Expanded Bed Adsorption of γ-aminobutyric Acid from E. coli broth by CS16GC and IRC747 Resins

Expanded-bed adsorption (EBA) is an efficient downstream technology that enhances the techno-economic potential of bio-based industries. However, application of EBA for bulk biochemicals requires the use of industrial resins. Therefore, two cation exchangers, namely, gel-type CS16GC and porous IRC747, were tested to purify γ-aminobutyric acid (GABA) from unclarified E. coli fermentation broth. Experiments compared the impact of gel-type and macroporous resin properties on the EBA process performance. As an outcome, the gel-type resin exhibited higher GABA binding capacity of compared to that of macroporous resin. This was due to improved hydrodynamics and uniform flow distribution in the case of gel-type resin. Further, CS16GC effectively removed ≥ 99 % of impurities and achieved ≥ 97 % GABA yield.

Keywords: γ-Aminobutyric acid, Expanded-bed adsorption, Fermentation broth, Ion exchange, Organic acids

Received: June 08, 2018; revised: September 21, 2018; accepted: September 21, 2018
DOI: 10.1002/ceat.201800295

1 Introduction

Expanded-bed adsorption (EBA) is a technology that is efficient in processing biomass containing fermentation streams. EBA enables both solid/liquid (S/L) separation and primary product capture with relatively high product-specific selectivity [1]. The EBA process involves an adsorbent resin bed fluidized by upward feed flow, where the bed void allows particulate biomass to pass through and capture target molecules [1]. However, unlike the traditional fluidized bed, EBA employs a flow distribution system that helps in achieving improved height equivalent to theoretical plates (HETP) [2] (Fig. 1).

Similar to other technologies, it is critical to understand the underlying mechanisms to optimize a unit operation. In case of EBA, mass transfer of target molecules from liquid to solid phase and kinetics in the solid phase are two mechanisms that define binding/elution. The rate at which these phenomena occur is dependent on several parameters. For example, mass transfer is dependent on the molecular diffusion coefficient, i.e., it varies with the molecular weight of a compound, as well as on density and viscosity of the liquid stream and specific surface area, defined by bed void, particle diameter, pore diameter, and porosity. Adsorption kinetics depends on functionality of the adsorbent and target compound.

In the case of traditional ion-exchange resins, the adsorption kinetics for small molecules like organic acids or amino acids is rapid. Therefore, mass transfer often is the limiting mechanism [3, 4]. These fundamental mechanisms hold for EBA and further define its performance [5]. Therefore, for EBA resin selection, the physiochemical interactions between the resin and target compound, the hydrodynamic properties like resin particle diameter and density, feed viscosity and density, linear velocity, and axial dispersion are critical factors [6].

Depending on the resin used, different diffusion models are applicable for EBA [7]. When applying macroporous resins, both homogeneous film and porous diffusion models are appli...
cable, while only film diffusion is applicable for gel-type resins [7]. A micrographic picture of gel-type and macroporous resin types is given in Fig. 2. The difference between porous resins and gel-type resins is displayed in Fig. 3.

Figure 2. Micrographic pictures of (a) gel-type and (b) macroporous-type polystyrene resins [12].

In the current work, EBA technology is specifically developed for separation of small molecules like organic acids from unclarified fermentation broth. EBA application for small molecules with molecular weights < 1000 Da has been scarcely published till date. Wesselingh et al. firstly investigated fluidized-bed adsorption in 1980. A fluidized-bed column with perforated plates separating the column into several stages was tested using a gel type ion-exchange resin [8]. However, no tests were performed using biological feed streams. In 1990, Gailliot et al. discussed the application of fluidized-bed adsorption for separation of immunomycin from unclarified fermentation broth using porous ion-exchange resin types SP-207 and HP-20 [9]. In 1993, Agosto et al. investigated the separation of amino acids using fluidized-bed adsorption in a multistage column involving gel-type duolite c-20 resin [10]. However, the paper did not discuss any tests using fermentation broth as feed stream. In 1995, Thömnes et al. discussed the impact of hydrodynamics on the performance of fluidized-bed adsorption, during which a protein was considered to be the exemplary compound [11]. Post-1995, the application of EBA has been concentrated in protein purification.

