

E. Coli and Oxygen: A Motility Transition

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The motility of *Escherichia coli* is correlated with oxygen concentration. We show that oxygen penetrating into an anaerobic sample induces the coexistence of two domains of motile and nonmotile bacteria. This coexistence generates a bacterial accumulation at the border that propagates slowly with a constant velocity. To show that this front propagation follows general scaling arguments, we characterize the sharp and fast motile to nonmotile transition occurring when bacteria exhaust oxygen. Additionally, we develop a novel technique to quantify oxygen *in situ* without affecting bacteria.

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Life without air is one of Pasteur's main discoveries [1]. He pointed out that some "ferments" were able to live without oxygen by producing butyric acid and he called them "anaerobes." In contact with air, he noticed that these bacteria lost their motility. Thus as soon as anaerobiosis was discovered, a correlation was made between oxygen and the motility of bacteria. He also showed that some microorganisms are facultative anaerobes, using oxygen when it is present but performing fermentation otherwise, the so-called Pasteur's effect [2]. Much later Monod established that this regulation indeed occurs at a genetic level, introducing modern concepts of the regulation of gene expression [3]. *E. coli* is an example of a facultative anaerobe. The biochemical pathways involved in the aerobic and anaerobic states of *E. coli* have been thoroughly investigated [4–6]. In addition, *E. coli* motility has been widely studied [7]. However, the change in bacterial motility depending upon oxygen concentration has not been extensively studied (see [8] for reference) and the way oxygen affects bacterial motility quantitatively is not really known. With few exceptions [8–10], most of the experiments were performed aerobically. It was shown that, in homogeneous environments, the nature of the motion of *E. coli* consists of a succession of runs and tumbles at a frequency of ~ 1 Hz. In heterogeneous environments, the change in this frequency biases the motion and allows phenomena such as chemotaxis [10,11] or aerotaxis [12]. Thus, in a homogeneous environment, bacteria are considered as random walkers [13] [Fig. 1(b)] and their effective diffusion coefficient (D) is used to characterize their mean motility [14,15]. Here, we report a novel technique to spatially and temporally quantify dissolved oxygen *in situ* [16], which enabled us to characterize the change in bacterial motility as a function of local oxygen concentration. In an oxygen gradient, when oxygen penetrates into an anaerobic sample, we observed that bacteria spatially segregate and form a propagating front. To show that this front propagation follows general scaling arguments, we first characterized the motility

transition that occurs when aerobic bacteria run out of oxygen.

To investigate the change in bacterial motility depending on the presence of oxygen, we enclosed bacteria in a sealed sample and we let them consume oxygen until exhaustion. We devised experiments for measuring simultaneously D and the oxygen partial pressure pO_2 in sealed samples as a function of time. *E. coli* bacteria were grown aerobically in a M9 minimal medium supplemented with glucose and casamino acids to midexponential phase, then suspended in a potassium phosphate buffer supplemented with sodium *L* lactate and *L* methionine [15]. In this buffer, bacteria were able to swim but did not divide during the time of the experiment. pO_2 was quantified by measuring the fluorescence of a dye we developed (Ru-micelle) [16]. This molecule is quenched by oxygen and allows direct mea-

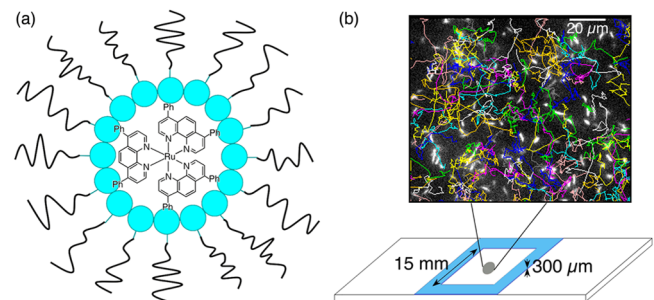


FIG. 1 (color online). (a) Schematic representation of the oxygen dye. The fluorescence of the ruthenium complex is quenched by oxygen. The encapsulation of the ruthenium complex in phospholipid micelles, "Ru-micelles," makes it biologically inert while conserving its sensitivity to oxygen. (b) Bacteria expressing yellow fluorescent protein were suspended in a minimal buffer and deposited in a square adhesive-sealed chamber. Samples were quickly prepared under argon and sealed with epoxy glue. Fluorescent bacteria were then tracked in the focal plane of the microscope, revealing a few thousand 2-dimensional random walks [27]. Movies of 10–20 s were acquired every 2–3 min at a $40\times$ magnification [15].

