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Challenges in Syngas Fermentation for Bioethanol Production: Syngas Composition

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Abstract. Energy challenges in developing countries are more significant if they continue to use fossil materials and impact air quality. Lignocellulosic biomass can be an alternative to new renewable sources to replace fossil materials. Indonesia produces various sources of lignocellulosic biomass, which can be used in multiple energy sources, such as bioethanol. The hybrid pathway is one of the routes for producing bioethanol. The first stage of the hybrid process is the conversion of biomass into CO, CO_2, and H_2 (syngas) gas through the gasification process. Then the syngas is converted into bioethanol through fermentation using microorganisms as biocatalysts. The bioethanol production line is the Wood-Ljungdahlii pathway. Factors that affect syngas are the type of biomass (chemical, physical, and morphological properties) and the gasification process (type of gasifier, temperature, gasification agent, and ratio equilibrium (ER)). This paper reviews the challenges in implementing syngas fermentation. In particular, variations in the composition of syngas as a substrate for fermentation.

Introduction

Energy challenges as a fundamental component of economic activity for developing countries are more significantly related to the dependence on dwindling fossil fuel sources and have a negative impact on air quality related to CO_2 emissions produced [1,2]. An alternative to meeting the long-term energy mix, minimizing the use of fossil energy sources, and reducing CO_2 emissions is to use biofuels produced from renewable resources such as biomass [3,4]. Biomass is one of the abundant renewable carbon sources and can be used as an alternative energy source to replace fossil fuels [5–7]. The advantage of using plant-based biofuels in the transportation sector is that it can reduce greenhouse gas emissions because the CO_2 content produced from the combustion process will be bound by plants for the photosynthesis process so that the CO_2 content does not increase in the atmosphere [3,8]. In addition, biomass is more efficient if converted into liquid fuels such as ethanol [9]. Raw materials in the manufacture of bioethanol can be sourced from the first-generation (starch-based raw materials), second-generation (lignocellulosic biomass), or third-generation (algae raw materials) [10–12].

Generally, there are three routes for bioethanol production: biochemical, thermochemical, and hybrid. The hybrid pathway combines thermochemical and biochemical processes: biomass is transformed into syngas through the gasification process, and then the syngas is fermented into short-chain organic acids and alcohols such as ethanol by acetogenic bacteria [13,14]. The syngas fermentation process offers an advantage over the chemical catalytic process in that the microorganisms can work in the presence of some impurities in the syngas and are flexible to the H_2/CO ratio [13]. In addition, the process conditions, such as temperature and pressure required in the fermentation process, are lower than the chemical process, reducing operating costs [13,15]. The characteristics of syngas as the output of biomass gasification maybe affected by various factors which further may also affect the performance of the syngas fermentation process. It is the goal of this paper to review the factors affecting the characteristics of syngas and the corresponding effects on the syngas fermentation performance.

Lignocellulosic Biomass

Lignocellulosic biomass can be obtained from agricultural residues, forest residues, and post-harvest processing [16,17]. Lignocellulosic biomass has the main components, namely cellulose $(C_6H_{10}O_5)_n$ and hemicellulose $(C_5H_8O_4)_m$ that may compose up to 60-80% of biomass, and lignin $(C_9H_{10}O_3(OCH_3)_{0.9-1,7})_y$ that may compose up to 10-25%, and minerals [12,18,19]**.** The selection of lignocellulosic biomass is essential because significant differences in the biomass chemical, physical, and morphological properties will affect the syngas composition [19,20]. High carbon and oxygen content in biomass can produce a higher percentage of combustibles in syngas [21]. The water content of more than 30% reduces the calorific value of syngas because the energy consumed is used to remove water content; besides that, the humidity will inhibit changes in the oxidation temperature of the gasifier so that it can increase the tar content [22]. The high ash and mineral content causes the formation of slag, which causes obstacles to biomass feed to the gasifier [23]. The larger the size of the biomass feed will reduce the reaction surface, thereby inhibiting mass and heat transport for the downdraft gasifier [24,25]**.**

Gasification

Gasification is a thermochemical conversion process technology for energy production that has been used for a long time; conventionally, the raw materials used are fossil-based such as coal and natural gas, but other alternative raw materials that can be used are biomass, municipal solid waste (MSW), and plastic [26,27]. The advantages of biomass compared to fossil sources in the gasification process are that it contains less nitrogen, sulfur, and heavy metals, lower gasification temperature, lower pollutant emissions, and higher reactivity [28,29].

