Syngas fermentation for production of ethanol

N A Istiqomah 1*, M T A P Kresnowati 1,2 and T Setiadi 1,3

1 Department of Chemical Engineering, Faculty of Industrial Technology Institut Teknologi Bandung, Jl. Ganesa 10, Bandung 40132, Indonesia
2 Department of Food Engineering, Institut Teknologi Bandung, Jl. Ganesha 10, 40132 Bandung, Indonesia
3 Centre for Environmental Studies (PSLH), Institut Teknologi Bandung, Jl. Sangkuriang No. 42A, 40135 Bandung, Indonesia

noviani105@students.itb.ac.id

Abstract. The global demand for fossil fuels has increased sharply in the past 50 years. Plant biomass is one of the fourth largest non-fossil renewable energy sources after geothermal, solar, and wind energy. Biomass can be first converted into another energy form, such as ethanol, to provide a more efficient energy source. Syngas fermentation enables converting all biomass components, including lignin, into ethanol, thereby increasing the biomass quantity. The development of the syngas fermentation process will be reviewed in this article, which focuses on the types of microorganisms used, the effect of syngas composition, and the gas-liquid mass transfer to be used as a reference for optimizing the syngas fermentation process.

1. Introduction
Syngas conversion into bioenergy through fermentation has been carried out since 1983, and this research spike occurred in 2010 until now. Syngas is a gas mix containing CO, H2, and CO2 produced from gasification of raw material lignocellulose and other carbon raw materials [1].

The conversion of synthetic gas to liquid biofuel and biochemistry can be carried out in two ways, first, using a metal catalyst through Fischer-Tropsch Synthesis (FT) and second, utilize a microbial catalyst known as syngas fermentation [2]. The weaknesses in FT Synthesis are the high cost of the metal catalysts, Inert gases and contaminants can be potentially toxic when reacting with catalysts, operating using high temperatures and pressures, the ratio and quality of CO and H2 gases must be consistent [3].

(Homo) acetogen is the name for a microbial catalyst that can ferment syngas. There are more than 20 genera and more than 100 different species of acetogens [4]. Acetogens can produce various types of chemicals and biofuels and have advantages over FT synthesis, including a higher specificity, a more flexible ratio of CO and H2, not using expensive metal catalysts so that the potential for metal poisoning is small, operations are carried out in ambient conditions [3]. However, fermented syngas still has challenges that need a solution, namely H2 and CO's low solubility in the water phase and low product conversion.

2. Syngas Characteristics
The operation process and the type of raw material from the gasification determine the gas's composition. The gas composition can be a mixture of CO2, CO, H2, N2, CH4, and a small amount of
NOx, SOx, tar, char, and hydrocarbons [34]. The composition of the gas affects the results of syngas fermentation. If pure syngas is used, the resulting product will be high.

Syngas impurities can be nitrogen oxide (NO), tar, H2S, and carbonyl sulfide (COS). Nitrogen oxide above 40 ppm will affect cell growth and hydrogenase activity. Cell growth can be inhibited by the presence of tar [35]. However, acetogen tolerance to toxic impurities, H2S (<2%), and COS are still better than inorganic catalysts [36].

3. Microbiology and Metabolic Pathways

Syngas fermentation carried out by acetogens can produce various useful products, besides anaerobic conditions can reduce the risk of contamination. The fixation of CO2 to acetyl-CoA in acetogen takes place effectively through the Wood Ljungdahl Pathway (WLP) so that WLP is known as acetyl-CoA reductive pathway [5].

The fermentation stage is generally divided into two stages: the acidogenesis and solventogenesis stages [6]. Acidogenesis stage was a growth stage when cells would grow, accompanied by acetate and butyrate as products. This process causes the pH of the medium to drop. As more acetate is formed, the cell growth rate will continue to decline until it eventually becomes zero. In this time, the acetogens respond by consuming the acetic acid. Once acetate starts to be toxic to cells, it is converted to solvent, which in this case, is ethanol. This process causes the pH of the solution to rising again. The process will continue until the medium's nutrients run out, or the acid crash phenomenon occurs. In this phenomenon, cells are already inactive due to too much acid and have poisoned the cells.

![Wood-Ljungdahl Pathway (WLP)](image)

**Figure 1.** Wood-Ljungdahl Pathway (WLP), adapted from [7,8].
Energy for the sustainability of WLP comes from ATP from the formation of acetate from acetyl-CoA, as figure 1 depicts. Production of ATP occurs during the cell growth phase. When the cells transition to the stationary phase, acetyl-CoA is reduced to ethanol. Apart from ethanol, acetyl-CoA can form other products, such as isopropanol, butyrate, butanol, hexanol, and butanediol [9]. The difference in the resulting product depends on the type of acetogen used. Information regarding the types of acetogens, fermentation conditions (pH and temperature optimal), and the resulting products with CO, H₂, and CO₂ as substrates can be seen in table 1.

