Ariennir yn Rhannol gan **Lywodraeth Cymru**
Part Funded by **Welsh Government**

Combining scale-down techniques and design of experiments to identify operating regions for the process-scale yield of intracellular enzymes.

Background

This Knowledge Transfer Partnership is a collaboration between Biocatalysts Ltd and UCL bringing together advanced fermentation technologies with new ways of translating yield and purification processes from the bench to production scale.

Biocatalysts Ltd develop and manufacture speciality enzymes from gram to tonne scale for a variety of industries, e.g. food, flavour & fragrance, life science, pharma and fine chemicals. The company offers a rapid, low-cost speciality enzyme service from discovery to global supply of regulatory compliant enzymes.

Objective

To design a robust flocculation process for the enhanced yield of intracellular proteins

Method

Initial scale down approach

Experiments were carried out by diluting homogenate in modulating agent solution and then flocculating, centrifuging and assaying to attain protein and activity data alongside pellet weight.

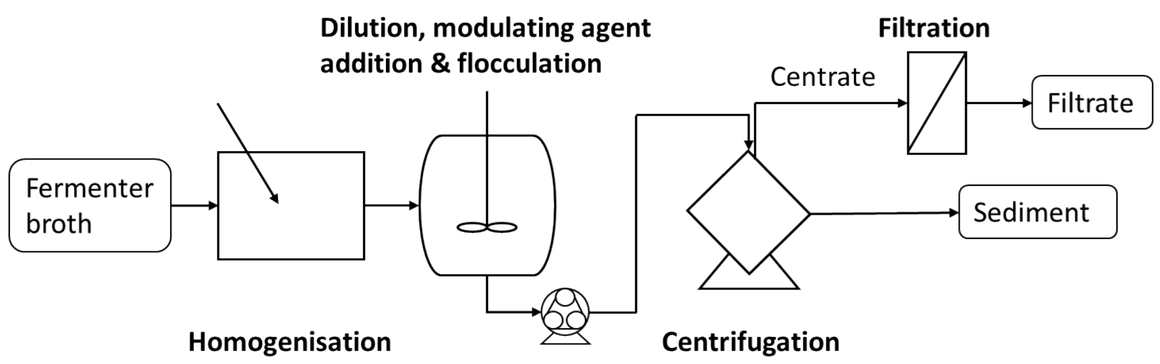

Figure 1: Process flow diagram of primary yield steps in enzyme production. Each sequence is a series of batch stages.

USD (Ultra Scale-Down) Platform

Aligned to Figure 1, the creation of an USD platform is underway to:

a) Provide deeper process understanding of factors affecting protein and enzyme yield and purification.

b) Mimic conditions at large-scale to allow prediction of factors affecting product yield.

1. USD FLOC VESSEL
2. USD SHEAR DEVICE*
3. USD CENTRIFUGATION
4. USD FILTRATION PLATFORM*

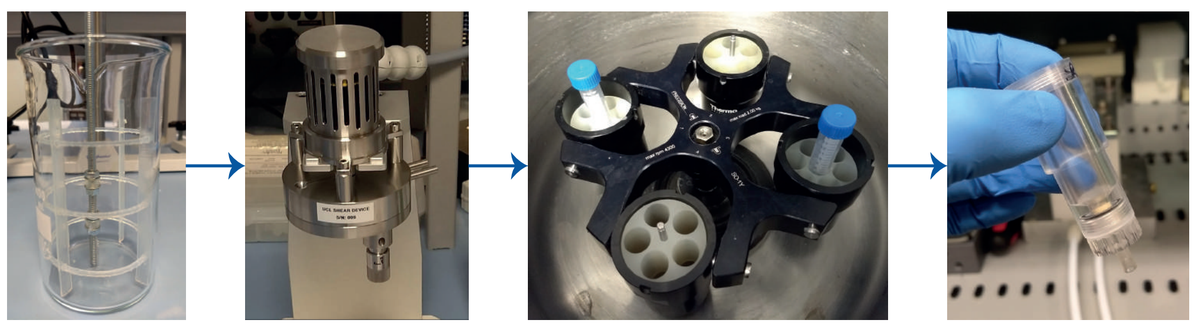

Figure 2: Establishment of an USD platform for primary yield at Biocatalysts Ltd

1. USD floc vessel: mimicking the geometry and engineering principles of process-scale operation.
2. USD shear device: varying disc speed to mimic stress during floc transport, especially in the centrifuge feed zone.
3. Varying volume, spin speed and time to mimic process-scale centrifugation.
4. Varying pressure difference to mimic full-scale filtration.

Process Characterization

The yield (Y) at process scale was estimated using:

$$Y = \frac{[C_c] \times DF}{[C_h]} \times F_D \times F_C \times 100$$

where C is concentration, subscript c is centrate, subscript h is homogenate, DF is dilution factor, FD is estimated proportion lost due to entrapment in the sediment and FC is estimated proportion lost due to liquor carry over with the sediment during solids discharge from the centrifuge.

