

Degassing of Pore Water Methane during Sediment Incubations†

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Laboratory experiments were used to examine the degassing of CH₄ from a muddy sediment. Sediment containing dissolved CH₄ showed a hyperbolic time course of CH₄ release when allowed to degas in stoppered 20-ml vials. Equilibration required ca. 24 h for 5 ml of sediment. The rate of CH₄ release was found to be dependent on the ratio of exposed sediment surface area to sediment volume. The water content of the sediment was a factor in the total amount of CH₄ released but did not affect the rate of degassing. Addition of water to sediment samples (to form a slurry) accelerated CH₄ release, with a 1:1 dilution giving ca. 80% of maximum release after only 2 min. Shaking (vortexing) the sediments also facilitated CH₄ exchange, with 2 min of vigorous agitation giving 77% of maximum release. The organic content of the sediment did not affect either the amount or the rate of CH₄ degassing. Rubber stoppers exposed to CH₄ were found to absorb CH₄ rapidly and to subsequently release it in proportion to the concentration to which they were exposed. Artifacts may be associated with CH₄ production measurements if sediment and stopper degassing are not considered. It is recommended that any study of methane production or distribution include preliminary experiments to determine the degassing kinetics for the specific sediment system being used.

There is considerable interest in the role of methane in the carbon cycle of aquatic environments (7, 12). Methanogenesis is a terminal sink for electrons generated during the anaerobic degradation of organic matter (8, 15) and is important in the diagenesis of recently deposited sediments. Methanogenic bacteria proliferate below the surface layer in organic-rich sediments in which oxygen penetration is limited.

In our studies of methane production, in which we used whole sediments from various marine and freshwater environments, we noted that methane was released for extended periods (up to and longer than 24 h) when "killed" sediments were placed in sealed containers (unpublished data). Similar release was observed in "unkilled" controls; however, these samples continued to release CH₄ after the amount released from killed samples had leveled off. Initial rates of total methane release were often very much greater than the rates observed after the methane release from controls had leveled off. We believe that the initial rate of CH₄ release was due to degassing of pore water CH₄ and that the lower rate observed after the controls had ceased to release methane more closely approximated the actual biological methane production. Since dissolved CH₄ concentrations from 1 to 10 mM in sediments have been reported (3, 6, 10, 14), degassing of pore water CH₄ may be important when methane production measurements are sought. In several studies, CH₄ degassing has been recognized (5, 8), but to date this phenomenon has not been rigorously examined.

We report the results of laboratory experiments which were used to identify several factors which may affect the apparent rate of release of methane and the amount of methane released from sediments isolated in sealed containers. Factors controlling CH₄ degassing are probably directly applicable to other gases of biological and chemical interest, not only in terms of degassing but also with regard to gas diffusion into a sediment system (11).

In addition, we report data on the uptake and release of

methane by rubber stoppers, which are routinely used in microbiological assays.

MATERIALS AND METHODS

Sediment samples used for laboratory degassing experiments were obtained near the mouth of the Carmans River estuary, located on the south shore of Long Island, N.Y. This estuary has been studied extensively by Carpenter and Dunham (*Limnol. Oceanogr.*, in press). Freshly collected field samples from this site in the Carmans River had been observed to have high levels of dissolved CH₄ and active methanogenesis (unpublished data). Fresh sediment appears brown-black in color, is relatively homogeneous to a depth of at least 20 cm, and consists entirely of fine silts and clays. The organic content is high, with a loss on ignition of 17.5 to 19%. The porosity of fresh sediment ranges from 0.80 to 0.84.

Sediment from the 5- to 16-cm depth interval was allowed to dry for several days in a core tube and was then stored in a Whirlpak bag until used. The sediment remained damp during storage and had a porosity of 0.66 at the time this study was performed. No precautions were taken to maintain anaerobic conditions during storage.

At the start of an experiment, partially dried Carmans River sediment was mixed with distilled water to give a water content similar to that in fresh samples. This sediment was then loaded into a 5-ml plastic syringe with the end cut off. Care was taken to fill the syringe to the desired volume, leaving no air spaces. The sediment was then extruded into a 20-ml glass scintillation vial (Wheaton or Rochester Scientific Co.). So that the gas exchange area was similar (ca. 4.5 cm²) for all sediment volumes used, the vials were tapped on the bench to allow the sediment to settle to a flat surface, or in some cases a syringe plunger was used to form a flat surface on the top of the sediment. The vials were then sealed with no. 1 black rubber stoppers (A. H. Thomas Co.).

