Enhancing mass transfer and ethanol production in syngas fermentation of Clostridium carboxidivorans P7 through a monolithic biofilm reactor

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HIGHLIGHTS

- Syngas fermentation process is limited by gas-to-liquid mass transfer.
- A novel monolithic biofilm reactor (MBR) for efficient mass transfer was developed.
- MBR with slug flow resulted in higher $k_L a$ than bubble column reactor (BCR).
- MBR enhanced ethanol productivity by 53% compared to BCR.
- MBR was demonstrated as a promising reactor configuration for syngas fermentation.

ABSTRACT

Syngas fermentation is a promising process for producing fuels and chemicals from lignocellulosic biomass. Currently syngas fermentation faces several engineering challenges, with gas-to-liquid mass transfer limitation representing the major bottleneck. The aim of this work is to evaluate the performance of a monolithic biofilm reactor (MBR) as a novel reactor configuration for syngas fermentation. The volumetric mass transfer coefficient ($k_L a$) of the MBR was evaluated in abiotic conditions within a wide range of gas flow rates (i.e., gas velocity in monolithic channels) and liquid flow rates (i.e., liquid velocity in the channels). The $k_L a$ values of the MBR were higher than those of a controlled bubble column reactor (BCR) in certain conditions, due to the slug flow pattern in the monolithic channels. A continuous syngas fermentation using Clostridium carboxidivorans P7 was conducted in the MBR system under varying operational conditions, with the variables including syngas flow rate, liquid recirculation between the monolithic column and reservoir, and dilution rate. It was found that the syngas fermentation performance – measured by such parameters 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 on the biofouling or abrading of the biofilm attached on the monolithic channel wall. At a condition of 300 mL/min of syngas flow rate, 500 mL/min of liquid flow rate, and 0.48 day^{-1} of dilution rate, the MBR produced much higher syngas (CO/H₂) utilization efficiency and much greater metabolite (ethanol/acetic acid) productivity than what was obtained using a traditional bubble column reactor. The study demonstrates the great potential of MBR as a promising reactor configuration for syngas fermentation with high mass transfer efficiency, low energy consumption, and high metabolite productivity.

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1. Introduction

The conversion of lignocellulosic biomass into fuels has been widely studied on a biochemical platform in which biomass is converted through pretreatments and enzymatic hydrolysis into reduced sugars, which are subsequently fermented into alcohols. As an alternative to the biochemical platform, the biomass can be gasified into syngas (mainly CO and H₂), which can then be fermented into fuels. Compared with the biochemical platform, the gasification-syngas fermentation platform pathway eliminates the costly pretreatment and enzymatic hydrolysis process [1]; it is also independent of feedstock types and compositions as all components of biomass including lignin can be utilized [2]. As a result, syngas fermentation has been chosen as an attractive conversion route by several companies, such as INEOS Bio, Coskata,
and LanzaTech, for pilot- and commercial-scale cellulolic ethanol production [3].

While these developments are promising, challenges such as low mass transfer efficiency and the presence of inhibitory compounds in syngas still exist for syngas fermentation. Low mass transfer efficiency has long been a major bottleneck [4]. Continuous stirring tank reactor (CSTR) is commonly used in syngas fermentation. A widely-used approach for enhancing gas-to-liquid mass transfer in CSTR is to increase the agitation and/or gas flow rates [5]. However, high agitation rates often lead to high energy consumption and thus, this approach is not economically feasible for scale-up. An energy-efficient alternative for improving mass transfer is the use of an agitation-free bubble column [6,7] or airlift reactor [8]. This type of reactor, if combined with a microbubble diffuser [9], can greatly enhance mass transfer efficiency [10].

Syngas fermentation in a bubble column, however, has its own limitations, such as low cell density and cell wash-out at high dilution rates [6]. A monolith biofilm reactor, which integrates a monolithic packing material within a bubble column, provides an ideal way to maintain high cell density with high mass transfer efficiency.

