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# The preservation of biofilms on macroalgae by osmium vapour

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**Abstract**

The aims of this study were to determine if osmium vapour treatment prior to glutaraldehyde fixation could preserve the biofilms found on macroalgae and to gain insight into the structure of the extracellular polymeric substance (EPS or slime layer) of the biofilm. The microscopic surface features of twelve different species of macroalgae from Palm Beach, KwaZulu-Natal, South Africa were compared after being subjected to the different fixation procedures. Treating the seaweed samples with osmium tetroxide ($OsO_4$) prior to fixation with glutaraldehyde significantly enhanced the preservation of the EPS of the biofilms. The EPS was found to be complex and multi-layered with two types of EPS being distinguished, a fluffy or downy variety, and a flat sheet-like type, of which the latter varied in thickness.


*Keywords:* Biofilm; Seaweeds; Macroalgae; Bacteria; Osmium tetroxide; Glutaraldehyde; Extracellular polymeric substance; Slime layer; Preservation; Scanning electron microscopy (SEM)

## 1. Introduction

Biofilms are ubiquitous, being found in habitats as diverse as the oceans and on mammalian teeth, and consist mostly of various types of bacteria. At maturity, some biofilms often incorporate other life forms, e.g. algae, fungi and/or invertebrates depending on the habitat. These complex communities contain symbionts that work together to utilise resources optimally and even protect each other from antimicrobial agents (Mayer et al., 1999; Flemming et al., 2000). This is achieved by the formation of a slime layer or extracellular polymeric substance (EPS) that consists mainly of water, polysaccharides and proteins, with DNA, RNA, ions and lipids as minor components (Marsh and Bowen, 2000; Sutherland, 2001; Lawrence et al., 2003).

Interactions between the carbohydrates, proteins and nucleic acids in the EPS are thought to maintain its cohesiveness and integrity. Sutherland (2001) states that lipids function as biosurfactants and that their presence is considered to cause a loss of material from the EPS. Other workers mention the presence of lipoproteins, but not their possible function (Lawrence et al., 2003). However, McKeekin et al. (1979) found that biofilms on chicken skin were better preserved by a lipid-stabilising pretreatment of osmium tetroxide ($OsO_4$) vapour before the specimens were fixed and dehydrated for scanning electron microscopy (SEM) viewing (Komorowska et al., 1982).

Fixation in glutaraldehyde (without $OsO_4$ fixation) and drying damages the biofilm by removing the EPS and the underlying cells (Richards and Turner, 1984). Freezing hydrated samples prior to SEM viewing enables one to bypass

Table 1
Species of colonial diatom and macroalgae sampled for scanning electron microscope observation

| Division/class | Family | Genus and species |
|---|---|---|
| Heterokonta | Bacillariophyceae | *Nitzchia martiana* |
| Chlorophyceae | Codiaceae | *Codium duthieae* |
| | | *Halimeda cuneata* |
| | Caulerpaceae | *Caulerpa filiformis* |
| Rhodophyta | Corallinaceae | *Amphiroa bowerbankii* |
| | | *A. ephedraea* |
| | | *Cheilosporum multifidum* |
| | Gelidiaceae | *Gelidium abbottiorum* |
| | Hypneaceae | *Hypnea rosea* |
| | | *H. spicifera* |
| | Ceramiaceae | *Spyridia hypnoides* |
| | Rhodomelaceae | *Osmundaria serrata* |

