Pergamon

0038-0717(95)00149-2

Soil Biol. Biochem. Vol. 28, No. 3, pp. 393–398, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0038-0717/96 $15.00 + 0.00

POTENTIAL DENITRIFICATION ACTIVITY ASSAY IN SOIL—WITH OR WITHOUT CHLORAMPHENICOL?

MIKAEL PELL,* BO STENBERG, JOHN STENSTRÖM and LENNART TORSTENSSON

Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, 750 07, Uppsala, Sweden

(Accepted 17 October 1995)

Summary—A common way to characterize denitrification in soil is to determine the potential denitrifying activity (PDA). Our objectives were to compare different techniques of mathematically describing experimental data obtained in the PDA assay, both with and without use of chloramphenicol (CAP), and to quantify the effect of CAP on the process. The PDA assay was carried out in the presence of acetylene in slurries of three agricultural soils containing 1 mM glucose and 1 mM KNO_3. When CAP was not used in the assay, growth related curves of N_2O-formation were obtained for all three soils. These data were used to calculate the initial rate by: (1) assuming the initial phase to be linear and using the four first data points for linear regression; and by (2) using a growth-associated product formation equation. The good fit to the data that was obtained with the latter method suggests that PDA is a continuous process without distinct phases. Moreover, our results clearly show that denitrifying activity is inhibited by CAP even at the lowest concentration tested, 20 mg l^{-1}. The inhibiting effect increased with increasing concentrations of CAP. The PDA was 17–42% lower at 1 g CAP l^{-1} compared with assays without CAP. This shows that not only synthesis of new enzymes is affected but also that the activity of already existing enzymes is decreased. Results from our study strongly suggest that single concentrations of CAP must not be used in PDA assays. An alternative strategy could be to use multiple CAP concentrations and then extrapolate to the rate at 0 g CAP l^{-1}. However, we recommend assays without CAP and that data should be fitted to the growth-associated product formation equation. By using this method, values of the PDA and the growth rate of the denitrifying bacterial population are objectively obtained.

INTRODUCTION

A common way of characterizing denitrification in soil is to determine the potential denitrifying activity (PDA) which reflects the amount of denitrifying enzymes in the system. The general idea of PDA is to optimize the conditions so that only the amount of denitrifying enzymes will be rate-limiting for the process. This can be done by using a soil slurry to eliminate problems with limiting substrate diffusion (Myrold and Tiedje, 1985; Ambus and Christensen, 1993) and by incubating the slurry anaerobically with additions of optimal amounts of a terminal electron acceptor, usually NO_3^-, and an easily-available C and energy source, such as glucose.

One reason for the popularity of the PDA assay is that the technique can easily be performed in the laboratory. Moreover, by altering the environmental conditions, rate-limiting factors for the process can be determined (Parkin *et al.*, 1985; Peterjohn, 1991; Klingensmith and Van Cleve, 1993) and denitrifying mechanisms elucidated. The PDA has been used to describe the temporal and spatial variability of the process on a field scale (Christensen *et al.*, 1990; Parsons *et al.*, 1991) and has been included as a variable in denitrification models (Bergstrom and Beauchamp, 1993). Seasonal variations and correlation to field measurements of denitrification losses of N have been investigated (Pinay *et al.*, 1993; Schipper *et al.*, 1993). Moreover, the microenvironment has been investigated by determination of PDA in size fractions of soil aggregates (Lensi *et al.*, 1995). The method has also been used to evaluate effects of heavy metals (Bradley and Chapelle, 1993), organic solvents (Yeomans and Bremner, 1989) and pesticides (Bollag and Kurek, 1980) on denitrification.

The most widely used and accepted method today for assessing PDA is the one originally proposed by Smith and Tiedje (1979) and then further formalized in a procedure developed by Tiedje *et al.* (1989). In this acetylene-based method, chloramphenicol (CAP) is used to inhibit synthesis of new enzymes, thereby prolonging the constant product formation rate of N_2O. The rationale behind the CAP technique was to develop a simple and fast method to establish the initial production rate. However, there are reports showing that CAP not only inhibits synthesis of new enzymes but also decreases the activity of existing enzymes (Pell *et al.*, 1991; Brooks *et al.*, 1992; Wu and Knowles, 1995). This dilemma could be avoided by excluding CAP from the PDA assay and by a

*Author for correspondence.

