Polymers in Two-Dimensional Bacterial Turbulence

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We experimentally investigate the effects of polymer additives on bacterial turbulence in two-dimensional (2D) films of swarming Serratia marcescens. We find that even minute amounts (≤ 20 ppm) of polymers can suppress velocity fluctuations and increase the size and lifetime of large-scale coherent flow structures. In addition, we report an upscale transfer of enstrophy and energy using the recently developed filtering techniques. Unlike in classical 2D turbulence, both enstrophy and energy fluxes move primarily towards large scales in bacterial turbulence; such fluxes are greatly modified by polymer additives.

Relatively simple life forms, like bacteria and protozoa, can exhibit complex behaviors, such as swarming [1, 2], quorum sensing [3, 4], and biofilm formation [5–7]. At sufficiently high cell densities, microorganisms can communicate chemically [8, 9] and hydrodynamically [10, 11], and move together in a coordinated manner known as collective motion [12]. An intriguing phenomenon is the emergence of turbulent-like features in bacterial suspensions; examples include large-scale coherence [10, 13], strongly fluctuating velocity [14, 15], and anomalous diffusivity [2, 16]. Due to the qualitative similarity to turbulence at high Reynolds numbers, these behaviors are often referred as “bacterial turbulence” [17–19]. Both theoretical and numerical studies based on continuum theory [18–20] as well as discrete swimming particles [21–23] have shown that hydrodynamic effects alone can capture some of the main features of bacterial turbulence, even without biochemical interactions.

Microorganisms often live in fluid environments where (bio)polymers are present [24]. For instance, bacteria can secrete slime to reduce friction while swarming across a solid surface [25], and produce protective exopolymeric matrix during the formation of biofilms [26]. How the presence of polymer molecules in the fluid media affect the swimming behavior of single microorganisms has been investigated for the past decade or so; both enhancement [27–30] and hindrance [31–34] in swimming speed have been found depending on the often nonlinear interaction between the swimmer kinematics, velocity fields, and fluid rheological properties.

Less explored, however, are the effects of polymers on the collective behavior of swimming microorganisms. A numerical study on the collective dynamics of rod-like swimmers shows that fluid elasticity can suppress velocity fluctuations and break down large-scale flow structures [35]. Simulations based on mean field theory suggest that fluid elasticity can mediate hydrodynamic interactions and lead to larger coordinated structures [36]. Recently, large oscillatory vortices are found in bacterial suspensions inside droplets containing viscoelastic fluids (DNA suspensions); the observed spatial-temporal order is found for fluids with sufficiently high levels of elasticity [37]. Despite recent advances, there is still a dearth of investigations on the effects of polymers on the collective motion of microswimmers, particularly in the (ultra)dilute regime where polymer molecules have a relatively minor effect on the bulk fluid properties. As a result, our understanding of the collective behaviors of living organisms in polymeric fluids remains incomplete.

In this manuscript, we experimentally investigate the effects of polymer additives on the collective dynamics of swarming bacteria in quasi-2D liquid films. Our results show that even minute amounts of polymers (≤ 20 ppm) can significantly enhance bacterial collective motion and promote large-scale coherence. Velocimetry data show that the size and lifetime of the flow structures are nearly doubled in the presence of polymers, and velocity fluctuations are suppressed. Energy spectra show a power law of −5/3, reminiscent of the inverse energy cascade scaling...
in 2D Newtonian turbulence. Surprisingly, our calculations show that the primary directions of both energy and enstrophy fluxes are inverse (upscale) in bacterial turbulence. The inverse enstrophy flux increases substantially with the addition of polymers, which is a potential mechanism for the increase in large-scale coherence.

Swarming experiments are performed on ATCC 274 strain of *Serratia marcescens*, a rod-shaped bacterium that is on average 2 μm long and 0.8 μm in diameter. When cultivated on soft agar plate (see Supplemental Material [38] for details), the bacteria differentiate into swarmer cells with additional (10 to 100) flagella and elongated bodies of ≥ 5 μm [39]. Polymeric solutions are prepared by diluting a carboxymethyl cellulose (CMC, 7 × 10^5 MW) stock solution in phosphate-buffered saline (PBS) to final concentrations of 5, 10, and 20 ppm. Note that the highest polymer concentration is much below the overlap concentration c* (≤ 0.2% c*). A 2-μL drop of PBS or CMC solution containing swarming *S. marcescens* [38] is placed in a thin-film apparatus [29, 34, 38], and stretched into an approximately 1-cm² large and 40-μm thin film. Images are taken using bright-field microscopy and a CMOS camera (Flare 4M180) at 24 frame/s. Velocity fields of swarming bacteria are obtained using particle imaging velocimetry (PIV, see [40]), with a total number of 6400 or 80 × 80 interrogation windows, each of a size of 25 × 25 pixel or 7.0 × 7.0 μm².

