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Review Paper

A review of conversion processes for bioethanol production with a focus on syngas fermentation

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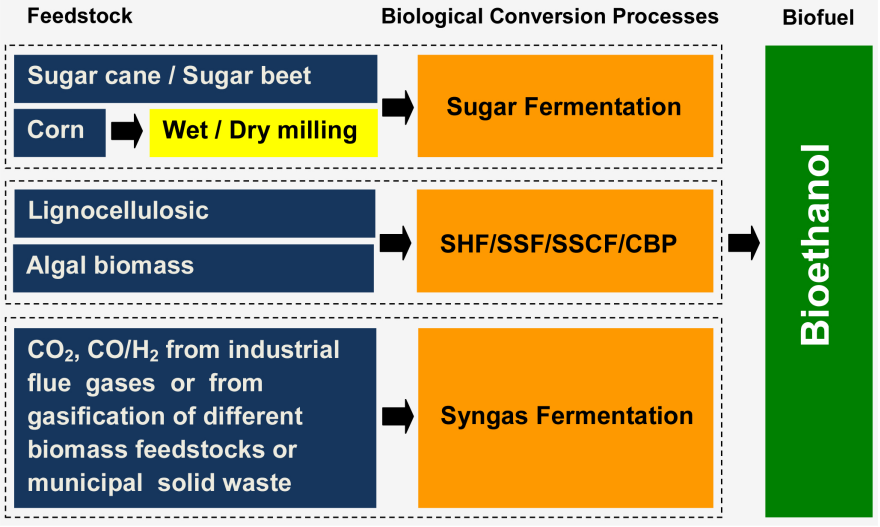
HIGHLIGHTS

- Summary of biological processes to produce ethanol from food based feedstocks.
- Overview of fermentation processes for ethanol production from biomass.
- Process development and reactor design are critical for feasible syngas fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioethanol production from corn is a well-established technology. However, emphasis on exploring non-food based feedstocks is intensified due to dispute over utilization of food based feedstocks to generate bioethanol. Chemical and biological conversion technologies for non-food based biomass feedstocks to biofuels have been developed. First generation bioethanol was produced from sugar based feedstocks such as corn and sugar cane. Availability of alternative feedstocks such as lignocellulosic and algal biomass and technology advancement led to the development of complex biological conversion processes, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), consolidated bioprocessing (CBP), and syngas fermentation. SHF, SSF, SSCF, and CBP are direct fermentation processes in which biomass feedstocks are pretreated, hydrolyzed and then fermented into ethanol. Conversely, ethanol from syngas fermentation is an indirect fermentation that utilizes gaseous substrates (mixture of CO, CO_2 and H_2) made from industrial flue gases or gasification of biomass, coal or municipal solid waste. This review article provides an overview of the various biological processes for ethanol production from sugar, lignocellulosic, and algal biomass. This paper also provides a detailed insight on process development, bioreactor design, and advances and future directions in syngas fermentation.

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Contents

1. Introduction

Renewable energy can be derived from sunlight, wind, water, geothermal, and biomass, which are considered sustainable and environmentally friendly. Conversely, non-renewable energy is derived from fossil fuels such as coal, oil and natural gas, which do not regenerate at sustainable rates (Twidell and Weir, 2003). Most of the world's energy demand is currently met using fossil fuels. The Energy Information Administration (EIA) reported that 70% of all oil consumed in the United States was used for transportation (EIA, 2015a). According to EIA's 2014 net imports data 27% of petroleum consumed in the U.S. was imported from foreign countries (EIA, 2015b). Factors such as high gas prices, rising concerns over national energy security and dependency on foreign oil imports, and environmental impacts of high oil usage have led to an increased focus on biofuel production (German et al., 2011).

Ethanol was the first biofuel produced from food-based feedstocks such as corn and sugarcane. The United States, being the largest producer of corn, have successfully commercialized corn ethanol production (Dien et al., 2002). However, the use of corn for biofuels raised debate over its potential interference with the food market. This gave rise to the use of non-food based feedstocks such as agricultural and forest residues, municipal wastes, lignocellulosic, and algal biomass for bioethanol production. Unlike crude oil, biomass feedstocks are diverse in their composition. Hence, different conversion processes have been developed to produce a variety of biofuels. This review article focuses on conversion processes pertinent to bioethanol production using different biomass feedstocks. Further, this article discusses the developments of syngas fermentation for ethanol production.

1.1. Bioethanol from sugar/starch

First generation bioethanol is produced from corn and sugarcane using a well-established technology (Sims et al., 2008). The steps involved in production of ethanol from sugar and starch crops are shown in **Figure 1**.

Sugar crops such as sugar cane, sugar beet and sweet sorghum mostly consist of glucose, fructose, and sucrose as their major components (Bai et al., 2008). These fermentable sugars are extracted by grinding or crushing followed by fermentation to ethanol. Further, ethanol is separated from the products stream by distillation followed by dehydration.

Grains such as corn and wheat contain starch, which is a polysaccharide of glucose units linked by α (1-4) and α (1-6) glycosidic bonds (Pandey, 2010). Starch is not directly fermented by yeast. After milling the grains and extracting starch, starch is hydrolyzed into glucose using α-amylase and glucoamylase (Nigam and Singh, 1995). Glucose is then fermented to ethanol.

Production of ethanol from starch is performed by either dry grind or wet milling process (Bothast and Schlicher, 2005). The main difference between these two processes is the extraction method of glucose and co-products formed (Sims et al., 2008). In dry grind, whole corn is milled to produce ethanol along with high protein animal feed called dry distillers' grains with solubles (DDGS). In wet milling, steeping of corn is followed by separation of germ, fiber, and starch. Wet milling produces value added by-products such as corn sweeteners, oil, and corn gluten meal in addition to ethanol. Wet milling requires high capital cost and is less efficient in producing ethanol than dry grind process (Rausch and Belyea, 2006 ; Rodríguez et al., 2010). The high capital cost of wet milling process is due to separation of various corn components to co-produce value added by-products in addition to 2.5 gallons of ethanol per bushel of corn. However, whole corn is utilized in dry grind facilities maximizing capital return per gallon of ethanol. About 2.8 gallons ethanol are produced per bushel of corn via the dry grind process (Bothast and Schlicher, 2005). Most corn ethanol plants in the U.S. are dry grind facilities (USGC, 2012). One disadvantage of dry grind process is that the value of DDGS has decreased due to an increase in dry grind facilities. Thus, modified dry grind facilities have been proposed to recover germ and fiber from the corn grains and improve byproduct value (Rodríguez et al., 2010). The cost efficiency of ethanol production from food based feedstocks and impacts on change in land usage has been criticized (Rathmann et al., 2010). Such drawbacks of first generation bioethanol gave rise to the need for ethanol production from non-food based feedstocks such as biomass.

1.2. Bioethanol from cellulosic feedstocks

The non-food based feedstocks used for production of second generation ethanol comprises of cellulosic biomass such as dedicated energy crops (e.g., switchgrass, miscanthus) and agricultural and wood residues (e.g., woodchips, cornstover, sugarcane bagasse, and sawdust) (Naik et al., 2010). Cellulosic biomass mainly consists of cellulose, hemicellulose, and lignin polymers interlinked in a heterogeneous matrix (Kitani and Hall, 1989). Cellulose is a linear polysaccharide consisting of several β(1-4) linked D-glucose units. Hemicellulose is a heteropolymer of xylose, mannose, galactose, rhamnose and arabinose. Lignin is a complex polymer of cross-linked aromatic compounds. Lignin acts as a protective barrier and hinders the depolymerization of cellulose and hemicellulose to fermentable sugars. Unlike first generation ethanol production, the process for conversion of cellulosic feedstocks to ethanol is complex (Stöcker, 2008; Szczodrak and Fiedurek, 1996). Cellulosic biomass is first pretreated either chemically or enzymatically to breakdown the polymeric units and increase the accessibility of C5-C6 sugars for microbial fermentation to produce ethanol. An overview of the biological conversion processes for ethanol production is discussed in sections 2 and 3.

