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ORIGINAL ARTICLE

Hydrogen emission by three wood-feeding subterranean termite species (Isoptera: Rhinotermitidae): Production and characteristics

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Abstract Hydrogen emission by wood-feeding termites, *Coptotermes formosanus*, *Reticulitermes flavipes* and *Reticulitermes virginicus*, was investigated upon a cellulosic substrate as their food source. The emission rates among the three species tested were significantly different and *R. virginicus* demonstrated the greatest H_2 emission at 4.78 ± 0.15 μmol/h/g body weight. In a sealed test apparatus, H_2 emission for each termite species showed a quick increase at the initial incubation hours (3–6 h), followed by a slower growth, possibly due to the feedback inhibition by gas accumulation. Further investigation revealed that continuous H_2 emission could be maintained by reducing the H_2 partial pressure in the sealed container. The bioconversion of cellulose to molecular H_2 by the subterranean termites tested could reach as high as 3 858 ± 294 μmol/g cellulose, suggesting that the termite gut system is unique and efficient in H_2 conversion from cellulosic substrate.

Key words biofuel, hydrogen, methane, subterranean termite, symbiotic micro-organism

Introduction

Termites are abundant social insects and cover about two-thirds of the Earth's terrestrial surface (Sanderson, 1996; Sugimoto *et al*., 1998). With the association of gut symbionts, termites can decompose significant portions of cellulose (74%–99%) and hemicellulose (65%–78%) components of wood lignocellulose into glucose, other hexose and pentose sugars, which are mainly fermented to CO_2, H_2 and acetate. Carbon dioxide and hydrogen may then be released to the atmosphere or possibly converted to CH_4 and acetate through the activities of methanogenic and acetogenic bacteria in the termite gut system (Sugimoto *et al.*, 1998; Ohkuma, 2003).

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Hydrogen, in some references, is considered to play an intermediate role in linking the fermentative breakdown of carbohydrate with methanogenesis and reductive acetogenesis (Ohkuma, 2003). Most of the phylogenetically lower termites (characterized by having symbiotic protists in their hindguts) show H_2–CO_2 homoacetogenesis, indicating that reductive acetogenesis dominates the hydrogen sink reaction (Breznak & Switzer, 1986).

Hydrogen emission from termites is postulated to be insignificant due to the fact that molecular H_2 is largely transferred from H_2-producing micro-organisms to acetogens or methanogens within the termite's hindgut. This process internally consumes most of the produced H_2 (Breznak & Switzer, 1986; Brauman *et al.*, 1992; Brune, 1998; Sugimoto *et al*., 1998). However, hydrogen emission by some termites has been reported and their emission rates could vary from 122.0–5883.3 μmol/h/g body weight (Sugimoto *et al*., 1998; Kawaguchi *et al.*, 2006; Inoue *et al.*, 2007). Hydrogen and methane are

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simultaneously produced by termite gut symbionts which include the protist inhabitants in the hindgut of the phylogenetically lower termites or the bacteria in the hindgut of higher termites (typically without symbiotic protists in their hindguts) (Taguchi *et al.*, 1992; Breznak & Brune, 1994; Sugimoto *et al*., 1998; Leadbetter *et al.*, 1999; Inoue *et al.*, 2007). It was also reported that H_2 emission rates and the ratio of CH_4/H_2 varied with different termite species (Sugimoto *et al*., 1998; Pester & Brune, 2007). Wood-feeding termites, especially most of the lower termites, demonstrated a relatively higher H_2 emission capability with a lower ratio of CH_4/H_2 than higher termites (e.g., soil-feeding and fungus-feeding termites) (Brauman *et al.*, 1992; Sugimoto *et al*., 1998; Pester & Brune, 2007). For lower termites, hydrogen production is mainly attributed to the dense population of cellulolytic protists in the hindguts. Recently, two iron hydrogenase genes were successfully identified from the protozoan, *Pseudotrichonympha grassii*, in the hindgut of *Coptotermes formosanus*, which directly clarified the molecular basis for H_2 production of this species (Inoue *et al.*, 2007).

