



**IFA International Workshop on Enhanced-Efficiency Fertilizers  
Frankfurt, Germany, 28-30 June 2005**

**NITRIFICATION INHIBITION FOR NITROGEN  
EFFICIENCY AND ENVIRONMENT PROTECTION**

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## “Nitrification inhibition for nitrogen efficiency and environment protection”

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### Introduction

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Nitrogen is the most yield-limiting nutrient element in crop production in most soils worldwide. It is the fertilizer element required in greatest quantity as a soil amendment and is the easiest lost from the soil of the three major soil nutrients—nitrogen, phosphorus, and potassium. Also, being the most costly nutrient, nitrogen demands the greatest management skills in successful and economical crop production.

Most of the Earth’s supply of nitrogen is in the atmosphere (87%) and sediments (12%). The waters of oceans and lakes contain about 0.52% of the total nitrogen, the terrestrial biosphere 0.01%, and terrestrial soils 0.0055% (Stevenson and Cole, 1999).

Not so long ago, agricultural scientists and farmers were mostly concerned with cycling nitrogen in organic matter of the soil and supplementing the soil nitrogen through animal manures, nitrogen mined from the Earth’s evaporite deposits, and atmospheric N<sub>2</sub> fixation by legume plants. Modern technology in manufacturing of synthetic nitrogen, i.e., the Haber process, allowed the large-scale tapping of N<sub>2</sub> in the atmosphere as the major source of nitrogen for crop production, but it clearly linked the cost of nitrogen fertilizers to that of fossil fuels. Furthermore, concern for the environment has brought into focus certain forms of nitrogen as pollutants in both water and the atmosphere.

Nitrogen added to the soil in any form is readily transformed through a number of biological and chemical processes, depending on the form, ultimately becoming nitrate through the process of nitrification. During these transformation processes, nitrogen is vulnerable to loss along several pathways that may decrease nitrogen-use efficiency and result in undesirable environmental consequences. These concerns intensify the demand for more careful management of nitrogen for economic efficiency and protection of the environment.

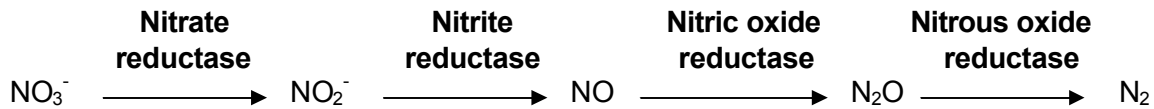
### Nitrogen Losses and Environmental Concerns

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There are a number of pathways, mostly involving nitrogen transformations, which potentially result in losses from the nitrogen cycle and have ecological consequences. The losses discussed here are the most likely to be observed and are generally somewhat amenable to control through management practices.

- **Soil erosion** removes nitrogen in many forms by transporting mineral and organic soil particles, soil microorganisms, and both inorganic and organic forms of nitrogen dissolved in the surface runoff water that produces the erosion.
- **Leaching**, usually of NO<sub>3</sub><sup>-</sup>, is one of the most important nitrogen-loss pathways, particularly in humid climates. Large quantities of nitrogen can be lost from soil by leaching, resulting in both undesirable economic and environmental consequences.

- **Denitrification** is the major reductive fate of  $\text{NO}_3^-$ , the product of nitrification, in most soil environments. It is a multi-step process catalyzed by distinct enzymes for each step, the latter three producing gaseous forms of nitrogen.



- **Ammonia volatilization** occurs during hydrolysis of urea and can result in losses of 10 to 50% of the nitrogen from surface-applied urea fertilizers. Ammonia volatilization due to urease activity is given as one explanation for the loss of 3 to 50% of the nitrogen in unincorporated animal manures, such as poultry litter, when applied to near-neutral soils.
- **Nitrogen immobilization** represents a significant temporary loss in availability of nitrogen. Rapid microbial growth, especially on a high C:N-ratio substrate (about 30 and higher), is conducive to immobilization of inorganic nitrogen as the microorganisms assimilate the available nitrogen into amino acids. Although immobilization temporarily renders part of the inorganic nitrogen unavailable, it retards nitrogen loss from the soil.

