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Carbon and hydrogen isotope fractionation by microbial methane oxidation: Improved determination

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Abstract

Isotope fractionation is a promising tool for quantifying methane oxidation in landfill cover soils. For good quantification an accurate determination of the isotope fractionation factor (α) of methane oxidation based on independent batch experiments with soil samples from the landfill cover is required. Most studies so far used data analysis methods based on approximations of the Rayleigh model to determine α. In this study, the two most common approximations were tested, the simplified Rayleigh approach and the Coleman method. To do this, the original model of Rayleigh was described in measurable variables, methane concentration and isotopic abundances, and fitted to batch oxidation data by means of a weighted non-linear errors-in-variables regression technique. The results of this technique were used as a benchmark to which the results of the two conventional approximations were compared. Three types of batch data were used: simulated data, data obtained from the literature, and data obtained from new batch experiments conducted in our laboratory. The Coleman approximation was shown to be acceptable but not recommended for carbon fractionation (error on $\alpha - 1$ up to 5%) and unacceptable for hydrogen fractionation (error up to 20%). The difference between the simplified Rayleigh approach and the exact Rayleigh model is much smaller for both carbon and hydrogen fractionation (error on $\alpha - 1 < 0.05\%$). There is also a small difference when errors in both variables (methane concentration and isotope abundance) are accounted for instead of assuming an error-free independent variable. By means of theoretical calculations general criteria, not limited to methane, ^{13}C, or D, were developed for the validity of the simplified Rayleigh approach when using labelled compounds.

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1. Introduction

Methane (CH_4) contributes to the greenhouse effect. The contribution of CH_4 to the climate change is estimated at 22% of the contribution of all greenhouse gases (Lelieveld et al., 1998). Landfills are an important source of CH_4 since about 7% of CH_4 emissions to the atmosphere are estimated to originate from landfills (IPCC, 2001).

In a landfill cover soil layer, part of the CH_4 is oxidized to CO_2. When CH_4 emissions are estimated in greenhouse gas emission inventories, this effect is usually not taken into account due to the difficulties to measure CH_4 oxidation. Nevertheless, CH_4 oxidation is significant, with estimates ranging from 10% as a year-round average for a landfill in New Hampshire (Czepiel et al., 1996) to 50% (Whalen et al., 1990) and higher.

A promising method to quantify CH_4 oxidation is by isotope fractionation. Bacteria oxidize CH_4 with ^{12}C slightly faster than CH_4 with ^{13}C (Barker and Fritz, 1981). The result is an increase of the ^{13}C/^{12}C ratio of the remaining CH_4. This increase can be used to estimate CH_4 oxidation. Liptay et al. (1998), Chanton et al. (1999) applied this technique on several landfills in Northeast United States, and found

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oxidation efficiencies in general agreement with the annual mean value of 10% reported by Czepiel et al. (1996).

Chanton and Liptay (2000) estimated 20% oxidation for clay and mulch topsoil but this was under a warmer climate than the studies of Czepiel et al. (1996), Liptay et al. (1998). Much higher oxidation percentages up to 80% were also reported (Bergamaschi et al., 1998). Large seasonal variations (0–94% oxidation) have been reported for Swedish landfills (Börjesson et al., 2001). Barlaz et al. (2004) used the isotope method to compare CH_4 oxidation efficiencies of soil and compost as landfill cover materials. Other applications of the method were reported by Christophersen et al. (2001), Scheutz et al. (2003).

The isotope method has been used in other ecosystems as well. Ambus et al. (2002) used an isotope method to demonstrate that the occurrence of a threshold concentration for CH_4 oxidation from the atmosphere by soils is actually the result of CH_4 production. However, isotope data in many ecosystems like marches are much more difficult to interpret than in landfill cover soils, because CH_4 oxidation often occurs in isolated rhizospheric pockets where CH_4 oxidation is nearly complete, leaving no trace on the isotope signature of the emitted CH_4 (Happell et al., 1993).

The enrichment of ^{13}C in CH_4 is measured as isotopic abundance:

$$\delta^{13}C = \left(\frac{R}{R_{st}} - 1\right) \cdot 1000‰ \quad (1)$$

with R the isotope ratio $^{13}C/^{12}C$ of the sample and R_{st} the isotope ratio of the reference standard (VPDB for carbon).

When the isotope ratios are measured at the waste, where CH_4 is produced, and at the top of the landfill, the fraction of CH_4 oxidized can be calculated with the equation (Blair et al., 1985):

$$f_{ox} = \frac{\delta E - \delta A}{1000 \cdot (\alpha - \alpha_{trans})} \quad (2)$$

with δE the isotopic abundance of the emitted CH_4, δA the isotopic abundance of the produced CH_4, α the fractionation factor for CH_4 oxidation (i.e., the preference for oxidizing $^{12}CH_4$ above $^{13}CH_4$) and α_{trans} the fractionation factor for CH_4 transport. Eq. (2) was suggested by Blair et al. (1985) for open systems, and is appropriate for landfill cover soils, where CH_4 can move freely to escape to the atmosphere, or to be oxidized by soil methanotrophs.

The fractionation factor for CH_4 transport is usually assumed to be equal to 1, which means that no fractionation due to transport is assumed. However, De Visscher et al. (2004) found that α_{trans} can be as high as 1.014, due to molecular diffusion of CH_4.

Before Eq. (2) can be used to evaluate CH_4 oxidation from isotope data, it is important that the fractionation factor is determined accurately by means of independent batch tests with soil samples from the cover soil. For the calculation of α from such batch data, the Rayleigh (1896) equation applies. In most studies, α is calculated by the simplified Rayleigh approach, which is based on the Rayleigh (1896) equation. For CH_4, the approximate method of Coleman et al. (1981), which is also based on the Rayleigh (1896) equation, has often been used to calculate α based on batch oxidation experiments.

