Agropedology 2022, 32 (01), 110-121
doi.org/10.47114/j.agroped.2022.jun8

Heterotrophic Nitrification - Effects of N-Serve on Ammonia Oxidation and Nitrification in Acid Soils of the Nilgiris

D. Rajkumar* and S. Gobika

Department of Agriculture Engineering, RVS Technical Campus, Coimbatore-641 402

Abstract: Nitrification process in the acid soils (pH 6.7, 5.6 and 4.3) of the Nilgiris was studied. Accumulation of nitrate was found to be higher in the soils with pH 6.7 and 5.6 than the soil with pH 4.3. Accruement of nitrite was observed along with nitrate only in the soil with pH 4.3 after 42 days of incubation. Addition of N-Serve, an inhibitor of autotrophic ammonium oxidizers and chlorate, (an inhibitor of nitrite oxidizers) completely inhibited the nitrate and nitrite formation in the soils of pH 5.6 and 6.7, but only 50% reduction in the formation of nitrite and nitrate was observed in the soil of pH 4.3. Nine heterotrophic organisms isolated from the soil with pH 4.3 were tested for their heterotrophic nitrifying ability in glucose peptone liquid medium. Among nine, two fungal isolates identified as *Fusarium* sp. and *Penicillium* sp. were found to produce significant quantities of nitrite and nitrate. The amount of nitrite and nitrate production by these fungi varied with different cultural conditions *viz.,* nitrogen sources and pH.

Key words: *Heterotrophic nitrification, acid soils, Fusarium sp., Penicillium sp*

Introduction

Nitrogen is an essential nutrient for the growth and development of crop plants. The nitrogenous fertilizers applied to the soil as well as soil nitrogen are subjected to biological oxidation producing nitrate nitrogen which is assimilated by plants. A good nitrifying soil is usually considered as a fertile and productive one. Nitrification is mediated by two major groups of microorganisms, autotrophs and heterotrophs. In contrast to autotrophic nitrification, heterotrophic nitrification is not considered as a significant characteristic of heterotrophs, as it is not considered to be coupled with energy metabolism and cell growth . A much more heterogeneous group of bacteria and fungi are involved in heterotrophic nitrification; (Kuenen and Robertson 1988; De Boer and Kowalchuk 2001 and Sun *et al.* 2019). As heterotrophic nitrification does not appear to yield energy for growth, these organisms must have other reasons for carrying out the reactions. Focht and Verstraete (1977) and Shoupeng *et al. (*2021) suggested that heterotrophic nitrifiers could utilize certain intermediates of nitrogen oxidation as growth factors or as biocidal factors to assist in their competition and survival (Zhang *et al.* 2020). Nitrification by heterotrophic microorganisms *in vitro* is well documented, but the ecological significance of such a process in nature is uncertain Liu *et al.* (2019). However, they may be of significance in acidic forest soils, where their large numbers or higher biomass might compensate for their relative inefficiency (Myrold and Posavatz 2007; Hayatsu *et al.* 2008). In some acidic coniferous soils, heterotrophic nitrifiers have in fact been

* Corresponding author : (Email: drdrajmicro@gmail.com)

considered to be responsible for nitrification (Killham 1990). *In-situ* occurrence of heterotrophic nitrification has only been reported in specific soil habitats such as organic soils, (Tate 1977) acidic environments (Adams 1986; Schimel *et al.* 1984) and saline conditions, (Yokoyama *et al.* 1992) where autotrophic activity is likely inhibited. Major factor which influences on nitrification is pH (Prosser 1990). The role of autotrophic nitrifiers in the soil nitrogen cycle and the process of nitrification is aerobic with optimal activity at mesophilic temperatures (Martilainen 1985) and neutral to alkaline pH, with no growth or activity at acid pH. The pH of the Nilgiris soils is normally well below 5.0. The type of nitrification process and organisms involved in this reaction of the Nilgiris soil is not yet studied so far. Hence, an attempt was made to assess the type of nitrification process and the organisms involved in acid soils of Nilgiris.

Materials and Methods

To study the nitrification process and the microorganisms involved in the nitrification of acid soils of the Nilgiris, soil samples (0-20 cm) with pH of 4.3, 5.6 and 6.7 were collected from three different locations namely Ithalar village (pH 4.3), Kundah Bickatty village (pH5.6), and Nanjanadu village (pH 6.7). The soil samples were air dried under shade and ground to pass through a 2 mm sieve. The sieved soil samples were stored in plastic bags and used as and when required for determining the microbiological processes (Pramer and Schmidt 1964).