Second generation bioethanol from cellulosic feedstocks was successfully demonstrated in pilot scale plant (Menetrez, 2014). Recently in 2014, 25 million gallons per year capacity commercial scale cellulosic ethanol plants were commissioned by POET-DSM and Abengoa Bioenergy (Lane, 2015; POET-DSM, 2014). Further, DuPont's 30 million gallon per year cellulosic ethanol plant is expected to start production in 2015. While commercialization of second generation ethanol plants looks promising, the

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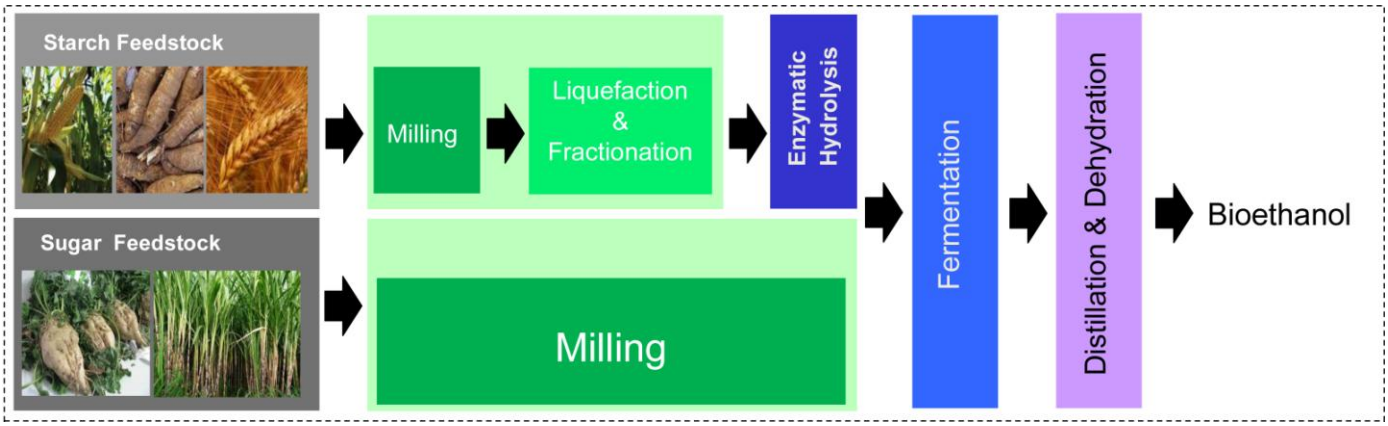

Fig.1. Bioethanol production from first generation biomass (Adapted from Sims et al., 2008).

sustainability of these plants will largely depend on the market availability of the feedstocks at reasonable prices. For the cellulosic ethanol industry to flourish, biomass feedstocks should be available at large scale and low cost. Most of the cellulosic feedstocks meet this requirement (Carriquiry et al., 2011). One of the main challenges of cellulosic ethanol commercialization is the impact of the change in land usage (Searchinger et al., 2008). The production of dedicated energy crops requires vast land area. However, land management practices are necessary to reduce any indirect carbon and nitrogen gas emissions that pose a threat to produce harmful greenhouse gases (GHGs) (Tilman et al., 2006). This disadvantage of cellulosic biomass gave rise to considering algal biomass as a potential feedstock for biofuels production.

1.3. Bioethanol from algal biomass

Algal biomass can be used to produce a variety of biofuels such as hydrogen, diesel, isobutene, and ethanol (Cruz et al., 2014; Mussatto et al., 2010; Nayak et al., 2014; Posten and Schaub, 2009). Microalgae are unicellular plants that are either autotrophic or heterotrophic and can grow in diverse environment (Mata et al., 2010). Autotrophic algae harness sunlight and fix atmospheric CO_2 into carbohydrates such as starch and cellulose via photosynthesis. On the other hand, heterotrophic algae species can utilize small organic carbon compounds that are turned into lipids, protein, and oils (John et al., 2011). Conversely, macroalgae are large multicellular marine algae obtained from natural and cultivated resources. Harvested macroalgae (red, brown and green) are mainly used to produce hydrocolloids that constitute 10-40% of their biomass. Macroalgae has a low concentration of lipids and primarily contains 35-74% carbohydrates and 5-35% proteins (Ito and Hori, 1989). Conversely, most of the microalgae such as *Botryococcus braunii*, *Chlorella sp.*, *Nannochloris sp.*, *Nitzschia sp.*, *Schizochytrium sp.* have at least 20-50% oil content (Chisti, 2007). Several studies have reported the production of bioethanol from both micro- and macro-algal biomass (Fasahati et al., 2015; Harun et al., 2010; Harun et al., 2014; John et al., 2011; Jung et al., 2013). Starch and cellulose are extracted from algae biomass using mechanical shear or by enzyme hydrolysis, after which they are utilized for bioethanol production (John et al., 2011). Enzymatic hydrolysis of cellulose from algae is simpler than from plant biomass due to negligible or no presence of lignin in algae. Various species of algae were reported to contain different starch and biomass content after oil extraction (John et al., 2011). Ethanol production from algal starch is similar to conversion processes of starch or sugars to ethanol discussed in section 1.1. The conversion technologies of algal and plant based cellulosic biomass to ethanol are similar, which are discussed in sections 2 and 3 of this review article.

Algae can grow on non-arable lands and do not change land usage. Further, CO_2 produced in industrial flue gases can be used to produce algal biomass (Brennan and Owende, 2010). Another main advantage of algal biomass is that it does not require fresh water for cultivation. Waste waterfrom industrial and domestic sewage can also be used for the cultivation of algal biomass (Mussatto et al., 2010).

The major obstacle for the commercialization of algal biofuels is process economics. Harvesting corresponds to 20-30% of total cultivation costs (Demirbas and Fatih Demirbas, 2011). Cultivation of microalgae through open ponds is economical but has inherent disadvantages such as low productivity, water loss, low CO_2 utilization, and high affinity to be contaminated by other algal strains (Chisti, 2007; John et al., 2011; Posten and Schaub, 2009). The disadvantages of open ponds led to development of closed photobioreactors, which facilitate higher productivity, less contamination, and less water loss. However, photobioreactors suffered from CO_2, O_2 and pH gradients, wall growth, fouling, hydrodynamic stress, and high scale up costs (John et al., 2011). While macroalgae has recently gained renewed interest as bioethanol feedstock; its process economics are not fully addressed. Nevertheless, a recent quantitative sustainability assessment on macroalgae reported it to have a potential as a sustainable bioethanol feedstock (Park et al., 2014).

Conversion of non-food based feedstocks to bioethanol and other products can be broadly classified into chemical and biological processes. Further, biological conversion of biomass can be through direct or indirect fermentation. Bioethanol can be produced through direct fermentation of the biomass via hydrolysis-fermentation and through indirect fermentation via syngas fermentation. In this article, ethanol production through hydrolysis-fermentation is briefly discussed followed by a detailed review of syngas fermentation process an indirect biomass conversion process to produce bioethanol. Discussion on thermochemical conversion processes can be found elsewhere and is out of scope of this review article (Dutta et al., 2011; Perales et al., 2011).

2. Hydrolysis fermentation

Biological conversion of lignocellulosic biomass to ethanol consists of three main steps namely pretreatment, hydrolysis and fermentation. Different pretreatment methods have been employed to disrupt the cell wall and expose the cellulose, hemicellulose fibers for further processing. Pretreatment methods are mainly divided into (i) physical (milling and grinding), (ii) physiochemical (steam pretreatment/auto hydrolysis, hydrothermolysis, and wet oxidation), (iii) chemical (alkali, dilute acid, oxidizing agents, and organic solvents), (iv) biological or a combination of these methods (Alvira et al., 2010; Mood et al., 2013). After biomass pretreatment, the cellulose and hemicellulose are broken down into monomers by acid or enzymatic hydrolysis (Sun and Cheng, 2002). Next, fermentation is carried out to convert these monomeric sugars into alcohols using yeast or bacteria (Liu et al., 2015a; Liu et al., 2015b; Pessani et al., 2011).

