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Article

Modeling of *Clostridium tyrobutyricum* for Butyric Acid Selectivity in Continuous Fermentation

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Abstract: A mathematical model was developed to describe batch and continuous fermentation of glucose to organic acids with *Clostridium tyrobutyricum*. A modified Monod equation was used to describe cell growth, and a Luedeking-Piret equation was used to describe the production of butyric and acetic acids. Using the batch fermentation equations, models predicting butyric acid selectivity for continuous fermentation were also developed. The model showed that butyric acid production was a strong function of cell mass, while acetic acid production was a function of cell growth rate. Further, it was found that at high acetic acid concentrations, acetic acid was metabolized to butyric acid and that this conversion could be modeled. In batch fermentation, high butyric acid selectivity occurred at high initial cell or glucose concentrations. In continuous fermentation, decreased dilution rate improved selectivity; at a dilution rate of 0.028 h^{-1}, the selectivity reached 95.8%. The model and experimental data showed that at total cell recycle, the butyric acid selectivity could reach 97.3%. This model could be used to optimize butyric acid production using *C. tyrobutyricum* in a continuous fermentation scheme. This is the first study that mathematically describes batch, steady state, and dynamic behavior of *C. tyrobutyricum* for butyric acid production.

Keywords: butyric acid; steady-state model; batch fermentation; continuous fermentation; *Clostridium tyrobutyricum*

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1. Introduction

Organic acids can be produced from biomass-derived sugars through fermentation. One of these products, butyric acid, has many applications in the food and perfume industries. Pure butyric acid is used in food flavors, and esters of butyric acid are widely used as additives in the perfume industry [1,2]. Butyric acid is also used to produce the biodegradable polymer β-hydroxybutyrate and in the production of several drugs [3]. Butyric acid might also find application as an intermediate in the production of one of the next generation of biofuels, biobutanol, through two-step fermentation as described by Ramey [4]. Butyric acid is currently produced chemically, which starts from the oxosynthesis of propylene [5]. The fermentation process to produce butyric acid is difficult because it forms multiple products at low concentrations, which greatly increases downstream separation costs.

There are several *Clostridial* strains which can convert sugars to butyric acid [6–14]. Of these strains, *Clostridium tyrobutyricum* is attractive because it only requires a simple medium (yeast extract) for growth, and can produce butyric acid in high yield, selectivity and concentration [15]. *C. tyrobutyricum* is a rod-shaped, Gram-positive bacterium which grows under anaerobic conditions. It produces butyric acid and acetic acid as its main fermentation products from sugars, and also produces hydrogen and carbon dioxide as gaseous by-products. The production of acetic acid diminishes the feasibility of the fermentation process with *C. tyrobutyricum* by decreasing the selectivity and yield for butyric acid. Some research has been done to improve this fermentation by trying to inhibit or eliminate the production of acetic acid. Several fermentation modes and operation parameters were investigated [10–12,16]. During these studies, the selectivity varied from 71% to 89% in batch fermentation, depending on the glucose concentration. For continuous fermentation, the selectivity varied from 60% to 96%, depending on the dilution rate. It was also found that continuous fermentation with partial cell recycle gave high selectivity at low dilution rate and high cell recycle ratio [17]. A novel extractive fermentation, with 10% alamine 336 in oleyl alcohol as a solvent, selectively extracted butyric acid from the fermentation with a butyric acid selectivity of 91% [15]. Further, selective separation using electrodeionization has given high selectivity at relatively high productivities [18]. Gene manipulation methods have also been used to disrupt the gene associated with the acetic acid formation pathway. In this way, more butyric acid was produced, although acetic acid production was not eliminated [3,19,20].

The cell growth rate of *C. tyrobutyricum*, glucose concentration within the system and nutrient supply have significant influences on selectivity. A possible explanation for the influence of cell growth rate on selectivity is that the production of acetic acid produces more ATP, which is needed for rapid cell growth [3,10,13]. Additionally, the influence of glucose concentration and supply modes are dependent upon the cell growth rate. For example, in a continuous fermentation of *C. tyrobutyricum*, the selectivity for butyric acid decreases with increased dilution rate [11]; the relationship between dilution rate and cell growth rate exists because the dilution rate equals the specific cell growth rate for continuous fermentation [21]. The selectivity for butyric acid increases with increasing glucose concentration. This could be explained by product inhibition of cell growth at higher product concentrations, which then prefers the production of butyric acid. A continuous fermentation with total cell recycle showed a selectivity for butyric acid of 96.8%, which was much higher than batch fermentation results, because cell recycle inhibited cell growth [12]. Although experiments have

shown the impact of cell growth rate, a comprehensive simulation explaining batch and continuous behavior has not been published for this fermentation.

1.1. Theory

The objective of this paper was to develop a mathematical model to describe cell growth rate, product formation, and substrate consumption for the fermentation of glucose to butyric acid with *C. tyrobutyricum*. By solving these coupled differential equations with initial cell, glucose and products concentrations, a time course for the batch fermentation could be simulated. By introducing the mass balance for continuous fermentation, a steady state simulation for continuous fermentation could also be obtained.

The classical Luedeking-Piret equation shows how the rate of product formation changes with cell mass and cell growth rate [22,23], which illustrates that cell growth rate is important to describe most fermentation processes. The Monod equation expresses the change in cell growth rate [24]. These two equations, combined with an equation describing substrate consumption, are often used for fermentation simulation, mainly for the production of lactic acid, glutamic acid, and ethanol [22,23,25–28]. Although these fermentations produce a single product, the Luedeking-Piret equation is also applicable to multiple-product fermentations [23]. Therefore, it is possible to develop a mathematical model to predict the production of butyric acid and acetic acid, and then the selectivity for butyric acid in batch and continuous fermentations, by combining the Luedeking-Piret equation, the Monod equation, and an equation describing substrate consumption.

