5- BIOFOULING AND BACTERIAL BIOFILMS

A casual look at a typical dock piling, seawall, ship hull, water intake pipe, or anything else manmade (Figure 1-3) that's put into the ocean environment gives you an appreciation for those industries that must contend with problems of marine biofouling.

**FIGURE 1** Fouling of propeller and rudder of a vessel.

**FIGURE 2** A wooden pile damaged by borers.

**FIGURE 3** (a) Fouling of the inner wall of a pipe by hydroids. (b) Fouling of stationary structures.

**Biofouling is generally defined** as the settlement and accumulation of living organisms on man-made physical structures placed in an aquatic or marine environment. The term usually refers to sessile (stationary) macroscopic organisms like macroalgae (Figure 5), barnacles, bivalves, bryozans, sea squirts, and the like (Figure 4), but biofouling occurs very rapidly on a microscopic scale as well. In fact, the formation of so-called microbial 'biofilms' is considered an essential first step in the establishment of a marine fouling community.
FIGURE 4 Marine animals inhabiting surfaces of hard bodies. (1) Sponge; (2) hydroid polyp; (3) coral sea pen; (4) polychaetes of the family Serpulidae; (5–6) cirripedes: acorn barnacles Balanus (5) and goose barnacles Lepas (6); (7) bryozoans; (8–11) mollusks: mussel Mytilus (8), oyster Ostrea (9), abalone Haliotis (10), shipworm Teredo navalis and its tunnels in wood (11); (12–14) echinoderms: starfish Asterias rubens (12), sea urchin (13), sea cucumber (14); and (15) ascidian.

FIGURE 5 Marine macroalgae. (1–2) Green algae Ulva (1) and Enteromorpha (2); (3) red alga Ahnfeltia; (4) brown alga Laminaria.
Bacterial biofilms
A biofilm is an aggregation of bacteria on a surface that is surrounded or held together by extracellular polymeric substances. Bacterial biofilms (Figure 6) are the root cause of biofouling in most industrial systems. It is the first process in the typical fouling sequence. Biofilms, result from the very tight adherence of bacteria to surfaces. Bacteria produce extracellular polymeric substances (Figure 6), in part, to help them attach to surfaces and bind to one another. Although bacteria are at risk and grow very poorly, when floating in water, they grow extremely well on surfaces. Bacterial and microalgal colonization of surfaces is accompanied by settlement of invertebrate larvae and algal spores, eventually leading to "hard fouling" and the need for costly cleaning.

![FIGURE 6 SEM images showing the attachment of microorganisms by means of polymers. (a) Bacteria; (b) diatoms.](image)

A significant amount of research has focused on exploring the sort of intimate associations that exist between bacteria and various surfaces, particularly in marine environments. Understanding factors that promote the transition of bacteria (and other fouling organisms) from a planktonic to a sessile state is essential to the development of effective biofouling monitoring and treatment programs.

Economic importance of biofouling
The biofouling issue is more than aesthetic. The biological fouling of submerged materials is of great economic importance to several industries, and is responsible for:

- increased frictional resistance (drag) of fouled ship hulls
- structural corrosion of engineered materials
- restricted flow through fouled aquaculture cage netting
- mechanical blockage of intake and outfall pipes
- losses in heat-transfer efficiency of marine cooling systems
- increased costs for maintaining/replacing all of the above.
While it is difficult to put a figure on the worldwide cost of combating biofouling, the fact that the US Navy alone spends about $1 billion every year on the problem hints at the economic scope involved. Fouling of a ship's hull can decrease fuel efficiency by as much as 20-30%. This efficiency reduction represents a substantial financial liability since fuel accounts for up to 50% of marine transportation costs. Additional costs come from hull cleaning, paint removal and reapplication, and meeting environmental compliance standards.

**Successional biofouling sequence**

Marine biofouling communities are the products of *successional sequences* (Figure 8) occurring over time on the surfaces of engineered materials as following:

a. Almost immediately upon immersion, manmade materials start to become 'conditioned' by the marine environment. Dissolved organic molecules coat the exposed surfaces within minutes as the result of physical adsorptive processes, thus naturally conditioned.

b. The introduced surfaces become appealing to bacterial colonizers who take up residence within hours. Diatoms and other unicellular algae, cyanobacteria (blue-green "algae"), and similar organisms also contribute to the establishment of this initial microfouling biofilm or 'slime' coat.

c. It is this slime coat that makes the manmade surfaces appealing to the macroscopic fouling organisms like barnacles, mussels, and sea squirts that cause most of the problems.

