Modeling the synthetic gas fermentation for bioethanol production

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Abstract. The productivity of bioethanol from the synthetic gas anaerobic fermentation by Clostridium ljungdahlii is still very low when compared to other bioethanol fermentation methods. The low mass transfer rate of CO, CO2, and H2 gases to the liquid fermentation broth has been considered a major bottleneck in the overall process. Another possible bottleneck is the low concentration of biomass as the real catalyst for bioethanol production. A repeated batch fermentation configuration is proposed to solve the biomass concentration problem. This paper presents the evaluation of the repeated batch configuration for syngas anaerobic fermentation. A model for syngas fermentation has been developed and was used to simulate the effects of repeated batch configurations on bioethanol productivity. The results indicated more than a 50% increase in bioethanol productivity can be achieved by running this fermentation configuration.

1. Introduction
The production of ethanol from lignocellulosic materials can be conducted via three paths, i.e. the biological method, the thermochemical method, and the combined thermochemical and biological method. In the biological method, lignocellulosic materials are processed through pretreatment, enzymatic hydrolysis to convert the polymeric carbohydrate into monomeric sugars, and fermentation to convert simple sugar into ethanol. In the thermochemical method, lignocellulosic materials are first converted into syngas by gasification, which is followed by conversion into ethanol via the thermochemical pathway, which is carried out in exothermic reactions at high pressure (200 bar) and high temperature (300°C) [1]. In the combined thermochemical and biological method, the syngas produced from the gasification of lignocellulosic materials is fed as the carbon source for fermentation that converts CO and CO2 into ethanol. Syngas fermentation can be conducted by employing anaerobic microorganisms such as Clostridium ljungdahlii, C. carboxidivorans, C. ragsdalei, C. autoetanogenum, C. aceticum, Acetobacterium woodii, or Alkalibaculum bacchi [1–3].

The ethanol produced from the syngas fermentation process is still relatively low when compared to other processes. Liu et al. [4] pointed out that the bottleneck was due to the low gas mass transfer during the fermentation process. Shen et al. [5] reported that hollow fiber membrane addition could diminish mass transfer rate hinderance due to the low gas solubility. Similarly, Keryanti et al. [6] showed that the application of a hollow fiber membrane supported bioreactor improved the mass transfer of gases to the fermentation broth [6]. Despite the high gas mass transfer, low CO and CO2/H2 utilizations were observed and implied that the low biomass concentration might be the bottleneck [7].
There have been some attempts in order to increase cell concentration. Shen et al. [8] and Lee et al. [9] employed fructose addition as an initial carbon source. Philips et al. [10] maximized microorganism growth with certain media formulations. Two steps-fermentation with different reactors was conducted by Richter et al. [11]. The first step was focused on microbe growth, while the second step was focused on the fermentation process.

The manuscript presents a theoretical model development on syngas fermentation on hollow fiber membrane supported bioreaction. The model was verified with data from previous work conducted by Keryanti et al. [12]. The developed model was then used to simulate syngas fermentation in a repeated batch configuration. Repeated batches is the reuse of some components in the process for use in the next process. This component can be a cell or a medium. The concentration of cells in the fermenter can be increased by a repeat batch system. Some advantages of the repeated batch fermentation configuration have been reported, including: improved cell adaptation, elimination of the lag phase, and process cost efficiency [12,13]. Sakai et al. [14] reported an increase in ethanol in the CO2/H2 gas fermentation process by algae Morella sp. Through a repeated batch fermentation method with cell recycling.

2. Materials and method

The model consists of 3 main processes (Fig. 1), that are the solubilization of CO and CO2/H2 from the gas feed into fermentation broth, the conversion of soluble CO and CO2/H2 into acetate and biomass (acetogenesis phase), the conversion of acetate to ethanol (solventogenesis phase). The limiting factor in syngas fermentation is the diffusion resistance of the gaseous substrate at the gas-liquid interface [15]. As a result, cell mass and productivity will be low. To enhance the mass transfer of CO and CO2/H2 from the gas feed into the fermentation broth, a hollow fiber membrane (HMF) module was added before the fermentor as was explained by Keryanti et al. [6,7]. HMF has been shown to increase the solubility of gases in solution by increasing the gas-liquid contact area. This will affect the value of the mass transfer coefficient that increases the substrates consumed by microbes. The more substrates consumed by microorganisms, the better for the metabolism of these microorganisms. Simple growth of microorganisms:

\[
\text{Substrate} \rightarrow \text{cell biomass} + \text{Metabolic Products} \quad (1)
\]

In C. ljungdahlii the products of the fermentation process are acetic acid and cell biomass. C. ljungdahlii was assumed to produce acetic acid following the growth associated product mechanism during the acetogenesis phase and to produce ethanol following the non growth associated product mechanism during the solventogenesis phase.