Cell growth over time during fermentation can be divided into the lag phase, the exponential growth phase, the stationary phase, and the death phase. The rate of cell growth in the exponential phase can be written as:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu \times X \tag{1}$$

where X is the concentration of cells (dry cell mass) in the reactor in g L^{-1}; t is the time in hours; and μ is the specific cell growth rate in h^{-1}.

A simplified equation to describe the specific cell growth rate is the Monod equation, assuming glucose is the limiting growth component of the fermentation [23]:

$$\mu = \frac{\mu_0 S}{K_s + S} \tag{2}$$

where S is the concentration of substrate in the reactor, in g L^{-1}; μ_0 is the maximum specific cell growth rate in g L^{-1} h^{-1}; and K_s is the semi-empirical substrate saturation constant in g L^{-1}.

The Monod equation allows for good prediction in a low inhibition environment. If there is a strong inhibition with a maximum cell concentration, it is necessary to introduce a cell inhibition term. X_m is the maximum cell concentration that can be reached and the constant m represents how strongly the inhibition affects the growth rate equation [21], shown as:

$$\mu = \frac{\mu_0 S}{K_s + S} \times \left(1 - \frac{X}{X_m}\right)^m \tag{3}$$

After describing the cell growth rate with a modified Monod equation, the production formations of butyric acid and acetic acid must be described. The Luedeking-Piret equation with empirical constants α_i and β_i correlates product formation, dP_i/dt with cell growth rate, dX/dt and cell concentration, X, respectively:

$$\frac{dP_i}{dt} = \alpha_i \times \frac{dX}{dt} + \beta_i \times X \quad (4)$$

In cultivation with a composite medium (a medium specially designed for this organism), a 95%–100% carbon source is used for product formation, meaning that almost all the carbon is going to products and little is going to cell maintenance [8]. Thus, the rate of substrate utilization, dS/dt, may be shown as:

$$\frac{dS}{dt} = -\frac{1}{Y_{B/S}} \times \frac{dP_B}{dt} - \frac{1}{Y_{A/S}} \times \frac{dP_A}{dt} \quad (5)$$

where $Y_{B/S}$ is the butyric acid yield factor in g butyric acid/g glucose; $Y_{A/S}$ is the acetic acid yield factor in g acetic acid/g glucose; and dP_A/dt and dP_B/dt are the rates of formation of acetic and butyric acid, respectively.

In batch culture the cell growth, product formation and substrate consumption change with time and then terminate after a period of time. In continuous culture, steady state is reached, and cell growth rate can be obtained from the mass balance [23]:

$$\mu = \frac{F}{V_R} = D \quad (6)$$

where D is the dilution rate in h^{-1}; F is the feed stream flow rate in L h^{-1} and V_R is the volume of the reactor in L.

1.2. Definitions

Selectivity was defined as the ratio of the weight of butyric acid to the weight of all acids produced. $Y_{P/S}$ was used to define how much glucose is converted to products, both desirable (organic acids) and undesirable (carbon dioxide). It should be noted that the stoichiometry of producing butyric acid dictates that 1 mole of carbon dioxide is made for every mole of butanol. A $Y_{P/S}$ of 1 meant all of the glucose was converted to products. The yield of butyric acid was defined as the ratio of the weight of butyric acid produced to the weight of glucose consumed. The conversion of glucose was defined as the ratio of the consumed glucose to the initial glucose.

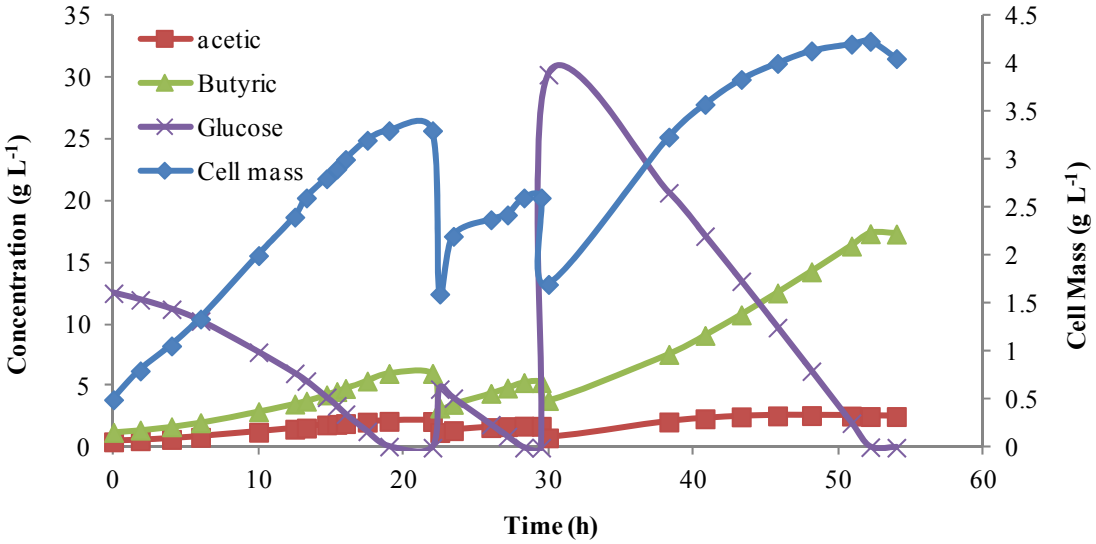
2. Results and Discussion

2.1. Batch Fermentation Performance with C. tyrobutyricum

To evaluate the parameters in the mathematic models describing the batch fermentation, repeated batch fermentations were conducted. The time course of the batch fermentations was recorded and compared to other published data. Results from batch experiments are shown in Figure 1. The initial glucose concentration was varied by feeding medium with different glucose concentrations, and the initial cell mass was varied by purging different volumes of fermentation broth. Note, the non-linear

