Alcanivorax borkumensis Biofilms Enhance Oil Degradation By Interfacial Tubulation

ONE SENTENCE SUMMARY

Oil eating bacteria adapts its interfacial properties to accelerate the rate of consumption

AUTHORS

M. Prasad[1], N. Obana[2,3], S.-Z. Lin[4], S. Zhao[1], K. Sakai[5,6], C. Blanch-Mercader[7], J. Prost[7,8], N. Nomura[1,3], J.-F. Rupprecht[4*], J. Fattaccioli[5,6*], A. S. Utada[1,3*]

AFFILIATIONS

[1]Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan
[2]Transborder Medical Research Center, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan
[3]Microbiology Research Center for Sustainability (MiCS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, TARA center, Ibaraki 305-8572, Japan
[4]Aix Marseille Univ, Université de Toulon, CNRS, CPT (UMR 7332), Turing Centre for Living systems, Marseille, France
[5]PASTEUR, Département de Chimie, École Normale Supérieure, PSL Université, Sorbonne Université, CNRS, 75005 Paris, France
[6]Institut Pierre-Gilles de Gennes pour la Microfluidique, 75005 Paris, France
[7]Laboratoire Physico-Chimie Curie UMR168, Institut Curie, Paris Sciences et Lettres, Centre National de la Recherche Scientifique, Sorbonne Université, 75248 Paris, France
[8]Mechanobiology Institute, National University of Singapore, 117411 Singapore

Correspondence: utada.andrew.gm@u.tsukuba.ac.jp, jacques.fattaccioli@ens.psl.eu, jean-francois.rupprecht@univ-amu.fr

ABSTRACT

During the consumption of alkanes, *Alcanivorax borkumensis* will form a biofilm around an oil droplet, but the role this plays during degradation remains unclear. We identify a shift in biofilm morphology that depends on adaptation to oil consumption: longer exposure leads to the appearance of dendritic biofilms optimized for oil consumption effected through tubulation of the interface. In situ microfluidic tracking enables us to correlate tubulation to localized defects in the interfacial cell ordering. We demonstrate control over droplet deformation by using confinement to position defects, inducing dimpling in the droplets. We develop a model that elucidates biofilm morphology, linking tubulation to decreased interfacial tension and increased cell hydrophobicity.

Obligately hydrocarbonoclastic bacteria (OHCB) are a group of cosmopolitan marine bacteria with an unusual ecology: they can survive by consuming hydrocarbons as a sole carbon and energy source (*1*). These metabolic specialists are found at very low densities due to the lack of hydrocarbons but are thought to play a global role metabolizing naturally occurring alkanes (*2*) in the ocean. However, they become the dominant bacteria, outcompeting generalists, at the site of oil spills (*1*, *3*, *4*). OHCB are thought to degrade a significant fraction of spilled oil worldwide (*1*, *5*), which has generated interest for their potential as agents of bioremediation (*5–9*).

Alcanivorax borkumensis SK2 is an aerobic and rod shaped OHCB (*10*) that is often used as a model organism for prevalence (*1*, *2*) and its genetic tractability (*6*, *11*). Like most bacteria, it transitions between planktonic and biofilm lifestyles, which is now recognized as integral to bacterial biology (*12*). Biofilms are often dense 3-dimensional communities encased in self-secreted extracellular polymeric substances, which adhere them to surfaces. Biofilms begin with the colonization of a surface. This can lead to high 2-dimensional cell densities, which in the case of rod-shaped bacteria, induces nematic liquid crystal order. Bacteria have been shown to escape confinement in dense packings (*13*, *14*) by leveraging regions where the nematic order is undefined, called topological defects (*15*). Unlike most bacteria, since *A. borkumensis* forms biofilms on a liquid, it is not clear how the interfacial fluidity affects cell packing and biofilm morphology formation during oil consumption.

