The development of microfouling on four commercial antifouling coatings under static and dynamic immersion

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Abstract

Since the composition and abundance of microfouling communities that develop on antifouling coatings reduce the performance and efficiency of ship operations, microfouling was investigated during static and dynamic seawater immersion of four commercial antifouling coatings, of which three were biocide based (tributyltin self-polishing, copper self-polishing, copper ablative) and one was biocide free (silicone fouling release). The total bacterial counts were similar on all coatings after static immersion, but after dynamic immersion the largest decrease in numbers was seen on the fouling release coating. The bacterial population on the fouling release surface was also more heterogeneous than on the biocide-based coatings. After static immersion, diatom populations were dominated by Amphora, Navicula and Synedra; after dynamic immersion, no diatoms could be detected on the copper ablative coating and only Amphora was left on the fouling release coating.

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1. Introduction

Microbial communities that form on antifouling coatings create a roughness that can significantly reduce the performance and efficiency of boats and ships. These biological growths are generically described as slime films (ASTM D 3623-78a, 1993; Swain and Schultz, 1996), however, there is only limited information about their composition and how they vary with respect to the type of antifouling coating and whether the vessel is in port or underway at sea. This research set out to identify and quantify the bacteria and diatoms that became established on four commercial antifouling coatings under static seawater immersion (representing a ship in port) and dynamic immersion (representing a ship at sea).

Several papers have described the effect of slime films on the hydrodynamic performance of a surface. Lewthwaite et al. (1985) found that a 1-mm thick slime layer that developed on a 23-m fleet tender caused an 80% increase in skin friction coefficient, together with a 15% loss in ship speed, compared with values obtained for the clean hull. Bohlander (1991) ran full-scale power trials on a frigate and found that biofouling, mainly in the form of microfilms, caused an increase of 8–18% in drag. Schultz and Swain (1999, 2000) observed penalties in local skin friction coefficients of 33–187% on flat plates fouled with biofilms. An understanding of the development of microfouling communities is therefore an important aspect in the selection and management of antifouling coatings for ship operations. This has become especially important as regulations are now in effect (International Maritime Organization, 2003) that will force a change from the highly successful self-polishing copolymer organotin systems to alternative copper-based and silicone fouling-release technologies.

Bacteria and diatoms form the main components of slime films in marine environments. Both attach to and reproduce on all but the most toxic surfaces (Marsalek et al., 1979; Baier, 1980; Callow et al., 1986). Their subsequent multiplication and production of exopolymers form a thin layer of organic matter, which traps nutrients
from the water column and provides protection for other microorganisms living within the biofilm (Costerton et al., 1978, 1995; Van Loosdrecht et al., 1990). Furthermore, it has been shown that microfouling plays a major role in mediating settlement and metamorphosis of invertebrate larvae (Kirchman and Mitchell, 1983; Maki et al., 1988; Henschel and Cook, 1990; Rodriguez et al., 1995; Weiszork et al., 1995). In particular, the study of different bacterial species has shown the importance of exopolymeric substances in the activation or inhibition of macrofouling settlement (Young and Mitchell, 1973; Crisp, 1974).

The processes involved in bacterial adhesion to surfaces are species specific and are determined by the ability to secrete exopolymeric substances, the physiological state of the organisms, and physicochemical features of the surface (Dexter et al., 1975; Callow and Fletcher, 1994). Diatom fouling is dominated by a restricted number of genera and previous studies have shown that the raphid diatoms *Navicula* and, especially, *Amphora* are the most common on conventional antifouling paints, whilst *Achnanthus* is very common on copper-free and tributyltin (TBT)-based coatings (Robinson et al., 1985; French and Evans, 1986; Callow, 1986). Laboratory studies suggest that *Amphora* and *Navicula* are also likely to be the most abundant types of diatom on non-toxic and fouling-release coatings (Wigglesworth-Cooksey et al., 1999; Finlay et al., 2002).

### 2. Materials and methods

#### 2.1. Antifouling coatings

The commercial antifouling coatings were three containing biocides and one without biocide (Table 1). These were (a) a tributyltin self-polishing copolymer (Sn-SPC), which, although no longer commercially available, acted as a standard, since this type is still considered the best performing formulation; (b) a copper self-polishing copolymer based on (Cu-SPC), one of the replacement technologies for Sn-SPC; (c) an ablative copper-based system (Cu-Abl) representing traditional antifouling technology; and (d) the biocide-free fouling release system (FR) based on polydimethylsiloxane. Each system was applied over an epoxy barrier coat to aluminum test panels (0.254 m × 0.305 m).

