

Review

Influence of Surfactants on Interfacial Microbial Degradation of Hydrophobic Organic Compounds

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Abstract: This review explores the role of surfactants in enhancing or inhibiting the biodegradation of hydrophobic organic compounds (HOCs) by microorganisms. Bioavailability, the extent to which pollutants can be accessed and metabolized by microbes, is a key factor in determining degradation efficiency. Hydrophobic organic compounds, like polycyclic aromatic hydrocarbons, have limited bioavailability due to their low aqueous solubility, hindering microbial uptake. Microorganisms utilize strategies such as biofilm formation and direct adhesion to hydrophobic surfaces to overcome this limitation. Surfactants, both synthetic and biosurfactants, have been explored to improve HOC bioavailability by increasing solubility through micelles or emulsions. However, surfactants can also affect microbial adhesion by altering cell surface properties, leading to mixed results in degradation efficiency. Biosurfactants, which are more environmentally friendly, show promise in enhancing biodegradation without the toxic effects of synthetic surfactants. The review highlights the complex interactions between surfactants, microbial adhesion, and HOC biodegradation, emphasizing the need for tailored surfactant formulations to optimize bioremediation. Future research should focus on balancing the surfactant concentration and microbial surface properties to enhance biodegradation in contaminated environments.

Keywords: surfactants; hydrophobic organic compounds; microbial degradation; biofilms; Pickering emulsions



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1. Introduction

The biodegradation of environmental organic pollutants is largely determined by their bioavailability, which refers to the extent and rate at which a contaminant can be taken up and metabolized by microorganisms [1]. In this review, bioavailability is defined, in a narrow sense, as the extent to which pollutants can be readily absorbed by microorganisms. Bioavailability is a critical factor influencing the efficiency of microbial degradation, as it dictates the accessibility of the pollutant to microbial cells [2]. For hydrophobic organic compounds (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and alkanes, bioavailability is particularly limited due to their low aqueous solubility [3]. This low solubility restricts the amount of HOCs that can be dissolved in water, thereby reducing the concentration of these compounds available for microbial uptake [4]. As a result, HOCs often persist in the environment, leading to the long-term contamination of soil and water systems.

Microbial adhesion to hydrophobic surfaces is a common strategy employed to overcome the limited bioavailability of HOCs. By adhering to oil–water interfaces or hydrophobic surfaces, microorganisms can directly access and degrade these pollutants, bypassing

the need for dissolution in the aqueous phase [5]. This direct contact mode of degradation is facilitated by the hydrophobic properties of microbial cell surfaces, which enable them to adhere to and colonize on hydrophobic substrates [6]. The formation of biofilms on these surfaces further enhances the degradation efficiency by providing a structured environment that supports microbial activity and protects cells from the toxic effects of HOCs [7].

In addition to microbial adhesion, the use of surfactants, both synthetic and biosurfactants, has been widely explored as a strategy to enhance the bioavailability of HOCs [8]. Synthetic surfactants are industrially produced and commercially available, while biosurfactants are naturally produced by microorganisms and are environmentally friendly [9]. Surfactants can increase the apparent solubility of HOCs by forming micelles or microemulsions, thereby facilitating a mass transfer from the aqueous phase to microbial cells [9]. However, while the emulsifying properties of surfactants have received considerable attention, their impact on microbial adhesion and interfacial biodegradation processes has been less studied [10]. Surfactants can alter the surface properties of microbial cells, potentially affecting their ability to adhere to hydrophobic surfaces and access to HOCs [11]. This dual role of surfactants, enhancing solubility while potentially inhibiting adhesion, highlights the complexity of surfactant–microbe interactions in the context of HOC biodegradation.

This review aims to provide a comprehensive overview of the influence of surfactants on the interfacial microbial degradation of HOCs. Specifically, it will explore the mechanisms by which surfactants affect microbial adhesion to hydrophobic surfaces, the role of biofilms and Pickering emulsions in enhancing degradation efficiency, and the potential inhibitory or stimulating effects of surfactants on interfacial biodegradation processes. By synthesizing recent findings in this field, this review seeks to advance our understanding of the complex interplay between surfactants, microbial adhesion, and HOC biodegradation, and to identify future research directions for optimizing surfactant-enhanced bioremediation strategies.

2. Microbial Uptake on Hydrocarbons

2.1. Uptake from the Aqueous Phase

Three major uptake modes of HOCs by microorganisms were proposed, as shown in Figure 1. In natural environments, the majority of microorganisms are hydrophilic, thriving in aqueous habitats where they can freely take up dissolved substrates [12]. However, the bioavailability of HOCs is significantly limited due to their low aqueous solubility, resulting in minimal amounts of these compounds being directly accessible to microbial cells in the water phase [13,14]. This limitation is a critical factor contributing to the persistence of HOCs in soil and water systems, leading to long-term environmental contamination [13].