jump in the cell mass with addition of more glucose is expected as the reactor returns to equilibrium. The total reaction volume was kept constant at 700 mL, although several times reactor volume was removed and new media was added (indicated by a drop in cell mass). Butyric acid concentrations, as shown in Figure 1, increase and decrease in proportion to cell mass, reaching a high of above 15 g L^{-1}. Acetic acid byproduct was relatively steady under 5 g L^{-1}. The selectivity of butyric acid varied from 76% to 90%, which was consistent with the work of Michel-Savin *et al.* [10] and Fayolle *et al.* [16], indicating that the media used was suitable for butyric acid production.

Figure 1. Kinetics of batch fermentation of glucose by *Clostridium tyrobutyricum* producing butyric acid and acetic acid at pH 6.2 in PYG medium.



These results showed that cell mass grew almost linearly when the glucose concentration was high, but with the consumption of glucose, cell growth rate slowed. In comparing different fermentation batches, independent of the initial glucose concentrations, the cell concentration approached, but never surpassed 5.0 g L^{-1}. This was probably because the chief product, butyric acid, strongly inhibits cell growth [3,10–12,15]. Work by Du *et al.* [18] showed this cell mass could be increased dramatically with continuous product removal. These batch results were later used for model parameter estimation.

2.2. Continuous Fermentation Performance with C. tyrobutyricum

Continuous fermentation experiments were carried out at different dilution rates (0.02 to 0.14 h^{-1}) and different glucose concentrations (17 g L^{-1}, 25 g L^{-1} and 50 g L^{-1}). As shown in Table 1, cell washout occurred when the dilution rate was between 0.107 and 0.14 h^{-1}, independent of the glucose concentration indicating a limit on the maximum specific cell growth rate. The cell concentration remained below 5.0 g L^{-1}, which was consistent with the cell concentrations in the batch fermentations. When the glucose concentration was 50 g L^{-1}, not all of the glucose was consumed, thus requiring a lower dilution rate. Dilution rate also had an influence on butyric acid selectivity. Independent of glucose concentration, the butyric acid selectivity increased with decreasing dilution rate, most likely as a result of low specific cell growth rate at low dilution rate.

Table 1. Experimental results for continuous fermentation with and without cell recycle with *Clostridium tyrobutyricum* at pH of 6.2.

Feed Conc. (g L^{-1})	Dilution Rate (h^{-1})	Glucose (g L^{-1})	Selectivity	Butyric Acid Yield	Cell Mass (g L^{-1})	Glucose Conversion
17.0	0.037	0.040	0.895	0.436	4.50	0.998
17.0	0.052	0.040	0.860	0.416	4.60	0.998
17.0	0.080	0.030	0.840	0.407	4.80	0.998
17.0	0.140			Washout		
25.0	0.023	0.030	0.952	0.420	4.80	0.998
25.0	0.069	2.00	0.800	0.350	4.80	0.92
25.0	0.107			Washout		
50.0	0.028	19.2	0.958	0.393	4.70	0.616
50.0	0.063	16.8	0.820	0.297	4.90	0.664
50.0	0.120			Washout		
17.0	0.078	0.040	0.973	0.460	5.10	0.998

2.3. Continuous Fermentation of C. tyrobutyricum with Total Cell Recycle

An extreme condition available for use to evaluate the influence of cell growth rate on continuous fermentation was total cell recycle by ultrafiltration or microfiltration. Total cell recycle greatly inhibited cell growth, and kept the cell growth rate close to zero. However, it could never be truly continuous as the fermentation vessel would eventually fill with dead cell mass. Theoretically, the cell growth rate for total cell recycle equals the cell death rate [21]. Continuous fermentation experimentation with cell recycle was conducted at a glucose concentration of 17 g L^{-1} and a dilution rate of 0.078 h^{-1}, yielding a butyric acid selectivity of 97.3%, as shown in Table 1. Normally, the cell concentration in a fermenter with cell recycle would be much higher than without cell recycle [12]. For this particular fermentation, however, the cell concentration was only slightly higher, which again showed that product concentration increased cell growth inhibition.

2.4. Batch Fermentation Modeling

In order to accurately model the fermentation characteristics, the adjustable parameters from Equations (3)–(5) were determined. Thus, Equation (4) was rearranged to yield:

$$\frac{1}{X}\frac{\mathrm{d}P_i}{\mathrm{d}t} = \alpha_i \times \mu + \beta_i = \alpha_i \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}t} + \beta_i \quad (7)$$

To obtain α_i and β_i, $(1/X) \times (\mathrm{d}P_i)/\mathrm{d}t$ and $(1/X) \times \mathrm{d}X/\mathrm{d}t$ were obtained using the Matlab curve fit toolbox. As shown in Figures 2 and 3 which contains all the data in Figure 1, Luedeking-Piret equations for butyric acid and acetic acid formation were generated:

$$\frac{\mathrm{d}P_B}{\mathrm{d}t} = 0.18 \times X \quad (8)$$

$$\frac{\mathrm{d}P_A}{\mathrm{d}t} = 0.98 \times \frac{\mathrm{d}X}{\mathrm{d}t} \quad (9)$$

Figure 2. Specific rate of butyric acid formation as a function of specific cell growth rate for batch fermentation at pH 6.2.

