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DENITRIFICATION BY *AZOSPIRILLUM BRASILENSE* AND *SINORHIZOBIUM SP* IN THE PRESENCE OF THE NITRIFICATION INHIBITOR

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ABSTRACT

Investigation the effects of nitrification inhibitor viz, 3,4dimethylpyrazolephosphate (DMPP on denitrification and N_2O/N_2 productions by N-fixers bacteria (*Azospirillum brasilense* Az204 and *Sinorhizobium sp* TNAU14 a model experiment was carried out in laboratory conditions. As a model experiments consisted of 3 treatments, control, 1% v/v C_2H_2 and 1 µgDMPP mL^{-1} in combination with the inoculation of *Azospirillum brasilense* or *Sinorhizobium sp*. The concentration of N_2O and CO_2, the population density, and the level of nitrate, nitrite, and ammonium in the medium solution were analyzed. The results showed that the kinetic of N_2O production by *Azospirillum brasilense* in the presence and absence of 1 % acetylene ranged about 2–18 µg N_2O $mL^{-1}day^{-1}$, and 2–17 µgN_2O mL^{-1} day^{-1}. The rate of N_2O production by *Sinorhizobium sp* was about 4–15 µgN_2OmL^{-1} day^{-1} and 4–12 µgN_2OmL^{-1} day^{-1} in the presence and absence of 1% v/v acetylene.The N_2O portion of total-denitrification los by *Azosprillum brasilense* or *Sinorhizobium sp* was about 70–98% and 80–95% and decreased until about 50–51% due to the decreasing of nitrate supply. The application of 1 µg DMPP mL^{-1} decreased both N-fixers population.The CO_2-production by *Azospirilum brasilense* decreased significantly. DMPP may influence the population of non-target-microorganism in soils.

Key words : Azosprillium brasilense, Sinorhizobium sp, nitrification inhibitor

PENGARUH INHIBITOR NITRIFIKASI TERHADAP AKTIVITAS DENITRIFIKASI OLEH *AZOSPIRILLUM BRASILENSE* DAN *SINORHIZOBIUM SP*

ABSTRAK

Kajian pengaruh inhibitor nitrifikasi 3,4 -dimethylpyrazolephosphat (DMPP) terhadap denitrifikasi dan produksi N_2O/N_2 oleh bakteri pemfiksasi nitrogen (*Azospirillum brasilense* Az_20_4 and *Sinorhizobium sp* TNAU14) telah dilakukan dalam bentuk percobaan model pada kondisi laboratorium. Percobaan terdiri dari 3 perlakuan yaitu kontrol: pemberian 1% v/v C_2H_2 dan pemberian 1 µg DMPP mL^{-1} yang dikombinasikan dengan inokulasi *Azospirillum brasilense* atau *Sinorhizobium sp*. Pengukuran dilakukan terhadap konsentrasi gas yang dihasilkan yaitu N_2O dan CO_2, kerapatan populasi, kandungan nitrat, nitrit, dan ammonium dalam larutan media. Hasil penelitian menunjukkan bahwa produksi N_2O oleh *Azospirillum brasilense* dengan perlakuan dan tanpa perlakuan 1% asetylen masing-masing sekitar 2–18 µg N_2O $mL^{-1}hari^{-1}$, dan 2–17 µg N_2O mL^{-1} $hari^{-1}$. Produksi N_2O oleh *Sinorhizobium sp* berkisar 4–15 µg N_2O mL^{-1} $hari^{-1}$ dan 4–12 µg N_2O mL^{-1} $hari^{-1}$ yaitu dengan pemberian dan tanpa pemberian 1% asetylen. Kehilangan nitrat dalam bentuk emisi N_2O melalui denitrifikasi oleh *Azosprillum brasilense* atau *Sinorhizobium sp* berkisar 70–98% dan 80–95% dan menurun hingga sekitar 50–51% akibat penurunan suplai nitrat. Perlakuan 1 µg DMPP mL^{-1} pada media pertumbuhan dapat menurunkan jumlah populasi kedua bakteri pemfiksasi nitrogen. Produksi CO_2 oleh *Azospirilum brasilense* menurun secara signifikan. DMPP dapat mempengaruhi populasi mikroorganisma non target di dalam tanah.

