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THE TECHNOLOGY OF THE SECOND GENERATION OF BIOETHANOL
ТЕХНОЛОГІЯ БІОЕТАНОЛУ ДРУГОГО ПОКОЛІННЯ

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Abstract. *In this article we describe the practilal realazing of the second and final part of the technology of biomass gasification in the bioethanol (fuel ethanol) production. This part is the conversion of syngas to ethanol using the microorganisms. The living conditions of microorganisms depends from meny conditions: temperature, pH, ingibitors and/or activators influence. This all conditions give as realize the industrial volume production of bioethanol from cellulose.*

Key words: *bioethanol, biomass, microorganisms, fermentation, synthesis gas (syngas), acids, acetate-producing process.*

Introduction

The biomass gasification process is an alternative approach for pro-ducing ethanol from lignocellulosic biomass. This method involves controlled burning of biomass to produce synthesis gas, or syngas, and then conversion of syngas to ethanol.

There are two methods to convert biomass-derived synthesis gas to ethanol. The first method is to use biocatalysts and the second method is to use metal-based chemical catalysts. Biocatalysis, or fermentation route, is a relatively new technology. In this approach microorganisms that can convert CO, CO_2 and H_2 in the syngas to biofuels are used as the biocatalyst and are exposed to syngas by bubbling the gas through an aqueous culture media solution containing microorganisms. A large variety of microorganisms capable of producing carboxylic acids, hydrocarbons, alcohols, and carbonyl compounds from syngas are known, but in this chapter we will focus mainly on the ethanol producing biocatalysis process. Recent progress in this approach is discussed in four review articles. These reviews include two by Munasinghe et al. [1,2], one by Mohammadi et al. [3] and also a 2011 review written by Wilkins and Atiyeh on microbial production of ethanol from carbon monoxide, where CO metabolism and recent genomic studies are discussed [4].

Advantages and Disadvantages of Biocatalysis

When compared to the metal-based chemical catalysis route, the fermentation method has its advantages as well as some disadvantages.

Advantages

In contrast to chemical catalysts, biocatalysts operate at moderate temperatures close to ambient temperature, and pressures close to atmospheric pressure, which

result in substantial energy savings in large industrial-scale operations. Additionally, the reactor designs are simpler and no high-temperature, pressure-resistant special materials are required in the fabrication of the reactors. Moreover, operation at ambient temperature avoids the thermodynamic equilibrium relationship and causes the irreversibility of biological reactions, which consequently should result in high conversion efficiencies. High reaction specificity is achieved in fermentation-based methods in comparison to chemical catalytic processes due to the high enzymatic specificity. Biocatalysts are known to have higher tolerance for sulfur-containing gases like hydrogen sulfide (H_2S) and carbonyl sulfide (COS), and also for smaller amounts of mercaptans or organic sulfur compounds, as well as for chlorine and chlorine-containing compounds. Furthermore, most microorganisms are even capable of adapting to contaminants like tar within certain limits. It is interesting to note that the growth of some anaerobic bacteria can be stimulated in the presence of sulfur compounds, as sulfur acts as reducing agent which reduces the redox potential of the medium. Even though most microorganisms can tolerate these impurities, the syngas requires some clean-up before the fermentation process to maintain the maximum bacterial activity. With metal catalysts, even a trace amount of sulfur gases present in the syngas can poison the chemical catalytic conver-sion; therefore, elaborate gas cleaning techniques are required in the chemical catalysis process, which contribute to the high cost of ethanol. Furthermore, biocatalysts are less sensitive to the composition of syngas and usually do not require a fixed CO/H_2 ratio, whereas metal-based chemical catalysts need a specific ratio of gas components to yield a desired product.

Disadvantages

There are major drawbacks in this technology as well, like intrinsic poor solubility of CO and H_2 components of syngas in aqueous broths, which will result in low substrate uptake by microbes, thus, leading to poor conversion efficiencies and low ethanol yields. For example, Kuniyana et al. have reported their findings on a pilot plant-scale experiment conducted in a 100 L fermenter, and they have indicated that the conversion efficiency of CO and H_2 from the gaseous phase is only 20% at a continuous gas flow rate of 0.9 L per minute (LPM) at 37°C [5]. Additionally, Henstra et al. have shown that increasing the temperatures has a negative impact on the solubilities of CO and H_2 and will result in a decrease in the mass transfer rate of these gases to cells [6]. Dissolution of a gas in a liquid phase is a complex process, and then there are several intermediate steps involved in transporting syngas components into the microbial cells. These steps include the diffusion through the bulk gas to the gas-liquid interface, moving across the gas-liquid interface, transport into the bulk liquid surrounding the microbial cells, and the diffusive transport through the liquid-solid boundary. In an assessment of various steps Klasson has identified that gas-liquid interface mass transfer is the major resistance for gaseous substrate diffusion [7].

The cost of fermentation media is also an important factor in a large-scale operation of a fermenter. For instance, Kundiyana et al. have reported that morpholinoethanesulfonic acid (MES) used as a buffering agent in syngas fermentation media accounts for approximately 97% of the cost of “C. ragsdalei”

standard media. The buffering of the media is an essential feature of the fermentation broth, as pH of the media controls the balance of acidogenesis (acetic acid production) to solventogenesis (ethanol production). Therefore development of economical buffering systems is also a central issue in scaling up the process.

