The effects of elevated CO₂ concentration on competitive interaction between aceticlastic and syntrophic methanogenesis in a model microbial consortium

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INTRODUCTION

In nature and engineered environments, many species of microorganisms coexist by interacting with each other. Comprehension of interspecies interactions is essential for describing the features of complex microbial ecosystems, and competition among microorganisms occupying similar trophic niches is a conventional and significant aspect of such interspecies interaction. Coexistence of multiple microorganisms with similar trophic niches is regarded as one of the major factors to confer functional stability and resiliency on microbial ecosystems (Loreau et al., 2001; Deng, 2012). It is important to grasp how the population of each microorganism changes depending on a specific environmental disturbance. Most microbial ecological research has assessed the effects of specific environmental factors on competitive interactions among multiple microbial species by observing the transition of abundances of each microorganism responding to environmental disturbances. Although this approach has produced many excellent outcomes, existence of non-target microorganisms and uncontrollable environmental factors in the systems often hamper precise understanding of the effects of specific environmental factors on the competitive interactions among target microorganisms.

Construction of microbial model consortia, in which interspecies interactions in ecosystems are reproduced by defined co-culture of isolated microorganisms, is appreciated as a worthwhile method to investigate microbial interactions (Haruta et al., 2009; De Roy et al., 2014; Großkopf and Soyer, 2014). For instance, the complex phenomenon of bacterial competition as being similar to rock-paper-scissors among colisin-producing, colisin-resistant, and colisin-sensitive strains was untangled by constructing model co-culture systems (Kerr et al., 2002; Nahum et al., 2011). Kato et al. (2005, 2008) constructed model microbial consortia composed of 4–5 bacterial strains, in which all members stably coexisted for long period of time, and demonstrated...
that existence of both positive and negative interspecies inter-
actions among the members make these consortia stable. The
construction of model consortia is a specific and beneficial feature
of microbiological research fields, which will also be effective for
proof-of-concept studies for theories in the field of macro-ecology
(Haruta et al., 2009, 2013).

Methanogenesis from organic compounds is a complex micro-
bial process accomplished by catabolic interactions among differ-
ent trophic levels of microorganisms (Schink, 1997; Jones et al.,
2008; Kato and Watanabe, 2010). Among the sequential biodegra-
dation processes, acetate is the most important intermediary
metabolite (Schink, 1997). Methanogenic acetate degradation pro-
cceeds by either aceticlastic methanogenesis or syntrophic acetate
oxidation. The aceticlastic pathway is solely mediated by aceticlas-
tic methanogens (Jetten et al., 1992). On the contrary, syntrophic
acetate oxidation pathway requires cooperative interactions of
two different types of microorganisms: acetate is first oxidized
to H₂ and CO₂ by syntrophic acetate-oxidizing bacteria (SAOB),
and then hydrogenotrophic methanogens convert the products
to CH₄ (Zinder and Koch, 1984). As the acetate oxidation reac-
tion is endoergonic under the standard conditions and is feasible
only under extremely low H₂ partial pressure, acetate oxidation by
SAOB requires H₂ elimination by hydrogenotrophic methanogens
(Karakashev et al., 2006; Hattori, 2008). These two different
acetate-degrading methane-producing pathways and organisms
involved can co-exist, but diverse environmental factors, such as
temperature, pH, salinity, toxic compounds, and concentra-
tions of substrates determine one pathway and organisms
to dominate over the other (Nüsslein et al., 2001; Shigematsu
et al., 2004; Karakashev et al., 2006; Hao et al., 2013; Kato et al.,
2014).

In our previous studies, we demonstrated that the syntrophic
pathway is the dominant methanogenic acetate degradation path-
way in underground, thermophilic petroleum reservoirs (Mayumi
et al., 2011). We further demonstrated that aceticlastic pathway
becomes dominant under high CO₂ concentrations, which mimicked carbon capture and storage field conditions (Mayumi
et al., 2013), whereas syntrophic acetate oxidation dominated
over aceticlastic reactions under low CO₂ concentrations. Since
CO₂ is either substrate or product of aceticlastic methanogen-
esis, acetate oxidation, and hydrogenotrophic methanogenesis,
high CO₂ concentration alters the thermodynamics of each
methanogenic reaction, which may cause the observed tran-
sition between syntrophic and aceticlastic methanogenic path-
ways. However, all the data were based on the analyses of
complex microbial communities in field samples thus many
other factors that affect the community shift could not be
ruled out.

