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Attached-growth bioreactors for syngas fermentation to biofuel

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Attached-growth bioreactors for syngas fermentation to biofuel

by

Yanwen Shen

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Iowa State University
Ames, Iowa
2013

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Lignocellulosic biomass is a renewable resource for sustainable production of biofuels and chemicals. Syngas fermentation, a hybrid process integrating the thermochemical (i.e. gasification of feedstock to syngas) and biochemical (i.e. microbial fermentation of syngas) conversions, has been considered as a promising technology for production of lignocellulosic-biomass-derived ethanol. The challenge to commercialize syngas fermentation, though, is to enhance the gas-to-liquid mass transfer rate due to the low solubilities of carbon monoxide (CO) and hydrogen (H₂) in an energy-efficient manner. Conventional suspended-growth bioreactors, such as continuous stirred tank reactor (CSTR) and bubble column reactor (BCR), suffer from inefficient mass transfer and unwanted cell washout at high dilution rate, resulting to low productivities. The present study explored the feasibility of applying three innovative attached-growth bioreactor systems, hollow fiber membrane biofilm reactor (HFM-BR), monolithic biofilm reactor (MBR) and rotating packed bed biofilm reactor (RPB-BR), in continuous syngas fermentation, in order to enhance mass transfer of CO and to maximize ethanol production, by optimizing selected operational parameters.

The highest ethanol productivity of HFM-BR, MBR and RPB-BR was achieved at 3.44 g/L/day, 2.35 g/L and 6.70 g/L/day with optimized fermentation operational condition, respectively. HFM-BR showed the highest CO $k_{L\alpha}$ (1096.2 hour⁻¹) among the three bioreactor systems; however, the costly membrane and biofouling issue are the drawbacks to conduct continuous syngas fermentation with high ethanol productivity for extended period of time. MBR showed modest performance of CO mass transfer rate and ethanol productivity, but it has inherent advantages such as high mechanical strength and less biofouling problem. With
installation of an in-situ washing device, the microchannel-clogging problem could potentially be resolved, indicating its capability of extended periods of continuous fermentation. RPB-BR gave the highest ethanol productivity with a simple mechanical design, inexpensive packing media and stable operation. The present study demonstrated the great potential of attached-growth bioreactors as efficient systems to obtain syngas fermentation with high productivity of ethanol, making cellulosic ethanol biorefinery move one step forward to technical and economic feasibility. Ultimately, it is believed that this study will contribute to our nation’s independence from petroleum fuels.
CHAPTER 1. GENERAL INTRODUCTION

1. Introduction

Lignocellulosic biomass is the most abundant biorenewable resource on earth (Ragauskas et al. 2006; Zhang et al. 2007; Zhang et al. 2006) with the great potential for sustainable production of biofuels and chemicals. This biomass is composed of sugar-based polymers (cellulose, hemicellulose) and an aromatic polymer (lignin). There are two conventional routes for conversion of lignocellulosic feedstock into biofuels, particularly ethanol (cellulosic ethanol): the biochemical route and the thermochemical route. The biochemical conversion generally involves three sequential steps: (1) feedstock size reduction and pretreatment to disrupt lignin wrapping and to extract sugar-based cellulose and hemicellulose, thus making them more accessible to enzymes; (2) enzymatic hydrolysis, by which cellulose and hemicellulose are hydrolyzed to fermentable sugars (e.g. glucose, xylose); and (3) sugar fermentation to desired biofuels (e.g. ethanol, butanol) or bio-based chemicals (e.g. lactic acid, succinic acid). In the thermochemical route, biomass is firstly gasified into synthesis gas (syngas) and then syngas is converted into fuels and chemicals performed by using metal catalysts known as Fischer-Tropsch process or by using biocatalysts (microorganisms) known as syngas fermentation. Syngas is a flammable gas mixture primarily composed of carbon monoxide (CO), hydrogen (H₂) and carbon dioxide (CO₂).

As a hybrid thermo/biochemical process, syngas fermentation is a promising technology for cellulosic ethanol production and has many inherent advantages compared to the biochemical route and Fischer-Tropsch process (Bredwell et al. 1999; Munasinghe and Khanal 2010a), such as:
(1) the whole components of the lignocellulosic biomass, including sugar-based polymers and lignin, can be converted into syngas as an intermediate for subsequent microbial fermentation;

(2) it eliminates an extensive pretreatment procedure involving heat and fine chemicals for reducing the recalcitrant structure;

(3) it does not need costly enzymes for hydrolysis;

(4) it is very flexible with the syngas composition and does not require a specific CO to H₂ ratio as Fischer-Tropsch process does;

(5) compared to metal catalysts, microorganisms have remarkably high product-specificity;

(6) compared to metal catalysts, most syngas-fermenting microorganisms have much higher tolerance to sulfur contaminants in syngas, such as hydrogen sulfide (H₂S), carbonyl sulfide (COS) and sulfur dioxide (SO₂), thus reducing the risk of catalysts poisoning;

(7) syngas fermentation is usually operated under ambient condition; in other words, no high temperature or high pressure is needed.

2. Microbiology of syngas fermentation

A wide diversity of microorganisms can be involved in syngas fermentation with their capabilities of utilizing CO and/or CO₂/H₂ as the metabolic building block, including both aerobic and anaerobic species. Aerobic carboxydotrophic bacteria can grow on CO as the sole carbon and energy source via molybdopterin-carbon monoxide dehydrogenase (Mo-CODH), an enzyme catalyzing CO oxidation with oxygen as an electron acceptor (Meyer and Schlegel 1983). Since the turnover number of Mo-CODH is almost 1000-fold lower than that of anaerobic nickel-containing CODH (Ni-CODH) enzymes (Gnida et al. 2003), carboxydotrophic bacteria
are applied in CO detoxification in environment. From a biofuel prospect, we are more interested in those anaerobic acetogenic, hydrogenogenic and methanogenic microorganisms (Table 1) capable of producing a variety of fuels and chemicals from syngas.

3. Biological reactions, metabolic pathway and energy conservation of syngas fermentation

3.1 Biological water-gas shift reaction

A group of hydrogenogenic bacteria catalyze biological water-gas shift (BWGS) reaction, in which CO reacts with water to produce H$_2$ and CO$_2$. In anaerobic environment, electrons and protons are derived from CO oxidization (equation 1) catalyzed by Ni-CODH enzyme; protons are subsequently reduced to molecular hydrogen by the CO-tolerant hydrogenase coupled with electron capture (equation 2). Meanwhile, ATP synthesis is driven by ATP synthase, via which protons are translocated across the cytoplasmic membrane.

$$\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{e}^- + 2\text{H}^+ \quad (1)$$

$$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2 \quad (2)$$

Overall, the combined activities of two enzymes (Ni-CODH and CO-tolerant hydrogenase) lead BWGS reaction (equation 3). The produced CO$_2$ is partially assimilated into biomass and cell components and the remaining CO$_2$, along with H$_2$ are released from the reacting system.

$$\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2 \quad \Delta G^\circ (298.15 \text{ K}) = -20.1 \text{ kJ/mol} \quad (3)$$

Among BWGS-catalyzing hydrogenogenic bacteria (Table 1), two purple nonsulfur bacteria, *Rhodospirillum rubrum* and *Rubrivivax gelatinosus*, have been extensively studied because they provide great potential industrial applications: H$_2$ yielding ability via CO-metabolism and intracellular accumulation of polyhydrobutyrate (PHB) (Do et al. 2007), a
polymer precursor for biodegradable plastic production. Under anaerobic conditions, these microorganisms are able to grow out in the dark using CO as the sole carbon and energy source (Kerby et al. 1995). In the presence light, the photo-reactive pigments contained in the biomass enable absorption of light energy and conversion to chemical potential, which is then involved in CO-H₂ metabolism.

3.2 Reductive acetyl-CoA pathway

Reductive acetyl-CoA pathway is one of the five pathways for autotrophic CO₂ fixation (Thauer 2007), which is also known as “Wood-Ljungdahl pathway” in recognition of Dr. Harold G. Wood and Dr. Lars G. Ljungdahl, two pioneers who elucidated the biochemistry and enzymology of CO/CO₂ fixation by anaerobic acetogenic bacteria (Hu et al. 1982; Ljungdahl 1986; Pezacka and Wood 1984). Although the pathway can be presented in a cyclic form (Ragsdale and Pierce 2008), reductive acetyl-CoA pathway is the only linear process where CO₂ is not fixed in a cyclic mode depending on multi-carbon intermediates. The reductive acetyl-CoA pathway is a terminal electron-accepting process providing a mechanism for assimilation of CO₂ into biomass (Ljungdahl 1986). Acetyl-CoA serves a precursor for cellular molecules and ATP and it can be biologically synthesized from CO and/or CO₂ in the methyl and carbonyl branch of reductive acetyl-CoA pathway (Henstra et al. 2007b; Kopke et al. 2011a; Wilkins and Atiyeh 2011).

The methyl branch (Figure 1) involves various enzymes for a 6-electron reduction of CO₂ to a methyl group (Ljungdahl 1986; Ragsdale 2004; Ragsdale and Pierce 2008), whereas CODH reduces CO₂ to CO in the carbonyl branch. Down the methyl branch, formate dehydrogenase catalyzes the reduction of CO₂ to formate, which is fused with tetrahydrofolate (THF) by formyl-
THF synthase, yielding formyl-THF coupled with ATP consumption. Then formyl-THF is reduced to methyl-THF by a series of THF-dependent enzymes including methenyl-THF cyclohydrolase, methylene-THF dehydrogenase and methylene-THF reductase, with methenyl-THF and methylene-THF as intermediates. A methyltransferase subsequently binds the methyl-THF to a corrinoid iron-sulfur protein to form the methyl group. Acetyl-CoA is eventually formed when the methyl group is fused with CO molecule and coenzyme A (CoA) by the activity of the bifunctional CODH/ACS enzyme (Ragsdale and Pierce 2008).

The reducing equivalents required for CO₂ fixation through acetyl-CoA pathway come from either H₂ (Figure 1) under autotrophic growth or NADH/Fd₆red (reduced ferredoxin) under heterotrophic growth (Muller 2003). Most syngas-fermenting acetogens can utilize CO for acetyl-CoA buildup (Table 1) since CO serves as both carbon and energy source (Figure 1). The redox potential of CO₂/CO was reported as -524 ~ -558 mV at pH 7.0 (Grahame and DeMoll 1995; Thauer 1990), providing extremely low potential electrons for reducing cellular electron carriers like ferredoxin and flavodoxin, which indicates that CO is approximately 1000-fold more potent than NADH (Ragsdale 2004).

As a key precursor, acetyl-CoA is further utilized by acetogens for cell mass development and synthesis of metabolic products such as acetate and ethanol (Table 1). Other products (e.g. lactate, n-butyrate, n-butanol, 2,3-butanediol) can also be produced using native or genetically engineered acetogens according to the main interest of syngas fermentation. The overall stoichiometric reactions of acetate and ethanol formation are listed as in equation (4) ~ (10):

\[
\begin{align*}
4\text{CO} + 2\text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COOH} + 2\text{CO}_2 & \Delta G^\circ (298.15 \text{ K}) = -148.7 \text{ kJ/mol} \\
6\text{CO} + 3\text{H}_2\text{O} & \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 4\text{CO}_2 & \Delta G^\circ (298.15 \text{ K}) = -217.9 \text{ kJ/mol} \\
2\text{CO} + 2\text{H}_2 & \rightarrow \text{CH}_3\text{COOH} & \Delta G^\circ (298.15 \text{ K}) = -108.5 \text{ kJ/mol}
\end{align*}
\]
\[
\begin{align*}
3\text{CO} + 3\text{H}_2 & \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 & \Delta G^\circ (298.15 \text{ K}) = -157.6 \text{ kJ/mol} \\
2\text{CO} + 4\text{H}_2 & \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} & \Delta G^\circ (298.15 \text{ K}) = -137.5 \text{ kJ/mol} \\
2\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} & \Delta G^\circ (298.15 \text{ K}) = -68.3 \text{ kJ/mol} \\
2\text{CO}_2 + 6\text{H}_2 & \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 3\text{H}_2\text{O} & \Delta G^\circ (298.15 \text{ K}) = -97.3 \text{ kJ/mol}
\end{align*}
\]

It should be noted that acetate and ethanol can be produced with CO as the sole carbon and energy source (equation 4, 5), or with CO as the carbon source and CO/H\(_2\) as energy source (equation 6,7,8) or with CO\(_2\) as carbon source and H\(_2\) as energy source (equation 9, 10). Thus, the theoretical acetate or ethanol yield from CO or CO\(_2\) is dependent on the composition of syngas mixture. For example, only one third of carbon consumed in CO can theoretically be converted to ethanol while the remaining two third of carbon is lost in the form of CO\(_2\) formation when CO is the only substrate utilized as the sole carbon and energy source (equation 4). When H\(_2\) is involved in the reaction with H\(_2\) to CO molar ratio of 2 (equation 8), the theoretical ethanol yield from CO is 100% based on carbon mass balance. In this sense, it is preferable to obtain reducing equivalent from H\(_2\) and not to sacrifice CO for energy generation, in which way CO can be incorporated into the desired fuels and chemicals. Excess H\(_2\) supply has been demonstrated to improve ethanol production in *Clostridium ljungdahlii* syngas fermentation (Gaddy et al. 2007), whereas hydrogen consumption was found to significantly affect ethanol and acetate yields in *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11 syngas fermentation (Ahmed and Lewis 2007; Skidmore et al. 2013).

Energy conservation mode in reductive acetyl-CoA pathway is still not completely understood. Acetate production via acetyl-CoA pathway is balanced in ATP, as one unit of ATP produced by substrate-level phosphorylation in acetate kinase reaction with one unit of ATP consumed for formyl-THF formation (Figure 1). Therefore, membrane-associated electron
transport has been elucidated as the mechanism of energy conservation in acetogens carrying out acetyl-CoA pathway. Cytochrome and menaquinone in H⁺-dependent acetogens such as *Moorella thermoacetica* and *Moorella thermoautotrophica*, have been found to generate a membrane-crossing proton gradient via a H⁺F₁F₀ ATPase for ATP synthesis, together with ferredoxin, flavodoxin, flavoproteins and rubredoxin (Das and Ljungdahl 2003; Gottwald et al. 1975; Ivey and Ljungdahl 1986). According to its genome sequence (Pierce et al. 2008), it was predicted that *Moorella thermoacetica* contains membrane-associated energy-conserving enzyme complex catalyzing the reduction of H⁺ to H₂ with reduced ferredoxin. Recently the reversible electron-bifurcating [Fe-Fe]-hydrogenase complex (HydABC) was found to be involved in energy metabolism of *Moorella thermoacetica* (Huang et al. 2012; Wang et al. 2013) and electron bifurcation was hypothesized as the energy conservation mechanism. Nevertheless, no experimental evidence has been available to support this hypothesis.

A different mechanism was discovered for Na⁺-dependent acetogens which lack a membranous electron translocation system containing cytochromes. Volker Müller and his research group investigated the energetics of *Acetobacterium woodii* as a model of Na⁺-dependent acetogens and firstly proposed that the methyltransferase transferring methyl from methyl-THF to CODH/ACS via CoFeS-protein is a membrane-bound, Na⁺-translocating enzyme complex, serving as a Na⁺ extrusion site for ATP synthesis by means of a Na⁺F₁F₀ ATPase, and their work has been reviewed elsewhere (Müller, 2003). However, the discovery of a ferredoxin : NAD⁺-oxidoreductase, the Rhodobacter nitrogen fixation (Rnf) complex, in *Acetobacterium woodii* as a novel coupling site (Muller et al. 2008), might shed light on the long-sought-after mechanism of bioenergetics in acetyl-CoA pathway. The recent researches on genome sequencing of *Acetobacterium woodii* (Biegel et al. 2009; Poehlein 2012) revealed that
Rnf complex is probably the only ion-pumping enzyme coupled to the *Acetobacterium woodii* acetyl-CoA pathway: with ferredoxin (Fd) as a central electron carrier, the entire metabolism is optimized to maximize $\text{Fd}^2/\text{NAD}^+$ ratio for powering the Rnf complex and to overcome the energy barriers by using soluble electron bifurcating [Fe-Fe]-hydrogenase, methylene-THF and a hydrogen-coupled formate dehydrogenase. *Acetobacterium woodii* harbors an energy-conserving Rnf complex with six (A, B, C, D, E, G) subunits (Biegel and Muller 2010) but not an energy-converting [NiFe]-hydrogenase complex (Poehlein 2012).

Despite that *Moorella thermoacetica* and *Acetobacterium woodii* have been studied as two model organisms in elucidating the energy conservation coupling with acetyl-CoA pathway, *Clostridium ljungdahlii* may represent a third model of homoacetogenic metabolism. Na$^+$-translocating ATPase is not present in *Clostridium ljungdahlii* (Kopke et al. 2010). Furthermore, although its genes encode H$^+$-translocating ATPase, *Clostridium ljungdahlii* does not contain any membrane-bound cytochromes or quinones to generate a proton gradient (Kopke et al. 2010). However, *Clostridium ljungdahlii* harbors genes encoding the Rnf-complex, functioning as a proton translocation pump, which has been proposed to be a possible means of energy conservation (Kopke et al. 2010). A recent functional in vivo study demonstrated that the Rnf complex of *Clostridium ljungdahlii* is a proton-translocating ferredoxin:NAD$^+$ oxidoreductase as the only proton pump to generate the proton-motive force necessary for ATP synthesis during autotrophic growth on CO$_2$/H$_2$ (Tremblay et al. 2012). Therefore, unlike *Acetobacterium woodii* containing an Rnf complex functioning as a Na$^+$-motive ferredoxin:NAD$^+$ oxidoreductase generating a Na$^+$ gradient across the membrane, or *Moorella thermoacetica* containing membrane-bound cytochromes or quinones generating a proton potential, *Clostridium ljungdahlii* has a novel mechanism for energy conservation in acetyl-CoA pathway.
4. Strain improvement and metabolic engineering for syngas fermentation

The ideal microbial biocatalyst used for commercial-scale syngas fermentation to fuels and chemicals should have the desirable traits including: high product selectivity, high substrate-utilization efficiency, high product yield, low product inhibition, strong toxic tolerance, prolonged metabolic viability and being environmentally friendly. In addition to other engineering obstacle like mass transfer limitation, microbial production of fuels and chemicals via syngas fermentation has been limited by nature’s biochemical repertoire for many years. Furthermore, genetic manipulation of Clostridia (one of the most common species applied in syngas fermentation) has been difficult due to the limiting factors such as a strong restriction-modification system, strong nuclease activity causing heterologous DNA degradation and thick membrane layer of Clostridia (Heap et al. 2007; Shao et al. 2007). However, with the advancement of synthetic biology tools, it became possible to integrate heterologous pathways in syngas-fermenting microorganisms via metabolic engineering.

*Clostridium ljungdahlii* has been genetically manipulated as a novel microbial platform to produce 1-butanol from syngas (Kopke et al. 2010). Six 1-butanol synthesis pathway genes, *thlA, hbd, crt, bcd, adhE* and *bdhA* (encoding thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butanol/butyraldehyde dehydrogenase and butanol dehydrogenase, respectively) from *Clostridium acetobutylicum* were expressed in *C. ljungdahlii* using a *Clostridium-Escherichia coli* shuttle vector pIMP1plasmid. Thus this recombinant *C. ljungdahlii* was able to produce 2 mM 1-butanol in batch-culture syngas fermentation, though most of the 1-butanol was converted to 1-butyrate at the end of growth probably due to the presence of aldehyde/alcohol dehydrogenase enzymes contained in the cell body. Kopke et al.
(2010) opened a new window for heterologous fuel production from *C. ljungdahlii* syngas fermentation; however, the efficiency of plasmid transformation was low by their method. Recently an efficient electroporation protocol was reported to improve the genetic manipulation of *C. ljungdahlii* (Leang et al. 2013), demonstrating its utilization as a chassis for autotrophic production of biocommodities.

In recent years, patent filings by INEOS Bio (www.ineosbio.com) Coskata (www.coskata.com) and LanzaTech (www.lanzatech.com), which are companies exploring commercialization of gas fermentation to liquid fuels and chemicals, have revealed the metabolic engineering of syngas-fermenting microorganisms to produce energy-condensed fuels (e.g. 1-butanol, isobutanol, 2,3-butanediol, methyl ethyl ketone and isoprene) with high yield and to increase product inhibition tolerance. For example, LanzaTech patented genetic manipulation of *Clostridium autoethanogenum* to produce 1-butanol as the main fermentation (Kopke and Liew 2011) and to increase ethanol tolerance (Simpson et al. 2011) using CO-rich steel mill waste gas as the sole carbon and energy source.