The microbial uptake of HOCs in the aqueous phase is primarily dependent on the concentration gradient of the dissolved compounds (Figure 1a). Many microorganisms exhibit chemotactic behavior towards HOCs, indicating their sensitivity to concentration gradients and their preference for higher substrate concentrations [15]. However, the low solubility of HOCs in water means that only a small fraction of the total substrate is available for microbial uptake at any given time [16]. Consequently, the degradation efficiency of HOCs in the aqueous phase is often low, as the limited dissolved substrate concentration cannot sustain high microbial activity [17].

Furthermore, many microorganisms rely on passive diffusion for substrate uptake, which is highly inefficient for HOCs due to their low solubility and the resulting slow mass transfer rates [13]. As a result, even when microorganisms are capable of degrading HOCs, the overall degradation rate is often limited by the slow diffusion of the substrate from the bulk aqueous phase to the cell surface [17]. This inefficiency in substrate uptake is one of

the key reasons why HOCs can persist in the environment for extended periods, leading to chronic pollution [13].

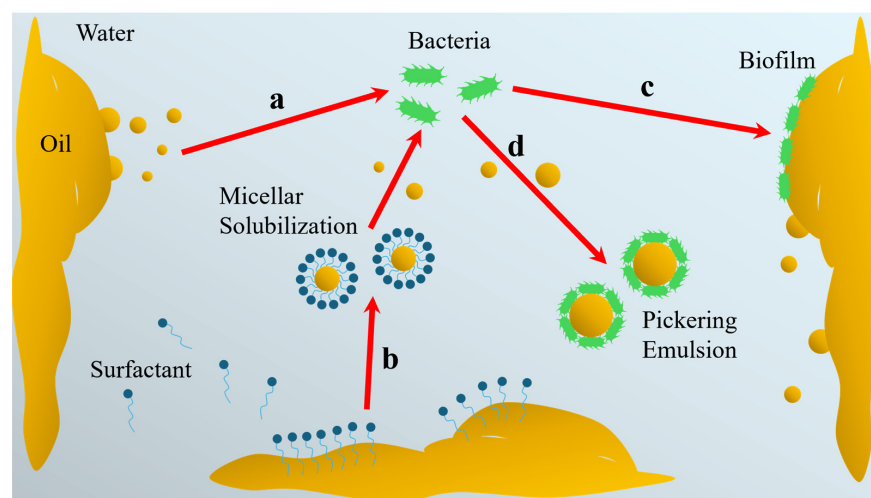


Figure 1. Microbial uptake of hydrocarbons. (a) Uptake from the aqueous phase, where HOCs are dissolved in water and taken up by hydrophilic microorganisms; (b) surfactant-mediated uptake, where surfactants form micelles or microemulsions to increase the apparent solubility of HOCs, facilitating a mass transfer to microbial cells; (c) direct contact mode, namely biofilm formation on hydrophobic surfaces, where microorganisms adhere to oil–water interfaces and degrade HOCs directly; and (d) direct contact mode, namely bacteria-stabilized Pickering emulsions, where hydrophobic bacteria stabilize emulsions and enhance interfacial biodegradation.

2.2. Surfactant-Mediated Uptake

Surfactant-mediated uptake is a key strategy employed by microorganisms to enhance the bioavailability of HOCs (Figure 1b), such as PAHs and alkanes [18]. This process relies on the emulsifying properties of surfactants, which increase the apparent solubility of HOCs in aqueous environments, facilitating a mass transfer to microbial cells [19]. The solubility of HOCs is enhanced through micelles or microemulsions, improving their accessibility to microbial degradation pathways [10,20]. Nonionic surfactants like Triton X-100, for example, significantly enhance phenanthrene solubility by forming micelles that encapsulate the compound [20]. However, a surfactant's effectiveness depends on its concentration and type. Low concentrations enhance biodegradation, while higher concentrations may inhibit microbial activity due to toxicity [10].

Selecting appropriate surfactants is crucial, as their biocompatibility directly influences microbial activity and biodegradation efficiency [3]. Cationic and anionic surfactants, while effective in solubilizing HOCs, can negatively impact microbial cell viability and metabolic activity due to toxicity [13,20]. In contrast, nonionic surfactants, such as Triton X-100 and Tween 80, are more biocompatible and widely used in bioremediation [10,13]. For instance, a mixture of Brij 30 and Tergitol TMN-3 (40 g/L, 1:1) enhances PAH solubility by forming micelles in a cloud point system, increasing phenanthrene solubility to 400 mg/L [9]. However, high surfactant concentrations can reduce microbial activity; for example, Triton X-100 above 100 mg/L reduces phenanthrene degradation by 30% due to cell desorption from oil–water interfaces [10].

Biosurfactants have gained attention due to their superior biocompatibility and environmental safety. Naturally produced by microorganisms, biosurfactants like rhamnolipids enhance HOC biodegradation without the adverse effects of synthetic surfactants [13,20]. Rhamnolipids produced by *Pseudomonas* species, for example, increase the solubility and biodegradation rates of PAHs, such as phenanthrene and pyrene, by forming stable micelles that facilitate mass transfer to microbial cells [20,21]. Additionally, biosurfactants

are biodegradable and less toxic, making them ideal for surfactant-enhanced bioremediation [20,22].

