

III.B.3. Biofuels from Syngas

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Abstract

The production of biofuels from syngas is an emerging technology that can utilize a wide variety of biomass. This route of biofuels production has the advantage of utilizing the entire biomass including the lignin content, which is usually difficult to break down. Biomass is converted to syngas via a process called gasification, which involves partial oxidation of the biomass at high temperatures. Biomass-syngas can then be converted to biofuels such as methanol, ethanol and hydrogen via the metal-catalytic or bio-catalytic methods.

This section provides an overview of syngas production as well as the conversion of syngas to biofuels. The current processes of syngas production and the types of gasifiers used are discussed. The chemistry of gasification, reactions involved, and the impurities formed during this process are also discussed in this section. Syngas can be converted to biofuels using metal catalysts or bio-catalysts. The metal catalytic conversion usually involves either the Fischer-Tropsch Synthesis (FTS) or methanol synthesis, as described. The FTS process results in hydrocarbon products which can be further refined to produce fuels while methanol synthesis results in methanol, which is a renewable fuel or fuel additive. The metal-catalytic conversion is a reliable technology for the conversion of syngas to biofuels. However, the process is limited by severe reaction conditions as well as the high tendency of catalyst poisoning. An alternative method is the bio-catalytic conversion of syngas. This section discusses hydrogen and ethanol production by this method. Commonly used microbial catalysts and the reactions involved are discussed. This section also describes some of the bioreactor types used in these processes. A summary of hydrogen and ethanol yields found in the literature is also provided in this section. Though this method overcomes some of the drawbacks of metal-catalytic conversion, research is still required to overcome bottlenecks such as low productivity and long reaction times.

Keywords: Syngas, biofuels, gasification, Fischer-Tropsch Synthesis (FTS), methanol, ethanol, hydrogen

III.B.3.a. Introduction – Synthesis gas or syngas is a gas mixture primarily consisting of carbon monoxide (CO), carbon dioxide (CO₂), hydrogen (H₂) and nitrogen (N₂). Syngas can be produced from various sources, such as natural gas, coal, petroleum coke and biomass (Dayton, 2003). Though many of these processes are well-established, the focus is steadily shifting towards the production of syngas from biomass due to its abundant availability and renewable nature. In this process, called gasification, biomass is subjected to partial oxidation at temperatures above 800°C to generate syngas. One of the main advantages of gasification is that a wide variety of raw materials can be utilized such as prairie grasses, wood chips, solid municipal wastes and paper wastes. This process is also well-suited to raw materials such as softwoods that are normally difficult to handle (Dayton, 2003). A second main advantage is that gasification can break down cellulosic, hemicellulosic and lignin bonds that are difficult to break down using fermentative or enzymatic reactions. This provides a greater conversion efficiency of biomass to energy (McKendry 2002/5a).

Biomass-syngas can be converted to several useful fuels such as hydrogen, methanol, and ethanol. Chemicals such as ammonia and acetic acid can also be produced from syngas. Hydrogen is typically produced by a water-gas shift reaction, while methanol is produced by the metal-catalytic conversion of syngas. Ethanol can be produced from syngas either via the metal-catalytic or the bio-catalytic route. Production of ethanol from syngas is an emerging technology with great potential as it reduces the need to use food feedstocks, such as corn, for ethanol production. Figure 1 shows a schematic of the common processes of syngas conversion to biofuels. Co-firing (gasification of a coal/biomass mixture) and biomass gasification are the primary sources of biomass-syngas.

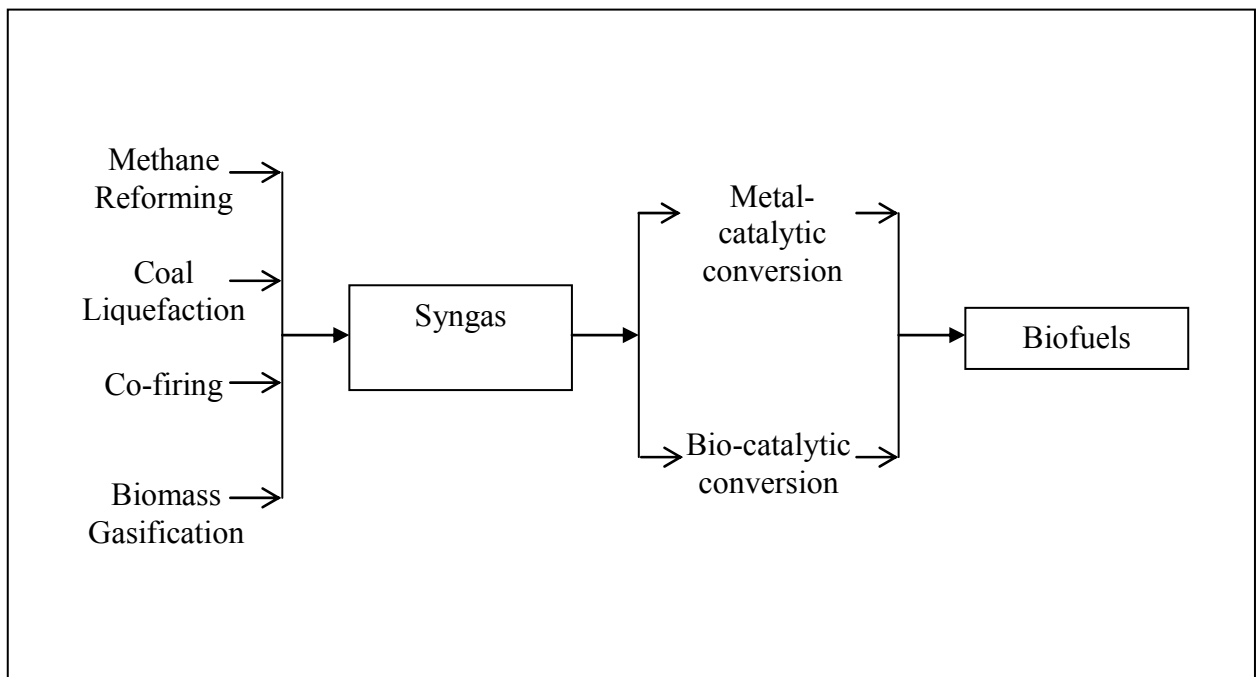


Figure 1. Processes of biomass-syngas production and conversion to biofuels.

III.B.3.b. Syngas Production

III.B.3.b. (i). Current Process & Equipment— Gasification is a partial combustion process that converts carbonaceous materials into CO, CO₂ and H₂. Complete combustion of these materials in stoichiometric (or greater) amounts of oxygen would yield combustion products of CO₂ and H₂O. However, in the gasification reaction, lower than stoichiometric amounts of oxygen (in the form of air, pure oxygen or steam) are fed to the reactor at high temperatures (greater than 700° C) and therefore the products are only partially reduced. These partially reduced products are advantageous as they can be used as building blocks in other processes to produce liquid fuels.

Gasification occurs in three main steps: 1) Initial heating to dry out any moisture embedded in the fuel, 2) Pyrolysis (heating up to 300-500 °C in absence of oxidizing agents) yielding gas, tars, oils and solid char residue, and 3) Gasification of solid char, tars and gas to yield the primary components of syngas (Bridgwater 2003).

There are four main types of gasifiers currently used commercially: counter-current fixed bed, co-current fixed bed, fluid bed and entrained flow.

Counter-current fixed bed (Updraft): A fixed bed of carbonaceous fuel (coal or biomass) with a counter current flow of steam, oxygen and/or air flows up through the fuel bed. The ash is then removed dry or as slag. In order to form a fixed bed that is permeable to the flow of the oxygen source, the fuel must have high mechanical strength and be non-caking. Gas exit temperatures are low, which is good for thermal efficiency, but this increases the tar and methane impurities in the gas (McKendry 2002/5b, Bridgwater 2006).

Co-current fixed bed (Downdraft): This is similar to the counter-current gasifier described above. However, the steam, oxygen and/or air flows co-currently down with the fuel bed. Because the gas passes through the hot char at the bottom of the bed before exiting, some impurities such as tars are entrained in the char and the final product has a higher purity. The exit temperature of the gas is higher, resulting in a lower overall efficiency (McKendry 2002/5b, Bridgwater 2006).

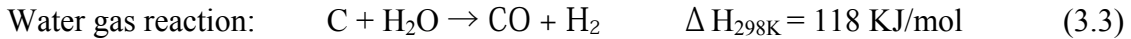
Fluid bed: The fuel is fluidized in oxygen/air and steam. The ash is removed dry as it becomes defluidized. Fuel throughput is higher than for a fixed bed, and has the advantage of uniform temperature distribution achieved in the gasification zone resulting in cleaner reactions. However, conversion is lower and recycle streams are often necessary. Fluidized beds work particularly well for biomass, as it has a lot of highly corrosive ash that would harm the fixed bed reactors (McKendry 2002/5b, Bridgwater 2006).