Kata kunci: *Azosprillium brasilense, Sinorhizobium sp*, inhibitor nitrifikasi

INTRODUCTION

Nitrous oxide is a biogenic greenhouse gas emitted to the atmosphere from soils and it stimulated by agricultural management practices. Optimization of agricultural resources for improved and sustainable agriculture involves the use of nitrification inhibitors. Nitrous oxide (N_2O) is produced by biological processes in the soil such as denitrification and nitrification (Stark and Richards, 2008). The use of nitrification inhibitors has been shown to be a useful technique to reduce N_2O emissions and NO_3- leaching from soil, when mineral or high organic-N fertilizers are applied. Further, the application of these compounds that retard nitrification is used to improve N recovery and N use efficiency in agricultural soils, while the same time limiting the environmental impacts of N loss and thus improving sustainability (Fillery, 2007). It have beneficial effect on nitrous oxide emission to the atmosphere or denitrification (Weiske *et al.* 2001; Di *et al.* 2006; 2007), and reducing nitrate leaching into ground water (Di *et al.* 2004; 2005) and affect N retention in the root zone and microbial biomass and activity in the rhizosphere and as a result increase plant growth (Douma *et.al,* 2005; Malla *et. al,* 2005, Moir, *et al.* 2007). The use of nitrification inhibitors (NIs) show a reduction of about 60% in NO_3-leaching, 70% in N_2O emissions and an increase of more than 20% in crop and pasture yield can be achieved (Pasda, *at . al,* 2001: Sahrawat, 2004; Douma *et al.* 2005; Di. *et al.* 2007; Moir *et al.* 2007; Singh & Verma, 2007). Nitrification results in the formation of highly mobile nitrate, which is susceptible to loss from root zone by leaching and/or gaseous emissions of di-nitrogen or nitrous oxide through denitrification. As the loss of soil N in solution or gaseous form can cause pollution as well as N deficiencies in crops and pastures, the prospect of actively regulating these soil processes has major implication for improving effiency of fertilizer nitrogen in agriculture and for plant productivity (Chen *et al.* 2008; Li *et al.* 2008: Stark & Richards, 2008). The reduced nitrification can have significant impacts on the soil carbon cycle and, for example, decrease organic decomposition. N species (i.e. ammonium vs. nitrate) may a more important driver of carbon cycling and ecosystem functioning than the quantity of N present in the system (Austin *et al.* 2006, Stark & Richards, 2008).

Nitrification inhibitors use in agriculture should be recommended in low concentration and capable to control nitrate supply to crop so that avoid the excess of nitrate supply in soils. The inhibitor has the specific influence that is only inhibit the nitritation and not for nitratation so that accumulation can be avoid. The Inhibitor should be *bacteriostatic* and not a *bactericide* which killing certain microorganism in soils like *Nitrosobacter* sp, *Nitrosococcus* sp. Ammonium-recommended fertilizers are the most widely used source of N for crop production and keeping the applied fertilizer N in NH_4^+ form by using nitrification inhibitors (NIs) is a well documented strategy for reducing N loss and to minimize negative environmental impacts of the fertilizer-use. Three compounds have been commercialized as NIs for agricultural use including (i) nitrapyrin (2-chloro-6-trichloromethyl-pyridin, trade name N-Serve), (ii) dicyandiamide or DCD (trade name Didin, Alzon or and Ensan), and (iii) more recently DMPP (a pyrazole derivative, 3,4-dimethylpyrazole phosphate; trade name ENTEC) (Zerulla *et al*. 2001; Weiske *et al*. 2001; Barth *et. al* 2006; 2008; Ali, *et al.* 2008). The application DMPP under field conditions reduced the emission of N_2O and CO_2 significantly (Weiske *et al.* 2001). However, the site effect on non target microorganism activity in soils is still unclear, particularly on diazothrophs N-fixer bacteria.

Recently, increasing attention is being paid to diazothrophs bacteria, this due to their ability in fixing nitrogen and produce the plant growth promoting factor (phytohormones) and as well as on their ability to denitrify simultaneously (Biro, *et al.* 2000; Cassan, *et al.* 2009: Naher, *et al.* 2009). It was also reported that arbuscular mycorrhizal fungi (AMF) colonization and AM fungi activity

