Chemosphere, Vol.26, Nos.1-4, pp 617-622, 1993
Printed in Great Britain

0045-6535/93 $6.00 + 0.00
Pergamon Press Ltd.

# NUTRITIONAL FACTORS AFFECTING METHANE EMISSION FROM TERMITES

C. Rouland[1*], A. Brauman[2], M. Labat[3], and M. Lepage[4]

[1] Laboratoire de Zoologie, Université Paris XII, 94010-Creteil cedex, France
[2] Laboratoire de Microbiologie, Orstom, B.P. 181, Brazzaville, R.P. Congo
[3] Laboratoire de Microbiologie, Orstom, SFAX, Tunisie
[4] Laboratoire d'Ecologie, E.N.S., 46 rue d'Ulm 75 006, Paris, France

(Received in USA 17 December 1991; accepted 14 May 1992)

## ABSTRACT

Because of their numbers and diet, termites are important potential producers of atmospheric methane. This methane production is due to the anaerobic degradation of plant material by a symbiotic microflora localized in the termite's hindgut.

The bacterial population and the methane production are correlated to the termite's nutrition mode. The xylophageous termites possess primarily acetogenic bacteria and thus are low methane producers; on the other hand, the soil-feeding and some of the fungus-growing termites have numerous methanogenic bacteria in their guts and produce high quantities of methane but no acetate.

It is, therefore, essential to take into account the distribution of the different termite alimentary groups before assessing their global annual methane production.

## 1. INTRODUCTION

In tropical areas termites are known to have a substantial effect upon organic matter turnover (Boyer, 1973; Mielke, 1978) and humus formation (Mishra and Sen-Sarma, 1980) as a consequence of their abundance and nutrition mode. Three classes of diet could be distinguished (Wood, 1978; Grassé, 1982):

- xylophageous termites eat wood at different stages of decomposition;
- soil-feeding termites eat organic matter mixed with mineral particles;
- fungus-growing termites develop a symbiotic relationship with a fungus, *Termitomyces sp.* (Heim, 1977). The fungus grows on structures (fungus comb) within termite nests and degrades the various plant material (leaves, roots, grass, deal wood, etc.) collected by the termite workers. Termites feed only at the lower stratum of the fungus comb, which is degraded by *Termitomyces* (Grassé and Noirot, 1958; Grassé, 1982; Rouland et al., 1988a, b, c, 1991).

The degradation of plant materials by termites is mainly due to symbiotic relationships established with anaerobic bacteria localized in the hindgut (Breznak, 1975; Brauman et al., 1987). This anaerobic metabolism is characterized by methane emission, a typical product of intestinal fermentation. The methane emission by termites, first suggested by Cook (1932), has since been widely emphasized. On account of their wide distribution, their contribution to the tropospheric methane concentration has been estimated by different authors (Zimmerman et al., 1982; Seiler et al., 1984; Collins and Wood, 1984; Fraser et al., 1986; Khalil et al., 1990). The mode of extrapolation varied widely (5 to 40% of the global methane production).



ORSTOM Documentation
010000508

-4 JUIL. 1995

ORSTOM Fonds Documentaire
N° : 41.782 ex1
Cote : B

The figures obtained have been the subject of controversy since the first estimates by Zimmerman et al. (1982) were claimed by Collins and Wood (1984) as too high. Zimmerman et al. calculated a global production of 150 Tg $CH_4$ $year^{-1}$ by extrapolating $CH_4$ emissions obtained in the laboratory, while other authors agree upon a figure around 20 Tg $CH_4$ produced $year^{-1}$. But the workers generally did not take into account the interspecific (diets) and intraspecific (castes) variability of the termite populations.

Previous work has shown that the digestive metabolism of termite workers varied widely according to their diet (Rouland et al., 1986, 1989a). The purpose of this paper is to clarify the relation between the termites' diet and their methane emission.

## 2. MATERIALS AND METHOD

### 2.1. Biological Material

The species tested came from the Republic of Congo: *Trinervitermes rhodesiensis*, *Pseudacanthotermes militaris*, *Ps. spiniger*, and *Macrotermes bellicosus* have been collected in the Niari savanna, the other species in the Mayombe forest.

### 2.2 Respirometric Measurements

Termite workers were extracted from their nests and samples of 1 to 1.5 g were immediately placed into respirometric units using a microrespirometer (Jeulin, Evreux, France) at a constant temperature of 29°C for 2 hours. Every 15 minutes the consumption of $O_2$ and the emission of $CO_2$ was measured.

