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EFFECT OF TEMPERATURE ON DENITRIFICATION IN SOILS MEASURED BY ACETYLENE INHIBITION TECHNIQUE UNDER LABORATORY CONDITIONS

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A method to measure denitrification in soils based on the observation that in the presence of acetylene the sole product of denitrification in soil is N_2O has been established. The soil samples collected from two research stations of Denmark varying in texture were incubated under helium atmosphere with 75% water holding capacity moisture and after injection of 10% acetylene in the head space of glass bottles. The bottles so treated were kept at a range of temperature, from 10-70° in incubator. After 5 days in the gas samples taken from head space and analysed for N_2O and CO_2 to determine the rate of denitrification and respiration respectively. It was observed that denitrification increased with increasing temperature and was maximum at 50°. Denitrification was comparatively greater in sandy loam soil than coarse sand. Rate of respiration of micro-organisms was same in atmosphere with or without acetylene.

Key words: Denitrification, Temperature, Incubation.

INTRODUCTION

The process of denitrification causes losses of nitrate nitrogen from soils. Until recently the losses of nitrogen were measured through nitrogen balance sheet [1] and no method for its direct measurement was available. However Rolston and co-workers [2], worked out a direct method of measuring denitrification nitrogen losses from soils by measuring the production of N_2O. The report that acetylene inhibits further reduction of N_2O to N_2 during denitrification has been verified [3]. This has made possible a direct measurement of the rate of denitrification in soil. further studies have proved acetylene does not effect the rate of microbial activity and denitrification [2]. Thus this method is considered rapid and reliable for measuring the rate of denitrification in soils.

Numerous factors are known to affect the rate of denitrification in soils under anaerobic conditions [4]. The work of Nommik [5] indicated that temperature was an important factor for biological denitrification. The optimum temperature for biological denitrification in soils has been reported to be in the range 60-65° [6], which is surprizingly high. In order to investigate and confirm the effect of temperature on the process of denitrification being measured directly by newly developed acetylene inhibition technique, this project was planned. This research work will confirm the effect of temperature on the process and will also assess the applicability of the technique in research, concerning nitrogen losses from soils.

MATERIALS AND METHODS

A composite soil sample of sandy loam texture from Roskilde Agriculture research station and coarse sand sample from Store Jyndevad, another Agriculture experimental station of Denmark was collected from 0-20 cm surface layer. The sampling plots had been under barley cultivation over last seven years on both stations. The samples taken were stored at field moisture conditions in a polythene container at 5° until used. The analytical work was conducted in the laboratories of Department of general microbiology Royal Veterinary and Agriculture University Copenhagen Denmark. The physico-chemical characteristics of soils under study are presented in Table 1.

Air dried soils were ground and sieved through 2 mm sieve, and the equivalent of 50 g oven dry soil were weighed into 250 ml glass bottles. After addition nitrate at the rate of 100 µg N g^{-1} Y in solution the moisture level was adjusted to 75% of water holding capacity. The bottles were then sealed with sub seal stoppers, evacuated and

Table 1. Physico-chemical characteristics of soils under investigation.

Character		Samling site: Store Jyndevad	Roskilde
1. Texture	Clay	3:	10:
	Silt	4: Coarse	17: Sand
	Fine sand	19: sand	49: loam.
	Coarse sand	72:	21:
2. Organic matter (%)		2.3	2.5
3. Total Nitrogen (%)		0.11	0.18
4. pH ($Cacl_2$)		5.6	6.9
5. C.E.C. (meg/100 g soil).		9.3	14.4
6. Exchangeable K mg (100 g soil).		10.7	14.8
7. Phosphorus soluble (mg/100 g soil)		21.8	24.3
8. Population of denitrifiers (Number per gram dry soil).			
1. Nitrate reducing bacteria.		48.9 x 10^6	73.5 x 10^6
2. Denitrifying bacteria		34.0 x 10^5	60.0 x 10^5

All figures are average of three replicates.

filled with pure He to create anaerobic conditions. The process of evacuation and filling with He was repeated at least 5 times. Then 10% of the gas was then replaced with acetylene and the bottles were incubated at different temperatures. Three replicates were used for each temperature. The system remained closed throughout the incubation period. Sterilized soil controls were incubated at each temperature (N_2O was not evolved from these bottles). In addition to that soil samples with or without acetylene were also incubated for the specified period at 25°. This was done to evaluate the activities of micro-organisum, based on evolution of CO_2 in the presence or absence of acetylene. At the end of 5 days samples of the gas phase were taken for determination of N_2O and CO_2 by chromatography. Gas samples of 0.2 ml were taken with gas tight syringes and injected in to a Perkin Elmer sigma 4 gas-chromatograph with an electron capture detector, equipped with a column (3 mm x 4 m) containing pora-pak Q. Carrier gas was mixture of Argon (95%) and methane (5%) at a flow rate of 30 ml $min.^{-1}$ Oven and detector temperature were 100 and 200° respectively.