Monoliths are structures of parallel straight channels separated by thin walls. Compared with other biofilm-based reactors, such as trickling-bed reactors, the pressure drop in the monolithic reactor is much lower because the flow in the channel does not have bends and obstructions. The flow characteristics of monolith reactors have been thoroughly studied [11,12]. The monoliths have been widely applied as catalyst support for multi-phase reactions such as hydrogenation and oxidation in industry [13–15]. Within the field of biological processes, monoliths can be used as economic supporting material for microbial immobilization, providing benefits such as high surface area and superior mechanical strength [12,16]. The potential channel clogging problem can also be minimized by using appropriate operational conditions such as gas/liquid flow rates, substrate concentration and monolith channel size (diameter) [15]. Compared to bubble column reactors, monolithic reactors provide the unique feature of cell attachment [16,17] while maintaining the desirable benefits of bubble columns such as high mass transfer efficiency [18].

To date, limited research has been reported on the use of monolithic reactors for biological processes. Researchers have evaluated organic acid production using *Gluconobacter suboxydans* [19], hydrogen production through sucrose dark fermentation of *Clostridium butyricum* [20], hazardous waste biodegradation [21–23], and waste-gas biofiltering for volatile organic compounds removal [24,25]. However, the potential application of the monolith in syngas fermentation has not been reported. The objective of this study is to investigate the feasibility of using monolith-based biofilm reactors in syngas fermentation for enhanced CO mass transfer and ethanol production.

2. Materials and methods

2.1. Set-up of monolithic biofilm reactor system

A cordierite-based ceramic monolith cylinder (Applied Ceramics, Inc., Doraville, GA) was used. The monolithic cylinder has dimensions of 9.3 cm in diameter and 30.5 cm in length with 200 cpsi (cells per square inch). Each cell channel has a cross section of 1.5 mm × 1.5 mm with 0.3 mm wall thickness. The column has a surface area of 1850 m$^2$/m$^3$ and a 70% void fraction.

The schematic diagram of the monolithic biofilm reactor setup is shown in Fig. 1. The monolithic cylinder was housed in a plexiglass column (4-inch diameter). The monolithic column was fixed inside the plexiglass column by two identical block rings (3-inch inner diameter, 4-inch outer diameter) located at the top and bottom of the monolithic column, respectively. The plexiglass column was connected to a reservoir modified from a BioFlo 110 bench fermenter vessel (New Brunswick Scientific, Edison, NJ). The total working volume of the system (vessel and column) was 8 L. The vessel temperature was maintained at 37 °C with a heating blanket. The monolithic column was covered with an insulation sheet to minimize heat loss. During the syngas fermentation, the broth was circulated between the column and the vessel. Artificial syngas (20% CO, 5% H$_2$, 15% CO$_2$, 60% N$_2$) was fed through two Lee’s® Wooden Air Diffusers (Lee’s Aquarium & Pet Products, San Marcos, CA). The gas and liquid flowed concurrently in the column.

A bubble column reactor was also developed as a control to evaluate the mass transfer and syngas fermentation performance of MBR. The monolithic packing in the plexiglass cylinder was removed; the column remained connected to the reservoir vessel. The geometric parameters of the column and the operational conditions were identical to MBR.

2.2. Determination of volumetric mass transfer coefficient ($k_a$)

The CO $k_a$ value in the monolithic reactor was determined under anaerobic conditions at 25 °C. High purity (99.5%) CO was sparged into the monolithic column through a microporous diffuser. Water was circulated between the column and the vessel through a peristaltic pump. The gas and liquid flowed concurrently at certain flow rate. Liquid samples were periodically taken from the sampling port located at the liquid overflow line. The dissolved CO concentration was determined based on myoglobin-bioassay [26]. The detailed procedures were described elsewhere [5] and the CO $k_a$ value was determined as follows:

$$\ln \left( \frac{C - C_0}{C - C_f} \right) = (k_at)$$

where $C$, $C_0$, and $C_f$ are the saturated concentration, initial concentration, and actual concentration of CO in aqueous phase; $k_a$ is the gas transfer coefficient (cm/s); $a$ is the gas–liquid interfacial area per working volume (cm$^{-1}$); and $t$ is the sampling time. In this work, the shortest sampling time interval is 10 s. To achieve a precise sampling and $k_a$ measurement within such a short time, we pre-inserted eleven 10-µL high performance gastight syringes (#0 to #10) into the sampling port. Syringe #0 was used to take sample at $t = 0$ (the time when CO was just injected into the reactor), syringes #1 to #10 were then used to sequentially take samples on every 10 s basis. Once the samples were taken out of the reactor, they were immediately subject to the myoglobin-bioassay protocol to determine $C_f$ value. After the initial eleven samples, the sampling was continued but the time interval was extended to every 20–100 s. The operation was continued until $C_f$ value stabilized.

2.3. Syngas fermentation in MBR

2.3.1. Strain, medium, and inoculum preparation

*Clostridium carboxidivorans* strain P7 (ATCC BAA-624) was used. The cells were maintained under anaerobic conditions in 125 mL serum bottles containing 80 mL of modified 1754 PECT medium. The medium contains (per liter): 10 g fructose, 0.5 g yeast extract, 5 g morpholinoethanesulfonic acid (MES), 30 mL mineral solution, 10 mL trace metal solution, 10 mL Wolfe’s vitamin solution, and 10 mL 4% cysteine-sulfide reducing agent [27]. Resazurin solution (0.1%) was added as a redox indicator. The medium (excluding the vitamin solution and reducing agent) was autoclaved at 121 °C for 20 min and cooled to room temperature. Then N$_2$ was used to purge the medium for 5 min to remove dissolved oxygen. The vitamin solution and reducing agent were then added to the
medium using a 0.22 µm sterile filter under aseptic and anaerobic conditions. The medium pH was adjusted to 6.0 prior to inoculation. Culture media were then purged with artificial syngas (20% CO, 5% H₂, 15% CO₂, 60% N₂) with headspace pressure of 15 psi at the time of inoculation and every 24 h afterwards. The bottles were incubated in a shaker at 37 °C and 200 rpm. A total of 800 mL seed culture was inoculated into the MBR reservoir.

2.3.2. Syngas fermentation in MBR system

The MBR system was operated in batch mode until biomass attachment was observed. The composition of the initial medium was the same as that used in the subculture. For sterilization of the system, the vessel with media was autoclaved at 121 °C for 20 min, while the column was soaked in 75% (v/v) ethanol for 24 h then flushed with sterilized water. After sterilization, the medium in the vessel was purged with artificial syngas (20% CO, 5% H₂, 15% CO₂, 60% N₂) for 3 h. The fermentation broth was retained in the vessel for 48 h before being recirculated between the column and the vessel. At the time of recirculation, the syngas sparging was also switched from the vessel to column; the outlet gas from the column was directed to the headspace of the reservoir and exhausted from the condenser (Fig. 1). The pH was initially controlled at 6.0, but it was allowed to freely drift to 4.5–5.5 once the liquid recirculation was started. As a control, the methods used for the batch operations of the bubble column and MBR were kept uniform.

The reactor was operated in batch mode for 15 days for biofilm buildup with syngas flow rate and liquid flow rate being set at 200 mL/min and 500 mL/min, respectively, and then switched to continuous operation with gas flow rate and liquid flow rate set according to experimental design, during which spent broth was withdrawn from and fresh media fed to the reservoir every 4 h. The composition of the feed medium was the same as that of the initial medium without fructose. The reactor was operated at various settings of syngas flow rate, liquid recirculation rate, and dilution rate. The steady-state condition under each operation was considered to be established after at three volume changes (the total volume of liquid flowing through the fermenter), with a variation of daily measurement of exhausted gas composition as well as the ethanol and acetates concentration less than 5%.