### 2.3 Bacterial Numeration

All the experiments were realized anaerobically according to the method of Hungate (1969). Termite workers were dissected in an anaerobic chamber. Ten guts of each species were homogenized in 1 mL of a Widdel culture medium (Widdel, 1987). For the enumeration, five tubes were realized for each dilution from $10^{-1}$ to $10^{-6}$. The medium used to enumerate the hydrogenophilic bacteria is a Widdel medium with $H_2/CO_2$ and acetate; the medium for the enumeration of the acetoclastic bacteria contained only acetate. After incubation at 35°C for one month, the methane emission was measured using a gas chromatograph (C.P.G., Delsi 30 E) equipped with flame ionization.

### 2.4 Methane Measurements

Ten termites were taken out of their nests and enclosed in a Hungate tube. The methane production was analyzed by the C.P.G. previously described. Sampling and injection were periodically repeated at intervals of 10 minutes for one hour. Details of the sampling system and analytical procedure are given in Brauman (1989).

## 3. RESULTS

### 3.1 Respiratory Metabolism (Table 1)

With the exception of *Microcerotermes parvus*, the xylophageous and the fungus-growing termite species exhibited a higher $O_2$ consumption than the soil-feeding species.

The emission of $CO_2$ by soil-feeding species is also clearly lower than the emission by the termites of the other alimentary groups.

The ratio $CO_2/O_2$ is very similar for the wood-eating and the fungus-growing termites. Values obtained

range between 0.74 and 1. Ratios for soil-feeding termites are very different, with low values ranging from 0.4 to 0.67.

Table 1. Consumption of $O_2$ and emission of $CO_2$ and $CH_4$ by African termite species: 4 wood-eating termite species (*Microcerotermes parvus, Nasutitermes lujae, N. arborum, Trinervitermes rhodensiensis*), 6 fungus-growing termite species (*Pseudacanthotermes militaris, Pseudacanthotermes spiniger, Macrotermes bellicosus, Macrotermes muelleri, Macrotermes nobilis, Microtermes sp.*), and 5 soil-feeding termite species (*Noditermes sp., Crenetermes albotarsalis, Cubitermes speciosus, Thoracotermes macrothorax, Astratotermes sp*). BW: Big Workers.

For $CO_2$ and $O_2$, the results are mean values of three experiments; for $CH_4$ the results are mean values of duplicate gas phase chromatographic analysis for n = 20 injections (0.1 ml).

| Species | $O_2$ µl/g/h | $CO_2$ µl/g/h | $CO_2/O_2$ | $CH_4$ µmol/g/h |
|---|---|---|---|---|
| *Microcerotermes parvus* | 178 | 145 | 0.81 | 0.14 ± 0.03 |
| *Nasutitermes lujae* | 593.6 | 577.6 | 0.97 | 0.15 ± 0.015 |
| *Nasutitermes arborum* | 548.2 | 534.6 | 0.98 | 0.13 ± 0.022 |
| *Trinervitermes sp.* | 478 | 465 | 0.97 | 0.021 ± 0.04 |
| *Pseudacanthotermes militaris* (BW) | 648 | 537 | 0.83 | 0.88 ± 0.28 |
| *Pseudacanthotermes spiniger* (BW) | 337.5 | 262.5 | 0.78 | 0.42 ± 0.1 |
| *Macrotermes muelleri* (BW) | 549 | 519 | 0.95 | 0.35 ± 0.09 |
| *Macrotermes bellicosus* (BW) | 853.3 | 630 | 0.74 | 0.42 ± 0.08 |
| *Macrotermes nobilis* (BW) | 620 | 486 | 0.78 | n.d. |
| *Microtermes sp.* | 317.6 | 282.3 | 0.89 | n.d. |
| *Noditermes sp.* | 204.8 | 130.2 | 0.64 | 0.64 ± 0.07 |
| *Crenetermes albotarsalis* | 129.6 | 47.2 | 0.36 | 0.93 ± 0.12 |
| *Cubitermes speciosus* | 222.1 | 87.24 | 0.39 | 0.89 ± 0.15 |
| *Thoracotermes macrothorax* | 207.1 | 132.9 | 0.64 | 1.09 ± 0.12 |
| *Astratotermes sp.* | 118 | 79 | 0.67 | 0.53 ± 0.06 |

3.2 Bacterial Numeration (Table 2)

A specific numeration of the hydrogenophilic microflora has been made on seven species, characteristic of the different diets.

In xylophageous species the digestive metabolism was characterized as fermentative due to a particularly abundant anaerobic microflora, mainly acetogenic. The acetate produced came not only from cellulose fermentation but also from the utilization of $H_2$ by homoacetogenic bacteria.

In soil-feeding species, bacterial density is lower than in xylophageous species, but it is characterized by a very abundant methanogenic microflora and the absence of acetogenic bacteria.

The density of the fermentative microflora is also lower in fungus-growing termites than in xylophageous ones. The function of this microflora seems to be very different among the species studied. In *M. muelleri* the large methanogenic microflora found seems to suggest that digestive fermentations are important in the metabolism of this species. In *Microtermes sp.* the low populations of the numerated microflora suggested that the digestive metabolism in this species is not really dependent on its symbiotic microflora.

