Trinath Pathapati*

Dennis N. Rutze

Pieter de Wit

Piet den Boer

Menne Zaalberg

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

Bio-based industries need efficient downstream solutions to process complex streams. This was addressed through a technology integration approach, where expanded-bed adsorption (EBA) is integrated with simulated moving-bed (SMB) technology. Current work involved adaptation of an SMB apparatus and control principle to implement expanded-bed level control. As an outcome, EBA-SMB technology was successfully applied for purification of γ-aminobutyric acid (GABA). This resulted in two-fold increase in productivity and a GABA purity $\geq 92\,\%$ in one step from unclarified fermentation broth, compared to $\geq 93\,\%$ purity in case of clarified broth and packed-bed SMB. These results proved that EBA-SMB technology is able to enhance process efficiency and economics of bioprocesses.

Keywords: Expanded-bed adsorption, Fermentation broth, Ion exchange, Simulated moving bed, Technology integration

1 Introduction

Product stream from a fermentation step is water-based ($> 80\,\mathrm{wt}\,\%$) 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 unit operations to achieve people, planet, and profit demands [1].

As depicted in Fig. 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.

1.2 Adsorption and Chromatography Resins

Adsorption technology involves a solid stationary phase interacting with a mobile liquid stream containing one or more target compounds. Adsorption is controlled by different interactions as indicated in Fig. 2. Ion exchange (IX) is one of the common adsorption mechanisms and includes weak acid cation (WAC), strong acid cation (SAC), weak base anion (WBA), and strong base anion (SBA) exchange resins [4].

Polymeric IX resins can be further distinguished into gel-type and macroporous resins based on particle porosity. Pore sizes of gel-type and macroporous polystyrene resins range between 20–50 Å and 200–2000 Å, respectively [5].

1.3 Expanded-Bed Adsorption

Adsorption input streams are usually clarified using centrifugation or filtration steps to prevent clogging of packed beds. These steps result in yield losses and modification of stream properties that can pose challenges to succeeding downstream operations [6]. On the other hand, EBA addresses these limitations by integrating both solid-liquid separation and primary product capture in one unit operation [7]. In an EBA column, the resin bed is fluidized by upward feed flow and the bed void allows particulate biomass to flow through the bed and to selectively capture target molecules [8] (Fig. 4).

Resin selection is one of the critical aspects of EBA and is based on hydrodynamic properties like resin particle diameter and density, feed viscosity and density, linear velocity, and axial dispersion [9]. The effect of particle size distribution on the EBA performance is dependent on the mass transport phenomenon and the properties of feed stream and target molecule [10]. Depending on the resin used, both homogeneous film and porous diffusion models are applicable for EBA [5].

Trinath Pathapati, Dennis N. Rutze, Pieter de Wit, Dr. Piet den Boer, Menne Zaalberg

trinath.pathapati@xendo.com

Xendo B.V., Bio Science Park, Schipholweg 73–75, 2316 ZL Leiden, The Netherlands.
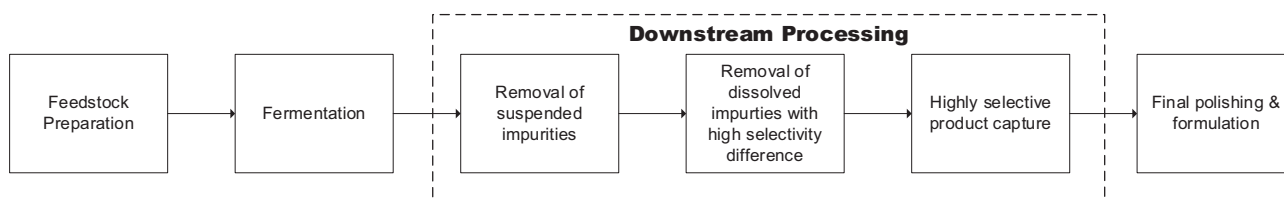
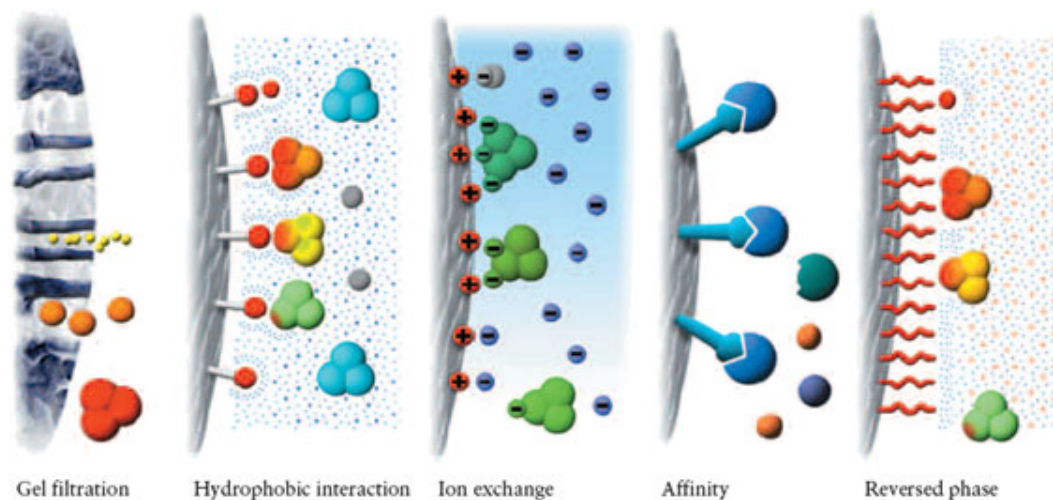**Figure 1.** Typical flow of a fermentation-based process.