#### 2.2. Seawater exposure methods

The test coatings were first immersed under static conditions for 60 days in March 2004 in the Indian River Lagoon, Melbourne, Florida. Each test surface was suspended at a depth approx. 50 cm below the water surface and enclosed in a fish exclusion cage to prevent disturbance of the fouling communities by grazing and predation (Preedeekanit et al., 1998). This represented conditions similar to a ship in port. After static immersion, the same panels (without any cleaning) were directly placed in dynamic immersion for 15 days. This represented conditions experienced by an antifouling coating on a ship under way. Dynamic immersion was in a polyethylene tank (1.8 m diam.) filled to a depth of 0.5 m with sea water, which was rotated by paddles to create peripheral velocities of 4–5 m s⁻¹. The panels were attached to the periphery of the tank, so that there was turbulent flow over the panels with boundary layer conditions similar to that of the forward part of a ship hull moving at that velocity. The seawater in the tank was continuously replenished with a once-through supply at a rate of approx. 2 L min⁻¹.

#### 2.3. Fouling and surface evaluation

The degree of fouling and surface condition was determined by visual inspection following the guidelines in ASTM D 3623 (1993). The fouling was rated according to the percentage of the intact portion of coating that was covered by a particular type of fouling organism. The fouling types identified in these experiments included slimes, barnacles and tubeworms.

#### 2.4. Microfouling

The slime films were sampled to identify their microbial constituents. A representative area of the coating was chosen and thoroughly rinsed with filtered (0.2 μm) sterilized seawater. This eliminated sediment and microorganisms that were not permanently attached to the test panel. From each panel duplicate samples of material from 3 cm² surface were removed with a sterile scalpel blade and transferred to 30 ml filtered (0.2 μm) sterilized seawater. All samples were held in a cooler at 4 °C and returned directly to the laboratory for analysis.

##### 2.4.1. Microscope count (total cell count)

This method was used to count both bacteria and diatoms. Samples were stained in 0.01% acridine orange and an appropriate dilution of each sample was deposited in a Leavy Counting Chamber (Hauser Scientific, Horsham, PA, USA). These were then counted under epifluorescence light microscopy.

##### 2.4.2. Coulter counter

A Coulter Counter (Coulter Electronics, Haleah, FL, USA) was used to determine bacterial concentration in each sample. Dilutions of the samples were prepared in phosphate buffered saline for counting (Isotone II diluent, Coulter® balanced electrolyte). The instrument was set to count the particle between 0.5 and 1.5 μm in diameter.

### Table 1

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Coating type</th>
<th>Commercial name</th>
<th>Active Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sn-SPC</td>
<td>Tributyltin self-polishing copolymer</td>
<td>Hemps 79051&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tributyltin oxide + cuprous oxide + tributyltin methacrylate</td>
</tr>
<tr>
<td>Cu-SPC</td>
<td>Copper self-polishing copolymer</td>
<td>Sea Quantum Classic&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cuprous oxide</td>
</tr>
<tr>
<td>Cu-Abl</td>
<td>Copper ablative</td>
<td>Interspeed 642&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Cuprous oxide</td>
</tr>
<tr>
<td>FR</td>
<td>Polydimethylsiloxane fouling release</td>
<td>Bior L&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturers:

<sup>a</sup>Hempel Paint.
<sup>b</sup>Jotun.
<sup>c</sup>International<sup>®</sup> Marine Coatings.
<sup>d</sup>Kansai Paint.
2.4.3. Bacterial culture and characterization

The duplicate samples from each panel were mixed together (2 ml each) and the mixture was serially diluted 10-fold using sterilized seawater, and 0.1 ml aliquots from each tube were plated on Marine Agar 2216 (Difco). For each coating only the three dilutions giving the highest counts were kept for selection. The main bacterial populations were characterized by selecting from the marine agar plates 20 of the predominant colonies, which were visually selected to represent the diversity of all colonies present on these plates and then plated on fresh marine agar plates. The bacteria were characterized to generic level according following Bergey's Manual (1997). Tests employed for presumptive identification included Gram-stain characteristics, observation under phase contrast microscope, oxidase, catalase, motility, colonial characteristics on marine agar, nitrate reduction, oxidative-fermentative utilization of glucose in tubes containing 2% NaCl, methyl red and Voges-Proskauer tests for acid production, medium sulfur indole motility, MacConkey agar (2% NaCl), mannitol salt and thiosulfate citrate bile sucrose agar.

2.4.4. Diatom characterization

Diatoms were observed under a microscope and images recorded using a digital camera to enable identification to generic level by comparison with photographs and descriptions found in diatom databases.

3. Results and discussion

3.1. Fouling and surface evaluation

The percentage cover of the surface by different fouling types was visually assessed after the static and then dynamic immersion periods (Table 2, Fig. 1). After static immersion for 60 days all four test coatings had >80% fouling cover, which was dominated by slime films.