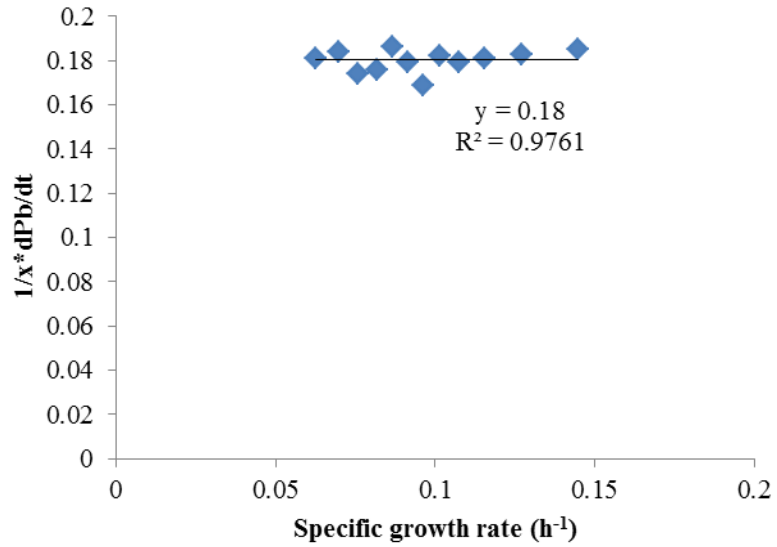
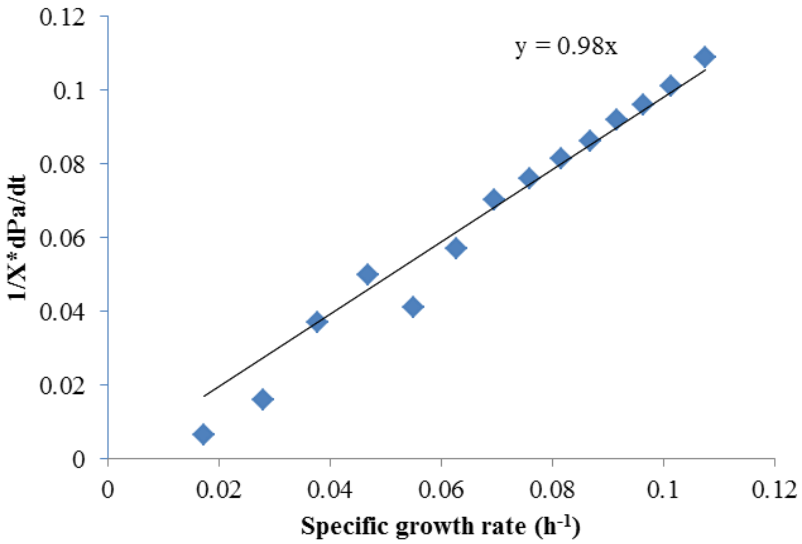


Figure 3. Specific rate of acetic acid formation as a function of specific cell growth rate for batch fermentation at pH 6.2.



Both of these equations showed very good fits (R^2 > 0.975), strongly indicating butyric acid was non-growth rate-related and the production of acetic acid was growth-rate-related. The strength of these correlations indicated that low growth conditions (such as late in batch fermentation or in high rates of cell recycling) were best for high selectivities of butyric acid.

To simplify the Monod equation, an assumption was made that cell inhibition was low. The following equation represented cell growth when initial glucose concentration was low:

$$\frac{dX}{dt} = 0.15 \times \frac{S}{5+S} X \quad (10)$$

which indicated that the maximum specific cell growth rate would not exceed 0.15 h^{-1}, which was estimated as the limit of the data in Table 1. When the extended Monod model was needed, at higher glucose concentrations, a K_s of 4 and m = 1/3 (based on literature) were used, and X_m was found to

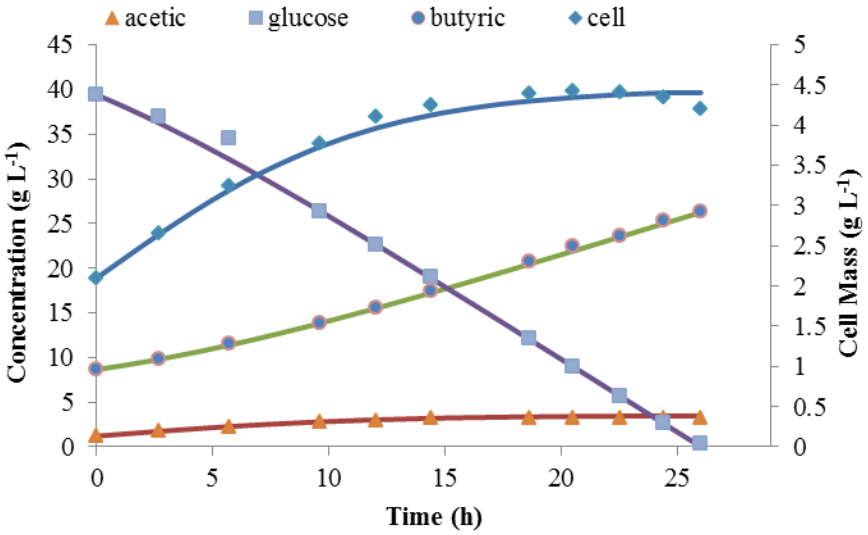
be 5 g/L; this was the maximum cell growth rate for this media. In this work we modified K_s at different concentrations for a better fit but one K_s could be used. These numbers would need to be modified if a different medium with different growth conditions was used.