Gel filtration Hydrophobic interaction Ion exchange Affinity Reversed phase

Figure 2. Simplified (artist impression) different mechanisms for chromatographic separations commonly used in protein chromatography [14].
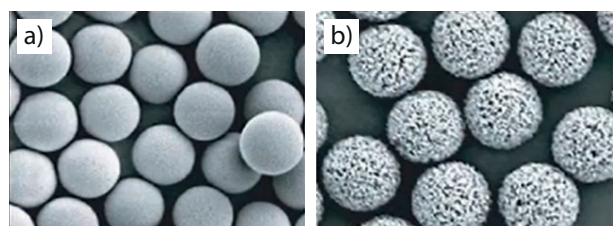

Figure 3. Micrographic pictures of (a) gel-type and (b) macroporous-type polystyrene resins [15].
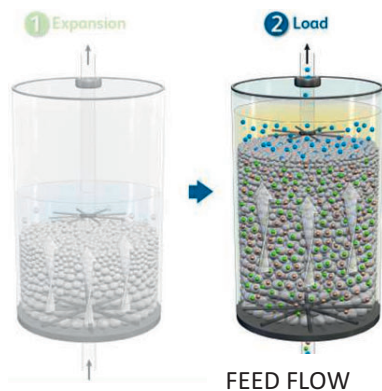

Figure 4. Schematic representation of EBA using separate inlet and outlet pumps [15].

1.4 Simulated Moving Bed

In the batch process, fluid flows through the resin bed and equilibrium is attained between resin and process fluid. This results in a mass transfer zone that gradually moves through the bed. The product "breaks through" as the mass transfer zone reaches the exit of the resin bed. The resin then needs to be washed, eluted, and regenerated before it can be loaded again. The aim of the SMB process is to keep the mass transfer zone effectively at the same location by moving the resin beds in the opposite direction of the input streams. This approach ensures optimal resin utilization, superior resolution, leading to more efficient and compact separation processes. In a true moving-bed concept, the resin flows countercurrently to the liquid flow, whereas in an SMB concept the resin flow rate is simulated by periodically shifting the different inlet/outlet ports in the direction of the fluid flow.

2 Process Design by Technology Integration

Technology integration is a potential driver towards techno-economically viable process solutions. One of the best examples is the concept of biorefinery, where several subprocesses are integrated to valorize both feedstock and side streams into biomaterials, biochemical, and biofuels [3, 11]. A similar integration approach when introduced at a unit operation level can

improve the step efficiency. The technology integration approach in the current article involved six steps:
– Understanding the basis of the process and defining the problem.
– Identifying suitable process technology to address the problem.
– Detailed understanding of the operational fundamentals of chosen technologies.
– Defining technology-specific critical process parameters-
– Designing hardware and software for optimal performance of integrated technology.
– Testing of the integrated technology.

2.1 Problem Definition for an Exemplary System

Purification of γ-aminobutyric acid (GABA) from *Escherichia coli* sugar fermentation broth was chosen as the exemplary system to develop the EBA-SMB process. GABA was considered a representative component for renewable materials that are produced using state-of-the-art biotechnology. Fig. 5 shows the molecular structure of GABA.
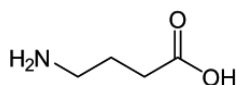

Figure 5. Schematic GABA molecule structure.

Using the current process, it is required to purify GABA from unclarified fermentation broth in continuous mode and to achieve > 90 % product purity, while at the same time ensuring that: (i) the system enabled critical process requirements, (ii) the system control was able to observe and react to changes in process conditions without impacting the process performance, and (iii) a control strategy is established and maintained a steady-state operation.

