Modelling and Simulation of Biobutanol Fermentation by
Clostridium saccharoperbutylacetonicum N1-4

Elvi Restiawaty\textsuperscript{a, b, e*}, Ardiyan Harimawan\textsuperscript{c, d}, Novaldio Rizki\textsuperscript{c}, Fauz Irfan Rafi\textsuperscript{c}

\textsuperscript{a} Research Group of Chemical Engineering Process Design and Development, Faculty Industrial Technology, Institut Teknologi Bandung
\textsuperscript{b} Department of Bioenergy Engineering and Chemurgy, Faculty Industrial Technology, Institut Teknologi Bandung
\textsuperscript{c} Department of Chemical Engineering, Faculty Industrial Technology, Institut Teknologi Bandung
\textsuperscript{d} Research Group of Chemical Engineering Product Design and Development, Faculty Industrial Technology, Institut Teknologi Bandung
\textsuperscript{e} Biosciences and Biotechnology Research Center, Institut Teknologi Bandung

*Corresponding Author’s E-mail: erstiawaty@che.itb.ac.id

Abstract. Biobutanol exhibits high octane number, non-hygroscopic, non-corrosive and can be mixed with gasoline without engine modification. Furthermore, the use of biobutanol as a fuel can reduce the negative impact of environmental due to the use of fossil-based fuel. Therefore, research on the development of biobutanol is still very much needed, including research on modelling and simulation of biobutanol production by fermentation. With modelling and simulation, we can predict various biobutanol production behaviour. This paper deals with the kinetics models of biobutanol production as developed by Shinoto based on the C. acetobutylicum metabolic pathway which consists of the glycolysis, acidogenesis and solventogenesis pathways with several simplification of reactions. In this study, modelling was carried out by adapting the rate parameter value of $k_{13}$ indicating the rate constant of cell death. The adapted models were applied to determine the effect of the initial concentration of glucose and organic acids on the production of butanol and compared to the experimental data from previous research. Model simulations were performed by using MATLAB\textsuperscript{®} software. The butanol yield was affected by the initial glucose concentration. The highest butanol yield of 77\% was obtained at a glucose concentration of 22 g/L. The simulation results exhibit that the biobutanol yield decreases when the glucose concentration is higher than 22 g/L. These results are consistent with previous experimental data. The simulation results also show the effect of the concentration of acetic acid in the fermentation medium and can be optimized. From the simulation results, the presence of 7.5 g/L acetic acid can increase the biobutanol production by 20\%. However, previous experimental data showed that a concentration of 2.5 g/L acetic acid was preferred. The simulation results also show the effect of the addition of butyric acid and lactic acid on the production of biobutanol.
1. Introduction

The issue of alternative energy is currently a hot topic of discussion due to the crisis of fossil-based fuels. Biofuel as an alternative energy can be produced from renewable resources. The use of renewable resources certainly has a positive impact on the environment and can produce environmentally friendly fuels. Global biofuel consumption is still quite low, i.e. about 3.8%, of which 60% is dominated by ethanol production in 2017\(^1\). Although bioethanol is well developed, some of the properties of this fuel still have drawbacks. Furthermore, the development of biobutanol is currently being carried out considering that biobutanol has advantages over bioethanol. Biobutanol exhibits a higher energy density, quite similar to gasoline in octane number and air-fuel ratio, so that it is able to be used as a substitution of gasoline without engine modification\(^2\).