is enhanced by diazotrophs (*Azospirillum* and *Rhizobium*) (Barassi, *et al.* 2007: Mia & Shamsuddin, 2010: Molina-Favero, *et al.* 2008:) The highly energy consumption during the nitrogen fixation may be provided from nitrate respiration. The energy gain by using nitrate as an alternative electron acceptor under anaerobic condition is nearly as high during oxygen respiration (Stephan, *et. al,* 1984: Ottow & Benckiser 1994). Consequently, the combination of highly energy consuming of nitrogen fixation with the ATP providing nitrate respiration seems to be widespread under N-binding bacteria. Recently work showed that the nitrogen fixing denitrifiers are detected under N-diazotrophic families of *Aquaspirillum, Azospirillum, Azoarcus, Bacillus, Bradyrhizobium, Sinorhizobium, Pseudomonas, Rhodobacter* and *Rhodopseudomonas* (Yassin & Patwardhan, 2007; Cassan, *et al.* 2009: Mia & Shamsuddin, 2010). The quantification of potential N_2O released by N-fixing-denitrifying bacteria is important in estimating the contribution of agricultural practice to the global N_2O-emission. In addition, the percentage of a climate relevant trace gas (N_2O) or green house effect gas on the total denitrification losses is still unclear.

Objective of the present study was to evaluate under laboratory conditions the effect of the nitrification inhibitor viz., 3,4-dimethylpyrazole-phosphate (DMPP) on the activity of N-fixer-denitrifiers bacteria *Azospirillum brasilense* Az204 and *Sinorhizobium sp* TNAU14 (population number, kinetic of N_2O- and CO_2-emission and as well as on the N_2O/N_2-ratio).

MATERIALS AND METHODS

Used NI concentrations

The used nitrification inhibitor DMPP (purity 99.9 %), was obtained from the BASF, Ludwigshafen, Germany. In experiments utilizing this NI, stock solution of the inhibitor was prepared in distilled water by mixing the inhibitor in solution, whereas for experiment with NI as pure active ingredient as well as the control. Portions of this stock solution were used to achieve the desired level in soil. Recommended rates for the application of DMPP is 0.36 µg g^{-1} dry soils, respectively. In agriculture the recommended NI concentrations correspond to 90 kg N ha^{-1} N-fertilizer application.

Culture enrichments

The *Azospirillum brasilense* Az 204 and *Sinorhizobium sp* TNAU used were obtained from culture collection of Department of Agricultural Microbiology Tamil Nadu University of Agriculture (TNAU), Coimbatore, India and was isolated from sugarcane rhizosphere, while the *Sinorhizobium sp* TNAU 14 was isolated from peanut nodule (*Arachys hypogea*). Both *Azospirillum* and *Sinorhizobium* stock culture were maintenanced on solid yeast-manitol growth medium for Sinorhizobium and solid Lactat-biotin-nicotin acid-pantothenic growth medium for Azospirillum at 4 ^oC (Dreyfus *et al.* 1988). The enrichment was successfully by inoculated those culture in to 250 ml Flask volume containing 100 ml nutrient mediums (Table 1). The amount of about 10^8 cell mL^{-1} was obtained after 3 days of incubation at 25 ^oC and was used as inoculants in model experiments.

Model experiments and analyses

The experiments were consisted of three treatments, as follows; (1) control, (2) 1% v/v C_2H_2 and (3) 1 µg mL^{-1} DMPP in combination with the inoculation of *Azospirillum brasinlense* Az204 or *Sinorhizobium sp* TNAU 14 and provided with three replication. One milliliter of inoculants (about 10^8 cell mL^{-1}) of Azospirillum or Sinorhizobium was transferred inoculated in to 2,5 L flask (Fisherband, Fisher Scientific, Germany) equipped with firmly capped and rubber septum (Verneret, French) containing 100 ml medium which was treated formerly with 1 µg mL^{-1} DMPP or about 3 times of field concentration used (3-4 dimethylpyrazolephosphate) under sterile condition. Subsequently the bottle was flushed with N_2-gases (about 10-min) to obtained N_2-atmospheric conditions (anaerobic conditions): Finally, the 1% v/v C_2H_2 was added in to the suitable flask