In the present study, the effect of CO₂ concentrations on
methanogenic microorganisms were assessed by using a defined
inorganic medium and a defined methanogenic consortium which
is comprised of three organisms, i.e., SAOB, hydrogenotrophic
methanogen and aceticlastic methanogen, namely, which con-
tains two different acetate-degrading methanogenic pathways. The
experiments allowed to precisely show the CO₂ concentrations
to be a crucial factor affecting the dominance of respective pathways
and organisms.

**MATERIALS AND METHODS**

**MICROORGANISMS AND CULTURE CONDITIONS**

*Methanosaeta thermophila* DSM6194T (Kamagata and Mikami,
1991) and *Thermacetogenium phaeum* DSM12270T (Hattori et al.,
2000) were obtained from the Deutsche Sammlung von Mikroor-
ganismen und Zellkulturen GmbH (Braunschweig, Germany).
*Methanothermobacter thermautotrophicus* strain TM was isolated
from a thermophilic anaerobic methanogenic reactor in Japan
(Hattori et al., 2000). Routine cultivations were conducted at
55°C with 68-ml capacity serum vials containing 20 ml of a bicarbonate-buffered inorganic medium (pH 7.0; Kato et al.,
2014) under an atmosphere of N₂-CO₂ [80/20 (v/v)] without
shaking. Pyruvate (40 mM) or 200 kPa H₂-CO₂ [80/20 (v/v)]
was supplemented as energy and carbon sources for the pure
cultures of *T. phaeum* and *Methanothermobacter thermautotrophicus*, respectively. Sodium acetate (40 mM) was utilized as an
energy and carbon source for the pure culture of *Methanoseta
thermophila*, the defined co-culture of *T. phaeum* and *Methan-
othermobacter thermautotrophicus*, and the tri-culture of the
three strains. The tri-culture was constructed by simultaneous-
ously inoculating 1 and 2 ml of the early-stationary phases of
pure culture of *Methanoseta thermophila* and the defined co-
culture of *T. phaeum* and *Methanothermobacter thermautotrophicus*, respectively, into the 20 ml of the medium. Although the
long term stability of the tri-culture was not been tested, coex-
istence of the three microorganisms in the batch culture was
confirmed.

**CULTURES WITH DIFFERENT CO₂ CONCENTRATIONS**

Three culture conditions were prepared to examine the effects
of CO₂ concentrations on the microorganisms. For each condition,
the media were supplemented with different concentrations of
sodium bicarbonate and the gas phases were replaced with N₂/CO₂
mixed gas with different volume ratios, as described in Table 1.
The medium was bubbled with the respective deoxygenated gas
with 100 ml min⁻¹ for 5 min and immediately capped with a
butyl rubber stopper and an aluminum cap. The medium pH was
adjusted to 7.0 by adding 1N NaOH solution before the cultivation,
and the fluctuation of pH value throughout the cultivation was
less than 0.2. For pH measurement, 100 µl of the medium was
sampled with syringes and the pH value was determined using a
compact pH meter B-212 (Horiba). The concentration of CO₂ in
the aqueous phase [cₕ (M)] was calculated according to Henry’s
law (cₕ = kₕ), where k is the Henry’s low constant (0.019 for
CO₂ at 55°C) and p is the partial pressure of CO₂ in the gas phase
(atm). Then the bicarbonate concentrations were calculated based
on the equilibrium formula (H₂CO₃ = H⁺ + HCO₃⁻) with the

**Table 1 | Media with different initial [ΣCO₂] used in this study.**

| [ΣCO₂]initial (mmol l⁻¹) | NaHCO₃ added (mM) | Partial pressure of the gas phase CO₂ (atm) | Calculated [HCO₃⁻]initial (mM) |
|------------------------|------------------|------------------------------------------|-------------------------------|
| 5.0                    | 5                | 0                                        | 0.8                           |
| 50.7                   | 35               | 0.2                                      | 8.1                           |
| 113.4                  | 35               | 1                                        | 18.1                          |
equilibrium constant of $4.47 \times 10^7$. The culture experiments were conducted in triplicate and the student's t-test was used for the statistical analyses.