Syngas Biofuels Inc. (www.syngasbiofuelsenergy.com) developed proprietary electrofusion as an efficient transformation technology (Berzin and Tyurin 2012; Tyurin et al. 2012) for chromosomal recombination of *Clostridium* strains. Hence, they had 12 publications on electrofusion-based metabolic engineering of a series of newly-isolated *Clostridium* strains to produce syngas-fermentation-derived fuels and chemicals with highly exclusive production, including ethanol, acetone, 1-butanol, methanol, mevalonate and 2,3-butanediol, as described in Table 2. Despite high product yield achieved, however, those recombinant *Clostridium* strains had to be cultured with the amendment of selective antibiotics to maintain their stabilities and hence they cannot be utilized as commercial syngas-fermenting biocatalysts. Furthermore, no
genome sequencing information can be found in any publically accessible culture collection
database for those host strains (Clostridium sp. MT351, MT653, MT683, MT896, MT962,
MT1121, MT1243, MT1824, MT1962), so we are concerned about the validity and
reproducibility of the results.

To date strain improvement work in syngas fermentation (Table 2) has been
predominantly focused on Clostridia species as the natural host. However, this strict anaerobe
might not be the only chassis and high-titer production of some energy-condensed products (e.g.
n-butanol) might be very hard to achieve by using Clostridia due to the product inhibition (e.g. n-
butanol tolerance 13 g/L). Therefore, an alternative microbial platform, such as E. coli and S.
cerevisiae, might be developed for syngas fermentation with the advancing synthetic biology
tools by integration of butanol synthesis pathway into those fast-growing, high-alcohol-tolerant
organisms.

5. Syngas fermentation process parameters

5.1 Fermentation temperature

For syngas fermentation, temperature not only plays an important role on cell growth and
metabolisms, but also affects the solubility of the syngas constituent gases (e.g. CO, H2). As
shown in Table 1, most of the microorganisms applied for syngas fermentation are mesophiles
with optimum growth temperature between 30 ~ 40 °C. Little has been reported on using
thermophiles with optimum growth temperature between 55 ~ 60 °C in syngas fermentation.
This is probably because very few thermophilic species are naturally capable of converting
syngas into bulk fuels or fine chemicals. Nevertheless, conducting syngas fermentation with
thermophilic culture has some engineering merits, such as reduced occurrence of contamination
and better reuse of the heating generated from syngas cooling process (commercial syngas leaves the gasifier at extremely high temperature between 700 to 800 °C).

**5.2 Media pH**

Media pH is one of the most important parameters for syngas fermentation, because it significantly affects the cell growth, metabolic process and product distribution. Most microorganisms used for syngas fermentation in production of fuels (e.g. ethanol, butanol, isopropanol) and chemicals (e.g. acetate, butyrate) have optimum media pH for cell growth between 5.5 to 6.5, depending on the specific strains (Table 1). In the case of acetogenic bacteria (e.g. *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, *Clostridium drakei*, *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11) their metabolism on CO/CO$_2$/H$_2$ gaseous substrate is generally separated into two phases, namely acidogenesis for organic acids production and solventogenesis for alcohols production. An examination of the acetyl-CoA pathway (Figure 1) reveals that acidogenesis is balanced in ATP whereas solventogenesis results in net consumption of ATP. Therefore, it has been well acknowledged that acidogenesis is associated with cell growth and on the other hand, solventogenesis is non-growth-associated. In this sense, operating syngas fermentation at growth-optimum pH (5.5 ~ 6.5) promotes rapid cell growth and organic acids production. The acid accumulation leads to pH drop, at which point the fermentation switches from acidogenesis to solventogenesis. During the solventogenetic phase, the cells are in slow-growth condition but still have metabolic activity for solvent production (Cotter et al. 2009). Media pH shift has been proven to be an effective strategy to promote ethanol production in multi-stage syngas fermentation, during which the acidogenesis (with rapid cell growth) and solventogenesis (with slow cell growth) are separated. For example, Klasson et
al. (1992) performed a two-stage syngas fermentation using *Clostridium ljungdahlii* with the first reactor pH 5.0 and second reactor 4.0~4.5 to promote ethanol production in the second reactor at the expense of acetate. By applying the pH shift, they obtained a 30-fold improvement of ethanol productivity compared to that in a single continuous stirred tank reactor (CSTR).

### 5.3 Syngas composition

The effects of gas composition on syngas fermentation could be discussed from two aspects. The first aspect relates to ratio of CO to H\(_2\) in syngas. Although several microorganisms can utilize CO as the sole carbon and energy source without H\(_2\), it is believed that supplying the fermentation with sufficient H\(_2\) is beneficial for biofuel production (Gaddy et al. 2007). As shown in Figure 1, the electrons and protons required for acetyl-CoA pathway could be obtained from H\(_2\) oxidation via hydrogenase or from CO oxidation/CO\(_2\) reduction via CODH enzyme. In order to maximize the product yield from CO, it is preferred to obtain electrons from H\(_2\), because this will leaves more carbon in CO to be utilized for organic metabolites production but not being sacrificed for electrons generation. Feeding a continuous syngas fermentation with excess H\(_2\) has been demonstrated to enhance ethanol production in Clostridium ljungdahlii culture (Gaddy et al. 2007).

The second aspect is about the impacts of the syngas impurities. Common impurities of the biomass-derived syngas are sulfur gases (H\(_2\)S, COS), NH\(_3\), NO, ethane, ethylene, tar, ash and char (Munasinghe and Khanal 2010a), which have been found to cause cell dormancy, inhibition on hydrogenase and cell growth and metabolic shift between acidogenesis and solventogenesis in acetogens (Ahmed et al. 2006; Ahmed and Lewis 2007; Datar et al. 2004; Xu et al. 2011). Effective approaches to mitigate the negative impacts of syngas impurities include placing
ultrafilter, chemical absorbing using agents like potassium permanganate, sodium hydroxide or sodium hydrochloride. Also, improvement of gasification efficiency will relieve the inhibition.

5.4 Media formulation

Many constituents in the liquid media for syngas fermentation have been shown to influence the cell growth and metabolic process, including yeast extract (Guo et al. 2010; Klasson et al. 1993a; Vega et al. 1989a), calcium pantothenate, cobalt, vitamin B₁₂ (Gaddy et al. 2007; Kundiyana et al. 2011), reducing agent (Panneerselvam 2010) and trace elements (Saxena and Tanner 2011). Substituting the defined costly media nutrient with inexpensive organic supplementation will reduce the cost of fermentation media and hence enhance the process economics. The researchers at Oklahoma State University have successfully replaced the yeast extract in the liquid media with corn steep liquor for Clostridium ragsdalei P11 (Maddipati et al. 2011) and with cotton seed extract for Clostridium carboxidivorans P7 syngas fermentation (Kundiyana et al. 2010a) with significant ethanol production.

6. Mass transfer limitation in syngas fermentation

Gas-to-liquid mass transfer limitation has been identified as the major engineering bottleneck in syngas fermentation (Bredwell et al. 1999; Klasson et al. 1993a; Worden et al. 1997). While oxygen mass transfer has been the rate-limiting step for aerobic fermentation process, this limitation is expected to be even more severe for syngas fermentation, as the water solubilities of CO and H₂ are only 60% and 4% (on mass basis) of that of oxygen, respectively.

Two rate-limiting steps, namely mass transfer limitation and kinetic-growth limitation, are expected to affect the productivity of microbial fermentation process. With a high cell
density in fermentation broth, the process is likely to be mass transfer limited, in which condition the mass transfer rate is not high enough to keep in pace with the cell growth demand, and hence the substrate concentration in liquid phase is always zero. Mass transfer limitation makes the availability of substrate become low to be consumed by microorganisms, leading to the low productivity. On the other hand, kinetic-growth limitation is usually found when the cell density is low and/or the cells’ metabolic activities are low. In this condition, the substrate uptake rate is lower than the supply rate of substrate, and thus the substrate concentration in liquid phase is not zero. Both of the above rate-limiting steps may occur in syngas fermentation (Mohammadi et al. 2011). Therefore, a bioreactor configuration enabled to achieve efficient mass transfer and high cell density in economically-feasible manner plays an important role for syngas fermentation scale-up in production of cellulosic ethanol. Volumetric gas-liquid mass transfer coefficient ($k_{L}a$) is commonly used as the criterion to compare the mass transfer efficiency among various reactor configurations, which is determined by using equation (11) and (12):

$$\frac{dC}{dt} = (k_{L}a) \cdot (C^{*} - C)$$

(11)

$$\ln \left( \frac{C^{*} - C_0}{C^{*} - C} \right) = (k_{L}a) \cdot t$$

(12)

where $C^{*}$ is the saturated dissolved gas concentration in the aqueous phase, $C_0$ is the dissolved gas concentration in the aqueous phase at time zero, $C$ is the measured gas concentration in the aqueous phase at time $t$, and $t$ is the sampling time.

Table 3 summarized the CO $k_{L}a$ values measured in various reactor configurations. A literature review of the bioreactor configurations designed and operated for syngas fermentation was briefly discussed as below.
6.1 Continuous stirred tank reactor (CSTR)

CSTR is one of the most common reactors used in syngas fermentation, in which microorganisms are suspended in the mixed fermentation broth. In CSTR, syngas is continuously sparged through the gas diffuser; large bubbles dispersed in the fermentation broth are immediately broken down into the smaller bubbles by the mechanical agitation. Two approaches are usually employed to enhance $k_La$ values of syngas constituent gases: (1) to feed the syngas at a high supply rate (i.e. increasing the specific syngas flow rate) and (2) to increase the gas-liquid interfacial area by increasing the agitation speed. For example, by increasing the specific CO flow rate from 0.14 to 0.86 vvm (gas volume flow per unit of liquid working volume per minute) and increasing agitation speed from 200 to 600 rpm, CO $k_La$ in a CSTR was increased from 10.8 hr$^{-1}$ to 155 hr$^{-1}$ (Riggs and Heindel 2006). It should be noted that agitation and gas flow rate are linearly proportional to power-per-volume ($P/V$) and superficial gas velocity ($u_G$), respectively. Equation (13) explains the dependence of $k_La$ on the $P/V$ and $u_G$ under mass-transfer limited condition (van't Riet 1979).

$$k_La = K \cdot \left(\frac{P}{V}\right)^{\alpha} \cdot (u_G)^{\beta}$$  (13)

where $\alpha$ and $\beta$ are constants depending on the reactor geometry, impeller type and configuration and the characteristics of continuous phase.

However, increasing the agitation is not economically feasible for commercial-scale reactors for syngas fermentation due to the extensive energy cost, whereas increasing high syngas flow rate causes wastage of gaseous substrate. Therefore, alternative approaches have been explored to achieve a more energy-efficient mass transfer. Bredwell and Worden (1998) proposed microbubble sparging as a potential method to enhance mass transfer with low power consumption. Microbubbles (or so-called colloidal gas aphrons) are surfactant-stabilized small
bubbles with diameter of 50~60 µm, which are much lower than the average diameter (3~5 mm) obtained with conventional bubble dispersing method (Kaster et al. 1990). Since $k_{\text{L}a}$ is inversely proportional to the bubble diameter under mass-transfer limited condition, microbubble dispersion has great potential to attain high mass transfer rate. Such potential was demonstrated for *Butyribacterium methylotrophicum* syngas fermentation in CSTR by Bredwell and Worden (1998), in which case CO $k_{\text{L}a}$ of 91 hr$^{-1}$ was reported for microbubble sparging, as compared to that of 14 hr$^{-1}$ for conventional sparging. Moreover, the power consumption using microbubble sparging was estimated to be only 0.01 kW/m$^3$ of fermentation capacity, which is very energy-efficient. Another approach to enhance mass transfer in an energy-efficient manner was adding small particles on the order of nanometers to micrometers. The enhancement of mass transfer rate results from the “grazing effect”, in which those small particles transport additional amount of gas to the liquid bulk through adsorption in the gas-liquid diffusion interface layer and desorption in the liquid (Beenackers and van Swaaij 1993; Olle et al. 2006). Zhu et al. (2009) enhanced CO $k_{\text{L}a}$ by 190% and 470% in ultrapure water amended with MCM41 nanoparticles with mercaptopropyl functional groups and electrolytes, respectively. Later the authors demonstrated an approximate 200% improvement of H$_2$ yield in *Rhodospirillum rubrum* syngas fermentation due to the enhanced CO mass transfer rate brought by adding 0.6 wt% of MCM41 nanoparticles functionalized with 5% molar ratio of mercaptopropyl group (Zhu et al. 2010). However, the scalability of this approach is doubted with the following concerns: First, the nanoparticles are costly and how to recycle them is unknown. Second, additional separation or purification procedures are expected to be required to recover the final products (e.g. ethanol, acetate) from the fermentation broth containing large quantities of nanoparticles. Last but not least, the environmental impacts of those nanoparticles remain unclear.
6.2 Bubble column reactor (BCR)

BCR is another reactor configuration widely applied in industrial fermentation process. The mixing gaseous substrate is achieved by gas sparging without mechanical agitation and the gas hold-up time in BCR is longer than that in CSTR because of the bubble column geometry (usually height to diameter ratio > 10). In this sense, BCR has been considered as an economically viable alternative to achieve moderate mass transfer rate with low power consumption for gas-liquid biological reactions. Advantages of BCR systems include low maintenance and operational costs, while back mixing and coalescence of gaseous substrate are their main drawbacks.

BCRs have received less attention than CSTR for syngas fermentation. Chang et al. (2001) determined CO \( k_La \) of 72 hr\(^{-1} \) in a BCR at CO flow rate of 0.4 vvm and obtained the acetate concentration of 40 mM or productivity of 5.8 g/L/day in *Eubacterium limosum* KIST612 continuous syngas fermentation with cell-recycle. They evaluated the effect of CO partial pressure on cell growth and acetate production and found that the CO mass transfer rate was lower than required for the maximum cell growth but higher than that to meet the maintenance requirement. Rajagopalan (2002) reported ethanol concentration of 0.16 wt% and productivity of 0.99 g/L/day in continuous syngas fermentation using *Clostridium carboxidivorans* strain P7. Without cell recycle, they did not achieve high cell concentration in the course of syngas fermentation, which might explain the relatively low productivity. Similar results were reported by Datar et al. (2004), in which the authors observed unwanted cell washout at dilution rate > 0.55 day\(^{-1} \).
6.3 Other bioreactor configurations

Besides CSTR and BCR, other bioreactor configurations have been used for syngas fermentation, such as trickle bed reactor (TBR) and moving bed biofilm reactor (MBBR).

In TBR, the liquid fluid flows in downward direction over a bed of solid packing media for microorganisms accumulation, while the gaseous substrate may flow in upward or downward direction, forming “countercurrent” or “cocurrent” gas-liquid flow. The term “trickle” in TBC depicts the hydrodynamic characteristics of its operation, in which liquid intermittently flows over solid catalysts (microorganisms in the case of syngas fermentation) in the form of films, rivulets or droplets. Klasson et al. (1992) compared the mixed-culture (*Rhodospirillum rubrum*, *Methanobacterium formicicum*, and *Methanosarcina barkeri*) syngas fermentation in a packed bubble reactor (PBC), a CSTR and a TBR packed with ceramic saddles. The CO $k_{L}a$ of PBC and TBC were 2.1 hr$^{-1}$ and 55.5 hr$^{-1}$, respectively, and at a given syngas-loading rate, CO conversion in the TBR was 2-fold higher than in a CSTR. The research group in Oklahoma State University recently presented the feasibility of semi-continuous syngas fermentation in production of ethanol and acetate by *Clostridium ragsdalei* strain P11 in a TBR (Atiyeh et al. 2013), in which the gas was fed continuously and the fermentation broth was internal-circulated within the reactor. They reported over 90% of CO utilization and 70% H$_2$ utilization by the bacteria.

Compared to conventional reactors like CSTR, BCR and TBR, MBBR is a more recent development. It was originally designed by a group of researchers in Norway for application in municipal wastewater treatment. A classical MBBR compromises a vessel/tank containing the culture broth, a gas injection system placed at the bottom of the vessel/tank for gas diffusion and turbulence enhancement and a large quantities (usually above 50% filling volume) of carriers for attachment of microorganisms grown as biofilm. The biofilm carriers are the core technology of
MBBR, which are able to provide a very high surface area per volume for biofilm development. Costaka (www.costaka.com), a company exploring commercial-scale syngas fermentation, recently filed a patent on using a pilot-scale (36 m³ capacity with 18 m³ liquid working volume) MBBR for continuous syngas fermentation using Clostridium ragsdalei strain P11 (Hickey 2009). The author reported ethanol concentration of 30 g/L and attached biomass concentration of 5 g/L dry weight with fermentation broth withdrawal rate at 22 L/min (i.e. dilution rate 1.76 day⁻¹) by the end of 30-day continuous operation.

7. Research objectives

To sum up, the bioreactor configuration plays an important role in syngas fermentation. The present study investigated the feasibility of using three innovative biofilm-based reactors, namely hollow fiber membrane biofilm reactor (HFM-BR), monolithic biofilm reactor (MBR) and horizontal rotating packed bed bioreactor (RPB-BR), for continuous syngas fermentation. The overall research objective was to maximize ethanol production of syngas fermentation by optimizing several key operational parameters. The specific objectives of the present study were:

1. Design and fabricate the bench-top scale HFM-BR, MBR and RPB-BR;
2. Determine CO kLa under abiotic condition in the HFM-BR under varied gas and liquid velocities;
3. Evaluate the effects of operational parameters (syngas flow rate, liquid recirculation rate and dilution rate) on syngas fermentation performance in the HFM-BR;
4. Determine CO kLa under abiotic condition in the MBR under varied gas and liquid velocities and compare the results to those of a BCR with the identical geometry;
5. Evaluate the effects of operating parameters (syngas flow rate, liquid flow rate and dilution rate) on syngas fermentation performance in the MBR and compare the results to those of the BCR;

6. Determine CO $k_{l,a}$ under abiotic condition in the RPB-BR under varied rotational speed and headspace pressure;

7. Evaluate the effects of operational parameters (rotational speed, headspace pressure and dilution rate) on syngas fermentation performance in the RPB-BR.
REFERENCES


Gaddy JL; 2000. Biological production of ethanol from waste gases with Clostridium ljungdahlii. US patent 6136577 A.