Obviously, with nitrogen being the most costly of the major crop nutrients, any loss without it first contributing to crop production is an economic loss to the grower. Such losses lower the nitrogen-use efficiency and are largely responsible for the generally low recovery (30 to 60%) of fertilizer nitrogen applied for crop production. Some of these losses, including soil erosion,  $\text{NO}_3^-$  leaching, denitrification, and  $\text{NH}_3$  volatilization, can be managed to varying extents. Nitrification is strongly implicated in  $\text{NO}_3^-$  leaching and denitrification losses, and it contributes indirectly to the release of gaseous forms of nitrogen that are implicated in global warming and ozone destruction.

## Nitrification

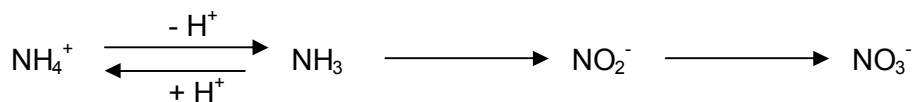
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### The Process

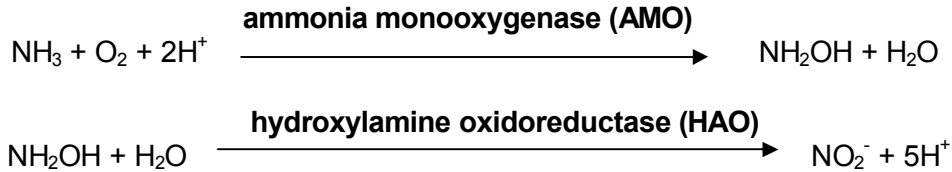
Nitrification is the general term for aerobic oxidation of reduced nitrogen ( $\text{NH}_3$ ) to  $\text{NO}_2^-$  and  $\text{NO}_3^-$  by either autotrophic or heterotrophic pathways (Coyle and Frye, 2005).

### *Autotrophic Nitrification*

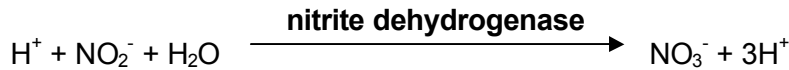
Autotrophic nitrification is a two-step process carried out by chemolithotrophic bacteria, which obtain energy by oxidizing  $\text{NH}_3$  to  $\text{NO}_3^-$  as follows:



The first step is performed by NH<sub>3</sub>-oxidizing bacteria such as *Nitrosomonas*, and involves the sequential oxidation of NH<sub>3</sub> to hydroxylamine (NH<sub>2</sub>OH) and then to NO<sub>2</sub><sup>-</sup> as shown here.



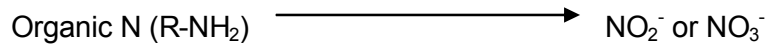
The second step in the pathway (shown below) is performed by NO<sub>2</sub><sup>-</sup>-oxidizing bacteria typified by the genus *Nitrobacter*. Ordinarily, nitrite oxidation is rapid, and NO<sub>2</sub><sup>-</sup> rarely accumulates to any significant extent in soil.



Autotrophic nitrification is the dominant type of nitrification occurring in agricultural soils. Nitrification is an acidifying process and use of urea and other ammoniacal forms of nitrogen, as well as animal manures, will lower the soil pH with time because of nitrification.

### ***Heterotrophic Nitrification***

Heterotrophic nitrification is the oxidation of organic nitrogen and NH<sub>3</sub> by various chemoheterotrophic bacteria and fungi, such as *Arthrobacter*, *Streptomyces*, and *Aspergillus*, that obtain energy from organic carbon. Heterotrophic nitrification produces NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in soils typically too acid to permit autotrophic nitrification.



### **The Importance**

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Autotrophic nitrification frequently produces nitrous oxide and nitric oxide as gaseous intermediates, which are greenhouse gases. Nitrous oxide (N<sub>2</sub>O) is much more effective than carbon dioxide at absorbing thermal energy, and nitric oxide (NO) has been shown to destroy stratospheric ozone. The major concern with nitrification is, however, the production of NO<sub>3</sub><sup>-</sup>, which is readily leached from soil and is subject to loss through denitrification. The nitrate is problematic as a contaminant in both surface water and groundwater. Inhibiting nitrification can slow leaching and denitrification losses and ameliorate the ecological consequences often associated with nitrogen fertilizer and animal manure use.