### Table 1. Acetogen for syngas fermentation to produce ethanol.

| Organism                          | pH<sub>opt</sub> | T<sub>opt</sub> (°C) | Products                                      | References |
|----------------------------------|------------------|----------------------|-----------------------------------------------|------------|
| Clostridium drakei               | 3.6-6.8          | 25-30                | Ethanol, acetate, butyrate                    | [10–14]    |
| Clostridium scatologenes         | 5.4-7.5          | 37-40                | Ethanol, acetate, butyrate                    | [10,11]    |
| Clostridium autoethanogenum      | 5.8-6            | 37                   | Ethanol, acetate, lactate, 2,3-butanediol     | [15–18]    |
| Butyribacterium methylotrophicum | 6                | 37                   | Ethanol, acetate, butyrate, butanol           | [19–21]    |
| Clostridium ljungdahlii          | 6                | 37                   | Ethanol, acetate, lactate, 2,3-butanediol     | [22–25]    |
| Clostridium ragsdalei/P11        | 6,3              | 37                   | Ethanol, acetate, lactate, 2,3-butanediol     | [16,26]    |
| Clostridium coskatii             | 5.8-6.5          | 37                   | Ethanol, acetate                              | [27]       |
| Alkalibaculum bacchi             | 8.0-8.5          | 37                   | Ethanol, acetate                              | [28,29]    |
| Clostridium carboxidivorans/ P7  | 6,2              | 38                   | Ethanol, acetate, butyrate, butanol, lactate  | [11,30]    |
| Clostridium difficile            | 6.5-7.0          | 35-40                | Ethanol, acetate, butyrate                    | [31–33]    |

### 4. Factors that Affect Syngas Fermentation

#### 4.1 Temperature and pH

The temperature factor affects microorganisms' growth and the mass transfer of gas to liquid fermentation syngas medium. Each microorganism used has an optimum temperature for its respective growth. However, operation at optimum growth temperature does not ensure that the expected product is formed. The temperature used in syngas fermentation depends on the type of microbe and the product expected.

For example, *C. carboxidivorans* P7 grown at an optimum temperature of 37 °C, the product produced in high quantities is acetic acid, while ethanol is obtained in low amounts. The condition occurs because the production of acetic acid is linear with cell growth. When fermentation operation at a temperature of 25 °C, cell growth is not fast, but the ethanol produced is much more [37].

Another effect of temperature is its effect on gas transfer to the liquid medium of fermentation. The solubility of gases in the liquid will decrease as the temperature increases. However, increasing the temperature will decrease the solution's viscosity, which reduces in the size of the bubbles so that the area of contact between the gas-liquid phases will increase so that the kLa (gas-liquid transfer coefficient) value will increase. The use of temperature in syngas fermentation is determined based on the microorganisms used. Mesophilic microorganisms grow in a temperature range of 37 to 40 °C, while
thermophilic microorganisms are at a temperature of 55 °C and extreme-thermophilic at a temperature of 80 °C [1].

Like temperature, each microorganism has an optimum pH value. However, in general, a decrease in pH triggers solventogenesis (formation of ethanol and butanol). The use of different fermenters can be done to get optimal results. The first reactor aims to support growth and acid formation so that it is maintained at pH 6, then flowed to the second fermenter, which is maintained at pH 5 so that acid and cells from the first fermenter are used to form ethanol [38].

4.2 Growth media culture
The growth medium must meet microbes’ needs to grow optimally, including vitamins, minerals, trace elements, and reducing agents. The growth medium used, pH, and temperature are determined by the microorganisms used and the product expected. Special media are readily available at the American-Type Culture Collection (ATCC).

4.3 Mass transfer
In syngas fermentation, the gas substrate acts as a source of carbon and energy to produce by-products. The gas transfer steps to microbial cells include gas diffusion from the bulk gas to the gas-liquid interface, then movement across the gas-liquid interface, transportation to the bulk liquid around the microbial cell, and diffusive via the liquid-solid frontier [34].

The syngas fermentation process’s biggest problem is the transfer of mass gas to the liquid fermentation medium. The mass transfer problem is more significant than aerobic processes because syngases' solubility is much less than oxygen. CO Solubility is only about 76% of oxygen, while H₂ is only about 55% [39], and low substrate solubility results in low product productivity.

The mass transfer rate is quantified in the following equation:

\[ \frac{dc}{dt} = k_L a (C^* - C_L) \]  

In that equation, \( \frac{dc}{dt} \) is the amount of gas that moves from the gas to liquid phase per unit time, \( k_L \) (ms⁻¹) is the mass transfer coefficient, \( a \) (s⁻¹) is the specific mass transfer area, \( C^* \) is the concentration of saturated gas in liquids, \( C_L \) is the concentration of the gas dissolved in the liquid, which is considered to be in equilibrium with the gas phase concentration according to Henry's law. Based on these equations, the components that can be modified to increase the mass transfer rate increase the area of mass transfer or enlarging the driving force. If using gas with low solubility, the \( k_La \) depends on the size and number of bubbles by controlling the agitation speed, the gas and liquid medium's flow rate, and the reactor configuration [40].