In the bioprocess industry, biobutanol can be produced from fermentable sugars or biomass hydrolysate through acetone-butanol-ethanol (ABE) fermentation using *Clostridia* bacteria. In the early growth phase, *Clostridia* produces hydrogen, carbon dioxide, acetate, and butyrate which causes a decrease in the pH of the culture media in a batch reactor system so that this phase is referred to as the acidogenic phase. Then, cell metabolism undergoes a shift to the solventogenic phase after the culture enters the stationary growth phase. In this phase there is re-assimilation of acid, which occurs with continued consumption of carbohydrates, usually resulting in an increase in the pH of the culture medium. In this solventogenic phase, acetyl-CoA and butyryl-CoA are the main intermediaries in the production of ethanol and butanol, respectively \(^3\). There have been many studies using several solvent clostridial strains that can naturally produce ABE, such as *Clostridium acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum*, and *C. saccharoperbutylacetonicum* \(^4\)-\(^6\) and some use genetically engineered clostridial mutations for the production of biobutanol \(^7\)-\(^9\). This study focuses on the biobutanol production from fermentable sugar using *C. saccharoperbutylacetonicum* N1-4. This strain is a mesophilic bacterium and can grow in an oxygen-free environment (anaerobic conditions). *C. saccharoperbutylacetonicum* N1-4 exhibits a good growth ability on both simple and complex sugars, such as glucose, sucrose, or starch \(^10\).

The fermentation of biobutanol has been widely investigated both experimentally \(^11\)-\(^16\) and computer simulation \(^11\). Zhou et al. \(^15\) studied the influence of acetate and lactate, meanwhile Al-Shorgani et al. \(^16\) studied the influence of butyrate. Shinto et al. \(^11\) have proposed mechanistic models of *C. saccharoperbutylacetonicum* N1-4 in mathematical equation forms that based on Michaelis-Menten’s enzymatic reaction kinetics, except for the cell death reaction. Shinto et al. \(^11\) varied the initial glucose concentration in their experiments to develop their mechanistic model of butanol fermentation. However, when the Shinto’s model was applied to consider the laboratory work by Tanaka \(^13\) and Ishizaki \(^14\), in which high glucose concentration was used (90 g/L), it was not appropriate to validate the data. Those laboratory works indicated that glucose was still remained in certain concentration at the end of fermentation, meanwhile simulation of Shinto’s model indicated that glucose was fully utilized. According to the Shinto model, the consumption rate of glucose correlates with the concentration of residual glucose, butanol, and biomass. In Shinto’s mechanistic models, the biomass concentration is considered to be the most influencing one, since biomass concentration is consisted in every single kinetics rate equation. In other hand, the biomass concentration change is affected by the acetyl-CoA and butanol concentrations, and also death rate of the biomass. Furthermore, acetyl-CoA and butanol are intertwined with other compounds. It is leading to death rate as the most independent factor of biomass concentration, since it is only directly influenced by the biomass concentration itself.

The objective of this study is to investigate the production of biobutanol in a batch reactor by varying some process variables through modelling and simulations. The simulation is also able to estimate the performance of a system in several conditions \(^17\). In this study, modeling was carried out by adapting the Shinto’s model. The adapted models were applied to determine the effect of the initial concentration of glucose (\(S_0\)) and organic acids on the production of butanol and compared to the experimental data from previous research.
2. Methodology

2.1. Simulation algorithm

The outline algorithm of this research consisted of literature studies, evaluating the models statistically, and applying the model to identify the effect of glucose and organic acids. This research began with a literature study on the metabolism mechanism of *C. saccharoperbutylacetonicum* N1-4 and kinetic models of the reaction mechanism by Shinto *et al.* [11], which is provided in Figure 1. In this study, the rate constant of biomass death rate (value of $k_{13}$ in Equation A.13) was tuned by several iterating.

The adapted mathematical models were validated with the previous laboratory experimental data investigated by Shinto *et al.* [11], Thang *et al.* [12], Tanaka *et al.* [13], and Ishizaki *et al.* [14]. Accuracy of the model was evaluated based on residual $R^2$ statistical method with the help of MATLAB® as the simulation tool. The statistical results are then used as an indicator of the adapted parameter to give more accurate results (higher $R^2$), with the same method. Once it is deemed accurate enough, the adapted model was applied to experiment variations, along with other experimental data.

