Research

Cite this article: Stricker L, Guido I, Breithaupt T, Mazza MG, Vollmer J. 2020 Hybrid sideways/longitudinal swimming in the monoflagellate *Shewanella oneidensis*: from aerotactic band to biofilm. *J. R. Soc. Interface* **17**: 20200559. <http://dx.doi.org/10.1098/rsif.2020.0559>

Received: 14 July 2020

Accepted: 6 October 2020

Subject Category:

Life Sciences—Physics interface

Subject Areas:

biophysics

Keywords:

electroactive bacteria, aerotaxis, biofilm, collective behaviour

Author for correspondence:

Laura Stricker

e-mail: laura.stricker@mat.ethz.ch

[†]These two authors contributed equally to the present work.

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5178293>.

Hybrid sideways/longitudinal swimming in the monoflagellate *Shewanella oneidensis*: from aerotactic band to biofilm

Laura Stricker^{1,†}, Isabella Guido^{2,†}, Thomas Breithaupt², Marco G. Mazza^{2,3} and Jürgen Vollmer⁴

¹ETH Zürich, Department of Materials, Polymer Physics, 8093 Zurich, Switzerland

²Max Planck Institute for Dynamics and Self-Organization (MPIDS), 37077 Göttingen, Germany

³Loughborough University, Interdisciplinary Centre for Mathematical Modelling and Department of Mathematical Sciences, Loughborough, Leicestershire LE11 3TU, UK

⁴University of Leipzig, Institute of Theoretical Physics, 04103 Leipzig, Germany

LS, 0000-0002-7893-6939; IG, 0000-0002-1781-3767

Shewanella oneidensis MR-1 are facultative aerobic electroactive bacteria with an appealing potential for sustainable energy production and bioremediation. They gather around air sources, forming aerotactic bands and biofilms. Here, we experimentally follow the evolution of the band around an air bubble, and we find good agreement with the numerical solutions of the pertinent transport equations. Video microscopy reveals a transition between motile and non-motile MR-1 upon oxygen depletion, preventing further development of the biofilm. We discover that MR-1 can alternate between longitudinal fast and sideways slow swimming. The resulting bimodal velocity distributions change in response to different oxygen concentrations and gradients, supporting the biological functions of aerotaxis and confinement.

1. Introduction

A functional metabolism is a crucial factor for the growth and survival of biological cells. It involves the conversion of extracellular molecules into chemical energy supplies. Many microorganisms are able to react to a decrease in such energy supplies by moving towards a microenvironment that can replenish them (energy taxis) [1]. Chemotaxis (energy taxis driven by chemical gradients) is ubiquitous in natural environments, regulating symbiotic interactions [2]. Aerotaxis is the migration of living systems towards areas with favourable oxygen concentrations for their metabolism [3]. Aerotactic bacteria [4–6] accumulate next to air sources forming aerotactic bands [3]. The strategies used by bacteria to effectively sample space in their quest for a better environment depend on their morphology [7]. Typically, bacteria with one flagellum move fast with episodic 180° reversals or reverse-flicks [8]. Bacteria with multiple flagella swim slower, adopting a run-and-tumble strategy [9].

In the present work, we develop a comprehensive framework for the study of aerotaxis, encompassing a novel experimental set-up, a mathematical model, and the analysis of bacterial trajectories. Our set-up reproduces a minimalistic ecological niche, where the microorganisms modify the environment and adapt their behaviour in return. We use the Gram-negative facultative aerobic *Shewanella oneidensis* MR-1 as model bacterial strain [10]. These bacteria shift electrons from an electron donor towards an electron acceptor available in the environment (e.g. solid metals or oxygen) during their respiration [11–13]. Hence, they are presently considered the cornerstone for the development of sustainable technologies for energy production (microbial fuel cells) [14,15] and wastewater treatment [16], as well as biosynthesis of metal

Figure 1. Experimental set-up: (a) closed set-up and (b) open set-up.

nanoparticles [17], heavy metals reduction [18] and biosensors [19]. MR-1 are extremely versatile and responsive to available resources. Their genome has been sequenced [20], allowing intensive physiological and biochemical studies on their rich signal-transduction regulatory systems [21]. Despite the effort put into their genetic and microbiological characterization, their collective behaviour has not been intensively investigated. The present study fills this gap by providing a quantitative characterization of the collective strategies adopted by MR-1 to optimize resources, namely the formation of aerotactic bands [6] and air–liquid biofilms (pellicles) [22,23]. Previous studies have shown that active motility is required for pellicle formation in MR-1 [22–24]. Understanding motility and collective motion is the first crucial step towards technological exploitation.

Here, we investigate the process leading from accumulation to biofilm formation and we clarify how each step is related to different motility types, in terms of velocities and directional changes. We assess the change of their locomotion strategies in response to different ambient conditions (oxygen concentration and gradient), discovering a surprisingly rich behaviour for a monotrichous bacterium. Most remarkably, besides the expected run-reverse [25] and run-reverse-flick patterns [26], we find that they can transition between longitudinal (fast) and sideways (slow) swimming. This mechanism, explainable in light of the recent discovery that some *Shewanella* can move with the flagellum wrapped around their bodies [27], allows them to access motility schemes typical of both mono- and multiflagellate bacteria. The resulting velocity distributions are bimodal and continuously changing with the local environment. Our work discloses how the dynamic tuning of the uncommon hybrid locomotion patterns of MR-1 regulates their collective behaviour, from the accumulation in aerotactic bands to the inception of biofilm formation.

2. Material and methods

2.1. Cultivation

We use *Shewanella oneidensis* MR-1 bacteria (Zentrum für Angewandte Geowissenschaften, Universität Tübingen). The bacteria

are cultivated in aerobic incubation at 25°C with 150 r.p.m. shaking, first in Luria–Bertrani broth (Roth, Karlsruhe, Germany), then in minimal medium (MM) [28] with 20 mM sodium lactate (Roth, Karlsruhe, Germany) as the substrate, following the procedure described in [29]. The latter culture is harvested in the early exponential growth ($OD_{600} = 0.2 - 0.5$) and used for the experiments. All the figures are realized using cultures with $OD_{600} = 0.3$, unless otherwise specified. Further details are provided in the electronic supplementary material.