The gasification process is drying, pyrolysis, oxidation, and reduction [24]. Drying, this process is carried out at a temperature of 100-200 ^{0}C until the water content reaches 10% to 20% [24]. As a result, energy efficiency is increased, and syngas quality is improved by reducing the moisture content [30]. Pyrolysis occurs without air, gas, or other gasification media, which causes the breakdown of hydrocarbon molecules into smaller gas molecules, tar, and leaves solid carbon biomass [31]. Pyrolysis occurs at a temperature of 200-700 ^{0}C [32]. Oxidation is a reaction between solid biomass and oxygen from agent gasifying and biomass to form CO_2, CO, and H_2O, formed from hydrogen in oxidation biomass [24]. The oxidation of carbon and hydrogen is an exothermic reaction that produces a large amount of heat. When oxygen is available in sufficient stoichiometric quantities, partial carbon oxidation will occur, forming carbon monoxide [24]. The reactions that occur in the oxidation process are as follows:

$$C + O_2 \rightarrow CO_2 \qquad \Delta H = -394 \text{ kJ/mol} \qquad (1)$$
$$C + 0{,}5\, O_2 \rightarrow CO \qquad \Delta H = -111 \text{ kJ/mol} \qquad (2)$$
$$H_2 + 0{,}5O_2 \rightarrow H_2O \qquad \Delta H = -242 \text{ kJ/mol} \qquad (3)$$

Reduction occurs when there is little oxygen in the system, a reduction reaction occurs in the temperature range of 800 – 1000 ^{0}C [33]. The reaction is as follows:

Reaksi *water-gas*	$C + H_2O \leftrightarrow CO + H_2$	$\Delta H = 131{,}4$ kJ/mol	(4)
Bounded reaction	$C + CO_2 \leftrightarrow 2\,CO$	$\Delta H = 172{,}6$ kJ/mol	(5)
Reaksi *hydrogasification*	$C + 2H_2 \leftrightarrow CH_4$	$\Delta H = -75$ kJ/mol	(6)

Gasification conditions affect the performance of the gasification process, such as the gasifier type, temperature, gasification agent (air, O_2, or steam), and the equivalent ratio of supplied air demand to stoichiometry (ER) [27,28,34]. The ER represents the real air–to–biomass ratio in terms of stoichiometry [35]. There are three gasifier types categories: Fixed Bed, Fluidized Bed, and entrained flow. Fixed bed types include downdraft gasifiers, and updraft gasifiers, while fluidized bed types include circulating fluidized bed gasifiers and bubbling bed gasifiers [36]. A fixed bed gasifier is the simplest gasifier, with long solid residence time, low gas velocity, biomass that does not need to be uniform, and low dust (ash) content [37]. The disadvantages of fixed bed gasifiers are that they produce high tar content and operate at high temperatures due to the biomass size [38]. Fluidized bed gasifiers are suitable for large-scale biomass gasification because the mixing and uniform temperature result in increased carbon conversion and are the most widely used for biomass gasification today [39].

The higher temperature in the gasification process causes an increase in the calorific value of syngas because the high temperature is suitable for the oxidation process and produces less tar, but if the temperature is too high, the gasifier will crack faster [40,41]. In addition, the type of gasifier agent affects the composition of the syngas, and if the air is used as a gasifying agent, it will produce nitrogen compounds in the syngas, which is higher when compared to the gasifying agent of steam or oxygen [42].

Gasification converts raw materials into carbon monoxide and other synthetic gases to become fuel gases or other chemicals using gasification agents such as oxygen, steam, or air [22]. The main components of syngas are carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2). In addition, it may contain other gases such as water (H_2O), ammonia (NH_3), nitrogen oxides (NO_X), methane (CH_4), ethene (C_2H_4), ethane (C_2H_6), ethine (C_2H_2), benzene (C_6H_6), naphthalene ($C_{10}H_8$), hydrogen cyanide (HCN), sulfur dioxide (SO_2), hydrogen sulfide (H_2S), carbonyl sulfide, and condensable volatiles [15]. Impurities from the gasification process include tar and particulates [43]. The composition of syngas from various biomass and variations in gasification operating conditions can be seen in Table 1.1.Overall, the composition of syngas may be effected by the the types of biomass used as the raw materials, the types of gasifier, the types of gasification agent, and the applied ER.

