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High Pressure Moving Bed Biofilm Reactor for Syngas Fermentation

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Two well-studied strategies to enhance syngas fermentation are elevated headspace pressure, increasing gas-liquid (GL) mass transfer, and moving bed biofilms (MBB) to overcome kinetic growth limitation. A combination of these two methods has not received much attention. This study evaluates the integration of these two strategies in one single reactor. The hydrogen (H_2) GL mass transfer is the primary bottleneck in syngas fermentation due to the lowest solubility among syngas components. The syngas composition is mimicked here as a mixture of H_2 gas and sodium bicarbonate ($NaHCO_3$) salt. Initially, fermentation is performed at 15 bar H_2 headspace pressure in the suspended culture, then the experiment is repeated with MBB integration, and the performance is critically evaluated. The study with integrated biofilm shows a 33 % improvement in H_2 gas uptake rate (200 mmol/L·day) and 48 % enhancement in acetate synthesis rate (37.4 mmol/L· day). This study concludes that MBB could successfully be integrated into an elevated pressure syngas fermenter, enhancing the fermentation process efficiency.

1. Introduction

Increasing energy demand and climate change are the foremost current world challenges. Even though the world is actively engaging in the clean energy transition, 80 % of the total global energy demand has been base on fossils fuel for the last three decades (World Energy Outlook Analysis, 2020). Growth in energy production increases carbon dioxide (CO_2) emission and causes a significant impact on climate change. Moreover, waste disposal practices (landfills, open burning, incineration, gasification, pyrolysis) and industrial processes as cement and metals production are the substantial sources of greenhouse gasses. Carbon dioxide is the component that indeed needs to be either captured or utilised to stop climate change. Several capture and separation technologies are available; however, those technologies call for expensive and energy-intensive steps. Therefore we need climate-friendly solutions to capture and utilise CO_2 in more sustainable ways.

Homoacetogenesis is one of such sustainable approach. It is a biochemical process performed by autotrophic bacteria, which reduces CO_2 into acetate (Kanchanasuta et al., 2016) through an intermediate enzymatic pathway called acetyl-CoA (Eq 1). It is also called the Wood-Ljungdahl pathway to honour Harland G. Wood and Lars G. Ljungdahl, the scientists who introduced this process in 1986 (Drake, 2012). The CO_2 reduction process requires electrons as an energy source. H_2 and carbon monoxide (CO) can serve as electron donors. Moreover, CO can contribute as a carbon source also. This is where the syngas plays a pivotal role in the acetyl-CoA path because syngas is a mixture of CO, H_2 and CO_2, that can be directly used as the substrate for the homoacetogenic fermentation process. Gasification and pyrolysis are the primary sources of syngas. Emitted CO_2 from power plants and other process-related industries can also be used as substrate with alternative electron sources, such as H_2 produced from water electrolysis by utilising renewable electricity.

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad \Delta G^0 = -74.3\ \mathrm{kJ/mol} \tag{1}$$

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Though homoacetogenesis was discovered several decades ago, industrial level applications are still under maturation. Gas-Liquid (GL) mass transfer and kinetic-growth limitation are the primary engineering bottlenecks that have slow down the scale-up process. Especially, the lowest solubility of H_2 demands more energy-intensive strategies. The mass transfer rate is insufficient to cope with cell growth demand, resulting in low biomass growth and product synthesis, leading to an inefficient carbon reduction. The autotrophic homoacetogens specific growth rate is relatively low (Karekar et al., 2019; Regueira et al., 2018), results in poor cell density, which is referred to as a kinetic-growth limitation, which leads to inefficient product synthesis. Increasing H_2 gas headspace pressure is a well-known (Stoll et al., 2018; Van Hecke et al., 2019) lower-cost strategy than using energy-intensive agitators to counteract GL mass transfer limitation.

At the same time, increasing the cell density with biofim substrates can overcome kinetic-growth limitations. Hollow fibre membrane biofilm, monolithic biofilm, rotating packed bed biofilm and moving bed biofilm (MBB) reactors are a few examples of attached-growth biofilm syngas fermentation reactors (Shen, 2013). MBB reactors are a salient strategy that can surpass both GL mass transfer and kinetic growth limitations. Biocarriers' surface area facilitates biofilm growth, which helps to attain higher cell density (Wang et al., 2019), and movement in biocarriers induce an efficient GL mass transfer. Moreover, the MBB integration within the syngas fermentation reactor has many other process advantages, such as lower hydraulic retention time, higher sludge retention time, higher resistance to the toxic substances that come along with the syngas mixture.