The mechanism of surfactant-mediated uptake involves micelles that encapsulate HOCs, increasing their solubility and transporting them to microbial cells, where they can be absorbed and metabolized [10,13]. This process enhances HOC bioavailability and reduces diffusion distance to microbial cells, accelerating biodegradation rates [23].

2.3. Direct Contact Mode

2.3.1. Biofilm

The microbial degradation of HOCs is significantly enhanced through biofilm formation (Figure 1c), which provides a structured environment that facilitates the uptake and degradation of pollutants [24]. Biofilms, unlike planktonic cells, exhibit a higher degradation efficiency and greater resilience to toxic conditions, making them essential for HOC bioremediation [6,25]. The ability of microorganisms to form biofilms on hydrophobic surfaces is closely linked to their cell surface hydrophobicity (CSH), which plays a pivotal role in initial adhesion and biofilm development [11].

Biofilm formation on hydrophobic interfaces, such as oil–water interfaces, depends on the physicochemical properties of both microbial cells and the substrate. High CSH promotes bacterial adhesion to hydrophobic surfaces, facilitating biofilm formation [25,26]. For instance, *Pseudomonas furukawaii* PPS-19, a marine biofilm-forming bacterium, exhibits strong hydrophobicity under various physicochemical stressors, correlating with its ability to adhere to and degrade petroleum hydrocarbons [25]. Similarly, *Alcanivorax borkumensis*, a hydrocarbonoclastic bacterium, forms biofilms around oil droplets, optimizing oil degradation through interfacial tubulation, driven by the bacteria's adaptation to hydrophobic substrates [6]. These findings suggest that biofilm-forming bacteria with high CSH are particularly effective in degrading hydrophobic pollutants, as they can directly metabolize HOCs at oil–water interfaces.

The role of CSH in biofilm formation is further supported by studies comparing biofilm-forming bacteria with different hydrophobic properties. For example, *Pseudomonas aeruginosa*, with high CSH, rapidly adsorbs to oil–water interfaces and forms stable biofilms, while *Staphylococcus epidermidis*, with lower CSH, forms efficient biofilms through biosurfactant secretion that modifies interfacial properties [11]. This suggests that CSH is critical, but biosurfactants and extracellular polymeric substances (EPS) also influence biofilm formation and function.

Biofilms provide a protective environment that enhances microbial survival and metabolic activity in the presence of toxic HOCs. For example, *P. furukawaii* PPS-19 showed a 94.3% degradation rate of n-dodecane and the 81.5% degradation of pyrene under optimal conditions [25]. The EPS matrix of biofilms not only sequesters hydrophobic compounds but also reduces their toxicity by limiting direct exposure to cells [27]. The EPS produced by *P. furukawaii* PPS-19 demonstrated an emulsifying activity of 66.23% on petrol, superior to commercial polymers like xanthan [25]. This protective effect is particularly evident in PAH degradation, where biofilm-forming bacteria degrade pollutants more efficiently than planktonic cells, even in the presence of solubilizers, like Triton X-100 and β -cyclodextrin [27].

Biofilm formation on hydrophobic surfaces is a key strategy for enhancing HOC uptake and degradation. The interplay between CSH, biosurfactant production, and the protective nature of biofilms underscores the importance of interfacial biocatalysis in bioremediation. Future research should focus on optimizing these microbial strategies to improve biofilm-mediated degradation in environmental applications.

2.3.2. Pickering Emulsions

Pickering emulsions, first identified by Ramsden in 1903 and later refined by Pickering in 1907, are clearly stabilized by solid particles, rather than conventional surfactants [28]. Pickering emulsions are stabilized by particles known as Pickering particles, which can be inorganic, polymeric, or biological in nature [29–31]. A critical property of these particles is their hydrophobicity, often quantified using the contact angle (θ) [32]. The value of θ determines the type of emulsion formed: when θ is less than 90° , oil-in-water emulsions are favored, while a θ of greater than 90° tends to form water-in-oil emulsions [33].

The size of bacteria has been found to be comparable to that of colloidal particles, enabling them to act as Pickering emulsifiers [30]. For instance, *Mycobacterium neoaurum*, with its hydrophobic cell surface, was used to stabilize Pickering emulsions in a water–oil two-phase system for the microbial transformation of hydrophobic cholesterol into two key intermediates [17,34]. The bacteria adsorbed at the oil–water interface, allowing for the simultaneous utilization of hydrophobic and hydrophilic substrates, which significantly enhanced the biocatalytic activity compared to the conventional aqueous system (Figure 1d). This demonstrated that Pickering emulsions could be used to enhance interfacial catalytic processes, such as the microbial transformation of pharmaceutical precursors and the biodegradation of hydrophobic organic pollutants [35].

