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Biofouling and Mitigation Methods: A Review

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Abstract: Biofouling accumulates living organisms on surfaces in contact with the water and causes significant economic, structural, and microbial problems on ship hulls, piers, oil rigs, power plants, pipework, water treatment facilities as well as medical devices. In order to mitigate problems associated with biofouling, many toxic and non-toxic antifouling methods have been developed. Unfortunately, most of the methods used to control biofouling are either harmful to the environment or, in some cases, considered effective. Thus, antifouling research's main objective is to develop green, sustainable, viable, widely applicable, and environmentally friendly antifouling technology. In this review, chemical, physical, and biological mitigation methods to prevent biofilm formation employed in the past and present have been discussed along with the current literature. Chemical antifouling methods generally contain antifouling (AF) paints with biocides including copper, silver, thiocyanate, Copper powder, Irgarol 1051, Zinc pyrithione, and Tributyltin (TBT). The physical antifouling control methods employ physical force or surface modifications such as low drag, low adhesion, wettability (super hydrophobicity or super hydrophilicity), as well as microtextured structures that minimize microorganism adhesion and/or accumulation on contact surfaces, hindering the formation of biofouling. The use of nature-inspired antibiological and biomimetic surfaces like shark skin, whale skin, dolphin skin, and lotus leaves are promising for the effective control of biofouling and present opportunities for developing non-polluting technologies.

Keywords: anti-biofouling; microorganism; surface-adhesive; non-toxic coatings.

1. Introduction

Fouling is described as unwanted deposits on the surface of water contacting materials. It is a historical problem; early Phoenicians and Carthaginians mentioned fouling 2000 years ago [1]. After the rise and expansion of technological developments following the industrial revolution, fouling problems were recognized in a wide range of industries, including marine and medical areas [2], nuclear power plants [3], heat exchangers [4], pipelines, underwater cooling systems, ship hulls [5], and even implant technologies [6]. Fouling is the primary source of several problems, e.g., increase in maintenance cost, fuel consumption, resistance to the flow of working fluids, food quality and hygiene, corrosive damage, impediment of heat transfer, and inflammation in the body following implantation, limiting the performance and operating lifetime of the equipment in some cases for aforementioned industries. Besides all these industries, biofouling indirectly affects and plays a crucial role in climate change and global warming [7].

Fouling is examined under three categories: inorganic-, bio-, and organic-fouling [8]. Foulants, on the other hand, consist of a variety of materials and/or particles such as macromolecules, microorganisms, colloids, salt crystals, etc. The inorganic fouling process is not fully understood yet [9]. However, it is suggested that, in industrial cooling and heating processes, due to physical and chemical effects like heat, pressure, and friction, minerals within the water or cooling liquid such as magnesium and calcium salt, iron oxide, waterborne mud, airborne dust, become stacked at the surface of the materials and cause inorganic fouling. On the other hand, biofouling occurs when micro-sized organic particles/microorganisms such as microalgae, bacteria, and diatoms are first adsorbed onto material surfaces and then grow as a biofilm, secondary and tertiary colonizers such as bryozoans, barnacles. Macro-algae attach to this biofilm layer and form macro-sized biofouling on submerged materials [8].

An antipollution coating technology that offers no pollution or toxicity is necessary for environmental concerns. Today many studies focus on mitigating the harmful effects of antifouling and developing more effective and environmentally friendly coating systems[1]. Unfortunately, chemical coatings do not only affect the target organisms on the contact surface but also the non-target organisms in the environment [10]. Antifouling chemicals containing tributyltin (TBT) are typically successful [11]. In TBT-based coating, seawater penetrates the soluble pigment particles over time, causing controlled hydrolysis, which removes the TBT component. This reaction proceeds layer by layer until the coating paint is completely dissolved [12]. Although TBT-based coatings improve protection, chemical coating methods are very harmful due to their toxic effects on the environment [13]. Therefore, the TBT-based coating paints have been banned since 2008 in many countries after International Maritime Organization (IMO) report [14].

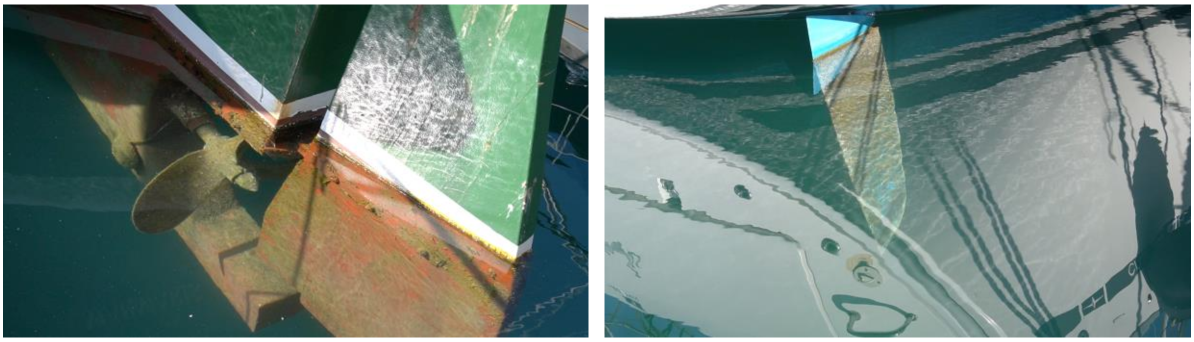
In contrast, physical antifouling methods such as water washing, ultra-sonic cleaning, high-frequency vibration, and mechanical cleaning are not harmful. However, as these physical methods cannot be applied to every surface and exhibit very poor antifouling performance compared to the chemical coatings, physical antifouling methods are limited. Therefore, the present review focuses on reducing the harmful effects of antifouling technology and enhancing the contact surface characteristics by modifying hydrophilicity, hydrophobicity, microtexture, and wettability, developing self-cleaning, low-adhesion superhydrophobic surfaces, and artificially replicating solutions exhibited by animals and plants in nature against biofouling.

