Enhanced extraction of butyric acid under high-pressure CO₂ conditions to integrate chemical catalysis for value-added chemicals and biofuels

Jaesung Chun, Okkyoung Choi and Byoung-In Sang*

Abstract

Background: Extractive fermentation with the removal of carboxylic acid requires low pH conditions because acids are better partitioned into the solvent phase at low pH values. However, this requirement conflicts with the optimal near-neutral pH conditions for microbial growth.

Results: CO₂ pressurization was used, instead of the addition of chemicals, to decrease pH for the extraction of butyric acid, a fermentation product of Clostridium tyrobutyricum, and butyl butyrate was selected as an extractant. CO₂ pressurization (50 bar) improved the extraction efficiency of butyric acid from a solution at pH 6, yielding a distribution coefficient (D) 0.42. In situ removal of butyric acid during fermentation increased the production of butyric acid by up to 4.10 g/L h, an almost twofold increase over control without the use of an extraction process.

Conclusion: In situ extraction of butyric acid using temporal CO₂ pressurization may be applied to an integrated downstream catalytic process for upgrading butyric acid to value-added chemicals in an organic solvent.

Keywords: Clostridium tyrobutyricum, Butyric acid, Extraction process, Carbon dioxide, High pressure

Background

Short chain fatty acids (SCFA) including butyric acid have potential to be promising platform chemicals for the production of many chemicals and biofuels. Through the chemical catalytic reaction, butyric acid can be converted into hydrocarbons that can be used for the vehicle fuels, such as gasoline, diesel, and jet fuel and for the various application in the fragrance, cosmetic, paint, solvent, and coating industries. Since butyric acid is produced in petrochemical process by chemical synthesis from xxx crude oils currently, there is a need to produce butyric acid from renewable carbon sources to replace its chemical synthesis and to provide the flexibility needed to accommodate regionally specific biomass [1–3]. In particular, butyric acid can be produced with acetic acid during the acidogenic phase, followed by the solventogenic phase, in Clostridia fermentations [4, 5], and can be converted to the useful platform chemicals, which can be integrated with the existed petrochemical process by chemical catalytic or enzymatic esterification, putative enzymatic decarboxylation, and catalytic decarboxylation [6].

Butyric acid production with fermentation is one of the oldest and most-studied processes, and various genera have been investigated for their feasibilities of industrial application. Clostridium tyrobutyricum, Clostridium acetobutyricum, Clostridium thermobutyricum are some of the most generally investigated and industrially used strains [7]. C. tyrobutyricum has been the preferred strain for butyric acid production [8, 9], and produced 55.2 g/L of butyric acid with 3.22 g/L/h of productivity using pretreated molasses [10] and 58.8 g/L with a productivity of 1.9 g/L/h using a combination of sweet sorghum stalks and beet molasses [11]. For the industrial scale production of butyric acid, separation and recovery technology...
of butyric acid from fermentation broth is recognized as a major challenge due to the process operation technological hurdles, but also due to product inhibition of butyric acid from fermentation broth by the toxicity of butyric acid at relatively low concentrations [12]. To be concentrated from the fermentation broth, butyric acid can be recovered by the use of nanofiltration membrane, liquid–liquid extraction, electrodialysis [13], and adsorption [14]. Since the contribution of downstream processing costs including the separation and recovery technologies is typically 30–40% of the total production costs, development of a competitive separation and recovery process is important to enable microbial production of butyric acid [15]. Type of inorganic acid or base to adjust the optimum pH for butyric acid production is also considered and these may determine how process steps can be integrated, how side streams may be reused in the process, and which separation and recovery processes can actually be used. Therefore, separation and recovery processes are required in a biorefinery to separate and purify the products and intermediates for the next stage of processing such as chemo-catalytic conversion for value-added chemical or fuel production, and to remove the inhibitory effects of butyric acids produced during fermentation. For in situ product recovery of butyric acid from fermentation broths, extractive fermentation has been attempted, and adsorption and extraction showed fairly good performance in the continuous acid recovery from anaerobic fermentation [16–18].