For hydrophilic microorganisms that are inherently incapable of stabilizing Pickering emulsions, surface modifications or the addition of nanoparticles, such as silica, can enhance their adherence to oil–water interfaces, thereby strengthening interfacial catalysis [36–38]. The surface modification of bacteria with hydrophobic compounds, like dodecanol or chitosan, has been reported to increase their hydrophobicity, resulting in stable emulsions and enhanced hydrocarbon degradation rates [36,39]. Furthermore, polymers such as carboxymethyl cellulose (CMCE) have been shown to effectively promote the stabilization of Pickering emulsions by *Moraxella* sp. CFP312. In combination with CMCE, hydrophilic CFP312 cells were able to utilize phenanthrene in the oil phase at the interface, thereby enhancing the biodegradation process [14]. Additionally, encapsulating bacteria within inorganic particles, such as calcium phosphate doped with magnetic nanoparticles, has been utilized to create Pickering interfacial biocatalysts for one-step enzymatic reactions and hydrocarbon degradation [37].

3. Mechanisms of Microbial Adherence to Hydrocarbons

3.1. Microbial Cell Surface Hydrophobicity

In most cases, bacterial cells are well-dispersed in aqueous environments. However, certain bacteria, such as *M. neoaurum*, disperse more effectively in non-polar solvents like tetradecane, indicating the presence of surface hydrophobicity [17]. This hydrophobicity, critical for cell adhesion at oil–water interfaces, is quantified by the hydrophobicity index (HI), where a higher log HI value correlates with increased cell surface hydrophobicity [40]. For instance, modifying whole-cell biocatalysts with betaine enhances their hydrophobicity [41], which in turn improves their ability to adhere to hydrophobic substrates and degrade HOCs.

While Gram-positive and Gram-negative bacteria differ in their cell wall structures [42], such as the outer membrane in Gram-negative bacteria and the thick peptidoglycan layer in Gram-positive bacteria, these structural differences do not directly determine CSH. Instead, CSH is influenced by genetic and environmental factors, including the production of surface proteins, EPS, and biosurfactants. For example, Gram-negative bacteria like *P. aeruginosa* and *A. borkumensis* exhibit high CSH due to hydrophobic surface proteins and EPS, aiding adhesion to oil–water interfaces [6,11]. Similarly, Gram-positive bacteria like *Mycobacterium* species show high CSH when they produce mycolic acids or other hydrophobic surface

components [10,17]. Therefore, while Gram classification may influence CSH, genetic and environmental factors play a more significant role in interfacial biodegradation processes.

More broadly, the hydrophobicity of microbial cell surfaces is commonly indexed by the contact angle, a widely accepted parameter for assessing cell surface properties [35]. The contact angle can be measured using the bacterial lawn method, where a higher θ value indicates stronger cell surface hydrophobicity. This parameter will be further elaborated in subsequent sections.

The hydrophobicity of microbial cells is also influenced by genetic factors. For example, altering plasmid copy numbers and sequences regulates the CSH of *Sphingomonads* [43]. Additionally, the accessory protein PlaS from *Serratia marcescens* has been found to increase the permeability of both the outer and inner cell membranes, leading to reduced membrane fluidity and surface hydrophobicity [44]. Conversely, the upregulating of genes involved in the biosynthesis of surface proteins, poly-L-glutamine layers, and mycolic acids, such as *secD*, *glnA*, *pks13*, and *fadD32*, enhances the cell surface hydrophobicity and biodemulsifying activity of *Dietzia natronolimaea* S-XJ-1 cultured with liquid paraffin [45].

Cell adhesion at oil–water interfaces is predominantly governed by CHS, influenced by genetic and environmental factors. Understanding these mechanisms can optimize microbial interactions at oil–water interfaces, leading to the more efficient biodegradation of hydrophobic pollutants. Future research should explore genetic engineering approaches to enhance the CHS, improving HOC degradation in contaminated environments.

3.2. Theory of Microbial Adherence to Oil–Water Interface

3.2.1. Derjaguin–Landau–Verwey–Overbeek (DLVO) Theory

The adhesion of microbial cells at the oil–water interface can be effectively characterized using classical DLVO theory, which describes colloidal stability. As microbial cells ($<5\ \mu\text{m}$) behave as colloidal particles, their interactions at various interfaces are well explained by this framework [46]. However, traditional DLVO theory omits short-range Lewis acid–base interactions [47], leading to the development of extended DLVO (XDLVO) theory. XDLVO theory incorporates three components regarding the interactions between colloidal particles: non-polar Lifshitz–van der Waals forces, polar Lewis acid–base interactions, and electrostatic interactions [48].

