

Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol

Haris Nalakath Abubackar, María C. Veiga and Christian Kennes
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Abstract

Bioconversion of syngas/waste gas components to produce ethanol appears to be a promising alternative compared to the existing chemical techniques. Recently, several laboratory-scale studies have demonstrated the use of acetogens that have the ability to convert various syngas components (CO, CO₂, and H₂) to multicarbon compounds, such as acetate, butyrate, butanol, lactate, and ethanol, in which ethanol is often produced as a minor end-product. This bioconversion process has several advantages, such as its high specificity, the fact that it does not require a highly specific H₂/CO ratio, and that biocatalysts are less susceptible to metal poisoning. Furthermore, this process occurs under mild temperature and pressure and does not require any costly pre-treatment of the feed gas or costly metal catalysts, making the process superior over the conventional chemical catalytic conversion process. The main challenge faced for commercializing this technology is the poor aqueous solubility of the gaseous substrates (mainly CO and H₂). In this paper, a critical review of CO-rich gas fermentation to produce ethanol has been analyzed systematically and published results have been compared. Special emphasis has been given to understand the microbial aspects of the conversion process, by highlighting the role of different micro-organisms used, pathways, and parameters affecting the bioconversion. An analysis of the process fundamentals of various bioreactors used for the biological conversion of CO-rich gases, mainly syngas to ethanol, has been made and reported in this paper. Various challenges faced by the syngas fermentation process for commercialization and future research requirements are also discussed.

Introduction

With the increase in population and growing industrialization of many countries, there has been a tremendous rise in the demand for energy in the world. A 17-fold increase in world-wide energy consumption was reported in the last century. This energy demand is overcome by utilizing primarily the petroleum reserves, which are on the verge of extinction and are estimated to be depleted in less than 50 years at the present consumption rate. The processing of these fossil fuels and their usage leads to enormous release of hazardous and toxic gases to the environment, which is harmful to mankind as well as to the environment. The increasing concentrations of these gases has negative impacts such as severe floods and droughts, rising sea levels, and extreme weather conditions. Growing concern about global warming leads researchers to search for sustainable and safer alternative renewable fuels.

Ethanol is one of the most promising alternative biofuels. Fuel ethanol is an oxygenated, water-free, high octane (108) alcohol which has been recognized as a potential alternative fuel as well as an additive to gasoline. As an additive, it can replace methyl tertiary butyl ether (MTBE), which is used as an oxygenate and also to raise the octane number, by which the groundwater pollution due to MTBE usage can be eliminated. Today, ethanol can be used as blends with mineral gasoline at typical ratios of 10, 15, or 20% (E10, E15, and E20). It can even be used pure or almost pure as an alternative transportation fuel (E85).⁶ Since it burns cleaner than petroleum products, by using 10% ethanol blend (E10), a reduction of 25–30%, 6–10%, 7% and 5% respectively of harmful emissions of gases as CO, CO₂, VOCs, and NO_x can be achieved. In addition, ethanol is biodegradable and contains 35% oxygen, which reduces particulate and NO_x emissions upon combustion compared to conventional fuels.

Bioethanol is derived from renewable sources of feedstock such as sugar, starch, or lignocellulosic materials. Current processes include either direct or indirect fermentation of sugars or catalytic conversion of producer gas. In direct fermentation, feedstocks such as sugar-based crops (e.g. beets, sorghum, and cane) or starch-based crops (e.g. corn, wheat, barley, and potatoes) are converted into alcohols by yeasts or bacteria. This technology is well established at industrial level and currently, about 90% of the world bioethanol production comes from fermenting sugars or starch crops, known as first-generation technology. The high value of these crops as a food commodity either for human consumption or for feeding livestock and the issue of low utilization efficiency of crop parts per hectare of land used questions the feasibility of this technology. A potential solution for these issues, known as second-generation technology, is to utilize lignocellulosic feedstocks, such as agricultural or municipal wastes, wood, straw, grasses and crop residues. Lignocellulose is the most abundant renewable organic material on earth, composed of three major components: cellulose (40–50%), hemicelluloses (20–40%), and lignin (10–40%). It is the major structural component of all plants. In indirect fermentation, cellulosic as well as hemicellulosic biomass originating from trees and grasses are hydrolyzed chemically or enzymatically to simple sugars. The available sugars are then fermented to yield ethanol. A large proportion of lignin mostly present in straw and wood, along with cellulose and hemicellulose, is highly resistant to microbial attack. Gasification technology can be used to convert the biomass into a mixture of gases, called producer gas. Producer gas can subsequently be converted to ethanol either by using a chemical process (Fischer-Tropsch Synthesis, FTS) or by means of anaerobic microbial catalysts.

Bioethanol production is based on rather inexpensive feedstocks, such as biomass and waste organic matter. It in turn reduces the nation's dependency on imported fossil fuels and thus helps the economy. All this biomass-based production creates employment opportunities by utilizing trivial lands for the cultivation of inexpensive dedicated feedstocks, and the waste can be considerably regenerated for the production of an ecofriendly fuel. Similarly to syngas, CO-rich waste gases can also be used for bioethanol production. The paper summarizes the microbial aspects of ethanol production, ethanologenic homoacetogens, parameters affecting the syngas fermentation and various bioreactors reported in literature. Challenges and R&D needs for syngas fermentation processes are also explained.

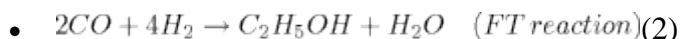
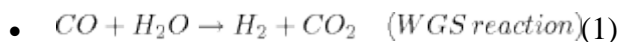
Ethanol production from syngas

Syngas, or synthesis gas, a mixture of principally CO and H₂, can be produced by gasification of solid fuels, such as coal, petroleum coke, oil shale, and biomass; by catalytic reforming of natural gas; or by partial oxidation of heavy oils, such as tar-sand oil. The syngas composition mainly depends upon the type of resources used, their moisture content, and the gasification process.

Gasification is the thermochemical process of converting carbonaceous materials, such as coal, petroleum or biomass, in the presence of a controlled amount of oxidant (air/O₂), into a gas mixture consisting mainly of CO, H₂, CH₄, CO₂, and N₂. The intrinsic chemical energy of the solid feedstock is thereby extracted and converted into both the thermal and chemical energy of the gas. It is a flexible and well commercialized efficient technology. Usually the process takes place in a gasifier and the composition of the syngas depends mainly on factors such as gasifier type (fixed bed, fluidized bed, etc.), feedstock properties (moisture, ash, dust and tar content, particle size, etc.), and operational conditions (temperature, pressure, etc.). Gasification of biomass involves three steps: (1) drying step to remove the moisture out of the feedstock; (2) pyrolysis at 300–500°C in the absence of oxidizing agents to produce gases, tars, bio-oils, and solid char; and finally (3) gasification of the products of pyrolysis in the presence of an oxidizing agent to yield the various components of producer gas. By optimizing the gasification operation, the composition of the producer gas can be narrowed to mainly CO and H₂, which are the main components for the ethanol production. Also by maintaining adiabatic conversion, the resource energy can be conserved to a higher extent. For example, gasifying at temperatures of 1500–1800°C and 1100°C, respectively of coal and biomass, produces syngas with CO and H₂ as main products.¹⁹ In addition, by using pure oxygen to feed the gasifier, the nitrogen concentration in the producer gas can be reduced.

The synthesis gas thus obtained can be converted chemically to ethanol and a variety of chemicals through the Fischer-Tropsch (FT) cycle. A variety of fuels and chemicals such as methanol, acetic acid, methane and heavy waxes can be produced by this technique. This method of production is a multistep, energy-intensive process carried out at elevated pressure and temperature using different chemical catalysts, which include metal iron, cobalt, or rhodium. These conditions make catalytic conversion faster than bioconversion processes. In this process, the catalytic water gas shift (WGS) reaction takes place, converting CO and H₂O to H₂ and CO₂, thus increasing the H₂/CO ratio, which is essential for the stoichiometry of reaction as well as for reducing the catalytic deactivation (Eqn 1). For protecting the sensitive FT catalyst, other products,

such as tar, oil, and water-soluble contaminants, present in the producer gas have to be removed. The sulfur contaminants present in the syngas have to be reduced to less than 60 ppb and the limits on level of NO_x and NH₃ to avoid FT catalyst poisoning are in the order of 0.1 and 10 ppm, respectively. Following the purification, the syngas containing CO and H₂ is converted to ethanol using different catalysts and processing conditions (Eqn 2).



Even though this process takes place at high reaction rates, it has many limitations. Mainly, the various processes such as WGS reaction, FT reaction, and purification take place under different process conditions, converting FT synthesis into a complex and expensive method. Moreover, the catalyst used should be specific and will deactivate when the concentration of sulfur, as well as carbon deposition, increases. The yield of liquid fuels from this process is also not high.

