

**Full Length Research Paper**

Estimation of Nitrogen Mineralization by Nitrifying Bacteria BtRs8 and NBtRs2 in Bt and NBt Cotton Rhizospheric Soil

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Abstract

Nitrogen mineralization by nitrifying Bacteria BtRs8 and NBtRs2 was detected in Bt and NBt rhizospheric soil of cotton cultivated fields for 10-12 consecutive years as a mono crop from Khammam district of Telangana. These soil sample were used as a source material for by estimating the initial ammonium and oxidized forms, the nitrite and nitrate levels. Nitrite (-N) was estimated by diazotization method. Absorbance of pink colored solution was read at 520 nm in a UV Vis Spectrophotometer. The nitrate (NO₃⁻-N) produced due to the activity of microorganisms in NCC medium was determined. The amount of nitrate was calculated by referring to a calibration curve

Keywords: Nitrogen mineralization, Nitrifying Bacteria, Bt and NBt cotton fields nitrite and nitrate estimation.

Introduction

Cotton is the most important commercial crop of India contributing up to 75% of total raw material needs of textile industry and provides employment to about 60 million people. India has the largest area under cotton cultivation with relatively low productivity primarily due to the large area under rainfed cultivation with inadequate supply of inputs. Nitrifying bacteria that live universally in nature is becoming more and more popular. It has become clear that nitrifying bacteria are microorganisms deeply involved with environment protection and maintenance of resources (Velmourougane and Sahu 2013).

Nitrification is an important step in the nitrogen cycle in soil the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates (Focht and Verstraete, 1977). The nitrogen is the key element of the biogeochemical cycle that describes the transformation of nitrogen and nitrogen containing compounds in nature (Sun et al., 2007; Sarkar et al., 2009). Estimation of the rate of nitrogen mineralization is the gain of information on available nitrogen to plants. The immediate product of nitrogen mineralization is ammonia (ammonium ion), generated by ammonification which in turn gets converted to nitrate by nitrification depending on the prevailing redox conditions and suitable microorganisms i.e., wide spectrum of bacteria and fungi which are naturally involved in nitrogen mineralization

Methodology**Estimation of nitrogen mineralization by nitrifying Bacteria BtRs8 and NBtRs2 in Ammonium Calcium Carbonate (ACC) medium**

Nitrogen mineralization was detected by estimating the initial ammonium and oxidized forms, the nitrite and nitrate levels. Aliquots of sterilized media were inoculated with 1 ml of bacterial suspension and incubated at 37°C in an orbital shaker at 120 rpm. Increase or decrease in nitrite nitrogen was recorded regularly by diazotization method (Barnes and Folkard, 1951) to check the activity of ammonium oxidation or nitrite oxidation respectively.

Estimation of ammonium in ACC

The ammonium ion (-N) in the ACC medium was analyzed by Nesslerisation method (Jackson, 1971). To suitable aliquots of the medium, 0.5 ml of the Nessler's reagent was added and the volume was made up to 7 ml. The developed yellow colour was read at 495 nm in a UV-Vis spectrophotometer - 117 (Systronics). The amount of ammonium was calculated by referring to a calibration curve prepared with standard solution of known ammonium concentrations.

Estimation of nitrite in ACC

Nitrite (-N) was estimated by diazotization following the method of Barnes and Folkard (1951). Suitable aliquots from the culture were pipetted into test tubes and 1 ml of 1 % Sulphanilamide in 1N HCl was added and shaken thoroughly. To the so formed coloured diazo compound, 1 ml of 0.12 % N-(1-naphthyl) ethylene diaminedihydrochloride in distilled water was added. Absorbance of pink

colored solution was read at 520 nm in a UV-Vis spectrophotometer - 117 (Systronics). The amount of nitrite was calculated by referring to a calibration curve prepared with the standard solution of known nitrite concentrations.

Estimation of nitrate in Nitrite Calcium Carbonate (NCC) medium

The nitrate (NO₃⁻-N) produced due to the activity of microorganisms in NCC medium was determined by the method of Ranney and Bartlett (1972). Three drops of brucine reagent (2 g brucine dissolved in 50 ml ethanol) were added to suitable aliquots of the culture tubes followed by 2 ml of concentrated Sulfuric acid. The solution was mixed by vortexing and placed in the dark for 30 minutes to ensure full colour development, after which the volume was made up to 10 ml with distilled water and the developed yellow color was read at 410 nm in a UV-Vis spectrophotometer - 117 (Systronics). The amount of nitrate was calculated by referring to a calibration curve prepared with the standard solution of known nitrate concentrations.

Results

Nitrogen mineralization by the selected bacterial isolates

Estimation of nitrite production levels (µg/g) in BtRs8, NBtRs2 and Stnd organisms

Bt and NBt were inoculated separately and incubated 12 days at 37°C. Nitrite production by Bt isolates increased with incubation time up to the 9th day 76µg/g and later there was a gradual decrease. However, NBt and standard isolates had shown an increased in nitrite production upto 7th day 97µg/g, 98µg/g were recorded and later showed a gradual decrease in nitrite production.