Table 1. Some physical and chemical characteristics of the three soils used

Soil	pH_{H2O}	Dry weight (%)	CEC (mequiv 100 g^{-1})	Organic matter (%)	Clay (%)
Mellby	5.9	89.1	18.0	3.9	9.9
Lanna	7.1	87.9	26.7	4.4	29.4
Ekhaga	6.9	70.4	33.2	8.1	57.0

description of the growth-related and non-linear product formation data then generated.

Our objectives were to compare different techniques of mathematically describing experimental data in the PDA assay, both with and without the use of CAP, and to quantify the effect of CAP on the process.

MATERIALS AND METHODS

Soils

For the experiments, a sandy soil (Mellby), a silty clay (Lanna) and a very heavy clay soil (Ekhaga) were used (Table 1). Fresh soils were sieved (4 mm) and stored airtight at -20 ± 2°C until used.

Potential denitrifying activity (PDA)

The PDA assay was performed mainly according to the C_2H_2 inhibition method described by Smith and Tiedje (1979) and modified by Tiedje *et al.* (1989). Thawed and mixed soil portions of 25 g were placed in 250-ml Duran-flasks and made into slurries by adding 25 ml of a substrate solution containing 1 mM glucose, 1 mM KNO_3 and various concentrations of CAP ranging from 0 to 3.0 g l^{-1}. A manifold system with 9 outlets was used to evacuate (− 100 kPa) and flush the flasks with N_2 (+ 100 kPa) 4 times. After venting the head space to atmospheric pressure, C_2H_2 was injected to a partial pressure of 10 kPa to inhibit the N_2O reductase. Slurries were incubated at 24°C on a rotary shaker at 225 rev min^{-1} for 380–610 min. Head-space samples of 0.5 ml were withdrawn every 30–60 min. The samples were transferred to 10-ml gastight glass vials and stored until analysis of N_2O.

Analysis of N_2O

The N_2O was analysed on a gas chromatograph (Chrompak CP 9000) equipped with a Poraplot Q wide-bore capillary column (25 m × 0.53 mm) and a ^{63}Ni electron-capture detector. The injection temperature was 125°C, the oven temperature 38°C and the detector temperature 350°C. The carrier gas (He) and the make-up (Ar 95% and CH_4 5%) flow rates were 6 and 37 ml min^{-1}, respectively. Concentrations of N_2O were corrected for the amount dissolved in the liquid using a Bunsen coefficient of 0.571 (Tiedje, 1982).

Calculation of PDA

Regressions were made using the commercially-available software Sigma Plot for Windows ver. 2.0 (Jandel Scientific).

When N_2O production was linear, data were fitted to product equation (1):

$$p = p_0 + qN_0t; \quad (1)$$

$$\frac{dp}{dt} = qN_0. \quad (1')$$

When the rate of product formation increased over time, data were fitted by non-linear regression to product equation (2) that takes exponential growth into consideration (Stenström *et al.*, 1991):

$$p = p_0 + \frac{qN_0}{\mu}(e^{\mu t} - 1); \quad (2)$$

$$\frac{dp}{dt} = qN_0e^{\mu t}. \quad (2')$$

In equations (1) and (2), p is the amount of product at time t, p_0 is the amount of product at the start of the incubation, q is the specific enzyme activity, N_0 is the number of bacteria at the start and μ is the specific growth rate constant. According to equations (1′) and (2′), the initial denitrification rate equals qN_0 when $t = 0$.