Metabolic Pathways

The microorganisms used in this approach to produce alcohol fuels from CO, H_2 and CO_2 gas mixture are the type of anaerobic bacteria called acetogens. An acetogen is a general term used for microorganisms that generate acetate as a product of anaerobic respiration. This acetate-producing process follows the Wood-Ljungdahl metabolic pathway and historical perspective of this pathway, as well as metabolic versatilities of acetogens, are discussed in a classic review by Drake et al. [8]. In this pathway CO_2 is reduced to CO, which is then converted to acetyl coenzyme A. Enzymes CO dehydrogenase and acetyl-CoA synthase are the enzymes involved in the end process, and the former catalyzes the reduction of the CO_2 and the latter combines the resulting CO with a methyl group to give acetyl CoA [9]. In the conversion of syngas to ethanol, acetic acid is also generally produced as a co-product and two anabolic processes, acetogenesis and solventogenesis, are involved. Acetogenesis pro-duces acetic acid, while solventogenesis produces ethanol. The two processes do not happen simultaneously, but rather acetogenesis precedes solventogenesis.

Acetyl-CoA pathway leading to the production of ethanol and acetic acids from CO, CO_2 and hydrogen in the syngas is shown in Figure 1 [6,10–12]. This pathway is an irreversible, non-cyclic path that takes place under strictly anaerobic conditions and governs acetogenic bacterial fermentation. The net ATP formation to provide energy for the growth of cells is zero for this pathway. Furthermore, the proposed acetyl-CoA synthesis pathway consists of two branches as shown in Figure 1. The left branch is known as the methyl branch, whereas the right as the carbonyl branch.

Through these paths CO_2 is reduced to methyl and carbonyl level via several enzyme-dependent reactions outlined in the scheme in Figure 1.

In the methyl branch of the acetyl-CoA pathway, CO_2 is first reduced to formate ($HCOO^-$). This reaction is reversibly catalyzed by the formate dehydrogenase (FDH) enzyme, whose function is to reduce CO_2 to formate [12]. The generated formate is then the precursor for the methyl group synthesis of the acetyl-CoA path-way. The formate is activated by tetrahydrofolate (H_4folate) to form 10-formyl-H_4folate catalyzed by 10-formyl-H_4folate synthetase [12]. The enzyme cyclohydrolase catalyze the further conversion of this intermediate to yield 5,10-methenyl-H_4folate. In the next NADPH-dependent reduction, the 5,10-methenyl-H_4folate is converted to 5,10-methylene-H_4folate by the methylene- H_4folate dehydrogenase enzyme. Then, the enzyme methylene- H_4folate reductase reduces this intermediate to (6S)-5-methyl- H_4folate. At the final stage of the methyl synthesis, CH_3– H_4folate is transferred to the cobalt center of the corrinoid/iron–sulfur protein. The corrinoid protein must be reduced to accept a methyl group from 5-methyl- H_4folate. This reduction is carried out by reduced ferredoxin which may be gen-erated using pyruvate and pyruvate–ferredoxin oxidoreductase or CO and CODH as shown in the

equation below [12].

$$[Co_3+\text{-}E] + 2 \text{ ferredoxin red} \rightarrow [Co+\text{-}E] + 2 \text{ ferredoxin ox} \quad (1)$$

In the next step, the reduced corrinoid protein is methylated by transmethylase through the following reaction [12]:

$$[Co+\text{-}E] + CH_3\text{-}H_4\text{folate} \rightarrow [CH3\text{-}Co\text{-}E] + H_4\text{folate} \quad (2)$$

Within the carbonyl branch of the acetyl-CoA pathway, a car-bonyl group is produced which is then merged with the methyl group to produce acetyl-CoA.

The enzyme carbonyl dehydrogenase (CODH) plays a central role in the carbonyl branch, or the right branch, of the pathway. Ni-Dependent carbon monoxide dehydrogenase (Ni-CODH) is a key enzyme in the scheme, and its role can be classified as: (1) monofunctional CODH, which catalyzes the oxidation of CO to CO2, which as a result could be reduced to formate and then methyl group in acetyl-CoA pathway, and (2) bifunctional CODH, which reduces CO_2 to CO as the carbonyl group in acetyl-CoA synthesis and also mediates the evolution of acetyl-CoA alongside the acetyl-CoA synthase (ACS) [13].

During the closing stage of acetyl-CoA synthesis, CO (carbonyl moiety) condenses with the Co-methyl group (methyl moiety) of the methylated corrinoid protein and coenzyme A to yield acetyl-CoA. This reaction is catalyzed in the presence of carbonyl dehy-drogenase (CODH)/acetyl-CoA synthase (ACS) as shown in the reaction below [12,13].

$$[CH_3\text{-}Co\text{-}E] + CO + HS\text{-}CoA \rightarrow CH_3COS\text{-}CoA + [Co\text{-}E] \quad (3)$$

The acetyl-CoA produced is the perfect precursor for the synthesis of a number of cell materials including nucleotides, amino acids, carbohydrates, and lipids [12]. Acetyl-CoA can be used as a source of cellular carbon or cellular energy depending on anabolic or catabolic pathway involved in these processes. In the anabolic pathway, acetyl-CoA is carboxylated to pyruvate in the presence of pyruvate synthase. Then, the pyruvate is converted to phosphoenolpyruvate, which is considered as an intermediate in the evolution of cell materials. In contrast, in catabolic pathway the acetyl-CoA under-goes some reaction to generate ATP and acetate.

