Introduction

CO_2 capture and storage (CCS) is a strategic foundational technology essential for achieving carbon neutrality. During the development of oilfields, approximately 30-40% of residual oil remains in the reservoir following CO_2-enhanced oil recovery. During the stage of CO_2 storage, the processes of anaerobic hydrocarbon biodegradation and CO_2 utilization can facilitate the conversion of carbon into methane, which serves as a clean energy source for further development within the reservoir. On one hand, methane dissolves in crude oil to enhance fluidity, thereby increasing the crude oil recovery rate. On the other hand, the density difference between methane gas and CO_2 can induce gravitational differentiation, resulting in an accumulation of methane in higher structural regions that may be exploited at an appropriate future time (Song et al., 2023). For instance, CO_2 injection was employed for enhanced oil recovery in the Olla oilfield over a span of 29 years, it was observed that 13-19% of injected CO_2 had been converted into methane (Tyne et al., 2021). Hydrogenotrophic methanogens and acetoclastic methanogens are prevalent throughout subsurface environments. To gain deeper insights into microbial methanogenesis during CO_2 storage in a low-temperature oil reservoir maintained at 30°C, we conducted continuous anaerobic incubations with microbial communities derived from produced water under varying conditions. Our findings reveal both the stimulatory effect of CO_2 on methane production and elucidate patterns regarding hydrocarbon evolution. This study is crucial for advancing underground CCS applications.

Methods

- Incubation and analysis of DNA

Wellhead produced fluid samples of Well I were extracted from the low-temperature block of the Yumen Oilfield in Gansu Province, China, where the reservoir temperature is 30°C. The microbial community derived from oil production water was continuously incubated anaerobically under various conditions: 1) CO_2 + H_2, 2) CO_2, and 3) no addition. Please refer to Table 1 for further details.

Table 1 Experimental groups

No.	Samples	Gas volume
1	oil + water production + CO_2 + H_2	30 mL (CO_2:H_2=1:4)
2	oil + water production + CO_2	15 mL
3	oil + water production	-

Our incubation setup was designed to replicate conventional anaerobic experiments utilizing Hungate technology, with a total volume of 120 mL. Each bottle contained 14 g of 40-70 mesh quartz sand, 50 mL of oil production water (incubation broth), and 2 g of crude oil, resulting in a headspace volume of 60 mL. After replacing the atmosphere with nitrogen, the bottles were promptly sealed with rubber plugs (SANSHIN, Japan) and subsequently secured with aluminum seal caps (Chemglass, USA). The bottles were then statically incubated for a duration of 800 days at a temperature maintained at 30 °C. Following this incubation period, total microbial DNA was extracted from the incubation water. Bacterial DNA was amplified targeting the 16S rRNA gene, purified, and sequenced using the HiSeq platform. The genomic DNA extraction from bacteria was performed according to the protocol provided by the FastDNA® Spin Kit for soil (MP Biomedicals). High-throughput sequencing of the 16S rRNA gene was conducted by Beijing Novogene Biotech Co., Ltd.

- Gas Chromatography Analysis of Saturates

The procedure for separating saturated hydrocarbons was carried out in accordance with the oil and gas industry standard of the People's Republic of China, SY/T 5779-2008 (Analysis of Saturated Hydrocarbons in Rock Chloroform Extracts and Crude Oil). This analytical method employs gas chromatography to assess hydrocarbons present in petroleum and sediment. The testing was conducted by the Central Laboratory of Geological Sciences at the Research Institute of Petroleum Exploration & Development, PetroChina.

Results

- Methane production

After 800 days of normal pressure culture at 30°C, the methane production in the CO_2+H_2 group, CO_2 group, and non-addition group accounted for 13.3%, 5%, and 2.3% of the gas-phase components in the culture system, respectively (Figure 1). The addition of CO_2 significantly enhanced methane production, yielding approximately twice the amount produced from anaerobic degradation of crude oil. In oil reservoirs, there is a deficiency of H_2. During crude oil degradation, hydrogen synthesized by hydrogen-producing bacteria is rapidly consumed by methanogenic bacteria, leading to methane production via the hydrogenotrophic methanogen pathway. By externally supplying H_2, the utilization of CO_2 can be accelerated. Consequently, methane production in the CO_2+H_2 group exceeds that observed in the CO_2 group by more than twofold.