Enumeration of nitrifying bacteria

Bacteria, fungi, and actinomycetes population in the fresh soil samples were enumerated following standard serial dilution plate count method using Nutrient agar for bacteria, Martin's Rose Bengal agar for fungi and Kenknights agar for actinomycetes. The populations of nitrifying bacteria in the fresh soil samples were estimated by Most Probable Number (MPN) technique as described by (Belser and Mays 1982) using Pramer and Schmidt medium .

Quantification of nitrification

Hundred gram of air dried soil samples having pH 4.3, 5.6, and 6.7 were placed in 500ml Erlenmeyer flasks. These soil samples were supplemented with 500 mg kg^{-1} ammonium sulphate and the moisture level of the soil was adjusted to 60 per cent of its water holding capacity by adding distilled water and incubated at room temperature. Periodically distilled water was added to compensate the evaporation loss. At 0, 14^{th}, 28^{th}, and 42^{th} day of incubation, 10 g soil sample in triplicate flasks were withdrawn then ammonium, nitrite and nitrate were extracted and analyzed calorimetrically using Spectrophotometer GS 5705. The extraction of nitrogen from the soil, procedure involves the use of 1 *N* Sodium sulphate as the extractant (in a modification of the extractant) used by Onken and Sunderman (Onken and Sunderman 1977). The extractant first developed for simultaneous extraction of ammonium, nitrite, nitrate and urea, consisted of 1 *N* sodium sulphate and 5 mg kg^{-1} phenyl mercuric acetate (to hydrolyse urea). As urea was not used in the present study only 1 *N* sodium sulphate without phenyl mercuric acetate solution was used as the extractant. For extraction, 50ml of 1 *N* sodium sulphate solution was added to 10 g soil sample and stirred for 30 minutes on wrist action shaker. After filtration of suspension through buchner funnel, the clear filtrate was analysed for ammonium, nitrite and nitrate. Sodium sulphate (1*N*) was as efficient as Morgan's solution in terms of quantitative recovery of ammonium, nitrite and nitrate.

Ammonium in the soil extracts was estimated by nesselerization (Jackson 1958). The soil extract was treated with Nessler's reagent and the resulting colour was read at 410 nm in a colorimeter. Nitrite in the soil extract and culture medium was estimated calorimetrically employing sulphanilamide and N-1-naphthyl ethylene diamine dichloride. Equal amounts of these reagents were added to the extracts and the resultant pink colour was read at 520 nm in a colorimeter after appropriate dilution. Nitrate in the soil extract and culture medium was estimated by 2, 4- disulphonic acid method (Bremner 1965) at 420 nm in a colorimeter.

Effect of N- Serve on ammonium oxidation in soils

To determine whether autotrophic or heterotrophic nitrifying organisms were involved in the formation of nitrite and nitrate, the soil samples were examined in the presence of specific inhibitor of autotrophic ammonium-oxidizing bacteria, N-Serve (Nitrapyrin, 2- cholro- 6- trichloromethyl pyridine). N-Serve was diluted in 0.1 ml ethanol and introduced to 100 g soils to provide 10 ppm concentration. The control soils received only 0.1 ml ethanol. The ethanol added was allowed to evaporate for 24 hours and the soils were added with ammonium sulphate at 500 mg kg^{-1} concentration. After 40 days of incubation at room temperature, the ammonium, nitrite and nitrate in the soil samples were extracted and analysed as per the methodology of Jackson (1958) and 2, 4- disulphonic acid method (Bremner 1965).

Effect of N- Serve and chlorate on nitrification in soil with pH 4.3

To study the involvement of heterotrophic organisms involvement in nitrification in the acid soils of pH 4.3, the ammonium sulphate (500mg kg^{-1}) amended soil samples were treated with sodium chlorate (10mg kg^{-1}) and N-Serve as described above. After 30 days of incubation, ammonium, nitrite and nitrate in the soil samples were extracted and analysed (Jackson 1958; Bremner 1965). Control soils were also maintained.