Figure 1 shows experimental velocity and vorticity fields for the buffer (PBS) and the 20 ppm CMC solution (see movies in [38]); instantaneous streamlines are plotted on top of the fields to better visualize structures. The flow fields show that the addition of polymers significantly increases the swimming speed of *S. marcescens*; the maximum velocity magnitude is nearly doubled from 10 μm/s in the buffer to 20 μm/s in the polymeric solution [Fig. 1(a) and 1(b)]. While it has been previously found that a single microbe can swim faster in polymeric solutions [27–30], the flows induced by bacterial collective motion here are not merely scaled up with a higher swimming speed. If that were the case, one would expect the flow structures to remain roughly of the same size. Here, on the other hand, we find that the flow structures length-scale increases with the addition of polymers, as shown by the vorticity fields [Fig. 1(c) and 1(d)]. This indicates that bacterial collective motion in these ultra-dilute polymeric fluids have distinct underlying flow structures from those in Newtonian fluids.

The effects of polymers on flow structures in swarming bacteria are further quantified by calculating the probability density functions (PDFs) of the velocity magnitude fields. We find that the addition of 20 ppm of CMC (≤ 0.2% c*) more than doubles the maximum swimming speed [Fig. 2(a)] and roughly triples the mean speed ̄u [Fig. 2(a), inset]. We note that these PDFs are not simply rescaled, rather they follow different statistical distributions. As polymer is added to the swarms, the PDFs of the velocity magnitudes tend to a Rayleigh distribution [black curve in Fig. 2(a)]. The Rayleigh distribution is a feature of the magnitude of a vector with two independent Gaussian components. This result suggests that the PDFs of velocity components are Gaussian for the 20 ppm CMC case (and non-Gaussian for the buffer case).

To test this hypothesis, we compute the PDFs of the in-plane velocity components u_x and u_y for the 0 (PBS) and 20 ppm CMC cases [Fig. 2(b)]. For better contrast, the velocity components u_x* and u_y* are normalized to have a mean of zero and a standard deviation of unity. Importantly, we find no noticeable difference between the PDFs of x- and y-velocity components, suggesting the in-plane motion of bacteria is statistically isotropic. In the buffer case (0 ppm), the velocity distributions are broadened, with heavy tails at high velocities. A generalized Gaussian function fitting, $N \exp\left(-c|u_x^*|^{\beta}\right)$, reveals that the PDFs are super-Gaussian with $\beta \approx 1.4$. In contrast, such tails are absent in the polymeric case (20 ppm), and the PDFs are approximately Gaussian with $\beta \approx 2.0$. The polymer additives seem to reduce the velocity distributions tails by suppressing velocity fluctuations.

The suppression of tails in the velocity PDFs can be characterized by the kurtosis of velocity components [Fig. 2(b), inset]. The kurtosis is 3 for a Gaussian distribution, greater than 3 for super-Gaussian and less than 3 for sub-Gaussian distributions. We find that as the polymer concentration increases, the kurtosis of velocity components decreases from ~ 4.5 in the buffer to ~ 3 in the polymeric fluid (20 ppm). The decrease in kurtosis suggests that polymers suppress outlier velocities and weaken the intermittency of fluctuations in 2D bacterial turbulence. This is likely due to polymer molecules mediating the hydrodynamic interaction between nearby bacteria, which reduces the likelihood of local swimming velocity deviating from the mean swimming velocity. The decrease in
velocity fluctuation with polymers is consistent with the observation in previous numerical simulations [35]. Note that polymer additives have an opposite effects in classic 2D turbulence, where the sudden release of polymer elastic energy increases intermittency and the kurtosis of velocity distributions [41–43].