The kinetics of the biochemical reaction in the fermentation reaction is built on the model obtained from Benalcázar et al. [16] and Gaddy et al. [17]. The feed composition and operating conditions are defined based on research by Keryanti et al. [7]. The fermentation process was conducted at 37°C, the optimum temperature for Clostridium ljungdahlii.

![Figure 1. Model system.](image)
2.1 Data and Kinetics

The transfer of CO and CO₂ from the gas feed into the fermentation broth can be expressed as:

\[ r_{\text{solubilization}} = \frac{K_{L,a}}{H} \times (P^g - P^l) \times V \]  

(2)

in which

- \( K_{L,a} \) = Mass transfer coefficient
- \( H \) = Henry's constant
- \( P^g \) = Gas partial pressure
- \( P^l \) = Gas partial pressure in bulk phase, assumed to be 0
- \( V \) = Working volume of fermentor

| Component | Unit          | Data                                      |
|-----------|---------------|-------------------------------------------|
| CO        | L atm mol⁻¹  | 1249                                      |
| CO₂       | L atm mol⁻¹  | 4011                                      |
| H₂        | L atm mol⁻¹  | 1370.7                                    |
| Flow rate | L hour⁻¹     | 7.65                                      |
| \( K_{L,a} \) | hour⁻¹ | 300.5 \( K_{L,a} \) = Mass transfer coefficient |
| Gas partial pressure | atm | 0.25 \( K_{L,a} \) = Mass transfer coefficient |

Table 1. Data for the stoichiometric.

Acetogenesis phase

From the calculation, the recommended stoichiometry for acetogenesis is:

\[ 0.0117 \text{ CO} + 0.271 \text{ CO}_2 + 0.027 \text{ H}_2 \rightarrow 0.087 \text{ CH}_3\text{COOH} + 0.033 \text{ CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2} + 0.6498 \text{ H}_2\text{O} \]  

(3)

Solventogenesis phase

The equation for the reaction in the solventogenesis process refers to Benalcázar et al. [16]:

\[ \text{CH}_3\text{COOH} + \text{H}_2 \rightarrow \text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O} \]  

(4)

Kinetics equations based on the formula found in SuperPro Designer:

\[ \text{Rate} = [\alpha \mu_{\text{max}}(S1 - \text{Term})(S2 - \text{Term})(S3 - \text{Term}) + \beta](B - \text{Term}) \]

Where is, S1-Term fill with monod and S2-Term fill with Inhibition,

Monod:

\[ \mu = \frac{S}{K_s + S} \]  

(5)

Inhibition:

\[ \mu = 1 - \frac{[S]}{K_{\text{mic}}} \]  

(6)

in which

- \( \mu \) = Specific biomass growth rate
- \( S \) = Substrat
- \( K_s \) = Monod constant
- \( K_{\text{mic}} \) = Inhibition constant

The process runs in batches with the air flow rate carried out continuously. The data, assumptions, and parameters used in the fermentation process are presented in table 2.
Table 2. The data, assumptions and parameters used in the fermentation process.

| Data                                | Value  |
|-------------------------------------|--------|
| Fermentor volume                    | 1.5 L  |
| Circulation rate                    | 0.053 vvm |
| Inoculum and feed ratio             | 1:1000 |
| Syngas composition % v/v (CO:CO₂:H₂:N₂) | 25:20:15:40 |
| Syngas rate                         | 0.080 vvm |
| Time                                | 9 days |

In the kinetic reaction section, the value of $\mu_{\text{max}}$ was set to be 0.02 hour⁻¹ for the acetogenesis. These values were obtained from a study by Keryanti et al. [7]. According to Phister et al. [18], acetate and ethanol in a certain amount and time can inhibit microorganisms. The inhibition value for acetate is 4500 mg/L and for ethanol is 54500 mg/L. The model was developed and simulated using SuperPro Designer version 8.5. an image of the model can be seen in figure 2.

Figure 2. (a) Experimental set up [7], (b) Model system.

