

RESEARCH ARTICLE

Regulation on C2-C8 carboxylic acid biosynthesis from anaerobic CO₂ fermentation

Wanling Wu^{1,2} | Zhiqi Li¹ | Guangqing Liu² | Ling Zhou³ | Wen Wang^{1,2} 

¹College of Chemical Engineering, Beijing University of Chemical Technology, Beijing, China

²Biomass Energy and Environmental Engineering Research Center, Beijing University of Chemical Technology, Beijing, China

³Modern Agricultural Engineering Key Laboratory at Universities of Education Department of Xinjiang Uygur Autonomous Region, Tarim University, Alar, Xinjiang, China

Correspondence

Wen Wang, College of Chemical Engineering, Beijing University of Chemical Technology, No. 15 Baisanhuan East Road, Chaoyang District, Beijing 100029, China.

Email: anne_wangwen@163.com

Ling Zhou, Modern Agricultural Engineering Key Laboratory at Universities of Education Department of Xinjiang Uygur Autonomous Region, Tarim University, Alar, Xinjiang Uygur Autonomous Region, 843300, China.

Email: zhoul-007@163.com

Funding information

Beijing Nova Program, Grant/Award Number: Z201100006820022; National Natural Science Foundation of China, Grant/Award Numbers: 52270096, U20B2022; Key Science and Technology Development Program of Xinjiang Corps, Grant/Award Number: 2021DB006

Abstract

Bioconversion of CO₂ into liquid fuels or chemicals, preferred medium chain carboxylic acids (caproic and caprylic acid), is an attractive CO₂ utilization technology. The present study aims to investigate the effects of different ratios of H₂/CO₂ on regulating the distribution of C2-C8 carboxylic acid products, while the headspace pressure of 1.5 bar was set to amplify the effect of different ratios. The H₂/CO₂ ratio of 4:1 was more suitable for preparing acetic acid, where the highest acetic acid yield was 17.5 g/L. And the H₂/CO₂ ratio of 2:1 showed excellent chain elongation ability with the highest n-caprylic yield of 2.4 g/L. Additionally, the actual H₂/CO₂ ratios of 4:1 reactors were higher than that in 2:1 may be course chain elongation often accompanied by H₂ production. The 16S rRNA genes analysis shows that the genus *Terrisporobacter* and *Coriobacteriales* may be related to acetic acid production enriched in H₂/CO₂ ratio 4:1 reactors, and the genus *Clostridium* and *Paenibacillaceae* may associate with the chain elongation pathway were enriched in H₂/CO₂ ratio 2:1 reactors.

KEYWORDS

anaerobic microbiology, caproic acid, caprylic acid, chain elongation, CO₂ fermentation

1 | INTRODUCTION

Since the industrial revolution, the global temperature has kept escalating, mainly attributed to anthropogenic greenhouse gas emissions [1]. CO₂ is the most significant contributor to greenhouse gas, accounting for more than

70% of global emissions, while the rest consists of methane, nitrous oxide, and fluorinated gases [2]. The total global CO₂ emissions reached 3.49×10^{10} t in 2021, with a continuous growth trend [3]. Therefore, carbon neutrality is the essential prerequisite to development for maintaining ecological life prosperity and environmental balance. Various technologies for carbon capture, utilization and storage are being investigated in different fields to support carbon neutrality [4]. In particular, CO₂ utilization contributes

Abbreviations: MCCAs, medium chain carboxylic acids; SCCAs, short chain carboxylic acids; W-L pathway, Wood-Ljungdahl pathway.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Engineering in Life Sciences* published by Wiley-VCH GmbH.

to carbon fixation while obtaining high-value chemicals, which is a more compelling research perspective. The common techniques for CO₂ utilization include thermochemical catalysis, photochemical catalysis, and electrochemical catalysis [5–7]. These processes have achieved impressive outcomes but are usually accompanied by the disadvantage of high costs and high energy consumption, which remains a major challenge for CO₂ utilization [4, 8].

Besides traditional chemical catalysis, enzyme catalysis has attracted much attention recently due to its high specificity and mild reaction conditions [9, 10]. Various microorganisms can grow on CO₂ as the carbon source, presenting diverse metabolic functions and high-value products [11, 12]. Six major natural CO₂-fixation pathways have been reported, of which, the reductive acetyl-CoA pathway (also known as the Wood-Ljungdahl pathway, W-L pathway) is the shortest and most efficient of all [13–15]. In the W-L pathway, anaerobic microorganisms, like homoacetogen, could convert CO₂/H₂ to acetyl-CoA and then to acetic acid or ethanol [15]. But the economic value of acetic acid/ethanol is limited, and their high solubility increases the cost of subsequent separation. Studies have shown that specific microorganisms can metabolize acetyl-CoA to medium chain carboxylic acids (MCCAs, C6–C12) via the chain elongation pathway [15]. Compared to acetic acid/ethanol, the longer carbon chains of MCCAs provide higher hydrophobicity and thus facilitate separation. Additionally, MCCAs have lower O/C ratios and consequently higher energy densities. MCCAs could be used for various value-added derivatives, such as pharmaceuticals, fragrances, food additives, resins and rubbers, etc. [16, 17]. Therefore, converting CO₂ to MCCAs by anaerobic microorganisms will create greater economic value while supporting carbon neutrality.

