Advanced nanostructures for the control of biofouling: The FP6 EU Integrated Project AMBIO

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The colonization of man made structures by marine or freshwater organisms or "biofouling" is a problem for maritime and aquaculture industries. Increasing restrictions on the use of toxic coatings that prevent biofouling, create a gap in the market that requires new approaches to produce novel nonbiocidal alternatives. This review details the systematic strategy adopted by an FP6 EU Integrated Project "AMBIO" to develop fundamental understanding of key surface properties that influence settlement and adhesion of fouling organisms. By this approach the project contributes to the understanding of fundamental phenomena involved in biofouling, and to the development of environmentally benign solutions by coating manufacturers within the consortium. © *2008 American Vacuum Society.* [DOI: 10.1116/1.2844718]

I. INTRODUCTION

Biofouling, the colonization of submerged man-made or natural surfaces by unwanted biological organisms, is a major problem for many marine industries. Shipping and leisure vessels, membrane filters, heat exchangers, underwater sensors, and aquaculture systems are all subject to its detrimental effects (Fig. 1). It is estimated that the world fleet consumes an additional 300 million tons of fuel annually as a result of hull fouling.[1]

The historical paradigm for controlling marine biofouling was to use biocidal products within coating systems to kill colonizing organisms. With the legal implementation of the International Maritime Organisation Treaty[2] on biocides in 2008 the use of components such as tributyltin (TBT) will be increasingly restricted. Developing environmentally benign fouling-resistant products to fill the gap in the market requires a greater understanding of how the physical and chemical characteristics of a surface influence its tendency to foul. While biofouling and its effects are easily recognizable at the macroscopic level, the processes that lead to it, surface location, exploration, and adhesion, all occur at small length scales particular to the colonizing organisms. The dimensions of fouling organisms during their settling stages (cells, spores, or larvae) are typically $1-100$ μm, but the recognition of surface cues through the relevant sensory structures probably occurs at much smaller length scales (μm, nm, or even the molecular level).

AMBIO is an Integrated Project with a consortium of 31 Partners, funded under the 6th Framework Programme of the European Union.[3] It brings together excellence in polymer chemistry, surface science, and marine biology with experienced coating manufacturers and end-users from industries, small and medium sized enterprises, and research institutes across Europe. The overall aim of the AMBIO project is to develop innovative, nonbiocidal solutions against biofouling. For this purpose, a knowledge base is built up which connects interfacial properties with adhesion of marine organisms to directly inform the development of new materials and surface designs. These activities are combined with state-of-the-art surface- and nanoanalytics capable of characterizing the coatings *in situ* in aqueous "seawaterlike" environments. The identification and selection of successful coating technologies proceed via laboratory and field-testing to scale-up and demonstration activities. It is anticipated that exploitation of the results leading to marketable end-user products would follow after the R&D program is completed. The strength of the AMBIO approach lays in the multidisciplinay character of the consortium, the open access to advanced analytical tools, and in its "knowledge driven" strategy for engineering novel fouling-resistant solutions.

II. KNOWLEDGE DRIVEN RESEARCH

A few of the partners within AMBIO focus on the development of "model" surfaces. These surfaces are not prepared directly for coating applications, but rather to investigate and identify fundamental physicochemical properties relevant for inhibition of colonization (settlement) or for increased release of fouling organisms (fouling-release). The ultimate fouling-resistance/release performance of a coating relies normally on a combination of several surface and materials properties, some of which are illustrated in Fig. 2.

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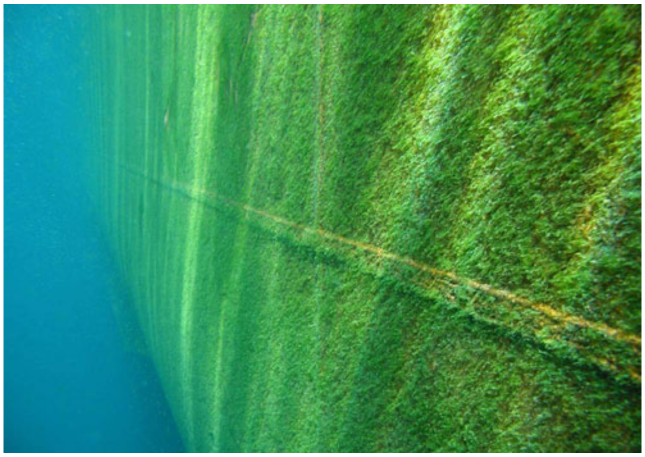

FIG. 1. Fouling a ship hull by the green seaweed *Ulva* (image courtesy of D. Williams, International Paints Ltd.).