using 70 ml volume of air tight syringes (Becton & Dickinson, Irland) and those treatments were incubated for 12 days at 25 °C. The concentration of N_2O and CO_2 in the headspace were measured regularly using a gas chromatography equipped with electron capture detector (Perkin Elmer 8500, Überlingen, Germany; Porapak Q Alltech, column 3 + 1m precolumn, 1/8", 80-100 mesh, N_2 carrier gas, detector 350 °C, injector 40 °C, oven 50 °C, flow 30 ml min^{-1}). Before each measurement, the flask was flushed about 5 min with N_2 gas and the measurement was done after 30 min incubation. About 40 ml of gas sample was taken using air tight syringes (Plastikpak-Syringes, Becton and Dickinson, Irland) for N_2O and CO_2 analyses. The released N_2O and CO_2 were calculated after integration of peak area using the computer program peak simple gaschromatography data system for GC and LC (SRI Instruments, USA) using and the kinetic of N_2O- and CO_2-production expressed in µg mL^{-1} d^{-1} were calculated according to Gay-Lussack for ideal gas, n = (P x V)/(R x T). The N_2O or CO_2 retained in medium solution were estimated according to Regina *et al.* (1998). The population density was calculated according to the MPN method (Lorch *et al.* 1995) and as well as the chemical properties (Nitrate, nitrite, and ammonium content) in the medium solution were analyzed regularly during the incubation times (DEV, 1981:1983, Navone, 1964).

Quantification of actual N_2O-emission and total denitrification losses ($N_2O + N_2$)

Denitrification as measured via the acetylene-inhibition-method. This method can be used to determine either actual denitrification under field conditions or potential denitrification under optimized laboratory conditions (anaerobiosis, addition of substrates, optimum temperatures). The N_2O released in absent of acetylene was pointed as actual denitrification, where as its concentration in present of 1% v/v acetylene was pointed as total denitrification (Pell, *et al.* 1998)

RESULTS AND DISCUSSION

Results

Potential of N_2O-emission and Nitrate Reduction

In Fig. 1 the N_2O-emission potential (control) and total denitrification (N_2O + N_2, in the present of 1% C_2H_2) of N-fixing *Azospirillum brasilense* Az204 (A) and *Sinorhizobium sp* TNAU14 is presented, where as the nitrate reduction is presented in Fig. 2.

This results revealed that either *Azospirillum brasilense* Az204 or *Sinorhizobium* were able to use the nitrate as electron acceptor. The N_2O-emission of the treatments inoculated with *Azospirillum brasilense* Az204 was increased rapidly and reached the highest emission (about 16-18 on 2-3th days , while the highest N_2O-

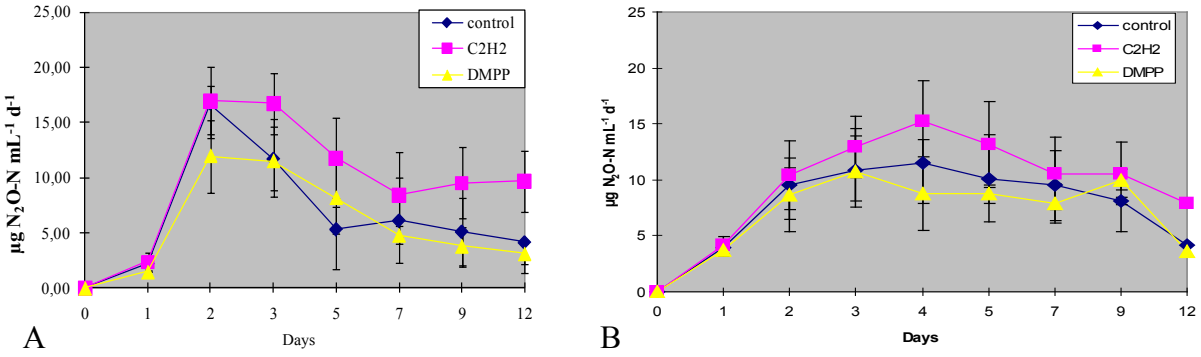

Figure. 1. The N_2O-release of *Azospirillum brasilense* Az204 (A) and *Sinorhizobium sp* TNAU14 (B) in the present of C_2H_2 and DMPP in model experiments (25 °C, N_2-atmosphere)

Table. 1 Actual N_2O-release (without acetylene) and totral denitrification (in the present of 1% acetylene) during 12 days of incubation time (N_2-Atmosphare, 25 °C) by *Azospirillum brasilense* Az204- und *Sinorhizobium sp* TNAU14.

