THE DENITRIFICATION PROCESS IN SOIL

C.M. Cho
Department of Soil Science
University of Manitoba

Introduction

The denitrification process has been defined as biological reduction of NO_3^- and NO_2^- to volatile gases such as N_2O and N_2. The definition excluded the biological reduction of NO_3^- to NO_2^- even though the production of NO_2^- and the formation of N_2O and N_2 are carried out by the same organisms under the same process - reduction. A modified definition of denitrification should include the reduction of NO_3^- to NO_2^- in order to quantitatively describe the process.

Nitrogen has nine oxidation states (some claim there are 10). The reduced form, NH_4^+ is stable under reduced condition and the oxidized form, NO_3^-, is stable under oxidized condition. Reduction of O_2 and NO_3^- by microorganisms can be expressed as

$$\text{carbohydrate} + O_2 \longrightarrow CO_2 + H_2O \qquad (1)$$

$$\text{carbohydrate} + NO_3^- \longrightarrow CO_2 + H_2O + N_2 \qquad (2)$$

In eq. (1), the oxidation state of O_2 was 0 before the reaction and it was reduced to -2 state. In the case of NO_3^- (eq. (2)), the oxidation state of N was +5, and it was reduced to 0 state. Thus, both oxygen and nitrogen were reduced while carbon in carbohydrate was oxidized.

Treating the denitrification process as a reduction process has a number of advantages. The process can be related to the rate of respiratory activity and can be compared with O_2 consumption or biological oxygen demand. However, in order to answer questions related to agriculture such as "when, where and how much of denitrification" it is necessary to learn more about the fundamental process of denitrification.

In this presentation, some of the advances in the denitrification process carried out in the laboratory will be summarized. Conditions under which the denitrification takes place, analysis of the process based on rate of respiratory activity, variability of the magnitude of the denitrification process and probable magnitude of denitrification in a soil in the field will be discussed.

Experimental Observations and Discussions

I. Conditions for denitrification

According to eq. (1) and (2) both O_2 and NO_3^- can act like e^- acceptor. A question arises whether the reactions expressed by eq. (1) and (2) can occur simultaneously. In other words, can NO_3^- compete with O_2 as e^- acceptor? In order to obtain an answer to the above question the following experiment was carried out. Fifteen grams of Wellwood soil and 15 ml of $Ca(NO_3)_2$ solution labelled with ^{15}N were incubated in a closed container (41 ml). One set was vertically placed so that there was 1 cm of stagnant water above the soil while the other set was shaken horizontally. Gas samples were taken from the incubation atmosphere and analyzed for O_2, CO_2, N_2O and N_2 with a mass spectrometer throughout the incubation (20°C) period.

The comsumption of O_2 and production of CO_2, N_2O and N_2 by the soil when the suspension (100 ppm N) was not shaken are shown in Fig. 1.

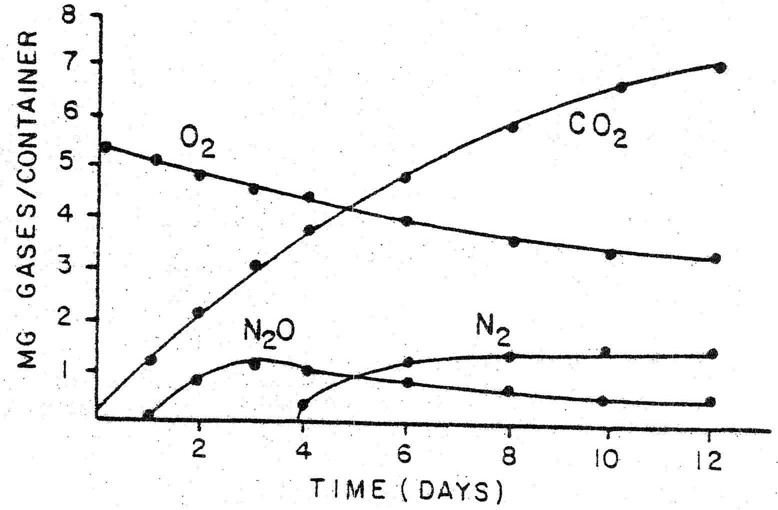

Fig. 1. Oxygen consumption, CO_2, N_2O and N_2 production; NO_3^- -N 100 ppm unshaken. T = 20°C.