In the extractive fermentation, protonated species of butyric acid at low pH values below its pKa of 4.82 at 25 °C improve its extraction efficiency. However, this requires the use of cyclic pH changes to transition between optimal microbial growth conditions (pH 6–7) and partitioning into the solvent phase (pH <4). The addition of acid to the downstream partitioning process causes the accumulation of ions in the culture medium and hinders microbial growth by increasing the osmotic stress on bacteria [16, 19]. CO₂ sparging has been used to achieve temporarily lower pH values for enhancing performance of the extractive fermentation without leaving the accumulation of salts from the large additions of acid and base for pH shifts and without expense of fermentation productivity. Elevated CO₂ pressures with repeated 1-h cyclical exposure up to 60 bar of pCO₂ result in more effective pH swings (up to pH 3.8 in 5 g/L yeast extract) compared with atmospheric CO₂ sparging without having an inhibitory effect on C. tyrobutyricum [16, 20]. Recent efforts have been made to upgrade fermentation products to value-added chemicals and to further integrate chemical catalysis with extractive fermentation [6, 21–23]. One such attempt is the use of a transition-metal catalyst for alkylation in ABE fermentation for conversion to a higher-molecular-mass fuel [21]. Another is the upgrading of butyric acid to butanol by hydrogenation [24] or by esterification [25, 26], and hydrogenolysis [27–29] (Fig. 1).

To operate a continuous process integrated with extractive fermentation and catalytic process, the properties of extractant in the extractive fermentation should be considered for downstream processes such as catalytic upgrading of a fermentation product to value-added chemicals. For example, tertiary amines are easily extractible but, due to its corrosive nature and high reactivity with chemical catalysts, it requires special attention as extractants may not react with catalysts in future steps. We also considered the process by which butyric acid is integrated with value-added chemicals by investigating the use of CO₂-mediated pH swings, and chose an extractant for liquid–liquid extraction. Butyl butyrate was selected as an extractant for butyric acid because it is not an amine-type chemical or a corrosive substance that could react with the catalyst downstream [30].

Here, we show for the first time the use of high CO₂ pressure for the liquid–liquid extraction of butyric acid from fermentation medium. While a previous study of the application of high CO₂ pressure was conducted through direct absorption between cells and polymeric absorbents [20], this study used the solvent extraction under high CO₂ pressure and minimized microorganism toxicity of solvent by separation of the cells and the

![Fig. 1 Pathway used to upgrade butanol from butyric acid](image)
extraction process through cell recovery through the membrane. The aim of this study was to investigate the increase of butyric acid extraction efficiency in liquid–liquid extraction through a temporary decrease in pH using high CO₂ partial pressure.

Results and discussion
Extraction of butyrate using butyl butyrate under high pCO₂
Butyl butyrate was selected from among oleyl alcohol, dodecanol, and mixtures of trioctylamine or ditri-decylamine as a solvent for the extraction of butyric acid from the fermentation medium because it is not an amine-type chemical, nor a corrosive substance that can react with the catalyst downstream during the conversion of butyrate into various chemicals and fuels [30] (refer to Additional file 1: Figure S1 for the different extraction efficiencies of solvents). If amine-type solvents are used as extractants, it is necessary to remove the extractant before the catalytic process, which incurs an additional cost and integrates as a continuous process extractive fermentation and the catalytic process.

The distribution coefficient (D) of butyrate using butyl butyrate was found to be dependent on pH; the distribution coefficient increased up to 2.11 ± 0.19% at pH 4.0, from 0.08 ± 0.03% at pH 6.0 (Fig. 2a), due to an increase in undissociated acid forms as pH values decrease below the pKa of butyric acid (4.8). The dependence of distribution coefficients on CO₂ partial pressure is shown in Fig. 2b. From the distribution coefficients in both Fig. 2a, b, the pH value under 50 bar CO₂ in liquid extraction with an equal volume of butyl butyrate can be inferred as approximately as 4.9 because the distribution coefficients at 50 bar is 0.42, similar to the expected value at pH 4.9.