Most knowledge of bacteria-mediated oil degradation comes from chemical, genetic, and metagenomic analysis of ocean samples (*7*, *16*, *17*) and microcosm tests using crude oil and sea water (*18–20*). Recent work has begun to clarify the important initial step of bacterial colonization (*21*) and to characterize biofilm formation (*22–25*) on oil drops in the size range commonly found dispersed in the ocean after an oil spill. Biofilms can deform oil drops (*18*, *24*, *25*) and increase their hydrodynamic drag (*23*), potentially mediating the formation of organic-oil aggregates. These aggregates, also known as marine oil snow, have been identified as a mechanism by which large quantities of partially biodegraded oil was transported to the seafloor in the aftermath of the Deepwater Horizon disaster (*4*, *23*, *26*). However, the mechanism of biofilm formation, which depends on the interfacial properties of individual cells, and its relation to oil degradation remains unclear (*18*).

Experimental setup and microfluidic device

To address these questions, we developed a microfluidic device that allows the trapping and real-time imaging of numerous bacteria-covered oil droplets. This platform allows us to capture the full dynamics of biofilm development starting from individual bacteria through the complete consumption of oil droplets.

In the ocean, *A. borkumensis* subsists primarily on naturally occurring organic acids and alkanes (*2*); however, during oil spills, it blooms to exploit the hydrocarbons in the crude oil mélange. To study the biofilm dynamics as *A. borkumensis* adapts (*27*) to using solely alkanes, we initially cultivate using pyruvate and then switch to an artificial seawater medium supplemented with hexadecane (C16) (fig. S1A). We sample bacteria from liquid cultures cultivated up to 5 days by harvesting the cells and generating cell-laden oil microdroplets with fresh C16, which we incubate in individual microfluidic traps (see supplementary text S1). Our device, which is highly permeable to oxygen, facilitates in situ culturing, enabling the longitudinal investigation of biofilm development simultaneously on numerous droplets (Fig. 1A and fig. S2A). Trapped drops initially have ~20-50 cells attached and reach confluency in ~12 h, which we define as t_0 (fig. S3A).

Bacteria exhibit two distinct phenotypes and consumption rates

Testing different liquid cultures at 1 and 5 d reveals different phenotypes that manifest vastly different biofilm morphologies and oil consumption rates. When sampled after 1 d, *A. borkumensis* forms a spherical biofilm (SB) that grows outward from the oil where the oil droplet remains mostly spherical as it is consumed (Fig. 1B, fig. S3B, and movie S1). In contrast, bacteria sampled from 5 d culture can develop into thin biofilms with a local nematic ordering of the interfacial cells, which eventually buckle and then tubulate (*24*, *25*) the droplet interface (Fig. 1C) that we call dendritic biofilms (DB) (see supplementary text S2). The biofilm buckles the interface to accommodate a population that is continuously increasing in number. The magnitude of the deformations grows as the oil is consumed, which ultimately shreds the droplets into tiny fragments (fig. S3C and movie S2).

To quantify the oil consumption rate for each phenotype we measure the oil volume (V) over time using confocal microscopy. SBs consume >90% of their oil droplet volume in approximately 72 h, whereas DBs achieve the same level of consumption in ~20 h. We find that the oil volume of SBs decreases as a polynomial function of time, whereas for DBs, the decay is much faster (Fig. 1D,E and fig. S3D-F). In both cases, the decrease is consistent with a model of oil consumption effected exclusively by bacteria at the interface (see supplementary text S3).

The good agreement between our analytical models and data allows us to estimate the single-cell consumption rates of oil by the SB and DB cells as 0.7 and 0.8 fL/h, respectively. This small difference in consumption rate is consistent with their similar division times (fig. S1D). For comparison, the volume of a single cell is ~1 fL, meaning that these bacteria consume a volume of oil close to their own, every

hour. Despite the similarity in consumption rates on a per-cell basis, the normalized surface-to-volume ratio (S^*/V^*) shows that DBs are significantly more efficient: S^*/V^* doubles in 72 h for SBs, whereas it diverges in less than 24 h for DBs (Fig. 1E, inset). The S^*/V^* ratio provides a means for comparing the relative efficiencies of the two phenotypes and highlights the fact that these differences arise from the rapid increase in interfacial area caused by DB biofilms. In both cases, the shape of the interface defines the dynamics of volume decrease. For SBs, the interfacial area defined by the spherical droplet determines the number of cells (N) that can pack onto the interface to have access to the oil. Conversely, for DBs, it is the increase of N at the interface due to cell division that determines the interfacial area. Thus, the rate of consumption decreases over time for SBs, while it increases continuously for DBs.