Isolation of heterotrophic microorganisms and quantification of nitrifying ability

Bacteria, fungi, and actionomycetes isolated from the three soil samples having pH of 6.7, 5.6 and 4.3 were further characterized based on the colony characteristics and the predominant colonies were selected and transferred to agar slants *viz.*, nutrient agar, potato dextrose agar and Kenknight agar for bacteria, fungi and actinomycetes, respectively. These organisms were tested for their ability to form nitrite and/or nitrate after growth for 14 days under static or shaken conditions in a liquid glucose peptone medium described by Eylar and Schmidt (1959). Glucose and peptone solutions were sterilized separately from the salt solution and added aseptically into 250 ml Erlenmeyer flasks containing 100 ml of sterile glucose peptone medium, the test organisms were inoculated and incubated at 28°C under static (fungus and actinomycetes) or shaken (bacteria) conditions. After 14 days of incubation the bacterial cultures were centrifuged at 5000 g for 30 minutes and the supernatants were used for analysis. The fungal and actinomycetes cultures were filtered through Whatman No.42 filter paper and the supernatants / filtrates were decolourized with charcoal and used for analysis. The samples were tested qualitatively for nitrite and nitrate.

The fungal isolates which are shown to produce significant amount of nitrite and/ or nitrate were identified as described by Alexopoulos *et al.* (1996). In order to test the ability of the fungal isolates to produce nitrite and/ or nitrate from nitrogen sources other than peptone, in the glucose peptone medium, peptone was replaced with ammonium sulphate (0.5%), urea (0.2%), yeast extract (0.5%) and casein (0.5%). These media were sterilized and inoculated with fungal cultures as agar discs and incubated for 14 days under static condition. The culture filtrates were analysed for nitrite and nitrate. To study the effect of pH on ammonium oxidation by *Penicillium* sp., the medium employed by Hirsch *et al*. (1961) was slightly modified and used. The modified medium consisted of K_2HPO_4 (1.0g); $MgSO_4$ .7 H_2O (0.2g); $CaCl_2$ (0.1g); $FeSO_4.7H_2O$ (0.001g); $MnSO_4$ (0.001g); $ZnSO_4$ (0.001g); Glucose (10.0g); $(NH_4)_2$ HPO_4 (2.0g); and distilled water (1000ml). The medium was prepared twice the normal concentration and mixed with equal volumes of 0.07 *M* aconitate buffer, 0.1*M* phosphate buffer, 0.1 *M* tris (hydroxymethyl)-aminomethane buffer, or 0.02*M* borate buffer. Aconitate buffer was used below pH 6.0, phosphate from pH 6.0 to 7.6 and tris (hydroxymethyl) – aminomethane for pH 8.0. The fungus was grown at 28°C in Erlenmeyer flasks containing 100 ml of above medium for 14 days. Final pH, nitrite and nitrate contents of the medium were measured. Further study was undertaken to find out the relationship between the rate of depletion of energy source and the formation of nitrate in the culture of *Penicillium* sp. Two sets of glucose peptone medium were prepared and 100 ml of the medium was dispensed into

the 250 ml Erlenmeyer flasks and sterilized. In one set of medium, the energy source glucose was added at 10 g L^{-1} concentration and in another set it was added at 7 g L^{-1} concentration. The *Penicillium* culture was inoculated as agar discs into the medium. At periodical interval, duplicate flasks in each set were withdrawn and filtered using Whatman No. 42 filter paper. The filtrates were analyzed for glucose and nitrate contents. The glucose content and nitrate content was determined. The glucose in the culture filtrate was determined by the method described by . The culturate was centrifuged and 0.2 ml of supernatant was pipetted into a test tube. To that 1 ml of alkaline copper tartarate reagent was added and placed over boiling water bath for 10 minutes. After cooling, 1 ml of arsenomolydate reagent and 6 ml of distilled water was added. The absorbance was measured after 10 minutes at 620 nm. Standard curve was prepared by using the above procedure and the amount of glucose present in the sample was calculated.

Statistical analysis

The data were analysed as factorial experiment in Completely Randomized Design (Anderson and McLean 1974).