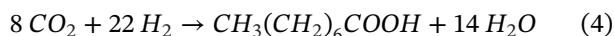
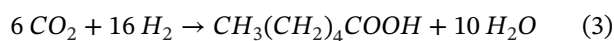
However, more studies are currently focused on producing acetic acid instead of MCCAs by the microbial fixation of CO₂ [18]. With the carbon chain lengthens, the reaction steps and key enzymes increase significantly, making the production of MCCAs more difficult than acetic acid [19]. Additionally, CO₂ molecules are highly stable and not prone to chemical reactions, which increase the difficulty of CO₂ chain elongation. Therefore, it is essential to investigate the appropriate conditions for producing MCCAs by CO₂ fermentation. H₂ is a typical electron donor in CO₂ fermentation, providing electrons and energy for microbial metabolism [15]. In terms of the theoretical reaction equations (Equations 1–4) for the production of acetic acid and longer chain carboxylic acids, as the carbon chain length increases, the amount of H₂ consumed to fix the same CO₂ increases [20]. The H₂/CO₂ ratio more suitable for acetic acid or MCCAs may differ, which may be critical in regulating carboxylic acid products. In addition, CO₂ solubility is related to the chemical reaction equilibrium and

Practical Application

The present study is mainly applied to the artificial biological treatment technology of industrial waste gas CO₂, simultaneously obtaining high-value carboxylic acid products. This study could achieve a maximum CO₂ consumption rate of 1.26 L/d per liter of microbial solution. Meanwhile, the highest acetic acid production rate could reach 2.36 g/L/d, the highest n-caproic acid production rate could reach 0.6 g/L/d, and the highest n-caprylic acid production rate was 0.25 g/L/d. The present study provides a new method that adjusts the distribution of C2–C8 carboxylic acid products by regulating the H₂/CO₂ ratio. The H₂/CO₂ ratio of 4:1 was more suitable for the preparation of acetic acid, where the highest acetic acid yield was 17.5 g/L. And the H₂/CO₂ ratio of 2:1 showed excellent chain elongation ability with the highest n-caprylic yield of 2.4 g/L. Summarily, this biotechnology for CO₂ fixation has the potential for industrial applications.

phase equilibrium [21]. Therefore, some of the CO₂ provided may be dissolved, and the actual H₂/CO₂ ratio will be incompatible with that provided.

Based on the above, this study aimed to (1) investigate the regulation of C2–C8 carboxylic acids by different headspace H₂/CO₂ ratios, and a headspace pressure of 1.5 bar was set to amplify the inter-reactor difference; (2) expound the metabolic pathway regulation from CO₂ to C2–C8 carboxylic acids; (3) analyze the clustering and composition of the microbial community.



2 | MATERIALS AND METHODS

2.1 | Inoculum and feedstock

The inoculum was anaerobic granular sludge derived from a mesophilic sewage treatment plant in Beijing, China. The characteristics of the inoculum were as follows: total

TABLE 1 Experimental conditions

Reactor	Headspace	H ₂ (ml/d)	CO ₂ (ml/d)	Pressure (bar)
B-1	N ₂	–	–	1.0
B-1.5	N ₂	–	–	1.5
4:1-1	H ₂ :CO ₂ = 4:1,v/v	365	86	1.0
4:1-1.5	H ₂ :CO ₂ = 4:1,v/v	548	137	1.5
2:1-1	H ₂ :CO ₂ = 2:1,v/v	288	141	1.0
2:1-1.5	H ₂ :CO ₂ = 2:1,v/v	432	212	1.5

solids (TS) 19.8 ± 0.5 g/L, volatile solids (VS) 15.2 ± 0.9 g/L, and pH 6.8 ± 0.2 . The anaerobic granular sludge was heat pretreated under 120°C for 40 min to inhibit the activity of methanogens. BA medium was used as feedstock as previously described [22].

2.2 | Experimental setup

The experiments were set up with six sets of batch culture reactors, with each added inoculum (90 ml) and BA medium (60 ml) in the beginning. The total and headspace volumes were 580 and 430 ml, respectively. All reactors were controlled at 37°C in the air bath shaker with a shaking speed of 150 rpm. The pH was adjusted to 7 in all the reactors by 4 M NaOH and 2 M HCl. 2-Bromoethanesulfonate (BES; 100 mM) was added to ensure methanogenesis inhibition. The headspace gas composition was detected and re-purged set daily for 5 min during the experiment. All the reactors were operated in triplicate. The experimental conditions are shown in Table 1.

2.3 | High-throughput 16S rRNA gene sequencing and analysis

Samples were obtained from all the reactors on day 32 when all the bottles reached steady-state. An FastDNA Spin Kit for Soil (MP Biomedicals, USA) was used to extract microbial DNA from the samples according to the manufacturer's protocol, and the genomic DNA was qualitatively checked using 1% agarose gel electrophoresis. The extracted DNA was amplified by polymerase chain reaction (PCR) using primers 338F 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3'. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The

obtained sequences were quality checked and normalized to approximately 30,000 sequences. Phyla or genera making up less than 1 % of the total composition are indicated as "others."

2.4 | Analytical methods

The pH value was detected by a le438 pH electrode (Mettler Toledo, USA). The headspace pressure was detected by a 3151 WAL-BMP-Test system pressure gauge (WAL Mess-und Regelsysteme GmbH, Germany).

The gas composition was analyzed by gas chromatography (GC) equipped with a TCD detector. The gas composition, including CO₂, H₂, and CH₄, was measured by a gas chromatograph (Agilent 7890B, USA) equipped with a thermal conductivity detector and an analytical column of Agilent Haysep Q. The dissolved CO₂ concentration was detected by a digital titrator (HACH, USA). The concentration of short chain carboxylic acids (SCCAs, C2-C5, including acetic acid, propionic acid, butyric acid, and valeric acid) and mainly MCCAs (C6-C8, including caproic acid, heptanoic acid, and caprylic acid, C9-C12 were not detected in the present study) were detected and analyzed by a gas chromatograph (Agilent 7890A, USA) equipped with a flame ionization detector and DB-WAXETR capillary column (30 m × 0.53 mm × 1.00 μm). Detailed information can be found in our previous study [22].

2.5 | Data analysis

Analysis of variance (ANOVA) and Student's t-test were used to test the significance of the results, and $p < 0.05$ was considered statistically significant. The gas volume reported in this study was calibrated to standard temperature (273 K) and pressure (1 atm).

3 | RESULTS AND DISCUSSION

3.1 | SCCA and MCCA biosynthesis performance

The variations of carboxylic acid concentrations in all reactors are shown in Figure 1A–F. As shown in Figure 1, the types and productions of carboxylic acid in reactors fed with the same H₂/CO₂ ratio showed a similar variation trend, while the partial pressure had certain but less impact.