Four process configurations for ethanol production are possible based on the degree to which the above mentioned steps are consolidated as shown in **Figure 2**.

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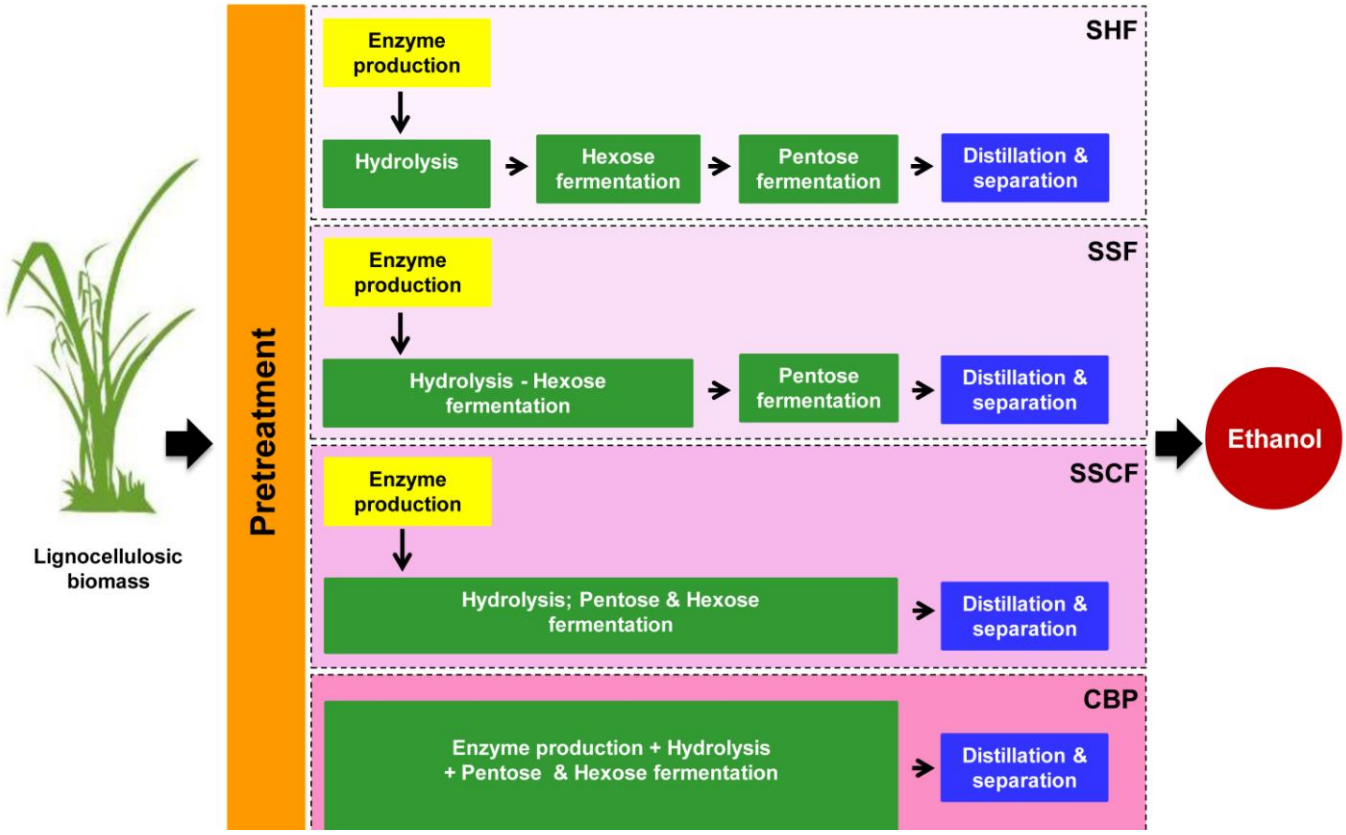

Fig.2. Bioethanol lignocellulosic biomass process configurations (i) Separate Hydrolysis & Fermentation (SHF) (ii) Simultaneous Saccharification & Fermentation (SSF) (iii) Simultaneous Saccharification & Co-Fermentation (SSCF) (iv) Consolidated Bioprocessing (CBP) (Adapted from Hamelinck et al., 2005).

Process integration reduces capital cost and makes the biofuel production process more efficient and economically viable (Cardona Alzate and Sánchez Toro, 2006; Hahn-Hägerdal et al., 2006; Hamelinck et al., 2005). In Separate Hydrolysis and Fermentation (SHF) configuration, the enzyme production, hydrolysis of biomass, hexose and pentose fermentation are carried out in separate reactors (Lynd et al., 2002). In SHF, hydrolysis and fermentation can occur at their optimum conditions. However, the accumulation of glucose and cellobiose during hydrolysis inhibit the cellulases and reduce their efficiencies (Margeot et al., 2009).

The disadvantages of SHF led to the development of Simultaneous Saccharification and Fermentation (SSF) process (Wright et al., 1988). In SSF, both cellulose hydrolysis and hexose fermentation occur in the same reactor. This results in relieving the end product inhibition on the cellulases as the sugars are immediately consumed by the fermenting microorganism (Hahn-Hägerdal et al., 2006). However, SSF process has some limitations. There is a trade-off between the cost of enzymes production and hydrolysis fermentation process (Lynd et al., 2002). In SSF, the rate of enzyme production limits the rate of alcohol production. In addition, cellulases used for hydrolysis and the fermenting microorganisms usually have different optimum pH and temperature conditions. It is important to have compatible conditions for both the enzyme and the microorganism. Another issue with SSF is that most microorganisms used for fermentation of glucose cannot utilize xylose, a hemicellulose hydrolysis product (Lin and Tanaka, 2006).

In Simultaneous Saccharification and Co-fermentation (SSCF) process, glucose and xylose are co-fermented in the same reactor. Strains of *Saccharomyces cerevisiae* and *Zymomonas mobilis* are genetically engineered to co-ferment both glucose and xylose (Dien et al., 2003; Hahn-Hägerdal et al., 2007; Öhgren et al., 2006; Zhang et al., 1995).

Another method of process integration is the Consolidated BioProcessing (CBP), in which one single microorganism is used for hydrolysis and fermentation steps. This potentially reduces the capital costs and increases process efficiency (Lynd et al., 2002). However, microorganisms which can both produce enzymes for hydrolysis of biomass and then ferment released sugars are still in the early development stage (Lynd et al., 2005).

The main advantage of biochemical conversion technologies is the high product selectivity of the biocatalyst (Foust et al., 2009). The enzymes that catalyze the biochemical reactions produce highly specific products. Hence, metabolic engineering and synthetic biology can be used to alter the metabolic pathway and regulate only specific enzymes to increase the desired product yields (Fischer et al., 2008; Percival Zhang et al., 2006). Another advantage of the biochemical processes is that they are usually operated at ambient temperature and pressure, unlike the chemical processes. However, lignin is not utilized in biochemical processes. Ethanol production from lignocellulosic feedstocks using biochemical processes is more difficult compared to corn ethanol production (Lynd et al., 2008). This is attributed to the high costs associated with pretreatment and enzymatic hydrolysis. Research areas that should be addressed to increase the economic feasibility of biochemical conversion processes include (i) improving effectiveness of biomass pretreatment, (ii) increasing enzymatic hydrolysis yields, (iii) decreasing enzyme cost, (iv) reuse of enzymes, (v) genetically modifying microorganisms for efficient fermentation of pentose and hexose sugars, and (vi) producing high value co-products to improve process economics.