Hunkeler (2002) explained that the simplified Rayleigh approach is only applicable for studies at natural abundance level, which means that the Rayleigh approach can be used in batch studies for the determination of α for CH_4 oxidation. Scott et al. (2004) compared different regression methods which use a linearized version of the simplified Rayleigh approach and concluded that there is no improvement in comparison with the classical linear regression for parameter estimation of α. The errors associated with the method of Coleman et al. (1981) have not been evaluated yet.

The aim of this paper is to test the accuracy of the method of Coleman et al. (1981) and the simplified Rayleigh approach by comparison with a benchmark method based on the original Rayleigh (1896) model. The methods were applied to simulated batch data, as well as on real data obtained from the literature and obtained in our laboratory. We report the relative error of each method as $(\alpha - 1)$, because, following Eq. (2), this is also the relative error on f_{ox} resulting from an incorrect estimate of α.

2. Theoretical background

For a closed system the method of Rayleigh (1896) can be used to describe the effect of CH_4 oxidation on the $^{13}C/^{12}C$ isotope ratio:

$$\frac{d^{13}C}{d^{12}C} = \frac{k_{13}}{k_{12}} \cdot \frac{^{13}C}{^{12}C} \quad (3)$$

with ^{12}C and ^{13}C the carbon isotope concentrations of the remaining CH_4 and k_{12} and k_{13} the oxidation rate constants of these isotopes, $d^{13}C$ and $d^{12}C$ refer to infinitesimal changes of ^{12}C and ^{13}C concentrations and should not be confused with $\delta^{13}C$.

The ratio k_{12}/k_{13} is the fractionation factor α. In some studies, α is defined as the inverse, k_{13}/k_{12} (King et al., 1989; Ambus et al., 2002). If α is constant the solution of equation (3) is:

$$\frac{^{13}C/^{12}C}{^{13}C_0/^{12}C_0} = \left(\frac{^{12}C}{^{12}C_0}\right)^{\frac{1-\alpha}{\alpha}} \quad (4)$$

with $^{12}C_0$ and $^{13}C_0$ the initial carbon isotope concentrations of the CH_4. The derivation of Eq. (4) is given in Appendix A.

The ratio k_{12}/k_{13} is the fractionation factor α. In some studies, α is defined as the inverse, k_{13}/k_{12} (King et al., 1989; Ambus et al., 2002). If α is constant the solution of equation (3) is:

$$^{12}C = \frac{CH_4}{1 + \frac{R_{st} \times \delta^{13}C}{1000} + R_{st}}$$
$$^{13}C = \frac{CH_4 \cdot \left(\frac{\delta^{13}C}{1000} + 1\right)}{\frac{1}{R_{st}} + \frac{\delta^{13}C}{1000} + 1} \quad (5)$$

With Eqs. (5) the ^{13}C and ^{12}C concentrations can be substituted in Eq. (4) to obtain measurable variables:

$$\frac{\delta^{13}\mathrm{C}+1000}{\delta^{13}\mathrm{C}_0+1000}=\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\cdot\frac{\frac{1}{R_{\mathrm{st}}}+\frac{\delta^{13}\mathrm{C}_0}{1000}+1}{\frac{1}{R_{\mathrm{st}}}+\frac{\delta^{13}\mathrm{C}}{1000}+1}\right)^{\frac{1-\alpha}{\alpha}} \tag{6}$$

with $\mathrm{CH}_{4,0}$ the initial CH_4 concentration. Because this equation is rigorously derived from Eq. (3) assuming constant α, without approximations, this equation is referred to as the exact Rayleigh model.

The exact Rayleigh model can be simplified by approximating the ^{12}C concentration by the total CH_4 concentration because the natural ^{13}C concentrations are small (1.1%).

Combining Eqs. (4) and (1) with $\mathrm{CH}_4 \approx {}^{12}\mathrm{C}$ gives:

$$\frac{\delta^{13}\mathrm{C}+1000}{\delta^{13}\mathrm{C}_0+1000}=\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\right)^{\frac{1-\alpha}{\alpha}} \tag{7}$$

Eq. (7) is best known as the Rayleigh approach (e.g., Liptay et al., 1998; Snover and Quay, 2000; De Visscher et al., 2004). In this paper it is referred to as the simplified Rayleigh approach to avoid confusion with the exact Rayleigh approach.

Coleman et al. (1981) made further approximations. After rearranging the left-hand side of Eq. (7) and taking the logarithm, they approximated the left-hand side as follows:

$$\ln\left(1+\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{\delta^{13}\mathrm{C}_0+1000}\right)\approx\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{\delta^{13}\mathrm{C}_0+1000} \tag{8}$$

because $\ln(1+x)=x-\frac{x^2}{2}\ldots\approx x$ for a small x. This approximation was considered acceptable because the difference between the δ values during an experiment is small.

Eq. (8) was further simplified because the initial abundance is small:

$$\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{\delta^{13}\mathrm{C}_0+1000}\approx\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{1000} \tag{9}$$

This gives the equation of Coleman et al. (1981), which has been used by many researchers (e.g., Chanton and Liptay, 2000; Börjesson et al., 2001):

$$\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{1000}=\frac{1-\alpha}{\alpha}\cdot\ln\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\right) \tag{10}$$

Essentially the same technique was used by Miller et al. (2001) for methyl halides.