Growth of *Methanosaeta thermophilia* and *Methanothermobacter thermautotrophicus* in pure and mixed cultures was determined by measuring methane production. Growth of *T. phaeum* pure culture was determined by measuring acetate production from pyruvate. The partial pressure of CH$_4$ was determined using a gas chromatograph GC-2014 (Shimadzu) as described previously (Kato et al., 2014). The partial pressure of H$_2$ was determined using a trace reduction gas analyzer TRA-1000 (ACE Inc.) according to the manufacturer’s instruction. The concentrations of organic acids were determined using a high performance liquid chromatography (D-2000 LaChrom Elite HPLC system, HITACHI) equipped with Aminex HPX-87H Ion Exclusion column (BIO-RAD) and L2400 UV detector (HITACHI).

**FLUORESCENT IN SITU HYBRIDIZATION (FISH)**

Microbial cells of the tri-cultures in the early stationary phases were collected by centrifugation, fixed with 4% paraformaldehyde in phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$, pH 7.2) and left for 6 h at 4°C. The samples were washed three times with PBS, immobilized on glass slides, and dehydrated by successive passages through 50, 70, 80, 90, and 100% ethanol (3 min each). The following oligonucleotide probes complementary to specific regions of 16S rRNA were utilized for hybridizations: (i) Alexa488-labeled EUB338, specific for the domain *Bacteria* (Amann et al., 1990) and (ii) TexRed-labeled ARCH917, specific for the domain *Archaea* (Loy et al., 2002), (iii) Alexa594-labeled MSMX860, specific for the order *Methanosarcinales* (Raskin et al., 1994), and (iv) Alexa488-labeled MB311, specific for the order *Methanobacteriales* (Crocetti et al., 2006). Hybridizations were performed at 46°C for 3 h with hybridization buffer (0.9 M NaCl, 0.1 M Tris-HCl, pH 7.5) containing 5 ng µl$^{-1}$ of each labeled probe. The specificity of each probe was confirmed by FISH observations using pure cultures of the three microorganisms used in this study even with the hybridization buffer not containing formamide. The washing step was done at 48°C for 30 min with washing buffer (0.2 M NaCl, 0.1 M Tris-HCl, pH 7.5). The samples hybridized with the probes were observed with a fluorescent microscope Provis AX70 (Olympus).

**QUANTITATIVE RT-PCR (qRT-PCR)**

Microbial cells were harvested from the mid-logarithmic phases by centrifugation at 10,000 X g and 4°C. Total RNA was isolated using ISOGEN II reagent (Nippon Gene, Japan) combined with a bead-beating method, as described previously (Kato et al., 2014). Total RNA was purified using an RNeasy Mini kit (Qiagen) with DNase treatment (RNase-free DNase set, Qiagen) as described in the manufacturer’s instructions. The purified RNA was spectroscopically quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The PCR primers used for quantitative RT-PCR (qRT-PCR) were designed with Primer3 software (http://bioinfo.ut.ee/Primer3/) and are listed in Table 2. Quantification of 16S rRNA copy numbers in the defined mixed culture were performed by one-step real-time RT-PCR using a Mx3000P QPCR System (Stratagene) and RNA-direct SYBR Green Realtime PCR Master Mix (Toyobo) as described previously (Kato et al., 2014). At least three biological replicates were subjected to qRT-PCR analysis, and at least two separate trials were conducted for each sample. Standard curves were generated with serially diluted PCR products ($10^2$–$10^8$ copies ml$^{-1}$) amplified using the respective primer sets and were used to calculate the copy number of rRNA in the total RNA samples.

**RESULTS AND DISCUSSION**

**EFFECTS OF CO$_2$ CONCENTRATIONS ON THE MODEL METHANOCENIC CONSORTIUM**

As the model consortium performing methanogenic acetate degradation, we utilized a defined mixed culture of an aceticlastic methanogen (*Methanosaeta thermophilia*), a hydrogenotrophic methanogen (*Methanothermobacter thermautotrophicus*), and a SAOB (*T. phaeum*; Table 3). These microbial species were originally isolated from a thermophilic methanogenic digester (Kamagata and Mikami, 1991; Hattori et al., 2000) and are regarded as representative species for the methanogenic acetate degradation reactions that occur in various natural environments such as high-temperature petroleum reservoirs (Pham et al., 2009; Mayumi et al., 2011, 2013) and thermophilic methanogenic digesters (Sekiguchi et al., 1998; McHugh et al., 2003; Hori et al., 2011).

