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Wetting/spreading on porous media and on deformable, soluble structured substrates as a model system for studying the effect of morphology on biofilms wetting and for assessing anti-biofilm methods

Dominika Zabiegaj[1], Farzaneh Hajirasouliha[1], Angela Duilio[2], Stefano Guido[3,4], Sergio Caserta[3,4], Margaritis Kostoglou[5], Maria Petala[6], Thodoris Karapantsios[5] and Anna Trybala[7]

Abstract

Biofilm is a layer of syntrophic microorganisms stick to each other and to the surface. The importance of biofilms is enormous in various industrial applications and human everyday life. The effects of biofilm could be either positive or negative. Positive effects are encountered in industrial processes, bioremediation, and wastewater treatment. Negative effects are more common with the marine industry being one of the sectors, which confronts severe corrosion problems caused by biofouling on the surfaces of equipment and infrastructures. In space industry, microbial contamination and biofouling adversely affect both crew health and mission-related equipment, the latter including hardware, water systems, piping, and electrical tools. The capacity of biofilms to grow in space environment was confirmed already in 1991. One of the most important surface properties of biofilms is wettability, which dictates not only how a liquid spreads over the uneven external surface of biofilms but also how it penetrates into their porous and morphologically complex structure. To investigate wetting and spreading onto biofilms, model materials are often used to simulate different morphological and functional features of biofilms in a controlled way, for example, soft, deformable, soluble, structured, porous materials. Here, we review recent advances in wetting and spreading on porous and soft deformable surface together with biofilms wetting properties and its importance in space industry. We conclude with a discussion of the main directions for future research efforts regarding biofilm wetting.

Addresses

[1] Smart Materials and Surfaces Laboratory, Department of Mechanical and Construction Engineering, Faculty of Engineering and Environment, Northumbria University, NE1 8ST, Newcastle Upon Tyne, United Kingdom

[2] Dipartimento di Scienze Chimiche, Università Federico II, Naples, 80126, Italy

[3] Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale (DICMAPI), Università di Napoli Federico II, P.le Tecchio, Napoli, 80125, Italy

[4] CEINGE Biotecnologie Avanzate, Azienda Ospedaliera Universitaria Federico II, Via Sergio Pansini, Naples, 80131, Italy

[5] Laboratory of Chemical and Environmental Technology, Department. of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece

[6] Laboratory of Environmental Engineering & Planning, Department. of Civil Engineering, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece

[7] Department of Chemical Engineering, Loughborough University, Loughborough, LE113TU, UK

Corresponding authors: Corresponding authors: Zabiegaj, Dominika (dominika.zabiegaj@northumbria.ac.uk); Trybala, Anna (A.Trybala@lboro.ac.uk)

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Introduction

Biofilms represent the most widely diffused and successful microbial way of life. The ability of bacteria to produce complex biofilm matrix, known as extracellular polymeric matrix (EPS), promotes colonization of biotic and abiotic surfaces, inducing stability in the growth environment and resistance against antibiotic and stress conditions [1].

Bacterial biofilms, in various aspects, can be beneficial for nature and humankind, as certain plants use a coat of

harmless biofilms, that is, these produced by *Bacillus subtilis* to protect themselves from pathogenic microorganisms [2,3]. *Microalgal/cyanobacterial* biofilms are used in industrial processes and bioremediation [4—6], wastewater treatment in photobioreactors [7—9], and nontoxic leaching of copper from ore that relies on bacterial biofilms [8,9]. Biofuels such as bioethanol can be produced through bioprocessing associated with biofilm as an energy-efficient option without secondary pollution. *C. thermocellum* biofilm and Polymicrobial biofilms of *Bacillus subtilis* and *Staphylococcus aureus* are examples of the strains used in this field [10]. Food industry can benefit from biofilms, as biofilms of a probiotic bacteria, *Lactobacillus plantarum*, grown on nanofiber membranes are used as a starter culture for producing fermented milk [11]. Although biofilms are certainly actuating many industries, there are frequent cases where their presence and development might result in severe damages. In most industrial and medical settings, bacterial biofilms have a negative impact on the function of processes and devices [12]. Bacterial biofilms are the cause of almost 80% of the recurrent and chronic microbial infections that happen in human body. They can also be a source of inflammation when they grow on the medical device surfaces such as implants. Microbial contamination and subsequent formation of biofilms on surgical implants frequently cause chronic infections that are difficult to eradicate. The risk of the infection depends on the type of the medical device, its invasiveness level, the site of insertion in human body, and the time during which it is applied to the anatomical site. When there is no external device, host immune defenses clear the tissue contaminations spontaneously. But when a foreign body, such as an implant is inserted into the target sites of human body, a local tissue response is triggered. This response alters the immune defense and creates a *locus minoris resistentiae*. This causes a vulnerability toward the bacterial attacks. Biofilms, being resistant to most of antimicrobial agents, spontaneous cure does never occur, and currently, the available treatment for biofilm-related infections consists in the administration of conventional antibiotics at high doses for a long-term period. *Staphylococcus aureus* is one of the major implant-infecting bacteria. This strain shows a high rate of antibiotic resistance, similar to *Staphylococcus epidermidis*, *Streptococcus* spp., and *Enterococcus* spp., which are also examples of bacterial strains causing orthopedic infections [13,14] and are responsible for diseases that are difficult to fight [15,16]. In the case of Staphylococcal biofilms, they can be eliminated by rifampin combination therapy and Gram-negative biofilms by fluoroquinolones, but the treatment duration is 3 (hip prosthesis) and 6 (knee prosthesis) months, very often leading to implant exchange [17,18].

Marine industry is one of the sectors that encounters severe corrosion caused by biofouling on the surfaces of equipment and infrastructures [19]. Colonization in marine biofouling can be performed by various organisms, such as bacteria, diatoms, spores of macroalgae, protozoa, and larvae of macrofoulers. More importantly, with the growth of international trade in recent decades and especially of transoceanic maritime transport, littoral states have been confronted with ecological problems of a new order related to the contribution of living organisms foreign to the local environment [20,21]. In aquatic and coastal environments, invasive species, such as the bacterium *Vibrio anquillarum*, have been recognized as one of the serious threats to global biodiversity and identified as one of the four greatest risks to the oceans with land-based sources of marine pollution, overexploitation of living marine resources, and alteration or physical destruction of marine habitats [22,23].

In space industry, microbial contamination and biofouling adversely affect both crew health and mission-related equipment, including hardware, water systems, piping, and electrical tools [24,25]. Onboard the International Space Station (ISS) biofilm formation [26] and consequently microbial contamination continue to pose mission risks to crew well-being as the opportunistic pathogens in water systems and crew cabin present a serious health threat [27—30]. On the other hand, the formation of biofilms on mechanical systems can seriously challenge the hardware reliability, as they can also cause biofouling and material degradation, which can lead to system failure during long-term missions [31—33]. Especially, with increasing spacecraft complexity, crew numbers, duration of missions, and multiple flights for each spacecraft, new challenges have arisen for long-term control of microbial contamination and biofilm development in systems reused mission-to-mission, particularly in the water storage/distribution systems [28]. The growth of biofilms was confirmed in water and waste line samples, already in June 1991, after the STS-40 mission. On the Space Shuttle Columbia, despite continuous addition of iodine, bacterial biofilms such as *Burkholderia cepacia*, *Bacillus* spp, and *Sphingomonas paucimobilis* were found during the standard servicing protocols. Moreover, onboard the ISS [34], analysis of water samples from potable water sources has been performed already before the arrival of the first permanent crew. The results showed that the predominant microbial isolates were Gram-negative bacteria such as *Cupriavidus metallidurans*, *Sphingomonas paucimobilis*, *Methylobacterium fujisawaense*, and *Wauteria basiensis*. This demonstrates the potential problems with the extended use of closed-loop systems and current control mechanisms.

Consequently, an increasing interest of several scientific communities is put to biofilms formation, growth, their microbial behavior, and, finally, the development of efficient methods to eradicate bespoke biomaterial [35,36]. Typical biofilm control strategies either aim at

preventing bacterial attachment and thus biofilm formation, chemically inactivating the bacteria within the biofilm [37—39] or removing the whole biomaterial from surfaces by mechanical forces [12]. However, these traditional biofilm mitigation approaches are limited because of bacterial persistence and biocide resistance. Genetic modification of bacteria could represent a further possible strategy for fighting biofilm development. The modification of genes involved in biofilm formation and their development may have positive effects on these processes. Gene products with a negative effect can also be considered an excellent target to inhibit events needed for biofilm formation. The negative function of yeast-form cell wall protein 1 in the adherence step might represent a positive function in biofilm dispersal and desegregation [40]. A different strategy to counteract biofilms development consists in the inhibition of genes that regulate key factors for biofilm production. In *Salmonella Typhimurium*, the activation of the Rcs phosphorylation pathway results in the inhibition of the expression of genes encoding surface adhesins, thus leading to the inhibition of biofilm formation [41].

As the biofilm covers the surface of a material, a new surface with new properties is created. The wetting properties of such a newly formed surface are important in both exploiting the advantages of biofilms and preventing any detrimental consequences of their unfavorable effects. Surface parameters and wettability of biofilms are gaining increasing attention especially now that among the emerging technologies for combating biofilms, new surface coatings show promise for preventing biofilm formation [42]. This approach aims to interrupt the critical initial step of biofilm formation (cell attachment) through surface modification.