Methanotrophs also have a preference for H over D. Consequently two methods are available for the quantification of CH_4 oxidation: carbon and hydrogen isotope fractionation. The same derivations as above apply to the H fractionation.

Mariotti et al. (1981) developed an equation similar to Eq. (10) and used it for N fractionation.

The models used in the comparison are based on Eqs. (6), (7), (10). The equations were rearranged (Eq. (11)–(13)) to always obtain $\ln(\mathrm{CH}_4/\mathrm{CH}_{4,0})$ on the left hand side: Rayleigh model:

$$\ln\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\right)=\frac{\alpha}{1-\alpha}\ln\left(\frac{1000+\delta^{13}\mathrm{C}}{1000+\delta^{13}\mathrm{C}_0}\right)+\ln\left(\frac{1000+\delta^{13}\mathrm{C}+\frac{1000}{R_{\mathrm{st}}}}{1000+\delta^{13}\mathrm{C}_0+\frac{1000}{R_{\mathrm{st}}}}\right) \tag{11}$$

Simplified Rayleigh model:

$$\ln\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\right)=\frac{\alpha}{1-\alpha}\ln\left(\frac{1000+\delta^{13}\mathrm{C}}{1000+\delta^{13}\mathrm{C}_0}\right) \tag{12}$$

Coleman model:

$$\ln\left(\frac{\mathrm{CH}_4}{\mathrm{CH}_{4,0}}\right)=\frac{\alpha}{1-\alpha}\cdot\frac{\delta^{13}\mathrm{C}-\delta^{13}\mathrm{C}_0}{1000} \tag{13}$$

From Eq. (11), α can be obtained from experimental data only by nonlinear regression. This is the exact Rayleigh approach.

Plotting Eq. (12) with $\ln(1000+\delta^{13}\mathrm{C})$ on the X axis and $\ln(\mathrm{CH}_4)$ on the Y axis yields a straight line with slope $\frac{\alpha}{1-\alpha}$. Therefore, α can be obtained by linear regression of experimental data in the same plot. This is the approximate Rayleigh approach.

Using Eq. (13), α can also be obtained by linear regression of the data plotting $\delta^{13}\mathrm{C}$ on the X axis and $\ln(\mathrm{CH}_4)$ on the Y axis. This is the Coleman method.

The Coleman method and the simplified Rayleigh approach can also be applied by interchanging the X and Y axes.

3. Materials and methods

3.1. Experiments

3.1.1. Experimental set-up

The fractionation factor α was determined with a batch experiment. Soil samples were taken from two landfills: Hooge Maey (Antwerp, Belgium) and Armhoede (Lochem, The Netherlands). A soil sample (100 g) taken from a landfill cover soil layer was put in a bottle of 215 ml 24 h before the experiment, 1 ml CH_4 was injected to activate the soil and the bottle was closed with a rubber stopper. Just before the experiment the bottle was opened for aeration. The bottle was closed again and 2 ml CH_4 was added. At regular time intervals, gas samples were taken to measure $\delta^{13}\mathrm{C}$ and the gas concentration. The CH_4 concentrations were measured with a Chrompack CP 9000 gas chromatograph with a FID detector. The isotopic abundances were measured with an ANCA-TGII isotope ratio mass spectrometer (PDZ Europa Ltd.).

Sampling frequency depended on the activity of the soil samples. For GC analysis it was typically 20 min. For IRMS analysis a sample was taken at the beginning of the experiment and when the CH_4 concentration was approximately half the concentration of the previous IRMS sample. In a typical experiment, about 20 samples (100 µl) were taken for GC analysis and 5 samples (varying volumes, to obtain 2 µl CH_4) were taken for IRMS analysis.

3.1.2. Measurement variance

It may be expected that errors on concentrations measured with a gas chromatograph are not constant over the measured range of concentrations but increase with increasing concentrations. To obtain an unbiased calibration of a model it is necessary to account for this effect.

Standards were prepared by injecting a known volume of pure CH_4 into empty 215-ml bottles. From these standards, samples were taken repeatedly, and injected into the GC. The number of samples taken from each bottle was limited so the variance created by depletion of CH_4 in the bottle was negligible compared to the measurement variance. In Fig. 1 the standard deviation of CH_4 concentration measurements together with the confidence interval is shown. The data shows that the measurement variability is roughly proportional with the concentration, with an average relative error of 1.7%. A parameter estimation with constant weights based on the logarithm of the concentration is consistent with a measurement error proportional to the concentration. From Fig. 1 it is clear that this is a justified assumption. It is concluded that Eqs. (11)–(13) can be used without further transformations.

3.2. Literature data

Data from literature (Coleman et al., 1981; Snover and Quay, 2000) were also used to illustrate the differences between the models for hydrogen fractionation.

Snover and Quay (2000) measured the uptake of atmospheric CH_4 with static flux chambers on native grassland and forested arboretum in Washington State (USA). Before the start of the experiment a sample of ambient air was taken for isotopic analysis. A second sample was taken when the methane concentration dropped to 25–55% of the initial concentration in the chamber.

