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Amperometric Detection of Nitric Oxide with Microsensor in the Medium of Seawater and Its Applications

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Abstract: Amperometric detection of nitric oxide with ISO-NOPMC NO microsensors (WPI) is systemically studied in the six media including seawater. The linear range of the microsensor for NO was from 10^{-6} to 10^{-9} mol/l and the detection limit was 4.2×10^{-10} mol/L(S/N=2). With this method, we provide preliminary evidence that NO production could be a general attribute of marine alga (*Haeterosigma akashiwo*). Experiment conducted with inhibitor of uncoupler 2,4-DNP (2,4-dinitropheno) revealed that NR (nitrate reductase) activity is responsible for NO formation.

Keywords: Amperometric detection, ISO-NOPMC NO microsensors, Nitric oxide, Seawater, *Haeterosigma akashiwo*

Introduction

Since its identification as the endothelium-derived relaxing factor (EDRF) [1-2], nitric oxide (NO) has been proved effective as a neurotransmitter and an immune mediator. Some pathological processes, such as diabetes, ischemia and atherosclerosis are found to be connected with abnormalities of the EDRF [3].

The measurement of NO in solutions is very difficult because of its low stability and high fugacity. Most of several NO detection methods [4] involve indirect detection of the NO oxidation products removed from biological media. These direct methods also include electrochemical methods which are the best because they are simple, relatively fast, highly sensitive, applicable *in vivo* and capable of real time performance [5-6].

The electrochemical detection of NO in biological media has been reported [7-11]. However, up to now, there are few reports concerning practical electrochemical measurement of NO in seawater. The aim of this work is to use amperometric method and ISO-NOPMC microsensor [12] to detect NO in the six media including seawater for the first time.

Much research has been made about the influence of dissolved inorganic nitrogen (DIN) in seawater, including NH_4^+, NO_3^-, and NO_2^-, on the marine phytoplankton growth. However, NO was always neglected in the past nitrogen studies [13]. Since NO is the necessary intermediate at the nitrogen cycle and has important effect in organism, the research on the physiological effect of NO in algal growth is significant [14-15]. In this paper, amperometric detection of nitric oxide with ISO-NOPMC NO microsensors (WPI) is also applied for studying NO production of *Haeterosigma akashiwo*. The results of the study will help to explore NO in ocean ecosystem.

2. Experimental

2.1. Chemicals

Pure NO gas (99.9%, *Zhuo Zheng* gas limited company, Guangzhou), pure nitrogen gas (99.999%，*He Li* Industrial Gas Center, Qingdao); $CuCl_2 \cdot 2H_2O$ was obtained from Sigma-Aldrich (St. Louis, USA). Natural seawater (from nearshore of Qingdao) filtered by 45 μm membrane filter. All other products were of analytical grade and used as received.

NO standards were prepared by making serial dilutions of saturated NO solutions: doubly distilled water (2 mL) was bubbled with nitrogen for 30 min to remove oxygen. Then the solution was bubbled with pure NO gas for 30 minutes. The concentration of a saturated NO solution is 1.4 mM [16]. Standards were freshly made for each experiment and kept in a glass flask with a rubber septum. Dilutions of the saturated solution were made using doubly distilled water.

Martin artificial seawater was made by Martin recipe. Fleming artificial seawater was made by Fleming recipe. Alga Solution was made by F/2 recipe. The regents were analytical purity. *Heterosigma akashiwo* was from the Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences.

2.2. Electrochemical instrumentation

Electrochemical experiments were performed using ISO-NO Mark II NO meter (World Precision Instruments (WPI), Inc., Sarasota, USA) connected with an ISO-NOPMC microsensor. The analog signal from the ISO-NO Mark II NO meter was digitized using a DUO18 two-channel data-acquisition system (World precision instruments (WPI), Inc., Sarasota, USA) connected to a Pentium III PC computer.