Waktu (hari)	N_2O (µg N_2O-N ml^{-1})		Total denitrifikasi (N_2O + N2) (µg N_2O-N mL^{-1})		Persentase N_2O (%)	
	Azosprillum brasilense	*Sinorhizobium sp*	*Azosprillum brasilense*	*Sinorhizobium sp*	*Azosprillum brasilense*	*Sinorhizobium sp*
1	2.19	4.00	2.37	4.17	92.53	95.8
2	16.67	8.49	16.98	9.42	98.17	90.1
3	11.65	10.87	16.70	12.92	69.79	84.1
5	5.31	11.54	11.77	15.25	45.11	75.7
7	6.09	10.11	8.42	13.18	72.36	76.7
9	5.10	8.16	9.52	10.56	53.55	77.3
12	4.13	4.12	9.67	7.94	42.70	51.9

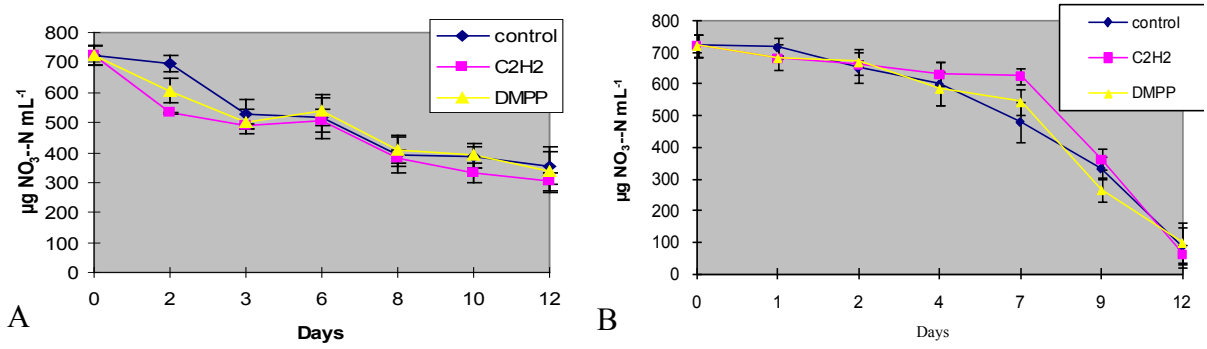

Figure 2. The reduction nitrate by *Azospirillum brasilense* Az204 (A) and *Sinorhizobium sp* TNAU14 (B) in the present of C_2H_2 and DMPP in model experiments (25 °C, N_2-atmosphere)

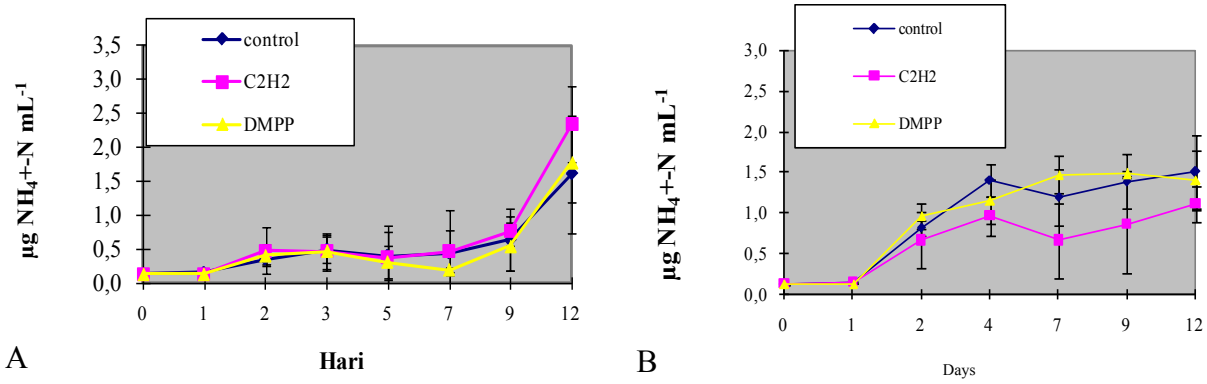

Figure 3. The ammonium formation by *Azospirillum brasilense* Az204 (A) and *Sinorhizobium sp* TNAU14 (B) in the present of C_2H_2 and DMPP in model experiments (25 °C, N_2-atmosphere)

emission (15-16 µg N_2O-N mL^{-1} medium) of the treatments inoculated with *Sinorhizobium sp* TNAU14 was reached on 3-4th days after incubation. The N_2O percentage of total denitrification were relatively high (80-90%) at the beginning of the experiments and it's decreased slowly until 40-50% (Tab. 1). This behavior was depended on nitrate availability (Fig. 2). The relatively high of nitrate supply (about 700 µg NO_3-N mL^{-1} at the beginning of the experiments) lead to the higher production of N_2O) and the total denitrification losses (the quality of denitrification gas) was dominated by N_2O.

