College London, UK

Key Words: Miniature bioreactor, engineering characterisation, fed-batch, *E. coli* cultures, antibody fragment

At industrial scale, microbial cultivations are usually performed in fed-batch mode to allow for high cell density cost-effective processes. Miniature bioreactors are becoming widely used in the biopharmaceutical industry as a tool for high throughput strain evaluation and fermentation process development. However, there are relatively few examples of miniature bioreactors capable of fed-batch operation and of supporting the high oxygen demand. There are several challenges that need to be addressed to establish high cell density fed-batch cultivation at microscale: attaining high oxygen mass transfer rates, achieving good mixing for the duration of the culture and implementation of an industrially relevant feeding strategy requiring low volume additions.

In this work a shaken, single-use 24-well miniature bioreactor (Pall, Micro 24 MicroReactor System) has been characterised in terms of volumetric oxygen mass transfer coefficient (k_La) and liquid phase mixing time (t_m) to assess the feasibility of high cell density microbial cultures. The impact of shaking frequency, total gas flow rate and fill volume on oxygen transfer and fluid mixing were investigated and the optimum operating conditions were determined. To enable fed-batch cultivation in the miniature bioreactor system a bespoke feeding system for direct, continuous feed delivery has been developed that works at feed flow rates of 20µL h^{-1} and above. This feeding system allows for 24 fed-batch cultures to be run in parallel.

Within the operating ranges of the miniature bioreactor system, it was found that oxygen transfer was dependant on both shaking frequency and gas flow rate, but was independent of fill volume; the oxygen mass transfer coefficient, k_La increased with both increasing shaking frequency and gas flow rate over the range 3-101h^{-1}. The liquid phase mixing time, t_m under non-aerated conditions increased with shaking frequency and decreased with fill volume over the range 0.8-15.3s. It has been demonstrated that the miniature bioreactor system is well mixed under the range of operating conditions evaluated.

The bespoke feed delivery system was used to perform fed-batch cultures of an industrial *E. coli* strain producing an antibody fragment under operating conditions defined from the engineering characterisation studies. Fermentations were performed on a semi-complex medium containing glycerol with direct feeding of a glycerol solution initiated around 15 hours. It was found that direct feeding enhances biomass production by 30-40% and product expression by 45-65% in comparison to non-fed cultures. The feeding system developed in this work allows for industrially relevant microbial processes to be implemented at the microscale.