Pure CH₄ (1 ml) was added to each vial with a gastight syringe after an equal volume of headspace had been removed to keep the pressure in the vial at 1 atm (101 kPa). Sediment samples were exposed to CH₄ for at least 24 h at

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room temperature on the laboratory bench to allow equilibration between the headspace and the sediment. After exposure, the concentration of CH_4 in each vial was determined. At this time, the stoppers were removed and the vials were flushed with a stream of N_2 for 8 to 10 s. Air was then allowed to enter the vials for 5 s, after which new stoppers were used to seal the vials, and the time was taken as time zero. The vials were incubated without disturbance at 25°C . The methane concentration in the headspaces of the vials was measured over a time course by using a Perkin-Elmer Sigma 2-B gas chromatograph equipped with a flame ionization detector. Measurements were made until the concentration of methane in the headspace reached a constant value.

The sediment was incubated under an air atmosphere to prevent methane production. For all experiments, control sediment samples which were not exposed to CH_4 were used. In no case was methane produced during the course of an experiment. It was therefore assumed that the increase in CH_4 concentrations in the vials was due to non-biological degassing of CH_4 and not to active methanogenesis.

The effects of water addition on methane degassing were examined by adding 0, 1, 2, 3, 4, and 5 ml of distilled, methane-free water to a series of 5-ml samples of sediment which had been exposed to the same concentration of methane. After water was added, each vial was shaken for 5 s to form a slurry. The effect of agitation was investigated by shaking the vials on a Vortex mixer at high setting for various lengths of time immediately after sealing them with new stoppers. For the above treatments, the amount of methane released was analyzed at the 2-min time point and expressed as a percentage of the maximum amount of CH_4 released.

The effect of the water content in the sediment (porosity) was tested by mixing sediments with various amounts of distilled water, while keeping the total volume constant at 5 ml.

The influence of organic matter on CH_4 degassing was examined by using ashed and unaltered Carmans River sediment (17.5% loss on ignition) mixed in various proportions to obtain a range of organic contents.

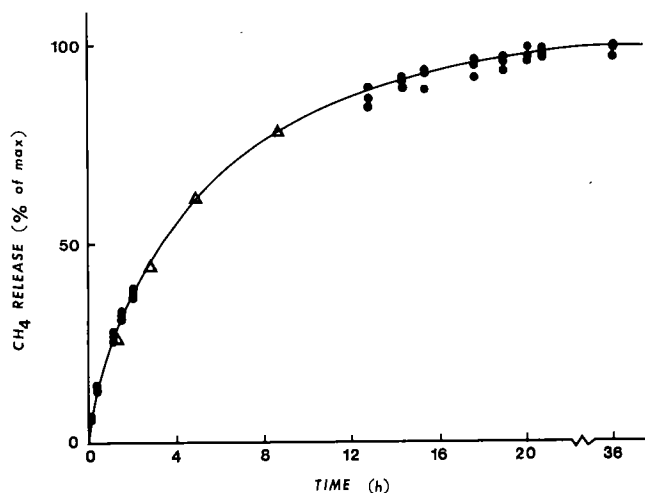


FIG. 1. Time course of diffusive CH_4 release from 5 ml of sediment expressed as a percentage of maximum release. Samples were incubated in 20-ml scintillation vials at 25°C without disturbance. Symbols: ●, Individual vials from one experiment; Δ, mean values from duplicate vials from a separate experiment. Sediment pore water initially contained ca. 0.06 mM CH_4 .

The absorption and release of CH_4 by stoppers was examined by exposing no. 1 natural rubber (A. H. Thomas Co.) and butyl serum (Wheaton) stoppers to known concentrations of methane in scintillation vials and serum vials, respectively, for 24 h. To measure CH_4 release, the stoppers were quickly transferred to new vials and the evolution of methane was monitored with time. The total amount (in nanomoles) of methane released from the stoppers was compared with the concentration of methane to which they had been exposed. A series of stoppers was exposed to the same concentration of CH_4 for different lengths of time to determine the rate of CH_4 absorption.

Methane standards were prepared periodically from pure CH_4 and standardization was cross-checked with commercially obtained standards (Alltech Association). Porosity was determined by drying a known volume of sediment at 105°C and taking the weight loss as the volume of water.