Oxygen in the incubation atmosphere decreased gradually and CO_2 was produced. Nitrous oxide was produced from the first day of incubation even though there was ample O_2 in the system. After N_2O reached the maximum at the third day N_2 started to appear. The rates which represent the slopes of various curves were not constant as visually seen from Fig. 1. Approximate rate of O_2 consumption was 0.22 μ mol/container-hr. and the initial rate of N_2O production was 0.89 μ mol/container-hr.

When the incubation container (75 ppm N) was vigorously shaken horizontally, quite different patters of consumption and production were obtained (Fig. 2). The O_2 in the container decreased linearly with time. The production of CO_2 was also linearly related with time until all O_2 in the system was consumed, thereafter, the rate of CO_2 production changed to a different value. The difference in the rates of CO_2 production between aerobic (O_2 in the system) and anaerobic conditions may be related to the activities of total aerobes and facultative anaerobes in the soil. Only after the system became anaerobic (all O_2 depleted) did N_2O start to appear with nearly a constant rate of production. The N_2O reached a maximum and then decreased very rapidly with the formation of N_2. The rate of O_2 consumption was 4.98 μ mol/container-hr, about 23 times the rate of O_2 consumption when the container was not shaken. The production rate of N_2O was 1.25 μ mol/container-hr, 1.4 times the unshaken rate.

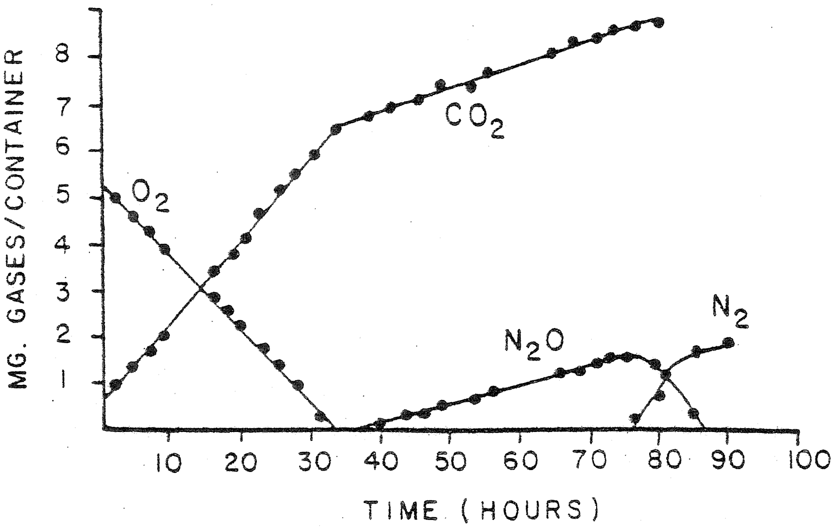

Fig. 2. Oxygen consumption CO_2, N_2O and N_2 production; NO_3^- -N 75 ppm; shaken. T = 20°C.

The results of Fig. 2 clearly demonstrated that NO_3^- can not compete with O_2 as e⁻ acceptor, even though it appeared like that NO_3^- could compete with O_2 according to the results of Fig. 1. In other words, denitrification does not occur whenever the system is aerobic. The reason why denitrification reaction occurred under unshaken condition (Fig. 1) even though there was ample O_2 in the system requires further explanations which will be treated next.