Adsorption of CAP

Adsorption of CAP to the soil particles was analysed in Ekhaga soil slurries with the same amounts of soil and water as those used for the PDA assay. After CAP additions ranging from 0.02 to 2.5 g l^{-1} and 30-min incubation on a rotary shaker at 225 rev min^{-1}, samples were collected and centrifuged at 4000 rev min^{-1} for 10 min. The short time for equilibration (30 min) was chosen in order to get information on how much CAP was adsorbed during the initial part of a PDA assay. Dissolved CAP was determined by reverse-phase liquid chromatography (Waters WISP 710B). The UV detector (Waters 490E) was adjusted to 276 nm. A radial PAK NOVA PAK C18 column (100 × 8 mm) was used. The eluent was 0.1 M methanol–water (50 : 50) and the flow rate was 1.0–1.5 ml min^{-1}. Adsorbed CAP was calculated as the difference between total and dissolved CAP. The data were fitted to the Freundlich adsorption equation:

$$\frac{x}{m} = KC_s^{1/n}, \quad (3)$$

where x / m is the amount of CAP adsorbed per amount of soil, K is the adsorption constant, C_s is the

Table 2. Potential denitrification rates in four soils calculated with different methods

Method	Mellby	Lanna	Ekhaga	Brookston*
	($ng\ N_2O-N\ g^{-1}$ dw min^{-1})			
Without CAP				
A. Initial rate from linear regression	4.9 ± 0.8 (0.951)†	8.7 ± 0.6 (0.990)	50 ± 3‡ (0.993)	7.5 ± 0.3 (0.996)
B. Initial rate from product equation (2)	6.6 ± 0.6 (0.994)	8.6 ± 0.6 (0.992)	45 ± 3‡ (0.997)	7.2 ± 0.2 (1.000)
With CAP				
C. Zero-order kinetics with 1 g CAP l^{-1}	5.5 ± 0.2 (0.994)	6.6 ± 0.1 (0.997)	29 ± 1 (0.998)	ND§
D. Rate vs CAP relation interpolated to 1 g CAP l^{-1}	5.0 ± 0.0 (0.934)	5.4 ± 0.1 (0.568)	30 ± 0 (0.890)	ND
E. Rate vs CAP relation extrapolated to 0 g CAP l^{-1}	5.9 ± 0.1 (0.934)	6.3 ± 0.4 (0.568)	34 ± 0 (0.998)	ND

*Calculated from data reported by Smith and Tiedje (1979, in their Fig. 2).
†Regression parameter value ± SE (n = 1), and coefficient of determination (R^2) within parentheses.
‡Numbers of replicates, n = 3.
§ND not determined.

concentration of CAP in the solution and n is a constant.

RESULTS

The initial rates of PDA were calculated using five different methods, A–E (Table 2).

When CAP was not used in the assay, non-linear curves were obtained for all three soils [Figs 1(a), 2(a) and 3(a)]. These data were used to: (Method A) calculate the initial rate by assuming the initial phase to be linear and using the 4 first data points for linear regression; and (Method B) calculate qN_0 by equation (2). The highest activity (min^{-1}) was found in the Ekhaga soil, (45–50 ng N_2O-N g^{-1} dw) followed by the Lanna soil (8.6–8.7 ng N_2O-N g^{-1} dw) and the Mellby soil (4.9–6.6 ng N_2O-N g^{-1} dw). The specific growth rates (μ) obtained by the product equation (2) were 0.084 ± 0.013 h^{-1} (numbers of replicates, n = 3), 0.079 ± 0.016 h^{-1} (n = 1) and 0.070 ± 0.020 h^{-1} (n = 1) (parameter value ± SE) for the Ekhaga, the Lanna and the Mellby soils, respectively.