Results and Discussion

Microbial characteristics of soils

Results pertaining to microbial population in different soils are presented in table 1. The soil having pH 6.7 recorded highest population of NH_4^+ oxidizers (16.0 x 103 g^{-1}) and NO_2-oxidizers (8.2 x 103 g^{-1}). The soil having pH 4.3 had lowest population of NH_4^+ oxidizers (0.54 x 103 g^{-1}) and NO_2^- oxidizers (0.02 x 103 g^{-1}). The total bacterial count was highest in soil with pH 6.7 (100.9 x 105 g^{-1}) and lowest in soil with pH 4.3 (1.20 x 105 g^{-1}). The highest fungal population was observed in soil with pH 4.3 (93.9 x 103 g^{-1}) and lowest in the soil with pH 6.7 (38.6 x 103 g^{-1}). The actinomycetes population was highest in soil with pH 6.7 (30.0 x 103 g^{-1}) and lowest in soil with pH 4.3 (1.3 x 103 g^{-1}). The reason is that the pH requirement for the optimal activity of fungi, bacteria and actinomycetes was acidic, neutral and near neutral to slightly alkaline respectively .

Table 1. Microbiological properties of soils

Soil type	Population/ g of dry soil				
	Autotrophic NH_4^+ -oxidizers (No.x 10^3)	**Autotrophic NO_2^- oxidizers (No.x 10^3)**	**Total bacteria (No.x 10^5)**	**Total fungi (No.x 10^3)**	**Total actinomycetes (No.x 10^3)**
pH 4.3	0.54	0.02	1.20	93.90	1.30
pH 5.6	16.00	5.50	10.50	60.00	10.90
pH 6.7	16.00	8.20	100.90	38.60	30.00

Nitrification in the acid soils

In the soil with pH 6.7, nitrate formation was observed and it was found to increase with incubation period (Table 2). Nitrite was not detected throughout the incubation period. Ammonium concentration decreased with the incubation period except on the 14th day of incubation in which an increase in the content of NH_4^+ - N was observed. Similar results were obtained in the soil having pH 5.6 on incubation with ammonium sulphate but the rate of NH_4^+ - N oxidation into NO_3- N was less as indicated by the amount of NH_4^+- N and NO_3- N detected. Nitrite formation was not detected in this soil. In the soil having pH 4.3, the amount of NO_3 - N detected was comparatively less. An amount of 54 µg $g^{-1}$$NO_3$ - N was detected in soil with pH 4.3 after 42 days of incubation as compared to 77 µg $g^{-1}$and 63 µg g^{-1}in soils with pH 6.7 and 5.6 respectively. In addition to NO_3 - N, NO_2 – N, 2 µg g^{-1}, 7 µg $g^{-1}$and 10 µg $g^{-1}$was also detected in soil with pH 4.3

on 14th, 28th and 42nd day of incubation. Nitrate was accumulated over a period of incubation in all the soil samples. But nitrite accumulation was observed only in the soil sample having the pH of 4.3. Nitrite and nitrate accumulation increased with increase in the period of incubation. This indicated that nitrification process occurs in the above acid soils. But, accumulation of nitrite in soil is observed less frequently and known to occur in extreme environments . (Ramakrishna *et al.* 1978; Stroo *et al.* 1986; Thamdrup 2012)

Table 2. Nitrification in soils

Incubation period (days)	Ammonium -N ($\mu g\ g^{-1}$)			Nitrite- N ($\mu g\ g^{-1}$)			Nitrate – N ($\mu g\ g^{-1}$)		
	pH 6.7	pH 5.6	pH 4.3	pH 6.7	pH 5.6	pH 4.3	pH 6.7	pH 5.6	pH 4.3
0	126.2	117.5	119.5	0.0	0.0	0.0	7.0	6.2	4.1
14	162.8	168.5	133.6	0.0	0.0	2.0	60.1	32.2	12.1
28	72.7	94.4	113.7	0.0	0.0	7.1	68.1	54.2	41.2
42	54.5	72.5	81.2	0.0	0.0	10.1	77.1	63.1	54.3

Nitrification in the acid soils with N-Serve

In the control, nitrate formation was observed in three soils after 40 days of incubation but nitrite only in the soils having pH 4.3. Addition of N-serve completely inhibited the NO_3 - N formation in the soils of pH 5.6 and 6.7. The presence of 32.5 $\mu g\ g^{-1}$ of NO_3- N and 4.5 $\mu g\ g^{-1}$ of NO_2 -N was observed in the soil with pH 4.3 after 40 days of incubation (Table 3). Since N-Serve is a selective inhibitor of ammonium oxidizer, formation of nitrite in N-Serve added soil might be due to heterotrophic organisms. Acidic pH below 4.5 is highly inhibitory on the population and activities of autotrophic ammonium oxidizing bacteria (Alexander 1978). The accumulation of significant amount of nitrite in the N-Serve added soil might be due to oxidation of nitrite formed by the heterotroph, into nitrate by autotrophic nitrite oxidizers or by the activity of the heterotrophs itself.