Table 2
Percentage biofouling cover of antifouling surfaces after static immersion for 60 days and dynamic immersion for 15 days

<table>
<thead>
<tr>
<th>Immersion Type of coating</th>
<th>Sn-SPC</th>
<th>Cu-SPC</th>
<th>Cu-Abl</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Static</td>
<td>Dynamic</td>
<td>Static</td>
<td>Dynamic</td>
</tr>
<tr>
<td>Fouling %a</td>
<td>80</td>
<td>77</td>
<td>95</td>
<td>15</td>
</tr>
<tr>
<td>Slime %</td>
<td>78</td>
<td>75</td>
<td>95</td>
<td>15</td>
</tr>
<tr>
<td>Barnacle %</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tubeworm %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*aFouling = slime + barnacle + tubeworm.

Fig. 1. Appearance of test panels after static immersion for 60 days (upper) and dynamic immersion for 15 days (lower).
However, a small number of juvenile barnacles became established on the Sn-SPC coating and a few calcareous tubeworms settled on the FR coating. After dynamic immersion, the fouling cover was considerably reduced on the Cu-SPC, Cu-Abl and FR coatings, but there was only a small reduction in fouling cover on the Sn-SPC system.

3.2. Bacterial fouling

No previous field study has been published that describes the differences in the bacterial fouling occurring on different types of antifouling paint under static and dynamic immersion, but it is known that the associations of bacteria which develop on surfaces are strongly influenced by the environment in which they reside (Carson and Allsopp, 1983; Dempsey, 1981; Railkin, 2004). Cultural methods for identifying bacteria are always restrictive, because not all the bacteria can grow under chosen cultural conditions. Therefore, the most important findings of this investigation were not the types of bacteria which were found, but the differences that there were in bacterial composition and abundance depending on coating type and the hydrodynamic condition.

After static immersion for 60 days, both Gram-positive and Gram-negative bacteria were found on all test surfaces (Table 3). Two genera present on all coatings were *Micrococcus* and *Pseudomonas*. *Alcaligenes* was also found.

### Table 3
Viable bacteria on test surfaces after static immersion for 60 days and after dynamic immersion for 15 days

<table>
<thead>
<tr>
<th>Immersion</th>
<th>Sn-SPC</th>
<th>Cu-SPC</th>
<th>Cu-Abl</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Static</td>
<td>Dynamic</td>
<td>Static</td>
<td>Dynamic</td>
</tr>
<tr>
<td>Gram-positive:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus</em></td>
<td>11</td>
<td>20</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>11</td>
<td>20</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gram-negative:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alcaligenes</em></td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>6</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Characterization to genus; values represent numbers of colonies of individual types among the 20 colonies selected.

![Graph](image-url)  
Fig. 2. Surface counts of bacteria after static immersion for 60 days and dynamic immersion for 15 days. Each value is the mean of 30 counts on 2 replicate samples (microscope) and the mean of 6 counts on 2 replicate samples (Coulter Counter).
on all except Cu-Abl Vibrio sp. was present on FR, and Proteus sp. on Sn-SPC. The total bacterial counts showed similar population densities, $10^{-8} - 10^9$ bacteria cm$^{-2}$, for all test surfaces, with the Sn-SPC surface having the lowest numbers (Fig. 2). Corresponding microscope and Coulter counts were similar.

The bacterial populations changed after the coatings were subjected to dynamic immersion. Micrococcus was the main viable bacterium found on all surfaces, but Pseudomonas had disappeared from the biocide-based surfaces. The viable bacteria on the FR coating were more heterogeneous and comprised Micrococcus, Pseudomonas, Streptococcus and Staphylococcus. Counts were reduced on all surfaces. The greatest reduction occurred on the FR, where a > 100-fold reduction in bacterial numbers was observed, and the least change occurred on the Sn-SPC coating.

### 3.3. Diatom fouling

Only pennate diatoms were observed and they were represented by the two classes (Table 4), Raphidineae (raphids) and Araphidineae (araphids). After 60 days of static immersion there were large populations of diatoms in the slime film of all test coatings (Fig. 3). The coatings with the lowest numbers of diatoms were the FR and Cu-SPC surfaces ($\approx 5 \times 10^5$ diatoms cm$^{-2}$). The Sn-SPC and Cu-Abl surfaces had the largest counts, approx. $2 \times 10^6$ diatoms cm$^{-2}$. Amphora, Navicula and Synedra were found on all the surfaces. Species diversity was greatest.