Table 1. Estimation of Nitrite production levels (µg/g) in Bt, NBt and Stnd

Incubation period (in days)	Concentration of nitrite (µg/g)		
	Bt	NBt	Stnd
2	28	31	32
3	34	42	46
4	42	58	60
5	49	67	70
6	59	86	88
7	64	97	98
8	70	84	86
9	76	68	69
10	56	58	59
11	48	52	53
12	34	38	39

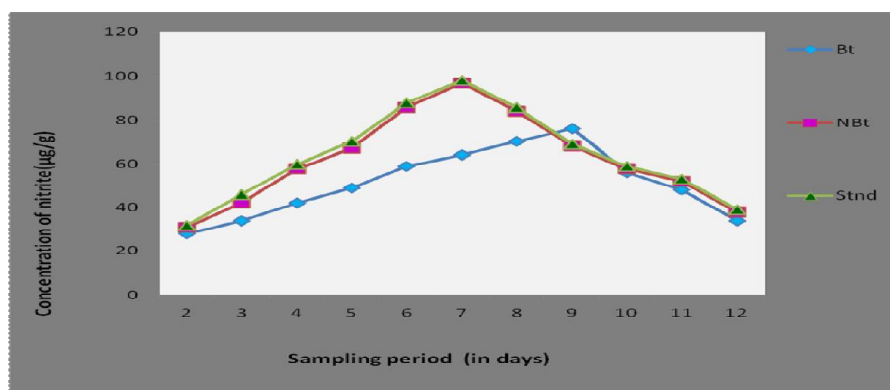


Fig. 1: Estimation of nitrite production (µg/g) in Bt Rs8, NBtRs2 and Stnd

Estimation of Nitrate production (µg/g) in BtRs8, NBtRs2 and Stnd

Bt and NBt were inoculated separately and incubated at 37°C for nitrate production over a period of 14 days. Results were recorded for all three organisms, i.e. Bt, NBt and Stnd, increased production of nitrate i.e. Was recorded for Bt isolate 154 µg/g with incubation

time upto 12th day and later there was a gradual decrease. However, NBt and Standard organism showed increased nitrate production 178µg/g, 180µg/g respectively upto 9th day and later showed decrease in Nitrate production.

Table 2: Estimation of Nitrate production (µg/g) in BtRs8, NBtRs2 and Stnd

Incubation period in day	Concentration of nitrate (µg/g)		
	Bt	NBt	Stnd
2	38	39	42
3	60	68	72
4	80	93	94
5	98	118	120
6	106	129	132
7	128	145	146
8	132	165	167
9	140	178	180
10	148	172	173
11	152	156	157
12	154	134	135
13	132	128	125
14	112	112	113

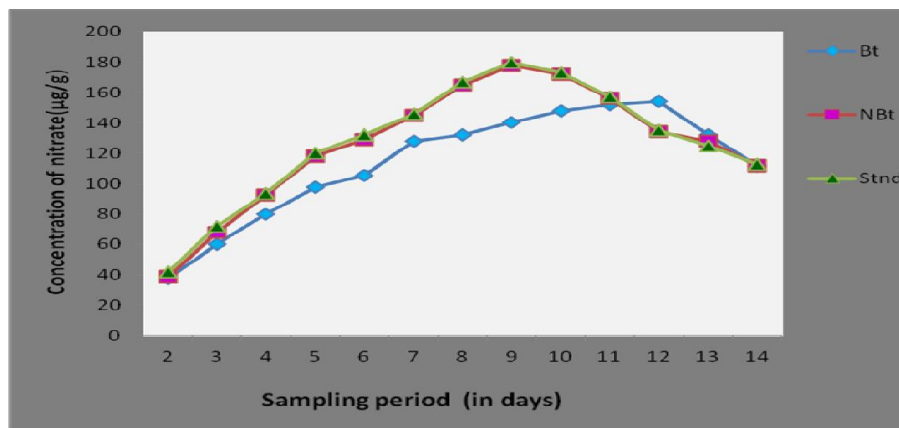


Fig. 2: Estimation of Nitrate production (µg/g) in Bt, NBt and Stnd

Discussion

In the present study after selection of suitable media, for production of nitrites and nitrates the isolates were tested by using standard protocols (Barnes and Folkard, 1951; Jackson, 1971; Ranney and Bartlett, 1972). The change in concentration of exchangeable forms of NH₄⁺, N₂, NO₂⁻, N⁻ and NO₃⁻ N during the nitrification process was studied up to 14 days. It was observed that nitrite production in ACC medium by *Bacillus cereus* increased with incubation time up to 9th day and later a gradual decrease was observed. The nitrate production in NCC medium was observed to increase up to 12th day and later there was a gradual decrease. Similar results were reported by (Ramakrishna and Sethunathan, 1983).

Nitrite production was observed over 12 days period up to the levels (97µg/g, 98µg/g) with NBt soil, standard organism and (76 µg/g) for Bt soil organism by day 9, and subsequently these levels decreased during the following days. Nitrate production was observed over 14 days period up to the levels of (178µg/g, 180µg/g) for NBt, standard organism (154µg/g) and for Bt soil organism by 12th day, and subsequently these levels decreased during the following days.

Conclusion

Comparatively NBtRs2, and Standard organisms shows better in nitrites and nitrates production while compare with BtRs8 cotton soil isolate.

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