Table 3. Effect of N – Serve on nitrification in acid soils

Soil type	$\mu g\ N\ g^{-1}$ soil after 40 days of incubation					
	Control			N- serve added		
	NH_4 -N	NO_2- N	NO_3- N	NH_4 -N	NO_2- N	NO_3- N
pH 4.3	93.3	7.2	63.3	115.1	4.5	32.5
pH 5.6	66.2	0.0	54.6	130.5	0.0	0.0
pH 6.7	48.0	0.0	84.2	144.4	0.0	0.0

Effect of N-Serve and chlorate on nitrification in soil with pH 4.3

Nitrite and nitrate was accumulated at a level of 5.5 µg $Ng^{-1}$and 36.0 µg Ng^{-1} respectively after 30 days of incubation due to the addition of N-Serve and chlorate. Similar level of nitrite accumulation was also found in control soil and not the nitrate (Table 4). As chlorate is a specific inhibitor of autotrophic nitrite oxidizers (Schimel *et al.* 1984), nitrate formed may probably due to the activities of heterotrophic microorganisms, as the autotrophic activity was eliminated by the addition of N-Serve. Many heterotrophic microorganisms isolated from acid soils were shown to produce nitrite and / or nitrate under laboratory condition (Eylar and Schmidt 1959; Stroo *et al.* 1986)

Table 4. Effect of N-serve and chlorate on nitrification in an acid soil with pH 4.3

Treatments	µg N g^{-1} soil after 30 days of incubation		
	Ammonium-N	Nitrite-N	Nitrate-N
Control	86.5	6.4	72.3
N-serve + Sodium chlorate	102.5	5.5	36.0
S.Ed	2.001	0.075	0.116
CD (5%)	4.075	0.118	0.481

Ability of microorganisms isolated from the acid soil with pH 4.3 to form nitrite and nitrate when grown on glucose peptone medium

Two bacterial, two actinomycetes, and five fungal colonies were isolated and tested for their ability to form nitrite and nitrate in glucose peptone medium. None of the bacterial and actinomycete isolates produced nitrite and nitrate in the medium. Among the five fungal isolates (Plate 1), two isolates produced lesser nitrite, while the other two isolates showed accumulation of significant quantities of nitrite and nitrate. These cultures were identified as *Fusarium* sp. and *Penicillium* sp. which produced 3.2 and 5.2 µg ml-1 of nitrite-N and 9.0 and 18.4 µg ml-1 of nitrate-N respectively after 14 days of incubation under static condition (Fig. 1). *Penicillium* sp. produced more amounts of nitrite and nitrate than the *Fusarium* sp. Many bacteria, actinomycetes were shown to produce nitrite and / or nitrate from organic and inorganic nitrogen sources (Eylar and Schmidt 1959). Among these fungi is the 9 predominant group of organism which showed more significant heterotrophic nitrification (Laughlin *et al.* 2008). Among the fungal group, Aspergillus is the frequently reported genera having the ability to produce nitrite and nitrate (Hirsch *et al.* 1961). Fungal genera other than Aspergillus reported for their heterotrophic nitrifying ability are the Penicillium, Cephalosporium etc. (Hirsch *et al.* 1961; Laughlin *et al.* 2008). In the acid forest soils, fungi are the organisms responsible for the entire nitrifying capability of the soil (Stroo *et al.* 1986).