Entrained flow: Fuel is fed either as a dry pulverized solid or a fuel slurry co-currently with oxygen (and sometimes air). Gasification takes place in a dense cloud of fine particles. This process is particularly useful for coals which can be easily pulverized into

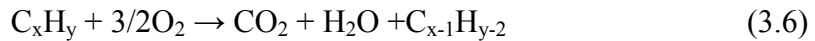
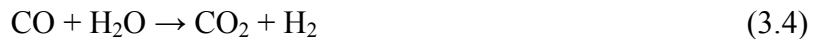
fine particles. This gasifier has the highest operating temperature and pressure, which decreases the amount of tars and methane (McKendry 2002/5b, Bridgwater 2006).

Gasifier manufacturers in a recent survey indicated 75% of commercial gasifiers were downdraft, 20% were fluid beds, 2.5% were updraft and 2.5% were other variations (Van Loo and Koppejan 2003).

III.B.3.b.(ii). Gasification Chemistry– There are three main reactions taking place in a gasifier (McKendry 2002/5b):



These processes can also react further to produce other products and impurities in smaller concentrations. Some of the main side reactions are listed below (Kuo 2005).



Notably, the production of H₂ from the water gas shift reaction (Equation 3.4) can lead to the production of methane as fuel (Equation 3.5), although this is not the focus of this review.

Different feedstocks will produce different ratios of exit gas concentration. The results of a fluidized-bed gasification study (using air-steam as the gasification agent) for a variety of feedstocks is shown in Table 1 (McIlveen-Wright, et al. 2006).

In addition, the products vary depending on the oxidizing agent used (air, pure O₂ or steam). Air (because of the high inert N₂ content) increases the exit volume of the gas, thus increasing the size of cleaning and storage of syngas downstream. However, the air process must be balanced with the extra cost of pure O₂ or steam using as the oxidizing agent. Use of steam produces a higher H₂ content because of the water gas-shift reaction, although the higher energy cost of steam must be taken into account (Bridgwater 2006).

The study shown in Table 1 was also performed using air with a gasification catalyst (20-30 wt% silica sand and dolomite) instead of steam. As shown in Table 2, the gas compositions vary from the steam gasification study. The air process has less methane and a lower hydrocarbon volume percentage.

Table 1. Gas composition (air-steam gasification experiment performed at INETI) (McIlveen-Wright, et al. 2006).

Gas composition (% volume/volume)				
Fuel mixture (with Puertollano coal)	100% coal	20% biomass and 80% coal	20% plastic and 80% coal	10% biomass, 10% plastic and 80% coal
CO ₂	26.5	24.9	22	24.7
CO	19.4	23.3	15.2	20
H ₂	45	42	37.2	41
CH ₄	7.4	7.5	13.5	9.2
C ₂ H ₆	1.7	2.3	12.1	5.1
Char production ratio (g/g daf)	480	350	350	350
Gasifier temperature (°C)	846	846	845	846
Carbon loss in the ash (%)	8	7.7	7.7	7.2

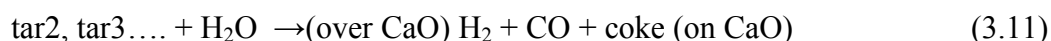
Table 2. Gas composition (air-catalyst gasification experiment performed at University of Saragossa) (McIlveen-Wright, et al. 2006).

Gas composition (% volume/volume)				
Fuel mixture (with Puertollano coal)	100% coal	20% biomass and 80% coal	20% plastic and 80% coal	10% biomass, 10% plastic and 80% coal
CO ₂	31.9	31	33.5	36.7
CO	34.7	35.2	30.6	25.3
H ₂	30.6	28.5	29.4	34.6
CH ₄	1.7	4.2	5.1	2.2
C ₂ H ₆	1.1	1.1	1.5	1.2
Char production ratio (g/g daf)	135	133	147	144
Gasifier temperature (°C)	850	850	850	850
Carbon loss in the ash (%)	7.2	6.3	7.5	6

III.B.3.b.(iii). Impurities– The gasification-fermentation of biomass to ethanol is a relatively new technology and most of the research being conducted is still laboratory

scale that makes use of synthetic syngas (mixed from commercial gases). However, biomass-generated syngas also contains other constituents such as methane, acetylene, ethylene, ethane, nitric oxide, tars, and ash. Many of these “impurities” of syngas may have complex effects on the catalysts (whether chemical or microbial) being used. Continuing research is particularly necessary to develop a better understanding of how impurities affect microbial catalysts and associated enzymatic functions.

Solutions to impurities often include a gas clean-up stream downstream of the gasifier. Other solutions are imbedded within the design of the gasifier itself, for example, the downdraft fixed bed and fluidized bed offer lower tar ratios. The addition of catalysts (such as CaO) to the reactor can also decrease tars via the following reaction (Corella, et al. 2006):



Different feedstocks can also produce different impurities. For instance, gasification of coal leads to more NO_x and SO_x impurities, whereas gasification of biomass leads to higher hydrocarbon impurities (Kuo 2005, McIlveen-Wright, et al. 2006).

III.B.3.c. Metal Catalytic Conversion of Syngas to Fuels

III.B.3.c.(i). Introduction – Catalytic conversion using metal catalysts is currently a primary industrial method to produce fuels from syngas. During the conversion, CO and H₂ are converted into fuels, such as liquid hydrocarbons and methanol, resulting from a series of metal-catalytic reactions. The most important conversion methods are Fischer-Tropsch Synthesis (FTS) and Methanol synthesis.

Fischer-Tropsch Synthesis (FTS), the production of liquid hydrocarbons from CO and H₂ mixtures over a transition metal catalyst, was developed by two German researchers Franz Fischer and Hans Tropsch in the 1920s. Following their initial discoveries, considerable effort has been carried out for developing catalysts and reactor designs for this process. The first FTS plant began operation in Germany in 1938, playing an important role in supplying the fuel needs of Germany during World War II when its fuel supplies were cut off. From 1955 to the early 1990s, Sasol (South Africa) was the only big-scale FTS operation in the world. Sasol’s three coal-to-liquid (CTL) facilities, Sasol I, II and III came on line in 1955, 1980 and 1982 respectively. In 1990s, the yield of Sasol was approximately 140,000 bbl/day (Steynberg et al., 1999; Dry, 2002). Prior to 2005, there were two other small FTS plants. The Moss gas plant, which was a 22,500 bbl/day plant, began operation in South Africa in 1992 and Shell commissioned a 14,000 bbl/day plant in Bintutlu, Malaysia in 1993. Currently, more than fifty gas-to-liquid (GTL) facilities are under consideration all over the world. Large corporations, such as Shell, Exxon, Sasol-Chevron etc, have also developed individual GTL patents.

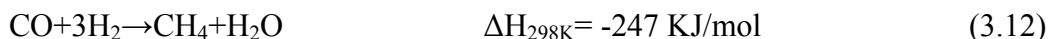
Methanol production is another important industrial process for syngas. Methanol can be used as a renewable fuel, either directly for fuel cells or blended with petroleum as an

additive to improve octane rating. In addition, higher alcohols, alcohols with two or more carbon atoms, can be produced by metal-catalytic conversion as by-products of FTS and methanol synthesis from syngas. Higher alcohols, which are less toxic and corrosive, are considered better gasoline additives than methanol.

Theoretically, syngas produced from biomass, coal or natural gas can all be used as feedstock for metal-catalytic conversion processes. Coal syngas accounts for the largest amount in industry because of the low price of coal. However, the emerging biomass technology in the last several decades has resulted in increasing interest in biomass syngas for fuel production.

III.B.3.c.(ii). Processes – The industrial processes for catalytic conversion from syngas to fuels are similar, but use different catalysts. Five major steps are involved during the conversion process: (1) The contaminants in raw syngas have to be removed before entering the reactor because the contaminants may induce catalyst deactivation and/or affect the reaction rate and conversion efficiency, (2) Because of different ratios of H₂/CO in syngas, the ratio may need to be adjusted to fit catalyst and reaction conditions, (3) The gas mixtures are compressed to the required operating pressure. For methanol synthesis, pressures usually vary from 50 to 100 bar. And for FTS, the operating pressures vary from atmospheric to 150 bar resulting from different catalysts, (4) The compressed gases are passed through the reactor containing the catalysts at the appropriate temperature, and (5) The products are collected and separated at the exit of the reactor and the unconverted gases are recycled to decrease waste.

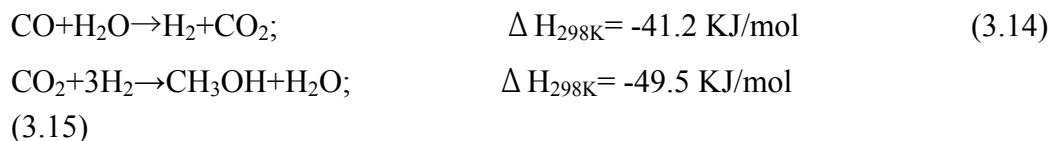
III.B.3.c.(iii). Chemistry – The chemistry of FTS processes involves the following main reactions, the latter of which produces the liquid hydrocarbon fuel:



Currently, four main series of catalysts are considered effective in FTS, including Fe, Co, Ru and Ni. Sasol and Mossgas plants in South Africa use Fe catalysts, and Shell in Bintulu, Malasia uses a Co catalyst. Recently, research of catalysts in FTS has become a hot-spot and several papers have been published in this area (Zhang et al., 2005; Liang et al., 2005).

