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14. ABSTRACT *Enteromorpha* is the most important macrofouling alga and the diatom *Amphora* is a dominant member of slimes that develop on all coatings including foul-release silicones. The objective of this research was to use these two species as models to understand fundamental aspects of adhesion and to apply this mechanistic understanding to a practical, laboratory scale evaluation of novel anti-fouling materials provided by other contractors. Two methods to apply hydrodynamic shear forces to adhered organisms were developed and used to characterize baseline adhesive properties on defined, model surfaces. A 5-point evaluation protocol was the developed for novel test surfaces, incorporating quantitative settlement, adhesion and removal assays. Test surfaces provided by 7 contractors were iteratively evaluated and the results reported for further development. Six papers were published in refereed journals.

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biofouling, adhesion, algal spores, diatoms, *Enteromorpha*, *Amphora*, test protocols, anti-fouling and foul-release coatings

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FINAL REPORT

GRANT NO. N00014-99-0311

PRINCIPAL INVESTIGATORS: Prof. James A Callow and Dr. Maureen E Callow

INSTITUTION: University of Birmingham, UK

GRANT TITLE: Evaluation of the strength of attachment of *Enteromorpha* zoospores to novel polymer surfaces

AWARD PERIOD: 1st April 1999 - 31st March 2002

OBJECTIVES: *Enteromorpha* is the most important alga that fouls ships and submarines and the diatom *Amphora* is a dominant member of slimes that develop on all coatings including foul-release silicones. The overall objective of this research was to use these two species as models to understand fundamental aspects of adhesion and to apply this mechanistic understanding to a practical evaluation of novel anti-fouling materials provided by other contractors.

APPROACH: Spores of the green alga *Enteromorpha* and cells of the diatom *Amphora* are allowed to settle onto polymer coatings applied to glass microscope slides. The number of adhered biomass (spores, sporelings or cells) is quantified before and after exposure to flowing seawater in either a fully developed turbulent flow water channel or an automated water jet. The percentage of biomass detached is compared against two standard surfaces: clean, acid-washed glass, and a polydimethyl siloxane (PDMS) (Dow Corning Silastic[R] T2) provided by Prof. A. Brennan (UoF, Gainsville, Florida).

To evaluate test surfaces for minimal adhesion and foul-release properties against *Enteromorpha* we applied a comprehensive 5-point evaluation protocol incorporating the following: toxicity test; spore settlement test; strength of adhesion after 1 h (flow apparatus) or 4h (water jet), growth of a biofilm (7-10 day old sporelings); adhesion strength of sporelings.

To systematically evaluate surface properties that may influence release we used model surfaces in the form of self-assembled monolayers (collaboration with Lopez group, University of New Mexico) and modified PDMS(polydimethylsiloxane) elastomers, including two commercial products.

Environmental Scanning Electron Microscopy (ESEM) was used to visualize adhesive pads of *Enteromorpha* spores.

ACCOMPLISHMENTS: The operation of the prototype flow channel for removal of microfouling species at different scales was extensively characterised in collaboration with its designer, Dr M.Schultz (Schultz *et al.*, 2000, Schultz *et al.*, 2002). A higher capacity pump was fitted to facilitate detachment of 1 h settled *Enteromorpha* spores. The water jet provided by Prof. G. Swain, was semi-automated and computerised and its operation was optimised for laboratory-scale investigations of soft-fouling species (Finlay *et al.*, 2002b). The two methods are complementary and provide a critical technology for evaluation of foul-release materials.

The baseline adhesion properties of the two algal species were characterised (Finlay *et al.*, 2002a, Finlay *et al.*, 2002b, Schultz *et al.*, 2000). It was shown that adhesion strength of *Enteromorpha* spores and *Amphora* cells to a standard glass surface is a function of time as the adhesive progressively 'cures' and/or cohesive bonds develop. For *Enteromorpha*, surface pressures of ~250 kPa (water jet) were required to quantitatively remove attached spores after 4 h contact with a surface but after 8h spores did not detach, even at pressures in excess of 250 kPa. The interfacial forces exerted as water impinges on the surface and the derivation of adhesion strength values in terms of critical wall shear stress have been computed and compared with those obtained by other methods (Finlay *et al.*, 2002b). A surface pressure of 250 kPa approximates to 325 Pa wall shear stress. Calculation using the power-law formula predicts that detachment forces of this magnitude are unlikely to be realized at operating speeds for most vessels and that most *Enteromorpha* spores would not detach from untreated hulls.

Spores settled in groups were shown to be more strongly adhered than single spores, which suggests that the adaptive value of gregarious settlement behaviour may lie in the greater resistance of groups to detachment forces in a naturally turbulent environment (Finlay *et al.*, 2002a). A practical consequence of gregariousness is that group size effects need to be monitored in the evaluation of novel surfaces.

