140-Fold Increase of Protein Achieved in *Pichia* Fermentation

NBS microbiologists recently assisted researchers at the University of California in achieving a 140-fold increase in protein production from a recombinant strain of *Pichia pastoris* yeast. Top yields by the researchers thus far had been 1.4 mg/L of a thrombomodulin fragment when culturing *Pichia* in shake flasks. By switching to a BioFlo 3000® fermentor, NBS scientists were able to overcome the two main problems which plagued earlier studies — insufficient oxygen, and a temperature increase of as much as 25°C generated by the organism’s prolific growth. Although fermentors commonly achieve yields of ten or more times that of shakers, these extraordinary results were largely due to the unique capabilities inherent in the BioFlo 3000 fermentor.

**An Ideal Pichia Culturing System**

The versatile BioFlo 3000 batch, fed-batch and continuous fermentor offers several design advantages. Uniquely, this fermentor comes with a built-in two gas controller for optimizing dissolved oxygen (DO) during fermentation, (as well as a four-gas controller for use during cell culture). Using any of the system’s four operating modes, agitation may be increased and/or pure oxygen added throughout the run to maintain the desired DO level.

Second, the vessel’s stainless-steel water-jacket provides superior heat induction capability. A PID controller regulates culture temperature for optimum protein production – in this case at 30°C.

In addition, five pumps provide programmable addition and harvesting functions, which ensured tight control over methanol, antifoam and nutrient addition in these studies. Furthermore, BioCommand®, NBS’ proprietary Windows®-based software can be used to automate process control, log and graph data on-line, or optimize product yields. NBS will even supply software control algorithms and protocols expressly written for *Pichia pastoris* to BioFlo 3000 users upon request.

**Materials & Methods**

In these studies, a Muts strain of *Pichia* was grown in fed-batch and continuous culture. AFS BioCommand helped to control and later optimize nutrient feeding and oxygen supplementation. Production of the thrombomodulin fragment in the fed-batch studies reached an average of 200 mg/L, a 142-fold increase over shake flasks. In the final study, a continuous culture process utilizing an on-line separation system to harvest products and recycle the cells enabled an extended production phase, resulting in yields four to five times that of the fed batch cultures.

**About Pichia**
*Pichia pastoris* (current name Komagataella pastoris) has been touted as an exciting new expression system, and an excellent alternative to *E. coli*, because the proteins produced are glycolated, typically folded correctly and secreted directly into the culture medium. For article reprints or product literature contact your NBS sales representative. To discuss contract process development, contact Dr. Julia Cino, at our Edison, NJ headquarters at 800-631-5417 or write to us at bioinfo@nbsc.com.

Caption:
With a built-in gas controller for regulating oxygen supplementation, the BioFlo 3000 readily meets the high-oxygen demands of *Pichia pastoris* yeast. This PC-compatible fermentor includes sensors, pumps, interchangeable vessels and impellers to adapt to the needs of virtually any animal, insect, microbial or plant cell line.