2.4. Analyses

The inlet and exhaust gas compositions (CO, H₂, and CO₂) were analyzed by a real-time non-dispersive infrared gas monitor (De Jaye Technologies, Des Moines, IA). The ethanol and acetic acid concentrations in the broth were determined using methods reported previously [28]. Upon completion of syngas fermentation, multiple small pieces of monolithic channel chips with biomass immobilized were observed using scanning electron microscopy (SEM). Chips were fixed with 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C for 48 h. Samples were then rinsed with deionized water and post-fixed in 2% aqueous osmium tetroxide, dehydrated in a graded ethanol series up to 100% ultra-pure ethanol, and dried using a Denton DCP-2 critical point dryer (Denton Vacuum, Moorestown, NJ). Dried samples were mounted on stubs and sputter coated (Denton Desk II Sputter Coater, Denton Vacuum, Moorestown, NJ) with palladium–gold alloy. The samples were imaged using a JEOL 5800LV SEM (JEOL Ltd., Peabody, MA) at 10 kV with a SIS ADDA II for digital image.
3. Results and discussions

3.1. CO mass transfer coefficient of monolithic reactor and bubble column reactor

The mass transfer efficiency of the monolithic biofilm reactor is highly dependent upon the flow characteristics of the monolithic microchannel, which are in turn a function of various operational parameters such as physical properties of gas and liquid (density, viscosity, and surface tension), microchannel geometry (shape of cross-section, diameter), flow mode (concurrent, countercurrent, up flow, down flow), and gas and liquid velocities ($U_G$ and $U_L$) inside the channel. When the channel cross section area is fixed, the $U_G$ and $U_L$ values depend on the volumetric gas and liquid flow rates, respectively. In this study, therefore, the measures of mass transfer efficiency of the MBR were correlated with the functions of gas flow rate and liquid flow rate (Fig. 2). The $U_G$ and $U_L$ values corresponding to the volumetric flow rate values were also plotted in Fig. 2. The mass transfer coefficient of the bubble column reactor is presented as a comparison.

As shown in Fig. 2A, the $k_L a$ for both MBR and BCR increased with increasing gas flow rates. At lower gas flow rates (<300 mL/min), the $k_L a$ value of MBR was almost identical to that of BCR in the lower flow rate range. When the gas flow rate exceeded 300 mL/min, MBR produced significantly higher $k_L a$ values than BCR ($p < 0.05$). Fig. 2B shows that $k_L a$ values under varying liquid flow rates were relatively stable compared with the wide variation in $k_L a$ values found under varying gas flow rates. At lower flow rate (<500 mL/min), MBR and BCR had similar $k_L a$ value; while at higher liquid flow rates, the $k_L a$ values of MBR were higher than those of BCR.

The superior mass transfer efficiency found in the MBR can be explained by the unique characteristics of fluid flow in the monolithic channels. In general, the flow pattern inside the monolithic channels can be characterized as bubbly, slug, or churn/annular flow [11,12]. Bubbly flow happens at very low gas flow rates. The bubbles in the channels are usually spherical and do not coalesce, similar to what is observed in a conventional BCR [11]. The similar $k_L a$ values found in MBR and BCR at low gas flow rates may be the result of the occurrence of bubbly flow in this flow-rate range (Fig. 2A).

With increasing gas flow rate, the fluid exhibits a slug flow in which gas bubbles are separated by liquid slugs, preventing bubble coalescence; the bubbles cover the entire channel cross section and the length of the bubbles is greater than the channel diameter. When syngas fermentation happens under this flow regime, gaseous substrate is transferred from gas phase to biofilm through the thin liquid film sandwiched between the two phases (Fig. 3). As surface tension pushes the bubbles towards the biofilm, a very thin liquid film remains between the gas bubbles and the biofilm. This unique feature of gas–liquid interaction greatly improves the mass transfer efficiency by increasing the gas–liquid interfacial area while reducing the thickness of the liquid boundary layer [18]. This feature makes the slug flow pattern in MBR desirable for syngas fermentation.
Unlike the effects of gas flow rate on $k_{l}a$ (Fig. 2A), increasing the liquid flow rate only moderately increased the $k_{l}a$ values (Fig. 2B), this was probably because the higher liquid flow rate only increases turbulence of bulk liquid in the reactor, which did not enhance the mass transfer efficiency as effective as the formation of slug flow occurring in the monolithic channels.