As a surface is characterized by a number of different properties, the design of antifouling or fouling-release coatings is a highly complex task and it is unlikely that a surface with favorable properties would be found by simple "trial and error" approaches. In this, model surfaces can contribute significantly to the chances of success by isolating the influence of a single surface property. Selective variation of one surface property, while keeping the others constant, is a powerful tool to identify cues responsible for biofilm formation, settlement, and fouling-release. These results ultimately lead to a knowledge base which allows the development of tailored antifouling and fouling-release surfaces for different purposes. As shown schematically in Fig. 2, a coating can be composed of different chemistries in the bulk as well as having differing surface termination. Thicker coatings can be viewed as volume materials with bulk mechanical properties, sometimes carrying a net bulk charge. All surfaces, no matter if on top of a thick polymer coating or if a self-assembled monolayer, reveal a range of physicochemical properties such as elastic modulus, surface charge, or surface free energy. The latter parameter manifests as wetting properties, which originate from a combination of surface chemistry and morphology. Beside wetting and hydration, specific biological interactions can be provoked by the surface chemistry. These interactions involve sensing of mechanical properties, membrane penetration, and specific receptor interactions. Some chemical interactions are highly selective due to the specific stereo-chemical nature of the molecules involved; immobilized enzymes, for example, with specific activity against the adhesives of model fouling organisms, are investigated within AMBIO. Detailed knowledge of the mechanisms of bioadhesion and protein resistance of engineered surfaces within the biomedical field has contributed to the selection of some model surfaces such as mono-, oligo-, and polysaccharides, oligo- and polyethylene glycols, polyelectrolytes, or oligopeptides. Inspired by these material classes, self-assembly approaches based on thiol or silane chemistries are used for molecular surface engineering to probe the biological responses of marine organisms.

Two examples of the use of self-assembled monolayers to correlate wetting properties to protein resistance and adhesion of marine algal species are given in Fig. 3. Previous works on oligosaccharides[4] and oligoethylene glycols[5] have shown that the protein resistance of surfaces can be correlated with their wetting properties [Fig. 3(b)]. Adhesion of proteins is favored on hydrophobic surfaces with the amount of adsorbed protein decreasing with decreasing contact angle until it drops below the detection limit of the analytical techniques used to monitor the adsorption.[4,5] The low protein affinity at intermediate contact angles of $\approx 65°$ is due to the onset of strong hydration of the surfaces in this contact angle range, known as the Berg limit.[6,7] This concept is valid for a range of adhesion processes at the aqueous/solid interface, including the described trend for fibrinogen adsorption on self-assembled monolayers based on different chemistries (Fig. 3). Within the AMBIO project it was possible to demonstrate that this concept can be extended toward the adhesion of two algal species, zoospores of *Ulva linza* and cells of a diatom, *Navicula perminuta*. Hydrophilic self-assembled monolayers of hydroxyl terminated hexaethylene-glycol are resistant to settlement (attachment) of *Ulva zoospores* and cells of *Navicula*. Introduction of hydrocarbons with increasing chain length as terminators to the ethylene glycols renders the surface properties more hydrophobic and results in a concomitant increase in algal attachment (Fig. 3).[8] Thus, physicochemical concepts of protein resistance can, in this case, be used to predict the adhesion of micro- and macrofouling algae.

Besides these intrinsic chemical properties, a tailoring of the surface morphology can be achieved by self-assembly, nanolithography, photolithography, and micro-contact printing (some examples are shown in Fig. 2). Surface roughness and aspect ratio can be varied as well as the order parameter. While certain self-assembled structures reveal low order parameters, often on different length scales (e.g., hierarchically organized polyelectrolyte surfaces), photolithography, chemical lithography, and micro-contact printing are suited to precisely tune structures and produce regular patterns of well defined shape at the nano- to micrometer length scale. The structure sizes prepared and evaluated within AMBIO range from two-dimensional molecular nanostructures created by chemical nanolithography to three-dimensional hydrogel microstructures with high aspect ratios.