II. Magnitude of Denitrification

Comparisons of the magnitude of rates of O_2 consumption between shaken and unshaken conditions, and that of N_2O production between shaken and unshaken conditions clearly indicate that the rate of reduction caused by the soil (or the rate of e⁻ acceptor demand) is higher under shaken condition. From the experimental results similar to that shown in Fig. 2 it became obvious that the rates of O_2 consumption and CO_2, N_2O production and N_2O disappearance obtained under vigorous shaking were found to be the maximum values which did not change any further by changing the experimental conditions. The constant, maximum value of the reduction process is due to constant rate of e⁻ production which is controlled by the rate of energy supply of the soil. The maximum value of electron production rate, EPR max, is a characteristic parameter of the soil and it can change to a different value if temperature is altered or a fresh energy source is added to the soil. The lower rate of reduction under unshaken conditions is due to lower rate of e⁻ production. The lower rate of e⁻ production under unshaken condition is caused by the lower rate of e⁻ acceptor supply even though the soil has potential EPR corresponditing to EPR max. In other words, the rate of denitrification under unshaken condition is e⁻ acceptor-supply-controlled while the rate of denitrification under well shaken condition is energy-supply-controlled.

The term called gaseous nitrogen production rate, GNPR, will be defined and its relation with the rate of nitrate supply will be discussed. Mathematically the term GNPR can be written as

$$\mathrm{GNPR} = \frac{d}{dt}\left[(N_2O - N) + (N_2 - N)\right] \qquad (3)$$

where t stands for time. Under anaerobic condition when the rate of e^- acceptor supply is controlling the actual EPR, NO_3^- arriving to the site of denitrification is all converted to either N_2O or to N_2, assuming NO_2^- does not accumulate. Therefore, under such condition,

$$\frac{d}{dt}\left[(N_2O - N) + (N_2 - N)\right] = \mathrm{GNPR} = \text{Rate of } NO_3^- \text{ supply} \qquad (4)$$

Under unshaken condition, rate of NO_3^- supply is diffusion-controlled. If the concentration of NO_3^- at the site of denitrification is assumed to be nearly zero, then the diffusive rate of NO_3^- supply becomes directly proportional to the NO_3^- concentration in bulk soil solution. Consequently the denitrification rate expressed by GNPR becomes dependent upon NO_3^- - concentration and behaves like a first order reaction.

If the rate of supply of NO_3^- is gradually increased by raising the concentration of NO_3^- or by decreasing the moisture content so that diffusion path is shortened, GNPR becomes controlled not by the rate of supply of NO_3^-, but by the rate of e^- production at the site of denitrification. The GNPR remains constant regardless of how greatly the NO_3^- concentration is increased. The behavior of GNPR becomes that of zero order reaction. Thus it is seen that the magnitude of denitrification varies from almost zero to a fixed maximum value depending upon the concentration of NO_3^-. The similarity of above-mentioned behavior and an enzymatic reaction which is expressible by Michaelis-Menten type kinetics is worth noting.

In Fig. 3 the relation between GNPR and the rate of NO_3^- supply is depicted. The values of GNPR and rate of NO_3^- supply are the same up to GNPR is equal to 1, which was arbitrarily chosen to correspond to EPR max of the soil. The value of GNPR does not increase any further even though rate of NO_3^- supply is increased if the reaction is

$$2NO_3^- \xrightarrow{10e^-} N_2 \qquad (5)$$

since the maximum EPR has been reached. If, however, the reaction is

$$2NO_3^- \xrightarrow{8e^-} N_2O \qquad (6)$$

then GNPR increases a further 25% as the rate of supply of NO_3^- is increased, thereafter GNPR remains constant. The reason why there is 25% more gaseous N produced if the final product is N_2O instead of N_2 is because the reduction of NO_3^- to N_2O requires less e^- than that requred for conversion of NO_3^- to N_2. In other words, the efficiency of e^- for reaction (6) is greater than that of reaction expressed by (5).

