

Test Conducted at the

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By:
Professor Brett Neilan

Qualifications

B.AppSc (Biomedical Science)

The University of Technology, Sydney, Australia.

PhD in Molecular Biology and Genetics (1995)

Title: The molecular phylogenetics of toxic, bloom-forming
Cyanobacteria. School of Microbiology and Immunology.

The University of New South Wales, Sydney, Australia.

“Oxygen Spray-O2 evolution” a product from “Reach for Life”

Conducted by: Professor Brett Neilan

Phase 1: Antimicrobial activity of the liquid test product against common skin-colonizing microorganisms.

Aim

To evaluate the inhibition of microbial growth and/or microbial death elicited by Oxygen Spray-O2 evolution (OSO2) in its liquid form, against the common skin colonizing microorganisms (bacteria and fungi), *Staphylococcus aureus*, *Streptococcus pyogenes*, *Candida albicans* and *Micrococcus luteus*.

Methods

- 1) Ten millilitres of nutrient media were inoculated with a loop-full of each test organism and incubated overnight to obtain a dense culture (~ 1 million cells/mL).
- 2) Six, fifty millilitre sub-cultures of each organism were prepared (~ 5000 cells/mL).
- 3) The test product was added to the sub-cultures (in duplicate) at 0, 10 or 50% vol/vol.
- 4) One hundred microlitre aliquots were removed from each duplicate sub-culture following 5 and 30 minutes incubation with the test product. Aliquots were inoculated onto nutrient agar plates and incubated overnight.
- 5) Colony forming units (cfu) were recorded for each plate the following morning.

Results

The results of the experiment are presented in Table 1. OSO₂ inhibited most of the test organisms in a dose dependant fashion, with the higher dosage (50% OSO₂) inhibiting all test organisms by >95%. The extended incubation time (30 min) did not significantly increase the inhibitory effects of the product.

S. aureus and *S. pyogenes* were not inhibited by the low dosage (10%) of OSO₂, however, the higher dose (50%) resulted in 99-100% inhibition of *S. aureus*, and total inhibition of *S. pyogenes*.

Low dosage with OSO₂ inhibited *C. albicans* by >97%, while the higher dose resulted in greater than 99% inhibition of fungal growth.

Low dosage with OSO₂ inhibited *M. luteus* by ~54%, while the higher dose resulted in >95% inhibition of bacterial growth. The extended incubation time (30 min) combined with a high dosage of the product, resulted in total inhibition of *M. luteus* cultures.

Table 1. Inhibition of microbial growth by OSO₂*

OSO ₂ dose	<i>S. aureus</i>		<i>S. pyogenes</i>		<i>C. albicans</i>		<i>M. luteus</i>	
	5 min	30 min	5 min	30 min	5 min	30 min	5 min	30 min
10%	0	0	0	0	97	>99	67	40
50%	100	>99	100	100	>99	>99	95	100

*0 corresponds to no inhibition, 100 corresponds to complete inhibition.

Conclusions

OSO₂ significantly inhibited the growth of all microbial organisms when administered to test cultures at 50% of the total culture volume. *M. luteus* displayed the highest sensitivity to low dosage with OSO₂, while *S. aureus* and *S. pyogenes* were completely resistant.

While the administered dose of OSO₂ appeared to be an important factor for determining the degree of microbial inhibition, extending the incubation period from 5 to 30 min had little effect in most cases.