Regarding SCCAs production, acetic acid was of the domination. Of all the reactors, the highest production of acetic acid was obtained in 4:1-1.5 (17.5 g/L), followed by

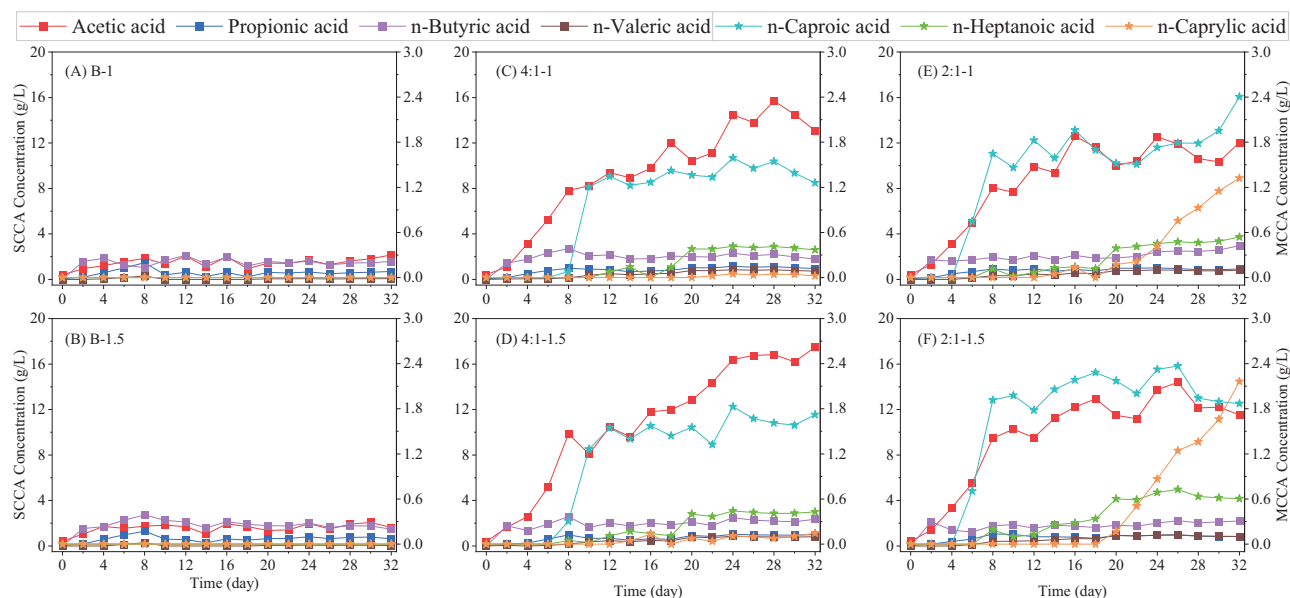


FIGURE 1 Variation of SCCA and MCCA concentrations in each reactor

15.7 g/L in 4:1-1. On the other side, under the same total pressure, the acetic acid production in 4:1-1.5 was 1.2 times more than those in 2:1-1.5, and the acetic acid production in 4:1-1 was 1.3 folds of those in 2:1-1. These data indicated the series of reactors fed with H_2/CO_2 ratio of 4:1 could offer a higher acetic acid production capacity.

Regarding MCCAs production, n-caproic acid and n-caprylic acid were the main products. In H_2/CO_2 ratio 2:1 reactors, n-caproic acid production was noticed since day 6, rapidly reaching 1.6 and 1.9 g/L in 2:1-1 and 2:1-1.5 on day 8. And the maximum concentrations both reached about 2.4 g/L. In contrast, n-caproic acid production in H_2/CO_2 ratio 4:1 reactors showed a longer lag phase (about 10 days) and reached lower concentrations. The highest yields of n-caproic acid production were only 65%–75% of those in H_2/CO_2 ratio 2:1 reactors. C8 was also detected in all experimental reactors, but its concentration could be ignoble in H_2/CO_2 ratio 4:1 reactors. N-caprylic acid was produced by n-caproic acid and received two carbons via once chain elongation cycle [23]. At day 20, n-caprylic acid was detected in the H_2/CO_2 ratio 2:1 reactors. In 2:1-1.5 reactors, the maximum concentration of n-caprylic acid reached 2.2 g/L, which was 15.7 times more than that in 4:1-1.5 reactors. 1.3 g/L of n-caprylic acid was generated in 2:1-1 reactors, 26.4 times more than that in 4:1-1 reactors. Therefore, H_2/CO_2 ratio of 2:1 could promote the chain elongation process, and higher partial pressure could further enhance the process, facilitating the production of C6-C8 carboxylic acids.

It could be seen from above that H_2/CO_2 ratio of 4:1 was more favorable for acetic acid production, while H_2/CO_2 ratio of 2:1 facilitated n-caproic and n-caprylic acids.

3.2 | CO_2 and H_2 consumption performance

The daily and cumulative consumption of the H_2 and CO_2 in each reactor is shown in Figures 2 and 3A. No methane was detected during the whole experiment.

H_2 and CO_2 consumption ratios were not in agreement with the supply ratio. As shown in Figure 2, in 4:1-1.5, the H_2 average consumption rate (266 ml/d) and cumulative consumption (8523 ml) were the highest of all reactors, with 1.1-1.3 times higher. The better hydrogen consumption performance in 4:1-1.5 may be related to the higher headspace pressure to facilitate the gas-liquid transfer. Correspondingly, in the 2:1-1.5 reactor, high hydrogen consumption was also observed at the beginning of the reaction (2–5 days), with an average consumption of 347 ml/d, 1.1 times and 1.4 times of 4:1 and 2:1, respectively. As the reaction proceeded, the total H_2 consumption of the 4:1 reactor caught up to 7634 ml, eventually exceeding 2:1-1.5, even though the total supply of H_2 was lower. Thus, the effect of the H_2/CO_2 ratio was more significant than the headspace pressure on the H_2 consumption.