III. STATE OF THE ART SURFACE CHARACTERIZATION

The fast progress in the knowledge-driven development of coatings necessitates close contact and rapid feedback from biological evaluation and physicochemical characterization of the surface properties. In the project, all groups preparing functionalized surfaces and coatings have been given access to a range of analytical tools for characterization of surface composition, nanostructure, and dynamics. Standard analytical methods, such as ellipsometry and contact angle measurements for thickness and wetting/surface free energy determination, respectively, electrokinetics for

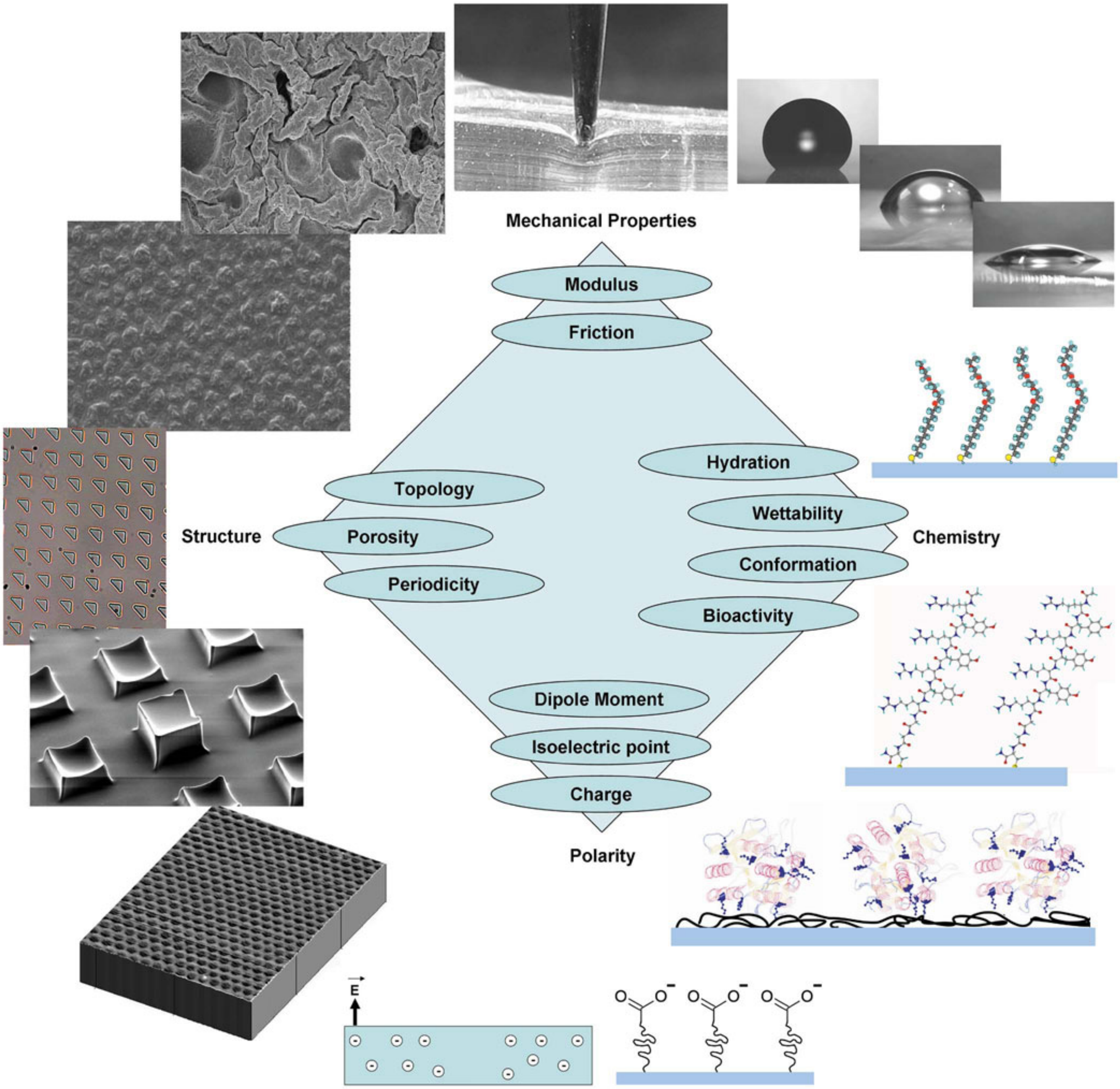

FIG. 2. Selected examples of surface architectures, chemistries, and characteristic properties explored within AMBIO (enzyme picture kindly provided by A. Cordeiro, IPF, Dresden).

ζ-potential determination, scanning probe, and electron microscopy are routinely utilized to enhance interpretation of the biological assays. Of particular importance are methods for underwater characterization. Upon immersion in natural seawater, the properties of hard surfaces are changed primarily via biofilm formation (in the short term), while for many soft coatings, such as copolymer-based films and hydrogels, water uptake causes structural rearrangement and morphological changes on the timescale of biofilm formation, emphasizing the importance of a proper understanding of the timescales involved in surface dynamics. Methods for underwater surface characterization include vibrational spectroscopies as infrared-ATR, Raman, nonlinear optical techniques as sum-frequency generation (SFG), atomic force microscopy (AFM), quartz crystal microbalance (QCM), imaging surface plasmon resonance (SPR), and ellipsometry. Efforts to build on the understanding of bioadhesion and protein resistance from the biomedical field have to be carried out carefully and rely heavily on real-time monitoring of surface interactions (adsorption-desorption processes) provided via ellipsometry, QCM, reflectometric interference spectroscopy (Rifs), and SPR. Moreover, an in-depth structural characterization of materials includes the use of large-scale facilities such as synchrotron and neutron sources.