The moving bed biofilm concept is a Norwegian invention, patented by Kaldnes Miljøteknologi in 1991 (Ødegaard, 2019). Initially, it was designed for nitrogen removal in wastewater treatment. However, over time MBB has been used in various applications such as organic substances removal, phosphorous removal and recovery (Rudi et al., 2019) and reject water treatment (Sivalingam et al., 2020). As a result of continuous advancement, Robert Hickey integrated the MBB reactor into a fermentation reactor to convert syngas components to liquid products, patented in 2009 (Hickey, 2009). Hickey used a pure culture of *Clostridium ragsdalei* ATCC No. BAA-622 as the fermentation medium and operated at 0.1 bar outlet pressure while the liquid pressure was between 1 to 5 bar.

Using pure cultures in a syngas fermentation medium gives efficient product specificity but maintaining a sterilised environment, to keep only the specific culture, add an extra cost, which becomes a significant challenge when upscaling. Therefore, using mixed cultures is getting more attention where the product specificity is not the primary concern, instead of carbon fixation. In this research, anaerobic sludge from the local municipal wastewater treatment plant is used as the mixed culture fermentation, easily available with relatively no cost.

Even though elevating headspace pressure and the use of MBB carriers in syngas fermentation has been individually investigated to enhance performance. A combination of these technologies has rarely been studied. As far as the authors know, this is the first time an investigation integrates elevated H_2 pressure (15 bar) into a MBB reactor mixed culture fermentation process.

This study consists two-phase batch experiments. Phase 1 deals with H_2 fermentation at an elevated pressure of 15 bar. In phase two, the pressure reactor is modified as a moving bed biofilm syngas fermentation reactor. The phases 1 and 2 are named as pressure syngas fermentation reactor (PSFR) and biofilm pressure syngas fermentation reactor (B-PSFR) . The results are compared and evaluated in terms of hydrogen consumption rate and product formation.

2. Material and methods

2.1 Fermentation medium preparation and bio carrier adaptation

Sludge from the biogas digester at Knarrdalstrand wastewater treatment plant, Porsgrunn, Norway, was used as mixed culture. The sludge underwent several pretreatment steps to obtain a homoacetogenic rich culture. Firstly, the coarse impurities such as plastics and woody debris were removed by sieving at 600 microns. The sludge was then incubated for seven days at 35 °C for further thickening and to get rid of remaining easily degradable organic matters. The thickened sludge was heat-treated at 105 °C for 48 hours to eliminate methanogens and to enrich spores forming acetogens. Subsequently, the heat-treated sludge was cooled down to ambient temperature. Finally, salt (10 mL/L), vitamin (1mL/ L), and mineral (1mL/ L) solutions were mixed with the inoculum to provide nutritional supplements for microbial growth. The nutrient base media composition was adapted from a similar study (Sivalingam et al., 2021), shown in Table 1.

Table 1: Nutrient base media compositions

Vitamin solution (g/L)	Mineral solution (g/L)	Salt solution (g/L)
Biotin: 0.02	$MnSO_4 \cdot H_2O$: 0.04	NH_4Cl: 100
Folic acid: 0.02	$FeSO_4 \cdot 7H_2O$: 2.7	NaCl: 10
Pyridoxine hydrochloride: 0.1	$CuSO_4 \cdot 5H_2O$: 0.055	$MgCl_2 \cdot 6H_2O$: 10
Riboflavin: 0.05	$NiCl_2 \cdot 6H_2O$: 0.1	$CaCl_2 \cdot 2H_2O$: 5
Thiamine: 0.05	$ZnSO_4 \cdot 7H_2O$: 0.088	
Nicotinic acid: 0.05	$CoCl_2 \cdot 6H_2O$: 0.05	
Pantothenic acid: 0.05	H_3BO_3: 0.05	
Vitamin B12: 0.001		
p-aminobenzoic acid: 0.05		
Thioctic acid: 0.05		

6.8 g/L sodium bicarbonate ($NaHCO_3$) was added to the inoculum as the carbon source. Once the fermentation medium is placed into the reactor, 10 mL of homoacetogens riched sludge was added as seed to trigger the startup process. The pH was neither adjusted nor controlled throughout the experiments. The characteristics of the treated inoculum are presented in Table 2.