2.2 Identifying a Suitable Technology

Based on physiochemical properties of the feed stream described in Tab. 1, some downstream alternatives were evaluated

Table 1. Feed composition.

Stream type	Fermentation broth
GABA concentration [g L^{-1}]	70–110
Biomass dry weight concentration [g L^{-1}]	1–16
Sugars [g L^{-1}]	0.1–2^1
Organic acids [g L^{-1}]	0.5–5
Glycerol [g L^{-1}]	0.1–1.4
Density [g L^{-1}]	1010–1100
Viscosity [mPa s]	1.1–1.3
pH	4–6.5

and EBA was identified to be suitable with two critical process benefits, namely, reduction of clarification steps, and that low/negligible backpressure enables high flow rates, resulting in enhanced productivity and easy scalability.

2.3 Fundamental Approach for EBA Resin Choice

SAC-type resins exhibited selective binding of GABA in packed-bed mode. Therefore, a suitable SAC resin was identified for separating GABA in EBA mode. During resin screening, it was considered that application of EBA to purify small molecules, e.g., amino acids or organic acids, required different assumptions compared to protein purification. This is due to the fact that purifications of small molecules are better described using a homogeneous diffusion model on a gel-type resin compared to a porous diffusion model for proteins on a porous resin [12, 13]. In Fig. 6, $u^{1)}$ is the linear velocity within the column and μ is the viscosity of the liquid.

This approach resulted in the use of an industrially available gel-type resin instead of a tailor-made macroporous EBA resin for GABA purification. It was also assumed that homogeneous film diffusion was less sensitive to axial dispersion (D_{ax}), particle size (r_p), and column verticality compared to intraparticle porous diffusion [14]. Properties of the used resin are described in Tab. 2.

Table 2. Resin properties.

Property	Value
Composition	50–55 % sulfonated polymer of styrene, divinylbenzene, and ethylstyrene Na$^+$ form 45–50 % water
Porosity	Gel type
Density/specific gravity [g L^{-1}]	1150–1200
Operating pH	0–14
Maximum operating temperature [°C]	120
Particle diameter [μm]	300
Particle size distribution [%]	> 95
Ion-exchange capacity [eq L^{-1}]	1.5–1.7

2.4 Critical Process Parameters

The critical process parameters for EBA include:
– optimal flow distribution generating multiple theoretical plates (height-equivalent theoretical plates, HETP)
– optimal bed expansion ensuring liquid void for flow-through of suspended solids

---

1)   List of symbols at the end of the paper.

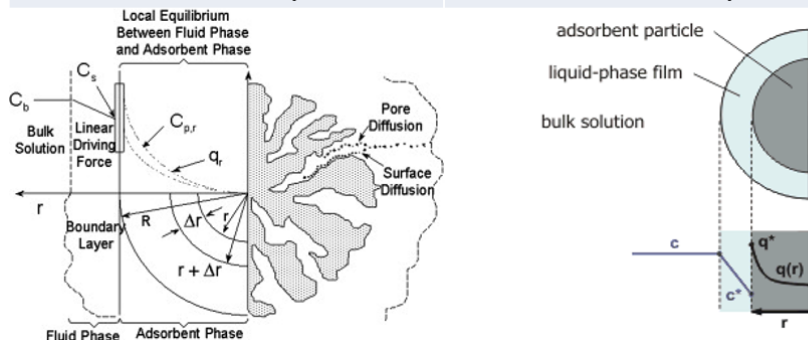| Porous resins	Gel-type resins
Larger surface area	Smaller surface area
Low exchange capacity	High exchange capacity
Sensitive to D_{ax} and r_p due to film and pore diffusion	Less sensitive to D_{ax} and r_p due to film diffusion
Sensitive to u and μ	Sensitive to u and μ

Figure 6. Difference between porous resins and gel-type resins [7, 12].

– resin bed height and flow rate ensuring optimal residence time for kinetics.

In addition, the SMB process efficiency of a bind and elute system is dependent on:
– number of columns in specific zone defining the total number of mass transfer zones
– total number of columns
– switch time defining the resin flow rate.

3 Hardware and Software Development (Experimental Setup)

3.1 SMB Design

A state-of-the-art 8-column lab-scale SMB system was designed and constructed. The columns were connected to a modular valve block, which consisted of 88 solenoid valves with precise control over the flow paths. Independent control of these valves granted flexibility to define the operating conditions per each column. The XPure-S software (XPure is Xendo's trademark for SMB continuous chromatography systems) then executed experimental recipes with a maximum of eight zones at the same time. 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 were defined in the recipe. Further, the control software also monitored sensor information, e.g., pH, conductivity, bed level, to control pump and valve settings.