Table 1. Mesophilic and thermophilic microorganisms fermenting syngas into fuels and chemicals

<table>
<thead>
<tr>
<th>Mesophilic bacteria (acetogen)</th>
<th>Substrate</th>
<th>T&lt;sub&gt;OPT&lt;/sub&gt; (°C)</th>
<th>pH&lt;sub&gt;OPT&lt;/sub&gt;</th>
<th>Product(s)</th>
<th>Genome sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acetobacterium woodii</em></td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;/H&lt;sub&gt;2&lt;/sub&gt;, CO</td>
<td>6.8</td>
<td>30.0</td>
<td>Acetate</td>
<td>Available</td>
<td>(Diekert and Ritter 1983) (Genthner and Bryant 1987) (Poehlein 2012)</td>
</tr>
<tr>
<td><em>Acetogenium kivui</em></td>
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<td>6.6</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Acetate</td>
<td>NA</td>
<td>(Gaddy 2000)</td>
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<td><em>Acetogenium longum</em></td>
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<td>30-33</td>
<td>7.8</td>
<td>Acetate, n-butyrate</td>
<td>NA</td>
<td>(Kane and Breznak 1991) (Tocheva et al. 2013)</td>
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<td><em>Alkalibaculum bacchi</em></td>
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<td>37</td>
<td>8.0-8.5</td>
<td>Acetate, ethanol</td>
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<td>(Allen et al. 2010) (Liu et al. 2012)</td>
</tr>
<tr>
<td><em>Peptostreptococcus productus</em></td>
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<td>7.0</td>
<td>Acetate</td>
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<td>(Lorowitz and Bryant 1984) (Misoph and Drake 1996) (Liu et al. 2008)</td>
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<td><em>Butyribacterium methyloptrophicum</em></td>
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<td>Acetate</td>
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<td>(Abrini 1994) (Kopke et al. 2011b) (Bruno-Barcena et al. 2013)</td>
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<td>Species</td>
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<td>pH Range</td>
<td>Products</td>
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<td>(Bruant et al. 2010)</td>
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<td>(Paul et al. 2010)</td>
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<td>Eubacterium limosum</td>
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<td>$pH_{\text{OPT}}$</td>
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<td>Genome sequence</td>
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<td><em>Citrobacter sp. Y19</em></td>
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<td>$pH_{\text{OPT}}$</td>
<td>Product(s)</td>
<td>Genome sequence</td>
<td>Reference</td>
</tr>
<tr>
<td><em>Moorella thermoacetica</em></td>
<td>CO$_2$/H$_2$, CO</td>
<td>55</td>
<td>6.5-6.8</td>
<td>Acetate</td>
<td>Available</td>
<td>(Kerby and Zeikus 1983) (Drake and Daniel 2004) (Pierce et al. 2008)</td>
</tr>
<tr>
<td><em>Moorella thermoautotrophica</em></td>
<td>CO$_2$/H$_2$, CO</td>
<td>58</td>
<td>6.1</td>
<td>Acetate</td>
<td>NA</td>
<td>(Wiegel et al. 1981) (Savage et al. 1987)</td>
</tr>
<tr>
<td><strong>Table 1. continued</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Thermoanaerobacter kivui</strong></td>
<td>CO₂/H₂</td>
<td>66</td>
<td>6.4</td>
<td>Acetate</td>
<td>NA</td>
<td>(Leigh and Wolfe 1981) (Daniel et al. 1990)</td>
</tr>
<tr>
<td><strong>Thermophilic bacteria (sulfate-reducing bacteria)</strong></td>
<td>Substrate</td>
<td>T&lt;sub&gt;OPT&lt;/sub&gt; (°C)</td>
<td>pH&lt;sub&gt;OPT&lt;/sub&gt;</td>
<td>Product(s)</td>
<td>Genome sequence</td>
<td>Reference</td>
</tr>
<tr>
<td><em>Desulfotomaculum carboxydivorans</em></td>
<td>CO, sulfate</td>
<td>55</td>
<td>6.8-7.2</td>
<td>H₂, H₂S</td>
<td>Available</td>
<td>(Parshina et al. 2005b)</td>
</tr>
<tr>
<td><em>Desulfotomaculum kuznetsovi</em></td>
<td>CO, sulfate</td>
<td>60</td>
<td>7.0</td>
<td>Acetate, H₂S</td>
<td>Available</td>
<td>(Parshina et al. 2005a) (Visser et al. 2013)</td>
</tr>
<tr>
<td><em>Desulfotomaculum thermobenzoicum</em> subsp. <em>thermosyntrophicum</em></td>
<td>CO, sulfate</td>
<td>55</td>
<td>7.0</td>
<td>Acetate, H₂S</td>
<td>NA</td>
<td>(Plugge et al. 2002) (Parshina et al. 2005a)</td>
</tr>
<tr>
<td><strong>Thermophilic bacteria (hydrogenogenic bacteria)</strong></td>
<td>Substrate</td>
<td>T&lt;sub&gt;OPT&lt;/sub&gt; (°C)</td>
<td>pH&lt;sub&gt;OPT&lt;/sub&gt;</td>
<td>Product(s)</td>
<td>Genome sequence</td>
<td>Reference</td>
</tr>
<tr>
<td><em>Caldanaerobacter subterraneus</em></td>
<td>CO</td>
<td>70</td>
<td>6.8-7.1</td>
<td>H₂</td>
<td>Draft</td>
<td>(Sokolova et al. 2001)</td>
</tr>
<tr>
<td><em>Carboxydothermus hydrogenoformans</em></td>
<td>CO</td>
<td>70-72</td>
<td>6.8-7.0</td>
<td>H₂</td>
<td>Available</td>
<td>(Svetlichny et al. 1991) (Wu et al. 2005)</td>
</tr>
<tr>
<td><em>Carboxydocella sporoproducens</em></td>
<td>CO</td>
<td>60</td>
<td>6.8</td>
<td>H₂</td>
<td>NA</td>
<td>(Slepova et al. 2006)</td>
</tr>
<tr>
<td><em>Carboxydocella thermautotrophica</em></td>
<td>CO</td>
<td>58</td>
<td>7.0</td>
<td>H₂</td>
<td>NA</td>
<td>(Sokolova et al. 2002)</td>
</tr>
<tr>
<td><em>Thermincola carboxydiphila</em></td>
<td>CO</td>
<td>55</td>
<td>8.0</td>
<td>H₂</td>
<td>NA</td>
<td>(Sokolova et al. 2005)</td>
</tr>
<tr>
<td><em>Thermincola ferriacetica</em></td>
<td>CO</td>
<td>57-60</td>
<td>7.0-7.2</td>
<td>H₂</td>
<td>NA</td>
<td>(Zavarzina et al. 2007)</td>
</tr>
<tr>
<td><em>Thermolithobacter carboxydivorans</em></td>
<td>CO</td>
<td>70</td>
<td>7.0</td>
<td>H₂</td>
<td>NA</td>
<td>(Sokolova et al. 2007)</td>
</tr>
<tr>
<td>Thermophilic archaea</td>
<td>Substrate</td>
<td>( T_{\text{OPT}} ) (°C)</td>
<td>( \text{pH}_{\text{OPT}} )</td>
<td>Product(s)</td>
<td>Genome sequence</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------</td>
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<tr>
<td><em>Archeoglobus fulgidus</em></td>
<td>CO, sulfate</td>
<td>83</td>
<td>6.4</td>
<td>Acetate, formate, ( \text{H}_2\text{S} )</td>
<td>Available</td>
<td>(Klenk et al. 1997) (Henstra et al. 2007a)</td>
</tr>
<tr>
<td><em>Methanotermobacter thermautotrophicus</em></td>
<td>CO</td>
<td>65</td>
<td>7.4</td>
<td>( \text{CH}_4 )</td>
<td>Available</td>
<td>(Daniel et al. 1977) (Smith et al. 1997)</td>
</tr>
<tr>
<td><em>Thermococcus onnurineus</em> NA1</td>
<td>CO</td>
<td>80</td>
<td>6.5</td>
<td>( \text{H}_2 )</td>
<td>Available</td>
<td>(Lee et al. 2008) (Kim et al. 2010) (Kim et al. 2013)</td>
</tr>
<tr>
<td><em>Thermococcus</em> sp. strain AM4</td>
<td>CO</td>
<td>82</td>
<td>6.8</td>
<td>( \text{H}_2 )</td>
<td>Available</td>
<td>(Sokolova et al. 2004b) (Oger et al. 2011)</td>
</tr>
</tbody>
</table>

* NA: not available
### Table 2. Genetically engineered acetogens and their target product

<table>
<thead>
<tr>
<th>Strain platform</th>
<th>Product (target)</th>
<th>Genetic engineering</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium ljungdahlii</em></td>
<td>1-Butanol</td>
<td>Plasmid overexpression of <em>Clostridium acetobutylicum</em> butanol synthesis pathway genes thlA, hbd, crt, bcd, adhE and bdhA</td>
<td>2 mM 1-butanol production from syngas</td>
<td>(Kopke et al. 2010)</td>
</tr>
<tr>
<td><em>Clostridium ljungdahlii</em></td>
<td>Acetate</td>
<td>Deletion of the bifunctional aldehyde/alcohol dehydrogenases adhE1 and adhE2</td>
<td>Increased acetate production at expense of ethanol</td>
<td>(Leang et al. 2013)</td>
</tr>
<tr>
<td><em>Clostridium autoethanogenum</em></td>
<td>1-Butanol</td>
<td>Plasmid overexpression of <em>Clostridium acetobutylicum</em> butanol synthesis pathway genes thlA, hbd, crt, bcd, etfA and etfB</td>
<td>25.66 mM 1-butanol production from steel mill waste gas</td>
<td>(Kopke and Liew 2011)</td>
</tr>
<tr>
<td><em>Clostridium autoethanogenum</em></td>
<td>Ethanol</td>
<td>Plasmid overexpression of the self-containing groES and groEL</td>
<td>Ethanol tolerance increased up to 60 g/L</td>
<td>(Simpson et al. 2011)</td>
</tr>
<tr>
<td><em>Moorella thermoacetica</em></td>
<td>Lactate</td>
<td>Plasmid overexpression of <em>Thermoanaerobacter pseudethanolicus</em> lactate synthesis pathway gene T-ldh</td>
<td>Transformant exhibited higher lactate dehydrogenase (LDH) activity than wild type strain.</td>
<td>(Kita et al. 2013)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Formate</td>
<td>Expression of formate hydrogenase-encoded gene FDH$<em>{H</em>{-}CloCa}$ from <em>Clostridium carboxidivorans</em> strain P7$^{T}$</td>
<td>Reduction of CO$_2$ to formate using recombinant <em>E. coli</em> expressing FDH</td>
<td>(Alissandratos et al. 2013)</td>
</tr>
<tr>
<td><em>Clostridium</em> sp. MT683</td>
<td>Ethanol</td>
<td>Electrofusion-derived inactivation of pta and overexpression of synthetic aldh from <em>Clostridium ljungdahlii</em> with selective antibiotics amendment</td>
<td>Exclusive 576 mM ethanol production from syngas (60% CO, 40% H$_2$) with elimination of acetate production</td>
<td>(Berzin and Tyurin 2012)</td>
</tr>
</tbody>
</table>
Table 2. continued

<table>
<thead>
<tr>
<th>Clostridium sp.</th>
<th>Type</th>
<th>Methodology</th>
<th>Productivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT653</td>
<td>Ethanol</td>
<td>Electrofusion-derived inactivation of pta and overexpression of synthetic adh from Eubacterium linosum KIST612 with selective antibiotics amendment</td>
<td>Exclusive 590 mM ethanol production from syngas (64% CO, 36% H₂) with elimination of acetate production</td>
<td>(Berzin et al. 2012b)</td>
</tr>
<tr>
<td>MT896</td>
<td>Acetone</td>
<td>Electrofusion-derived inactivation of pta via integration of a cassette comprising synthetic erm(B) from Moorella thermoacetica and thio and hmgCoAs from Clostridium ljungdahlii; inactivation of aldh via integration of synthetic cat from Moorella thermoacetica and hmgCoAl and adc from Clostridium acetobutylicum with selective antibiotics amendment</td>
<td>Exclusive 1.8 M acetone production from syngas (60% CO, 40% H₂) with elimination of acetate and ethanol production</td>
<td>(Berzin et al. 2012c)</td>
</tr>
<tr>
<td>MT1121</td>
<td>Ethanol</td>
<td>Cre-lox66/lox71-based elimination of pta by electrotransformation technology</td>
<td>Exclusive 610 mM ethanol production from syngas (60% CO, 40% H₂) with elimination of acetate production</td>
<td>(Berzin et al. 2012a)</td>
</tr>
<tr>
<td>MT962</td>
<td>Ethanol</td>
<td>Elimination of plasmid pMT351 by microwave/electric pulse stimulation</td>
<td>358 mM ethanol production from syngas (60% CO, 40% H₂) at the expense of acetate</td>
<td>(Berzin et al. 2013a)</td>
</tr>
<tr>
<td>MT1962</td>
<td>1-Butanol</td>
<td>Cre-lox66/lox71-based elimination of pta and aldh, integration of synthetic genes of thio and hbd from Clostridium ljungdahlii, crt and bcd from Clostridium difficile, bad from Clostridium beijerinckii</td>
<td>Exclusive 297 mM 1-butanol production from syngas (60% CO, 40% H₂) with elimination of acetate and ethanol production</td>
<td>(Berzin et al. 2013b)</td>
</tr>
<tr>
<td><strong>Clostridium sp.</strong></td>
<td><strong>Product</strong></td>
<td><strong>Methodology</strong></td>
<td><strong>Production</strong></td>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>--------------------</td>
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<td>--------------</td>
</tr>
<tr>
<td><strong>MT1230</strong></td>
<td><strong>Ethanol</strong></td>
<td>Overexpression of six copies of the inserted <em>al-adh</em> genes using <em>Tn7</em>-tool at the expense of eliminated <em>pta</em>, <em>ack</em>, <em>spo0A</em> and <em>spo0J</em> with selective antibiotics amendment</td>
<td>1018 mM ethanol production from syngas (60% CO, 40% H₂) or 652 mM ethanol production from CO₂/H₂ gas blend</td>
<td>(Kiriukhin and Tyurin 2013a)</td>
</tr>
<tr>
<td><strong>MT1424</strong></td>
<td><strong>Methanol</strong></td>
<td>Overexpression of synthetic methanol dehydrogenase, formaldehyde dehydrogenase and formate dehydrogenase using <em>Tn7</em>-tool at the expense of eliminated <em>pta</em>, <em>ack</em>, <em>spo0A</em> and <em>spo0J</em> with selective antibiotics amendment</td>
<td>2.2 mM methanol production from 20%CO₂/80%H₂ gas blend</td>
<td>(Tyurin and Kiriukhin 2013a)</td>
</tr>
<tr>
<td><strong>MT1243</strong></td>
<td><strong>Mevalonate</strong></td>
<td>Overexpression of synthetic thiolase, HMG-synthase and HMG-reductase using <em>Tn7</em>-tool at the expense of eliminated <em>pta</em>, <em>ack</em>, <em>spo0A</em> and <em>spo0J</em> with selective antibiotics amendment</td>
<td>145 mM mevalonate production from syngas (60% CO, 40% H₂) or 97 mM mevalonate production from CO₂/H₂ gas blend</td>
<td>(Kiriukhin and Tyurin 2013b)</td>
</tr>
<tr>
<td><strong>MT1802</strong></td>
<td><strong>2,3-Butanediol</strong></td>
<td>Overexpression of synthetic formate dehydrogenase at the expense of eliminated acetate-production and sporulation using Cre-lox66/lox71-approach and overexpression of synthetic formate acetyltransferase, acetolactate synthase, acetolactate decarboxylase and alcohol dehydrogenase using <em>Tn7</em>-tool with selective antibiotics amendment</td>
<td>102 mM 2,3-butanediol production from syngas (60% CO, 40% H₂) or 80 mM mevalonate production from CO₂/H₂ gas blend</td>
<td>(Tyurin and Kiriukhin 2013b)</td>
</tr>
</tbody>
</table>
Table 3. CO $k_La$ values determined in various bioreactor configurations

<table>
<thead>
<tr>
<th>Syngas composition</th>
<th>Biological system (i.e. with microorganisms grown)</th>
<th>Reactor type / working volume</th>
<th>Agitation (rpm)</th>
<th>Microorganisms</th>
<th>$k_La$ (hour$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO/CO$_2$/CH$_4$ (63.43/15.96/20.61)</td>
<td>CSTR / 325 mL</td>
<td>400</td>
<td>$P. productus$</td>
<td>35.45</td>
<td>(Vega et al. 1989b)</td>
<td></td>
</tr>
<tr>
<td>CO/H$_2$/CO$_2$/Ar (55/20/10/15)</td>
<td>CSTR / 1250 mL</td>
<td>400</td>
<td>$R. rubrum$</td>
<td>101</td>
<td>(Cowger et al. 1992)</td>
<td></td>
</tr>
<tr>
<td>Syngas mix</td>
<td>CSTR / NM</td>
<td>300</td>
<td>$R. rubrum, M. formicicum, M. barkeri$</td>
<td>28.1</td>
<td>(Cowger et al. 1992)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trickle bed / NM</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Packed column / NM</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Trickle bed / NM</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO/H$_2$/CO$_2$/Ar (55/20/10/15)</td>
<td>CSTR / 1250 mL</td>
<td>300</td>
<td>$R. rubrum$</td>
<td>14.9</td>
<td>(Klasson et al. 1993b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td></td>
<td>21.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>22.8</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td>23.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>700</td>
<td></td>
<td>35.5</td>
<td></td>
<td></td>
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<tr>
<td>CO</td>
<td>CSTR with conventional sparging / 1.5 L</td>
<td>200</td>
<td>$B. methylotrophicum$</td>
<td>14.2</td>
<td>(Bredwell and Worden 1998)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSTR with microbubble sparging / 1.5 L</td>
<td></td>
<td></td>
<td>90.64</td>
<td></td>
<td></td>
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<tr>
<td>Syngas mix</td>
<td>CSTR / NM</td>
<td>300</td>
<td>SRB $^{[3]}$ mixed culture</td>
<td>31</td>
<td>(Bredwell et al. 1999)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$C. ljungdahlii$</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSTR with microbubble sparging / NM</td>
<td>300</td>
<td>SRB mixed culture</td>
<td>104</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Trickle bed / NM</td>
<td>NA</td>
<td>SRB culture</td>
<td>121</td>
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<td>$C. ljungdahlii$</td>
<td>137</td>
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<tr>
<td>CO/H₂/CO₂/Ar (55/20/10/15)</td>
<td><strong>Reactor type</strong></td>
<td><strong>Agitation (rpm)</strong></td>
<td><strong>Working volume (L)</strong></td>
<td><strong>k₁a (hour⁻¹)</strong></td>
<td><strong>Reference</strong></td>
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<td>------------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Pure CO</td>
<td>Stirred tank</td>
<td>200</td>
<td>7.0</td>
<td>10.1 ~ 31.7</td>
<td>(Riggs and Heindel 2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td></td>
<td>16.6 ~ 54.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td></td>
<td>28.1 ~ 72.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>34.6 ~ 122.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td>61.2 ~ 154.8</td>
<td></td>
<td></td>
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<tr>
<td>CO/H₂/CO₂/N₂ (20/10/18/52)</td>
<td>Stirred tank</td>
<td>400</td>
<td>7.0</td>
<td>72.0 ~ 123.5</td>
<td>(Kapic et al. 2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>131.4 ~ 183.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td>147.6 ~ 212.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>650</td>
<td></td>
<td>172.8 ~ 248.4</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>700</td>
<td></td>
<td>187.2 ~ 288.0</td>
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</tr>
</tbody>
</table>

Abiotic system (i.e. in absence of microorganisms)

<table>
<thead>
<tr>
<th>Syngas composition</th>
<th>Reactor type</th>
<th>Agitation (rpm)</th>
<th>Working volume (L)</th>
<th>k₁a (hour⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure CO</td>
<td>Column diffuser</td>
<td>NA</td>
<td></td>
<td>2.5 ~ 40.0</td>
<td>(Munasinghe and Khanal 2010b)</td>
</tr>
<tr>
<td></td>
<td>Bulb diffuser</td>
<td>NA</td>
<td></td>
<td>31.7 ~ 78.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bubble column</td>
<td>NA</td>
<td></td>
<td>29.5 ~ 50.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bubble column with agitation</td>
<td>150</td>
<td>3.0</td>
<td>33.5 ~ 53.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air-lift with bulb diffuser</td>
<td>NA</td>
<td></td>
<td>49.0 ~ 91.1</td>
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</tr>
<tr>
<td></td>
<td>Air-lift with single point gas entry</td>
<td>NA</td>
<td></td>
<td>16.6 ~ 45.0</td>
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</tr>
<tr>
<td>CO/H₂/CO₂ (50/30/20)</td>
<td>HFM-BR</td>
<td>NA</td>
<td>2.4</td>
<td>21 ~ 385</td>
<td>(Lee et al. 2012)</td>
</tr>
<tr>
<td>----------------------</td>
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<td>------</td>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>Pure CO</td>
<td>HFM-BR</td>
<td>NA</td>
<td>3.0</td>
<td>85.7 ~ 946.6</td>
<td>(Munasinghe and Khanal 2012)</td>
</tr>
</tbody>
</table>

[1] NM: not mentioned  
[2] NA: not applicable  
[3] SRB: sulfate reducing bacteria
CHAPTER 2. SYNGAS FERMENTATION IN A HOLLOW FIBER MEMBRANE BIOFILM REACTOR: EVALUATING MASS TRANSFER RATE AND ETHANOL PRODUCTION

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ABSTRACT

Gasification followed by syngas fermentation is a unique hybrid process for converting lignocellulosic biomass into fuels and chemicals. Current syngas fermentation faces several challenges with low gas-liquid mass transfer being one of the major bottlenecks. The aim of this work is to evaluate the performance of hollow fiber membrane biofilm reactor (HFM-BR) as a reactor configuration for syngas fermentation. The volumetric mass transfer coefficient ($K_{La}$) of the HFM-BR was determined at abiotic conditions within a wide range of gas velocity passing through the hollow fiber lumen and liquid velocity passing through the membrane module shell. The $K_{La}$ values of the HFM-BR were higher than most reactor configurations such as stir tank reactors and bubble columns. A continuous syngas fermentation of Clostridium carboxidivorans P7 was implemented in the HFM-BR system at different operational conditions, including the syngas flow rate, liquid recirculation between the module and reservoir, and the dilution rate. It was found that the syngas fermentation performance such as syngas utilization efficiency, ethanol concentration and productivity, and ratio of ethanol to acetic acid depended not only on the mass transfer efficiency but also the characteristics of biofilm attached on the membrane module (biofouling or abrading of the biofilm). The HFM-BR results in a highest ethanol concentration of 23.93 g/L with an ethanol to acetic acid ratio of 4.79. Collectively, the research
shows the HFM-BR is an efficient reactor system for syngas fermentation with high mass transfer.