#### Table 4

Diatom populations identified on the test surfaces after static immersion for 60 days and after dynamic immersion for 15 days

<table>
<thead>
<tr>
<th>Immersion</th>
<th>Sn-SPC</th>
<th>Cu-SPC</th>
<th>Cu-Abl</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Static</td>
<td>Dynamic</td>
<td>Static</td>
<td>Dynamic</td>
</tr>
<tr>
<td>Araphidineae:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragilaria</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Synedra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raphidineae:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achnanthes</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Amphora</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Navicula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitzschia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Key: X, abundant; XX, extremely abundant. See Fig. 3 for number of diatoms.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Diatom counts after static immersion for 60 days and dynamic immersion for 15 days. Each value is the mean of 30 counts on 2 replicate samples from each panel.
on the Cu-Abl and lowest on the Cu-SPC surface. The main diatom populations on the FR coating were of *Amphora, Synedra* and *Fragilaria*, with only a few *Navicula* present. *Fragilaria* was only found in groups or colonies forming a succession of individuals joined together. It was found that the diatoms present on the fouling release surfaces were larger, than those present on biocide-based coatings (average 20–40 μm vs. 10–20 μm).

After dynamic immersion for 15 days there was a reduction in diatom numbers for all coatings. Diatoms could not be detected on the Cu-Abl surface, and the other test surfaces exhibited an eight-fold reduction in diatom concentrations. *Amphora* and *Synedra*, remained present on both self-polishing coatings, but *Navicula* was absent from the Sn-SPC. *Amphora* and *Navicula* have been reported as present on antifouling coatings in previous studies (Callow et al., 1986; French and Evans, 1986). *Achnanthes* was present on both Sn-SPC and Cu-Abl surfaces after static immersion but remained only on Sn-SPC after dynamic immersion. This probably reflects the resistance of *Achnanthes* to TBT, but not to copper (Callow, 1986).

*Amphora* was the only diatom that remained attached to the FR surface after dynamic immersion. From laboratory experiments, Wigglesworth-Cooksey et al. (1999) inferred that *Amphora* is an important component of diatom populations that form on low-surface energy coatings. Moreover, *Amphora* has two raphes which may allow it to adhere more strongly and efficiently to this type of surface. The FR surface is a very smooth and hydrophobic surface, contrasting with the biocide-based coatings, which are relatively rough and hydrophilic. Previous research has shown that for *Amphora* the hydrophobic surfaces stimulate production of more exopolymeric substances, thus reducing locomotion and increasing strength of attachment (Wigglesworth-Cooksey et al., 1999; Finlay et al., 2002). However, the inability to move on hydrophobic surfaces does not appear to be characteristic of all diatoms (Edgar and Pickett-Heaps, 1984). The present results have showed that *Amphora* formed the main population on FR and was the only diatom attached strongly enough to remain after dynamic immersion.

The ability of bacteria and diatoms to survive on antifouling surfaces is well documented. It is generally explained by the exopolymeric substances that isolate the organism from the biocide. Many marine bacteria grow in biofilms and produce organic secretions which capture nutrients from the environment (Costerton et al, 1995) and protect the bacteria living within the biofilm from antibacterial compounds (Van Loosdrecht et al., 1990; Gilbert et al., 1995; Donlan and Costerton, 2002; McBain et al., 2004). Diatoms secrete large amounts of exopolymeric substances from a slit (raphhe) or apical pore in the frustule (Hoagland et al., 1993, Wustman et al. 1997). Therefore, exopolymeric substances are important in isolating or buffering the organisms from the coating.

This study has shown that there are distinct differences in the quantity and composition of slime films that form on different types of commercial antifouling coatings. The interplay at the coating surface is unique for each coating and immersion environment. Factors associated with the coating include the presence or absence of biocide, the type of biocide, the rate of biocide release, the coating matrix type, and the surface energy. Factors associated with the immersion environment include the biological, chemical, physical and hydrodynamic (static or dynamic immersion) conditions. In the investigation, all coatings were subjected to identical immersion conditions and therefore differences in microbial fouling communities reflected differences in the coatings.

Substrate wettability, as indicated by the critical surface tension, influences the rate of attachment of marine bacteria to a variety of substrates (Baier, 1972; Dexter et al., 1975; Taylor et al., 1997). Dexter (1979) showed that adhesion of bacteria was lowest on hydrophobic surfaces with energies of 25 mJ m⁻². This is close to the values found on the FR surfaces (unpublished results). The present results show that the release of bacteria from the FR surface was 10 times greater than for the biocide-based coatings and that diatoms were also released from these coating. The results support the idea that the free energy of the surface is one of the main factors which influence the adhesion of micro-organisms to surfaces (Callow and Fletcher, 1994).

In summary, the present study has found that there are marked differences in the composition and density of diatoms and bacteria in the microfouling communities that develop on different commercial antifouling surfaces and that these communities are also impacted by the hydrodynamic conditions under which they operate. Previous research has shown that the slime films will increase the skin friction coefficient of the coatings and hence reduce the performance of shipping vessels. Knowledge of the biofilms that form on different antifouling coatings, combined with an understanding of how these communities contribute to drag will enable better management of ship hulls in the form of coating selection, hull cleaning and dry dock scheduling.

**Acknowledgments**

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