The adhesive pad of *Enteromorpha* spores was imaged in the absence of fixation and dehydration artefacts using environmental scanning electron microscopy (ESEM)(Callow *et al.*, 2002). The pad was revealed as a swollen, featureless, hydrophilic material and adhesive 'footprints' were seen remaining on glass surfaces subjected to shear. These studies lay the foundation for analysis of adhesive release mechanisms on foul-release materials.

Numerous interactions with other contractors in the ONR programme have been initiated. A standard specification for the supply of test material was developed, defining the need to prepare smooth, flat surfaces of controlled thickness, that are properly bonded to the substrate, with the appropriate number of replicates for statistical reliability. Some contractors required several attempts to meet these requirements.

Candidate coatings materials from 8 contractors (M.Chaudhury, Lehigh; C. Ober, Cornell; M. Detty, Buffalo; J. DeSimone, Chapel Hill; K. Wooley, Chapel Hill; Beckham, Athens; Gianellis, Cornell; Brennan, Gainsville) representing some 80 test surfaces (916 individual samples) were either fully or partially evaluated for minimal adhesion and foul-release properties against *Enteromorpha,* using the 5-point evaluation protocol (constraints on time and availability of samples precluded extensive parallel evaluations with *Amphora*). Reports on these evaluations were promptly submitted to the originating contractors, leading in most cases to further interactions and iterations as key hypotheses were developed and tested. This process is continuing through new ONR funding.

In general terms, the results of these evaluations of novel surfaces show that the bioassay systems do provide a robust, reproducible and relatively rapid means of assessing the performance of disparate materials. Results of specific evaluations are made known to ONR by the suppliers of the surfaces and through the annual Contractors' Meetings. One interaction has resulted in a publication (Youngblood *et al.*, 2002) and several more are planned.

In studies on the influence of surface and other coating properties on adhesion of fouling species, a generalization is that adhesion strength is proportional to surface energy, i.e cells/organisms stick more strongly to high energy surfaces. We explored this relationship for *Enteromorpha* and *Amphora* by studying the influence of wettability (related to surface energy) through the use of self-assembled monolayers (SAMs) in collaboration with the Lopez group, University of New Mexico. Published results (Finlay *et al.*, 2002a) show that *Enteromorpha* spore adhesion conforms to this general relationship since adhesion strength was lowest on low energy (hydrophobic, less wettable)surfaces. However, the diatom *Amphora* showed the opposite relationship, cells detached more readily from the more wettable surfaces. Although results on these model surfaces have to be interpreted cautiously, the latter result is consistent with the known problem of hydrophobic foul-release silicones being commonly fouled by diatom slimes that do not release even from high speed vessels (> 30 knots). We also monitored the percentage of *Amphora* cells that were motile on the hydrophilic vs hydrophobic surfaces. Cells were unable to move on surfaces with a water contact angle above approximately 60° and this may be related to the difficulty in removing them from foul-release surface.

There is considerable current interest in the influence of other materials properties of elastomers in the control of hard-fouling species such as barnacles. Preliminary studies in collaboration with Dr M Chaudhury (Lehigh University, PA) on the influence of modulus and thickness have shown that for soft-fouling species like *Enteromorpha,* which initiate fouling at the microscopic scale, there is little influence of either of these properties on spore adhesion except at very low modulus values, approaching that of the spore system itself (approx. 0.5 MPa). This result may suggest that a third factor, lubricity of the surface, may be important. We are currently investigating this further.

CONCLUSIONS: *Enteromorpha* and *Amphora* are significant 'soft-fouling' species and our studies have shown that they provide good models in which to study adhesion and fouling processes. Our research has illustrated the importance of carrying out fundamental studies on adhesion processes using model species and substrates to provide the information needed to guide the development of good quantitative assays of performance of test materials and to understand the results.

Laboratory scale evaluations are an important step in the evaluation of new materials with reduced adhesion or foul-release properties because quick assessments can be made under controlled conditions, the scale is appropriate to small quantities of test material, short term durability can be evaluated, and the mode of action of the surface material can be studied, thus increasing knowledge and predictive quality.

The development of soft-fouling by algae on a ship hull is only the final outcome of a whole range of sub-processes that include; location of a surface by a spore, the initial primary settlement and adhesion process, the development of cohesive and adhesive strength by the attached spore, the growth of the attached spore to form a biofilm of young plants that will eventually sporulate to complete the cycle, the adhesion properties of these older biofilms. Potentially an anti-fouling surface could influence any of these sub-processes; therefore, evaluation of candidate materials for antifouling coatings requires a comprehensive approach to the biological properties of the system, and this is provided for *Enteromorpha* by our 5-point evaluation protocol. Surfaces for full-scale field testing can be selected on the basis of these rapid laboratory screens.

SIGNIFICANCE: *Enteromorpha* is the most important of the algae that foul ships and submarines and its incidence is increasing as tin-based 'toxic' paints that effectively control it, are withdrawn (*Enteromorpha* is resistant to the alternative copper-based paints). ONR is therefore seeking new environmentally benign technologies to control fouling by this and other soft-fouling species.

PATENT INFORMATION: None

AWARD INFORMATION: None

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