An alternative method of converting syngas to ethanol is through bioconversion. Micro-organisms, mostly anaerobic, can be used as biocatalysts to produce valuable metabolites, such as organic acids and alcohols, from syngas. These products include, but are not limited to, acetic, propionic, butyric, formic, and lactic acids as well as methanol, ethanol, propanol, and butanol. As a biofuel, ethanol is considered the desired metabolite and the process has to be optimized to maximize its production. Later the desired product is recovered from the broth either by distillation or extraction or a combination of both or by any other efficient recovery process to yield fuel graded ethanol (Fig. 1). Syngas fermentation is a simple process which takes place at near ambient temperature. Although it is characterized by a slower reaction rate, it has several advantages over the conventional chemical catalytic process. First, it has a high specificity, which leads to a higher yield, simplifies the downstream processing, and reduces the concentration of toxic byproducts. Secondly, the biocatalyst used is cheap, has high tolerance to sulfur, and is capable of adapting to contaminants, such as tars. Thus, the need of costly gas purification steps prior to conversion can be avoided. However, an appropriate filtering system can be used to negate the inhibitory effects of some toxic compounds present in the gas mixture. An advantage of the presence of sulfur compounds is that they can stimulate the growth of anaerobic bacteria by reducing the redox potential of the medium. Thirdly, bioconversion does not require a fixed H₂/CO ratio. Hence, one reactor vessel is enough to carry out the process by utilizing suitable micro-organisms. Finally, the biocatalyst generally dies when exposed to air, the process is odorless, doesn't create any health hazard, and generates less environmental pollution. The reaction process is limited by the mass transfer of gaseous substrates to the medium as well as the need of maintaining rather sterile anaerobic conditions. A continuous supply of nutrients is needed to increase the efficiency of the bioconversion process. Certain industrial processes, such as petroleum refining, steel milling, and methods for producing carbon black, coke, ammonia, and methanol discharge enormous amounts of waste gases containing mainly CO and H₂ into the atmosphere either directly or through combustion. Biocatalysts can be exploited to convert these waste gases to chemicals and fuels as, for example, ethanol, in a similar way as in the case of syngas fermentation.

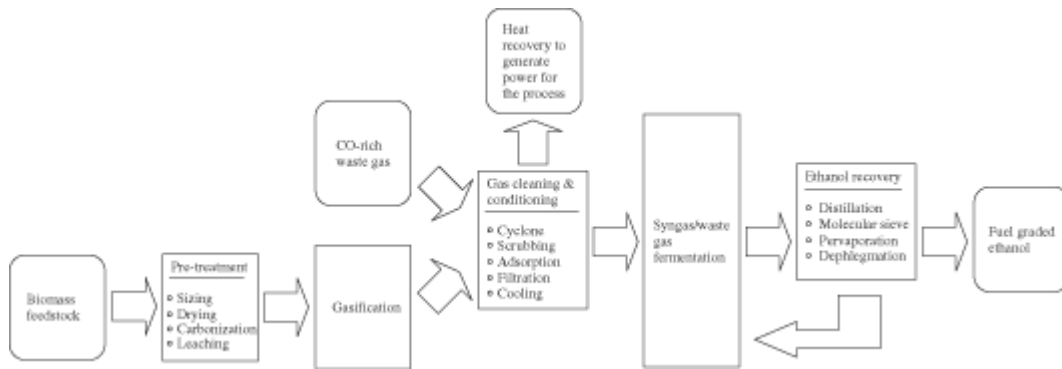


Figure 1. Syngas/CO-rich waste gas bioconversion process overview.

Biochemical pathway for ethanol production

The pathway which autotrophic anaerobes usually follow for the production of ethanol is the acetyl-CoA biochemical pathway or Wood-Ljungdahl pathway (Fig. 2). This pathway is present in several organisms including homoacetogenic bacteria and methanogenic archaea. It contains an eastern branch and a western branch.³³ The eastern branch comprises several reductive steps, where CO₂ is reduced to produce the methyl group of acetyl-CoA. The western branch, which is unique in anaerobes, either generates CO from CO₂ or directly takes CO from the media which then serves as the carbonyl group for the acetyl-CoA synthesis.

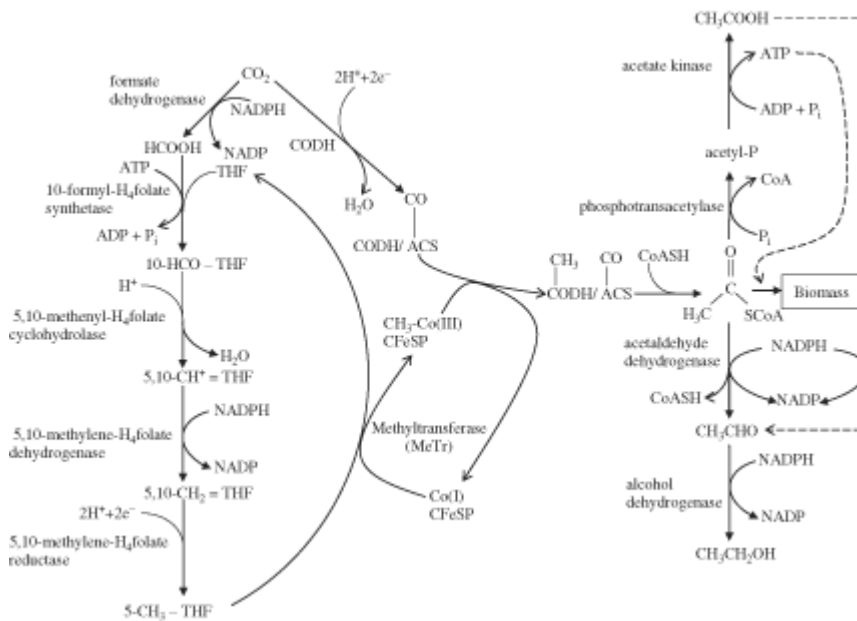
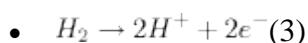
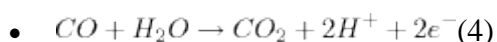


Figure 2. The Wood-Ljungdahl pathway for acetogenic microbes (CO to acetyl CoA) and reduction of acetyl-CoA to ethanol. Abbreviations: THF – Tetrahydrofolate; CFeSP – Corrinoid iron sulfur protein; CODH/ACS – CO dehydrogenase/acetyl-CoA synthase.

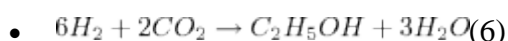
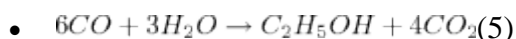
The reducing equivalents for the process are generated from H₂ by hydrogenase enzymes.



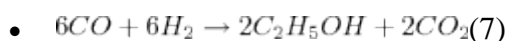
If H₂ is insufficient or inhibition of the hydrogenase enzyme occurs, then the reducing equivalents are produced via oxidation of CO to CO₂ using CODH.³⁷



It is worth observing that the sum of Eqn 4 and the reverse of Eqn 3 is the water gas shift reaction used to adjust the H₂/CO ratio during the chemical syngas conversion. The availability of CO as carbon source for ethanol synthesis thus decreases (Eqn 4) which can be interpreted using the Eqns 5 and 6.



It can be seen from Eqn 5 that only one-third of the available carbon source (CO) can be theoretically converted to ethanol. This is because CO is used to produce the reducing equivalents by oxidation to CO₂ via CODH in the absence of H₂ or in the state of inhibition of the hydrogenase enzyme. Moreover from Eqn 6 it can be deduced that CO₂ can be used to make ethanol if H₂ is present in the syngas.



Finally from Eqn 7, for an equimolar mixture of CO and H₂, two-thirds of the carbon substrate (CO) can be converted to ethanol since sufficient reducing equivalents are provided by hydrogen with the help of hydrogenase enzymes with a subsequent increased carbon conversion rate.

Eastern branch

The eastern branch is an H₄folate-dependent pathway which involves several reductive steps to convert CO₂ to (6*S*)-5-CH₃-H₄folate. The first step is the conversion of CO₂ by formate dehydrogenase to formate, which is condensed with H₄folate to form 10-formyl-H₄folate catalyzed by 10-formyl-H₄folate synthetase. A cyclohydrolase then converts the latter intermediate to 5,10-methenyl-H₄folate. The next step is an NAD(P)H-dependent reduction, where the methylene-H₄folate dehydrogenase converts the 5,10-methenyl-H₄folate to 5,10-methylene-H₄folate, which is reduced to (6*S*)-5-CH₃-H₄folate by methylene-H₄folate reductase.⁴¹ Thus, the conversion of CO₂ to the precursor of the methyl group of acetyl-CoA involves six electron reductions.

Western branch

The methyl group of the CH₃-H₄folate is transferred into the cobalt centre of the corrinoid/iron-sulfur protein (CFeSP) by the action of the methyltransferase (MeTr). This heterodimeric protein CFeSP44 is active when the cobalt centre is in active Co(I) state. The Co(I) then undergoes transformation into inactive Co(III) state by attaching a methyl group from the CH₃-H₄folate. The most important step in the MeTr mechanism is the activation of the methyl group because of the higher stability of CH₃-N bond in CH₃-H₄folate. The most studied mechanism of activation of the methyl group is by protonation of the N5 group of the CH₃-H₄folate thus leading to the electrophilic

activation of the methyl group. Hence the first organometallic intermediate is formed as methyl-Co(III)-CFeSP.