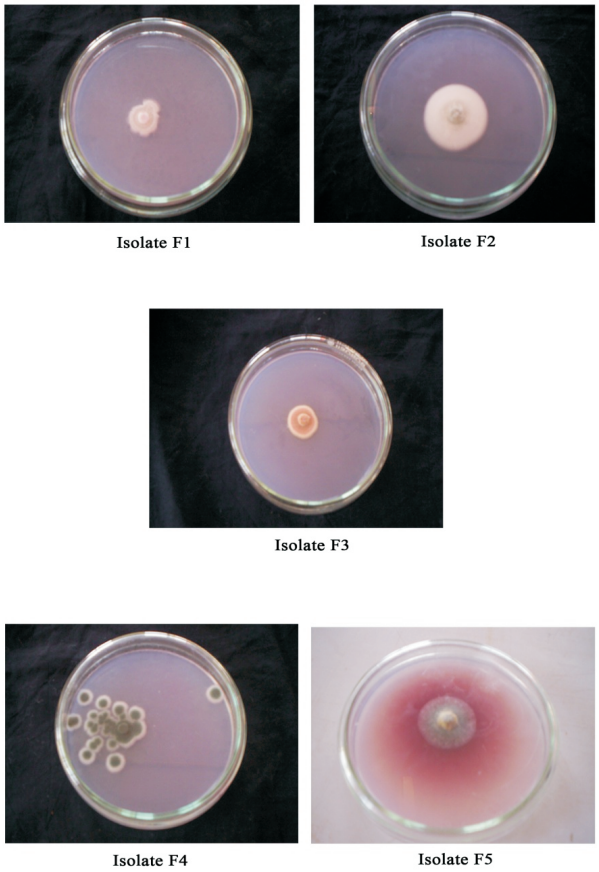

Plate 1. Fungal cultures isolated from soils of pH 4.3

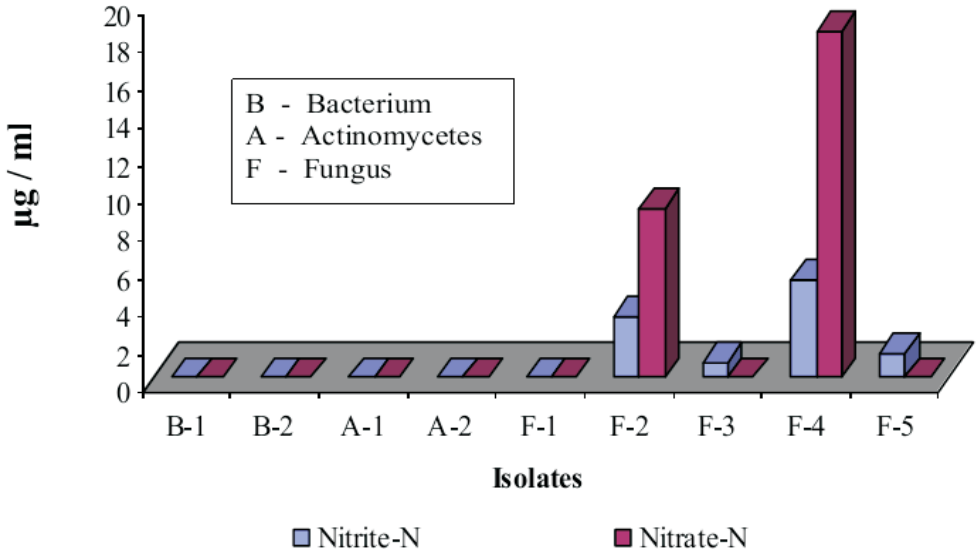

Fig. 1. Ability of microorganisms isolated from acid soil with pH 4.3 to form nitrite and nitrate when grown on glucose peptone medium

Production of nitrite and nitrate by shake and static cultures of fungi

The influence of shaking on the nitrite and nitrate producing ability of the fungal isolates were tested. The shaking had differential influence on the organisms and also on the product formation. *Fusarium* sp. under shake culture condition produced 4.5 and 12.0 µg ml^{-1} of nitrite-N and nitrate-N respectively. Whereas under static culture their formation was declined to 2.1 µg ml^{-1} of nitrite-N and 6.3 µg ml^{-1} of nitrate-N. But with *Penicillium* sp., production of nitrite (7.2 µg N ml^{-1}) was higher under static culture condition compare to shake culture condition (3.6 µg N ml^{-1}). Interestingly, nitrate formation (20.1 µg N ml^{-1}) was observed only in static culture (Table 5).

Table 5. Production of nitrite and nitrate by shake and non shaken cultures of fungi grown in glucose peptone medium

Fungi	Shake culture		Non shaken culture	
	NO_2^- N (µg ml^{-1})	NO_3^- N (µg ml^{-1})	NO_2^- N (µg ml^{-1})	NO_3^- N (µg ml^{-1})
Fusarium sp	4.5	12.0	2.1	6.3
Penicillium sp	3.6	0.0	7.2	20.1
S.Ed	0.06	-	0.018	0.075
CD (5%)	0.13	-	0.050	0.022

Effect of nitrogen sources on production of nitrite and nitrate by fungal isolates under static culture condition

Among the nitrogen sources, casein supported higher production of nitrite and nitrate by both the fungi. Maximum amount of nitrite (9.2 µg N ml^{-1}) and nitrate (21.2 µg N ml^{-1}) was produced by *Penicillium* sp. when casein was used as nitrogen source (Fig. 2). Casein was followed by peptone in enhancing the production of nitrite and nitrate by both the organisms. As the heterotrophic nitrification potential varied with amino acids used as substrate the amino acid composition of casein might be a cause for more production of nitrite and nitrate.