surement of its concentration within the sample [Fig. 1(a)]. The pO_2 initially present in the sample was $\sim 3 \pm 0.1\%$ (corresponding to the ambient level of oxygen in water) and the bacterial concentration was $\sim (3 \pm 0.3) \times 10^8$ cells/cm³. The bacterial solution was placed in a sealed chamber [Fig. 1(b)]. Because of the oxygen consumption by bacteria, a continuous recording of pO_2 exhibited a linear and continuous decrease from $\sim 2.6 \pm 0.1\%$ to a negligible value within $\sim 30 \pm 10$ min [Fig. 2(a)]. This time period can change slightly depending upon the initial pO_2 and the bacterial concentration in the sample. The linear trend of the decay yielded a consumption rate of oxygen by the bacteria in this buffer of approximately $\sim (3.5 \pm 0.3) \times 10^4$ dioxygen molecules/bacterium (in richer medium bacteria can consume about 10 times more oxygen; see, for instance, [17]).

Additionally, bacteria were also tracked and their trajectories were used to calculate the mean square displacement, the linear fit of which yielded D [Fig. 2(a), inset] [15]. In closed samples, we observed that bacteria had an initial constant diffusion coefficient $D_M \approx (2.8 \pm 1) \times 10^{-11}$ m²/s for the first $\sim 35 \pm 10$ min; they were in a “motile state” [Fig. 2(a)]. Then, within approximately ~ 10 min, all the bacteria started simultaneously to slow down and their diffusion coefficient dropped to $D_{NM} \approx (4.5 \pm 1.6) \times 10^{-13}$ m²/s. Thereafter bacteria exhibited only a passive diffusive behavior similar to particles of a hydrodynamic radius of ~ 1 μ m (approximately the size of *E. coli*) undergoing a Brownian motion; i.e., bacteria were in a “nonmotile state.”

The diffusion coefficient D is a macroscopic parameter revealing the mean motion of bacteria; the motile state of bacteria, however, can also be characterized by its average speed V and its tumbling time τ_{tumb} . Thus, by calculating V , we could deduce τ_{tumb} from the formula $D = V^2 \tau_{\text{tumb}}$ [18] [Fig. 2(b)]. We observed that for the first $\sim 30 \pm 10$ min the average speed remained constant around $\sim 16 \pm 2$ μ m/s and then started to decrease until it reached a minimal value $\sim 3 \pm 0.6$ μ m/s. This slowing down of V was concomitant with complete oxygen depletion and bacteria switched from a motile to a nonmotile state within a time $\tau_s \approx 15 \pm 5$ min [Fig. 2(b)]. We measured a delay of ~ 5 min between the decays of V and D , which suggests that τ_{tumb} increased while V decreased, thus maintaining D constant for another ~ 5 min.

From this experiment, we observed that the diffusivity of bacteria was unaffected by the concentration of oxygen in the solution, as long as it was available. However, as soon as the oxygen was below detectable levels, effects on motility were observed. This reveals that the metabolism of *E. coli* is highly correlated to the presence of oxygen and bacteria undergo a kind of adaptation. In this case however, differing from the adaptation observed by Monod [3], the motility adaptation was not regulated by the synthesis of new proteins. Indeed, bacteria were suspended in a minimal buffer that allowed them to survive but not to sustain protein expression. The sharp motility transition occurred