**KEYWORDS:** Syngas fermentation; lignocellulosic biomass; mass transfer; hollow fiber membrane biofilm reactor; ethanol

1. Introduction

Significant research have been conducted for producing biofuels from lignocellulosic biomass through biochemical conversion process, in which biomass is converted into reduced sugars through pretreatments and enzymatic hydrolysis, followed by yeast or bacterial fermentation of the sugars into alcohols. An alternative approach of producing fuel from biomass is to gasify the biomass into syngas (mainly CO and H₂) which is then fermented into alcohols. Compared with the biochemical conversion process, the gasification – syngas fermentation pathway has several advantages such as (1) the capability of using all components of biomass including lignin; (2) feedstock composition independence; (3) elimination of complex pretreatment and high enzyme costs; (4) independence of H₂:CO:CO₂ ratio in syngas fermentation (Munasinghe and Khanal 2010a).

Syngas fermentation, however, is still limited by several factors such as low efficient gas-liquid mass transfer, inhibitory compounds in syngas (ethylene, ethane, acetylene, tar, sulfur, and ash), and high medium cost. The low mass transfer efficiency has long been considered as a major bottleneck (Bredwell et al. 1999; Klasson et al. 1993; Worden et al. 1997). Traditional reactors such as continuous stirred tank reactor, bubble column, and airlift reactors are widely used in syngas fermentation (Bredwell et al. 1999; Datar et al. 2004; Rajagopalan 2002), where
mass transfer can be improved through increasing agitation or gas flow rate. These strategies, however, are usually not economical due to high energy consumption or shear stress to the microorganisms (Bredwell and Worden 1998). Increasing gas flow rate also resulted in a significant loss of syngas in the exhaust line (Bredwell et al. 1999; Klasson et al. 1992).

Various reactor designs have been studied in order to improve mass transfer such as hollow fiber membrane reactor (Lee et al. 2012; Munasinghe and Khanal 2012) and trickle-bed reactor (Orgill et al. 2013). The hollow fiber membrane biofilm reactor (HFM-BR) has shown a great potential for achieving high mass transfer efficiencies. In HMF-BR, gaseous substrate flows through the lumen of hollow fiber membrane and diffuses through the microporous membrane without forming bubbles; microorganisms grown as biofilm on the other side of the membrane are supplied nutrients from the aqueous phase. The hollow membrane module provides a large surface area for both gas-liquid transfer as well as cell attachment in relatively small reactor volume. Lee et al. (2012) studied the effects of membrane surface area, water velocity, and gas void fraction on the $K_{La}$ in an external type HFM-BR. Munasinghe and Khanal (2012) examined the $K_{La}$ as a function of inlet gas pressure and water recirculation rate of a multi-layered composite HFM-BR. Both studies reported high mass transfer coefficients.

To date, the HMF-BR has been used in a various biological conversion processes such as wastewater treatment (Gilmore 1999; Hwang et al. 2010; Pellicer-Nacher et al. 2010; Soreanu et al. 2010), hydrogenotrophic denitrification (Lee and Rittmann 2002), volatile organic compounds treatment (Kumar et al. 2008; Kumar et al. 2010a) and CO$_2$ sequestration by microalgae (Cheng et al. 2006; Kumar et al. 2010b). However, syngas fermentation based on HFM-BR system has been rarely reported, although the volumetric mass transfer coefficient ($K_{La}$) has been well characterized in abiotic conditions. While mass transfer of HFM-BR plays
an important role, the performance of a “real” syngas fermentation process may also be affected by other factors such as the characteristics of the biofilm (fouling and/or abrading) under different operational conditions, and the dilution rate in continuous operation. The objective of this work is to fill this gap by evaluating the cell growth, syngas consumption, and metabolites production of syngas fermentation of *Clostridium carboxidivorans* P7 in a microporous polypropylene (PP) based HFM-BR.

2. Materials and Methods

2.1. Set up of HFM-BR system

A Liqui-Cel® membrane contactor Mini-Module® (Membrana, Charlotte, NC) was used as membrane module. The module contains 7,400 microporous hydrophobic polypropylene X50 hollow fibers with 40% porosity, 0.04 µm pore size and 21 cm in length. The active surface area of the module was 1.4 m². The outer- and inner-diameter of the fiber are 300 µm and 220 µm, respectively. As shown in Figure 1, the membrane module was connected to a reservoir (8-L working volume) modified from a BioFlo-110 bench top fermenter vessel (New Brunswick Scientific, Edison, NJ). The agitation speed of the vessel was 200 rpm. The fermentation broth was recirculated between the module and vessel. The temperature of the vessel was kept at 37°C with a heat blanket; the module was covered with an insulation sheet to minimize heat loss. Artificial syngas (20% CO, 5% H₂, 15% CO₂, and 60% N₂) was fed into the lumen of the fibers. The pressure of the gas outlet was maintained at 15 psi by a back-pressure regulator.
2.2. Determination of volumetric mass transfer coefficient (K\textsubscript{L,a})

The K\textsubscript{L,a} of CO in HFM-BR system was determined under abiotic conditions at 25 °C. CO gas (99.5% pure) was fed inside the fiber lumen. Tap water was recirculated between the membrane module and the reservoir through a peristaltic pump. The gas flow rate and liquid flow rate were varied to evaluate the effects of gas velocity and the liquid velocity on K\textsubscript{L,a} value. Pressure in HFM lumen was regulated at 15 psig by a back-pressure value and liquid in reservoir was agitated at 200 rpm to maintain homogenization. Liquid samples were periodically withdrawn from the reservoir for immediate determination of dissolved CO concentration using myoglobin-bioassay (Kundu et al. 2003; Munasinghe and Khanal 2010b; Riggs and Heindel 2006). The K\textsubscript{L,a} value was determined as follows (Ahmed 1992b):

\[
\ln \left( \frac{C^* - C_0}{C^* - C_L} \right) = \frac{Q_L}{V} \cdot \left[ 1 - e^{-k_L a \frac{L}{u_L}} \right] \cdot t
\]

where \(C^*, C_0,\) and \(C_L\) are respectively the saturated concentration, initial concentration, actual concentration of CO in aqueous phase, \(Q_L\) is water recirculation rate through the shell side of the module, \(V\) is working volume of reactor system (module + vessel), \(K_L\) is the CO transfer coefficient (cm/sec), \(a\) is the gas-liquid interfacial area per volume of reactor (cm\textsuperscript{-1}), \(L\) is fiber length, \(u_L\) is liquid velocity passing through the module, and \(t\) is sampling time. For the membrane module used in this work, the values of \(a\) (1.75 cm\textsuperscript{-1}) and \(L\) (21 cm) are fixed.

2.3. Syngas fermentation using HFM-BR

2.3.1. Strain, medium, and inoculum preparation

\textit{Clostridium carboxidivorans} P7 (ATCC BAA-624) was used as the syngas fermenting microorganism. The cells were grown in 125 mL serum bottles with 80 mL working volume.
ATCC 1745 PETCH medium was used for subculture. The medium contains (per liter) 10 g fructose, 0.5 g yeast extract, 5 g morpholinoethanesulfonic acid, 25 mL mineral solution, 10 mL trace metal solution, 10 mL Wolfe’s vitamin solution, and 10 mL 4% cysteine-sulfide reducing agent (Tanner 2007). The medium (except vitamin and reducing agent) was autoclaved at 121°C for 20 min. After cooling down, the medium were purged with N₂ for 5 min to remove dissolved oxygen. Vitamin solution and reducing agent were then added to the bottles through 0.22 µm filter under aseptic anaerobic condition. The media pH was adjusted to 5.9 ~ 6.0 prior to inoculation.

The serum bottles were purged with syngas (50% CO, 30% H₂, 20% CO₂) up to 15 psi headspace pressure at the time of inoculation and every 24 hours afterward, incubated in a shaker with 200 rpm at 37°C. After 3 passages of subcultures, a total of 800 mL culture solution in serum bottles was inoculated into the reservoir of the HFM-BR system.

2.3.2. Syngas fermentation in HFM-BR

The syngas fermentation in HFM-BR was initially operated in batch mode. The medium was the same as for the subculture medium. For sterilization, the reservoir vessel with the media was autoclaved at 121°C for 20 min; the membrane module was soaked in 75% ethanol for 24 hours and then flushed with sterile distilled water. Before inoculation, the medium was purging with nitrogen gas for 3 hours followed with artificial syngas (20% CO, 5% H₂, 15% CO₂, 60% N₂) for another 3 hours. In the first 48 hours, the cells were retained in the vessel, without recirculation between the reservoir and the membrane module. The pH was controlled at 6.0. After 48 hours of batch culture, the liquid recirculation between vessel and module was started with pH being allowed to freely fluctuate within 4.5-5.5. Syngas was continuously fed into fiber
lumen of the module, directed to headspace of the reservoir, and exhausted from the condenser (Figure 1).

Once the suspended cells are completely attached on the membrane surface of the module and the thickness of the biofilm reaches equilibrium, the reactor was switched to continuous mode by feeding fresh media to and withdrawing spent broth from reservoir vessel every 4 hours. The composition of the feed medium was the same as those in subculture media except fructose was eliminated. The HFM-BR was operated at different setting of syngas flow rate, liquid recirculation rate, and dilution rate. The steady-state under each operation condition was considered to be established after at three volume changes (the total volume of liquid flowing through the fermenter), with a variation of exhausted gas composition less than 5%.

2.4. Analyses

The concentrations of CO, CO₂ and H₂ in the inlet and outlet gas of the reactor system were analyzed by a real-time gas monitor; CO and CO₂ measurement were based on non-destructive infrared sensors, while H₂ measurement was based on a thermal mass sensor (De Jaye Technologies, Des Moines, IA). To analyze fermentation metabolites (ethanol, acetic acid, 1-butanol and 1-butyric acid), the broth sample from the vessel (~1.5 ml) was acidified with 6 N HCl (200 ML), centrifuged at 18,440 g for 15 min, and filtered through a 0.22 μm membrane. The supernatants were analyzed for various compounds using a Varian 450 GC-FID (Varian Inc., Walnut Creek, CA) equipped with a Zebron ZB-WAXplus capillary column (Phenomenex, Torrance, CA). The sample was injected with a split ratio of 20:1 with the injector temperature of 200°C. Helium was used as carrier gas (25 mL/min). The detector temperature was 220°C. The oven temperature was controlled at 35°C for 5 min, ramped to 130°C at 10°C/min, and further to 230°C at 30°C/min.
3. Results and Discussions

3.1. Characteristics of CO mass transfer coefficient

The CO mass transfer efficient (K_{La}) of the HFM-BR system was characterized under different gas velocities passing through the fiber lumen and liquid velocity passing through the membrane module shell. As shown in Figure 2, increasing both gas and liquid velocity resulted in an increased K_{La} value. These trends indicate that the CO transfer from gaseous to liquid phase across the microporous membrane is governed by the resistance of both gas and liquid boundary layers located on the each side of the membrane (Ahmed 1992a; Yang and Cussler 1986); increasing gas and liquid velocity reduces the thickness boundary layer and, thus, increased the mass transfer efficiency. Previous studies using HMF for oxygen transfer have shown that higher liquid velocity resulted in reduced thickness of the boundary layer and hence enhanced gas transfer efficiency.

Various hollow fiber membrane reactors have been studied for their mass transfer efficiency. As shown in Table 1, K_{La} value was a function of a variety parameters such as the membrane module configuration, membrane material, liquid velocity, gas flow rate (i.e. the gas velocity), inlet gas pressure, and specific membrane surface area. Lee et al. (2012) reported that the specific membrane surface area, gas flow rate, and liquid velocity all affecting the K_{La} value of an external hollow fiber membrane reactor. The K_{La} value in our system (1096.2 hour^{-1}) was higher than that obtained by Lee et al. (385.0 hour^{-1}) (Table 1); however, considering the 3-fold specific membrane surface area of our system as compared to that reported by Lee et al. (2012), the specific CO mass transfer efficiency (k_{L}) of the two reactors were comparable. Table 1 also shows that the configuration of the membrane module (internal vs. external) played an important
role for mass transfer efficiency. For example, Munasinghe and Khanal (2010) reported a low $K_{L,a}$ value (1.08 hour$^{-1}$) of an internal module system; i.e., the module was submerged into a column reactor. When an external configuration was used, however, a magnitude-order higher $K_{L,a}$ value (946.6 hour$^{-1}$) was achieved (Munasinghe and Khanal 2012). Orgill et al. (2013) also reported a high $K_{L,a}$ value with a “stand alone” system, due to the high value of specific membrane surface area (a) in use.

To further compare the mass transfer efficiency of hollow fiber membrane system to other reactor configurations, Figure 3 summarizes the $K_{L,a}$ value as a function of specific CO flow rate reported for various syngas fermentation reactors. It clearly shows hollow fiber membrane reactor led to a higher $K_{L,a}$ value, indicating the potential of HFM-BR (especially the external type) to achieve an efficient mass transfer even with low gas flow rate. In the following studies, syngas fermentation in an external HFM-BR system was performed to evaluate the effects of several key factors on fermentation performance.

3.2. Syngas fermentation of *C. carboxidivorans* P7 in HFM-BR in batch culture

The syngas fermentation performance of *C. carboxidivorans* P7 in a batch mode in HFM-BR system is shown in Figure 4. As shown in Figure 4A, during the first 2 days when there was no liquid recirculation, the suspension cell growth followed a common exponential growth pattern for acetogenic clostridia (Bruant et al. 2010; Liou et al. 2005). The medium pH trends to drop but was brought back to around 6.0 in order to promote cell growth. The liquid recirculation between the membrane module and the reservoir vessel was started at the end of day 2. It was found that the suspended cells in the reservoir were gradually attached onto the membrane surface for biofilm buildup until reaching an equilibrium thickness while the suspended cell mass
in the vessel reduced. Meanwhile, pH of the medium was not further controlled during this stage for promoting ethanol formation. Accordingly, pH reduced to acidic range (Figure 4A).

Figure 4B showed the production of ethanol and acetic acid during batch syngas fermentation. In the first two days, acetic acid was produced as the predominate metabolite, which tends to reduce the medium pH (Datar et al. 2004). The highest acetic acid concentration obtained (8.2 g/L) was higher than those reported previously (Bruant et al. 2010; Datar et al. 2004; Ukpong et al. 2012), due to the inclusion of fructose in the culture medium. Without pH control from day 2 afterward, the accumulation of acetic acid tended to drop the pH, which in turn triggered a change in cell metabolism from acidogenesis to solventogenesis (Worden et al. 1991). As a result, ethanol production gradually increased; while acetic acid production ceased (Figure 4B). The acetic acid concentration was actually decreased at this stage, probably due to conversion of acetic acid to ethanol by *C. carboxidivorans* (Ramachandriya et al. 2011; Ukpong et al. 2012).

In addition to ethanol and acetic acid, small amount of butanol (less than 0.45 g/L) was also observed in syngas fermentation of *C. carboxidivorans* P7 (data not shown) since this strain is one of the very few CO-autotrophic acetogenic bacteria that is capable of producing butanol (Liou et al. 2005). This strain has been reported to possess genes encoding acetyltransferase, dehydrogenase and dehydratase enzymes involved in the conversion of acetyl-CoA to butanol via butyryl-CoA intermediate (Bruant et al. 2010).
3.3 Syngas fermentation of *C. carboxidivorans* P7 in HFM-BR in continuous culture

3.3.1 Effects of syngas flow rate on fermentation performance

Continuous syngas fermentation was performed at different syngas flow rate to the membrane module. As shown in Figure 5A, when increasing syngas flow rate from 50 to 300 mL/min, both CO and H$_2$ consumption rate increased while their utilization efficiency maintained at a relatively high level, indicating that syngas fermentation was substrate-limited within this range of flow rate. Further increasing flow rate from 300 to 500 mL/min did not significantly ($p > 0.05$) increase gas consumption rate; meantime, syngas utilization efficiency decreased sharply. The results indicate that at this high range of flow rate, the syngas supply may have exceeded the cells’ maximum capability of syngas utilization. The surface area of the HFM module determines the maximum amount of biomass that can be cultivated, which in turn determines the maximum syngas utilization rate of HFM-BR system. In a practical operation of HFM-BR system, therefore, the syngas flow rate should be carefully adjusted to match the module size so to maximize the syngas utilization efficiency.

The H$_2$ consumption by *C. carboxidivorans* P7 demonstrated the presence of hydrogenase in this strain (Datar et al. 2004; Ramachandriya et al. 2011). As *C. carboxidivorans* P7 obtains reducing equivalents ([H$^+$] and electrons) in Wood-Ljungdahl pathway through either CO oxidation by CO dehydrogenase (CODH) and/or H$_2$ oxidation by hydrogenase (Ragsdale and Pierce 2008), the utilization of H$_2$ can spare more CO available for product formation, thus, the overall product yield can be improved (Ahmed and Lewis 2007; Skidmore et al. 2013).

Figure 5B showed the concentration and productivity of ethanol and acetic acid as a function of syngas flow rates. The yield and productivity had a similar trend because of the same dilution rate used. Both ethanol and acetic acid production increased with increasing gas flow
rate from 50-200 mL/min. When gas flow rate exceeded 200 mL/min, ethanol production decreased with syngas flow rate, while acetic acid production continuously increased (Figure 5B).

Figure 5C showed the yield of ethanol and acetic acid based on CO input (mol product-C/mol CO-C). Overall, the yield of ethanol and acetic acid revealed an opposite trend, which was also reported in previous studies using the same strain (Datar et al. 2004; Ukpong et al. 2012). This might be attributable to the conversion of acetic acid to ethanol with the presence of aldehyde oxidoreductase, and aldehyde/alcohol dehydrogenase enzymes (Bruant et al. 2010). Syngas flow rate at 200 mL/min resulted in the highest ethanol yield (0.24 molC/molC), approximately 71% of theoretical yield (0.33 molC/molC) (Ramachandriya et al. 2011). Meantime, the yield of acetic acid (0.05 mol C/mol C) reached to the lowest value. Collectively, Figure 5C indicated that syngas flow rate is an important factor influencing the metabolites distribution of C. carboxidivorans P7.

3.3.2 Effects of liquid recirculation rate on syngas fermentation performance

Liquid recirculation rate (Q_L) between the HFM module and the reservoir vessel is another important parameter influencing syngas fermentation. As the cross-sectional area of HFM module (A) was fixed, Q_L is directly proportional to the liquid velocity (u_L) passing through the module, which has shown a significantly effect on the mass transfer efficiency (Figure 2B). In addition to mass transfer, liquid recirculation rate is also crucial to fermentation performance through maintaining an appropriate thickness of the biofilm in the module. In general, a high recirculation rate tends to abrade the biofilm due to the correspondingly high shear stress; while a low recirculation rate tends to cause membrane biofouling in HFM module.
The effects of liquid recirculation rate on syngas fermentation are presented in Figure 6. As shown in Figure 6A, CO consumption rate and the utilization efficiency increased with liquid recirculation rate increasing from 50 to 200 mL/min and leveled off when liquid recirculation rate exceeded 200 mL/min. H₂ consumption rate and utilization efficiency maintained at a relatively constant level (ca. 80%) from 50 – 300 mL/min of liquid recirculation, but decreased when liquid recirculation further increased to 500 mL/min.

Figure 6B showed the ethanol and acetic acid production as a function of liquid recirculation rate. The ethanol concentration and productivity reached the highest level at 200 mL/min of liquid recirculation, and decreased when the recirculation rate further increased. Acetic acid production increased as the liquid recirculation rate increased from 50 to 300 mL/min but leveled off afterward. Figure 6C showed the yield of ethanol and acetic acid from CO as well as their molar ratio. Overall, the ethanol yield achieved the highest level at 200-300 mL/min of liquid recirculation rate but decreased at 500 mL/min of liquid recirculation, while the acetic acid yield was relatively stable. As a result, the highest ethanol to acetic acid molar ratio was achieved at 200 ml/min.

The above results indicated that the liquid recirculation rate had complex effects on the syngas fermentation performance of HFM-BR. For example, the increased liquid recirculation rate (i.e. directly proportional to liquid velocity) promotes the mass transfer efficiency (Figure 2B), which might be the major factor contributing to the enhanced CO/H₂ utilization efficiency (Figure 6A) as well as the ethanol/acetic acid production when liquid recirculation rate was kept at lower range (Figure 6B). On the other hand, when the liquid recirculation rate was at a high level (i.e., 300-500 mL/min), immobilized biomass became unstable and tended to be abraded from the HFM module. At 500 mL/min of liquid recirculation, patchy biofilm were observed to
be peeled off from the membrane module, while the suspended cells in the reservoir vessel increased correspondingly. This detachment was either caused by the physical shear stress or the retarded formation of the biomass-EPS (extracellular polymeric substances) complex (Rickard et al. 2004). Moreover, the newly grown layer of biofilm may produce more acetic acid than ethanol because acetic acid is a preferred metabolite when cells are in growth stage, this metabolisms switch may eventually result in a reduced ratio of ethanol to acetic acid at higher liquid recirculation rate (Figure 6C).