Tubulation is facilitated by topological defects

We correlate the onset of the rapid increase in surface area for DBs to the emergence of nematic order of the interfacial cells. Active systems with nematic order have shown the ability to utilize topological defects to achieve surface deformation, both theoretically (*28*, *29*) and experimentally (*13*, *14*, *30*) (see supplementary text S4). Nematic topological defects of charge ±1/2 and ±1 are shown schematically in fig. S4A. At 2-4 h post-confluency, we observe the appearance of conical protrusions of cells that originate from the core of aster (+1) topological defects in the nematic director field, which is the average orientation of the bacteria (see Fig. 2A,B, fig. S4B, and supplementary text S3). As the biofilm matures, more protrusions appear while existing protrusions elongate into branched bacteria-covered tubes (Fig. 1C (16 h) and Fig. 2C).

Differential labeling of the oil and cells shows that the tubes are not filled with water; instead, they are filled with oil (fig. S5A). This indicates that cell adhesion to the oil stabilizes the tubes against collapse, thereby preventing the deformed oil from regaining a spherical shape. Furthermore, careful inspection of the confocal images of the tubes reveals that the bacteria are well aligned to the tube axis, which becomes clear in the director field of the cells on the tube (Fig. 2D). We characterize cell alignment along the tube with the local average scalar product between the director field and the tube axis, finding values close to 1; this indicates a high degree of alignment between the cells and the tube axis (Fig. 2E and fig. S5B,C).

Due to this alignment, we hypothesize that the rate of increase of the tube length is proportional to the number of cells on the tube, which should increase exponentially if all cells were to divide. We measure tube elongation on different droplets, finding that it increases rapidly and, in a manner, consistent with exponential elongation, supporting this hypothesis (Fig. 2F and fig. S5D). Furthermore, from the fit to our

data, we extract a tube length-doubling time of ~3.4 h, which is 2-fold larger than the cell division time (t_{div}) of 1.65 h (fig. S1D). This difference likely arises from the imperfect alignment of cells along the tubes and the expulsion of cells from the interface, which is visible around the tubes (Fig. 2C).

Biofilm phenotypes are associated with a decrease of interfacial tension

The large difference in biofilm morphology between the two phenotypes suggests differences in their interfacial properties. *A. borkumensis* secretes amphiphilic molecules that are thought to aid in the assimilation of oil (*10*, *31–33*). Furthermore, autolysis is thought to be important during *A. borkumensis* biofilm formation (*11*), which could liberate membrane-bound biosurfactants into the oil. These molecules can lower the oil-water interfacial tension (γ), making interfacial deformation easier. To measure changes in interfacial properties of the phenotypes, we fractionate SB and DB liquid cultures into three components: cells, conditioned media, and conditioned C16 to independently measure γ for each (fig. S6A). We find that γ for each of the respective fractions are depressed relative to control values; however, the DB-conditioned oil decreases the most. The γ for DB-conditioned oil decreases from 30 to 8 mN/m and is about half the value for SBs (Fig. 3A, fig. S6B-D, and table S1). Surprisingly, when we microfluidically test SB and DB cells using the DB-conditioned C16 instead of fresh C16, we find no change in observed phenotype (fig. S6E). Thus, despite the ~4-fold lower γ of DB-conditioned oil, the SB cells are unable to deform the oil-water interface, indicating that a lower γ is not sufficient to produce the DB phenotype.