3. Syngas fermentation

Syngas fermentation is an indirect conversion process for the production of alcohols, organic acids and other products. Unlike hydrolysis fermentation processes, syngas fermentation is referred to as an indirect fermentation because the feedstocks are not directly fed in the fermentor to form products. Feedstocks are first gasified into syngas, which is then cleaned and cooled before it is fed into the fermentor to make products. Non-food based feedstocks such as agricultural residue, municipal solid wastes, energy crops, coal, and petcoke can be gasified to produce syngas. Syngas is mainly a

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mixture of CO, CO_2, and H_2. However depending on the type of feedstock and gasification system used, small amounts of tars, CH_4, C_2H_2, C_2H_4 , H_2S, NH_3, carbonyl sulfide (COS), hydrogen cyanide (HCN) and nitric oxide are also detected in the syngas (Ahmed et al., 2006; Xu et al., 2011; Xu and Lewis, 2012). Tars can foul equipment and along with other contaminants such as nitric oxide, H_2S and HCN can inhibit growth and enzyme activity. For example, presence of 150 ppm of nitric oxide in the biomass-derived syngas inhibited hydrogenase (H_2ase) activity of *C. carboxidivorans* P7 (Ahmed et al., 2006). However, the same study reported that *C. carboxidivorans* P7 adapted and grew in the presence of tars in the syngas. Although some contaminants such as H_2S and NH_3 can be used as nutrients by syngas fermenting microorganisms, high levels of NH_3 can inhibit growth and enzyme activity. NH_3 in syngas is converted into ammonium ion (NH_4^+) in the fermentation medium and an increase in NH_4^+ in the medium to 0.7 M caused 50% inhibition of H_2ase activity of *Clostridium ragsdalei* (Xu and Lewis, 2012). A review of biomass derived syngas contaminants and suggested gas cleanup technologies are presented in Woolcock and Brown (2013). These include electrostatic separation, filtration, wet scrubbing, adsorption, thermal and catalytic cracking.

In addition to the syngas produced from gasification of biomass, industrial waste gas streams containing CO, CO_2 or H_2 can also be converted by acetogens to biofuels and chemicals. Under anaerobic conditions, acetogens such as *C. ljungdahlii, C. carboxidivorans, A. bacchi* and *C. ragsdalei* serve as biocatalysts (Liou et al., 2005; Liu et al., 2012; Phillips et al., 1994; Wilkins and Atiyeh, 2011).

In syngas fermentation, acetogens metabolize CO, CO_2, and H_2 to alcohols and organic acids. The overall biochemical reactions to convert syngas to ethanol and acetic acid are shown below (Klasson et al., 1990a; Vega et al., 1990).

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \quad (1)$$
$$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O \quad (2)$$
$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (3)$$
$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (4)$$

CO and/or H_2 can supply the electrons used in the enzymatic reactions. However, CO and CO_2 are used as a carbon source. As per the stoichiometry, if only CO is used as the sole carbon and energy source then the carbon conversion efficiency to ethanol will only be 33%, while, 67% of the carbon are lost as CO_2 as per **Eq.1**. However, if both CO and H_2 are utilized then **Eq.1** and **Eq. 2** are combined into **Eq. 5**.

$$3CO + 3H_2 \rightarrow C_2H_5OH + CO_2 \quad (5)$$

When equimolar amounts of CO and H_2 are provided, the maximum carbon conversion efficiency to ethanol increases to 67%. On the other hand, when CO and H_2 are utilized solely to make acetic acid, then the carbon conversion efficiency to acetic acid is 100% as indicated in **Eq. 6**.

$$2CO + 2H_2 \rightarrow CH_3COOH \quad (6)$$

It is important to note that if only CO is utilized to produce acetic acid then only 50% carbon conversion efficiency can be achieved. The carbon conversion efficiency is high when electrons are supplied by H_2 and CO is utilized as the carbon source. However, H_2 utilization decreases because hydrogenase activity is inhibited by CO (Terrill et al., 2012; Ukpong et al., 2012). This results in CO utilization as both carbon and energy source decreasing the overall conversion efficiency of the process (Ahmed and Lewis, 2007). While the stoichiometry provides an estimate of the maximum theoretical yields of products from the substrates, the actual production rates and yields vary depending on the microorganism, gas mixture, medium components and fermentation conditions (Gao et al., 2013; Phillips et al., 2015; Zeikus, 1980).

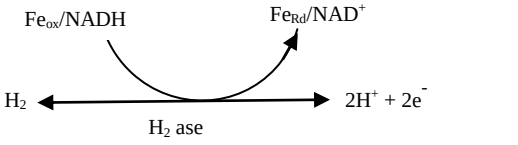
3.1. Biocatalysts

CO can be anaerobically metabolized by photosynthetic, acetogenic, carboxydotrophic, and methanogenic microorganisms to produce hydrogen, methane, acetate, butyrate, ethanol, and butanol as end products (Abrini et al., 1994). Among the different anaerobes, acetogens have been of prime interest due to their ability to grow chemolithotrophically (i.e., use inorganic reduced compounds as energy source) and produce ethanol and butanol along with acetate and butyrate from CO, CO_2, H_2, formate, and methanol (Mohammadi et al., 2011).

Moorella thermoacetica (formerly called *C. thermoaceticum*) is the most extensively studied acetogen (Fontaine et al., 1942). This microorganism was used to determine the acetyl-CoA pathway enzymology in the laboratories of Harland Goff Wood and Lars Gerhard Ljungdahl (Drake et al., 2008). To date, there are more than 100 acetogenic species isolated from a variety of habitats such as sediments, soils, sludge, and intestinal tracts of animals (Drake et al., 2008). Most of the microorganisms currently known to ferment syngas to ethanol are predominantly mesophilic with operating temperatures in the range of 30-40 °C (Munasinghe and Khanal, 2010a). The most widely studied mesophilic microorganisms are *C. aceticum, Acetobacterium woodii, C. ljungdahlii C. carboxidivorans, C. autoethanogenum* and *C. ragsdalei* (Abubackar et al., 2015; Phillips et al., 1993; Phillips et al., 2015; Ukpong et al., 2012; Younesi et al., 2005).

Acetogens metabolize single carbon source compounds via the acetyl-CoA pathway, also called the Wood-Ljungdahl pathway to (i) synthesize acetyl moiety of acetyl-CoA from CO_2, (ii) conserve energy, (iii) assimilate CO_2 to cell carbon (Ljungdhal, 1986; Wood et al., 1986). Acetyl-CoA is a major metabolic intermediate in acetogens and can be utilized to produce ethanol, butanol, hexanol, acetate, butyrate, hexanoate, and cell mass (Phillips et al., 2015). A list of selected syngas fermenting microorganisms, alcohol and organic acid concentrations, ethanol yield from CO and ethanol productivity are shown in **Table 1**.

The Wood-Ljungdahl pathway is a linear and reductive pathway unlike cyclic CO_2-fixing processes such as the Calvin and tricarboxylic acid cycles (Madigan et al., 2003). Acetogens cannot utilize the Calvin cycle that is employed by photosynthetic and chemosynthetic autotrophs because it lacks ribulose diphosphate carboxylase enzyme (Wood et al., 1986). The Wood-Ljungdahl pathway is considered to occur in both oxidation and reduction directions. Conversion of CO_2 to acetate is a reduction process. However, acetate can be converted back to CO_2 through oxidation (Ragsdale, 1997). Acetogens conserve energy by reduction of CO, and/or CO_2, and H_2 to acetate. In the Wood-Ljungdahl pathway, synthesis of the acetyl-CoA occurs through two branches, the methyl branch and carbonyl branch. Acetyl-CoA can then be converted to other products including acetate, ethanol, and cell mass (Drake and Daniel, 2004). The pathway for the conversion of acetyl-CoA to acetate is called acetogenesis and the conversion of acetyl-CoA to ethanol is called solventogenesis. The electrons necessary for the reduction reactions in the pathway come from oxidation of H_2 by hydrogenase and/or from oxidation of CO by carbon monoxide dehydrogenase (CODH) as shown in **Eqs.7** and **8**.



