Bioprocess and Biosystems Engineering (2018) 41:1283–1294
https://doi.org/10.1007/s00449-018-1956-6

RESEARCH PAPER

Mechanistic simulation of batch acetone–butanol–ethanol (ABE) fermentation with in situ gas stripping using Aspen Plus™

Kwabena Darkwah[1] · Sue E. Nokes[2] · Jeffrey R. Seay[3] · Barbara L. Knutson[1]

Received: 15 March 2018 / Accepted: 11 May 2018 / Published online: 22 May 2018

Abstract

Process simulations of batch fermentations with in situ product separation traditionally decouple these interdependent steps by simulating a separate "steady state" continuous fermentation and separation units. In this study, an integrated batch fermentation and separation process was simulated for a model system of acetone–butanol–ethanol (ABE) fermentation with in situ gas stripping, such that the fermentation kinetics are linked in real-time to the gas stripping process. A time-dependent cell growth, substrate utilization, and product production is translated to an Aspen Plus batch reactor. This approach capitalizes on the phase equilibria calculations of Aspen Plus to predict the effect of stripping on the ABE fermentation kinetics. The product profiles of the integrated fermentation and separation are shown to be sensitive to gas flow rate, unlike separate steady state fermentation and separation simulations. This study demonstrates the importance of coupled fermentation and separation simulation approaches for the systematic analyses of unsteady state processes.

Keywords Cell growth model · In situ separation · Fermentation · Biochemical reactors · Process simulation

Introduction

Process systems engineering (PSE) employ sophisticated mathematical models to quantify and optimize the production capacity of the chemical/biochemical and refinery industries [1]. Examples of commercial PSE tools include Aspen Plus™ (contained in Aspen Engineering Suite™, Aspen Technology; Cambridge, MA, USA), gPROMS (PSE; London, UK), UniSim® Design Suite (Honeywell International, Inc.; Morris Plains, NJ, USA), Extend (Image That, San Jose, CA, USA), and SuperPro Designer® (Intellingen; Scott's Plain, NJ, USA) [2]. Aspen Plus™ has become an industry standard for steady state process simulation [3]. However, Aspen Plus™ has limitations when simulating unsteady state processes, such as a batch acetone–butanol–ethanol (ABE) fermentation process with in situ, simultaneous product removal using gas stripping. Consumer demand for bio-based products is increasing, and PSE tools that can accurately simulate real-time product removal from batch fermentation are needed for properly designing biochemical production processes.

The alcohol products of the acetone–butanol–ethanol (ABE) fermentation process, butanol and ethanol, have properties that make them of interest for liquid transportation fuels [4–6]. Additionally, acetone and butanol are used as solvents and for the production of other chemicals [7]. Final ABE concentrations of 1–2 wt% butanol [4] or approximately 20 g/L ABE [8], ABE yields of 0.28–0.33 g/g-glucose and reactor productivities approaching 0.3 g/L/h are typical [8]. In situ product recovery techniques, such as gas stripping, adsorption, pervaporation, liquid–liquid extraction, perstraction, and reverse osmosis [4, 7, 9] have been shown to generally enhance the performance of the ABE fermentation process, putatively resulting from the removal of inhibitory products such as butanol and ethanol [6]. For example, Ezeji et al. [7] reported a 133 and 210% enhancements in the total ABE produced and ABE productivity,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00449-018-1956-6) contains supplementary material, which is available to authorized users.

✉ Barbara L. Knutson
bknut2@uky.edu

[1] Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506-0046, USA

[2] Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, KY 40546-0276, USA

[3] Department of Chemical and Materials Engineering, University of Kentucky, Paducah, KY 42002-7380, USA

respectively, when the integrated batch ABE fermentation and in situ gas stripping was compared to the batch fermentation. Gas stripping as a chemical separation method selectively removes volatile components (e.g., acetone, butanol, and ethanol) by continuously bubbling a gas through the aqueous fermentation broth [4, 6, 7]. Gas stripping is a relatively simple technology which has the option of using the fermentation product gasses (carbon dioxide and hydrogen) or another inert gas as the stripping gas and can be operated at fermentation temperatures [10].

Fermentation processes in general, are simulated by determining the rate at which microorganisms extract carbon and nutrients from the substrate within the fermentation broth and the rate at which they produce additional cells, metabolic energy, and metabolic products within a controlled environment as shown in Eq. 1 [11]:

$$\left[\frac{\delta(\text{Substrate})}{\delta t}\xrightarrow{\text{Cells}}\frac{\delta(\text{Cell biomass})}{\delta t}+\frac{\delta(\text{Metabolic energy})}{\delta t}+\frac{\delta(\text{Metabolic products})}{\delta t}\right]. \quad (1)$$

In our model system, metabolic products include acetone, butanol, and ethanol, which will partition into both the liquid and vapor phases of the reactor, affecting reactor liquid/vapor volume, and the product concentrations in the liquid and vapor phases. This partitioning depends on the thermodynamics of the system. Aspen Plus has algorithms to predict the partitioning of each chemical species, assuming the system is at steady state. The ability of Aspen Plus to describe the partitioning of ABE fermentation products from the liquid fermentation broth into the vapor headspace of the reactor and into the gas stripping stream is the main advantage of simulating the fermentation using Aspen Plus environment relative to programs that do not incorporate thermodynamics models.

Sequential steady state process simulations, which decouple the product concentration-dependence of the fermentation and separation unit operations, have been used in Aspen Plus software to simulate batch ABE fermentation with product recovery by gas stripping [12–14] (Fig. 1a). The ABE fermentation was described as a steady state reactor where stoichiometric equations with fixed product yields relative the substrate concentration (e.g., glucose) were assumed adequate to describe the kinetics [12–14]. The autocatalytic production of cells were either ignored [14–16] or represented with stoichiometric equations in which cell maintenance or growth was at a fixed rate relative to the formation of other products [13], thus eliminating the critical influence of product concentration on cell growth and inhibition. Aspen Plus then determines the partitioning of the fermentation products and the new liquid/gas volumes at the completion of the batch fermentation in the continuous steady state flow sheet environment [17].