Table 2. Fermentative bacterial numeration in 7 African termite species: 2 wood-eating termite species (*Microcerotermes parvus*, *Nasutitermes lujae*), 2 fungus-growing termite species (*Macrotermes muelleri*, *Microtermes sp.*), and 3 soil-feeding termite species (*Crenetermes albotarsalis*, *Cubitermes speciosus*, *Thoracotermes macrothorax*).

| Species | B.F. $10^8$/ml | B.H.A. $10^6$/ml | B.M.H. $10^6$/ml | B.M.F. $10^6$/ml | B.M.A. $10^6$/ml |
|---|---|---|---|---|---|
| *Microcerotermes parvus* | 10.4 | 81.5 | 1.6 | 2 | 5.2 |
| *Nasutitermes lujae* | 14.2 | 115 | 1.5 | 7.4 | 2.7 |
| *Macrotermes muelleri* | 1.5 | 0.05 | 16.6 | 0.05 | 0.06 |
| *Microtermes sp* | 2.2 | 0 | 0.2 | 0 | 0.23 |
| *Crenetermes albotarsalis* | 1.6 | 0.045 | 12.3 | 23.5 | 7.8 |
| *Cubitermes speciosus* | 5.6 | 0.08 | 30.4 | 33.7 | 1.6 |
| *Thoracotermes macrothorax* | 1.9 | 0.04 | 23.6 | 1.2 | 7.7 |

B.F. Fermentative anaerobic bacteria
B.H.Ac $H_2CO_2$ acetogenic bacteria
B.M.$H_2$ Hydrogenophilic methanogenic bacteria
B.M.F. Formatotrophic methanogenic bacteria
B.M.Ac Acetoclaste methanogenic bacteria

3.3 Methane Production (Table 1)

The direct measurement of methane emission by termites shows clearly that its production depends greatly upon the termites' diet. Soil-feeding and some fungus-growing termites are producing methane at a high rate (0.6 to 1.09 $\mu mol.g^{-1}.h^{-1}$), while the xylophageous termites exhibited little or no methane production. The study of the respiratory metabolism indicates that the values of the ratio $CO_2/O_2$ of the different termite species is conversely proportional to the methane emission rates.

## CONCLUSIONS-DISCUSSION

This study emphasized the role of the diet in the methane emission by termites. The wood-eating termites have very high respiratory activity. They are able to degrade most of the plant polysaccharides (Rouland et al., 1986). This hydrolysis takes place in the proctodeum by fermentative processes. The fermentation of plant material is realized by an abundant homoacetogenic microflora and involved an important acetate production (Breznak and Switzer, 1986) and a very low methane emission.

The soil-feeding termites showed low respiratory activity and low $CO_2$ emission; they also have an exceptionally low $CO_2/O_2$ ratio. Their fermentative microflora is mainly composed of methanogenic bacteria; they are the most important methane-producers. The high methane production by the soil-feeding termites could be correlated to the nature of their nutriment. These termites are able to degrade aromatic products, as has been shown by the isolation of a bacterial strain degrading benzoate from the digestive tract of *Cubitermes speciosus* (Brauman, 1989). The interspecies transfer of $H_2$ from fermentative to methanogenic bacteria could permit them to hydrolyze these very reduced molecules.

The fungus-growing termites showed a high respiratory activity, like the wood-eating termites, but thei

fermentative microflora consist mainly of methanogenic bacteria. They are good methane-producers but present great variation between species. Previous work (Rouland et al., 1986, 1988a, 1989b; Veivers et al., 1991) have shown that the plant material in fungus-growing termites is mainly hydrolyzed aerobically in the midgut; the real importance of the fermentative bacteria in the digestive metabolism of these termites is not yet explained.

These results clearly demonstrate that it is very important to take into account the diet of the different termite species in order to estimate their global methane production.

## REFERENCES


Boyer, P. (1973), Action de certain termites constructeurs sur l'évolution des sols tropicaux, Ann. Sc. Nat. Zool. Paris 15, 329-498.

Brauman, A. (1989), Etude du métabolisme bactérien de termites supérieurs à régimes alimentaires différenciés, Thèse d'Université, Aix-Marseille II, 168p.

Brauman, A., M. Labat, P. Methener, C. Rouland, and J.L. Garcia (1987), Etude de la microflore hétérotrophe de termites supérieurs en fonction de leur régime alimentaire, in Diversité microbienne, A. Coll. Soc. Fr. Microiol., 18-21.

Breznak, J.A. (1975), Symbiotic relationship between termites and their intestinal microbiota, In: Symbiosis, D.H. Jenning and D.L. Lee, Editors, Cambridge University Press.