Table 1.1 The composition of syngas from biomass gasification

Gasifier	Biomass	ER	Gasification agent	Temp (°C)	Composition (%volume Dry)					Ref
					CO	H_2	CO_2	CH_4	N_2	
Fluidized bed	Wood Chips	0.3	Air	800	18.0	13.2	11.5	4.1	41.1	[44]
		0.3	O_2		35.7	27.9	10.5	0.76	0.9	
		n/a	Steam		14.0	31.1	14.1	7.3	0.0	
		n/a	Steam (dry)		18.5	41.0	18.6	9.7	0.06	
Fluidized bed	Cypress sawdust	0.54	Air	700-800	6.9	5.6	18.1	1.4	68.0	[45]
	Mixed pine bark-spruce	0.22	Air		21.4	5.4	14.7	4.6	53.9	
Bubbling fluidized bed	Rice husk	n/a	Air	702	21.3	4.4	11.3	4.3	57.1	[46]
				737	16.9	4.8	15.9	3.7	57.1	
Fixed bed downdraft	Olive	n/a	Air	1190	17.4	13.2	12.4	9.5	54.9	[47]
	Peach			1170	17.7	15.0	13.5	5.8	51.7	
	Pine			1140	16.0	12.0	11.4	8.9	59.4	

Syngas can be converted through the Fischer-Tropsch (FT) process using a chemical catalyst to produce diesel, methanol, or ethanol [13]. The FT process is generally conducted at high operating temperatures and pressures and requires stable H_2/CO ratios. Gaseous impurities may become toxic during the process [13]. Syngas can also be converted using a microorganism catalyst to alcohols, carboxylic acids [48,49], and 3-butanediol [50].

Syngas fermentation

The production of bioethanol via syngas fermentation offers an advantage when compared to the conventional lignocellulosic hydrolysis followed by sugar fermentation that it can increase product yields from the same amount of raw materials because all biomass components, including lignin, can be converted into syngas in the gasification process. On the other hand, the low solubility of syngas to fermentation broth may presents as the main challenge for the process [51–54]. Some efforts have been made to address this issue, among others by developing a dedicated fermentor design equiped by a hollow fiber membrane contactor [55].

The syngas fermentation is facilitated by the acetogenic bacteria that can convert CO, CO_2, and H_2 with flexible molar ratios and withstand some impurity gases in syngas [49,56]. Syngas fermentation is a biological process in which CO and CO_2 from syngas are converted to acetyl-CoA with the help of acetogenic bacteria and subsequently to acetic acid, ethanol, and many other products via the Wood-Ljungdahl pathway [57]. The microorganisms used are anaerobic microorganisms which are classified as autotrophic. Autotrophs utilize C1 compounds in syngas, including CO and/or CO_2 as a carbon source and H_2 as an energy source [58].

The Wood-Ljungdahl pathway reduces CO_2 to CO and formic acid or directly to formyl groups. The formyl group is reduced to a methyl group and then combined with carbon monoxide and co-enzyme A to produce acetyl-CoA [59]. This path can be seen in Fig.1.