2.2. Experimental set-up

We have two types of set-up, featuring an air-tight closed chamber ('closed set-up', figure 1a) and a chamber open to the air ('open set-up', figure 1b).

Closed set-up. We build the closed chamber using a microscope glass slide, a coverslip and a spacer, with the following procedure. We prepare the glass slide and the coverslip by washing them in consecutive steps in acetone, isopropanol and water, to prevent adhesion of the bacterial flagellum to the surface. By means of a double-coated adhesive tape 30, 50 or 100 μm thick (no. 5603, no. 5605 and no. 5015P, Nitto Denk Corporation, Japan), we fabricate a mask 40×40 mm with an internal square-shaped chamber 15×15 mm and we attach it to the glass slide $76 \times 52 \times 1$ mm (Marienfeld, Germany). Using a pipette, we deposit a few droplets of the bacterial culture inside the internal chamber. We then cover the chamber with a coverslip 18×18 mm (no. 1.5, Menzel-Gläser, Germany) and uniformly distribute a thick layer of silicon vacuum grease (Dow Corning, Midland, MI, USA) all around the mask and the coverslip, to prevent air leakages into the chamber. One or more bubbles remain trapped inside the chamber. We discard the samples with bubbles with eccentricity higher than 0.5 or closer than 10 bubble radii to each other or to the chamber walls. To produce samples without air bubbles, for the control experiments and the tracking of trajectories, the coverslip is deposited in a slower fashion.

Open set-up. We build the open chamber using the microfluidic device $\mu\text{-Slide I Luer}$ (Ibidi, Martinsried, Germany), in combination with a glass coverslip D 263 M Schott (2.5×7.5 cm). After assembly, the device consists of a rectangular channel $50 \text{ mm} \times 5 \text{ mm} \times 450 \mu\text{m}$, with the extremes connected to the air through two outlets. The culture medium with the bacteria is introduced inside the channel with a syringe, creating a meniscus, located approximately in the middle of the channel. The outlet on the liquid side is closed with a plastic cap, and made

air-tight by applying a thick layer of silicon vacuum grease (Dow Corning, Midland, MI, USA). The other outlet, on the air side, is left open.

2.3. Imaging

For the dark-field imaging, we use an upright microscope Leica DM 2500M (10×, 20× and 40× magnification). For the phase-contrast imaging, we use an inverted microscope Leica DMi8 (100× magnification). The image acquisition of the pictures is done with a Canon EOS 600D camera. The videos for the particle tracking are taken in the closed set-up (30 μm thick) with a sCMOS Camera pco.edge 4.2 (PCO AG, Germany) at 100 fps (20× and 40× magnification). In particular, all presented figures are obtained with 20× magnification, while 40× magnification was used to verify the precision of the tracking procedure.

2.4. Particle tracking

For the considered chamber thickness, the trajectories are quasi-two-dimensional, hence inhibiting artefacts appearing in the analysis of three-dimensional trajectories [30]. We analyse the trajectories of the bacteria, with the two-dimensional particle tracking MATLAB software u-Track [31] and we individually check them by visual inspection. The videos are pre-processed with an in-house code, developed in MATLAB, to remove the sessile bacteria and the occasional light reflections in the background. The results of the tracking are analysed using another in-house software, written in MATLAB. For the details of the pre- and post-processing algorithms and the optimal parameters used for the tracking, we refer the reader to [32]. Details on the statistical analysis are provided in the section ‘Statistical analysis’ in the electronic supplementary material. Examples of the videos used for the analysis of trajectories can be found in the electronic supplementary material.

2.5. Model and equations

We develop a model based on a continuum field description, consisting of a system of coupled differential equations. We consider an idealized axially-symmetric geometry, with a cylindrical air bubble immersed in an infinite liquid medium. The gas inside the bubble (air) is treated as a binary mixture of perfect gases, i.e. nitrogen and oxygen, diffusing into the liquid, causing the bubble to shrink. The oxygen is depleted by the bacteria. The nitrogen is only passively diffusing and does not interact with the bacteria, but it is included to prevent the total dissolution of the bubble. The model describes the evolution of the bubble radius $R(t)$ and the concentration fields of oxygen $c(r, t)$, nitrogen $n(r, t)$ and *Shewanella* $s(r, t)$, where t is the time and r the radial coordinate. The flux of bacteria is modelled as the superposition of a diffusive flux, due to their random motility, and a chemotactic flux, responsible for the drift along the oxygen gradient [33,34]. The bacterial growth and the transition between the aerobic and anaerobic state are recorded; bacterial death is neglected, as it is irrelevant on the timescales of the experiment. The mechanism is described in the section Results. Adsorption at the air–liquid interface for the partial adsorption of the bacteria at the air–liquid interface is incorporated in the boundary conditions. The temperature of the system T_∞ is homogeneous and constant in time. We summarize here the equations of the model and refer the reader to ‘Model derivation’ and ‘Estimate of parameters’ in the electronic supplementary material for details.

Time-evolution of the bubble radius

$$\dot{R} = \frac{T_\infty}{p_g - \frac{\sigma}{2R}} \left[D_{O_2} \mathcal{R}_{O_2}^* \frac{\partial c}{\partial r} \Big|_{R(t)} + D_{N_2} \mathcal{R}_{N_2}^* \frac{\partial n}{\partial r} \Big|_{R(t)} \right], \quad (2.1)$$

where the dot denotes a time derivative, p_g is the total pressure inside the bubble, σ the surface tension, D_{O_2} , D_{N_2} the diffusive constants and $\mathcal{R}_{O_2}^*$, $\mathcal{R}_{N_2}^*$ the specific gas constants of oxygen and nitrogen.