A simple approach to increasing the area of mass transfer is to increase the propeller's rotational speed, but this is less applicable because a massive rotational speed will damage cells in the fermenter, high energy costs, and decreased productivity [41]. Minimizing the driving force's influence can be done by increasing the gas’s residence time through an increase in the gas flow rate, especially CO, as the primary carbon source. However, CO can inhibit cell hydrogenase enzyme activity, so another alternative is needed to solve mass transfer [4].

4.4 Reactor configuration
The mass transfer effectiveness is also related to the bioreactor configuration. Parameters in designing bioreactor efficiency include high mass transfer rate, bioreactor size and ease of scale-up, high cell density, and low budget operation and maintenance [1].

A mass transfer without agitation can be done to minimize power, including by modifying the bubble flow pattern in the Bubble Column Reactor (BCR) [44], using packaging media in the Trickle Bed
Reactor (TBR) [45], and the use of microbubble generators in Hollow Fiber Membrane Reactors (HFMR). HFMR has the highest mass transfer effectiveness to date, with a \( k_{La} \) CO value of 1096.2 h\(^{-1}\) [43]. The \( k_{La} \) values for various reactors can be seen in Table 2.

5. Conclusion

Syngas fermentation can be carried out by acetogens consisting of various species and can produce various useful products through the Wood-Ljungdahl Pathway (WLP). The growth medium used, pH, and temperature are determined by the microorganisms used and the product expected. Bottleneck from syngas fermentation lies in the low mass transfer of gas to the liquid fermentation medium. Transfer mass gas-liquid depends on the size and number of bubbles by controlling the agitation speed, the gas and liquid medium's flow rate, and the reactor configuration. HFMR is the right choice for syngas fermentation because it has the highest mass transfer. The use of HFMR for a large scale still has several challenges because it is predicted to require high costs. The use of membranes from reusable materials to reduce costs is an efficient alternative to commercial membranes.

Further research on alternative materials based on sustainability, cost, and durability needs to be done. The efficiency of the HFMR can still be improved by making internal adjustments. Application features such as height to diameter ratio and the use of micro sparger can be studied further.
| Type of Reactor | Reactor Scale | Microbe | Substrate (CO:H₂:CO₂:N₂) | Gas Flow | pH | Cell Density (g/L) | Yield Ethanol (g/L) | Kₐ (h⁻¹) | Reference |
|----------------|---------------|---------|---------------------------|----------|----|-------------------|---------------------|---------|----------|
| Batch          | 125 mL        | C. ljungdahlii | 55:20:10:Ar 15 | 200 μL | 37 | 5.5 and 6.8 | 0.55 | 0.17-0.29 | [46]     |
| CSTR           | 250 mL        | C. ljungdahlii | 20:10:20:50 | 5.7 and 10 ml/min | 37 | 5.5 and 6.8 | 0.5 | 0.9 | [47]     |
| Batch          | 5 L           | C. ljungdahlii and A. bacchi | 20:10:20:50 | 5.7 and 10 ml/min | 37 | 5.5 and 6.8 | 0.5 | 0.9 | [47]     |
| CSTR           | 3.3 L         | C. ljungdahlii | 20:5:15:60 | 200 sccm | 37 | 5.5 and 6.8 | 0.5 | 0.9 | [47]     |
| Batch          | 250 mL        | C. ljungdahlii | 20:5:20:55 | 0.4-5.05 | 37 | 6.8 | 0.5 | 0.9 | [47]     |
| CSTR           | 1.2 L         | C. autoethanogenum | CO 100% | NA | 30 | 4.75 and 5.75 | 0.4 | 0.9 | [47]     |
| Trickle bed    | 500 mL        | A. bacchi | 200 mL | 200 sccm | 37 | 5.5 and 6.8 | 0.5 | 0.9 | [47]     |
| CSTR           | 2 L           | C. carboxidivorans | 38:28:52:8:5.5 | 20:15:60 | 33 | 5.5 | 0.15 | 0.8-3.75 | [43]     |
| STR-external HFM | 800 mL | C. carboxidivorans | 20:5:15:60 | 300 mL/min | 37 | 5.5 | 0.2 | 0.9 | [43]     |
| STR-external HFM | 800 mL | C. carboxidivorans | 20:5:15:60 | 300 mL/min | 37 | 5.5 | 0.2 | 0.9 | [43]     |
| STR-external HFM | 800 mL | C. carboxidivorans | 20:5:15:60 | 300 mL/min | 37 | 5.5 | 0.2 | 0.9 | [43]     |
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