The efficacy in H_2 emission largely depends on termite species, food source, the symbiotic micro-organisms in termite hindguts, as well as the mechanisms associated with H_2 production (Messer & Lee, 1989; Ebert & Brune, 1997; Schmitt-Wagner & Brune, 1999; Kawaguchi *et al.*, 2006; Tanaka *et al.*, 2006). Effects of environmental, nutritional and physiological stresses on termites would potentially influence hydrogen/methane emission profiles via symbionts in termite hindguts. Factors influencing the intestinal micro-organisms or living conditions of termites would also affect the gas emission from termites. Such factors include chemical treatment to selectively remove H_2- or CH_4-consuming symbionts (Messer & Lee, 1989), termite diet (Rouland *et al.*, 1993; Kawaguchi *et al.*, 2006), the atmosphere constituents in the headspace of test containers (Tsunoda *et al.*, 1993; Schmitt-Wagner & Brune, 1999; Pester & Brune, 2007; Pester *et al.*, 2007), as well as the size of a test population (Tsunoda *et al.*, 1993).

Hydrogen emitted from termite guts could represent a novel source of biohydrogen and a unique mechanism in generating hydrogen from cellulose. Biohydrogen production from lignocellulosic substrates is scarcely found in other natural ecosystems (Sugimoto *et al*., 1998; Inoue *et al.*, 2007). The termite digestive system, which generates H_2 in a significant amount from the degradation of lignocellulose, has not received much attention for its potential values in biohydrogen technologies. In the literature, most early investigations mainly focused on CH_4 production from termite guts and its potential impact on global warming (Fraser *et al.*, 1986; Messer & Lee, 1989; Brauman *et al.*, 1992; Darlington *et al.*, 1997; Sugimoto *et al*., 1998). Only a few termite species have been investigated for H_2 emission (Ebert & Brune, 1997; Inoue *et al.*, 2007; Pester & Brune, 2007).

In this report, the profiles of H_2 and CH_4 emission were established for the three wood-feeding lower termites, *C. formosanus*, *Reticulitermes flavipes*, and *Reticulitermes virginicus*. A modified CH_4/H_2 index was introduced to compare the characteristics of energy gas emission patterns among the three termite species tested. Finally, the effect of accumulated gas on continuous H_2 emission by a group of termites in a sealed container was also investigated.

Material and methods

Termites

Worker and soldier termites of *C. formosanus*, *R. flavipes* and *R. virginicus* were collected from Poplarville, Mississippi, US. Each termite species was reared at 27–28°C and > 90% RH in an individual plastic container (31.8 × 25.6 × 9.7 cm, Tri-State Plastics, Latonia, KY, USA) in the dark with moist sand and the originally infested wood blocks.

Test apparatus

A wide-mouth glass bottle (diameter 4.3 cm, height 9.4 cm, inner volume 106 mL; VWR International, West Chester, PA, USA, hereafter referred to as "apparatus") with a butyl rubber stopper (diameter 3.2 cm) was employed as test apparatus to hold termites. One piece of Whatman No 4 filter paper (42.5 mm wide, Whatman, Banbury, UK, > 99% cellulose) moistened with 150 μL distilled water was placed in each apparatus to serve as food and moisture for termites. Filter paper used in this experiment was dried at 70°C for 1 h and weighed individually before and after the trial to estimate cellulose consumption by the termites during the incubation period.

To test the air tightness of the apparatuses after being sealed with a butyl rubber stopper, three bottles were randomly sampled. One mL gaseous H_2 was injected into each of the three sealed empty bottles with an air-tight pressure-lock syringe (VICI, Baton Rouge, LA, USA). Then, 1-mL gas sample was immediately taken from each bottle for H_2 analysis. After incubation for an additional 72 h at 27°C, 1 mL gas was sampled again from each of the three bottles. All samples were subjected to H_2 analysis with gas chromatography (GC). Results showed

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that no significant difference in H_2 concentration was detected ($P < 0.001$) at the beginning and the end of the 72 h incubation with 1 mL H_2. Thus, the experimental apparatuses used were completely airtight with no detectable H_2 leakage.

Test procedure and gas sampling

Twenty worker termites (3rd instar or above) of each species were weighed before use and placed in the apparatus with a piece of wet filter paper, and then sealed with a rubber stopper. The apparatus with termites was maintained in an incubator at 27°C. At each of the following incubation intervals, 1, 3, 6, 24, 48, and 72 h, three apparatuses were randomly selected for gas sampling. One-mL gas samples from the headspace of each apparatus were taken with a pressure-lock syringe. After gas sampling, these three apparatuses were excluded from the test. Gas samples were also taken from three sealed apparatuses without termites to serve as base levels of H_2 and CH_4. All gas samples were subjected to GC analysis for H_2 and CH_4 at Mississippi State Chemical Laboratory, Starkville, MS, US.