2 Resin Selection

The first step for developing an EBA process is to choose a suitable resin. In the current case, the following assumptions were made for identifying a resin that can purify GABA from E. coli fermentation broth in expanded bed mode:

1. The low molecular weight of GABA along with rapid mass transfer and adsorption kinetics compared to proteins results in similar capacities of GABA binding to gel-type and macroporous resins.
2. Homogeneous film diffusion is less sensitive to axial dispersion ($D_{\text{ax}}$), particle size ($r_p$), and column verticality compared to intraparticle porous diffusion [7].

Based on the above assumptions, low-density (1100–1300 g L$^{-1}$), high-capacity (> 1.0 eq L$^{-1}$), and relatively low-cost commercial ion-exchangers based on divinylbenzene (DVB) matrix were chosen instead of high-density (2400–3600 g L$^{-1}$), low-capacity (0.4 eq L$^{-1}$), expensive agarose-based EBA resins.

However, the current approach of using DVB resins results in maximum operating flow rates lower than that of high-density EBA resins. Nevertheless, the high capacity, faster mass transfer kinetics for small molecules and low resin costs were analyzed to prove the economic advantage of using high-capacity, low-density DVB resins.

3 Materials and Methods for EBA Studies

3.1 Exemplary System

Purification of $\gamma$-aminobutyric acid (GABA) from E. coli sugar fermentation broth was chosen as the exemplary system to develop the EBA process. GABA was considered to be a representative component for renewable organic acids produced using state-of-the-art biotechnology. Fig. 4 shows the molecular structure of GABA.

3.2 Experimental Approach

In the first stage of the study, the following (functionality) resins were tested for affinity towards GABA (as a zwitterion GABA exhibits both cationic and anionic properties, dependent on pH),

- Strong acid cation resins (SAC),

Figure 3. Difference between porous resins and gel-type resins [3, 4].
Figure 4. Schematic GABA molecular structure.

- Strong and weak base anion resins (SBA and WBA).
- Non-functionalized resins (polymeric adsorbent).

Based on the affinity constants \((K_i)\) determined per resin, for GABA separation, a porous and gel-type DVB resin with similar density, affinity, and capacity were further tested in EBA mode. The chosen performance indicators and requirements for EBA included:

- Optimal flow distribution that generated uniform bed fluidization.
- Bed expansion ensuring uniform liquid void sufficient for suspended solids to flow through.
- Hydrodynamic and physicochemical properties of the feed and resin should enable sufficient binding of the target compound.
- Achieve a product purity comparable to that of a multistep solid-liquid (S/L) separation and fixed-bed adsorption.
- Form an economically viable alternative to the multistep S/L separation and fixed-bed adsorption.

3.3 Feed Composition

Physiochemical properties of the GABA feed stream used for testing the EBA technology for the separation of small molecules are summarized in Tab. 1.

3.4 Resin Screening

All resin screening experiments were done in packed-bed mode on bench-scale using an Äkta Purifier equipped with UV, conductivity, and pH detection. A tracer solution, which does not interact with the resin, was used to study the column integrity. The tracers included 2% acetone and 0.8 M NaCl. Furthermore, the retention volume of GABA was compared with that of the tracer solution to determine the affinity constant \((K_i)\); see Tab. 2. The resins that exhibited high affinity were then used for EBA testing with real fermentation broth.

Table 2. Resin test method for determination of \(K_i\).

| Step          | Solution                  | Amount | Flow rate [m h\(^{-1}\)] |
|---------------|---------------------------|--------|-------------------------|
| Loading       | Synthetic GABA 120 g L\(^{-1}\), 0.67 g/100 mL NH\(_4\)\(^+\) | 0.5 mL | 5                       |
| Wash          | Demineralized water       | 1 BV   | 5                       |
| Elution       | NH\(_4\)OH 5 wt %         | 3 BV   | 5                       |
| Wash          | Demineralized water       | 2 BV   | 5                       |
| Regenerate    | H\(_2\)SO\(_4\) solution 4 wt % | 4.5 BV | 5                       |
| Wash          | Demineralized water       | 4 BV   | 5                       |

\[ K_i = \frac{RV_i - RV_0}{RV_0} \] (1)

Eq. (1) gives the definition of \(K_i\) (equilibrium constant); herein, \(RV\) is the GABA retention volume and \(RV_0\) is the retention of the volume tracer compound.