XDLVO theory explains electrostatic repulsions and hydration forces at hydrophilic surfaces [49], successfully predicting the interactions of uncharged colloids in aqueous media [50]. Nevertheless, both theories assume perfectly smooth particle surfaces, neglecting any roughness or surface structures. In reality, microbial cells exhibit complex surface characteristics due to the presence of extracellular structures, which can significantly affect the measured adhesion forces compared to theoretical predictions [30]. These surface features cause discrepancies between theoretical predictions and measured adhesion forces through bridging or steric effects from surface polymers [51].

The steric effect primarily occurs during bacterial adhesion, when surface polymers obstruct bacteria approaching the oil–water interface [30]. Conversely, bridging attraction occurs when long surface polymers connect bacteria to the oil phase via strong short-range hydrogen bonding interactions, facilitating adhesion. At long distances, bridging effects dominate, while steric effects prevail at shorter distances [52,53].

Interfacial properties control emulsion aging mechanisms, like flocculation, coalescence, and Ostwald ripening [51]. The adhesion of *Rhodopseudomonas acidophila* to hydrophobic surfaces was found to be governed by both electrostatic repulsions and hydration forces, with a Hamaker constant of $2.27 \times 10^{-23}\ \text{J}$ [54]. The application of DLVO theory, in combination with experimental data on the surface characteristics of *R. acidophila*, provides valuable insights into the electrostatic and hydrophobic forces that influence microbial

adhesion [54]. Surface charge and hydrophobicity critically modulate attachment to hydrophobic substrates, essential for optimizing bioremediation processes. Surfactants and particles reduce interfacial tension, complicating distinctions between their effects based solely on equilibrium measurements [55]. Recent reviews highlight non-specific interactions between bacteria and surfaces, comparing bacteria to Pickering particles and exploring the stabilization mechanisms of colloidal structures by natural or surface-modified bacteria [30].

3.2.2. Free Energy Theory of Bacterial Cell Aggregation

The theory of microbial adhesion at the oil–water interface is based on the free energy of bacterial cell aggregation, which measures the thermodynamic favorability of bacterial adhesion and aggregation. This concept is essential in predicting and controlling bacterial behavior in oil–water biphasic systems, particularly for bioremediation, biodegradation, and industrial processes involving emulsions [30,56].

The free energy of bacterial aggregation in water (ΔG_{bwb}) is quantified using surface thermodynamic parameters (Equation (1)), including the Lifshitz–van der Waals (LW) component (γ^{LW}) and electron-donor (γ^-) and electron-acceptor (γ^+) components [57].

$$\Delta G_{bwb} = -2(\sqrt{\gamma_b^{LW}} - \sqrt{\gamma_w^{LW}})^2 - 4(\sqrt{\gamma_b^+ \gamma_b^-} + \sqrt{\gamma_w^+ \gamma_w^-} - \sqrt{\gamma_b^+ \gamma_w^-} - \sqrt{\gamma_b^- \gamma_w^+}) \quad (1)$$

where subscript *b* and *w* represent bacterial cells and water, respectively [48].

These parameters, derived from contact angle measurements on bacterial lawns, are used to calculate the interfacial tension between bacterial cells and the aqueous phase [56]. The ΔG_{bwb} is calculated using the extended Young's equation, which accounts for both apolar and polar interactions [57].

In microbial adhesion, a negative ΔG_{bwb} indicates thermodynamically favorable interactions, promoting bacterial aggregation and adhesion to the oil–water interface. A positive ΔG_{bwb} suggests that bacteria are hydrophilic and less likely to interact with the interface [56]. The γ^- value is a critical factor in bacterial hydrophobicity, which influences adhesion behavior [30,57]. Lower γ^- values correspond to higher hydrophobicity, enhancing cell–interface and cell–cell interactions, promoting partitioning and aggregation, and facilitating demulsification [56].

The influence of surfactants further complicates these thermodynamic properties. Surfactants can alter bacterial hydrophobicity depending on type, concentration, and bacterial surface characteristics [57]. This modulation has implications for controlling bacterial adhesion in environmental and industrial applications. Understanding these thermodynamic properties is crucial for developing strategies to manage bacterial behavior in oil–water systems, with potential applications in biotechnological processes and pollution mitigation.

3.3. Methods to Quantify Microbial Adhesion at Oil–Water Interfaces

3.3.1. Microbial Adhesion to Hydrocarbons (MATH)

The MATH assay, initially developed by Rosenberg et al., quantifies microbial cell adhesion to hydrophobic liquids (e.g., hydrocarbons) by measuring turbidity changes in the aqueous phase after vortexing with a hydrocarbon [58]. It assesses cell surface hydrophobicity and microbial adhesion to hydrocarbons in fields like environmental bioremediation and medical microbiology [59]. However, its classical form has limitations due to hydrocarbon droplets interfering with absorbance measurements, causing inaccurate adhesion estimates [60]. To address this issue, a modified MATH assay was proposed, in which microscopic examination and direct cell counting are incorporated to accurately determine cell concentrations without interference from hydrocarbon droplets [60]. This modification

ensures the more reliable quantification of microbial adhesion to hydrocarbons, especially in cases where the formation of quasi-stable hydrocarbon droplets is observed.

