EXPRESSION AND DOWNSTREAM PURIFICATION OF INSULIN MOLECULES IN *PICHIA PASTORIS*

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In the next decade and beyond global demand for insulin is expected to rise significantly requiring additional manufacturing capacity. The next generation of insulin manufacturing plants will likely be based on new and robust expression and bioprocess platforms that are flexible, safe, simple to implement in a manufacturing setting and capable of step improvements in productivity and cost compared to current manufacturing techniques. Towards this end, *Pichia pastoris* expression systems has been evaluated for insulin due to its capacity to secrete a variety of heterologous proteins and its ability to grow to high cell densities. Under the well-characterized, tightly regulated AOX1 promoter, yields of 1.5 to nearly 3 g/L of purified insulin have been reported.[1,2,3] However, methanol is a very volatile substance requiring specialized facilities, which can hamper large-scale production. Downstream processing of insulin precursors also requires use of organic solvents which can also burden manufacturing. We report development of an insulin process using a constitutive promoter expression system in place of the inducible AOX1 promoter, and a simplified downstream purification process using precipitation. Fermentations were carried out in 2 L scale bioreactors and culture supernatant collected after 65 hours. A design of experiment (DoE) was performed to identify optimal conditions for polyelectrolyte precipitation of the recombinant protein using polyvinyl sulfonic acid (PVS).[4] The resulting pellets were then analyzed via SDS-PAGE and HPLC.

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