BWT X type biocarriers (Dimensions 14,5 x 14,5 x 8,2 mm and Protected surface: 650 m^2/m^3) from Biowater Technology As, Tønsberg, Norway was used as the biofilm media. Homoacetogens have a low growth rate; therefore, the biocarriers (# 70, 200 mL bulk volume) were adapted for two weeks into a homoacetogens rich medium. The adaptation could help microorganisms establish a necessary foundation on carrier surfaces, consequently shortening the experimental period.

Table 2: Characteristics of the treated inoculum.

Parameters	pH	TS* (g/L)	VS‡ (g/L)	TCOD# (g/L)	NH_4-H (g/L)	Alkalinity (g/L as $CaCO_3$)
Values	8.5	14.3	7.5	9.4	0.8	1.97

* Total solids, ǂ Volatile solids, # Total Chemical Oxygen Demand

2.2 Experimental setup and procedure

This research was performed in two phases. In phase 1 (PSFR), a 1.8 L stainless steel stirred-tank pressure reactor (NR-1500, Berghof, Eningen, Germany) was used as a fermenter (Figure 1, Left). The reactor was equipped with a digital manometer (LEO-3, Keller, Winterthur, Switzerland) and connected to a computer via a RS485 interface. The Control Center Series-30 (Keller, Winterthur, Switzerland) software package recorded the headspace pressure changes. The reactor lid has a fixed mounted mechanical stirrer (BG 65X50, Dunkermotoren, Bonndorf, Germany) which run continuously at 200 rpm. All the experiments were performed at 25°C. At the start of phase 1, the reactor was filled with 1 L treated inoculum with supplements. The fermentation medium was purged with N_2 gas for 5 min, followed by H_2 gas for 2 min, to ensure anaerobic conditions. The headspace was then pressurised to 15 bar (Laboratory H_2 gas 5.5 = ≥ 99.9995 %, Linde Gas AS, Oslo, Norway). The headspace pressure 15 bar was determined from the authors' laboratory study. There, different H_2 headspace pressure varies from 1 to 25 bar were tested and figured out 15 bar is the optimum (Results are not presented here) for this particular mixed culture fermentation medium. The phases one and two experiments were continued until a static pressure was observed (approximately a week).

In phase 2 (B-PSFR), the pressure reactor used in phase 1 was modified as a moving bed biofilm syngas fermenter (Figure 1, Right). The adapted MBB carrier was incorporated into the fresh treated suspended fermentation medium. The working volume was maintained at 1 L same as phase 1. Successively, all other steps performed in phase 1 were repeated. The biocarriers were observed with a Nikon Stereo Microscope SMZ745T, and the Infinity Analyze software measured biofilm thickness.

2.3 Analytical methods

pH, TS, VS, ammonium, alkalinity and TCOD were performed based on the standard method of the America Public Health Association (APHA, 1995). Volatile fatty acids (VFAs) were analysed by gas chromatography (PerkinElmer, Clarus 500, Massachusetts, USA), the instrument was equipped with a capillary column (SCION Instruments, Livingston, Scotland; length 25 m x diameter 0.25 mm x film 0.2 µm) and Flame Ionization Detector. The carrier gas was H_2 at a flow rate of 45 mL/min. The injector and detector temperatures were 270 °C and 250 °C, respectively. The initial oven temperature was constant at 80 °C for 0.7 minutes and then increased to 200 °C by 25 °C/min followed by 20 °C/min ramp-up rate until reach 240 °C.