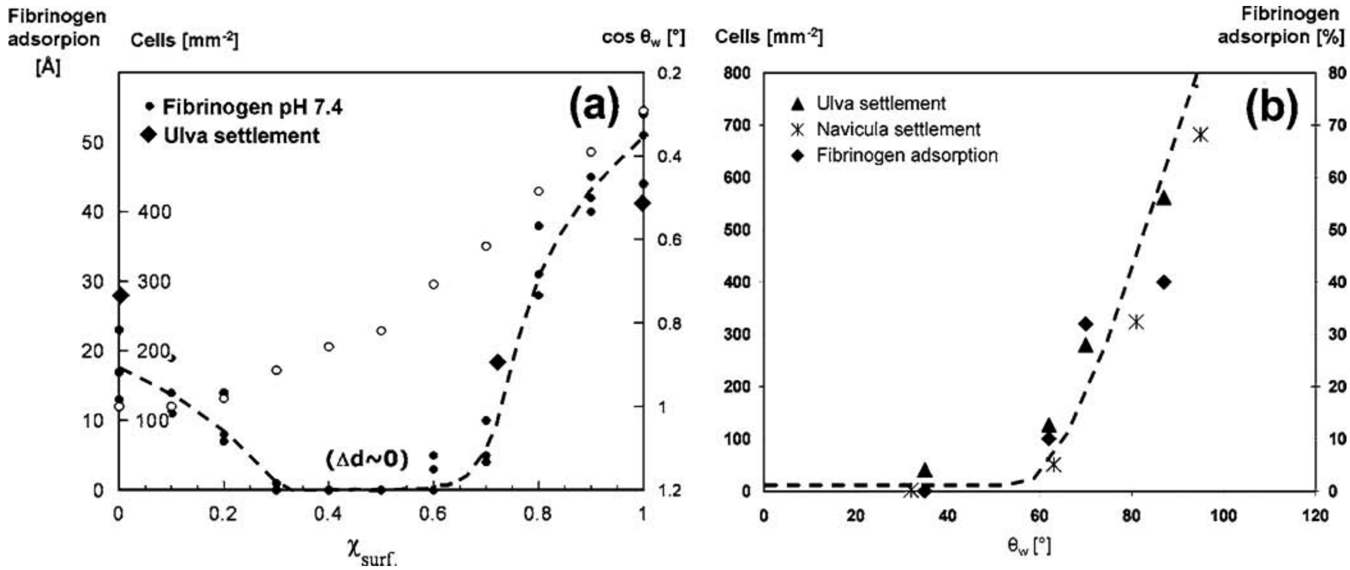

FIG. 3. Examples of the connection between wetting, protein resistance, and the attachment of two species of fouling algae (*Ulva linza* zoospores and cells of the diatom *Navicula perminuta*). (a) Fibrinogen adsorption (filled circles), settlement of *Ulva* zoospores (filled diamonds), and the cosine of the water contact angle (open circles) on mixed self-assembled monolayers of varying surface fractions of methyl- and hydroxyl-functionalized galactoside-terminated alkylthiols (increasing fraction of methyl-termination to the right) (Ref. 4). (b) Fibrinogen adsorption, settlement of *Ulva* zoospores and cells of *Navicula* on a series of oligoethylene glycol-terminated self-assembled monolayers (Refs. 5 and 8).

IV. BIOLOGICAL CHALLENGE

The antifouling and fouling-release properties of both "model" and "practical" surfaces within the AMBIO project are characterized with respect to a range of fouling organisms. The selection of the model organisms was based on a twofold requirement. They must represent the major fouling groups with which surfaces would be challenged in the natural environment, namely microfoulers or "slime" (comprising bacteria and microscopic unicellular algae), soft macrofoulers (e.g., macroalgae, anemones and hydroids), and hard macrofoulers (e.g., barnacles, mussels, and tubeworms). Additionally, a range of colonization and adhesion strategies at a range of length scales was required. Microfoulers are represented within AMBIO by a freshwater bacterium, *Pseudomonas fluorescens*, by three marine bacteria, *Marinobacter hydrocarbonoclasticus, Vibrio alginolyticus*, and *Cobetia marina*, and by the diatom *Navicula perminuta,* a microalga prevalent in biofilms on existing fouling-release coatings. These microfouling organisms adhere to surfaces by the production of extracellular polymeric substances, which are composed of polysaccharides and proteoglycans. The macrofouling alga, *Ulva linza*, is an intertidal species and the most common ship-fouling alga.