XPURE
innovative continuous chromatography

February 28, 2019 BLOG

EBA-SMB: Single Step Purification Of Amino Acids From Bacterial Broth

This blog will present a new development regarding the application of Expanded Bed Adsorption (EBA) technology for small molecules.

Amino acid – Gamma-aminobutyric Acid (GABA)

Biotechnological fermentation processes are nowadays common in the industry to produce a broad range of biological molecules. Obviously, the bio-based industries need efficient, cost-effective, downstream solutions to process these complex streams.

The topic for this blog is the purification of small molecules like amino acids from the unclarified bacterial broth using a combination of different technologies. To date, the use of Expanded Bed Adsorption (EBA) for processing unclarified broth is state-of-the-art technology and its description can adequately be found in the literature. In an EBA column, the resin bed is expanded by upward feed flow and the (then actuate) bed void allows particulate biomass to flow through the resin bed and selectively capture target molecules. This makes dedicated clarification steps such as centrifugation and filtration redundant. However, a further improvement of the technology that has been regarded as feasible, is the integration of EBA with the simulated moving bed (SMB) technology (Ref. 1). To address this challenge the purification of a model compound, -aminobutyric acid (GABA) was investigated.

What is new: use of gel type adsorbent in Eba mode processing

Resin selection is one of the critical aspects of developing an EBA process. Relevant resin hydrodynamic properties include particle diameter and density. Typically, these beads are agarose-based and have a heavy core. Only a few specific EBA resin types are available, which are also quite expensive. These aspects are major hurdles for the application of the EBA technology in the manufacturing of bulk chemicals where low production costs are key.

The current study focusses on the purification of -amino butyric acid (GABA) from a bacterial, E. coli, fermentation broth. It is known that strong acid cation exchange (SAC) resins exhibit selective binding of GABA (Ref. 2). The resin screening for current investigation was not limited to specific, porous EBA resins. Surprisingly, it has been

discovered that gel-type (non-porous) resin(s) could also be used for EBA applications; although the density is at the low end (e.g. 1.2 g/mL) linear flow rate up to 600 cm/h were attained. In this case, industrially available resin type (Finex, CS16GC) is furthermore characterized by high ion exchange capacity (e.g. 1.6 eq/L). In Fig. 1 pictures of gel-type and macroporous-type polystyrene resins are shown.

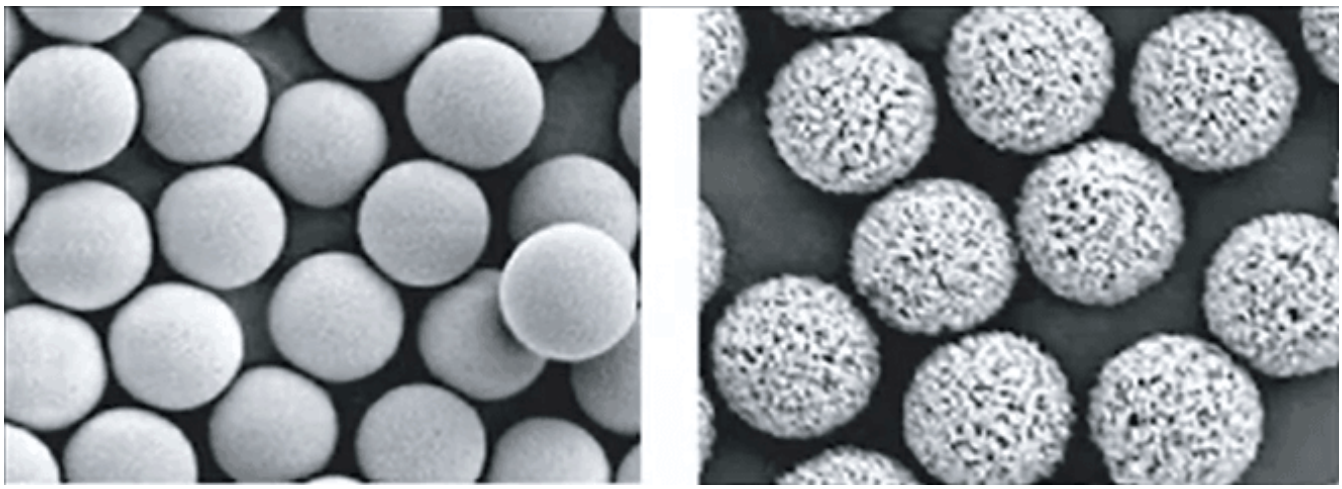

Figure 1.

GABA purification and eba-mode processing

First, the GABA purification process using the gel-type strong acid cation exchange (SAC) resin was defined for a “single EBA column process”. The following process steps were identified:

- Adsorption (loading GABA-containing unclarified broth)
- Wash (H_2O)
- Elution (NaOH)
- Wash (H_2O)
- Regeneration (H_2SO_4),
- Wash (H_2O)

Obviously, critical process requirements for EBA processing (e.g. maintaining the target bed expansion) should be fulfilled. Important parameters incl. buffer concentrations, target buffer volumes, and flow rates were established. Flow rate ranges for individual process buffers and unclarified broth were studied and optimized to prevent the occurrence of unwanted changes in the expanded resin bed. Detrimental for EBA process in SMB mode is the overflow of resin from the column top resulting in loss of resin or even clogging of valves.

Technology integration: EBA in SMB mode

For the integration of the EBA and SMB process, a state-of-the-art 8-column lab-scale system has been designed and built (see Figure 2). An important feature is the flexibility to define the operating conditions per column.