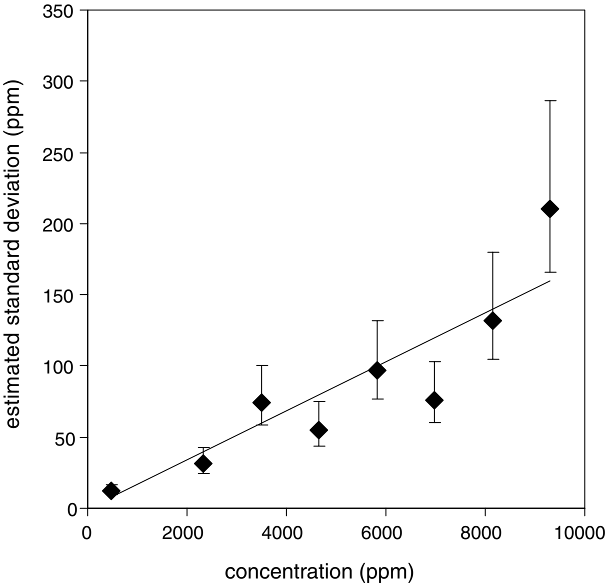

Fig. 1. Estimated standard deviation of concentration measurements at different methane concentrations. Error bars are 95% confidence intervals.

In the experiment of Coleman et al. (1981) bacteria were collected from different sources and incubated in flasks after addition of methane (18–30%). Periodically samples where taken to measure the CH_4 concentration and isotopic composition.

3.3. Parameter estimation

From the experiments the fractionation factor could be calculated with the above mentioned models (Eqs. (11)–(13)) because they were all closed systems. In the experiments of Snover and Quay (2000) there was also fractionation by diffusion through the soil. In that case the calculated fractionation factor was a combination of bacterial fractionation and fractionation by diffusion. The data of Coleman et al. (1981) and our data refer to bacterial fractionation, because these experiments were incubations in flasks where diffusion plays a minor role.

Parameters (e.g., α) are estimated by minimisation of a function, the objective function, J. In the case of an unweighted regression (simple linear regression in the case of Eqs. (12) and (13), simple nonlinear regression in the case of Eq. (11)), the objective function is the sum of squares of the residuals:

$$J(\alpha) = \sum_{k=1}^{N} (Y_k - \hat{Y}_k(\alpha))^2 \qquad (14)$$

with Y_k the measured value of the dependent variable and $\hat{Y}_k$ its calculated value for the kth measurement.

With this objective function it is assumed that the independent variable (e.g. $\delta^{13}C$ in Eqs. (11)–(13) is free of error.

However, both variables used in the models, CH_4 concentration and the $\delta^{13}C$ value, are measured and not error-free. This is a so called errors-in-variables problem: both variables have a measurement error. According to Dochain and Vanrolleghem (2001), the objective function to be minimised in this case is:

$$J(\alpha) = \sum_{k=1}^{N} \varepsilon_k^{\mathrm{T}}(\alpha) V_k^{-1} \varepsilon_k(\alpha) \qquad (15)$$

with V_k the measurement error covariance matrix and ε_k the residual vector:

$$\varepsilon_k(\alpha) = \begin{bmatrix} Y_k - \hat{Y}_k(\alpha) \\ X_k - \hat{X}_k(\alpha) \end{bmatrix} \qquad (16)$$

with X_k the independent, and Y_k the dependent variable of the kth measurement.

Here the measurement error covariance matrix can be simplified because there is no correlation between the measurement error of $\delta^{13}C$ and the error of the methane concentration.

$$V_k^{-1} = \begin{bmatrix} \frac{1}{\sigma_Y^2} & 0 \\ 0 & \frac{1}{\sigma_X^2} \end{bmatrix} \qquad (17)$$

with σ_Y^2 and σ_X^2 the measurement variance of the variables.

Hence the objective function becomes:

$$J(\alpha) = \sum_{k=1}^{N} \frac{1}{\sigma_Y^2}(Y_k - \hat{Y}_k(\alpha))^2 + \sum_{k=1}^{N} \frac{1}{\sigma_X^2}(X_k - \hat{X}_k(\alpha))^2 \quad (18)$$

For the estimation of the fractionation factor with the errors-in-variables method, knowledge about the measurement errors is needed. With our setup the measuring errors are: $\sigma_{\delta^{13}C} = 0.6‰$ and $\sigma_{CH_4} = 0.017CH_4$ with CH_4 the methane concentration.

3.4. Determination of parameter estimation error

The parameters defining the $100(1 - a)\%$ confidence region (e.g., $a = 0.05$ for the 95% confidence region) are found where the objective function equals the critical value (Dochain and Vanrolleghem, 2001):

$$J_{crit} = J_{opt} \cdot \left(1 + \frac{p}{N_{data} - p} F_{a;p,N_{data}-p}\right) \quad (19)$$

With J_{crit} the critical value and J_{opt} the minimum value of the objective function, p the number of parameters (2 in the present case), N_{data} the number of data points and $F_{\alpha;p,N_{data}-p}$ the value of the F distribution with p and $N_{data} - p$ degrees of freedom and a confidence level a.

3.5. Parameter estimation on simulated data

With the exact model of Rayleigh, CH_4 concentrations and isotopic abundances were generated for different values of α. Each simulated data set consisted of 5 samples points. Each sample had half the CH_4 concentration of the previous sample to reflect the experiments (see Section 3.1.1). With these virtual experimental data, α was estimated again with the approximated models. An ordinary least squares estimation could be used here because there is no measurement error in the simulations. The parameter estimations were performed for typical values of α for C and H fractionation in landfill cover soils.

The error of each method is reported as percentage: $\left(\frac{\alpha_{approx} - \alpha_{true}}{\alpha_{true} - 1} \cdot 100\right)\%$. In fractionation studies the deviation of α from 1 is of importance because the methane oxidation efficiency as calculated from isotope data (Eq. (2)) is inversely proportional to $(\alpha - \alpha_{trans}) \approx (\alpha - 1)$. Therefore $(\alpha - 1)$ was used to compare the three models.