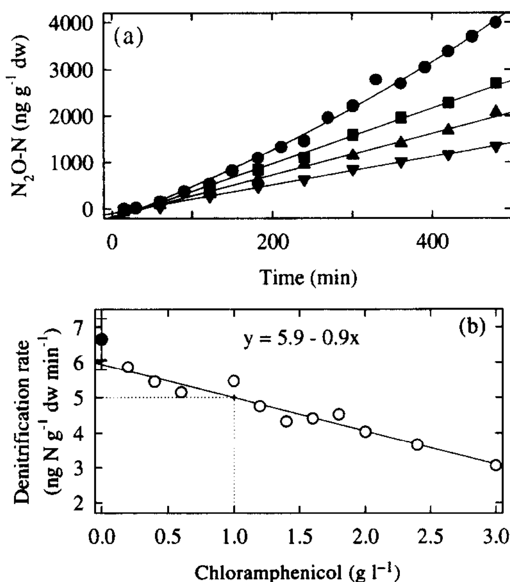

Fig. 1. Potential denitrifying activity in the Mellby soil. (a) Production of N_2O vs time at 0 (●), 0.2 (■), 1.4 (▲) and 3.0 (▼) g CAP l^{-1}. (b) Initial rate (qN_0) vs CAP concentration with the qN_0 value ± SE(1) as obtained from equation (2), and SE for the y-intercept. Dotted line shows the interpolated rate at 1 g CAP l^{-1}.

When CAP was used at 1 g l^{-1} in the medium, zero-order kinetics were obtained. With one exception, linear regression of these data (Method C) resulted in lower qN_0 values than those obtained from the assays excluding CAP (Table 2). The exception was the initial rate obtained from linear regression of the first 4 data points in the Mellby soil. Excluding this result, the rates were 24–42% lower with CAP than without it and the 4 first data points used for linear regression, and 17–36% lower than those calculated from product equation (2).

Excluding CAP results in bacterial growth during the assay, while high concentrations of CAP in the medium totally inhibit growth. However, concentrations of CAP in the range from 20 to 120 mg l^{-1} resulted in an initial linear phase of about 100 min, after which *de novo* synthesis of reduction enzymes and growth seemed to start [Fig. 3(a)]. When these low concentrations of CAP were used, qN_0 was calculated by linear regression of the initial phase, while at higher concentrations the whole data set was included in the linear regression. Results from all soils showed a strong negative, linear correlation with increasing concentration of CAP in the medium [Figs 1(b), 2(b) and 3(b)]. The regression parameters obtained were also used to derive qN_0 by interpolation at 1 g l^{-1} CAP (Method D). Moreover, the intercept of the resulting regression line (Method E) could represent qN_0 at 0 g l^{-1} CAP. The values so obtained by extrapolation were 11–27% lower for all soils compared with when equation (2) was applied to data obtained without use of CAP (Table 2).

The constants, K and n in the Freundlich equation (3) were 1.2 l kg^{-1} dry soil and 1.1, respectively (Fig. 4). Thus, adsorption of CAP to the Ekhaga soil after 30 min was low and almost linear.

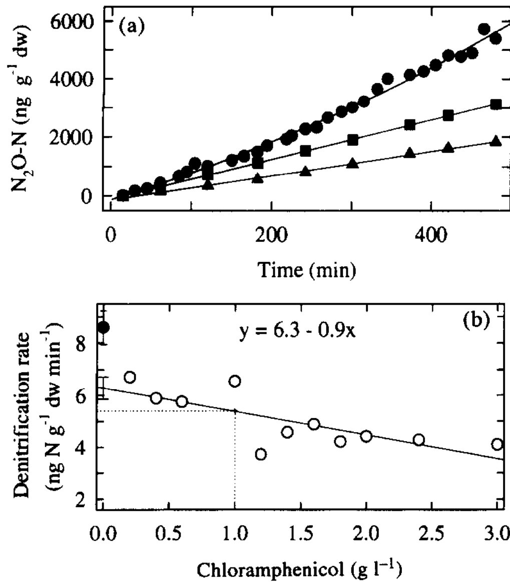

Fig. 2. Potential denitrification activity in the Lanna soil. (a) Production of N_2O vs time at 0 (●), 0.2 (■) and 3.0 (▲) g CAP l^{-1}. (b) Initial rate (qN_0) vs CAP concentration with the qN_0 value ± SE(1) as obtained from equation (2), and SE for the y-intercept. Dotted line shows the interpolated rate at 1 g CAP l^{-1}.