In addition, the decreasing of nitrate content was followed by the reduction the N_2O-emission and the dynamic nitrate reduction and the N_2O-production of both N-fixers bacteria showed relative similar pattern.

Nitrite and Ammonium Formation

The pattern of the ammonium (Figure 3) and nitrite (Figure 4) formation either inolucated with *Azospirillum brasilense* Az204 or *Sinorhizobium* were relative different. The nitrite concentration of treatments inoculated with *Azospirillum brasilense* Az204 were relative low. In contrast, the nitrite contents of treatments inoculated with *Sinorhizobium sp* TNAU14 were clearly increased. This may lead to a differently denitrification potential . An increasing of the ammonium contents in medium of both treatments may be derived from nitrogen fixation. Consequently, both N-fixers have the ability to denitrify and to bind the nitrogen simultaneously under a relative high of nitrate content.

CO_2-Emission and Population Density

In Figure5. the CO_2-production of *Azospirillum brasilense* Az204 (A) and *Sinorhizobium sp* TNAU14 (B) in the present of acetylene and DMPP is presented. Fig. 6 showed that even pattern of CO_2-emission were relative similar, but the inoculation with *Sinorhizobium sp* lead to much higher production of CO_2. The relative higher production was supported by the high population density of *Sinorhizobium* sp (Fig. 6). The population of Sinorhizobium sp TNAU was much higher than *Azospirillum brasilense* Az204. This may be expected, because the *Sinorhizobium* is belong to fast growing rhizobacteria .

The effect of DMPP on the nitrogen fixer activity

The activity of *Azospirillum brasilense* Az204 and *Sinorhizobium sp* TNAU 14

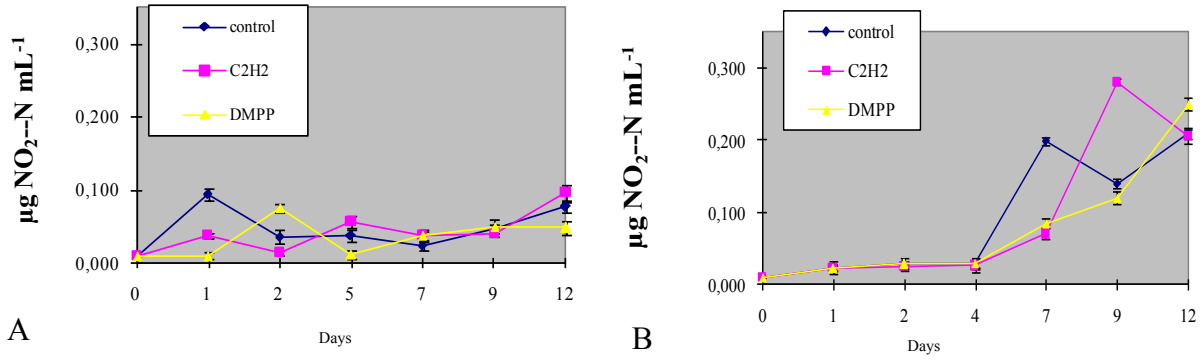

Figure 4. The nitrite formation by Azospirillum brasilense Az204 (A) and Sinorhizobium sp TNAU14 (B) in the present of C2H2 and DMPP in model experiments (25 oC, N2-atmosphere)

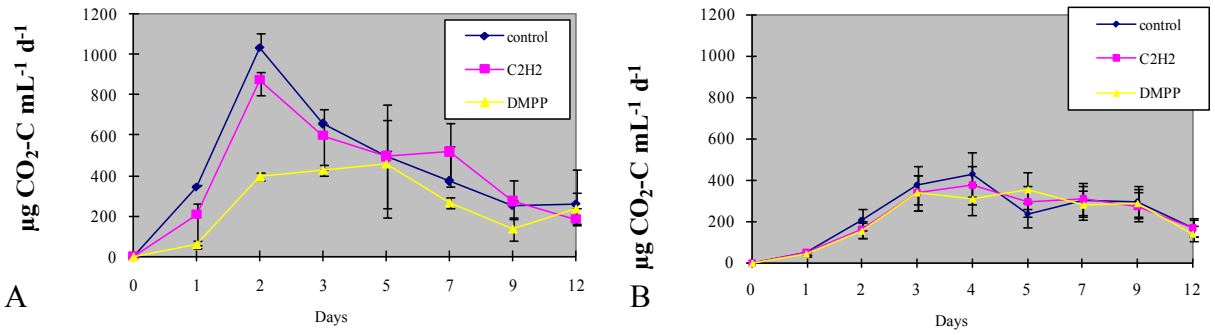

Figure 5. The CO2-emission of of N-fixing Azospirillum brasilense Az204 (A) and Sinorhizobium sp TNAU 14(B) in the present of acetylene or DMPP model experiments (25 oC, N2-atmosphere)