3.3.3 Effects of dilution rate of syngas fermentation performance

Dilution rate is another important factor influencing the continuous operation of syngas fermentation. Unlike syngas flow and liquid recirculation affecting the mass transfer efficiency, membrane biofouling and biofilm attachment/detachment phenomenon, dilution rate did not impose impacts on those physical properties related with the HFM module because continuous syngas fermentation was performed by adding fresh medium in and withdrawing spent medium from the reservoir vessel (Figure 1). However, the existence of the HFM module enables cells to be retained in the module rather than being washed out with the medium; therefore, syngas fermentation in the HFM-BR system was able to be operated at a high dilution rate. For example, the dilution rate used in this work can reach as high as 0.96 day$^{-1}$, two-fold higher than possible in suspended growth syngas fermentation reactors using the same strain (Datar et al. 2004).

Figure 7 illustrated the syngas fermentation performance of HFM-BR operated at different dilution rates. As shown in Figure 7A, CO and H$_2$ utilization efficiencies remained relatively stable with dilution rate ranging from 0.12-0.48 day$^{-1}$. At higher dilution rate (i.e., 0.96 day$^{-1}$), however, the CO and H$_2$ consumption rate and their utilization efficiency revealed an
opposite trend. Figure 7B showed that elevated dilution rate reduced ethanol and acetate concentrations due to the “diluting effect”. The ethanol productivity was relatively stable in the range of 0.12-0.48 day\(^{-1}\), and increased significantly \((p < 0.1)\) when dilution rate increased to 0.96 day\(^{-1}\). In contrast, acetate productivity showed an increasing trend over the entire range of dilution rate. Figure 7C showed the yield of ethanol and acetic acid on CO and their molar ratio. Over the entire dilution rate range, the ethanol yield maintained stable, while the acetic acid yield increase with dilution rate; the ratio of ethanol to acetic acid monotonically decreased.

Overall, the effects of dilution rate on syngas fermentation performance were consistent with previous studies (Datar et al. 2004; Klasson et al. 1992; Mohammadi et al. 2012; Vega et al. 1989). It shows that the metabolism of *C. carboxidivorans* P7 in HFM-BR was strongly affected by dilution rate. For example, at lower dilution rate, the biofilm growth was slow as the nutrient supply was limited; as a result, the cell metabolism was dominated by solventogenesis. When the dilution rate increases, the fresh medium imposed an increased supply of nutrients particularly yeast extract, which support the P7 cell growth (Klasson et al. 1992). Consequently, the cells metabolism tends to migrate to growth-associated acidogenesis. This non-growth to growth stage transition is also reflected in the pH change, indeed, with the dilution rate increases, the medium pH steadily increase towards to neutral range which is more favorable cell the growth (data not shown).

### 3.4 Comparison of syngas fermentation of HFM-BR with other type of reactors

Table 2 summarized syngas fermentation performance (syngas conversion efficiency, ethanol concentration, and ethanol to acetic acid molar ratio) obtained in various reactor configurations. As shown in the table, most of the studies were conducted in suspended growth
reactors (CSTR or bubble column reactor). The HFM-BR system used in this study resulted in an ethanol concentration of 23.93 g/L and a ratio of ethanol to acetic acid of 5.20. These results were higher than (or at least comparable to) most of previous results (Table 2). It should be noted that there is still room for further increasing ethanol production in our system through various strategies as adapted by other researches listed in Table 2, such as retaining high cell density (Gaddy et al. 2007; Klasson et al. 1991; Vega et al. 1989), maintaining acidic pH, using two-stage reactor systems to separate acidogenesis and solventogenesis (Gaddy et al. 2007; Klasson et al. 1991), and limiting some nutrients supply (Gaddy et al. 2007). Selecting the syngas fermenting strain is also very important (Table 2). For example, Klasson et al. (1993) reported a quite high ethanol titer (48 g/L) with *C. ljungdahlii* syngas fermentation by pH control and medium nutrient manipulation.

4. Conclusions

This work demonstrated the effectiveness of using hollow fiber membrane biofilm reactor (HFM-BR) as a unique system for syngas fermentation. Although the HFM-BR has been well studied for its enhanced mass transfer characteristics in abiotic conditions, to our best knowledge, this is the first comprehensive report on the use of an HFM-BR system for syngas fermentation. The results indicate that the syngas fermentation performance is affected not only by the mass transfer characteristics, but also the biological characteristics of the strain as well as the physical properties of the membrane module (biofouling or abrading of the biofilm). A continuous HFM-BR based syngas fermentation of *Clostridium carboxidivorans* P7 resulted the highest ethanol concentration of 23.93 g/L and ethanol productivity of 3.44 g/L-day. This ethanol production values was much higher than the suspended culture of the same strain. Future work is
needed to evaluate the scalability of the system so a commercial syngas fermentation using HFM-BR could be feasible.
REFERENCES


Gaddy JL; 2000. Biological production of ethanol from waste gases with Clostridium ljungdahlii. US patent 6136577 A.


Table 1.
Maximum $K_{La}$ value in various HFM-BR configurations for syngas fermentation

<table>
<thead>
<tr>
<th>Configuration</th>
<th>HFM material</th>
<th>Gas fed</th>
<th>$u_L$ (cm/s)</th>
<th>$q_G$ (vvm)</th>
<th>$P_G$ (psi)</th>
<th>Specific membrane surface area (cm$^{-1}$)</th>
<th>$K_{La}$ (hour$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal</td>
<td>PE/PU/PUE</td>
<td>CO</td>
<td>NA $^{d}$</td>
<td>no gas outlet (dead-end)</td>
<td>35.0</td>
<td>2.00</td>
<td>1.08</td>
<td>Munasinghe and Khanal 2010b</td>
</tr>
<tr>
<td>External</td>
<td>PE/PU/PUE</td>
<td>CO</td>
<td>1.27</td>
<td>no gas outlet (dead-end)</td>
<td>30.0</td>
<td>2.00</td>
<td>946.6</td>
<td>Munasinghe and Khanal 2012</td>
</tr>
<tr>
<td>External</td>
<td>PP</td>
<td>50% CO, 30% H$_2$, 20% CO$_2$</td>
<td>2.20</td>
<td>0.029</td>
<td>16.6</td>
<td>0.56</td>
<td>385.0</td>
<td>Lee et al. 2012</td>
</tr>
<tr>
<td>Stand alone $^a$</td>
<td>PDMS</td>
<td>Air with 20.9% O$_2$</td>
<td>NA</td>
<td>40 ~ 80</td>
<td>0.7</td>
<td>100</td>
<td>1062</td>
<td>Orgill et al. 2013</td>
</tr>
<tr>
<td>External</td>
<td>PP</td>
<td>CO</td>
<td>1.14</td>
<td>0.625</td>
<td>15.0</td>
<td>1.75</td>
<td>1096.2</td>
<td>This study</td>
</tr>
</tbody>
</table>

$^a$ the HFM module was used as sole reactor system, without liquid recirculation between reservoir and module
$^b$ PE: polyethylene; PU: polyurethane; PP: polypropylene; PDMS: polydimethylsiloxane
$^c$ membrane surface area divided by working volume
$^d$ not available
Table 2. Syngas fermentation performance carried out in various reactor configurations

<table>
<thead>
<tr>
<th>Reactor configuration</th>
<th>Syngas composition</th>
<th>Biocatalyst</th>
<th>pH</th>
<th>Ethanol production</th>
<th>Ethanol : Acetate ratio</th>
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<td>CSTR</td>
<td>CO/H₂/CO₂/Ar (56.1/18.5/10/15.4)</td>
<td><em>C. ljungdahlii</em></td>
<td>4.0</td>
<td>2.8 g/L</td>
<td>1.25</td>
<td>Vega et al. 1989</td>
</tr>
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<td>Two-stage CSTR</td>
<td>NA [1]</td>
<td><em>C. ljungdahlii</em></td>
<td>4.5</td>
<td>3.0 g/L</td>
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<td>Klasson et al. 1991</td>
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<tr>
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<td><em>C. ljungdahlii</em></td>
<td>4.5</td>
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<td>4.9</td>
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<td>5.3</td>
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<td>BCR</td>
<td>CO/H₂/CO₂/CH₄ (14.7/4.4/16.5/4.2)</td>
<td><em>C. carboxidivorans</em> P7</td>
<td>5.2~5.4</td>
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<td>10.5 g/L</td>
<td>57.25</td>
<td>Richter et al. 2013</td>
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<td>5.5 (1&lt;sup&gt;st&lt;/sup&gt;)</td>
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<td>57.25</td>
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<td>57.25</td>
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<td>4.5 ~ 6.0</td>
<td>23.93 g/L</td>
<td>4.79</td>
<td>This study</td>
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[1] NA: not available
Figure 1. HFM-BR configuration for syngas fermentation
Figure 2. Volumetric mass transfer coefficient ($K_{La}$) of CO as a function of (A) gas velocity, $u_G$ (with liquid velocity fixed at 2.7 cm/sec) and (B) liquid velocity, $u_L$ (with gas velocity fixed at 4.8 cm/sec). Data are means of three replicates and error bars show standard deviations.
Figure 3. Volumetric mass transfer coefficient ($K_{L,a}$) of CO measured in various syngas fermentation reactor configurations.
Figure 4. Time course of batch syngas fermentation in HFM-BR (A) medium pH and suspended cell dry weight, (B) ethanol and acetic acid concentration.
Figure 5. Syngas fermentation performance in HFM-BR at varied syngas flow rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetic acid molar ratio. Liquid recirculation rate was fixed at 200 mL/min; dilution rate was fixed at 0.12 day\(^{-1}\). Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 6. Syngas fermentation performance in HFM-BR at varied liquid recirculation rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetic acid molar ratio. Syngas flow rate was fixed at 300 mL/min; dilution rate was fixed at 0.12 day\(^{-1}\). Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 7. Syngas fermentation performance in HFM-BR at varied dilution rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetic acid molar ratio. Syngas flow rate was fixed at 300 mL/min; liquid recirculation rate was fixed at 200 mL/min. Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
SYNGAS FERMENTATION IN A MONOLITHIC BIOFILM REACTOR: ENHANCING MASS TRANSFER AND ETHANOL PRODUCTION

A manuscript to be submitted to Biotechnology and Bioengineering

Yanwen Shen, Robert C. Brown, Zhiyou Wen

ABSTRACT

Syngas fermentation is a promising hybrid process for production of lignocellulosic-biomass-derived fuels and chemicals. However, several engineering challenges need to be addressed with gas-to-liquid mass transfer limitation being the major bottleneck. Here we explore the applicability of a monolithic biofilm reactor (MBR) in syngas fermentation. The volumetric mass transfer coefficient \( k_{L,a} \) of carbon monoxide (CO) in MBR was determined under abiotic condition with varied superficial gas and liquid velocities. Continuous syngas fermentation using \textit{Clostridium carboxidivorans} strain P7 was conducted and optimized by investigating the effects of syngas flow rate, liquid flow rate and dilution rate. The optimal operating condition (syngas flow rate 300 mL/min, liquid flow rate 500 mL/min and dilution rate 0.48 day\(^{-1}\)) was selected which led to the highest ethanol productivity (2.4 g/L/day). Syngas fermentation performance in MBR was compared to that in a bubble column reactor (BCR) with the identical reactor geometry and operating condition. MBR showed superior syngas fermentation performance than BCR, with regard to 57\% enhancement in CO consumption rate, 60\% increase in ethanol productivity and 36\% increase in acetate productivity. This study demonstrated the great potential of MBR as a promising reactor configuration for syngas...
fermentation characterized by efficient mass transfer, low energy consumption and high metabolite productivity.

**KEYWORDS:** Syngas fermentation; monolithic column; biofilm reactor; mass transfer; *Clostridium carboxidivorans*; ethanol

1. Introduction

Lignocellulosic biomass is considered as a promising renewable feedstock for biofuel production. Two working platforms have been developed to convert lignocellulosic biomass to biofuel: (1) sugar platform (saccharification + fermentation) and (2) syngas platform (Fisher-Tropsch process: gasification + metal-catalytic conversion). As an alternative platform, syngas fermentation is a hybrid thermo-biochemical process, where biomass is firstly gasified to syngas and then converted to fuels and chemicals by syngas fermenting microorganisms. Syngas fermentation has attracted much attention due to its inherent advantages (Munasinghe and Khanal 2010a) and consequently, is already utilized industrially for pilot and commercial scale cellulosic ethanol production (INEOS Bio, Coskata, LanzaTech). However, several engineering challenges need to be addressed to improve biofuel productivity, including gas-to-liquid mass transfer limitation as the bottleneck problem (Bredwell et al. 1999).

Continuous stirring tank reactor (CSTR) is extensively applied in syngas fermentation and increasing agitator’s power-to-volume ratio is a common approach to enhance gas-to-liquid mass transfer efficiency (Riggs and Heindel 2006). However, this approach is not economically feasible for bioreactor scale-up, because high agitation speed means excessive power costs. In contrast, an energy-efficient alternative is syngas microbubble dispersion (Bredwell and Worden...
1998), which generates micro-bubbles with diameter of 10 ~ 100 µm, compared to bubbles with diameter of 3 ~ 5 mm formed by conventional gas diffuser (Kaster et al. 1990). Consequently, efforts have been made for efficient mass transfer by conducting syngas fermentation in agitation-free bioreactor configurations such as bubble column reactor (Datar et al. 2004) and airlift reactor (Munasinghe and Khanal 2010b) with microbubble diffuser. However, performance limitations were observed due to low biomass concentration and cell wash-out phenomenon at high dilution rate (Datar et al. 2004; Mohammadi et al. 2012). As alternative to suspended growth reactors, attached growth reactors are enabled to achieve high cell densities even at high dilution rate by biofilm formation on the supports (Qureshi et al. 2005).

Monolithic biofilm reactor (MBR) can be a promising attached growth bioreactor to achieve efficient mass transfer for syngas fermentation. Monoliths, usually made of metal or ceramic materials, are structures of parallel straight channels separated, which have been widely used in multi-phase catalytic processes. Monoliths can be tailored to fulfill the needs for an economically feasible and microbial immobilization support for biological application with many inherent advantages, including low pressure drop, high specific surface area and superior mechanical strength (Kreutzer et al. 2005). Taylor flow (Figure 1A) is one of the dominant flow regimes in MBR, where gas and liquid move through the microchannel as separate slugs. With the biofilm attached onto the channel surface, gaseous substrate is transferred from gas phase to biofilm through the thin liquid film layer sandwiched between the two phases (Figure 1B). Gas-liquid slug flow facilitates the availability of both gases substrate and nutrients in liquid for biofilm development and mass transfer efficiency; with the thickness of liquid boundary layer reduced, improved mass transfer efficiency is obtained. Many literatures reported the biological application of MBR in solvent/acid production (Ariga et al. 1986; Shiraishi et al. 1989),
hydrogen production (Fritsch et al. 2008), wastewater biodegradation (Ebrahimi et al. 2006; Quan et al. 2003; Sun and Shan 2007; Zhang et al. 2002) and waste-gas biofilter (Jin et al. 2008; Rene et al. 2010). However, applying MBR for syngas fermentation has not been explored. The objective of this study is to investigate the feasibility of employing MBR in syngas fermentation to enhance CO mass transfer and ethanol production.

2. Materials and Methods

2.1. Set-up of MBR system

The ceramic monolith, made of cordierite, was supplied by Applied Ceramics Inc (Doraville, GA) and it has the following characteristics: cylinder column with geometry-square vertical channels, 3.66 inches in diameter, 12 inches in length, 200 cpsi (cells per square inch) in cell density, 1.5 mm × 1.5 mm in channel cross-section of a single cell, channel wall thickness 0.3 mm, geometric surface area 1850 m²·m⁻³, 70% void fraction. The monolith was housed in a Plexiglass column and two block rings were placed at the top and bottom of the monolith to prevent gas or liquid by-pass. The schematic diagram of the monolithic biofilm reactor (MBR) system setup was shown in Figure 2. The monolith column was connected to a reservoir modified from a BioFlo 110 bench top vessel (New Brunswick Scientific, Edison, NJ) and the working volume of the system was 8 L. Fermentation broth was circulated between the monolithic column and vessel. The vessel temperature was maintained at 37 °C with a heating blanket and the monolithic column was covered with an insulation sheet to minimize heat loss. Artificial syngas (20% CO, 5% H₂, 15% CO₂, 60% N₂) was fed through two wooden 50-μm microporous diffusers placed at the bottom of the monolithic column.
As a control, a bubble column reactor was developed to evaluate syngas fermentation and CO mass transfer performance, which has the identical dimension and operating conditions to MBR.

2.2 Determination of volumetric mass transfer coefficient ($k_{L,a}$)

The CO $k_{L,a}$ value in MBR was determined under abiotic conditions at 25 °C. High purity (99.5%) CO (Praxair USA, Danburg, CT) was sparged into the monolithic column using wooden microporous diffusers at certain flow rate. Water circulation was carried out using a digital peristaltic pump (Masterflex L/S 7524-40, Cole Parmer, Vernon Hills, IL) and maintained a certain liquid flow rate. Liquid samples were periodically withdrawn from the sampling port located at liquid overflow line with 10 µL gastight high performance syringes (Hamilton Gastight1701, Reno, NV) to measure the dissolved CO concentration in aqueous phase using myoglobin-bioassay (Kundu et al. 2003). The detailed procedures were described elsewhere (Riggs and Heindel 2006) and the CO $k_{L,a}$ value was determined using the following equation:

$$\ln \left( \frac{C^* - C_0}{C^* - C_L} \right) = (k_{L,a}) \cdot t$$

where $C^*$ is the saturated CO concentration in aqueous phase, $C_0$ is the initial CO concentration in aqueous phase, $C_L$ is the real-time dissolved CO concentration in aqueous phase, $k_L$ is the gas transfer coefficient (cm/sec), $a$ is the gas-liquid interfacial area per working volume of reactor (cm$^{-1}$) and $t$ is the sampling time.
2.3 Syngas fermentation in MBR

2.3.1 Strain, medium and inoculum preparation

*Clostridium carboxidivorans* strain P7 (ATCC BAA-624) was obtained from American Type Culture Collection (ATCC, Manassas, VA) and maintained under strictly anaerobic conditions in 125 mL serum bottles containing 80 mL of modified 1754 PETC medium (per liter): 10 g fructose, 0.5 g yeast extract, 5 g MES, 25 mineral solution, 10 mL trace metal solution, 10 mL Wolfe’s vitamin solution and 10 mL 4% cysteine-sulfide reducing agent (Tanner, 2007). Resazurin solution (0.1%) was added as a redox indicator. The medium (excluding vitamin solution and reducing agent) was autoclaved at 121 °C for 20 min and cooled to room temperature. Then 100% N$_2$ was continuously sparged through the medium for 5 min to remove dissolved oxygen. Vitamin solution and reducing agent were added to the medium using 0.22 µm sterile filter under aseptic and anaerobic condition. The medium pH was adjusted to 6.0 prior to inoculation.

Culture media in serum bottles were purged with artificial syngas (20% CO, 5% H$_2$, 15% CO$_2$, 60% N$_2$) at headspace pressure of 15 psi at the time of inoculation and every 24 hours afterwards, incubated in a shaker at 37 °C, 200 rpm. After 3 passages of subcultures, a total of 800 mL seed culture in serum bottles was inoculated into the MBR reservoir at rate of 10% (v/v).

2.3.2 Syngas fermentation in MBR system

The MBR system was initially operated in batch mode until biomass attachment was observed. The batch culture medium in the bioreactor was the same as that for subculture passage. The reservoir vessel containing media was autoclaved at 121 °C for 20 min, while the monolithic column was sterilized using 75% (v/v) ethanol for 24 hours, after which it was
drained and washed thoroughly with sterilized distilled water. Following sterilization, fermentation medium was continuously purged with N\textsubscript{2} and artificial syngas (20\% CO, 5\% H\textsubscript{2}, 15\% CO\textsubscript{2}, 60\% N\textsubscript{2}) for 3 hours. The bacteria were retained in the reservoir vessel within the first 48 hours in batch culture and the reactor pH 6.0 was maintained to promote cell growth (Liou et al. 2005). Afterwards, the fermentation broth was circulated between monolithic column and reservoir. Reactor pH was allowed to fluctuate between 4.5 and 5.5 to favor solventogenesis (Klasson et al. 1992). Syngas was continuously fed through the monolithic column with the outlet gas directed to headspace of the reservoir and exhausted from the condenser (Figure 2).