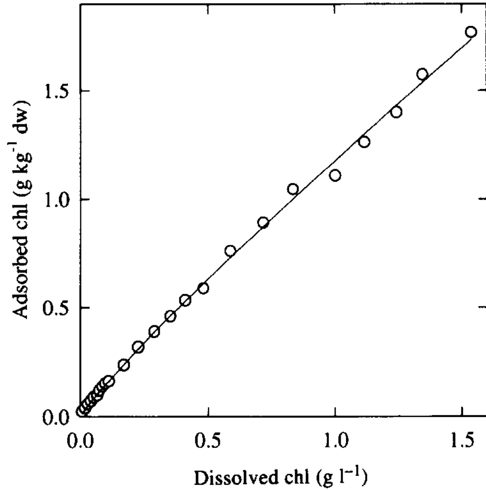

Fig. 4. Adsorption of chloramphenicol in the Ekhaga soil slurries. The line represents data fitted to the Freundlich adsorption equation (3).

DISCUSSION

There are principally three techniques adopted in the literature to overcome problems with interpretation of the non-linear curves generated in PDA measurements: (1) defining a certain period for incubation and only taking start and end samples for analysis of the product (Drury *et al.*, 1991), thus, the shape of the N_2O production curve is ignored; (2) using short incubations and approximating this period as zero-order kinetics (Erich *et al.*, 1984); and (3) using CAP to inhibit *de novo* synthesis of enzymes and, thus, prolonging the linear product formation phase (Smith and Tiedje, 1979).

The first technique gives seriously biased results and has therefore not been considered here.

In our study, the second technique (Method A) gave the highest rates (Table 2). This is also the case for the Brookston loam soil as calculated from the data reported by Smith and Tiedje (1979) (Fig. 5 and Table 2). The low value for the Mellby soil was

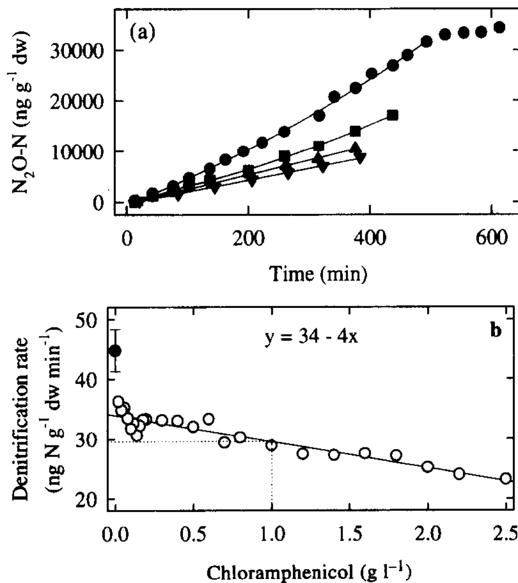

Fig. 3. Potential denitrifying activity in the Ekhaga soil. (a) Production of N_2O vs time at 0 (●), 0.02 (■), 0.7 (▲) and 2.5 (▼) g CAP l^{-1}. (b) Initial rate (qN_0) vs CAP concentration with the qN_0 value ± SE(1) as obtained from euation (2), and SE for the y-intercept. Dotted line shows the interpolated rate at 1 g CAP l^{-1}.

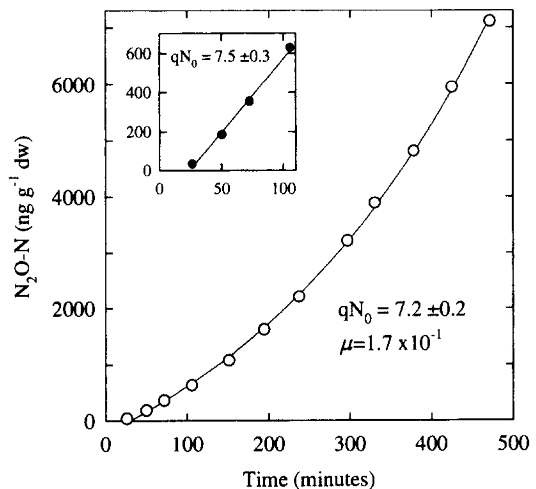

Fig. 5. Potential denitrification activity in Brookston loam soil data from Smith and Tiedje (1979, in their Fig. 2). The line represents data fitted to equation (2) and the inserted figure shows the initial phase approximated with zero-order kinetics. Values given are calculated initial rates (ng N_2O-N g^{-1} dry soil min^{-1}) and specific growth rate constant, μ (h^{-1}).

probably the result of an outlier. If one of the two first data points included in the 4-point regression of the Mellby soil is excluded, a considerably higher rate is obtained. This shows the fragility of the approach when a few subjectively chosen data points are used to calculate initial rates.