![Figure 1. Metabolic pathway in Clostridium [11]](image)

Mechanistic equations of reaction rates proposed by Shinto *et al.* [11] are presented below.

\[
\begin{align*}
\text{Glucose} & \quad \text{R1} \quad \text{ATP} \\
\text{Fructose} & \quad \text{R2} \quad \text{ATP} \\
\text{Glyceraldehyde-phosphate} & \quad \text{R3} \\
\text{Lactate} & \quad \text{R4} \quad \text{ATP} \\
\text{Pyrurate} & \quad \text{R5} \quad \text{ATP} \\
\end{align*}
\]

\[
\begin{align*}
\frac{d[\text{Glucose}]}{dt} &= -r_1 & (A.1) \\
\frac{d[\text{Lactate}]}{dt} &= r_2 & (A.2) \\
\frac{d[\text{G3P}]}{dt} &= r_3 & (A.3) \\
\frac{d[F6P]}{dt} &= r_4 - r_5 & (A.4) \\
\frac{d[\text{Pyruvate}]}{dt} &= r_5 - r_6 & (A.5) \\
\end{align*}
\]
\[ r_1 = \frac{V_{\text{max},1}[\text{Acetate}][\text{Biomass}]}{K_{m1} + [\text{Acetate}]} \times F \]  
\[ r_2 = V_{\text{max},2} \left( \frac{1}{1 + \left( \frac{K_{m2}}{[\text{Acetate}]} \right)} \right) \left( 1 + \frac{1}{K_{m2}} \right) [\text{Biomass}] \]  
\[ r_3 = \frac{V_{\text{max},3}[\text{Acetate}][\text{Biomass}]}{K_{m3} + [\text{Acetate}]} \times F \]  
\[ r_4 = \frac{V_{\text{max},4}[\text{Acetate}][\text{Biomass}]}{K_{m4} + [\text{Acetate}]} \times F \]  
\[ r_5 = \frac{V_{\text{max},5}[\text{Butanol}][\text{Biomass}]}{K_{m5} + [\text{Butanol}]} \times F \]  
\[ r_6 = \frac{V_{\text{max},6}[\text{Acetone}][\text{Biomass}]}{K_{m6} + [\text{Acetone}]} \times F \]  
\[ r_7 = \frac{V_{\text{max},7}[\text{Butyrate}][\text{Biomass}]}{K_{m7} + [\text{Butyrate}]} \times F \]  
\[ r_8 = \frac{V_{\text{max},8}[\text{BCoA}][\text{Biomass}]}{K_{m8} + [\text{BCoA}]} \times F \]  
\[ r_9 = \frac{V_{\text{max},9}[\text{BCoA}][\text{Biomass}]}{K_{m9} + [\text{BCoA}]} \times F \]  
\[ \frac{d[\text{Lactate}]}{dt} = r_1 - r_4 \]  
\[ \frac{d[\text{ACoA}]}{dt} = r_6 + r_7 - r_8 - r_9 - r_11 - r_12 \]  
\[ \frac{d[\text{Biomass}]}{dt} = r_{12} - r_{13} \]  
\[ \frac{d[\text{Acetate}]}{dt} = r_6 - r_7 - r_3 \]  
\[ \frac{d[\text{Ethanol}]}{dt} = r_{11} \]  
\[ \frac{d[\text{AAcCoA}]}{dt} = r_9 - r_8 - r_13 - r_5 \]  
\[ \frac{d[\text{AaCoA}]}{dt} = r_6 + r_5 - r_6 \]  
\[ \frac{d[\text{Butyrate}]}{dt} = r_{12} - r_9 - r_7 \]  
\[ \frac{d[\text{Butyrate}]}{dt} = r_6 \]  
\[ \frac{d[\text{Butyrate}]}{dt} = r_{12} - r_9 - r_7 \]  
\[ \frac{d[\text{AaCoA}]}{dt} = r_9 \]  