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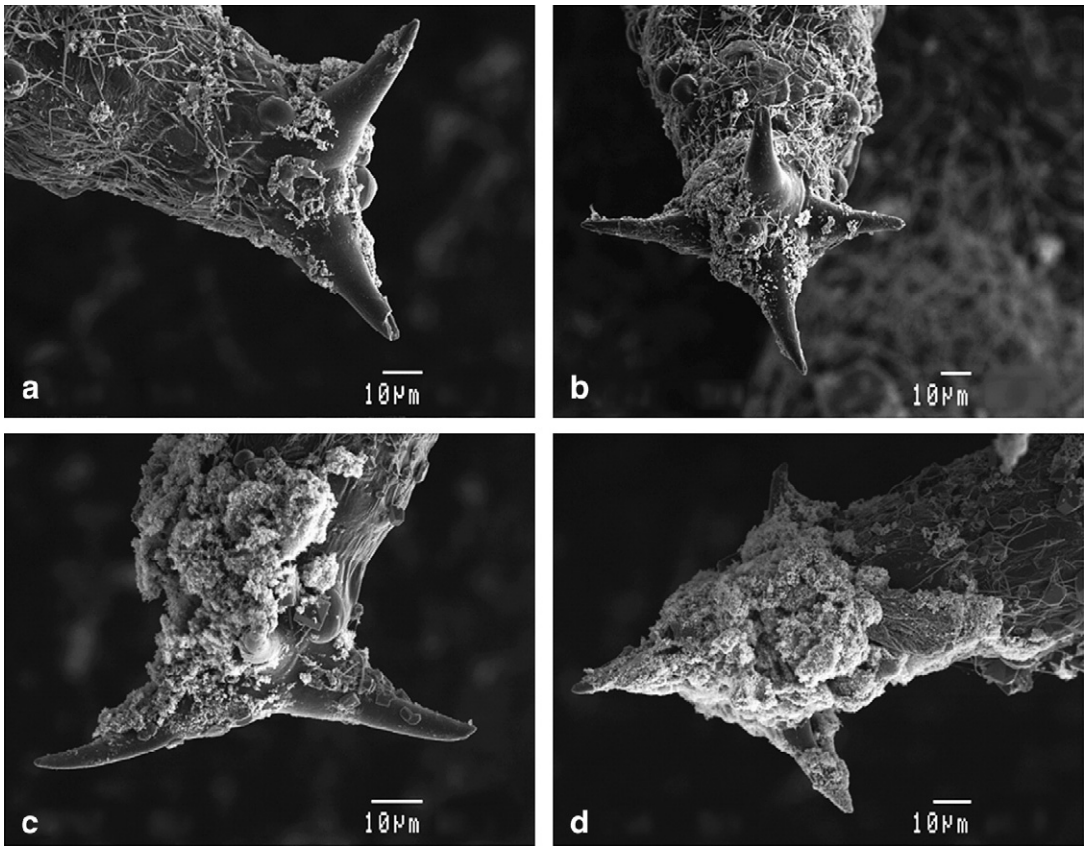


Fig. 1. Scanning electron micrographs of the tips of *Spyridia hypnoides* comparing sample untreated (a and b) with those treated with osmium tetroxide ($OsO_4$) vapour (c and d) before fixation in glutaraldehyde.

this problem, but with such cryotechniques only the surface of the biofilm is visible giving no indication of what lies beneath. More recently, non-destructive techniques such as confocal laser scanning microscopy and scanning transmission X-ray microscopy have been used to analyse the interior of biofilms in much greater detail (Lawrence et al., 2003; Larson and Passy, 2005). With these techniques it is possible to view the locations of labelled biochemicals *in situ* and *in vivo*, and thus gain a greater understanding of biofilms.

The aim of this study was to determine if an osmium vapour treatment prior to glutaraldehyde fixation could preserve the biofilms found on macroalgae and to gain insight into the structure of the EPS.

## 2. Materials and methods

All seaweeds samples collected from Palm Beach, KwaZulu-Natal, South Africa (30° 59′ 30″ S, 30° 16′ 30″ E) in June 2002

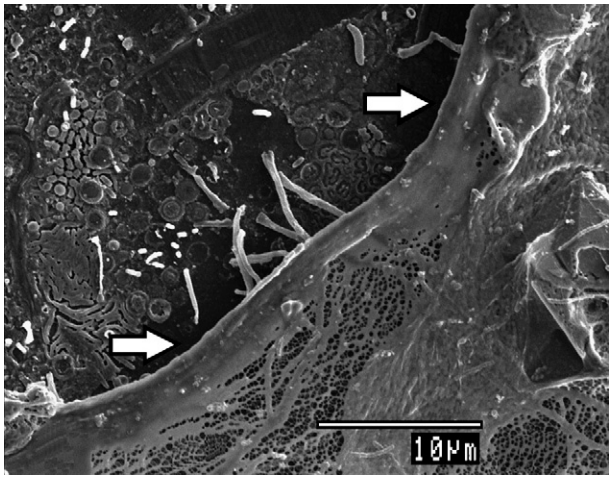


Fig. 2. Micrograph of the surface of *Caulerpa filiformis* showing the mucilage layer (arrows) exposing the underlying biofilm.

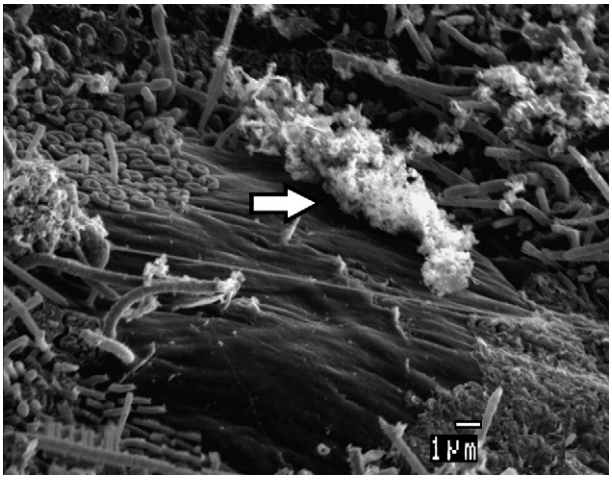


Fig. 3. Micrograph of the surface of *Gelidium abbottiorum* showing remnants of the downy-type extracellular polymeric substance (EPS, arrow).