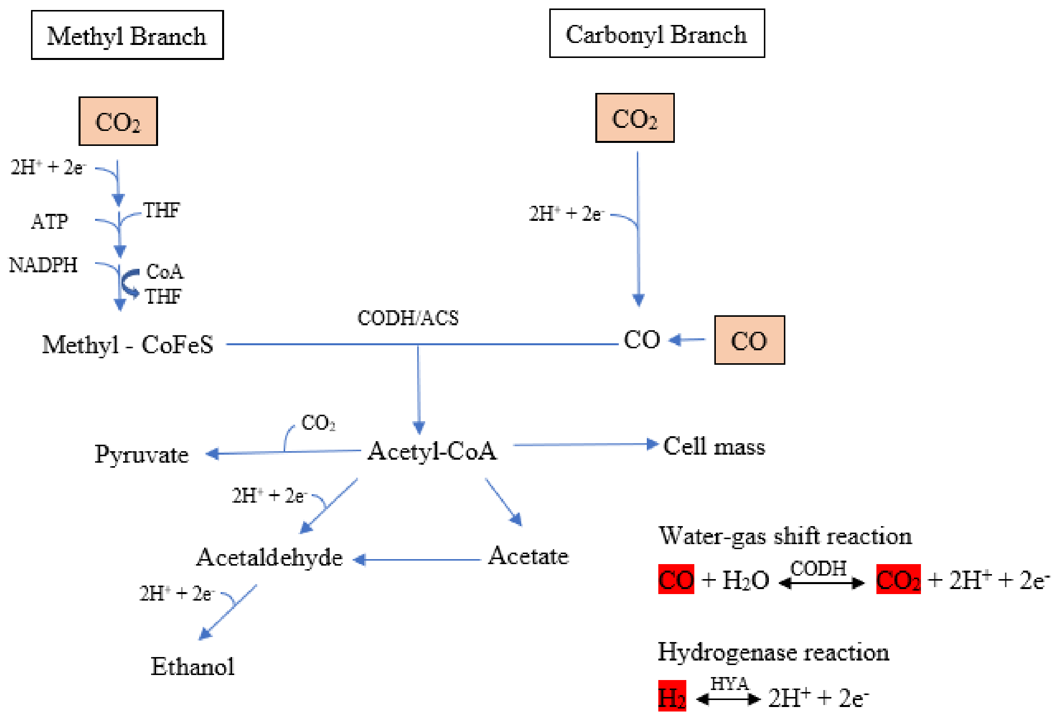

Fig.1 Wood – Ljungdahl Pathway [9,15,49]

The initial step before the WLP process is to convert 1 mole of H_2 into 2 moles of reducing agent [H] by the enzyme hydrogenase (HYA). Meanwhile, a water-gas shift reaction converts one mole of CO and H_2O into CO_2 and 2 moles of reducing agent [H] by carbon monoxide dehydrogenase (CODH). The reducing equivalent [H] is used to fix carbon molecules from CO and CO_2 into cell biomass and other metabolites via WLP. [60,61]. There are two main steps in the formation of acetyl-CoA. The first step is to reduce CO_2 in the methyl group through several reduction reactions using several enzymes.

In contrast, in the carbonyl group, CO_2 compounds are reduced to CO using the carbon monoxide dehydrogenase (CODH) enzyme. In the second step, the methyl and carbonyl groups are combined with the enzyme acetyl-CoA synthase (ACS) and carbon monoxide dehydrogenase (CODH) to produce acetyl-CoA. Then form acetic acid and ethanol. Some acetyl-CoA is used to create microbial cell mass [62].

Syngas Fermentation Conditions

The syngas fermentation process is influenced by the type of microorganism, bioreactor type, syngas composition, and gas-liquid mass transfer [63].

Types of microorganisms. The microorganisms used for ethanol production are *Clostridium ljungdahl, Clostridium autothenogenum, Clostridium ragsdelai,* and *Alkalibaculum bacchi* [15]. *C. ljungdahl* energy sources are H_2 and CO_2 or CO, whereas *C. autothenogenum* uses CO as the only source of carbon and energy [64]. *Alkalibaculum bacchi* will produce more ethanol if the CO composition is greater [65].

Bioreactor type. Bioreactors with a high reaction surface area will support a high mass transfer ratio and get a high syngas conversion [56]. Bubble diameter will be one of the critical parameters in gas-liquid mass transfer in suspended growth bioreactors [56]. Several reactors used in the production of ethanol are stirred tank bioreactor (STB), Bubble column reactor (BCR), Trickling bed reactor (TBR), Moving bed biofilm reactor (MBBR), and membrane bioreactor (MBR) [56]. STB uses mechanical stirring to reduce bubbles, BCR, which is applied in the process, does not use a stirrer but makes the syngas stay longer in the reactor, TBR is a reactor design with the main influencing parameters, there is the size of the reactor gasket, liquid recirculation rate, and gas flow rate, MBBR is a reactor where microbes grow on the surface of certain media then form a biofilm layer that increases mass transfer [66]. One type of MBR is a hollow filter membrane bioreactor (HFMR) which can produce micro solid bubbles [67]. The maximum concentration of ethanol obtained was more significant when using STR with HFM, which was 1.09 g/L compared to 0.35 g/L without HFM [55].

Gas-liquid Transfer mass. Gas-liquid mass transfer is needed to balance the kinetic needs of the cell without inhibiting metabolism so that the fermentation process can run efficiently [68] The limited mass transfer causes a reduction in the availability of substrate for the growth of microorganisms in the growth medium [69]. The solubility of syngas in the fermentation broth will affect the availability of substrate for the growth of microorganisms in the medium [70]. The volumetric mass transfer coefficient (K_La) is often used to measure the solubility of gases in the liquid phase [71]. The K_La for CO, CO_2, and H_2 gases are respectively 300.5, 425,9, dan 277,1 hour $^{-1}$ [72]. Simulations were carried out to calculate the utilization of CO and CO_2 with the utilization of CO and CO_2 of 0.92 mol and 2.03 mol with a consumption rate of 5.15 g/L-day and 17.87 g/L-day resulting in the conversion rate of dissolved CO in the fermentation broth is 23% while the dissolved CO_2 in the fermentation broth is 100% because the solubility of CO_2 is more significant than CO [72,73]

Effect of Impurity Gases in Syngas. Overall, the syngas fermentation process is said to be more resistant to the presence of impurities in the syngas than the chemical catalytic process [63]. However, the literature also mentioned that some impurities could inhibit the activity of acetogenic bacteria even at low concentrations by limiting enzyme activity and cell growth or by changing physicochemical conditions such as osmolarity, redox potential, pH, etc. [63].