4. Results and discussion

4.1. Theoretical differences between the models

4.1.1. Simplified Rayleigh approach

Figs. 2 and 3 show the difference between the true α and the calculated α. The differences remain small (<0.05%) for both C and H fractionation, which confirms the finding of Hunkeler (2002) that the simplified Rayleigh approach can be used at natural abundance level. The difference increases with increasing values of α, $\delta^{13}C_0$ or δD_0. This is because the fractionation is more pronounced when α increases.

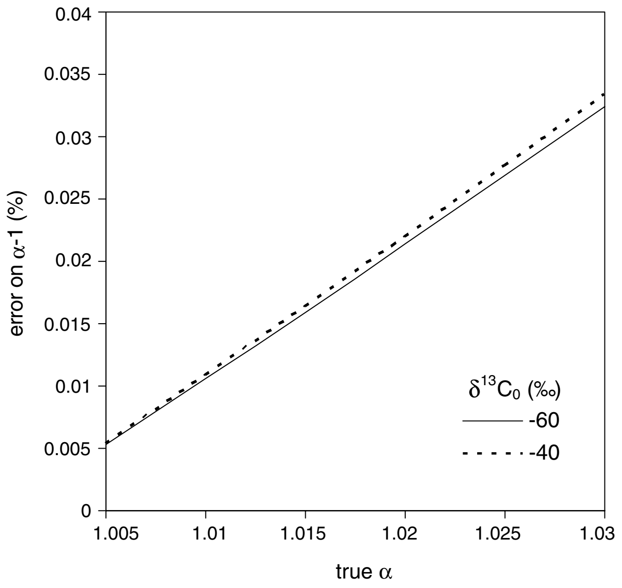

Fig. 2. Influence of the approximation of the Rayleigh model on the parameter estimation of α for C fractionation.

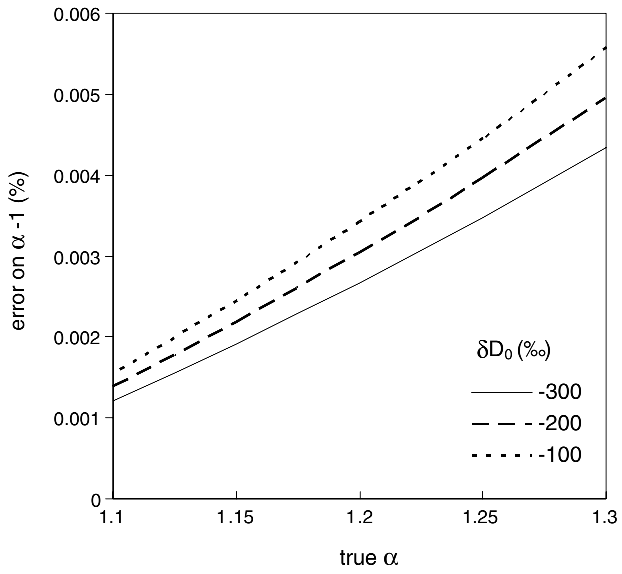

Fig. 3. Influence of the approximation of the Rayleigh model on the parameter estimation of α for H fractionation.

4.1.2. Coleman model

Again, a parameter estimation can be performed on virtual data generated by the exact model.

The results are shown in Figs. 4 and 5. The difference decreases as α or $\delta^{13}C_0$ increases. In the case of C fractionation the error can be up to 5% (Fig. 4). In the case of H fractionation the differences are larger and under some conditions exceed 20% (Fig. 5). This can be explained by the more negative δD_0 for hydrogen. For some combinations of α and δD_0 the error vanishes (Fig. 5).

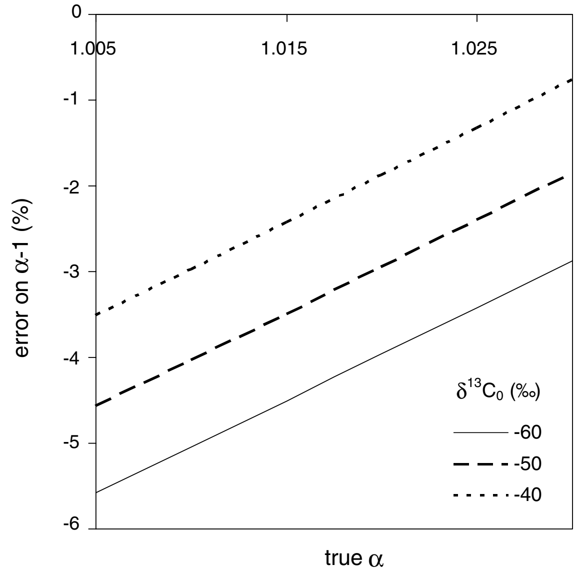

Fig. 4. Influence of the approximations in the model of Coleman et al. (1981) on the parameter estimation of α for C fractionation.

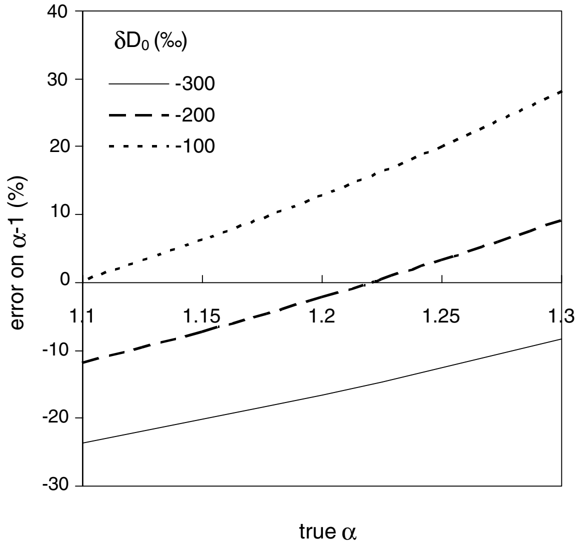

Fig. 5. Influence of the approximations in the model of Coleman et al. (1981) on the parameter estimation of α for H fractionation.