In contrast to these previous simulation approaches, batch fermentation processes are unsteady state processes: the substrate concentration decreases and the product concentrations increase over the course of the fermentation. In actual operation, gas stripping operates simultaneously with the fermentation, so that the concentration of the products in the fermentation broth at any given time depend on both the amount of products produced by the microorganism in that time step, and the amount of product removed through the gas stripping process in that time step. Current unit operation models in Aspen Plus lack the ability to readily incorporate typical mechanistic models that describe unsteady

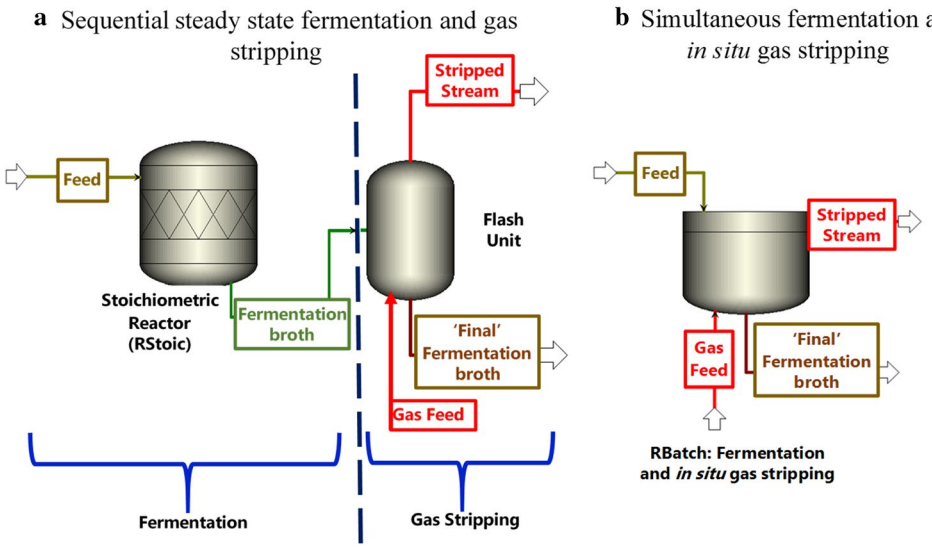

Fig. 1 Schematic of sequential ABE fermentation with gas stripping **a** as traditionally simulated in Aspen Plus; and **b** simultaneous ABE fermentation with in situ gas stripping as simulated in the modified Aspen Plus model

state fermentation processes and couple these unsteady state fermentations with in situ separations. To accurately simulate an integrated fermentation with in situ gas stripping, it is essential to know how much product will be removed from the broth, as the concentration of the product in the broth affects the product production rate and the amount of product the stripping gas can remove. Thus, in a simultaneous fermentation and in situ gas stripping model (Fig. 1b), the fermentation and separation kinetics must be recalculated at the end of each time step, because these two processes are coupled on a time-dependent basis through the fermentation product concentrations.

The objective of this study was to enable mechanistically accurate simulation of the product concentration-dependent kinetics of both the ABE fermentation and the simultaneous in situ gas stripping process in Aspen Plus. A cell-based kinetics mathematical model, a system of ordinary differential equations (ODEs) describing the ABE fermentation developed by Votruba et al. [18], was used as a model system to develop techniques in Aspen Plus to simulate an unsteady state batch fermentation. The batch reactor in Aspen Plus, RBatch block, was linked to a Fortran user kinetics subroutine (calculating the rates of generation or consumption of each component) with a gas continuously fed to the reactor to simulate the unsteady state batch fermentation and in situ gas stripping process. To verify the accuracy of the procedure developed, the system of ODEs describing the batch ABE fermentation were integrated in MATLAB and the results compared with the simulation results of the unsteady state batch ABE fermentation in Aspen Plus. The simulation results were compared to experimental trends observed in the available literature for ABE batch fermentation and in situ gas stripping as a function of gas flow rates. Furthermore, the integrated batch fermentation and in situ gas stripping simulations were compared with traditional separate simulations of a steady state fermenter with gas stripping of the final fermentation broth.

Methods

Initialization

Figure 2 provides an overview of the structure used to incorporate RBatch (unsteady state reactor subroutine) into the steady state environment of Aspen Plus. The initial batch charge

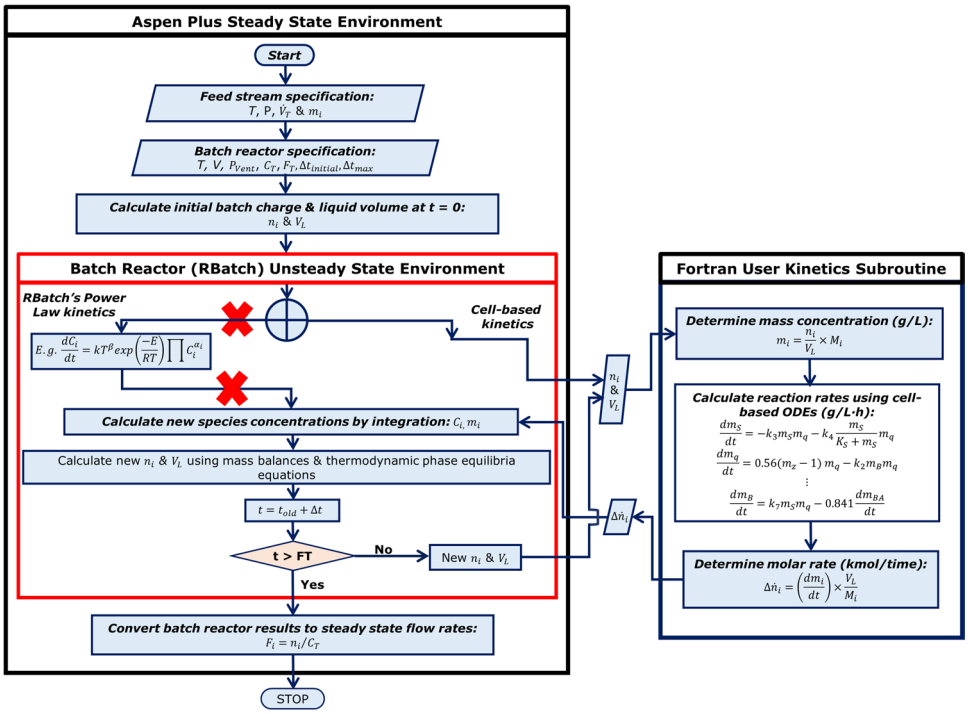

Fig. 2 Flow chart showing the interface between the Aspen Plus steady state environment and the unsteady state batch reactor linked to the new cell-based kinetics subroutine. The notation is: components (i), temperature (T), pressure (P), $\dot{V}_T$ (total volumetric flow rate of feed stream, L/time), m (mass concentration, g/L), P_{vent} (pressure at which venting begins), C_T (total cycle time), F_T (total fermentation time), $\Delta t_{initial}$ (initial time step size), Δt_{max} (maximum time step size), n (moles), V_L (liquid volume in reactor, L), C (molar concentration, mol/L), E (activation energy), R (universal gas constant), α (order of reaction), β (temperature exponent), M (molar mass, g/mol), $\Delta\dot{n}$ (change in the molar rate, mol/time), k, k_i, k_2, k_3, k_4, k_7, K_S (kinetic parameters), S, q, B, BA (glucose, cells, butanol, butyric acid), t (new time), Δt (variable time step), F (molar flow rate, mol/time)

(50 g/L glucose, 0.03 g/L cell biomass in a 1 L aqueous solution) was introduced to the reactor using a steady state feed over the course of 1 min, ensuring that charge occurred at the start of the batch fermentation. The batch reactor was simulated at a constant temperature of 39 °C and 1 atm pressure, ending at a total fermentation time of 32 h. The time step and maximum step size for the integration in Aspen Plus were both set to 0.01 h from their default values of 0.1 h.