3.5 EBA Column Dimension

The selected resins, i.e., a gel-type and a macroporous, were gravity-packed to a settled bed height of 20.5 cm in a column with a total height of 45 cm and an internal diameter (ID) of 1.95 cm. The column design was performed to ensure the desired flow distribution at the inlet and maintain minimum back pressure. A reliable flow distribution system was created by using a short bed of inert high-density type YTZP, 0.6–0.8 mm ceramic beads at the column inlet. A 0.5-mm mesh was used at the column inlet to stop the beads from flowing out of the column and still enable unclarified broth to flow through. The high density of the ceramic beads also allowed them to remain at the bottom of the column. In the current case, fixed top columns with no air compartment in the column were used.

3.6 Packed-Bed Column Dimension

A column with 1.5 cm ID and 22 cm total height was packed with resin to a bed height of 20 cm under a downward operating flow rate of 300 cm h\(^{-1}\) for 20 min.
3.7 Evaluating EBA vs. Packed Bed and Gel-Type vs. Porous Resin

Six experiments were performed, two in EBA mode with gel-type resin (EXP001, EXP002), two in EBA mode with macroporous resin (EXP003, EXP004), and two in packed-bed mode with gel-type resin (EXP005, EXP006). The results obtained were used to determine the extent to which the process requirements described in the experimental approach have been achieved. The experimental conditions tested are described in Tab. 3.

### 4 Results and Discussion

#### 4.1 Resin Screening Tests

An overview of the screened resins is presented in Tab. 5. Based on the results, it was concluded that IEX SAC resins (7, 8, 10, and 11) showed high affinity for GABA. From the four, the CS16GC gel-type and IRC747 UPS macroporous resins were chosen for testing in EBA mode due to their high $K_i$, similar capacity, and a resin-specific gravity > 1.1 g mL$^{-1}$. The properties of these two resins are listed in Tab. 4.

The above resins exhibited stable bed expansion while fluidized with demineralized water at 3 m h$^{-1}$. CS16GC resulted in an expansion factor (ratio of expanded bed height to settle bed height) of about 1.35, while IRC747 had an expansion factor of 1.3. The resins tested for their affinity towards GABA are summarized in Tab. 5.

### Table 3. Experimental conditions for testing EBA performance.

| Step   | Stream          | Amount (BV) | Flow rate [m h$^{-1}$] |
|--------|-----------------|-------------|------------------------|
| Loading| Fermentation broth | 1           | 3                      |
| Wash   | Demineralized water | 3–4         | 3                      |
| Elution| 8 wt% NaOH | 4           | 3                      |
| Wash   | Demineralized water | 4           | 3                      |
| Regenerate | 4 wt% H$_2$SO$_4$ | 3–4         | 3                      |
| Wash   | Demineralized water | 3–4         | 3                      |

### Table 4. Properties of EBA resin choices.

|                     | CS16GC | IRC747 UPS |
|---------------------|--------|------------|
| Porosity            | Gel type | Macroporous |
| Density/specific gravity [g L$^{-1}$] | 1150–1200 | 1100–1140 |
| Operating pH        | 0–14    | 0–14       |
| Maximum operating temperature [°C] | 120     | 80         |
| Average particle diameter [μm] | 300     | 500        |
| Ion-exchange capacity [eq L$^{-1}$] | 1.7–1.8 | 1.7–1.9    |

