CULTURAL HERITAGE CONSERVATION SCIENCE AND SUSTAINABLE DEVELOPMENT
EXPERIENCE RESEARCH INNOVATION

LES SCIENCES DE LA CONSERVATION DU PATRIMOINE ET LE DÉVELOPPEMENT DURABLE
ACQUIS, RECHERCHE, INNOVATION

Assessment of the effect of nitric oxide-based treatments on biofilm formation: A comparison with biocides used in paint formulations and the treatment of cultural heritage

Kyi, Caroline [1-3] Rouse, Emma[1, 2] Sloggett, Robyn [1, 3] Cather, Sharon [4] and Schiesser, Carl H.[1,2]

1. ARC Centre of Excellence for Free Radical Chemistry and Biotechnology.
2. School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia.
3. Centre for Cultural Materials Conservation, School of Historical and Philosophical Studies, The University of Melbourne, Victoria 3010, Australia
4. The Courtauld Institute of Art, Somerset House, Strand, London WC2R 0RN

The University of Melbourne

Introduction

Biocides are chemical substances used in the treatment of damaging biological growth. They are commonly added as 'preservatives' to paint formulations to prevent biofouling. They are also applied in the control of organisms responsible for the biodecay of cultural material.

The demand for sustainable, low-toxic alternatives to conventional biocide use, requires a more sophisticated approach to biocidal systems (Denyer & Stewart 1998). We have investigated how the anti-bacterial properties of the free-radical molecule nitric oxide (NO•), when used in combination with commercial biocides, can enhance their efficacy.

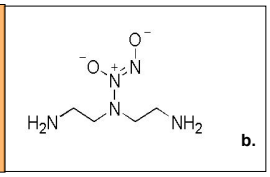
Bacterial reduction of nitrate to nitric oxide

Nitrate reduction

$NO_3^- + 2\,e^- + 2H^+ \Leftrightarrow NO_2^- + H_2O$

Nitrite reduction

$NO_2^- + 2H^+ + e^- \Leftrightarrow NO + H_2O$ **a.**



b.

Figure 1: (a) Bacterial nitrate reduction generates nitric oxide, (b) The nitric oxide donor (Z)-1-[N-(2-aminoethyl)-N-2 ammonioethyl)amino] dizen-1-ium1,2-diolate (DETA/NO•)

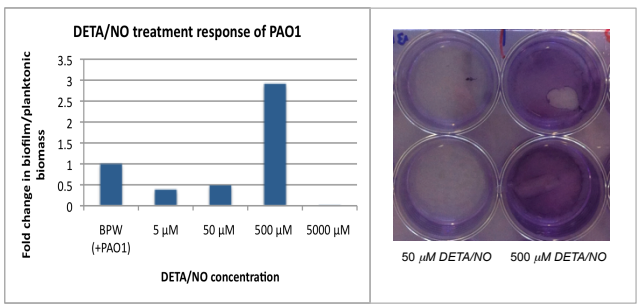


Figure 1: At 50 and 50 µM DETA/NO decreases in the ratio of biofilm to planktonic cells are seen for PAO1. At 500 µM concentration (12 hour treatment) there is an increase in the ratio of biofilm to planktonic cells as stressed cells form protective biofilms. At 5000 µM a cytotoxic response is observed. Accompanying image of biofilms treated with 50 and 500 µM DETA/NO stained with crystal violet.

Therapeutic applications of NO• have led to the synthesis of a range of nitric oxide donor compounds (Keefer 2005). These can control the formation of anti-bacterial resistant communities of microorganisms, known as biofilms (Costerton 1987). When nitric oxide donors are administered to induce biofilm dispersal, cells released from biofilms show increased motility and an enhanced response to biocide treatments (Barraud et al., 2006).

Paradoxically, NO• is also produced by nitrate reducing bacteria (Figure 1a). The biochemistry of this process is also associated with increases in cell motility (Van Alst et al., 2007). We investigated how biofilm development, and the response of treated biofilms were influenced by the nitric oxide donor (DETA/NO) (Figure 1b) and NO• generated by bacteria.

We demonstrated that nitrate-reducing species of bacteria were present, and able to be cultured from samples of stone, canvas and paper. Assays were performed to compare the treatment response of a mixed population of organisms, originating from the samples (CMO), to a known nitrate reducer *Pseudomonas aeruginosa* PAO1.

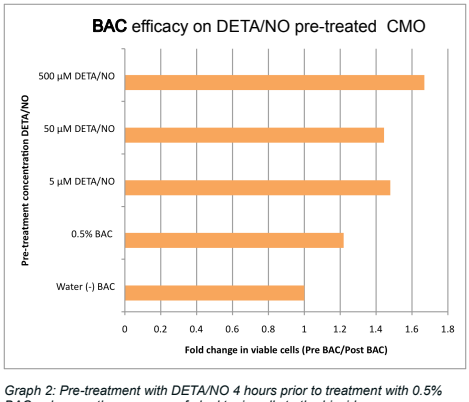
Treatment of young established biofilms with nitrate, caused a reduction in biofilm formation and an increase in non-biofilm associated (planktonic) cells. The trend is concentration dependant. The therapeutic range is 5-50 µM for PAO1 and 50-500 µM for CMO. Similar results were observed for DETA/NO treatments (Graph 1). This trend is also time dependant. Concentrations of 5-50 µM were effective, with increases in the duration of treatment producing biofilm enhancing or cytoxic effects (Graph 1 and accompanying image)

To test for enhanced susceptibility to biocides, Dowicil 75 (Dow Chemicals) a paint preservative and Benzalkonium chloride (BAC) (Sigma-Aldrich) a biocide used in the treatment of stone were combined with NO• based treatments. Increases in susceptibility were not observed when DETA/NO or nitrate were added simultaneously with either biocide. However, increases were recorded when NO• based treatments were administered 4 hours in advance of the biocide. The DETA/NO-BAC combination having the most pronounced effect (Graph 2).

Treatment with a nitric oxide donor or bacterial generation of NO• can reduce biofilm formation and increase the susceptibility of planktonic cells to biocide treatment. To optimise combined NO•/ biocide systems, further examination of concentration and treatment exposure times is underway.

References

BARRAUD, N., HASSETT, D. J., HWANG, S., RICE, S. A., KJELLEBERG, S. & WEBB, J. S. 2006 Involvement of nitric oxide in biofilm dispersal of Pseudomonas aeruginosa. *J. Bacteriol.*, 188, 7344-7353.
COSTERTON, J. W., CHENG, K. J., GEESEY, G. G., LADD, T. I., NICKEL, J. C., DASGUPTA, M. & MARRIE, T. J. 1987. Bacterial biofilms in nature and disease. *Annu. Rev. Microbiol.*, 41, 435-462.
DENYER, S. P. & STEWART, G. S. A. B. 1998. Mechanisms of action of disinfectants. *International Biodeterioration & Biodegradation*, 41, 261-268.
KEEFER, L. 2005. Nitric oxide (NO)- and nitroxyl (HNO)-generating diazeniumdiolates (NONOates): emerging commercial opportunities. *Curr. Top. Med. Chem.*, 5, 625-636.
VAN ALST, N., PICARDO, K., IGLEWSKI, B. & HAIDARIS, C. 2007. Nitrate sensing and metabolism modulate motility, biofilm formation, and virulence in Pseudomonas aeruginosa. *Infect. Immun.*, 75, 3780-3790.



Graph 2: Pre-treatment with DETA/NO 4 hours prior to treatment with 0.5% BAC enhances the response of planktonic cells to the biocide.