2. Biofouling

2.1. Marine biofouling.

Marine biofouling is one of the well-known and most significant problems in the marine industry. It is defined as the accumulation and growth of marine organisms on surfaces immersed in the sea/non-sterile environment [15]. Biofouling develops sequentially from an initial condition layer of absorbed organic and inorganic matter in the aquatic environment. Through microbial film formation, it progresses to a community of macroscopic aquatic species such as plants and animals [16]. Colonization creates a more favorable environment for survival and reproduction. Most aquatic fouling organisms prefer hard surfaces for settlement. It was estimated that about 127 000 aquatic species live on hard surfaces while 30 000 on soft surfaces [17]. A wide range of micro and macroorganisms can contribute to marine biofouling: more than four thousand fouling organisms have been identified worldwide. In addition to natural hard surfaces like stones and rocks, fouling organisms attach to anthropogenetic surfaces such as ship hulls, navigation equipment, oceanographic sensors, aquaculture systems, stationary structures, industrial pipelines, and fixed submerged systems [18]. Fouling can be divided into two main categories based on the types of organisms that attach to the contact surface. Microfouling results from microscopic organisms such as bacteria, diatoms, fungi, and microalgae, and it is essential for the settlement of more complex macrofouling organisms [19]. Macrofauling organisms are further divided into two types based on the body structure of the colonizing organisms. Soft macrofouling organisms include macroalgae, soft corals, anemones, tunicates, and sponges with no solid supporting structure [19]. Macroalgae such as *Ulva australis* and *Ectocarpus* are common marine soft macrofoulers [8]. Hard macrofouling organisms include animals with a hard supporting structure, such as barnacles, tubeworms, bivalves, and polychaetes which are difficult to remove once established.

Ever since man sailed, the oceans' marine biofouling became a problem for ship hulls, as shown in Figure 1 [20]. The unwanted accumulation of the micro/macro-organisms on ship hulls causes extra drag force, increases total weight, and increases hydrodynamic drag, which requires more power and increased fuel consumption [21]. A biofouling film with a thickness of 1 mm causes a 15% loss in speed [8]. It has been reported that a 5% biofouling cover underneath a ship hull results in a 14 % increase in greenhouse gases emission. Therefore, periodic dry-docking and hull cleaning are required for a biofouling management plan for all ships. Fouling also reduces the heat transfer efficiency of heat exchangers and causes loaded stress corrosion on fixed submerged materials such as oil towers and bridge pillars [22]. In addition, due to the negative effects of biofouling on fishing gears and nets, it has been reported that the final market prices of economically important aquatic species such as oysters and salmon increased [23]. It is estimated that the global economic cost of biofouling in various industries ranges between US$ 1.5 - 3 billion per year [17].



(a) **(b)**

Figure 1. Marine biofouling examples (**a**) on a boat propeller; (**b**) on a rudder blade surface.

A wide range of parameters such as pH of the water, the temperature of the medium, salinity, pressure, nutrient levels, flow rates, the intensity of solar radiation, and geographic coordinates affect biofilm formation [24,25]. In addition, offshore and nearshore fouling types exhibit different characteristics. However, global rather than local differences in the species composition of biofouling are of great concern. The expansion of international transportation and shipping became an important vector for spreading non-indigenous marine organisms.

Ports and harbors are considered hot spots for introducing exotic species due to the biofouling of ship hulls [26]. There is a growing body of evidence on the reproduction potential of exotic species transported via ship hulls out-competing native species, which may negatively impact marine ecosystems [18,26].

2.2. Stages of marine biofouling.

Marine biofilm formation is a highly dynamic combination of chemical, physical, and biological processes that occurs within seconds of the initial contact between a solid material and an aquatic ecosystem rich with nutrients and bacteria. Freely floating bacteria/microorganisms could rapidly attach to the solid surface and start colonizing to form biofilms. During its very early stage of formation, the effectiveness of the settlement depends on both the bacterial characteristics and the substratum properties facilitated by the Brownian motion of weak physical forces such as the Van der Walls and electrostatic interactions [27]. After settlement, the bacteria strengthen their attachment and grow with the extracellular polymeric substance (EPS) secretion becoming more persistent on the surface [28]. Bacteria can interact cell-cell, extend an invitation to other organisms and organize individual bacteria for a proper location using Quorum Sensing (QS) [29]. Most of the macro foulants, except a few (e.g., bryozoans and barnacles), require a preceding slimy biofilm layer to settle over the substratum.

Marine biofouling accumulates on surfaces in five succeeding stages: initial attachment, irreversible attachment, initial growth, final growth, and dispersion (Figure 2) [30]. Since even a mature biofilm is never static, the relationship between habitat and biofilm community is crucial. Environmental factors such as pH [31], salinity [32], turbulent flow [31], and temperature [31] easily affect the structure, formation time, topology, or physiology of the biofilm. The first step is a reversible settlement formed by the adhesion of nutrients like proteins and microorganisms. This stage, supported by weak physical forces, starts to form within seconds. Scarcely, they reach a thickness of less than 100 nm [33].

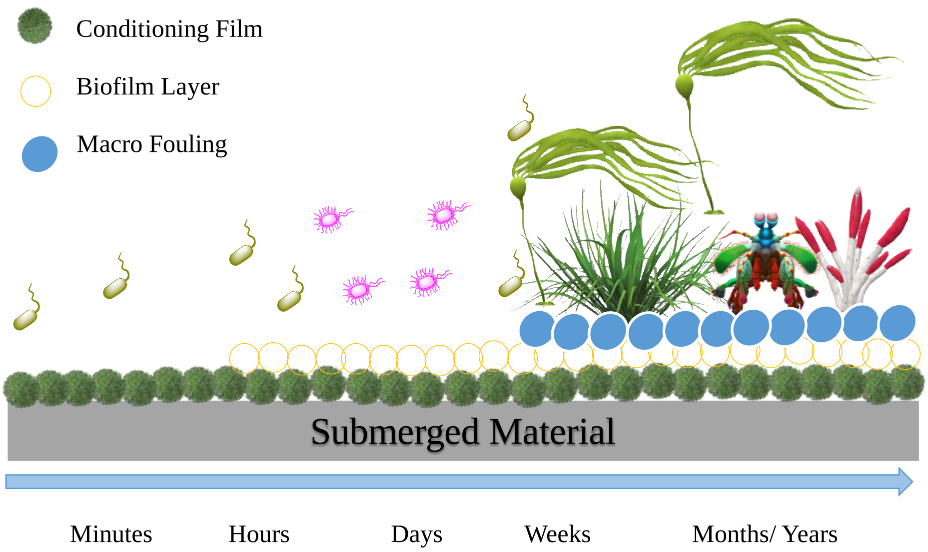


Figure 2. Schematic illustration of the marine biofouling stages.