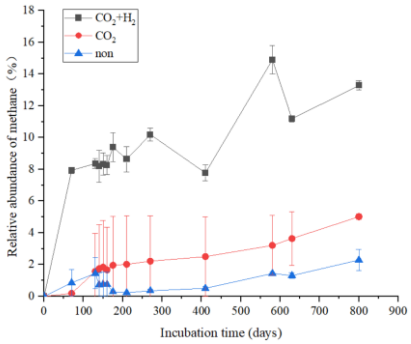
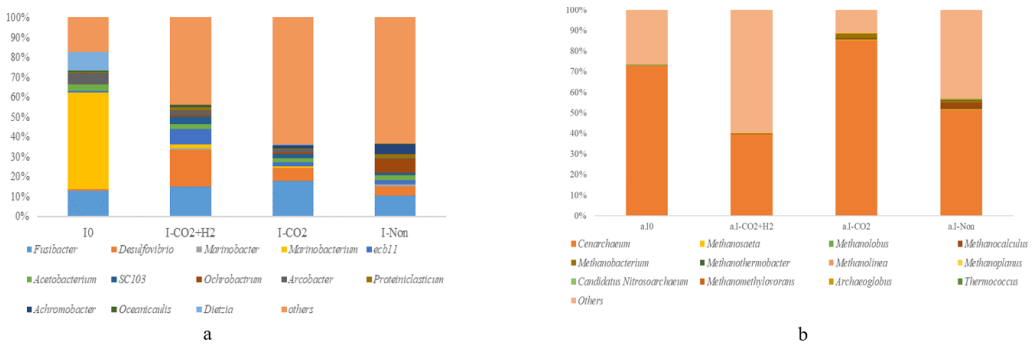


Figure 1 *Accumulative methane production in the enrichment cultures.*

- Microbial community of bacteria and archaea

Compared to the initial time point, the relative abundances of the bacterial genera *Marinobacterium*, *Dietzia*, and *Arcobacter* all exhibited a decline after 800 days of cultivation (Figure 2a). Specifically, the abundance of *Marinobacterium* was recorded at 48.51% at the initial time point; however, it decreased to a range between 0.2% and 2.23% across different samples following 800 days of cultivation. The abundance of *Dietzia* started at 9.10% initially but diminished to a range from 0.17% to 0.26% after this period. Similarly, *Arcobacter*'s abundance was noted at 5.59% initially and subsequently decreased to between 0.63% and 2.36%. In addition to these three genera, other dominant microbiota present at the initial time point included *Fusibacter* (12.88%) and *Acetobacterium* (3.34%). After an extensive cultivation period of 800 days, in the CO_2+H_2 group, which demonstrated high methane production, the predominant bacterial genera were *Desulfovibrio* (18.67%) and *Fusibacter* (14.76%). In contrast, within the CO_2 group exhibiting moderate methane production, *Fusibacter* (18.00%) and *Desulfovibrio* (6.32%) emerged as dominant genera. Furthermore, in the non-addition group, *Fusibacter* (10.62%) along with *Ochrobactrum* (6.92%) were identified as the primary bacterial genera present.



a b

Figure 2 *Microbial community composition of (a)bacteria and (b) archeara from sequencing*

At the genus level, the archaeal communities cultured at initial time point and after 800 days of anaerobic culture both had *Cenarchaeum* as the dominant genus (Figure 2b). In the CO_2+H_2 group, The *Cenarchaeum* genus had an abundance of 39.62%. In the CO_2 group, the genus *Cenarchaeum* accounted for 85.23%, and the content of the genus *Methanobacterium* was higher than that in other groups, with an abundance of 2.31%. In the non-addition group, the genus *Cenarchaeum* accounted for 51.47%, and the abundance of the genus *Methanocalculus* was the highest among the three experimental groups, at 3.18%. *Methanocalculus natronophilus* sp. nov. It is a newly discovered hydrogenotrophic methanogenic archaea (Zhilina et al., 2013).