Modifications to RBatch within Aspen Plus to incorporate mechanistic models

The batch reactor unit process, RBatch, was used in Aspen Plus to simulate unsteady state batch process. RBatch uses holding tanks to interface the unsteady state batch operation with the steady state flowsheet environment in Aspen Plus (Fig. 3). RBatch can accommodate multiple input streams and can simulate a continuous feed stream (i.e., the gas feed for stripping). This RBatch configuration allows the potential simulation of unsteady state fermentation with in situ gas stripping, where fermentation products in the reactor are repartitioned into liquid and vapor phases after each time step to provide time-dependent information. In the commercial configuration of RBatch, the time-accumulated products (liquid and gas) are released into the steady state flowsheet environment as time-averaged streams.

The ability of RBatch to handle multiple input streams and repartition components in the reactor into liquid and vapor phases after each time step made it a good candidate for modification to simulate the unsteady state batch fermentation with in situ gas stripping (Fig. 1b). However, the reactions in Aspen Plus were built for chemical reactions that follow the Power Law as shown in Eq. 2 [17], such that the RBatch can only handle Power Law reaction kinetics:

$$r = kT^{\beta}\exp(-E/RT)\prod\left(C_i\right)^{\alpha_i}, \tag{2}$$

where r is the rate of reaction (typically in moles/volume/time). The concentration-dependence of the reaction rate is expressed as the product of the concentration of each reactant i, C_i, taken to its reaction order, α_i. The rate constant for the reaction at a given temperature, k, is adjusted to additional temperatures (T), using the temperature exponent (β) and the activation energy (E). R is the universal gas law constant.

The mechanistic mathematical models used to describe batch fermentation express the reaction rates in terms of reaction rate parameters and components concentrations (for example, Eq. 3 describes glucose consumption rate in units of mass for the ABE model [18]), but do not typically conform to the Power Law model:

$$\frac{dm_S}{dt} = -k_3 m_S m_q - k_4 \frac{m_S}{K_S + m_S} m_q, \tag{3}$$

where m_s, m_q, are substrate concentrations (g/L), cell biomass concentration (g/L) and k_3, k_4, and K_S are kinetic parameters, respectively. Therefore, a subroutine was written in Fortran (calculating the rates of production/consumption of each component), and dynamically linked to the batch reactor (RBatch) in Aspen Plus to simulate the unsteady state batch ABE fermentation process (Fig. 2). The Fortran user kinetics subroutine was written based on the ODEs of the selected fermentation model (described in "Batch fermentation simulation in Aspen Plus"), compiled into a written subroutine (creating a readable Aspen Plus file from the written subroutine) and supplied as a compiled readable file to Aspen Plus to run the simulation dynamically (Supplementary Material, sections SM 1 and SM 2).

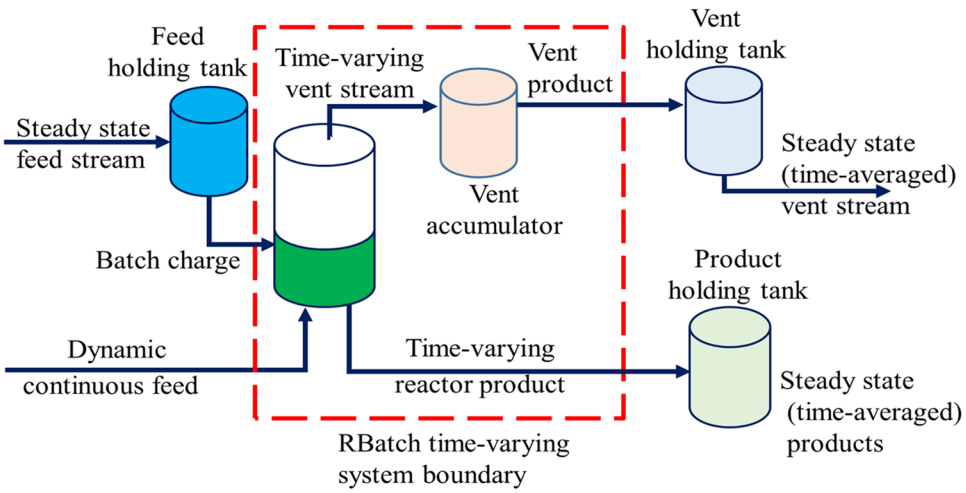

Fig. 3 The configuration of RBatch shows how the unsteady state fermentation reactions and product removal by venting in the batch reactor are linked to the time-averaged steady state flowsheet environment of Aspen Plus [17]

Batch fermentation simulation in Aspen Plus

The mathematical model developed by Votruba et al. [18] for a batch culture of *Clostridium acetobutylicum* was selected to simulate the ABE fermentation process in the Aspen Plus environment. The ABE model is mechanistic and in the form of mass balances and rate equations for substrate consumption, the production of extracellular products (acetone, butanol, ethanol, acetic acid, butyric acid, carbon dioxide, and hydrogen), the autocatalytic production of cell biomass, and product and substrate inhibitions of cell growth. The model was validated experimentally [18]. The mathematical equations for the fermentation kinetics and model parameters are presented in Appendices A and B, respectively. Cell biomass (m_q in Eq. 9) in Appendix A was simulated within Aspen Plus as a user-defined solid component with the physical properties assumed to be those of water. This assumption is valid because the cells are non-volatile, nonpolar, and do not participate in the vapor–liquid equilibrium (VLE) calculations [19].

Thermodynamic models used in the simulation

The phase composition, concentrations of each component in the reactor (vapor, liquid, solid) and the stripped stream (vapor), and other estimated properties including the volume of the liquid, solid and vapor components are evaluated using the thermodynamic models in Aspen Plus to satisfy material and energy balances. For components in a mixture that have either dissimilar sizes, shapes, and/or intermolecular forces, the system forms a non-ideal mixture. The activity coefficient-based models are generally well accepted and used routinely to model non-ideal liquid mixtures at low pressures. The activity coefficient represents the deviation of the mixture from an ideal system, where the greater the value of the activity coefficient from unity, the more non-ideal the system is [17]. The ABE fermentation mixture was modeled as a mixed aqueous and organic stream, with solid (due to the microbial cells), liquid and vapor phases at a low pressure of 1 atm. The nonrandom two-liquid–Hayden O'Connell (NRTL–HOC) property model was selected as the thermodynamic model for the simulation [6]. The NRTL activity coefficient model was selected to account for the non-ideality of the liquid mixture as a function of temperature and composition [19, 20]. The fermentation system contains the carboxylic acids, butyric and acetic acids, which form a strong association in the vapor phase. The HOC equation of state calculates the thermodynamic properties of these acids in the vapor phase by incorporating the chemical theory of dimerization, thus accounting for the association. The fermentation mixture also contains the light gasses, carbon dioxide (CO_2) and hydrogen (H_2). Nitrogen gas (N_2) is used in gas stripping at concentrations less than 5%, at a temperature above the critical temperatures of the pure components (CO_2, H_2, and N_2) and in subcritical solvents [19]. These components were, therefore, simulated as Henry's components in Aspen Plus to account for dissolved product gas components in liquid fermentation mixture [6].