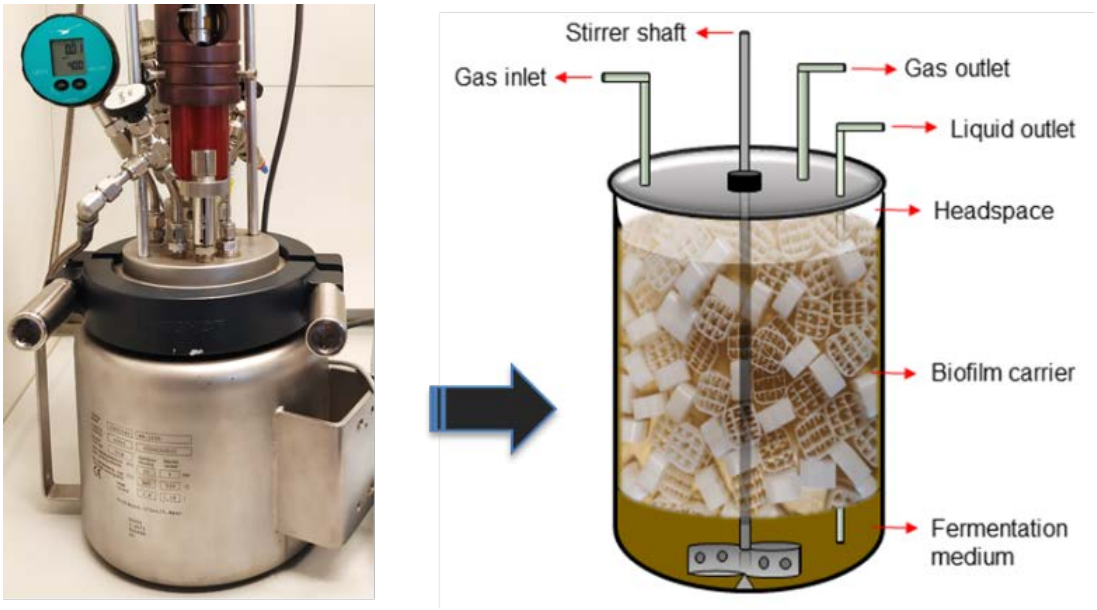

Figure 1: Left: Pressure reactor (PSFR), Right: Sketch of pressure reactor with MBB (B-PSFR).

3. Results and discussion

Figure 2a describes the pressure time series and cumulative H_2 consumption profiles for both PSFR and B-PSFR. The PSFR took seven days to reach the static headspace pressure, while B-SFR took only 4.5 days. However, the lag phase duration is the same, 2.5 days. The B-PSFR depicted the highest gas uptake rate (200 mmol/L·day) and sizeable cumulative gas consumption (218 mmol), respectively, 33 % and 20 % higher than PSFR. This is a clear evidence that MBB integration significantly improved the GL mass transfer and minimised the kinetic limitation. The gas uptake rate of PSFR is 150 mmol/L·day. This is a considerably large number compared to other experiments performed at lower headspace pressure (Stoll et al., 2018). According to Henry's law, the enhancement in headspace pressure increased the GL mass transfer, resulting a rise in gas solubilisation and utilisation.

Figure 2b shows the acetic acid concentration change in both PSFR and B-PSFR. Acetic acid was the only detected VFA as the fermentation product. The increment in acetic acid concurs with cumulative H_2 consumption profiles. There was a rapid rise in concentration from day 3 to 4, after that flattened out. The B-PSFR showed a predominant acetic acid synthesis (2.7 g/L); it is 40 % higher than PSFR (without biofilm). Not only the concentration but the acetic acid production rate (48 %) was also remarkably higher in B-PSFR (37.4 mmol/L· day). The notable acceleration in acetic acid production rate affirms that biofilm integration as a promising strategy for making the H_2 fermentation process more efficient.