[9] It colonizes surfaces by means of quadriflagellate motile zoospores, which actively select a settlement site and adhere by secreting a preformed glycoprotein adhesive. The antifouling properties of surfaces for hard macrofoulers are characterized using the settlement stage of the tropical barnacle, *Balanus amphitrite*. Cyprid larvae explore a surface on specialized antennular appendages prior to selecting a settlement site and secreting proteinaceous permanent cement. For promising practical chemistries the adhesion strength of adults of *B. amphitrite* is a critical measure of the fouling-release potential of the coating on the way to scale-up and field testing (Fig. 4, model organisms).

In addition to conventional laboratory assays, which result in "static" information (i.e., degree of settlement, adhesion and/or percentage removal), the study of settlement dynamics is used to reveal subtle variations in how colonizing organisms sense and respond to different surfaces. The explo-

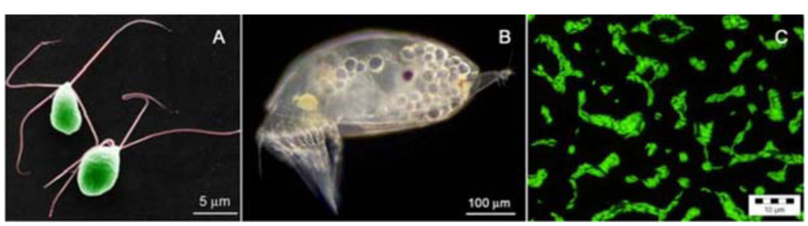

FIG. 4. Model organisms. (a) False color, scanning electron micrograph of quadriflagellate zoospores of *Ulva linza*. (b) Light micrograph of a cypris larva of *Balanus amphitrite*. (c) Fluorescence micrograph of a *Marinobacter hydrocarbonoclasticus* biofilm stained with Syto-13. *Ulva* image courtesy of J. A. Callow (University of Birmingham), cyprid image courtesy of R. R. Kirby (University of Plymouth) and A. S. Clare (University of Newcastle), and *M. hydrocarbonoclasticus* image courtesy of F. D'Souza (TNO Science & Industry, Den Helder, Netherlands).