2.3. Electrochemical measurement of NO

The applied potential of the sensor was 0.86 V (vs. Ag/AgCl). The ISO-NOPMC sensor was allowed to polarize in 0.1 mol/L $CuCl_2$ for at least 1 hour before use. During polarization, the background current of the sensor, observed as the base line on a current-time chart, will decrease slowly. The sensor is ready for use when the background current has reached a stable value (less than 2000 pA). Deoxygenated solution (10 mL) was placed in the electrochemical cell and aliquots of standard saturated or diluted NO solution were added with micrometer syringe. Electrochemical responses of the ISO-NOPMC microsensor to NO were evaluated by current-time plot on ISO-NO Mark II NO meter. Data were recorded under constant stirring conditions of solution at room temperature. The main electrolytes in Martin artificial seawater, Fleming artificial seawater, alga culture solution, filtered seawater and natural seawater used for electrochemical detection are NaCl +$MgSO_4$. When distilled water without any electrolyte was used as samples, the sensor can not detect NO properly since ion strength will affect the background current.

3 Results and discussion

3.1 Sensitivity and linear range of the NO microsensor

The study about the response of ISO-NOPMC NO microsensors to nitric oxide in distilled water, Martin artificial seawater, Fleming artificial seawater, alga culture solution, filtered seawater and natural seawater was carried out. The results indicate that the linear responses of NO sensor to NO ranging from 10^{-9} to 10^{-5} mol/L in above media are good (R>0.98). Although in distilled water the response current to the same NO concentration is higher (the detection limit is 3.5×10^{-10} mol/L (S/N>3)), the sensor can not detect NO properly since ion strength will affect the background current. In the other media, the detection limits are about 4.2×10^{-10} mol/L (S/N=2). As the medium component will affect the responses of the microsensors, NO sensors should be calibrated in the same environment in which the experimental measurement is to be made as possible. Fig.1-2 show the response curves of NO sensor to the NO in seawater and the corresponding linear equations.

Fig.3 shows the linear equations of NO sensor in six media. In distilled water and artificial seawater, the linear range of the microsensors ranges from 10^{-5} mol/L NO to 10^{-9} mol/L NO. In artificial seawater the slope of the linear equations at NO concentration of 10^{-6} mol/L has small variation for the influence of the ions in the solutions. In algal solution, filtered seawater, natural seawater, the slope of the linear equations at NO concentration of 10^{-6} mol/L has large variation for the influence of the organic and colloid matter in the solutions. In result, the linear range in the three solutions is from 10^{-6} mol/L NO to 10^{-9} mol/L NO or from 10^{-5} mol/L NO to 10^{-6} mol/L NO.