The reactor was operated under batch culture for 15 days for biofilm buildup and then switched to semi-continuous operation: fermentation broth was withdrawn from reactor followed by fresh liquid medium feed 6 times per day at 4-hour intervals. The composition of the feed medium was the same as that of batch culture except that fructose was eliminated. The reactor was operated continuously during different running phases with specific syngas loading, liquid circulation rate and dilution rate.

2.4 Analytical methods

The inlet syngas and exhaust gas compositions (CO, H\textsubscript{2} and CO\textsubscript{2}) were analyzed by DJGAS, a real-time non-dispersive infrared gas monitor (De Jaye Technologies, Des Moines, IA). Liquid sample (1.5 mL) was collected from withdrawn fermentation broth and pretreated prior to gas chromatography (GC) analysis: acidified with 6 N HCl (200 \mu L), centrifuged at 18440 \textit{g} for 15 min and filtered through a 0.22 \mu m membrane. The filtrate was analyzed for ethanol and acetic acid concentration using a Varian 450 GC-FID (Varian Inc., Walnut Creek, CA) equipped with a Zebron ZB-WAXplus capillary column (Phenomenex, Torrance, CA). The
carrier gas was helium (25 mL/min). The sample was injected with a split ratio of 20:1 and the injector temperature was 200 °C, while the detector temperature was 220 °C. The oven temperature profile was 35 °C for 5 min, after which the temperature was ramped to 130 °C at rate of 10 °C/min and then ramped to 220 °C at rate of 30 °C/min.

Pieces of biomass immobilized monolithic channel chip were observed using scanning electron microscopy (SEM) until completion of syngas fermentation. Chips were fixed with 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C for 48 hours. Samples were then rinsed deionized water and post-fixed in 2% aqueous osmium tetroxide followed by dehydration in a graded ethanol series up to 100% ultra-pure ethanol and dried using a Denton DCP-2 critical point dryer (Denton Vacuum, LLC, Moorestown, NJ). Dried samples were mounted on stubs and sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ) with palladium-gold alloy. Finally the samples were imaged using a JEOL 5800LV SEM (Japan Electron Optics Laboratory, Peabody, MA) at 10kV with a SIS ADDA II for digital image capture (Olympus Soft Imaging Systems Inc., ResAlta, Golden, CO).

3. Results and Discussions

3.1 CO mass transfer coefficient

The effects of superficial gas (u_G) and liquid velocity (u_L) on CO mass transfer coefficient (k_La) in MBR was compared under monolith gas-liquid cocurrent upflow (MCU) and bubble column (BC) operating mode (Figure 3). The u_G effect on CO k_La was investigated by fixing u_L constant at 0.28 cm/sec and varying u_G from 0.26 to 2.60 cm/sec. Figure 3(A) revealed that CO k_La is positively correlated to u_G under both MCU and BC modes with u_L fixed at 0.26 cm/sec. The flow pattern in a monolithic microchannel is highly dependent on parameters,
including physical properties of gas and liquid (e.g. density, viscosity and surface tension), geometry of the microchannel (e.g. shape of cross-section, diameter), flow operation mode (e.g. cocurrent, countercurrent, upflow, downflow) and gas ($u_G$) and liquid velocities ($u_L$). When $u_L > 1.7$ cm/sec and $0.3 < u_G/u_L < 2.0$, slug flow is the dominant flow pattern (Kreutzer et al. 2005). Moreover, at low liquid velocity ($0.01 < u_L < 0.5$ cm/sec) and moderate gas velocity ($0.5 < u_G < 5.0$ cm/sec), slug flow was also found to be the two-phase flow regime in the monolith bioreactor under gas-liquid cocurrent upflow operation mode (Kawakami et al. 1987; Kawakami et al. 1989). Though the flow regime was not visually identified in this study, it might be estimated based on a developed flow regime map for laminar flow ($Re < 2100$). Figure 3(C) indicated that the flow regime falls into the region of slug flow with $u_L$ fixed at 0.260 cm/s and $u_G$ varied from 0.260 to 2.604 cm/s (Figure 3A) based on the flow map developed by Ambatipati (2005), where boundaries of bubbly/slug flow and slug/annular flow were quantified by correlation between Weber number ($We_L$) and gas-phase Reynolds number ($Re_G$). In lower $u_G$ regime ($u_G > 1$ cm/sec), CO $k_{L,a}$ under MCU mode is significantly ($p < 0.05$) higher than that under BC mode, which was attributable to the slug flow in microchannels (Vandu et al. 2005): with surface tension pushing the bubbles towards channel wall, a very thin liquid film remains between the gas bubble and channel wall, whereas the liquid separates the gas bubbles as slug, preventing bubble coalescence. Compared to bubble column reactor, despite that monolithic reactor requires energy consumption in the first place to sparge gas into the column for fine gas bubbles generation, no external energy is needed to retain or to further break up the bubbles inside the microchannels. In lower $u_G$ regime ($u_G < 1$ cm/sec), MCU does not show significant advantage ($p > 0.05$) over BC mode with regard to CO $k_{L,a}$ value (Figure 3A). So it could be possible that
bubbly flow occurs under MCU mode when \( u_G < 1 \) cm/sec, in which case a monolithic reactor is considered as a bubble column reactor (Liu et al. 2005).

Figure 3(B) showed that \( CO \) \( k_{La} \) increases with the increase of \( u_L \) under MCU and BC modes and that MCU operation mode gave the \( CO \) \( k_{La} \) values 41% ~ 54% higher than the BC mode when \( 0.260 < u_L < 0.521 \) cm/sec. This might also result from the slug flow formed under MCU mode: at \( 0.156 < u_L < 0.156 \) cm/sec, data plotted on the flow map (Figure 3C) fall into the slug flow region. Overall, Figure 3 indicated that gas and liquid velocities are two important parameters to determine the two-phase flow regime and that slug flow occurring in a monolithic reactor has superiority over a bubble column reactor to enhance mass transfer efficiency.

### 3.2 Comparison of liquid-batch syngas fermentation in MBR and BCR

The profiles of medium pH, suspended cell dry weight, ethanol and acetic acid concentrations in MBR and BCR were compared over 15-day liquid-batch syngas fermentation (Figure 4). Within the first 2 days when P7 cells were remained in reservoir vessel with pH controlled at 6.1~6.2, cell growth curves observed in both MBR and BCR (Figure 4A) showed a typical exponential growth pattern for acetogenic clostridia. No apparent initial growth delay was found herein since 10 g/L fructose was included in the liquid media at the start to eliminate the substrate-diffusion limitation for faster cell growth. By the end of day 2, fermentation broth was recirculated between monolithic column and reservoir to allow cell attachment on the surface of monolithic microchannels and pH was not controlled unless it dropped below 4.0. For MBR, suspended cell mass gradually decreased (day 3 ~ 8) and eventually stayed stable at approximately 0.3 g DW/L (Figure 4A), indicating that \( C. \) carboxidivorans P7 immobilized into the monolithic column. Medium pH continuously to 4.36 (day 6), then rebounded up to 5.04 (day
11) and afterwards leveled off. As for BCR, after peaking at 1.3~1.4 g DW/L (day 2~3), suspended cell mass went down slowly to the valley value (0.9 g DW/L, day 11) and slightly increased afterwards, which coincided with the pH rebound (Figure 4A). The maximum suspended cell dry weight obtained in MBR and BCR during liquid-batch condition was higher than what was reported previously for bioreactor-scale P7 syngas fermentation (Ahmed et al. 2006; Datar et al. 2004; Rajagopalan 2002; Ukpong et al. 2012). This might be due to the fructose (10 g/L) added at the start and higher headspace gas pressure (15 psi) used in the present study. It has been reported that higher CO and H2 partial pressure facilitates cell growth (Abubackar et al. 2012; Hurst and Lewis 2010; Skidmore et al. 2013).

Ethanol and acetate are the major metabolites produced during liquid-batch period in MBR and BCR (Figure 4B). For MBR, within the first 2 days when pH was maintained at cell-growth-favorable level, acetic acid was produced as the predominant metabolite (Figure 4A). Acetate accumulation (up to 5.9 g/L) ceased on day 6 and its concentration continuously decreased until day 11, after when a minimal increase (from 3.5 to 4.1 g/L) in acetate concentration was noticed (Figure 4B). Acidogenesis coupled with pH drop in the first phase is essential for the subsequent solventogenic phase (Worden et al. 1991). An examination of the acetyl-CoA pathway in C. carboxidivorans strain P7 syngas fermentation showed that ethanol production from acetyl-CoA results in consumption of protons and electrons with the presence of acetaldehyde/alcohol dehydrogenase (Bruant et al. 2010): one mole of ethanol produced from acetyl-CoA consumes 4 moles of [H+] and 4 moles of electrons. In this experiment, the fermentation switched from acidogenesis to solventogenesis on day 3, signified by the linear increase of ethanol concentration from day 3 until the end of the liquid-batch operation (Figure 4B). This coincided with the decrease in acetate concentration (Figure 4B), corresponding to pH
increase (Figure 4A). Similar trends (i.e. a decrease in acetate concentration along with an increase in ethanol concentration) have also been observed in previous studies on P7 syngas fermentation (Datar et al. 2004; Hurst and Lewis 2010; Ramachandriya et al. 2011). The genomic analysis (Bruant et al. 2010; Paul et al. 2010) revealed that C. carboxidivorans strain P7 contains genes encoding domains of aldehyde:ferredoxin oxidoreductase, indicating that P7 cells could metabolize acetate reduction to acetaldehyde and further to ethanol with reduced ferredoxin present.

For BCR, the time course of acetate concentration shows a similar trend to that in MBR (Figure 4B) but keeps at a lower level: it reached its peak concentration (4.6 g/L) on day 4 and then decreased until the end of liquid-batch operation. Significant ethanol was produced from day 4. However, no substantial ethanol production was observed in BCR after day 10, in contrast with the linear increase in ethanol concentration found in MBR during the same period. The higher ethanol productivity achieved in MBR could be attributed to the enhanced syngas mass transfer efficiency in MBR. Moreover, from a thermodynamic point of view, P7 cells tends to produce ethanol from CO instead of acetate reduction because the electron production via CO utilization is more thermodynamically favorable than H₂ in syngas fermentation (Hu et al. 2011).

$$6\text{CO} + 3\text{H}_2\text{O} \rightarrow \text{C}_2\text{H}_5\text{OH} + 4\text{CO}_2 \quad \Delta G^\circ = -217.9 \text{ kJ/mol}$$

$$\text{CH}_3\text{COOH} + 2\text{H}_2 \rightarrow \text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O} \quad \Delta G^\circ = -9.6 \text{ kJ/mol}$$

### 3.3 Liquid-continuous syngas fermentation in MBR

#### 3.3.1 Effects of syngas flow rate on fermentation performance

Syngas fermentation performance was investigated at different syngas flow rate (Figure 5). As shown in Fig. 5A, both CO and H₂ consumption rates increased with syngas flow rate
increased from 50 to 300 mL/min and leveled off when the flow rate further increased to 500 mL/min. The corresponding CO and H\textsubscript{2} utilization efficiencies increased with syngas flow rate increased from 50 to 200 mL/min, leveled off at 300 mL/min and dramatically decreased at 500 mL/min. The low CO and H\textsubscript{2} consumption rate was attributed to substrate-limiting situation, because within this syngas flow rate range, syngas loading rate was lower than the cell’s gas-uptake capacity. From 300 to 500 mL/min, CO and H\textsubscript{2} consumption rates decreased, possible because of the increased pressure drop at 500 mL/min.

Figure 5B showed the concentration and productivity of ethanol and acetic acid at different syngas flow rate. With the constant dilution rate (0.12 day\textsuperscript{-1}) operated, the products’ concentration and productivity revealed similar trend as the syngas flow rate varied. Both ethanol and acetic acid concentrations increased with syngas flow rate increased from 50 to 300 mL/min, while slightly decreased ($p < 0.05$) at 500 mL/min. The low ethanol and acetic acid concentrations in low gas flow rate range were probably due to the correspondingly low mass transfer efficiency. Despite that biomass in MBR was predominantly in the form of biofilm immobilized on the surface of monolithic microchannels, suspended cells also contributed to syngas utilization and metabolites formation. In low gas flow regime, where gas velocity is significantly lower than liquid velocity, bubbly flow instead of Taylor flow occurred in MBR, making MBR operated like a slurry bubble column reactor, where fermentation is under gas-to-liquid mass transfer limitation. From 100 to 200 mL/min, a 100% increase of syngas flow rate resulted in 162% improvement of ethanol and 126% improvement of acetic acid concentration, which might be explained by enhanced mass transfer efficiency achieved at higher gas flow rate, forming segmented bubbles through the immobilized cells in monolith column. The thin liquid film sandwiched between biomass and gas phase efficiently enhanced gas-to-liquid mass transfer.
efficiency. This result demonstrated the important role of syngas flow rate played in MBR syngas fermentation.

Figure 5C showed the ethanol and acetic acid yield from CO and ethanol to acetic acid molar ratio at different syngas flow rate. Overall, ethanol yield from CO was roughly twice of acetic acid yield and kept at a constant level (~ 0.18 mol C/mol C).

3.3.2 Effects of liquid flow rate on fermentation performance

Liquid flow rate is also a very important parameter affecting syngas fermentation performance in MBR (Figure 6), as it influences not only the mass transfer efficiency (Figure 3B), but also the biofilm growth characteristics. Substrate mass transport limitation and surface shear force have been found to be the two prevailing favors affecting the biofilm growth pattern in a monolith microchannel (Ebrahimi et al. 2005). As shown in Figure 6A, CO consumption rate and CO utilization efficiency increased with liquid flow rate increasing from 200 to 500 mL/min while decreased when liquid flow rate exceeded 500 mL/min. H₂ utilization efficiency fluctuated in the range of 200 ~ 500 mL/min liquid flow rate but decreased when liquid flow rate was further increased. Ethanol concentration and productivity peaked at 500 mL/min of liquid flow rate (Figure 6B). Acetic acid concentration and productivity showed the same trend. Figure 6C showed that both ethanol and acetic acid yields maintained at a relatively constant level (0.18 molC/molC-CO and 0.08 molC/molC-CO, respectively) over all the applied liquid flow rate conditions. Ethanol to acetic acid molar ratio remained stable (ca. 2.2) at the range of 200 ~ 500 mL/min liquid flow rate and increased when liquid flow rate further increased up to 1000 mL/min. The maximum ethanol to acetic acid molar ratio was obtained at 1000 mL/min. The above results indicated that liquid flow rate was an important parameter to manipulate the syngas fermentation regarding syngas utilization and metabolite production, though it did not
significantly affect the metabolite yield from CO. In the lower liquid flow rate regime (200 ~ 500 mL/min), the positive correlation between liquid flow rate and metabolite concentration/productivity indicated that gas-to-liquid mass transfer might be rate-limiting during syngas fermentation, because CO $k_{L}$ increases with liquid velocity (i.e. liquid flow rate divided by cross-sectional area) increased from 0.11 cm/sec (i.e. 200 mL/min) to 0.28 cm/sec (i.e. 500 mL/min). In the higher liquid flow rate regime (500 ~ 1000 mL/min), the decreasing trend indicated that the fermentation performance might be primarily controlled by intrinsic kinetics of P7 cells, which was affected by liquid flow rate. The increased liquid flow rate leads to higher shear force, leaving the potential risk of losing active biomass attached on the microchannel surface. Foaming phenomenon was noticed in the course of bioreactor operation with high liquid recirculation (> 800 mL/min). The large amounts of stable foam could have caused syngas entrapment, leading to dissolved CO, H$_2$ and CO$_2$ level fluctuates substantially in fermentation broth.

### 3.3.3 Effects of dilution rate on fermentation performance

Previous studies found out that liquid retention time (i.e. inverse of dilution rate) was the most import parameter determining the growth of biofilm/suspended cell, especially that of the hetero-culture microbial communities (Ebrahimi et al. 2006; Tijhuis et al. 1994), since suspended-growth cells would eventually be washed out of the bioreactor if the dilution rate is higher than their maximum growth rate, which favors the development of biofilm-growth cells. Figure 7 shows that dilution can also be used to control the fermentation performance carried out by the single-culture microorganisms. As illustrated in Figure 7A, CO consumption rate and utilization efficiency were improved when dilution rate was increased from 0.12 to 0.48 day$^{-1}$,
while they decreased with dilution rate further increased to 0.96 day$^{-1}$. Hydrogen consumption rate and utilization efficiency maintained at high level (ca. 85%) with dilution rate ranging from 0.12 to 0.48 day$^{-1}$ and decreased at 0.96 day$^{-1}$ dilution rate. The concentration of ethanol and acetate decreased with the increase of dilution rate (Figure 7B), which was obvious due to the “diluting effect”. However, ethanol productivity increased in the range of 0.12 ~ 0.48 day$^{-1}$ and decreased at 0.96 day$^{-1}$. Acetic acid productivity shows a similar trend (Figure 7B). When dilution rate was increased from 0.48 to 0.96 day$^{-1}$, acetic acid productivity decreased 11%, which was lower than that of ethanol productivity (31%). In contrast, yield from CO of ethanol and acetic acid appeared quite stable (Figure 7C), except that acetic acid yield increased at 0.96 day$^{-1}$, resulting in the corresponding decrease of ethanol to acetic acid molar ratio. The overall worse syngas fermentation performance observed at 0.96 day$^{-1}$ might be explained by the wash-out phenomenon of suspended P7 cells. Despite that cordierite ceramic material is proven to be a great support for anchoring bacteria, we still observed suspended P7 cells (OD 0.3 ~ 0.8) in the bioreactor, which also contribute to syngas fermentation. When they are washed out of the bioreactor, the total amount of biomass fermenting syngas is decreased. On the other side, as a biofilm reactor, MBR demonstrated its capability to be operated at high dilution rate (0.96 day$^{-1}$) with moderate ethanol productivity (Figure 7C). However, as a control system, dilution rate of bubble column reactor could only be raised up to approximately 0.6 day$^{-1}$ (data not shown) since unwanted cell wash-out phenomenon occurred at dilution rate higher than that threshold.

3.4 SEM imaging of monolith column

The MBR system was dissembled after all the fermentation experiments were completed. Figure 8A is the top view of monolithic column. The zoom-in top views of the monolithic
column before and after P7 biofilm development during syngas fermentation period were shown in Figure 8(B) and (D), while the existence of P7 cells on surface of monolithic channel surface with biofilm growth was evident by the scanning electron microscope (SEM) imaging (Figure 8C). It was observed that the biofilm pattern varied in monolithic channels, which might attribute to the maturity of biofilm and gas/liquid flow characteristics. During the early stages of biofilm development, biofilm was found to start from the middle (but not corner) of the square-section monolithic channel side due to the fact that biofilm growth in the corners would be limited by the substrate transfer resistance. Once the mature biofilm forms, a more regular biofilm shape (including monolithic channel corners) will be developed resulting from the kinetic balance between biofilm growth and detachment (Ebrahimi et al. 2005; Tijhuis et al. 1994).

3.5 Comparison of syngas fermentation in MBR and BCR

In order to compare the syngas fermentation in MBR and BCR, the fermentation condition leading to the highest ethanol productivity in MBR (Figure 7B) was selected as the liquid-continuous operating condition for the two bioreactors: syngas flow rate 300 mL/min, liquid flow rate 500 mL/min and dilution rate 0.48 day⁻¹. The comparison results were shown in Table 1.

Overall, better syngas fermentation performance was achieved in MBR, considering syngas (CO and H₂) consumption rate and ethanol and acetate productivities as the criteria.

According to the biomass development characteristics, bioreactors can be classified into the suspended-growth reactors and attached-growth reactors. Monolithic bioreactor provides an alternative configuration since it can be utilized for both suspended- and biofilm-growth process. For suspended growth application, monolith offers high interfacial mass transfer capacity and
liquid holdup to improve product yield. Fritsch et al (Fritsch et al. 2008) achieved 35% enhancement in hydrogen production rate and 30% increase in hydrogen yield by using a monolith column reactor for Clostridium butyricum dark fermentation on sucrose, as compared to a conventional bubble column reactor. With suspended-growth cells development, the high specific surface area of the monolith column increases bubble formation in the liquid supersaturated with hydrogen, enhancing mass transfer the gaseous end product out of the liquid. For attached growth application, the monolithic structure provides a high surface area per unit of volume (A/V) available for biocatalysts attachment. For example, the 200-cpsi monolith column used in the present study has an A/V degree of 1850 m²/m³, which is much higher than BCR (10 ~ 500 m²/m³, dependent on the configuration) (Charpentier, 1981). Moreover, the formation of biofilm on the microchannel surface enables the bioreactor to be operated at higher dilution rate. Despite the decrease in ethanol and acetate productivities, no fermentation performance down was observed at dilution rate of 0.96 day⁻¹ in MBR in this study (Figure 7B). However, it was unable to operate BCR syngas fermentation at such a high dilution rate due to the unwanted cell washout phenomenon (data not shown). Additionally, compared with membrane-based reactor, an MBR system has the potential to be operated for extended periods of fermentation with unique advantages such as high mechanical strength without membrane fouling problem.