Interfacial behavior is also affected by cell hydrophobicity, which, together with γ controls the extent that oil wets the cells (fig. S7A). Cell hydrophobicity is thought to increase the longer cells consume oil, potentially through accumulation of membrane-bound hydrophobic biosurfactants (*33*); however the relationship with phenotype is unclear (*19*, *34*). Although the bacteria appear to lay flat on the surface at t_0, the contact angle is difficult to estimate from confocal images (fig. S7B). Thus, to estimate cell hydrophobicity of both phenotypes, we measure the 3-phase contact angle (θ) between a water drop deposited on a bacterial lawn submerged in oil (*35*) (fig. S7C-E); larger θ's indicate greater hydrophobicity. SB cells, which are isolated after 1 d of culture, have a $\theta \approx 80°$, whereas DB cells, which are isolated after 5 d, have a $\theta \approx 100°$ (Fig. 3B). This suggests that the midplanes of SB and DB cells are ±10% above and below the interface, respectively (fig. S7A).

The higher hydrophobicity of DB cells also indicates that they have a larger interfacial adhesion strength than SB cells, for a constant γ (see supplementary text S4). To directly compare the respective adhesion strengths of SB and DB, we force them to compete for interfacial area on oil microdroplets, noting that

both phenotypes have similar division times (fig. S1D). We generate cell-laden droplets using mCherry-expressing SB cells and GFP-expressing DB cells in a 3:1 ratio, and record fluorescence intensity as the biofilm develops. In this test, both phenotypes experience the same γ. We find that although the DB cells are initially in the minority, they dislodge the established SB, becoming dominant in ~5 h (Fig. 3C,D). This confirms our expectation that DB cells, which are more hydrophobic do indeed have a larger adhesion energy to the interface than SB cells.

Rod-shaped gammaproteobacteria like *Pseudomonas aeruginosa* have been shown to colonize and remodel oil-water interfaces with their biofilms, similar to what we observe in the early stages (<3 h) of DB formation (*36*, *37*). However, those biofilms lack the large-scale deformations produced by DBs (Fig. 1C, >5 h). Using our γ, we estimate the biofilm compression modulus to be ~200 Pa (*38*), which is much smaller than the ~1 MPa growth pressure of *P. aeruginosa* (*39*). Assuming a similar growth pressure for *A. borkumensis*, cell division supplies enough stress to easily deform the interface. Thus, DBs generate the biofilm phenotypes we observe only if enough cells remain adhered to the interface to drive the tubulation process. Conversely, the lack of deformations in the SB phenotype is the consequence of insufficient cell hydrophobicity combined with a stiffer interface, leading to cell detachment followed by biofilm formation around the droplet.

Membrane theory predicts the transition from SB to DB phenotype

Based on these observations, we develop a coarse-grain membrane model to describe the interfacial dynamics of the growing interfacial biofilm. The model explains the transition between SB and DB phenotypes in terms of a competition between the interfacial tension and the spontaneous curvature of the biofilm, which is its intrinsic tendency to bend in a preferred direction. Interfacial tension resists expansion of the surface by the biofilm, while the spontaneous curvature of the biofilm governs the shape of the expanding surface. Tubes are generated when the energetic cost of increasing surface area is lower than the cost of bending; they expand exponentially, according to:

$$\eta \frac{1}{L}\frac{dL}{dt} = -\left(\sigma_{t,H} + \sigma_{n,H} - \frac{\kappa_B}{r_0 r_{eq}}\right), \quad (1)$$

where L is the tube length, η is the viscosity of the biofilm layer, $\sigma_{t,H}$ and $\sigma_{n,H}$ are the respective tensions along the circumferential and normal directions of the tube, κ_B is the bending rigidity of the membrane in the circumferential direction of the tube, $1/r_0$ is the spontaneous curvature of the biofilm, and r_{eq} is the tube radius (see supplementary text S6 and fig. S14).