The improved $k_{l}a$ values of MBR vs. BCR at certain gas flow and liquid flow ranges (Fig. 2A) indicate that the flow pattern may be slug flow in these ranges [29,30]. In previous research on the use of monolithic reactors for immobilized glucose oxidase fermentation, Kawakami et al. [29,30] also hypothesized a slug flow pattern in the monolithic channels based on observed $k_{l}a$ values. The gas flow velocity range ($0.5 < U_{c} < 5.0$ cm/s) reported by the authors was similar to the gas flow velocity observed in this study.

It should be noted that when the gas flow rate in the monolithic channels is further increased, churn/annular flow results, with longer gas bubbles and very short liquid slugs; at extreme condition, the entire channel is occupied by gas with thin wavy liquid film flows along the walls of the channel. In this work, however, this flow pattern was not observed.

Overall, the results shown in Fig. 2 indicate that gas and liquid flow rates are important parameters for mass transfer efficiencies of monolithic reactors. At certain high ranges of gas flow and liquid flow rates, the MBR has significantly higher mass transfer efficiencies than the BCR, and this advantage is mainly the result of the slug flow characteristics in the monolithic channels. However, it should be noted that the energy consumption of the MBR and BCR need to be evaluated before choosing the appropriate system for developing a cost effective syngas fermentation system.

### 3.2 Syngas fermentation of **C. carboxidivorans P7** in MBR and BCR in batch culture

The syngas fermentation performance of *C. carboxidivorans P7* in a batch mode in MBR and BCR is shown in Fig. 4. As shown in Fig. 4A, cells grew rapidly without a lag phase in both MBR and BCR, which was probably due to the inclusion of fructose in the medium. At day 2–3, the cell density decreased to a certain degree, probably due to the cell lysis resulting from an unfavorable acidic pH. At this time, the broth circulation was started between the monolithic column and reservoir in order to allow cell attachment on the surface of the monolithic channels; the medium pH was also allowed to freely drift into acidic ranges. The suspended cells in the MBR reservoir gradually attached onto the monolithic channels, resulting in a gradual decrease in suspended cell biomass. Throughout the entire culture period, there remained some suspended cells in the MBR, but much less than those in BCR, indicating the majority cells in the MBR were attached in the monolith column.

Medium pH continuously dropped to 4.36 (day 6), then rebounded up to 5.04 (day 11), and afterwards leveled off. Similar to that found in the MBR system, the cell biomass in BCR peaked at day 2–3, and then slowly diminished. The pH profile of BCR was also similar to that of the MBR system.

Ethanol and acetate are the major metabolites produced by *C. carboxidivorans P7* (Fig. 4B). In the first 2 days of both MBR and BCR testing, when pH was maintained around 6.0–6.2 (a level favorable for cell growth), acetic acid was the predominant metabolite and little ethanol was produced. After day 2, when pH was allowed to drop into acidic range, which favors solventogenesis, ethanol production increased and acetic acid production decreased through the culture period. This correlation between the pH change and ethanol/acetate changes has also been reported in previous studies [6,28,31,32]. At later culture stages, the acetic acid concentration itself was actually reduced; this was probably due to the consumption of acetic acid as a substrate for further ethanol production. The genomic analysis [33,34] revealed that *C. carboxidivorans P7* contains genes that encode domains of aldehyde: ferredoxin oxidoreductase, indicating that P7 cells can metabolize acetate reduction to acetaldehyde and further to ethanol with reduced ferredoxin.