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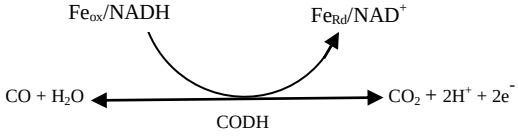

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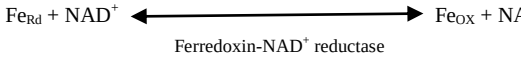

(9)

The reducing power donated by H_2 or CO are carried by electron carrier pairs NADH/NAD^+ , NADPH/$NADP^+$ or ferredoxin (Ljungdhal, 1986) as shown in **Eq. 7** through **Eq. 9**. While electrons are carried by the electron carrier pairs, adenosine triphosphate (ATP) transports the chemical energy within the cells for metabolism. The hydrolysis of the phosphate bonds releases energy and converts ATP to adenosine diphosphate (ADP).

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Table 1.
Alcohol and organic acid concentrations, yields and productivities during syngas fermentation using various biocatalysts.

Biocatalysts	Reactor/gas composition [a]	Products (g/L)	Yield from CO [b](%)	Productivity [c] (mg/L·h)	References
Clostridium ljungdahlii	CSTR with cell recycle (55% CO, 20% H_2, 10% CO_2 and 15% Ar)	Ethanol: 48 Acetate: 3.0	70.2	168.0	(Phillips et al., 1993)
	CSTR without cell recycle (55% CO, 20% H2, 10% CO2 and 15% Ar)	Ethanol: 6.50 Acetate: 5.43	38.9	48.8	(Mohammadi et al., 2012)
	Two stage CSTR & bubble column with cell recycle (60%CO, 35% H_2 and 5% CO_2)	Ethanol: 19.7 Acetate: 8.6	100	306.4	(Richter et al., 2013)
Clostridium carboxidivorans	Bubble column reactor without cell recycle (25% CO, 15% CO_2, 60% N_2)	Ethanol: 1.6 Acetate: 0.4 Butanol: 0.6	39.6	42.7	(Rajagopalan et al., 2002)
	HFR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 24.0 Acetate: 5.0	72.0	112.5	(Shen et al., 2014a)
	Bubble column reactor (20% CO, 15% CO_2, 5% H_2 60% N_2)	Ethanol: 3.2 Acetate: 2.35	51.0	64.1	(Shen et al., 2014b)
	MBR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 4.9 Acetate: 3.1	51.0	97.9	
	Serum bottles (70% CO, 20% H_2, 10% CO_2)	Ethanol: 3.0 Acetate: 0.5 Butanol: 1.0 Hexanol: 0.9	ND [d]	21.4	(Phillips et al., 2015)
Clostridium ragsdalei	CSTR (20% CO, 15% CO_2, 5% H_2, 60% N_2)	Ethanol: 9.6 Acetate: 3.4	60.0	26.7	(Maddipati et al., 2011)
Mixed culture *of Alkalibaculum bacchi* & *C. propionicum*	CSTR without cell recycle (28% CO, 60% H_2, 12% N_2)	Ethanol: 8.0 Acetate: 1.1 Propanol: 6.0 Butanol: 1.1	30.6	40.0	(Liu et al., 2014a)
Clostridium autoethanogenum	CSTR without cell recycle (100% CO)	Ethanol: 0.9 Acetate: 0.9	ND	4.5	(Abubackar et al., 2015)

[a] CSTR: continuous stirred tank reactor; HFR: hollow fiber membrane reactor; MBR: monolithic biofilm reactor
[b] Ethanol yield = (mol EtOH consumed/mol CO consumed)*100%/(1 mol EtOH/6 mol CO)
[c] Ethanol productivity
[d] ND: not determined

The redox reactions involved in the Wood-Ljungdahl pathway to form ethanol, acetate, and cell mass are shown in Figure 3.

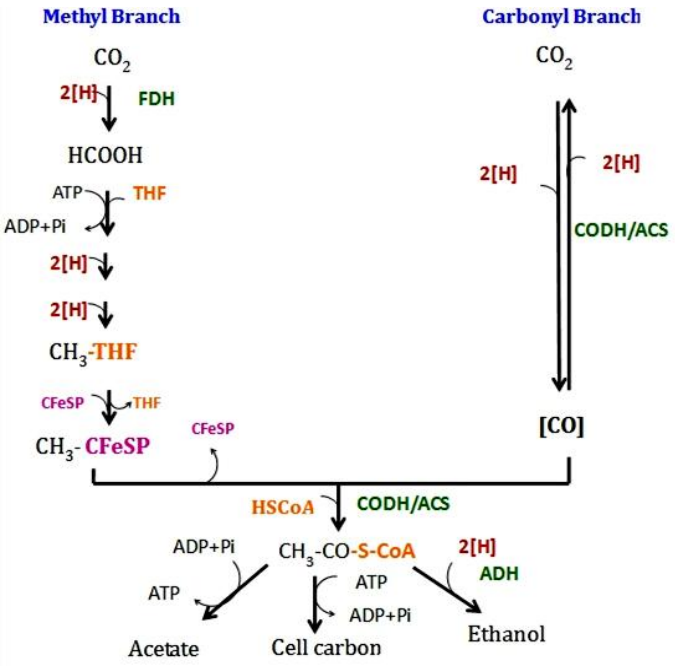

Fig.3. Overview of Wood-Ljungdahl pathway. Green text indicates enzymes, orange text indicates coenzymes and pink text indicates co-protein involved in the metabolic pathway. FDH: formate dehydrogenase; CODH/ACS: bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase; ADH: alcohol dehydrogenase; CFeSP: corrinoid iron(Fe)-sulfur(S) protein; THF: tetrahydrofolate (vitamin B9, folic acid derivative); HSCoA: thiol (SH) functional group Coenzyme A; CH_3-CO-S-CoA: acetyl-Coenzyme A intermediate (Adapted from Drake and Daniel, 2004).

3.2. Advantages and disadvantages

One of the main advantages of syngas fermentation is that it utilizes all the biomass components unlike saccharification fermentation where lignin cannot be fermented (Lewis et al., 2008; Phillips et al., 1994). Syngas fermentation can result in high yields (Bredwell et al., 1999; Vega et al., 1989; Worden et al., 1991). Syngas fermentation also occurs at ambient temperatures and pressures. . Further, microbial catalysts are not poisoned by trace amount of sulfur gases like metal catalysts during chemical conversion processes (Ahmed and Lewis, 2007). In addition, no xenobiotic products are expected to be formed during syngas fermentation (Worden et al., 1991).

The main disadvantages of syngas fermentation are (i) low solubility and mass transfer limitations of the CO and H_2 gaseous substrates, (ii) slow reactions resulting in long residence times, (iii) low metabolic energy is produced when the microorganisms grow on gaseous substrate instead of sugar substrates resulting in slow growth, low cell density and low solvent production (Barik et al., 1988; Vega et al., 1989).

3.3. Process development

A schematic of the hybrid gasification-syngas fermentation process is shown in Figure 4. The hybrid conversion process involves gasification of biomass and other feedstocks followed by fermentation and purification of bioethanol. Industrial flue gases can be directly fed into the fermentor.