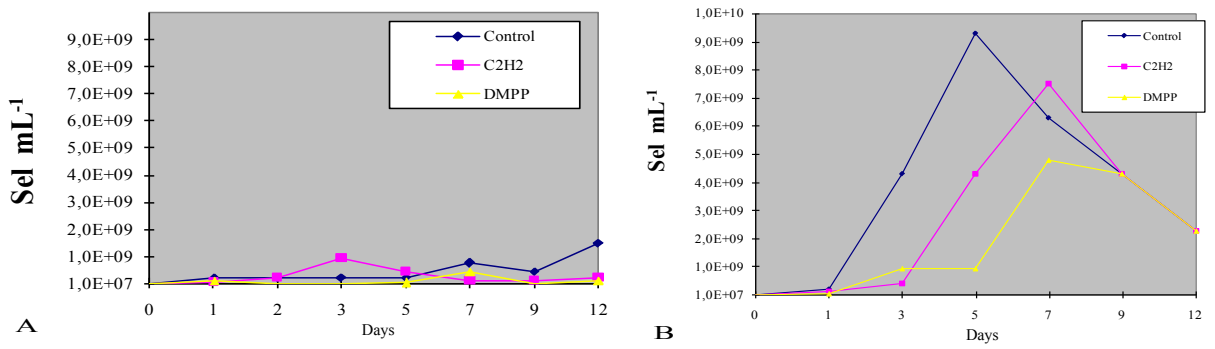

Figure 6. The population density of Azospirillum brasilense Az204 (A) and Sinorhizobium sp TNAU14 (B) in the present of DMPP and acetylene in model experiments (25 oC, N2-atmosphere)

in the present of 1 µg mL^{-1} (about 3 times recommended field application) DMPP (dimethyl pyrazolephosphate) are presented in Figure. 1, 5 and Figure. 6, respectively. The N_2O emission in present of DMPP was relative lower than control either inoculated with *Azospirillum brasilense* or *Sinorhizobium sp*. These results were supported by the CO_2-formation and population density. The population density and as well as the CO_2 formation of both N_2-fixers in the present of DMPP were relative lower than control. Consequently, the application of DMPP may give a negative side effect on other microbes (*non target microorganisms*). However, It effect under field conditions will be influenced on the soil physical and chemical properties and as well as the biological interactions.

Discussion

Thus far, the potential side effects of nitrification inhibitors on the soil ecosystem have not been established in detail. The existing literature suggests that their use does not negatively affect the soil microbial community, and there is evidence that nitrification inhibitors have no effects on microbial biomass, respiration and enzymatic activities (Di *et al.* 2007: Mahmood, *et al.* 2005:Muller *et al.* 2002). Molecular analysis of the soil bacterial community indicated that application of nitrification inhibitor (dicyandiamide :DCD) to soil did not affect the composition of the predominant bacterial phyla present in soil (Callaghan, *et al.* 2010). In agreement with these findings, Egamberdiyeva *et al*. (2001) reported increased numbers of oligonitrophilic bacteria and cellulose degradation activity and a decrease in the number of nitrifying and denitrifying bacteria after application of potassium oxalate as nitrification inhibitor, while at the same time availability of fertilizer N to plants was increased. They concluded that the combination of potassium oxalate and mineral fertilization showed promising potential concerning nitrification inhibition. While their study did not assess the specific effects of synthetic nitrification inhibitors on soil microbial populations. Austin *et al*. (2006) showed that reduced nitrification can have significant impacts on the soil carbon cycle and, for example, decrease organic matter decomposition, when applying nitrapyrin to an undisturbed semi-arid steppe. Their results indicated that N species (i.e. ammonium vs. nitrate) may be a more important driver of carbon cycling and ecosystem functioning than the quantity of N present in the system. This underlines the significance of different forms of N in terms of carbon turnover in soils and highlights the need for further studies into the effects of chemical nitrification inhibitors on all nutrient turnover processes and their interactions in soil ecosystems.