The development of materials capable of preventing or inhibiting bacterial attachment on medical devices might represent an important alternative to the use of biocide substances. Several different approaches that involve physical and chemical surface modification have been proposed. The engineered surfaces can be coated with molecules capable of inhibiting bacterial adhesion or with active antimicrobial agents. Moreover, surface treatment with natural disruptive agents and modification of surface topographical parameters should also be considered to disrupt the biofilm matrix [43]. Furthermore, the essential oils from aromatic plants were screened for their ability to prevent biofilm formation and to disrupt preformed biofilms against clinical and *Methicillin-Resistant Staphylococcus aureus* (MRSA) strains [44]. Finally, very recently, hydrolytic enzymes secreted by bacterial cells such as dispersin B have been used to degrade the components of the biofilm polymeric matrix of *S. epidermidis*, *Burkholderia cenocepacia*, and *Achromobacter xylosoxidans*, leading to active dispersal of the biofilm with a reduction of the biomass [17].

Besides chemicals, also physical strategies have been addressed toward biofilm disruption; low cytotoxicity magnetic nanoparticles in combination with magnetic fields were shown to provide a deep penetration into the biofilm damaging the biofilm matrix and causing detachment [45]. Finally, modification of surface topographical parameters is able to reduce the attachment of microorganisms on materials for long time, providing a local and well-characterized distribution of topographical patterns [46].

Biofilm resistant coatings can eliminate or reduce the need for disinfectants, reduce the environmental marine pollution, and avoid the development of biocide resistant "superbugs," thus offering distinguishable advantages for biofilm prevention during long-duration missions. The microscopic organisms tend to move toward the material surfaces and form aggregations on these nutrient-rich surfaces because of the concentration gradient of nutrients. As this bacterial movement is stimulated by a directional exogenous factor, it is called taxis. The taxis caused by the nutrients is chemotaxis. The adsorption of chemical materials and the attachment of the microorganisms form a film onto the surface. In comparison with the substrate, this new thin layer has different surface characteristics, such as surface charge, hydrophilicity/hydrophobicity, surface tension, surface free energy, roughness, and wettability. This system is a nonideal surface containing pores and microgrooves and possessing deformable structure. It means that their interfacial characteristics such as wettability cannot be evaluated by equations and models used for ideal flat solid surfaces [47—49]. Therefore, wetting properties and/or spreading characteristics of biofilms along with their adsorption capabilities and adhesive parameters on porous media are noteworthy to be studied as a matter of priority.

To optimize the design of the future space exploration vehicle for long-term missions, new technologies, in which the superficial and wetting properties have to be considered, are needed to control the habitat microbial environment over multiple years.

Biofilm structure

Bacteria generally grow in one of two ways: planktonic, freely existing in bulk solution, and sessile, as a unit attached to a surface or within the confinement of a biofilm. A biofilm consists of a microbial community sheltered in matrix of extracellular polymeric substances (EPS) that include proteins, polysaccharides, and surface-associated microorganisms, such as bacteria, fungi, algae, protozoa, and extracellular DNA [50,51]. Together with EPS, pili, flagella, and other adhesive fibers secreted by the microorganisms act as a stabilizing scaffold for the three-dimensional (3D) biofilm structure. Flagella and pili are the structures on the outer

surfaces of bacteria. These organelles enable the bacteria to interact with their environment. There is a potential influence of bacterial motility in contaminated liquids and their accumulation on specific regions of the surface on biofilm formation and structure, even if these aspects are still not fully investigated. Nutrients, in the matrix, are trapped for metabolic utilization by the resident bacteria, and water is efficiently retained through H-bond interactions with hydrophilic polysaccharides [52,53]. Enzymes secreted by the bacteria modify EPS composition in response to changes in nutrient availability, thereby tailoring biofilm architecture to the specific environment [38]. Thus, the structural components of the matrix give rise to a highly hydrated, robust structure with high tensile strength that keeps bacteria in close proximity, enabling cell-to-cell interactions and DNA exchange, while protecting the biomass from environmental stresses, creating an inhomogeneous, porous thin layer, which represents a new surface with newfound properties.

Biofilm formation

Biofilm formation follows five stages: (1) reversible attachment of the bacteria to the substrate, followed by (2) irreversible attachment of cells to a solid substrate, the key step in biofilm formation, (3) first maturation through which microcolonies grow and become thick, (4) second maturation, in which microcolonies get the maximum size, and (5) detachment [54]. Colonization is the first action in this process to overcome repulsive forces between bacteria and the surface, allowing the initial contact and translocation. Mechanisms governing bacterial adhesion at the single-cell level are different, depend on cell type, surface physic and chemistry, and the liquid environment. It is not possible to draw a general description about how adhesion is achieved at the single-cell level; however, a wide discussion of the phenomena, including an analysis of the physical forces experienced by a cell before reaching the surface, has recently been discussed by Berne et al. [55]. Once single cells are attached to the surface, they start to multiply and form communities. Some other bacterial cells interact with surface, divide, and leave. A multigeneration memory of this mechanism allows future

Table 1

Bacterial motility mechanisms [61].

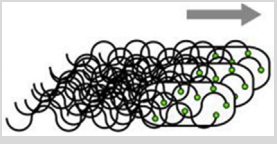
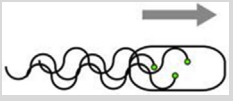
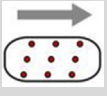
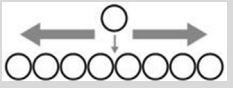
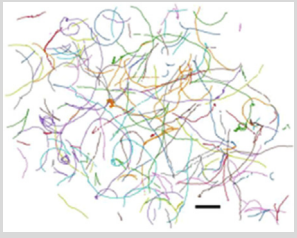
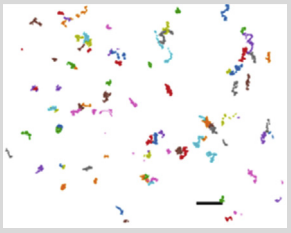
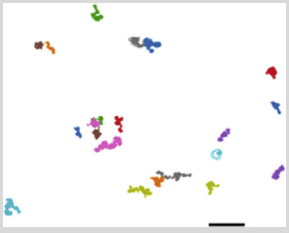
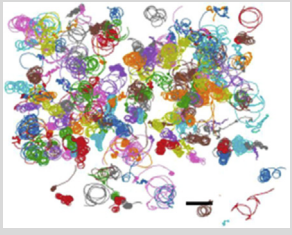
Swarming motility (flagella)	Defined as a rapid multicellular bacterial surface movement powered by rotating flagella	
Swimming motility (flagella)	Movement powered by rotating flagella but takes place as individual cells moving in liquid environments	
Twitching motility (pilius retraction)	Surface motility powered by the cyclic extension and retraction of type IV pili that confers slow cell movement often with a jerky or "twitchy" appearance	
Gliding motility (focal adhesion complexes)	A catch-all definition for active surface movement that occurs along the long axis of the cell without the aid of either flagella or pili	
Sliding motility (spreading by growth)	Passive form of surface spreading that does not require an active motor but instead relies on surfactants to reduce surface tension enabling the colony to spread away from the origin driven by the outward pressure of cell growth	

Table 2

Description of different bacteria trajectories at the oil–water interface (from Figure 2 in Ref. [62]).

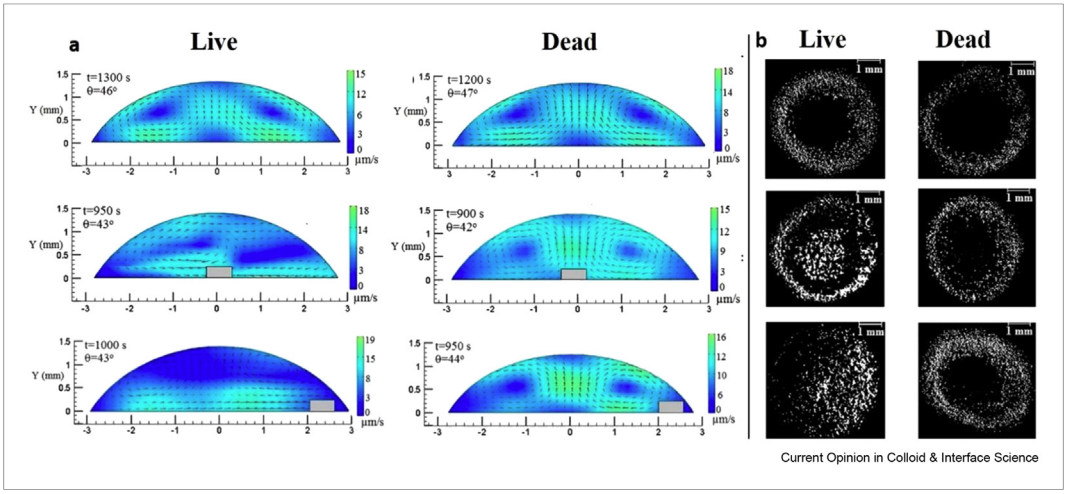
Motility type	Population frequency	Description	Trajectories
Interfacial visitors	10–20%	Are not adhered but swim toward and away from the interface, changing their heights by several micrometers.	
Brownian diffusive bacteria	30%	Are similar to inert passive colloid trapped at the interface. The bacteria are probably in a sessile, inert state or are trapped in a configuration that denies the molecular motor access to ions that fuel its rotation, for example, by immersion of the flagella in the oil phase.	
Pirouettes	(rare, ~5%)	Rotate quickly in nearly fixed positions.	
Curly paths	~40%	Swim in curly paths more than any other mode of motion; the trajectories are quite stable except in the event that they collide with other bacteria and become trapped in a cluster.	

Scale bars are 20 μm.

generations to return to the surfaces and progressively better adapting to surface sensing and attachment [56]. To protect and strengthen colony adhesion to the surface, an extracellular matrix is formed.

