CULTURAL HERITAGE CONSERVATION SCIENCE AND SUSTAINABLE DEVELOPMENT
EXPERIENCE RESEARCH INNOVATION

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

'CULTURED' MICROORGANISMS: TOWARDS THE DEVELOPMENT OF NEW TREATMENT METHODOLOGIES

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Introduction

Biodeterioration and biofilms in conservation: It is well known that both aesthetic and structural damage occurs to cultural heritage as a result of the growth and metabolism of microorganisms (figure 1).[1,2] Microfloral contamination of cultural materials occurs primarily as a community of organisms called a biofilm (figure 2). Compared to lone planktonic microorganisms, these surface-associated assemblages are encased in a self-made bioorganic-coating (exopolymeric substance (EPS)) that provides 100-fold protection against environmental hazards.[3] A wide spectrum of biodeterioration treatment practices are currently used by conservators. Preventative conservation techniques that involve the control of relative humidity and temperature, while useful in controlled museum environments, are not applicable in areas such as immovable heritage and disaster response mitigation. Likewise, reactive biocide-based conservation treatments have their own pitfalls. As a result of the specialized survival techniques of colonized organisms, effective treatments are difficult to implement and sustain without the use of toxic and expensive chemicals.

Project scope

Bottom-up approach - towards new conservation-directed biodeterioration treatments: It has been shown that biofilm formation and dispersal is controlled through direct cell-to-cell communication.[4] Nitric oxide (NO•) is a signalling molecule (autoinducer) that acts as an elicitor of biofilm dispersal and an inhibitor of biofilm formation,[5] however its high reactivity and gaseous nature prevents it being an effective treatment option. Aminoxyl radicals have a similar functionality to NO•, however due to their electronic and structural stability they can be handled as inert compounds making their use ideal in a biodeterioration treatment methodology. It is hypothesized that aminoxy radicals may mimic the same mechanistic pathways as NO• to elicit the same effect but over a longer period of time, given their electronic and structural stability. This project reports the effect of aminoxyl radical candidates on biofilm formation and dispersal.

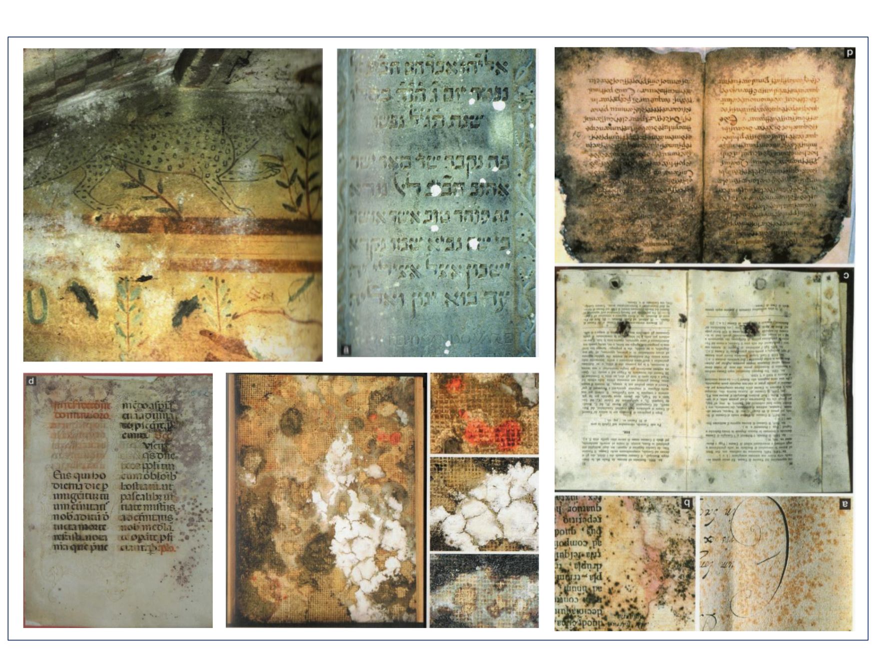


Figure 1: Examples of biodeteriorated cultural heritage.[6]