4.2. Illustrative parameter estimations

To illustrate the simulation results some parameter estimations were done with the different approximated models described above on data from actual batch experiments.

In Tables 1 and 2 the results of parameter estimations for experiments with soil of the landfill of Armhoede and Hooge Maey are shown. The fractionation factor was estimated with an errors-in-variables method and also with a simple linear regression where all error is attributed to either $\delta^{13}C$ or CH_4 to check if it was necessary to apply the errors-in-variables method.

The errors made by approximating the CH_4 concentration by the ^{12}C concentration are smaller than 0.05%. This justifies the use of the simplified Rayleigh approach. However, Hunkeler (2002) concluded that this approximation can only be used for isotopes at natural abundance level and not for studies with labelled compounds.

The approximation of Coleman et al. (1981) results in errors of up to 3%. For both experiments the α for C fractionation calculated with the model of Coleman et al. (1981) lies within the confidence interval of the α calculated with the model of Rayleigh, but α is systematically underestimated. Consequently, systematic use of the Coleman model can potentially lead to a systematic overestimation of CH_4 oxidation in landfill cover soils.

Mariotti et al. (1981) compared the same approximation of Coleman et al. (1981) with the simplified Rayleigh approach, but for N fractionation. In the study of Mariotti et al. (1981), the differences between the models where of the same magnitude as for C fractionation in this study but the confidence interval was larger.

In this study the experiments consisted of 5 samples at most. If more samples are taken or more experiments in the same conditions are carried out, the confidence interval will be smaller and as a result, the differences between the models will become more statistically significant.

The result of the errors-in-variables method approaches the result obtained when it is assumed that all error is in the $\delta^{13}C$ measurements. This could be expected because the errors on the $\delta^{13}C$ measurements relative to the range observed in an experiment are larger than the errors in the CH_4 concentration. For example for Hooge Maey:

$$\frac{\sigma_{\delta^{13}C}}{(\delta^{13}C_0, \delta^{13}C_t)} = \frac{0.6\text{‰}}{45\text{‰}} = 0.013$$

$$\frac{\sigma_{\ln CH_4}}{(\ln CH_{4,0}, \ln CH_{4,T})} = \frac{\frac{1}{CH_4}\sigma_{CH_4}}{2.7} = 0.006$$

The numerators of the latter equation are based on the assumption that the errors are sufficiently small to apply differential calculus: $d(\ln CH_4) = d(CH_4)/CH_4$. Based on analysis of simulated data, Scott et al. (2004) do not recom-

Table 1
Calculated α_C values, 95% confidence interval half-width and difference between $\alpha - 1$ values calculated with different models for data from Hooge Maey

Dependent variable	Rayleigh	Simplified Rayleigh	Coleman	Difference (%)[a]	
				Simplified Rayleigh	Coleman
$\delta^{13}C$	1.01731 ± 0.00052	1.01731 ± 0.00064	1.01709 ± 0.00057	0.040	−1.3
$\ln CH_4$	1.01732 ± 0.00052	1.01733 ± 0.00064	1.01710 ± 0.00057	0.020	−1.3
Both variables	1.01731 ± 0.00051	1.01732 ± 0.00051	1.01709 ± 0.00046	0.020	−1.3

[a] Difference between approximated models and Rayleigh model (% on $\alpha - 1$).

Table 2
Calculated α_C values, 95% confidence interval half-width and difference between $\alpha - 1$ values calculated with different models for data from Lochem

Dependent variable	Rayleigh	Simplified Rayleigh	Coleman	Difference (%)[a]	
				Simplified Rayleigh	Coleman
$\delta^{13}C$	1.0189 ± 0.0018	1.0189 ± 0.0020	1.0184 ± 0.0019	0.023	−3.0
$\ln CH_4$	1.0190 ± 0.0020	1.0190 ± 0.0020	1.0184 ± 0.0019	0.021	−3.0
Both variables	1.0189 ± 0.0019	1.0189 ± 0.0019	1.0184 ± 0.0018	0.020	−3.0

[a] Difference between approximated models and Rayleigh model (% on $\alpha - 1$).

mend the errors-in-variables method. However, the analysis of Scott et al. (2004) corresponds to making assumptions on the values of $\sigma_{\delta^{13}C}$ and σ_{CH_4} (e.g. $\sigma_{\delta^{13}C} = \sigma_{CH_4}$ or $\sigma^2_{\delta^{13}C}\sigma^2_{CH_4} = \sum(\delta^{13}C - \overline{\delta^{13}C})^2/\sum(CH_4 - \overline{CH_4})^2$), whereas our analysis is based on independent measurements of $\sigma_{\delta^{13}C}$ and σ_{CH_4}. When independent measurements of $\sigma_{\delta^{13}C}$ and σ_{CH_4} are available, the errors-in-variables method is the preferred method.

For the dataset of Hooge Maey and Lochem, the difference between the fractionation factor when all error is associated with $\delta^{13}C$ and the assumption that all error is in $\ln CH_4$ is small (<0.1% based on $\alpha - 1$) but larger than the difference between the simplified Rayleigh approach and the exact Rayleigh equation. In this case it is better to apply an errors-in-variables method or to assign all error to the $\delta^{13}C$ measurements even if only the simplified Rayleigh approach is used.