### Table 5. Overview of resins tested for affinity towards GABA and the determined $K_i$.

| Proposed resin for trial | Resin | Supplier | Particle size [μm] | RV tracer [mL] | RV GABA [mL] | Result $K_i$ | DVB [%] | [eq L$^{-1}$] |
|--------------------------|-------|----------|-------------------|---------------|--------------|--------------|---------|-------------|
| 1                        | Amberlite XAD761 | More polar XAD | DOW | 21–60 | 6 | 6 | No affinity | NA | NA |
| 2                        | Amberlite FPX68 | Microporous aromatic polymer | DOW | 350–600 | 5 | 5 | No affinity | NA | NA |
| 3                        | DOWEX Monosphere 99Ca/320 | Metal interaction | Dow | 300–330 | 5 | 4.8 | No affinity | NA | NA |
| 4                        | Lewatit MDS 1368 Na/320 | Ion exclusion | Lanxess | 300–330 | 5 | 4 | No affinity | NA | NA |
| 5                        | ODS-AQ-HG | Hydrophobic | YMC | 50 | 7 | 8 | No affinity | NA | NA |
| 6                        | AS510GC | Anion exchange | Finex | 300 | 5 | 5 | No affinity | NA | NA |
| 7                        | CS16GC | SAC | Finex | 300 | 5 | 60 | 11 | 8 | >1.8 |
| 8                        | Amberlite 252 H | SAC | Dow | 590–840 | 4.8 | 36 | 6.5 | 12 | >1.7 |
| 9                        | Lewatit K1221 | SAC | Lanxess | 500–622 | NA | NA | NA | 4 | 1.2 |
| 10                       | Lewatit K2629 | SAC | Lanxess | 500–600 | 4 | 36 | 8 | 18 | 1.7 |
| 11                       | Amberlite IRC 747 UPS | Na$^+$/H$^+$ IEX | Dow | 550–560 | 4 | 55 | 12.4 | 1.75 |
| 12                       | Lewatit VPOC 1026 | Polymer impregnated with D2EPA | Dow | 520–660 | 4.3 | 5 | 0.14 | NA | NA |
4.2 Gel-Type vs. Porous Resin

Three sets of duplicate experiments were performed under the operating conditions described in Tab. 3. The chromatograms of UV and conductivity signals for EBA experiments using gel-type and macroporous resins are depicted in Fig. 5.

It has to be noted that the slight shift in peaks between EXP001 and EXP002 is due to the shorter adsorption wash of the three settled-bed volumes (BV) in case of EXP002 compared to the four BV in case of EXP001. From the chromatograms, it was evident that in EBA mode the UV peak height (between 350 and 600 mL) in case of the CS16GC gel-type resin is 5000 mAU compared to a peak height of 1800 mAU in case of the IRC747 porous resin. However, the UV signal was not solely due to GABA and also implied other impurities. Therefore, to confirm the performance of gel-type and porous resin in EBA mode, the fractions collected were analyzed using a HPLC Aminex 87 C column for GABA, Aminex 87 H column for sugars and organic acid impurities and dry matter content by incubating the samples at 105°C.

From the analytics it was estimated that the CS16GC resin exhibited an average binding capacity of about 74 g GABA L⁻¹, whereas the IRC 747 resin had an average binding capacity of about 8 g GABA L⁻¹ under the same binding and elution conditions. Further, a product yield of 97 % was achieved using the gel-type resin and the peak fraction contained a product titer of 63.5 g GABA L⁻¹. This indicated that the gel-type CS16GC resin is more suitable for application in EBA mode compared to the IRC747 macroporous resin. The poor performance of the IRC747 was due to preferential flow channeling observed during expansion with unclarified broth, demonstrated in Fig. 6. Flow channeling resulted in low HETP of about 8 plates m⁻¹ for IRC747 compared to about 40 plates m⁻¹ in case of CS16GC, under similar conditions. The channeling was mainly due to the low density and surface properties of the IRC747 resin.

4.3 EBA vs. Packed Bed

Similar to gel-type vs. porous resin, the peak fractions obtained from the packed-bed experiments of gel-type resin were analyzed for GABA and other impurities. In Fig. 7, it was determined that the CS16GC resin exhibited a binding capacity of 93 g GABA L⁻¹ BV in packed-bed mode. The sugar and organic acid impurities were reduced to as low as 0.01 g L⁻¹ in case of both EBA and packed-bed mode.