From Henry’s law (Eq. 1), the concentration of CO₂ dissolved in water at 50 atm of pressure and 298.15 K is 1.7 M.

\[ C_{aq} = kP_g \]  

(1)

The Henry’s law constant, \( k \) is 3.4 × 10⁻² mol L⁻¹ atm⁻¹ of CO₂ in water at 298.15 K, and \( P_g \) is the partial pressure of gas. The first acid equilibrium of CO₂ is predominant and to account for the fact that CO₂ (aq) is in equilibrium with H₂CO₃ (aq) and that the proton and bicarbonate concentrations are equal,

\[ K_{A1} = \frac{[H^+][HCO_3^-]}{[CO_2(aq)]} = \frac{[H^+]^2}{[CO_2(aq)]} = 4.45 \times 10^{-7} \]  

(2)

From Eqs. 1 and 2, the pH is 3.06 at 50 atm, the partial pressure of CO₂ (pCO₂). The reason the pH value under these conditions is higher than 3.06, the calculated pH at pCO₂ 50 atm (50.7 bar), is the buffering effect of medium (Additional file 1: Figure S3). The buffering effects of medium components such as yeast extract and phosphate have been observed in previous studies [20, 31]. Ammonium acetate present in the medium seems to strongly resist pH changes, and the filtrate of culture broth exhibited the strongest buffering capacity; a change in pH of ΔpH = 0.08 was found under CO₂ purging at ambient pressure (Additional file 1: Figure S3). When the amount of the butyl butyrate is twice the volume of the culture medium, the extraction efficiency of butyrate using butyl butyrate under 50 bar CO₂ was 45.7% (data not shown). Previous studies have shown that the removal of butyric acid by polyether Pebax 2533 (solid–liquid extraction) improves from 3 to 40% upon acidifying a pH 6 solution with 60 bar of CO₂ [32]. The high measured extraction efficiency of butyrate in our study indicates that the use of high pCO₂ is more efficient in the liquid–liquid phase rather than in the solid–liquid phase extractions.
The extractive fermentation of *C. tyrobutyricum*

A high pCO₂ was used for the extraction of butyric acid, a glucose fermentation product produced from *C. tyrobutyricum*. The filtrate produced from microfiltration was used for butyric acid extraction. Figure 3 shows the time profile of microbial growth and the concentration changes of glucose (substrate) and butyric acid (product) without (Fig. 3a) or with (Fig. 3b, c) liquid–liquid extraction using high pCO₂ (CO₂ pressure–liquid extraction). The total amount of butyric acid produced through CO₂ pressure–liquid extraction is the sum of butyric acid in the aqueous and organic phases. The rate of glucose consumption and butyric acid production increased compared with that produced without extraction (Fig. 3a, b). However, the optical density decreased after approximately 20 h of incubation under CO₂-pressurized liquid extraction (Fig. 3b). This phenomenon is caused by residual butyl butyrate in the aqueous phase of approximately 1.9 g/L, as measured using GC-FID (for more detail see Additional file 1: Figure S4). As a result, butyl butyrate induced microbial death, resulting in a decrease in optical density. Due to the decrease of microbial growth, production of butyric acid also decreased. The toxicity of butyl butyrate on microbial growth of 1 g/L was measured (Additional file 1: Figure S4). Therefore, an extraction reservoir was used to remove residual butyl butyrate from the aqueous phase (Fig. 4). Tetradecane was used to prevent the inflow of butyl butyrate to the aqueous phase. Figure 2c shows the increase of microbial growth associated with a higher rate of glucose consumption (5.55 g/L h) and butyrate production (3.99 g/L h) after tetradecane treatment.