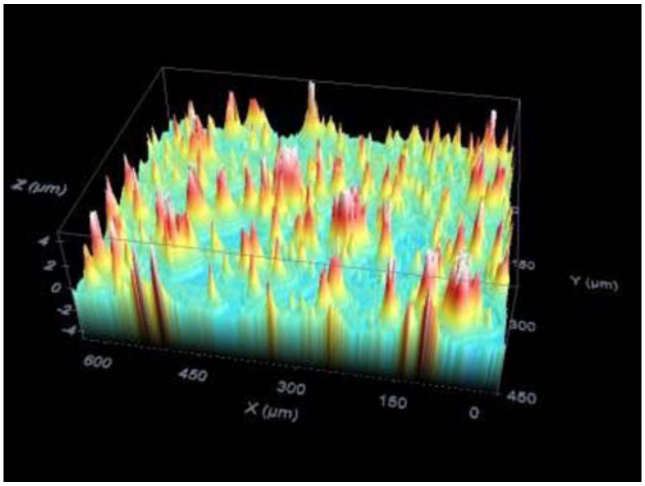

FIG. 5. Confocal microscopy picture of a nanocomposite surface (courtesy of C. Rentrop, TNO Science and Industry).

ration of surfaces by barnacle cyprids can be visualized and analyzed by microscopic tracking experiments.[10] Small changes in surface properties have been found to critically influence the observed motion patterns. Zoospores of *Ulva* show a highly complex, three-dimensional exploration behavior. To record these patterns, digital in-line holography[11,12] is used. From the holographic scattering data, the motion patterns of marine organisms can be extracted. Furthermore, three-dimensional motion data and the behavior in the vicinity of surfaces can be analyzed and quantified.[13] The three-dimensional nature of the traces obtained yields quantifiable information on the behavior of colonizing spores, offering a deeper insight into possible sensing mechanisms.

V. PRACTICAL COATING DESIGN

The engineering of innovative fouling-resistant surfaces includes new materials, new formulations, and new mechanisms for application. In the AMBIO project, polymer chemists and chemical engineers use the knowledge derived from the model surfaces to develop innovative, new materials. Polymeric material classes investigated in the project range from low surface energy polymers, which are more traditional in the biofouling community (e.g., those based on silicone elastomers and fluoropolymers), toward mid to high surface energy surfaces, which are commonly used in the biomedical community (e.g., based on polyethylene glycols). Applied as nanocomposite or nanohybrids, the combination of polymer chemistry and self-organized morphology is aiming to act in a synergistic manner against biofouling. Interesting examples of nanocomposite materials are carbon-nanotube-filled silicone coating developed at the University of Mons-Hainaut. A second example, developed by TNO, is a silica-based, sol-gel coating modified with nanoclays to generate surface morphologies (confocal microscopy picture in Fig. 5). Results to date indicate that in some cases the introduction of surface textures by the use of fillers clearly improves the fouling-release properties of the coatings for some test organisms compared to the sole use of the matrix material [TNO/University of Mons-Hainaut (unpublished data)]. In other cases the fouling-release properties are adversely affected, so generalization should be avoided. Besides using nanoparticles, inorganic micro-composites are prepared by physical deposition under vacuum conditions, which produce novel surface morphologies at different length scales. Those practical coatings, which show promise in laboratory-scale evaluation with model organisms, proceed to field trials carried out using rafts either in sheltered harbors or in the open water. Here coating prototypes are assessed for their ability to inhibit adhesion of the natural mixture of macromolecules, bacteria, and microorganisms present in the ocean. Field trial performance informs the scale-up and potential future commercialization of coating formulations in collaboration with specific end-user partners in the project. For particular applications, such as heat exchangers or underwater optical windows, practical testing is done by specialized end-user groups. Feedback from end-user groups is vital to inform the scale-up of promising new technologies and maximize the potential for future commercialization of novel, environmentally benign foul-resistant products.

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