Hydrogen emission with partial gas removal

Hydrogen emission by termites is reported to be constant (Inoue *et al.*, 2007). Our preliminary trial showed that H_2 emission did not continue after the H_2 reached a certain concentration in a sealed test container. Therefore, we hypothesized that the accumulated gases had a feedback inhibition on H_2 emission and when the H_2 equilibrium was broken by removing some gas from the sealed apparatus, it may possibly promote a continuous H_2 emission by termites. To test this hypothesis, half volume of gas, 53 mL, was removed with an air-tight pressure-lock syringe from the headspace of each apparatus after 3 h incubation, of which 1 mL was used for H_2 analysis. After incubation for an additional 3 h, another 1 mL gas sample was taken from the same apparatus for H_2 analysis. For comparison, the control treatment was set up exactly the same except that no gas was removed at 3 h incubation post-treatment. One-mL gas sample was taken only at the end of the 6 h incubation period. At each incubation interval, three replicates were sampled for each termite species. Gas samples were analyzed with GC for H_2. Total hydrogen emission of the two 3-h incubation intervals was compared with that of the 6-h uninterrupted incubation of the control group.

Hydrogen emission with modified test apparatus

To provide extra space for gas emission when emitted gas by termites accumulated to a given level, the apparatus was modified by connecting a vacuumed plastic gas sampling bag (50 mL, Jensen Inert Company, Coral Springs, FL, USA) to the apparatus with a syringe needle (51 mm, 22 gauge, Hamilton, Reno, NV, USA) through the butyl rubber stopper. Four hundred *C. formosanus* workers with two pieces of wet filter paper (Whatman No 4, 42.5 mm wide), which served as termite food and moisture, were pre-administered into each bottle prior to sealing. The modified apparatus was then maintained in an incubator at 27°C. After 48 h of incubation, 1 mL gas samples were separately taken from both the bottle and the connected gas bag with pressure-lock syringes. Total H_2 production of the modified apparatus was calculated by summing the H_2 from the bottle and the gas bag and compared with that of the regular apparatus, in which 400 termite workers were also used. Both the regular and modified apparatus treatments were performed in triplicate.

Hydrogen conversion rate

The hydrogen conversion rate was defined as the capability for H_2 emission (μmol) from each gram of filter paper (cellulose) consumed by a termite species (μmol/g filter paper). It was calculated by dividing the H_2 emission rate (μmol/h/g body weight) by the food consumption rate (g filter paper/h/g body weight).

Gas analysis

Concentrations of H_2 and CH_4 in each gas sample were analyzed using a Varian 3400 GC with N_2 as the carrier gas (25 mL/min, injector at 150°C, detector at 150°C, column at 50°C). The GC was fitted with thermal conductivity detector (TCD, Varian, Palo Alto, CA, US) and a steel column (3 m × 3.2 mm) filled with a molecular sieve 13 × 45/60 mesh.

Data analysis

The effects of termite species on H_2 and CH_4 emission in the test apparatus at various sampling intervals and the effect of partial gas removal from test apparatus on H_2 emission for the three termite species tested were analyzed using the analysis of variance (ANOVA) (PROC MIXED model procedure, SAS, 2004; SAS Institute Inc., Cary, NC, US), respectively. Tukey's Honestly

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Significant Difference (HSD) test was used to separate means at $\alpha = 0.05$. The effect of the modified apparatus on H_2 emission of *C. formosanus*, the air tightness of the apparatus, and the food conversion rate were analyzed with one-way ANOVA model.

Results

Emission of H_2 and CH_4

Hydrogen emission was detected for all three termite species tested and recorded at each observation interval except the first incubation hour (Fig. 1). There was a quick increase phase in H_2 emission at the initial 3–6 h of incubation followed by a slow growth phase after 24 h, which indicated a significant time dependence on H_2 emission ($F_{5,35} = 151.95$, $P < 0.001$). The recorded hydrogen concentration reached a plateau at 10.07 ± 0.62 nmol/mL for *C. formosanus*, while for *R. flavipes* and *R. virginicus*, the H_2 concentration still maintained a slow increase after 24 h of incubation. The difference in the capability of H_2 emission among the three termite species tested was significant ($F_{2,35} = 29.66$, $P < 0.001$), where *R. flavipes* demonstrated the highest H_2 concentration (15.63 ± 1.70 nmol/mL) at 72 h (Fig. 1).