2.2. Repeated batch
The operating conditions were similar to the previous batch with an additional fermentation media volume of 50%. The cells and the medium were separated by a microfilter which was subsequently remixed by using a mixer with new medium prior to the next batch. The value of the rejection coefficient (RC) in the microfilter is 0.5 for biomass with 80% recovery for the concentration factor. Fermentation was performed for 9 days on each batch and repeated three times. This model is approached by separating each batch into one reactor.

Figure 3. SuperPro Designer model system.
3. Result and discussion

3.1. Acetic Acid, Biomass and Ethanol

In the early stages, CO$_2$ will be synthesized into acetyl-CoA. In general, microorganisms that utilize CO also have the ability to utilize CO and CO$_2$/H$_2$ through the same series of enzymes and transformation mechanisms [19]. Acetyl-CoA resulting from CO synthesis is a precursor for various products such as acetate, ethanol, butyrate, butanol, pyruvate, and 2,3-butadienol [18]. In the metabolic pathway of C. ljungdahlii, acetyl-CoA will turn into biomass (cells). Acetyl-CoA in the presence of phosphate will also be converted to acetyl-phosphate then with the enzyme acetatekinase will be converted into acetic acid. A series of C. ljungdahlii processes to produce biomass and acetate constitute the acetogenesis phase. Figure 4 shows the comparison of the acetate, biomass and ethanol recovery results between the lab experiments conducted by Keryanti et al. [7] and the SuperPro designer simulation during the fermentation process.

![Figure 4](image)

Blue square: Keryanti (2019). Orange circle: This Study

**Figure 4.** (a) Acetic acid recovery, (b) Biomass recovery, (c) Ethanol recovery.

In figure 4 (a), the simulation results show that acetic acid increases from day 1 to day 5, whereas in the Keryanti’s [7] experiment results, acetic acid increases from day 2 to peak on day 5. This could be because the simulation does not consider the lag phase, whereas in the experiment in the lab, days 1 and 2 are the lag phase of microorganisms, where the microorganisms will adapt to a new environment. The lag phases can't be accommodated by Superpro. The lag phase of each microorganism will have a different time. The lag phase is an early stage which is also the adaptation stage of the microorganisms in the macro. There is no activity during this stage, but the microorganisms carry out micro-activities in the form of adjusting to the new environment, which also includes macromolecular improvements of the previous stage [20]. The final result of the acetic acid formed is not much different. In the lab experiment, the acetic acid formed is 0.98 g/L while the result from the simulation is 1.15 g/L. Acetate concentration increases with increasing cell concentration, so that acetate is a growth-associated product.

The initial cell concentration entered into the experimental fermentation process in the lab was 0.13g/L, while for the simulation the initial concentration was set at 0.1 g/L. The simulation results and experimental results in the laboratory show that the cell concentration increases exponentially on day 2 to day 5. Keryanti’s shows the first day was the lag phase for C. ljungdahlii, so there were no significant changes. It shows in figure 4 (b) the comparison of the results obtained by cell concentration between the lab experiments conducted by Keryanti et al. [7] and the SuperPro Designer simulation during the fermentation process. The maximum peak of cell concentration in both laboratory experiments and simulations with SuperPro Designer occurred on day 5, with the yields that were not so far away, 0.64 g/L and 0.54 g/L. In laboratory experiments, it can be seen that the cell concentration has dropped on day 6. This shows that the accumulation of acetic acid is so great that it can reduce the pH value internally in the presence of H$^+$ ions [21]. This process causes a change in metabolism from the acetogenic phase at neutral pH to the solventogenic phase at acidic pH. The microorganisms will begin to produce NAD(P) to be used to produce solvent compounds in order to maintain their sustainability.
In contrast to the lab experiment results on the 5th day, the simulation results in figure 4 (b) show that the concentration value tends to be static with no increase or decrease in cell concentration. This can be caused because the SuperPro Designer simulation does not take into account the cell death constant, such that the cell acquisition is only based on reaction stoichiometry. At the end of the fermentation process, the biomass concentration for the laboratory experiment and simulation was obtained at 0.32 g/L and 0.54 g/L.