Non-food based biomass feedstocks can be partially combusted to produce syngas, which is cleaned and then fed into a fermentor to produce ethanol, acetate, and cell carbon in the presence of acetogenic biocatalysts (Table 1). Various process parameters such as temperature, pH, gas composition, gas partial pressures, medium components, reducing agents, and gas-liquid mass transfer affect the cell growth and product distribution during syngas fermentation (Abrini et al., 1994; Hurst and Lewis, 2010; Munasinghe and Khanal, 2010a). The ability to predict and control the onset of solventogenesis is important for improving ethanol yields and productivity. Even though the effect of some of the above operating parameters on the ethanol yield and productivity using different clostridia species was studied; there are

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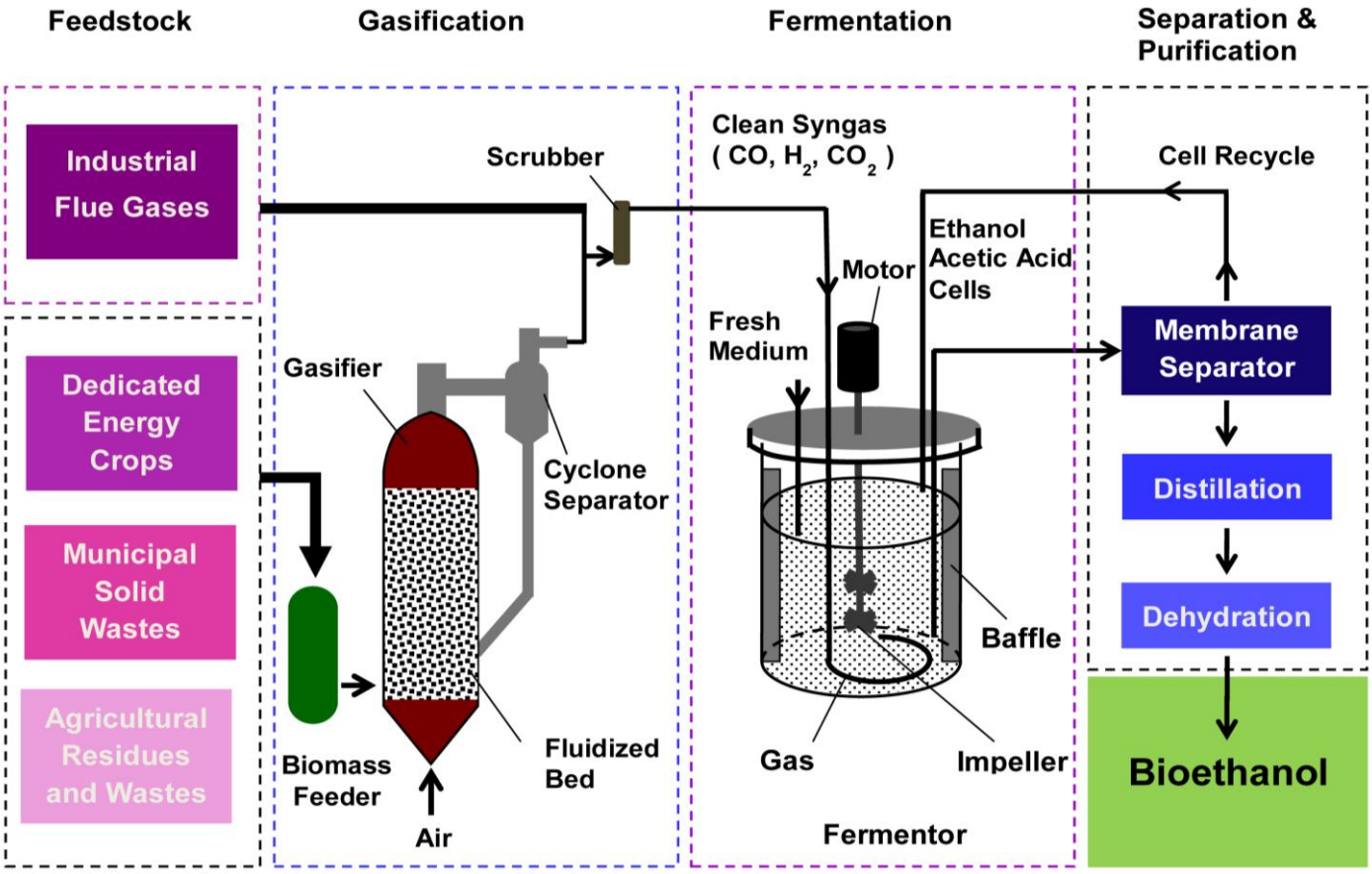

Fig.4. Bioethanol hybrid gasification-syngas fermentation conversion process for the production of ethanol and acetic acid from various feedstocks.

opportunities for further optimization of these parameters to make ethanol production from syngas more feasible at commercial scale.

3.3.1. Temperature

Fermentation temperature impacts the cell growth, enzyme activities and gas solubility. Acetogenic species such as *C. ljungdahlii, C. ragsdalei, C. carboxidivorans,* and *A. bacchi* used in syngas fermentation are mesophiles with an optimum temperature between 37 and 40°C (Gaddy and Clausen, 1992; Huhnke et al., 2010; Liou et al., 2005). However, thermophiles such as *Carboxydocella sporoproducens, Moorella thermoacetica, M. thermoautotrophica* have an optimum temperature between 50 and 80°C (Daniel et al., 1990; Henstra et al., 2007; Savage et al., 1987; Slepova et al., 2006). Thermophilic conditions usually result in reduction of gas solubility, however the rate of gas transfer is considered to increase due to low viscosity of the medium (Munasinghe and Khanal, 2010a).

3.3.2. pH

Fermentative bacteria maintain a pH gradient across the membrane and regulate the internal pH which is essential for stability and functioning of metabolic enzymes (Gutierrez, 1989) . Studies with *C. acetobutylicum* reported that when acetate and butyrate production decrease external pH, acids accumulate inside cells and lower their internal pH to maintain a constant pH gradient (Gottwald and Gottschalk, 1985). However, accumulation of high concentrations of undissociated acid inside the cells stresses them and decreases the pH gradient. Thus, the cells counteract by producing solvents (Ahmed, 2006; Gottschal and Morris, 1981; Gottwald and Gottschalk, 1985).

In syngas fermentation, the external pH in the fermentation medium is a widely studied physiological parameter to optimize cell growth and solvent production. The optimum external pH range for cell growth of most of the syngas fermenting microbes usually varies from 5.5 to 6.5 (Abrini et al., 1994; Liou et al., 2005; Tanner et al., 1993). The optimum external pH for solvent production was reported to be around 4.5 to 4.8 (Ahmed et al., 2006; Sakai et al., 2004; Worden et al., 1991). Recently a moderately alkaliphilic bacterium called *A. bacchi* has shown capabilities to grow on syngas at an optimum pH between 8 and 8.5 and produce ethanol at pH range between 6.5 and 7 (Allen et al., 2010; Liu et al., 2012). In syngas fermentation studies, the changes in external pH were correlated with the substrate metabolism and release of metabolic by-products (Devi et al., 2010; Hu, 2011; Kundiyana et al., 2011b; Liu et al., 2014a). However, future studies to understand how internal pH changes and the pH gradient across the cell membrane effect syngas fermentation are important to improve solvent production and reduce acid stress on cells.

3.3.3. Gas partial pressure

The concentration of CO in syngas has a significant impact on the overall process efficiency and utilization of other syngas components (namely CO_2 and H_2). Hu (2011) reported that electron production from CO is thermodynamically favorable compared to H_2 independent of pH, ionic strength and gas partial pressure. In a syngas fermentation using *C. carboxidivorans,* the increase of CO partial pressure from 35.5 to 70.9 kPa and from 35.5 to 202.7 kPa was reported to decrease hydrogenase activity by 84% and 97 %, respectively (Hurst, 2005). In addition, CO partial pressure of 8.5 kPa was reported to inhibit hydrogenase activity in *C. ragsdalei* cells by 90% (Skidmore, 2010).