RESULTS AND DISCUSSION

Degassing kinetics showed a high initial rate, followed by a decreasing slope which ultimately approached zero (Fig. 1). This hyperbolic degassing curve was found in all our experiments and is similar to that obtained from field samples. The shape of the curve was independent of the amount of CH_4 in the sediment (data not shown). Only the amount of methane released changed. The data cannot be fit by a simple diffusion model because of the non-steady-state condition of the CH_4 gradient between the sediment and headspace. The total amount of CH_4 released from the sediments agreed well with the calculated amounts of dissolved methane based on the Bunsen absorption coefficient, the partial pressure of CH_4 during exposure, and the volume of water contained in the sediments (see Table 1). More than 99% of the CH_4 in the sediment was transferred to the headspace (4).

Equilibration of sediment samples with the headspace of vials required much longer times than did a static sample of distilled water (data not shown), which took ca. 1 h to achieve maximum release. Vigorous agitation of water samples resulted in essentially instantaneous equilibration. Although it appears that most of the pore water CH_4 is evolved over the first 12 h, significant amounts may be released thereafter. For the case of 5 ml of sediment in a scintillation vial, the non-biological release of methane during the period 12 to 20 h can appear quite linear (Fig. 1) and may be mistaken for methane production. The average slope of the actual data points during this time indicates a rate of release of $0.56 \text{ nmol ml}^{-1} \text{ h}^{-1}$ ($r^2 = 0.97$) (Fig. 1). Initial rates of degassing (0 to 2 h) are ca. 12 times higher than the rates over the 12- to 20-h period. The pore water concentration of CH_4 in this and other experiments was ca. 0.06 mM , calculated from the CH_4 partial pressure during equilibration. Concentrations of up to 10 mM dissolved methane have been reported for aquatic sediments (3, 9). Since degassing kinetics appear independent of the initial pore water concentration, sediments with 10 mM initial CH_4 concentrations if allowed to degas in the type of system described here would yield apparent linear rates of CH_4 production of ca. $93 \text{ nmol ml}^{-1} \text{ h}^{-1}$ over the 12- to 20-h period. This represents a relatively high rate of methanogenesis.

In an early experiment, empty vials were used as controls for any CH_4 which might remain after the flushing procedure used. Although all the methane was removed during flushing, the amount of methane in these vials appeared to increase with time. The rubber stoppers were suspected as the source of methane. Time courses for CH_4 release from rubber

stoppers were very similar to those for CH_4 release from sediments (Fig. 1), with equilibration occurring at ca. 24 to 28 h.

The amount of CH_4 released from stoppers was found to be directly proportional to the concentration of CH_4 to which they were exposed. Figure 2 illustrates this relationship for two types of stoppers which are routinely used in methane production experiments. The slopes of the two regression lines are almost identical (0.94 for black rubber stoppers and 0.91 for butyl stoppers). The difference in the intercepts is probably due to different surface areas of the two types of stoppers and different solubilities of CH_4 in butyl and natural rubber. Stoppers absorb CH_4 rapidly over the first 6 h, with ca. 50% of the maximum taken up by this time, and are saturated by ca. 18 to 24 h (Fig. 3). The total amount of CH_4 released from a black rubber stopper is about four times that which would be released from 5 ml of sediment if they were both equilibrated to the same concentration. If rubber stoppers are used during sediment incubations, the headspace should not simply be flushed to remove the degassed CH_4 . After the containers are flushed, the stoppers will release methane at a relatively high rate, depending on the exposure time and concentration. A double layer of Borden plastic wrap (polyvinyl chloride) or Saran wrap (polyvinylidene) greatly reduced uptake and release of CH_4 from stoppers, despite being pierced several times by a needle. A single layer of plastic wrap reduced uptake and release but was torn easily when the stopper was seated and gave variable results. Van Kessel (13) has found that Saran wrap is an effective barrier against the diffusion of several gases, including methane. The actual amount of methane which is absorbed and released by stoppers can be ca. 3% of the total CH_4 in the vial when black rubber stoppers and a 16-ml headspace are used. For serum stoppers, ca 1.6% of the total CH_4 in the vial (6-ml headspace) was absorbed and released. For all our sediment degassing experiments, new stoppers were used to avoid degassing from stoppers.