One of the main enzymes in the Wood-Ljungdahl pathway is CO dehydrogenase. This Ni-CODH is classified into two groups: (1) Monofunctional; and (2) Bifunctional CODH. Monofunctional CODH catalyses the oxidation of CO to CO₂, which is then reduced to formate and finally to the methyl group of acetyl-CoA. The bifunctional CODH converts CO₂ to CO, which serves the carbonyl group of acetyl-CoA, and also catalyses the formation of acetyl-CoA along with acetyl-CoA synthase (ACS). Following the synthesis at the C-cluster of CODH, CO then migrates to the Ni_p site of A-cluster in ACS forming organometallic intermediate; Ni-CO. The next step in the pathway involves the transfer of the methyl group from the methylated CFeS protein to the CODH/ACS complex. Thus the third organometallic complex, the methyl-Ni complex is formed. In the next step, condensation of methyl and carbonyl groups at the Ni_p form an acetylmethyl, the final organometallic intermediate. Finally, in the Wood-Ljungdahl pathway, CoA together with ACS thiolysis the acetylmethyl to form acetyl-CoA. Acetyl-CoA is converted by the cell to cell mass, acetate and ATP during the growth stage and to ethanol and NAD(P) during the non-growth stage.

Autotrophic bacteria for the conversion of syngas or waste gas to ethanol

The production of ethanol by anaerobic bacteria using syngas was first reported around the 1990s. However, chemicals such as acetic acid, butanol, and in some cases butyric acid and lactic acid were also produced along with ethanol. Various ethanol producing homoacetogens and their characteristics are listed in Table 1. These unicarbonotrophic microbes exhibit great potential for use in the conversion of syngas and follow the acetyl-CoA pathway for cell growth and product formation. Though these microorganisms grow well on multicarbon compounds, their potential to utilize CO, CO₂, and H₂ gases without additional feedstocks as co-substrates to produce various chemicals and fuels is important and well documented in the literature.

Table 1. Characteristics of different syngas/CO-rich waste gas fermenting bacteria for ethanol production.

Characteristics	<i>C. ljungdahlii</i>	<i>C. carboxidivorans P7^T</i>	<i>B. methylotrophicum</i>	<i>C. autoethanogenum</i>	<i>C. drakei</i>	<i>C. ragsdalei P11</i>	<i>A. bacchi CP11^T</i>
Origin	Chicken yard waste	Agriculture settling lagoon	Sewage digester	Rabbit feces	Acidic sediment	Duck pond sediment	Saturated soil
Size (µm)	0.6 x 2 – 3	0.5 x 3	0.8 ± 0.2 x 2.7 ± 0.54	0.5 x 3.2	0.6 x 3 – 4	0.7 – 0.8 x 4 – 5	0.5 – 0.8 x 1.5 – 2.2
Temperature range (°C)	30 – 40	24 – 42	10 – 50	20 – 44	18 – 42	18 – 37	15 – 40
Optimum temperature (°C)	37	37 – 40	37 – 40	37	30 – 37	37	37
pH range	4.0 – 7.0	4.4 – 7.6	6 – 9	4.5 – 6.5	4.6 – 7.8	4.0 – 8.5	6.5 – 10.5
Optimum pH	6.0	5.0 – 7.0	7.5	5.8 – 6.0	5.5 – 7.5	6.3	8.0 – 8.5
G + C (mol %)	22 – 23	31 – 32	49.8 ± 0.2	26 ± 0.6	30 – 32	29 – 30	34
Doubling time on H ₂ /CO ₂ (h)	2.7	5.8	19		3.5	4b	
Reference	Tanner <i>et al.</i> 114	<i>et Liou et al.</i> 62	Zeikus <i>et al.</i> 64	Abrini <i>et al.</i> 70	Liou <i>et al.</i> 62	<i>et Huhnke et al.</i> 77	<i>et Allen et al.</i> 76

- a
Marburg strain;
- b
Doubling time on CO.

The majority of the original works have focused on increasing the culture stability throughout the growth on syngas and increasing the alcohol production over acids. In most cases, a general trend in the shift from acetogenic to solventogenic product spectrum was observed as the fermentation pH is reduced. Hence, fermentation pH was found to have great influence in the regulation of syngas metabolism. Since most of the ethanol producing micro-organisms mentioned here were isolated recently, most of the research work with those microbes was done by only few research groups in the 1990s (Table 2).

Table 2. Ethanol production using gaseous substrate by various homoacetogenic bacteria.

Micro-organism	Bioprocess mode	Culture elapsed time (h)	Syngas composition (v/v%)	pH	Ethanol concentration (g L ⁻¹)	Alcohol/acid (mol mol ⁻¹)	Reference
<i>Clostridium ljungdahlii</i>	Continuous stirred tank bioreactor with cell recycle	560	CO = 55, H ₂ = 20, CO ₂ = 4.5, Ar = 15		48	21	58
	Two CSTR in series	16a	CO = 55.25, H ₂ = 18.11, CO ₂ = 10.61, Ar = 15.78	4.0	3b	1.5b	59
<i>Butyribacterium methylotrophicum</i>	Continuous stirred tank bioreactor		CO = 100	6c	0.056	0.131	68
	Batch experiments with serum bottles	144	CO = 35, H ₂ = 40, CO ₂ = 7.3, 25		0.02	0.018d	69
<i>Eubacterium limosum</i> KIST612	Continuous bubble column reactor with cell recycle	233e	CO = 100	6.8	0.092b	0.061f	75
<i>Clostridium carboxidivorans</i> P7 ^T	Continuous bubble column reactor	10a	CO = 14.7, CO ₂ = 16.5, N ₂ = 56.8, H ₂ = 4.4g	6h	1.6		61
	Batch experiments with cell culture flask	6.5a	CO = 20, CO ₂ = 15, H ₂ = 5, N ₂ = 60i	5.7	0.337	0.392	63
<i>Clostridium autoethanogenum</i>	Continuous modified bioreactorj	72	CO = 20, CO ₂ = 20, N ₂ = 50, H ₂ = 10	6	0.066k	0.062 ^k	73
<i>Moorella</i> HUC22-11 sp.	Batch experiments with serum bottles	156	H ₂ = 80, CO ₂ = 20	6.3m	0.069	0.026	74
	Repeated batch experiments with cell recycle using fermentor	430	H ₂ = 80, CO ₂ = 20	5.8	0.317	0.023	115

- a Culture elapsed time in days (d);
- b Approximate value in the reactor;
- c Other products at pH 6: butyrate, acetate and butanol;
- d Other products: acetic acid, butyric acid and lactic acid;
- e Dilution rate of 0.15 h⁻¹;
- f Other products: butyrate 6 mM, acetate 16.5 mM;
- g Rest contains CH₄ = 4.2%, C₂H₄ = 2.4%, C₂H₆ = 0.8%;
- ^h The broth pH at the ethanol concentration of 1.6 g L⁻¹;
- h, i 130 ppm of NO was added into the medium;

- j Modified spinner flask: the spinner is replaced with stainless steel tube with stainless steel porous gas dispersion cylinder connected to it;
- k At a flow rate of 10 mL min⁻¹;
- l Thermophile which grows at a temperature of 55 °C;
- m Initial pH.

Clostridium ljungdahlii

C. ljungdahlii is, by far, the most widely studied ethanol-producing homoacetogen. Various research works have been completed using this organism, mainly focusing on increasing the ethanol yield or improving the ethanol to acetate ratio. Vega *et al.* demonstrated the effects of yeast extract concentration in syngas fermentation on the stability of the process in both batch and continuous mode. Furthermore, with various initial substrate pressures in batch studies, the variation in the uptake and utilization of different substrates (CO or H₂) was briefly explained. In a continuous process with constant gas flow rate of 3.5 mL min⁻¹ (18.5% H₂, 15.4% Ar, 56.1% CO and 10% CO₂), a molar ratio of ethanol to acetate of 1:0.8 was obtained with a liquid flow rate of 10.85 mL h⁻¹ and by reducing the yeast extract concentration (0.01%).⁵⁷ Using *E. coli* as a model, medium composition was optimized by Philips *et al.* where B-vitamin concentration was reduced and yeast extract was eliminated for *C. ljungdahlii*. Ethanol concentrations of 50 and 25 g L⁻¹ were then obtained respectively with and without cell recycle. An ethanol-to-acetate ratio ranging from 1.6 to 21 mol mol⁻¹ was reported in the latter research during CSTR studies with cell recycle.⁵⁸ Using two-CSTR in series, Klasson *et al.* achieved a 30-fold increase in ethanol production rate (250–300 mmol g⁻¹ cell d⁻¹) compared to a single CSTR. By promoting cell growth in the first reactor followed by an increase in the ethanol production in the second reactor, a product ratio of 4 mol ethanol mol⁻¹ acetate was attained. The influence of nutrient limitation, pH, and dilution rate was studied to improve the product distribution. One major limitation in the overall CO bioconversion rate is the very low water solubility of carbon monoxide at ambient temperature and pressure. The influence of using a pressurized system in decreasing the gas-liquid mass transfer resistance in syngas fermentation and thus obtaining high CO uptake rate was postulated by Younesi *et al.*⁶⁰ The CO₂ concentration profile during the study revealed that CO was the preferred inorganic carbon source for *C. ljungdahlii*. A maximum product ratio of 0.54 mmol ethanol mmol⁻¹ acetate was obtained at syngas (55% CO, 20% H₂, 10% CO₂ and 15% Ar) total pressure of 1.8 atm in batch study using Wheaton serum bottles.⁶⁰