Breznak, J.A., and J. Switzer (1986), Acetate synthesis from $H_2$ plus $CO_2$ by termite gut microbes, Appl. Env. Microbiol. 52, 623-630.

Collins, N.M., and T.G. Wood (1984), Termite and atmospheric gas production, Science 224, 84-86.

Cook, S.F. (1932), The respiratory gas exchange in *Termopsis nevadensis*, Bio. Bull. Mar., Woods Hole 63, 246-256.

Fraser, P.J., R.A. Rasmussen, J.W. Creefield, J.R. French, and M.A.K. Khalil (1986), Termites and global methane: another assessment, J. Atmos. Chem. 4, 295-310.

Grassé, P.P. (1982), Anatomie. physiologie. reproduction des termites, In: Termitologia, Masson, Ed., 676p.

Grassé, P.P., and C. Noirot (1958), La meule des termites champignonnistes et al signification symbiotique, Ann. Sc. Zool. Bio. Anim. 11, 113-129.

Heim, R. (1977), Termites et champignons, Boubée, Editor, Paris, 207p.

Hungate, R.E. (1969), A roll-tube method for cultivation of strict anaerobes, In: Methods in Microbiology, J.R. Norris and D.W. Ribbon, Editors, Academic Press, London, vol. 3, 117-132.

Khalil, M.A.K., R.A. Rasmussen, J.R.J. French, and J.A. Holt (1990), The influence of termites on atmospheric trace gases: $CH_4$, $CO_2$, $CHCl_3$, $N_2O$, $H_2$ and light hydrocarbons, J. Geophys. Res. 95, 3619-3634.

Mielke, H.W. (1978), Termitaria and shifting: the dynamic roles of the termites in soils of tropical wet-dry Africa, Tropical Ecology 19, 117-122.

Mishra, S.C., and P.K. Sen-Sarma (1980), Humic acids in faecal matter, nests material and fungus comb of termites, Bull. Ent. 21, 122-125.

Rouland, C., C. Chararas, and J. Renoux (1986), Etude comparée des osidases de trois espèces de termites à régime alimentaire différent, C.R. Acad. Sc. Paris 302(9), 341-345.

Rouland, C., A. Civas, J. Renoux, and F. Petek (1988a), Purification and properties of cellulases from the

termite *Macrotermes mülleri* and its symbiotic fungus, Comp. Biochem. Physiol. 91B, 449-458.

Rouland, C., A. Civas, J. Renoux, and F. Petek (1988b), Synergistic activities of the enzymes involved in cellulose degradation purified from *Macrotermes mülleri* and from its symbiotic fungus *Termitomyces sp.*, Comp. Biochem. Physiol. 91B, 459-465.

Rouland, C., J. Renoux, and F. Petek (1988c), Purification and properties of two xylanases from the termite *Macrotermes mülleri* and its symbiotic fungus *Termitomyces sp.*, Insect Biochem. 18, 709-715.

Rouland, C., and C. Chararas (1989a), Les osidases digestives présentes dans l'intestin moyen, dans l'intestin postérieur et dans les glandes salivaires du termites *Crenetermes albotarsalis*, C. R. Acad. Sc. Paris 308, 281-285.

Rouland, C., A. Brauman, S. Keleke, M. Labat, P. Mora, and J. Renoux (1989b), Endosymbiosis and exosymbiosis in the fungus growing termites, in Microbiol. Poecil, R. Lesel, Ed., Elsevier Science, Amsterdam, 79-82.

Rouland, C., F. Lenoir, and M. Lepage (1991), The role of the symbiotic fungus in the digestive metabolism of several species of fungus-growing termites, Comp. Biochem. Physiol. 99 (4), 657-663.

Seiler, W., R. Conrad, and D. Scharffe (1984), Field studies of methane emission from termites nests into the atmosphere and measurements of methane uptake by tropical soils, J. Atmos. Chem. 1, 171-186.

Veivers, P.C., R. Mühlemann, M. Slaytor, R.H. Leuthold, and D.E. Bignell (1991), Digestion, diet and polyethism in two fungus-growing termites: *Macrotermes subhyalinus* and *Macrotermes michaelseni*, J. Insect Physiol. 9, 675-682.

Widdel, F. (1987), Microbiology and ecology of sulfate or sulfur reducing bacteria, in Environmental Microbiology of Anaerobes, A.J.B. Zehnder, Ed., John Wiley, New York.

Wood, T.G., and W.A. Sands (1978), The role of termites in ecosystems, In: Production Ecology of Ants and Termites, M.V. Brian (Ed.) Cambridge University Press, 68-73.

Zimmerman, P.R., J.P. Greenberg, S.O. Wandiga, and P.J. Crutzen (1982), Termites: a potentially large source of atmospheric methane, carbon dioxide and molecular hydrogen, Science 218, 563-565.