As for CO_2 , the CO_2 consumption of H_2/CO_2 ratio 2:1 reactors was higher than that of the 4:1 reactors. Meanwhile, the cumulative consumption of 2:1-1.5 (2906 ml) was the highest, 112%–154% of the other reactors, and the average consumption rate reached 90 ml/d. In addition, the cumulative CO_2 consumption for 2:1-1, 4:1-1, and 4:1-1.5 was 2594, 2483, and 1881 ml, respectively. Therefore, the CO_2 consumption became correspondingly larger when the absolute amount of CO_2 provided was higher.

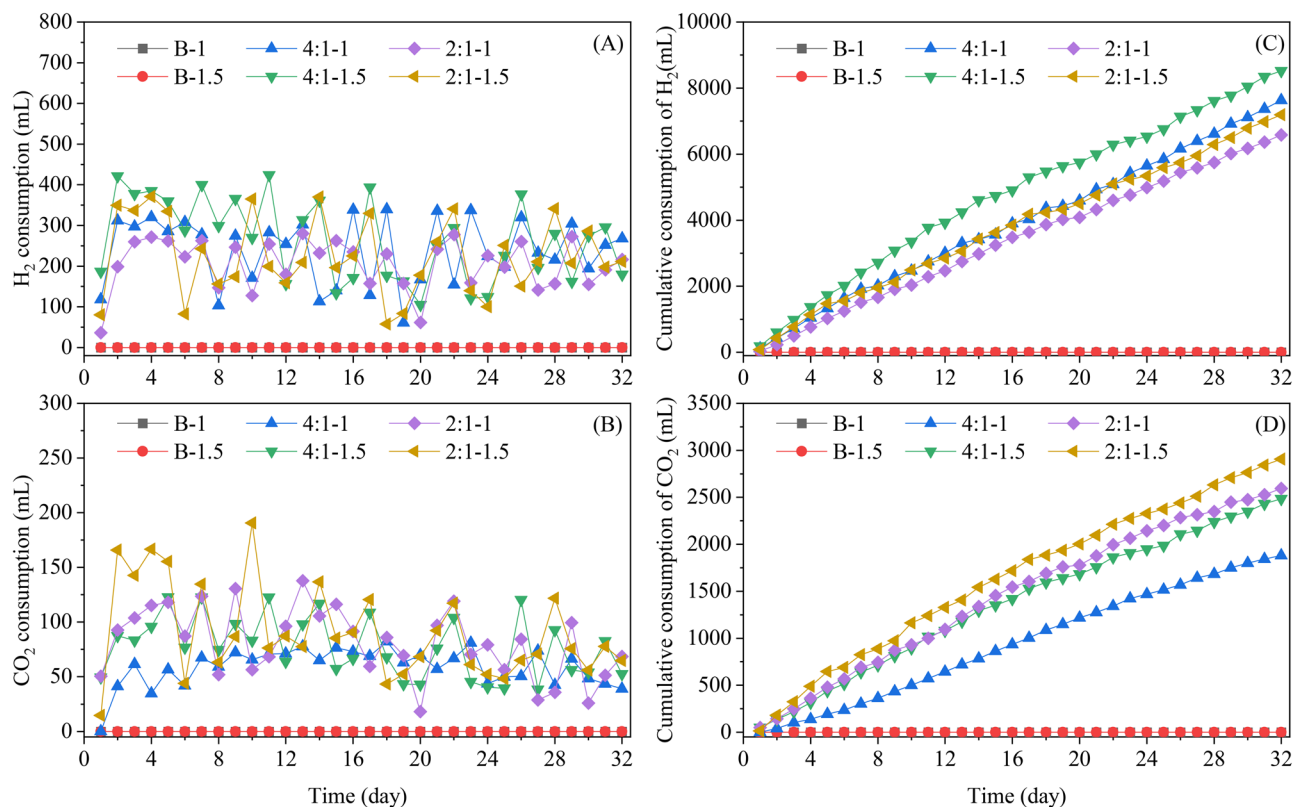


FIGURE 2 (A and B) represent the daily consumption (ml) of H_2 and CO_2 , respectively. (C and D) represent the cumulative consumption (ml) of H_2 and CO_2 , respectively

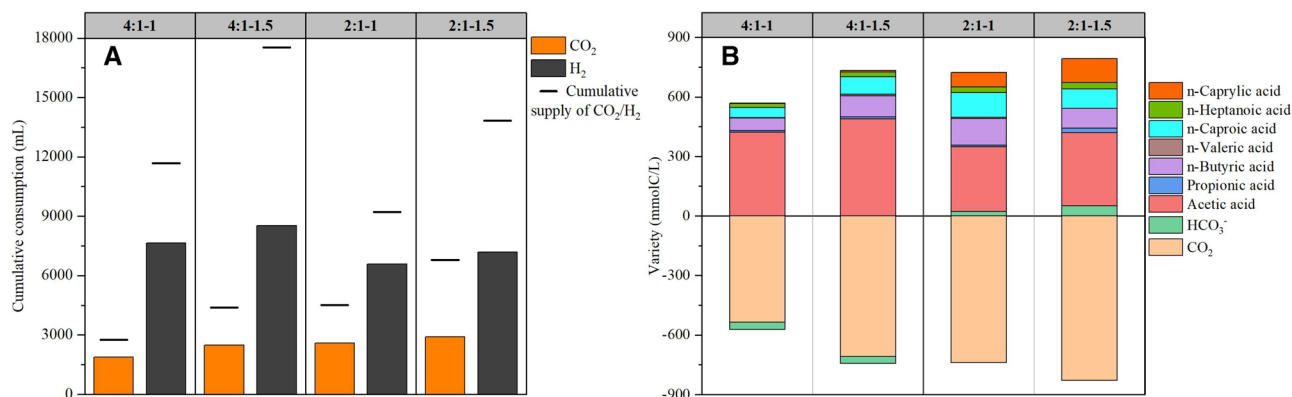


FIGURE 3 (A) shows the cumulative consumption of CO_2 and H_2 , with the horizontal lines in the graph showing the cumulative supplied amount. (B) The carbon balance in each reactor