- Saturates analysis

The percentage contents of hydrocarbons under different carbon numbers, as showed in Figure 3a. For short-chain alkanes (C_8-C_{14}), the initial content was lower than that observed in each experimental group after cultivation. After 800 days of incubation, the percentage contents of short-chain saturated hydrocarbons increased by 10.48%, 9.26%, and 9.95% in the CO_2+H_2 group, the CO_2 group, and the non-addition group, respectively, compared to the initial time point. In contrast, for medium-chain alkanes (C_{15}-C_{24}), the initial content exceeded that of each experimental group after cultivation. After 800 days of incubation, the percentage contents of medium-chain saturated hydrocarbons decreased by 6.24%, 4.91%, and 4.07% in the CO_2+H_2 group, the CO_2 group, and the non-addition group, respectively, compared to the initial time point. Regarding long-chain alkanes (C_{25}-C_{31}), minimal changes were observed across all three experimental groups after 800 days of incubation when compared to the initial time point, as illustrated in Figure 3b. From this perspective, anaerobic degradation of petroleum by reservoir microorganisms present in produced fluid from Well I resulted in an increase in the relative content of short-chain saturated hydrocarbons while simultaneously decreasing that of medium-chain saturated hydrocarbons; this indicates that the degradation process primarily targeted medium-chain alkanes.

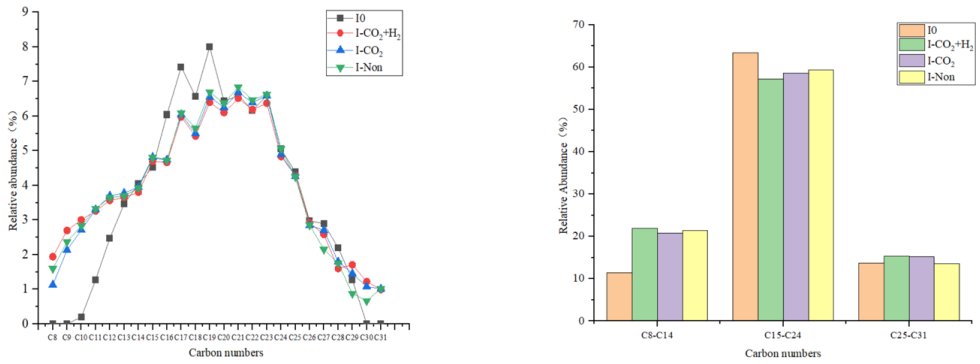


Figure 3 *a) Saturated hydrocarbon chromatograms of different experimental groups after incubation, b) The distribution of saturates in short, medium and long chains in different experimental groups.*

Discussion

In this study, the methane produced by the CO_2 group constituted for 5% of the gas-phase components in the culture system, which was 2.2 times the abundance ratio of methane in the gas-phase components of the non-addition group. This indicated that the reservoir microorganisms utilized in this experiment primarily employed a hydrophilic methanogenic pathway that utilizes CO_2. Among bacteria, *Fusibacter* and *Desulfovibrio* were identified as dominant genera within the CO_2 group. Notably, *Fusibacter paucivorans* gen. nov. has been previously reported, it was isolated from high-salinity oilfield production wells in Africa (Ravot et al., 1999). Additionally, among strains belonging to the genus *Desulfovibrio*, five species have demonstrated catalytic capabilities for H_2 synthesis. Regarding archaea, *Methanobacterium* showed a higher relative abundance in the CO_2 group compared to other groups. It has been documented that *Methanobacterium* sp. Mb1 represents hydrophobic methanogenic archaea (Maus et al., 2013). Further investigations revealed that during co-culture processes involving *Desulfovibrio* and *Methanobacterium* strains, *Methanobacterium* YSL can directly acquire electrons

from *Desulfovibrio* JY, facilitating the reduction of CO_2 to methane (Zheng et al., 2021). The abundances of both *Desulfovibrio* bacteria and *Methanobacterium* archaea within this study's CO_2 group were notably high and consistent to the previous research.