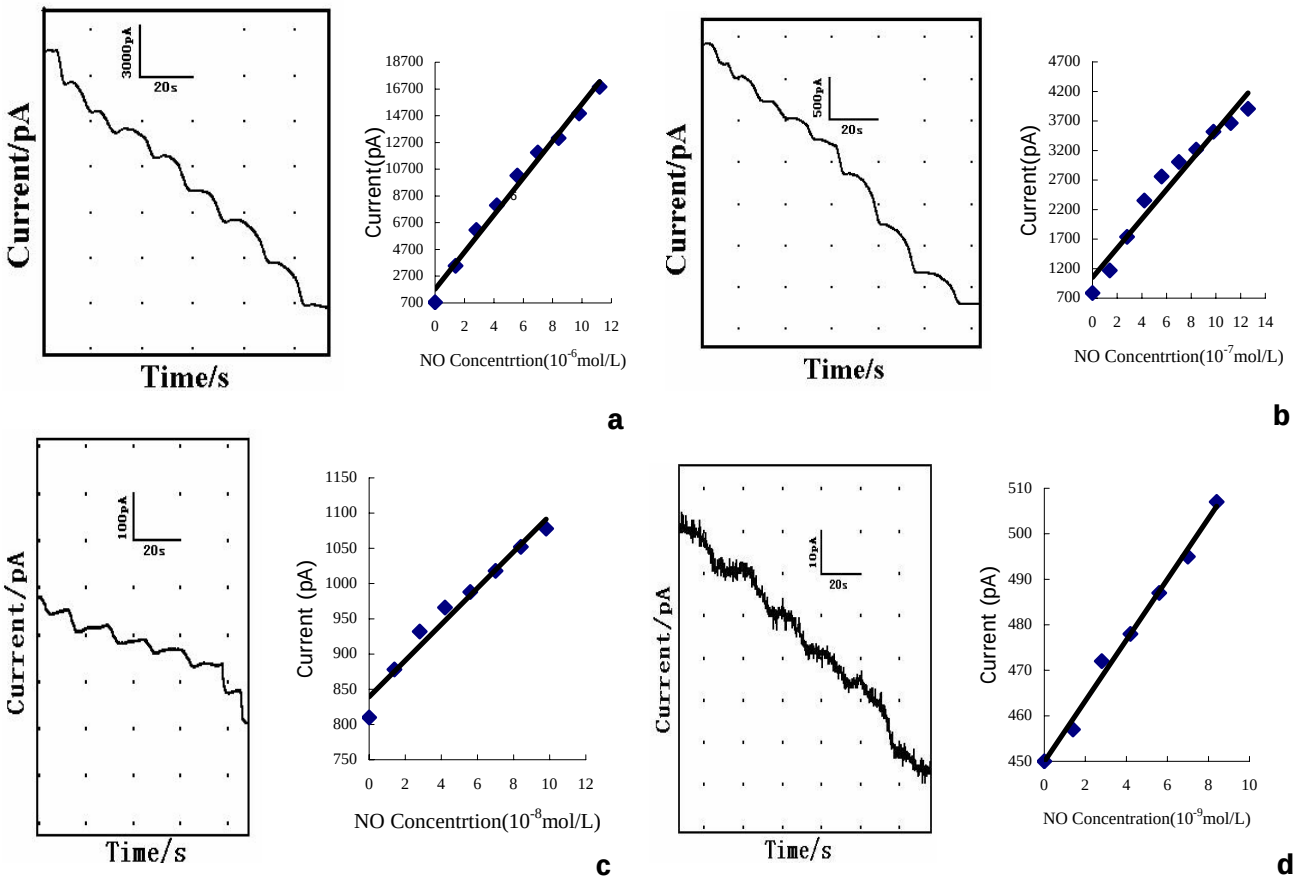

Figure 1. Current-time curves of the ISO-NOPMC microsensor response to successive additions of NO and plots of the response *vs.* NO concentration in natural seawater. Each [NO] increment is 1.4×10^{-6} (a), 1.4×10^{-7} (b), 1.4×10^{-8} (c) and 1.4×10^{-9} mol/L (d).

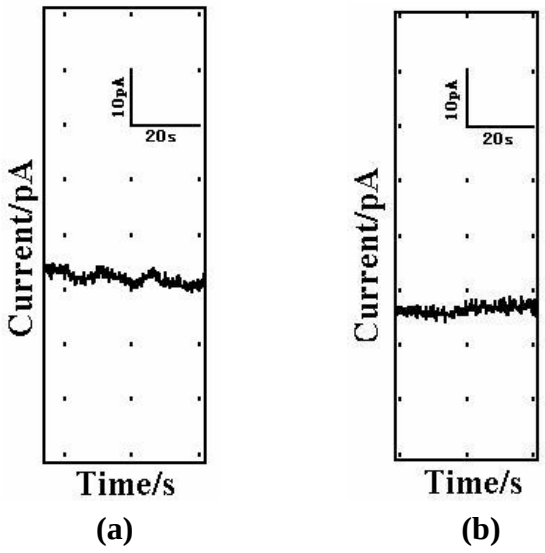

Figure 2. Current-time curve of the ISO-NOPMC microsensor response to successive additions of 4.2×10^{-10} mol/L NO (a) and the noise curve (b) in natural seawater.