The conversion of acetyl-CoA to acetate is catalyzed by phos-photransacetylase, and this reaction is carried out via formation of acetyl-phosphate ($CH_3COO\text{–}\ PO_3^{2-}$) as the intermediate. In the next reaction, acetyl-phosphate is transformed to acetate, while a molecule of ADP is phosphorylated to ATP in the presence of acetate kinase. This phase of metabolism results in acetate production, and is frequently known as the acidogenic phase as well. Conversion of acetyl-CoA to acetate and ATP is carried out during the growth phase of the microorganism. While the evolution of ethanol and NADP is performed during the non-growth phase. Therefore the growth is slow and no ATP is evolved in the solventogenic phase where etha-nol is produced from acetyl-CoA. In this phase of the fermentation process, the reducing potential in the form of NADPH is utilized by the organism to form acetaldehyde (CH_3CHO) in the

presence of enzyme acetaldehyde dehydrogenase. In the last step of the production of alcohol, the acetaldehyde generated is converted to ethanol by the enzyme alcohol dehydrogenase where NADPH is converted to NADP+ during the process as shown in the equation below.

$$CH_3CHO + NADPH + H^+ \rightarrow CH_3CH_2OH + NADP^+ \quad (4)$$

In order to avoid the consumption of reducing equivalents by other metabolic pathways such as aerobic respiration, it is very important to maintain a strict anaerobic environment during the acetyl-CoA pathway shown in Figure 1. Furthermore, through the proposed metabolic pathway, intermediate acetyl-CoA performs two major roles: firstly, it acts as a precursor for the cell macromolecule, and secondly, it serves as an energy source.

Microorganisms Used in Syngas Fermentation

As this is a relatively new area of science a limited number of microorganisms are presently known for the fermentation of syngas to ethanol and other biofuels. Some of the most commonly used microorganisms that can produce a significant proportion of ethanol in the fermentation process are as follows:

Clostridium ljungdahlii [14,15] Clostridium carboxidivorans P7 [16]
Clostridium ragsdalei (also called Clostridium strain P11) [17–21]
Clostridium autoethanogenum [22]
Peptostreptococcus products [23]

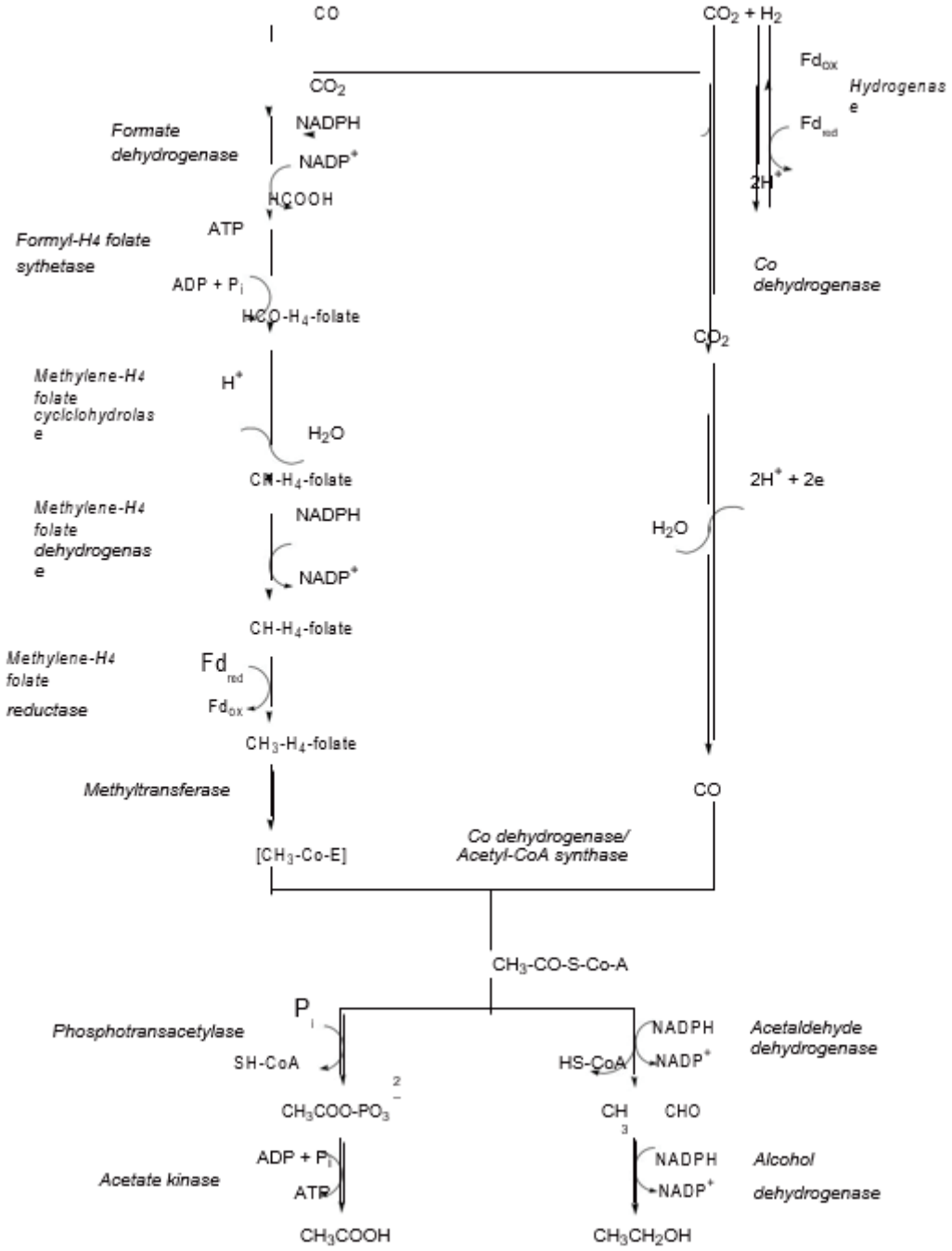
These microorganisms can proficiently operate in the pH 4.0 to 7.0 range, at temperatures close to ambient temperature, and utilize the reductive acetyl-CoA pathway for growth and production of acetic acid and ethanol as major products from CO, H_2 and CO_2 [11,24,15]. Alcohol and acetate yields from a selected sample of microorganisms and experimental conditions used in recent literature examples are shown in Table 1.