The MATH assay has been further discussed in the context of biophysical methods for studying bacterial behaviors at oil–water interfaces [61]. It has been highlighted that the assay is a valuable tool for probing the affinity of bacteria for hydrocarbons, particularly in the context of biodegradation processes [62]. The simplicity and adaptability of the MATH assay have been emphasized, although care must be taken to control experimental conditions, such as ionic strength and buffer composition, to minimize electrostatic effects and ensure accurate measurements [59].

The MATH assay, both in its original and modified forms, provides a quantitative approach to studying microbial adhesion at oil–water interfaces. While the classical method remains widely used, the modified version offers improved accuracy by addressing the issue of hydrocarbon droplet interference, making it a robust tool for investigating microbial hydrophobicity and adhesion in various applications [61].

3.3.2. Contact Angle

Among the methods used to assess microbial adhesion, the contact angle measurement is a key technique widely employed to characterize the surface hydrophobicity of microorganisms [63]. Hydrophobic cells exhibit greater adhesion than hydrophilic cells, with adhesion levels correlating significantly with measured contact angles [64]. However, the reliability of this method has been debated.

The contact angle, while indicative of bacterial hydrophobicity, has limitations. Gallardo-Moreno et al. highlighted the challenges in defining hydrophobicity and surface Gibbs energy due to the strong dependence of contact angles on dried bacterial lawns, measurement time, and environmental conditions, such as the medium's pH [65]. This precludes the accurate estimation of absolute values and surface Gibbs energy, making contact angles only useful as relative indicators of hydrophobicity, similar to microbial adhesion tests based on solvents.

In microbial lawns, contact angle measurements are influenced by the moisturizing medium and equilibration time. Eschlbeck and Kulozik demonstrated significant variations in contact angles under different conditions (Figure 2a) [66]. The equilibration time ensures a uniform moisture content across the bacterial layer, crucial for consistent results. Xie et al. showed that the contact angles of bacterial lawns stabilized 3–4 h after drying [17], which is vital for the accurate assessment of microbial adhesion at oil–water interfaces.

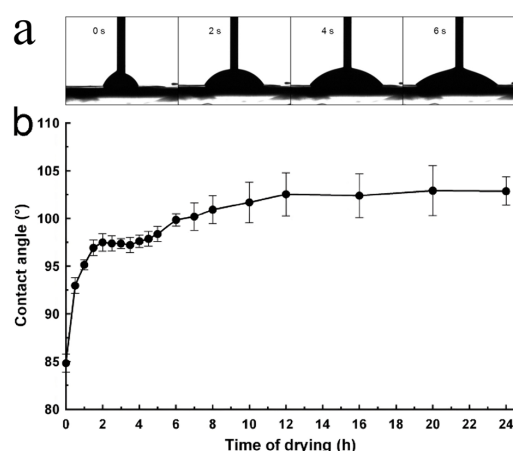


Figure 2. Contact angle varying with time. (a) Advancing contact angle after the deposition of the drop (0 s) and during the extension of the drop (2 and 4 s) until the drop starts to sink in (6 s) [66] (Copyright 2017 Elsevier). (b) The relationship between the three-phase contact angles of cell lawns in air and the time of drying [17] (Copyright 2021 Royal Society of Chemistry).

While the contact angle measurement provides a quantitative method for evaluating microbial adhesion, caution is necessary due to its sensitivity to various factors. Standardizing the method, including the cell count, equilibration medium, and timing, is essential for reproducible results. This approach will help researchers better understand the physico-chemical properties that influence bacterial interactions with interfaces, leading to more effective strategies in controlling microbial adhesion and biofilm formation.

3.3.3. Microscopy

The observation of microbial adhesion at the oil–water interface is crucial for understanding biofilm formation and Pickering emulsion stabilization. Various microscopy techniques (Figure 3), including optical microscopy, confocal laser scanning microscopy (CLSM), and electron microscopy, are used to visualize and quantify microbial interactions at these interfaces [10,62]. Each method provides unique insights into the microbial adhesion's structural and dynamic aspects.

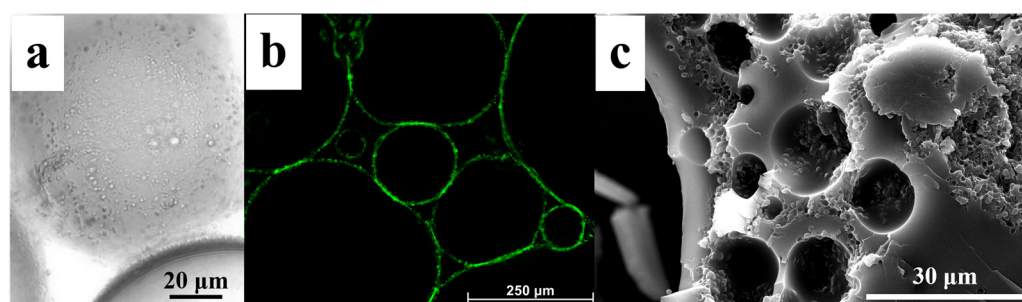


Figure 3. Microscopy images of emulsions stabilized by *Mycobacterium* cells [10] (Copyright 2024 American Chemical Society). (a) Optical microscopy image; (b) CLSM image; (c) SEM image.