To the best of our knowledge, the present study is the first to carry out liquid-continuous syngas fermentation in an MBR system by optimizing operating parameters (gas flow rate, liquid flow rate and dilution rate). We believe that the application of MBR in syngas fermentation is of future impact because no additional energy input (e.g. stirring) is required to obtain mass transfer enhancement and the corresponding improvement of metabolite productivity.
4. Conclusions

The present work provided an insight into the application of a monolithic biofilm reactor (MBR) in syngas fermentation for ethanol production. To the best of our knowledge, this is the first comprehensive investigation on utilizing an MBR system for syngas fermentation, including examination of CO mass transfer, liquid-batch culture and liquid-continuous culture with optimization of bioreactor operating parameters (syngas flow rate, liquid flow rate and dilution rate). Considering the ethanol productivity as the criteria, the optimal syngas fermentation was obtained in the MBR system under the operating condition of syngas flow rate 300 mL/min, liquid flow rate 500 mL/min and dilution rate 0.48 day\(^{-1}\), resulting in ethanol productivity of 2.4 g/L/day, which was 60% higher than that achieved in a bubble column reactor with the identical reactor geometry and fermentation operating condition.
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Table 1. Comparison of liquid-continuous syngas fermentation performance in MBR and BCR under condition of syngas flow rate 300 mL/min, liquid flow rate 500 mL/min and dilution rate 0.48 day\(^{-1}\)

<table>
<thead>
<tr>
<th>Performance</th>
<th>MBR</th>
<th>BCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO utilization efficiency (%)</td>
<td>84.9 ± 2.2</td>
<td>54.2 ± 3.9</td>
</tr>
<tr>
<td>CO consumption rate (mmol/L/day)</td>
<td>600.5 ± 15.1</td>
<td>383.3 ± 27.3</td>
</tr>
<tr>
<td>H(_2) utilization efficiency (%)</td>
<td>90.0 ± 5.8</td>
<td>70.9 ± 4.1</td>
</tr>
<tr>
<td>H(_2) consumption rate (mmol/L/day)</td>
<td>159.2 ± 10.7</td>
<td>125.6 ± 7.2</td>
</tr>
<tr>
<td>Ethanol concentration (g/L)</td>
<td>4.9 ± 0.5</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Ethanol yield from CO (mol C/mol C)</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Ethanol productivity (g/L/day)</td>
<td>2.4 ± 0.2</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Acetate concentration (g/L)</td>
<td>3.1 ± 0.4</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Acetate yield from CO (mol C/mol C)</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Acetic acid productivity (g/L/day)</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Ethanol/Acetate molar ratio</td>
<td>2.1</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Figure 1. (A) Bubble train flow and Taylor flow in monolithic channels (Kreutzer et al. 2005); (B) Mass transfer from gas to biofilm through thin liquid film sandwiched between the gas phase and the biofilm
Figure 2. MBR configuration in co-current upflow operation for syngas fermentation
Figure 3. Volumetric mass transfer coefficient ($k_{L,a}$) of CO in MBR system under monolith cocurrent upflow and bubble column operating mode as a function of (A) gas velocity, $u_G$ (with liquid velocity fixed at 0.26 cm/sec) and (B) liquid velocity, $u_L$ (with gas velocity fixed at 0.52 cm/sec); (C) Correlation between liquid-phase Weber number ($W_{e,L}$) and gas-phase Reynolds number ($Re_G$), where the boundaries between flow regimes in the flow map were quantitatively identified according to Ambatipati, 2005
Figure 4. Time course of batch syngas fermentation in MBR and BCR (A) medium pH and suspended cell dry weight; (B) ethanol and acetic acid concentration
Figure 5. Syngas fermentation performance in MBR at varied syngas flow rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Liquid flow rate was fixed at 500 mL/min; dilution rate was fixed at 0.12 day⁻¹. Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 6. Syngas fermentation performance in MBR at varied liquid flow rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Syngas flow rate was fixed at 300 mL/min; dilution rate was fixed at 0.12 day\(^{-1}\). Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 7. Syngas fermentation performance in MBR at varied dilution rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Syngas flow rate was fixed at 300 mL/min; liquid flow rate was fixed at 500 mL/min. Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 8. (A) Top view of monolith; (B) top view of monolith (zoom in); (C) SEM imaging of *C. carboxidivorans* biofilm attached on microchannel surface; (D) *C. carboxidivorans* P7 biofilm formation after syngas fermentation
CHAPTER 4 APPLICATION OF A HORIZONTAL ROTATING PACKED BED BIOFILM REACTOR (RPB-BR) FOR SYNGAS FERMENTATION TO BIOFUEL

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ABSTRACT

Enhancement of gas-to-liquid mass transfer rate and remaining high cell density are among the greatest technical and economic barriers to commercialize the syngas fermentation, a promising thermo/biochemical hybrid process in production of fuels and chemicals. Conventional suspended-growth bioreactors commonly used for continuous syngas fermentation suffer from inefficient mass transfer and unwanted cell washout at high dilution rate, resulting to the low productivities. An innovative horizontal rotating packed bed biofilm reactor (RPB-BR) was developed in the present study to address the above engineering issues. The volumetric mass transfer coefficient ($k_{L\alpha}$) of carbon monoxide (CO) was determined in RPB-BR under abiotic condition at different rotational speed and headspace pressure. For Clostridium carboxidivorans P7 syngas fermentation with liquid-continuous operation, three selected operational parameters, rotational speed, headspace pressure and dilution rate, were investigated of their effects on fermentation performance. Under optimal condition (rotational speed 50 rpm, headspace absolute pressure 29.7 psi and dilution rate 0.96 day$^{-1}$), ethanol was continuously produced with productivity of 6.0 g/L/day. The present study demonstrated the great potential of RPB-BR as an efficient system to perform high-productivity syngas fermentation, making cellulosic ethanol biorefinery move one step forward to technical and economic feasibility.
1. Introduction

Synthesis gas (syngas) is a gas mixture consisting primarily of carbon monoxide (CO) and hydrogen (H₂), serving as an important building block in petrochemical and refining industries. It is usually generated from inexpensive carbonaceous feedstock (i.e. coal, pet coke, natural gas, biomass and municipal solid waste) through gasification process (Klasson et al. 1992). Syngas can be utilized as a growth substrate by a group of microorganisms in production of fuels and chemicals. Such a hybrid thermochemical (gasification)/biochemical (microbial fermentation) conversion process is defined as syngas fermentation, which has great potential for commercial development. Compared to sugar-platform-derived biochemical process, syngas fermentation is able to utilize the whole biomass feedstock including the lignin components, not requiring complex pretreatment or costly enzymes. Compared to Fisher-Tropsch process, syngas fermentation also offers advantages: microbial fermentation can be operated at lower temperatures and pressures which are close to ambient conditions; syngas-fermenting microbes do not require a fixed CO/H₂ ratio, have higher product-specificity and are far more tolerant to syngas impurities such as sulfur and tars, which easily cause metal catalysts poisoning (Ragauskas et al. 2006).

However, although syngas fermentation is technically feasible, several engineering obstacles need to be overcome to make this process become more economically viable (Ragauskas et al. 2006). Gas-to-liquid mass transfer has been recognized as a rate-limiting step
in syngas fermentation process (Bredwell et al. 1999; Do et al. 2007; Klasson et al. 1993a; Worden et al. 1997). Mass transfer limitation renders insufficient syngas uptake by microorganisms in fermentation culture and eventually leads to low productivity. On the other hand, assuming that fermentation is not mass transfer limited, high cell density must be retained in the system to maintain sufficient gaseous substrate consumption rate, otherwise the system will be under kinetic-growth-limited condition, in which case the productivity and syngas bioconversion are limited by the cell density in the system. Both of the two rate-limiting conditions have been found during syngas fermentation (Vega et al. 1989a; Vega et al. 1989b). Therefore, an appropriate bioreactor configuration able to enhance mass transfer and to attain high cell density is important for syngas fermentation. Moreover, several other criteria proposed for designing and operating a syngas fermentation bioreactor include low power consumption, low maintenance costs, moderate operational flexibility and stable operation for prolonged periods of time.

To achieve these goals, a horizontal rotating packed bed biofilm reactor (RPB-BR) was designed, fabricated and operated for syngas fermentation to ethanol at bench-top scale in the present study. The attached-growth rotating biological contactors (RBCs) are widely employed in full-scale municipal wastewater treatment plants for secondary process due to their advantages such as easy construction, compact design, simple process control, excellent oxygen-transfer efficiency and resistance to shock loads (Cortez et al. 2008; Patwardhan 2003). The horizontal RPB-BR design for syngas fermentation aims to maintain these benefits while optimized for syngas-fermenting biofilm development and ethanol production. With regard to support media of RBCs, random packed cage configurations have been successfully used as substitutes for conventional individual rigid discs or disc packets in wastewater treatment plants because they
provide larger support area for biofilm attachment within the same reactor size, contribute to higher mass transfer efficiency due to the increased turbulence and have lower energy consumption and fabrication cost (Mathure and Patwardhan 2005; Wanner et al. 1990; Ware et al. 1990). Random-packed-cage type RBCs with various packing media have been applied extensively in wastewater treatment (Nahid et al. 2001; Sirianuntapiboon 2006; Sirianuntapiboon and Chuamkaew 2007), but rarely in syngas fermentation.

AnoxKaldnes™ K1 biofilm carriers were originally used in moving bed biofilm reactor (MBBR) for municipal wastewater treatment (Odegaard et al. 1994). These biofilm carriers offer a high protected surface area per volume unit of carrier (500 m²/m³) with open structure allowing for high mass transfer rate. Also, they are made of high-density polyethylene and are durable enough to sustain the wear and tear from constant carrier collisions. Hickey (2009) reported syngas fermentation for ethanol production using Clostridium ragsdalei strain P11 grown as biofilm attached on K1 carriers suspended in a MBBR. Therefore, K1 biofilm carriers have great potential to be used as packing media in a RPB-BR system for syngas fermentation.

The objective of this study was (1) to determine CO mass transfer in a horizontal RPB-BR with K1 carriers as packing media at different gas flow rates and (2) to maximize ethanol productivity of syngas fermentation in this horizontal RPB-BR system under continuous operation by optimizing the operational parameters including rotational speed, headspace gas pressure and dilution rate.

2. Materials and Methods
2.1. Set-up of RPB-BR system

The horizontal RPB-BR system (Figure 1) consisted of a glass tank (3.3-L total capacity) modified from a BioFlo 3000 fermentor vessel (New Brunswick Scientific, Edison, NJ) with a cylindrical cage made of 316L stainless steel (diameter 7.62 cm, length 22.5 cm) mounted on a stainless steel shaft. Twelve 316L stainless steel threaded rods were equidistantly placed along the each disc’s inner circumference on which the wire-mesh was wrapped. The cage was packed with AnoxKaldnes™ K1 carriers (diameter 9.1 mm, length 7.2 mm) and was rotated by using detachable AC gearmotors (Rex Engineering Corporation, Titusville, FL) with fixed output speed (5, 10, 20, 50, 75 and 100 rpm). The working volume of the system was 1.5 L, giving a cage submergence of 45%. Here submergence was referred to the ratio of the distance from the cage edge submerged in the liquid to the cage diameter expressed in percentage. A microporous seamless-tubing sparger (Mott Corporation, Farmington, CT) with pore size of 1 µm was used as the gas distributor. The vessel temperature was maintained by using a water-filled Tygon® tubing-jacket whereby the water temperature was regulated by an external thermal circulator. The system pressure was controlled by a back-pressure regulator placed at the exhaust gas line.

2.2 Determination of volumetric mass transfer coefficient (k_{L,a})

The CO k_{L,a} of the horizontal RPB-BR system was determined under abiotic conditions at 25 °C using high purity (99.5%) CO gas and tap water. The rotational speed (5, 10, 20, 50, 75 and 100 rpm) and headspace absolute pressure (14.7, 16.7, 19.7, 24.7 and 29.7 psi) were varied to evaluate their effects on the CO k_{L,a} at different gas flow rate (0.1, 0.2, 0.4, 0.6 and 1.0 vvm). Three trials were conducted for each operating condition to obtain the average k_{L,a} value. Nitrogen was bubbled through the reactor containing 1.5 L tap water prior to CO sparging for
each trial. Liquid samples were periodically taken from the reactor using 10 µL gastight high performance syringes (Hamilton Gastight 1701, Reno, NV) for immediate measurement of the dissolved CO concentration in aqueous phase using myoglobin-bioassay (Kundu et al. 2003). The detailed procedures were described elsewhere (Riggs and Heindel 2006) and the CO $k_{L}a$ value was determined using the following equation:

$$\ln \left( \frac{C^* - C_0}{C^* - C_L} \right) = (k_{L}a) \cdot t$$

where $C^*$ is the saturated CO concentration in aqueous phase, $C_0$ is the initial CO concentration in aqueous phase, $C_L$ is the real-time dissolved CO concentration in aqueous phase, $k_L$ is the gas transfer coefficient (cm/sec), $a$ is the gas-liquid interfacial area per working volume of reactor (cm$^{-1}$) and $t$ is the sampling time.

2.3 Inoculum, medium and bioreactor condition

*Clostridium carboxidivorans* strain P7 (ATCC BAA-624) was used as the microorganism for syngas fermentation in this experiment. The seed culture was grown and maintained anaerobically in 125-mL-serum bottles containing 50 mL of modified 1754 PETC medium composed of (per liter): 10 g fructose, 0.5 g yeast extract, 5 g MES, 25 mineral solution, 10 mL trace metal solution, 10 mL Wolfe’s vitamin solution and 10 mL 4% cysteine-sulfide reducing agent (Tanner, 2007). Resazurin solution (0.1%) was added as a redox indicator.

The horizontal RPB-BR system containing 1.35 L liquid medium (the same as that used for seed culture) was autoclaved at 121 °C for 20 min. Following sterilization, nitrogen gas was continuously purged through the bioreactor for 2 hours after when vitamin solution and reducing agent were filtered into the medium. The bioreactor was inoculated with 150 mL seed culture at 10% vv$^{-1}$ rate. The bioreactor was initially operated under gas-continuous, liquid-batch mode for
biofilm buildup in the packing cage. Artificial syngas (20% CO, 5% H₂, 15% CO₂ and 60% N₂) was fed into the bioreactor through the microporous sparger continuously at 300 mL/min and absolute inlet pressure of 20.7 psi, while exhaust gas exited the system through the condenser at atmospheric pressure (Figure 1). Composition of the artificial syngas was comparable to the “real” syngas produced from the 20 kg/hr fluidized bed reactor gasifying switchgrass at BioCentury Research Farm of Iowa State University. The reactor temperature was maintained at 37 °C. The pH was controlled at 6.0 within the first 48 hours by using 2 N NaOH to promote cell growth (Liou et al. 2005) and afterwards was allowed to drop without control until pH 4.5 to favor ethanol production (Klasson et al. 1992). After 15-day liquid batch mode, the bioreactor was switched to semi-continuous operation: fermentation broth was withdrawn from reactor followed by replenishing fresh liquid medium 6 times per day at 4-hour intervals. The composition of the fresh medium for continuous operation was the same as that for batch culture except that fructose was eliminated. The bioreactor was operated continuously during different stages with specific rotational speed, headspace pressure and dilution rate.

2.4 Analytical methods

The inlet syngas and exhaust gas compositions (CO, H₂ and CO₂) were analyzed by DJGAS, a real-time non-dispersive infrared gas monitor (De Jaye Technologies, Des Moines, IA). Liquid sample (1.5 mL) was collected from withdrawn fermentation broth and pretreated prior to gas chromatography (GC) analysis: acidified with 6 N HCl (200 µL), centrifuged at 18440 g for 15 min and filtered through a 0.22 µm membrane. The filtrate was analyzed for ethanol and acetic acid concentration using a Varian 450 GC-FID (Varian Inc., Walnut Creek, CA) equipped with a Zebron ZB-WAXplus capillary column (Phenomenex, Torrance, CA). The
carrier gas was helium (25 mL/min). The sample was injected with a split ratio of 20:1 and the injector temperature was 200 °C, while the detector temperature was 220 °C. The oven temperature profile was 35 °C for 5 min, after which the temperature was ramped to 130 °C at rate of 10 °C/min and then ramped to 220 °C at rate of 30 °C/min.

Statistical analysis was determined using JMP® Pro version 10.0.2 (©SAS Institute Inc., Cary, NC) with and p-values were shown in the text, which were calculated at a 95% confidence level.

2.5 Scanning electron microscopy (SEM) image of biofilm attached on K1 carriers

Characterization of biofilm attached on K1 carriers was performed by using SEM imaging until completion of syngas fermentation. One single K1 carrier was randomly selected and fixed with 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C for 48 hours. Samples were then rinsed deionized water and post-fixed in 2% aqueous osmium tetroxide followed by dehydration in a graded ethanol series up to 100% ultra-pure ethanol and dried using a Denton DCP-2 critical point dryer (Denton Vacuum, LLC, Moorestown, NJ). Dried samples were mounted on stubs and sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ) with palladium-gold alloy. Finally the samples were imaged using a JEOL 5800LV SEM (Japan Electron Optics Laboratory, Peabody, MA) at 10kV with a SIS ADDA II for digital image capture (Olympus Soft Imaging Systems Inc., ResAlta, Golden, CO).

3. Results and Discussions
3.1 CO mass transfer characteristics

The effects of rotational speed and headspace pressure on CO $k_{\text{L,a}}$ value of the horizontal RPB-BR at different gas flow rate (vvm) were shown in Figure 2. With headspace absolute pressure fixed at atmospheric level (i.e. 14.7 psi), CO $k_{\text{L,a}}$ increased with the increase of cage rotational speed in all cases of CO gas flow rate varied from 0.1 to 1.0 vvm (Figure 2A). This was mainly attributed to the reduced liquid-boundary-layer-side transport resistance from the thinner liquid film and diffusion depth due to the increased liquid turbulence created at higher cage rotational speed (Mathure and Patwardhan 2005; Patwardhan 2003). Although this experiment focused on the effects of rotational speed on $k_{\text{L,a}}$ of CO fed into the horizontal RPB-BR, the trends are similar to those observed for oxygen transfer in conventional disc-type rotating biological contactor (Boumansour and Vasel 1998; Kim and Molof 1982; Kubsad et al. 2004; Mathure and Patwardhan 2005; Suga and Boongorsrang 1984), that $k_{\text{L,a}}$ is positively correlated to rotational speed at a fixed submergence.

With cage rotational speed fixed at 50 rpm, CO $k_{\text{L,a}}$ slightly increased with the increase of reactor headspace absolute pressure (Figure 2B). Here the reactor headspace absolute pressure refers to as CO partial pressure ($P_{\text{CO}}$) since CO was the only constituent gas sparged into the reactor. It could be seen that the increase of rotational speed contributed more to the enhanced $k_{\text{L,a}}$ than increasing reactor headspace pressure did, regardless of CO gas flow rate. Take the case of 0.4 vvm gas flow rate for example, doubling $P_{\text{CO}}$ (from 14.7 to 29.7 psi) only brought 14.5% enhancement of CO $k_{\text{L,a}}$. This indicated that the gas-to-liquid transport process is predominantly controlled by mass transfer resistance from the liquid film. In a previous study employing bubble column reactor continuously sparged with CO at gas flow rate of 0.4 vvm, for syngas
fermentation (Chang et al. 2001), no significant $CO_{k_{l,a}}$ enhancement was observed with $P_{CO}$ increased from 41.5 kPa (6.0 psi) to 99.3 kPa (14.4 psi).