Although the Carbene Mechanism proposed by Fischer and Tropsch in 1926 is supported by the vast majority of studies (Fischer and Tropsch., 1926; Biloen et al., 1979; Brady and Pettit, 1981; Van Dijk et al., 2003) and significant progress has been made in the last several decades in the development of FTS mechanisms (Pichler and Schultz., 1970; Sarup and Wojciechowski., 1989; Zennaro et al., 2000), the kinetics of FTS are not very clear and the research regarding mechanisms is still in the very early stages (Zou et al., 2005).

The production of methanol fuel is a combination of two exothermic equilibrium reactions: (Klier et al., 1982; Hansen, 1997)



In history, Zn/Cr₂O₃ catalyst and a high pressure of about 300 bar were the most effective technology for this process until the Cu/ZnO/Al₂O₃ catalyst was proved more active at much lower temperature and pressure in 1960s. Presently, most plants use the active Cu catalyst with an optimum temperature of about 200°C and an optimum pressure of 80-100 bar. A typical commercial catalyst may contain approximately 75% Cu (relative to Cu+Zn) (Hansen, 1997).

III.B.3.c.(iv). Advantages and Current bottlenecks – The metal-catalytic conversion of syngas to fuels is a reliable technology for alternative fuels and is somewhat favorable for environmental protection. However, there are still some barriers for catalytic conversion. The severe reaction conditions with high temperature and pressure limit the applications and require high energy inputs. In addition, the heat removal duty resulting from the strong exothermal reactions during the process (Reactions 3.11-3.15) is still a serious challenge. Also, the selectivity of catalytic conversion is low and some catalysts are very sensitive to contaminants and are easily poisoned. All of these challenges contribute to the high cost of synthesis fuels.

The economy, national security and environmental issues are all vital to evaluate the catalytic conversion from syngas to fuels. To some extent, it was the world-wide oil embargo that resulted in the commercial FTS plants in South Africa. With the continuous rise of crude oil prices, which increased from about \$23 per barrel in 2002 to \$61 per barrel in 2006 in The New York Mercantile Exchange, Inc., the interest in production of fuels from syngas is increasing because the higher cost of the synthesis fuel is compensated by the deficiency of inexpensive petroleum.

III.B.3.d. Bio-catalytic Conversion of Syngas to Fuels

III.B.3.d.(i). Introduction – An alternative route to metal-catalytic conversion is the bio-catalytic conversion of syngas to fuels. Several genera of microbial catalysts are capable of consuming syngas as part of their metabolism and producing useful end-products including hydrogen, alcohols and acids. This is a promising technology, as it circumvents problems such as solids handling as well as poisoning of metal catalysts by trace contaminants in the syngas (Ragauskas et al., 2006). Microbial catalysts have a higher tolerance for syngas contaminants and are even capable of adapting to contaminants like tar within certain limits (Ahmed et al., 2006).

III.B.3.d.(ii). Hydrogen Production – Hydrogen can be produced from syngas using a biological water-gas shift reaction. Photosynthetic bacteria such as *Rhodospirillum*

rubrum, *Rhodopseudomonas palustris*, *Rhodopseudomonas gelatinosa* and *Rubrivivax gelatinosus* CBS, are capable of converting CO to H₂ using the water-gas shift reaction (Amos, 2004; Jung et al., 1999; Klasson et al., 1993; Wolfrum and Watt, 2002).



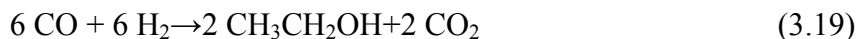
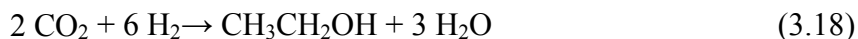
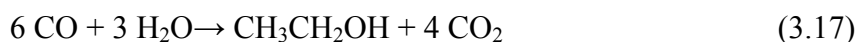
These bacteria usually contain the enzymes carbon monoxide dehydrogenase (CODH) and hydrogenase to carry out the above reaction. Some of the bacteria capable of carrying out the water-gas shift reaction are described as follows:

Rps. palustris is a purple, non-sulfur phototrophic bacterium that grows in the presence of light and metabolizes CO to produce H₂ in the absence of light (Jung et al., 1999). *Rps. palustris* is a nutritionally versatile microorganism that can also degrade a wide variety of aromatic compounds under aerobic and anaerobic conditions (Harwood and Gibson, 1988).

Rsp. rubrum is a purple-red non-sulfur bacterium that is known for its high CO uptake rates and hydrogen yields (Najafpour et al., 2004). This bacterium is spiral shaped, and is usually found in mud, sewage and pond water. *Rsp. rubrum* can utilize organic acids as well as CO for its metabolism. Najafpour et al. (2004) reported that the presence of acetic acid in the medium affected CO uptake by this organism and that 1-2 g/l was the optimum range of acetic acid that results in high H₂ yields.

R. gelatinosus CBS is also a purple non-sulfur bacterium which has a curved rod shape. Once the hydrogen producing pathway is induced in this organism, it can proceed equally well in both light and darkness. In the absence of light, the organism can utilize CO as the sole carbon source (Maness et al., 2002).

III.B.3.d.(iii). Ethanol Production – Several anaerobic bacteria have been isolated that have the ability to ferment syngas to ethanol, acetic acid and other useful end products. *Clostridium ljungdahlii* and *Clostridium autoethanogenum*, were two of the first known organisms to convert CO, CO₂ and H₂ to ethanol and acetic acid (Abrini et al., 1994; Vega et al., 1990). Known as acetogens, these microbes have the ability to reduce CO₂ to acetate in order to obtain energy and produce cell mass. The overall stoichiometry for the formation of ethanol using three different combinations of gaseous substrates is as follows (Vega et al., 1989):



Acetogenic bacteria are obligate anaerobes that utilize the acetyl-CoA pathway as their predominant mechanism for the reductive synthesis of acetyl-CoA from CO₂ (Drake, 1994). They may be Gram positive or Gram negative, rod-shaped or coccoid, and motile

or non-motile. Being a versatile group of microorganisms, they can use gases like CO₂/H₂ and CO as well as sugars and other substrates (Drake, 1994; Wood et al., 1986b, 1986c).

Clostridium ljungdahlii, the first autotrophic microorganism known to ferment a mixture of CO, CO₂ and H₂ (synthesis gas) to ethanol was isolated in 1987, (Klasson et al., 1992). *C. ljungdahlii* is a gram-positive, rod-shaped anaerobe which is capable of fermenting sugars like xylose and fructose in addition to synthesis gas. Being an acetogen, this organism favors the production of acetate during its active growth phase while ethanol is produced primarily as a non-growth-related product (Klasson et al., 1992). An effect of pH on growth and product formation was also observed in this organism. It was observed that the production of acetate was favored at a higher pH (5-7) whereas the production of ethanol was favored at lower values of pH (4-4.5).

Eubacterium limosum is an acetogen which has been isolated from various habitats like the human intestine, rumen, sewage and soil. It has a high growth rate under high CO concentrations and can ferment synthesis gas to produce acetate, ethanol, butyrate and isobutyrate (Chang et al., 1999; Chang et al., 2001; Chang et al., 1998).

Peptostreptococcus productus is a mesophilic, gram-positive anaerobic coccus, found in the human bowel and is capable of metabolizing CO₂/H₂ or CO to produce acetate (Lorowitz and Bryant, 1984). Studies have shown that although acetate is one of the primary end-products of its metabolism, *P. productus* can also form additional products in response to CO₂ limitations (Misoph and Drake, 1996).

Clostridium autoethanogenum is a strictly anaerobic, gram-positive, spore-forming, rod-like, motile bacterium which metabolizes CO to form ethanol, acetate and CO₂ as end products. It is also capable of using CO₂ and H₂, pyruvate, xylose, arabinose, fructose, rhamnose and L-glutamate as substrates (Abrini et al., 1994).

Clostridium carboxidivorans P7^T is a novel solvent-producing anaerobic microbial catalyst, which was isolated from the sediment of an agricultural settling lagoon. It is motile, gram-positive, spore-forming and primarily acetogenic, forming acetate, ethanol, butyrate, and butanol as end-products. The optimum pH range for this strain is 5.0-7.0 and the optimum temperature range is 37-40 °C (Liou et al., 2005).

“Acetogens are obligately anaerobic bacteria that can use the acetyl-CoA pathway as their predominant (i) mechanism for the reductive synthesis of acetyl-CoA from CO₂, (ii) terminal electron-accepting, energy-conserving process, and (iii) mechanism for the synthesis of cell carbon from CO₂” (Drake, 1994). The acetyl-CoA pathway, also known as the Wood-Ljungdahl pathway in honor of its discoverers, Harland Wood and Lars Ljungdahl, is an autotrophic pathway of CO₂ fixation. Like all other anaerobes, acetogens require a terminal electron acceptor other than oxygen. In the acetyl-CoA pathway, CO₂ serves as an electron acceptor and H₂ serves as the electron donor. The synthesis of acetyl-CoA from CO₂ and H₂ requires an eight-electron reduction of CO₂ and can be considered to consist of the following three steps (Wood et al., 1986a):

1. Formation of the carbonyl precursor of acetyl-CoA by the enzyme carbon monoxide dehydrogenase (CODH)
2. Formation of the methyl precursor of acetyl-CoA
3. Condensation of the above two precursors to form acetyl-CoA.