The carbon balance of the H_2/CO_2 ratio 4:1 and 2:1 reactors is shown in Figure 3B. Meanwhile, the cumulative supply and consumption of H_2/CO_2 , carbon direction and conversion ratio of each reactor were further calculated and shown in Table 2 for supporting the analysis of CO_2 and H_2 consumption performance. The final pH of the reaction system is shown in Figure S1, and the pH of all reactors was maintained between 6.25 and 6.75 during the main experimental period. As shown in Figure 3B

and Table 2, the results of all reactors showed clear carbon direction and high carbon conversion, suggesting that carboxylic acids were the main product in all reactors. In the H_2/CO_2 ratio 4:1 reactors, where acetic acid was more predominant, the actual H_2/CO_2 ratio and CO_2 conversion ratio were higher than that in 2:1 reactors. Therefore, more H_2 was consumed per unit volume of CO_2 fixed in H_2/CO_2 ratio 4:1 reactors. Also, bicarbonate depletion in the microbial substrate was observed in the 4:1 ratio reac-

TABLE 2 Cumulative supply and consumption of H₂/CO₂, carbon direction and conversion ratio of each reactor

Reactor		4:1-1	4:1-1.5	2:1-1	2:1-1.5
Cumulative supply (ml)	H ₂	11,680	17,536	9216	13,824
	CO ₂	2752	4384	4512	6784
	ratio ^a (H ₂ /CO ₂)	4	4	2	2
Cumulative consumption (ml)	H ₂	7634	8523	6578	7194
	CO ₂	1881	2483	2594	2906
	ratio ^a (H ₂ /CO ₂)	4.1	3.4	2.5	2.4
Carbon direction ^b (%)	C2	74.4	66.9	46.4	49.9
	C3	1.7	1.5	1.1	2.8
	C4	10.8	14.5	19.2	13.4
	C5	0.4	0.8	0.9	0.1
	C6	9.1	12.1	17.7	13.1
	C7	3.4	3.1	4.1	4.3
	C8	0.2	1.1	10.6	16.4
	Conversion ratio ^c (%)	99.3	98.6	97.7	96.7

^aRatio (H₂/CO₂) = V_{H₂} (ml)/ V_{CO₂} (ml).

^bCarbon direction = C_n (mmolC/L) / Total Carboxylic acid (mmolC/L).

^cConversion ratio = Total Carboxylic acid (mmolC/L)/ Total CO₂ consumption (mmolC/L).

tor, which means that the reactors consumed so much H₂ and not enough CO₂ for the reaction. And then, the chemical reaction equilibrium and phase equilibrium have to be activated to replenish the carbon source. In the H₂/CO₂ ratio 2:1 reactors, the actual H₂/CO₂ ratio were 2.5 and 2.4 in 2:1-1 and 2:1-1.5, respectively. It seems that the increase of longer carbon chain acid in the reactor will instead cause a decrease in H₂ consumption, which was inconsistent with the reaction equation (Equations 1–4) that showed H₂ consumption increase with the carbon chain length increase mentioned before. Therefore, the H₂/CO₂ ratio significantly affected the actual metabolic process of the microorganism.

In summary, it was clear from the headspace H₂/CO₂ consumption performance that more H₂ consumption facilitates the preparation of C2 carboxylic acid and more CO₂ consumption enhances the production of C4-C8 carboxylic acids.

3.3 | Metabolic pathway regulation from CO₂ to C2-C8 carboxylic acids

In this study, the microbial metabolic pathway for CO₂ to carboxylic acid was constructed based on previous studies [15, 24] to determine the regulatory role of different H₂/CO₂ ratios in producing C2-C8 carboxylates, as shown in Figure 4.

Under anaerobic conditions, microorganisms could fix CO₂ to acetyl-CoA or acetic acid by the W-L pathway [15], in which H₂ provides six-electrons via the methyl branch

and two-electrons via the carbonyl branch. Then, a part of acetyl-CoA enters the chain elongation cycle, and the chain length of the carboxylic acid increases by two carbons with each cycle. Notably, in each chain elongation cycle, Enoyl-CoA converts to Acyl-ACP with the help of oxidized ferredoxin. The oxidized ferredoxin will be regenerated by ferredoxin-dependent hydrogenase. Meanwhile, H⁺ gets electrons and thus produces H₂ [23]. Therefore, in H₂/CO₂ ratio of 4:1 reactors with higher hydrogen partial pressures, the process of Enoyl-CoA to Acyl-ACP may be inactive, thus reducing the chain elongation efficiency, which may account for the lower production of MCCAs in 4:1 reactors. In addition, as the chain elongation produces a certain amount of H₂, it may lead to smaller H₂/CO₂ for the actual reaction as the yield of the MCCAs concentration increases, which is consistent with the lowest H₂/CO₂ (Table 2) in 2:1-1.5 reactors with the highest MCCAs concentration.

3.4 | Microbial community composition analysis

To better understand the effects of different H₂/CO₂ ratios on the microbial community compositions, all reactors samples were detected by high-throughput sequencing of the 16S rRNA genes at the steady state of the experiment. And principal co-ordinates analysis (PCoA) based on Bray-Curtis distance was used to determine the response of the microbial community structures to different H₂/CO₂ ratios (Figure S1) [22].