Within a few minutes, microorganisms like bacteria (e.g., *Pseudomonas putrefaciens*, *Vibrio alginofyticus*) and diatoms (e.g., *Achnantes brevipes*, *Amphiprora paludosa*, *Amphora coffeaeformis*, *Lichmophora abbreviata Nifzschia psuilla*) start to settle on the substratum and secrete the EPS, which hold the community together and start the irreversible attachment phase.

One mL of seawater contains approximately $1x10^6$ bacteria [33], and every biofilm contains more than 4000 different co-existing bacteria [33-35], which rapidly start to change the physicochemical structure of the surface following settlement. In the third stage, spores of macroalgae such as *Enteromorpha intestinalis*, *Ulothrix zonata*, and protozoans such as Vaginicola sp., Vorticella sp. begin to settle on the surface within a period of one week and contribute to the macro formation. The accumulation, growth, and dispersion of macromolecules are considered the final stages of marine biofilm formation and can be observed over several years.

2.3. Medical biofouling.

Medical devices can be classified under two major subjects, temporary and indwelling, based on their usage area [8]. For the short-term, temporary medical devices such as biosensors, catheters, pacemakers, contact lenses, drug-delivery devices, bone plates, ventilation tubes, and needles are used [38]. On the other hand, indwelling medical devices are used for long-term applications and include biosensors, heart valves, bone plates, fasteners, orthopedic implants, dental implants, pacemakers, and long-drug-delivery devices [36-38]. The infection rate of indwelling medical devices is much higher than that of temporary devices, accounting for almost 80% of the total infection rate [39]. The most significant issue for all these medical devices is sterilization and biofilm formation. Biofilms could permanently accumulate on tissues or medical implants, causing infections and, therefore, playing a crucial role in mortality. According to the National Institute of Health (NIH, USA) report, only in the USA, more than 2 million hospital-acquired infections are due to medical device-related biofilms [40]. Biofilm formation could lead to a wide range of different infections. For example, a urinary catheter is one of the archetypical temporary medical devices which is highly vulnerable to the risk of infection because the medium it is in contains a high rate of bacteria such as *S. epidermidis, E. faecalis, E. coli, P. mirabilis, P. aeruginosa, K. pneumonia,* and other gram-negative microorganisms [41]. Every year, more than 900 000 case occurs only in the US related to urinary catheter and represent %40 of the nosocomial infections [42]. Bloodstream infections are another type of vascular-related infection region, causing 12-25% mortality and cost 25 000$ per case [42-44]. Half of the nosocomial endocarditis has been linked to infected intravascular catheters, also affected by biofilms [45]. Besides, the biofilm layer causes infections in the human body and is responsible for medical device failure most of the time [46,47]. All these infections and failures are evidence of efforts to mitigate the biofilm layer for temporary and indwelling medical devices and living tissues and develop specific antimicrobial compositions with antibiofilm activity.

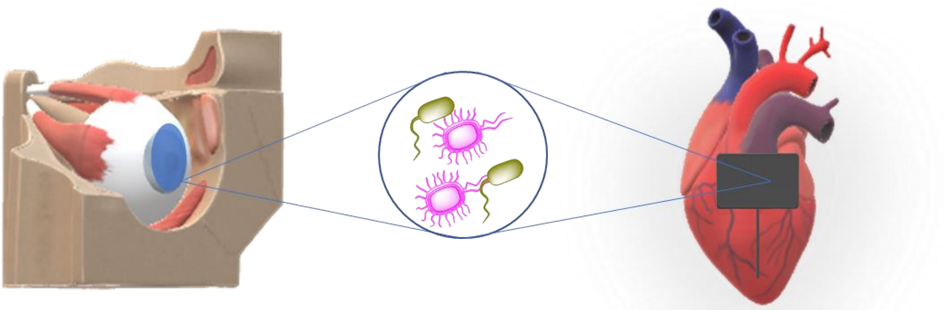


Figure 3. Schematic illustration of biofilm formation on medical devices.

With the insertion of the medical device into the aquatic inner body medium, the surface of the device is covered with various biomolecules, proteins, and polymers. This provides an ideal environment for the attachment and growth of microorganisms [48]. Substratum, conditioning films on the substratum, hydrodynamics of the aqueous medium, and various cell surface properties are considered important for settlement [48]. More than 25 different bacteria, fungi, and protozoa contribute to biofilm formation creating a favorable and proper medium for their colonization [49]. A pathogenic biofilm can resist both the immune system and antibiotics and can lead to host infections, making it difficult to fight bacteria with drugs [47]. For example, *Pseudomonas aeruginosa* [50], *Escherichia coli* [51], and *Staphylococcus epidermidis* [52] possess high resistance to drugs. Many studies show that bacteria within a biofilm are 1000 times more resistant to the host immune system against antimicrobials and/or antibiotics compared to freely suspended counterparts [48]. The infecting bacteria produce exopolysaccharides to form a proper biofilm. The negatively charged exopolysaccharide plays a crucial role in protecting bacterial cells from cationic antibiotics by limiting their effects [53]. The formation and resistivity rate of biofilm to the antibiotics are also related to wall composition, surface structure, and phenotypic variation in enzymatic activity [54]. The formation of biofilm affects the antibiotics and contributes to the slow antimicrobial penetration, the stress response, substrate limitation, and the presence of persistent cells [55].

2.4. Membrane biofouling.

Membranes are versatile and used for a wide range of different filtration processes such as wastewater treatment, water reuse, and water recycling. In membrane filtration, water and wastewater are filtered through a porous barrier that allows some components to pass and others not. Membrane biofouling is a major problem when a solution with molecular or biological species accumulates on the membrane's surface or pores, resulting in a decreased yield in processes like membrane bioreactors (MBRs), reverse osmosis, and ultrafiltration [56]. Membrane fouling occurs as inorganic, organic, or biofouling. Here, only the biofouling process will be examined, representing 45% of the fouling phenomenon [57]. If the deposited particle is larger than the pore size, the accumulated formation is called the "cake layer," which is not distributed homogeneously on the membrane surface [58]. If the particle size and the pore size are similar, it causes irremovable fouling. The major contribution to membrane fouling is through deposition of cake layer compared to membrane resistance and irremovable fouling [59]. Membrane biofouling is a little different in comparison to marine biofouling. Existing shear force due to aeration reduces biofilm formation. Besides, in the active sludge, bacterial concentration is higher than in marine biofilms, resulting in faster biofilm formation [58]. Furthermore, hydrodynamic flow within the membrane can occur as a permanent or cross-flow, which speeds up the early-stage cells, increases bacterial feeding, and improves gas exchange to the biofilm layer, resulting in superior attachment and material transport for better quorum sensing (QS) [60].