## Nitrification Inhibitors

Nitrification inhibitors slow the nitrification process by interfering with the activity of the *Nitrosomonas* group of bacteria that transform  $\text{NH}_3$  to  $\text{NO}_2^-$ , thus effectively slowing the formation of  $\text{NO}_3^-$ . The availability status of the nitrogen is essentially unchanged, but the  $\text{NH}_4^+$  is said to be stabilized, and the nitrogen is less susceptible to leaching and denitrification losses because it remains longer as  $\text{NH}_4^+$ . Therefore, ammoniacal nitrogen fertilizers are appropriately referred to as “enhanced-efficiency fertilizers,” a term adopted by the Association of American Plant Food Control Officials (AAPFCO) to describe such fertilizers along with slow-release fertilizers.

Several chemicals have been patented as nitrification inhibitors (Table 1), but few have been proven agronomically and economically effective. Until recently, only two—nitrapyrin and dicyandiamide (DCD)—had been successfully marketed as nitrification inhibitors. The first nitrification inhibitor to be approved by the U.S. Environmental Protection Agency (USEPA) was nitrapyrin, manufactured by Dow Chemical Company and marketed as N-Serve®. Nitrapyrin is 2-chloro-6-(trichloromethyl) pyridine. It may be used with any  $\text{NH}_4^+$ -N fertilizer, including anhydrous ammonia, urea, urea-ammonium nitrate solutions (UAN), ammonium nitrate, ammonium sulfate, and animal manures.

**Table 1:** Some patented nitrification inhibitors

Chemical name	Common name	Developer	Inhibition by day 14 %
2-chloro-6-(trichloromethyl)pyridine	Nitrapyrin	Dow Chemical	82
4-Amino-1,2,4-triazole-HCl	ATC	Ishihada Industries	78
2,4-Diamino-6-trichloromethyltriazine	CL-1580	American Cyanamid	65
Dicyandiamide	DCD	Showa Denko	53
Thiourea	TU	Nitto Ryuso	41
1-Mercapto-1,2,4-triazole	MT	Nippon	32
2-Amino-4-chloro-6-methylpyrimidine	AM	Mitsui Toatsu	31

*Adapted from Paul and Clark (1989).*

Nitrapyrin has a relatively high vapor pressure ( $2.8 \times 10^{-3}$  mm Hg at 23 °C). Because of its tendency to volatilize, the manufacturer initially recommended incorporation into the soil immediately after application. Research in Kentucky (Frye et al., 1981) showed that nitrapyrin was effective when sprayed directly onto urea or ammonium nitrate granules, which were then surface-applied to the soil for no-tillage maize (*Zea mays* L.) production (Table 2). We believe that the immediate proximity of nitrapyrin and  $\text{NH}_4^+$  ions together in the soil at the sites of potential nitrification facilitated its effectiveness in inhibiting the process, even when surface-applied.

**Table 2:** Grain yield of no-tillage maize (*Zea mays* L.) as affected by nitrapyrin\* in Kentucky

Nitrogen Rate, kg ha <sup>-1</sup>	0	90	135	180
	----- Yield, Mg ha <sup>-1</sup> -----			
Without nitrapyrin	3.41	5.96	5.13	7.45
With nitrapyrin	----	7.91	8.29	7.73

\*Nitrapyrin sprayed directly onto granular ammonium nitrate at rate of 0.56 kg ha<sup>-1</sup> and surface-applied broadcast.

Dicyandiamide, also known as cyanoquanidine, is a water soluble, organic amide that is both a slow-release nitrogen source and a nitrification inhibitor. The composition of DCD is C<sub>2</sub>N<sub>4</sub>H<sub>4</sub>, and it contains at least 65% N. It is a fine-grained, non-hygroscopic, crystalline powder, which is easily incorporated into solid, solution, or suspension fertilizers. Dicyandiamide was not officially approved by the USEPA as a nitrification inhibitor until the late 1990s, but it was used in Europe and was researched as a nitrification inhibitor in the United States much earlier.