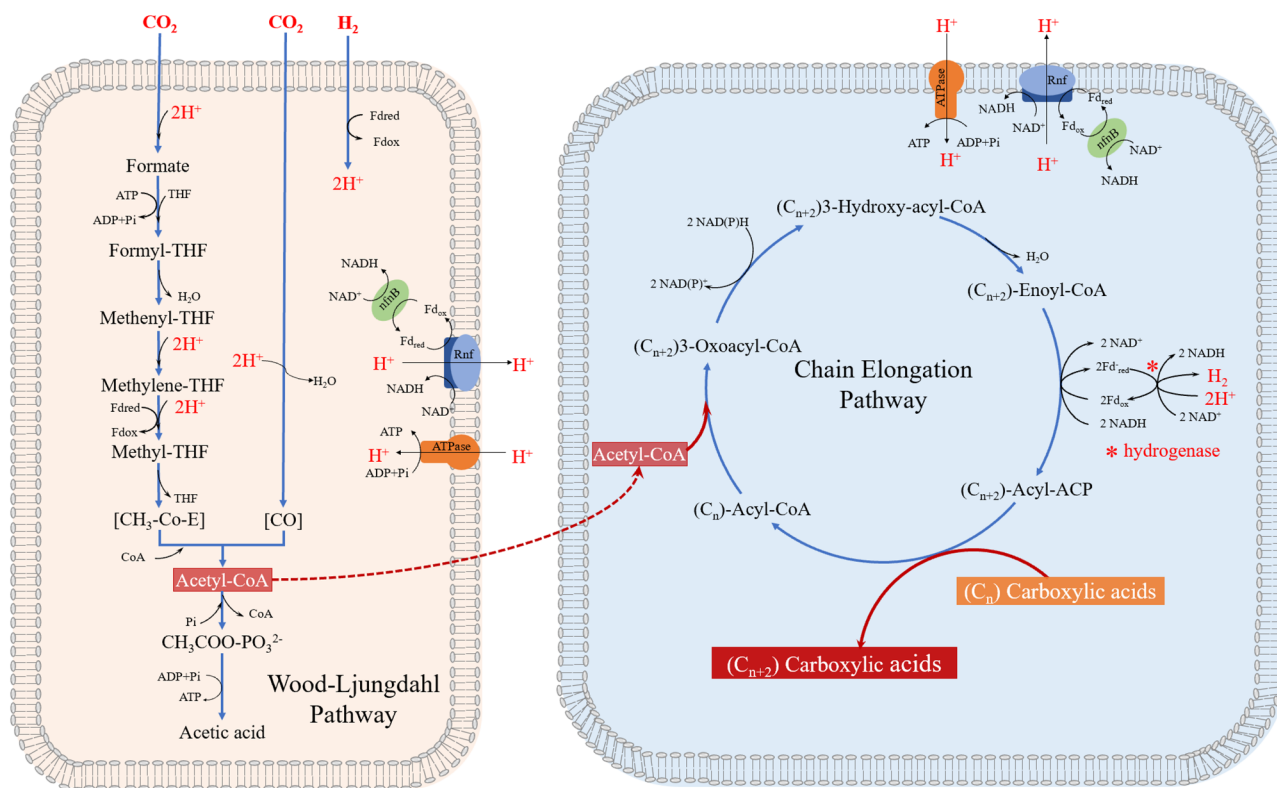


FIGURE 4 The metabolic pathways of Wood-Ljungdahl Pathway and chain elongation pathway (reverse β oxidation) are shown

As shown in Figure S1, the H_2/CO_2 ratio 4:1 reactors were clustered together while 2:1 were aggregated, suggesting the different H_2/CO_2 ratios could significantly change the microbial community structure, and the same ratio exhibited more similar properties. Additionally, B-1 and B-1.5 were far apart, indicating that the headspace pressure notably influenced microbial community structure. But this effect was insignificant in 4:1 and 2:1 reactors, meaning the impact of pressure was less than that of different ratios, which is consistent with the gas consumption results (Figure 2) in the previous discussion.

The relative abundance distributions at the phylum and genus levels are shown in Figure 5A,B, respectively. And the relative abundances at the class level are shown in Table S1. The phylum level classification showed that *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, *Proteobacteria*, and *Spirochaetota* were dominant in all reactors. In the reactors that fed with H_2/CO_2 , the relative abundance of *Firmicutes* and *Actinobacteriota* increased significantly. The relative abundance of *Firmicutes* was 77% in 2:1-1.5, the highest of all reactors, and 70% in 2:1-1. Most currently known microorganisms responsible for chain elongation belong to *Firmicutes* [16]. Therefore, the increase of *Firmicutes* might relate to regulating MCCAs production by the 2:1 ratio. The relative abundance of *Actinobacteriota* was highest in 4:1-1.5 (23%), followed by 4:1-1 (16%). It has been shown that various species in the *Actinobac-*

teriota could utilize the W-L pathway for acetic acid production [25].

At the genus level, *Clostridium sensu stricto 1* accounted for significant abundance in *Firmicutes* at H_2/CO_2 ratio 2:1 and 4:1 reactors. The relative abundance of *Clostridium sensu stricto 1* in 2:1-1.5 (23%) was the highest of all reactors, and next was 2:1-1 (19%). The *Clostridium sensu stricto 1* was often detected in anaerobic environments, related to the production of n-butyric acid by fermentation [26]. In H_2/CO_2 ratio 2:1 reactors, it was more relevant to the chain elongation process for producing n-caproic and n-caprylic acid. Meanwhile, *Paenibacillaceae* was also significantly enriched in H_2/CO_2 ratio 2:1 reactors, which belongs to the *Bacilli* class that was often found in n-caproic acid production reactors in previous studies [27]. In addition, many genera belonging to the *Clostridia* class under *Firmicutes* phylum were more enriched (as shown in Table S1) in the H_2/CO_2 ratio 2:1 reactors than all reactors, such as *Christensenellaceae R-7 group*, *Eubacterium*, *Anaerostipes* and so on. *Clostridia* class was the most frequently reported as containing lots of potential functional species (like *Clostridium kluyveri*) for chain elongation [28]. Therefore, more microorganisms associated with chain elongation were significantly enriched in H_2/CO_2 ratio 2:1 reactors. Correspondingly, in H_2/CO_2 ratio 4:1 reactors, *Terrisporobacter* and *norank f norank o Coriobacteriales* were remarkably enhanced compared to