Communication between the batch reactor and user kinetics subroutine and running the RBatch in Aspen Plus

While the integration time is less than the total fermentation time specified for the batch reactor, new calculated values (moles of each component and the liquid volume) are passed from the RBatch to the kinetics subroutine for the next step calculation. The RBatch unit operation is able to generate time-dependent data for a batch fermentation process in Aspen Plus because the integration process uses discrete time points. Once the fermentation/stripping is complete, the total accumulated material in the reactor and the vent accumulator are converted into steady state flow rates, calculated as the ratio of the total accumulated mass in the vent accumulator or the reactor at the end of fermentation to the total cycle time. The contents of the vent accumulator is the sum of the continuous time-varying vapor that leaves the reactor [17], and this is used as the vent product stream.

Unsteady state fermentation and in situ gas stripping simulations

To link the unsteady state batch ABE fermentation and in situ gas stripping process in Aspen Plus, varying feed rates of nitrogen gas (0.8, 1.6, 3, 5, 6.4 L/min per L of fermentation broth) were fed continuously to the reactor at specified start times (relative to the beginning of the batch fermentation at $t = 0$ h) with a vent. For the RBatch with a vent, a reactor volume of 1.009 L (allowing for a headspace requirement for vapors) was specified. A vent opening pressure of 1 atm was specified, triggering the RBatch subroutine to calculate the reactor pressure. To simulate actual batch ABE fermentation and in situ gas stripping laboratory experiments where gas stripping was initiated after a specified batch fermentation time (e.g., 20 h [9]) or product concentration in the fermenter (e.g., 3–4 g/L of ABE [7]), gas stripping was started after 15 h of fermentation when the ABE concentration was about 5.7 g/L. For simplicity, it was assumed that there was complete recovery of the stripped liquid components (acetone, butanol, ethanol, acetic acid, butyric acid, and water in the condensate).

Control simulation of separate steady state fermentation and gas stripping processes

Batch fermentation with in situ gas stripping has been simulated in Aspen Plus by separating the steady state batch

fermentation from the gas stripping. The biological reactions in the reactor were described stoichiometrically, and the final product concentrations from the stoichiometric reactions were then used as inputs to a flash unit to simulate the gas stripping process. In this study, we used this configuration as our control case, and we simulated a steady state batch Aspen Plus ABE fermentation with initial conditions of 50 g/L glucose and 0.03 g/L cell biomass. A steady state stoichiometric reactor (RStoic block) was used to simulate the fermentation (Appendix C presents the stoichiometric equations). The stoichiometric parameters for the simulation were selected so that the final product yields from the RBatch block linked with the Fortran user kinetics subroutine were 0.319, 0.495, 0.080, 0.120, 0 (mole of product/mole of glucose fed) for acetone, butanol, ethanol, acetic and butyric acids, respectively. The resulting fermentation broth calculated using the steady state RStoic block was fed to an isothermal flash unit (39 °C) with different N_2 gas flow rates (0.8, 1.6, 3, 5, 6.4 L/min per L of fermentation broth) to simulate the gas stripping process.

Validation of Aspen Plus batch fermentation results (no gas stripping) using MATLAB simulation

The results of the integration of the system of ODEs describing the ABE fermentation process in MATLAB were compared with the batch simulation results in the modified Aspen Plus (in the absence of gas stripping) to verify the accuracy of the procedure. The batch reactor (RBatch block) in Aspen Plus solves the mass, energy and composition equations for each fermentation time step using the variable-step-size Gear algorithm as the integration method [17]. Ode15s in MATLAB is a variable-step and variable-order solver that can be set to use the backward differentiation formulas (BDF), also known as the Gear's method. MATLAB ode15s was, therefore, configured to use the BDF (Gear's method) with the corresponding integration parameters used in the RBatch block so that the same integration method was used in both MATLAB and Aspen Plus. In the absence of gas stripping or a significant gas headspace in the fermenter, the concentration of the volatile components in the liquid phase fermentation broth in the Aspen Plus batch reactor is minimally affected by partitioning, such that the MATLAB results can be used to verify the procedure developed for RBatch in Aspen Plus without gas stripping.

Calculation of ABE fermentation performance parameters

Productivity, yield, percent mass recovery, and selectivity are used to describe the performance and operation of the simulated ABE fermentations in the various configurations that were modeled. The parameters were calculated as follows:

$$\text{Productivity (g/L/h)} = \frac{\text{MCR} + \text{MCV}}{\text{VLIQS} \times t}, \tag{4}$$

$$\text{Yield (g/g)} = \frac{\text{MCR} + \text{MCV}}{\text{GS}}, \tag{5}$$

$$\text{Percent recovery (\%)} = \frac{\text{MCV}}{\text{MCR} + \text{MCV}} \times 100, \tag{6}$$

$$\text{Selectivity} = \frac{y(1-x)}{x(1-y)}, \tag{7}$$

where MCR is the accumulated mass in the reactor (g), MCV is the accumulated mass in the stripped stream in grams (vent accumulator, condensate), VLIQS is the total volume (L) of the liquid and solids contents in the reactor, t is the fermentation time (h). GS is the total grams of sugar utilized (calculated as the difference between the initial mass of glucose and the mass of glucose at the end of fermentation), y and x are the mass fractions in the stripped vapor stream (assuming complete recovery of acetone, butanol, ethanol, water, butyric and acetic acids and neglecting CO_2, H_2 or N_2) and the accumulated mass fraction in the reactor (acetone, butanol, ethanol, water, butyric and acetic acids) at the same time point, respectively.

Results and discussion

Validation of Aspen Plus batch fermentation results (no gas stripping) using MATLAB simulation

The average NRTL liquid phase activity coefficient values obtained from Aspen Plus for glucose, butyric acid, acetic acid, acetone, butanol, ethanol, water, hydrogen, and carbon dioxide are 0.97, 22.47, 2.61, 5.99, 26.36, 4.61, 1.00, 0.97, and 0.97, respectively. The activity coefficient values for butyric acid, acetic acid, acetone, butanol, and ethanol are significantly greater than one, indicating a highly non-ideal liquid mixture and supporting the choice of NRTL activity coefficient model to describe the thermodynamic properties of the liquid fermentation broth. Figure 4 shows the simulation results of the modified RBatch block in Aspen Plus compared to the results using MATLAB to integrate the system of ODEs describing the batch ABE fermentation. The results for all components are provided in the Supplementary Material Fig. S1. MATLAB and Aspen Plus simulation of batch fermentation in the absence of gas stripping are indistinguishable, with the exception of acetone production. Figure 4a depicts a typical Monod microbial cell growth kinetics with exponential growth and a