Advection–diffusion equation for the nitrogen concentration [35,36]

$$\frac{\partial n}{\partial t} + \frac{R\dot{R}}{r} \frac{\partial n}{\partial r} = D_{N_2} \nabla^2 n. \quad (2.2)$$

The second term on the left-hand side (LHS) describes advection due to the bubble shrinkage, while the right-hand side (RHS) describes gas diffusion.

Advection–diffusion equation with consumption for the oxygen concentration

$$\frac{\partial c}{\partial t} + \frac{R\dot{R}}{r} \frac{\partial c}{\partial r} = D_{O_2} \nabla^2 c - A_0 s \frac{c}{C_s + c}, \quad (2.3)$$

where A_0 is a constant indicating the mass of oxygen consumed per mass of *Shewanella* produced, and C_s is the half-saturation constant of *Shewanella*. The last term on the RHS describes the consumption of oxygen due to bacterial respiration.

Advection–diffusion–aerotaxis equation for the Shewanella concentration

$$\frac{\partial s}{\partial t} + \frac{R\dot{R}}{r} \frac{\partial s}{\partial r} = \nabla \cdot [\mu(c) \nabla s - \chi_0 s \alpha(c) \nabla c] + v s \frac{c}{C_s + c}. \quad (2.4)$$

Here, the last term on the RHS describes the bacterial growth, with v the maximum specific growth rate. The terms under divergence on the RHS denote the bacterial flux, χ_0 is the chemotactic sensitivity, $\mu(c) = \mu_0 H(c - c_T)$ the random motility coefficient, with μ_0 its maximum value, H is the Heaviside function and c_T the oxygen concentration corresponding to the aerobic/anaerobic transition; $\alpha(c)$ is a function expressing the dependence of the aerotactic response on the local oxygen concentration

$$\alpha(c) = \frac{K_D}{(K_D + c)^2} H(c - c_T) [1 - H(c - c_{opt})], \quad (2.5)$$

with K_D the receptor dissociation constant, c_{opt} the optimal oxygen concentration for the bacteria, $H(c - c_T)$ and $H(c - c_{opt})$ the Heaviside functions centred around c_T and c_{opt} , respectively, replaced by their smeared versions in the numerical treatment [37]. Our heuristic formulation is in line with the classical formulations [38,39], accounting for downregulation (the effect of attractant concentration on the number of expressed cell surface receptors) and receptor saturation, but it additionally incorporates the suppression of aerotaxis when the bacteria reach their favourite concentration range, and the transition to anaerobic functioning at low oxygen concentration (extended data figure 8). For the boundary conditions at infinity, we take all concentration fields with null spatial derivative. At the bubble wall, for oxygen and nitrogen, we assume equilibrium with the gas inside the bubble, by means of Henry’s Law; for *Shewanella* we assume partial adsorption, regulated by the oxygen, in line with the experimental findings (see Results. Adsorption at the air–liquid interface). Hence, we impose the bacterial flux J at the bubble wall

$$J|_{R(t)} = r_a s \epsilon(c)|_{R(t)}, \quad (2.6)$$

with r_a the adsorption constant and $\epsilon(c)$ an increasing function of the oxygen concentration; for simplicity $\epsilon(c) = c$.

2.6. Numerical method

The system is solved with a pseudospectral collocation method, previously developed and tested in [35,40], implemented with an in-house code written in Fortran. Details on the implementation are given in ‘Numerical method’ in the electronic supplementary material.

Figure 2. Time evolution of the aerotactic band formed around a confined bubble. The initial bubble radius is $R_0 = 328 \mu\text{m}$. The closed set-up is imaged in dark-field at times (a) 20 min, (b) 25 min, (c) 30 min, (d) 37 min, (e) 42 min, (f) 50 min after closure of the sample.

3. Results and discussion

All the experiments are performed with *S. oneidensis* MR-1 bacteria. The main set-up, the ‘closed set-up’ (figure 1a), consists of an air-tight closed chamber filled with the liquid bacterial culture. An entrapped micrometric air bubble provides a limited oxygen supply. The air diffuses from the bubble into the liquid, where the bacteria reduce the oxygen during their respiration, depleting it. This configuration is used to create a time-variable oxygen concentration gradient in the sample. A second set-up, the ‘open set-up’ (figure 1b), consists of a microfluidic device, partially filled with the bacterial culture, open to the air on one side and closed on the other side. The air side acts as an unlimited oxygen supply. This configuration is used to create a time-constant oxygen concentration gradient. Imaging is done with dark-field and phase-contrast microscopy.

3.1. Dynamic evolution of the aerotactic band

In the closed set-up, MR-1 form an aerotactic band around the bubble (electronic supplementary material, video S1, figure 2). We describe here the formation and evolution of the band. Initially, the bacteria have a uniform concentration and a high motility in the whole sample. After 20 min, they

start to aggregate around the bubble, forming a distinct band (figure 2a,b). The bacteria move inside the band and in the area between the bubble and the band, while the rest of the sample enters a non-motile subdiffusive state (electronic supplementary material, video S2), as revealed by the time evolution of the mean squared displacement (MSD) (extended data figure 1). As the bacteria consume the oxygen, the band progressively advances towards the bubble (figure 2c), leaving behind a depletion layer (figure 2d) with non-motile bacteria. Eventually, the band reaches the bubble (figure 2e) and disappears (figure 2f). At this point, all the bacteria are in the non-motile state. Depending on initial conditions, the band forms in 5–40 min and disappears 15–60 min after closing the sample. The formation of aerotactic bands has been reported also for other bacteria [41–47]. For different species, this phenomenon originates from different physiological responses of cells. In particular, a direct response to oxygen concentrations results in accumulation at the highest oxygen concentration (*Bacillus subtilis* [48]). Conversely, indirect monitoring of the effect of oxygen on the cell metabolism results in bands located at a preferred oxygen concentration [49], like for *Escherichia coli* [47], *Azospirillum brasilense* [43], *Spirochaeta aurantia* [44] and *Shewanella* [50].