***Clostridium carboxidivorans* P7^T**

C. carboxidivorans P7^T, named on the basis of its ability to readily utilize CO, is an obligate anaerobe isolated from an agricultural settling lagoon. It was shown that this strain is able to produce ethanol, acetic acid, and butanol when grow on ‘clean’ bottled syngas without hydrogen in a bubble column.¹² The effects of biomass-generated producer gas on cell stability, hydrogen utilization, and acid/alcohol production in a 4-L bubble column reactor were also assessed by Datar *et al.*⁶¹ It was observed that cells are very sensitive to chemical species, such as nitric oxide and acetylene, produced along with the syngas generated from switchgrass. A minor amount of butyrate (max. conc. 1.2 g L⁻¹) was also reported along with the usually observed products mentioned above. The stoppage of hydrogen uptake as well as a decline in cell concentration after introduction of biomass-generated syngas reveal the need for further studies to identify chemicals contributing to the above findings. By using a 0.025 µm filter instead of 0.2

μm , the previously observed decline in cell concentration was able to negate. But hydrogen consumption ceased irrespective of the filter size. Further studies revealed that the presence of NO below 40 ppm in syngas will not cause any negative impact on cell growth, hydrogenase enzyme activity, and product re-distribution in *C. carboxidivorans* P7^T.

Butyribacterium methylotrophicum

B. methylotrophicum is able to use methyl radicals and was first isolated from a sewage sludge digester in Marburg, Federal Republic of Germany. The neotype strain of the species is called the Marburg strain and grows on multicarbon compounds in addition to one-carbon compounds and acetate, typically used by other methylotrophs. A comparison of the efficiency of cell synthesis during the growth of *B. methylotrophicum* on heterotrophic (glucose) and unicarbonotrophic ($\text{H}_2:\text{CO}_2$, and methanol) substrates has been done by Lynd and Zeikus. A final acetate concentration of 16 mM was produced during the growth on $\text{H}_2:\text{CO}_2$ (2:1) and a minor amount of butyrate was detected after the growth, with a doubling time of 9.0 h. Another strain of *B. methylotrophicum*, the CO strain, was the first anaerobic microbe to show its ability to grow unicarbonotrophically on CO as the sole carbon and energy source. It was designated CO strain for its growth on 100% CO.⁶⁶ This strain, when grown in batch culture with a continuous supply of CO in the headspace, yielded an acetate/butyrate ratio of 32:1 at a pH of 6.8. A general trend in the formation of more reduced products like alcohols was observed when the fermentation pH was reduced from 6.8 to 6 in the continuous study with a dilution rate of 0.015 h^{-1} . A gradual increase in the ethanol concentration in the continuous culture from 0.028 to 0.056 g L^{-1} was observed with a pH shift from 6.8 to 6 with a doubling time of 12 h. Nearly half of the available carbon in the substrate was found to be lost via CO_2 formation and acetate was the main reduced product formed in both the batch and continuous studies mentioned above. A more recent study investigated on the effect of supplementing CO with CO_2 and H_2 , using a CO adapted strain. The study revealed that bottles supplemented with CO_2 showed increased final product concentrations.⁶⁹ During this batch study, an ethanol concentration of 0.02 g L^{-1} and a total carbon yield to products of 110% was obtained using syngas ($\text{CO}:\text{CO}_2:\text{H}_2 = 35:25:40$) along with other products such as acetic acid (1.1 g L^{-1}), butyric acid (0.3 g L^{-1}) and a minor amount of lactic acid.

Clostridium autoethanogenum

C. autoethanogenum was originally isolated from rabbit feces using CO as the sole carbon and energy source. Electron microscopic studies using an old culture revealed that after a long period of incubation, the cell morphology changed from rod-shaped to continuous chains of encapsulated filaments having a size of $0.6 \times 42.5 \mu\text{m}$ along with the normal cells. Less syngas fermentation research has been completed using this micro-organism. With the objective of examining the effects of nitrogen-limited media on resting cells of *C. autoethanogenum* in ethanol production, Cotter *et al.*⁷¹ formulated six different non-growing media by varying or excluding some of the following nitrogen sources: yeast extract, trypticase peptone, and/or NH_4Cl and using xylose as substrate. In that study, a high ethanol production of 9.43 mM and ethanol to acetate ratio of 1:4.5 was reported in yeast extract excluded media, which is greater than the values obtained in growing cultures (5.11 mM and 1:7.8, respectively). This result supports the findings that yeast extract limitation can enhance ethanol production in

homoacetogenic bacteria. Importantly, a high level of culture stability was observed throughout the experiment in the medium containing 0.1 g L^{-1} yeast extract.⁷¹ The same research group achieved a 1:13 ethanol to acetate ratio when using syngas as substrate in liquid-batch continuous gas fermentation with a xylose adapted culture. Three different flow rates of 5, 7.5, and 10 mL min^{-1} of bottled synthesis gas were used in that study allowing the conclusion that despite increasing growth and product formation, the gas flow rate has no role in the product distribution in *C. autoethanogenum*.

Other strains

Isolates of *Moorella* species HUC22-1 were demonstrated to produce ethanol and acetate as main products formed from 130 mM CO_2 and 270 mM H_2 at 55°C , with an ethanol concentration of 1.5 mM after 156 h study. Moreover, this is the first example of an ethanol-producing thermophile that converts H_2 and CO_2 during growth. Another strain, *Eubacterium limosum* KIST612, isolated from an anaerobic digester, which was found to have high ability to grow at elevated CO partial pressure, was able to produce trace amounts of ethanol along with butyrate and acetate using 100% CO as substrate. A number of other anaerobic bacteria were also shown to utilize syngas as carbon and energy source and in particular, to convert syngas mainly to ethanol along with some other metabolites. Some of them are new isolates, including *Alkalibaculum bacchi* CP11^T, *Clostridium drakei*, and *Clostridium ragsdalei* P11.

Parameters affecting the bioconversion of syngas to ethanol

If one wants to produce bioethanol as a fuel product, conditions should be optimized to form that metabolite over any other during the bioconversion of CO-containing syngas or waste gases.

Effect of pH

As with other biochemical processes, fermentation pH is found to have a strong influence in regulating the metabolism of the substrate, namely CO-containing syngas or waste gas. There is a significant relationship between pH and the product composition. A general trend observed in most of the syngas fermentation studies is the shift in the product spectrum from acidogenic to solventogenic phase when lowering the fermentation pH. Since every organism is metabolically active over a limited range of pH, decreasing the pH has a negative impact on the cell growth. This is one major obstacle in the optimal conversion of syngas to fuel ethanol, as lowering the pH to produce highly reduced products as ethanol will also reduce the overall productivity of the process. In most studies, it was observed that lowering the pH causes a decrease in electron and carbon flow from the substrate toward the cell mass. At the same time, a decrease in acid production and an increase in alcohol production at the expense of the acid were observed. In a more recent study with *C. ljungdahlii*, an expected increase in ethanol production by lowering the pH of the broth from 6.8 to 5.5 was not observed. The culture in more acidic medium (pH 5.5) reached overall cell and ethanol concentrations of 388 mg L^{-1} and 1.81 mM , respectively, which was lower than at pH 6.8 (562 mg L^{-1} and 3.81 mM , respectively).⁷³ Nevertheless, fermentation pH is one of the important factors to be considered for the overall success of the syngas fermentation process.

Effect of media composition

During syngas fermentation, micro-organisms consume syngas constituents as carbon and energy sources; however, they also need various mineral nutrients to maintain a high metabolic activity. Special compounds, such as vitamins, may also be needed. Earlier reports suggest that even though growth ceases, a reduction in B-vitamins concentration along with eliminating yeast extract favored an increase in ethanol to acetate ratio. Eliminating yeast extract causes an improvement in product ratio of up to 300%; however, a minimum concentration of 0.01% yeast extract is necessary to provide the required trace nutrients for the structural integrity in *C. ljungdahlii*. Some studies have been reported on different nutrient sources, which induce sporulation along with an improvement in solvent production. It was found that compared to yeast extract and other nutrients, cellulobiose-containing culture media show an increase in cell concentration of greater than 20%, as well as ethanol concentration and ethanol to acetate ratio values greater than 4 and 3 times respectively compared to the values obtained in the presence of yeast extract.