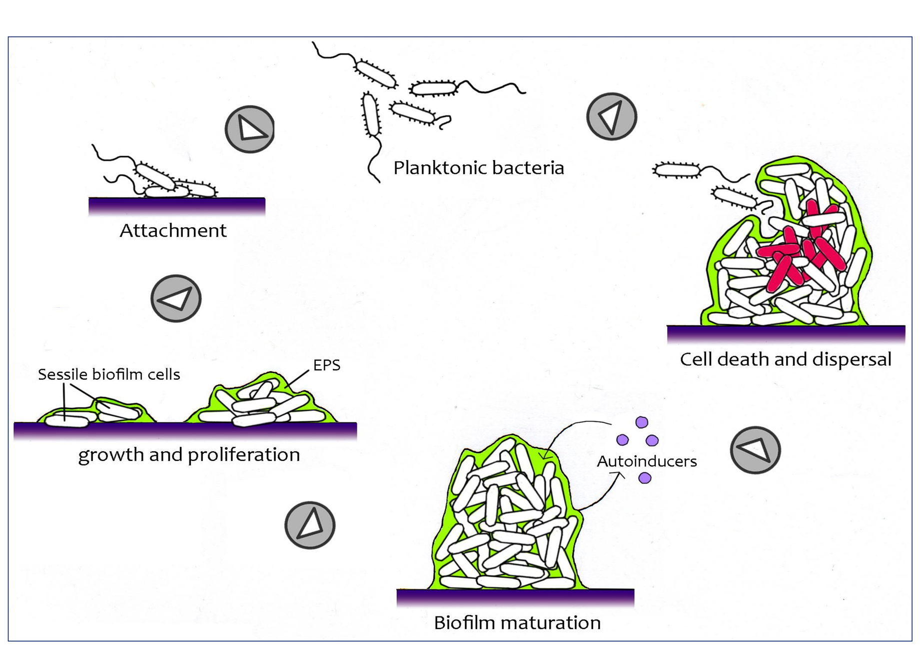
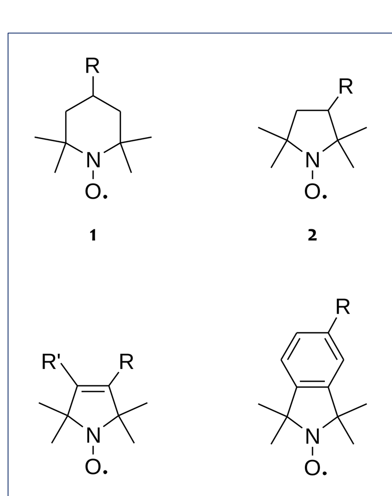


Figure 2: Pictorial representation of biofilm development.



	R =										
	H	O	OH	COOH	$COO^- Na^+$	CN	$CONH_2$	CH_2OH	CO-D-glucose	$S(CH_2)_2$-D-glucose	NO_2
Piperidinoxyl **1**	Tempo	Tempone	Tempol	Carboxy Tempo	Carboxy Tempo sodium salt	Cyano Tempo	-	-	-	-	-
Pyrrolidinoxyl **2**	-	-	-	Carboxy Proxyl	Carboxy Proxyl Sodium salt	-	-	-	3-Gluco Proxyl	-	-
Pyrrolinoxyl **3**	-	-	-	TPOCA	TPOCA sodium salt	-	CTPO	HMTPO	-	4-Gluco ETPO	-
Isoindolinoxyl **4**	-	-	-	-	-	-	-	-	-	-	Nitro TMIO

R' = H unless otherwise indicated

Figure 3: Chemical formulae and naming of aminoxyl radical candidates.

Aim

To investigate the ability of aminoxyl radical candidates to inhibit biofilm formation and/or elicit dispersal in established biofilms.