3.2 EBA Column Design

Column design was performed to ensure the desired flow distribution at the inlet and maintain minimum backpressure. A reliable flow distribution system was created using a short bed of inert ceramic beads at the column inlet. The high-density type YTZP, 0.6–0.8 mm ceramic beads were obtained from Chemcobeads, China. A 0.5-mm mesh was used at the column inlet to stop the beads from flowing out of the column. The high density of the ceramic beads allowed them to remain at the bottom of the column. In the current case, fixed top columns were selected.

As it was critical to ensure optimal bed expansion, an ultrasound sensor was used to measure the bed level according to Thelen et al. [15]. The sensor installed at the column outlet measured the distance between the surface of the expanded bed and the outlet. By this measurement, the XPure-S software controlled the pump flow rate to maintain the desired bed expansion (feedback loop in Fig. 7). A description of different sensors that send a signal to the controller per each EBA column is given in Fig. 7.

3.3 EBA-SMB System

The EBA-SMB system was an adaptation of an SMB unit described in the SMB design chapter. The detailed specifications of the EBA-SMB apparatus are given in Fig. 8 and Tabs. 3 and 4.

Table 3. EBA column dimension used for experimental studies.

Parameter	Value
Total column height [cm]	65
Expanded bed height	Varied with flow rate
Column inner diameter [cm]	2
Settled bed height [cm]	32
Flow distribution mechanism	3 cm ceramic bead bed
Expansion factor	Varied with flow rate
Bed level detection	Using ultrasound sensor mounted to the fixed top adapter at the column outlet