Preliminary conclusions: The results obtained from the batch experiments clearly proved that the gel-type resin CS16GC
exhibited better binding properties in EBA mode compared to IRC 747 macroporous resin. The DVB-based macroporous resin failed to address the primary requirement to enable uniform bed fluidization. Further, the product purity using gel-type EBA was comparable to that of multistep S/L separation and packed-bed adsorption. However, the EBA mode resulted in 20% loss in CS16GC resin binding capacity compared to packed-bed mode under similar operating conditions. This was due to the lower specific surface area in EBA mode as a result of a higher bed void compared to the packed bed. Therefore, in the next stage, the process economics for the GABA case using EBA and packed-bed operations were evaluated to achieve a holistic conclusion.

4.4 Process Economics

The process economics were evaluated for two scenarios. Scenario 1 as displayed in Fig. 8 involved a set of S/L separations and packed-bed adsorption. Scenario 2 included direct loading of unclarified broth through an EBA column as demonstrated in Fig. 9.

As the input stream, 60 m$^3$h$^{-1}$ fermentation broth containing 85 g L$^{-1}$ GABA was assumed, processing 37 kt a$^{-1}$ GABA in 7200 production hours (assuming 65 maintenance days). The selling price of GABA was assumed to be 7500 € t$^{-1}$, which resulted in an annual revenue of 275 M€. Further, it should be noted that the current economic evaluation was performed using estimates based on practical knowledge, and similar assumptions were made for both scenarios to make them comparable. Additionally, the capital expenditure (CAPEX), operating expenditure (OPEX) and cost of goods (COGs) do not include costs incurred due to the fermentation and final formulation steps like crystallization and drying.

4.4.1 CAPEX

CAPEX estimation was based on the bare equipment cost (BEC), which were calculated from the unit costs assumed in Tab. 6. Changing the process from packed bed to expanded bed resulted in a reduction of BEC from 14.5 M€ to 9.5 M€. The major cost category in typical breakdown of CAPEX is the fixed capital investment (FCI). Guthrie’s method was chosen for the estimation of FCI due to its level of detail and reli-

Figure 6. Flow channeling of (a) macroporous vs. (b) gel-type resin when feeding unclarified fermentation broth.

Figure 7. UV and conductivity chromatograms of runs performed using gel-type (EXP001; EXP002) resin in packed-bed mode with clarified fermentation broth as feed.

Figure 8. Unit operations involved for clarification of fermentation broth and capture of GABA according to EXP005; EXP006.
ability. Based on Guthries’s method for a process handling dilute and large streams > 50 m³h⁻¹, percentage factors of BEC were assumed for inside battery limits (ISBL) costs. Outside battery limit (OSBL) cost was assumed to be 40% of the ISBL cost. As the plant was assumed to be established in a site with existing infrastructure for utilities, the start-up costs were considered minimal at 8% of FCI. The contribution of the working capital (20% of sales revenue) to the total capital investment was ignored assuming continuous production and sales. As a result, CAPEX of scenario 1 was calculated to be 60.9 M€ over 39.9 M€ for scenario 2, thus resulting in about 35% reduction in CAPEX due to implementation of EBA.

4.4.2 OPEX

OPEX was estimated based on raw materials, utilities, and consumables costs for scenario 1 and 2 as described in Tab. 7. The total cost in Tab. 7 per year remained the same for EBA and packed-bed cases. This was because the EBA process due to lower binding capacity required more resin, NaOH, and H₂SO₄.

In both scenarios, the cost of consumables and raw materials were the major contributors, as the resins and membranes had to be replaced in regular intervals and a large amount of NaOH is required for elution. In the current case, it was also assumed that 60% of NaOH and H₂SO₄ stream is recycled for both packed bed and EBA at a cost of 5 €t⁻¹ waste stream.

From the calculated economic figures, the COGs decreased from 1831.5 €t⁻¹ to 1663.3 €t⁻¹ GABA on implementing EBA. This resulted in an additional cost savings of about 6.2 M€ with 35% lesser CAPEX, thereby explaining the techno-economic potential of an EBA process using gel-type resin. In addition, higher GABA yields due to implementation of one step EBA in place of multistep S/L separation and packed-bed adsorption can further contribute to the economic advantage.