Membrane biofouling reduces the flow rate and causes an increase in the transmembrane pressure (TMP) [59]. TMP is critical for MBRs. Membrane biofouling leads to three different TMP: short-term fast, long-term slow, and sudden dramatic increase (TMP jump) [61]. Decreasing, delaying, or terminating the TMP jump is vital for the MBRs. Research indicates that multiple foulants make the TMP jump, such as dead bacterial species and EPS release at the inner part of the membrane [61].

Foulants are classified into three categories [62], according to their behaviors on the membrane: Removable fouling occurs at the early stage of the fouling and is easily cleaned with physical treatment; Irremovable fouling is a type of fouling in which physical cleaning is inadequate and needs to be supported by chemical cleaning. Chemical cleaning may reduce the life of the membrane and cause environmental issues in the long term, and 3. Irreversible fouling is the most persistent form of foulants and requires chemical reagents to be mitigated.

The fouling mechanism in Nanofiltration (NF) and Ultrafiltration (UF) is unknown. Like UF/NF, filter cleaning periods could be extended 3-6 months using high-pressure-driven systems. For the low-pressure Microfiltration (MF) and Ultrafiltration (UF) membranes, chemical cleaning treatment can be used weekly [63].

3. Other Industries

Microorganisms are ubiquitous in our environment and affect a wide range of industries. A list of biofouling problems observed in different industries and potential mitigation methods are given in Table 1, such as oil towers [64], heat exchangers [65], nuclear power plants [66], and food production [67], and dentistry [68]. The economic effects of biofouling in industrial systems are much more significant than expected as waterborne bacteria accumulate in large quantities, and a biofilm forms on the surfaces of pipes, tanks, packages, fresh foods, and other equipment. A wide range of factors also affects biofilm formation, including nutrient availability, velocity and turbulence, temperature, pH, surface conditions, and the sizes of particles [69]. Biofilm causes biocorrosion and biofouling, which degrades the microbiological quality of water. Corrosion and weathering may induce further operational problems such as degradation of pharmaceutical or microelectronic products, decreased heat exchanger potency, accidental stainless-steel corrosion, and premature mineral material loss.

From the harvesting stage to the processing stage, biofilm formation is critical in the food industry [70]. Foods and drinks themselves are nutrients that attract bacteria, facilitate nutrient transfer, and provide an appropriate environment for biofilm formation, even faster than bacterial cells in the aqueous medium. The increased amount of nutrients in food-contact surfaces, such as albumin, gelatin, fibrinogen, and pepsin, serves as a conditioning film in food processing systems [70]. Bacterial attachment to food products or commodity touch surfaces causes major hygienic issues as well as financial damages related to food spoilage. Low sterilization conditions and air-bone micro-flora enhance bacterial activity. Besides the food itself, biofilm formation may occur on the floors, conveyor belts, rubber seals, stainless steel surfaces, etc., and may create hygiene problems.

Oil production is another process that is tremendously affected by biofouling [71]. As a result of harsh conditions in oil production, bacterial diversity is not as common as in the aqueous environment. Biofouling affects oil reservoirs, tanks, refineries, and downstream processes like injection systems, subsea manifolds, emergency firewater systems, and offshore oilrig legs [64].

Nuclear power plants/heat exchangers are subject to biofouling due to their cooling liquid supply from natural water resources. Biofilms can develop in most water bodies, including ultrapure water in stainless steel pipes and even microorganisms that can thrive in extremely radioactive environments of nuclear power plants [72]. Many species of organisms are transported via cooling systems, and the higher temperatures on the intake side are suitable for bacteria growth and, therefore, aggravate biofouling-related problems. These organisms frequently grow on cooling pipes' inner surface, posing serious problems such as higher pumping load, corrosion, and decreased heat-exchange efficiency. Dead cells and microorganisms are often transferred by flow and clog small pipes or valves, increasing friction and energy consumption, reducing pipe pressure, lowering heat transfer efficiency, and adversely influencing the cycling process. Microfouling is seen in the condenser and process water heat exchangers, while macrofouling is seen more in the pre-condenser device [72]. According to some estimates, damages inflicted by biofouling cost $0.93 million for a 550 MW power plant each year [73].

Table 1. Industries/fields and related major problems caused by biofouling and potential mitigation methods.

Type	Methods	Problem	Solution	Reference
Food Production				
Packing	Chemical	Bacterial formation on Packing surfaces	Nanocomposite	[70]
Conveyor Belt	Physical	Bacterial formation on Conveyor belts	Ultrasound	[74]
Medical				
Pacemaker	Physical	Pacemaker Infections	Titan Casing	[74]
Contact Lens	Physical	Contact Lens Infections	Nanocomposite	[75]
Orthopedic Implant	Physical	Orthopedic Implant Infection	Super-paramagnetic Nanoparticles	[76]
Marine				
Ship Hull	Physical	Increased fuel consumption	Silicon-Based Coating	[77]
Fishing Nets	Chemical	Biofilm Formation on Fishing Nets	Conductive Coating	[78]
Other Industries				
Cooling System	Chemical	Reduced Flux	Chlorination	[79]
Nanofiltration Membrane	Physical	Reduced Flux	UV Irradiation	[80]

4. Surface Factors

Biofouling formations rely on a wide range of different variables. Surface factors like wettability [81], color [82], and microtexture [83] are some of the most important factors. Surface wettability can exhibit different behaviors such as hydrophilicity, hydrophobicity, super hydrophilicity, and superhydrophobicity that affect the colonization of the foulants. A hydrophobic surface exhibits low wettability and low surface energy, whereas a hydrophilic surface exhibits high wettability and high surface energy [83]. The contact angle with the surface of the substratum classifies these behaviors where contact angles less than 10^o are classified as super hydrophilic, and over 150^o are exhibit superhydrophobic behaviors [83]. For the ideal surfaces, which exhibit strictly smooth, chemically homogeneous, and stable characterization behaviors, the contact angle of the liquid drop is given as

$$cos\ cos\ (\theta) = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \quad (1)$$

where θ is the contact angle and interfacial energies acting between the solid-liquid (γSL), solid-vapor (γSV), and liquid-vapor (γLV) interfaces.