Biochemical Reactions in Syngas Fermentation

The biochemical conversion of CO, CO_2 and H_2 in syngas to ethanol following the acetyl-CoA pathway under anaerobic conditions produces acetic acid and ethanol as the major products. The over-all stoichiometry for ethanol and acetic acid production from these gaseous reactants can be represented by Equations 12.6–12.9 [31].

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \quad \Delta G° = -216.0 \text{ kJ/mol} \quad (5)$$
$$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O \quad \Delta G° = -97.1 \text{ kJ/mol} \quad (6)$$
$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad \Delta G° = -135.0 \text{ kJ/mol} \quad (7)$$
$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad \Delta G° = -54.8 \text{ kJ/mol} \quad (8)$$

The combination of Equations 6 and 7 shows that two-thirds of the carbon from CO is converted to ethanol. During the acetyl-CoA pathway, hydrogen provides the required reducing equivalents and electrons when hydrogenase enzyme is present in the fermentation media as shown in Equation 12.10.



$$H2 \rightarrow 2H+ + 2e \quad (9)$$

Figure 1. The acetyl-CoA pathway for acetogenic microorganisms [6,10–13].

If the hydrogenase enzyme is inhibited or hydrogen is not pres-ent in the fermentation broth, the required electrons are obtained from CO in the presence of carbonyl dehydrogenase (CODH) enzyme. In other words, CO is used in supplying electrons, rather than in the biofuel production. This obviously results in a drastic reduction in alcohol yields. It is therefore vital to maintain healthy concentrations of both hydrogen and CO in the culture medium during the fermentation. It is very important to operate under optimum growth conditions of the syngas-fermenting

microorganisms whose conditions can vary from organism to organism. Many of the known microbes tend to produce significant fraction of acetate products in addition to alcohol products as well. Therefore, in order to shift the product formation from acetogenesis to solven-togenesis, researchers have investigated nutrient limitations, pH shifts, reducing agent addition, hydrogen addition and many other modifications.

Table 1

Production of ethanol from fermentation of syngas using various acetogenic bacteria

Organism	Gas substrate (v/v)	Culture mode	Ferm. time	Temp. (°C)	pH	Cell density (g/l)	Conv. efficiency	Product(s) (Reference)
Clostridium Ragsdalei	$CO/H_2/CO_2/N_2$ (20:5:15:60)	CSTR	59d	37	5	1.13	na	ethanol 25.26 g/L acetate 4.82g/L 2-propanol 9.25g/L butanol 0.47g/L [5]
Clostridium Ragsdalei	$CO/H_2/CO_2/N_2$ (20:5:15:60)	Batch	15d	32	6	0.95	na	ethanol 1.89 g/L acetate 1.45g/L [25]
Clostridium ljungdahlii	$CO/H_2/CO_2/N_2$ (20:5:15:60)	Batch	15d	37	na	1.2	XCO:100%	ethanol 0.6 g/L acetate 1.3g/L [26]
Clostridium ljungdahlii	$CO/H_2/CO_2/N_2$ (55:20:10:15)	Cell recycle	560 h	36	4.5	4	XCO:90% XH_2:70%	ethanol 48 g/L acetate 3g/L [27]
Clostridium ljungdahlii	$CO/H_2/CO_2/N_2$ (14:17:4:65)	TBR	na	37	5.1	na	XC0:57% XH_2:78.6%	ethanol 2.74 g/L acetate 6.4g/L [28]
Clostridium ljungdahlii	$CO/H_2/CO_2/Ar$ (55:18:111:16)	CSTR	na	37	4–5	na	na	ethanol 1.0 g/L acetate 3.0g/L [29]
Clostridium ljungdahlii	$CO/H_2/CO_2/N_2$ (20:10:20:50)	Liquid batch	44h	37	6.8	0.562	na	ethanol 0.29mmol/ gh acetate 0.127mmol/ gh [30]
Clostridium autoethanogenum	$CO/H_2/CO_2/N_2$ (20:10:20:50)	Liquid batch	72h	37	na	0.15	na	ethanol 1.45mmol/ gh acetate 23.3mmol/ gh [30]

The Effects of Operation Parameters on Ethanol Yield

Several operation parameters can affect the ethanol yield. The most important ones are: culture media, pH, carbon source, inhibitors, gas impurities, and gas pressure.