Materials and methods

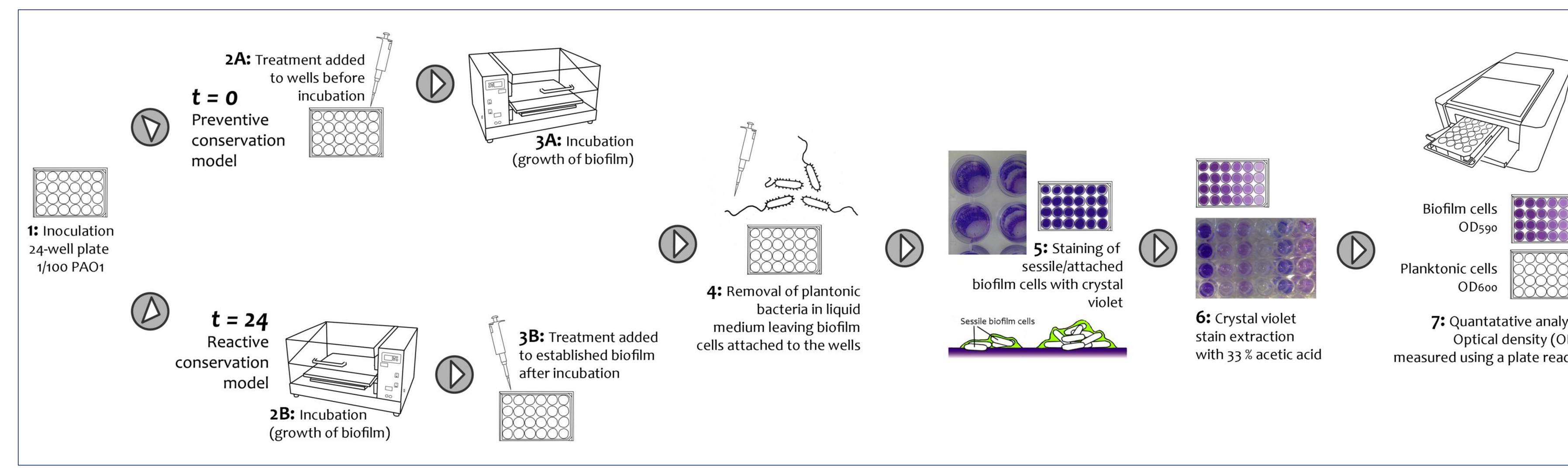


Figure 4: Pictorial representation of experimental set-up.

Model organism: *P. Aeruginosa* (PAO1) was chosen as the model organism in this study because it has a ubiquitous distribution in nature, and has been identified in association with biodeterioration of cultural heritage.[2,7]

Treatment: Aminoxyl candidates investigated in this study are easily accessible with a range of functional groups, and as a consequence, pH, solubility and cell permeability. Aminoxyl radicals candidates belong to the piperidinoxyl **1**, pyrrolidinoxyl **2**, pyrrolinoxyl **3** and isoindolinoxyl **4** classes and are listed in Figure 3.