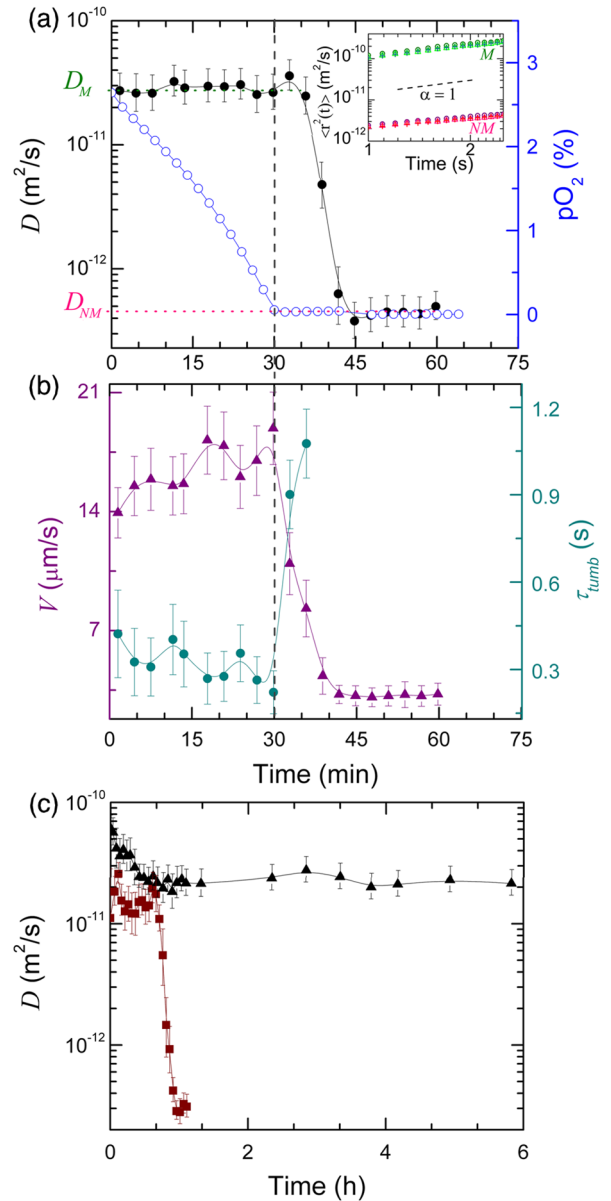


FIG. 2 (color online). Motility of bacteria in a sealed sample as a function of time. (a) Oxygen partial pressure pO_2 (blue open circles) decreases during the first $\sim 30 \pm 10$ min to reach a negligible and stable value. Mean diffusion coefficients D (black solid circles) are obtained from a linear fit of the mean square displacement (MSD). Inset: MSD of both motile (M) and non-motile (NM) bacteria. The dashed line with a slope $\alpha = 1$ is a guide to the eyes. (b) Calculated average speed V (purple triangles) and deduced tumbling time τ_{tumb} (cyan circles). τ_{tumb} is only represented during the first ~ 35 min while bacteria were actively diffusive. (c) Diffusion coefficient D measured in a buffer supplemented with: 250 mM threonine (red squares) and 250 mM serine (black triangles).

within a time τ_s of $\sim 15 \pm 5$ min after $\sim 30 \pm 10$ min of regular swimming. These time scales are much shorter than the time required for protein expression under the conditions of the experiment and in those bacteria whose metabolism was particularly slow. Although we did not

perform proteomic studies, the sudden change in bacterial motility suggests that the transition is not mediated by a change in protein expression.

The motility may also be affected in the presence of other energy sources. For instance, Adler and co-workers [8,19] showed that serine is the only amino acid that anaerobic *E. coli* can metabolize. This specificity of serine is due to the presence of an enzyme that *E. coli* is able to express anaerobically and that converts serine into useful energy [20]. To test this statement, we ran the same experiments with amino acids in the bacterial solution [Fig. 2(c)] and especially with threonine and serine, two amino acids differing only by a methyl group on the side chain. We observed that in a buffer containing 250 mM threonine, bacteria transitioned to a nonmotile state after $\sim 35 \pm 10$ min, i.e., as soon as oxygen was exhausted. However, in the presence of 250 mM serine, bacteria were still motile after at least 6 h. In agreement with previous observations [8], we noticed a change in the effective diffusion coefficient from $\sim (6 \pm 1.8) \times 10^{-11}$ m²/s to $\sim (2 \pm 0.7) \times 10^{-11}$ m²/s during the first $\sim 30 \pm 10$ min. This experiment confirmed that the presence of serine in the buffer allowed bacteria to sustain a proton-motive force and to keep swimming. In contrast, in a buffer supplemented with a different amino acid, the bacterial behavior did not change compared to a buffer free of amino acid.