**Table 1.** Kinetic parameters estimated by Shinto et al. \[^{[11]}\]

| Reaction | \( k \) (h\(^{-1}\)) | \( V_{\text{max}} \) (h\(^{-1}\)) | \( K_m \) (mM) | \( K_i \) (mM) | \( K_a \) (mM) | \( K_b \) (mM) | \( K_{a,b} \) (mM) |
|----------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| \( R_1 \) | 3.20 | 46.0 | 55.6 | 67.5 |
| \( R_2 \) | 40.0 | 10.0 |
| \( R_3 \) | 120 | 26.5 |
| \( R_4 \) | 7.50 | 177 |
| \( R_5 \) | 9.70 | 500 |
| \( R_6 \) | 180 | 1.50 |
| \( R_7 \) | 0.30 | 50.0 |
| \( R_8 \) | 19.0 | 40.0 | 70.0 |
| \( R_9 \) | | 26.5 | 51.0 |
| \( R_{10} \) | 20.0 | 1.00 |
| \( R_{11} \) | 7.45 | 30.0 |
| \( R_{12} \) | 8.10 | 1.10 | 23.0 |
| \( R_{13} \) | 0.017 |
| \( R_{14} \) | 10.0 | 5.20 |
| \( R_{15} \) | 80.0 | 15.0 | 50.0 |
| \( R_{16} \) | 12.0 | 10.0 |
| \( R_{17} \) | 35.0 | 4.90 | 2.20 |
| \( R_{18} \) | 100 | 6.10 |
| \( R_{19} \) | 3.15 | 5.00 | 67.5 | 2.20 |
2.2. Statistical method

Shinto et al. [11] used Pearson correlation coefficient \( R^2_{\text{Pearson}} \) to measure the accuracy of their model on experimental data. \( R^2_{\text{Pearson}} \) can be determined using a formula written in Equation (1), \(^{[18]}\).

\[
R^2_{\text{Pearson}} = \frac{\sum(y - \bar{y})(\hat{y} - \bar{\hat{y}})}{\sqrt{\sum(y - \bar{y})^2 \sum(\hat{y} - \bar{\hat{y}})^2}}
\]  

(1)

where \( y \) is an experiment datum, \( \hat{y} \) is a simulation datum corresponding to \( y \), \( \bar{y} \) is an average of the experiment data, and \( \bar{\hat{y}} \) is an average of the simulation data \(^{[18]}\). In contrast to the method of determining accuracy by Shinto et al. \(^{[11]}\), the model accuracy on experimental data in this study was examined using residual determination coefficients \( R^2 \). The value of \( R^2 \) can be calculated using Equation (2) \(^{[18]}\).

\[
R^2 = 1 - \frac{\sum(y - \bar{y})^2}{\sum(y - \bar{\hat{y}})^2}
\]  

(2)

The main difference of \( R^2_{\text{Pearson}} \) and \( R^2 \) is the average simulation value \( (\bar{\hat{y}}) \) that \( R^2_{\text{Pearson}} \) includes the \( \bar{\hat{y}} \), while \( R^2 \) does not. Overall, \( R^2 \) illustrates how much total error between the simulation and the experimental results, compared to total variance of the experiment results itself. Variance states the extent of data distribution. A negative value of \( R^2 \) means that the simulation deviates too much from the distribution of the experimental data. For summarizing some values of \( R^2_{\text{Pearson}} \) or \( R^2 \) in this study, average formula \( (\text{Avg}) \) is used as follows:

\[
\text{Avg} = \frac{\sum_{i=1}^{n} R^2_i}{n}
\]  

(3)

with \( R^2_i \) as the individual values of \( R^2_{\text{Pearson}} \) or \( R^2 \), and \( n \) as number of \( R^2_i \).