A multitude of proteins plays essential roles at different stages of this process. Some proteins contribute to biofilm accumulation, whereas others are involved in the mediation of primary attachment to surfaces or the matrix development. Each stage of the biofilm formation process depends on the microbial genera, species, characteristics of the attachment surface, environmental conditions, external stress, and physiological status of the microorganism [57]. Bacteria involved in the biofilm

Figure 1



Fluid flow measured inside an evaporating droplet using the Particle Image Velocimetry (**PIV**) technique [76] shows that the presence of a chemo-attractant can influence the spatial distribution of bacteria: (**a**) velocity vectors are superimposed over velocity contour, during droplet evaporation comparing the case of live (swimming) and dead bacteria; (**b**) gray images compare the deposition pattern of live and dead cells after complete drying of the droplet, top image are in the absence of sugar, in the case of middle images, sugar was deposited at the center of the droplet (gray rectangle), in the bottom line, sugar is on the right side of the droplet.

matrix are more tolerant to antibiotics than planktonic cells. This antibiotic resistance can be related to the increased transmission of resistance markers, efflux pumps, physical protection, and acquired resistance. Biofilms have also dynamic structural properties, and rapid alterations in their gene expression lead to modification of their surface antigens [58].

Bacterial motility

Biofilms are usually investigated in static conditions that, however, are very far from reality, as in the vast majority of cases, biofilms form under fluid flow, with the flow playing a significant role in the production, composition, and architecture of the biofilm [34,59]. The fluid flow drives bacteria motility, favoring surface colonization.

Bacterial mobility is enabled by two different types of structures, flagella, fimbriae, and pili. Flagella are lash-like appendages that protrude from the cell body and are made of three basic parts: a filament, a hook, and a basal body; cells can have one or more flagella. Fimbriae and pili are thin protein tubes originating from the cytoplasmic membrane that is rapidly polymerized and depolymerized, assembling protein subunits called pilin [60]. Both are able to stick bacteria to surfaces, but pili are typically longer and fewer in number than fimbriae. They are found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. At the end of the tube is the adhesive tip structure based on glycoprotein or glycolipid receptors. These structures are necessary for the movement toward surfaces, allowing microcolonies formation and initial bacterial adhesion [33].

Different motility mechanisms can be identified [61]; a brief summary is reported in Table 1.

When there is a cell transition from swimming to swarming, the number of flagella on the cell surface increases. Organisms with alternative flagellar systems become hyperflagellated in the transition from the single polar to multiple peritrichous flagella. Chemotaxis and surface sensing can influence directionality and motility mechanisms.

Analysis of trajectories of *Pseudomonas aeruginosa* PA01 (monotrichous bacteria, propelled by a single flagellum located at the pole at one end of the cell body) in an oil/water emulsion [62] evidenced four distinct characteristic motions, summarized in Table 2:

By using advanced microscopy techniques, such as dual-view light-sheet microscopy, it is possible to monitor spatial trajectories of individual cells and the collective motion that lead the biofilm expansion. Trajectories of early born cells (0–7 h) are more trapped at the substrate with respect to cells born later (12–15 h) [63].

In the initial phase (0–5 h), the biofilm grew predominantly in the lateral plane, and cells showed a Brownian and random walk. As the biofilm develops (5–10 h),

individual cells shown persistent and straight trajectories, which dominate the bulk of the biofilm at the later stage (10−15 h). Biofilm expansion is driven by cell division, extracellular matrix secretion, and osmotic swelling. The Brownian-to-ballistic transition of cell motion coincided with the transition from predominantly lateral biofilm expansion to accelerated vertical expansion, a transition from two dimension (2D) to 3D.

Spreading of the bacteria-laden droplets on solid substrates

Secchi et al. developed a mathematical model of bacteria swimming in flow using microfluidic strategy and *Pseudomonas aeruginosa* and *Escherichia coli* as models and provided a new tool to predict the location and magnitude of bacterial attachment to surfaces [64]. Hydrophobic coating [65,66] can prevent biofilm formation on different surfaces, affecting wettability and surface properties. Other studies investigated the possibility of inhibiting contamination of medical implants by treating titanium surfaces with radiofrequency cold plasma [67].

Inhibition of bacterial mobility and/or swimming decreases biofilm formation in many pathogenic strains. Inactivation of the PA5001 gene in *P. aeruginosa* generated a nonmobile strain, resulting in the alteration and disruption of biofilm matrix [68]. A similar effect was observed in *P. aeruginosa* after treatment with plant-derived phenolic compounds; the swarming motility and, consequently, biofilm production were reduced by about 50% [69]. In *Enterococcus faecalis*, confocal laser scanning microscopy (CLSM) and Scanning Electron Microscope (SEM) analysis demonstrated that treatment with phenyllactic acid (PLA) affects cell motility and reduces EPS production inhibiting bacterial adherence and biofilm formation [70]. Several antimicrobial peptides were demonstrated to affect biofilm formation at different stages and through different mechanisms of action. Human cathelicidin LL-37 peptide inhibits *P. aeruginosa* biofilm formation by downregulating genes related to the quorum sensing (QS) system, decreasing the ability of bacterial cells to attach on surfaces and stimulating twitching motility mediated by type IV pili. The CRAMP antimicrobial peptide is able to inhibit fungal biofilm formation, and a CRAMP short fragment, the AS10 peptide, was shown to inhibit biofilm growth of *P. aeruginosa*, *E. coli*, and *Candida albicans* [71]. A novel synthetic cationic peptide, defined as 1037, is able to affect biofilm formation by downregulating several genes related to flagella decreasing swimming motility in PA14, PAO1, and *Burkholderia cenocepacia* and suppressing the expression of a variety of genes involved in biofilm formation [72]. AMPs can also cause disruption of the biofilm matrix. The hepcidin 20 peptide can reduce the mass of the extracellular matrix altering the *S. epidermidis* biofilm architecture by targeting polysaccharide intercellular adhesin (PIA) [54].

Bacteria motility can induce the formation of aggregates and affect interfacial properties in the case of multiphase systems (droplets). Motile bacteria can aggregate in polymer-rich environment via polymer-induced depletion forces. In the presence of nonadsorbing polymers such as polyethylene glycol (PEG), bacteria aggregate through depletion interactions, which occur when two bacteria approach each other and reach a depletion zone where the polymer is excluded from the space between them: this force is expressed as an osmotic pressure difference generated from the variation in polymer concentration between the depletion zone and the bulk solution. For nonmotile bacteria, the only driving forces for aggregation are polymer-induced depletion forces. For motile bacteria, motility forces and depletion forces competition determine a steady-state aggregation behavior at sufficient polymer concentrations and long-time scales. Porter et al. [73], by measuring size distribution of bacterial aggregates using confocal microscopy, showed that motility influences the polymer-induced depletion aggregation of bacteria at short time scales (10 minutes). In dilute polymer concentrations, aggregation of nonmotile bacteria is observed but no aggregation of motile bacteria because the depletion forces are simply not strong enough to compete with the swimming forces. In the semidilute regime, in a viscous environment, when a critical PEG concentration threshold is reached, the aggregation starts also for motile bacteria.

Bacterial motility can heavily affect interfacial properties also in the case when bacteria are present in a droplet of liquid wetting a surface.

A water drop can slide on a tilted plane of agar gel when the driving force (gravitational) overcomes the capillary pinning force, that is, when the value of the Bond number (Eq. (1)) reaches a critical value:

$$Bo = \frac{\rho V\, gsin(\alpha)}{V^{1/3}\gamma} \tag{1}$$

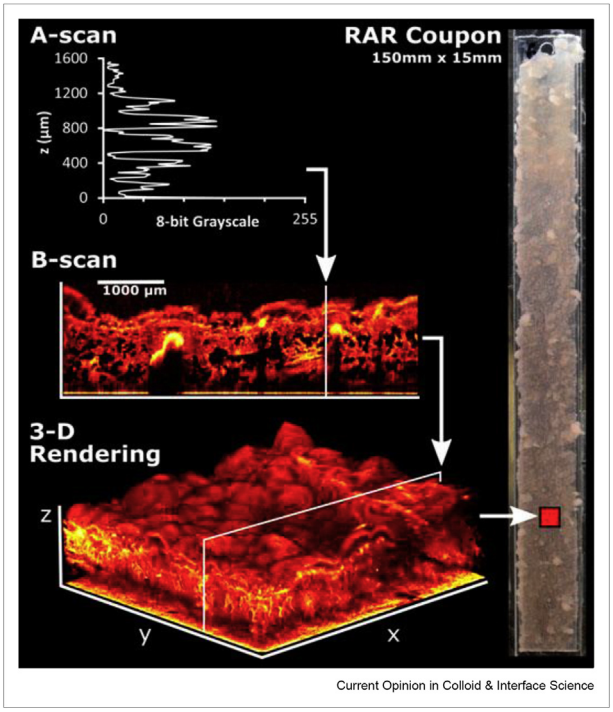
where ρV and $V^{1/3}$ are the drop mass and typical width, $gsin(\alpha)$ is the effective gravity, and γ is the surface tension.

Bacteria can unpin such droplets, leading in practice to the collective "surfing" of the entire colony. Hennes et al. [74] observed the sliding of bacteria-laden droplets with an initial Bond number of Bo = $3 \cdot 10^{-3}$, whereas water drops only start sliding for Bond numbers larger than Bo = 0.25.

Bacteria influenced the Bond number of the drop in the following ways:

1. Pump water from the environment can increase drop volume

Figure 2



Two- and three-dimensional images of the biofilm of ammonia-oxidizing bacteria (AOB) on polycarbonate coupons in the rotating annular reactor (RAR) using optical coherence tomography (OCT). To produce the three-dimensional rendering image of the morphology, multiple adjacent A-scans as the vertical one-dimensional profiles with grayscale intensity are collected and assembled to generate B-scan as the two-dimensional images. Then B-scans are used to render three-dimensional images [79].