Methane (CH_4) emission by *R. flavipes* and *R. virginicus* was observed during the 72 h incubation period, where

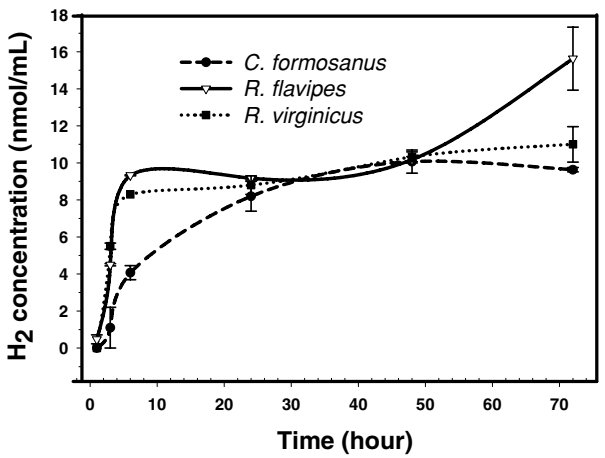

Fig. 1 H_2 emission (nmol/mL) recorded in the test apparatus during the period of 72 h incubation by *Reticulitermes flavipes*, *R. virginicus*, and *Coptotermes formosanus*. Error bars indicate 1 s.e.m. The minimum level for quantitative determination of H_2 concentration is 0.1 nmol/mL, with the minimum detection capability at 0.05 nmol/mL for H_2 by the gas chromatography machine used.

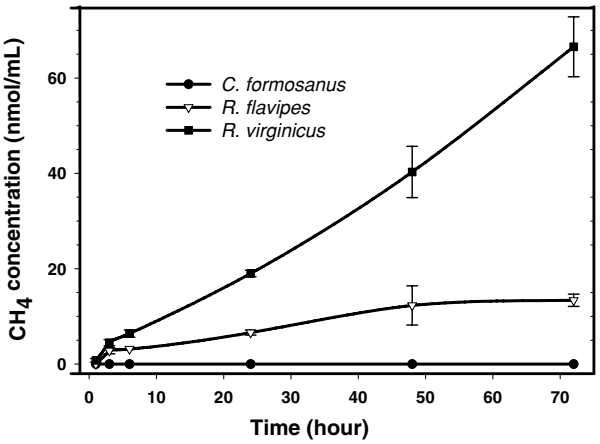

Fig. 2 CH_4 emission (nmol/mL) recorded in the test apparatus during the period of 72 h incubation by *Reticulitermes flavipes*, *R. virginicus*, and *Coptotermes formosanus*. Error bars indicate 1 s.e.m. The minimum level for quantitative determination of CH_4 concentration is 0.2 nmol/mL, with the minimum detection capability at 0.1 nmol/mL for CH_4 by the gas chromatography machine used.

CH_4 was released from termite guts simultaneously with molecular H_2. However, no detectable CH_4 emission was recorded for *C. formosanus* during the 72 h observation period (Fig. 2). Thus, the capability of methane emission among the three termite species differed significantly for the period we investigated ($F_{2,35} = 163.88$, $P < 0.001$). In contrast to the H_2 emission profiles of the termite species tested, the patterns of methane emission demonstrated a steady growth in CH_4 concentration during the 72 h incubation period (Fig. 2). Time dependence was significant on termite CH_4 emission during the incubation period ($F_{5,35} = 62.00$, $P < 0.001$).