The kinetic parameters for each condition are summarized and compared in Table 1. The results of extractive fermentation under 50 bar pCO₂ were divided into two conditions; without/with the removal of butyl butyrate from the aqueous phase. The final titer of butyrate was changed after CO₂ pressure–liquid extraction and was measured at 27.4 g under control conditions vs. 36.5/45.1 g under extractive fermentation conditions at 50 bar pCO₂ (Table 1). However, the productivity of butyric acid increased from 2.3 up to 3.99 g/L h. Previous studies of solid–liquid phase extraction under 60 bar showed that the productivity was decreased (0.44 vs. 0.50 g/L h), while the final titer was increased (74 vs. 68.4 g) in the batch system [20]. The productivities reported in previous studies of *C. tyrobutyricum* are shown in Table 2. The productivity in fed-batch fermentation was below 2 g/L h. The value we measured was slightly higher, 2.30 g/L h, and after extractive fermentation under 50 bar pCO₂, it increased to 3.99 g/L h (Table 1). Fed-batch fermentation was performed with two glucose feedings (each 80 g/L) (Fig. 5). Total butyrate production was 80.9 g, and the productivity of butyrate was 4.10 g/L h, which are comparable to the values measured in previous studies (Table 2). The increased production of butyrate has been demonstrated in studies of endproduct inhibition in *C. tyrobutyricum* [33]. The yield of butyrate is relatively constant (~ 0.3 g/g), but the
The production of butyrate increased at higher dilution rates [33, 36]. The in situ extraction of butyric acid increased the production rate (productivity) of butyric acid generated by *C. tyrobutyricum*.

The upgrading of carboxylic acids to their corresponding aldehydes, alcohols and hydrocarbons require carefully balanced oxygen removal reactions, such as several catalytic routes including dehydration, hydrogenolysis and hydrogenation [37]. For the production of larger molecules appropriate for diesel and jet fuels, C–C coupling reactions such as ketonization or esterification reactions can also be exploited [25, 38, 39]. In addition, butyl butyrate produced by esterification with butyric acid can be used for the production of butanol by hydrogenolysis with hydrogen [27]. The intermediate step of esterification allows milder conditions to be used compared

### Table 1 Comparison of butyrate production and extraction efficiency in batch fermentation

|                    | Control                                           | Liquid–liquid extraction under 50 bar pCO₂ | Without the recovery of butyl butyrate from the aqueous phase | With the recovery of butyl butyrate from the aqueous phase |
|--------------------|--------------------------------------------------|-------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------|
| Butyric acid production (g) | 27.4                                            | 36.5                                       | 45.1                                                          |
| Extraction efficiency (%)      | –                                                | 62.1                                       | 57.8                                                          |
| Butyric acid productivity (g/L h) | 2.30                                          | 3.39                                       | 3.99                                                          |
| Y (g butyric acid/g glucose)    | 0.38                                            | 0.30                                       | 0.38                                                          |

### Table 2 Comparison of butyrate productivity in extractive fed-batch fermentation with previous studies of *C. tyrobutyricum*

| Productivity (g/L h) | References                                           |
|----------------------|------------------------------------------------------|
| Fed-batch fermentation | 0.82 Michel-Savin et al. [33]                        |
|                      | 1.25 Fayolle et al. [34]                              |
|                      | 1.41 Song et al. [35]                                 |
|                      | 1.9 Sjöblom et al. [11]                               |
| Extractive fermentation | 2.15 Du et al. [18]                                  |
|                      | 7.37 Wu and Yang [17]                                 |
|                      | 4.10b This study                                     |

* Immobilized cells of *C. tyrobutyricum*
* Extractive fed-batch fermentation
to direct catalytic conversion to butanol. Subsequent hydrogenolysis of butyl butyrate to butanol was tested with a commercially available Cu/ZnO/Al₂O₃ catalyst prepared with 1.0 wt.% palladium under a downstream reactor pressure of 10 bar, temperature of 150–200 °C [28]. After hydrogenolysis, one part of the butanol is then used as a product and one part is used for the esterification reaction to produce butyl butyrate. By associating the above process with our work, the biohydrogen produced during the fermentation as well as the use of butyl butyrate for the extraction of butyric acid could in principle be used for the hydrogenolysis reaction making the process more sustainable. Therefore, when the extractive fermentation with butyl butyrate for butyric acid production is used under 50 bar pCO₂, the catalytic upgrading of butyric acid with particular focus on butanol as a target product is not required to supply the additional pressure and solvents. In order for catalytic upgrading to be commercially applicable, efficient recovery processes of the carboxylic acid in combination with cost-effective catalytic systems must be developed. Integrated recovery and upgrading systems with butyl butyrate under high CO₂ pressure are highly attractive and minimize waste and energy consumption.