Results

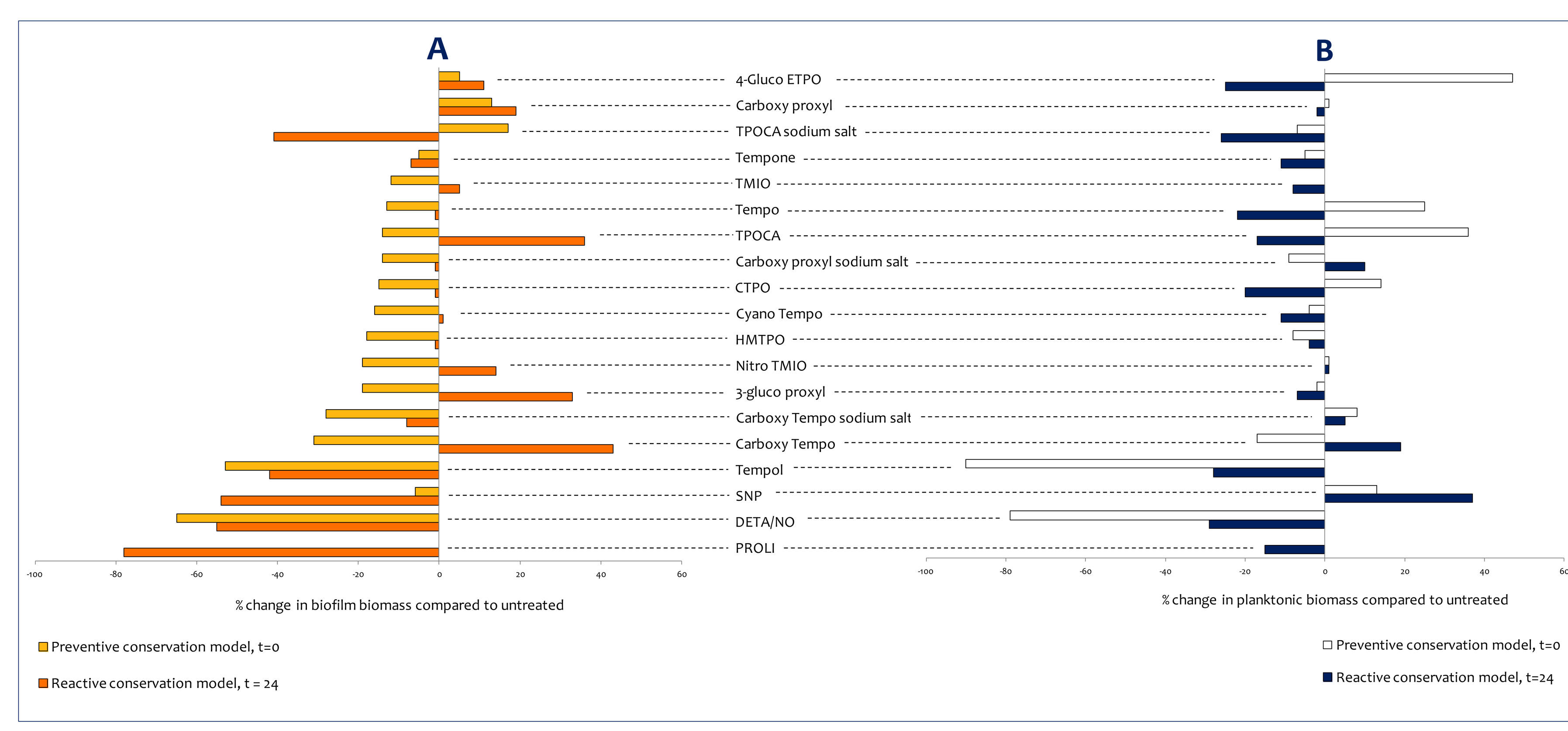
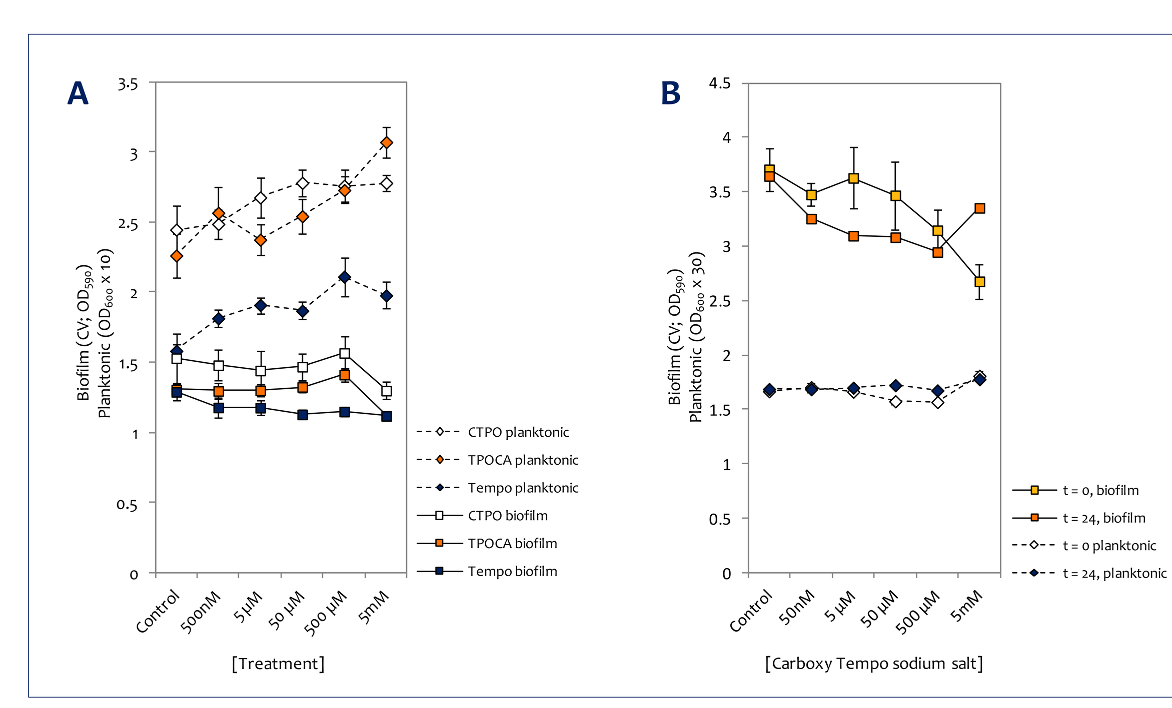