3. Results and Discussion

3.1. Model scripting evaluation

At the beginning of this study, an evaluation of script writing in MATLAB® from the models made by Shinto et al. \(^{[11]}\) needed to be done. This was because Shinto et al. wrote a model script into different software, namely WinBEST-KIT. This evaluation was intended to ensure that the model script used in this study conforms to the model script written by Shinto et al. \(^{[11]}\). The evaluation was done by comparing the value of Pearson correlation coefficient \( R^2_{\text{Pearson}} \) obtained from Shinto et al. \(^{[11]}\) and from the simulation results in this study. The experimental data are also in accordance with the data from Shinto et al. \(^{[11]}\). Table 2 shows the comparative values of \( R^2_{\text{Pearson}} \) between the value obtained from Shinto et al. \(^{[11]}\) using WinBEST-KIT and from this study using MATLAB®.

| Table 2. Comparison of \( R^2_{\text{Pearson}} \) values between literature and this study for changes in the concentration of compounds involved in butanol fermentation obtained by Shinto et al (2007). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **References**  | **Pearson correlation coefficients on the concentration trend of compounds** |
| Glu.*           | 0.972           | 0.975           | 0.970           | 0.644           | 0.850           | 0.901           |
| Ace.*           | 0.983           | 0.984           | 0.976           | 0.679           | 0.831           | 0.905           |
| But.*           | 0.984           | 0.986           | 0.976           | 0.679           | 0.831           | 0.905           |
| Act.*           | 0.970           | 0.976           | 0.679           | 0.831           | 0.905           | 0.905           |
| Btr.*           | 0.644           | 0.679           | 0.831           | 0.905           | 0.905           | 0.905           |
| Bms.*           | 0.850           | 0.831           | 0.905           | 0.905           | 0.905           | 0.905           |
| **Avg.**        | 0.905           | 0.905           | 0.905           | 0.905           | 0.905           | 0.905           |

*Abbreviation used in this study: Glu. stands for glucose, Ace. for acetone, But. for butanol, Act. for acetate, Btr. for butyrate, Bms. for biomass, and Avg. for average.*
The difference in average error value between this study and the literature using Shinto’s models is quite small, i.e. 0.04 (0.44%). The small error was accepted because the software used to simulate Shinto’s models in this study was different with the Shinto’s software. In addition, the data used in this study was data extracted from the visual graph provided by Shinto et al. \[11\], thus allowing the data to be read not completely accurate. It was concluded that the small error value indicates good-accuracy of the model-scripting, thus the simulation model used in this study can be considered the same as what Shinto et al. \[11\] have proposed.

3.2. Model adapting evaluation

As explained in model scripting evaluation, Shinto et al. \[11\] used Pearson correlation coefficient as a statistical method for justification of their model accuracy, but this method is considered inappropriate. Instead, a better and more common alternative is residual determination coefficient method (\(R^2\)) \[18\]. Re-evaluation of the model using \(R^2\) have given negative values of \(R^2\) on butyrate and biomass trend.

To achieve higher value of \(R^2\), the model was adapted by tuning the parameter \(k_{13}\) or cell death rate constant. This parameter is involved in a simpler first-order reaction kinetics equation, unlike other equations that follow the Michaelis-Menten kinetics which tends to be more complicated. In addition, this parameter is directly related to the biomass-concentration model. The formula of cell death kinetics rate is given in Equation A.13 with \(k_{13}\) as a cell death rate constant (h\(^{-1}\)) and [Biomass] as concentration of cell biomass (g/L). Shinto et al. \[11\] obtained \(k_{13} = 0.017\) h\(^{-1}\).

In this study, tuning is done through trial-and-error by iterating \(k_{13}\) in the range 0.035 – 0.07 h\(^{-1}\). The results is depicted in figure 2 and show that the iteration with \(k_{13} = 0.05\) h\(^{-1}\) is the best tune based on visual rating. Hereinafter, this adapted model is called ‘Shinto bms2’, while the reference model without tuning is ‘Shinto original’.