CH_4/H_2 index and the patterns of energy gas emission

To define the characteristics of termite gas emission for CH_4 and H_2, a modified CH_4/H_2 index (see the caption of Fig. 3) was introduced. This index was distinct among the three termite species tested. Each species demonstrated a unique pattern in gas emission. As shown in Fig. 3, the CH_4/H_2 indexes for *R. flavipes* were characterized by maintaining a growth trend at values much greater than 1 after 24 h incubation, suggesting that more CH_4 was emitted and accumulated in the test apparatus than H_2. However, for *R. virginicus*, the CH_4/H_2 index was much lower than that of *R. flavipes* and fluctuated only from 0.4 to 1.2. This implied that *R. virginicus* had a similar emission rate of CH_4 and H_2 at the same time. Since no detectable CH_4 was noticed for *C. formosanus*, the CH_4/H_2

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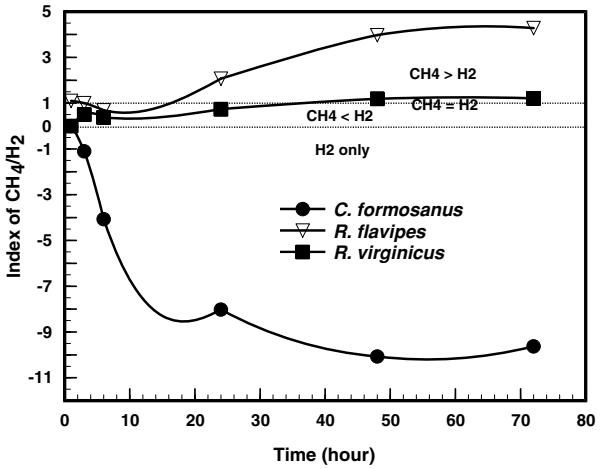

Fig. 3 Comparison of the profiles of H_2 and CH_4 emission with the modified index of CH_4/H_2 for *Reticulitermes flavipes*, *R. virginicus* and *Coptotermes formosanus* during the period of 72 h incubation. A modified CH_4/H_2 index from Sugimoto *et al.* (1998) was introduced to define the characteristics of termite gas emission for CH_4 and H_2. Absolute concentration of CH_4 and H_2 in each gas sample was used for this calculation. It was defined as follows: subtract H_2 from CH_4 when either H_2 or CH_4 concentration (nmol/mL) was zero; divide H_2 by CH_4 when the concentrations (nmol/mL) of both H_2 and CH_4 were above zero; name the CH_4/H_2 index to be zero if there was no detectable H_2 and CH_4. Therefore, more CH_4 emission was measured than H_2 when index was > 1 (denoted as $CH_4 > H_2$); CH_4 emission was equal to H_2 when index was 1 (denoted as $CH_4 = H_2$); less CH_4 was produced than H_2 when index was from 0 to 1 (denoted as $CH_4 < H_2$); only H_2 was detected when index was negative (denoted as H_2 only).

index fell below zero, indicating that *C. formosanus* predominantly emitted H_2 gas during the observation period (Fig. 3).

Effect of gas accumulation on continuous H_2 emission

When a portion of the gas volume was removed from the test apparatus at 3 h (midway through the 6 h incubation), the total H_2 production during the 6 h incubation period for each termite species was significantly enhanced compared to the control groups with no gas removal. The gas removal treatment from the test containers resulted in a 39.3%, 40.9%, and 60.6% more H_2 emission by *R. flavipes*, *C. formosanus*, and *R. virginicus*, respectively (Fig. 4). Clearly, gases accumulated in a limited and sealed container significantly affected continuous H_2 emission by termites ($F_{3,12} = 32.12$, $P < 0.001$).

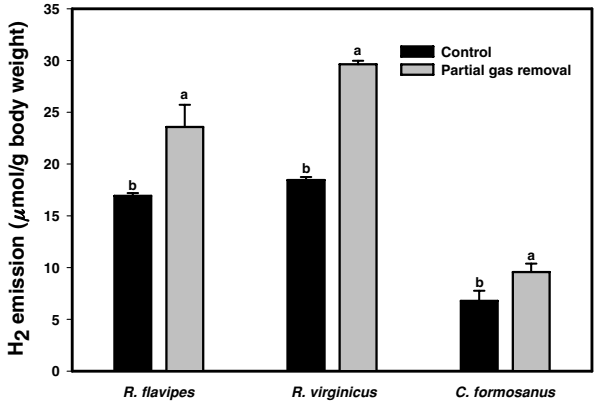

Fig. 4 Effect of partial gas removal from test apparatus after 3 h incubation on continuous H_2 emission by *Reticulitermes flavipes*, *R. virginicus* and *Coptotermes formosanus* during a total of 6 h incubation period (Letters represent mean separation of H_2 emission rates between gas removal and control, bars with the same letter did not differ significantly at $\alpha = 0.05$; PROC MIXED model data analysis). Control represents no partial gas removal from test apparatus. Error bars indicate 1 s.e.m.