Conclusions
CO₂ pressure–liquid extraction increased the extraction efficiency of butyric acid from a culture broth of C. tyrobutyricum. The extraction efficiency was higher (62.1%) than that previously found in studies of CO₂ pressure–solid phase extraction. The removal of butyric acid through extractive fermentation led to an increase in the productivity of butyric acid from 2.30 to 3.99 g/L h, and reached 4.10 g/L h through fed-batch fermentation.

Extraction of butyrate using butyl butyrate under high pCO₂
The dependence of distribution coefficient on pH was preliminarily tested under various pH values of 4, 4.5, 5, 5.5, 6, and 6.5. The pH was adjusted using 3 M HCl. The distribution coefficient \( D \) was calculated as per Eq. 3.

\[
D = \frac{C_{\text{sol}}}{C_{\text{aq}}}
\]

Here, \( C_{\text{sol}} \) is the concentration of butyric acid presented in the solvent phase, and \( C_{\text{aq}} \) is the concentration in the aqueous phase (fermentation broth) after extraction.

The extractive fermentation of C. tyrobutyricum
Figure 4 illustrates the processes and equipment used in this study. Fermentation was performed by connecting a flat type membrane and a hydrophilic PVDF microfiltration (MF) module (0.45 μm, 0.1 m², Millipore, USA) for cell recovery. All cultures were grown anaerobically at 37 °C, 150 rpm. The pH was initially set at 7 and controlled at pH 6 after inoculation. Batch or fed-batch fermentation was initially conducted in 3-L fermenter with a 1.5-L working volume. For preculture, 100 mL stock cultures were used to inoculate 75 mL of P2 medium for about 12 h.

Bioreactors were arranged sequentially and performed fermentation, cell recycling, and butyric acid extraction functions. For the removal of butyl butyrate, one reservoir was prepared with tetradeane (Fig. 4). The assembly used consisted of three or four jacketed bioreactors treating a fermentation culture volume of 1.5 L. The bioreactor was inoculated with 5% C. tyrobutyricum. Fermentation was allowed to proceed in batch mode for 12 h, and both cell recycling and extraction were begun. Culture broth was circulated at 20 mL/min, keeping 900 mL working
volume in the fermenter, 300 mL in the reservoir, and 300 mL in the extractor. Cells were recovered by the membrane module as a rate of 400 mL filtrate/min using microfiltration feeding at 500 mL culture-broth/min.

A 1-L extraction vessel containing 600 mL of butyl butyrate (extractant) was used to remove butyrate generated during continuous cultivation from fermentation. The extraction vessel was pressurized with CO2 at 50±5 bar. Culture broth (300 mL) and extractant (600 mL butyl butyrate) were mixed by continuous agitation at 100 rpm, and the extractor volume was maintained at 900 mL. The flow rate of output from the extractor was 20 mL/min. The pressure of the extractor was regulated by CO2 bombe and a back-pressure valve.

A reservoir was prepared with tetradecane (200 mL) for the removal of butyl butyrate from the medium to prevent its introduction into the medium. The reservoir was agitated at 100 rpm to allow full mixing. The working volumes of all of the bioreactors used were kept constant by removing extra medium with peristaltic pumps.

To operate fed-batch fermentation, additional glucose and yeast extract were added intermittently to the culture using a concentrated solution; when the glucose level fell below 5 g/L, it was replaced to adjust the initial concentration of glucose (80 g/L) and other medium components, using a 300 mL bolus. At the same time, 600 mL of butyl butyrate in the extractor was replaced for the treatment of a second batch of fermentation.