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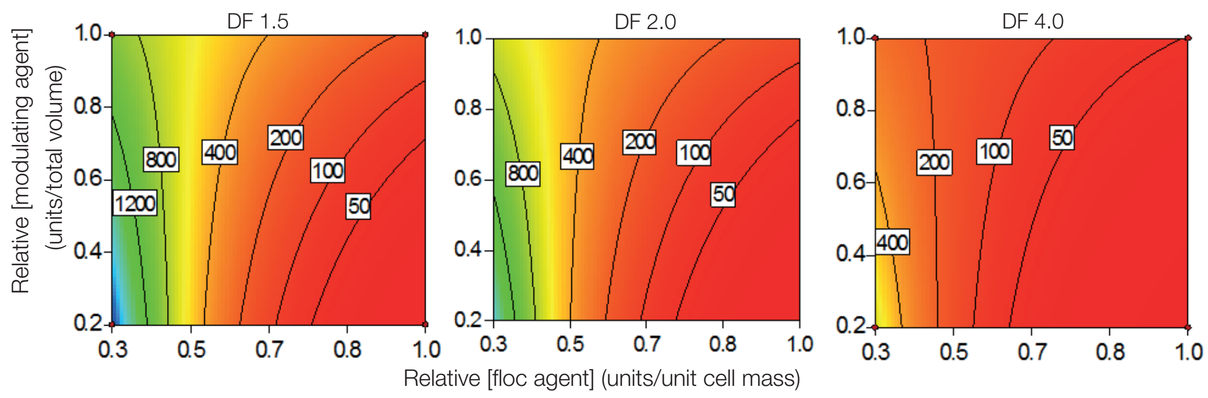
**Rayat A.C.M.E., Chatel A., Hoare M., Lye G.J. (2016). Ultra scale-down approaches to enhance the creation of bioprocesses at scale: impacts of process shear stress and early yield stages. Curr Op Chem Eng 14, 150-157. https://www.ucl.ac.uk/biochemical-engineering/research/research-tools/ultra-scale-down*

Results

Figure 3: DoE study of resultant turbidity after flocculation (red is desirable) shows the effect of modulating agent concentration, flocculant concentration, and DF on supernatant turbidity.



DoE Statistical Analysis: p values (p<0.05 is considered significant)

Factor	Measured protein concentration	Measured enzyme concentration	Measured solids	Measured turbidity
Relative [floc agent]	>0.1	>0.8	>0.4	<0.0001
DF	<0.0001	<0.0001	<0.0001	<0.02
Relative [modulating agent]	<0.0101	>0.2	0.0520	<0.02

Correction of protein, enzyme, solids and turbidity measurements for dilution leads to greater overall solids ($p<0.0001$) and less protein yield ($p=0.053$) with no significant changes in enzyme yield or overall turbidity.

The impact of modulating agent on predicted protein yield is included in model due to significant effects observed elsewhere. All combinations of variables are considered insignificant ($p>0.45$) except the combined impact of modulating agent and flocculant on turbidity ($p<0.002$).

- Increasing flocculant concentration strongly decreases turbidity but has little effect on predicted protein yield.
- Increasing modulating agent concentration increases turbidity at high flocculant and possibly increases predicted protein yield.
- Increasing dilution decreases turbidity and increases predicted protein yield.
- The methods used for determining floc and modulating agent concentrations lead them to be independent of DF.

Figure 4: Operating regions to achieve required process-scale protein yield for a specified filter demand (filter demand = turbidity x DF)

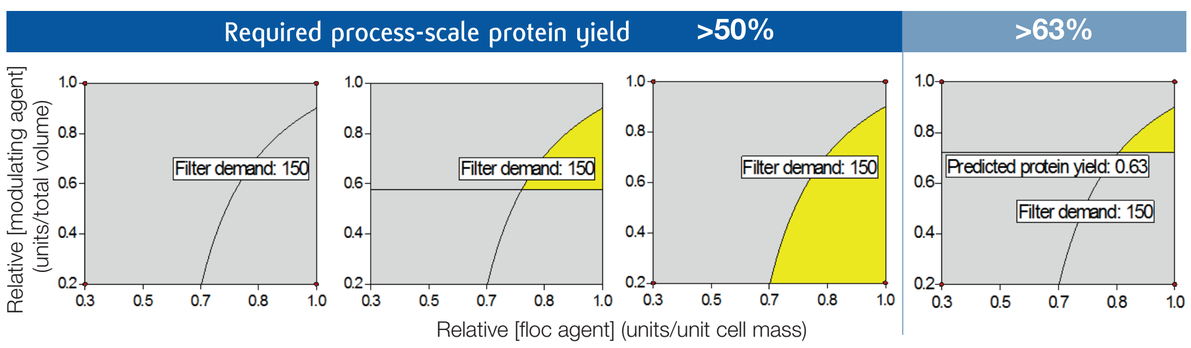


Figure 4 shows possible operating regions depending on conditions selected as determined by the predicted yield at process scale and by the estimated filter demand for clarification of the centrate.

- Increasing DF results in larger operating regions and allows a higher predicted protein yield.
- A predicted process-scale yield of 63% for protein is expected to result in a process-scale enzyme yield of >80% (studies not shown here).

Conclusions

The combined use of a flocculating agent, a modulating agent and dilution is predicted to lead to greater product yields at process scale.

It appears that the demand on process-scale filtration will be unaffected by use of this strategy. However, the increased use of process reagents and tankage will need to be assessed.

Future Work

Increase use of the USD platform to study impact of changes in process-scale design e.g. effect of shear in the centrifuge feed zone, different method of solids handling.

Extend the range of flocculant and modulating agent concentration to better model their impact.

Explore operating windows for different enzymes.