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Effect of Metasystox-R on Marine *Nitrosomonas* sp. as a Nitrification Inhibitor

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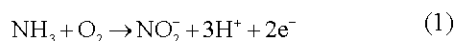
Abstract: Metasystox-R is a systemic soluble liquid insecticide for the control of aphids on brassica vegetable crops, cotton and lupins and it is possible enter to the marine environment and may be have a hazard effects for the marine organisms and nitrification processes. Effect of Metasystox-R on ammonia oxidizing activity by marine *Nitrosomonas* sp. was investigated by determining nitrification inhibitor assay in the cell suspension. Results showed that $8 \mu\text{g mL}^{-1}$ of Metasystox-R with $\text{PI}_{50} = 4.48$ significantly inhibited nitrite production by marine *Nitrosomonas* sp. These results suggested marine *Nitrosomonas* sp. may be one of the target bacteria which was inhibitor and decreasing nitrification in the marine environment.

Key words: Metasystox-R, nitrification inhibitors, marine *Nitrosomonas* sp.

INTRODUCTION

Major transformation of soluble inorganic compounds occur on fresh water and sea water. Remains of cell types of biota (detritus) sink to bottom of the water body, decomposing to ammonia (Austin, 1989).

The process of nitrification is carried out by Nitrifying bacteria (Hu *et al.*, 2003; Murakami *et al.*, 1995). *Nitrosomonas* and *Nitrobacter* which was widely distributed in sea water and significance in nitrogen cycle. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite (Kim *et al.*, 1997; Bock *et al.*, 1989; Lopez *et al.*, 2003). In the first step of nitrification, ammonia-oxidizing bacteria such as *Nitrosomonas* sp. oxidize ammonia to nitrite according to Eq. 1 (Watson *et al.*, 1981).



We have already reported the nitrification inhibitory activity of some compounds such as 2-chloro-6-(Trichloromethyl) pyridine, 2-amino 4 methyl-6-trichloromethyl-1,3,5 triazine (MAST), on the nitrification activity by *Nitrosomonas europaea* ATCC 25978 and *Nitrosomonas* sp. TK794 (Ohki *et al.*, 1999; Murakami *et al.*, 1993; Takagi *et al.*, 1994).

Inhibitory activity of some compounds have been investigated with intact nitrified bacteria cells and

inhibitor compound, to conclude that the inhibitors effect the ammonia oxidizing process to nitrite. In these 15 years, more than 20 species of ammonia-oxidizing bacteria have been isolated from waste water and sea water (Matsuba *et al.*, 2003; Mizoguchi *et al.*, 1998) which was mostly belong to *Nitrosospira*, *Nitrosococcus* and *Nitrosolobus* species.

Metasystox-R (ODM) is an organophosphorus compound. ODM systemic insecticide for control of aphids on brassica vegetable crops, cotton and lupins and establishment of maximum residue limits for oxydemeton-methyl in brassica vegetable crops, cotton, lupins and animal commodities (Australian Pesticides and Veterinary Medicines Authority, 1998). The ecotoxicological of Metasystox-R indicates high to moderate toxicity to fresh water and sea water fish and very high toxicity to aquatic invertebrates (Anderson, 1987). Otherwise enter Metasystox-R into water, hazard to fish and daphnia and other aquatic invertebrates. We have investigated to obtain effects of Metasystox -R as inhibitor on the marine *Nitrosomonas* sp. as a compound inhibitory of nitrification.

MATERIALS AND METHODS

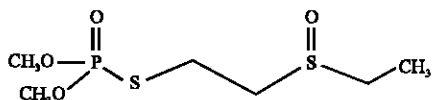
The experiments were carried out in the laboratory of (NTB) faculty of marine science, Tehran, since 14 June 2007.