Field experiments with DCD as a nitrification inhibitor were conducted extensively during the 1980s on several field and vegetable crops throughout the United States (Frye et al., 1989). The higher ratio of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> in the soil following applications of ammoniacal fertilizers with DCD than without DCD showed that DCD effectively inhibited nitrification, usually for several weeks depending on conditions. Inhibiting nitrate formation, however, was not often reflected in crop yield responses. If DCD increased yields, generally it occurred only with the lower nitrogen fertilizer rates, indicating that preventing nitrogen loss only at the lower end of the yield-response curve where nitrogen is a limiting factor had a measurable positive effect on crop yields.

Ammonium thiosulfate (ATS) is (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>3</sub> containing about 19% nitrogen and 43% sulfur in its solid form. Its normal form as a commercial fertilizer is a 60% aqueous solution containing 12% nitrogen and 26% sulfur. The solubility of ATS in water is 1.48 parts ATS per 1.0 part H<sub>2</sub>O, and it is compatible in neutral or alkaline phosphate-containing liquid fertilizers, as well as aqueous ammonia and other nitrogen solutions. Ammonium thiosulfate has been shown to inhibit the nitrification process to some extent (Goos, 1985) and was defined as a nitrification inhibitor in 2000 by the AAPFCO.

Recently, BASF in Germany has developed a new nitrification inhibitor 3,4-dimethylpyrazole-phosphate (DMPP). Research appears to indicate that it is highly effective, easily incorporated into granular fertilizers facilitating its use, and presents no cause for environmental concerns. Crop experiments in the field have shown its beneficial effects on yields, crop quality, and economic efficiency. The product is registered in and is being marketed successfully in several European countries (Zerulla et al., 2001).

Widespread research on nitrapyrin and DCD shows that these chemicals are usually very effective in suppressing nitrification in soil for several weeks after application. The ratio of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> is usually much higher in soils and NO<sub>3</sub><sup>-</sup> is lower in drainage water from soils following application of ammoniacal fertilizers treated with these inhibitors than the same applications without a nitrification inhibitor. Our results in Kentucky indicated effective inhibition in the range of 4 to 6 weeks.

A yield response to a nitrification inhibitor, however, occurs only if nitrogen was lost by leaching or denitrification, and then only if those losses resulted in a nitrogen deficiency sufficient to reduce crop yields

without the nitrification inhibitor. Rarely are yield increases observed if nitrification inhibitors are used with application rates of nitrogen more than slightly above optimum.

The environmental benefits of decreasing  $\text{NO}_3^-$  leaching and the production of nitrous and nitric oxides by denitrification through inhibition of nitrification would nonetheless be present, and even more important, where an excess of N was applied.

### **Deterrents to use of Nitrification Inhibitors**

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In the United States, nitrification inhibitors have not been widely adopted by growers and farm managers. Several factors have contributed to this generally low level of interest in nitrification inhibitors.

- **Inexpensive Nitrogen Fertilizer**

In most of the years since nitrification inhibitors have been marketed and studied by soil scientists and agronomists, nitrogen fertilizer has been relatively inexpensive. Thus, growers have tended to use somewhat excessive amounts, which were usually still profitable. Growers often spoke of applying more than the optimum rate as “insurance” against higher-than-normal nitrogen losses from the soil because of excessive rainfall. In most years, the additional nitrogen was more economical than using a nitrification inhibitor.

- **Delayed and split applications**

During the early spring, soil water content is often high. Summer annual crops are in the early stages of development, and there is little demand for nitrogen. Therefore, early spring application often leaves nitrogen very vulnerable, and even normal rainfall may result in high losses. This, of course, would be an excellent opportunity to use a nitrification inhibitor to avoid much of these losses. Instead, growers have more readily adopted the practice of totally delaying application of any nitrogen fertilizer for 4 to 6 weeks for maize production, or splitting it with a starter application at planting and the rest applied 4 to 6 weeks after planting the maize.

- **Volatility of Nitrapyrin**

The manufacturer of nitrapyrin, Dow Chemical Company, stated that it must be incorporated into the soil because of its volatile property. In moldboard-plow tillage, this often meant an extra trip over the field with a disk harrow, requiring more time and fuel in the tillage and planting operations. In no-tillage, it meant that nitrapyrin could not be used except if knifed into the soil with anhydrous ammonia or UAN using a special no-tillage applicator.