The effect of sediment porosity (water content) on CH_4 degassing was examined (Table 1). The porosities used were

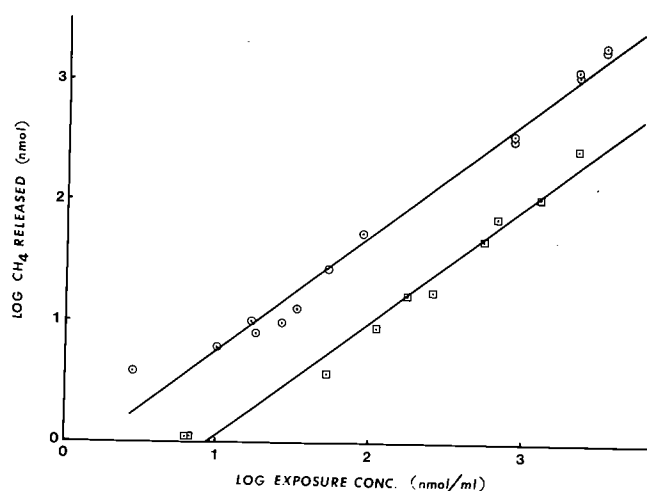


FIG. 2. Relationship between CH_4 concentration during exposure and the amount of CH_4 subsequently released for two types of rubber stoppers: \odot , no. 1 black rubber; \square , gray butyl serum. Black rubber stoppers and butyl stoppers were exposed and allowed to degas in scintillation vials (21.1-ml headspace) and serum vials (6.2-ml headspace), respectively.

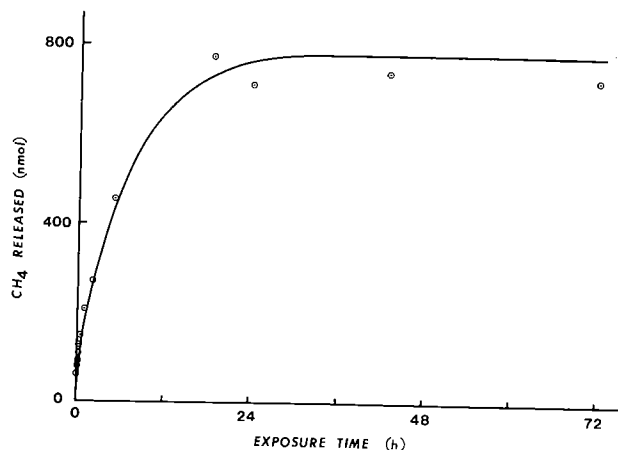


FIG. 3. Time of exposure of no. 1 black rubber stoppers to CH_4 versus amount of CH_4 subsequently released. All stoppers were exposed to 5% (by volume) CH_4 . The lowest point represents 1 min of exposure. Stoppers not exposed to CH_4 did not release CH_4 .

0.76 to 0.85, which in this case represented a range from a relatively dry sediment to a thick slurry. The total amount of CH_4 evolved increased with increasing water content. However, the time to reach maximum release indicates that porosity has little effect on the rate of CH_4 degassing in the range of porosities used. A slight trend toward more rapid degassing is evident with the higher water content, but the effect is not striking.

Various amounts of water were added to 5 ml of methane-exposed sediment, and the vials were shaken briefly after water addition. The results shown in Table 2 indicate that addition of water to the sediment causes methane to be released rapidly and that the initial release appears to be proportional to the amount of water added. Addition of 5 ml of water to 5 ml of sediment (a 50:50 slurry) results in ca. 80% release in only 2 min. After the pulse release resulting from water addition and shaking, CH_4 evolved slowly in accordance with the diffusion kinetics described above. Slurries prepared by homogenizing sediment and water which are kept anoxic during preparation by bubbling with nitrogen generally do not have degassing problems since CH_4 is stripped out during bubbling and often display a lag in the appearance of CH_4 (2, 5a).

Since methane is relatively insoluble in water, it was expected that vigorous agitation of sediment samples containing dissolved CH_4 would facilitate the exchange of CH_4 to the headspace. The effect of shaking time on the amount of CH_4 released, expressed as a percentage of the maximum release of CH_4 from 5 ml of sediment, is presented in Fig. 4. Samples were taken at the same time point (2 min) for each vial, regardless of shaking time, so that comparisons could

TABLE 1. Effect of porosity on CH_4 degassing from 5 ml of sediment

Porosity ^a	Amt of CH_4 released (nmol)	Calculated dissolved CH_4 (nmol)	Time to reach maximum value (h)
0.761	184	230	28.2
0.784	208	237	28.1
0.801	222	242	27.8
0.831	248	251	27.2
0.854	263	258	26.4

^a Porosity: volume of water/volume of sediment.