So, a key concern with some of the antifouling compounds being discovered is that they may target specific organisms in the successional biofouling sequence to be effective against a wide spectrum of fouling organisms.

**FIGURE 8** A classical scheme of succession of a biofouling community. Dashed lines show collateral ways of succession, bold lines show climax communities.
Environmental Impacts

Biological fouling is a major problem that results in significant environmental impacts, both directly and indirectly through the misuse and misapplication of biocides. The first anti-fouling ship paints were developed as far back as the mid-1800s. These primarily used copper as a broad-spectrum biocide responsible for keeping ship hulls free of fouling organisms. The copper had a short effective lifespan, even after decades of improvements, copper-based paints could not be developed that had a service life of more than about a year-and-a-half, after which time a ship would have to be hauled out and dry-docked for reapplication. Modern copper-based paints are slightly better, having a service life of up to four years.

Since the mid-1960s, industry preference has been for the use of tributyl tin (tributyl tin, or TBT) as the active biocide in antifouling marine paints. The key advantage of TBT over copper was that paints could be developed which would protect ships for five years or more. The increase in service life is due largely to the capacity of TBT to slowly and continuously leach from the painted hull to kill any organisms that might have gotten a foothold. The resulting improved ship performance and less frequent repainting led to considerable monetary savings.

Another key benefit of the new TBT paints was their capacity for 'self polishing.' Thin microlayers of older paint are sloughed off of moving vessels over time, continually exposing fresh layers of active biocidal material.

From an environmental perspective, the constant sloughing of the TBT coatings (along with a relatively long half life in the marine environment) is also the very feature that makes them unsuitable for continued use in the world fleet. By the 1980s it had become apparent TBT had accumulated and become a severe contaminant of many bays and estuaries, especially those close to shipping ports.

Among the documented ecological effects of TBT contamination are reproductive failure in commercially important shellfish populations, physical deformity in oysters, and sex reversal or hermaphroditism in some snail species. TBT can also bioaccumulate in apex predators such as dolphins, sea otters, and squid, potentially leading to concentrations of TBT more than 100 times greater than in the surrounding water. From the standpoint of human health, there is a growing concern about prolonged exposure of shipyard workers to TBT paint. There is also no safe way to dispose of TBT waste generated when antifouling paints are removed as part of regular maintenance and repainting.

Concern over the negative impacts of TBT on the natural environment became so great that the International Maritime Organization placed a partial ban on the use of tributyltin paints that began in 2003 and is scheduled to phase out all TBT-containing paints by 2008.
Marine biotechnology (MBT) and Next Generation Antifouling Strategies

Although the phased TBT ban has been applauded by environmentalists, there is not yet an ecologically safe alternative that is totally suitable as a replacement. Many fleets are returning to the older copper-based coatings. But, aside from not being as effective as tributyltin, the copper paints are themselves toxic to many forms of marine life and have been banned in parts of the world. At best, the return to copper is an interim antifouling strategy until better alternatives are developed and evaluated. One route being pursued in the search for environmentally safe antifouling compounds is to **look at the way marine organisms naturally overcome fouling problems.** This has led to a number of exciting discoveries with potential industrial application. Natural products with antifouling properties have been identified from species of seaweeds, seagrasses, sponges, soft corals, and others. Note that most of these organisms are sessile (non-moving) species that would be negatively impacted if other organisms were to settle onto or overgrow them. There is clearly an adaptive advantage conferred upon those organisms able to produce natural fouling deterrent substances.

The natural antifouling substances isolated from marine organisms are typically evaluated by means of bioassays that determine whether the compounds are effective in inhibiting settlement of specific test organisms.

**The red macroalga *Delisea pulchra***

Many marine plants and animals remain free of attached bacteria, either because they produce repelling compounds or because their surface structure neutralizes bacterial adhesives. The Australian red macroalga *Delisea pulchra* (literally, "delicate beauty") almost never developed the bacterial biofilms that coat practically every other living and nonliving surface in the ocean and set the stage for later colonization by the macrofouling community. It produces a novel class of chemical compounds called **furanones** that prevent the development of biofilms. The researchers are now focused on finding a way to incorporate these compounds into anti-fouling paints and other coatings.