Chemicals: Metasystox-R (oxydemeton-methyl) (ODM) $\text{C}_6\text{H}_{15}\text{O}_4\text{PS}_2$

Table 1: Composition of the Nitrosomonas medium

Compounds	Concentration
(NH ₄) ₂ SO ₄	1.00 (g)
KH ₂ PO ₄	0.50 (g)
NaCl	2.00 (g)
MgSO ₄	0.20 (g)
FeSO ₄ ·7 H ₂ O	0.05 (g)
CaCO ₃	6.00 (g)
Distilled water	400.00 (mL)
Sea water	600.00 (mL)

The pH was adjusted at 7.6



Metasystox-R, from Bayer AG, Company Germany were used as nitrification inhibitor in this study.

Bacteria and their incubation: Marine strain of *Nitrosomonas* sp. were used as ammonia-oxidizing bacteria which is supply from SERA company which is used as a nitrifying bacteria in the sea water. This strain was incubated at 29°C with shaking at 200 rpm in the dark in 100 mL Erlenmeyer flasks containing 30 mL of liquid media (Murakami *et al.*, 1995; Takagi *et al.*, 1994) was cultured in the Nitrosomonas Medium (Watson *et al.*, 1981; Austin, 1989). The composition of Nitrosomonas medium mention in the Table 1.

Nitrification inhibitory activity

Preparation of cell suspension: Cell suspension from *Nitrosomonas* sp. was cultured in the Nitrosomonas medium (Table 1). Ten milliliters of the preculture growth for 10 days were transferred into 500 mL Erlenmeyer flasks containing 100 mL of the new liquid media (Murakami *et al.*, 1995; Takagi *et al.*, 1994). The flasks were shaken at 200 rpm in the dark at 29°C for 10 days. About 300 mL of their culture media were centrifuged at 8600 x g for 15 min at 4°C. Then the bacteria pellet was washed twice with sterilized 20 mM phosphate buffer (pH 8, P-buffer) by centrifugation at 7200 x g for 15 min at 4°C. The harvested wet cells resuspended with P-buffer to 5 mL (Murakami *et al.*, 1995).

Determination of nitrification inhibition assay in cell suspension: For determination Nitrification inhibitory assay in cell suspension substrate solution and different concentration of Metasystox-R (ODM) were used.

Substrate solution: Ammonia sulfate (100 µg nitrogen per milliliter in P-buffer) was used to the substrate solution for the ammonia-oxidation (Murakami *et al.*, 1995).

Different concentration of Metasystox-R: Ten milligrams of Metasystox-R were emulsified in water with a few drops of DMSO to make a 5 mL of inhibitor solution.

Cell suspension: The cell suspension described in 3.1 was diluted with P-buffer which were about 1×10⁶ cells mL⁻¹.

Nitrification inhibition assay in cell suspension: Into a 2 mL test tube, 1 mL of each substrate solution, 1 mL of the diluted cell suspension and 0.016, 0.008, 0.004 and 0.0004 mg mL⁻¹ of Metasystox-R solution was added and the test tube was allowed to stand in 37°C water bath for 30 min to incubate. The nitrite concentration was determined at the start of reaction (0 min) and after 30 min incubation (Murakami *et al.*, 1995) by means of OD value at 540 nm according to MOOPAM (1999) method which was determined sea water nitrite.

Effects of DMSO without Metasystox-R was also tested as control and also another control (P-buffer) was used diluted cell suspension and substrate solution without Metasystox-R.

The inhibitory rate at each concentration was obtained from the following equation (Murakami *et al.*, 1995).

$$\text{Inhibitory rate (\%)} = \frac{\{1 - (\text{Inhibitor (OD value at 30 min)} - \text{Inhibitor (OD value at 0 min)})\}}{\text{Control } \{(\text{OD value at 30 min}) - \text{control (OD value at 0 min)}\}} \times 100$$

The molar concentration of inhibitor which shows 50% inhibition against the nitrite production relative to the control was estimated (Takagi *et al.*, 1994).

The inhibitory introduced were expressed as PI₅₀, the molar concentration of the inhibitor, which shows 50% inhibition (molar I₅₀) against the nitrite formation by ammonia-oxidizing bacteria relative to the control. The nitrification inhibition introduced are expressed as PI₅₀ which was the negative logarithm of the molar I₅₀ (Matsuba *et al.*, 2003; Murakami *et al.*, 1995).