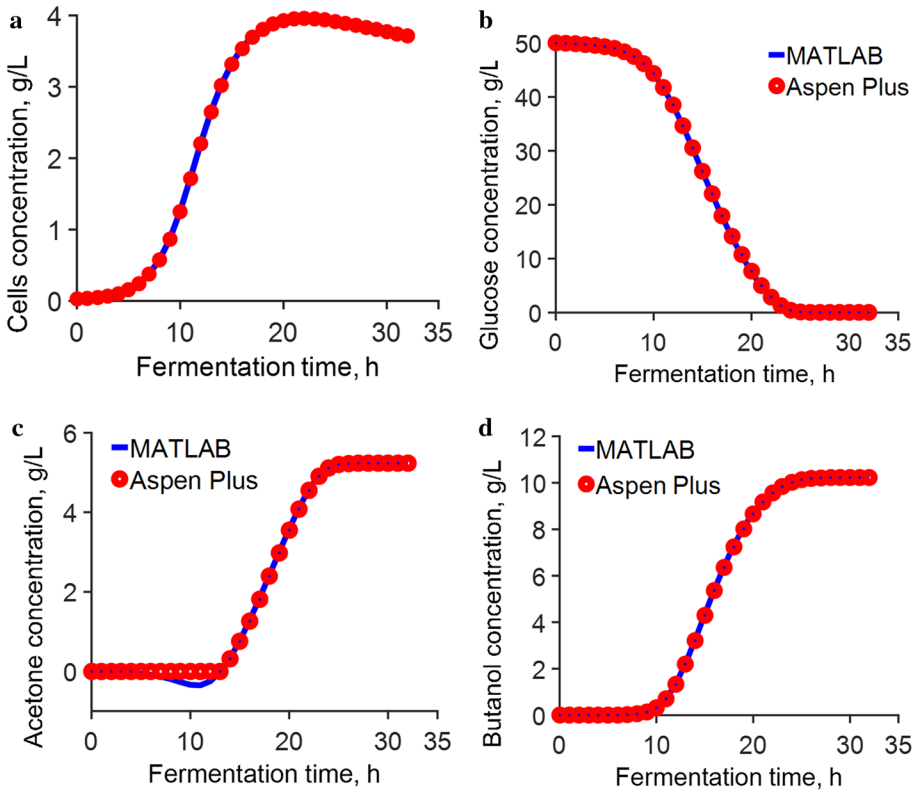

Fig. 4 Comparison of batch fermentation simulation results in Aspen Plus (RBatch) with the integration of the ordinary differential equations describing the batch fermentation process in MATLAB for cells (**a**), glucose (**b**), acetone (**c**), and butanol (**d**)

stationary phase followed by a death phase, with corresponding consumption of the substrate (Fig. 4b). Consistent with the metabolic pathway of *C. acetobutylicum*, the concentration profiles for acetic acid (Supplementary Material Fig. S1f) and butyric acid (Supplementary Material Fig. S1g) depict a trend of acid production (from 0 to 13 h) followed by consumption and reutilization (13–32 h) of these acids to produce solvents. Solvent production (acetone, butanol and ethanol) was, therefore, not significant until after about 13 h, in support of starting gas stripping after 15 h of batch fermentation. In MATLAB, negative concentrations were predicted for acetone between 0 and 13 h, which are physically unrealistic but present in the ODEs of the fermentation model (Fig. 4c). In Aspen Plus, the RBatch block solves the mass and energy component equations to satisfy the material and energy balances, and negative concentrations are avoided. Comparison of the MATLAB and Aspen Plus results validates the direct use of the ODEs incorporating the autocatalytic production of cells, substrate consumption, and production and inhibition of fermentation products in Aspen Plus to provide time-dependent simulations of batch fermenters.

Comparison of traditional Aspen Plus batch separate steady state fermentation and gas stripping with unsteady state batch fermentation and in situ gas stripping simulations

The trends from the steady state simulation are compared with the integrated batch ABE fermentation and in situ gas stripping in Fig. 5. In the steady state simulation, the selectivities (Fig. 5a) and condensate concentrations (Fig. 5c) of acetone, butanol, ethanol and ABE in the condensate are not a strong function of gas flow rate. Condensate refers to the contents of the stripped stream (accumulated in the vent accumulator). The steady state selectivities, percent recovery and condensate concentration of acetone decrease slightly with increasing gas flow rate whereas the selectivities and condensate concentrations of butanol and ethanol increase slightly with increasing gas flow rate (Supplementary Material Table S1).

In contrast, the simulation linking batch fermentation with in situ gas stripping (unsteady state) predicts that selectivities (Fig. 5b) and recoveries of acetone, butanol,

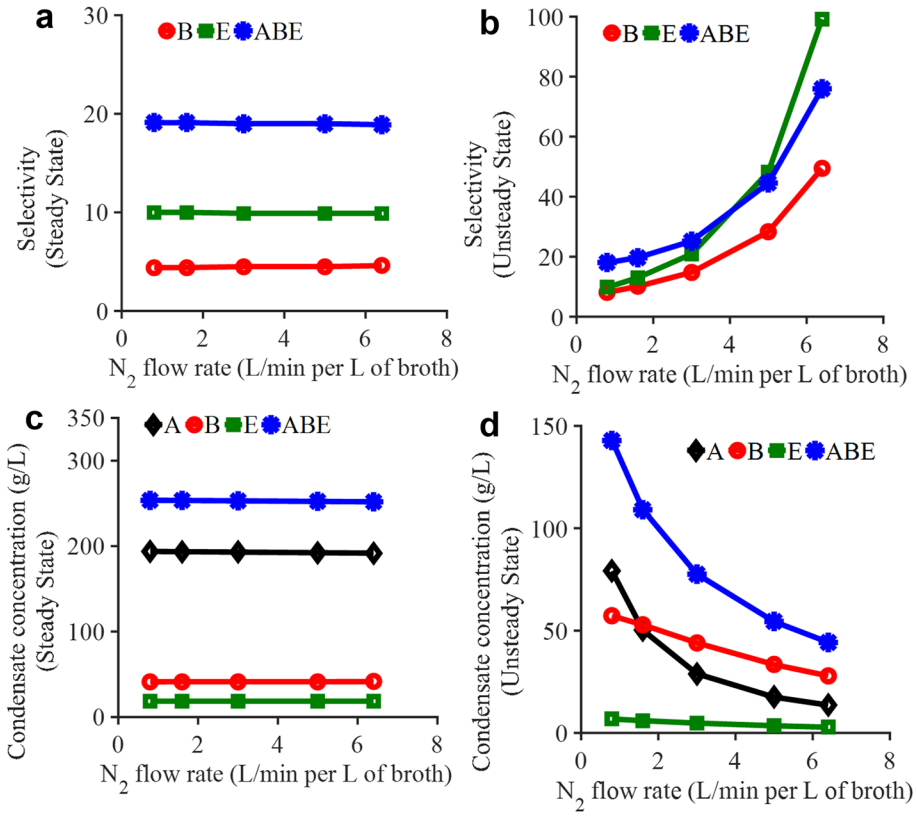

Fig. 5 Selectivities and condensate concentrations at the end of 32-h batch fermentation and in situ gas stripping of acetone (A), butanol (B), ethanol (E) and the combined ABE products (ABE) using traditional steady state (stoichiometric reactor and flash unit) and unsteady state (RBatch) in Aspen Plus with different gas flow rates. The notations are: selectivity from the steady state simulation (**a**), selectivity from the unsteady state simulation (**b**), concentration of the condensate from the steady state simulation (**c**) and concentration of the products from the unsteady state simulation (**d**)

