Scale up and process development of recombinant enzymes used for mRNA vaccine manufacturing

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The RNA vaccine manufacturing process requires the mRNA molecule synthesis, and this process step requires various recombinant enzymes. None of these key enzymes are produced in South Africa, moreover, they represent approximately 80% of the production costs of mRNA vaccines, and are therefore not only critical to enable local vaccine manufacturing, but also for doing so cost-effectively. (Kis & Rizvi, 2021).

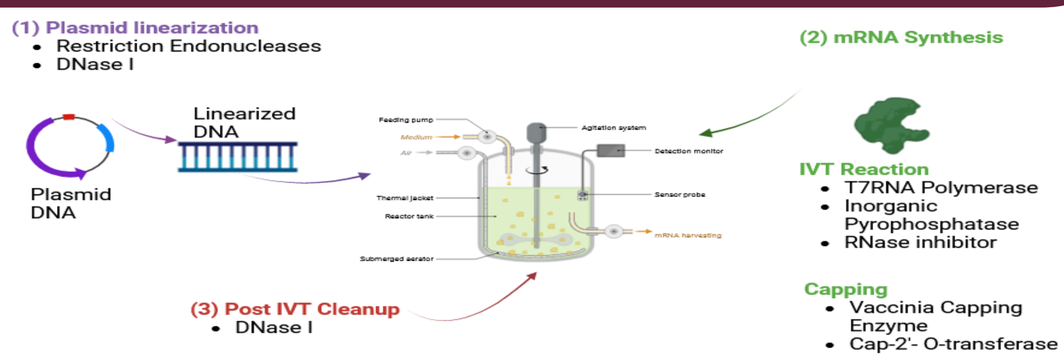

Figure 1: mRNA production adapted from Kis et al., 2020. (Image created in Biorender.com)

Aim

To develop a scalable procedure that can be readily tailored for current Good Manufacturing Practices (cGMP)-compliant production to produce T7RNAP, Pyrophosphatase and vaccinia capping enzyme.

Objectives

- To use new strains of *Escherichia coli* and *Pichia pastoris* to express T7RNAP, Vaccinia capping enzyme, and Inorganic pyrophosphatase
- To identify, test and select upstream and downstream unit operations for the scale up of the recombinant enzyme production process.
- To investigate, the feasibility of scaled up process in a 5 L bioreactor and associated purification steps
- To troubleshoot and optimize productivity and yields of recombinant enzymes in a 5 L bioreactor
- To scale-up the optimized process to 20 L bioreactor cultivation and the associated purification steps required

Upstream processes

Possible variables for fermentation:

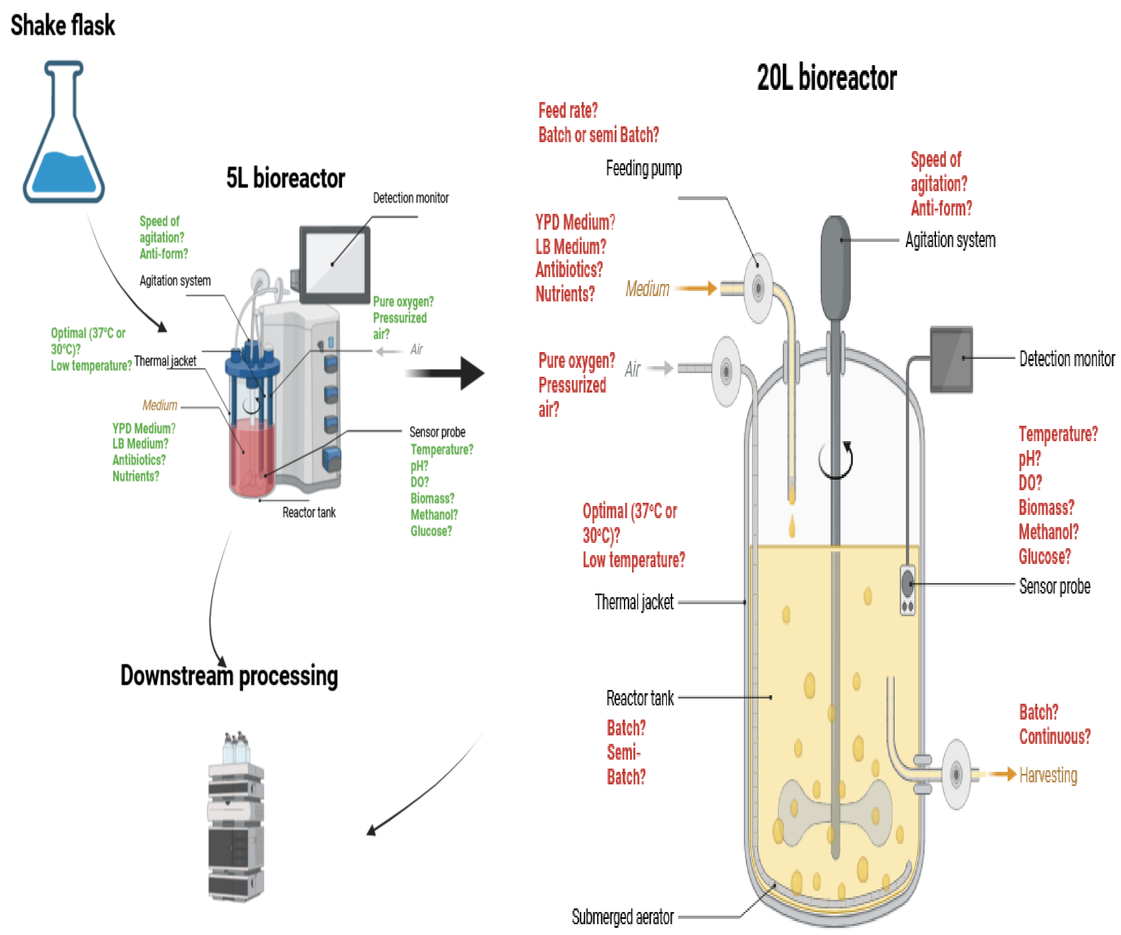

Figure 2: Upstream heterologous protein production. Adapted from Macauley-Patrick et al., (2005). (Image created in Biorender.com)

Downstream processes

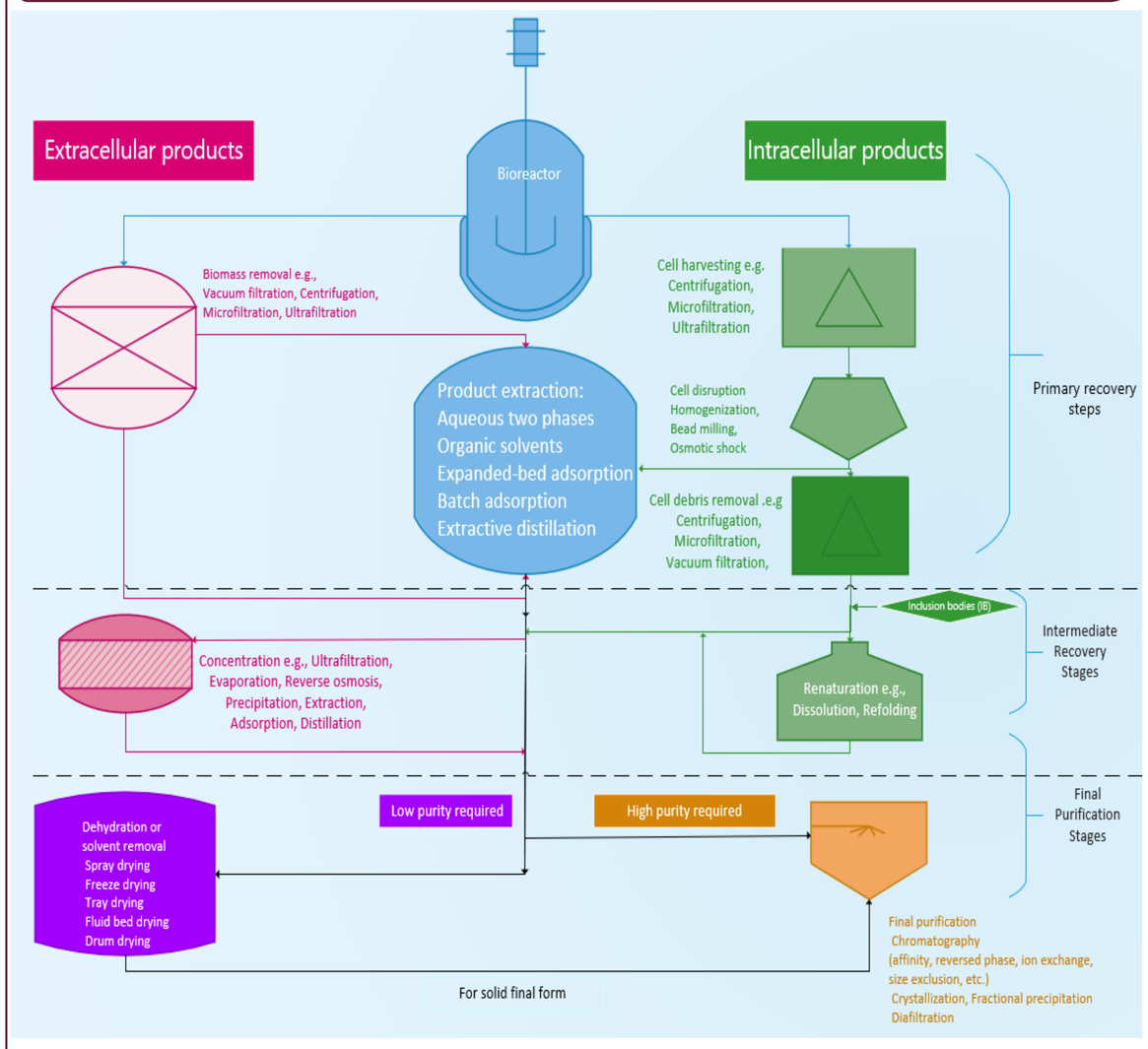

Figure 3: Simplified downstream protein purification. Adapted from Harrison, Todd, Rudge & Petrides, (2003)