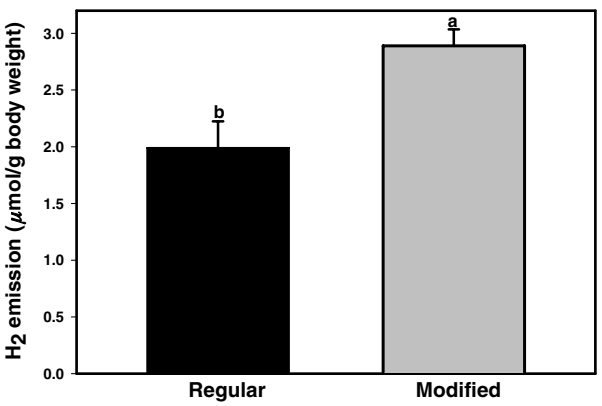

Fig. 5 Comparison of the H_2 emission rates of *Coptotermes formosanus* between modified test apparatus (extra space available, i.e., a gas bag was connected with the test apparatus) and regular test apparatus (without extra space). Letters represent mean separation of H_2 emission rates, bars with the same letter did not differ significantly at $\alpha = 0.05$; one-way ANOVA data analysis. Error bars indicate 1 s.e.m.

Hydrogen emission of *C. formosanus* was 45.2% higher in the modified test apparatus (extra space available for gas emission to reduce the hypothesized feedback inhibition by gas accumulation) than that in the regular test apparatus (fixed and volume-limited without supplying extra space) ($F_1 = 11.32$, $P = 0.044$) (Fig. 5).

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Table 1 H_2 emission and its conversion rate from cellulosic substrate by three wood-feeding termites, *Reticulitermes flavipes*, *R. virginicus* and *Coptotermes formosanus*[†].

Measurement	*C. formosanus*	*R. flavipes*	*R. virginicus*
Food consumption rate (mg filter paper/h/g body weight)	0.64 ± 0.06 b	1.04 ± 0.03 a	1.26 ± 0.14 a
H_2 emission rate (μmol/h/g body weight)[‡]	1.37 ± 0.15 c	3.07 ± 0.23 b	4.78 ± 0.15 a
H_2 conversion rate (μmol/g filter paper)	2 138 ± 88 b	2 941 ± 156 b	3 858 ± 294 a

[†]Data were analyzed using PROC MIXED of SAS. Means in each raw followed by the same letter did not differ significantly at $\alpha = 0.05$, Tukey's Highly Significant Difference.

[‡]The maximum emission rates during the 72 h incubation period were used.

Cellulose food consumption and H_2 conversion rates

There was no observable change in termite feeding behavior during the 72 h incubation period and their survival rates were maintained at 95%–100%. Food consumption rates for the three termite species tested on the filter paper substrate (> 99% cellulose content) ranged from 0.64–1.26 mg/h/g body weight (Table 1).

Significant differences were found in the conversion rates ($F_2 = 18.73$, $P = 0.003$) from the degradation of the cellulosic filter paper to H_2 among three termite species tested, where *R. virginicus* showed the highest H_2 conversion rates at 3 858 ± 294 μmol/g cellulosic substrate (Table 1). In a sealed apparatus environment, the highest H_2 emission rate for each of the termite species occurred between the initial 3–6 h during the 72 h observation period and it varied from 1.37–4.78 μmol/h/g body weight depending on termite species (Table 1).