The fractionation factors of two datasets with δD measurements from Coleman et al. (1981), Snover and Quay (2000) were also recalculated. The measurement errors of the dataset of Coleman et al. (1981) were unknown so the error in variables method could not be applied to these data. In Tables 3 and 4 only the two simple regression methods are shown. From the data of Snover and Quay (2000), α must be calculated analytically because only two isotope measurements were performed (Table 5). Snover and Quay (2000) repeated the experiments several times so the fractionation factors in Table 5 are mean values.

The approximation of Coleman et al. (1981) gives larger errors for H fractionation than for C fractionation, which is consistent with the simulation experiments. The differences between the approximation of Coleman et al. (1981) and the exact Rayleigh model are up to 15% (Tables 3–5), which is unacceptable for a good quantification of isotope fractionation. Again the fractionation factor calculated with the simplified Rayleigh approach is close to the one calculated with the original Rayleigh model.

For the experiments of Coleman et al. (1981), Snover and Quay (2000), the difference between assuming all error in $\ln CH_4$ or in the $\delta^{13}C$ measurements is small like in parameter estimates for Hooge Maey and Lochem (Tables 1 and 2).

Note that the confidence interval for the dataset of Coleman with culture A at 26 °C is very large (see Table 4). In this experiment only three points were plotted in the graphs of Coleman et al. (1981), which is not sufficient to accurately estimate the error of α.

The consequences of the errors in the approximations for the quantification of CH_4 oxidation can be calculated

Table 3
Calculated α_C values and 95% confidence interval half-width with different models for data from Coleman et al. (1981)

Sample	Dependent variable	Rayleigh	Simplified Rayleigh	Coleman	Difference (%)[a]	
					Simplified Rayleigh	Coleman
A 26 °C	$\delta^{13}C$	1.0243 ± 0.0012	1.0243 ± 0.0014	1.0239 ± 0.0014	0.053	−1.4
	$\ln CH_4$	1.0243 ± 0.0013	1.0243 ± 0.0014	1.0240 ± 0.0014	0.027	−1.4
B 11.5 °C	$\delta^{13}C$	1.01322 ± 0.00033	1.01322 ± 0.00053	1.01283 ± 0.00054	0.022	−3.0
	$\ln CH_4$	1.01323 ± 0.00034	1.01323 ± 0.00053	1.01284 ± 0.00054	0.014	−2.9

[a] Difference between approximated models and Rayleigh model (% on $\alpha - 1$).

Table 4
Calculated α_D values and 95% confidence interval half-width with different models for data from Coleman et al. (1981)

Sample	Dependent variable	Rayleigh	Simplified Rayleigh	Coleman	Difference (%)[a]	
					Simplified Rayleigh	Coleman
A 26 °C	$\delta^{13}C$	1.33 ± 0.18	1.33 ± 0.18	1.281 ± 0.033	0.38	−15
	$\ln CH_4$	1.33 ± 0.44	1.33 ± 0.18	1.281 ± 0.033	0.0045	−15
B 11.5 °C	$\delta^{13}C$	1.1404 ± 0.0074	1.141 ± 0.012	1.1230 ± 0.0075	0.40	−12
	$\ln CH_4$	1.1413 ± 0.0087	1.141 ± 0.012	1.1232 ± 0.0075	0.0018	−13
B 26 °C	$\delta^{13}C$	1.305 ± 0.019	1.307 ± 0.025	1.2687 ± 0.0063	0.77	−12
	$\ln CH_4$	1.308 ± 0.022	1.308 ± 0.025	1.2687 ± 0.0063	0.0041	−13

[a] Difference between approximated models and Rayleigh model (% on $\alpha - 1$).

Table 5
Calculated α_C and α_D values and 95% confidence interval half-width with different models for data from Snover and Quay (2000)

Sample	Rayleigh	Simplified Rayleigh	Coleman	Difference (%)[a]	
				Simplified Rayleigh	Coleman
WSU site 1					
α_C	1.0162 ± 0.0040	1.0162 ± 0.0040	1.0155 ± 0.0038	0.017	−4.4
α_D	1.088 ± 0.010	1.088 ± 0.010	1.0807 ± 0.0080	0.0013	−7.8
WSU site 2					
α_C	1.0174 ± 0.0046	1.0174 ± 0.0046	1.0167 ± 0.0044	0.019	−4.3
α_D	1.125 ± 0.013	1.125 ± 0.013	1.117 ± 0.015	0.018	−6.3
ARB					
α_C	1.01786 ± 0.00019	1.01786 ± 0.00019	1.01709 ± 0.00014	0.019	−4.3
α_D	1.069 ± 0.010	1.069 ± 0.010	1.064 ± 0.010	0.0010	−7.7

[a] Difference between approximated models and Rayleigh model (% on $\alpha - 1$).

with Eq. (2). If $\alpha_{trans} = 1$, the relative error in the estimated CH_4 oxidation percentage will be the same as the relative difference given in Tables 1 and 2.

However, if $\alpha_{trans} = 1.01$, which is realistic following De Visscher et al. (2004), the relevant error is the relative error of $\alpha - 1.01$, which will be more than two times the relative error of $\alpha - 1$ when C fractionation is used for the determination. So even for C fractionation the Coleman model can lead to unacceptable inaccuracies in the determination of α.

One could argue that even an error of 20% on CH_4 oxidation estimates is acceptable, given the fact that the natural variability of landfill gas fluxes and CH_4 oxidation is usually greater than 20%. However, the errors reported here are systematic. Unlike the random variation encountered in ecosystem gas exchange measurements, systematic errors due to incorrect data analysis do not diminish by averaging large numbers of measurements.