In the syngas fermentation reaction, the high availability of electrons from the oxidation of CO and H₂ causes ethanol production to follow a mixed-growth associated pattern [22]. As shown in figure 4 (c), the results of the Keryanti’s [7] lab experiment, ethanol is not produced during the initial lag phase, which is on the 1st and 2nd day of fermentation (non-growth-associated). However, the ethanol began to be made on the 3rd day following cell growth (growth-associated) and continues to increase even though the cell population decreases due to acetate that is started to be consumed (non-growth-associated). The simulation results display similar data trends, but as previously discussed, the simulation using the superPro Designer does not consider a lag phase in the fermentation process. The process in the simulation is considered to run immediately, even though it can be seen that the ethanol gain on the 1st and 2nd days is very small. From the simulation, it is obtained that the ethanol concentration values on day 1 and day 2 were 0.04 g/L and 0.1 g/L, respectively. The final results obtained from the experiment lab and simulation with SuperPro Designer are not too far away, 1.1 g/L and 1.4 g/L.

The results of the fermentation products (acetate, biomass and ethanol) showed a similar trend between Keryanti’s [7] lab results and simulation with SuperPro Designer shown in figure 4. This showed that the model simulation with SuperPro Designer can be used as a reference for the syngas fermentation process. For acetic acid, ethanol, and biomass, the sum-square error between lab and modeling results was determined statistically. The sum square error values for Acetic Acid, Ethanol, and Biomass are 0.35, 0.015, and 0.017, respectively. Besides the same trend, its recoverable values are among the laboratory experiments [7] and the simulation results are similar. CO, CO₂ Conversion and Yield Analysis.

Conversion calculations for CO and CO₂ utilized are carried out referring to the equation in Keryanti’s [7] report. In this simulation, the utilization values of CO and CO₂ are 0.1 mol and 0.3 mol, with conversion rates of CO and CO₂ are 22.5% and 100%, respectively. The simulation results show that CO₂ is consumed on the 6th day, CO conversion has reached 100%. This is also due to the solubility of CO₂ which is much higher than CO. This acquisition is close to the results of Keryanti’s lab research. One of the things that influences the utilization of CO is the specific flow rate. In this case, the specific syngas flow rate is 0.053 vvm. Keryanti et al. [7] reported that the flow rate used is susceptible to 0.053-0.08 vvm. The gas supply has exceeded the maximum capability of gas utilization by cells so that the fermentation process is no longer limited by mass transfer but by cell growth kinetics. This statement is also supported by Shen et al. [5] which carried out specific flow rate variations.

Product yield can be calculated from data on CO and CO₂ utilization and the concentration of the product produced. The simulation results showed that the yield of acetate, biomass and ethanol from CO was 0.091 mol/mol, 0.527 mol/mol and 0.028. While the yields of acetate, biomass, and ethanol from CO₂ were 0.026 mol/mol, 0.155 mol/mol, and 0.008 mol/mol, respectively. The circulation rate will affect the yield during the fermentation process. The circulation rate will determine the residence time of the gas in the liquid. The higher the circulation rate, the higher the contact time with the gas, and it will increase the KLa value [8]. However, it is necessary to pay attention to the maximum limit in determining the flow rate. As in Shen et al., [5] which varied the flow rate. It can be concluded that when the flow rate is carried out at a susceptible 300-500 mL/min, there is a decrease in the productivity of acetate and ethanol. This is because at a medium circulation rate that is too high, the immobilized biomass on the surface of the hollow fiber membrane becomes unstable and tends to be eroded from the membrane [7].

3.2. Repeated Batch
The initial simulation result was applied to the repeated batch system. 50% of the previous batch biomass was mixed with the new inoculum prior to the next batch by adjusting the Rejection Coefficient of biomass value to 0.5 in the superPro program microfilter setting. The biomass value obtained from the
initial process to be employed in the next process was 0.324 g/L. The second batch’s conditions were similar to the previous batch’s. The result yielded a final concentration of ethanol obtained on the 9th day of 2.64 g/L. 2.18 g/L of acetate was obtained on the 3rd day of fermentation. The acquired final biomass amount was 0.75 g/L. Figure 5 (a) shows that fermentation with a batch system will reach the solventogenesis phase faster than the initial batch. In the initial batch it takes 5 days to reach the solventogenesis phase, while in the repeat batch it only takes 2 days. The acetate rapidly reached its peak amount due to the lag phase that was not experienced by repeated batch cells, thus they could directly perform fermentation to produce acetate. The acetate amount gradually decreased after the 3rd day, showing that the fermentation process entered the solventogenesis step. During the repeated batch, the ethanol production followed mix-growth caused by the electron availability produced by CO and H2 [22]. The final ethanol recovery with a repeated batch system produces higher ethanol than the initial batch. From 1.40 g/L to 2.64 g/L.