Influence of pH on ammonium oxidation by Penicillium sp.

Maximum amount of nitrite and nitrate was accumulated when the pH of the medium at the time of inoculation was 7.0. About 7.6 and 15.9 µg ml^{-1} of nitrite-N and nitrate-N respectively was accumulated at neutral pH. Initial pH of the medium below and above this severely affected the production of nitrite and nitrate. Virtually nitrite formation was completely inhibited in the medium possessing pH 8.0 at the time of inoculation. After the growth of culture, the pH of the media got reduced to all pH levels tested (Table 6). The results are in 10 similar to that of (Hirsch *et al.* 1961) were he observed higher production of nitrite and nitrate by *Aspergillus flavus* at neutral pH.

Table 6. Influence of pH on ammonium oxidation by *penicillium sp.*

Initial pH	Final pH	NO_2^- N formed (µg ml^{-1})	NO_3^- N formed (µg ml^{-1})
3.0	2.9	1.7	2.0
5.0	4.8	5.8	12.0
6.0	5.8	6.3	14.5
7.0	6.6	7.6	15.9
8.0	7.2	0.0	5.0

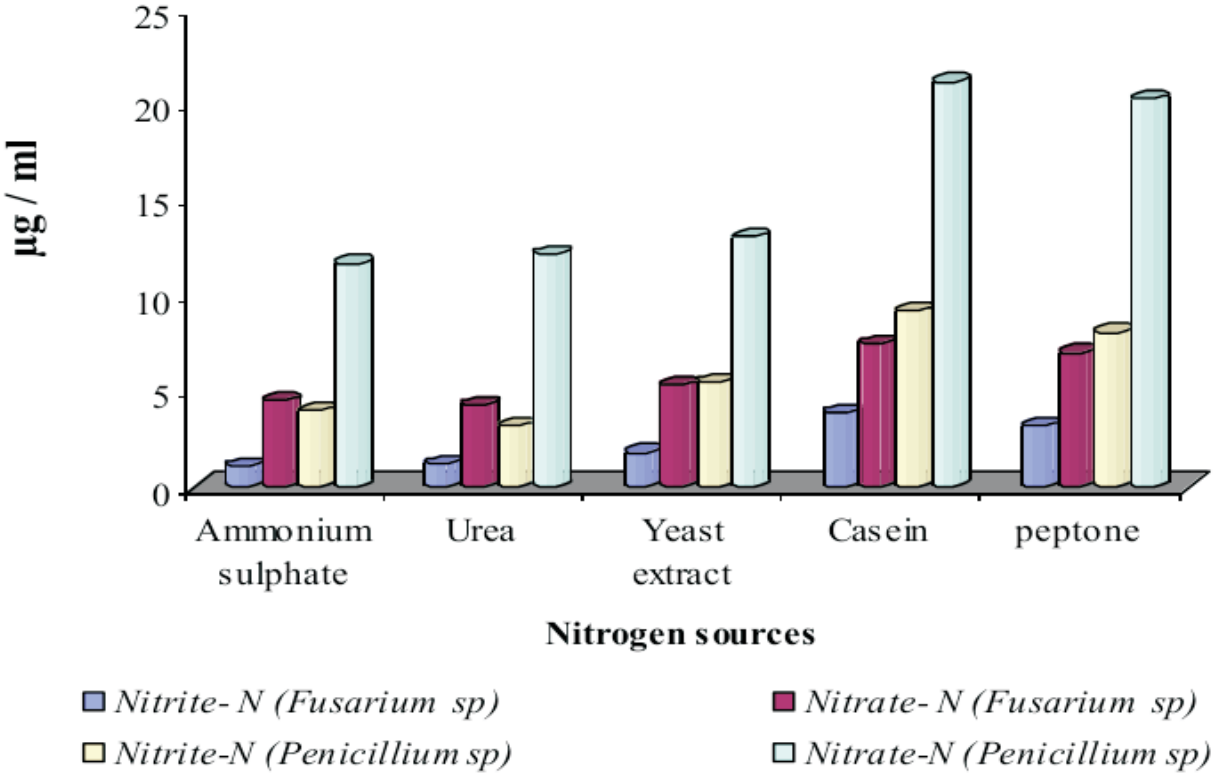

Fig. 2. Production of nitrite and nitrate by fungi in medium with various nitrogen sources under static culture condition