Within the software, recipes can be generated that address

- the total number of columns in an SMB cycle
- number of column positions
- inlet and outlet valve configuration per position
- pump flow rate
- sensor control and
- switch time per position.

As mentioned, a critical aspect is to ensure optimal bed expansion. For this purpose, ultrasound sensors were installed at each column outlet that measured the expanded bed level. By this measurement, the software-controlled the pump flow rate to maintain the desired bed expansion for the individual columns. The pH profile of the product stream exiting the different SMB columns passing through the elution zone was assessed to represent consistent product quality at a cyclic steady state of operation. The quality was defined by the removal of biomass and other soluble impurities (Ref. 3).

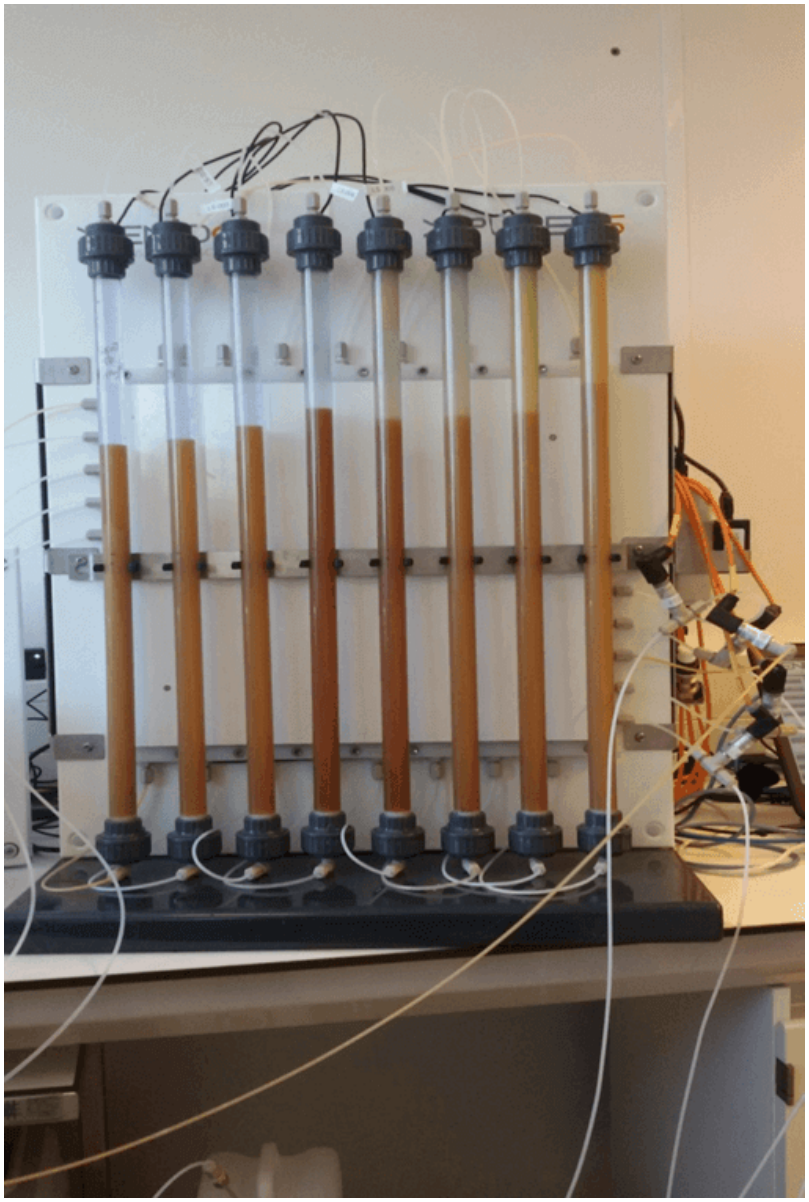

Figure 2.

Conclusion

The promise of reduction of unit operations, increased product yield and productivity, and reduced buffer consumption using EBA technology for complex feed streams has been recognized for many years. It has now been demonstrated that further process improvement by integration of the EBA and SMB technology for purification of amino acid, GABA, from the unclarified bacterial broth was successful. A relevant factor is the use of gel-type resin (CS16GC) exhibiting a higher binding capacity as compared to that of macroporous resin. The integrated one-step EBA-SMB process resulted in a GABA purity of 92% and > 98% removal of biomass. The results show that integrated EBA-SMB technology enhances process efficiency and the economics of bioprocesses. It is anticipated that further improvement can be realized by increasing the number of columns.

References

Ref. 1. Ping Li, Pedro Ferreira Gomes, José M. Loureiro, and Alirio E. Rodrigues. Proteins Separation and Purification by Expanded Bed Adsorption and Simulated Moving Bed Technology. In Continuous Processing in Pharmaceutical Manufacturing (2014). Editor Ganapathy Subramanian.

Ref. 2. Trinath Pathapati, Dennis N. Rutze, Piet den Boer, Pieter de Wit, and Menne Zaalberg. Expanded Bed Adsorption of -Aminobutyric Acid from E. coli broth by CS16GC and IRC747 Resins. Chem. Eng. Technol. 2018, 41, No.12, 2427-2434..

Ref. 3. Trinath Pathapati, Dennis N. Rutze, Pieter de Wit, Piet den Boer, and Menne Zaalberg. Innovation of Expanded-Bed Adsorption by Integrating Simulated Moving-Bed Technology. Chem. Eng. Technol. 2018, 41, No.12, 2393-2401.