Smith and Tiedje (1979) divide the PDA course without CAP into two phases. They interpret phase I as the initial period with linear production of N_2O resulting from the activity of already existing enzymes, and the non-linear phase II as a result of growth and the synthesis of new enzymes. However, no objective method is given for finding the transition from phase I to phase II. In our study we used not only the initial phase but also the whole data set and fitted it to equation (2) that describes growth-associated product formation (Stenström *et al.*, 1991). The good fit of the data to product equation (2) obtained in our study (Table 2) suggests that PDA is a continuous process without distinct phases. This is also valid for the data from the Brookston loam soil assayed without CAP (Fig. 5 and Table 2). Besides an objectively-derived and biologically-relevant qN_0 value, the product formation equation also yields information on the growth rate, μ, of the denitrifying bacterial population. Martin *et al.* (1988) reported a specific cell denitrifying activity (q) of 1.7×10^{-16} g N $cell^{-1}$ min^{-1} at 0.1 g CAP l^{-1}. According to Figs 1(b), 2(b) and 3(b), the denitrifying enzyme activity (qN_0) was lowered by 12–28% at 0.1 g CAP l^{-1} compared to when no CAP was used. Hence, if the value for q (Martin *et al.*, 1988) is corrected for the CAP effect the estimated number of denitrifying bacteria (N_0) in our soils range from 3×10^7 to 2×10^8 cells g^{-1} dry soil. The μ value probably reflects the fastest growing subpopulation in the system. The growth rate constants obtained (0.070–0.084 h^{-1}) correspond to generation times of about 10 h. When using the data reported by Smith and Tiedje (1979), a μ value of 0.17 h^{-1} corresponding to a generation time of 4 h was obtained. Robert *et al.* (1990) found doubling times of 1.2–3.7 h for denitrifying bacteria isolated from different soils grown in NO_3 broth under anaerobic conditions. When these bacteria were inoculated into sterile soil, the growth rate generally decreased and when inoculated into a non-sterile soil it decreased further. However, it was not possible to predict which organism would grow best in non-sterile soil from the results of growth in sterile soil or media. Denitrifiers are found within most taxonomical or physiological groups of soil bacteria (Zumft, 1992). Most frequently found are bacteria of the genera *Pseudomonas*, *Alcaligenes* and *Bacillus*. Thus, the product formation equation gives two parameters, qN_0 describing the total activity of existing enzymes and μ describing the growth and synthesis of new enzymes. This may provide an effective tool when evaluating effects of toxic chemicals on denitrifying bacteria in soil, which in turn probably are good representatives of the bacterial community.

Technique 3 above (Method C), probably is the most widely used technique for assessing PDA. Our results clearly show that the denitrifying activity is inhibited by CAP even at the lowest concentration tested, 20 mg l^{-1}. The adsorption of CAP to soil particles in a very heavy clay soil such as Ekhaga is low during the initial part of the assay. However, it is not obvious whether it is the adsorbed CAP, the CAP in the solution, or both that is the active inhibitory substance. The almost linear adsorption of CAP means that the shapes of the curves in Figs 2 and 3 would not change if data were plotted vs adsorbed or dissolved amounts of CAP instead of the total amounts added.