Nitrification during growth of Penicillium sp

In order to find out whether the nitrification process as indicated by nitrate production is occurring during active growth or completion of the growth of the fungus (as indicated by the complete utilization of energy source) was assessed employing glucose peptone medium with two different concentrations of glucose. The glucose was completely utilized at 5th day of incubation when it was added at 7 g L^{-1} concentration. The nitrate formation coincides with the depletion of the energy source *i.e.,* glucose. When the glucose concentration increased to 10 g $L^{-1}$the growth was fast as indicated by the depletion of glucose on 3rd day itself and the nitrate started accumulating thereafter (Fig. 3a, 3b). In contrast to the autotrophic nitrifiers, heterotrophic nitrifiers do not derive any energy by the oxidation of reduced nitrogen compounds. Only on the cessation of active growth, accumulation of nitrite and nitrate was observed. As noted by Van Gool and Schmidt (Van Gool and Schmidt 1973) with *Aspergillus flavus*, the formation of nitrate is evident only after the biosynthesis of cellular material is essentially complete. The rate of nitrate formation was clearly a function of the rate of growth.

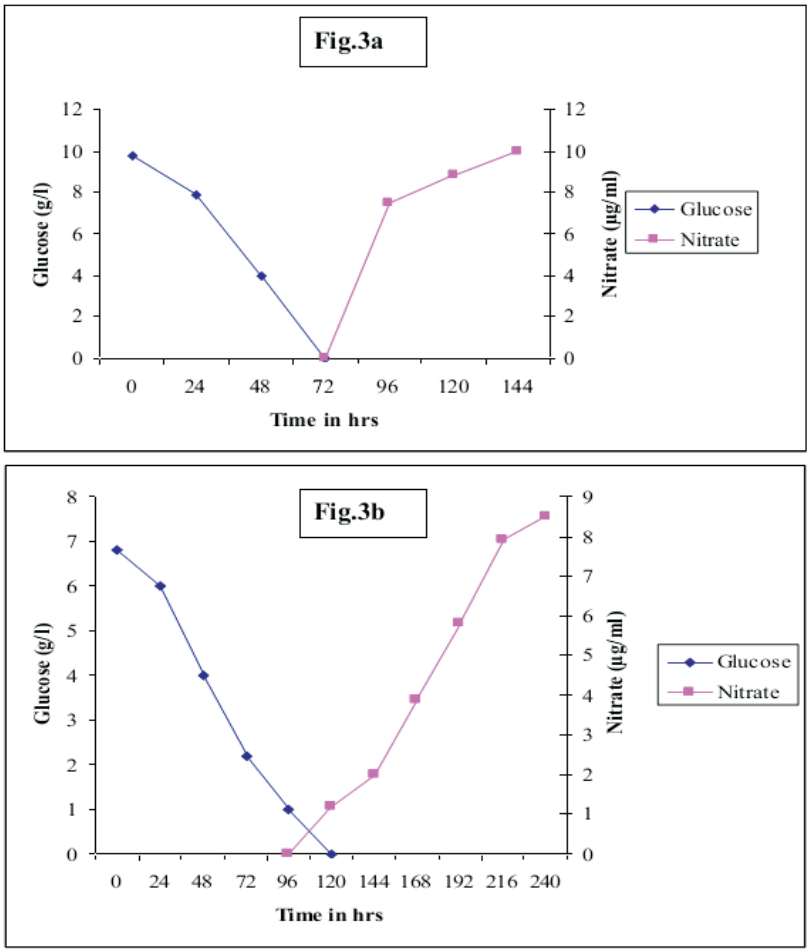

Fig. 3a, 3b. Nitrification during growth of *Penicillium* sp

Conclusion

The study concludes that nitrification in the acidic condition of Nilgiri soils at low pH by the heterotropic organisms. Among the different heterotropic organisms, two fungus species *viz., Penicillium* and *Fusarium* produces a significant quantities of nitrate and nitrites forms as the source of nitrogen for the absorption of nutrients, thus proceeds to the better growth and development of crops in acidic soils of Nilgiri.

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Received: February 2022 *Accepted: April: 2022*