In this study, anaerobic methanogenesis resulted in a greater degradation of medium-chain alkanes. In the previous study, after 185 days of cultivation, it was observed that under aerobic conditions, the biodegradation of hydrocarbons occurred at a rapid pace, with small-molecule straight-chain alkanes being preferentially degraded. When nitrate ions were employed as electron acceptors under anaerobic condition, medium-chain alkanes (C_{14}-C_{34}) are utilized preferentially, followed by long-chain alkanes (C_{35}-C_{39}) (Hasinger M., 2012). Consistent with the findings of this study, it suggests that the anaerobic degradation processes carried out by certain reservoir microorganisms exhibit selectivity for the preferential degradation of medium-chain alkanes.

Conclusions

This study presents a preliminary assessment of the capacity of reservoir microorganisms in a low-temperature oil reservoir to convert CO_2 into methane after CO_2 injected. This study revealed the stimulatory effect of CO_2 on methane production. The findings demonstrate that shallow reservoirs possess hydrogenotrophic methanogenic potential via the CO_2 utilization pathway, characterized by the presence of typical hydrogenotrophic methanogenic bacteria and archaea within the microbial community. During anaerobic degradation processes involving alkanes, medium-chain alkanes exhibit preferential selectivity. This research holds significant theoretical implications for future on-site applications of CO_2 storage in oil fields. Our investigation sheds light on microbial methanogenesis associated with CO_2 storage in low-temperature oil reservoirs, which is crucial for advancing underground CCS.

Acknowledgements

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References

Song XM, Wang F, Ma DS, Gao M, Zhang YH. 2023. Progress and Prospects of Carbon Dioxide Capture, Oil Recovery and Storage Technologies in CNPC. Petroleum exploration and development, 50(1):206-218.

Tyne RL, Barry PH, Lawson M, Byrne DJ, Warr O, Xie H, Hillegonds DJ, Formolo M, Summers ZM, Skinner B, Eiler JM, Ballentine CJ. 2021. Rapid microbial methanogenesis during CO_2 storage in hydrocarbon reservoirs. Nature, 600:670-674.

Zhilina TN, Zavarzina DG, Kevbrin VV, Kolganova TV. 2013. *Methanocalculus natronophilus* sp. nov., a new alkaliphilic hydrogenotrophic methanogenic archaeon from a soda lake, and proposal of the new family Methanocalculaceae. Microbiology, 82 (6): 698-706.

Ravot G, Magot M, Fardeau ML, Patel BC, Thomas P, Garcia JL, Ollivier B. 1999. Fusibacter paucivorans gen. nov., sp., nov., an anaerobic, thiosulfate-reducing bacterium from an oil-producing well. International Journal of Systematic Bacteriology, 49, 1141-1147.

Maus I, Wibberg D, Stantscheff R, Cibis K, Eikmeyer FG, König H, Pühler A, Schlüter A. 2013. Complete genome sequence of the hydrogenotrophic archaeon *Methanobacterium* sp. Mb1 isolated from a production-scale biogas plant. Journal of Biotechnology, 168:734-736.

Zheng S, Li M, Liu Y, Liu FH. 2021. *Desulfovibrio* feeding *Methanobacterium* with electrons in conductive methanogenic aggregates from coustal zones. Water Research, 1174490.

Hasinger M, Scherr KE, Lundaa T, Bräuer L, Zach C, Loibner AP. 2012. Changes in *iso*- and *n*-alkane distribution during biodegradation of crude oil under nitrate and sulphate reducing conditions. Journal of Biotechnology, 157, 490-498.