ethanol and ABE (Supplementary Material Table S2) increase significantly with increasing gas flow rate per L of the fermentation broth whereas the respective condensate concentrations (Fig. 5d) decrease with increasing gas flow rate. The unsteady state selectivity of butanol, ethanol, and the ABE mixture overall increase significantly with increasing gas flow rate, whereas the steady state selectivities decrease slightly (with the exception of butanol), but are largely insensitive to gas flowrate. Acetone, which was almost exhausted in the fermenter at high gas flow rates, had significantly higher selectivities (results not shown). At high gas flow rates relative to the fermentation broth volume, the volatile components in fermenter may be exhausted, resulting in these high selectivities. In general, high selectivities can be observed at conditions of low product concentration in the fermenter, thus selectivity is sensitive to both the start time of the gas stripping (controlling the initial accumulation of the product) and the gas flow rate in the integrated batch fermentation and in situ gas stripping. Correspondingly, the condensate concentrations of butanol, ethanol, acetone, and the ABE mixture decrease significantly in the unsteady state simulation relative to the steady state concentrations, which are relatively insensitive to gas flow rate. Again, this observation can be attributed to the time-dependent decrease in concentration of the ABE fermentation products in the fermenter over the course of gas stripping. Water is the most abundant volatile component in the fermentation broth. In the unsteady state simulation, the product in the condensate is diluted by the significant amount of water that is also volatilized in the gas stripping process, which increases with gas flowrate. Thus, there exists a trade-off between product recovery from the fermentation broth and ABE concentration in the corresponding condensate when selecting a gas flow rate.

Simulations and representations of the fermentation coupled with the gas stripping process based on unsteady state models, such as the cell-based dynamic mathematical models, offer opportunities to further investigate and understand the interaction and relationship among the typical parameters (e.g., selectivities, recoveries and condensate concentrations, total ABE produced, productivity, and yield) that describe integrated fermentation and in situ gas stripping process.

Comparison of performance of batch ABE fermentation and in situ gas stripping simulations to available literature

A broad range of gas flow rates relative to fermentation broth volume, stripping or operating temperatures, and initiation times of gas stripping have been investigated experimentally for the gas stripping of ABE fermentation [4]. The models used to describe solventogenic *Clostridia* species (for example, *C. acetobutylicum, C. beijerinckii, C. saccharobutylicum*

and *saccharoperbutylacetonicum* [13, 21]) generally lack applicability to other microorganisms, making direct comparisons of in silico analyses and available laboratory ABE fermentation experimental data difficult. To systematically analyze the effect of the broad range of gas flow rates employed in the ABE batch fermentation and in situ gas stripping, the trends in the simulation results were, therefore, compared with the observed trends in literature.

The total ABE produced (sum of ABE in the reactor and stripped stream), productivity and yield for an integrated ABE batch fermentation and in situ gas stripping with 0 (no gas stripping), 0.8, 1.6, 3, 5 and 6.4 L/min N_2 per L of fermentation broth are presented in Fig. 6. While there is a trade-off between the concentration of ABE in the stripped stream and ABE selectivity (Fig. 5) with increasing gas flowrate, the total ABE produced, productivity and yield were improved up to 105, 110, 119, 130 and 150% for the integrated batch process employing 0.8, 1.6, 3, 5 and 6.4 L/min N_2 per L of broth, respectively, relative to the simulated results of the batch ABE fermentation without gas stripping. The improvement in the total ABE produced and productivity in this study are comparable to the 133 and 210% enhancements in the total ABE produced and productivity, respectively, reported by Ezeji et al. [7] in their laboratory integrated batch ABE fermentation and in situ gas stripping with an initial glucose concentration of 60 g/L and a gas flow rate of 3 L/min per L of broth started after 15 h. When ABE product in removed in situ by gas stripping, product inhibition is reduced and ABE performance is generally improved [7]. However, the cells in the fermenter may be concentrated at high gas flow rate per L of fermentation broth because of the loss of volatile components (ABE and water) in the fermenter. The concentrated cells at high gas flow rate per L of fermentation broth may lead to higher ABE produced, productivity and yield.

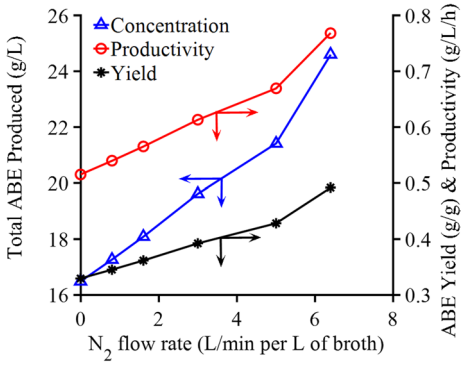

Fig. 6 Total ABE produced (total concentration in the fermenter and stripped stream), productivity and yield from the simulated batch ABE fermentation and in situ gas stripping with different gas flow rates

ABE fermentations are characterized by low product concentration (< 20 g/L ABE), low reactor productivities (< 0.3 g/L/h) and low ABE yield (0.28–0.33 g/g) as a result of product toxicity (especially due to butanol

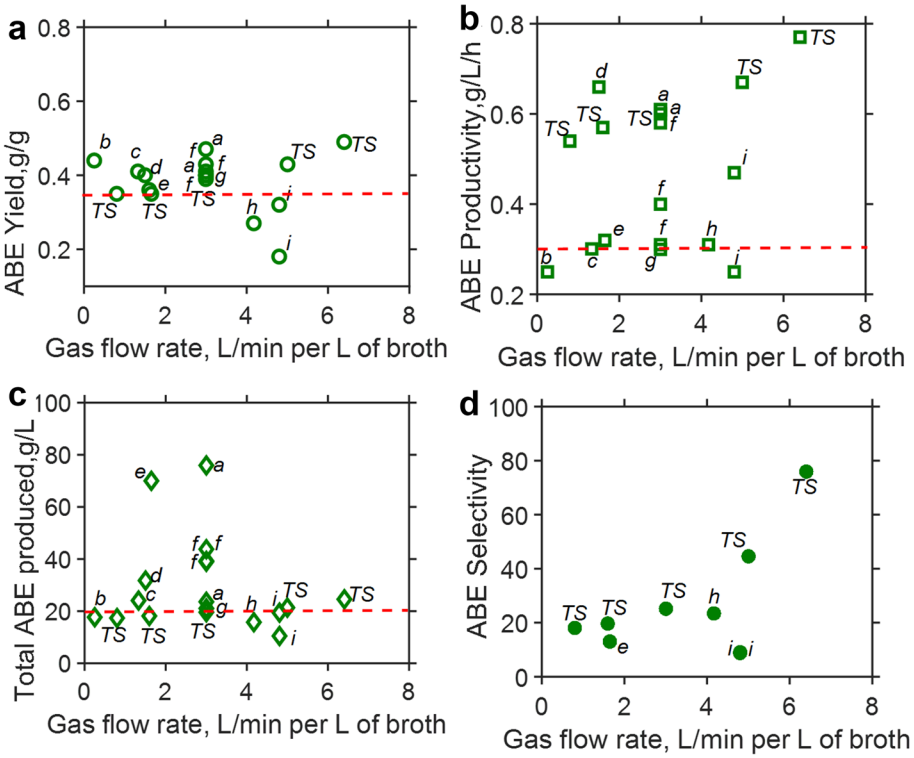

Fig. 7 ABE yield (**a**), ABE productivity (**b**), total ABE produced (**c**) and ABE selectivity (**d**) versus normalized gas flow rates (L/min per L of fermentation broth) from batch fermentation and in situ gas stripping literature data from refs *b* [27], *c* [28], *d* [29], *e* [26], *a* [7], *f* [25], *g* [30], *h* [24], *i* [23], and Aspen Plus unsteady state batch fermentation and in situ gas stripping simulation (*TS*) and performance benchmark dashed line) for a typical batch fermentation of 0.35 g/g ABE yield, 0.30 g/L/h ABE productivity and 20 g/L total ABE produced (chosen based on data from Qureshi and Blaschek) [8]