Fig. 4B also shows that MBR and BCR had similar acetic acid and ethanol production profiles, except that no substantial ethanol was produced after day 10 in BCR while ethanol production sustained throughout the culture period in MBR. In this work, the gas and liquid flow rate were 200 ml/min and 500 ml/min, respectively; however, re-visit Fig. 2A shows that the $k_{l}a$ values of MBR and BCR under this gas/liquid flow level were almost identical. Therefore, instead of enhanced mass transfer efficiency, the high ethanol production in MBR was probably due to more cells attached in the monolith column as compared to suspended cells in the BCR.

The attachment of bacterial cells on the monolith materials was further observed (Fig. 5). Fig. 5A and B shows the top view of a monolithic column and an enlarged view of the cell channels, respectively. The monolithic microchannels after P7 batch fermentation are shown in Fig. 5C, which clearly indicates a nice development of biofilm along the channel wall surface. The biofilm growth was evident in the scanning electron microscope imaging (Fig. 5D).

### 3.3 Syngas fermentation of **C. carboxidivorans P7** in MBR in continuous culture

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

Continuous syngas fermentation of *C. carboxidivorans* was performed at various syngas flow rates. As shown in Fig. 6A, both CO and H$_2$ consumption rates increased with gas flow rates increasing from 50 to 300 ml/min, When gas flow further increased from 300
to 500 mL/min, however, the gas consumption did not further increase. The CO and H₂ utilization efficiencies reached their highest levels at 200–300 mL/min syngas flow rate, but dramatically decreased above 500 mL/min. Here the CO and H₂ utilization efficiency were defined as:

\[
E = \left( \frac{C_{\text{in}} \times F_{\text{in}} - C_{\text{out}} \times F_{\text{out}}}{C_{\text{in}} \times F_{\text{in}}} \right) \times 100\%
\]  

where \(C_{\text{in}}\) and \(C_{\text{out}}\) represent the gas (CO or H₂) content (v/v) in inlet gas and exhaust gas streams, respectively; \(F_{\text{in}}\) and \(F_{\text{out}}\) represent the volumetric flow rate of inlet and exhaust gas, respectively.

Fig. 6B shows the production of ethanol and acetic acid at various syngas flow rates. Acetic acid and ethanol concentrations and their productivities increased as the syngas flow rate increased from 50 to 300 mL/min. Further increases in the syngas flow rate above 300 mL/min did not improve ethanol or acetic acid production. These results, together with the syngas consumption rate trends (Fig. 6A), indicate that syngas fermentation at the low gas flow rate (50–300 mL/min) was substrate-limited; an increased gas flow rate resulted in a better mass transfer, and thus, better syngas consumption and metabolite formation. At higher flow rates (300–500 mL/min), however, the syngas supply may have exceeded the cells' maximum capacity for syngas consumption and metabolite formation.

Fig. 6C shows the ethanol and acetic acid yields from CO and the ethanol to acetic acid molar ratios at various syngas flow rates. The yield is defined as the amount (mole) of carbon contained in the product over the amount (mole) of carbon contained in the consumed CO. Overall, ethanol yields from CO were roughly twice that of acetic acid yield and remained around 0.18 mol C/mol C.

### 3.3.2. Effects of liquid flow rate on fermentation performance

The liquid flow rate in monolithic channels influences not only mass transfer efficiency, but also biofilm characteristics. Ebrahimi et al. [17] have identified substrate mass transfer and surface shear force as two prevailing factors affecting the biofilm growth pattern in a monolithic channel. The effects of liquid flow rate on syngas fermentation in MBR are shown in Fig. 7. Fig. 7A shows that the consumption rate and utilization efficiency of CO increased while those of H₂ leveled off with liquid flow rates from 200 to 500 mL/min. When the liquid flow rate exceeded 500 mL/min, the gas consumption as well as the utilization of the two gases decreased. The production of the ethanol and acetic acid shows a similar trend, with the peak values being achieved at 500 mL/min of liquid flow (Fig. 7B). Fig. 7C shows that both ethanol and acetic acid yields maintained at a relatively constant level over the liquid flow rate range studied. Ethanol to acetic acid molar ratios remained around 2.20 within the range of 200–500 mL/min liquid flow and increased with liquid flow rates exceeding 500 mL/min.