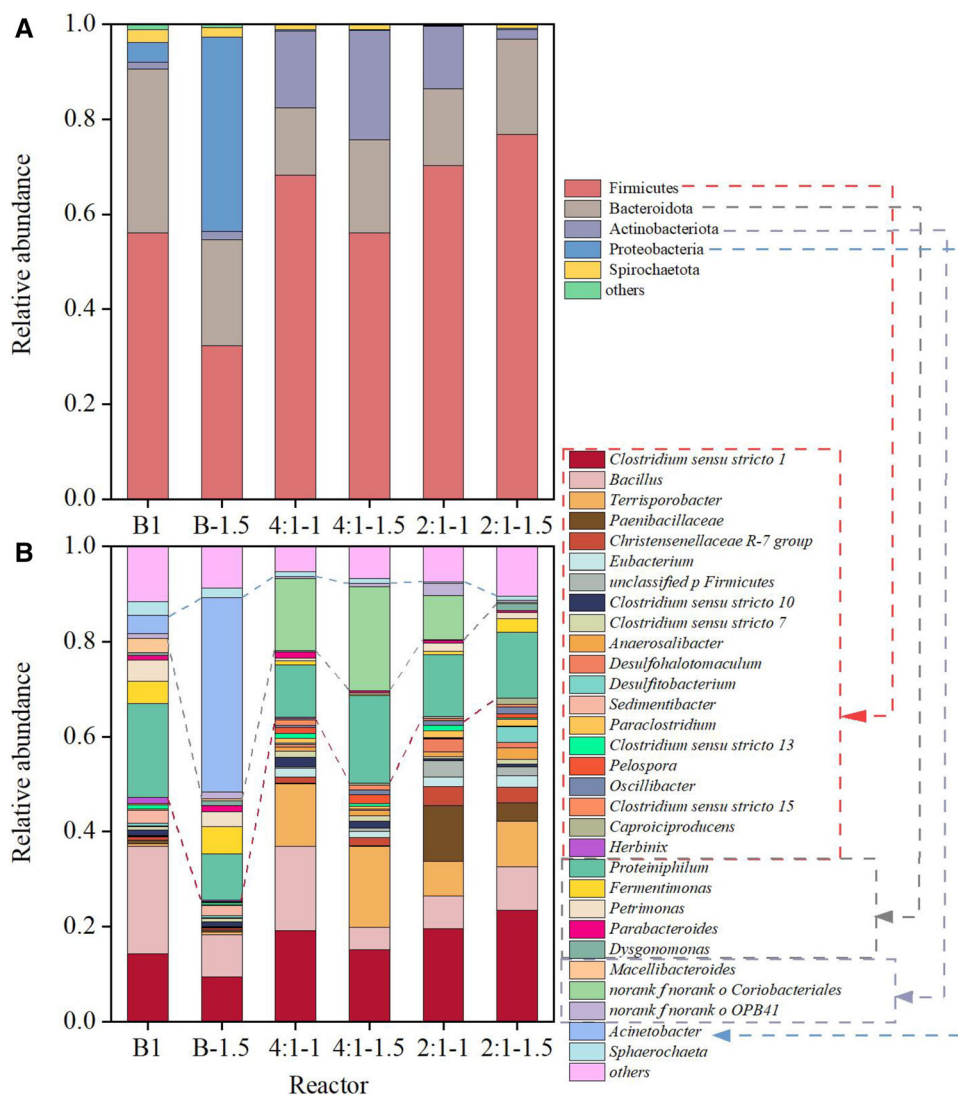


FIGURE 5 Taxonomic classification of the microbial community: comparison of the relative abundances at (A) phylum and (B) genus levels of each sample

all reactors. *Terrisporobacter* has been reported to perform excellent ability to consume H_2/CO_2 to acetic acid [29]. Meanwhile, *norank f norank o Coriobacteriales* belong to *Coriobacteriia* class that also could complete the H_2/CO_2 to acetic acid process [25]. Therefore, the H_2/CO_2 ratio 4:1 condition was more suitable for acetic acid production.

4 | CONCLUDING REMARKS

The purpose of present study was to investigate the effects of different H_2/CO_2 ratios on regulating the production of C2-C8 carboxylic acids, while the headspace pressure of 1.5 bar was set to amplify the effect of different ratios. The results demonstrated that acetic acid was the predominant product in SCCAs, while n-caproic and n-caprylic acids were the major MCCAs. The H_2/CO_2

ratio 4:1 reactors performed a better acetic acid production capacity, with the highest acetic acid concentration (17.5 g/L) in the 4:1-1.5 reactor. The H_2/CO_2 ratio 2:1 reactors were more suitable for chain elongation to produce n-caprylic acid. The maximum n-caprylic acid concentration (2.1 g/L) was obtained in the 2:1-1.5 reactor, which was 15–108 folds of the H_2/CO_2 ratio 4:1 reactors. From the consumption of H_2/CO_2 , the actual H_2/CO_2 ratio in the reactor with more acetic acid production was higher than that with more MCCAs. Thus, microbial metabolic pathways were analyzed for the consumption of H_2/CO_2 , showing that the W-L pathway requires more hydrogen than the chain elongation pathway, and each chain elongation cycle was accompanied by H_2 production. The 16S rRNA genes analysis showed the different H_2/CO_2 ratios significantly changed the microbial community composition, and the same H_2/CO_2 ratio tended to cluster together,

thus the effect of the headspace ratio was more significant than pressure. The abundance of genus *Terrisporobacter* and *Coriobacteriales* have enriched in H₂/CO₂ ratio 4:1 reactors, which may be related to acetic acid production. Meanwhile, the abundance of genus *Clostridium* and *Paenibacillaceae* have enriched in H₂/CO₂ ratio 2:1 reactors associated with the chain elongation pathway. Consequently, the present study may provide new insight into CO₂ utilization via high-value conversion to MCCAs by anaerobic microorganisms, contributing to carbon neutrality goals.