Optical microscopy is widely used to observe microbial distribution and aggregation at the oil–water interface (Figure 3a). This method enables the real-time monitoring of cell adhesion and biofilm formation, offering qualitative and semi-quantitative data on cell density and spatial organization [17]. For instance, studies on *Marinobacter hydrocarbonoclasticus* showed that cells adhered to the hexadecane–water interface through Brownian motion, later forming clusters [67]. However, optical microscopy has a limited resolution, making it difficult to observe fine structural details.

CLSM offers a higher resolution and the three-dimensional imaging of microbial biofilms at the oil–water interface (Figure 3b). CLSM can be combined with fluorescent dyes to label biofilm components, such as EPS or the oil phase [68]. For example, CLSM visualized the bacterial cell distribution at the interface of phenanthrene-containing emulsions, where dense clusters of cells facilitated the direct uptake and degradation of phenanthrene [10].

Electron microscopy (Figure 3c), particularly scanning electron microscopy (SEM), provides a higher resolution for observing the fine structural details of microbial cells and their interactions with the interface [69]. However, SEM requires solid samples, posing challenges for imaging liquid emulsions. Researchers address this by polymerizing the oil phase or replacing it with a solid material, like paraffin. SEM was used to observe emulsion microstructures by replacing the oil phase with polymerized styrene [10,37]. Cryogenic electron microscopy (cryo-EM), which rapidly freezes samples to preserve their structure, allows for the direct observation of liquid emulsions but is costly and complex [70].

Each method contributes to a comprehensive understanding of microbial adhesion at the oil–water interface. Optical microscopy provides real-time insights, CLSM offers detailed three-dimensional imaging, and SEM reveals fine structural details. Together,

these techniques help elucidate the mechanisms by which microbial cells stabilize Pickering emulsions and enhance the biodegradation of hydrophobic substrates.

4. Mechanism of Surfactants Inhibiting Interfacial Biodegradation of Hydrophobic Organic Compounds

The application of surfactants in the remediation of hydrophobic organic pollutants, such as oil spills, has yielded seemingly contradictory results. Various studies have reported that surfactants can either enhance, inhibit, or have no effect on the bioavailability and biodegradation of HOCs [10,70,71]. For instance, Corexit 9500, a widely used chemical dispersant, has been shown to neither inhibit nor enhance the biophysical processes of bacterial attachment and growth on oil droplets [71]. Similarly, the effects of surfactants on the stability of Pickering emulsions stabilized by hydrophobic bacteria, such as *Mycobacterium*, have been observed to be either synergistic or antagonistic, depending on the surfactant type and concentration [8]. These conflicting outcomes suggest that the impact of surfactants on the biodegradation of HOCs is highly context-dependent, influenced by factors such as microbial cell properties, pollutant characteristics, environmental conditions, and the surfactant type [72]. It is worth noting that surfactants are rarely reported to have no impact on biodegradation, especially in interfacial processes, likely due to the difficulty in publishing such results.

4.1. Surfactants Inhibit Interfacial Biodegradation

Despite the variability in the reported effects, it has been increasingly recognized that surfactants, even those with high biocompatibility, can exert negative effects on the interfacial biodegradation of HOCs by hydrophobic microorganisms. Hydrophobic bacteria, such as *A. borkumensis* and *Mycobacterium*, typically adhere to oil–water interfaces, where they directly access and degrade hydrophobic substrates, leading to high biodegradation efficiency [10,70]. However, Corexit 9500, a widely used chemical dispersant, inhibited the attachment of *A. borkumensis* to hexadecane droplets by reducing bacterial adhesion by 50% over 3 days, thereby causing cell starvation [70]. This displacement is particularly evident when surfactants such as Corexit 9500 or Tween 80 are used, as they can form stable monolayers at the interface, inhibiting bacterial attachment and subsequent degradation [8,70].

Further evidence of surfactant-induced inhibition has been provided by studies on *Mycobacterium* sp. WY10, a hydrophobic bacterium capable of degrading PAHs, such as phenanthrene [10]. At low nonionic surfactant Triton X-100 concentrations (0.075–0.15 g/L), it was observed that the surfactant preferentially adsorbed onto the bacterial cells, increasing their hydrophobicity and causing them to desorb from the oil–water interface [10]. This desorption led to the destabilization of Pickering emulsions and a 40% reduction in the PHE biodegradation efficiency. Notably, high TX100 concentrations (5 g/L) paradoxically restored the biodegradation efficiency through oil emulsification, demonstrating a concentration-dependent dual effect [10]. The surfactant-induced increase in cell hydrophobicity not only disrupted the emulsion structure but also created a kinetic barrier to the substrate transfer, effectively decoupling bacterial activity from interfacial processes.