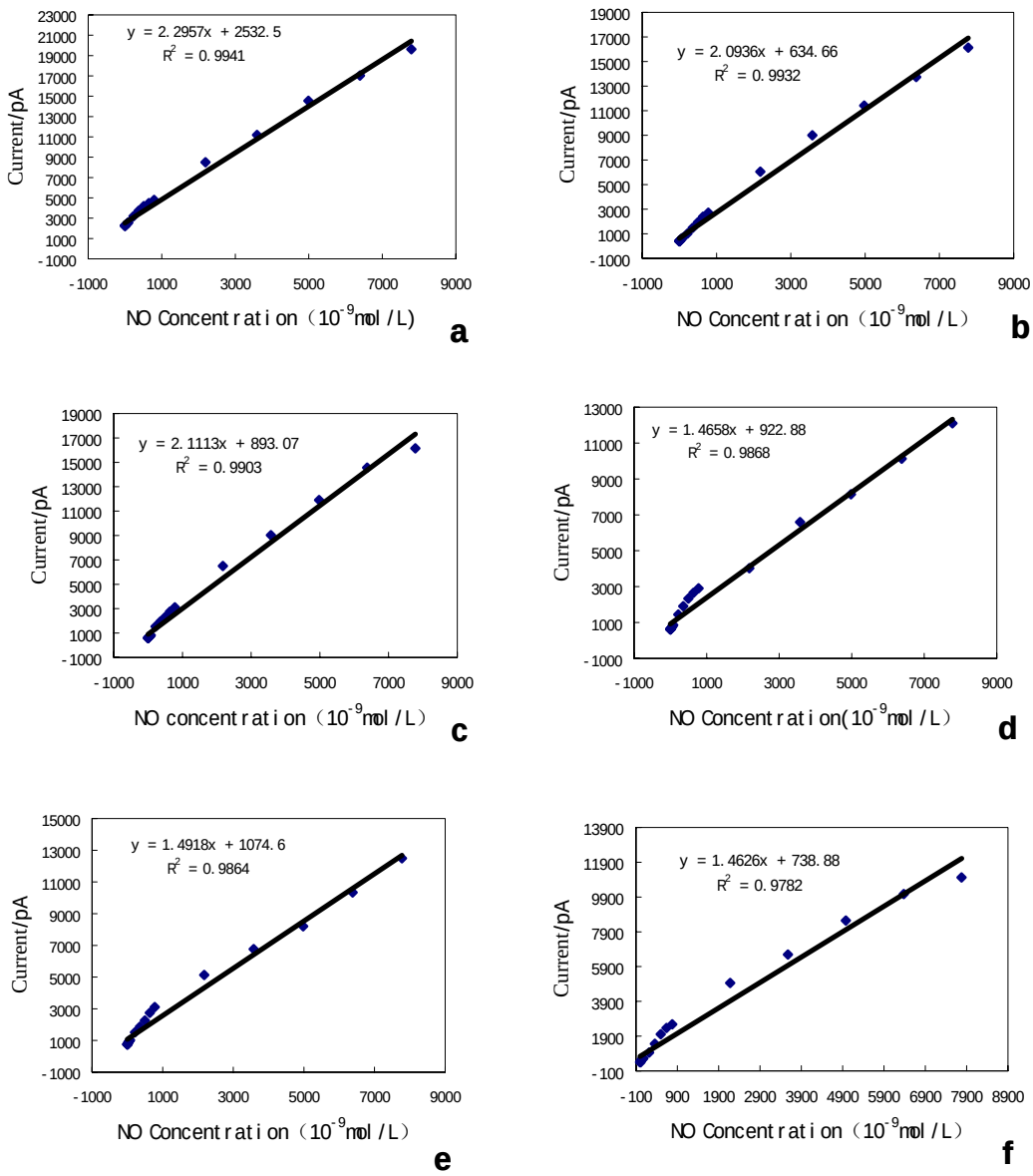

Figure 3. Relationship between NO concentration (10^{-9}-10^{-5} mol/L) in distilled water and electrode current measured at the ISO-NOPMC microsensor (a: distilled water, b: Martin artificial seawater, c: Fleming artificial seawater, d: filtered seawater, e: alga culture solution, f: natural seawater).

Since there are a lot of biomolecules in blood and tissue liquid, the sensors are usually calibrated in PBS buffer (8.0 g NaCl + 2.9 g Na_2HPO_4 + 0.2 g KCl + 0.2 g KH_2PO_4 + 0.1 g $MgCl_2 \cdot 6H_2O$ dissolved in 1 L aqueous solution, pH 7.4) in the biological detection of nitric oxide. But the above study proved the medium could influence the detection of NO, and NO sensors should be calibrated in the same environment in which the experimental measurement is to be made as possible. Natural seawater is a kind of fine electrolytic solution, and the detection limit of NO in seawater is similar with the reported detection limit of NO in PBS buffer [12]. Therefore, the method is fit for the practical detection of NO in natural seawater.

3.2 Lifetime and Precision of the sensors

The sensors used daily for at least 4 hours could work normally for about two months. After working for two months, the response time of the sensors will become long and the response current of the sensors will get low. The deviation is less than 2% for ISO-NO-Mark II in the range from 0 to 10,000 pA. The experiment of precision showed that the relative standard deviation was 6.3%.

3.3 NO production of alga

It was proved that NR from corn could produce NO from nitrite with NADH, and light can influence this reaction. The study of Rockel [17] shows that the main way by which plants produce NO is nitrite reduction with NR (nitrate reductase) (Fig. 4). Marine phytoplankton such as red tide algae and food algae can perhaps produce NO by the way.