The Effect of Culture Media

Culture media is probably the most important operation parameter in the optimization of the ethanol yield [25,32,33,21]. Kundiyana and coworkers recently reported two studies on the effect of culture media during the syngas fermentation utilizing Clostridium ragsdalei. In one study they reported [32] the effect of nutrient limitation and two-stage continuous fermenter design on productivities, whereas in the other study the role of the buffer presence in the media was evaluated [25]. In the first study three nutrients, calcium pantothe-nate, vitamin B_{12} and cobalt chloride ($CoCl_2$), on syngas fermentation using Clostridium ragsdalei were determined using serum bottle fermentation techniques. These studies indicated that three-way interactions between the three limiting nutrients, and two-way interactions between

vitamin B_{12} and $CoCl_2$ had a significant posi-tive effect on ethanol and acetic acid formation. In general, ethanol and acetic acid production ceased at the end of nine days, corre-sponding to the production of 2.01 and 1.95 g L^{-1} for the above inter-actions. Reactor studies indicated the three-way nutrient limitation in two-stage fermenter, which showed improved acetic acid and ethanol yields compared to treatments in single-stage fermenter. As a result of these studies Kundiyana and coworkers concluded [32] that their results further support the hypothesis that it is possible to modulate the product formation by limiting key nutrients during *C.ragsdalei* syngas fermentation.

As the fermentation medium is a critical factor in the biocatalysis route, there are several reports on using experimental and theoretical techniques in the optimization of pH and composition parameters. In one example, Plackett–Burman applied central com-posite designs to optimize the medium for ethanol production by Clostridium autoethanogenum in a medium containing NaCl, KH_2PO_4, $CaCl_2$, yeast extract, $MgSO_4$, and NH_4Cl [33]. The optimum ethanol yields predicted by response surface methodology (RSM) and an artificial neural network-genetic algorithm (ANN-GA) were 247.48 and 261.48 mg/L, respectively. Furthermore, Guo et al. reported that these values are similar to those obtained experimentally under the optimal conditions suggested by the statistical methods (254.26 and 259.64 mg/L). It is interesting to note that the fitness of the ANN-GA model was higher than that of the RSM model. Additionally, as a result of optimizations in the culture media, yields obtained in this study were substantially higher than those previously reported (60–70 mg/L) with the same organism [33], and this result clearly established the importance of using optimum nutrient concentrations in the fermentation broth.

Effect of Medium pH

Fermentation pH is another parameter related to the culture media capable of regulating the substrate metabolism and altering the physiological parameters, including the internal pH of cells, membrane potential and proton-motive force. As a result, the medium pH affects the product selectivity, composition release and meta-bolic byproducts. The effect of pH on syngas fermentation is discussed in recent publications and in reviews [1,2,3,25]. The optimum pH value varies with the organism, and there is a narrow range for every organism, in which the cells are metabolically active. Any large deviations in pH can lead to damage or death of the cells and consequently results in loss of biological activity. Lowering the fer-mentation broth pH can weaken the cell growth and affect the over-all productivity of the process because of the reduced flow of carbon and electron from the substrate toward the cell mass. However, in the case of acetogenic bacteria, this effect can be considered as an advantage because the reaction path can shift from acetogenic to sol-ventogenic phase, which supports the production of more reduced alcohol products such as ethanol. In such cases, the generated acetic acid, which is a weak organic acid, permeates through the cell mem-brane, as it is a lipophilic acid in the undissociated form. Acetic acid conducts H+ ions while diffusing through the cell membrane, thus reducing the intracellular pH. At the low internal pH values, exter-nal pH plays an important role in counteracting this situation [21].

Kundiyana and Wilkins have recently studied the effect of fermentation medium

pH together with other parameters like temperature, and presence or absence of media buffer [25]. They have found that the temperature dependence on the gas solubility is an important factor as well. This may be due to the fact that carbon monoxide and hydrogen components of syngas show decreased solubility with increasing temperature. These studies revealed that Clostridium species preferentially switch from acetogenesis to solventogenesis phase at pH below 5.0, and morpholinoethanesulfonic acid (MES) added as media buffer has been shown to increase lag time for ethanol production. Furthermore, this study showed syngas fermentation using Clostridium ragsdalei at 32°C in a media without a buffer was associated with higher ethanol concentration and reduced lag time as a result of switching to solventogenesis. The optimization study concluded that temperature above 40°C and pH below 5.0 were outside the most favorable range for growth and metabolism of the Clostridium ragsdalei [25]. Probably the most important finding from this study was that it is possible to conduct syngas fermentation using Clostridium ragsdalei in the 32 to 37°C without any buffer addition as seen in the results of Table 2.

Additionally, temperatures above 37°C greatly reduced *C. ragsdalei* cell growth and performance resulting in significantly lower ethanol production [25].