Figure 3. Time evolution of bacterial concentration profiles around a confined bubble. Solid lines depict experiments in the closed set-up, dashed lines numerical solution of the model; x is the distance from the interface, s the bacterial concentration, $R_0 = 193 \mu\text{m}$ the initial radius and $s_0 = 7.11 \times 10^7$ bacteria/ml the initial bacterial concentration. Each colour represents a different instant in time: $t_1 = 18$ min, $t_2 = 21$ min, $t_3 = 23$ min, $t_4 = 24$ min, $t_5 = 25$ min.

We provide a quantitative evaluation of the band dynamics in our experiment by tracking the time evolution of the concentration profiles of *Shewanella* around the bubble (figure 3). The bacterial density is estimated from dark-field images, as proportional to the local light intensity. We compare the experimental results (solid lines) with the predictions from an in-house numerical model (dashed lines) (electronic supplementary material), finding good qualitative and quantitative agreement. The model captures how the maximum bacterial concentration increases, as the band moves towards the bubble. The timescales for band formation and disappearance are reproduced with a maximum error of 5% and 15%, respectively.

In the open set-up, the observed phenomenon is analogous but a steady state is reached after 2 h: the *Shewanella* concentration profile becomes stable, with a band at a fixed distance from the air–liquid interface (extended data figure 3a). The bacteria in the band retain their motility and never enter the non-motile state. The observation is unaltered after 24 h. We conclude that the transition between motile and non-motile bacteria observed in the closed set-up corresponds to the transition between aerobic and anaerobic functioning.

3.2. Adsorption at the air–liquid interface

Shewanella form a biofilm called a pellicle at air–liquid interfaces [22,23]. We clarify here how the oxygen level regulates the process.

With dark-field imaging, we do not have direct access to the air–liquid interface itself, which is saturated by the high amount of scattered light. However, we can extract equivalent information by imaging the bacterial concentration in the liquid in its immediate neighbourhood. In the closed set-up, such a concentration remains constant during the initial phases of the band formation (figure 3, curves t_1 , t_2); later on, it increases while the band moves towards the interface (figure 3, curves t_3 – t_5). This behaviour indicates that, initially, the bacterial flux at the bubble wall is entirely adsorbed, while it gets progressively reflected as the oxygen supply decreases. Conversely, in the open set-up,

where the oxygen supply is unlimited and the oxygen concentration at the interface is constant, the nearby bacterial concentration remains unvaried throughout the whole process. This observation indicates that the incoming flux is entirely adsorbed by the interface. Phase-contrast microscopy reveals a layer of bacteria piled up at the air–liquid interface and surrounded by a depletion zone. In the closed set-up, entailing a limited oxygen supply, such a layer does not increase indefinitely, but stops growing presumably below a certain oxygen concentration (figure 4). Conversely, with an unlimited oxygen supply, the layer keeps growing (extended data figure 4). We conclude that the oxygen concentration regulates the adsorption of MR-1 at the air–liquid interface.

On occasion, small droplets are captured inside the bubble in the closed set-up. Their observation allows direct imaging of the pellicle formation process (extended data figure 5; electronic supplementary material, video S3). Here, two air–liquid interfaces are present: (i) between the bubble and the liquid, where a moving aerotactic band appears, and (ii) between the droplet and the bubble. On the latter interface, we observe the presence of bacterial clusters with active motion. They progressively grow in size, by incorporating colliding swimming bacteria (electronic supplementary material, video S4). The process slows down in time. Eventually, the bacteria stop moving and the clusters stop growing. This happens exactly when the band surrounding (i) reaches the bubble, i.e. when the lack of oxygen induces the anaerobic transition everywhere. We infer a connection between bacterial motility and pellicle formation, both regulated by the oxygen level. This conclusion is in line with previous findings that, on one side, active motility is required for pellicle formation in MR-1 [23,24], on the other side, oxygen promotes the transcription of genes encoding cell-to-cell adhesion [51], relevant for clustering upon bacterial contact.

3.3. Hybrid locomotion patterns

We show here that MR-1 feature hybrid locomotion patterns, even in the absence of oxygen gradients. The trajectories of the motile bacteria are analysed in a variation of the closed set-up where no bubble is present, immediately after closure of the sample (i.e. with high uniform oxygen concentration).

To evaluate the motility, we consider the probability distribution function (PDF) of the absolute values of instantaneous velocities, derived from the time sampling of trajectories. Around 15% of bacteria perform a stop-and-go type of motion: they alternate short pauses and runs (electronic supplementary material, video S5). Hence, there is a peak in the PDF, around $0 \mu\text{m s}^{-1}$ (extended data figure 6). We will show in the next section that this is consistent with the inception of biofilm formation. By removing the pauses and accounting only for the motile parts of trajectories, the PDF becomes bimodal, with two peaks around $25 \mu\text{m s}^{-1}$ and $75 \mu\text{m s}^{-1}$ (figure 5a). *A priori*, this could correspond to two different scenarios: two populations of bacteria with a constant velocity (fast or slow), or bacteria changing their velocity, by alternating fast and slow runs. We compare the PDF of the instantaneous velocities with the PDF of the average velocities along the trajectories (extended data figure 7). Coincidence between the two PDFs would indicate that there are two populations with constant velocity and no switching. We observe that the PDF of the average velocities

Figure 4. Biofilm precursor formed at the air–liquid interface, with limited oxygen supply. The closed set-up is imaged after the band has reached the bubble: (a) dark-field imaging shows a depletion layer, around the bubble, with a reduced bacterial density; (b) phase-contrast imaging reveals a layer of piled up bacteria at the bubble interface.

Figure 5. PDF of instantaneous velocities of MR-1 in the closed set-up without oxygen gradient (a) 1 min and (b) 10 min after sealing the sample. Each histogram includes approximately 4000 points, derived from time sampling of approximately 700 trajectories with constant time intervals of 0.07 s.

along the trajectories is still bimodal but the relative amplitude of the peaks has changed. Therefore, we conclude that both scenarios occur: there are fast and slow bacteria but also bacteria changing their velocity along a single trajectory, as confirmed by visual inspection.