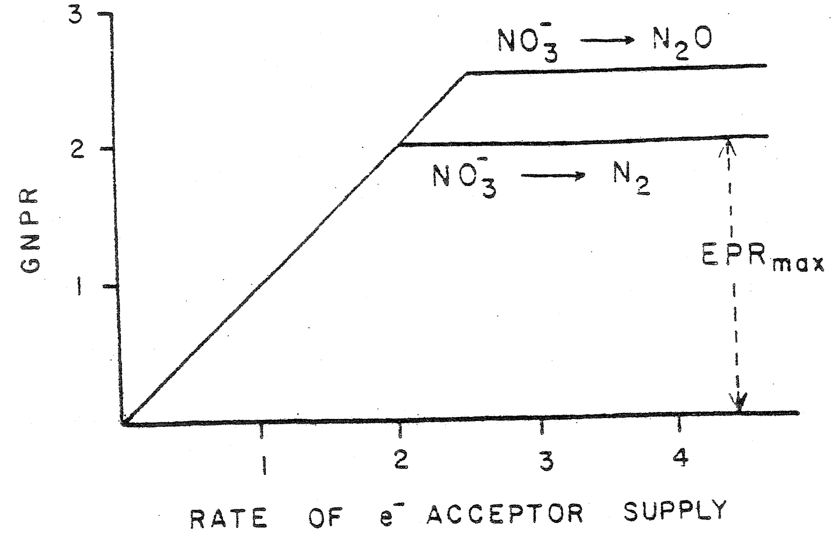

Fig. 3. The relation between gaseous nitrogen production rate and the rate of electron acceptor supply.

If the denitrification process produces both N_2O and N_2 then the magnitude of GNPR will lie anywhere between the lines of $NO_3^- \longrightarrow N_2$ and $NO_3^- \longrightarrow N_2O$ (Fig. 3) provided the rate of NO_3^- supply is not controlling the denitrification. If the reaction is diffusion-controlled then the rates of N_2O -N production and of N_2 -N production will be equal.

III. Denitrification in Soil Column

A soil column is not uniform with respect to biological activity and O_2 distribution. Generally the surface of a column is aerobic and somewhere below the surface it is anaerobic. A quantitative analysis of the O_2 distribution in reconstituted Wellwood soil column applying the steady state O_2 transport equation with empirical depth-dependent O_2 consumption rate seems to describe the experimental results reasonably well (Fig. 4).

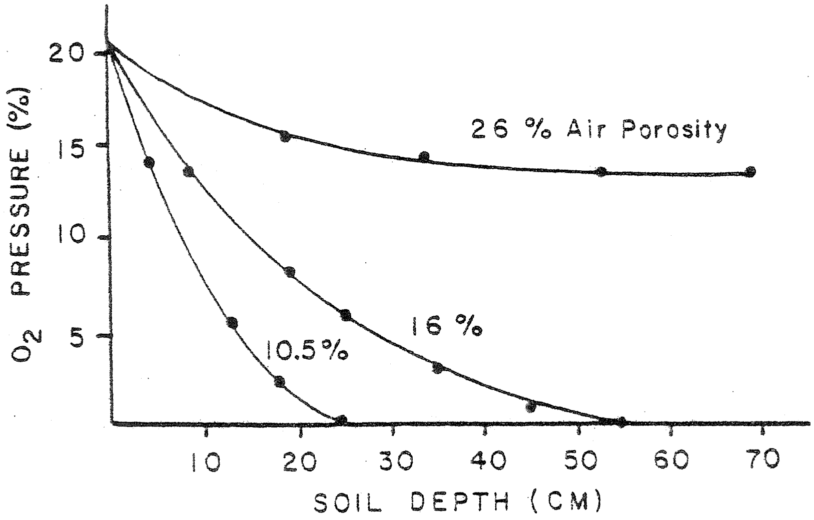

Fig. 4. Steady state O_2 concentration distribution in a reconstituted Wellwood soil profile at various air porosities. T = 20°C.

The depth of O_2 penetration or boundary between aerobic and anaerobic zones is rather shallow if air porosity is small but extends deep if air porosity is increased. Such O_2 profiles as shown in Fig. 4 were found to remain stationary if moisture content and temperature were kept constant.