Eq. 1 encompasses the existence of active extensile nematic stresses driven by bacterial growth, with an effective interfacial tension $\gamma_{eff}(\rho) = \frac{1}{2}(\sigma_{t,H} + \sigma_{n,H})$ that depends on the interfacial cell density (ρ) (see supplementary text S6). This implies that tubulation occurs at a critical value of the buckling density (ρ_B), which is consistent with our observation that tubes form after confluency (Fig. 1C). In our model, bacteria populate a circular oil-water interface with a density that increases logistically towards a homeostatic density (ρ_H), where division and loss are balanced. Depending on the ratio ρ_B/ρ_H, the phenotype changes: when $\rho_B/\rho_H > 1$, tubes are unable to form, resulting in the SB phenotype; when $\rho_B/\rho_H < 1$, the interface buckles and stable tubes form, producing the DB phenotype. Our model implies that physically lowering ρ by removing cells of a DB below the critical value should cause a deformed droplet to recover its unperturbed spherical shape. To test this hypothesis, we infuse surfactants into a device containing DBs. We use a formulation similar to Corexit 9500, which is used to disperse marine oil spills, and concentrations that range from 25-100x the critical micelle concentration (*37*). We note that our concentrations exceed estimated levels in the top 20 cm of the ocean after the Deepwater Horizon accident (*4*, *37*). Following a ~4 h lag, the biofilm abruptly washes away and the deformed droplets become spherical; this recovery is the consequence of positive interfacial tension in the absence of bacteria (fig. S8A,B and movie S3).

In addition to the SB and DB phenotypes described so far, we observe an intermediate phenotype when we isolate cells at an intermediate culture time (fig. S1A). These biofilms present dynamic oscillatory behavior (OB), alternating between the dendritic and spherical biofilms, with a period of ~12 h (Fig. 3E, fig. S9, and movie S4). The relatively short (~30 min) transition from tubulated to spherical is consistent with a sudden loss of tube stability due to a sudden increase in effective surface tension (see Eq. 1).

To elucidate the emergence of oscillations between spherical and dendritic phenotypes, we use a phase-field approach following an established literature on simulating interfacial dynamics of multiphasic systems (*40*). In our case, the simulated field is the local fraction of oil ($\varphi(x,y)$) that obeys a Cahn-Hilliard equation with an interfacial term $\kappa_1(\rho)(\nabla\varphi)^2/2$; here, $\kappa_1(\rho)$ is defined by the right hand side of Eq. 1 and sets the tube elongation rate. $\kappa_1(\rho)$ is thus a proxy for the active stress along the interface (see supplementary text S4). In our model, a linear relation of the form $\kappa_1(\rho) = \gamma - k_1 \rho$, where k_1 accounts for the force exerted by the biofilm on the oil is sufficient to understand the SB-to-DB transition. For the SB phenotype, the $\kappa_1(\rho)$ tension is positive for all densities precluding tube formation. The interface remains spherical until ρ surpasses the critical density, $\rho_B = \gamma / k_1$; here, $\kappa_1(\rho)$ becomes negative and tubulation occurs as the interface buckles. However, the morphological oscillations emerge only if we consider a

second-order expansion of $\kappa_1(\rho)$ in ρ, such that an 'optimal' cell density exists where tube elongation and the final tube length are maximal, which we denote ρ_S (Fig. 3F). Oscillations arise for the specific case when $\rho_B<\rho_S<\rho_H$ (see supplementary text S5); here, as cell density increases beyond the optimal value ρ_S, tube elongation ceases, and contraction starts. During this contraction, the surface shrinks faster than cells can be ejected from the interface, leading to an abrupt increase in bacterial density. We find that ρ overshoots ρ_H and causes $\kappa_1(\rho)$ to become positive (Fig. 3F, blue). This dynamical overshoot ultimately induces a catastrophic collapse of the tubes and is accompanied by a large reduction in the number of cells at the interface. Consistent with this prediction, in our experiments we observe a large and persistent flow of cells away from the interface soon after tube collapse (movie S4). Our biomechanical model recapitulates the transition from these three phenotypes in terms of a tubulation mechanism that depends on bacterial growth dynamics, with oscillations emerging through a dynamical phase transition mechanism (*41*) (Fig. 3G and movie S5). These oscillations are driven by continuous cell division that pushes density beyond the critical values for tube growth and collapse.

