**Innovation of Expanded-Bed Adsorption by Integrating Simulated Moving-Bed Technology.**

Trinath Pathapati1\*, Dennis N. Rutze1, Piet den Boer1, Menne Zaalberg1

*1 ProPharma Group (Previously called Xendo B.V.), Schipholweg 73 – 75, P.O. Box 255, 2300 AG Leiden, The Netherlands;*

*\*Corresponding author: Trinath.Pathapati@xendo.com*

**Highlights**

* Efficient downstream processing of complex biological streams
* Hybrid process by integration of EBA and SMB technologies
* Development of novel equipment for implementation of an EBA-SMB process
* Experimental results prove that the innovation resulted in enhance techno-economic efficiency

**1. Introduction**

Product stream from a fermentation step is water-based (> 80 wt %) and contains dissolved and suspended impurities. As the choice of technologies define the downstream process efficiency and resulting costs, it is critical to apply innovative and efficient unit operations to achieve people, planet and profit demands [1]. One or more unit operations are employed in each block of downstream processing. An approach to enhance efficiency of such a process is by integrating one or more operating principles into a single unit [2]. The current article emphasizes the integration of expanded-bed adsorption(EBA) and simulated moving-bed (SMB) technologies [3] for selective product capture from unclarified fermentation broth.

**2. Methods**

An EBA-SMB system with 8 columns, 5 inlets and 5 outlets per column was designed with a bed level detecting mechanism. The recipe consisted of defined zones including adsorption, adsorption wash, elution, elution wash, regeneration and regeneration wash that each EBA column went through during the SMB operation. After validating the system, experiments were performed to purify gamma-amino butyric acid (GABA) from unclarified fermentation broth containing up to 100g GABA/l and 16 g *Escherichia coli* biomass /l. The process conditions studied include,

1. Change in amount of feed fed/ml settled bed resin volume (SBV) to determine the maximum resin binding capacity that can be achieved
2. NaOH concentration in elution buffer to shows its impact on elution recovery and product titer
3. Number of columns in series in feed and elution zones for improved separation efficiency and recovery
4. Entrainment rejection (ER) to prevent yield losses
5. Fractionation of product rich elution stream

**3. Results and discussion**

**Figure 1.** Outlet pH profile while different columns move through the product collection position after 2 cycles

It was observed that decreasing the amount of feed/column and increasing the number of columns in feed zone from 1 to 2 and entrainment rejection had a positive impact. By this approach GABA bound in feed zone increased from about 50 % (EXP001, 002, 003) to about 74 % (EXP004). However, the decrease in BV of feed/column resulted in 39 % reduction of binding capacity. Further, it was identified that due to the 8-column configuration and other critical zones including adsorption wash and elution, the number of columns available for feed zone was limited to 2. Therefore, to achieve further increase in yield and higher binding capacities, it is required to configure more than 2 columns in feed zone. The experiments performed to study the impact of buffer strength proved that by increasing NaOH content from 5 to 8 wt% in elution buffer, GABA recovery increased from about 55 % (EXP001) to about 84 % (EXP003). This, along with fractionation resulted in increase of product titer from 33 g/L to 47 g/L. Further, from the impurity analysis, it was also determined that the one step EBA-SMB purification of unclarified fermentation broth resulted in a product stream containing about 40 g/l GABA with purity >92 % and >98 % removal of biomass.

**4. Conclusions**

The experimental results proved that the EBA-SMB technology processed complex biological feed streams in one-step by integrating both biomass separation and selective product capture. The desired product purity was achieved. EBA-SMB design was validated by processing feed stream for several SMB cycles. No leakages and pressure buildups were noted. The integration and control strategy not only enabled stable operation, but also provided flexibility for process optimization. Thereby indicating improved techno-economic feasibility for industrial scale implementation. Further, parameters like the number of columns in a zone, feed loaded/SBV and elution buffer strength were identified to be critical to achieve optimal process performance.

**References [Calibri 10]**

[1] A. Karau, C. Benken, J. Thommes, M. R. Kula, Biotechnology and Bioengineering 1997, 55 (1), 54-64. DOI: 10.1002/(SICI)1097-0290(19970705)55:1<54::AID-BIT7>3.0.CO;2-W

[2] A. Rajendran, G. Paredes, M. Mazzotti, Journal of Chromatography A 2009, 1216 (4), 709-738. DOI: 10.1016/j.chroma.2008.10.075

[3] E. d. Jong, G. Jungmeier, Industrial Biorefineries and White Biotechnology, null. (Eds: A.Pandey, R. Hofer, C. Larroche, M. Taherzadeh and M. Nampoothiri), Elsevier B.V., Amsterdam 2015, Ch. 01.