The provision of more electrons by the addition of reducing agents into the culture medium will help the metabolism of microbes to shift toward solventogenesis. This occurs due to the presence of more reducing equivalents for the microbes to convert acetyl-CoA to ethanol. Klasson *et al.* examined the feasibility of increasing the ethanol concentration as well as product ratio in *C. ljungdahlii* by using different concentrations of reducing agents such as sodium thioglycolate, ascorbic acid, methyl viologen, and benzyl viologen. The authors found that even though the growth ceased, a high ethanol concentration (3.7 mmol) and a high product ratio (1.1) were found in bottles containing 30 ppm benzyl viologen. The most commonly recommended reducing agents for various acetogens by ATCC and DSMZ are cysteine-HCl and $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$. The effect of various trace metal ions in the fermentation media on growth and ethanol production by *C. ragsdalei* was investigated and it was observed that increasing the concentration of Ni^{2+} , Zn^{2+} , SeO_4^- and WO_4^- positively affected ethanol production.

Effect of gas composition

Gasification of biomass generates primarily CO , CO_2 , CH_4 , H_2 , N_2 and small amounts of NO_x , O_2 , acetylene, phenol, COS , H_2S , light hydrocarbons such as C_2H_2 , C_2H_4 , and C_3H_8 , ash, char, and tars. Autotrophic microbes are capable of growing well on bottled synthesis gas composed of CO , CO_2 , and H_2 . However, biomass-generated producer gas fermentation may sometimes face problems in maintaining the culture stability and the efficiency of carbon conversion due to the presence of trace amounts of additional constituents, such as acetylene or NO . Acetylene and NO are known to be potent inhibitors of hydrogenase enzyme activity. Since hydrogenase activity is essential for the reaction with hydrogen to obtain electrons for the CO conversion process, the inhibition of the hydrogenase enzyme will force the cell to obtain electrons from CO using CODH enzymes. Thus, available carbon for ethanol production will be greatly reduced. Hence, a decrease in carbon conversion efficiency will be seen during the process. In a study using *Rhodospirillum rubrum*, it was found that CO -linked hydrogenase enzymes show a 50% inhibition in the presence of 10% (v/v) C_2H_2 . Recent studies with *C. carboxidivorans* P7^T showed that product redistribution also happened due to the presence of NO and the effects due to the presence of tar towards the cell

dormancy was eliminated by cleaning the syngas using a cyclone, scrubber (10% acetone) and a 0.025 μm gas cleaning filter prior to the introduction into the fermentor.

Effect of substrate pressure

The partial pressure of the various constituents in syngas or waste gases plays a crucial role in the metabolism of the microbes. Partial pressures of both CO (P_{CO}) and CO₂ (P_{CO_2}) significantly influence microbial growth and product distribution. CO is used as a carbon source and sometimes oxidized to produce reducing equivalents via carbon monoxide dehydrogenase in the absence of H₂. Moreover, since CO is usually the least soluble gas among the syngas or waste gas components, more attention needs to be given to overcome mass transfer limitation due to this gas. Hence, one way of reducing the gas-liquid mass transfer limitations is by increasing the initial pressure of the gaseous substrates. The net electron production from CO by CODH increases with an increase in P_{CO} and decreases with an increase in P_{CO_2} . In a study done with *C. carboxidivorans* P7^T, it was shown that the maximum cell concentration increased when increasing the P_{CO} . A decrease in acetic acid concentration with an increase in ethanol concentration was also reported in the later stages of experiments conducted at high P_{CO} (1.35 and 2 atm). This is due to the utilization of excess electrons produced at high P_{CO} for the conversion of acetic acid to ethanol. An increase of 440% in cell concentration in that study was reported for an increase in P_{CO} from 0.35 to 2.0 atm. But some microorganisms are also reported to be less resistant to high P_{CO} resulting in an increase in their doubling time when increasing P_{CO} . In batch experiments using *C. ljungdahlii*, it was reported that increasing the initial pressure of the syngas will cause lengthening of the lag-phase period⁵⁷ and significantly improve the substrate utilization while yielding high ethanol/acetate ratios.

Mass transfer

One potential bottleneck of syngas fermentation is mass transfer limitations.^{87,88} When the fermentation broth contains a high cell concentration, the system is said to be in a mass transfer limited state, which is due to the low aqueous solubilities of the sparingly soluble gaseous substrates, CO and H₂. Due to these diffusion limitations, availability of gaseous substrates for the micro-organisms becomes low, which eventually leads to reduced productivity. The yield from the process also becomes low when the system is under kinetic-limited conditions, which happens when either the cell concentration or the CO consumption rate is too low.⁸⁹ Both of these two rate-limiting conditions may occur during the course of syngas fermentation.

From the theoretical equations of syngas fermentation (Eqns 5 and 6), it is clearly observed that 6 moles of CO or H₂ have to transfer into the culture medium to produce 1 mole of ethanol. Moreover, on a molar basis, the solubilities of CO and H₂ are only 77 and 68%, respectively to that of oxygen at 35°C. Hence, more moles of syngas must be transferred per carbon equivalent consumed in order to enhance the yield and productivity.

Gas-liquid mass transfer is of prime importance and the various gas components present in the bioreactor have to overcome a series of transport resistances before being utilized by the biocatalyst. The overall mass transfer rate of a gaseous substrate to the liquid phase is given by the product of the mass transfer coefficient, available area for

mass transfer, and the driving force. The driving force for diffusion in this case is the difference between the actual partial pressure of the substrate in the bulk gas phase, P^g (atm), and the partial pressure of the substrate that would be in equilibrium with the substrate in the bulk liquid phase, P^l (atm). Thus, the overall mass transfer rate can be defined as;

- Overall mass transfer rate = $\frac{K_L a}{H}(P^g - P^l)$ (8)

where H is the Henry's constant ($L \text{ atm mol}^{-1}$) and $K_L a$ is the volumetric mass transfer coefficient (s^{-1}).

Since the solubility of the substrate in the culture medium or in the biofilm is low, the amount of substrate present in the liquid phase is negligible compared to the substrate in the gas phase. Thus the substrate balance in the gas phase is given by

- $-\left(\frac{1}{V_L}\right) \frac{dN_s}{dt}(P^g - P^l)$ (9)

where N_s (mol) is the molar substrate concentration in the gas phase and V_L (L) is the volume of the reactor. From Eqn 9, the mass transfer coefficient K_L ($m \text{ s}^{-1}$) for the gaseous substrate can be determined.

The Andrew or Haldane model has been used to determine the kinetic substrate utilization and inhibition in syngas fermentation. The specific consumption rate q_s , which is the substrate uptake per dry cell weight, is given by

- $q_s = \frac{q_s^{\max} P^l}{K_p + P^l + (P^l)^2/K_i}$ (10)

where q_s is the specific substrate consumption rate (h^{-1}), q_s^{\max} is the maximum specific substrate consumption rate (h^{-1}), K_p is constant (atm) and K_i is the substrate inhibition constant (atm).

Ungermaier and Heindel compared CO-water $K_L a$ and power demand in a stirred tank reactor using different impeller designs and schemes and it was found that the highest mass transfer coefficient was obtained with the dual Rushton impeller scheme. Compared with the standard (single) Rushton impeller scheme, the dual Rushton impeller scheme could enhance the mass transfer by up to 27%. However, the impeller performance, which is the measure of volumetric mass transfer coefficient per unit power input, was lowest for the dual Rushton. As discussed later, increasing the agitation speed as a way to improve the mass transfer consumes more power. Hence this method is not economically feasible for large-scale bioethanol production. Bredwell *et al.* reviewed various bioreactor studies on syngas fermentation using conventional stirred tank and columnar reactors and observed that the volumetric mass transfer coefficient in these bioreactors depends mainly on reactor geometry, configuration, process operating conditions and the liquid phase properties.

Various additives can be added to increase the gas-liquid mass transfer rates which include surfactants, alcohol, salts, catalyst and small particles.⁹³ Ethanol concentration

of 1% (w/v) in the fermentation broth was shown to increase the mass transfer rate up to 3-fold compared to clean water.⁹⁴ This is due to the change in surface tension, thereby formation of small gas bubbles and hence better surface area for mass transfer. A new approach to enhance the mass transfer is by using nanoparticles. Zhu *et al.*⁹³ found that surface hydroxyl and functional groups on the nanoparticles have influence in enhancing the CO–water mass transfer coefficient. The highest K_La enhancement of 1.9 times was obtained when mercaptan groups were grafted on the nanoparticles.

Bioreactors for syngas fermentation

The selection of an appropriate bioreactor configuration is important for efficient syngas fermentation, especially configurations that could overcome mass transfer limitations and achieve high cell density. Transfer of syngas components mainly CO and H₂ is a major concern due to their low aqueous solubility.

To obtain a high syngas conversion, a good bioreactor should provide a high specific surface area for the reaction to occur, and favor high mass transfer rates. The bubble diameter will be one of the key parameters in gas–liquid mass transfer in suspended growth bioreactors. The specific surface area for mass transfer is inversely proportional to the bubble diameter under mass transfer limited condition. Hence, dispersing a sparingly soluble substrate using microbubble dispersion would offer a high area for mass transfer and the decreased bubble size during this process would allow longer gas hold-up times in the reactor, due to its slow rise. Microbubbles or colloidal gas aphanes are surfactant-stabilized small bubbles of diameter 50–60 μm , created by intense stirring using a high shear impeller in a separate reactor. Sebba proposed that these bubbles are composed of a gas bubble surrounded by a surfactant-stabilized shell of water. They are comparatively stable and offer high surface areas. The multiple surfactant shell prevents the adjacent bubbles from coalescence by imparting electric repulsion between them. A 6-fold increase in the overall mass transfer coefficient of CO has been reported by Bredwell and Worden, with microbubble sparging compared to conventional sparging, using *B. methylotrophicum*. Furthermore, the power requirement to generate the microbubbles for syngas fermentation was estimated to be very low, of 0.01 kW m⁻³ of fermentation capacity.