2. Surfactant secretion can lower the surface tension (*B. subtilis secretes surfactin*), strongly enhancing the wettability of the agar gel.

In the case of E. Coli moving a sub-millimetric emulsion drop [75], each motile bacteria can induce force of magnitude f (Eq. (2)),

$$f \sim \eta l v_0 \tag{2}$$

where v_0 and l are the characteristic speed and size of the bacterium and η is the viscosity of the surrounding fluid. The energy required to create a "bump" of size comparable to the bacterial body in the drop surface ($\sim \gamma l^2$) is lower than the interfacial tension [73], whereas the energy that a bacterium spends by swimming the same distance is Eq. (3)

$$fl \sim \eta l^2 v_0 \tag{3}$$

The ratio between these two energies is of the order of the capillary number (Eq. (4)),

$$Ca = \frac{\eta v_0}{\gamma} \tag{4}$$

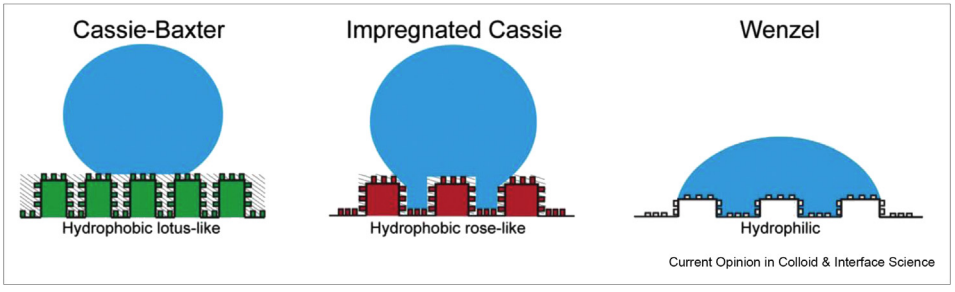
For a typical water–oil interface in the presence of surfactants, $\gamma \sim 1$ mN/m and $Ca \sim 10^{-5}$.

As a result, bacteria swimming near a typical water–oil interface feel a rigid boundary and thus behave like swimming near a solid wall rather than a free surface; they interact hydrodynamically and accumulate at the interface. This accumulation near the drop interface can enhance the interaction of the bacterial flows in the drop (Figure 1a) and the fluid surrounding the drop. It is shown that the drop movement and its direction is determined by the bacteria that move near the substrate, causing the drop to roll over the substrate. The turbulent-like motion of the bacterial bath constantly changes the direction and speed of the bacteria that swim near the bottom of the drop. This explains both the persistent movement of the droplets at short times and their random motion at long times.

Coffee ring effect

Swimming cells in a drop do not distribute randomly. Particles in an evaporating droplet accumulate at the interface and typically leave a ring-like pattern on the

Figure 3



Wetting behavior of the rough surfaces of different biofilms in contact with a water droplet [12].

underlying substrate after complete evaporation, a phenomenon commonly known as "coffee ring effect." When bacteria produce surfactants, the pattern of coffee ring deposition does not appear [76]. The presence of gradients of nutrients (such as sugar) induces the bacteria to move toward the nutrient site with resulting convective flows (Figure 1b). This may be attributed to the fact that bacterial chemotaxis near the base dominates over the internal fluid flow, while away from the sugar crystal, chemotaxis is relatively weaker. Chemotaxis can hence influence live bacteria deposition and motion in a drop.

This nonrandom distribution of bacterial and their accumulation in specific areas of the surface can be expected to influence surface contamination and biofilm formation, for example, by inducing surface tension gradients. A clear study about direct connection between bacterial motility inside a contaminated droplet and spreading of biofilm is not yet available to our knowledge, but we believe that investigation of wetting of bacteria-laden droplets on clear surfaces could represent a promising approach to study surface contamination by droplets.

Inclusion of bacteria in drops can be controlled using microfluidic concepts to create monodisperse double and triple emulsion drops that serve as 3D microenvironments for the containment and growth of bacterial biofilms. *B. subtilis* [77] was encapsulated in an aqueous suspension of planktonic bacterial cells to create w/o/w double emulsion drops with an outer diameter of $\approx 164 \pm 4$ μm. Within 24 hours, these planktonic cells multiply and differentiate into matrix-forming cells at the inner interface of these microscopic drops, forming 3D spherical biofilms on the inside of the oil shells. The inner water—oil (w/o) interface was stabilized with a silicone surfactant, which is a known film former, and provides a substrate on which the biofilm readily adheres.

An overall decrease in drop size is observed as the biofilm grows. The calculated inner water volume decreases by 45% over the first 12 hours and then remains constant. This corresponds to the peak in matrix production. Thus, this decrease in volume can be attributed to nutrient depletion, which creates an osmotically driven water flux from the inner aqueous phase to the outer continuous phase.

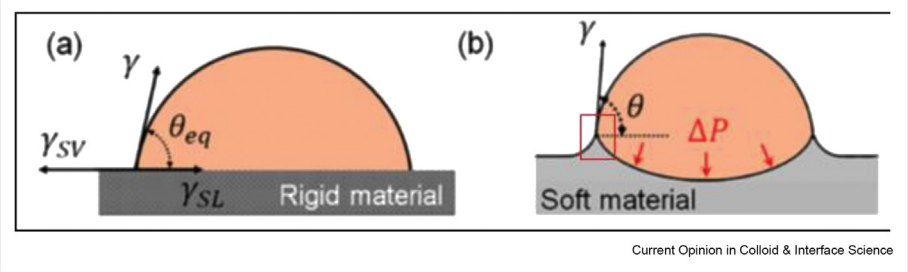
Wetting/spreading on porous media

Biofilm topography

As already mentioned, surfaces of materials in different environments will inevitably be coated by carbon compounds as nutrients. At first, proteins are adsorbed onto the surfaces, and this is followed by carbohydrate adsorption. A formed layer of nutrients is called conditioning film. As the microscopic organisms tend to move toward the material surfaces, they form aggregations on these nutrient-rich surfaces because of the concentration gradient of nutrients. Thus, the adsorption of chemical materials and the attachment of the microorganisms change the characteristics of the surface, such as surface charge, hydrophilicity/hydrophobicity, surface tension, surface free energy, roughness, and wettability [47].

Biofilm can be considered as a porous, thin layer in which the fraction of void space is a characteristic parameter for modeling the structure of the biofilm. Imaging techniques such as confocal laser scanning microscopy (CLSM, magnetic resonance microscopy, multiphoton-excitation laser scanning microscopy (MPLSM), and near-infrared optical coherence tomography (OCT) have been applied to indicate and analyze the morphological parameters, including porosity, pore size distributions, and roughness [78]. Figure 2 shows the 2D and 3D views of the biofilm morphology using the OCT system [78,79]. The pore radius in the structure of the biofilms is of micron (μm) order. On the basis of the experimental observations about this structure,

Figure 4



A liquid droplet deposited on the (**a**) rigid and (**b**) soft material. The red square in (b) is the wetting ridge [86,88].

pore-scale models are used for biofilm formation. These models consider the biofilm as a porous medium [80].

Besides the structure of the biofilm, its mechanical properties influenced by the morphology are also of a great importance for predicting the behavior of biofilms, their control, and even removal [81]. To ascertain these mechanical characteristics, both experimental measurements and modeling methods are used together. OCT technique has demonstrated the 2D deformation of the biofilms. This imaging method, together with poroelastic fluid–structure interaction numerical computations, results in developing a method for determining the elastic properties of the biofilm as a deformable structure [82]. Because of the porosity and elasticity of the structure, it is quite accurate to consider the biofilm as a porous medium and/or deformable substrate when it is in contact with other materials. This hypothesis about the biofilms is used to investigate their wetting properties as a significant interfacial characteristic when it comes to either the comprehensive range of applications or the necessity of removal of the biofilms.

Wetting of biofilms as porous substrates

Wetting is an indicator of the behavior of a unique liquid on the surface. For biofilms, this indicator depends on surface topography. The concept of wetting can be defined by the contact angle (CA), which quantifies the wettability. Therefore, measuring the CA on the porous and rough surfaces of the biofilms determines its wettability. As it was mentioned previously, the size of the pores radius in the biofilms is of micrometer order. This means that biofilms have micropatterned surfaces on which two states of wetting can be distinguished: (1) the Wenzel state and (2) the Cassie-Baxter state [83]. In the Wenzel state, the liquid fully wets the porous structure. On the basis of this assumption, the apparent CA, θ_{ap}, is calculated by Wenzel equation as follows:

$$\cos\theta_{ap} = r\cos\theta_{eq} \tag{5}$$

where r and θ_{eq} are the roughness ratio and Young's angle, respectively [84]. The equation for Young's angle is as follows:

$$\gamma\cos\theta_{eq} = \gamma_{SV} - \gamma_{SL} \tag{6}$$

where γ, γ_{SV}, and γ_{SL} are the representative of liquid–vapor, solid–vapor, and solid–liquid interfacial tensions, respectively. In the Cassie-Baxter state, the wetting is partial so that the liquid droplet sits on the top of the protrusions of the rough surface. The proposed equation by Cassie-Baxter is as follows:

$$\cos\theta_{ap} = f'\cos\theta_{eq} + f' - 1 \tag{7}$$

where f' is the area fraction of the liquid–vapor interface blocked by the rough structure [85].