While surfactants are often employed to enhance the bioavailability of HOCs in contaminated environments, their interaction with hydrophobic microorganisms can lead to unintended inhibitory effects [3]. By displacing bacteria from oil–water interfaces and altering cell surface properties, surfactants can disrupt the natural biodegradation processes that rely on direct interfacial contact [10]. These findings underscore the importance of carefully considering the type and concentration of surfactants used in bioremediation strategies, particularly in environments where hydrophobic bacteria play a key role in pollutant degradation.

4.2. Surfactants Enhance Interfacial Biodegradation

In contrast to scenarios where surfactants displace bacteria from interfaces, emerging evidence demonstrates that certain surfactants enhance interfacial biodegradation by modulating microbial cell surface properties to favor hydrophobic interactions. The interplay between surfactant-induced alterations in cell surface charge and hydrophobicity critically governs microbial adhesion to oil–water interfaces and subsequent substrate accessibility. For instance, biosurfactants such as rhamnolipids exhibit concentration-dependent dual roles in modifying cell surface architecture [73]. At sub-critical micelle concentrations (CMC, ≤ 100 mg/L), rhamnolipids increased the cell surface hydrophobicity of *Pseudomonas* sp. Ph6 by 30%, correlating with higher phenanthrene adsorption coefficients and nearly complete biodegradation (99.9%). Conversely, concentrations exceeding 200 mg/L disrupted membrane integrity, reduced EPS production, and suppressed biodegradation by 40%, illustrating the delicate balance between surfactant-mediated hydrophobicity optimization and cytotoxicity [73]. Similar non-monotonic effects were observed with synthetic surfactants: low concentrations of Tween 20 (0.1–0.2 CMC) reduced interfacial tension to promote *A. borkumensis* adhesion and hydrocarbon degradation, whereas excessive doses destabilized biofilms through the oversaturation of interfacial monolayers [74].

The structural congruence between synthetic surfactants and microbial biosurfactants may induce synergistic effects. Tween 20, structurally analogous to bacterial glycolipids, enhanced *Pseudomonas putida* biofilm activity 13-fold on hydrophilic carriers by lowering contact angles and increasing the EPS protein content, which elevated the n-hexane removal efficiency from 56.1% to 76.5% [75]. Such synergy arises when exogenous surfactants complement endogenous biosurfactant functions—modulating interfacial tension while preserving cell hydrophobicity. Conversely, surfactants with opposing physicochemical properties, like Span 80, suppressed biodegradation by increasing cell hydrophobicity beyond optimal levels, forcing bacteria into an oil-phase entrapment that limited interfacial access [8]. This dichotomy highlights the criticality of surfactant selection based on microbial surface characteristics: hydrophobic bacteria (*Xenorhabdus poinarii*, *Mycobacterium*) achieved 81.7% hydrocarbon adsorption when treated with biosurfactants that amplified native hydrophobicity, whereas hydrophilic strains (*Escherichia coli*) showed minimal improvement under identical conditions [76].

Notably, surfactant-mediated hydrophobicity modulation often transcends mere solubility enhancement. Rhamnolipids at sub-CMC concentrations (< 50 mg/L) accelerated *P. aeruginosa* adhesion to hexadecane from 33% to 78% within 100 h by stripping hydrophilic lipopolysaccharides from cell membranes, a process decoupled from bulk-phase solubilization [77]. This interfacial preconditioning enables bacteria to bypass diffusion-limited substrate uptake, directly accessing hydrophobic compounds through adhered cell clusters. Such mechanisms explain why even trace surfactant concentrations can disproportionately enhance biodegradation kinetics when strategically aligned with microbial surface properties [78]. Collectively, these findings establish that surfactants enhance interfacial biodegradation not as passive solubility enhancers but as dynamic modifiers of microbial surface thermodynamics, provided their concentration and chemistry are optimized to sustain interfacial colonization without disrupting cellular integrity.

5. Significance and Future Directions

The role of surfactants in the interfacial biodegradation of HOCs is multifaceted and highly dependent on the specific system and conditions involved. While surfactants have traditionally been used to enhance the solubility and bioavailability of HOCs, recent studies have highlighted their potential to inhibit the biodegradation process by disrupting the adhesion of hydrophobic bacteria to oil–water interfaces and altering their surface prop-

erties. This dual functionality underscores the need for a more nuanced understanding of surfactant–microbe interactions and their implications for bioremediation strategies. Future research should focus on developing tailored surfactant formulations that optimize biodegradation efficiency while minimizing inhibitory or enhancing promotional effects. Additionally, exploring the synergistic use of surfactants with other biostimulation techniques could offer innovative solutions for enhancing the degradation of HOCs in complex environmental settings.

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