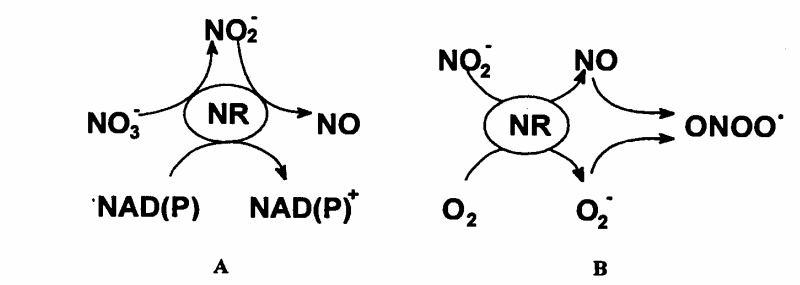

Figure 4. NO (A), O_2^- and $ONOO^-$(B) catalysed by NR in plants.

NR activity is usually higher under good photosynthetic conditions than that in the dark, and NR activity in the dark for 6 hours is only about 50% of that in illuminated leaves [18]. In our experiments, NO can be detected in day but can't be monitored in night. About 1.5×10^{-9} mol/L NO release of *Haeterosigma akashiwo* was monitored during a dark-light transient time (Fig. 5(a)). It indicates light intensity is important for NO produce of plants.

In the dark, NR activity can be activated by uncouplers or 5'-AMP (adenosine monophosphate) [19]. 2,4-DNP can active NR activity [20]. In Fig.5(b), *Haeterosigma akashiwo* fed with 2 mmol/L 2,4-DNP in the dark released 2.4×10^{-9} mol/L NO. These results demonstrate NO production from *Haeterosigma akashiwo* is correlated with NR activity.

4. Conclusion

The relation between the sensors currents and the NO concentrations is linear for NO concentrations ranging from 10^{-6} to 10^{-9}mol/L and the detection limits of them are about 4.2×10^{-10} mol/L (S/N=2) in natural seawater. Compared with our previous research on the detection of NO in seawater using polarography and homemade Nafion/Co(salen) modified platinum electrode [21-22], amperometric detection of nitric oxide with NO microsensors has high sensitivity (4.2×10^{-10} mol/L

NO), stability (relative standard deviation is 6.30‰). The method is a kind of practical NO determination method in seawater. Using this method, NO production of *Haeterosigma akashiwo* was proved.

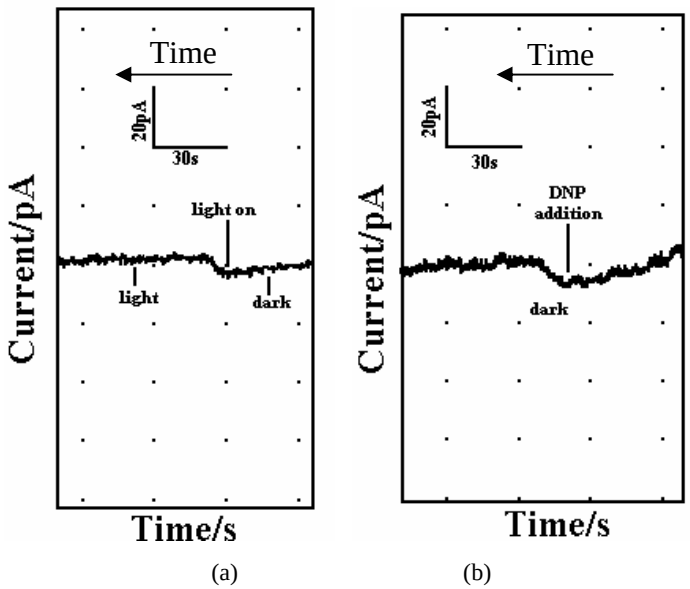

Figure 5. NO in alga solution during a dark-light transient (a) and effect of 2,4-DNP on NO production by dark-grown *Haeterosigma akashiwo* (b).