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The decrease in hydrogenase activity and H_2 utilization results in a decrease in overall gas conversion efficiency. However, a study on effect of CO partial pressure using CO:CO_2 (molar ratios of 1.7 to 4) gas mixture without H_2 reported that *C. carboxidivorans* switched from non-growth related to growth related ethanol production and grew 440% more when the partial pressure of CO was increased from 35.5 to 202.79 kPa (Hurst and Lewis, 2010). When fructose in the medium was replaced with CO , *C. carboxidivorans* was reported to shift the molar ethanol to acetate ratio from 0.3 to 8 (Liou et al., 2005). The presence of CO, which may have acted as an effective electron source, enabled ethanol production rather than acetate production by acetogens (Tanner, 2008).

It should be noted that syngas produced during gasification contains H_2 along with CO and CO_2. Thus ideally, for high product yields and efficient gas utilization, CO and CO_2 should be used as carbon source and H_2 should be used as the sole electron source (Hu et al., 2011; Skidmore, 2010). In a batch culture with *C. ljungdahlii*, when the total pressure of syngas was varied from 81.1 to 182.4 kPa, the ethanol to acetate molar ratio of 5:1 was achieved at total syngas pressure of 162.1 and 182.4 kPa (Najafpour and Younesi, 2006; Younesi et al., 2005). Younesi et al. (2005) reported that H_2 and CO_2 consumption occurred after CO was exhausted indicating CO as a preferred substrate for cell growth.

3.3.4. Medium components

Fermentation medium components such as vitamins, minerals, and metals act as cofactors or coenzymes that are necessary for enzymes to catalyze biochemical reactions (Phillips et al., 2014; Zabriskie and Mill, 1988). Additionally, syngas fermentation medium is often supplemented with yeast extract (YE) to provide the amino acids and nitrogenous compounds necessary for cell synthesis and with buffer solutions (such as 2-(N-morpholino)ethanesulfonic acid and [N-tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid) to maintain the medium pH (Liu et al., 2012; Saxena, 2008; Tanner et al., 1993). The addition of YE and buffer solution would be expensive and uneconomical for commercial syngas fermentation (Gao et al., 2013). Several studies were reported on the optimization of the nutrients for ethanol production using syngas fermentation. Studies with *C. ljungdahlii* showed that reducing or completely removing YE from fermentation medium increased ethanol concentration from 1 g/L to 48 g/L (Phillips et al., 1993; Vega et al., 1989). The increase in the concentrations of Ni^{2+}, Zn^{2+}, SeO_4^- and WO_4^- from 0.84 µM, 6.96 µM, 1.06 µM and 0.68 µM to 8.4 µM, 34.8 µM, 5.3 µM and 6.8 µM, respectively, improved ethanol production by *C. ragsdalei* by fourfold (Saxena and Tanner, 2011).

In another study with *C. ragsdalei*, limiting calcium pantothenate, vitamin B_{12} and cobalt chloride in two-stage continuous bioreactor resulted in 15 g ethanol/g cell compared to 2.5 g ethanol/g cell in a single-stage bioreactor (Kundiyana et al., 2011a). Standard YE medium was replaced with defined minimal medium, cotton seed extract (CSE) and corn steep liquor (CSL) to reduce medium cost and improve ethanol production (Gao et al., 2013; Kundiyana et al., 2010; Maddipati et al., 2011; Phillips et al., 2014). CSL medium, which is rich in vitamins, minerals and amino acids was shown to produce 40% more ethanol using *C. ragsdalei* (Maddipati et al., 2011). Also, the use of a completely defined minimal medium was shown to result in 36% higher ethanol yield than in standard YE medium at 5% of the cost of the YE medium (Gao et al., 2013).

3.3.5. Reducing agents

Reducing agents are artificial electron carriers that alter NADH/NAD^+ ratio. Reducing agents significantly decrease the redox potential of the fermentation medium (Frankman, 2009). Redox potential is a fermentation parameter that defines the ability of the solution to undergo oxidation reduction reaction (IFIS, 2009). In syngas fermentation using *C. ragsdalei*, a decreasing trend of redox potential during cell growth and increasing trend of redox potential during ethanol production was reported (Kundiyana et al., 2010; Maddipati et al., 2011). Solventogenesis is an electron intensive process that requires high levels of NADH (Rao et al., 1987). Addition of reducing agents was reported to increase the NADH levels in cells and direct electron flow towards ethanol production (Babu et al., 2010; Sim and Kamaruddin, 2008). Reducing agents such as neutral red were also reported to increase the activity of aldehyde dehydrogenase and alcohol dehydrogenase, which catalyze the aldehyde and ethanol production from acetyl-CoA intermediate (Girbal et al., 1995).

The addition of methyl viologen to the fermentation broth of *Thermoanaerobacter ethanolicus* and *C. acetobutylicum* resulted in the onset of ethanol production from glucose (Rao and Mutharasan, 1986; Rao et al., 1987). The addition of neutral red was reported to increase the activity of alcohol dehydrogenase and ethanol production from syngas by *C. carboxidivorans* (Ahmed et al., 2006). The addition of methyl viologen and dithiothreitol to fermentation medium with *C. ragsdalei* also showed enhancement in ethanol production (Babu et al., 2010; Panneerselvam et al., 2009).

3.4. Bioreactor design

A bioreactor should provide a controlled environment to enhance cell growth, substrate conversion and productivity of the biological process, and minimize the overall cost of production of desired products (Wilkins and Atiyeh, 2012). Continuous stirred tank reactors, bubble columns, packed columns, air-lift, trickle beds and hollow fiber reactors are some of the bioreactor configurations studied for alcohol production using syngas fermentation (Datar et al., 2004; Hickey et al., 2011; Kimmel et al., 1991; Kundiyana et al., 2010; Mohammadi et al., 2012; Shen et al., 2014a). Further, these reactors can be operated in different fermentation modes such as batch, fed-batch, continuous with and without cell recycle (Cotter et al., 2009; Grethlein et al., 1991; Lewis et al., 2007; Maddipati et al., 2011; Phillips et al., 1993). Klasson et al. (1990a) used two STRs in series and reported a 30 fold increase in ethanol productivity using *C. ljungdahlii*. Bredwell and Worden (1998) showed that the use of a microsparger in a STR for production of acetate, ethanol and butyrate by *Butyribacterium methylotrophicum* increased the mass transfer by six times with 50% of the flow rate used without a microsparger. The highest ethanol concentration of 48 g/L was produced in a continuous syngas fermentation in a CSTR with cell recycle (Phillips et al., 1993). A list of reactors used for syngas fermentation, syngas composition, ethanol yield and productivity is shown in **Table 1**.

In addition, higher ethanol production (20-24 g/L) was achieved in a two stage CSTR and bubble column with cell recycle and in the hollow fiber membrane reactor (HFR) with biofilm formation (Richter et al., 2013; Shen et al., 2014a). The increase in cell mass density and mass transfer increased ethanol production. However, bubble columns and monolithic biofilm bioreactor only produced about 3 g/L ethanol (Shen et al., 2014b).

Efficient syngas fermentation bioreactor designs should (i) provide gas-liquid mass transfer that balances the cells' kinetic requirement without inhibiting the cells' metabolic activity, (ii) sustain biocatalyst viability and high concentration, (iii) reduce operating and maintenance cost, (iv) be easily scaled up.

The ability to maintain high cell concentrations and high gas transfer rates in the reactor enhances productivity and reduces required reactor size. Gas-liquid mass transfer can limit the rate of syngas fermentation due to the low solubility of CO and H_2 in fermentation medium (Bredwell et al., 1999). The rate of mass transfer (*dn/dt*) is given as follows (Sherwood et al., 1975):

$$\frac{1}{V}\cdot\frac{dn}{dt} = -k_L a\cdot(C_i - C_L) \quad (10)$$

where, *dn/dt* is the rate of mass transfer (*mmol/h*); k_La is the overall mass transfer coefficient (h^{-1}); C_i is the concentration of the gas in gas liquid interface (*mmol/L*); C_L is the concentration of gas in the bulk liquid (*mmol/L*) and *V* is the working volume of the reactor (*L*). The rate of gas transfer can be increased by either increasing the mass transfer coefficient (k_La) or by increasing the driving force (C_i-C_L). The driving force can be increased by operating the reactor at high CO partial pressures (Klasson et al., 1993b). However, high concentrations of CO could be inhibitory to the microorganisms (Munasinghe and Khanal, 2010a). The mass transfer limiting conditions occur when the concentration of CO in the liquid is zero, at which the reaction rate is a function of the gas transfer rate.