At the end of incubation the samples were also extracted with 250 ml of 2 N KCL. Nitrate was determined in the filtrate by steam distillation [7] and nitrite was also determined by Griess Illoway's method [8].

The counts of bacteria were performed by a most probable-number method [9] using nitrate broth (Difco nutrient broth with 0.1% w/v KNO_3 added) as growth medium, distributed in Durham tubes. Five replicate tubes were inoculated from each soil dilution.

The inoculated tubes were incubated for three weeks at 25°. Then presence of gas in the inverted tubes was used as criterion for presence of denitrifiers, and presence of nitrite, dectected by the starch iodide spot test [10], served as indication of nitrate-reducing bacteria, i.e. bacteria able to reduce nitrate only to nitrite.

RESULTS AND DISCUSSIONS

The data on CO_2 evolution, which reflects the microbial activity/respiration in soils, in the presence and absence of acetylene (Table 2) indicated that presence of acetylene did not affect the microbial activity, as the amount of CO_2 measured under both conditions is same. Similar results were observed by Ryden *et. al.* [11] at 5% C_2H_2. level. These findings support the applicability of this technique in biological research under laboratory or field conditions particularly for measurement of biological nitrogen fixation and denitrification.

The data presented in Table 3 regarding nitrogen balance at the end of 5 days incubation indicated that denitrification is slow at 10°. It increased with increase in temperature to 50°. At 60°, denitrification decreased by 20%, whereas at 70° the process ceased as the added NO_3-N in the bottles remained as such until the end of incubation.

Table 2. CO_2 produced at 5th day of incubation in various soils under helium atmosphere in the presence and absence of 10% acetylene and at temperature of 25°.

Soil type	CO_2 U moles per flask	
	In presence of 10% acetylene	In absence of 10% acetylene
Sandy loam	370.00	368.00
Coarse sand	198.00	198.00

All figures are average of three replicate experiments

Table 3. Effect of temperature on denitrification in sandy loam and coarse sand soils.

Temperature	Incubation period	Nitrate N-balance (% age of total - NO_3- N in system)			
		Gaseous Nitrogen: as N_2O:		NO_3-N	
		Roskilde Sandy loam	Jyndevad Coarse sand	Roskilde Sandy loam	Jyndevad Coarse sand
10°	5 days	10+1	8 1	90+1	92+1
20"	" days	17+1	12+1	80+1	87+1
30"	" days	55+1	50+1	40+1	49+1
40"	" days	90+1	80+1	10+1	18+1
50"	"days	100+1	98+1	0+1	0+3
60"	" days	80+1	80+1	20+0	20+1
70"	" days	0+1	0+1	98+1	98+1

All figures are average of three replicates.

Keeney *et al.* [12] observed maximum denitrification at 60°-67° and cessation of process at 75°. In fact it is surprising to observe high denitrification rates at elevated temperatures. In this regard Foch and Verstraete [13], proposed that high optimal temperature for denitrification may be due to thermophillic species of *Bacillus*. They also reported that denitrification at elevated temperature may be due to biological and chemical reduction of NO_3 Several *Bacillus* sp. as well as some other Thermophillic sp. such as *Clostridium* are NO_3-respires (NO_3-NO_2). Thus nitrite may accumulate initially but it is unstable at elevated temperature and it is further reduced to form N_2O. Keeney *et. al.* [12] argued that reduction of NO_2-N to N_2O is by chemodenitrification at elevated temperatures. By reacting steam sterilized samples with NO_2-N at 40-60°, they observed evolution of NO from the break down of NO_2-N and NO formed during high temperature due to Chemo-denitrification is rapidly reduced further to N_2O. From these observation it can be concluded that denitrification at high temperatures is due to combination of biological as well as chemical reduction reactions. As thermophillic temperatures are approached thermophillic NO_3- respiration and chemodentrification reactions dominate.

The soils under investigation contained nitrate reducers as well as denitrifiers (Table 1). The population of both, the nitrate reducers as well as denitrifiers was comparatively larger in Roskilde sandy loam than Jyndevad Coarse sand

soil [14]. This could be the probable reason of comparatively higher denitrification rate in sandy soil, than coarse sand.

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