The inhibiting effect increases with increasing concentrations of CAP. This shows that not only the synthesis of new enzymes is affected (Smilack *et al.*, 1991) but also the activity of enzymes already existing is decreased. Other reported effects of CAP are the inhibition of glutamate dehydrogenase activity in plant cells (Sengar and Srivastava, 1992), but no effect on the NO_3 reductase was observed (Queiroz *et al.*, 1991). Moreover, CAP induces transcription of the major cold shock gene of *Escherichia coli* (Jiang *et al.*, 1993) and Tocher *et al.* (1994) showed that the reduction products of CAP are reactive with DNA bases, which may cause DNA damage. The effect of CAP on existing denitrifying enzyme activity was reported for soils by Pell *et al.* (1991), and for aquifer sediments and denitrifying groundwater bacteria by Brooks *et al.* (1992). Wu and Knowles (1995) showed that pure cultures of *Pseudomonas denitrificans* and *Flexibacter canadensis* are inhibited by CAP at the level of NO_3 reduction and the latter species also at the level of NO reduction. On the other hand, Dendooven *et al.* (1994) showed that the CO_2 and the N_2O production increased in soil incubated with CAP. They concluded that these effects were due to the diversion of C from protein synthesis to respiratory processes, and no effect on the activity of existing denitrification enzymes was observed. However, our results strongly suggest that single concentrations of CAP must not be used in PDA assays (Method C). An alternative strategy could be to use multiple CAP concentrations and then extrapolate to the rate at 0 g l^{-1} (Method E). The advantage of such a technique would be that linear data are easy to handle. However, the relation between qN_0 and the concentration of CAP may not be linear, especially at very low concentrations. Therefore, we recommend assays without CAP and that data should be fitted to equation (2). By using this method, values of the PDA (as qN_0) and the growth rate (as μ) of the denitrifying organisms are objectively obtained.

Acknowledgements—Financial support by the Swedish Environmental Protection Agency is gratefully

acknowledged. The work forms a part of the Integrated Soil Analysis (ISA) programme.

REFERENCES

Ambus P. and Christensen S. (1993) Denitrification variability and control in a riparian fen irrigated with agricultural drainage water. *Soil Biology & Biochemistry* **25**, 915–923.

Bergstrom D. W. and Beauchamp E. G. (1993) An empirical model of denitrification. *Canadian Journal of Soil Science* **73**, 421–431.

Bollag J. M. and Kurek E. J. (1980) Nitrite and nitrous oxide accumulation during denitrification in the presence of pesticide derivatives. *Applied and Environmental Microbiology* **39**, 845–849.

Bradley P. M. and Chapelle F. H. (1993) Arsenate inhibition of denitrification in nitrate contaminated sediments. *Soil Biology & Biochemistry* **25**, 1459–1462.

Brooks M. H., Smith R. L. and Macalady D. L. (1992) Inhibition of existing denitrification enzyme activity by chloramphenicol. *Applied and Environmental Microbiology* **58**, 1746–1753.

Christensen S., Simkins S. and Tiedje J. M. (1990) Spatial variation in denitrification—dependency of activity centers on the soil environment. *Soil Science Society of America Journal* **54**, 1608–1613.

Dendooven L., Splatt S. and Andersson J. M. (1994) The use of chloramphenicol in the study of the denitrification process: some side effects. *Soil Biology & Biochemistry* **26**, 925–927.

Drury C. F., Mckenney D. J. and Findlay W. I. (1991) Relationships between denitrification, microbial biomass and indigenous soil properties. *Soil Biology & Biochemistry* **23**, 751–755.

Erich M. S., Bekerie A. and Duxbury J. M. (1984) Activity of denitrifying enzymes in freshly sampled soils. *Soil Science* **138**, 25–342.

Jiang W., Jones P. and Inouye M. (1993) Chloramphenicol induces the transcription of the major cold shock gene of *Escherichia coil*, cspA. *Journal of Bacteriology* **175**, 5824–5828.

Klingensmith K. M. and Van Cleve K. (1993) Denitrification and nitrogen fixation in floodplain successional soils along the Tenant river, interior Alaska. *Canadian Journal of Forest Research* **23**, 956–963.

Lensi R., Clays-Josserand A. and Jocteur Monrozier L. (1995) Denitrifiers and denitrifying activity in size fractions of a Mollisol under permanent pasture and continuous cultivation. *Soil Biology & Biochemistry* **27**, 61–69.

Martin K., Parsons L. L., Murray R. E. and Smith M. S. (1988) Dynamics of soil denitrifier populations: relationships between enzyme activity, most-probable-number counts, and actual N gas loss. *Applied and Environmental Microbiology* **54**, 2711–2716.