The Effect of Carbon Source

Anaerobic microorganisms are able to grow either chemoorgano-trophically with carbon sources such as fructose, acetate, malate, glutamate, furmarate, succinate and pyruvate, or chemolithotrophi-cally on substrates such as CO and H_2/CO_2, which are considered as the main syngas constituents. During the syngas fermentation these anaerobic microorganisms utilize CO or CO_2 during the fermentation process to provide energy for bacterial growth and maintenance and also metabolize the production of alcohols and acetate. Cotter et al. first reported a comparison of a carbohydrate carbon source with syngas [30]. In this study the growth of C. ljung-dahlii was measured in syngas (50% N_2, 20% CO, 20% CO_2 and 10% H_2), as well as utilizing fructose as the carbon source. They found that bacterial growth on sugar-based carbon resulted in a dense cul-ture of about 1 g/L, whereas the syngas produced only 0.562 g/L. Moreover, the ethanol concentration of fructose-fed culture was 13 mM and in the syngas-fed culture was 3.8 mM. This type of large difference in growth performance was most likely due to the diffusion limitations at the gas–liquid interface and/or efficiency of the uptake and transport mechanism of the gaseous substrate. In addition to this, they observed [30] that syngas fermentation inoculated with cells pre-cultured on fructose resulted in a higher cell density (0.850 g/l) in comparison to the cells pre-cultured on syngas (0.562 g/l). It was inferred that such difference in culture performance was likely attributed to a greater availability of intracellular cofactors, enzymes, and maintenance energy in cells adapted to sugar substrate.

The Effect of Inhibitors and Impurities in Syngas

In biological processes, the growth and product formation rate of microorganism may be reduced or even inhibited by products, contaminants, and by impurities in the syngas. For example, production of organic acids is known to be associated with hydrogen formation. However, increase of the H_2 partial pressure in the gas phase, as

well as accumulation of H_2 in the fermentation medium, may inhibit the fermentation and acetogenesis due to the altera-tion of carbon flow in the biological pathway of organism [34]. In addition, higher concentrations of CO_2 can be a possible source of inhibition as well, as CO_2 dissolves in water making carbonic acid or its carbonate derivatives affecting the medium pH.

The impurities in the syngas can also affect the growth of micro-organisms and product yields [35,36]. Once an impurity transfers from the syngas into the bioreactor media, the impurity may directly affect the organism by causing cell toxicity by enzyme inhi-bition and this may affect product distribution as well, or indirectly affect the fermentation process by changing process conditions like pH, osmolarity, redox potential, etc.

Depending on the concentration of impurities, syngas can be cleaned up before the fermentation. Selection of commercial tech-nologies suitable for syngas cleanup is mainly based on the cost and the ability to meet the end-user specifications. The impurities in biomass-derived syngas can be categorized into solid impurities, tars and gaseous impurities [37,38]. Filters and cyclones are commonly utilized for removal of particulate matter or solid impurities. In general, tar removal technologies can be branched into primary methods inside the gasifier treatments and secondary methods after the gasifier treatments.

Table 2.

The experimental data for syngas fermentation utilizing *Clostridium ragsdalei.* Product yields are shown under different pH and temperature conditions at the end of a 15 day period (*n* = 3)

Treatment	Buffer (%)	pH	Temperature (°C)	Acetic acid gL^{-1}	Ethanol gL^{-1}	Cell density [a] (after 15 days)	pH
1	1	7.0	42.0	0.22	0.00	0.08	6.46
2	1	7.0	32.0	6.35	0.58	1.07	4.77
3	1	5.0	42.0	0.34	0.00	0.06	5.19
4	1	5.0	32.0	3.71	0.52	0.81	4.29
5	2	7.0	37.0	7.88	0.30	0.70	5.14
6	0	7.0	37.0	1.60	1.65	0.84	4.50
7	2	5.0	37.0	3.58	0.43	0.62	4.40
8	0	5.0	37.0	2.41	0.39	0.50	4.34
9	2	6.0	42.0	0.21	0.00	0.08	5.98
10	0	6.0	42.0	0.28	0.00	0.08	5.47
11	2	6.0	32.0	3.53	0.73	1.03	4.85
12	0	6.0	32.0	1.45	1.89	0.95	4.39
13	1	6.0	37.0	3.94	0.69	0.74	4.74

Generally primary inside the gasifier tar cracking methods can effectively convert the heavy and light hydrocarbons to negligible levels. In addition to this, scrubbing with water can be employed for removal of water soluble gaseous impurities such as

ammonia, HCl and chlorine and other trace impurities. Zinc oxide beds are also popular for removal of sulfur in the syngas.

The toxicity of impurities on bacterium are due to inhibition of various enzymes, and these enzyme inhibitory effects of common gaseous impurities NH_3, NO, NO_2, H_2S, COS, and SO_2 are summarized in Table 3.

Effect of NH_3

Ammonia in the syngas can affect enzymes alcohol dehydrogenase and amidase. Xu and Lewis have studied the effects of ammonia impurity in raw syngas on dehydrogenase activity [45]. In this work, it was shown that NH_3 rapidly converts to ammonium ion (NH_4^+) following exposure of fermentation broth to NH_3, and they found that accumulated NH_4^+ also inhibited dehydrogenase activity and cell growth. A kinetic model for dehydrogenase activity that included inhibition effects from NH_4^+ + was developed in this work, and KH_2 (Michaelis-Menten constant) and KNH_4^+ (the inhibition constant for NH_4^+) were included as model parameters. Experimental results showed that NH_4^+ behaves as a non-competitive inhibitor for dehydrogenase enzyme with KNH_4^+ of 649 ± 35 mol m^{-3}. As part of the work, Xu and Lewis have been able to distinguish the unique aspect of NH_4^+ inhibition by comparison with other species such as K^+ and phosphate ions, by proving that potassium and phosphate ions had no effect on hydrogenase activity. Since NH_4^+ can easily be accumulated in fermentation media and transport across the cell membrane, they concluded that it is crucial to remove NH_3 impurity from raw syngas to minimize the reduction in alcohol dehydro-genase activity.