To assess how the speed is related to the reorientation strategies, we analyse the angles between consecutive pieces of trajectories and we sort them based on the average speed preceding the turn (figure 6). We find three regimes with significant differences: slow (below $25 \mu\text{m s}^{-1}$), fast (above

Figure 6. PDF of consecutive angles performed by MR-1, in the closed set-up without oxygen gradient, for different instantaneous velocity ranges: (a) below $25 \mu\text{m s}^{-1}$, (b) between $25 \mu\text{m s}^{-1}$ and $60 \mu\text{m s}^{-1}$ and (c) above $60 \mu\text{m s}^{-1}$. The inserts depict typical locomotion patterns of the bacteria in such velocity ranges: (a) sideways, (b) run-reverse and (c) straight longitudinal runs. They represent the stroboscopic mapping of dark-field images with constant time lags of (a) 0.1 s, (b) 0.06 s, (c) 0.04 s. The arrows indicate the direction of motion.

$60 \mu\text{m s}^{-1}$) and intermediate ($25\text{--}60 \mu\text{m s}^{-1}$). Slow bacteria span all angles between 0° and 180° ; by visual inspection, we determine that more than 80% of these bacteria swim

Figure 7. PDF of instantaneous velocities of MR-1 at different locations. The initial bubble radius is $R_0 = 514 \mu\text{m}$. The closed set-up is imaged 20 min after sealing the sample: (a) at the bubble interface, (b) between the bubble and the aerotactic band and (c) on the band. Each histogram includes approximately 10 000 points, evaluated in the same way as in figure 5.

sideways with respect to their main body axis. Fast bacteria swim with straight or continuously curving trajectories, parallel to their longitudinal axis, and turn by small angles (up to 70°). Bacteria with intermediate velocities exhibit an intermediate behaviour, performing either small angles (0° – 80°) or reversals (140° – 180°); they mostly swim parallel to their main axis but can switch to sideways motion (electronic supplementary material, video S6).

This differentiation within the same population of mono-flagellate bacteria is remarkable, as it combines features of typical strategies used by morphologically different species to explore the surroundings. Monotrichous bacteria, such as approximately 70% of marine bacteria, are typically fast (up to $75 \mu\text{m s}^{-1}$), moving by straight or curved trajectories [25]. They change direction by inverting their flagellar rotation, inducing reversals (150° – 180° angles), like MR-1 in the intermediate velocity runs. Peritrichous bacteria, such as most enteric bacteria, are typically slower (approx. $30 \mu\text{m s}^{-1}$) and move by run-and-tumbling [25]. They swim in almost straight runs, with the flagella bundled together, stopping and tumbling, when one flagellum inverts its rotation disrupting the bundle. All angles occur upon reorientation, like in the slow runs of MR-1 [9].

Hybrid locomotion mechanisms have been reported for several bacterial species, as a way to enhance direction randomization [8]. Other monotrichous bacteria alternate 180° reversals with 90° flicks induced by the flagellum bending (run-reverse-flick) [8,26]. Though MR-1 can perform this type of motion, the absence of the peak at 90° shows that it

is not their main strategy. The predominance of sideways swimming in the slow regime, together with the possibility to switch between sideways and longitudinal motion, suggests that they generate torque at low velocities by modulating the coiling shape of the flagellum. This is in line with the recent discovery that some bacteria, including the conspecific *S. putrefaciens*, can swim with the flagellum wrapped around their body [27,52].

3.4. Impact of oxygen concentration and gradient

We study here how the ambient conditions influence the motility and the reorientation strategies of MR-1, determining different collective behaviours, namely band and biofilm formation. We perform two experiments to separately test the impact of the oxygen concentration and the oxygen gradient. To this aim, we use two variations of the closed set-up: without a bubble (i.e. uniform oxygen concentration, decaying in time) and with a bubble (i.e. with an oxygen gradient).

To evaluate the effect of the oxygen concentration, we examine the samples without bubbles for 15 min after sealing. As the bacteria deplete the oxygen, the peak at slow velocities in the PDF of instantaneous velocities progressively disappears (figure 5b). The PDF becomes unimodal with a peak around $80 \mu\text{m s}^{-1}$. This observation is compatible with the interruption of biofilm formation at low oxygen concentration. We conclude that, like *S. putrefaciens* [53], *S. oneidensis* adapts its speed in response to the local concentration of a chemoattractant. Hence, it not only performs

chemotaxis (reaction to a concentration gradient) but also chemokinesis (reaction to the concentration itself).

To investigate the effect of the oxygen gradient, we analyse the case with an entrapped bubble, featuring a moving band. Figure 7 displays the PDF of instantaneous velocities, at three different locations: (a) at the air–liquid interface, (b) between the bubble and the band (middle) and (c) on the band. The model shows that the oxygen concentration is always monotonically decreasing in the radial direction. At the interface and in the middle, the PDFs reproduce the trend observed without bubbles, for decreasing oxygen concentrations: the bimodal distribution in (a) turns unimodal in (b), where the oxygen concentration is lower. On the band, however, such a trend is disrupted: the peak at slow velocities reappears and the number of particles with an intermediate speed ($25\text{--}60\ \mu\text{m s}^{-1}$) increases. The PDFs of turning angles, sorted by velocity ranges, are unvaried with respect to the case without oxygen gradient. We conclude that different speed ranges correspond to different reorientation strategies, fulfilling specific biological functions. In particular, a peak at slow velocities, where *Shewanella* reorients in any direction, emerges in environmental conditions where there is no preferential direction of swimming. At the bubble wall, it relates to the inception of the biofilm formation. In the areas where the bacteria accumulate (aerotactic band), it reflects the fact that MR-1 is in its optimal oxygen concentration range. On the band, the increased number of bacteria swimming at intermediate velocities ($25\text{--}60\ \mu\text{m s}^{-1}$), favouring straight runs or reversals, guarantees the persistence in the region: when a bacterium exits the band, it either stops or immediately comes back. In the region between the bubble and the band, high velocities are dominant (above $60\ \mu\text{m s}^{-1}$), mostly associated with straight runs. This is typical of directional motion and reflects the aerotaxis towards the band. Summarizing, slow velocities are associated with sideways displacements and isotropic direction randomization, intermediate velocities present run-reverse patterns producing confinement, high velocities encompass straight runs, related to directional aerotactic motion.