Myrold D. D. and Tiedje J. M. (1985) Diffusional constrains on denitrification in soil. *Soil Science Society of America Journal* **49**, 651–657.

Parkin T. B., Sexstone A. J. and Tiedje J. M. (1985) Adaptation of denitrifying populations to low soil pH. *Applied and Environmental Microbiology* **49**, 1053–1056.

Parsons L. L., Murray R. E. and Smith M. S. (1991) Soil denitrification dynamics—spatial and temporal variations of enzyme activity, populations, and nitrogen gas loss. *Soil Science Society of America Journal* **55**, 90–95.

Pell M., Stenberg B. and Stenström J. (1991) Modified method for estimation of potential denitrification in soil. In *International Workshop on Methods of Research on Soil Structure / Soil Biota Interrelationships*, Wageningen, The Netherlands, 24–28 November 1991 (Abstr.).

Peterjohn W. T. (1991) Denitrification—enzyme content and activity in desert soils. *Soil Biology & Biochemistry* **23**, 845–855.

Pinay G., Roques L. and Fabre A. (1993) Spatial and temporal patterns of denitrification in a riparian forest. *Journal of Applied Ecology* **30**, 581–591.

Queiroz C. G. D. S., Alves J. D., Rena A. B. and Cordeiro A. T. (1991) The effect of chloramphenicol, propanol, pH and temperature on the *in vivo* activity of nitrate reductase in young coffee plants. *Revista Brasileira de Botanica* **14**, 73–78.

Robert E. M., Parsons L. L. and Smith M. S. (1990) Aerobic and anaerobic growth of rifampin-resistant denitrifying bacteria in soil. *Applied and Environmental Microbiology* **56**, 323–328.

Schipper L. A., Cooper A. B., Harfoot C. G. and Dyck W. J. (1993) Regulators of denitrification in an organic riparian soil. *Soil Biology & Biochemistry* **25**, 925–933.

Sengar R. S. and Srivastava H. S. (1992) Protein synthesis inhibitors and glutamate dehydrogenase activity in maize roots and shoots in light–dark conditions. *Proceedings of the National Academy of Sciences India Section B (Biological Sciences)* **62**, 419–424.

Smilack J. D., Wilson W. R. and Cockerill III F. R. (1991) Tetracyclines, chloramphenicol, erythromycin, clindamycin, and metronidazole. *Mayo Clinic Proceedings* **66**, 1270–1280.

Smith M. S. and Tiedje J. M. (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology & Biochemistry* **11**, 261–267.

Stenström J., Hansen A. and Svensson B. (1991) Kinetics of microbial growth associated product formation. *Swedish Journal of Agricultural Research* **21**, 55–62.

Tiedje J. M. (1982) Denitrification. In *Methods of Soil Analysis, Part 2. Chemical and Microbiological properties* (A. L. Page, R. H. Miller and D. R. Keeney, Eds), pp. 1011–1026. American Society of Agronomy, Madison, WI.

Tiedje J. M., Simkins S. and Groffman P. M. (1989) Perspective on measurement of denitrification in the field including recommended protocols for acetylene based methods. In *Developments in Plant and Soil Science, Vol. 39. Ecology of Arable Land. Perspectives and Challenges* (M. Clarholm and L. Bergström, Eds), pp. 217–240. Kluwer, Dordrecht.

Tocher J. H., Edwards D. I. and Thomas A. (1994) The reactivity of chloramphenicol reduction products with DNA bases. *International Journal of Radiation Oncology Biology Physics* **29**, 307–310.

Wu Q. and Knowles R. (1995) Effect of chloramphenicol on denitrification in *Flexibacter canadensis* and "*Pseudomonas denitrificans*". *Applied and Environmental Microbiology* **61**, 434–437.

Yeomans J. C. and Bremner J. M. (1989) Effects of organic solvents on denitrification in soil. *Biology and Fertility of Soils* **7**, 336–340.

Zumft W. G. (1992) The denitrifying prokaryotes. Chapter 23. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, Vol. 1 (A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K-H. Schleifer, Eds), 2nd edn, pp. 554–582. Springer-Verlag, New York.