What happens if urea or NO_3^- are applied to the soil and stationary O_2 profile such as shown in Fig. 4 develops? Results obtained with urea are shown in Fig. 5.

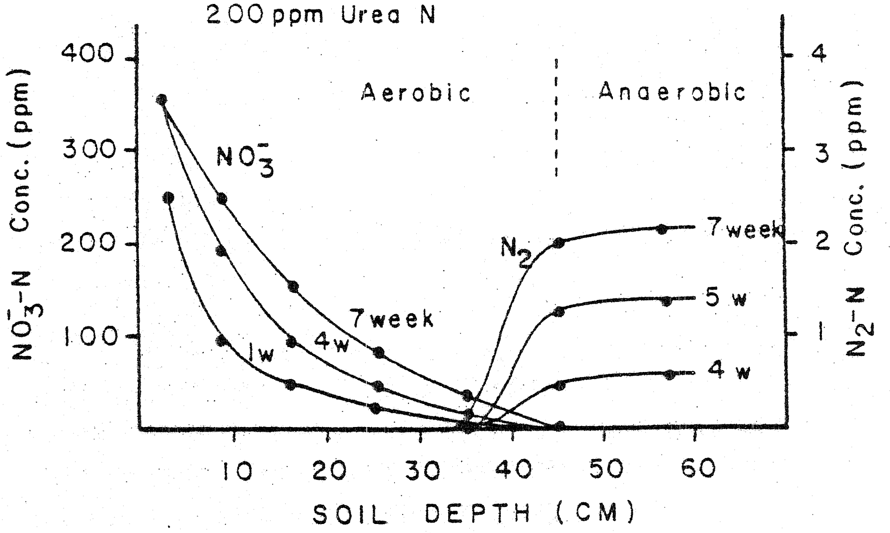

Fig. 5. Distribution of NO_3^- -N and N_2 gas in a reconstituted Wellwood Soil profile after application of urea; air porosity = 16%. T = 20°C.

Urea, applied uniformly throughout the column, was oxidized to NO_3^- and the concentration of NO_3^- increased with time. Since O_2 concentration and biological activity decreased with depth, the conversion of NH_4^+ from urea to NO_3^- also decreased with depth. Some NO_3^- crossed the aerobic-anaerobic boundary and was denitrified as evidenced by the accumulation of N_2 gas. Clearly the magnitude of denitrification was controlled by the rate of NO_3^- supply to the anaerobic zone from aerobic zone (or NO_3^- flux across the boundary) and this magnitude was not too great. Low concentration of NO_3^- and high pH of the soil at the site of denitrification were probably the reasons why N_2O was not detected. There was not enough NO_3^- near the zone of denitrification (near aerobic-anaerobic boundary) to compete with N_2O as e⁻ acceptor.

When NO_3^- (100 ppm N) was added uniformly throughout aerobic zone (up to 20 cm depth) the NO_3^- diffused downward and denitrified (Fig. 6). Since the concentration of NO_3^- was much greater in this case than the urea addition (Fig. 5), the amount of NO_3^- diffused more and consequently more denitrification took place (compare Fig. 6 and 5). There was no NO_3^- in the deeper depth of anaerobic zone indicating that the NO_3^- that crossed the aerobic-anaerobic boundary was all denitrified within