![Figure 5](image_url)

**Figure 5.** (a) Acetic acid and Ethanol recovery; (b) Biomass recovery.

Waesarat et al. [23] confirm that maximum ethanol was obtained from the repeated batch fermentation. Generally, based on figure 4, repeated batch system yielded higher results compared to the general fermentation process. Figure 5 (b) shows the biomass concentration during the fermentation process. On the 10th day, the initial fermentation process was completed, and 50% of the total biomass accumulated was taken (2.75 g/L) for a repeated batch. More biomass growth occurs due to cell rejuvenation by adding fresh medium to the fermenter and there is no lag phase so microorganisms can reproduce immediately. On the 14th day, the graph stagnates. This can happen because the SuperPro Design simulation does not include microbial death constants.

### 3.3. Comparison with other studies

In this case, a comparison of the results obtained with several different operating mode systems will be carried out to determine whether the system created is efficient and good. The case discussed is the creation of a modeling system from a lab experiment that has been carried out by Keryanti. The addition of a membrane module in this system is carried out by involving the solubility factor in stoichiometric
determination. From the simulation results, there is a slight difference in trends (figure 4) in the biomass acquisition after passing the peak point of day 6. This can be due to the fact that SuperPro Designer does not involve the cell death constant.

There are several factors that affect the performance of syngas fermentation, including the type of microorganism selected, the composition of the medium, the composition of syngas, syngas flow rate, operating conditions (pressure, pH control, temperature), and reactor configuration (type of reactor, addition of membranes, type of process operation). As Maedah et al. [21] demonstrated by performing syngas fermentation with the CSTR system. The results from the CSTR system obtained ethanol production reached 6.45 g/L. The continuous system will provide gas and media continuously in the fermentation system. Microorganisms will get a continuous supply of nutrients, so microorganisms will exist in an exponential phase. In the experiment, they also carry out initial treatment to keep the pH in the growth phase until they reach a certain density. At the solventogenesis stage by leaving the pH unattended to produce ethanol. Another experiment was carried out by Shen et al. [5] who modified a reactor equipped with HMF as a gas-liquid contactor and used C. carboxidovorans to obtain ethanol at a rate of 23.92 g/L with CO utilization reaching 53.6%. Shen used a membrane contractor 3 times larger than that used in the Keryanti experiment. A larger active surface area of the membrane can increase the liquefied gas contact. The KLa CO achieved in the Shen experiment was 1096.2 h⁻¹. While in this simulation, the KLa CO used is 300.5 h⁻¹.

### Table 3. Comparison of the results of the syngas fermentation process.

| Component     | Keryanti [7] | This Study        |
|---------------|--------------|-------------------|
| Biomass (g/L) | 0.64ᵇ        | 0.54ᵃ 0.75ᵇ      |
| Acetat Acid (g/L) | 1.20ᵇ        | 2.00ᵃ 2.17ᵇ      |
| Ethanol (g/L) | 1.10ᵇ        | 1.40ᵃ 2.64ᵇ      |

ᵃ The highest peak
ᵇ Final Result

### 4. Conclusion

In this study, a syngas fermentation simulation was carried out with the aid of the SuperPro Designer software using a kinetics reactor with a batch operation mode for 9 days and the simulation was carried out anaerobically. This simulation takes cases from the lab experiments that have been carried out by Keryanti et al. [7]. The operating conditions and supporting data were taken from the results of Keryanti’s research. The concentration of the cell entering the reactor is 0.098 g/L with the syngas composition CO:CO₂:H₂:N₂ with the ratio of 25:20:15:40. The simulation results showed that the values of acetate, ethanol and biomass on the 9th day of the fermentation process were 0.54 g/L, 1.15 g/L and 1.40 g/L. The utilization values of CO and CO₂ are 0.1 mol and 0.3 mol, with conversion rates of CO and CO₂ are 22.5% and 100%. Repeated batches were employed to increase cell concentration in the fermentor. Ethanol production was increased by 50%. The ethanol amount at the end of fermentation obtained by repeating the batch was 2.63 g/L. The simulation results have the same pattern as the experimental results from the lab, and the values of the simulation results show good agreement with the data results from the lab experiment.

### 5. Reference

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