concentrations > 13 g/L) to the microorganisms used in fermentation [8]. Figure 7 graphs the ABE productivity, yield, total ABE produced, and selectivity versus the gas flow rate per L of broth from available literature data along with the data predicted from the Aspen Plus RBatch unsteady state simulation for batch fermentation and in situ gas stripping. Generally, about 3 L/min per L of fermentation broth is the gas flow rate used most in batch fermentation and in situ gas stripping experiments while the lowest and highest gas recycle rates used were 0.25 and 4.8 L/min per L of broth. ABE yield (Fig. 7a), ABE productivity (Fig. 7b), total ABE produced (Fig. 7c) and ABE selectivity (Fig. 7d) for the literature data increase (significantly above their respective limits in batch fermentations) with gas flow rate up to about 3 L/min per L of broth. At higher gas flow rates, the ABE performance generally decreases with increasing gas flow rate. The performance of the simulation of integrated ABE fermentation and in situ gas stripping is consistent with literature data up to about 3 L/min per L of broth. Above 3 L/min per L of broth, the performance of the ABE fermentation predicted from the Aspen Plus simulation are above the experimental benchmark of ABE yield of 0.35 g/g, approximately 20 g/L total ABE produced, and productivity of 0.30 g/L/h. A review of the batch ABE fermentation and in situ product recovery by gas stripping in literature reveal that some studies compensate for the water and/or volume loss due to gas stripping by adding water at time intervals to maintain the liquid volume [22], whereas other studies do not [23–26]. The addition of water to compensate for either water or volume loss due to gas stripping or the absence thereof may be the source of discrepancy between the experimental literature data and the results from the unsteady state simulations with in situ gas stripping, in which water was not added.

Conclusion

This study has focused on simulation of batch fermentation as an unsteady state process by incorporating autocatalytic production of cells, time-dependent concentrations of the fermentation components, and substrate and product inhibitions in the framework of Aspen Plus, a universally accepted traditional process simulator of choice for refinery and chemical processes. This simulation approach allowed the batch fermentation process (described using a time-dependent fermentation model) to be coupled with in situ product recovery by gas stripping. In this way, the time-dependent phase composition and concentrations of components in a fermenter (solid, liquid, and vapor) and stripped stream (vapor) were predicted by the thermodynamic models in Aspen Plus to provide realistic simulations of integrated batch fermentation and in situ gas stripping experiments under different operating conditions. The performance of the integrated batch and in situ gas stripping is shown to be dependent on the gas flow rate employed, an artifact that is absent unless a time-dependent fermentation model is linked in situ to the gas stripping process. The traditional steady state separate fermentation and gas stripping may be inadequate for systematic analyses of bioprocesses, especially if fermentations are linked with in situ separations.

Our simulation approach predicts trends that are consistent with available literature data and offer insight into the performance of the ABE batch fermentation and in situ gas stripping at high gas recycle flow rates and outside the range investigated in available literature. The simulation approach in this research will allow the full suite of PSE tools to be applied to the ABE production process, providing a decision-support tool to aid the fermentation experimentalist. This research also provides a general platform to integrate biorefinery processes (fermentations) with chemical and refinery processes in the process simulation packages.

Acknowledgements This manuscript is based on the work funded by the USDA-NIFA Biomass Research and Development Initiative (BRDI # 68-3A75-7-608) and an NSF EPSCoR Track-2 RII, Award No. OIA1632854.

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Appendix A

Ordinary differential equations representation of the fermentation kinetics of a batch culture of *Clostridium acetobutylicum* [18]:

$$\frac{dm_z}{dt} = k_1 m_S \frac{K_I}{K_I + m_B} m_z - 0.56\left(m_z - 1\right)m_z, \tag{8}$$

$$\frac{dm_q}{dt} = 0.56(m_z - 1)m_q - k_2 m_B m_q, \tag{9}$$

$$\frac{dm_S}{dt} = -k_3 m_S m_q - k_4 \frac{m_S}{K_S + m_S} m_q, \tag{10}$$

$$\frac{dm_{BA}}{dt} = k_5 m_S \frac{K_I}{K_I + m_B} m_q - k_6 \frac{m_{BA}}{K_{BA} + m_{BA}} m_q, \tag{11}$$

$$\frac{dm_B}{dt} = k_7 m_S m_q - 0.841 \frac{dm_{BA}}{dt}, \tag{12}$$

$$\frac{dm_{AA}}{dt} = k_8 \frac{m_S}{K_S + m_S} \frac{K_I}{K_I + m_B} m_q - k_9 \frac{m_{AA}}{K_{AA} + m_{AA}} \frac{m_S}{K_S + m_S} m_q, \tag{13}$$

$$\frac{dm_A}{dt} = k_{10}\frac{m_S}{K_S + m_S}m_q - 0.484\frac{dm_{AA}}{dt}, \quad (14)$$

$$\frac{dm_E}{dt} = k_{11}\frac{m_S}{K_S + m_S}m_q, \quad (15)$$

$$\frac{dm_{CO_2}}{dt} = k_{12}\frac{m_S}{K_S + m_S}m_q, \quad (16)$$

$$\frac{dm_{H_2}}{dt} = k_{13}\frac{m_S}{K_S + m_S}m_q + k_{14}m_S m_q. \quad (17)$$