Mass transfer characteristics of various reactor configurations have compared by many researchers (Bredwell and Worden, 1998; Cowger et al., 1992; Jones, 2007; Klasson et al., 1990b; Klasson et al., 1991; Klasson et al., 1993a; Munasinghe and Khanal, 2010b; Munasinghe and Khanal, 2014; Orgill et al., 2013; Riggs and Heindel, 2006; Shen et al., 2014a; Yasin et al., 2014). In a STR, the mass transfer coefficient can be increased by increasing

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the agitation speed or the gas flow rate (Orgill et al., 2013). However, using high gas flow rates decreases the gas conversion efficiency. The increase in agitation speed has been widely used to increase the k_La in STRs. The hydrodynamic shear generated by the impeller reduces the bubble size and increases the interfacial area for mass transfer (Bredwell et al., 1999). However, the use of high agitation speed increases the power requirement for large reactors.

Ungerman and Heindel (2007) reported a dual impeller scheme with axial flow impeller at the top and lower concave impeller that resulted in a similar k_La and less power requirement compared to Rushton impellers. Bredwell and Worden (1998) used a microsparger that was shown to be energy efficient and increased the k_La by six fold compared to conventional gas sparging. In the case of an air lift reactor, the use of a 20 µm bulb diffuser was reported to provide higher mass transfer coefficient (91 h^{-1}) than air lift reactor configurations with column diffusers, gas spargers with mechanical mixing (Munasinghe and Khanal, 2010b). Also, it was claimed that due to the simple reactor configuration and low energy requirements, the scale up of air lift reactors with a 20 µm bulb diffuser will be easy and cheap compared to a conventional STR (Munasinghe and Khanal, 2010b).

Performances of different syngas fermentation reactors were compared during the production of hydrogen and methane using a mixed culture of *R. rubrum*, *M. formicicum* and *M. barkeri* (Klasson et al., 1990b; Klasson et al., 1991; Klasson et al., 1992). The TBR was reported to have better CH_4 productivity, CO gas conversion and mass transfer capabilities than the packed bubble column reactor (PBR). The mass transfer coefficients of 3.5 h^{-1} and 780 h^{-1} were reported for PBR and TBR, respectively (Klasson et al., 1990b). The TBR showed better mass transfer capabilities than PBR and STR for the production of acetate from syngas by *P. productus*.

A comparison between STR, TBR and five different HFR modules showed that the polydimethylsiloxane (PDMS-HFR) provided better gas liquid mass transfer (1063 h^{-1}) followed by the TBR (421 h^{-1}) and STR (114 h^{-1}) (Orgill et al., 2013). In addition, the use of 0.3 wt% methyl-functionalized silica nanoparticles was reported to enhance the mass transfer of syngas components into the medium leading to a significant increase in the levels of biomass, ethanol and acetic acid production (Kim et al., 2014). Besides assessing the mass transfer capabilities of different reactor configurations, researchers have recently focused on developing new techniques to measure the dissolve concentrations of CO and H_2 gases in the liquid phase to determine the CO and H_2 mass transfer coefficients (Munasinghe and Khanal, 2014).

An accurate and reliable technique would be essential to adjust the fermentation parameters (such as agitation speeds, gas and liquid flow rates) in order to meet the cells kinetic requirement. The increase in gas flow rate beyond cells kinetic requirements would decrease gas conversion efficiency, while increasing agitation speed and liquid flow rate would have detrimental effects on the cell viability and costs associated with power consumption in large-scale reactors.

3.5. Commercialization and future prospective

LanzaTech, Coskata, and INEOS Bio are among the companies that are currently pursuing commercialization of syngas fermentation for biofuels production (Liew et al., 2013). Coskata has a fully integrated demonstration facility in Madison, Pennsylvania (USA) and has recently isolated and patented a new strain *C. coskatii* (Zahn and Saxena, 2012). The company is focusing on fermentation of syngas produced from natural gas reforming or gasification of wood and coal (Coskata, 2011).

INEOS Bio has operated the first commercial cellulosic ethanol and power generation facility using syngas fermentation technology in Vero Beach, Florida (USA) since July 2013 (INEOS, 2013). However, soon after the commissioning of the plant it was stopped due to the very high sensitivity of the microorganisms to hydrogen cyanide in the syngas produced during gasification of vegetative matter (Lane, 2014). The company is currently installing scrubbers to reduce the hydrogen cyanide concentrations from 15 ppm to less than 5 ppm (Lane, 2014). The company utilizes patented bacteria to produce ethanol and generate power from vegetative and woody waste. The company was projected to produce 8 million gallons of ethanol per year and generate 6 MW of renewable electricity (INEOS, 2013).

LanzaTech is a New Zealand based company that utilizes CO-rich flue gases from steel making industries to produce ethanol using its proprietary *Clostridial* biocatalyst. It has a pilot plant facility in Glenbrook, New Zealand and a demonstration facility in Shanghai, China that has an operating capacity of 100,000 gallons ethanol per year. LanzaTech reported to expands to production of more products through syngas fermentation (LanzaTech, 2015).

The future of syngas fermentation technology depends on production of high value products beyond ethanol. Ethanol's low heating value, miscibility with water and inability to use the existing infrastructure for fuel transportation are just a few of the disadvantages that led to the focus towards advanced biofuels such as butanol and hexanol.

In addition, discovering new microorganisms, processes, and strain development, including synthetic biology are required to utilize the biological gas conversion technology to produce fuels and biobased products. Recent research indicates production of advanced biofuels such as butanol and hexanol from CO, CO_2 and H_2 through medium optimization (Phillips et al., 2015). Several studies also reported production of higher alcohols such as isopropanol, butanol, and hexanol using syngas fermentation (Liu et al., 2014b; Maddipati et al., 2011; Rajagopalan et al., 2002; Ramachandriya et al., 2011; Worden et al., 1991). In presence of CO as a reductant, *C. formicaceticum* and *M. thermoacetica* were reported to reduce acids to their corresponding alcohols (Fraisse and Simon, 1988; White et al., 1987). *C. acetobutylicum* was also shown to directly reduce acetate and butyrate to corresponding alcohols (Hartmanis et al., 1984).

It was recently reported that mono-cultures of *C. ljungdahlii* and *C. ragsdalei* as well as a mixed culture of *A. bacchi* and *C. propionicum* were able to convert added acids such as propionic, butyric, and hexanoic acids to their respective alcohols (Liu et al., 2014b; Perez et al., 2013). Additional development and optimization of biological gas conversion processes are expected to result in production of various products besides biofuels at commercial scale in the near future.

4. Conclusions

The production of ethanol using diverse conversion technologies and various renewable non-food feedstocks marks the beginning of sustainable energy future. Production of ethanol from sustainable non-food feedstocks in first generation biorefineries has been recently deployed at commercial scale. Biological conversion processes including hydrolysis-fermentation and syngas fermentation have been developed for the production of ethanol. Various process configurations are possible in the hydrolysis-fermentation route. Syngas fermentation is an indirect conversion process for production of alcohols and chemicals from CO, CO_2, and H_2. Advancement in metabolic engineering, strain and process development of syngas fermentation resulted in production of new products from syngas and enhanced product selectivity, productivity, and yields. Further research efforts should be focused on utilization of different types of non-food feedstocks, process integration, metabolic engineering, and discovering new highly productive microorganisms. Ultimately, the reduction in biofuels production cost improves their feasibility to become a viable alternative to fossil fuels.

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