Topographical characterizations can be conducted by a profilometer. Using light profilometry images obtained by this system, the developed interfacial area ratio is calculated. In a relevant study, the behavior of water droplet on different biofilm surfaces was investigated [12]. Three distinct states were demonstrated: hydrophilic, hydrophobic rose-like, and hydrophobic lotus-like biofilms. On rose petal-like surfaces, the water can penetrate into the microscopic pores of the underlying surface, which results in notable contact angle hysteresis. In this case, called impregnated Cassie state, the droplets remain attached when the surface is tilted. The impregnated Cassie state is a state between Wenzel and Cassie-Baxter states. In the case of lotus-like biofilms, when the surface is turned upside down or tilted, the droplet rolls off. Lotus-like behavior is the representative of the Cassie-Baxter state. Figure 3 shows the wetting behavior of different biofilms exposed to the water droplet [12].

The importance of the wetting concept of biofilms can be divided into three areas: (1) to control the behavior of biofilm during its interactions with other materials such as a reactant liquid that flows in the reactor during its operation, (2) to predict the interactions between the biofilm surface and chemical agents used for its removal, and (3) to modify the different surfaces to impart anti-biofilm characteristics. The last area, which is related to the wetting phenomena for biofilms, is different from the first two areas. In this case, wetting properties of a surface is manipulated by physical and/or chemical methods so that the final surface exhibits anti-biofilm or antibiofouling features. To clarify, changing a surface from a hydrophilic character to a hydrophobic one shifts the adhesion of microorganisms onto this surface.

Wetting of soft/deformable substrates

Similar to porous medium, soft/deformable substrates can be proposed as the second model for investigation of wetting properties of biofilms. Wetting on soft substrates is not captured by the laws dominating rigid wetting phenomena.

The structure of a soft biofilm is deformed by the deposition of a droplet on it. This happens because of the surface tension and Laplace pressure, ΔP, of the droplet. According to Young's equation, Eq. (6), there is an in-plain balance between the three interfacial tensions at the three-phase contact line (Figure 4a). The vertical component of liquid–vapor surface tension, $\gamma\sin\theta_{eq}$, remains unbalanced. So, a vertical net force is exerted to the solid surface at the three-phase contact line. In addition, Laplace pressure is applied to the liquid–solid interface (Figure 4b). This pressure is

inversely proportional to the curvature of the droplet. Consequently, a wetting ridge, δ, with a length scale of the order of elastocapillary length, L_{ec}, is formed at the three-phase contact line [86]. This ridge considerably changes the macroscopic spreading dynamics [87].

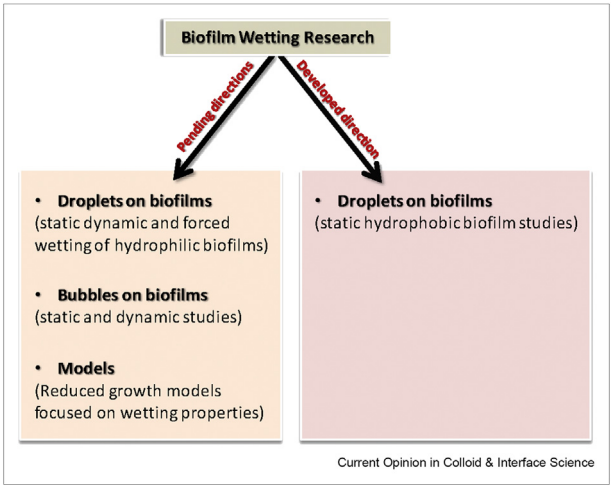
The elastic/shear modulus, G, for biofilms has been predicted to be between 0.7 and 7 kPa [82] which, according to Eq. (8), leads to a wetting ridge, δ, of submillimeter scale. Therefore, it can affect the wettability [86,89].

$$\delta \sim L_{ec} = \frac{\gamma}{G} \tag{8}$$

Both static and dynamic wetting properties of biofilms are affected by their deformation when they are in contact with the liquid droplets. In static wetting, deformations rebalance the interfacial tensions and modify the CA and CA hysteresis. In the case of dynamic wetting, the wetting ridge moves with the contact line. This movement results in additional energy dissipation and influences dynamic wetting [86].

In addition to the force balance near the three-phase contact line, there are other characteristics and features that must be noted in this case. The dynamic solid surface tension of the microstructure of the underlying polymer, which is a combination of EPS and microorganisms in case of biofilms, boundary conditions, moving contact lines, the mechanisms of dissipation inside the substrate, and the consequent macroscopic movement of the droplets are the other factors that must be revisited [87].

Figure 5



A schematic on the current and future research topics regarding biofilm wetting.

Wetting of biofilm-covered surfaces

The wetting of biofilm-covered surfaces is a complex phenomenon. Considering a droplet in the size scale of millimeters, it is of practical interest in many applications. Most of the droplet surface shape is still described by the classical Young–Laplace equation. However, in the region of the biofilm, for example, size order up to 100 μm, a new phenomenon appears. The first one refers to the partial adsorption of liquid to the biofilm. Its extent depends on the properties of the biofilm and of the liquid. The second phenomenon is the modification of the triple line location and of the CA distribution along this line. The former effect is similar to what is met in wetting of porous media, in particular, of a thin and loose porous media, discussed in Wetting/spreading on porous media section. The latter effect is related to the wetting of structurally and chemically heterogeneous surfaces about which there is very extensive literature [90]. It is clear that both the structure (the term "topology" is also used) and the composition of the biofilm affect its wetting behavior. A complete 3D experimental knowledge of these quantities is out of the question by today means so by necessity modeling must be invoked to expand our understanding of biofilm formation and structure/composition. A discussion of the available modeling approaches of biofilm structure and composition follows because the biofilm modeling will be in the future an indispensable tool to understand its wetting and to correlate wetting properties to biofilm formation conditions.

Biofilm formation is a "nucleation"—"growth" process that explains the highly nonuniform structure arising. The "nucleation" step is actually the microbial deposition and attachment stage. The physical-chemistry of this step has been recently reviewed in detail by Carniello [91]. Some key approaches to modeling of the biofilm growth is described here. A basic classification separates morphological (i.e. two or three spatial dimensions) from nonmorphological (i.e. zero or one spatial dimension) models. The landmark work on biofilm growth was reported by Kapellos et al. [92], which combines complete solution of flow field and nutrient concentration equations in the biofilm, considering it as a complex porous medium. As already mentioned, the biofilm composition is described as a combination of cells (at different states) and extracellular polymeric substance (EPS. Additional phenomena such as chemical mechanical stresses and quorum sensing are also taken into account. The biofilm shape evolution is determined by a cellular automata-like procedure. The detachment of biofilm pieces is also considered in the model. "Nucleation" is introduced by following trajectories of planktonic microbes. The transport properties in the biofilm are related to its local composition through an effective medium approach. The model is numerically solved by an in-house code. However, the

required computational effort is too high for any practical use of it. A 2D case simulation needed 5 days of computer time.

The computational effort is attempted to be reduced by ignoring stress effect and biofilm composition, introducing the concept of an effective viscosity to simulate the flow in the biofilm and implementing the code in a combination of MATLAB, COMSOL Multiphysics, and Java environments [93]. A simpler in-house cellular automata algorithm is implemented. The position of "nucleation" is randomly selected among the surfaces with local shear stress lower than a prescribed value. The previously mentioned modification made possible the simulation of 3D biofilm growth for several cases (simple in practical context because a single nutrient and a single microbe are considered) with the highly localized character of colonies to be evident. The most sophisticated biofilm model today is the one presented in Ref. [94] that considers multiphasic hydrodynamic theory and takes into account interactions among various bacterial phenotypes, extracellular polymeric substance, quorum sensing (QS molecules, solvent, and antibiotics. In the model, bacteria are classified into downregulated QS, upregulated QS, and non-QS cells based on their QS ability. The evolution of biofilm is determined by combining Cahn–Hilliard type equations for each substance. The model is capable of giving 3D results for the biofilm structure.

Another category of models sacrifices dimensionality to increase the sophistication of film composition description. In this case, the model is one dimensional (1D), so it is completely continuum (no need for cellular automata). In addition, no flow in the biofilm has to be resolved. Such a model in Ref. [95] covers the possibility for simultaneous existence of several microbial types and several nutrients. It is specifically focused on the release of planktonic bacteria from biofilm to the bulk liquid. This process is different from the detachment because these bacteria are produced throughout the biofilm volume because of the phenotypic change of the attached bacteria.

Finally, the last category refers to very abstractive 1D models, which are based on the diffusion–reaction equation of a single nutrient [96]. The difference from the previous case is that a series of simplifications (i.e. linearization of the reaction rate) brings the problem to a standard form in reaction–diffusion physics. A roughness elimination force is introduced through the notion of an artificial "surface tension" of the biofilm. A stability analysis of the model (assuming a deformed second dimension) is performed, leading to phase diagrams for stable (flat) and unstable (rough) film growth. Obviously, this type of modeling is only of academic and not of practical merit.

From the above, it can be inferred that the existing models are too simplified to use relevant information or too complex to be constructively used in the context of the wetting properties of biofilms. There is a need for reduced order models that have as state variable a finite set of descriptors determining the wetting behavior. In the case of a wetted biofilm, the simplest set could be its average thickness, its EPS content, and an integral roughness descriptor.