3.4 Experimental Testing

The experiments using the EBA-SMB system were performed in two stages, namely, validation of the EBA-SMB setup and
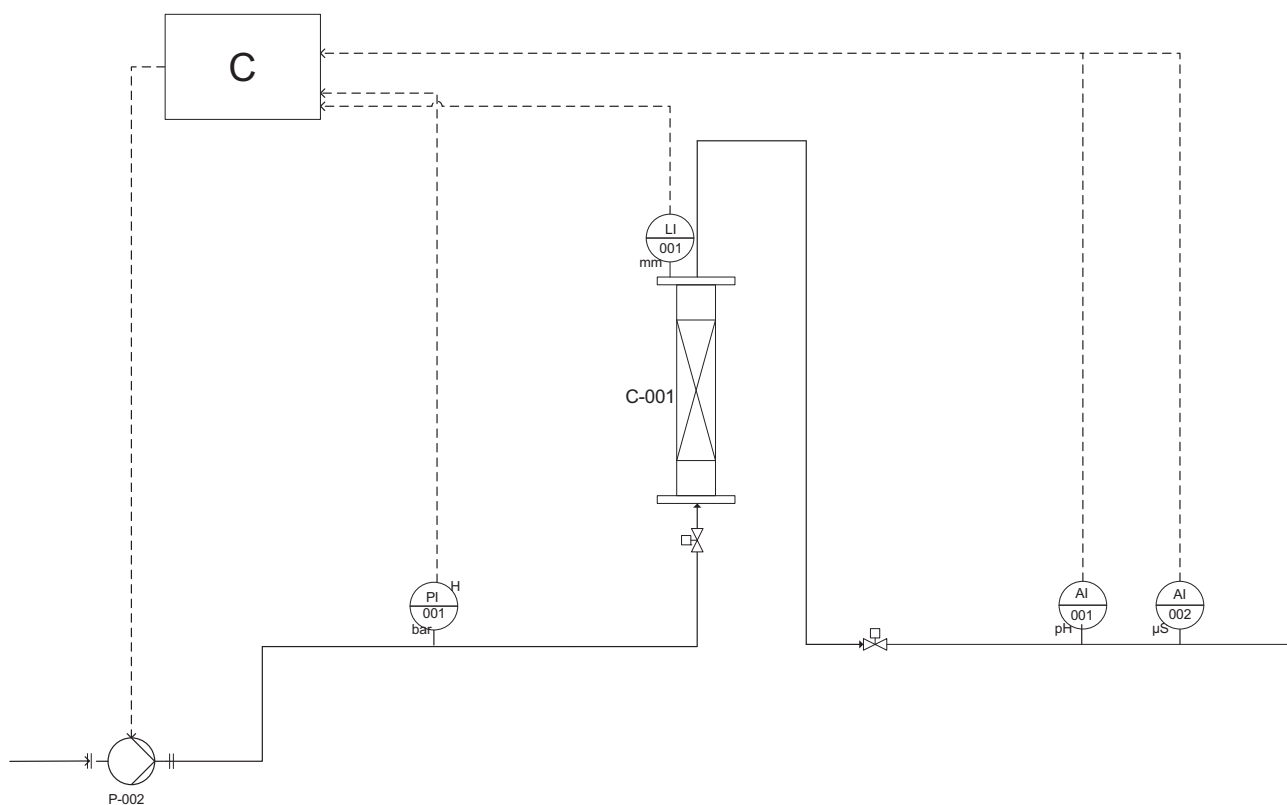
Figure 7. Sensors including level sensor LI per EBA column sending signals to the controller (C), which sends a signal to the inlet pumps.
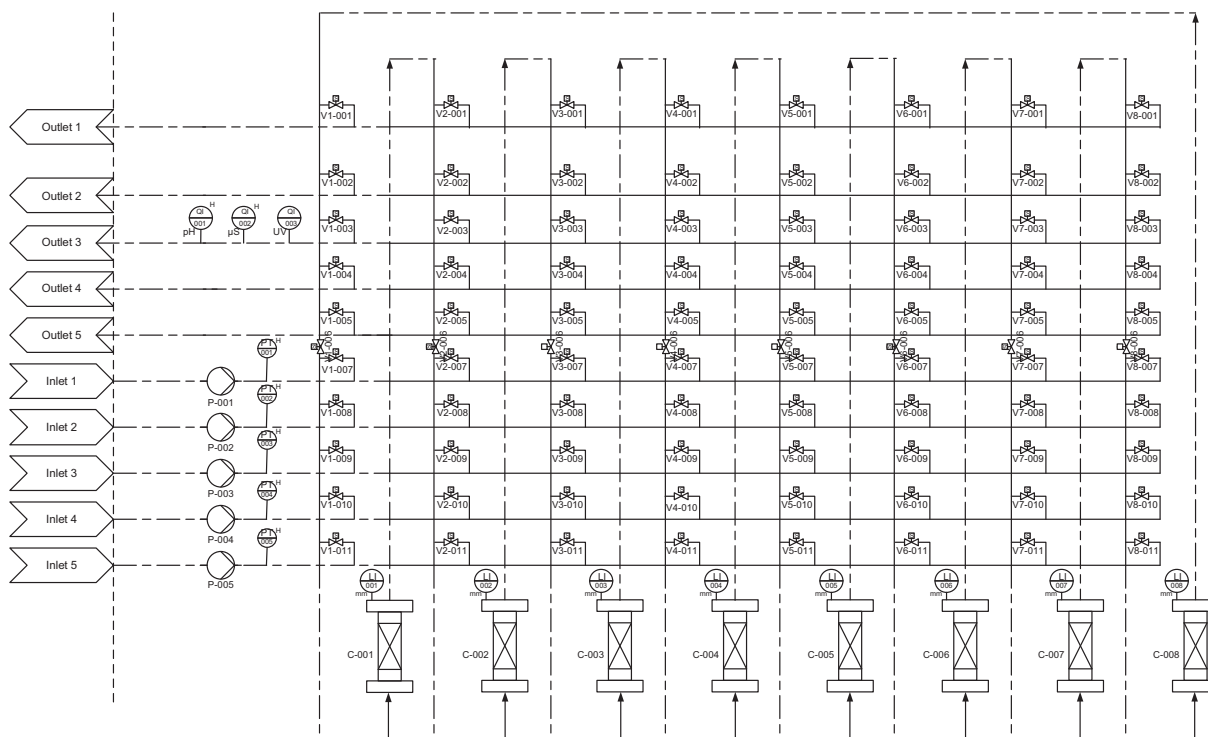

Figure 8. EBA-SMB setup, with eight columns, every column equipped with an LI (level sensors), five inlet valves, one series valve, and five outlet valves, inlet valve channels connected to five inlet pumps including pressure sensor and transmitter; outlet 3 is equipped with conductivity, UV, and pH sensors.

Table 4. EBA-SMB setup as displayed in Fig. 10.

No. of columns	8
No. of inlet valves/column	5
No. of outlet valves/column	5
No.of series valves/column	1
No. of inlet pumps for 8 columns	5 (specific inlet valve of every columnis connected to a common pump, e.g., inlet valve 1 of 5 of every column is connected to pump 1)
No. of inlet ports for pumps	5
No. of outlet ports for fractionation	5
EBA bed level measuring ultrasound sensors	8 (1 attached to every column outlet)
EBA bed level control box with control units	Contains 8 control units, one for each ultrasound sensor enabling signal conversion from ultrasound frequency to voltage (analogue) that the EBA-SMB software converts to cm (digital)

control software, and experimental proof-of-concept by purifying GABA from the unclarified fermentation broth.