4. Conclusion

The present work is a comprehensive study of the collective behaviour of *Shewanella oneidensis* MR-1 next to an air source, from accumulation to the air–liquid biofilm formation. We studied the dynamic evolution of the aerotactic band gathering around a bubble, both with experiments and theory. When the air source is not confined, the band remains at a constant distance from the air–liquid interface. Conversely, when the air source is confined and the oxygen is depleted by the bacteria, the band moves towards the interface, eventually reaching it and disappearing. The local oxygen concentration regulates the biofilm formation, by affecting the bacterial adsorption at the interface and their motility. Both are suppressed when the oxygen is entirely depleted. In particular, when the oxygen concentration drops below a certain threshold, the bacteria turn from motile to non-motile (aerobic to anaerobic transition), and the biofilm formation stops.

The system at hand is a minimal model of an ecological niche, where the inhabitants modify the environment and their behaviour dynamically adapts in return. We

characterized the collective locomotion strategies of MR-1 in response to the environmental conditions (oxygen concentration and gradient), by tracking their trajectories. They explore the surroundings with a combination of fast and slow runs, spanning a broad range of velocities. Their velocity distributions are bimodal and dynamically change with the ambient conditions, posing a challenge to the concept of average velocity of a bacterial population. Different velocities are associated with specific reorientation strategies, realizing different biological functions, such as aerotaxis or confinement. Fast runs are mostly straight or curved; their number increases when the bacteria perform aerotaxis in response to an oxygen gradient. Slow runs correspond to a higher direction randomization, with turns spanning all angles. They are associated with favourable conditions for the bacteria, such as their preferred oxygen concentration range in the aerotactic band, or to the inception of biofilm formation. Surprisingly for a monotrichous bacterium, they are mostly realized through sideways swimming. At intermediate velocities the bacteria alternate straight runs and reversals. In the aerotactic band, this behaviour supports confinement, correcting the trajectories leading away from the optimal oxygen concentration. The possibility to switch between fast (longitudinal) and slow (mostly sideways) swimming allows MR-1 to access typical velocity and reorientation ranges of both mono- and multiflagellate bacteria, tremendously increasing their biological competitiveness.

From an applied-science perspective, the present work establishes a model of biofilm formation that combines measurements on a versatile experimental set-up and theoretical modelling of the evolution of the bands formed by MR-1 around air bubbles. The matching with the model provides guidelines for parameter choices that can severely speed up prototyping of bioremediation devices and microbial fuel cells (e.g. with air-breathing electrodes [54,55]), where air–liquid interfaces may be exploited to accelerate accumulation of MR-1. Moreover, from a biophysical perspective the present work establishes the link between the intriguing hybrid locomotion strategies of MR-1 and their aerotactic collective behaviour. It shows how the adaptive tuning of their bimodal velocity distributions regulates the process leading from confinement in aerotactic bands to biofilm formation.

Data accessibility. The authors declare that the relevant data supporting the findings of this study are available as electronic supplementary material, Source Data. Any additional data that support the findings of this study as well as the in-house codes are available from the corresponding author upon request.

Authors' contributions. I.G., L.S. and M.M. designed the experiments. I.G. and L.S. performed the experiments. L.S. and J.V. developed the numerical model. T.B. and L.S. did the tracking. L.S., I.G. and J.V. did the statistical analysis and interpretation of the data. J.V. and M.M. contributed with comments. L.S., I.G. and J.V. wrote the draft. All the authors contributed to subsequent revisions.

Competing interests. The authors declare that they have no competing financial interests.

Funding. M.M. gratefully acknowledges support from the Deutsche Forschungsgemeinschaft (SFB 937, project A20). We acknowledge financial support from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007–2013/under REA grant agreement no. 628154, from the MaxSynBio Consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society, and from the Deutsche Forschungsgemeinschaft (SFB 937, project A20).

Acknowledgements. We thank Laura Turco for technical support in the experiments, Gal Scholnik for performing preliminary experiments, Andreas Kappler for providing the bacterium *S. oneidensis* MR-1 and Mathias Schröter for support in preliminary bacterial tracking.

We acknowledge discussions with Anupam Sengupta, Filippo Meno-lascina, Roman Stocker and Martin Kröger. We thank Hans Christian Öttinger, James Clewett and Martin Callies for feedback on the manuscript.