The most commonly used bioreactor configurations reported for conversion of syngas to ethanol include conventional stirred tank bioreactors, bubble columns, and membrane reactors; their schematics are illustrated in Fig. 3. Maximum cell and product concentrations obtained in various bioreactor studies are summarized in Table 3.

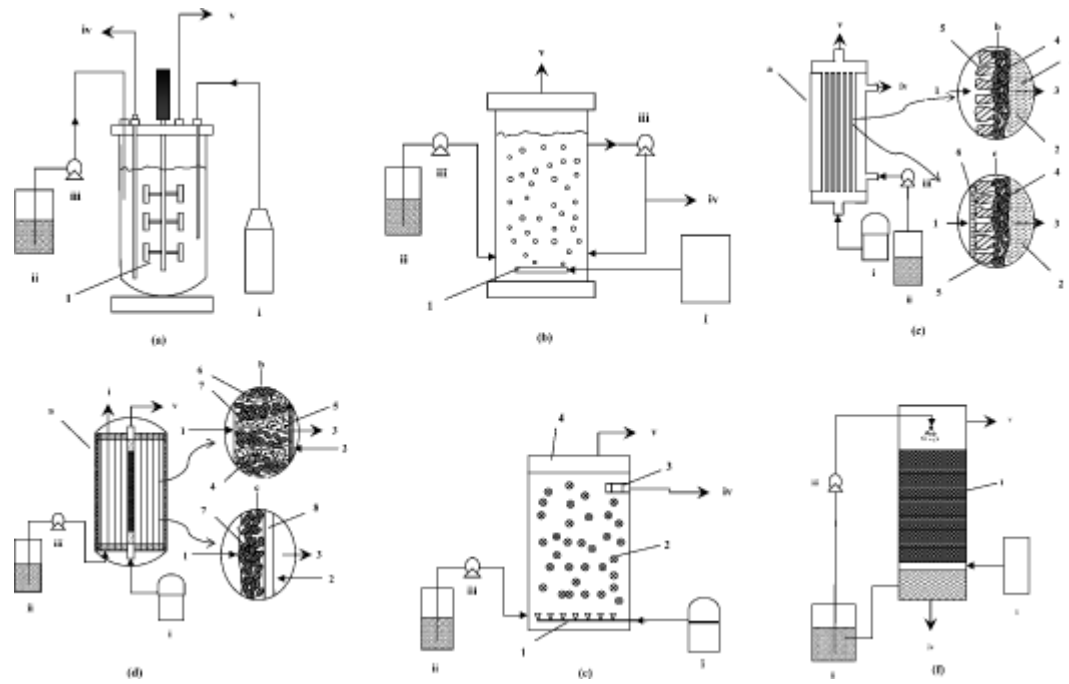


Figure 3. Schematic representation of various bioreactors for the conversion of syngas/CO-rich waste gas into ethanol. **(a)** Stirred tank bioreactor (STB): 1 – Agitator; **(b)** Bubble column reactor (BCR): 1 – Gas sparger; **(c)** Membrane bioreactor (MBR) with gas fed through the hollow fiber lumens while the liquid flows through the outer surface: a – Bioreactor vessel having plurality of membrane modules, b – Cross section of a microporous membrane present in modular membrane supported bioreactor (MMSB), c – Cross section of membrane present in membrane supported bioreactor (MSB), 1 – Gas inlet to the membrane, 2 – Liquid phase, 3 – Liquid products from the membrane, 4 – Microorganism (biofilm), 5 – Microporous membrane, 6 – Liquid impermeable layer; **(d)** Membrane bioreactor (MBR) with gas fed through the outer surface of the membrane fibers and the liquid flowing through the hollow fiber lumens: a – Bioreactor vessel having plurality of membrane modules, b – Cross section of an asymmetric membrane present in stacked array bioreactor (SAB) and horizontal array bioreactor (HAB), c – Cross section of an hydrophilic membrane having biofilm growth on the membrane surface, 1 – Gas inlet to the membrane, 2 – Medium inlet, 3 – Liquid products from the membrane, 4 – Biopores, 5 – Hydration layer, 6 – Biolayer, 7 – Microorganism (biofilm), 8 – Hydrophilic membrane; **(e)** Moving bed biofilm reactor (MBBR): 1 – Gas sparger, 2 – Biomass carrier, 3 – Carrier retainer, 4 – Gas recovery chamber; and **(f)** Trickle bed reactor (TBR): 1 – Packed bed. i – Gaseous feed into the reactor; ii – Nutrient feed into the reactor; iii – Pump; iv – Liquid products from the reactor and v – Gas outlet from the reactor.

Source: Figure (c) is adapted from Tsai *et al.*,⁹⁸ Hickey *et al.*, and Datta *et al.*, ; figure (d) from Tsai *et al.*, Tsai *et al.*, and Hickey *et al.*¹⁰⁴ and figure (e) from Hickey *et al.*

Table 3. Ethanol production using various components of syngas in bioreactors.

Bioreactors	Organism	Culture elapsed time (d)	Culture volume (L)	Stirring speed (rpm)	Gas retention time (min)	Dilution rate (h ⁻¹)	Cell concentration (g L ⁻¹)	Ethanol concentration (g L ⁻¹)	Reference
STB	<i>C. ljungdahlii</i>	1	0.6a	1000	1.4	0.208	7.1	12	102
	<i>C. carboxidivorans</i> P7 ^T	17	3	400	18.75	0.0069	0.215 ^b	0.75 ^b	27
	<i>Clostridium</i> strain P11	59	70	150	77.78	NA	0.87	25.26	107
	<i>B. methylotrophicum</i>	9	1.25	50	25	0.015	0.286	0.056	68
	<i>B. methylotrophicum</i>	56	1.5 ^a	200	NR	NR	9 ^b	TA	95
	<i>Moorella</i> Sp. HUC22-1	220c	0.5	500	8.34	NA	0.28 ^b	0.221	74
	<i>C. carboxidivorans</i> P7 ^T	100c	0.123	120	NR	NA	1.08	2	83
BCR	<i>E. limosum</i> KIST612	233c	0.2	NA	2.5	0.15	4.01	0.092	75
	<i>C. carboxidivorans</i> P7 ^T	10	4.5	NA	22.5	0.027	NR	0.16d	12
	<i>C. carboxidivorans</i> P7 ^T	20	4	NA	22.22	0.023	0.215 ^b	2.75 ^b	61
MBBR	<i>C. ragsdalei</i>	30	18000	NA	5.14	1.33	NR	30	94
MBR	<i>C. ragsdalei</i>	20	0.18 ^a	NA	NR	NR	NR	15	116

- Abbreviations: STB – Stirred tank bioreactor; BCR – Bubble column reactor; MBBR – Moving bed biofilm reactor; MBR – Membrane bioreactor with asymmetric hydrophilic membranes; TA – Trace amount; NA – Not applicable; NR – Not reported or not sufficient data to calculate.

- a
Reactor volume.

- b
Approximate values.

- c
Culture elapsed time in h.

- d
Ethanol concentration mentioned in wt%.

Note: Maximum cell concentration and maximum ethanol concentration reported during the respective studies is quoted here.

Stirred tank bioreactor (STB)

It has so far been one of the most studied reactor configurations for ethanol production, where the syngas or the gaseous substrate ultimately breaks into smaller bubbles, well dispersed in the liquid medium by the mechanical agitation caused by the rotating impeller. One way to increase the mass transfer of sparingly soluble gases like CO and H₂ is by increasing the impeller speed. Increasing the speed can increase the bubble break-up, but this requires a relatively high input of energy per unit volume. Consequently, this method of increasing the speed is not economically viable for large-scale production processes due to the excessive operational cost.

Continuous fermentation studies using a 750-mL stirred tank reactor without cell recycling, at different liquid dilution rates and yeast extract concentrations, resulted in achieving different cell densities and product distributions. The highest ethanol concentration (2 g L^{-1}) and molar product ratio of 1.2 was achieved in that study at a dilution rate of 0.031 h^{-1} and at 0.01% yeast extract concentration. With cell recycling in a STB (13.5 L) using the strain *C. ljungdahlii*, maximum cell and ethanol concentrations of 4 and 48 g L^{-1} were achieved, respectively, after 560 h of continuous operation.⁵⁸ These values are much higher compared to any other ethanol production studies using syngas. Recently, a successful installation and operation of a pilot scale fermentor (100 L) was reported by Kundiyana *et al.*⁹⁷ In that study, a 6-fold increase in ethanol production from syngas using *Clostridium* strain P11 was achieved by microbubble sparging.