A description of the pathway and the significance of some of the key enzymes involved are discussed in appendix A.

III.B.3.d.(iv). Bioreactors used in Syngas Conversion to Fuels – Trickle-bed reactors (TBR) have been used for the production of hydrogen from CO (Amos, 2004; Wolfrum and Watt, 2002). A TBR consists of a vertical tubular reactor, packed with solid material that the cells can attach to. The direction of fluid-flow is normally counter current, with the liquid trickling downwards and the gases flowing upwards.

Continuous stirred-tank reactors (CSTR) are commonly used in syngas fermentation. A CSTR has a continuous flow of gas through a constant liquid volume. The liquid typically consists of a dilute solution of essential nutrients for the microbial catalyst to grow and survive. Syngas is bubbled through a sparger and a relatively high agitation is required for enhanced mass transfer between the two phases (Klasson et al., 1992). Cell-recycle systems can be used in conjunction with the CSTR to increase cell-density within the reactor. In such a system, the fermentation broth is pumped through a recycle filter and the retentate (containing the cells) is separated from the permeate (cell-free media) and recycled to the bioreactor. This process prevents loss of cell-mass from the bioreactor during continuous operation and also allows the CSTR to be operated at dilution rates greater than the maximum growth rate of the microbial catalyst. Klasson et al. (1993) observed a 2.6-fold increase in cell-concentration with the use of cell-recycle in their studies.

Bubble-column reactors can be used for the production of hydrogen as well as ethanol from syngas. These reactors have a large height-to-diameter ratio (aspect ratio) in which high mass transfer can be obtained even without the use of additional agitation. Smaller bubble size and improved gas dispersion can be obtained by using porous fritted discs to disperse the syngas (Vega et al., 1990). However, bubble columns are associated with high pressure drops at large capacities.

Packed-bed reactors or Immobilized-cell reactors are columns packed with immobilized biocatalyst particles (Bailey & Ollis, 1986). Such reactors are used to obtain a low pressure drop, and are usually operated concurrently (Klasson et al., 1992). These reactors have the advantages of high cell-density of the microbial catalyst and easy separation of the cells from the fermentation broth. However, gas mass transfer is usually slow in such reactors.

III.B.3.d.(v). Ethanol and Hydrogen Yields – Several research groups have reported product yields during the conversion of syngas to biofuels. Table 3 summarizes some of the results from the literature, indicating the hydrogen and ethanol yields (from CO).

Table 3. Summary of H₂ and ethanol yields from CO. The second column indicates the microbial catalyst used in the respective studies. In some cases (*), the yield was calculated from the information provided in the publication.

Product	Microbial catalyst	Yield (mol ethanol/mol CO)	Reference
H ₂	<i>R. rubrum</i>	0.87	(Klasson et al., 1993)
H ₂	<i>R. rubrum</i>	0.98	(Najafpour et al., 2004)
H ₂	<i>Rx. gelatinosus</i>	0.35-1.4	(Amos, 2004)
Ethanol	<i>Clostridium</i> species	0.008*	(Vega et al., 1989)
Ethanol	<i>C. ljungdahlii</i>	0.249*	(Younesi et al., 2005)
Ethanol	<i>C. carboxidivorans</i>	0.15*	(Rajagopalan et al., 2002)
Ethanol	<i>C. carboxidivorans</i>	0.16*	(Liou et al., 2005)

III.B.3.d.(vi). Advantages and Current bottlenecks – The use of bio-catalytic routes to produce fuels from syngas has certain advantages over the metal-catalytic routes, though there are still some bottlenecks that need to be addressed.

Advantages:

- The processes can be operated at relatively low pressures and temperatures. Most biological catalysts operate at close to ambient temperatures and this reduces the costs associated with high temperatures required for metal-catalysts. In case of the water-gas shift reaction, it is advantageous to operate at ambient temperature as the reaction is not equilibrium-limited at this temperature (Wolfrum and Watt, 2002).
- As most of the biological water-gas shift reactions occur in the dark, the reaction can be carried out in closed reactors, which result in simple reactor designs, and reduced costs.
- Biological catalysts are often more tolerant to syngas contaminants than metal-catalysts, the later which are very susceptible to poisoning. Microbes are even capable of adapting to contaminants such as tars (Ahmed et al., 2006).
- The fermentation of syngas to ethanol and other products circumvents problems such as solids handling and disposal of unconverted lignin.

Bottlenecks:

- The biological water-gas shift reaction often suffers from slow cell-growth, resulting in longer reaction times. As the reaction is anaerobic and proceeds in the

dark, it does not provide as much energy for cellular metabolism as the photosynthetic and aerobic reactions.

- Syngas fermentation is known to be associated with issues such as gas mass transfer limitations and low alcohol productivity (Worden et al., 1997). This necessitates a good bioreactor design and high cell-densities of the microorganism to make the process economically feasible.
- Another bottleneck in syngas conversion to fuels is the enzymatic inhibition by syngas components such as carbon monoxide (CO) and nitric oxide (NO). NO and CO at high partial pressures can cause inhibition of hydrogenase, an enzyme involved in both the water-gas shift reaction and the fermentation of syngas to alcohols (Krasna et al., 1954; Tibelius and Knowles, 1984).

III.B.3.e. Conclusions

There has been a growing interest in biofuels such that researchers around the world are striving to develop economically viable processes to produce fuels from renewable sources. The answer may not lie in one process. A variety of processes and biofuels may be required to make the global shift towards the use of renewable energy. The production of biofuels from syngas is one such process with great potential. However, this is a relatively new technology that still requires research in order to overcome the current bottlenecks.

References:

- Abrini, J., Naveau, H., & Nyns, E. J. (1994). *Clostridium autoethanogenum*, Sp-Nov, an Anaerobic Bacterium That Produces Ethanol from Carbon-Monoxide. *Archives of Microbiology*, 161(4), 345-351.
- Acosta, F., Real, F., Ruiz de Galarreta, C. M., Diaz, R., Padilla, D., & Ellis, A. E. (2003). Toxicity of nitric oxide and peroxyxynitrite to *Photobacterium damsela* subsp. piscicida. *Fish & Shellfish Immunology*, 15(3), 241-248.
- Ahmed, A., Cateni, B. G., Huhnke, R. L., & Lewis, R. S. (2006). Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of *Clostridium carboxidivorans* P7^T. *Biomass and Bioenergy*, 30(7), 665-672.
- Amos, W. M. (2004). *Biological Water-Gas Shift Conversion of Carbon Monoxide to Hydrogen* (No. NREL/MP-560-35592). Golden, Colorado: National Renewable Energy Laboratory.
- Bailey, J. E., & Ollis, D. F. (1986). *Biochemical Engineering Fundamentals* (2nd ed.): McGraw-Hill Book Company.
- Biloen, P., Helle, J.N., & Sachtler, W.M.H. (1979). Incorporation of surface carbon into hydrocarbons during Fischer-Tropsch synthesis: Mechanistic Implication, *Journal of Catalysis*, 58(1), 95-107.
- Brady, R.C. & Pettit, R. (1981). On the mechanism of the Fischer-Tropsch reaction: The chain propagation step. *Journal of American Chemical Society*, 103, 1287-1289.
- Bridgwater A.V. 2003. Renewable fuels and chemicals by thermal processing of biomass. *Chemical Engineering Journal* 91,87-102.
- Bridgwater T. 2006. Review Biomass for energy. *Journal of the Science of Food and Agriculture* 86:1755-1768.
- Byung Hong Kim, P. B., Datta, R. & Zeikus, J.G. (1984). Control of Carbon and Electron Flow in *Clostridium acetobutylicum* Fermentations: Utilization of Carbon monoxide to Inhibit Hydrogen Production and to enhance butanol yields. *Applied Environmental Microbiology*, 48(4), 764-770.
- Chang, I. S., Kim, B. H., Kim, D. H., Lovitt, R. W., & Sung, H. C. (1999). Formulation of defined media for carbon monoxide fermentation by *Eubacterium limosum* KIST612 and the growth characteristics of the bacterium. *Journal of Bioscience and Bioengineering*, 88(6), 682-685.
- Chang, I. S., Kim, B. H., Lovitt, R. W., & Bang, J. S. (2001). Effect of CO partial pressure on cell-recycled continuous CO fermentation by *Eubacterium limosum* KIST612. *Process Biochemistry*, 37(4), 411-421.
- Chang, I. S., Kim, D. H., Kim, B. H., Shin, P. K., Sung, H. C., & Lovitt, R. W. (1998). CO fermentation of *Eubacterium limosum* KIST612. *Journal of Microbiology and Biotechnology*, 8(2), 134-140.
- Corella J., Toledo J.M., Molina G. 2006. Steam Gasification of Coal at Low-Medium (600-800°C) Temperature with Simultaneous CO₂ Capture in Fluidized Bed at Atmospheric Pressure: The Effect of Inorganic Species. 1. Literature Review and Comments. *Industrial & Engineering Chemistry Research*, 45, 6137-6146.
- Dayton, D.C & Spath, P.L (2003). *Preliminary Screening —Technical and Economic Assessment of Synthesis Gas to Fuels and Chemicals with Emphasis on the*