4.3. The validity of the simplified Rayleigh approach

In all of the examples discussed so far the simplified Rayleigh approach turned out to be a valid method for α determination. It would be useful to test the range of validity of this approach when labelled compounds (CH_4 or others) are used. A set of simulations similar to the ones discussed in Section 4.1.1. was performed with varying values of R_{st}, α, δI_0 (I = isotope) and the concentration range of the degrading compound in the batch experiment. For each simulation, α was calculated with the simplified Rayleigh approach, and compared with the set value. Two general observations were made. First, the concentration range had a limited influence on the error on α. The error increased with increasing concentration range (i.e., with decreasing final concentration). All further calculations were based on a final concentration of 1/16 of the initial concentration, consistent with the approach in Section 3.5. As most experiments described in the literature used a more narrow concentration span, we consider this to be a worst-case scenario. Second, it was observed that the error on α was independent of R_{st} if the initial heavy isotope fraction, $F\%$ (expressed as % of total compound), was kept constant. The maximum value of $F\%$ to obtain a set error of ε% (expressed as % of $\alpha - 1$) was determined. Fig. 6 shows the maximum allowable value of $F\%$ as a function of $\alpha - 1$, to stay within a set value of ε%. The range of validity of the simplified Rayleigh approach is strongly dependent on α. In strongly fractionating systems even a limited amount of labelled compound can lead to errors of 1% or more, whereas the simplified Rayleigh approach is always valid in weakly fractionating systems. As a rule, the error expressed as % of $(\alpha - 1)$ never exceeds $100(\alpha - 1)$. In Fig. 6 it can be observed that a change of ε% leads to a vertical displacement of the curve. From this the following empirical relationship for the calculation of F% was developed:

$$\ln(F\%) = \ln(\varepsilon\%) - 0.756337 - 1.4352\ln(\alpha - 1) - 0.084315(\ln(\alpha - 1))^2 - 0.00542(\ln(\alpha - 1))^3 \qquad (20)$$

Using Eq. (20) the necessity to use the exact Raleigh approach can be determined for any given batch system based on any given isotope.

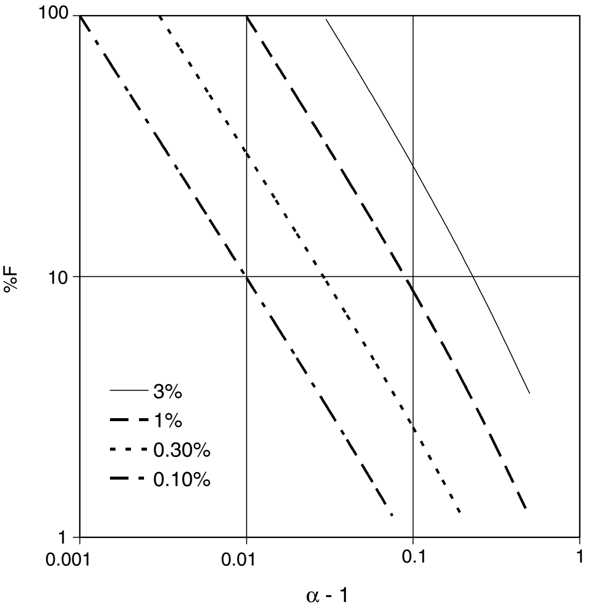

Fig. 6. Maximum allowable initial heavy isotope percentage (%F) in a labelled batch fractionation experiment versus $\alpha - 1$ for different values of the allowable error (expressed as % of $\alpha - 1$).

5. Conclusions

In this study the original model of Rayleigh for determining the fractionation factor α of methane oxidation was compared with the two most common approximations, the simplified Rayleigh approach and the Coleman method.

The differences caused by the simplified Rayleigh approach are small (<0.05% for C, <0.006% for H), so this approach can be considered valid when the experiment is performed with unlabeled methane. However, the further simplifications made by Coleman et al. (1981) led to large errors, especially for H fractionation (up to 5% for C, up to 20% for H).

Simulation results (Figs. 2–5) were confirmed with experimental data (Tables 1–4).When errors are assigned to both variables, the difference with the assumption that all error is in the CH_4 concentration is small (<0.1%) but can be larger than the error made by the simplified Rayleigh approach.

The advantage of the equation of Coleman et al. (1981) and the simplified Rayleigh approach is that α can be estimated by simple linear regression. However, nowadays it is perfectly feasible to use non-linear parameter estimation. In the case of labelled systems, this is especially important when the fractionation is strong. In contrast, the simplified Rayleigh approach is always valid when fractionation is weak.

Appendix A

This Appendix outlines the solution of the differential equation:

$$\frac{d^{13}C}{d^{12}C} = \frac{k_{13}}{k_{12}} \cdot \frac{^{13}C}{^{12}C} \tag{3}$$

Introducing, $\alpha = \frac{k_{12}}{k_{13}}$, and rearranging leads to:

$$\alpha \frac{d^{13}C}{^{13}C} = \frac{d^{12}C}{^{12}C}$$

If α is constant, both sides of the equation can be integrated from initial concentration ${}^{i}C_0$ ($i = 12, 13$) to final concentration ${}^{i}C$:

$$\alpha \ln\left(\frac{^{13}C}{^{13}C_0}\right) = \ln\left(\frac{^{12}C}{^{12}C_0}\right)$$

Taking the exponential of both sides, and rearranging, leads to:

$$\frac{^{13}C/^{12}C}{^{13}C_0/^{12}C_0} = \left(\frac{^{12}C}{^{12}C_0}\right)^{\frac{1-\alpha}{\alpha}} \tag{4}$$

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