3.4.1 System and Software Validation

The integration and control strategy was tested using a recipe made to process unclarified GABA fermentation broth. The stream composition, resin properties, and system design were according to Tab. 1, Tab. 3, and Fig. 8. The recipe is indicated to Tab. 5.

The recipe consisted of defined zones and positions that each EBA column went through during the SMB operation. However, it was found that additional compensation positions were required to implement bed level control in a specific zone. These compensation zones would account for varying switch times, when the pump flow rates were controlled to maintain the desired bed expansion. Therefore, experiments were performed with and without compensation positions to explain its impact on EBA-SMB operation.

3.4.2 Purification of GABA from Unclarified Fermentation Broth

After validating the system, experiments were performed to purify GABA from unclarified fermentation broth and the following process parameters were studied:
– change in amount of feed fed per mL settled bed resin volume (SBV) to determine the maximum resin binding capacity that can be achieved
– NaOH concentration in elution buffer to show its impact on elution recovery and product titer
– number of columns in series in feed and elution zones for improved separation efficiency and recovery
– entrainment rejection (ER) to prevent yield losses
– fractionation of product-rich elution stream.

The flow rates, zones, and stream properties for the experiments in Tab. 6 are similar to Tab. 5. The GABA concentration in the output samples was determined using an Aminex 87 C HPLC column and impurity analysis by dry matter measurement.

Table 5. Experimental conditions used for testing the EBA-SMB for an exemplary system.

Pump No.	Inlet No.	Zone	Stream	Flow rate [mL min^{-1}]	No. of SBV	Total number of columns	Total number of positions	Number of columns in series	Outlet No.
1	1	Regeneration	4 wt % H$_2$SO$_4$	20	2	1	1	NA	5
2	2	Elution	5 wt % NaOH	20	2	2	4	2	2 and 4
3	3	Adsorption	Unclarified fermentation broth	15	1	1	2/4	NA	5
4	4	Equilibration	Demineralized water	20	2	1	1	NA	5
4	4	Elution wash	Demineralized water	20	2	1	2	NA	3 and 4
5	5	Adsorption wash	Demineralized water	20	2	2	2/4	2	1

Table 6. Experimental conditions tested for purification of GABA from unclarified fermentation broth.

Exp. No.	Experimental description	Feed [mL mL$_{SBV}^{-1}$]	Elution stream [mL mL^{-1} feed]	Regeneration stream [mL mL^{-1} feed]	DEMI stream [mL mL^{-1} feed]
001	1 column feed without ER, 2 column 5 wt % NaOH elution with fractionation	1	2	2	6
002	1 column feed without ER, 2 column 8 wt % NaOH elution with fractionation	1	2	2	6
003	1 column feed without ER, 2 column 8 wt % NaOH elution modified fractionation compared to 003	1.2	2	2	4.56
004	2 column feed with ER, increased SBH, 2 column 8 wt % NaOH elution with fractionation	0.7	2	2	5.5

4 Results and Discussion

4.1 System and Software Validation

As described above, the preliminary experiments were done to validate the system functionality in EBA-SMB mode with bed level control. The recipe in Tab. 5 was initially tested without compensation position and with active bed level control. Shifting discontinuous patterns as observed in Fig. 9 explained that the usage of flow control resulted in varying durations (X-axis) of the same position (Y-axis) for different columns (Col1 to Col5). This further resulted in suboptimal progression of columns through the process and disturbed the steady state.

To counter this, compensation positions were introduced after each active bed level controlled position. These compensation positions with their adaptable switch duration ensured a proper flow of columns through the process and continuous operation of the system (Fig. 10). The results observed in this figure proved that the system meets the critical process requirement of EBA to maintain a desired bed expansion without disturbing the time-based switching functionality of an SMB system.

4.2 Purification of GABA from Unclarified Fermentation Broth

On proving the new EBA-SMB control strategy, the system operation was tested for several SMB cycles in EXP001. An SMB cycle is complete when a column runs through all the defined zones and positions described in Tab. 5. In case of EXP001, adsorption and adsorption wash zones were active bed level controlled with two columns/zone resulting in four positions/zone, two active bed level control positions, and two compensation positions.