5 Conclusions

Current studies proved that the CS16GC gel-type resin performed better than the IRC 747 in EBA mode to process unclarified E. coli fermentation broth containing GABA. This was mainly due to poor hydrodynamics in case of IRC 747, which resulted in low HETPs compared to CS16GC. The gel-type resin not only met the key process requirements for EBA by ensuring good flow distribution, product binding, and impurity removal, but also resulted in a step yield of > 97% GABA. The chromatograms and mass balance from analytics further proved that sufficient cleaning and regeneration of the CS16GC resin was possible. Based on the techno-economic evaluation, application of CS16GC resin for GABA is a proof-of-concept to expand the application of EBA technology for separation of bulk and fine bio-based chemicals. Therefore, it is interesting to study this approach for other exemplary systems.

Acknowledgment

The authors would like to thank the European Union for the grant support under SPIRE 2030 platform and BASF SE for the managing of and continuously supporting the project progress.

The authors have declared no conflict of interest.
Symbols used

\[ D_{ax} \quad [\text{m}^2\text{s}^{-1}] \quad \text{axial dispersion} \]
\[ r_p \quad [\text{\mu m}] \quad \text{particle radius} \]
\[ RV \quad [\text{mL}] \quad \text{GABA retention volume} \]
\[ RV_0 \quad [\text{mL}] \quad \text{retention of the volume tracer compound} \]
\[ u \quad [\text{m s}^{-1}] \quad \text{linear velocity} \]

Greek letter

\[ \mu \quad [\text{Pa s}] \quad \text{viscosity} \]

Abbreviations

BEC \quad \text{bare equipment cost} \]
BV \quad \text{settled-bed volume} \]
CAPEX \quad \text{capital expenditure} \]
COGs \quad \text{cost of goods} \]
CPP \quad \text{critical process parameter} \]
DVB \quad \text{divinylbenzene} \]
EBA \quad \text{expanded-bed adsorption} \]
FCI \quad \text{fixed capital investment} \]
GABA \quad \gamma\text{-aminobutyric acid} \]
ISBL \quad \text{inside battery limit} \]
IX \quad \text{ion exchange} \]
HETP \quad \text{height-equivalent theoretical plates} \]
LI \quad \text{expanded-bed level sensors} \]
OPEX \quad \text{operating expenditure} \]
OSBL \quad \text{outside battery limit} \]
PID \quad \text{proportional integral derivative} \]
SAC \quad \text{strong acid cation} \]
SBA \quad \text{strong base anion} \]
SMB \quad \text{simulated moving bed} \]
S/L \quad \text{solid/liquid} \]
WAC \quad \text{weak acid cation} \]
WBA \quad \text{weak base anion} \]

References

[1] A. Lihme, M. Hansen, M. Olander, E. Zafirakos, in Downstream Processing of Proteins (Ed: M. A. Desai), Humana Press, Totowa, NJ 2000, Vol. 9.
[2] www.therapurebio.com/plasmacap/expanded-bed-adsorption (Accessed on March 22, 2018)
[3] Q. Zhang, 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
[4] www.fast-software.de/documentation.html (Accessed on February 07, 2018)
[5] 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
[6] T. B. S. Giddey, J. South Afr. Inst. Min. Metall. 1980, 80 (12), 431–435.
[7] P. R. Wright, B. J. Glasser, Bioeng. Food Nat. Prod. 2001, 47 (2), 474–488. DOI: https://doi.org/10.1002/ai960470224
[8] A. Buijs, J. A. Wesselingh, J. Chromatogr. A 1990, 60 (5), 370–375. DOI: https://doi.org/10.1016/0021-9673(90)83888-1
[9] F. P. Gailliot, C. Gleason, J. J. Wilson, J. Zwarick, Biotechnol. Prog. 1990, 6 (5), 319–327. DOI: https://doi.org/10.1021/bp00005a009
[10] M. Agosto, N. H. L. Wang, P. C. Wankat, Ind. Eng. Chem. Res. 1993, 32 (9), 2058–2064. DOI: https://doi.org/10.1021/iel00021a030
[11] J. Thommes, M. Weiher, A. Karau, M. R. Kula, Biotechnol. Bioeng. 1995, 48 (4), 367–374. DOI: https://doi.org/10.1002/bit.260480409
[12] www.adareng.com/es/articulo/chromatography-types/n-41 (Accessed on March 22, 2018)