It appears that most research on wetting of biofilms focuses on the particular case of *Bacillus Subtilis* (BS), a *Gram-positive soil bacterium*, biofilms. These particular biofilms attract interest because they are nonwettable, not only with respect to water but for all liquids, including antimicrobial agents. Such omniphobic behavior creates the need for fundamental analysis to explain its origin on the one hand and for practical methods to overcome microbial resistance to biocides on the other. The landmark work on the subject is reported by Epstein [97]. In that study, the CA created between droplets of several liquids and biofilms is measured through a simple goniometer. The main comparison is performed with respect to a Teflon surface. Although both biofilm and Teflon are nonwettable by pure water (with the contact angle to be higher for the biofilm), the contact angle on Teflon decreases linearly with the percentage of ethanol concentration in the liquid, but the contact angle on biofilm remains constant up to 60% ethanol. Then it starts to decrease gradually, and at 100% ethanol, it reaches the contact angle of Teflon (highly wettable). The relevant figure has appeared extensively in the literature [98]. It is also shown that a similar behavior holds for isopropanol, methanol, and acetone. Parameters such as biofilm age, time of liquid exposure, and repeated liquid contact appear to have no effect on biofilm-liquid repellency. Experiments using several mutants of *Bacilus Subtilis* (to assess chemical contributions) and epoxy resin replicas (to assess structural contributions) lead to the conclusion that the biofilm nonwetting properties arise from both the polysaccharide and protein components of the extracellular matrix and are a synergistic result of surface chemistry, multiscale surface roughness, and re-entrant topography. Additional biological analysis focused on the further explanation of the chemical contribution to liquid repellency in Ref. [99]. The conclusion is that it is conferred by a small concealed protein called *BslA*, which self-assembles into an organized lattice at an interface. In the biofilm, the production of *BslA* is tightly regulated, and the resultant protein is secreted into the extracellular environment where it forms a very

effective communal barrier allowing the resident *Bacillus Subtilis* cells to shelter under the protection of a protein raincoat.

Conclusions

A microbial community sheltered in a matrix of extracellular polymeric substances called EPS, including polysaccharides, proteins, and extracellular DNA, create a layer of biofilm. Together with pili, flagella, and other adhesive fibers, EPS acts as a stabilizing scaffold for the 3D biofilm structure, which can be considered as a porous thin layer and which, because of nucleation process, yields a highly nonuniform structure. Creating a new surface with newfound properties is important in both exploiting the advantages of biofilms in various applications and preventing any detrimental consequences of their unfavorable effects. Their impact can be widely observed in the extended use of closed-loop systems and control mechanisms, affecting humankind safety or even life, especially in conditions where preventing any of detrimental consequences of their unfavorable effects is extremely difficult, that is, in microgravity conditions such as in the International Space Station or in Space Shuttles. There, the microbial contamination and biofouling events adversely pose mission risk, presenting a serious health threat to crew but also challenging the reliability of the mission-related equipment.

This is why one of the most important aspects of biofilms research, which should not be overlooked, regarding biofilms prevention or/and control strategies, are their surface properties and wettability. This is especially true now that among the emerging technologies for combating biofilms, new surface coatings show promise for preventing biofilm formation.

The study of wetting properties and surface interaction of droplets/bubbles can represent a useful and innovative tool to investigate the phenomena of surface contamination, including the prevention of biofilm formation and optimization of its removal. Two different aspects should be considered, both deserving further investigation in our opinion. On one side, the study of the interaction of bacteria-laden droplets on clean surfaces can be used to understand the basic mechanisms of bacterial contamination and biofilm spreading, including the possibility to prevent its formation by inhibiting cell attachment. A different, but not less interesting, approach concerns the interaction of droplets and bubbles on biofilm-covered surfaces. The investigation of this type of wetting can be applied in the study of biofilm structure, the prevention of its further growth, and its removal from already contaminated surfaces. A possible application would be the optimization of cleaning solutions and detergent formulations. We should mention a particular case would be that of the interaction of surface precontaminated by a biofilm with a droplet contaminated with a different cell line (eventually more dangerous with respect to the original host). Biofilm-coated surfaces can represent a favorable environment for further contamination; for this reason, biofilm removal is always recommended, even in the case of nondangerous contaminations.

Although the majority of actual biofilms are hydrophilic—because of the hydrophilicity of EPS—there is not a single study on their wetting properties. The argument behind it is that hydrophilic biofilms can be easily removed, so no concern exists on their wettability. Although, bacteria motility, biofilm superficial properties, and their mechanical properties influenced by their morphology are of a great importance in predicting the biofilms behavior and removal. The comprehension of their wetting behavior may serve as a tool to better understand their structure. This can be done not only by using static wetting properties, as in the case of hydrophobic biofilms, but also by testing their dynamic behavior in the spirit proposed in Ref. [100] for a patterned surface. Another important issue regarding wetting of biofilms is that the interaction of biofilms with bubbles has also not been studied. This may have practical interest because it has been proposed that introducing bubbles in cleaning water enhances its biofilm removal properties [101]. A wider investigation of this marginally studied aspect is needed. Figure 5 summarizes the main directions for future research efforts regarding biofilm wetting according to the present authors' point of view.

Moreover, knowing that biofilms are formed by reversible and irreversible attachment of cells to a solid substrate, followed by microcolony formation, maturation, and detachment, motility of biofilms should be subjected to studies in the flow condition, as it can induce the formation of aggregates and affect interfacial properties. Bacterial motility can heavily affect interfacial properties also in the case when bacteria are present in a droplet of liquid wetting a surface. In addition, considering that biofilms are inhomogeneous porous films, the porous medium, soft/deformable substrates could be used as models in the investigation of wetting properties of the biofilms.

Finally, having substantially understood the chemical effect on wetting resistance of biofilms, the next step is to further examine their structural effect [102]. In this respect, extensive BS biofilm structural characterization is conducted, using SEM images and light profilometry, and an attempt is made to correlate the resulting parameters to the wetting behavior. Depending on the nutrient type and location on the colony, three different wetting regimes are identified. The two are of nonwetting nature, and through correlation to the structural biofilm characterization, it is argued that the

one is of lotus-leaf (Cassie-Baxter state) type and the other of rose petal (impregnated Cassie state) type. Very interestingly, the realized state is affected by the nutrient availability. The next reasonable research step is to find ways to overcome the wetting resistance of certain biofilms [12]. Extensive experiments and measurement of topological structural parameters and contact angle for biofilm created by five types of bacteria are performed in Ref. [12]. The correlation between surface roughness, in terms of developed interfacial area ratio index, of biofilm, and its contact angle is clearly presented.

The previously mentioned observation motivated the following hypothesis: if the roughness features of a highly complex biofilm surface could be smoothened, such a biofilm surface should lose its strongly hydrophobic character. In this respect, it is found that a short treatment with ethanol solutions renders omniphobic biofilms omniphilic. It is also shown that the same effect can also be obtained by using less aggressive chemicals such as concentrated salt and sugar solutions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Di Somma A, Moretta A, Canè C, Cirillo A, Duilio A: *Inhibition of bacterial biofilm formation. Bacterial biofilms*. IntechOpen; 2020.
2. Morikawa M: **Beneficial biofilm formation by industrial bacteria Bacillus subtilis and related species**. *J Biosci Bioeng* 2006, **101**:1–8.
3. Hayat R, Ali S, Amara U, Khalid R, Ahmed I: **Soil beneficial bacteria and their role in plant growth promotion: a review**. *Ann Microbiol* 2010, **60**:579–598.
4. Wuertz S, Bishop PL, Wilderer PA: *Biofilms in wastewater treatment*. IWA publishing; 2003.
5. Halan B, Buehler K, Schmid A: **Biofilms as living catalysts in continuous chemical syntheses**. *Trends Biotechnol* 2012, **30**: 453–465.
6. Lear G: *Biofilms in bioremediation*. Norfolk, UK: Caister Academic Press; 2016.
7. Sheng G-P, Yu H-Q, Li X-Y: **Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review**. *Biotechnol Adv* 2010, **28**: 882–894.
8. Barros AC, Gonçalves AL, Simões M: **Microalgal/cyanobacterial biofilm formation on selected surfaces: the effects of surface physicochemical properties and culture media composition**. *J Appl Phycol* 2019, **31**:375–387.
9. Wu X, Cen Q, Addy M, Zheng H, Luo S, Liu Y, *et al.*: **A novel algal biofilm photobioreactor for efficient hog manure wastewater utilization and treatment**. *Bioresour Technol* 2019, **292**:121925.
10. Machineni L: **Lignocellulosic biofuel production: review of alternatives**. *Biomass Conversion and Biorefinery* 2019:1–13.
11. Hu M-X, Li J-N, Guo Q, Zhu Y-Q, Niu H-M: **Probiotics biofilm-integrated electrospun nanofiber membranes: a new starter culture for fermented milk production**. *J Agric Food Chem* 2019, **67**:3198–3208.
12. * García CF, Stangl F, Götz A, Zhao W, Sieber SA, Opitz M, *et al.*: **Topographical alterations render bacterial biofilms susceptible to chemical and mechanical stress**. *Biomaterials science* 2019, **7**:220–232.
The idea of modifying the biofilm wetting properties by modifying its surface topology is tested. It is found that after treatment with appropriate topology altering agents, the biofilm can be transformed from hydrophobic to hydrophilic enhancing the efficiency of antimicrobial solutions for its removal. Also, The effect of topographical changes of the biofilm surface on the wettability of these surfaces is demonstrated. These types of alterations were done by surface exposure toward different solutions.
13. Sharma D, Misba L, Khan AU: **Antibiotics versus biofilm: an emerging battleground in microbial communities**. *Antimicrob Resist Infect Contr* 2019, **8**:76.
14. Arciola CR, Campoccia D, Montanaro L: **Implant infections: adhesion, biofilm formation and immune evasion**. *Nat Rev Microbiol* 2018, **16**:397.
15. Hall-Stoodley L, Costerton JW, Stoodley P: **Bacterial biofilms: from the natural environment to infectious diseases**. *Nat Rev Microbiol* 2004, **2**:95–108.
16. Lens P, O'Flaherty V, Moran A, Stoodley P, Mahony T: *Biofilms in medicine, industry and environmental biotechnology*. IWA publishing; 2003.
17. Pinto RM, Soares FA, Reis S, Nunes C, Van Dijck P: **Innovative strategies toward the disassembly of the EPS matrix in bacterial biofilms**. *Front Microbiol* 2020:11.
18. Zimmerli W: **Orthopädische implantatassozierte infektionen [Orthopaedic implant-associated infections: update of antimicrobial therapy]**. *Orthopä* 2015, **44**:961–966.
19. Li Y, Ning C: **Latest research progress of marine microbiological corrosion and bio-fouling, and new approaches of marine anti-corrosion and anti-fouling**. *Bioactive materials* 2019, **4**:189–195.
20. Davidson IC, Scianni C, Minton MS, Ruiz GM: **A history of ship specialization and consequences for marine invasions, management and policy**. *J Appl Ecol* 2018, **55**:1799–1811.
21. Vinagre PA, Simas T, Cruz E, Pinori E, Svenson J: **Marine biofouling: a European database for the marine renewable energy sector**. *J Mar Sci Eng* 2020, **8**:495.
22. Bannister J, Sievers M, Bush F, Bloecher N: **Biofouling in marine aquaculture: a review of recent research and developments**. *Biofouling* 2019, **35**:631–648.
23. Otero M, Cebrian E, Francour P, Galil B, Savini D. *Monitoring marine invasive species in Mediterranean marine protected areas (MPAs): a strategy and practical guide for managers*, vol. 136. Malaga, Spain: IUCN; 2013.
24. Scharff RL: **Economic burden from health losses due to foodborne illness in the United States**. *J Food Protect* 2012, **75**:123–131.
25. Koenig D, Pierson D: **Microbiology of the space shuttle water system**. *Water Sci Technol* 1997, **35**:59–64.
26. Zea L, Nisar Z, Rubin P, Cortesão M, Luo J, McBride SA, *et al.*: **Design of a spaceflight biofilm experiment**. *Acta Astronaut* 2018, **148**:294–300.