Depending on the surface roughness, there are two different surface regimes known as Wenzel and Cassie-Baxter. According to the Wenzel regime, the measured contact angle (θ^*) differs from the actual contact angle (θ):

$$(\theta^*) = R.\ \ cos\ cos\ (\theta) = R.\frac{\gamma_{sv} - \gamma_{SL}}{\gamma_{LV}} \quad (2)$$

where R is the ratio between the actual surface area of the rough surface and the projected (apparent) area.

$$cos\, cos\, (\theta) = -1 + \emptyset_S\, [cos\, cos\, (\theta) + 1] = -1 + \phi_S\, [\frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} + 1] \quad (3)$$

where φS is the fraction of the surface in contact with the liquid; the remaining fraction $(1 - \phi_S)$ is in contact with air.

Contact angle hysteresis (CAH) determines the difference between the advancing and receding (uphill side) contact angles, low for Cassie–Baxter and high for Wenzel regimes. It is clear that the adhesion force is proportional to CAH. Therefore, all these aforementioned wettability properties affect biofilm formation. Surface properties exhibit unsuitable surface structures for such microorganisms and affect the surface choice. For example, *Ulva linza* prefers hydrophobic surfaces, whereas Balanus Amphitrite prefers hydrophilic surfaces.

In the case of existing surface ledges, the liquid drop may not be able to "wet" the surface because of the air bladders located between the surface and ledges and the Cassie-Baxter regime, which is illustrated in Figure 4.

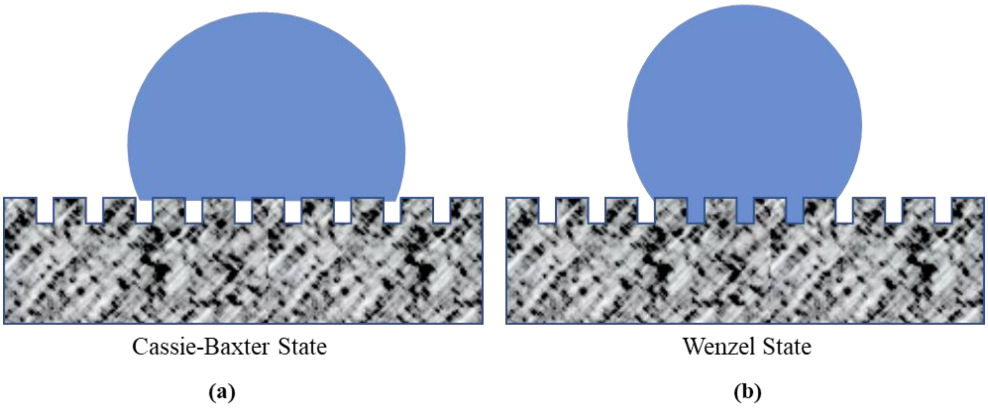


Figure 4. Sketch water drops on hydrophobic pillar structures (**a**) Cassie-Baxter State: Air is trapped underneath the liquid drop; (**b**) Wenzel State: Liquid drop completely penetrates between the grooves. Adapted from [84].

Surface color is another important factor for biofilm formation. When grown on a different colored medium such as white, red, blue, and black, various aspects of cyanobacteria performance, including biofilm growth, architecture, pigment production, levels of ATP, and reactive oxygen species, result in significant differences [84]. In another study [82], the darker colors increased biofilm formation compared to lighter colors for a short-term period. These findings indicate the importance of surface color on biofilm formation.

5. Extracellular Polymeric Substances (EPS)

Extracellular Polymeric Substances (EPS) secreted by the prokaryotic and eukaryotic microorganisms are one of the critical factors for biofilm formation [84]. EPS is a highly hydrated 3D biopolymeric component that specifies the biofilm's physical, chemical, and biological properties. EPS consists of extracellular DNA, polysaccharides (i.e., monosaccharides, uranic acids, amino sugars) [85], proteins (i.e., amino acids), nucleic acids (nucleotides) [86], phospholipids (fatty acids, glycerol, phosphate, ethanolamine, serine, choline, sugars) [87], humic substances (phenolic compounds, simple sugars, amino acids)87 and other polymeric compounds. EPS plays a crucial role in a wide range of different staminal functions such as adhesion to surfaces [87], aggregation of bacterial cells, formation of flocs and biofilms [88], cell-cell recognition [89], formation of a protective barrier from external stress factors [90], retention of water [90], sorption of exogenous organic compounds [90], sorption of inorganic ions and the interaction of polysaccharides with enzymes [90]. However, studies about the functions of various EPS materials during biofilm structural differentiation are still in their early stages. A better understanding of the structure of EPS could help a wide range of industries affected by biofilm formation in terms of their elimination and potential use.

6. Quorum Sensing

Quorum sensing (QS) is another key factor that affects biofilm formation and structure [90]. QS is simply an intercellular signaling "language" that makes it possible to communicate between individual bacteria [29]. In agreement with the fact that bacteria are closely associated with biofilms, QS has been shown to affect biofilm formation for various bacterial species. The discovery of bacteria's intercellular communication revealed that bacteria could coordinate activities once thought to be exclusive to multicellular organisms. The signals for communication can be related to cell density (auto-inducers) or bacteria-based at various growth stages [91]. They can regulate their environment and change gene expression to provide a competitive benefit for physiological activities such as symbiosis [91], virulence [92], competence [93], conjugation [93], antibiotic production [93], motility [94], sporulation [95], and biofilm formation [95]. When the cell density reaches the threshold level, binding proteins trigger the genes to control QS signals. For example, N-acyl homoserine lactose (HSL) is the signaling molecule for gram-negative bacteria, which requires a single enzyme, called LuxI, that binds to LuxR homologs to activate the expression of target genes [96]. At low cell densities, the concentration of the signal is low both inside and outside the cell, with minimal activation of LuxR. However, at high cell densities, acyl-HSL activates LuxR through binding and leads to the expression of downstream target genes [96].