Discussion

Wood-feeding termites possess a great potential to release a significant amount of H_2 as a byproduct from the breakdown of cellulose in their guts. The hydrogen conversion rates for the three termite species tested, in terms of the amount of H_2 released to the atmosphere, varied from 2–4 mmol H_2/g cellulose (Table 1), which was far lower than their intrinsic potential due to the internal H_2 consumption by methanogenic and acetogenic bacteria in their hindguts. Earlier reports showed that hydrogen concentration remained high in the gut center and decreased sharply toward the periphery due to the H_2 uptake by methanogenic or acetogenic bacteria (Ebert & Brune, 1997; Brune & Friedrich, 2000), and as a consequence, only a limited amount of H_2 was emitted to the atmosphere. Thus, the potential of hydrogen production and hydrogen conversion rates by termites was greatly underestimated. In contrast to the termite gut system, most fermentative anaerobic bacteria or some H_2-producing bacteria isolated from termite guts showed 4–5 times higher in the conversion rates from various mono-saccharides, or starch substrates (Table 2) (Taguchi *et al.*, 1993; Kumar & Das, 2000; Oh *et al.*, 2003; Lin & Lay, 2004; Kotay & Das, 2007), where the hydrogen was collected via a pure fermentative process without observable consumption. However, the hydrogen production emitted by these termites could be increased 5–7 times after an antibiotic treatment on their cellulosic diets (our unpublished data). This study also confirmed that the termite gut system could possibly maintain a continuous capability to emit molecular H_2 as long as the putative feedback inhibition induced by the termite's own gas emission could be minimized. In the completely sealed test apparatus of this study, maximum H_2 emission rates for the three termite species tested were obtained within the first 3–6 h of incubation (Table 1, Fig. 1). After that, the H_2 concentration in the test containers increased slowly, possibly due to the feedback inhibition effect from gas accumulation, and eventually H_2 concentration reached a plateau (Fig. 1). Similar results were also reported in fermentative H_2 production via anaerobic bacteria, in which the yield of H_2 was usually subjected to feedback inhibition by H_2 (Mousdale, 2008). Either by partially removing gases from a constrained test apparatus or by connecting a gas bag to serve as the extra space to the test apparatus prior to the incubation has achieved a similar response – enhancing the overall H_2 production emitted by termites (Figs. 4, 5). These investigations indicated that, by reducing H_2 partial pressure, the H_2 emission capability of termites could be maintained at a higher level. Thus, understanding why this H_2 equilibrium occurs and how the feedback inhibition takes place would potentially help us break the H_2 equilibrium for a continuous high yield of H_2 production released by termites.

Micro-organisms involved in hydrogen production in the termite gut may have a potential to become robust candidates for biohydrogen processes. The micro-organisms

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Table 2 Comparison of the substrate conversion rates by wood-feeding termites tested in this study and some fermentative bacteria from references.

Organism	Substrate	Maximum conversion rate (mmol H_2/g substrate)†	Reference
Termite			
Coptotermes formosanus	Cellulose	2.1	This report
Reticulitermes flavipes	Cellulose	2.9	This report
R. virginicus	Cellulose	3.9	This report
Bacteria isolated from *C. formosanus*			
Clostridium beijerinckii AM21B	Glucose	16.4	Taguchi *et al.*, 1993
	Starch	12.2	Taguchi *et al.*, 1993
Fermentative bacteria			
Enterobacter cloacae IIT-BT 08	Sucrose	17.5 (6)	Kumar & Das, 2000
Clostridium pasteurianum	Sucrose	14.0 (4.8)	Lin & Lay, 2004
Citrobacter sp. Y19	Glucose	15.3 (2.76)	Oh *et al*., 2003
Bacillus coagulans	Glucose	12.7 (2.28)	Kotay & Das, 2007

†The conversion rates were determined by the maximum amount of H_2 released to the atmosphere when termites fed on the cellulosic filter papers during the 72 h incubation period. The figures in each parenthesis are cited from the original references, where the unit used represents mol H_2/mol substrate.

that inhabit these lower termite digestive tracts, such as protozoa and bacteria, contribute to cellulose and hemicellulose digestion efficiency, and are most likely responsible for the subsequent emission of hydrogen and methane (Breznak & Brune, 1994; Sugimoto *et al.*, 1998; Ohkuma, 2003). Some H_2-producing bacteria or hydrogenase genes have been isolated from termite guts (Taguchi *et al.*, 1992, 1993; Inoue *et al.*, 2007). Although hydrogen is one of the byproducts during the breakdown of lignocellulose in lower termites, to date, this characteristic has received little attention. The termite gut as a special bioreactor may hold the key to developing efficient cellulose-based biohydrogen production because, in most cases, nowhere else in nature would these unique and efficient micro-organisms be found (Inoue *et al.*, 2007; Scharf & Tartar, 2008; Sun, 2008). Bioengineering techniques could also be helpful to construct an efficient H_2-producing strain for industrial-scale production using these hydrogen-producing microbes or related genes in the termite gut. Further research is also needed to identify how termite guts function as the bioreactors to facilitate cellulose degradation and subsequent H_2 emission.

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