27. Van Houdt R, Mijnendonckx K, Leys N: **Microbial contamination monitoring and control during human space missions**. *Planet Space Sci* 2012, **60**:115–120.

28. van Tongeren SP, Raangs GC, Welling GW, Harmsen HJ, Krooneman J: **Microbial detection and monitoring in advanced life support systems like the International Space Station**. *Microgravity Sci Technol* 2006, **18**:219.

29. Castro VA, Bruce RJ, Ott CM, Pierson D: *The influence of microbiology on spacecraft design and controls: a historical perspective of the shuttle and international space station programs*. SAE Technical Paper; 2006.

30. Bruce RJ, Ott CM, Skuratov VM, Pierson DL: **Microbial surveillance of potable water sources of the International Space Station**. *SAE Trans* 2005:283–292.

31. Wilson M, Cole H, Weir N, Oehler B, Steele J, Varsik J, *et al.*: *Selection of an alternate biocide for the ISS internal thermal control system ccoolant-phase II*. SAE Technical Paper; 2004.

32. Gu J-D, Roman M, Esselman T, Mitchell R: **The role of microbial biofilms in deterioration of space station candidate materials**. *Int Biodeterior Biodegrad* 1998, **41**:25–33.

33. Westheimer D, Tuan G: **Active thermal control system considerations for the next generation of human rated space vehicles**. In *43rd AIAA aerospace sciences meeting and Exhibit*; 2005:342.

34. Li W, Hummerick M, Khodadad C, Buhrow J, Spencer L, Coutts J, *et al.*: **Biofilm resistant coatings for space applications**. In *48th international conference on environmental systems*; 2018.

35. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L: **Targeting microbial biofilms: current and prospective therapeutic strategies**. *Nat Rev Microbiol* 2017, **15**:740.

36. Simoes M, Simões LC, Vieira MJ: **A review of current and emergent biofilm control strategies**. *LWT-Food Science and Technology* 2010, **43**:573–583.

37. Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R: **D-amino acids trigger biofilm disassembly**. *Science* 2010, **328**:627–629.

38. O'Toole A, Ricker EB, Nuxoll E: **Thermal mitigation of Pseudomonas aeruginosa biofilms**. *Biofouling* 2015, **31**:665–675.

39. Reilly S: **Biofilm and pathogen mitigation: a real culture change**. *Food Saf Mag* 2016, **22**:16.

40. Finkel JS, Mitchell AP: **Genetic control of Candida albicans biofilm development**. *Nat Rev Microbiol* 2011, **9**:109–118.

41. Latasa C, García B, Echeverz M, Toledo-Arana A, Valle J, Campoy S, *et al.*: **Salmonella biofilm development depends on the phosphorylation status of RcsB**. *J Bacteriol* 2012, **194**:3708–3722.

42. Sadekuzzaman M, Yang S, Mizan M, Ha S: **Current and recent advanced strategies for combating biofilms**. *Compr Rev Food Sci Food Saf* 2015, **14**:491–509.

43. Ghilini F, Pissinis DE, Miñán A, Schilardi PL, Diaz C: **How functionalized surfaces can inhibit bacterial adhesion and viability**. *ACS Biomater Sci Eng* 2019, **5**:4920–4936.

44. Rubini D, Banu SF, Nisha P, Murugan R, Thamotharan S, Percino MJ, *et al.*: **Essential oils from unexplored aromatic plants quench biofilm formation and virulence of Methicillin resistant Staphylococcus aureus**. *Microb Pathog* 2018, **122**:162–173.

45. Li J, Nickel R, Wu J, Lin F, van Lierop J, Liu S: **A new tool to attack biofilms: driving magnetic iron-oxide nanoparticles to disrupt the matrix**. *Nanoscale* 2019, **11**:6905–6915.

46. Khalid S, Gao A, Wang G, Chu PK, Wang H: **Tuning surface topographies on biomaterials to control bacterial infection**. *Biomaterials Science* 2020.

47. Kanematsu H, Barry DM: *Biofilm and materials science*. Switzerland: Springer; 2015.

48. Araújo GRdS, Viana NB, Gómez F, Pontes B, Frases S: **The mechanical properties of microbial surfaces and biofilms**. *The Cell Surface* 2019, **5**:100028.

49. Wang J, Wu Y, Cao Y, Li G, Liao Y: *Influence of surface roughness on contact angle hysteresis and spreading work*. 2020:1107–1112.

50. Clark ME, Edelmann RE, Duley ML, Wall JD, Fields MW: **Biofilm formation in Desulfovibrio vulgaris Hildenborough is dependent upon protein filaments**. *Environ Microbiol* 2007, **9**:2844–2854.

51. Hornemann JA, Lysova AA, Codd SL, Seymour JD, Busse SC, Stewart PS, *et al.*: **Biopolymer and water dynamics in microbial biofilm extracellular polymeric substance**. *Biomacromolecules* 2008, **9**:2322–2328.

52. Feng G, Cheng Y, Wang S-Y, Borca-Tasciuc DA, Worobo RW, Moraru CI: **Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough?** *npj Biofilms and Microbiomes* 2015, **1**:1–9.

53. Hollenbeck EC, Antonoplis A, Chai C, Thongsomboon W, Fuller GG, Cegelski L: **Phosphoethanolamine cellulose enhances curli-mediated adhesion of uropathogenic Escherichia coli to bladder epithelial cells**. *Proc Natl Acad Sci Unit States Am* 2018, **115**:10106–10111.

54. Tilahun A, Haddis S, Teshale A, Hadush T: **Review on biofilm and microbial adhesion**. *Int J Microbiol Res* 2016, **7**:63–73.

55. Berne C, Ellison CK, Ducret A, Brun YV: **Bacterial adhesion at the single-cell level**. *Nat Rev Microbiol* 2018, **16**:616–627.

56. Lee CK, de Anda J, Baker AE, Bennett RR, Luo Y, Lee EY, *et al.*: **Multigenerational memory and adaptive adhesion in early bacterial biofilm communities**. *Proc Natl Acad Sci Unit States Am* 2018, **115**:4471–4476.

57. * Recupido F, Toscano G, Tatè R, Petala M, Caserta S, Karapantsios TD, *et al.*: **The role of flow in bacterial biofilm morphology and wetting properties**. *Colloids Surf B Biointerfaces* 2020, **192**:111047.

The effect of flow on formation, morphology, and interfacial properties, that is, wetting, of the biofilms is investigated. Despite the dependence of morphology on the flow conditions, involving hydrodynamic forces, wetting characteristics poorly depends on the flow.

58. Banerjee D, Shivapriya PM, Gautam PK, Misra K, Sahoo AK, Samanta SK: **A review on basic biology of bacterial biofilm infections and their treatments by nanotechnology-based approaches**. *Proc Natl Acad Sci India B Biol Sci* 2019:1–17.

59. Thomen P, Robert J, Monmeyran A, Bitbol A-F, Douarche C, Henry N: **Bacterial biofilm under flow: first a physical struggle to stay, then a matter of breathing**. *PloS One* 2017, **12**, e0175197.

60. Craig L, Forest KT, Maier B: **Type IV pili: dynamics, biophysics and functional consequences**. *Nat Rev Microbiol* 2019, **17**:429–440.

61. Kearns DB: **A field guide to bacterial swarming motility**. *Nat Rev Microbiol* 2010, **8**:634–644.

62. Deng J, Molaei M, Chisholm NG, Stebe KJ: **Motile bacteria at oil–water interfaces: pseudomonas aeruginosa**. *Langmuir* 2020:6888–6902.

63. ** Qin B, Fei C, Bridges AA, Mashruwala AA, Stone HA, Wingreen NS, *et al.*: **Cell position fates and collective fountain flow in bacterial biofilms revealed by light-sheet microscopy**. *Science* 2020:71–77.

A dual-view light-sheet microscopy is developed for observation and investigation of the dynamics of biofilm development until it reaches a mature three-dimensional community. A fluid model is focused in this study, which describes the time evolutions and deformations of the biofilm structure when it grows.