3.3. Model evaluation using experiment results in other literatures

Evaluation of both Shinto original and Shinto bms2 models was carried out on other experimental data which have similar methodology, i.e., batch butanol fermentation with glucose as substrate using \(C.\ saccharoperbutylacetonicum\) N1-4, to enlarge the evaluation range. Those data were obtained from Thang et al. \[12\], Tanaka et al. \[13\], and Ishizaki et al. \[14\]. The results show that the Shinto bms2 model is more accurate than the Shinto original model based on negative \(R^2\) frequency (see Table 3).

![Figure 2. Tuning parameter evaluation for (a) \(S_0 = 12.7\) g/L (a) and (b) \(S_0 = 22\) g/L](image)

Some \(R^2\) values are negative, especially in the butyrate variables. The negative \(R^2\) value is caused by not-fitted results between experimental data and the model simulation. By assumption the raw data is
valid, there is two presumption causes of negative $R^2$ values. First, concentration of the related compounds during fermentation are low which resulting negative values after $R^2$ calculation. Second, the model itself is not suitable enough with actual condition, due to the model which is a simplification of complex metabolic mechanism. However, in general, the $R^2$ results after tuning (bms2) has more positive results counted than the original one, which is the new tuned-simulation more fit with the raw data. This indicates that the prior tuning indeed increases the accuracy of the model. Thus, Shinto bms2 was chosen as simulation model to determine the effect of initial concentration of glucose and organic acids to butanol production.

### Table 3. Evaluation results of the models using other literatures

| Model       | Data     | $S_0$ (g/L) | $R^2$ (residual determination coefficients) |   |
|-------------|----------|------------|---------------------------------------------|---|
|             |          |            | Glucose     | Acetone     | Butanol     | Acetate     | Butyrate    | Biomass    |
| Shinto      | Shinto   | 6.5 – 53.1| 0.923       | 0.811       | 0.918       | 0.788       | -0.997      | -0.890      |
| original    | Thang et al. | 66     | 0.907       | 0.891       | 0.848       | 0.898       | -5.463      | -3.389      |
|             | Tanaka et al. | 90    | -0.517      | -0.187      | -0.032      |            |             | -0.020      |
|             | Ishizaki et al. | 90   | -0.745      | -0.349      |            |             |             | 0.594       |
| Shinto      | Shinto   | 6.5 – 53.1| 0.949       | 0.808       | 0.947       | 0.754       | -1.456      | 0.421       |
| bms2        | Thang et al. | 66     | 0.954       | 0.880       | 0.924       | 0.724       | -7.338      | 0.128       |
|             | Tanaka et al. | 90    | 0.032       | 0.248       | 0.472       |            |             | -0.271      |
|             | Ishizaki et al. | 90   | 0.132       | 0.450       |            |             |             | -0.502      |

3.4. Effect of initial glucose concentration

Stoichiometrically, 1 mole of glucose produces 1 mole of butanol (0.411 g/g). However, the experiment shows the final butanol concentration never exceeds the theoretical concentration, because the metabolic reaction is more complex and produces other metabolites. Inhibition is another factor that also influences butanol production, especially when the butanol concentration does not increase when the initial glucose concentration is higher (figure 3). Higher initial glucose concentration causes higher final butanol concentration. But then, cell metabolism is increasingly inhibited by the butanol, reducing the butanol production itself. Regarding this phenomenon, Shinto bms2 model is only accurate for low initial glucose concentration, less than 66 g/L.

The same goes to butanol yield. The butanol yield also needs to be reviewed to determine the efficiency of glucose utilization by the bacteria. Experiments show that butanol yield never exceeds the stoichiometric yield (figure 3). The highest butanol yield of 77% was obtained at a glucose concentration of 22 g/L. The simulation results exhibit that the biobutanol yield decreases when the glucose concentration is higher than 22 g/L. These results are consistent with previous experimental data.