Effect of Nitric Oxide

Nitric oxide (NO) present in the syngas at concentrations greater than 0.004 mol% can inhibit the enzyme hydrogenase, and this is a reversible, non-competitive inhibitor activity. In addition, NO also had an adverse effect on cell growth and may contribute to increased production of acetic acid. Syngas fermentation using C. carboxidivorans has shown that NO concentrations less than 0.004 mol% had no effect on the efficiency of the process. Therefore, gas cleanup up to 0.004 mol% NO is sufficient in most of the syngas fermentation operations.

The Effect of Gas Pressure

The effect of partial pressure of gas components is another controlling factor that can affect the ethanol yield. In an early study with Clostridium ljungdahlii, varying initial CO partial pressures (up to 1 atm) in a closed bottle system was used to assess cell growth and product formation [26]. In this investigation it was shown that vary-ing CO partial pressure did not affect the maximum cell growth.

Additionally, ethanol production was similar for partial pressures up to 0.75 atm and acetate production had no apparent correlation with CO partial pressure. However, more recent work in this area was aimed at keeping partial pressure constant throughout the fer-mentation process. Since gas partial pressures do not remain con-stant in a closed bottle system as the metabolic process proceeds, later work utilized an experimental design to maintain constant CO and CO_2 gas phase partial pressures during the entire experiment. In a 2010 investigation Hurst and Lewis have studied the effect of CO partial pressure effects on the metabolic process

Table 3

The effects of common syngas impurities on enzymes, showing inhibitory concentrations.

Inhibitor	Name of enzymes	Amount	Reference
NH_3	Alcohol dehydrogenase (ADH), Amidase	NH_3 Inhibition at very high concentration of ADH	[39] [40]
NO	Hydrogenase, Alcohol dehydrogenase (ADH)	For hydrogenase, at 0.015 mol% level, 100% inhibition, at 0.004 mol% level	[16] [41]
NO_2	Formate dehydrogenase (FDH), Nitrate reductase	1 mol/m^3, 5% inhibition for FDH 1 mol/m^3, 20% inhibition of nitrate reductase activity	[36]
H_2S	Thiosulfate sulfurtransferase, L-ascorbate sulfurtransferase	At concentrations above 30 mol/m^3 for thiosulfate 1 mol/m^3, 97% inhibition for L-ascorbate oxidase	[42] [43]
COS	Carbon monoxide dehydrogenase	Rapid-equilibrium inhibitor largely competitive versus CO	[44]
SO_2	Ascorbic acid oxidase (AAO)	–	[36]

of syngas fermen-tation by keeping partial pressures constant throughout the process. In this work they assessed the effects of constant CO partial pressure (PCO), ranging from 0.35 to 2.0 atm, on cell growth, acetic acid production, and ethanol production using *Clostridium carbox-idivorans* $P7^T$. The key findings of this study could be summarized as follows: (a) the maximum cell concentration increased with increasing P_{CO}, increasing 440% with a P_{CO} increase from 0.35 to 2.0 atm, (b) ethanol production changed from non-growth-associated to growth-associated with increasing P_{CO}, (c) acetic acid production (gram acetic acid per gram cells) decreased for $P_{CO} \geq$ 1.05 atm rela-tive to $P_{CO} \leq 0.70$ atm, and (d) acetic acid appeared to be converted in the latter growth stages for P_{CO} of 1.35 and 2.0 atm.

The work of Hurst and Lewis as well as other groups have emphasized the importance of P_{CO} and the P_{CO} to P_{CO2} ratio on electron and ATP production. Since gasification processes that generate syngas could result in differing gas partial pressures, the process variations could significantly change growth and product forma-tion as evidenced by these metabolic changes.

Syngas Fermentation Reactors

Several reactor designs can be used for the fermentation process, and reactor configuration is closely related to the product yield and plays an important role in syngas fermentation. The most important parameters that we are looking for in designing an efficient fermentation bioreactor system are: high mass transfer rates, high cell densities, low operation and maintenance costs, and easy scale-up to very large industrial scales. In this section some of the common reactor designs that are in current use in syngas fermentation and undergoing further improvements will be discussed.

Continuous Stirred Tank Reactors (CSTR)

The continuous stirred-tank reactor (CSTR), also known as vat or backmix

reactor, is the most commonly employed bioreactor in syngas fermentation. This reactor has a continuous flow of gas bubbling through the liquid which typically consists of a dilute solution of essential nutrients for the microorganism to grow and survive on. A schematic representation of continuous stirred tank reactor (CSTR) used for fermentation of syngas to ethanol is shown in Figure 2.

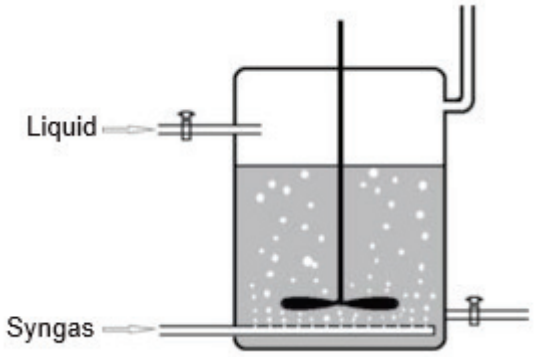


Figure 2 Schematic representation of the continuous stirred tank reactor (CSTR) used for fermentation of syngas to ethanol.