In terms of the arrangement of the auto-inducer molecules and the mechanism of signal processing and sensing, gram-positive bacteria's QS systems vary from gram-negative bacteria's canonical acyl-HSL-mediated QS. The auto-inducer molecule used in gram-positive bacteria is known as autoinducing peptide (AIP), in contrast to the acyl-HSL signal used in gram-negative bacteria [96]. AIP signals do not diffuse the inner or outer side of the cell. They are recognized through a two-component signal transduction mechanism in which the AIP binds to a membrane-bound histidine kinase sensor. The critical information is relayed to the cell through phosphorylation of reaction regulator proteins that eventually attach to the promoter of target genes to control gene expression. The concentration of the AIP signal outside the cell is low at low cell densities, and the Reaction Regulator (RR) is not activated. The binding of the AIP to a histidine kinase receptor results in phosphorylation of the RR and expression of downstream target genes at high cell densities. *Vibrio fischeri* (LuxI/LuxR), *Aeromonas hydrophila* (AhyI/AhyR), *Agrobacterium tumefaciens* (TraI/TraR) and *Burkholderia cepacia* (CepI/CepR) are among the organisms possessing LuxI/LuxR homologs [96].

In summary, QS allows a population of individuals to coordinate global behavior and thus lead to biofilm formation. To be considered QS as a signal, it should meet the following conditions [97]: Signals should occur at various stages of growth or in response to specific environmental changes; In extracellular environments, the QS signal could be aggregated and detected by a particular bacterial receptor; The occurrence of a critical threshold concentration of the QS signal can elicit a coordinated response; Beyond the physiological changes needed to metabolize or detoxify the molecule, the cellular response should be extensive.

7. Mitigation Methods

Driven by economic costs related to the detrimental consequences of biofouling, different strategies for limiting the accumulation of biofilms on surfaces have been developed [98,99]. Various mechanisms of biofouling mitigation methods are available, including biological controls, electrochemical methods, nutrient limitation, physical methods, biomimetic surface modifications, and their subclasses. The effectiveness of a particular biocide is affected by numerous factors. These include the features of the biocide, the kind and condition of the bacterium, the biofilm's characteristics, and the bacteria's surroundings. Also, the kinds of biocides, their concentrations, and the compounds' side reactions affect therapeutic effectiveness. The efficacy of disinfection is determined by environmental variables, including pH, solution temperature, and exposure time. Only chemical, physical and biomimetic methods will be examined within the scope of this review.

7.1. Chemical methods.

Chemical methods are prevalent prevention methods used in various industries and environments, including marine, membrane, food, and heat exchangers. Chemical reactions and diffusion phenomena have recently been discovered to be essential mechanisms in the performance of biocide-based antifouling (AF) paints [100]. They have been used for many years to inhibit microbial activity. A biocide is a toxic substance to microorganisms that cause cell death and prevent biofouling. Tributyltin (TBT)-based antifouling paints biocides have been acceptable for marine industries since the early 1960s [101]. However, due to non-preventable harmful effects to the marine environment such as imposex occurrences [18], TBT-based paints were banned from International Maritime Industries (IMO) in 2008 [102]. During the last two decades, copper-based antifouling (AF) paints have become the primary biocide used in most AF coatings. Copper (I) oxide is dominantly used worldwide besides Copper thiocyanate, Cu powder, Irgarol 1051, Zinc pyrithione, TCMS pyridine, Folpet, Zineb, and Chlorothalonil.

Two types of polymer matrix release agents are now available: insoluble matrix coating and soluble matrix coating [1]. An insoluble matrix coating that contains epoxy resin, acrylate, or chloro-rubbers and soluble antifouling agents cannot decompose, polish, or corrode in the aquatic environment [18]. Seawater penetrating the film has to diffuse through the interconnecting pores formed after the dissolution of the soluble pigments and keep dissolving to form a continuous antifouling effect. On the other hand, soluble antifouling matrix coatings integrate with rosin or its derivatives [103]. Contact with the aquatic environment triggers the reaction with the carboxyl groups and the sodium and potassium ions present in seawater. Soluble matrix has been shown to exhibit a better antifouling effect under dynamic conditions than static conditions [18].

However, even relatively low concentrations of copper can exhibit harmful effects on non-target organisms like fish. Diverse effects have been reported in several toxicity studies [104]. In 2005, 261 tons of copper were sold to the aquaculture industry just in Norway. AF paints that release copper at a rate of more than 200 ng/cm2 over the first 14 days were prohibited in Denmark [105]. Most current research focuses on reducing the negative impacts of heavy metals-containing self-polishing copolymers (SPCs) coatings and their environmentally detrimental outcomes [99,106-108].

The cooling water that is used in heat exchangers is also subject to biofouling, as mentioned in section 2.4, previously. Essentially, chemical control often includes the use of biocidal to kill microorganisms in cooling water or biocides to lessen their activity. Biocides are classified as oxidizing or non-oxidizing [109]. Chlorine, ozone, bromine, and peracetic acid are examples of oxidizing biocides, whereas acrolein, glutaraldehyde, isothiazolones, biodispersants, and heavy metal compounds are examples of non-oxidizing biocides [110]. Due to its efficacy against micro and macro-organisms and relatively lower cost, chlorine has been a favored biocide for many years. However, chlorine can generate carcinogenic chloromethane in the presence of organic substances in the natural environment [110].

Free chlorine reacts with water to form two acids:

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

The hypochlorous acid ionizes in water according to the equilibrium:

$$HOCl \leftrightarrow H^+ + OCl^-$$

So, here, the biocidal action is caused by the presence of hypochlorous acid rather than the hypochlorite ion.

7.2. Physical methods.

Contrary to the chemical control methods, physical control methods are highly eco-friendly applications and prevent potential chemical usage problems and the consequent environmental hazards. Unfortunately, physical methods are not effective when compared with the use of toxic biocides for long-term fouling control. Physical control methods in which the fouling layer is scraped and removed are shown in Figure 5.

(a) **(b)**

Figure 5. Example of **(a)** cleaning with high-pressure water, which is one of the physical cleaning methods; **(b)** bottom of the sailboat after cleaning process.

Besides, physical surface characteristics such as the structure and charge potential can be modified to control fouling.

7.3. Sponge rubber balls.

Sponge rubber balls are efficiently used in heat exchangers due to easy ball replacement, simple operation principles, and potential cost savings. Generally, for a better cleaning effect, the diameter of the balls chosen is approximately 1 to 2 mm larger than the inner tube diameter [111]. The cooling water flow transports the balls and spreads them over each tube, and the spread balls continuously hit/rub the exchanger with their momentum and remove the fouling layer. Unfortunately, sponge rubber balls are worn out after continuous use depending on the tube material and, therefore, need replacement over time.