The positive correlation between liquid flow rate and metabolite production in the lower liquid flow rate range (200–500 mL/min) indicates that gas-to-liquid mass transfer might be rate-limiting during syngas fermentation, as the \(k_{L}a\) increases with liquid flow rate increasing from 200 mL/min to 500 mL/min. In the high liquid flow rate range (500–1000 mL/min), the reduced syngas consumption and the ethanol/acetic acid production may be due to higher shear force, which causes biofilm abrading and loss of active biomass. Indeed, we observed that the cell density was at a rather low level (0.03–0.10 g/L) when the liquid flow rate was in the range...
of 200–500 mL/min, and reached to as high as 0.30 g/L when the liquid flow rate was in the range of 500–1000 mL/min.

3.3.3. Effects of dilution rate on syngas fermentation performance

Dilution rate is another important factor influencing the continuous operation of syngas fermentation. While gas and liquid flow affect the mass transfer efficiency and biofilm attachment/detachment in the monolith channels, the dilution rate did not impact those physical properties because continuous syngas fermentation was performed by adding fresh medium and withdrawing spent medium from the reservoir vessel (Fig. 1). Dilution rate has, however, been reported to affect the ratio of the biofilm/suspended cells as well as the microbial community characteristics [16,35]. The existence of the monolith also enables cells to be retained in the channels rather than being washed out; therefore, syngas fermentation in the MBR system can be operated at a high dilution rate.

Fig. 8 illustrates the syngas fermentation performance of MBR operated at varying dilution rates. As shown in Fig. 8A, CO consumption rate and utilization efficiency increased with dilution rates increasing from 0.12 to 0.48 day⁻¹, and then decreased as dilution rates further increased to 0.96 day⁻¹. H₂ consumption rates and utilization efficiencies maintained at a relatively stable level at lower ranges of dilution rates (0.12–0.48 day⁻¹) and then decreased at 0.96 day⁻¹. Fig. 8B shows that the concentrations of ethanol and acetate monotonically decreased with the increase of dilution rate due to the “diluting” effect. However, the productivity of these two metabolites reached their highest levels at 0.48 day⁻¹ and both acetic acid and ethanol productivity decreased above dilution rates of 0.96 day⁻¹. Fig. 8C shows that the yields of ethanol and acetic acid leveled off from 0.12 to 0.48 day⁻¹. The yield of acetic acid slightly increased, while the yield of ethanol decreased above 0.96 day⁻¹, resulting in a lower ratio of ethanol to acetic acid.

It should be noted that the slightly inferior performance at 0.96 day⁻¹ might be due to the elution of suspended cells in the MBR vessel, as a small portion of the suspended cells were observed during the operation of the MBR system. However, compared with the bubble column reactor, in which the cell washout was observed when the dilution rate reached to 0.6 day⁻¹ (data not shown), the MBR demonstrated its superior capability to retain cells at a high dilution rate (0.96 day⁻¹); this dilution rate (0.96 day⁻¹) was almost two-fold higher than the maximum dilution rate in bubble column reactor using the same strain [6,27].

3.4. Comparison of syngas fermentation in MBR and BCR

The results obtained in Section 3.3 shows that the optimal conditions for achieving the highest ethanol productivity was as follows: syngas flow rate 300 mL/min, liquid flow rate 500 mL/
using MBR for syngas fermentation, a syngas fermentation process syngas flow rate 300 mL/min, liquid flow rate 500 mL/min, and dilution rate 0.48 day\textsuperscript{\text{-1}}. Comparison of continuous syngas fermentation in bubble column reactor (BCR) and monolithic biofilm reactor (MBR), and the improvement of MBR vs BCR under condition of productivity; (C) product yield from CO and ethanol to acetate molar ratio. Syngas