In the extractive fermentation, extraction efficiency was calculated as shown in Eq. 4.

\[
\text{Extraction efficiency (\%)} = \frac{\text{Butyric acid in the solvent phase}}{\text{Total butyric acid in the extraction vessel}}
\]

The solvent in the extraction vessel was sampled by belows valve, and the amount of butyric acid in the aqueous phase was analyzed from fermentation broth leaving the vessel after extraction. The productivity was calculated from the 900 mL working volume in the fermenter at the late exponential phase.

**Analytical method**

Cell concentrations were estimated by optical density OD, at 600 nm. Butyric acid in acidified samples with 100 mM phosphoric acid was analyzed using gas chromatography (Agilent Technologies, Model 7890, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a 30 m × 0.25 μm × 0.25 μm HP-INNOWAX column and nitrogen as carrier gas. The concentration of glucose was reflectometrically measured using an RQflex 10 reflectometer (Merck Inc.).

---

**Additional file**

**Additional file 1:** Figure S1. The different extraction efficiencies of solvents used for butyric acid extraction. **Figure S2.** A comparison of extraction efficiencies at different extraction times. **Figure S3.** pH changes after CO2 purging. **Figure S4.** Microbial growth in fresh medium including 1 g/L butyric acid with or without tetradecane treatment.

**Authors’ contributions**

JC and BIS conceived and designed the studies. JC performed the fermentation studies and analyzed the data. The manuscript was written through contributions of all authors. All authors read and approved the final manuscript.

**Acknowledgements**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 16 January 2018 Accepted: 16 April 2018

Published online: 23 April 2018

---

**References**

1. Brar SK, Sarma SJ, Pakshirajan K. Platform chemical biorefinery: future green chemistry. Amsterdam: Elsevier; 2016.
2. Lee KM, Kim KY, Choi O, Woa HW, Kim Y, Han SO, et al. In situ detoxification of lignocellulosic hydrolysate using a surfactant for butyric acid production by *Clostridium tyrobutyricum* ATCC 25755. Process Biochem. 2015;50(4):630–5. https://doi.org/10.1016/j.procbio.2015.01.020.
3. Dvidar M, Kim S, Jeon BS, Um Y, Mitchell RJ, Sang BI. Co-culturing a novel Bacillus strain with *Clostridium tyrobutyricum* ATCC 25755 to produce butyric acid from sucrose. Biotechnol Biofuels. 2013;6(1):1.
4. Zigova J, Šturdík E. Advances in biotechnological production of butyric acid. J Ind Microbiol Biotechnol. 2000;24(3):153–60.
5. Dvidar M, Park JY, Mitchell RJ, Sang BI. The future of butyric acid in industry. Sci World J. 2012;2012:471417. https://doi.org/10.1100/2012/471417.
6. Sjöblom M, Matsakas L, Christakopoulos P, Rova U. Catalytic upgrading of butyric acid towards fine chemicals and biofuels. Femis Microbiol Lett. 2016; https://doi.org/10.1053/femse/fow054.
7. Jha AK, Li J, Yuan Y, Baral N, Al B. A review on bio-butyric acid production and its optimization. Int J Agric Biol. 2014;16(5):1019–24.
8. Michel-Savin D, Marchal R, Vandecasteele J. Control of the selectivity of butyric acid production and improvement of fermentation.
performance with \textit{Clostridium tyrobutyricum}. Appl Microbiol Biotechnol. 1990;32(4):387–92.

9. Mitchell RJ, Kim JS, Jeon BS, Sang BI. Continuous hydrogen and butyric acid fermentation by immobilized \textit{Clostridium tyrobutyricum} ATCC 25755: effects of the glucose concentration and hydraulic retention time. Bioresour Technol. 2009;100(21):5352–5.

10. Jiang L, Wang J, Liang S, Wang X, Cen P, Xu Z. Butyric acid fermentation in a fibrous bed bioreactor with immobilized \textit{Clostridium tyrobutyricum} from cane molasses. Bioresearch Technol. 2009;100(13):3403–9.