7.4. Ultrasonic methods.

An alternative method for cleaning the contaminated surfaces is provided by ultrasound techniques with frequencies ≥18 KHz. Ultrasound waves can propagate through the fouling medium, and the mechanical vibration creates an elastic medium of sound and pressure waves that transfer energy to the medium. The sound waves create oscillating regions with the support of cavitation. Cavitation bubbles lead to hot spots and increase the local temperatures and pressures up to 6000K under 10 µs [112]. A number of studies have investigated the effects of ultrasounds [112-115] and revealed that ultrasound technology that uses sound waves had been shown to play a crucial impact on different bacteria and barnacle species with their mortality and growth inhibitory at different frequencies. For example, the growth and microbiota of European sea bass (*Dicentrarchus labrax*) [115] can be controlled with ultrasound technology. Therefore, ultrasound technology can be used to control and mitigate biofouling.

7.5. Magnetic and electrical methods.

Magnetic/electric fields and electric current have also been used to prevent and control biofouling in different industrial areas such as medical, marine, filtration, and energy for many years. Using a lower electric current, prevention can be highly effective. One out of 5 of the biofilm layers of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida parapsilosis* biofilms grown on the inside surfaces of polyvinyl chloride (PVC) catheters were reduced under 4 days of 500 µA DC exposure [116]. Similar results were obtained with continuous, direct current at lower amperages and intermittent direct current against *S. aureus*, *S. epidermidis*, and *P. aeruginosa* biofilms [117]. In addition, successful results for protein-based biofouling that occurs in many chronically implanted devices were reported using polyimide-based flexible magnetic actuators [118]. Besides, in experiments using pulsed electric fields, electric stress on hydrozoans resulted in tentacle and body contraction to almost one-third of their original length [119]. All these studies showed the potential of magnetic/electric fields in controlling biofilms.

7.6. Biological methods.

As an alternative to physical and chemical methods, biological mitigation methods have been used to control biofouling in different fields with different mechanisms. Bacterial pathogenicity may prevent the inhibition of the quorum sensing ability. For example, meta-bromo-thiolactone (mBTL) inhibited *P. aeruginosa* quorum-sensing receptors, LasR, and RhlR and prevented biofilm formation [120]. In another study, subtilosin was evaluated as a QS inhibitor in gram-positive, gram-negative, and gram-variable bacteria. The results indicated that 15.1 µg/mL of subtilosin prevented about 60% biofilm formation and inhibited 85% of violacein production for *E. coli*, compared to the control [121].

The use of natural viruses that infect bacteria known as bacteriophages (commonly known as phages) is another biological control method for biofilm development. Many bacteriophage genomes contain genes for enzymes that can degrade biofilm matrix components [122]. A rare 7-11 phage used for AuNPs synthesis was used for anti-biofilm activity against *P. aeruginosa* (PAO1) [123]. In another study, engineered and injectable hydrogel bacteriophages delivered to the site of bone infections successfully reduced the presence of the live *P. aeruginosa* bacteria in both planktonic and biofilm phenotypes *in vitro* [124] and showed the potential medical application of bacteriophages. Finally, bacteriophages are also used for controlling foodborne disease (FBD) related pathogens in the food industry. For example, bacteriophages were used to mitigate *Salmonella* spp. biofilm formation on 3 different surfaces (stainless steel, glass, and polyvinyl chloride) which are used in food packaging [125]. Besides chemical and physical applications, biological control is vital in membrane bioreactors (MBRs). It was reported that metazoans affect the topological structure of biofilm and positively affect the membrane flux [126].

Furthermore, enzymes such as Lysozyme are also used to control biofilm formation, and 30 μg/mL of Lysozyme inhibited 19% of biofilm formation on clinically isolated 103 *Pseudomonas aeruginosa.* Another study also revealed that Lysozyme was very effective on biofilms by *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Gardnerella vaginalis* [127].

8. Advanced Surfaces

Microorganisms constantly look for settlement spots on surfaces such as crevices, grooves, recesses, and pits to colonize and survive. Because of these factors, as mentioned earlier, surface microtexture and material properties such as surface energy, wettability (hydrophobicity and hydrophilicity), surface charge, surface chemical composition, surface topography, surface elastic modulus, and coating thickness are very crucial for biofilm settlement and growth. Easy application on different substrate materials such as softwood fiber [128] polyurethane [129], Teflon-Si [130], PEG [131], surface materials like silica [128], SiO_2 [133], PEG hydrogel [131], and fabrication methods as dip coating [128], soft lithography [130], plasma etching for Si [130] and E-beam patterning [101] leads modifying the surface microtexture for biofilm prevention and the adverse effect on bacterial adhesion has become considerably more efficient, simple and environmentally friendly than in the past.

8.1. Superhydrophobic surfaces.

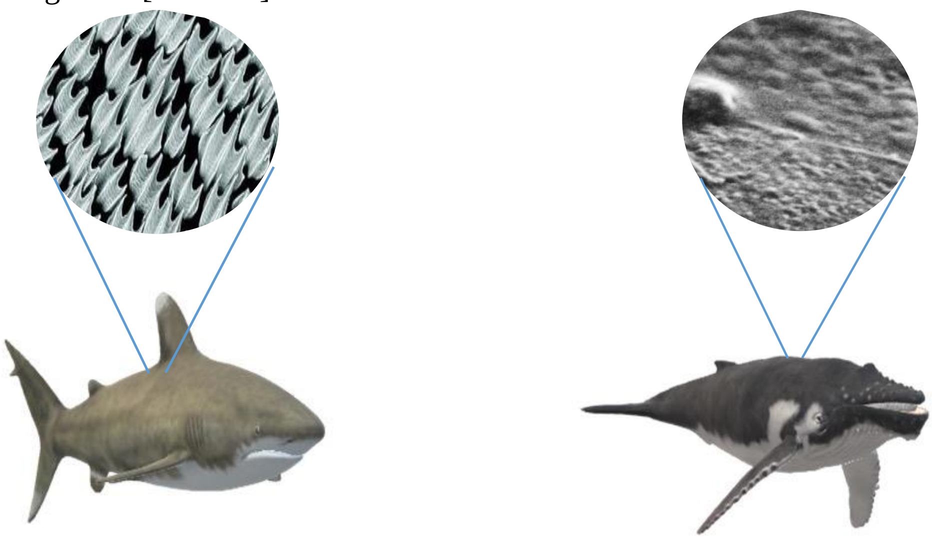
Through the contact angle mentioned in Section 3, low-adhesive superhydrophobic surfaces influence characteristics such as wettability and self-cleaning, which negatively affect the microorganism settlement to the substratum. Generally, microorganisms prefer to colonize on hydrophilic surfaces in contrast to hydrophobic ones. Plants like lotus (*Nelumbo nucifera*) [133], water fern (*Salvinia*) [127], and lady mantle (*Alchemilla mollis*) [134] combat biofilms with superhydrophobic surfaces. Nano-engineered artificial superhydrophobic surfaces are becoming promising advanced materials for industrial applications. Using 2D nanoporous and 3D nanopillar surfaces against *S. aureus* and *E. coli* settlement, bacterial adhesion was lowered [135]. Successful control over biofilm development was achieved by modulating hydrostatic pressure, wettability, and nutrient availability against *Komagataeibacter medellinensis* using 3D super hydrophobic molds [136]. Another study showed that the anti-biofouling property of a superhydrophobic surface was due to an entrapped air-bubble layer that reduced contact between the bacteria and the surface [137].