TABLE 2. Effect of water addition on CH₄ degassing from 5 ml of sediment

Treatment conditions ^a		Total CH ₄ released (nmol):		
Vortexing time (s)	Amt of water added (ml)	After 2 min	Maximum	% of maximum released after 2 min
0	0	11.9	251.7	4.7
5	0	22.7	204.3	11.1
5	1	50.3	263.2	19.1
5	2	61.4	260.2	23.6
5	3	90.7	260.2	37.5
5	4	198.5	285.3	69.6
5	5	192.0	240.4	79.9

^a Sediment samples were vortexed as indicated to mix the water and the sediment.

be made. Longer shaking times increased the amount of CH₄ released. However, vortexing continuously for 2 min resulted in only 77% of maximum release. After the shaking treatment, CH₄ degassed as shown in Fig. 1. It is evident that for this system, vortexing for 5 or 10 s releases only a small fraction of the total CH₄ and that substantial degassing will continue after this treatment.

The organic content of the sediment over a range from 1 to 17.5% loss on ignition had little effect (data not shown) on either the rate of CH₄ degassing or the total amount evolved. This suggests that adsorption onto organic matter was not responsible for the retarded exchange of CH₄ to the headspace.

The effect of sediment volume was examined by using 1, 2, 3, 4, and 5 ml of sediment with the same water content. Time courses of CH₄ release for this experiment are presented in Fig. 5. The total amount of CH₄ released increased with larger sediment volumes (Fig. 5A). This was expected, since the amount of pore water present in each sample should govern the absolute amount of dissolved CH₄ present. An important point to distinguish in Fig. 5A is the rate at which methane approaches the maximum value for different sediment volumes. Figure 5B shows the same data plotted as the percentage of maximum released versus time. From this figure, it is clear that with increasing sediment volume, the time taken to reach the maximum degassing increases con-

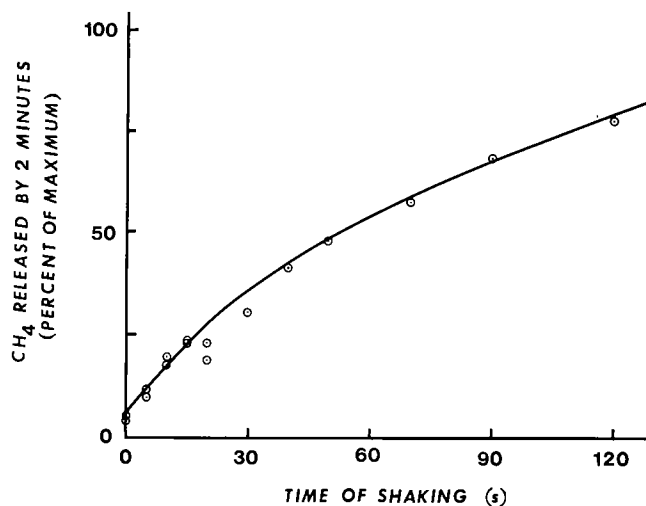


FIG. 4. Effect of shaking (vortexing) time on the percentage of maximum CH₄ release from 5 ml of sediment after 2 min.

siderably, and that significant degassing may occur over the time interval from 12 to 20 h. The time at which maximum degassing occurs has a negative exponential relationship with the sediment surface area/volume ratio (Fig. 6). Given a constant surface exchange area, the rate of equilibration of CH₄ depends on the volume of the sediment and on the diffusion coefficient, which is dependent on tortuosity and temperature (1). Muddy sediments, such as those used here, have a high tortuosity, which could account for the slow evolution of CH₄. Coarse sediments appear to release methane more rapidly (data not shown) than fine-grained sediments, probably as a result of a shorter diffusion path length. Although the effect of temperature was not examined, it is expected that lower temperatures would lead to slower gas equilibration because of a decrease in the diffusion coefficient and would affect the amount of gas released because of increased gas solubility.