Controlled Buckling of Confined Droplets

We leverage microfluidics to position the tube-generating topological defects through droplet confinement. By trapping droplets larger than the chamber height, we can grow biofilms on flattened drops (Fig. 4A - Upper inset**)**. These flat circular regions allow us to generate a centered defect, which concentrates growth stress to form a dimple. Interfacial tension, which initially excludes bacteria from the flat region, is eventually overcome by growth pressure (Fig. 4A, fig. S10A,B, and movie S6). As cells invade, their flow orients the director field, which forms a single aster defect (or two narrowly separated +1/2 defects) at confluency (Fig. 4B and fig. S10C,D) (*15*). As cell division continues, dimples form at the defects at the top and bottom of the drop (Fig. 4C, fig. S10B-inset, and fig. S10E). We use the theory of liquid crystal membranes to elucidate the role of the aster defect in generating deformation in the biofilm. The dimple height profile depends on two dimensionless parameters κ_F/κ_B, where κ_F is the elastic constant of distortions to the director field, and $\kappa_B/\gamma R_{dimple}^2$, where R_{dimple} is the radius of the dimple (see supplementary text S8). κ_F/κ_B captures the competition between the elastic energy of distortions to the liquid crystalline biofilm and its bending energy, whereas $\kappa_B/\gamma R_{dimple}^2$ captures the competition between biofilm bending energy and its surface tension. Buckling at the defect occurs when the energetic cost to deform the membrane exceeds its bending energy, $\kappa_F/\kappa_B > 1$; our fits yield a ratio of ≈ 2 (fig. S11 and table S2) (*29*, *42*). In agreement with experiments, the profiles in this parameter regime are qualitatively like pseudospheres (Fig. 4D and fig. S12) (*42*).

The ability of marine microorganisms to biodegrade hydrocarbons has been recognized for nearly a century (*1*), with recent improvements to metagenomic and imaging techniques further clarifying the possible mechanisms involved. In this study, we quantify the mechanism by which cultures of *A. borkumensis* pre-adapted to using an alkane leads to the appearance of a biofilm phenotype primed (*43*) for explosive growth. Leveraging microfluidics, we capture the dynamics of the optimized biofilms, correlating their appearance to measurable changes in the interfacial properties of the bacteria. These biofilms develop liquid crystalline order before buckling the interface at aster topological defects, morphing the spherical droplet into numerous branching dendrites. We develop a theoretical model that recapitulates each phenotype and explains the cause of the spectacular biofilm oscillations we observe. Furthermore, we use microfluidics to control the dimpling of the droplet and use the profiles to confirm the liquid crystalline elastic constant of the biofilm from theory.

Topological defects in layers of both prokaryotic and eukaryotic cells have been shown to be important in defect-driven morphogenesis (*13*, *14*, *29*, *30*, *44*). We find that the optimized *A. borkumensis* cells collectively utilize defect-driven buckling to escape the confines of the droplet interface and expand the biofilm laterally. Importantly, efficiency is achieved not through an increase of individual metabolic throughput, but rather by expanding the interface, allowing more cells to simultaneously feed.

Although we find that addition of oil-dispersant similar in composition to commercially available mixtures lead to the rapid detachment of the biofilm from the oil drops, numerous factors such as the dispersant concentration, oil composition, temperature and pressure, and nutrient concentrations likely affect biodegradation. Since our platform is an open system, all inputs can be dynamically controlled; thus, how biofilm formation changes depending on hydrocarbon composition, nutrient profile, as well as the dynamical response of bacteria to dispersant dose may be independently verified. These tests could also serve as a starting point in the investigation of artificially constructed multi-species consortia (*8*), which are more robust and effective than monocultures (*9*).