References

- Schweinitzer T, Josenhans C. 2010 Bacterial energy taxis: a global strategy? *Arch. Microbiol.* **192**, 507–520. (doi:10.1007/s00203-010-0575-7)
- Raina JB, Fernandez V, Lambert B, Stocker R, Seymour JR. 2019 The role of microbial motility and chemotaxis in symbiosis. *Nat. Rev. Microbiol.* **17**, 284–294. (doi:10.1038/s41579-019-0182-9)
- Taylor BL, Zhulin IB, Johnson MS. 1999 Aerotaxis and other energy-sensing behavior in bacteria. *Annu. Rev. Microbiol.* **53**, 103–128. (doi:10.1146/annurev.micro.53.1.103)
- Baracchini O, Sherris JC. 1959 The chemotactic effect of oxygen on bacteria. *J. Pathol. Bacteriol.* **77**, 565–574. (doi:10.1002/path.1700770228)
- Adler J. 1966 Chemotaxis in bacteria. *Science* **153**, 708–716. (doi:10.1126/science.153.3737.708)
- Li J. 2008 Molecular mechanisms of behavioral responses in *Shewanella oneidensis* MR-1. PhD thesis, Johns Hopkins University, Baltimore.
- Son K, Brumley DR, Stocker R. 2015 Live from under the lens: exploring microbial motility with dynamic imaging and microfluidics. *Nat. Rev. Microbiol.* **13**, 761–775. (doi:10.1038/nrmicro3567)
- Stocker R. 2011 Reverse and flick: hybrid locomotion in bacteria. *Proc. Natl Acad. Sci. USA* **108**, 2635–2636. (doi:10.1073/pnas.1019199108)
- Berg HC, Brown DA. 1972 Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. *Nature* **239**, 500–504. (doi:10.1038/239500a0)
- Venkateswaran K *et al.* 1999 Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int. J. Syst. Evol. Microbiol.* **49**, 705–724. (doi:10.1099/00207713-49-2-705)
- Gorby YA *et al.* 2006 Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc. Natl Acad. Sci. USA* **103**, 11 358–11 363. (doi:10.1073/pnas.0604517103)
- Subramanian P, Pirbadian S, El-Naggar MY, Jensen GJ. 2018 Ultrastructure of *Shewanella oneidensis* MR-1 nanowires revealed by electron cryotomography. *Proc. Natl Acad. Sci. USA* **115**, E3246–E3255. (doi:10.1073/pnas.1718810115)
- Benchari S, Ward MJ. 2005 Chemotactic responses to metals and anaerobic electron acceptors in *Shewanella oneidensis* MR-1. *J. Bacteriol.* **187**, 5049–5053. (doi:10.1128/JB.187.14.5049-5053.2005)
- Logan BE. 2009 Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* **7**, 375–381. (doi:10.1038/nrmicro2113)
- Di W, Defeng X, Xiaoxue M, Bingfeng L, Changhong G, Nanqi R. 2013 Electricity generation by *Shewanella* sp. HN-41 in microbial fuel cells. *Int. J. Hydrogen Energy* **38**, 15 568–15 573. (doi:10.1016/j.ijhydene.2013.04.081)
- Rabaey K, Rozendal R. 2010 Microbial electrosynthesis—revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* **8**, 706–716. (doi:10.1038/nrmicro2422)
- Ng CK *et al.* 2013 Influence of outer membrane c-type cytochromes on particle size and activity of extracellular nanoparticles produced by *Shewanella oneidensis*. *Biotechnol. Bioeng.* **110**, 1831–1837. (doi:10.1002/bit.24856)
- Ng CK, Cai TKC, Song H, Cao B. 2013 Reductive formation of palladium nanoparticles by *Shewanella oneidensis*: role of outer membrane cytochromes and hydrogenases. *RSC Adv.* **3**, 22 498–22 503. (doi:10.1039/C3RA44143A)
- Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K. 2006 Microbial fuel cells: methodology and technology. *Environ. Sci. Technol.* **40**, 5181–5192. (doi:10.1021/es0605016)
- Heidelberg JF *et al.* 2002 Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. *Nat. Biotechnol.* **20**, 1118–1123. (doi:10.1038/nbt749)
- Fredrickson JK *et al.* 2008 Towards environmental systems biology of *Shewanella*. *Nat. Rev. Microbiol.* **6**, 592–603. (doi:10.1038/nrmicro1947)
- Liang Y, Gao H, Chen J, Dong Y, Wu L, He Z, Liu X, Qiu G, Zhou J. 2010 Pellicle formation in *Shewanella oneidensis*. *BMC Microbiol.* **10**, 291. (doi:10.1186/1471-2180-10-291)
- Armitano J, Méjean V, Jourlin-Castelli C. 2013 Aerotaxis governs floating biofilm formation in *Shewanella oneidensis*. *Environ. Microbiol.* **15**, 3108–3118. (doi:10.1111/1462-2920.12158)
- Genfu W, Feifei J. 2019 Pellicle development of *Shewanella oneidensis* is an aerotaxis-piloted and energy-dependent process. *Biochem. Biophys. Res. Commun.* **519**, 127–133. (doi:10.1016/j.bbrc.2019.08.144)
- Johansen JE, Pinhassi J, Blackburn N, Zweifel UL, Hagström A. 2002 Variability in motility characteristics among marine bacteria. *Aquat. Microb. Ecol.* **28**, 229–237. (doi:10.3354/ame028229)
- Xie L, Altindal T, Chattopadhyay S, Wu XL. 2011 Bacterial flagellum as a propeller and as a rudder for efficient chemotaxis. *Proc. Natl Acad. Sci. USA* **108**, 2246–2251. (doi:10.1073/pnas.1011953108)
- Kühn MJ, Schmidt FK, Eckhardt B, Thormann KM. 2017 Bacteria exploit a polymorphic instability of the flagellar filament to escape from traps. *Proc. Natl Acad. Sci. USA* **114**, 6340–6345. (doi:10.1073/pnas.1701644114)
- Melzer S, Winter G, Jäger K, Hübschmann T, Hause G, Syrowatka F, Harms H, Tárnok A, Müller S. 2015 Cytometric patterns reveal growth states of *Shewanella putrefaciens*. *Microb. Biotechnol.* **8**, 379–391. (doi:10.1111/1751-7915.12154)
- Scholnik G, Schmidt M, Mazza MG, Harnisch F, Musat N. 2016 In situ analysis of a silver nanoparticle-precipitating *Shewanella* biofilm by surface enhanced confocal raman microscopy. *PLoS ONE* **10**, 1–23. (doi:10.1371/journal.pone.0145871)
- Figueroa-Morales N, Soto R, Junot G, Darnige T, Douarche C, Martinez VA, Lindner A, Clément E. 2020 3D spatial exploration by *E. coli* echoes motor temporal variability. *Phys. Rev. X* **10**, 021004. (doi:10.1103/PhysRevX.10.021004)
- Jaqaman K, Loeke D, Mettlen M, Kuwata H, Grinstein S, Schmid SL, Danuser G. 2008 Robust single-particle tracking in live-cell time-lapse sequences. *Nat. Methods* **5**, 695–702. (doi:10.1038/nmeth.1237)
- Breithaupt T. 2018 Analysis of the motility modes of *Shewanella oneidensis* MR-1. BSc thesis, Georg-August Universität, Göttingen, Germany.
- Keller EF, Segel LA. 1971 Model for chemotaxis. *J. Theor. Biol.* **30**, 225–234. (doi:10.1016/0022-5193(71)90050-6)
- Keller EF, Segel LA. 1971 Traveling bands of chemotactic bacteria: a theoretical analysis. *J. Theor. Biol.* **30**, 235–248. (doi:10.1016/0022-5193(71)90051-8)
- Stricker L, Prosperetti A, Lohse D. 2011 Validation of an approximate model for the thermal behavior in acoustically driven bubbles. *J. Acoust. Soc. Am.* **130**, 3243–3251. (doi:10.1121/1.3626132)
- Shpak O, Stricker L, Versluis M, Lohse D. 2013 The role of gas in ultrasonically driven vapor bubble growth. *Phys. Med. Biol.* **58**, 2523–2535. (doi:10.1088/0031-9155/58/8/2523)
- Xu JJ, Ren W. 2014 A level-set method for two-phase flows with moving contact line and insoluble surfactant. *J. Comput. Phys.* **263**, 71–90. (doi:10.1016/j.jcp.2014.01.012)
- Lapidus IR, Schiller R. 1976 Model for the chemotactic response of a bacterial population. *Biophys. J.* **16**, 779–789. (doi:10.1016/S0006-3495(76)85728-1)
- Rivero MA, Tranquillo RT, Buettnner HM, Lauffenburger DA. 1989 Transport models for chemotactic cell populations based on individual cell behavior. *Chem. Eng. Sci.* **44**, 2881–2897. (doi:10.1016/0009-2509(89)85098-5)