64. Secchi E, Vitale A, Miño GL, Kantsler V, Eberl L, Rusconi R, *et al.*: **The effect of flow on swimming bacteria controls the initial colonization of curved surfaces**. *Nat Commun* 2020, **11**:1–12.

65. Artini M, Cicatiello P, Ricciardelli A, Papa R, Selan L, Dardano P, *et al.*: **Hydrophobin coating prevents Staphylococcus epidermidis biofilm formation on different surfaces**. *Biofouling* 2017, **33**:601–611.

66. Piscitelli A, Cicatiello P, Gravagnuolo AM, Sorrentino I, Pezzella C, Giardina P: **Applications of functional amyloids from fungi: surface modification by class I hydrophobins**. *Biomolecules* 2017, **7**:45.

67. Monetta T, Bellucci F: **Strong and durable antibacterial effect of titanium treated in Rf oxygen plasma: preliminary results**. *Plasma Chem Plasma Process* 2014, **34**:1247–1256.

68. Li Y, Xia H, Bai F, Song X, Zhuang L, Xu H, *et al.*: **PA5001 gene involves in swimming motility and biofilm formation in Pseudomonas aeruginosa**. *Microb Pathog* 2020, **144**:103982.

69. Ugurlu A, Karahasan Yagci A, Ulusoy S, Aksu B, Bosgelmez-Tinaz G: **Phenolic compounds affect production of pyocyanin, swarming motility and biofilm formation of pseudomonas aeruginosa**. *Asian Pacific Journal of Tropical Biomedicine* 2016, **6**:698–701.

70. Liu F, Sun Z, Wang F, Liu Y, Zhu Y, Du L, *et al.*: **Inhibition of biofilm formation and exopolysaccharide synthesis of Enterococcus faecalis by phenyllactic acid**. *Food Microbiol* 2020, **86**:103344.

71. Di Somma A, Moretta A, Canè C, Cirillo A, Duilio A: **Antimicrobial and antibiofilm peptides**. *Biomolecules* 2020, **10**:652.

72. De La Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EB, Horsman S, *et al.*: **Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide**. *Antimicrobial agents and chemotherapy* 2012, **56**:2696–2704.

73. Porter MK, Steinberg AP, Ismagilov RF: **Interplay of motility and polymer-driven depletion forces in the initial stages of bacterial aggregation**. *Soft Matter* 2019, **15**:7071–7079.

74. Hennes M, Tailleur J, Charron G, Daerr A: **Active depinning of bacterial droplets: the collective surfing of Bacillus subtilis**. *Proc Natl Acad Sci Unit States Am* 2017, **114**:5958–5963.

75. ** Ramos G, Cordero ML, Soto R: **Bacteria driving droplets**. *Soft Matter* 2020, **16**:1359–1365.
A dense bacterial suspension inside a spherical droplet is used in a water-in-oil emulsion. The turbulent-like motion of the bacteria in the droplet is measured. So, the collective motion of bacteria is a cause of slippery rolling of the drop on the glass substrate.

76. ** Thokchom AK, Swaminathan R, Singh A: **Fluid flow and particle dynamics inside an evaporating droplet containing live bacteria displaying chemotaxis**. *Langmuir* 2014, **30**:12144–12153.
A droplet containing a suspension of bacteria is evaporated in this study. Particle image velocimetry is used for flow visualization. The results show that the chemotaxis changes the velocity fields and concentration patterns. This can be used to modify the coffee ring patterns.

77. Chang CB, Wilking JN, Kim SH, Shum HC, Weitz DA: **Monodisperse emulsion drop microenvironments for bacterial biofilm growth**. *Small* 2015, **11**:3954–3961.

78. Kleine D, Chodorski J, Mitra S, Schlegel C, Huttenlochner K, Müller-Renno C, *et al.*: **Monitoring of biofilms grown on differentially structured metallic surfaces using confocal laser scanning microscopy**. *Eng Life Sci* 2019, **19**:513–521.

79. Rosenthal AF, Griffin JS, Wagner M, Packman AI, Balogun O, Wells GF: **Morphological analysis of pore size and connectivity in a thick mixed-culture biofilm**. *Biotechnol Bioeng* 2018, **115**:2268–2279.

80. * Landa-Marbán D, Liu N, Pop IS, Kumar K, Pettersson P, Bødtker G, *et al.*: **A pore-scale model for permeable biofilm: numerical simulations and laboratory experiments**. *Transport Porous Media* 2019, **127**:643–660.
A multidimensional mathematical pore-scale model for the biofilm is developed. In this model, the structure of the biofilm is considered as a porous medium. This model is calibrated with laboratory experiments by doing the simulations based on experimentally derived parameters.

81. Kundukad B, Seviour T, Liang Y, Rice SA, Kjelleberg S, Doyle PS: **Mechanical properties of the superficial biofilm layer determine the architecture of biofilms**. *Soft Matter* 2016, **12**:5718–5726.

82. * Picioreanu C, Blauert F, Horn H, Wagner M: **Determination of mechanical properties of biofilms by modelling the deformation measured using optical coherence tomography**. *Water Res* 2018, **145**:588–598.
The deformation of biofilm is recorded by optical coherence tomography imaging with poroelastic fluid–structure interaction computations. As a result, a method was developed to improve the estimation of the elastic moduli of biofilms as deformable materials.

83. Rohrs C, Azimi A, He P: **Wetting on micropatterned surfaces: partial penetration in the Cassie State and Wenzel deviation theoretically explained**. *Langmuir* 2019, **35**:15421–15430.

84. Wenzel RN: **Resistance of solid surfaces to wetting by water**. *Ind Eng Chem* 1936, **28**:988–994.

85. ** Cassie A, Baxter S: **Wettability of porous surfaces**. *Trans Faraday Soc* 1944, **40**:546–551.
A fundamental study of the wettability of porous surfaces. The first idea of the interest put to the behavior of clothing surfaces encountering the rain drops. Formulations derived for apparent contact angles were confirmed by experimental data. Therefore, the results of this research are used as the principles of the porous solid surfaces exposed to liquid droplets.

86. ** Chen L, Bonaccurso E, Gambaryan-Roisman T, Starov V, Koursari N, Zhao Y: **Static and dynamic wetting of soft substrates**. *Curr Opin Colloid Interface Sci* 2018, **36**:46–57.
Summary of the static and dynamic wetting phenomena on the soft or deformable substrates such as polymer gels and biological tissues. Theoretical analysis, numerical simulations, and experimental observations are mentioned.

87. Andreotti B, Snoeijer JH: **Statics and dynamics of soft wetting**. *Annu Rev Fluid Mech* 2020, **52**.

88. Park SJ, Weon BM, San Lee J, Lee J, Kim J, Je JH: **Visualization of asymmetric wetting ridges on soft solids with X-ray microscopy**. *Nat Commun* 2014, **5**:1–7.

89. Bico J, Reyssat É, Roman B: **Elastocapillarity: when surface tension deforms elastic solids**. *Annu Rev Fluid Mech* 2018, **50**:629–659.

90. Bormashenko EY: *Wetting of real surfaces*. De Gruyter; 2013.

91. Carniello V, Peterson BW, van der Mei HC, Busscher HJ: **Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth**. *Adv Colloid Interface Sci* 2018, **261**:1–14.

92. Kapellos GE, Alexiou TS, Payatakes AC: **Hierarchical simulator of biofilm growth and dynamics in granular porous materials**. *Adv Water Resour* 2007, **30**:1648–1667.

93. Picioreanu C, Vrouwenvelder J, Van Loosdrecht M: **Three-dimensional modeling of biofouling and fluid dynamics in feed spacer channels of membrane devices**. *J Membr Sci* 2009, **345**:340–354.

94. Zhao J, Wang Q: **Three-dimensional numerical simulations of biofilm dynamics with quorum sensing in a flow cell**. *Bull Math Biol* 2017, **79**:884–919.

95. D'Acunto B, Frunzo L, Klapper I, Mattei M, Stoodley P: **Mathematical modeling of dispersal phenomenon in biofilms**. *Math Biosci* 2019, **307**:70–87.

96. Wang X, Stone HA, Golestanian R: **Shape of the growing front of biofilms**. *New J Phys* 2017, **19**:125007.

97. Epstein AK, Pokroy B, Seminara A, Aizenberg J: **Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration**. *Proc Natl Acad Sci Unit States Am* 2011, **108**:995–1000.

98. Lacombe RH: **Contact angle: the phenomena with endless applications**. In *Materials science and ttechnology conference*. USA: MATERIALS SCIENCE AND TECHNOLOGY NEWSLETTER; 2016:1–12.

99. Arnaouteli S, MacPhee CE, Stanley-Wall NR: **Just in case it rains: building a hydrophobic biofilm the Bacillus subtilis way**. *Curr Opin Microbiol* 2016, **34**:7–12.

100. Kim SH, Park U, Kim H: **Early stage of liquid drop spreading on tunable nanostructured surfaces**. *Exp Therm Fluid Sci* 2020:110126.

101. Burfoot D, Limburn R, Busby R: **Assessing the effects of incorporating bubbles into the water used for cleaning operations relevant to the food industry**. *Int J Food Sci Technol* 2017, **52**:1894–1903.

102. * Werb M, García CF, Bach NC, Grumbein S, Sieber SA, Opitz M, *et al.*: **Surface topology affects wetting behavior of Bacillus subtilis biofilms**. *npj Biofilms and Microbiomes* 2017, **3**:1–10.
Characterization of surface topology of biofilms of Bacilus Subtilis and correlation of the topological properties to wetting behavior. The wetting behavior of the particular biofilm is brought for the first time in the standard context of Interfacial Science regarding wetting modes.