Investigating the influence of the *Chlamydomonas reinhardtii* cell wall on downstream processing for recombinant protein production.

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**Introduction.**

Microalgae hold promise as a simple, low cost and benign production system for the manufacture of recombinant proteins. In particular, the chloroplast of *Chlamydomonas reinhardtii* has been used successfully to express a number of proteins, including human erythropoietin, fibropectin and proinsulin [Nakada et al., 2011]. To date, there have been few studies on the recovery of recombinant proteins from microalgae hosts with the aim of identifying process challenges for scale-up. The influence of host cell selection has significant implications on downstream processing. A less known question for C. reinhardtii is there an advantage to choose a cell wall-less mutant over a cell walled strain as a production system? Using a model system of C. reinhardtii engineered to produce endolysin-resistant proteins, process parameters relevant to downstream processing were investigated to understand the impact of host system on the yield and integrity of product. Cell walled (BC) and Cell wall-less (TC) mutants were engineered to express the endolysin Gpi-1. The use of Ultra Scale-Down (USD) technology has allowed for a side-by-side comparison of BC and TCi strains, highlighting the advantages of having a cell wall for particular downstream processing operations, and the lack of a cell wall for others. Knowing how to optimize these operations will enable effective scale-up for larger scale microalgae processes in future.

**Cell harvest.**

USD shear device (right) and device controller (left). Up to 40mL of sample can be injected into the device. A spinning disk inside can be used to create shear. For the clarification step, an Eclipse 5424R bench top centrifuge was used with 2ml microtube tubes.

**Cell rupture.**

**Filtration.**

**Conclusion.**

USD technologies [3 et al., 2013] enable small experiments to be used in order to try and predict large scale bioprocessing results with greater ease and reduced time. Using bench top shear devices and centrifuges in order to mimic the conditions undergone by the microalgae when entering large scale centrifugation, it was found that BCi were more resilient to the levels of shear that the cells are exposed to during the centrifugation process, resulting in less endolysin being released into the supernatant. Bench top focused applications were applied to effectively mimic the conditions of cellular disruption such as those encountered in larger batch or continuous cell homogenizers. USD experiments found that presence of a cell wall was predicted to require longer homogenization processing times in order to obtain the same degree of cell disruption achieved by cell wall-less variants in less time. Finally, the removal of cell debris post disruption was investigated using a USD depth filtration rig on a TECAN robotic lab workstation, it was found that using higher pressures and cell wall presence resulted in a greater volume of homogenate being able to pass through the filter before the filter pores became blocked.

These USD experiments have been replicated at pilot scale and similar results were achieved, proving the usefulness of USD experimentation when designing a bioprocess as a method to lower development costs and more rapidly establish parameters for operation.

**References.**