8.2. Zwitterionic surfaces.

Another commonly used method to control the accumulation of microorganisms onto surfaces is to graft surfaces with poly(ethylene glycol) (PEG) derivatives and, more recently, with zwitterionic polymers. It was reported that long-chain zwitterionic poly(sulfobetaine methacrylate) (pSBMA) surfaces reduced short-term adhesion of *S. epidermidis* and *P. aeruginosa* by 92 %and 96%, respectively, compared to glass [138]. It was reported that zwitterionic poly(carboxybetaine methacrylate) (pCBMA), grafted from glass surfaces coatings, reduced long-term biofilm formation of *P. aeruginosa* up to 240 h by 95% at 25 °C and for 64 h by 93% at 37 °C, and suppressed *P. putida* biofilm accumulation up to 192 h by 95% at 30 °C, with respect to the glass reference [139]. Also, dopamine-modified polymers,dopamine-terminated quaternary ammonium salt polymer (D-PQAs), and dopamine-terminated poly(sulfobetaine methacrylate) (D-PSBMA) on a silicon wafer were investigated with various lengths of *N*-alkyl showed resistance to *S. aureus,* which exhibited 10% to 91.9% bacteria killing efficiency on biofilm [140]. The zwitterionic polymers also possess promising medical applications as a surface anchoring group and can even be used as a dental antifouling coating.

8.3. Biomimetic surfaces.

Due to the diversity of biomimetic surface functionalities, natural surface inspiration is attractive for various engineering applications such as adhesion, self-cleaning antifouling, antibacterial phenomena, thermoregulation, wettability, and optics [141-149]. Furthermore, friction reduction and self-polishing surfaces are crucial for wild animals and plants, especially for reptiles and aquatic organisms, such as sharks, snakes, and lotus leaves to conserve energy and reduce drag force [150-154].



(a) **(b)**

Figure 6. Schematic illustration of (**a**) shark skin under an electron microscope; (**b**) pilot whale skin under an electron microscope. Adapted from the reference [145,148].

Aquatic mammals like whales are often coated with a biofouling layer, in contrast to sharkskin. In Figure 6, SEM images of the skin structure of a shark and a pilot whale are given for comparison. The shark skin is ornated with microstructured ribbons on dermal denticles, which minimize drag force and help avoid biofouling. A biomimetic shark skin prepared using the polydimethylsiloxane (PDMS)-embedded elastomeric stamping (PEES) method exhibited a drag reduction rate of 12.5% with better antifouling and anti-algae performance [155]. On the other hand, another study revealed that antifouling sharkskin patterns with antibacterial titanium dioxide (TiO2) nanoparticles (NPs) and photocatalytic shark-skin-patterned surfaces reduced attachment of Escherichia coli by ~70% compared with smooth films with the same chemical composition [156].

In a study inspired by biomimetic surfaces, glycocalyx-like hydrophilic methylcellulose (MeCe) polymer nanofilms grafted onto polydimethylsiloxane (PDMS; silicone) successfully reduced the initial adhesion of both *S. aureus* and P. aeruginosa biofilm-forming pathogens [157].

The lotus leaf surface is another source of inspiration for antifouling applications due to micro/nano-scale multiple structures on the surface of the lotus leaf and the surface wax crystal's low surface energy [158-161].

9. Conclusions and Outlook

In manufactured systems in which water is an integral component, biofilm formation on contact surfaces has proved to be a challenging problem. Numerous different toxic and non-toxic antifouling technologies have been developed to prevent the harmful effects of biofouling formation. Toxic antifouling technologies, mainly chemical mitigation methods, generally contain antifouling (AF) paints with biocides, including copper thiocyanate, Cu powder, Irgarol 1051, Zinc pyrithione, and Tributyltin (TBT). Although chemical methods are relatively successful compared to physical and biological methods, there is a need to develop environmentally friendly formulations due to the detrimental effects on the environment. Non-toxic antifouling technologies, on the other hand, include physical and biological methods, which generally remove the biofilm layer from the substratum surface with physical force or biological means. Although the latter methods are considered non-polluting, they are not as successful as the chemical antifouling methods, and therefore, there is a need to develop more efficient and environmentally friendly technologies for large-scale applications. The development of technology and increasing knowledge have finally brought antifouling into a bourgeoning new age. Today, there is a greater understanding of controlling contact surface characteristics such as low drag, low adhesion, wettability (super hydrophobicity or super hydrophilicity), and microtextured patterns, which minimize accumulation and attachment biofilm-forming organisms on surfaces and hinder biological fouling. Nature itself evolved the antifouling technologies over time. Advanced surface technologies can mimic the characteristics of animal skin or plant leaf surfaces on a nano/macro scale, such as sharkskin and lotus leaf, to mitigate fouling effectively and environmentally. However, a single biomimetic structure is not enough to combat a matrix of complex biological communities under uncontrolled conditions, and therefore, further improvements in surface engineering are required. The use of toxic chemical methods will be banned due to environmental and economic concerns. Therefore, successful large-scale, environmentally friendly, broad-spectrum, and sustainable surface topologies should be developed along with novel improvements in nano-engineering technology.

The existing chemical, physical, and biological antifouling methods have not successfully tackled biofouling problems effectively and sustainably. Therefore, new surface technologies in combination with current methods should be developed by considering ecological effects.

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Conflicts of interests

The authors declare that they have no known conflict of interest regarding this paper.

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