\begin{table}[h]
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\begin{tabular}{llll}
\hline
Parameters & BCR & MBR & Improvement (\%) \\
\hline
CO utilization efficiency (\%) & 54.2 ± 3.9 & 84.9 ± 2.2 & 56.6 \\
CO consumption rate (mmol/L/day) & 383.3 ± 27.3 & 600.5 ± 15.1 & 56.6 \\
H\textsubscript{2} utilization efficiency (\%) & 70.9 ± 4.1 & 90.0 ± 5.8 & 26.9 \\
H\textsubscript{2} consumption rate (mmol/L/day) & 125.6 ± 7.2 & 159.2 ± 10.7 & 26.7 \\
Ethanol concentration (g/L) & 3.20 ± 0.62 & 4.89 ± 0.45 & 52.8 \\
Ethanol productivity (g/L/day) & 0.17 & 2.35 ± 0.30 & 52.5 \\
Acetic acid productivity (g/L/day) & 0.08 & 1.46 ± 0.22 & 29.2 \\
Acetic acid productivity (mol C/mol C) & 1.8 & 2.1 & 16.6 \\
Ethanol/acetic molar ratio & 0.10 & 0.70 & 20.0 \\
\hline
\end{tabular}
\caption{Comparison of continuous syngas fermentation in bubble column reactor (BCR) and monolithic biofilm reactor (MBR), and the improvement of MBR vs BCR under condition of syngas flow rate 300 mL/min, liquid flow rate 500 mL/min, and dilution rate 0.48 day\textsuperscript{\text{-1}}.}
\end{table}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig8}
\caption{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.}
\end{figure}

min, and dilution rate 0.48 day\textsuperscript{-1}. To evaluate the advantages of using MBR for syngas fermentation, a syngas fermentation process was further performed in the BCR and the results are compared to those obtained in the MBR system. As shown in Table 1, CO/H\textsubscript{2} consumption rates and utilization efficiencies were much higher in the MBR than in the BCR; the MBR reactor also resulted in a higher ethanol/acetate acid titer and productivity. With respect to these parameters, the MBR demonstrates a superior performance for syngas fermentation.

The improvement of monolithic reactors as compared to traditional bubble column reactors was also reported in bio-hydrogen production, in which the monolith served as nucleation to facilitate the bubble hydrogen formation. For example, Fritsch et al.\cite{20} reported a 35% enhancement in hydrogen production rate and a 30% enhancement in hydrogen yields when using a monolith reactor for \textit{C.\ butyricum} dark fermentation as compared to a conventional bubble column reactor.

The advantage of using MBR for syngas fermentation was attributed to the unique feature of the monolithic materials. The slug flow pattern in the monolithic channels increases the gas–liquid interfacial area and thus the mass transfer capacity. The monolithic structure packing material also provides a high surface area per unit of volume available for cell attachment. For example, the 200-cpsi monolithic column used in the present study has an A/V degree of 1850 m\textsuperscript{2}/m\textsuperscript{3}. Moreover, the formation of biofilm on the monolithic packing material also has desirable mechanical properties such as high resistance to shear strength, pressure and temperature. Compared with other membrane-based reactors, such as hollow fiber membrane reactors, the biofouling problem in MBR is less severe due to the larger size of its monolithic channels.

\section{Conclusions}

This work demonstrated the effectiveness of monolithic biofilm reactors for syngas fermentation. To the best of our knowledge, this is the first comprehensive investigation on the use of MBR systems for syngas fermentation. This comprehensive investigation included the estimation of fluid flow patterns and CO mass transfers in abiotic conditions and the analyses of batch cultures and continuous cultures with optimization of various operating parameters (syngas flow rates, liquid flow rates, and dilution rates). MBR systems compare favorably to conventional BCR due to their higher mass transfer efficiency and desirable biofilm development capacity and the consequent high syngas consumption efficiencies and metabolites production.

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References