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SUPPLEMENTARY MATERIALS

Supplementary Information:

- Materials and Methods
- Supplementary Text
- Figs. S1 to S17
- Tables S1 to S3
- Movies S1 to S6
- References (46-63)

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FIGURE CAPTIONS

Fig. 1: Spherical and dendritic biofilm phenotypes on oil drops in a microfluidic trap. (A) Schematic of the microfluidic oil-drop trap device showing media-filled channels. Oil drops are trapped in the raised circular regions. Inlets on either side of the drop chamber connect to reservoirs that provide a gentle flow of media through the trap chamber. The white circles are pillars. Scale bar = 200 μm. (*center*) Schematic cross-section of an individual trap. (*right*) Bright-field image of a representative drop in a trap. The trap is outlined with a white dashed line, while the pocket is colored orange as a guide for the eye. Scale bar = 20 μm. (B) Representative time-lapse sequence of confocal images showing the development of the spherical biofilm (SB) phenotype. As the biofilm (green) grows, the oil droplet (central void) shrinks. Scale bar = 10 μm. (C) Maximum intensity projection confocal images showing the development of the dendritic biofilm (DB) phenotype on representative drops. Local nematic order is present at confluence (~0 h), buckling (0.1-0.5 h) and protrusions (2-4 h) appear later, and large-scale remodeling of the interface leading to the formation of tubes occurs much later (16 h). Scale bar = 10 μm. (D,E) Normalized surface area (S^*) and volume (V^*) of oil drops as a function of time for (∘) SB and (⋆) DBs (solid lines and filled regions: mean ± SD; $n_{SB} = 11$ drops and $n_{DB} = 12$ drops; representative experiment from ≥3 independent tests. See fig. S3 for additional data.). Filled symbols represent measurements, while open symbols are values calculated from measured quantities. The dashed and dotted lines are best fits of our analytical models of oil degradation for the phenotypes. (D,inset) Normalized drop radius (R^*) was used to estimate S^* and V^* for SBs. (E,inset) S^*/V^* as a function of time. R, S, and V are normalized by their initial values, respectively. Eq. 5, 6, 9, and 11 (supplementary text S3) were used to fit R^*, S^*, and V^* for SB and DBs, respectively.

Fig. 2: Dendrites originate from topological defects. Confocal images of representative droplets (A) early (~0 h) and (B) later (~12 h) in biofilm development. The images are color coded by depth. The dashed circles enclose +1 topological defects, while the arrows indicate protrusions. (B, inset) Magnified view of the central defect circled in yellow. (C) Confocal image of a bacteria-covered tube connected to a deformed droplet with corresponding orthogonal views (~20 h). (D) Director field of the visible cells in the dashed box in (C). The director field (yellow lines), tube axis (red line), the local tangent unit vector ($\mathbf{n}_{tube}$) along the tube axis, and the bin window (blue) are shown. (E) Axial order of the cells along the oil tube shown in (D). For a sliding window of width 1.5 μm along the tube, the local axial order is defined as the average of the individual scalar products between the director-field unit vectors ($\mathbf{n}_{cell}$) and local tangent unit vectors. $<\mathbf{n}_{cell} \cdot \mathbf{n}_{tube}> = 1$ for parallel alignment and 0 for perpendicular alignment (solid line and shaded region: mean ± SD). (F) Oil tube length (l_{tube}) plotted as a function of time (n=18 tubes; three independent tests). The dashed line is a fit to the average tube length using an exponential equation (adjusted *R*-square = 0.96). All scale bars = 10 μm.