Equation (5), describing substrate consumption, includes the parameters $Y_{B/S}$ and $Y_{A/S}$. These parameters could be obtained from reaction stoichiometry, showing that one mole of butyric acid was produced from one mole of glucose and two moles of acetic acid were produced from one mole of glucose [8]. Thus:

$$Y_{B/S} = \frac{M_B}{M_G} = \frac{88}{180} = 0.489 \text{ and } Y_{A/S} = \frac{2 \times M_A}{M_G} = 0.667 \text{ and } \frac{dS}{dt} = -2.045 \times \frac{dP_B}{dt} - 1.5 \times \frac{dP_A}{dt} \quad (11)$$

Matlab was used to solve these three coupled differential equations. These were solved simultaneously with the mass balance in order to assure carbon balance. Figure 4 shows the simulated time courses of batch fermentation against experimental data for the last batch fermentation, which was initiated with 39.6 g L^{-1} of glucose. The simulation predicted the kinetics of this batch fermentation very well. $Y_{P/S}$ was calculated from experimental results to be close to 1. This proved the assumption that for this composite medium, 95%–100% of the glucose was converted to products instead of cell mass. It also shows that this model can be used as the basis for continuous fermentation modeling.

Figure 4. Batch fermentation simulation and experimental data with *Clostridium tyrobutyricum* at initial glucose concentration of 39.6 g L^{-1} and pH of 6.2.



2.5. Steady State Continuous Fermentation Modeling

The equation describing product formation during the batch fermentation was used to find the steady-state mass balance for the continuous culture to obtain predicted output concentrations of products and selectivity for butyric acid. A mass balance on acetic acid is shown below, in Equation (13). From batch fermentation kinetics:

$$\frac{dP_A}{dt} = 0.98 \times \frac{dx}{dt} = 0.98 \times \mu \times X \quad (12)$$

This equation is empirically derived from data obtained by Figure 3. A transient simulation of the mass balance on acetic acid applied to the fermenter is:

$$F \times C_{A0} - F \times C_A + V_R \times \frac{\mathrm{d}P_A}{\mathrm{d}t} = V_R \times \frac{\mathrm{d}C_A}{\mathrm{d}t} \quad (13)$$

where F is the feed flow rate in and out in L h^{-1} and C_{A0} and C_A represent the starting and present concentration of acetic acid in the fermenter. When the continuous fermentation reached steady state, one would assume the concentration of acetic acid does not change with time. However, previous work in our group [18] has shown that acetic acid metabolizes to butyric acid and thus this assumption does not hold true. Thus:

$$\frac{\mathrm{d}C_a}{\mathrm{d}t} = \alpha_a \times \mu \times X + \beta_a \times X - D \times C_a \quad (14)$$

and the production of acetate was assumed to use the following format:

$$\frac{\mathrm{d}P_a}{\mathrm{d}t} = \alpha_a \times \frac{\mathrm{d}X}{\mathrm{d}t} - \beta_a \times C_a \times X \quad (15)$$

where α_a is assumed to be unchanged from the batch fermentation model; and β_a is evaluated by the following equation, which is a recombination of the continuous culture mass balance:

$$\beta_a = D \times \left(\frac{C_{a0} - C_a}{X} + \alpha_a\right)/C_a \quad (16)$$

A similar equation was derived for butyrate production:

$$\frac{\mathrm{d}C_b}{\mathrm{d}t} = \alpha_b \times \mu \times X + \beta_b \times X - D \times C_b \quad (17)$$

although the parameter β_b would be unchanged from the batch estimation since butyric acid did not further metabolize to any other products. Thus, the substrate consumption was defined as:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = D \times (S_0 - S) - \left(\frac{1}{Y_{\frac{a}{S}}} + \frac{1}{Y_{\frac{b}{S}}}\right) \times \frac{\mathrm{d}P_i}{\mathrm{d}t} \quad (18)$$

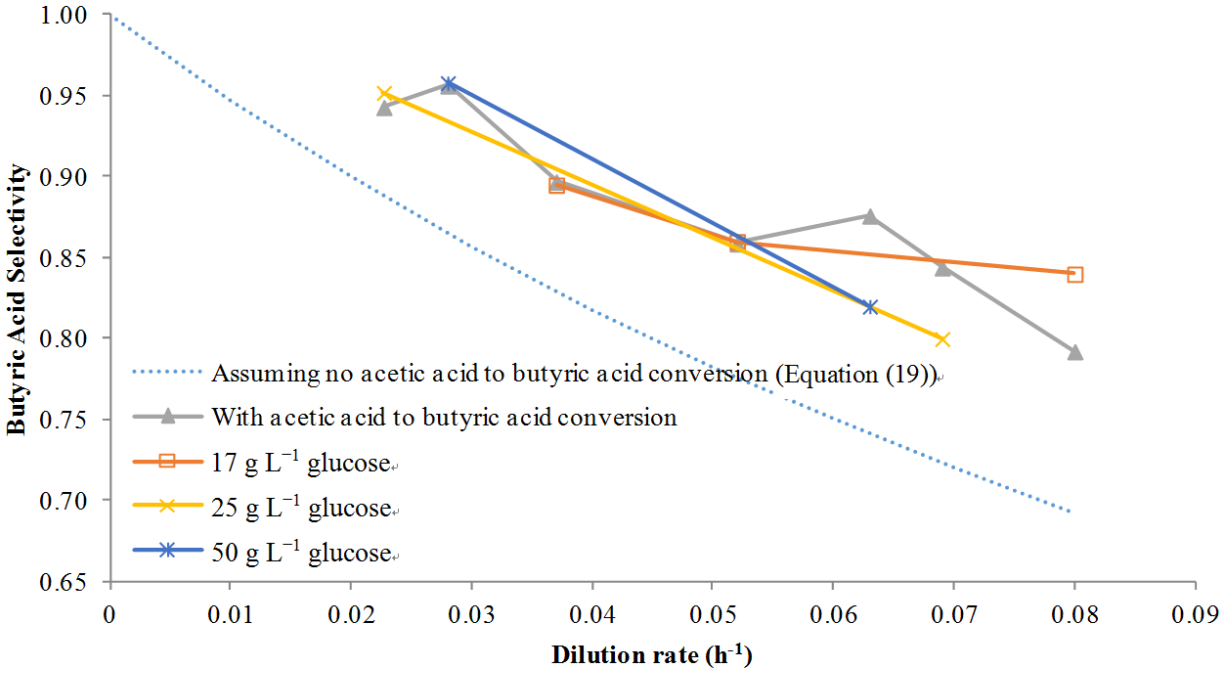
Finally, the selectivity was defined as the amount of butyric acid produced over the total amount of acids produced. If acetic acid was not metabolized to butyric acid this selectivity was given as:

$$S_B = \frac{C_B}{C_B + C_A} = \frac{\frac{X}{D} \times 0.18}{\frac{X}{D} \times 0.18 + 0.98 \times X} = \frac{0.18}{0.18 + 0.98 \times D} \quad (19)$$

However, if acetic acid was metabolized to butyric acid then Equations (14), (16)–(18) were solved simultaneously using Matlab and the selectivity was calculated on a point by point basis where the term β_a is concentration dependent with a big effect occurring at high concentrations. The results of using this model with several dilution rates at several different glucose concentrations are given in Figure 5. First, if no acetic to butyric acid conversion was assumed, the model clearly under-predicts the selectivity: butyric acid selectivity was predicted to be about 0.90 at a low dilution rate of 0.02 h^{-1}, for example, while the actual values at various concentrations of glucose are closer to 0.95. However, using the adjusted model it was found that the selectivity prediction was closer to the actual values at several different glucose feed concentrations. This provided proof that not only was acetic acid to butyric acid conversion taking place, but also that it was possible to model the conversion accurately

with few data points. Although Michel-Savin *et al.* [11] showed conversion of acetic acid to butyric acid, this phenomenon had not been accurately modeled until this work.

Figure 5. Comparison of experimental data and simulation of butyrate selectivity for continuous fermentation with *Clostridium tyrobutyricum* at pH of 6.2.



2.6. Predicting Optimum Operating Conditions

Since the model was proven at several different glucose concentrations, it was then used to predict optimum conditions for glucose concentration and dilution rate. The prediction of butyric acid selectivity, productivity, and glucose conversion are given in Figures 6–8, respectively. With all glucose concentrations, it was predicted that butyric acid selectivity was maximized at low dilution rates. This was because butyric acid was produced when cell growth rates were low. The best conditions to achieve these maxima are either a fed batch fermentation system, when cell density can remain high when feed conditions are low, or in a high cell recycle environment in a continuous system with cell recycling. To maximize productivity, glucose concentration must be kept high (>75 g L^{-1}) at all cell growth rates. However, a look at overall glucose conversion shows the opposite effect; that is, that glucose conversion is higher when glucose concentration is low. This opposite effect shows that with this fermentation, one always fights the battle of high selectivity and high conversion in optimizing these conditions. Thus, as has been proposed earlier, cell recycling [17] and product separation [18] can make it possible to get high selectivities, conversions, and productivities. These levels are >95% selectivity is certain cases.

Figure 6. Simulated effects of dilution rate and glucose feed concentration on butyrate selectivity. Color differences indicate gradient.