- Potential for Biomass-Derived Syngas*. Golden, Colorado 80401-3393: National Renewable Energy Laboratory.
- Diekert, G. & Wohlfarth, G. (1994). Metabolism of Homoacetogens. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 66(1-3), 209-221.
- Drake, H. L. (1994). *Acetogenesis*. New York: Chapman & Hall.
- Dry, M.E. (2002). The Fischer-Tropsch process: 1950-2000, *Catalysis Today*, 71(3-4), 227-241.
- Fischer, F. & Tropsch, H. (1926). Synthesis of petroleum from gasification products of coal at normal pressure, *Berichte der Deutschen Chemischen Gesellschaft [Abteilung] B: Abhandlungen*, 59B, 830-1, 832-6..
- Girbal, L., Croux, C., Vasconcelos, I., & Soucaille, P. (1995). Regulation of metabolic shifts in *Clostridium acetobutylicum* ATCC 824. *FEMS Microbiology Reviews*, 17(3), 287-297.
- Girbal, L., Vasconcelos, I., Saint-Amans, S., & Soucaille, P. (1995). How neutral red modified carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH. *FEMS Microbiology Reviews*, 16(2-3), 151-162.
- Gottschal, J. C., & Morris, J. G. (1981). The Induction of Acetone and Butanol Production in Cultures of *Clostridium-Acetobutylicum* by Elevated Concentrations of Acetate and Butyrate. *FEMS Microbiology Letters*, 12(4), 385-389.
- Grube, M., Gapes, J. R., & Schuster, K. C. (2002). Application of quantitative IR spectral analysis of bacterial cells to acetone-butanol-ethanol fermentation monitoring. *Analytica Chimica Acta*, 471(1), 127-133.
- Hansen, J.B. (1997). High conversion of synthesis gas into oxygenates. *Studies in Surface Science and Catalysis*, 61, 457-67.
- Harwood, C. S., & Gibson, J. (1988). Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodospseudomonas palustris* *Applied and Environmental Microbiology*, 54, 712-717.
- Hyman M.R. & Arp, D.J. (1988). Reversible and irreversible effects of nitric oxide on the soluble hydrogenase from *Alcaligenes eutrophus* H16. *Biochemical Journal*, 254, 469-475.
- Hyman M.R. & Arp, D.J. (1991). Kinetic analysis of the interaction of nitric oxide with the membrane-associated, nickel and iron-sulfur-containing hydrogenase from *Azotobacter vinelandii*. *Biochimica et Biophysica Acta*, 1076, 165-172.
- Jung, G. Y., Jung, H. O., Kim, J. R., Ahn, Y., & Park, S. (1999). Isolation and characterization of *Rhodospseudomonas palustris* P4 which utilizes CO with the production of H₂. *Biotechnology Letters*, 21(6), 525-529.
- Kashket, E. R., & Zhi-Yi Cao. (1995). Clostridial strain degeneration. *FEMS Microbiology Reviews*, 17(3), 307-315.
- Klasson, K. T., Ackerson, M. D., Clausen, E. C., & Gaddy, J. L. (1992). Bioconversion of synthesis gas into liquid or gaseous fuels. *Enzyme and Microbial Technology*, 14(8), 602-608.
- Klasson, K. T., Ackerson, M. D., Clausen, E. C., & Gaddy, J. L. (1993). Biological Conversion of Coal and Coal-Derived Synthesis Gas. *Fuel*, 72(12), 1673-1678.
- Klasson, K. T., Lundback, K. M. O., Clausen, E. C., & Gaddy, J. L. (1993). Kinetics of Light Limited Growth and Biological Hydrogen-Production from Carbon-

- Monoxide and Water by Rhodospirillum-Rubrum. *Journal of Biotechnology*, 29(1-2), 177-188.
- Klier, K. (1982). Methanol synthesis. *Advances in Catalysis*, 31, 243-313.
- Krasna, A. I. (1979). Hydrogenase: Properties and applications. *Enzyme and Microbial Technology*, 1(3), 165-172.
- Krasna, A. I., & Rittenberg, D. (1954). The inhibition of hydrogenase by nitric oxide. *Proceedings of the National Academy of Sciences*, 40(4), 225-227.
- Kuo K.K. (2005). *Principles of combustion* (pp.732). Hoboken, New Jersey:John Wiley & Sons, Inc.
- Kutzenok, A., & Aschner, M. (1952). Degenerative Processes in a Strain of Clostridium Butylicum. *Journal of Bacteriology*, 64(6), 829-836.
- Lemon, B. J., & Peters, J. W. (1999). Binding of exogenously added carbon monoxide at the active site of the iron-only hydrogenase (Cpl) from *Clostridium pasteurianum*. *Biochemistry*, 38(40), 12969-12973.
- Liang, X., Dong, X., Li, H., Lin, Guo., & Zhang, Hong. (2005). Carbon nanotubes as novel promoter of Co-Cu catalyst for synthesis of higher alcohols from syngas, *Journal of Xiamen University (Natural Science Edition)*, 44(4), 445-449.
- Liou, J. S.C., Balkwill, D. L., Drake, G. R., & Tanner, R. S. (2005). *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55(5), 2085-2091.
- Ljungdahl, L. G. (1986). The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annual Review of Microbiology*, 40, 415-450.
- Lorowitz, W. H., & Bryant, M. P. (1984). *Peptostreptococcus productus* Strain That Grows Rapidly with Co as the Energy-Source. *Applied and Environmental Microbiology*, 47(5), 961-964.
- Madigan, M. T., Martinko, J.M. & Parker, J. (2003). *Brock Biology of Microorganisms* (10th ed.). New Jersey: Prentice Hall.
- Maness, P.C., Smolinski, S., Dillon, A. C., Heben, M. J., & Weaver, P. F. (2002). Characterization of the Oxygen Tolerance of a Hydrogenase Linked to a Carbon Monoxide Oxidation Pathway in *Rubrivivax gelatinosus*, *American Society of Microbiology*, 68, 2633-2636.
- McIlveen-Wright, D.R., Pinto, F., Armesto, L., Caballero M.A., Aznar M.P., Cabanillas A., Huang, Y., Franco, C., Gulyurtlu, I. & McMullan J.T. 2006. A comparison of circulating fluidised bed combustion and gasification power plant technologies for processing mixtures of coal, biomass and plastic waste. *Fuel Processing Technology*, 87, 793-801.
- McKendry P. 2002/5a. Energy production from biomass (part 1): overview of biomass. *Bioresource Technology* 83, 37-46.
- McKendry P. 2002/5b. Energy production from biomass (part 3): gasification technologies. *Bioresource Technology* 83,55-63.
- Meyer, C. L., Mclaughlin, J. K., & Papoutsakis, E. T. (1985). The Effect of CO on Growth and Product Formation in Batch Cultures of *Clostridium acetobutylicum*. *Biotechnology Letters*, 7(1), 37-42.

- Meyer, C. L., & Papoutsakis, E. T. (1989). Increased Levels of ATP and NADH Are Associated with Increased Solvent Production in Continuous Cultures of *Clostridium acetobutylicum*. *Applied Microbiology and Biotechnology*, 30(5), 450-459.
- Meyer, C. L., Roos, J. W., & Papoutsakis, E. T. (1986). Carbon-Monoxide Gasing Leads to Alcohol Production and Butyrate Uptake without Acetone Formation in Continuous Cultures of *Clostridium acetobutylicum*. *Applied Microbiology and Biotechnology*, 24(2), 159-167.
- Misoph, M., & Drake, H. L. (1996). Effect of CO₂ on the fermentation capacities of the acetogen *Peptostreptococcus productus* U-1. *Journal of Bacteriology*, 178(11), 3140-3145.
- Najafpour, G., Younesi, H., & Mohamed, A. R. (2004). Effect of organic substrate on hydrogen production from synthesis gas using *Rhodospirillum rubrum*, in batch culture. *Biochemical Engineering Journal*, 21(2), 123-130.
- Pichler, H., & Schulz, H. (1970). Recent results in the synthesis of hydrocarbons from carbon monoxide and hydrogen. *Chemie Ingenieur Technik*, 42(18), 1162-74.
- Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., et al. (2006). The path forward for biofuels and biomaterials. *Science*, 311(5760), 484-489.
- Ragsdale, S. (1991). Enzymology of the Acetyl-CoA Pathway of CO₂ Fixation. *Critical Reviews in Biochemistry and Molecular Biology*, 26, 261-300.
- Rajagopalan, S., P. Datar, R., & Lewis, R. S. (2002). Formation of ethanol from carbon monoxide via a new microbial catalyst. *Biomass and Bioenergy*, 23(6), 487-493.
- Rao, G., & Mutharasan, R. (1989). NADH Levels and Solventogenesis in *Clostridium acetobutylicum* - New Insights through Culture Fluorescence. *Applied Microbiology and Biotechnology*, 30(1), 59-66.
- Sarup, B. & Wojciechowski, B.W. (1989). Studies of the Fischer-Tropsch synthesis on a cobalt catalyst. II. Kinetics of carbon monoxide conversion to methane and to higher hydrocarbons. *Canadian Journal of Chemical Engineering*, 67(1), 62-74.
- Schlegel, H. G., & Bowien, B. (1989). *Autotrophic bacteria*. Madison, WI, Berlin ; New York: Science Tech Publishers, Springer-Verlag.
- Seefeldt, L. C., & Arp, D. J. (1989). Oxygen Effects on the Nickel-Containing and Iron-Containing Hydrogenase from *Azotobacter vinelandii*. *Biochemistry*, 28(4), 1588-1596.
- Steynberg, A.P., Espinoza, R.L., Jager, B., & Vosloo, A.C. (1999). High-temperature Fischer-Tropsch synthesis in commercial practice. *Applied Catalysis, A:General*, 186(1, 2), 41-54.
- Tibelius, K. H., & Knowles, R. (1984). Hydrogenase activity in *Azospirillum brasilense* is inhibited by nitrite, nitric oxide, carbon monoxide and acetylene. *Journal of Bacteriology*, 160(1), 103-106.
- Van Dijk, H., Hoebink, J., & Schouten, J. (2003). A mechanistic study of the Fischer-Tropsch synthesis using transient isotopic tracing. Part 1: Model identification and discrimination, Part 2: Model quantification. *Topics in Catalysis*, 26(1-4), 111-119.
- Van Loo S, Koppejan J. 2003. *Handbook of Biomass Combustion and Co-firing*. The Netherlands:Twente University Press.