RESULTS AND DISCUSSION

Effect of Metasystox-R (ODM) on ammonia oxidizing activity by marine *Nitrosomonas* sp. from SERA company was investigated by determining nitrite amount in the cell suspension assay. Results showed that maximum inhibitory rate (62.5%) which was induced with 0.016 mg mL⁻¹ concentration of Metasystox-R and after diluted ODM had showed reduce inhibitory rate activity (Table 2). The results mentioned the ammonia oxidation

Table 2: The effect of Metasystox-R on ammonia oxidation in cell suspension of marine *Nitrosomonas* sp.

Sample No.	Concentration (mg mL ⁻¹)	0 min (OD) ^a value	30 min (OD) ^b value	Inhibitory rate (%)
P-buffer (control)	-	0.007	0.015	0.0
Metasystox-R	0.0160	0.075	0.078	62.5
	0.0080	0.064	0.068	50.0
	0.0040	0.050	0.055	37.5
	0.0004	0.011	0.017	25.0
DMSO	-	0.005	0.013	0.0

^a: OD value at the start of reaction indicate nitrite amount before adding inhibitory and substrate, ^b: OD Value after 30 min incubation with inhibitor and substrate, PI₅₀ value: Negative logarithms of the molar I₅₀ in soil-water and cell suspension

bacteria, marine *Nitrosomonas* sp. was too sensitive to the Metasystox-R and prevent oxidation of ammonia to nitrite in the culture medium with inhibitor activity (PI₅₀ = 4.48) with 8 µg mL⁻¹ significant inhibitory as shown in Table 2.

Metasystox-R was dissolved in DMSO and concentrations of DMSO and P-buffer did not inhibit the nitrite production nor the nitrite oxidation (inhibitory rate 0.0 %).

Our laboratory results showed that Metasystox-R had inhibitory activity on the marine *Nitrosomonas* sp. with PI₅₀= 4.48 which is show the moderate inhibitory activity.

MAST(2-Amino-4-methyl-6-trichloromethyl-1,3,5-triazine), Br-MAST(2-amino-4-tribromomethyl-6-trichloromethyl-1,3,5-triazine) and nitrapyrin (2-chloro-6-trichloromethyl-pyridine) are inhibitors of the ammonia-oxidation *Nitrosomonas europaea* ATCC 25978 with inhibitory activity (pI₅₀) of 5.75, 7.12, 5.66, respectively (Kasahara *et al.*, 2002). We known inhibitor with pI₅₀<4.35 were be week activity and exhibiting pI₅₀>6.4 more sensitive to *Nitrosomonas* sp. other experiment showed that *Nitrosomonas europaea* sensitive to the three inhibitors (MAST, Br-MAST, nitrapyrin) and compare this result with waste water and marine *Nitrosomonas* sp. was showed that less sensitive to the three inhibitors (Matsuba *et al.*, 2003; Okano *et al.*, 2004).

Comparing the PI₅₀ value in this experiment indicate that the nitrification inhibitory activity may be due to a direct action to marine ammonia-oxidizing bacteria and marine *Nitrosomonas* sp. with PI₅₀ = 4.48 which was significant inhibitory activity on marine *Nitrosomonas* sp. so results were observed that Metasystox-R with moderate to high effects on marine *Nitrosomonas* sp. and could be important effect on nitrogen cycle in the marine environment. The ecotoxicological profile of ODM indicates high to moderate toxicity to birds, moderate toxicity to mammals, moderate toxicity to

fish and very high toxicity to aquatic invertebrate (Australian Pesticides and Veterinary Medicines Authority, 1998).

Further investigation is under way to make clear the site of action of Metasystox-R nitrification inhibitors including isolation of microorganisms such as fresh water and marine *Nitrosomonas* and *Nitrosococcus* in our laboratory.

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