Bubble column reactor (BCR)

BCRs are considered to be a potential alternative to the conventional STBs, in which mixing of gaseous substrates is achieved by gas sparging without mechanical agitation, and are considered to be economically viable in terms of saving energy costs. Some advantages of bubble columns include low capital and operational costs, lack of moving parts, and satisfactory high heat and mass transfer rates. Increasing the flow rate for enhancing mixing will cause a heterogeneous flow to occur. Such a condition will eventually lead to back mixing of the gaseous components. Less research has been done using BCRs for ethanol production compared to STBs. Ethanol production by *E. limosum* KIST612 using CO was carried out in a 200- mL bubble column reactor in batch and continuous mode by Chang *et al.* In that study, a membrane module of pore size $0.2 \mu\text{m}$ was connected to the reactor for cell recycling. High ethanol yields were easily obtained from CO in a 4.5 L BCR, and these values were, respectively 6 and 2 times higher than for acetic acid and butanol for *C. carboxidivorans* P7¹.

Membrane bioreactor (MBR)

Various membrane-based bioreactors have recently been studied and/or patented for the conversion of syngas to soluble products. In these bioreactors, microbial cells are attached to the membrane surface to form a biofilm, thereby achieving a high cell retention and high cell concentration.

Modular membrane supported bioreactors (MMSB), also known as submerged membrane supported bioreactors (SMSR), consist of plurality of membrane modules having either microporous or non-porous or composite membranes made into hollow fibers. The syngas components are introduced into hollow fiber lumens and the biofilm containing micro-organisms is maintained on the outer surface, i.e. on the liquid contacting side of the membrane fibers. The process gas passes across the hollow fiber wall toward the biofilm, where the micro-organisms convert the gaseous substrates into ethanol, which is then mixed with the process liquids. Ethanol is recovered by using suitable recovery systems. One of the major disadvantages of this system is that the liquid may enter the pores owing to variation in pressure across the membrane, thus leading to a phenomenon known as pore-wetting. Performance of this type of membrane bioreactor to produce ethanol was studied and it was found that using microporous membranes with a biofilm of *C. ragsdalei* produced a concentration of 10 g L^{-1} after a 20-day continuous operation.

Membrane supported bioreactors (MSB) comprise membranes having a microporous layer to support a biofilm at the liquid contacting side (outer surface), while the gas contacting side (lumen) of the membrane is having a liquid impermeable layer, which may be a silicone coating. To maintain a stable gas–liquid transfer in this system configuration, it is not necessary to maintain a very precise pressure difference across the membranes as required for systems having only microporous membranes. In addition, the impermeable layer provides higher gas transfer across the membrane than offered by a composite membrane. In MSB, a sandwiched type combination of membranes having a liquid impermeable layer between two microporous membranes was used as an alternative to the double layer construction. In one study with *C. ragsdalei* using this sandwiched type membranes, ethanol production increased to a maximum (13.3 g L^{-1}) and then ceased due to pore-wetting.

The stacked array bioreactor (SAB) and the horizontal array bioreactor (HAB) make use of hydrophilic asymmetric membranes with biopores having an effective diameter greater than $1 \mu\text{m}$. As the name indicates, SAB consist of membrane modules in axially stacked arrangement, whereas in HAB, plurality of modules is arranged in a horizontal plane inside the bioreactor. Each membrane module consists of asymmetric membranes made into hollow fibers with a biolayer that retains micro-organisms on the outside and the hydration layer in contact with the liquid on the lumen side. Hollow fibers are packed to form membrane modules. The fermentation liquid flows through the inner side of the hollow fiber and permeates the biolayer. The syngas stream passes through the outer surface of the hollow fiber; contact with the immobilized cells inside the biopores is provided by the biolayer. The liquid products flow from the gas contacting side toward the lumen which is ultimately recovered from the process liquid. An approach to enhance the ethanol production is by periodically lavage the biolayer by decreasing the pressure on the shell side relative to the lumen side of the asymmetric membrane module. This was studied by growing *C. ragsdalei* inside the biopores. Datta *et al.* were able to enhance the ethanol production from 1.6 g L^{-1} to 4.2 g L^{-1} in that study.

In another membrane supported biofilm bioreactor, biofilm is retained on the biofilm exclusion surface present at the gas contacting side (outer surface) of the hydrophilic membrane. This biofilm exclusion surface has a pore size not greater than $0.5 \mu\text{m}$, preventing the biofilm from flowing across the membrane to the liquid contacting side. In one study, by using this approach, *C. ragsdalei* produced an ethanol concentration of 10 g L^{-1} after 20 days of continuous operation.

Moving bed biofilm reactor (MBBR)

The moving bed biofilm reactor (MBBR) employs the state of the art of cell retention on an inert biomass carrier, promoting greater gas dissolution and utilization of syngas components by using eminent gas transfer systems.⁹⁴ The MBBR comprises (1) a vessel for maintaining the culture broth and liquid product; (2) a gas injection system for delivering syngas into the vessel and also for providing additional mixing by creating eddy currents in the surrounding liquid; (3) an inert biomass carrier for supporting microbial growth; and (4) a carrier retainer for hindering biomass carrier to flow out through the outlet. Gas bubbles rise through the fermentation broth and convert into liquid products using the microbes attached on the suspended carrier. By using a slot or jet gas transfer system, the necessary syngas pre-treatment step to remove small

particulates can be avoided. Studies using an active culture of *C. ragsdalei* in a MBBR having a fermentor vessel of 36 m³ reported an ethanol concentration of 30 g L⁻¹ after 30 days of continuous operation.

Trickling bed reactor (TBR)

The trickling bed reactor (TBR) or biotrickling filter (BTF) is a commonly used reactor design for various gas treatments. Reactor packing material size, liquid recirculation rate, and gas flow rate are the main parameters which greatly influence the mass transfer rate in TBR. In this columnar reactor, plug flow is most readily achieved. In a study to compare the performance of three different types of bioreactors for syngas fermentation, Klasson *et al.* concluded that higher CO conversion rates (>80%) and higher productivities were achieved in a TBR than in a continuous STB and BCR. To our knowledge, no studies have been reported using this bioreactor for the fermentation of syngas to produce ethanol as one among the main products.

Product yield

A major advantage of microbial processes, as stated before, is the product specificity, yielding few byproducts and increased process yield. To get high productivity and yield, the cell concentration in the bioreactor has to be high; this is achieved by either cell recycling or by cell retention. Membrane-based bioreactor systems have recently been used, wherein the biofilm grows and attaches to the surface of the membrane as a biopolymer matrix, thereby preventing cell washout. As a fuel, ethanol is the most desired product of the syngas fermentation, while in most of the fermentation studies acetate productivity prevails over the ethanol production. Hence, in order to improve ethanol productivity or to increase the ethanol-to-acetate ratio, it is necessary to manipulate various fermentation parameters. Once a stable cell density is achieved, the following parameters can be adjusted individually or in combination to improve the ethanol productivity and to limit the acetic acid production: alteration of the medium constituents, liquid and gas feed rates, operating pH, temperature, pressure, and agitation rate or by providing excess H₂. By these ways, a reduction in redox potential and increased NADPH-to-NADP ratio in the fermentation broth is maintained, thereby promoting the reduction of acetic acid production compared to ethanol. Excess supply of H₂ means that ratio of H₂ fed to the sum of twice the CO converted and three times the CO₂ converted should be greater than 1 to promote ethanol production. In a patented study using *C. ljungdahlii*, it was observed that the biological pathway is directed in favor of ethanol production and less acetic acid production by first feeding gaseous H₂ in excess and then limiting the calcium pantothenate and cobalt concentrations in the nutrient medium. A doubling of ethanol concentration and reduction in acetate production in the fermentation broth was also reported when the iron concentration was increased 10-fold. Hence medium optimization is a prerequisite to favor ethanol over acetate production.

Cell separation and ethanol recovery

Micro-organisms grow either in planktonic form, or as a biofilm on a solid matrix usually on membranes. Cell retention, and thereby an increase in cell density, is possible by the formation of a biofilm attached on a solid support in the bioreactor. Conversely, in suspended-growth reactors, cells grow in suspension and are separated

from the product stream by employing solid/liquid separators, which includes membranous ultrafiltration units, hollow fibers, or spiral wound filtration systems or centrifuges.¹⁰⁸ Thus, the cells can return to the bioreactor.

The concentration of ethanol in the fermentation broth must be kept below a certain level in order to prevent microbial inhibition and to keep the cells metabolically active. Moreover, biomass-derived syngas fermentation usually produces low concentrations of ethanol (below 6%); hence, to economically recover ethanol, an efficient recovery process is required, which includes distillation followed by molecular sieve separation or pervaporation followed by dephlegmation technologies. Integration of vacuum distillation columns and vapor permeation units has numerous advantages, such as amenability to separate ethanol from the fermentation broth even when ethanol concentration is as low as 1% where approximately 99% by weight of dehydrated ethanol can be recovered by this process. Formation of toxic byproducts due to high temperature can be precluded, since vacuum distillation does not require high temperature. Hence, the majority of the distillation column bottoms can be recycled to the fermentor without any prior treatment. Another approach to enhance the concentration of ethanol in the feed to the vacuum distillation column is by flashing the feed before it enters the vacuum distillation column. Coskata Inc., Illinois uses a licensed membrane separation technology to separate the ethanol from water; thereby a reduction of 50% in energy requirement has been achieved compared to conventional distillation (www.coskata.com).