- Vasconcelos, I., Girbal, L., & Soucaille, P. (1994). Regulation of Carbon and Electron Flow in *Clostridium-Acetobutylicum* Grown in Chemostat Culture at Neutral Ph on Mixtures of Glucose and Glycerol. *Journal of Bacteriology*, 176(5), 1443-1450.
- Vega, J. L., Clausen, E. C., & Gaddy, J. L. (1990). Design of bioreactors for coal synthesis gas fermentations. *Resources, Conservation and Recycling*, 3(2-3), 149-160.
- Vega, J. L., Prieto, S., Elmore, B. B., Clausen, E. C., & Gaddy, J. L. (1989). The Biological Production of Ethanol from Synthesis Gas. *Applied Biochemistry and Biotechnology*, 20-1, 781-797.
- Wolfrum, E. J., & Watt, A. S. (2002). Bioreactor design studies for a hydrogen-producing bacterium. *Applied Biochemistry and Biotechnology*, 98, 611-625.
- Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986a). The Acetyl-CoA Pathway - a Newly Discovered Pathway of Autotrophic Growth. *Trends in Biochemical Sciences*, 11(1), 14-18.
- Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986b). The Acetyl-CoA Pathway of Autotrophic Growth. *FEMS Microbiology Reviews*, 39(4), 345-362.
- Wood, H. G., Ragsdale, S. W., & Pezacka, E. (1986c). A New Pathway of Autotrophic Growth Utilizing Carbon-Monoxide or Carbon-Dioxide and Hydrogen. *Biochemistry International*, 12(3), 421-440.
- Worden, R. M., Bredwell, M. D., & Grethlein, A. J. (1997). Engineering issues in synthesis-gas fermentations. *Fuels and Chemicals from Biomass*, 666, 320-335.
- Younesi, H., Najafpour, G., & Mohamed, A. R. (2005). Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii*. *Biochemical Engineering Journal*, 27(2), 110-119.
- Zennaro, R., Bartholomew, C.H., and Tagliabue, M. (2000). Kinetics of Fischer-Tropsch synthesis on Titania-supported Cobalt. *Catalysis Today*, 58(4), 309-319
- Zhang, C., Yang, X., Liu, D., Ying, W., Fang, D. (2005). Liquid fuel synthesis from syngas over Fe/AC catalyst, *Journal of East China University of Science and Technology (Natural Science Edition)*, 31(4), 413-416
- Zou, H., Bartholomew, C.H., Critchfield, B., Gokhale, A., and Mavrikakis, M. (2005). Microkinetic model for Fischer-Tropsch synthesis on Iron. Presented in *The 19th North American meeting of the north American Catalysis Society*. May 22-27, Philadelphia, PA.

Appendix A

The Methyl Branch of the Acetyl-CoA Pathway

The methyl branch of the acetyl-CoA pathway is shown on the left in Figure A.1. This part of the pathway results in the formation of the methyl-corrinoid protein, which then combines with the product of the carbonyl branch, to form acetyl-CoA. In the first step of this branch, CO₂ is reduced to formate (HCOO⁻) as shown in the following equation:



Formate Dehydrogenase: The above reversible reaction is catalyzed by the enzyme formate dehydrogenase (FDH). This enzyme is difficult to isolate due to its high sensitivity to oxygen (Ljungdahl, 1986). Though ferredoxin is the most commonly used electron acceptor, among acetogens, NADH often acts as the electron donor. For acetogens grown on CO, it has been suggested that CO must first be converted to CO₂ by the enzyme carbon monoxide dehydrogenase (CODH) and then reduced to formate by FDH (Ljungdahl, 1986).

Tetrahydrofolate Enzymes: Formate is then activated with tetrahydrofolate (THF) to form 10-formyl-THF, as shown in the figure, by the enzyme formyl-THF synthetase. This is an ATP-dependent condensation. This bound formyl group is then reduced by a series of 3 enzymes to a bound methyl group (methyl-THF). In the final step of this branch, the methyl group is transferred to a corrinoid containing protein, [Co]-E (Ragsdale, 1991).

The Carbonyl Branch and Carbon Monoxide Dehydrogenase

The carbonyl branch of the acetyl-CoA pathway is shown on the right in Figure A.1. This branch of the pathway results in the formation of a bound carbonyl group which is then merged with the bound methyl group formed in the methyl branch to form acetyl-CoA. Carbon monoxide dehydrogenase (CODH) plays a very important role in this branch of the pathway.

Carbon monoxide Dehydrogenase: This is considered one of the most important enzymes of the acetyl-CoA pathway due to its bi-functionality. It catalyzes the very first oxidation of CO to CO₂, the reduction of CO₂ to bound carbonyl and dominates the carbonyl branch of the pathway, finally mediating the synthesis of acetyl-CoA from the methyl and carbonyl groups. Due to this, CODH is also known as acetyl-CoA synthase and the pathway is often referred to as the carbon monoxide dehydrogenase pathway (Diekert & Wohlfarth, 1994). In the carbonyl branch, CO₂ is first reduced to [CO] (indicating carbon monoxide in an enzyme bound form) as shown in equation 3.21 and then bound to CODH.



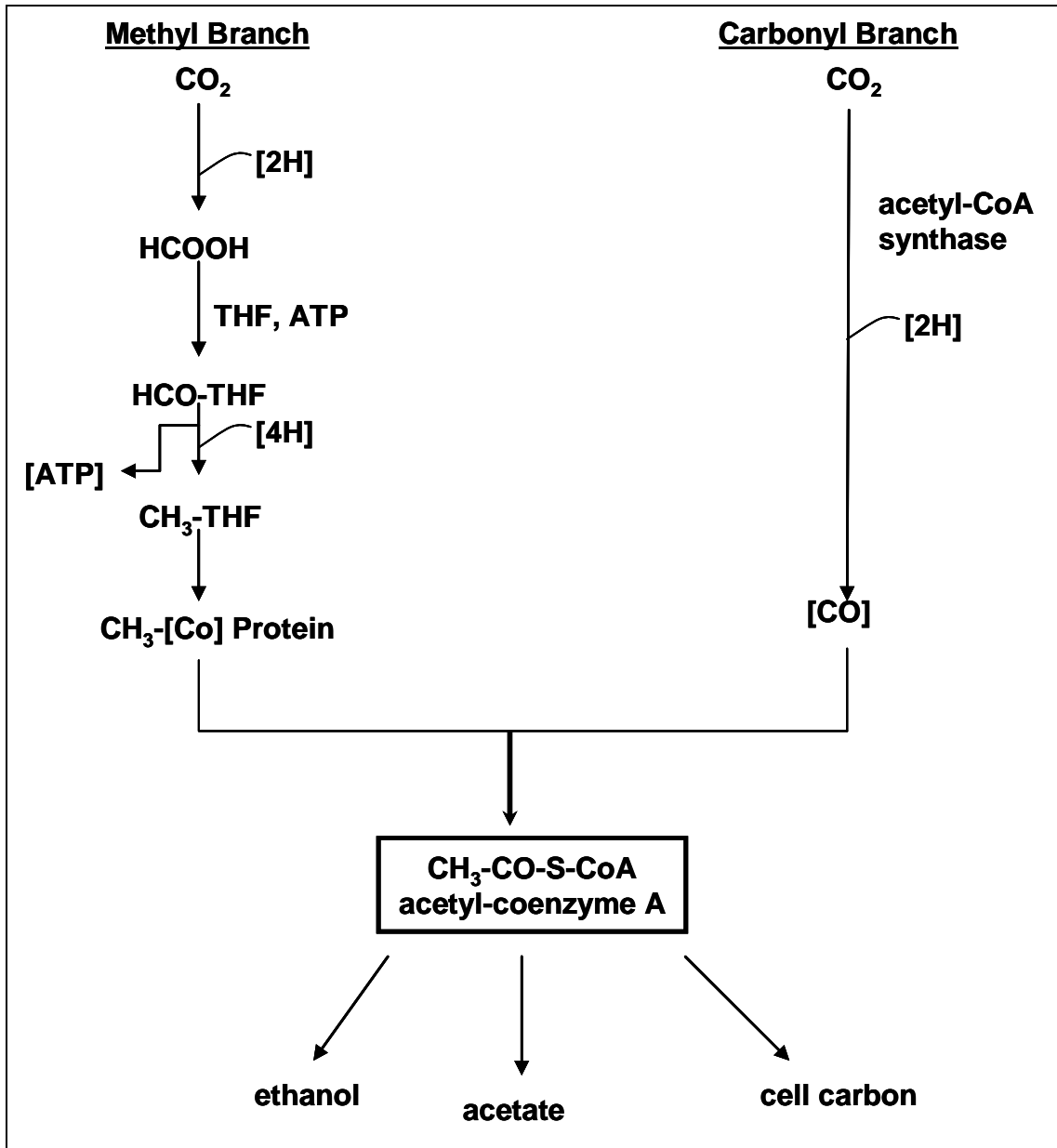
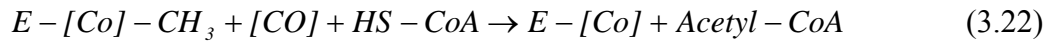