Figure 5: Effectiveness of tested compounds at 5 mM. A) Change in biofilm biomass compared to untreated. Bars extending to the left of this graph indicate a decrease in biofilm biomass whereas bars extending to the right indicate an increase in biofilm biomass. **B)** Change in planktonic biomass compared to untreated. Bars extending to the left of this graph indicate a decrease in planktonic biomass whereas bars extending to the right indicate an increase in planktonic biomass.

Figure 6: Most promising aminoxyl radical candidates. A) When treated with CTPO, TPOCA or Tempo using a preventive conservation model (t = 0), cells show a preference away from the establishment of a biofilm and towards a planktonic mode of growth; **B)** Carboxy Tempo sodium salt elicits a desirable response in biofilm and planktonic modes of growth in both the preventive conservation and reactive conservation models. In the preventive conservation model, cells prefer a planktonic mode of growth whilst in the reactive conservation model the cells underwent detachment and dispersal from the established biofilm. Error bars indicate 1 standard deviation (n = 8).



Conclusion

This study examined the effect of a series of aminoxyl radicals on biofilm formation and dispersal. While the majority of compounds investigated proved unsuitable as treatment agents, notably CTPO, TPOCA, Tempo and Carboxy Tempo sodium salt demonstrated the ability to direct cells away from the establishment of a biofilm and towards a planktonic mode of growth. These observations require further investigation and future translation to conservation practice.

References

1. Allsopp, D, Seal, K & Gaylarde, C 2004, *Introduction to Biodeterioration*, 2nd edn, Cambridge University Press, Cambridge; **2.** Ciferri, O 1999, 'Microbial degradation of Paintings', *Applied and Environmental Microbiology*, vol. 65, no. 3, pp. 89-885; **3.** Richards, JJ & Melander, C 2009, 'Controlling Bacterial Biofilms', *ChemBioChem*, vol. 10, pp. 2287-94.; **4.** Purevdorj-Gage, B, Costerton, WJ & Stoodley, P 2005, 'Phenotypica differentiation and seeding dispersal in non-mucoid and mucoid Pseudomonas aeruginosa biofilms', *Microbiology*, vol. 151, pp. 1569-76.; **5.** Barraud, N, Hassett, DJ, Hwang, S, Rice, SA, Kjelleberg, S & Webb, JS 2006, 'Involvement of Nitric Oxide in Biofilm Dispersal of Pseudomonas aeruginosa', *Journal of bacteriology*, vol. 188, no. 21, pp. 7344-53; **6.** Caneva, G, Nugari, MP & Salvadori, O (eds) 2008, *Plant Biology for Cultural Heritage: Biodeterioration and Conservation*, The J. Paul Getty Trust, Los Angeles.; **7.** Cappitelli, F & Sorlini, C 2008, 'Microorganisms Attack Synthetic Polymers in Items Representing Our Cultural Heritage', *Applied and Environmental Microbiology*, vol. 74, no. 3, pp. 564-9.