To adequately assess the effects of CO$_2$ concentration itself, media with different supplementation of CO$_2$/HCO$_3^-$ were prepared (Table 1). The initial concentrations of total CO$_2$/HCO$_3^-$ in the cultures, designated as [ΣCO$_2$]$_{initial}$, were 5.0, 50.7, or 113.4 mmol l$^{-1}$. The model consortium composed of *Methanosaeta thermophilia*, *Methanothermobacter thermautotrophicus*, and *T. phaeum* was cultivated under the three different [ΣCO$_2$]$_{initial}$ conditions to evaluate their methanogenic acetate degradation abilities (Figure 1). A stoichiometric production of CH$_4$ from acetate in a 1:1 molar ratio was observed in all culture conditions tested. Both acetate consumption and CH$_4$ production rates slightly decreased with increasing the [ΣCO$_2$]$_{initial}$ (Figures 1A,B). Interestingly, the partial pressure of H$_2$, which is an important intermediate of syntrophic
Table 3 | The metabolic reactions and the respective standard Gibbs free energy changes ($\Delta G^{\circ}$) of the microorganisms utilized in this study.

| Microbial species                  | Metabolic reactions                | $\Delta G^{\circ}$ (kJ mol$^{-1}$)$^a$ |
|------------------------------------|------------------------------------|---------------------------------------|
| Methanosaeta thermophila           | $\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-$ | -31.0                                 |
| Thermacetogenium phaeum            | $\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 4\text{H}_2 + \text{H}^+$ | +104.6                                |
| Methanothermobacter thermautotrophicus | $4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$ | -135.6                                |

$^a$The $\Delta G^{\circ}$ values were calculated according to the reference (Thauer et al., 1977).

acetate degradation, significantly decreased with increasing the $[\Sigma\text{CO}_2]_{\text{initial}}$ (Figure 1C). This observation suggests that syntrophic methanogenic microorganisms are influenced by elevated CO$_2$ concentrations.

To assess the influence of the elevated CO$_2$ concentrations on each methanogenic pathway, the relative abundances of each microorganism in the exponentially growing cultures of the model consortium with different $[\Sigma\text{CO}_2]_{\text{initial}}$ were evaluated by FISH and qRT-PCR analyses. The qRT-PCR analysis clearly demonstrated the decrease of the abundances of Methanothermobacter thermautotrophicus and T. phaeum in the higher $[\Sigma\text{CO}_2]_{\text{initial}}$ cultures (Figure 2). The FISH analysis also demonstrated that the relative abundances of Methanothermobacter thermautotrophicus and T. phaeum in the cultures with higher CO$_2$ concentrations are significantly lower than those in the low CO$_2$ cultures (Figure 3). These results indicate that the syntrophic methanogenic pathway is more strongly influenced by the elevation of CO$_2$ concentrations compared to the aceticlastic pathway.

FIGURE 1 | Effects of CO$_2$ concentrations on the metabolism of the model consortium composed of Thermacetogenium phaeum, Methanothermobacter thermautotrophicus, and Methanosaeta thermophila. Time courses of acetate (A), CH$_4$ (B), and H$_2$ (C) concentrations during cultivation on acetate with different $[\Sigma\text{CO}_2]_{\text{initial}}$ are shown. Data are presented as means of three independent cultures, and error bars represent SDs.

FIGURE 2 | Relative abundances of T. phaeum, Methanothermobacter thermautotrophicus, and Methanosaeta thermophila in the model consortium with different $[\Sigma\text{CO}_2]_{\text{initial}}$. The 16S rRNA copy numbers of each microorganism in the mid-exponential phases were determined by the qRT-PCR analysis. The abundance of each microorganism was normalized against those of the cultures with $[\Sigma\text{CO}_2]_{\text{initial}}$ of 5.0 mmol l$^{-1}$, and plotted against the respective $[\Sigma\text{CO}_2]_{\text{initial}}$ values. Data are presented as the means of three independent cultures, and error bars represent SDs.
EFFECTS OF CO\textsubscript{2} CONCENTRATIONS ON THE ACETICLASTIC AND SYNTROPHIC PATHWAYS

To confirm the differences in the suppressive effects of elevated CO\textsubscript{2} concentrations on the two methanogenic pathways, the pure culture of *Methanosaeta thermophila* and the defined co-culture of *Methanothermobacter thermautotrophicus* and *T. phaeum* were separately cultivated in the media with the different $[\Sigma CO_2]_{\text{initial}}$ (Figure 4). The growth of *Methanosaeta thermophila* was barely affected by the elevated CO\textsubscript{2} concentration: the methanogenic rate in the $[\Sigma CO_2]_{\text{initial}}$ of 113.4 mmol l\textsuperscript{-1} cultures decreased only about 10\% compared to the cultures with $[\Sigma CO_2]_{\text{initial}}$ of 5.0 mmol l\textsuperscript{-1} (Figures 4A,C). On the contrary, the methanogenic rate of the syntrophic co-culture in the $[\Sigma CO_2]_{\text{initial}}$ of 113.4 mmol l\textsuperscript{-1} dropped to less than half of that in the cultures with $[\Sigma CO_2]_{\text{initial}}$ of 5.0 mmol l\textsuperscript{-1} (Figures 4B,C). These observations confirm the assumption that the syntrophic acetate degradation pathway is more susceptible to elevated CO\textsubscript{2} concentrations than the aceticlastic pathway.