From Fig. 11, it is evident that the pH profile of the product stream coming out of different SMB columns passing through the elution zone lies in a similar window. This qualitatively implied consistent product quality at a

No compensation positions
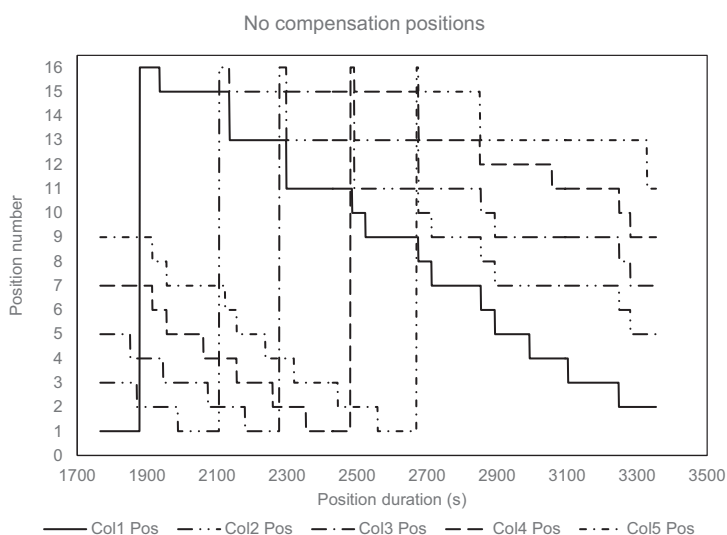
Figure 9. EBA-SMB experimental logged data describing the switching of eight columns in the absence of compensation position.

With Compensation positions

Figure 10. EBA-SMB experimental logged data describing the switching of eight columns in the presence of a compensation position.
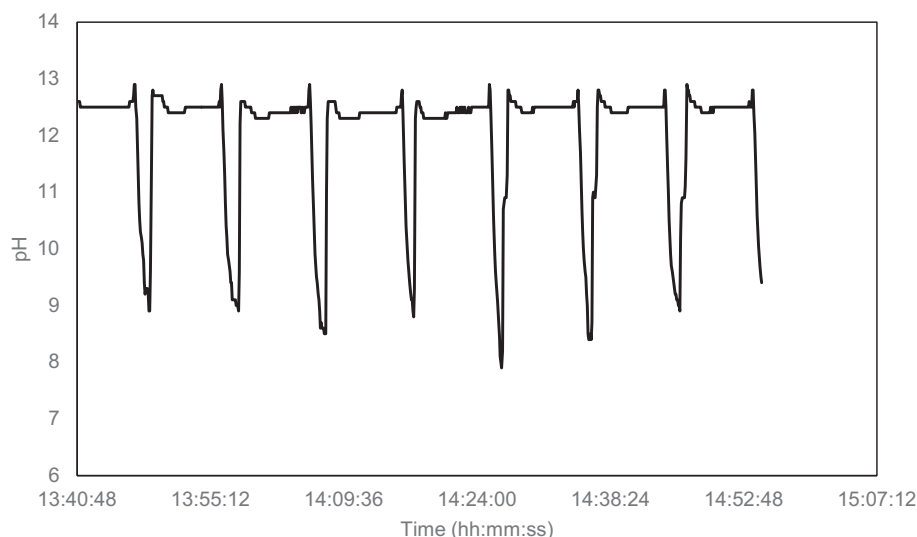
Figure 11. Outlet pH profile while different columns move through the product collection position after two cycles.

fied fermentation broth resulted in a product stream containing about $40\,g\,L^{-1}$ GABA with a purity of $>92\,\%$ and $>98\,\%$ removal of biomass.

Though the desired process requirements to purify GABA from unclarified broth in SMB mode was achieved, the overall GABA yield remained at about 64 % with 8-column EBA-SMB configuration. Therefore, it is proposed to modify the hardware and introduce more columns in the critical zones to achieve higher product yields.

5 Conclusions

The experimental results proved that the EBA-SMB technology processed complex biological feed streams in one step by integrating both solid/liquid separation and selective product capture. The desired product purity was achieved. The EBA-SMB design was validated by processing the feed stream for several 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.

The experiments also demonstrated that the integration and control system is sensitive to sensor inputs and therefore requires robust and stable sensors. 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, thus providing an operating window for further optimization.

cyclic steady state of operation after two cycles. The quality was defined by removal of biomass and other soluble impurities.

Further experiments described in Tab. 6 were performed to optimize the EBA-SMB process. The GABA binding capacities/ SBV (settled bed volume), product titer, and recoveries in case of optimization experiments are summarized in Tab. 7.