Appendix B

Parameter definition for the kinetic model and their respective values

k_1 kinetic constant in Eq. 8, = 0.009 L/g-substrate/h
k_2 kinetic constant in Eq. 9, = 0.0008 L/g-butanol/h
k_3 kinetic constant in Eq. 10, = 0.0255 L/g-biomass/h
k_4 kinetic constant in Eq. 10, = 0.6764 g-substrate/g-biomass/h
k_5 kinetic constant in Eq. 11, = 0.0136 g-butyric acid L/g-substrate/g-biomass/h
k_6 kinetic constant in Eq. 11, = 0.1170 g-butyric acid/g-biomass/h
k_7 kinetic constant in Eq. 12, = 0.0113 g-butanol L/g-substrate/g-biomass/h
k_8 kinetic constant in Eq. 13, = 0.7150 g-acetic acid/g-biomass/h
k_9 kinetic constant in Eq. 13, = 0.1350 g-acetic acid/g-biomass/h
k_{10} kinetic constant in Eq. 14, = 0.1558 g-acetone/g-biomass/h
k_{11} kinetic constant in Eq. 15, = 0.0258 g-ethanol/g-biomass/h
k_{12} kinetic constant in Eq. 16, = 0.6139 g-carbon dioxide/g-biomass/h
k_{13} kinetic constant in Eq. 17, = 0.0185 g-hydrogen/g-biomass/h
k_{14} kinetic constant in Eq. 17, = 0.00013 g-hydrogen L /g-substrate/g-biomass/h
K_I inhibition constant, = 0.833 g-butanol/L
K_S Monod constant, = 2.0 g-substrate/L
K_{BA} saturation constant, = 0.5 g-butyric acid/L
K_{AA} saturation constant, = 0.5 L/g-acetic acid/L
m_A acetone concentration, g/L
m_B butanol concentration, g/L
m_E ethanol concentration, g/L
m_{BA} butyric acid concentration, g/L
m_{AA} acetic acid concentration, g/L
m_S glucose concentration, g/L
m_q cell biomass concentration, g/L
m_{CO_2} carbon dioxide concentration, g/L
m_{H_2} hydrogen concentration, g/L
m_z marker of the physiological state culture, dimensionless

Appendix C

Stoichiometric equations (Eqs. 18–22) used together with stoichiometric coefficients relative to glucose [13–16]. The stoichiometric coefficients used in the stoichiometric reactor were 0.319, 0.495, 0.080, 0.120, 0 (mole of product/mole of glucose fed) for acetone, butanol, ethanol, acetic and butyric acids, respectively, calculated from the model of Votruba et al. [18]:

$$C_6H_{12}O_6 \rightarrow C_4H_{10}O\ (\text{butanol}) + 2CO_2 + H_2O, \quad (18)$$

$$C_6H_{12}O_6 + H_2O \rightarrow C_3H_6O\ (\text{acetone}) + 3CO_2 + 4H_2, \quad (19)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5O\ (\text{ethanol}) + 2CO_2 + H_2, \quad (20)$$

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2\ (\text{butyric acid}) + 2CO_2 + 2H_2, \quad (21)$$

$$C_6H_{12}O_6 \rightarrow 3C_2H_4O_2\ (\text{acetic acid}). \quad (22)$$

References

1. Koutinas M, Kiparissides A, Pistikopoulos EN, Mantalaris A (2012) Bioprocess systems engineering: transferring traditional process engineering principles to industrial biotechnology. Comput Struct Biotechnol J 3(4):1–9
2. Darkwah K, Knutson BL, Seay J (2018) A perspective on challenges and prospects for applying process systems engineering tools to fermentation-based biorefineries. ACS Sustain Chem Eng. https://doi.org/10.1021/acssuschemeng.7b03762
3. Sirigudi RR (2005) Biomass to ethanol: process simulation, validation and sensitivity analysis of a gasifier and a bioreactor. Thesis, Oklahoma State University
4. Abdehagh N, Tezel FH, Thibault J (2014) Separation techniques in butanol production: challenges and developments. Biomass Bioenergy 60:222–246
5. Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37:52–68
6. Ponce GH et al (2015) Simulation, analysis and optimization of sugar concentration in an in situ gas stripping fermentation process for bioethanol production. Chem Eng Trans 43:319–324
7. Ezeji TC, Qureshi N, Blaschek HP (2003) Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. World J Microbiol Biotechnol 19(6):595–603

8. Qureshi N, Blaschek H (2001) Recent advances in ABE fermentation: hyper-butanol producing *Clostridium beijerinckii* BA101. J Ind Microbiol Biotechnol 27(5):287–291
9. Ezeji TC, Qureshi N, Blaschek H (2013) Microbial production of a biofuel (acetone–butanol–ethanol) in a continuous bioreactor: impact of bleed and simultaneous product removal. Bioprocess Biosyst Eng 36(1):109–116
10. Vane LM (2008) Separation technologies for the recovery and dehydration of alcohols from fermentation broths. Biofuels Bioprod Biorefin 2(6):553–588
11. Shuler ML, Kargi F (2009) Bioprocess engineering basic concepts. Prentice Hall, New Jersey
12. Mesfun S et al (2013) Techno-economic evaluation of butanol production via black liquor fractionation. In: Proceedings of 12th international conference on sustainable energy technologies. Hong Kong: Faculty of Construction and Environment and Research Institute for Sustainable Urban Development, The Hong Kong Polytechnic University, China
13. Van der Merwe AB (2010) Evaluation of different process designs for biobutanol production from sugarcane molasses. Thesis, University of Stellenbosch
14. Wu M, Wang M, Liu J, Huo H (2007) Life-cycle assessment of corn-based butanol as a potential transportation fuel. ANL/ESD/07–10; Argonne National Laboratory: Chicago, Illinois, https://doi.org/10.2172/925379
15. Liu J, Wu M, Wang M (2009) Simulation of the process for producing butanol from corn fermentation. Ind Eng Chem Res 48(11):5551–5557
16. Nilsson R et al (2014) Techno-economics of carbon preserving butanol production using a combined fermentative and catalytic approach. Bioresour Technol 161(0):263–269
17. Aspen Technolgy Inc (2015) Aspen Plus V8.8 help and documentation
18. Votruba J, Volesky B, Yerushalmi L (1986) Mathematical model of a batch acetone–butanol fermentation. Biotechnol Bioeng 28(2):247–255
19. Carlson EC (1996) Don't gamble with physical properties for simulations. Chem Eng Prog 92(10):35–46
20. Hill D, Justice FC (2011) Understand thermodynamics to improve process simulations. Chem Eng Prog 107:20–25
21. Dürre P (2008) Fermentative Butanol Production. Ann NY Acad Sci 1125(1):353–362
22. Ezeji TC, Qureshi N, Blaschek HP (2004) Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 63(6):653–658
23. Ezeji TC et al (2005) Improving performance of a gas stripping-based recovery system to remove butanol from *Clostridium beijerinckii* fermentation. Bioprocess Biosyst Eng 27(3):207–214
24. Ennis B et al (1986) Continuous product recovery by in-situ gas stripping/condensation during solvent production from whey permeate using *Clostridium acetobutylicum*. Biotechnol Lett 8(10):725–730
25. Ezeji TC, Qureshi N, Blaschek HP (2007) Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping. J Ind Microbiol Biotechnol 34(12):771–777
26. Maddox IS, Qureshi N, Roberts-Thomson K (1995) Production of acetone-butanol-ethanol from concentrated substrate using clostridium acetobutylicum in an integrated fermentation-product removal process. Process Biochem 30(3):209–215
27. Lu C, Dong J, Yang S-T (2013) Butanol production from wood pulping hydrolysate in an integrated fermentation–gas stripping process. Bioresour Technol 143:467–475
28. Cai D et al (2016) Acetone–butanol–ethanol from sweet sorghum juice by an immobilized fermentation-gas stripping integration process. Bioresour Technol 211:704–710
29. Xue C et al (2013) Two-stage in situ gas stripping for enhanced butanol fermentation and energy-saving product recovery. Bioresour Technol 135(0):396–402
30. Qureshi N et al (2008) Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part I—batch fermentation. Biomass Bioenergy 32(2):168–175