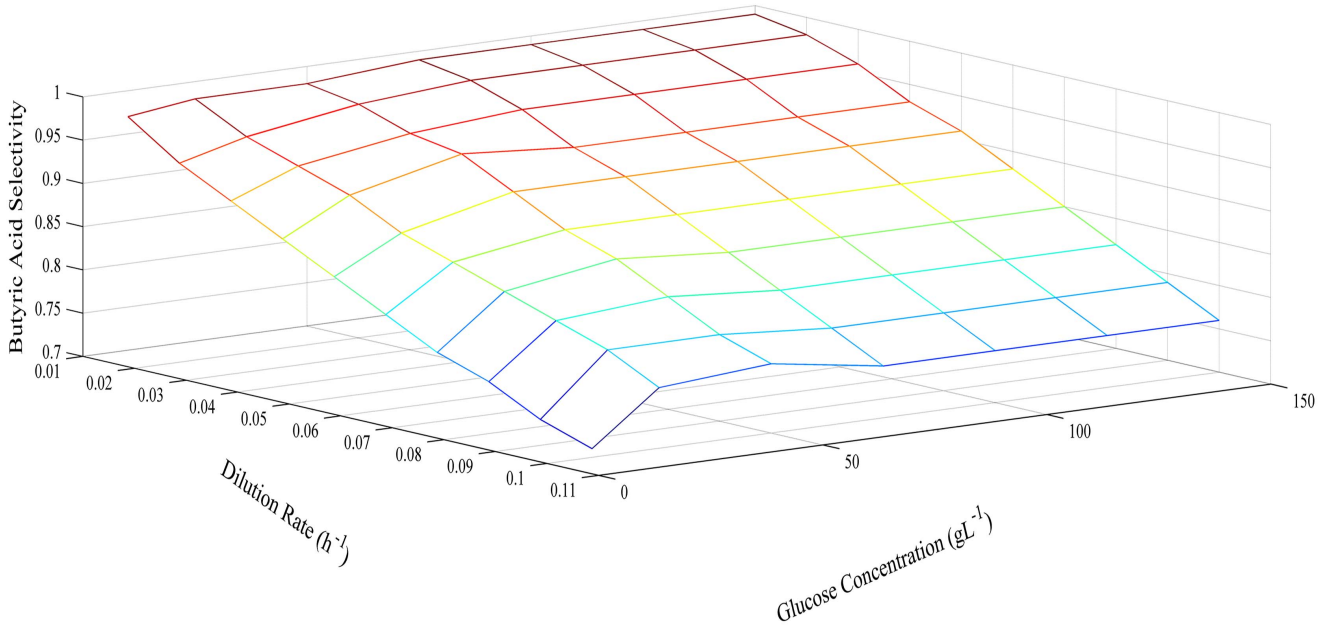


Figure 7. Simulated effects of dilution rate and glucose feed concentration on butyrate productivity. Color differences indicate gradient.

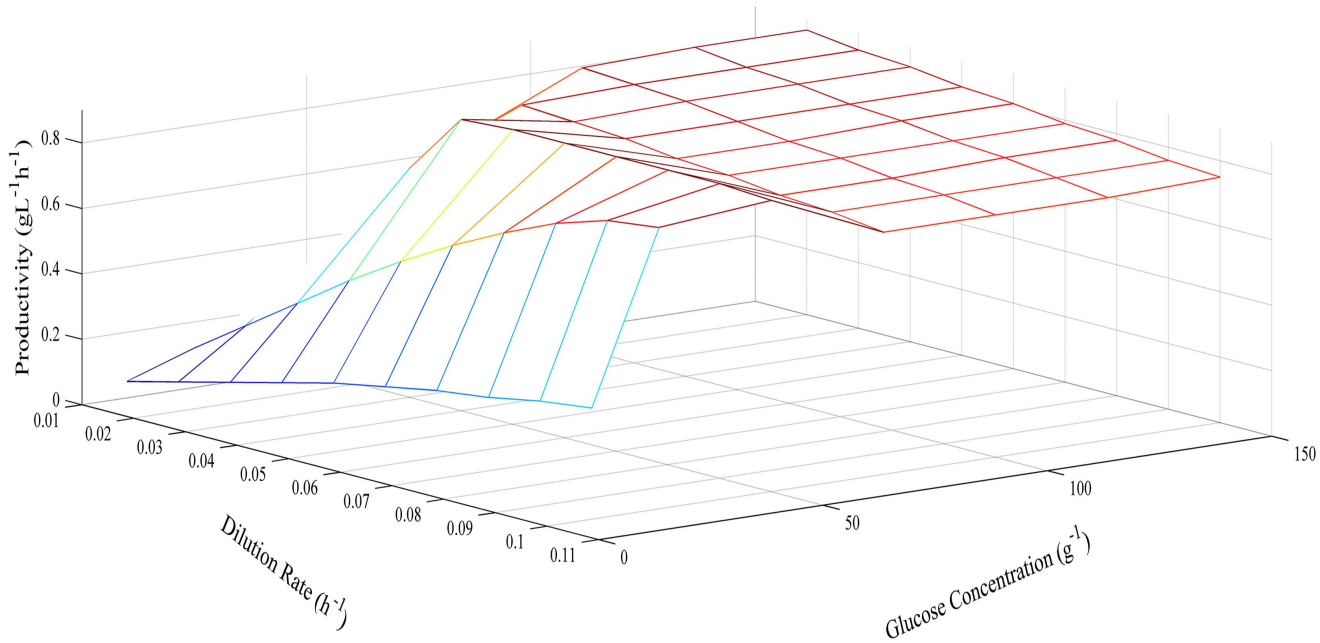
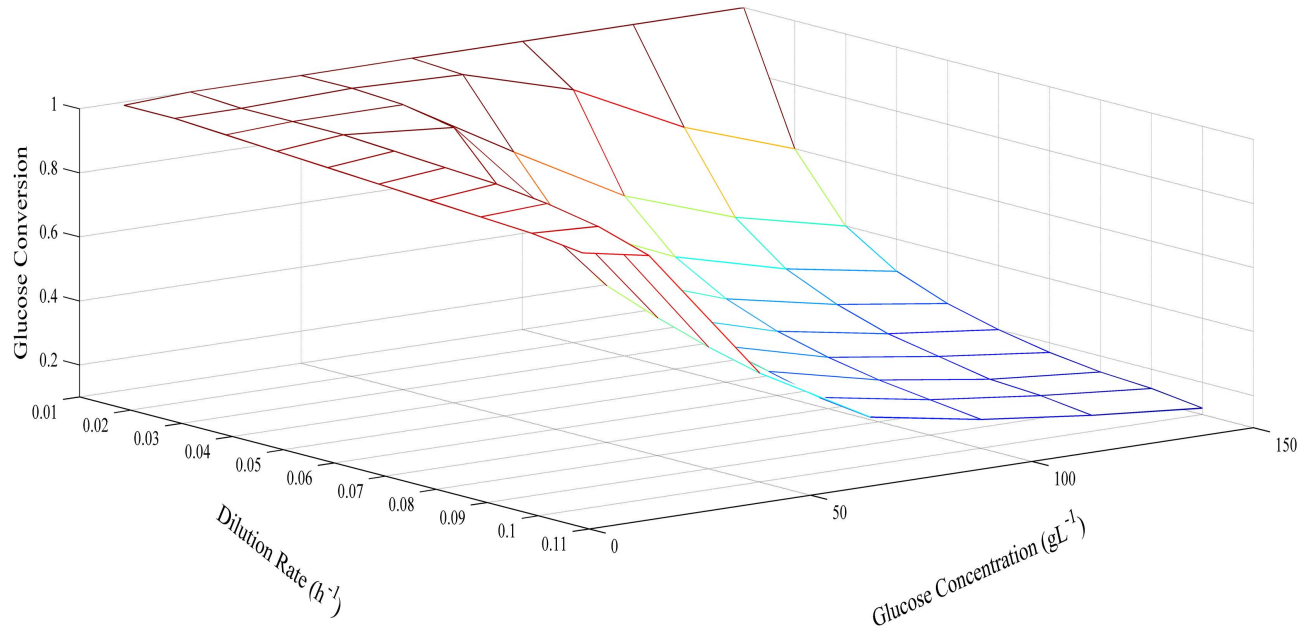