EFFECTS OF CO\textsubscript{2} CONCENTRATIONS ON THE PURE CULTURES OF *Methanothermobacter thermautotrophicus* AND *T. phaeum*

One possible explanation for the suppressive effects of CO\textsubscript{2} on the syntrophic methanogenesis is the susceptibility of *Methanothermobacter thermautotrophicus* and/or *T. phaeum* to some environmental alterations induced by increased CO\textsubscript{2} or to CO\textsubscript{2} itself. To evaluate this possibility, pure cultures of *Methanothermobacter thermautotrophicus* and *T. phaeum* were cultivated in media with different $[\Sigma CO_2]_{\text{initial}}$ (Figure 5). No significant differences were observed for the growth of both *Methanothermobacter thermautotrophicus* and *T. phaeum* under the different CO\textsubscript{2} conditions tested. These results suggest that elevated CO\textsubscript{2}
FIGURE 5 | Effects of CO$_2$ concentrations on the pure cultures of *T. phaeum* (A) and *Methanothermobacter thermautotrophicus* (B). Data are presented as the means of three independent cultures, and error bars represent SDs.

concentrations negatively affect the microbial activity only when *Methanothermobacter thermautotrophicus* and *T. phaeum* are in a syntrophic relationship.

**EFFECTS OF CO$_2$ CONCENTRATIONS ON THE THERMODYNAMICS OF EACH REACTION**

The other possible explanation for the suppression of syntrophic methanogenesis by elevated CO$_2$ concentration is alterations of thermodynamic conditions of each microbial reaction. A minimum energy required for biochemical energy conversion is estimated at around −20 kJ mol$^{-1}$ (Schink, 1997), while some anaerobic microorganisms have been reported to thrive under more thermodynamically restricted conditions (Jackson and McInerney, 2002; Nauhaus et al., 2002). The value was estimated from the energetics of ATP formation (around −70 kJ mol$^{-1}$ under the physiological conditions; Jetten et al., 1991; Trän and Unden, 1998) and the number of protons transported to ATP formation (between 3 and 4; Maloney, 1983; Stock et al., 1999). Since syntrophic methanogenesis from acetate is one of the least exergonic microbial metabolisms (Schink, 1997), it is no wonder that only slight perturbations on the thermodynamics induce deteriorations of the syntrophic methanogenesis.

To evaluate the influences of elevated CO$_2$ concentrations on the thermodynamic properties, ΔG values of metabolic reactions conducted by each microorganism in the model consortium were determined using the data-set of metabolite concentrations shown in **Figure 1**. The ΔG values of the acetoclastic methanogenesis conducted by *Methanoseta thermophila* were not significantly influenced by the elevated CO$_2$ concentrations (**Figure 6**). The average ΔG values during the logarithmic growth phase (day 2–5) with the [ΣCO$_2$]$^{\text{initial}}$ of 5.0, 50.7 and 113.4 mmol l$^{-1}$ were −47.7 ± 3.5, −44.9 ± 2.6, and −44.6 ± 2.0 kJ mol$^{-1}$, respectively, which are substantially lower than the ΔG value required for microbial energy acquisition.

The ΔG values of the hydrogenotrophic methanogenesis catalyzed by *Methanothermobacter thermautotrophicus* were also largely not altered with different CO$_2$ settings and were constantly lower than −20 kJ mol$^{-1}$ (**Figure 6**). The average ΔG values during the logarithmic growth phases with the [ΣCO$_2$]$^{\text{initial}}$ of 5.0, 50.7, and 113.4 mmol l$^{-1}$ were −24.6 ± 1.0, −27.2 ± 1.0, and −26.0 ± 1.2 kJ mol$^{-1}$, respectively. Since CO$_2$ is the substrate for hydrogenotrophic methanogenesis, lower ΔG values under the higher CO$_2$ conditions are expected. However, the decrease in H$_2$ partial pressures under the higher CO$_2$ conditions (**Figure 1C**)) compensates for the positive effects of increase in CO$_2$ concentration.