More recent studies demonstrated that the efficacy of DMPP was closely related to soil organic constituent and that the adsorption of DMPP to the soil fraction played a major role in controlling inhibition effect (Azam & Faroog, 2003: Austin, *et.*

al, 2006: Barth, *et al.* 2006:2008). Once the nitrification inhibitor is in the soil, it may be gradually broken down by soil microbes and its efficacy slowly disappears. A timely nitrification inhibition is whished and an incubation of DMPP in the sandy loam showed that after 12 days of incubation the DMPP-concentration significantly decreased and at day 35 only small amounts of DMPP were still detectable. A major role hereby may play the temperature and availability of organic carbon (Irigoyen, *et. al.* 2003: Sahrawat, 2004). However, performance of NIs can be highly variable in different agro ecosystems. Granulated DMPP-fertilizer application is apparently superior to liquid DMPP-application and under wet conditions, favorable for nitrate leaching, most effective in the sandy loam (Barth, *et al.* 2008: 2008: Irigoyen, *et al.* 2003: Li, *et al.* 2008).

In practice, however, DMPP as aggregates (with a grain diameter of about 4mm) formulated on ammonium-N applied, therefore it would not in a homogeneous distribution (Azam, *et al.* 2001; Barth, *et al.* 2001:Zerulla, *et al.* 2001: Di *et al.* 2006). It can be assumed that the granules of ENTEC (N-fertilizer and DMPP) after rain hydrolyzed gradually with the result that ammonium and possibly DMPP (also CIMPP) to diffuse rapidly among the granules into the soil. It took consequently in the field, temporally and spatially different concentration gradients of DMPP (or CIMPP) and ammonium, which for DMPP between 0 and about 100 µg g-1 dry soil hat a granulate distance (in the 0-5 mm zone of the granule center) are expected (Azam *et al.* 2001). A comparison of the inhibitory concentrations for further inhibition of nitritation in the laboratory showed that a complete inhibition of ammonium oxidation was temporally and spatially under the pellets and around them is around given the least, especially since about 80% of DMPPs remains in a loamy soil over a 10-days period in around during 0-5 mm of the granules (AZAM *et al.* 2001). However, it remains very difficult to know the real, locally and time and the process of granules to inhibit nitritation in the field, because the concentrations of DMPP and ammonium because of the different diffusion rates should change continuously. It is likely that DMPP in soils with usual pH values from 5 to 6.5 predominantly active cation (diffuse through Protonitation) and due to their molecular sizes significantly slower than the ammonium. In Laboratory experiments, which were carried out under standard conditions and at different temperatures (4, 15, 25 ^{o}C) and soil moisture (18 or 20% of the mWHC), let the example of a silty clay showed that only 5-15% of DMPPs after 10 days were in the 25-40 mm zone around the granules, which confirmed the very low mobility DMPPs (Azam *et al.* 2001, Di, *et al.* 2007). In soils, the ratio of ammonium was changed to DMPP in the course of the time probably constantly. The inhibitions of Nitritation in the field were depending on such conditions, which in model experiment are hardly possible to create them. From the above considerations' can be concluded that the nitrification inhibition must take place substantially in close contact with the DMPP-granules. To clear up this point, some more field experiments in different soil types should be conducted.

CONCLUSION

Based on the obtained results above, the following conclusion can be drawn; The kinetic of N_2O production by *A. brasilense* in the present of 1% v/v acetylene or in absent of acetylene ranged about 2-18 µg N_2O mL^{-1} day^{-1}, and 2-17 µg N_2O mL^{-1} day^{-1}, respectively, while the rate of N_2O production by *Sinorhizobium sp* was about 4-15 µg N_2O mL^{-1} day^{-1} and 4-12 µg N_2O mL^{-1} day^{-1}. The N_2O portion of total denitrification losses at the beginning of experiment (700 µg $N\text{-}NO_3^-$ mL^{-1}) by *Azosprillum brasilense* AZ204 or *Sinorhizobium sp* was about 70-98% and 80-95%, respectively and its decreased gradually until about 50- 51% due to the decreasing of nitrate supply. The application of 1 µg mL^{-1} DMPP (3 times of field concentration) has lead to the decreasing of both nitrogen fixers population, particularly; the CO_2-production (carbon mineralisation) by *Azospirilum brasilense* was decreased significantly.

DMPP may influence the population of non target microorganism in soils.

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