The liquid can be added and removed from this type of reactor while the reactor is under operation, and high agitation rate is needed to enhance the transfer rate of the CO, CO_2, and H_2 from the syngas to organisms. Higher agitation speeds lead to a higher mass transfer rate between the substrate gases and the microbes. However, in very large industrial-scale fermenters, higher agitation speeds increase the agitator's power consumption, thus increasing the operational cost of the plant. If the transfer is not fast enough, the production of cellular products will be limited to how fast the gas is transferred to the organism.

Microbial cell recycle systems can also 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 microbial cells is separated from the permeate (cell-free media) and recycled back 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 micro-bial catalyst. Recycling has been shown to provide up to about a 2.6-fold increase in cell concentration [14].

Packed Bed Reactors (Immobilized-Cell Reactors)

Packed-bed reactors, or immobilized-cell reactors, are columns packed with biocatalyst particles to which the microorganisms are immobilized. These reactors are usually operated concurrently where the liquid and gas flow in the same direction. Advantages of this reactor include high density of the microorganisms and easy separation of the microbial cells from the fermentation broth. However, the rate at which syngas components are transferred to the organism is usually slow.

Trickle Bed Reactors (TBR)

This is a vertical tubular reactor packed with solid material that the microorganisms can attach to as solid support. The term "trickle bed" entails the downward movement of a liquid and gas over a packed bed of catalyst particles. It is considered to be the simplest reactor type for performing catalytic reactions where a

gas and liquid (normally both reagents) are present in the reactor, and accord-ingly it is extensively used in processing plants. The direction of fluid flow is normally counter current, with the liquid trickling downwards as the gases flow upwards.

Comparative studies on different types of reactors in syngas fer-mentation are rare in the open literature. However, Kundiyana and Wilkins have reported their work on a 100L pilot plant-scale syngas fermenter [5] as well as a two-stage continuous fermenter design on productivities during Clostridium ragsdalei syngas fermentation [32]. These pilot-scale 100L fermenter studies were conducted in strictly anaerobic conditions, the fermentation system was maintained in a batch mode with continuous syngas supply, and the impact of improving the syngas mass transfer coefficient on the utilization and product formation was studied. Results indicated a six-fold improvement in ethanol concentration compared to serum bottle fermentation and the formation of other compounds as well, such as isopropyl alcohol, acetic acid and butanol, which are of commer-cial importance [5]. The two-reactor configuration experiment was conducted using two stirred-tank fermenters of equal volume in series in the partial-cell recycle mode [32]. The operational strat-egy of this reactor scheme involved operating the first reactor as a "growth reactor" and the second reactor as a "product reactor." These studies clearly demonstrated the advantages of two-stage reactor design over the single-vessel design.

Industrial-Scale Syngas Fermentation and Commercialization

The major deficiency in the current syngas fermentation route is gas-to-liquid mass transfer limitation, which is the most difficult barrier to overcome due to the poor solubility of the gaseous substrate, especially CO and H_2, in fermentation medium. The low ethanol yield in the process is also a result of solubility limitation. The slow reaction rate and the need for sterile condition to prevent media contamination are also some disadvantages involved in biological processes. But in the case of syngas fermentation, the presence of CO in the gas stream ensures sterility as it is toxic to most microorganisms.

Even though there are considerable challenges, the potential of the fermentation route to produce ethanol from syngas has been established by various successful laboratory-scale research studies and pilot plant studies. So far three major companies have reported the successful operation of large facilities for high-volume ethanol production via syngas fermentation technology [11], and these industrial facilities include:

1. Coskata, Inc., Madison, Pennsylvania, USA; 2009; 50,000 gallons/year semi-commercial plant
2. INEOS Bio, Vero Beach, Florida, USA; 2008; 8 million gallon/year
3. LanzaTech, Glenbrook, New Zealand; 2010

There are a number of areas that can be focused on for improving the ethanol yield, which include genetic engineering of microorganisms to develop better biocatalysts, innovative reactor designs that can improve the mass transfer, and cost-effective fermentation mediums that can enhance the syngas solubility in the liquid phase. It is encouraging to see that all these aspects are currently under rigorous study in academic and industrial laboratories.

Conclusion

The celluloseand other biomass as a raw material for the bioethanol production is one of the most perspective. Big volume, chip prise make the cellulose first for the increasing of bioethanol production and using.

The bioethanol producers can use biomass in two ways – directly threat and fermented or throw syngas.

The conventional of biomass into syngas by gasification processes is another route in the bioethanol production. Using of this technology depend from final price of production and, of course, its influence on the price of bioethanol.

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Анотація. *В роботі розглянуті питання промислової реалізації другого та завершального етапу переробки біомаси, зокрема целюлози, в біоетанол через варіант синтетичного газу з наступним зброджуванням сингазу мікроорганізмами. Розглянуто вплив різних факторів: температури, активної кислотності (pH), інгібіторів та активаторів на життєдіяльність мікроорганізмів, задіяних в біотрансформації сингазу, та, як результат, на вихів біоетанолу.*

Ключові слова: *біоетанол, біомаса, зброджування, синтетичний газ, кислоти, мікроорганізми, ацетатний процес.*

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