Release of CH₄ from sediments to a container headspace over time can be due to active production or the retarded exchange of pore water CH₄, or both. One could envision sediment pore waters containing substantial amounts of CH₄ as a result of diffusion from lower layers, yet having no

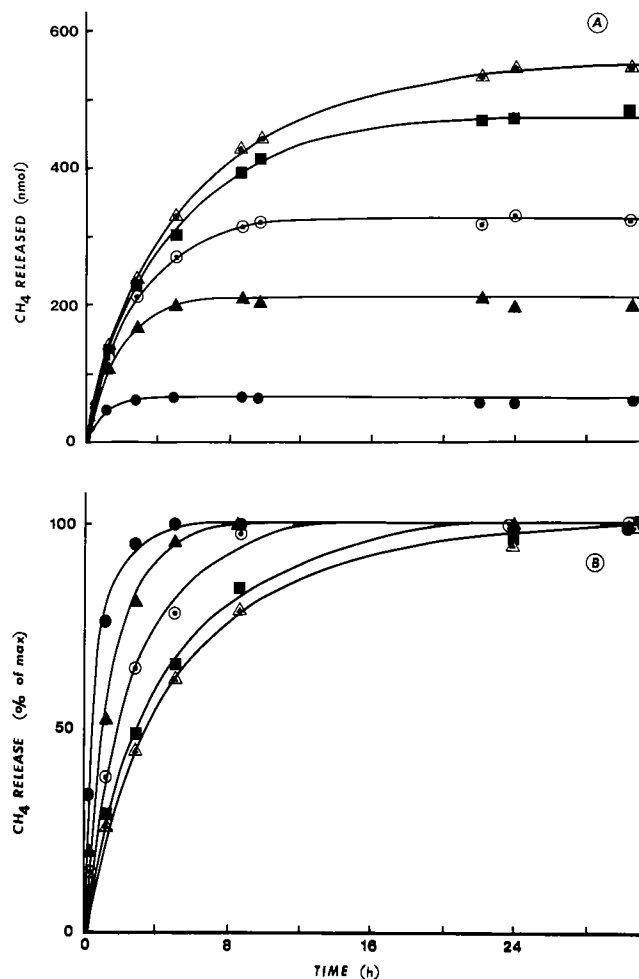


FIG. 5. Effect of sediment volume on the time course of (A) the total amount and (B) the percentage of maximum CH₄ evolution. The exposure concentrations were similar for all volumes used. Symbols: ●, 1 ml; ▲, 2 ml; ○, 3 ml; ■, 4 ml; △, 5 ml. Curves were fit to the data by eye.

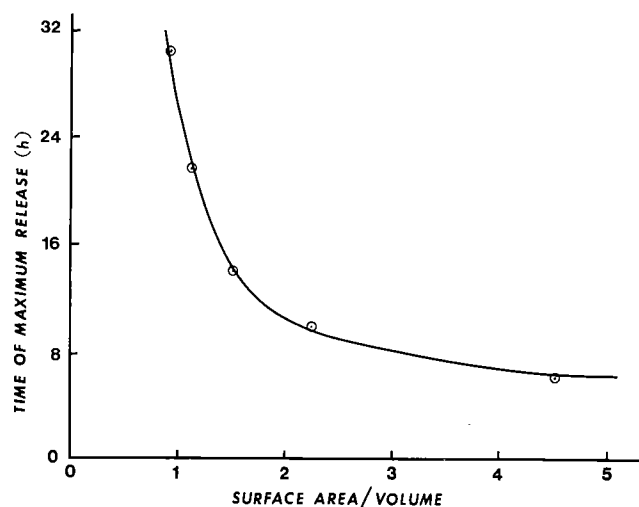


FIG. 6. Relationship between sediment surface area to volume ratio and the time of maximum degassing. Maximum release times were estimated from the curves in Fig. 5B.

active methanogenesis. Upon sampling, these sediments would evolve CH_4 in a manner similar to the laboratory experiments presented here. Equilibration of dissolved CH_4 with a headspace may be mistaken for CH_4 production. The results of our study show that the complete exchange of methane from muddy sediments to a container headspace does not occur as rapidly as is generally assumed. The long equilibration times probably result from the restricted diffusion of CH_4 due to high tortuosity and decreased diffusion coefficients in the sediment and from a small surface-exchange area relative to the sediment volume. Our data indicate that the rate of gas equilibration can be determined easily for a given system. It is recommended that degassing kinetics be considered in studies which involve the use of sediments isolated in containers for measurements of biological gas production or pore water gas concentrations.

The results presented here are probably applicable to other gases, including CO_2 , O_2 , N_2O , C_2H_2 , C_2H_4 , H_2S , and H_2 , which are relevant to chemical and biological studies. In addition, moist soils would also be expected to show similar gas exchange phenomena.

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