40. Stricker L. 2012 Acoustic cavitation and sonochemistry. PhD thesis, University of Twente, Twente, The Netherlands.
41. Barak R, Nur I, Okon Y, Henis Y. 1982 Aerotactic response of *Azospirillum brasilense*. *J. Bacteriol.* **152**, 643–649.
42. Grishanin RN, Chalmina II, Zhulin IB. 1991 Behaviour of *Azospirillum brasilense* in a spatial gradient of oxygen and in a 'redox' gradient of an artificial electron acceptor. *Microbiology* **137**, 2781–2785. (doi:10.1099/00221287-137-12-2781)
43. Elmas M, Alexiades V, O'Neal L, Alexandre G. 2019 Modeling aerotaxis band formation in *Azospirillum brasilense*. *BMC Microbiol.* **19**, 101. (doi:10.1186/s12866-019-1468-9)
44. Greenberg EP, Canale-Parola E. 1977 Chemotaxis in *Spirochaeta aurantia*. *J. Bacteriol.* **130**, 485–494. (doi:10.1128/JB.130.1.485-494.1977)
45. Caraway BH, Krieg NR. 1974 Aerotaxis in *Spirillum volutans*. *Can. J. Microbiol.* **20**, 1367–1377. (doi:10.1139/m74-211)
46. Fischer JP, Cypionka H. 2006 Analysis of aerotactic band formation by *Desulfovibrio desulfuricans* in a stopped-flow diffusion chamber. *FEMS Microbiol. Ecol.* **55**, 186–194. (doi:10.1111/j.1574-695X.2005.00024.x)
47. Douarche C, Buguin A, Salman H, Libchaber A. 2009 *E. coli* and oxygen: a motility transition. *Phys. Rev. Lett.* **102**, 198101. (doi:10.1103/PhysRevLett.102.198101)
48. Hou S, Larsen RW, Boudko D, Riley CW, Karatan E, Zimmer M, Ordal GW, Alam M. 2000 Myoglobin-like aerotaxis transducers in archaea and bacteria. *Nature* **403**, 540–544. (doi:10.1038/35000570)
49. Bibikov S, Biran R, Rudd K, Parkinson J. 1997 A signal transducer for aerotaxis in *Escherichia coli*. *J. Bacteriol.* **179**, 4075–4079. (doi:10.1128/jb.179.12.4075-4079.1997)
50. Zwirnmann H. 2015 Modelling the aerotaxis of *Shewanella oneidensis*. MSc thesis, Georg-August Universität, Göttingen, Germany.
51. McLean JS *et al.* 2008 Oxygen-dependent autoaggregation in *Shewanella oneidensis* MR-1. *Environ. Microbiol.* **10**, 1861–1876. (doi:10.1111/j.1462-2920.2008.01608.x)
52. Hintsche M, Waljor V, Großmann R, Kühn MJ, Thormann KM, Peruani F, Beta C. 2017 A polar bundle of flagella can drive bacterial swimming by pushing, pulling, or coiling around the cell body. *Sci. Rep.* **7**, 16771. (doi:10.1038/s41598-017-16428-9)
53. Barbara GM, Mitchell JG. 2003 Marine bacterial organisation around point-like sources of amino acids. *FEMS Microbiol. Ecol.* **43**, 99–109. (doi:10.1111/j.1574-6941.2003.tb01049.x)
54. Wang Z, Mahadevan GD, Wu Y, Zhao F. 2017 Progress of air-breathing cathode in microbial fuel cells. *J. Power Sources* **356**, 245–255. (doi:10.1016/j.jpowsour.2017.02.004)
55. Szczesny J, Markovic N, Conzuelo F, Zacarias S, Pereira IA, Lubitz W, Plumeré N, Schuhmann W, Ruff A. 2018 A gas breathing hydrogen/air biofuel cell comprising a redox polymer/hydrogenase-based bioanode. *Nat. Commun.* **9**, 1–9. (doi:10.1038/s41467-018-07137-6)