These aerobic bacteria deprived of oxygen were in a nonmotile state. We then allowed oxygen to penetrate through the edges of a square sample [Fig. 3(a)] containing an anaerobic bacterial buffer. In this experiment, we observed the whole sample and the same results were observed in the presence and in the absence of Ru-micelles in the bacterial solution. As a result of the oxygen penetration, after a transient regime of a few hours, a stable ring-shaped bacterial accumulation appeared [Fig. 3(b)]. The

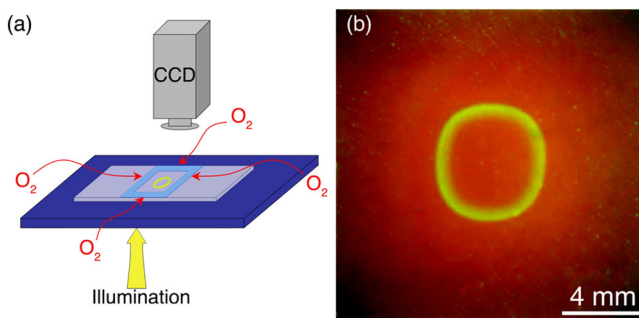


FIG. 3 (color online). (a) Open samples containing fluorescent bacteria and Ru-micelles were prepared under argon and kept under the glove box until bacteria have transitioned to a nonmotile state. Samples were then disposed onto a transilluminator and exposed to air to let oxygen penetrate through the frame into the anaerobic bacterial solution. Time-lapse images of the whole sample were acquired every 15 min. (b) Picture of a 15×15 mm sample: in yellow (or light gray) the bacterial fluorescence at ~ 527 nm and in red (or gray) the Ru-micelle fluorescence at ~ 615 nm. Ru-micelle fluorescence is quenched by oxygen, leading to a gradient of fluorescence intensity in the sample [15].

maximal bacterial concentration in the accumulation was $\sim (8.5 \pm 0.8) \times 10^8$ cells/cm³ (see [15]) (i.e., ~ 2.5 times more concentrated than elsewhere in the sample) and its width was $\sim 0.6 \pm 0.2$ mm [Fig. 4]. In this steady-state regime, the diameter of the ring shrunk at a constant velocity (inset [Fig. 4]) and the ring disappeared eventually [15,21,22]. A linear fit of the front position as a function of time yielded a velocity of $\sim 70 \pm 30$ nm/s [inset Fig. 4]. The front mainly consisted of nonmotile bacteria, delimiting a boundary between a domain of motile bacteria (outside the ring) and a domain of nonmotile bacteria (inside the ring).

With a diffusion coefficient $D_{O_2} \sim 3 \times 10^{-9}$ m²/s at 25 °C in water [23,24], oxygen should have been able to entirely fill the sample in less than 3 h and its mean square displacement would have increased linearly with time. Yet the nonmotile bacterial front invaded the sample with a constant velocity. Furthermore, at a velocity of $\sim 70 \pm 30$ nm/s, it took at least 15 h for the front to collapse and propagate through the whole sample. This is slow compared to the oxygen diffusion. We also observed that this velocity was independent of the bacterial concentration within a range of $\sim (2 \pm 0.2) \times 10^8$ to $\sim (6 \pm 0.3) \times 10^8$ cells/cm³.