**The seagrass *Zostera marina* (eelgrass)**

Sulphate of \( p \)-coumaric acid (zosteric acid), isolated from *Zostera marina* (eelgrass), is an effective agent for preventing fouling by bacteria, algal spores, and a variety of hard-fouling barnacles and tube worms. These facts indicate the possibility of a universal chemical anti fouling protection.
The gorgonarian coral *Sinularia* sp.

A group of Japanese researchers determined that several compounds belonging to a chemical class called the sesquiterpenes isolated from the gorgonarian coral *Sinularia* sp. were potent settlement inhibitors of larval barnacles (barnacles are among the most pernicious fouling organisms) of the species *Balanus amphitrite*. Such findings suggest these compounds may have relevance in commercial anti-fouling applications.

Worm Power in the War on Biofouling

The predatory nemertine worm (ribbon worm) *Amphiporus angulatus* produces a variety of compounds called pyridine alkaloids, many of which are bioactive and some of which are toxic to marine organisms. The toxic compounds are likely used by the worm to paralyze prey and in self-defense.

The alkaloid 2,3'-bipyridyl was identified to have an excellent ability to inhibit barnacle settlement on surfaces treated with the compounds. Unfortunately, it also exhibited high toxicity for crustaceans, making it unsuitable for targeted application as an eco-friendly marine antifouling paint.

A scientific team however, began by synthesizing a variety of compounds with structures similar to 2,3'-bipyridyl. Some of these compounds did in fact block settlement with dramatically reduced lethality. One of the most promising of these synthetic compounds, a substituted bipyridyl, showed no significant toxicity in tests with crustaceans.

Anti fouling strategies form the early life history stages

Organisms may be most susceptible to fouling release or deterrence strategies at their early life history stages i.e. the larval and spore stages of organisms. Scientists believe that development of novel antifouling strategies targeting the weaker-bonding temporary adhesive of the early life history stages is a logical and promising research avenue.

Researchers have found, for example, that the glycoprotein glue used by settling *Enteromorpha* algal spores cures over time, such that increasing force is required to dislodge spores as time goes on.

Similarly, settling barnacle larvae (called cyprids) initially enter a searching stage at settlement time to explore a substrate prior to committing and permanently attaching to it. During this exploratory stage, cyprids probe the substrate with a pair of chemosensory antennules (Figure 9). The antennules are tipped with attachment discs that secrete an adhesive that temporarily attaches the cyprid to the substrate. This temporary adhesive is not as strong as the cement used by the barnacle once it makes a substrate selection and attaches to metamorphose into a sessile juvenile.

![FIGURE 9 The sequence of morphological changes during metamorphosis in the barnacle *Semibalanus balanoides*. (a) Antennula.](image-url)
Scientists are examining molecular details of the adhesive chemistry of fouling marine organisms. The next task will be to discover or develop an array of enzymes selected for their ability to break down the adhesives of fouling organisms. The ultimate goal is the deployment of such enzymes in novel antifouling paints. A technology such as this should be very ecologically friendly. While the enzymes remain within the paint they would effectively thwart would-be settlers. Once they leach into the marine environment, however, the enzymatic proteins would be subjected to rapid breakdown by the microbial community.

**FIGURE 10** Larvae of sessile invertebrates. Polychaete larvae: (1) trochophore, (2) metatrochophore and (3) nectochaete of *Harmatoë imbricata*, (4) nectochaete of *Circeis spirillum*; cirripede larvae: (5) nauplius and (6) cypris of *Semibalanus balanoides*; mollusk larvae: (7) veliger and (8) pediveliger of the limpet *Testudinalia tessellata*, (9) veliger of *Littorina littorea*, (10) veliconcha of *Mytilus edulis*; bryozoan larva: (11) cyphonautes of *Electra pilosa*; echinoderm larvae: (12) bipinnaria and (13) brachiolaria of the starfish *Asterias rubens*, (14) pluteus of the sea urchin *Strongylocentrotus droebachiensis*. 