The butanol yield decreases with increasing initial glucose concentration. However, low initial glucose concentration is not an option to be applied to the industry because it produces low butanol concentration as well. Conversely, applying high initial glucose concentrations is also not appropriate because the butanol yield will be low and not economical well. Butanol productivity is the solution to determine the optimum initial glucose for butanol production. Productivity reviews the ratio of the final butanol concentration to the fermentation time when glucose runs out or reaches the end of utilization. Figure 3 shows the initial glucose concentration of 53 g/L could give the highest butanol productivity. This glucose concentration is in accordance with the use of sugar in the butanol industry, which uses molasses with sugar levels of 4-6% or approximately 40-60 g/L glucose [10].
3.5. Effect of initial organic acids concentration

3.5.1. Acetate. Acetate is the primary metabolite in the *Clostridia* metabolic pathway, so its presence is thought to affect the production of intermediate compounds and secondary metabolites. However, Zhou *et al.* [15] have found the addition of acetate did not increase butanol production linearly. Butanol concentration tends to be constant with optimum acetate concentration of 2.5 g/L, in contrast to simulation which shows an increase (figure 4). This difference is thought to be caused by different types of fermentation medium used. Zhou *et al.* [15] used the nitrogen-free medium, whereas Shinto *et al.* [6] used the TYA (tryptone, yeast-extract, acetate) medium which contains nitrogen. Acidity is also another factor that influences butanol production. More acidic or basic fermentation conditions (compared to optimum condition) can cause inhibition of butanol production, so an increase of initial acetate concentration results in the decrease of butanol production. This effect cannot be described by the simulation. Also, the initial acetate concentration of 2.5 g/L is sufficient to produce butanol with the highest concentration. The value itself is the acetate concentration in the TYA medium.

![Figure 4. Effect of initial concentration of (a) acetate and (b) lactate to butanol final concentration](image-url)
3.5.2. Lactate. Lactate is also an intermediate in the *Clostridia* metabolic pathway, which is directly related to pyruvate as the main precursor, so lactate is suspected to affect butanol production. Zhou et al. [15] have proved this, since their experiment results (figure 4) show the butanol production increases as the initial lactate concentration increases. At high initial lactate concentration, inhibition occurs because the fermentation medium is too acidic, so it inhibits the cell metabolism and therefore butanol production. The Shinto bms2 model cannot explain this phenomenon of inhibition, and the overall final butanol concentration from simulation is higher than the experimental data. This difference is thought due to differences in the type of medium used, as previously discussed.

3.5.3. Butyrate. Butyrate is also a primary metabolite. To produce butanol, butyrate is an important compound besides butyryl-CoA. Metabolic pathway from butyrate to butanol is relatively close, and butyrate is an inductor for butyryl-CoA, thus its effect is believed to be significant compared to other metabolites. Al-Shorgani et al. [16] reported that low initial glucose concentration (less than 15 g/L) can accommodate the addition of butyrate to increase butanol production, but not significant. When the initial glucose concentration is higher (more than 15 g/L), butanol production increases quite significantly, but only up to the initial butyrate-glucose ratio of 0.5 (figure 5). This shows that butyrate is also an inhibitor for butanol production, parallel to glucose.

![Figure 5](image-url)  
*Figure 5. Effect of initial butyrate to glucose concentration ratio to final butanol concentration, with initial glucose concentration of (a) 5 g/L, (b) 10 g/L, (c) 15 g/L, and (d) 20 g/L.*

4. Conclusion
The adapted model (Shinto bms2) was obtained by tuning the parameter value of $k_{13}$ and more accurate than the original one. Furthermore, the simulation results show that the initial concentration of glucose and organic acids (acetate acid, butyric acid, and lactic acid) can increase butanol production up to a certain concentration value. However, if the concentration of glucose and these acids exceeds the limit concentration, there will be an inhibition. The optimum concentration of initial acetate is 2.5 g/L, as it was prescribed in TYA medium (common medium for *Clostridia*). Without butyrate and lactate addition, the optimum butanol yield of 77% and final concentration of 7 g/L was obtained at initial
glucose concentration of 22 g/L. Meanwhile, the optimum concentration of initial lactate is 5 g/L, and the optimum ratio of initial butyrate to glucose is 0.5.

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