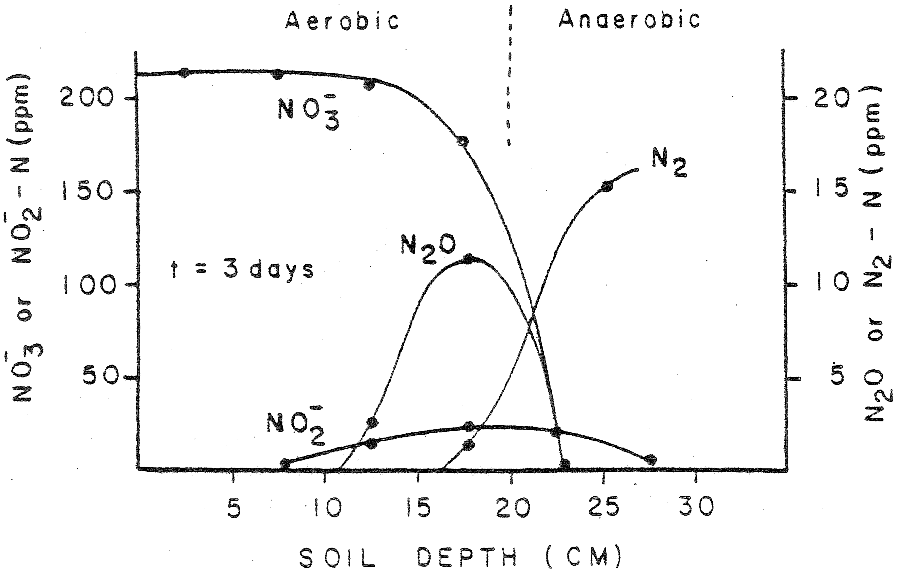

Fig. 6. Distribution of NO_3^- and denitrification products in a reconstituted Wellwood soil profile 3 days after the application of NO_3^-. T = 20°C.

the shallow layer of anerobic zone. There was considerable amount of N_2O at the boundary. Approximately 50% of the observed N_2O are in the aerobic zone. Nitrous oxide is stable in the presence of O_2, thus these N_2O will be able to escape to the soil surface and to the atmosphere. The remaining 50% of N_2O is in the anaerobic zone and it will be reduced further to N_2. The presence of high amount of N_2 indicates that N_2 was produced in the anaerobic zone. There has been a number of arguments regarding whether a soil is a sink or source of N_2O in nature. If a soil is under field capacity and the depth of aerobic zone is reasonably deep, then soil cannot be a sink of N_2O since N_2O cannot be consumed (reduced) at the surface where it is aerobic.

IV. Field Conditions

Soil under field conditions has, in addition to the non-uniformity in biological activity, non-uniform temperature and moisture distribution. Moisture content and air-filled porosity is negatively related. Since O_2 is transported through air-filled pore, the moisture content of a soil is very important in determining the depth of aerobic zone. Generally there is no direct relationship between air-filled porosity and the depth of aerobic zone. There are, however, several empirical relationships between diffusion coefficient of O_2 in porous media and the porosity. It is recognized that the diffusion coefficient increases faster than linearly as air porosity increases.

Consequently, the depth of aerobic zone, when biological activity and temperature distributions remained constant, increases very rapidly as soil moisture is decreased. Since the soil moisture distribution is so much governed by rainfall, the distribution is very difficult to predict as function of seasonal time.

Soil temperature distribution is caused by change in seasonal temperature. The surface of a soil, for example, is cold and subsoil is warm in January. However, the reversal of this distribution occurs as the season progresses to summer. Such temperature distribution affects the biological activity and consequently the 0_2 distribution in soil. Oxygen distribution of a soil with field capacity was estimated under constant moisture content using three empirical relations - (1) depth-dependent biological activity, (2) temperature effect upon biological activity and (3) temperature distribution of soil at various times of year. Table 1 shows the depth of the aerobic-anaerobic boundary as related to soil characteristics (microbial activity) at various times of a year. Biological activity was expressed as the oxygen consumption rate of the uppermost surface soil at 20°C, and it was expressed as ml of air by ml of soil per day. In reality 21% of the air contains 0_2, consequently approximately 1/5 of the values listed in the Table corresponds to ml of 0_2 at 1 atmosphere.