ACKNOWLEDGMENTS

This study was funded by Beijing Nova Program (Z201100006820022), National Natural Science Foundation of China (52270096, U20B2022) and Key Science and Technology Development Program of Xinjiang Corps (2021DB006).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ETHICS STATEMENT

The manuscript did not include animal and human subjects.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Wen Wang  <https://orcid.org/0000-0002-5100-8315>

REFERENCES

- Hansen J, Sato M, Ruedy R. Global temperature in 2021. *Acesso Em*. 2022;1. <http://www.columbia.edu/~jeh1/mailings/2022/Temperature2021.13January2022.pdf>. Diponivel Em. 13January2022. Pdf.
- Jin L, Chang Y, Wang M, et al. The dynamics of CO₂ emissions, energy consumption, and economic development: evidence from the top 28 greenhouse gas emitters. *Environ Sci Pollut Res*. 2022;29:36565-36574.
- Liu Z, Deng Z, Davis SJ, et al. Monitoring global carbon emissions in 2021. *Nat Rev Earth Environ*. 2022;3:217-219.
- Wang F, Harindintwali JD, Yuan Z, et al. Technologies and perspectives for achieving carbon neutrality. *Innovation*. 2021;2:100180.
- Roy S, Cherevotan A, Peter SC. Thermochemical CO₂ hydrogenation to single carbon products: scientific and technological challenges. *ACS Energy Lett*. 2018;3:1938-1966.
- Yaashikaa PR, Senthil Kumar P, Varjani SJ, Saravanan A. A review on photochemical, biochemical and electrochemical transformation of CO₂ into value-added products. *J CO₂ Util*. 2019;33:131-147.
- Kumar B, Brian JP, Atla V, et al. New trends in the development of heterogeneous catalysts for electrochemical CO₂ reduction. *Catal Today*. 2016;270:19-30.
- Valluri S, Claremboux V, Kawatra S. Opportunities and challenges in CO₂ utilization. *J Environ Sci*. 2022;113:322-344.
- Aleku GA, Roberts GW, Titchiner GR, Leys D. Synthetic enzyme-catalyzed CO₂ fixation reactions. *ChemSusChem*. 2021;14:1781-1804.
- Beller M, Bornscheuer UT. CO₂ fixation through hydrogenation by chemical or enzymatic methods. *Angew Chem Int Ed*. 2014;53:4527-4528.
- Erşan S, Park JO. Light-Independent biological conversion of CO₂. *Joule*. 2020;4:2047-2051.
- Fast AG, Papoutsakis ET. Stoichiometric and energetic analyses of non-photosynthetic CO₂-fixation pathways to support synthetic biology strategies for production of fuels and chemicals. *Curr Opin Chem Eng*. 2012;1:380-395.
- Fuchs G. Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? *Annu Rev Microbiol*. 2011;65:631-658.
- Berg IA, Kockelkorn D, Ramos-Vera WH, et al. Autotrophic carbon fixation in archaea. *Nat Rev Microbiol*. 2010;8:447-460.
- Spirito CM, Richter H, Rabaey K, et al. Chain elongation in anaerobic reactor microbiomes to recover resources from waste. *Curr Opin Biotechnol*. 2014;27:115-122.
- Angenent LT, Richter H, Buckel W, et al. Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemicals. *Environ Sci Technol*. 2016;50:2796-2810.
- Grootscholten TIM, Steinbusch KJJ, Hamelers HVM, Buisman CJN. Improving medium chain fatty acid productivity using chain elongation by reducing the hydraulic retention time in an upflow anaerobic filter. *Bioresour Technol*. 2013;136:735-738.
- Pan X, Zhao L, Li C, et al. Deep insights into the network of acetate metabolism in anaerobic digestion: focusing on syntrophic acetate oxidation and homoacetogenesis. *Water Res*. 2021;190:116774.
- Wenhao H, Pinjing He, Liming S, Fan Lü. Metabolic interactions of a chain elongation microbiome. *Appl Environ Microbiol*. 2018;84:e01614-18.
- Wang J, Yin Y. Biological production of medium-chain carboxylates through chain elongation: an overview. *Biotechnol Adv*. 2022;55:107882.
- da Silva EF, Svendsen HF. Computational chemistry study of reactions, equilibrium and kinetics of chemical CO₂ absorption. *Int J Greenhouse Gas Control*. 2007;1:151-157.
- Liu C, Luo G, Liu H, et al. CO as electron donor for efficient medium chain carboxylate production by chain elongation: microbial and thermodynamic insights. *Chem Eng J*. 2020;390:124577.
- Ding H-B, Tan G-YA, Wang J-Y. Caproate formation in mixed-culture fermentative hydrogen production. *Bioresour Technol*. 2010;101:9550-9559.
- Liu C, Ji J, Wu W, et al. Heterogeneous catalyst-microbiome hybrids for efficient CO-driven C₆ carboxylic acid synthesis via metabolic pathway manipulation. *ACS Catalysis*. 2022;12:5834-5845.
- Jiao J-Y, Fu L, Hua Z-S, et al. Insight into the function and evolution of the Wood-Ljungdahl pathway in actinobacteria. *ISME J*. 2021;15:3005-3018.

26. Yu J, Huang Z, Wu P, et al. Performance and microbial characterization of two-stage caproate fermentation from fruit and vegetable waste via anaerobic microbial consortia. *Bioresour Technol.* 2019;284:398-405.
27. Zagrodnik R, Duber A, Łężyk M, Oleskowicz-Popiel P. Enrichment versus bioaugmentation—Microbiological production of caproate from mixed carbon sources by mixed bacterial culture and *Clostridium kluyveri*. *Environ Sci Technol.* 2020;54:5864-5873.
28. Fu X, Ye R, Jin X, Lu W. Effect of nano zero-valent iron addition on caproate fermentation in carboxylate chain elongation system. *Sci Total Environ.* 2020;743:140664.
29. Zhu X, Chen L, Chen Y, et al. Effect of H₂ addition on the microbial community structure of a mesophilic anaerobic digestion system. *Energy.* 2020;198:117368.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Wu W, Li Z, Liu G, Zhou L, Wang W. Regulation on C2-C8 carboxylic acid biosynthesis from anaerobic CO₂ fermentation. *Eng Life Sci.* 2024;24:e2200069.

<https://doi.org/10.1002/elsc.202200069>