11. Sjöblom M, Matsakas L, Christakopoulos P, Rova U. Production of butyric acid by \textit{Clostridium tyrobutyricum} (ATCC25755) using sweet sorghum stalks and beet molasses. Ind Crops Prod. 2015;74:335–44.

12. Zhu Y, Yang ST. Adaptation of \textit{Clostridium tyrobutyricum} for enhanced tolerance to butyric acid in a fibrous-bed bioreactor. Biotechnol Prog. 2003;19(2):365–72.

13. Jones RJ, Massanet-Nicola J, Guwy A, Premier GC, Dinsdale RM, Reilly M. Removal and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional electrodialysis. Bioresour Technol. 2015;189:279–84. https://doi.org/10.1016/j.biortech.2015.04.001.

14. López-Garzón CS, Straathof AJ. Recovery of carboxylic acids produced by fermentation. Biotechnol Adv. 2014;32(5):873–904.

15. Straathof AJ. The proportion of downstream costs in fermentative production processes. In: Comprehensive biotechnology. 2nd ed. Burlington: Academic Press; 2011. p. 811–4.

16. Peterson EC, Daugulis AJ. Demonstration of in situ product recovery of butyric acid via CO2-facilitated pH swings and medium development in two-phase partitioning bioreactors. Biotechnol Bioeng. 2014;111(5):537–44. https://doi.org/10.1002/bit.25106.

17. Wu Z, Yang ST. Extractive fermentation for butyric acid production from glucose by \textit{Clostridium tyrobutyricum}. Biotechnol Bioeng. 2003;82(1):95–102. https://doi.org/10.1002/bit.10542.

18. Doe J, Lorenz N, Beitle R, Hestekin JA. Application of wafer-enhanced electrodeionization in a continuous fermentation process to produce butyric acid with \textit{Clostridium tyrobutyricum}. Sep Sci Technol. 2012;47(1):43–51.

19. Liu YP, Zheng P, Sun ZH, Ni Y, Dong JJ, Wei P. Strategies of pH control and glucose-fed batch fermentation for production of succinic acid by \textit{Actinobacillus succinogenes} CGMCC1593. J Chem Technol Biotechnol. 2008;83(5):722–9.

20. Peterson EC, Daugulis AJ. The use of high pressure CO2-facilitated pH swings to enhance in situ product recovery of butyric acid in a two-phase partitioning bioreactor. Biotechnol Bioeng. 2014;111(11):2183–91. https://doi.org/10.1002/bit.25285.

21. Anbarasan P, Baer ZC, Sreekumar S, Gross E, Binder JB, Blanch HW, et al. Integration of chemical catalysis with extractive fermentation to produce fuels. Nature. 2012;491(7423):235–9. https://doi.org/10.1038/nature11594.

22. Bond JQ, Upadhye AA, Olcay H, Tomssett GA, Jae J, Xing R, et al. Production of biodiesel jet fuel range ketone alkanes and commodity chemicals from integrated catalytic processing of biomass. Energy Environ Sci. 2014;7(4):1500–23. https://doi.org/10.1039/c3ee43846e.

23. Sreekumar S, Baer ZC, Gross E, Padmanaban S, Gouls K, Gunbas G, et al. Chemocatalytic upscaling of tailored fermentation products toward biodiesel. ChemSusChem. 2014;7(9):2445–8. https://doi.org/10.1002/cssc.201402244.

24. Lee JM, Upare PP, Chang JS, Hwang YK, Lee JH, Hwang DW, et al. Direct hydrogenation of biomass-derived butyric acid to n-butanol over a ruthenium–tin bimetallic catalyst. ChemSusChem. 2014;7(11):2998–3001. https://doi.org/10.1002/cssc.201402311.

25. Ju IB, Lim HW, Jeon W, Suh DJ, Park MJ, Suh YW. Kinetic study of catalytic esterification of butyric acid and n-butanol over Dowex 50WX8-400. Chem Eng J. 2011;168(1):293–302. https://doi.org/10.1016/j.cej.2010.12.086.