Figure A.1. Simplified schematic of the acetyl-CoA or Wood-Ljungdahl pathway of acetogens. (THF-tetrahydrofolate, [Co] protein-corrinoid enzyme)(Drake, 1994)

This bound carbonyl group is then merged with the bound methyl group from the methyl branch to form a bound acetyl-CODH moiety. In the final step, CODH condenses the bound acetyl with the free coenzyme A to form acetyl-CoA, as shown in equation 3.22.



Hydrogenase: Hydrogenase enzymes are expressed in organisms where their function is either hydrogen evolution, to dispose of electrons accumulated during fermentation, or hydrogen uptake, where the oxidation of hydrogen is coupled to the energy yielding process (Lemon and Peters, 1999). Studies have shown that CODH acts in combination with hydrogenase to form the carbonyl precursor of acetyl-CoA (Ljungdahl, 1986; Wood et al., 1986b). Equation 3.23 shows the reversible reaction catalyzed by hydrogenase.

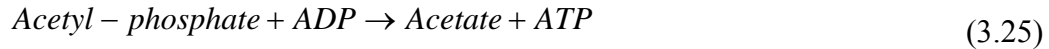
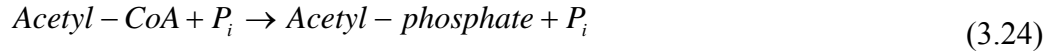


Hydrogenases are classified based on their metal content; depending on whether they contain nickel, iron, selenium or none of these and also what combination of metals they contain. Usually, the Ni containing hydrogenases are associated with hydrogen uptake and those containing Fe are associated with hydrogen evolution (Hyman MR, 1991). Often microorganisms contain several different hydrogenases, and in many cases the functions of these enzymes are difficult to determine. The catalytic activity of hydrogenase can be determined by assaying the enzyme by a method which measures its interaction with hydrogen (Krasna, 1979). One of the most popular assays is the reduction of an artificial electron acceptor by hydrogen. Dyes like methylene blue, methyl viologen, benzyl viologen etc., or other compounds like nitrate, nitrite, cytochromes, ferredoxin, hydroxylamine, sulfate, sulfite and NAD are some of the commonly used electron acceptors (Krasna, 1979).

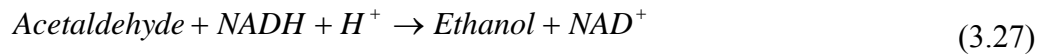
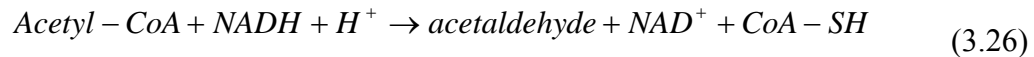
Inhibition of hydrogenase has been of particular interest due to the fact that it results in a change in the metabolic pathway of the microorganism. Gases like O₂ (Seefeldt and Arp, 1989), acetylene, CO, and nitric oxide (NO) are known inhibitors of hydrogenase (Acosta et al., 2003; Byung Hong Kim, 1984; Krasna et al., 1954; Tibelius and Knowles, 1984).

Fate of Acetyl-CoA

Acetyl-CoA is a versatile intermediate in the metabolic pathway of acetogens as it is a precursor of lipids, amino acids, nucleotides and carbohydrates (Ljungdahl, 1986). It is the source for cellular carbon as well as cellular energy. Cellular material is formed via the anabolic pathway, in which acetyl-CoA is reductively carboxylated into pyruvate by the enzyme pyruvate synthase (Diekert and Wohlfarth, 1994; Schlegel and Bowien, 1989). Pyruvate is then converted to phosphoenolpyruvate which is an intermediate in the conversion to cellular material. For the purpose of energy conservation, acetyl-CoA goes through the catabolic pathway in order to make ATP. This is the route by which acetyl-CoA is converted to acetate. Equations 3.24 and 3.25 describe the two steps of the acetate branch of the pathway.



In the first reaction, the CoA unit is removed from the acetyl-CoA and a phosphate group is added, resulting in the formation of acetyl-phosphate. This reaction is catalyzed by the enzyme phosphotransacetylase. In the second reaction, shown by equation 3.21, the acetyl phosphate is converted to acetate while a molecule of adenosine diphosphate (ADP) is phosphorylated to form ATP. This branch of the pathway is usually favored by the bacterium over the alcohol forming branch (described below) during its exponential growth phase as it provides the cell with energy in the form of ATP. This is often known as the acidogenic phase of the metabolism, which also results in a decrease in pH of the medium due to acid production (Rao and Mutharasan, 1989). The second phase of the fermentation is the solventogenic phase, in which ethanol is produced. This is characterized by slower growth, as there is no evolution of ATP in this case. Equations 3.26 and 3.27 describe the solventogenic branch of this pathway in which ethanol is produced.



In the solventogenic branch of the pathway, the organism utilizes the reducing potential available, in the form of NADH, to first form acetaldehyde by the enzyme acetaldehyde dehydrogenase, and then finally form ethanol by the enzyme alcohol dehydrogenase.

Many acetogens also produce four-carbon products like butanol and butyric acid by combining two molecules of acetyl-CoA to form acetoacetyl-CoA. This intermediate is then converted to butyryl-CoA which serves a purpose similar to acetyl-CoA. The reactions involved in the formation of butyric acid and butanol from butyryl-CoA are analogous to those seen above (3.28-3.31). The formation of butyric acid produces ATP while the formation of butanol results in the consumption of reducing equivalents. The corresponding equations are given below:

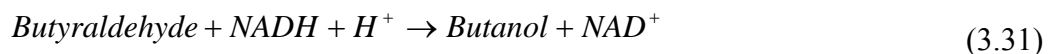
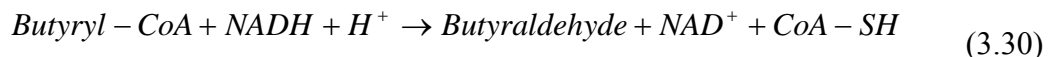
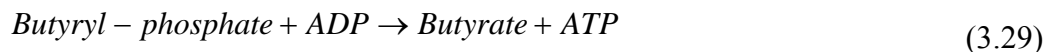
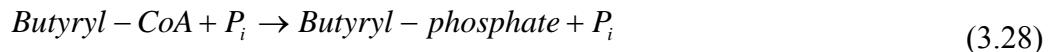


Figure A.2 shows the pathway of *C. acetobutylicum*, which is a well-researched acetogen, and represents the metabolism of most acetogens using the acetyl-CoA pathway. The enzymes responsible for the metabolism are shown.