Fig. 3: Theoretical model of tubulation. (A) Interfacial tension (γ) between biofilm-conditioned oil and fresh media measured using pendant drop tensiometry for spherical (SB) and dendritic biofilms (DB), respectively. The dashed line indicates γ between fresh oil and fresh medium (mean+SD; n_{SB}=3, n_{DB}=10, $n_{control}$=5 independent tests; * denotes p<0.05 (p=0.012), Welch's t-test with Holm-Bonferroni correction). (B) Three-phase contact angle (θ) between a water drop deposited on a bacterial lawn of SB and DB phenotypes, submerged in oil (□: mean, horizontal dashed lines: medians, and ● represents an outlier; n_{SB}=10, n_{DB}=13 independent tests; ***** denotes p<0.00001 (p=1.2×10^{-6}), Welch's t-test). (inset) Images of water drops on bacterial lawns, submerged in oil. (C) Time-lapse sequence of the competition between SB cells (magenta) and DB cells (green) for interfacial area on a trapped droplet. Droplets are generated in a suspension containing SB and DB cells at a ratio of 3:1 (see supplementary text for details). DB cells displace a monolayer of SB cells over the course of ~6 h. (D) Measurement of the fractional coverage of SB and DB as a function of time from confocal images (solid line and filled regions: mean ± SD; n=10 representative drops). (E) Oscillatory biofilm (OB) behavior demonstrated by a culture sampled for an intermediate duration between the SB and DB cultures. (F) Model of the tension (κ_1 in the phase-field model) experienced by the tip of a tube as a function of normalized interfacial cell density (ρ/ρ_H) (see supplementary text for details). We normalize cell density by the homeostatic interfacial cell density (ρ_H). Positive (negative) values of κ_1 indicate tube retraction (expansion). The slope at the y-intercept is k_1 = 3, 6, and 10 for the SB, OB, and DB models, respectively. The ρ_B is the critical buckling density, where $\kappa_1 < 0$. For SBs, $\rho_B = \infty$. The ρ_S is the optimal cell density, where κ_1 reaches a minimum, and beyond which the effect of spontaneous curvature is reduced. (insets) Phase-field simulation showing a circular droplet ($\kappa_1 > 0$) and a tubulated droplet ($\kappa_1 < 0$). (G) Schematic phase diagram of the biofilm phenotypes in terms of normalized densities: ρ_S/ρ_H and ρ_B/ρ_H. When $\rho_B/\rho_H > 1$, SBs form because the interfacial cell density can never become sufficiently large to induce buckling. For $\rho_B/\rho_H < 1$, cell division drives an increase in cell density beyond ρ_B. When $\rho_S/\rho_H < 1$, oscillations between the spherical and dendritic phenotypes can occur (OB). When, $\rho_S/\rho_H > 1$, stable dendrites (DB) occur.

Fig. 4: Defect mediated buckling of the surface of a confined droplet. (A-C) Time-lapse confocal image sequence showing the evolution of a dendritic biofilm on a 'flattened' drop, color coded by depth. (A) Prior to confluency (-40 min), interfacial tension excludes cells from the flattened regions at the top and bottom of the droplets. These regions are generated where the non-wetting droplets contact the glass floor and PDMS ceiling. See the (upper) inset for a droplet schematic and the (lower) inset for the top view of the droplet. (B) Confluent monolayer is formed at t_0 = 0 min (see inset and fig. S10). (inset) Top view of droplet with a circle enclosing the location of the nematic defect. (C) Dimple formation in the droplet at the defect. All scale bars = 10 μm. (D) Evolution of the dimple height (*h*) from *xy*-line profiles measured across the droplet midplane, at 20 min (early) and 26 ± 3 min (late) post-confluency. Dimple height is shown as a function of the radial coordinate (*r*). The fits to the data are based on Eq. (80) (supplementary text). The solid error bars and filled regions represent ±SD on the experimental data and fits, respectively (see supplementary text for details on the fitting procedure and error estimation). Early-profile data are the average of the *x*- and *y*-profiles (n=4) at *t* = 20 min, while the late-profile data are the average of the *x*- and *y*-profiles binned at 23, 26, and 29 min (n=12), respectively. To generate the dimensional values shown, we rescale the non-dimensionalized *h*-values by the dimple heights, 4.5 μm and 8.0 μm at early and late times, respectively, while *r* is rescaled by 26 μm at all times.