26. Severini F, Flannelly T, Nolan DO, Leahy JJ, Kwapisinski W. Development of heterogeneous acid catalysts produced from the carbonization of \textit{Miscanthus x giganteus} for the esterification of butyric acid to butyl butyrate with \textit{n}-butanol. J Chem Technol Biotechnol. 2016;91(7):2076–84. https://doi.org/10.1002/jctb.4804.

27. Ju IB, Jeon W, Park MJ, Suh YW, Suh DJ, Lee CH. Kinetic studies of vapor-phase hydrogenolysis of butyl butyrate to butanol over Cu/ZnO/Al2O3 catalyst. Appl Catal A. 2010;387(1–2):100–6. https://doi.org/10.1016/j.apcata.2010.08.009.

28. Kim SM, Lee ME, Choi JW, Suh DJ, Suh YW. Role of ZnO in Cu/ZnO/Al2O3 catalyst for hydrogenolysis of butyl butyrate. Catal Commun. 2011;12(14):1328–32. https://doi.org/10.1016/j.catcom.2011.05.006.

29. Zhang J, Leitus G, Ben-David Y, Milstein D. Efficient homogeneous catalytic hydrogenation of esters to alcohols. Angew Chem. 2006;118(7):1131–3. https://doi.org/10.1002/ange.200503771.

30. Datta D, Kumar S, Uslu H. Status of the reactive extraction as a method of separation. J Chem. 2015;2015:1–16. https://doi.org/10.1155/2015/853789.

31. García-González L, Teschert H, Geeraerd A, Elst K, Van Ginneken L, Van Impe J, et al. Mathematical modelling and in situ determination of pH in complex aqueous solutions during high-pressure carbon dioxide treatment. J Supercrit Fluids. 2010;55(1):77–85.

32. Peterson EC, Contreras-López D, Harris J, Scott Parent I, Daugulis AJ. Characterization of pH dependence in organic acid absorption with non-reactive and reactive polymers for application in two-phase partitioning bioreactors. Chem Eng J. 2016;287:503–10. https://doi.org/10.1016/j.cej.2015.11.068.

33. Michel-Savin D, Marchal R, Vandecasteele J. Butyrate production in continuous culture of \textit{Clostridium tyrobutyricum}: effect of end-product inhibition. Appl Microbiol Biotechnol. 1990;33(2):127–31.

34. Fayolle F, Marchal R, Ballerini D. Effect of controlled substrate feeding on butyric acid production by \textit{Clostridium tyrobutyricum}. J Ind Microbiol. 1990(63):179–83.

35. Song H, Eom M-H, Lee S, Lee J, Cho J-H, Seung D. Modeling of batch experimental kinetics and application to fed-batch fermentation of \textit{Clostridium tyrobutyricum} for enhanced butyric acid production. Biochem Eng J. 2010;53(1):71–6.

36. Herrero AA. End-product inhibition in anaerobic fermentations. Trends Biotechnol. 1983;1(2):49–53.

37. De S, Saha B, Luque R. Hydrodeoxygenation processes: advances in catalytic transformations of biomass-derived platform chemicals into hydrocarbon fuels. Bioresour Technol. 2015;178:108–18.

38. Gaertner CA, Serrano-Ruz JC, Braden DJ, Dumesic JA. Catalytic coupling of carboxylic acids by ketonization as a processing step in biomass conversion. J Catal. 2009;266(1):71–8.

39. Chuck CJ, Donnelly J. The compatibility of potential bioderived fuels with Jet A-1 aviation kerosene. Appl Energy. 2014;118:83–91. https://doi.org/10.1016/j.apenergy.2013.12.019.

40. Qureshi N, Blaschek HP. Butanol recovery from model solution/fermentation broth by pervaporation: evaluation of membrane performance. Biomass Bioenergy. 1999;17(2):175–84. https://doi.org/10.1016/S0961-0039(99)00030-6.