We found in Kentucky that nitrapyrin was a very effective nitrification inhibitor in no-tillage maize production if sprayed onto the surface of granular (prilled) ammonium nitrate or urea, as discussed above. The impracticality of that was the creation of a potentially explosive mixture of petroleum-based nitrapyrin and ammonium nitrate. The manufacturing, transportation, and storage of nitrapyrin-treated ammonium nitrate and urea discouraged Dow Chemical Company from pursuing such a practice. By then, the energy crisis of the 1970s and early 1980s had diminished, nitrogen fertilizer had become cheaper, and interest in nitrogen-fertilizer efficiency had waned.

- **Inconsistent Field Responses**

The circumstances under which crop yields responded to the use of nitrification inhibitors are dependent upon many factors determining nitrogen losses from soil relative to nitrogen supply to the crops, as explained above. Often growers who used nitrification inhibitors did not see a yield response.

- **Economic vs. Environmental Concerns**

The environmental benefits of nitrification inhibition in crop production are present regardless of yield response. Most growers, however, are more likely to voluntarily adopt a practice that promises economic benefits rather than one offers only environmental benefits.

- **U.S. Environmental Protection Agency Restrictions**

Dow Chemical Company obtained approval from the USEPA for nitrapyrin as a soil microbiocide. When SKW Trostburg in Germany proposed to market DCD in the USA as a nitrification inhibitor, with labeled claims as such, the EPA apparently held DCD to the same requirements for approval as nitrapyrin. This hurdle seemed to squelch the effort to market DCD as a nitrification inhibitor in the United States until the late 1990s when the EPA removed its objection to DCD being labeled as a nitrification inhibitor. Dicyandiamide was officially designated a nitrification inhibitor by AAPFCO in 2000 (Official Publication AAPFCO, No. 54, 2001).

### **Outlook for Nitrification Inhibitors**

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Higher energy prices have caused a rapid increase in the price of nitrogen fertilizers. That has rekindled an interest in nitrogen-use efficiency. Concern for environmental quality has been steadily rising for many years, with both voluntary and regulatory compliance coming into play more and more. Educational efforts, societal pressures, and the threat of greater regulations are moving more growers to adopt best management practices (BMPs) to help protect the environment.

The versatility and convenience of DCD as a nitrification inhibitor makes its use an attractive BMP for decreasing the potentially harmful effects associated with nitrogen fertilizers. Its effectiveness is even greater when used in tandem with an effective urease inhibitor to decrease volatilization of  $\text{NH}_3$  from surface-applied urea, particularly for no-tillage crop production. Perhaps the advent of 3, 4-dimethylpyrazole-phosphate (DMPP), joining nitrapyrin and DCD in the marketplace as effective nitrification inhibitors, will spur interest by both growers and researchers in nitrification inhibitors.

### **Conclusions**

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Nitrification is implicated in several nitrogen loss pathways that decrease use efficiency and contribute to environmental pollution. Ammoniacal nitrogen, when added to soil, is rapidly nitrified to nitrate under ordinary conditions. The nitrate is susceptible to leaching and denitrification losses from the soil. Nitrate is a pollutant in groundwater and surface water, contributing to eutrophication of streams and lakes and hypoxia of sea waters. Greater than 10 mg  $\text{NO}_3^-$ -N per liter in drinking water is considered to be a potential health hazard by the USEPA, particularly in infants.

Autotrophic nitrification, as well as denitrification, may release gaseous intermediate products into the atmosphere. Nitrous oxide ( $\text{N}_2\text{O}$ ) contributes to global warming by absorbing solar energy and nitric oxide (NO) destroys stratospheric ozone.

Inhibiting nitrification is an effective means of ameliorating the economic effects and ecological consequences of nitrogen losses from the soil. The combined effects of using a nitrification inhibitor together with an effective urease inhibitor enhances the appeal and practicality of both chemicals. Increasing nitrogen fertilizer prices, heightened concerns for pollution from nitrogen and its ecological effects, and a promising new nitrification inhibitor (DMPP) on the horizon most likely will renew interest in using nitrification inhibitors as a management tool, making their future the brightest ever.

## Further Reading

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