The $CO_{k_{l,a}}$ values obtained in this experiment are generally lower than those reported for stirred tank reactor (Riggs and Heindel 2006) and for hollow fiber membrane reactor (Lee et al. 2012) using the same gas flow rate. However, it should be noted that the practical mass transfer in the horizontal RPB-BR would potentially be enhanced with microorganisms grown on packing media when operated for syngas fermentation. Since the packing cage is partially submerged, syngas-fermenting microorganisms grown as biofilm formed on packing media are alternately exposed to the headspace syngas and to the liquid medium by cage rotation. In this sense, syngas mass transport occurs by (1) syngas diffusion through the liquid film over the biofilm surface when biofilm is exposed to headspace syngas; (2) direct syngas transfer at gas-liquid interface and (3) direct syngas absorption by microorganisms during gas exposure. Therefore, great potential could be expected for enhancement of mass transfer in RPB-BR with presence of microorganisms for syngas fermentation. The earlier investigation on the effects of biomass on oxygen transfer in aerobic RBC system demonstrated the significant enhancement in oxygen mass transfer coefficient ($k_{l,a}$) brought by microorganisms (Paolini 1986).

### 3.2 *Clostridium carboxidivorans* strain P7 biofilm morphology

SEM images (Figure 3) showed that *Clostridium carboxidivorans* strain P7 bacteria were developed as biofilm attached on the surface of K1 carrier, demonstrating the great potential of polyethylene-made K1 carrier used as random packing media for immobilization of syngas-fermenting bacteria. Rod-shaped P7 bacteria and various other deposits were observed. The latter include extracellular polymeric substances (EPS), filaments, cell-lysis aggregates and minerals.
The bulk “protected area” inside the cylinder was the most populated regions of the K1 carrier, while the longitudinal fins on the outside were not well colonized with P7 bacteria.

3.3 Syngas fermentation in RPB-BR during liquid-continuous culture

3.3.1 Effects of rotational speed on syngas fermentation performance

Syngas fermentation performance was investigated under varied cage rotational speed (Figure 4). As shown in Figure 4A, both CO and H\textsubscript{2} consumption rates increased with the rotational speed increased from 5 to 50 rpm. This trend was attributed to the enhanced mass transfer at higher rotational speed, as explained in the abiotic \( k_{L}a \) experiment (Figure 2A). However, the opposite trend was observed when rotational speed further increased above 50 rpm. This might be explained by the superiority of the fluid shear stress over the mass transfer effects. The thickness of viscous layer sandwiched between the biomass and the K1 carrier surface would be reduced as the rotational speed increases, rendering biofilm detachment possible, which would decrease the overall consumption of gaseous substrate. Moreover, although the presence of biomass enhances the gas mass transfer rate with respect to the rate obtained in abiotic condition, this enhancement effect was found to become less significant as rotational speed increased (Lu et al. 1997; Paolini 1986). The maximum gas consumption rates of CO and H\textsubscript{2} were attained at rotational speed of 50 rpm, corresponding to 17.1\% and 29.9\% CO and H\textsubscript{2} utilization efficiency, respectively.

Figure 4(B) showed the concentration and productivity of ethanol and acetate at different rotational speed. The trend of ethanol and acetate concentrations is similar to that of gas consumption with response to the variation of rotational speed. With a fixed dilution rate (0.12 day\(^{-1}\)), ethanol and acetate productivities have the same trend to their concentrations. Figure 4(C)
showed that the rotational speed did not have important effects on ethanol or acetate yield from CO. Moreover, there is no clear trend in ethanol to acetate molar ratio with the increase of rotational speed, as that ratio fluctuated markedly.

### 3.3.2 Effects of headspace pressure on syngas fermentation performance

Figure 5 showed the effects of headspace pressure on syngas fermentation performance. An interesting observation in Figure 5(A) was that increasing headspace pressure does not have significant impact on CO consumption rate but enhances hydrogen consumption evidently. Doubling the headspace pressure (from 14.7 to 29.7 psi) accounts for only 8.8% increase of CO consumption rate, but for 87.9% enhancement of H₂ consumption rate. Since the syngas mixture composition (20% CO, 5% H₂, 15% CO₂ and balance N₂) was maintained constant during the entire experiment, increasing total headspace pressure (Pₜ) of the reactor results in the increase of CO partial pressure (P₁₉₃), H₂ partial pressure (P₉₂) and CO₂ partial pressure (P₁₉₃₂). Pₜ of 14.7, 16.7, 19.7, 24.7 and 29.7 psi corresponds to P₁₉₃ of 2.94, 3.34, 3.94, 4.94 and 5.94 psi and P₉₂ of 0.74, 0.84, 0.99, 1.24 and 1.49 psi. The increasing trend of H₂ consumption rate was possibly attributed to the increased hydrogenase efficiency resulted from the higher P₉₂ applied in the fermentation. Recently (Skidmore et al. (2013)) found that increasing P₉₂ causes an increase in hydrogenase activity in the course of *Clostridium ragsdalei* strain P11 syngas fermentation.

Ethanol concentration did not evidently increase with Pₜ in the range of 14.7 to 24.7 psi (Figure 5B). No significant (p < 0.05) difference was found between all the possible pairs according to the comparison results from JMP MATCHED Pairs platform. On the other side, acetate production slight decreased with Pₜ increased from 14.7 to 19.7 psi whereas an opposite trend was observed at pressure above 19.7 psi (Figure 5B). The maximum ethanol concentration
(16.1 ± 1.1 g/L) and acetate concentration (11.0 ± 0.6 g/L) were both achieved at the highest $P_T$ (29.7 psi). Figure 5(C) showed that ethanol or acetate yield from CO did not change significantly ($p > 0.05$) over the increasing $P_T$; accordingly, ethanol to acetate molar ratio only slightly from 1.84 (at 24.7 psi) to 2.11 (at 19.7 psi). A previous study on batch culture P7 syngas fermentation (Hurst and Lewis 2010) revealed that (1) cell growth rate was not significantly affected by $P_{CO}$; (2) ethanol production did not have any apparent dependence upon $P_{CO}$ for $P_{CO} < 15.44$ psi; (3) ethanol production was non-growth-associated for $P_{CO} < 15.44$ psi; (4) acetate production was more cell-growth-associated at $P_{CO} < 10.29$ psi and lower $P_{CO}/P_{CO_2}$, which means that higher amounts acetate produced per cell mass produced was obtained at lower $P_{CO}$. Contrary to their findings, on the whole, Figure 5 indicated that the increase in bioreactor $P_T$ did not markedly affect the syngas fermentation. However, several key differences should be noted for the discrepancies. First, liquid-continuous syngas fermentation was carried out in this experiment as compared to the batch culture by Hurst and Lewis (2010). Second, neither $P_{CO}$ or $P_{CO}/P_{CO_2}$ ratio studied in this experiment falls into the range of Hurst and Lewis (2010) ($5.15$ psi $\leq P_{CO} \leq 29.39$ psi; $P_{CO}/P_{CO_2} = 1.7$ and 4.0): we used a much lower $P_{CO}$ ranging from 2.94 to 5.94 psi and a constant $P_{CO}/P_{CO_2}$ ratio of 1.33. Thirdly, since H$_2$ was present in the syngas, its oxidation via hydrogenase generates reducing equivalents required for metabolic process (Ragsdale 2004). Furthermore, oxidation of H$_2$ could possibly reduce ferredoxin to form a proton gradient and NADH for ethanol production as well as ATP synthesis for cell growth via Rnf complex, which was encoded on genome of C. carboxidivorans strain P7 (Bruant et al. 2010; Hemme et al. 2010; Paul et al. 2010). The Rnf complex has been proposed as the pump responsible for translocation of proton across membrane during autotrophic growth of Clostridium ljungdahlii (Kopke et al. 2010) and this hypothesis was recently demonstrated experimentally in vivo (Tremblay et al. 2010).
Considering the above factors and the complex interaction between $P_{CO}, P_{CO}/P_{CO_2}$ and $P_{H_2}$, further research should be performed in the future to provide some in-depth insights into their effects on cell growth and metabolic process.

### 3.3.3 Effects of dilution rate on syngas fermentation performance

Dilution rate has been considered as an important operational parameter for continuous production of fuels and chemicals in biofilm-based reactors (Halan et al. 2012; Qureshi et al. 2005). Providing that the majority of the microorganisms are grown as stable biofilm attached onto the packing media, this makes it possible to operate the RPB-BR at high dilution rate for continuous culture syngas fermentation without cell washout. Figure 6 illustrated the syngas fermentation performance in RPB-BR operated at different dilution rates. As shown in Figure 6(A), both CO and H$_2$ consumption rates increased with dilution rate from 0.12 to 0.96 day$^{-1}$ and leveled off when dilution rate was further increased to 2.00 day$^{-1}$. The corresponding CO and H$_2$ utilization efficiencies had a similar trend (Figure 6A). Ethanol and acetate concentrations decreased drastically with elevated dilution rate due to its “dilution effect” (Figure 6B). However, it’s noteworthy that their productivities increased almost linearly with dilution rate raised from 0.12 to 0.96 day$^{-1}$ and leveled off without when dilution rate exceed 0.96 day$^{-1}$. No fermentation failure was observed over the range of dilution rate studied (0.12 ~ 2.00 day$^{-1}$). In comparison, performance limitations due to undesired cell washout were found in a bubble column reactor (BCR) conducting C. carboxidivorans P7 syngas fermentation at dilution rate beyond 0.54 day$^{-1}$ (Datar et al. 2004) and in a continuous stirred tank reactor (CSTR) conducting C. ljungdahlii syngas fermentation at dilution rate beyond 0.43 day$^{-1}$ (Mohammadi et al. 2012). This indicated the horizontal RPB-BR was able to be operated at dilution rate approximately 4 to 5 times higher
than the conventional suspended growth bioreactors such as BCR and CSTR, making it possible to achieve high-productivity syngas fermentation. Figure 7C showed that ethanol yield from CO did not significantly ($p > 0.05$) change over the wide range of dilution rate, while acetate yield slightly increased with dilution rate. This resulted in a decreasing trend of ethanol to acetate molar ratio, which was likely due to the nutrient condition. Dilution rate determines the supply rate of nutrients in the fresh liquid media. Previous studies have demonstrated the important effects of various nutrients on cell growth and metabolic product distribution, including yeast extract (Guo et al. 2010; Klasson et al. 1991; Klasson et al. 1992; Vega et al. 1989c), calcium pantothenate, cobalt and vitamin B$_{12}$ (Gaddy et al. 2007; Kundiyana et al. 2011). Low dilution rate renders the cells in nutrient-limitation growth condition, favoring non-growth-associated solventogenesis; high dilution rate provides higher nutrient supply, promoting cell growth and growth-associated acidogenesis. Therefore, by applying the two-stage reactors, with the first for acidogenesis and the second for solventogenesis, dilution rate shift has been proven as an effective approach to facilitate ethanol production in *Clostridium ljungdahlii* syngas fermentation (Klasson et al. 1992; Richter et al. 2013). Overall, findings from Figure 7 indicated dilution rate being a crucial operational parameter to regulate the productivity and metabolite distribution in the course of continuous syngas fermentation.

The liquid-continuous syngas fermentation performance of the present study was compared to that from previous studies, with the comparison summarized in Table 1. The ethanol productivity (6.0 g/L/day) achieved using a horizontal RPB-BR in this study is higher than, or at least comparable to previous results using suspended-growth bioreactors like BCR and CSTR. Over half of the previous reports presented in Table 1 are recent patent fillings by companies exploring commercialization of syngas fermentation in production of bulk fuels and fine
chemicals, including Bioengineering Resources Inc. (now rebranded as INEOS Bio, www.ineosbio.com) and Coskata Inc. (www.coskata.com). To the best of the authors’ knowledge, the ethanol productivity reported by Gaddy et al. (2007) has been the highest (369 g/L/day) up to date, in which patent the cell recycle and high reactor pressure were employed. However, ethanol productivity of 6.0 g/L/day in this study is still believed to be promising, since there is great potential for further increase in ethanol production using our reactor though other strategies such as using multi-stage bioreactors in series with pH shift, dilution rate shift and appropriate nutrient limitation (Klasson et al. 1992; Kundiyana et al. 2011; Richter et al. 2013), increasing CO partial pressure (Hurst and Lewis 2010) and providing excess hydrogen (Gaddy et al. 2007).

4. Conclusions

Using an innovative horizontal RPB-BR system, we developed *Clostridium carboxidivorans* strain P7 syngas fermentation with high ethanol productivity (6.0 g/L/day) under liquid-continuous operation in this study. AnoxKaldnes™ K1 carriers have been demonstrated as effective packing media to form stable and active *C. carboxidivorans* P7 biofilm for efficient syngas uptake. By manipulating the selected operational parameters, rotational speed, reactor headspace pressure and dilution rate, syngas consumption and ethanol production could be optimized. The present work exploited a promising opportunity for achieving efficient syngas fermentation in a biofilm-based reactor with simple mechanical design, inexpensive packing media and stable operation for prolonged periods of time. The high efficiency of such a bioreactor system would potentially enhance the economy of gas-fermentation-derived biorefinery.
REFERENCES


Liou JS, Balkwill DL, Drake GR, Tanner RS. 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. Int J Syst Evol Microbiol 55(Pt 5):2085-91.


<table>
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<tr>
<th>Bioreactor configuration</th>
<th>Syngas feed</th>
<th>Microorganism</th>
<th>pH</th>
<th>Ethanol conc. (g/L)</th>
<th>Ethanol prod. (g/L/day)</th>
<th>Ethanol : Acetate ratio</th>
<th>Reference</th>
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<td>5.3</td>
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<tr>
<td>BCR (4 L)</td>
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<td>4.5</td>
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<td>150</td>
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<td>4.5</td>
<td>25</td>
<td>369</td>
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Table 1. continued

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<td>(Mohammadi et al. 2012)</td>
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<td>Clostridium ljungdahlii</td>
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<td>1&lt;sup&gt;st&lt;/sup&gt;: 0.5</td>
<td>7.3</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;: 0.08</td>
<td>(Richter et al. 2013)</td>
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<td>RPB-BR</td>
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<td>6.98</td>
<td>6.0</td>
<td>1.56</td>
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</table>

[1] Bubble column reactor
[2] Continuous stirred tank reactor
[3] High pressure AUTOKLAV™ reactor (Büchi)
[4] Liquid-Cel® MiniModule (Membrana) hollow fiber membrane module connected to BioFlo® 110 Fermentor
[5] MHF0504MBFT (Mitsubishi Rayon Engineering) hollow fiber membrane module connected to BIOSTAT Bplus Fermentor
[6] Moving bed biofilm reactor filled with AnoxKaldnes™ K1 carriers
[7] Romicon® hollow fiber membrane cartridge (Koch Membrane System) connected to BioFlo® 310 Fermentor
[8] MiniKros® hollow fiber membrane module connected to BioFlo® 310 Fermentor
[9] Xampler™ hollow fiber membrane cartridge (GE Healthcare) connected to BioFlo® 310 Fermentor
[10] Not Available
Figure 1. Configuration of a horizontal rotating packed bed biofilm reactor (RPB-BR) for syngas fermentation
Figure 2. Volumetric mass transfer coefficient ($k_{L,a}$) of CO in RPB-BR at varied gas flow rate (0.1 ~ 1.0 vvm) as a function of (A) rotational speed, with headspace absolute pressure fixed at 14.7 psi (i.e. atmospheric pressure) and (B) headspace absolute pressure, with rotational speed fixed at 50 rpm. Data are means of the values obtained from three trials for each operating condition error bars show standard deviations.
Figure 3. Scanning electron micrograph of *Clostridium carboxidivorans* strain P7 biofilm attached on a single K1 carrier showing its overall morphology and its connection to a complex matrix of filaments and extracellular polymeric substances dispersing throughout the K1 carrier surface (A, B, C, D, E, F) enlarged using higher magnification.
Figure 4. Syngas fermentation performance in RPB-BR at varied rotational speed in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Headspace absolute pressure was fixed at 14.7 psi; dilution rate was fixed at 0.12 day\(^{-1}\). Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 5. Syngas fermentation performance in RPB-BR at varied headspace absolute pressure in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Rotational speed was fixed at 50 rpm; dilution rate was fixed at 0.12 day\(^{-1}\). Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
Figure 6. Syngas fermentation performance in RPB-BR at varied dilution rate in terms of (A) gas consumption rate and utilization efficiency; (B) product concentration and productivity; (C) product yield from CO and ethanol to acetate molar ratio. Rotational speed was fixed at 50 rpm; headspace absolute pressure was fixed at 29.7 psi. Data are means of five replicates of consecutive samples at steady state and error bars show standard deviations.
CHAPTER 5 GENERAL CONCLUSIONS

1. General conclusion

Syngas fermentation has been considered as promising thermochemical/biological hybrid technology for sustainable production of biofuel and chemicals. However, the challenge of syngas fermentation, though, is the low gas-to-liquid mass transfer efficiency. Therefore, a bioreactor configuration that delivers high mass transfer rate in an energy-efficient manner for syngas fermentation remains an important engineering issue.

The present study focused on developing innovative attached-growth, biofilm-based reactors for continuous syngas fermentation at bench scale, namely hollow fiber membrane biofilm reactor (HFM-BR), monolithic biofilm reactor (MBR) and rotating packed bed biofilm reactor (RPB-BR). These bioreactor systems were designed, fabricated and investigated for volumetric mass transfer coefficient \(k_{La}\) of CO measurement under abiotic condition and for continuous syngas fermentation by using Clostridium carboxidivorans strain P7 culture.

The CO \(k_{La}\) of HFM-BR increased with gas velocity of CO fed through fiber lumen and liquid velocity flowing through shell-side of the HFM module. The CO \(k_{La}\) of HFM-BR was significantly higher than that of most continuous stirred tank reactor (CSTR) and bubble column reactor (BCR) reported previously. The selected operational parameters, syngas flow rate, liquid recirculation rate and dilution rate, were found to influence the performance of continuous syngas fermentation, with regard to syngas (CO and H\(_2\)) consumption, ethanol concentration and productivity, ethanol yield from CO and ethanol to acetate molar ratio. The highest ethanol productivity (3.44 g/L/day) was achieved at syngas flow rate of 300 mL/min, liquid recirculation rate of 200 mL/min and dilution rate of 0.96 day\(^{-1}\).
The CO $k_{L}a$ of MBR increased with gas velocity of CO and liquid velocity flowing through the monolith column. Compared to a BCR with identical reactor geometry and operational condition, MBR revealed higher CO $k_{L}a$ and lower energy consumption due to the unique hydrodynamic characteristics of monolithic microchannels of monolith structure. The selected operational parameters, syngas flow rate, liquid flow rate and dilution rate, were found to influence the performance of continuous syngas fermentation, with regard to syngas (CO and H$_2$) consumption, ethanol concentration and productivity, ethanol yield from CO and ethanol to acetate molar ratio. The highest ethanol productivity (2.35 g/L/day) was achieved at syngas flow rate of 300 mL/min, liquid flow rate of 500 mL/min and dilution rate of 0.48 day$^{-1}$. Compared to the continuous syngas fermentation using BCR conducted under the identical condition, MBR showed significantly superior fermentation performance.

The CO $k_{L}a$ of RPB-BR increased significantly with the cage rotational speed and slightly with the headspace pressure. Although the CO $k_{L}a$ achieved using RPB-BR was lower than what have been reported for CSTR and HFM-BR previously, the practical mass transport in RPB-BR was later demonstrated to be enhanced in presence of microorganisms grown as biofilm attached on the packing media. The selected operational parameters, rotational speed, headspace pressure and dilution rate, were found to influence the performance of continuous syngas fermentation, with regard to syngas (CO and H$_2$) consumption, ethanol concentration and productivity, ethanol yield from CO and ethanol to acetate molar ratio. The highest ethanol productivity (6.70 g/L/day) was achieved at rotational speed of 50 rpm, headspace absolute pressure of 29.7 psi and dilution rate of 2.00 day$^{-1}$. This result was significantly higher than what have been observed for continuous syngas fermentation using CSTR and BCR.
2. Outlook and recommendation for future research

All of the three attached-growth, biofilm-based reactor systems showed promising results of continuous syngas fermentation with enhanced mass transfer and ethanol production at bench scale. For practical application, factors, including cost of biofilm support material, extent of prolonged period of operation, simplicity of mechanical design and engineering flexibility, should be taken into account. Nevertheless, it is believed that development and optimization of such attached-growth bioreactors would potentially enhance the economy of gasification-fermentation-derived biorefinery.

The present study only covered carrying out continuous syngas fermentation by using defined culture media in single-stage operation. In the future, other strategies, such as multi-stage fermentation by connecting bioreactors in series with pH and dilution rate shift to separate acidogenesis and solventogenesis and manipulation of nutrient limitation, are suggested to be applied in the attached-growth bioreactors for further improvement of ethanol productivity. Moreover, feasibility of scaling-up the attached-growth bioreactor for continuous syngas fermentation should be tested to enable the commercial application of such bioreactor configurations.