Acknowledgements

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References

1. Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **1987**, *327*, 524-526.
2. Ignarro, L. I.; Buga, G. M.; Wood, K. S.; Chaudhuri, G. Endothelium –derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci.* **1987**, *84*, 9265-9267.
3. Xian, Y. Z.; Sun, W. L.; Xue, J.; Luo, M.; Jin, L. Iridium oxide and palladium modified nitric oxide microsensor. *Anal. Chim. Acta.* **1999**, *381*, 191-196.
4. Bedioui, F.; Trevin, S.; Devynck, J. The use of gold electrodes in the electrochemical detection of nitric oxide in aqueous solution. *J. Electroanal. Chem.* **1994**, *377*, 295-298.

5. Kikuchi, K.; Nagano, T.; Hayakawa, H. Detection of nitric oxide production from a perfused organ by a luminol system. *Anal Chem*, **1993,** *65,* 1794-1799.
6. Bedioui, F.; Trevin, S.; Devynck, J. Chemical modified microelectrodes designed for the electrochemical determination of nitric oxide in biological systems. *Electroanalysis* **1996**, *8(12),* 1085-1091.
7. Trevin, S.; Bedioui, F.; Devynck, J. New electropolymerized nickel porphyrin films application to the detection of nitric oxide in aqueous solution. *J. Electroanal. Chem.* **1996**, *408,* 261-265.
8. Jin, J. Y.; Miwa, T.; Mao, L.; Tu, H.; Jin, L. Determination of nitric oxide with ultramicrosensors based on electropolymerized films of metal tetraaminophthalocyanines. *Talanta* **1999**, *48,* 1005-1011.
9. He, X. C.; Deng, R.; Li, P.; Mo, J. Y. Electrocatalytic oxidation and determination of nitric oxide at Nafion-Co-schiff base-modified electrode. *J. Instrumental Analysis.* **2000**, *19(2),* 35-38 (in Chinese).
10. Mao, L. Q.; Lian, H. T.; Tian, Y. Preparation of ultramicroelectrode modified wiyh an electropolymerized film of ethylenebis (salicyldiminate) Cobalt and its application in the determination of nitric oxide. *Journal of Analytical Science* **1998**, *14 (4),* 273-277 (in Chinese).
11. Lantoine, J.; Trevin, S.; Bedioui, F.; Devynck, J. Selective and sensitive electrochemical measurement of nitric oxide in aqueous solution: discussion and new results. *J. Electroanal. Chem*. **1995**, *392,* 85-89.
12. Zhang, X. J.; Lin, J.; Cardosa, L.; Broderick, M.; Marley, V. A novel microchip nitric oxide sensor with sub-nM detection. *Electroanalysis* **2002**, *14(10),* 97-103.
13. Millero, F. J. *Chemical Oceanography, 2nd ed;* CRC press: Boca Raton, 1966; pp 178.
14. Ward, B. B.; Zafiriou, O. C. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. *Deep Sea Research* **1988,** *35 (7),* 1127-1142.
15. Zhang, Z. B.; Lin, C.; Liu, C. Y. The effect of nitric oxide on the growth of marine phytoplankton. *Journal of Ocean University of Qingdao* **2003**, *2(2),*185-188.
16. Lantoine, F.; Trevin, S.; Bedioui, F. Selective and sensitive electrochemical measurement of nitric oxide in aqueous solution: discussion and new results. *J. Electroanal. Chem.* **1995,** *392,* 85~89.
17. Rockel, P.; Strube, F.; Rockel, A. Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. *J. Exp. Bot.* **2002**, *53(366),* 103-110.
18. Yamasaki, H.; Sakihama, Y. Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. *FEBS Lett.* **2000,** *468(1),* 89-92.
19. Kaiser, W. M.; Weiner, H.; Huber, S. C. Nitrate reductase in higher plants: a case study for transduction of environmental stimuli into control of catalytic activity. *Physiologia Plantarum* **1999,** *105,* 385-390.
20. Mallick, N.; Rai, L. C.; Mohn, F. H. Studies on nitric oxide (NO) formation by green alga *Scenedesmus Obliquus* and the diazotrophic cyanobacterium *Anabaena Doliolum*. *Chemosphere* **1999,** *39(10),*1605-1610.
21. Ren, C. Y.; Zhang, Z. B.; Xing, L. The determination of nitric oxide in seawater by polarography. *Journal of Ocean University of Qingdao* **2003,** *33(5),* 801-808 (in Chinese).

22. Zhang, Z. B.; Xing, L.; Jiang, L. Q. The electrochemical determination of nitric oxide in seawater media with microelectrodes. *Sensors* **2003,** *3*, 304-313.