On the contrary, elevation of CO$_2$ concentrations significantly influenced the ΔG values of the acetate oxidation reaction performed by *T. phaeum* (**Figure 6**). While the average ΔG value during the logarithmic growth phases with the [ΣCO$_2$]$^{\text{initial}}$ of 5.0 mmol l$^{-1}$ (−23.1 ± 2.7 kJ mol$^{-1}$) was less than the borderline ΔG value of −20 kJ mol$^{-1}$, those with the [ΣCO$_2$]$^{\text{initial}}$ of 50.7 and 113.4 mmol l$^{-1}$ (−17.8 ± 1.3 and −18.7 ± 1.4 kJ mol$^{-1}$)
mol\(^{-1}\), respectively) exceeded the borderline. As acetate oxidation reaction produces 2 mol of CO\(_2\) from 1 mol of acetate, it is rational that this reaction is strongly influenced by the elevation of CO\(_2\) concentration. The decrease in the partial pressure of H\(_2\), the other metabolic product of acetate oxidation, is expected to compensate for the negative effects of increase in CO\(_2\). However, the decrease in H\(_2\) partial pressure would be limited by the minimum threshold for H\(_2\) consumption by *Methanothermobacter thermotrophicus*. The minimum thresholds for H\(_2\) utilization by hydrogenotrophic methanogens have been reported as around 5–10 Pa (Lovley, 1985; Thauer et al., 2008). However, considering the energy required for active growth, H\(_2\) partial pressure of around 10–15 Pa observed in the increased CO\(_2\) conditions in this study may be the minimum H\(_2\) threshold for the syntrophic interaction. Actually, if the H\(_2\) partial pressure in the cultures with \(\Sigma\)CO\(_2\) \(\text{initial}\) of 113.4 mmol l\(^{-1}\) at the log-logarithmic growth phase (day 5) becomes 10 Pa, the ΔG value becomes \(> -20 \text{kJ mol}^{-1}\) (\(-19.7 \pm 0.3 \text{kJ mol}^{-1}\)) from the actual value of \(-25.1 \pm 1.4 \text{kJ mol}^{-1}\) (with H\(_2\) partial pressure of 16.4 ± 1.7 Pa). These results clearly demonstrated that high concentrations of CO\(_2\) thermodynamically constrain the acetate oxidizing reaction, which results in the deterioration of syntrophic methanogenesis from acetate.

**CONCLUSION**

This is the first paper to evaluate the influence of elevated CO\(_2\) concentration on the two different methanogenic acetate degradation pathways, namely aceticlastic and syntrophic pathways, using a model microbial consortium. As expected from the observations based on in situ environments with complex microbial communities, high concentrations of CO\(_2\) suppressed the syntrophic pathway rather than the aceticlastic pathway. Thermodynamic calculations revealed that the acetate oxidation reaction is more intensely constrained by elevated CO\(_2\) concentrations. This study exemplified the importance of even slight changes in the ΔG values of microbial metabolisms in anaerobic biota. Furthermore, this study demonstrated that the construction of model microbial consortia is useful for assessing competitive interspecies interactions even in anaerobic, methanogenic environments.

**AUTHOR CONTRIBUTIONS**

Souichiro Kato, Tomoyuki Sato, and Yoichi Kamagata designed the research. Souichiro Kato, Rina Yoshida, Takashi Yamaguchi, Tomoyuki Sato, and Isao Yumoto carried out the experiments and analyzed the data. Souichiro Kato and Yoichi Kamagata wrote the manuscript.

**ACKNOWLEDGMENTS**

This work was supported by grants from the Japan Society for the Promotion of Science (JSPS). We thank Dr. Mia Terashima and Hiromi Ikekuchi for helpful comments by critical reading and technical assistance, respectively.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 August 2014; accepted: 13 October 2014; published online: 30 October 2014.

Citation: Kato S, Yoshida R, Yamaguchi T, Sato T, Yamoto I and Kamagata Y (2014) The effects of elevated CO2 concentration on competitive interaction between acetoclastic and syntrophic methanogenesis in a model microbial consortium. *Front. Microbiol.* 5:575. doi: 10.3389/fmicb.2014.00575

This article was submitted to Systems Microbiology, a section of the journal *Frontiers in Microbiology*.

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