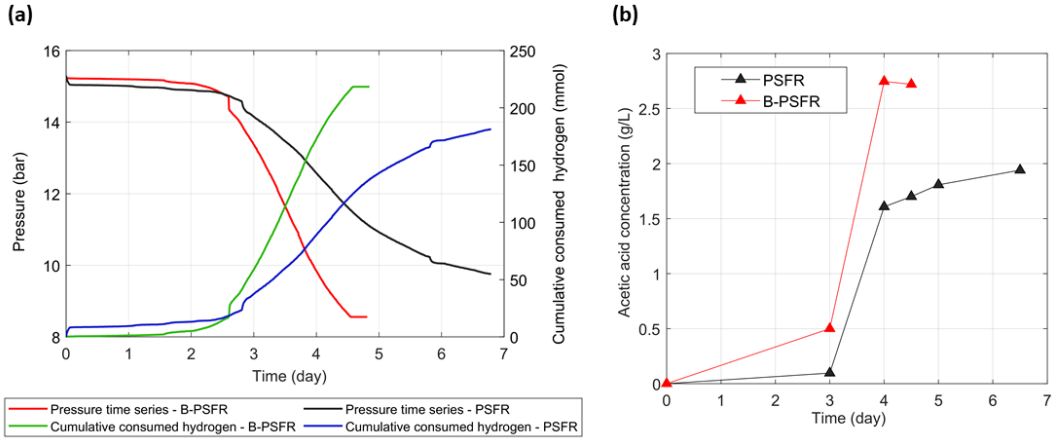

Figure 2: a - Pressure time series, and cumulative H_2 consumption profiles, b – Acetic acid profiles.

The H_2 utilisation and carbon capture efficiencies (HUE and CCE) are critical factors that determine the efficiency of a syngas fermentation process, calculated according to Eq (2) and (3) based on the stoichiometric Eq (1), tabulated in Table 3. The efficiency calculations proved that the MBB integration enhanced the HUE and CCE by 12 % and 32 %, respectively. The B-PSFR resulted in 112 % CCE. The percentage above a hundred is due to the carbon present in the fermentation medium as alkalinity. The treated inoculum contains inherent alkalinity, about 1.97 g/L as $CaCO_3$ (Table 2). The CCE calculation is based on added $NaHCO_3$ as the only carbon source.

$$\mathrm{HUE} = \frac{\mathrm{H_2\ equivalent\ to\ the\ produced\ acetic\ acid}}{\mathrm{Total\ consumed\ H_2}} \quad (2)$$

$$\mathrm{CCE} = \frac{\mathrm{CO_2\ \ equivalent\ to\ the\ produced\ acetic\ acid}}{\mathrm{Available\ CO_2}} \quad (3)$$

Table 3: The H_2 utilisation and carbon capture efficiencies.

Phases	HUE (%)	CCE (%)
Phase 1 - PSFR	71	80
Phase 2 – B-PSFR	83	112

The stereo microscopic images of the biocarriers are presented in Figure 3. The biofilm thickness was measured in different locations of the carriers; the average biofilm thickness was about 157 ± 20 µm. It was observed that the carriers' protected/inner surface area was populated with attached biofilm growth. The significant assemblage of biofilm and enhancement in the fermentation process at B-PSFR affirm that enhanced cell density reduced the kinetic growth limitation, heightening the fermentation efficiency.

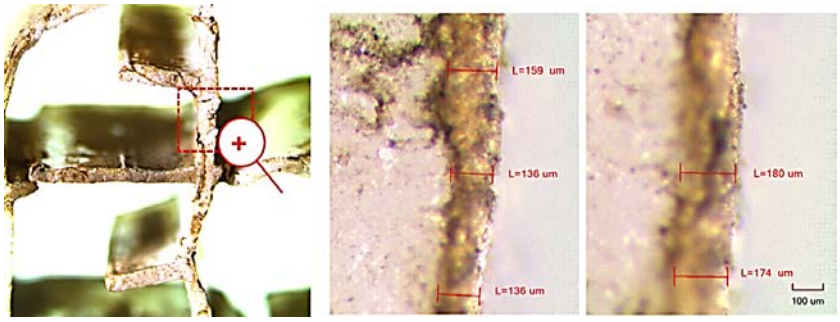

Figure 3: Stereo micrograph of the biocarrier and magnified version of attached growth on the carrier surface.

4. Conclusions

A high-pressure syngas fermentation reactor combined with MBB carriers demonstrated an enhancement in H_2-gas uptake (33 %), acetic acid synthesis rate (48 %) and carbon fixation efficiency (>20 %) compared to high-pressure suspended culture alone. The significant assemblage of biofilm on carriers enhanced the fermentation process at B-PSFR. The improved cell density of biofilms reduced the kinetic growth limitation compared to suspended cultures. This novel study demonstrated that combining both approaches accelerates the syngas fermentation process.

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