Volatile hydrocarbon compounds such as CH_4 in a concentration of 4,5% did not affect the gas utilization process by *C. carboxidivorans* [74]. In addition, the utilization of the same microbes using syngas containing 0.1% C_2H_2, 1.4% C_2H_4, and 0.35% C_2H_6 did not affect growth during the fermentation process [75].

Tar causes inhibition of cell growth until microbial microbes can adapt to tar. In addition, tar can also increase the ratio of ethanol to acetic acid because tar will inhibit the production process of acetic acid [75]. *C. butyricum* can be grown 500 times in a bioreactor using tar-free syngas compared to tar-containing syngas [76]. The conventional technology for separating tar from syngas is the scrubbing system [77]. Filtration can also remove ash, tar, and other particulates from the produced gas [78].

Some species can utilize certain impurities in the syngas fermentation process. NH_3 and H_2S gas can increase the growth and formation of alcohol (ethanol, 1-hexanol, and 1-butanol) during fermentation using *C. carboxidivorans* [79]. These bacteria can withstand NH_3 rapidly turning into ammonium ions (NH_4^+) in the fermentation medium, which causes inhibition of hydrogenase activity and the growth of acetogenic bacterial cells [80]

Nitrogen oxides, nitrates, and nitrites can reduce biomass growth and alcohol concentration during syngas fermentation using *Clostridium carboxidivorans* [81]. In addition, Nitric oxide, which is present in syngas at 150 ppm, inhibits the hydrogenase enzyme that consumes H_2 [75].

The presence of impurities affects the performance of syngas fermentation, it may either improve the ethanol productivity or reduce it. The net effects should be researched further for a specific syngas composition.

Effect of Composition Gas on Syngas Fermentation Process. The carbon source for acetogenic bacteria in the Wood-Ljungdahl is CO or CO_2, which CO_2 can be used as a carbon source in the presence of H_2 [28,63]. The energy source used is CO or H_2 [28,63]. One of the advantages of the fermentation process is that it is flexible to the H_2/CO ratio. Still, acetogenic prefer a low H_2/CO ratio because most organisms grow better on CO than on H_2 [49,82]. H_2 will increase the conversion of CO into organism growth and ethanol production because H_2 acts as a source of electrons [53]. If H_2 is reduced, the source of electrons used is CO; this causes reduced ethanol production because the amount of carbon used is reduced according to the required reducing equivalents [53].

The fermentation process using the microbe *C. ljungdahlii* with an H_2/CO ratio of 2.0 produced acetate of 35.21 mM and ethanol of 5.39 mM, while the H_2/CO ratio of 0.5 produced acetate of 22.64 mM and ethanol of 7.44 mM [83].

According to Henry's law, only a small amount of CO and H_2 are soluble in water, and their solubility depends on the partial pressure of various species [49]. Decreasing the partial pressure of CO from 2.0 to 0.35 atm reduced cell growth and reduced acetic acid to ethanol. In addition, the partial pressure ratio of CO to CO_2 will affect the production of electrons and ATP [84]. CO_2 can also stimulate acetogenesis, the CO:CO_2 ratio of 70:30 increases the concentration of acetic acid compared to only pure CO. The CO_2 produced during the solventogenesis process from CO conversion can be involved in the re(oxidation) of ethanol to acetic acid [85].

However, the microbes normally need some times to different process condition, significant changes in the gas composition may affect the performance of the fermentation. The time required by *C.ljungdahl* to reach the maximum cell concentration with a syngas compositin 55% CO, 30% H_2, 5% CO_2, and 10% Ar for 20 hours [86], while with a syngas composition of 25% CO, 15% H_2, 20 CO_2 and 40% N_2 it takes 72 hours [55]. On the other hand, due to process instability, the composition of syngas produced during the gasification process has changed in a certain range. Therefore, it becomes a challenge in the fermentation process.

Conclusion

The combination of the thermochemical-biological process to convert lignocellulosic biomass into bioethanol is the gasification of lignocellulosic biomass into syngas followed by fermentation of syngas into bioethanol is an emerging technology. The biomass type and the gasification process conditions will affect the gas composition and the resulting impurities, such as tar. These factors may affect fermentation performance and the corresponding bioethanol productivity. Further research needs to be conducted to address these problems.

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