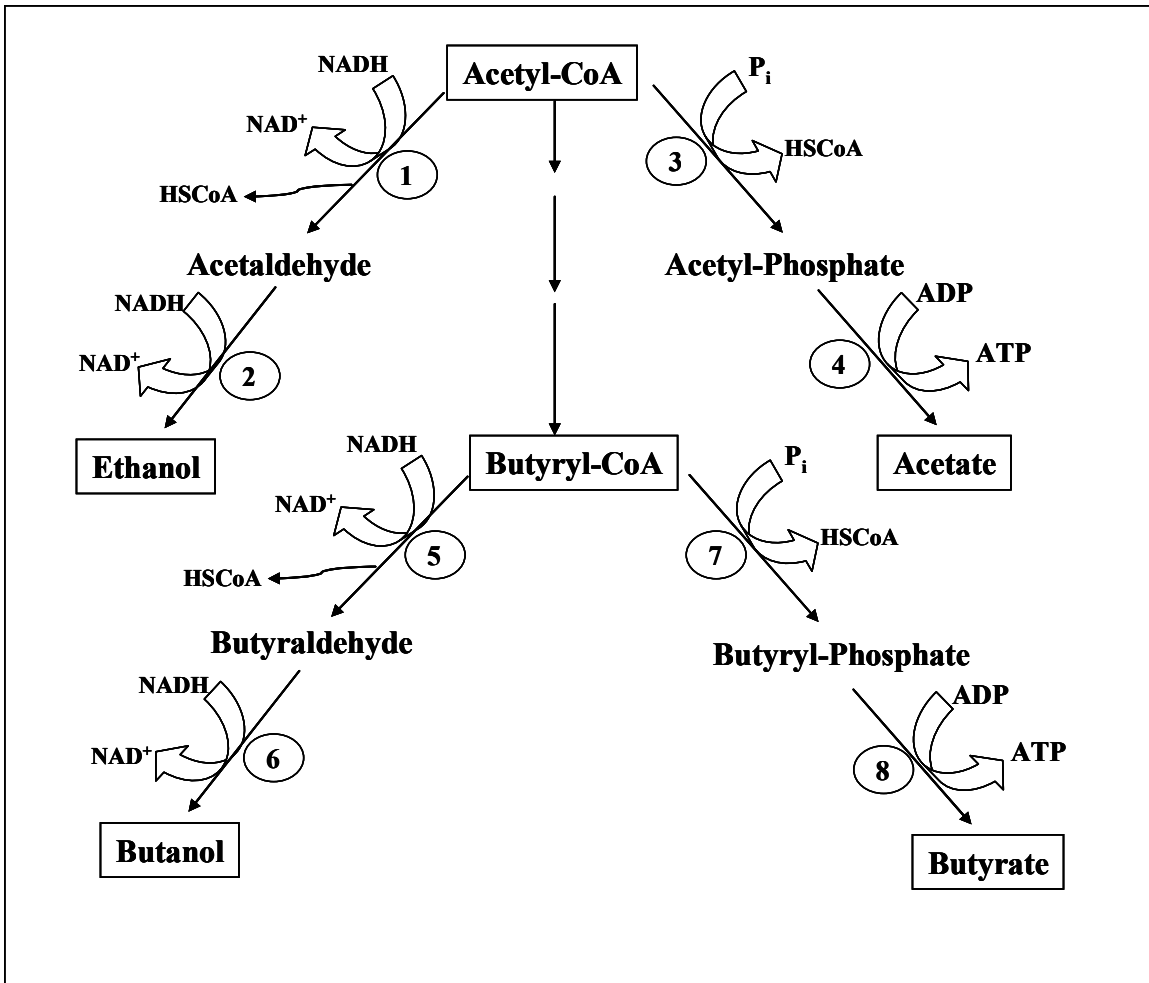


Figure A.2. Fate of acetyl-CoA in *Clostridium acetobutylicum* adapted from Vasconcelos et al., 1994. 1-acetaldehyde dehydrogenase, 2-alcohol dehydrogenase, 3-phosphotransacetylase, 4-acetate kinase, 5-butyraldehyde dehydrogenase, 6-butanol dehydrogenase, 7-phosphotransbutyrylase, 8-butyrate kinase.

Acetogenesis vs. Solventogenesis

The complex metabolism of acetogens like *C. acetobutylicum* has generated considerable interest among researchers, leading to several studies conducted to determine the factors involved in the transition between acetogenesis (or acidogenesis) and solventogenesis. Studies have shown that these bacteria usually show a “biphasic batch fermentation pattern” (Girbal et al., 1995). The bacteria produce acids such as acetate and butyrate during their exponential growth phase and then switch to alcohol production when the growth slows down before they enter the stationary phase. Several factors have been found to affect this switch from acidogenesis to solventogenesis, such as pH, ATP demand, availability of nutrients, availability of reducing equivalents, enzyme activities etc. In fermentations where many different products are possible, the amount of ATP produced per mole of substrate consumed depends on the product distribution (Meyer and Papoutsakis, 1989). For instance, in the acetyl-CoA pathway, the production of acetic acid results in ATP formation. Therefore, if the ATP demand of the cell is high, the pathway would preferably go towards acid production. On the other hand, if there is an excess availability of energy within the cell, the pathway would tend towards alcohol production so that the excess energy may be consumed.

Fermentation conditions and the state of the inoculum used have also been found to influence whether the microbial culture produces high levels of solvents. Grube et al. (2002) demonstrated that under strictly anaerobic conditions, using a spore inoculum led to almost three times the ethanol produced as compared to when a vegetative inoculum was used. It has also been seen that in some cases, an “acid crash” occurs wherein high amounts of acid may be produced and the culture then loses the ability to switch to solventogenesis. Clostridial strains are known to “degenerate” if, at the end of the exponential phase, they do not switch to solventogenesis. Degeneration has typically been observed when the inoculum is repeatedly derived from cells in their exponential stage (Kashket and Zhi-Yi Cao, 1995; Kutzenok and Aschner, 1952).

Due to the uncertainty in the switch from acidogenesis to solventogenesis, several research teams have studied methods of inducing solventogenesis in acetogens. The addition of acetate and butyrate to batch cultures was found to shorten the acidogenic phase and induce solventogenesis (Gottschal and Morris, 1981). It was proposed by Gottschal and Morris that this was due to the dissipation of the pH gradient (Δ pH) by acetate and butyrate as the intracellular pH could achieve the same low value as the culture medium. Klasson et al. (1992) showed that yeast extract, a component of the culture medium, also had an effect on the product ratio. They demonstrated that by decreasing the amount of yeast extract in the medium, a higher concentration of solvents can be achieved. Their studies also confirmed that solvent production was non-growth related, as the growth rate seemed to decrease with a decrease in yeast extract concentration. Klasson et al. (1992) also proposed that the addition of reducing agents can initiate solventogenesis, as the electrons can reduce NAD^+ to NADH, which provides reducing potential to form acetaldehyde and then ethanol in the alcohol pathway.

Meyer et al. (1985) demonstrated that reduced nitrogen-source availability in cultures of *C. acetobutylicum* induced solvent production. On the other hand, glucose-limited conditions caused high amounts of acids to be produced (Meyer et al., 1985). An excess availability of reducing equivalents has also been found to initiate solventogenesis (Girbal et al., 1995; Meyer et al., 1985). CO gassing and conditions of iron limitation are also known to increase alcohol production (Byung Hong Kim, 1984). Artificial electron carriers like methyl viologen, benzyl viologen and neutral red are known to alter the electron flow by forming NADH, which in turn promotes alcohol production (Girbal et al., 1995; Klasson et al., 1992). Girbal et al. (1995) demonstrated that adding 1mM neutral red to an acidogenic culture causes a deviation in the electron flow towards NADH production. This pool of NADH generated might be responsible for the change in the metabolism of the bacteria towards solventogenesis. Girbal et al. reported a 3-fold increase in ethanol production on the addition of 1mM neutral red to *C. acetobutylicum* cultures (Girbal, Croux et al., 1995; Girbal, Vasconcelos et al., 1995).

Hydrogenase Inhibition

In another study, Girbal et al. (Girbal, Croux et al., 1995) reported that under solventogenic conditions, the *in vitro* hydrogenase activities of the culture were lower than those under acidogenic conditions. Studies have also shown that when batch fermenters of *C. acetobutylicum*, grown on a glucose medium were sparged with carbon monoxide, the hydrogenase enzyme was inhibited and the alcohol production was enhanced (Byung Hong Kim, 1984; Meyer et al., 1985; Meyer et al., 1986).

However, the inhibition of hydrogenase in autotrophic organisms results in the inability of the microorganism to consume hydrogen. This in turn leads to the utilization of CO to form electrons, so that CO can only partially be utilized to make cell mass and products. Therefore, from the standpoint of a process to produce acids or alcohols, the inhibition of hydrogenase in autotrophic microorganisms is not very efficient. This has led to an interest among researchers to identify and characterize the inhibitors of hydrogenase. Gases like O₂ (Seefeldt and Arp, 1989), acetylene, CO, nitrite and nitric oxide (NO) are known inhibitors of hydrogenase (Acosta et al., 2003; Byung Hong Kim, 1984; Krasna et al., 1954; Tibelius and Knowles, 1984). Studies have also shown NO to inhibit hydrogenase activity in *Azotobacter vinelandii* (Hyman, 1991), *Proteus vulgaris* (Krasna et al., 1954), *Alcaligenes eutrophus* (Hyman, 1988) and *Azospirillum brasilense* (Tibelius and Knowles, 1984).

Alcohol Dehydrogenase - Function and Regulation

In the acetyl-CoA pathway, alcohol dehydrogenase (ADH) plays an important role in the formation of ethanol. It catalyzes the conversion of acetaldehyde to ethanol using NADH as a reducing equivalent. Assays to determine the activity of alcohol dehydrogenase can indicate whether the acetogen is in the solventogenic phase of ethanol production. ADH assays often use NADH as the reducing equivalent and acetaldehyde as the substrate. Studies have shown that the ADH activities of alcohologenic cultures are higher than those of acetogenic cultures. The addition of artificial electron carriers has been shown to

increase ADH activity, which in turn results in an increase in alcohol production. Girbal et al. demonstrated that the addition of 1mM neutral red led to a 3-fold increase in ethanol production and a 6.6-fold increase in ADH activity of *C. acetobutylicum* cultures (Girbal, Vasconcelos et al., 1995).