The results demonstrate that decreasing the amount of feed/ column and increasing the number of columns in the feed zone from one to two and ER had a positive impact. By this approach, GABA bound in the 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 the feed zone was limited to two. Therefore, to achieve further increase in yield and higher binding capacities, it is required to configure more than two columns in the feed zone

The experiments performed to study the impact of buffer strength proved that by raising the NaOH content from 5 to 8 wt % in the elution buffer the GABA recovery increased from about 55 % (EXP001) to about 84 % (EXP003). This along with fractionation resulted in an increment of the product titer from 33 to $47\,g\,L^{-1}$. Further, from the impurity analysis, it was also determined that the one-step EBA-SMB purification of unclari-

Table 7. Experimental results for purification of GABA from unclarified fermentation broth using EBA-SMB.

Exp. No.	Binding capacity [$g_{GABA}\,L_{SBV}^{-1}$]	Product titer [$g_{GABA}\,L^{-1}$]	GABA recovery in elution [%]	Binding in feed zone [%]	Overall GABA yield [%]
001	65.4	33	55.02	53	29.29
002	63	47	78.46	50	39.56
003	82	47	84.45	51	43
004	50	50	60	74	45

Symbols used

D_{ax}	$[m^2 s^{-1}]$	axial dispersion
r_P	$[\mu m]$	particle radius
u	$[m\,s^{-1}]$	linear velocity

Greek letter

μ	$[Pa\,s]$	viscosity

Abbreviations

BV	bed volume
CPPs	critical process parameters
EBA	expanded-bed adsorption
GABA	γ-amino butyric acid
IX	ion exchange
HETP	height equivalent theoretical plates
LI	expanded bed level sensors
PID	proportional integral derivative
SAC	strong acid cation
SBA	strong base anion
SBV	settled bed volume
SMB	simulated moving bed
S/L	solid/liquid
WAC	weak acid cation
WBA	weak base anion

References

[1] E. Annevelink, B. van Gogh, P. Bartels, J. Broeze, J. van Dam, J. Groot, N. Koenderink, M. van den Oever, J. Snels, J. Top, D. Willems, *How to Achieve Resource Use Efficiency in Integrated Food and Biobased Value Chains*, Wageningen Food & Biobased Research, Institute within the legal entity Stichting Wageningen Research, Wageningen **2016**.

[2] L. Michael, *Licentiate Thesis*, KTH Royal Institute of Technology, Stockholm **2014**.

[3] K.-M. Kim, J. W. Lee, S. Kim, F. V. Santos da Silva, A. Seidel-Morgenstern, C.-H. Lee, *Chem. Eng. Technol.* **2017**, *40*, 2163–2178. DOI: https://doi.org/10.1002/ceat.201700206

[4] *Ion Exchange Chromatography and Chromatofocusing Principles and Methods*, 11-0004-21, AA, Amersham Biosciences, Little Chalfont, UK **2004**.

[5] www.adareng.com/es/articulo/chromatography-types/n-41 (Accessed on March 22, 2018)

[6] A. A. Shukla, M. R. Etzel, S. Gadam, *Process Scale Bioseparations for the Biopharmaceutical Industry*, Taylor & Francis Group, New York **2007**.

[7] A. Lihme, M. Hansen, M. Olander, E. Zafirakos, in *Downstream Processing of Proteins*, Vol. 9, (Ed: M. A. Desai), Humana Press, Totowa, NJ **2000**.

[8] www.therapurebio.com/plasmacap/expanded-bed-adsorption (Accessed on March 22, 2018)

[9] T. B. S. Giddey, *J. South Afr. Inst. Min. Metall.* **1980**, *80 (12)*, 431–435.

[10] A. Karau, C. Benken, J. Thommes, M. R. Kula, *Biotechnol. Bioeng.* **1997**, *55 (1)*, 54–64. DOI: https://doi.org/10.1002/(SICI)1097-0290(19970705)55:1<54::AID-BIT7>3.0.CO;2-W

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

[12] Q. Zhanga, J. Crittenden, K. Hristovski, D. Hand, P. Westerhoff, *Water Res.* **2009**, *43 (7)*, 1859–1866. DOI: https://doi.org/10.1016/j.watres.2009.01.028

[13] www.fast-software.de/documentation.html (Accessed on February 07, 2018)

[14] P. R. Wright, B. J. Glasser, *Bioeng. Food Nat. Prod.* **2001**, *47 (2)*, 474–488. DOI: https://doi.org/10.1002/aic.690470224

[15] A. Rajendran, G. Paredes, M. Mazzotti, *J. Chromatogr., A* **2009**, *1216 (4)*, 709–738. DOI: https://doi.org/10.1016/j.chroma.2008.10.075

[16] www.spire2030.eu/prodias (Accessed on February 07, 2018)