Figure 8. Simulated effects of dilution rate and glucose concentration on glucose conversion rate. Color differences indicate gradient.



3. Experimental Section

3.1. Materials and Methods

3.1.1. Cultures and Medium

Stock culture of *C. tyrobutyricum* (ATCC 25755) was kept in bottles under anaerobic conditions at 4 °C. For the pre-culture, 10 mL stock cultures were used to inoculate 100 mL of PYG (Peptone, Yeast extract, Glucose) medium in an incubator at 37 °C for about 48 h. The medium used in the fermentation contained 5 g yeast extract, 1.5 g KH_2PO_4, 0.6 g $MgSO_4 \cdot 7H_2O$, 30 mg $FeSO_4 \cdot 7H_2O$, 3 g $(NH_4)_2SO_4$ and varying glucose concentrations per liter of deionized water (all from Aldrich-Sigma, St. Louis, MO, USA). The medium was autoclaved at 121 °C for 30 min before use.

3.1.2. Fermentation Modes

Batch and continuous fermentations were carried out in a 1 L stirred tank reactor (Applikon, Schiedem, The Netherlands). A schematic of the entire system is given and described in a previous paper [17]. The fermentation was controlled at a temperature of 37 °C and a pH of 6.2. The stirring rate was controlled at 200 rpm. The batch fermentation started with 700 mL of medium inoculated with 100 mL of pre-culture. Nitrogen was sparged into the culture at a flowrate of 20 mL min^{-1} to keep the fermenter anaerobic. Batch fermentations were continued by removing the fermentation broth and feeding new media with varying glucose concentrations. Fermentation was switched from batch to continuous mode by starting the feed pump and withdrawing culture. For continuous fermentation, the flowrate of the feed medium was controlled by a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA). A reaction volume of 700 mL was maintained by a liquid level control, which was

part of the fermentation system. Two 4 L bottles were used as the feed bottles, with all medium and connecting tubing sterilized in an autoclave for 30 min at 121 °C.

To investigate cell mass inhibition, a Koch Membrane ultrafiltration membrane cartridge (Wilmington, MA, USA) was used for cell recycle. This cartridge was made of polysulfone, under the brand of Romicon, with a 100,000 molecular weight cut-off (MWCO). The membrane cleaning and replacement procedure was described in a previous paper [17]. The permeate flowrate was controlled by a Masterflex peristaltic pump.

3.1.3. Analysis

High-performance liquid chromatography (HPLC) was used to analyze all the fermentation compounds, including glucose, acetic acid and butyric acid. The HPLC system consisted of a Waters Corporation 717 autosample injector, a Waters Corporation 1525 binary HPLC pump, an IC-PakTM ion-exclude column and a Waters Corporation 2414 refractive index detector (Milford, MA, USA). The solvent was 0.0005 mol L^{-1} sulfuric acid in water at a flowrate of 1.0 mL min^{-1} as described [29]. Cell concentration was measured by optical density at 600 nm with a DU 800 spectrophotometer (Beckman Coulter Inc., Brea, CA, USA). The dry cell mass was measured by employing an MB45 moisture analyzer (Ohaus, Parsippany, NJ, USA) at a temperature of 150 °C for 25 min. Before measuring the dry cell mass, the sample was centrifuged and rinsed with deionized water so that the sample was devoid of medium and products.

3.1.4. Kinetic Parameter Estimation

Matlab was used in the evaluation of kinetic parameters to solve the modeling equations. Kinetic parameter estimation utilized the curve fit toolbox to fit the batch fermentation results. This was done by solving Equations (14)–(18) simultaneously. A fourth-order Runge-Kutta solver was used to solve the coupled differential equations.

4. Conclusions

The developed model for continuous fermentation with *C. tyrobutyricum* accurately predicted fermentation kinetics, which includes cell growth, product formation, and substrate consumption. This model provides a specific understanding of the various factors that influence butyrate selectivity, productivity, and glucose conversion rate for the continuous fermentation with *C. tyrobutyricum*. The model also provides an understanding of the rate of conversion of acetate to butyrate, and how it influences butyrate selectivity. This model would be a useful tool to design a continuous fermentation process to produce butyrate at optimized conditions. Future work on this model should include adjustments for the effects of the separation technique used. The maximum selectivity of this organism, as shown in Table 1, is 97.3%.

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Conflicts of Interest

The authors declare no conflict of interest.

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