Survey on syngas bioconversion to ethanol in industry

Gasification of biomass followed by syngas fermentation to produce bioethanol is a developing technology. Very few companies have scaled up bioconversion technology at pilot scale. Coskata Inc., a US bioethanol company, developed bioethanol, known as FlexEthanolTM from biomass derived syngas via biofermentation. The proprietary process produces approximately 100 gallons of ethanol per ton of dry input material. A study by Argonne National Laboratory, Illinois has determined that Coskata's process can achieve a net energy balance of 7.7 and offer up to 80–90% reduction in lifecycle greenhouse gas emissions when compared to conventional gasoline. Its technology has been scaled up to a semi-commercial-scale plant located in Madison, Pennsylvania and the first commercial-scale plant will start operation by 2011. A New-Zealand-based clean technology company, LanzaTech, uses proprietary bacteria to convert industrial waste gases, i.e. mainly off-gas from steel industries, or biomass syngas into high octane premium fuel (www.lanzatech.co.nz). Using its proprietary technology, a pilot plant has been commissioned in 2008 at BlueScope steel plant, Glenbrook, to produce ethanol from steel mill flue gases. LanzaTech uses low-cost media as the sole fermentation media component and the process has been carried out with minimum waste gas conditioning. INEOS Bio, a UK/US-based bioenergy company uses a proprietary bioconversion process for converting a wide range of organic wastes, including household and commercial wastes into bioethanol (www.ineosbio.com). INEOS's bio pilot-scale facility in Fayetteville, Arkansas, has been in operation since 2003.

Challenges and R&D needs for commercialization of bioethanol production using gas fermentation

Feedstock

The feedstock for syngas production encompasses a wide spectrum of biomass materials, such as forest residues, agricultural and organic solid wastes, amongst others. Feedstock properties, for example, a high moisture content, have a negative influence on the CO fraction produced in the gasifier. In such cases, considerable energy is required for drying the biomass in order to keep the moisture content around 10–15%.¹¹¹ Every biomass contains ash and volatile compounds; the content varies from one feedstock to another. For instance, ash content in rice husk is about 15–25%, whereas in wood it is 2% or less.¹⁷ Gasification of such feedstock produces impurities that inhibit the syngas fermentation. Thus extensive gas-cleaning steps are required prior to feeding into the bioreactor, which substantially increases the overall production cost. However, the nitrogen and alkali contents of the biomass can be greatly reduced by upstream treatments, such as fractionation and leaching. It is quite obvious that an appropriate feedstock requires less pre-treatment and results in less syngas contaminant production, making ethanol production a process consuming less energy.

Gasification system and syngas purity

Various impurities are produced during gasification of biomass along with CO and H₂ which may cause problems in the subsequent bioconversion steps. The composition of the gas produced in the gasifier is greatly influenced by the gasifier configuration and the operating conditions. The equipment size can be decreased by feeding the gasifier with pure oxygen. But it will increase the overall cost of the process. The pyrolysis of volatile compounds releases tars, which not only affects the microbial activity during syngas fermentation but also gets deposited on the walls of the gasifier and gas transfer system, which ultimately decreases the performance of the gasifier. Using light hydrocarbons, the tar produced during the gasification can be substantially converted to syngas. About 90% of the tar generated in the gasifier is able to crack by this way. On the other hand, the feasibility of using light hydrocarbons derived from renewable energy sources and subsequent use of the produced syngas for microbial utilization to biofuels have yet to be explored.

Micro-organisms and media composition

Isolation of high yielding (>25 g L⁻¹) ethanologenic homoacetogens, which have greater tolerance to high ethanol concentrations in the fermentation broth, is necessary for successful commercialization of syngas fermentation. Moreover, culturing of anaerobic micro-organisms requires specialized techniques to maintain the system under oxygen-free conditions. Thermophilic micro-organisms having the above features might be interesting since less cooling of syngas would be required prior to feeding the bioreactor and an elevated temperature can improve the conversion rate. Another task is to enhance the ethanol production by modifying metabolically the available syngas fermenting microbes through genetic engineering.

There are many factors to be considered while selecting fermentation media for large-scale ethanol production such as, but not limited to, media complexity, cost, or presence

of chemicals that could improve ethanol productivity. Identifying unique media for specific micro-organisms which satisfy the above features is one of the important challenges faced by ethanol producers. Recently, it was reported that cotton seed extract (CSE) can be used as the sole fermentation medium for culturing *C. ragsdalei* P11 for ethanol production.

Mass transfer and scale-up

As discussed before, one of the main challenges faced during syngas fermentation is the gas–liquid mass transfer resistance. Various techniques to improve mass transfer of the syngas in STR have been discussed elsewhere. For commercial-scale bioreactors, however, more efficient and economical mass transfer systems have to be found.

For scale-up, a clear understanding and estimation of the volumetric mass transfer coefficient (K_{La}) is required. The achievement of a high syngas mass transfer rate with minimal power consumption and relatively low shear rates, whilst maintaining an anaerobic atmosphere, is a major challenge for syngas fermentation scale-up. More research is still necessary for syngas fermentation scale-up.

Product recovery

The low microbial resistance to ethanol in the fermentation broth is one major obstacle in developing this technology. Furthermore, the fermentation broth also contains other dissolved and undissolved compounds, such as cell extracts and unfermented soluble compounds, which also create separation problems during ethanol recovery. For these reasons, *in situ* ethanol separation is considered a better choice by coupling the fermentor vessel with various unit operations. Novel separation systems have still to be tested to overcome these challenges and thus increasing ethanol volumetric productivity.

Production costs

There are various parameters affecting the techno-economics of syngas fermentation. For instance, the cost of different feedstock regulates the overall production costs. In one recently published report, feedstock cost was shown to account for about 67% of the total production costs, even when dry biomass wood was used, without considering the depreciation factor. Besides feedstock, the need to maintain the selected pure biocatalyst can also have a sizable impact on the production costs. Xia and Wiesner compared the production costs involving two micro-organisms, and pointed out that, out of the two acetogens chosen, *C. ljungdahlii* showed better ethanol yield with production costs much lower than for *Moorella sp.* HUC22-1, excluding the operational cost and depreciation terms. This was attributed to the high ethanol production over acetate (3:1) of *C. ljungdahlii* over *Moorella sp.* HUC22-1 (1:28).

Although producing ethanol using syngas fermentation demands substantially less energy input, process modification and optimization steps are still at the development stage in order to achieve remarkably high process yields.^{11,111} From a literature viewpoint, very few studies have undertaken a systematic evaluation of the techno-economics involved in the syngas fermentation process, and more detailed studies

relating the costs to mass-energy balances, flow sheet modeling, and life cycle assessment should be initiated in order to obtain a valuable database.

Conclusions

Bioethanol production from biomass as well as from CO-rich waste gases or syngas fermentation is potentially viable. The presence of specific impurities, NO and acetylene, in syngas can have a severe antagonistic effect on the enzyme activity and its conversion pathway, and advanced filtration systems can be used as a pre-treatment step to remove these impurities. Literature reports on gene manipulation in the syngas fermenting microbes have been initiated only very recently. Alteration in the properties, such as ethanol tolerance level and production rate at the gene level, by recombinant DNA technology could improve the overall performance of this technology. The use of membrane-based bioreactors for syngas fermentation offers several advantages, in terms of providing a large surface area for both gas–liquid mass transfer and cell attachment, over conventional bioreactors. Yet, there is also a need to develop and evaluate hybrid and multistage bioreactor configurations keeping in view the low aqueous solubility of the syngas components and required high ethanol productivity. A systematic improvement, through retro-fitting and implementation of current technologies in these industries would guarantee investors and financial providers to reach their business goals without making risky investments.

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Haris Nalakath Abubackar

Haris is currently starting his second year PhD-studies at the Chemical Engineering Department of the University of La Coruña (UDC) in Spain, under the supervision of Prof. Christian Kennes. He has completed an M. Tech in Biotechnology from Karunya University in India and a B. Tech in Biotechnology from Met's School of Engineering, India. His present research focus is on the bioconversion of syngas and waste gases containing CO into biofuels and other chemicals.

María C. Veiga

Dr María C. Veiga is Professor at the Department of Physical Chemistry and Chemical Engineering of the University of La Coruña (UDC), Spain. Her main areas of activity are on wastewater treatment, waste gas treatment, and the production of biopolymers from renewable sources. Dr Veiga coordinates the Environmental Engineering Group at UDC.

Christian Kennes

Dr Christian Kennes is Full Professor of Chemical Engineering at the University of La Coruña (UDC) in Spain. He first undertook engineering studies in Brussels and later obtained his PhD degree from the University of Louvain in Belgium. He worked in the United States and the Netherlands before joining UDC in 1995. His main research topics presently focus on environmental technology and bioconversion processes.

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