Table 1. The depth of aerobic-anaerobic boundary at several values of biological oxygen consumption*

	Depth of the boundary (cm)		
	0.4**	2	4
Month	(ml air/ml-SOIL-day)		
May 1			464
June 1		54	37
July 1	624	39	29
August 1	160	36	27
September 1	148	38	29
October 1	173	47	35
November 1	268	71	56

*Soil was assumed to have 15% air-filled porosity

**The value indicates the oxygen consumption rate equivalent to that present in 0.4 ml of ambient atmosphere at 20°C by 1 ml of the surface soil per day.

When a soil has low biological activity, as exemplified by the value of 0_2 consumption rate of 0.4 ml/ml-day (Table 1), the soil profile is virtually aerobic till the end of June. The boundary moved up to approximately 150 cm depth in August and it gradually receded to the deeper depth. Soils with higher O_2 consumption rates were also aerobic throughout the profile up to May 1, then the boundary moved up to within 50 cm from the surface when warmer weather arrived. The boundary remained near 30 - 40 cm depth during the summer and receded to deeper depth as fall season approached. General picture regarding the presence of aerobic-anaerobic boundary and its cyclic movement due to seasonal change in soil temperature is very useful in predicting the possible denitrification that might take place in soil.

Fertilizer and mineralized nitrogen present in aerobic layer is stable. However, nitrate which crosses the aerobic-anaerobic boundary is denitrified. As long as the amount crossing the boundary is not in excess of the maximum EPR in the anaerobic zone, all NO_3^- which crosses the boundary will be denitrified. Since the amount of nitrate that crosses the boundary is probably related to the amount of NO_3^- in the aerobic layer the annual loss of nitrate due to denitrification under prairie conditions may not be too great if a crop is growing on the soil as crop removes NO_3^- from the soil solution. An important factor to be considered is the speed at which the aerobic-anaerobic boundary travels upward as soil temperature warms up. Any nitrate which was present in an aerobic zone that subsequently becomes anaerobic due to rise in soil temperature can all be denitrified. Thus the conservation of nitrogen in soil by minimizing the denitrification requires extensive knowledge of local soil and seasonal parameters as well as the rainfall patterns.

Conclusion

Quantitative description of the denitrification process is rather difficult with present state of knowledge. There are so many variables that govern the magnitude and speed of denitrifiction. However, gradual progresses in the knowledge enable us to analyze the process in a semi-quantitative way on certain aspects of denitrification.

Denitrification process occurs whenever O_2 is unavailable as terminal e^- acceptor of biological process. Oxidized nitrogen atoms accept e^- and are converted to reduced forms. Since N_2 is the terminal product, any compounds more oxidized than N_2 can act as e^- acceptor. Consequently there is competition for e^- among oxidized forms of N in various compounds such as NO_3^-, NO_2^- and N_2O. Formation of NO_2^- from NO_3^- and N_2O from NO_2^-, and subsequent competition for e^- among them for further reduction give rise to complex pattern of production and disappearance of various forms of nitrogen during the denitrification process.

The maximum rate of denitrification of a soil is governed by the biological activity of the soil and is characterized by the maximum rate of e^- production. The maximum rate can be realized only when the rate of e^- acceptor supply, whether it is O_2 or NO_3^-, is not limiting the rate of the whole process. If the rate of supply is smaller than the maximum rate of e^- production, the actual rate of e^- production is solely governed by the rate of e^- acceptor supply. Under such conditions, the rate of NO_3^- disappearance can be expressible as first order reaction:

Soil under field conditions has an oxidized and reduced layer within its profile. The depth of oxidized layer is controlled by soil moisture, temperature and probably organic matter content. The NO_3^- either added as fertilizer or produced from ammonium form within the oxidized layer can diffuse downward and denitrified. Consequently the quantity of denitrification is the same as the flux of NO_3^- crossing the aerobic-anaerobic boundary of the soil. Such quantity is rather appreciable in summer time when the boundary is close to the surface and is negligible during the winter and early spring period. Quantitative estimate of the denitrification under field condition is very difficult because air porosity which governs the depth of aerobic zone is very much governed by the climatic condition.