In the next step, we proceeded to determine the spatial distribution of oxygen within the sample in real time with Ru-micelles. The measurement of the oxygen concentration profile yielded a constant oxygen gradient in the aerobic part of the sample (Fig. 4). The front of nonmotile bacteria was located at the point where the level of oxygen

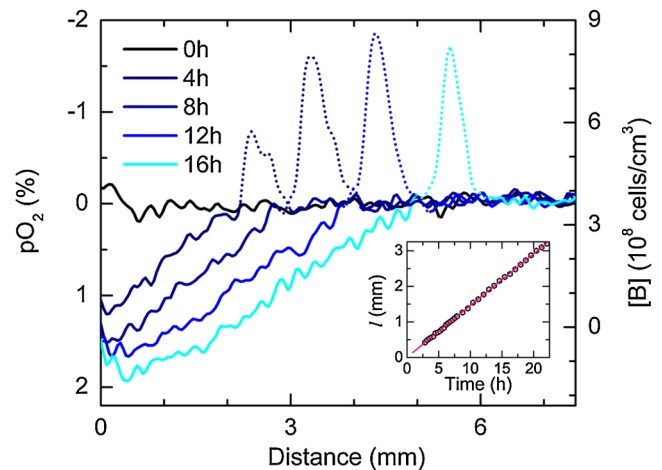


FIG. 4 (color online). Each curve corresponds to a measurement every 4 h from the time oxygen started to diffuse into the bacterial solution. Solid lines represent the oxygen partial pressure p_{O_2} given with an error of $\pm 10\%$ and dashed lines represent the bacterial concentration $[B]$ given with an error of $\pm 10\%$ (see [15]). Inset: Position l of a 1-dimensional front of bacteria as a function of time. 3 of the 4 edges of the sample were sealed. Oxygen was then allowed to penetrate only from one side leading to the formation of a 1-dimensional front, preventing any dimensional effects of the diffusion [15]. A linear fit yields a velocity of $\sim 70 \pm 30$ nm/s.

reached a negligible value. In the steady-state regime (front with a constant velocity), the first Fick's law gives [25]: $J_{O_2} = -D_{O_2} \nabla C_{O_2}$ where J_{O_2} is the oxygen flux and C_{O_2} is the oxygen concentration. Thus a linear variation in the oxygen concentration with an oxygen level zero at the front implies a constant flux J_{O_2} . Such a constant oxygen gradient can be maintained in the sample only if the front of bacteria is an oxygen sink.

An analytical description of the front profile would require the precise knowledge of many parameters. It is, however, possible to write a constitutive equation at a scaling level. In the steady-state regime and in the reference frame of the front moving at the velocity V_{front} , the flux $J_{NM \rightarrow M}$ of bacteria from the anaerobic to the aerobic region is $J_{NM \rightarrow M} = CV_{\text{front}}$ (C is the bacterial concentration, nearly constant on both sides of the front). This flux is a direct consequence of the presence of a gradient of diffusivity between the two regions: $J_{NM \rightarrow M} \sim C \frac{\partial D}{\partial x} \sim C \frac{\Delta D}{l}$ where l is the typical width of the front ($l \sim 0.6 \pm 0.2$ mm) and ΔD is the difference of the diffusion coefficients of the two domains. The balance between the two fluxes gives a characteristic front velocity: $V_{\text{front}} \sim \frac{D_M - D_{NM}}{l} \sim \frac{D_M}{l}$ which gives $\sim 50 \pm 35$ nm/s, in good agreement with the observation. The front length l can be calculated from the transition time τ_s ($\approx 15 \pm 5$ min) obtained from the homogeneous motility transition previously described. At the front interface, motile bacteria have a certain probability to penetrate in the anaerobic domain. Once in that region, the lack of oxygen forces them to slow down and stop, creating the anaerobic front. Their penetration depth is [26]: $l \sim 2(D_M \tau_s)^{1/2}$. This gives an average penetration depth of $\sim 0.3 \pm 0.1$ mm, in reasonable agreement with the measurement of the front width. It is then possible to rewrite $V_{\text{front}} = \frac{1}{2} \left(\frac{D_M}{\tau_s} \right)^{1/2}$. The velocity and the width of the front are defined by D_M and τ_s and are independent of the bacterial concentration.

In conclusion, we showed that a motility transition of *E. coli* is concomitant with the exhaustion of oxygen. We observed that this transition is sharp and fast between a motile and a nonmotile state and is governed by the oxygen concentration: it takes place only after the consumption of oxygen is complete. In the reverse transition, when oxygen penetrates into an anaerobic sample, a surprising accumulation of bacteria forms into a moving front. This front of nonmotile bacteria propagates very slowly at a constant velocity and regulates the penetration of oxygen.

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