Polymer mediation on local bacteria interaction may result in a long-range hydrodynamic effect, which could explain the increase in structure size shown in Fig. 1. The flow structure size can be quantified by the spatial integral length scale of vorticity, defined as: $C_\omega(r) = \langle |\omega(x)\rangle \cdot |\omega(x+r)| / \langle |\omega|^2 \rangle$ and $C_u(r) = \langle |u(x)| \cdot |u(x+r)| / \langle |u|^2 \rangle$ for comparison. Inset of Fig. 3(b) shows that the average vortex size increases by roughly 50%, from $\sim 30 \mu m$ in the buffer to $\sim 45 \mu m$ in the 20 ppm CMC solution. This increase in structure size with polymers is in contrast to previous numerical studies [35], where the induced polymer stress breaks down large-scale flow structures, but the cluster size of swimmers is increased. A much lower swimmer concentration was used in the simulations than in the current experiments, which may explain this discrepancy.

The lifetime of flow structures are examined by the temporal correlation functions of velocity $u$ and vorticity $\omega$, defined as: $C_u(\tau) = \langle u(t) \cdot u(t+\tau) \rangle / \langle |u|^2 \rangle$ and $C_\omega(\tau) = \langle \omega(t) \cdot \omega(t+\tau) \rangle / \langle |\omega|^2 \rangle$. To compensate for the increases in flow speed and structure size with polymers, the time lag $\tau$ for velocity correlation is rescaled by the eddy turnover time, $L_u / u$. An increase in velocity temporal correlations is found [Fig. 3(c)] up to half of an eddy turnover time ($\sim 5 s$), suggesting that polymers increase the average lifetime of flow structures. The vorticity fields are also increasingly correlated in time with the addition of polymers [Fig. 3(d)]. Here, the time lag $\tau$ is normalized by the enstrophy time scale, $\bar{\Omega}^{-1/2}$, where enstrophy is defined by the mean square vorticity, $\bar{\Omega} = \langle |\omega|^2 \rangle / 2$. The mean lifetime of flow structures can be measured by the vorticity integral time scale, defined as: $T_\omega = \int_0^\infty C_\omega(\tau) d\tau$. We find that the normalized mean lifetime is more than doubled in the 20 ppm case compared to the buffer [Fig. 3(d), inset]. Overall, these results imply that the interplay between polymer stresses and bacteria interaction leads to a longer flow memory in 2D bacterial turbulence.

Next, we explore how the presence of polymer affect the transfer of (kinetic) energy across different length scales in bacterial turbulence. We compute the energy spectra, $E(k) = 2\pi k \langle |\hat{u}(k)|^2 \rangle$, where $\hat{u}(k)$ denotes the Fourier transform of the velocity field. An increase in spectral power at all wavenumbers with polymers is found (Fig. 4) due to the enhancement in bacterial swimming speed. At large scales, energy spectra is found to follow an exponential decay, $\exp(-kL_c)$, for all polymer concentrations. Exponential decay in energy spectra has been observed in flows dominated by viscous dissipation [44] and expected here since the Reynolds number $Re \ll 1$. The length scale associated with the exponential decay, $L_c$, increases with polymer concentration (Fig. 4, inset).

FIG. 3. (a, b) Spatial correlation functions of (a) velocity and (b) vorticity. Inset: vorticity integral length scale $L_\omega$. (c, d) Temporal correlation functions of (c) velocity and (d) vorticity. The time lag $\tau$ is normalized by the eddy turnover time $L_u/u$ in (c), and by the enstrophy time scale $\bar{\Omega}^{-1/2}$ in (d). Inset: normalized vorticity integral time scale $T_\omega / \bar{\Omega}^{1/2}$.

FIG. 4. Energy spectra $E(k)$ for different polymer concentrations. Solid curves are exponential fits to low-wavenumber data, $\exp(-kL_c)$. A power law of $k^{-5/3}$ (dashed line) is drawn for comparison. Inset: characteristic lengthscale $L_c$ obtained from the exponential fits, versus polymer concentration.
This is consistent with our observations in the spatial correlations [Fig. 3(a) and 3(b)], although the value of \( L_c \) is larger (\( \sim 350 \) to 450 \( \mu m \)) than the integral length scale, since \( L_c \) is primarily associated with the exponential decay at the smallest wavenumbers.

A well-defined power law of \( k^{-5/3} \) for the energy spectra is found at small-scales of all cases (Fig. 4). In classic Newtonian 2D turbulence, the power law of \( k^{-5/3} \) suggests the existence of an inverse energy cascade [45, 46]. In bacterial turbulence, kinetic energy is injected by bacteria at the smallest length scale of the flow, which admits an inverse flux of energy towards large scales. The addition of polymers does not affect this inverse energy cascade scaling. Note that the power law of \( k^{-5/3} \) is different from the \( k^{-8/3} \) scaling that has been found in previous experimental studies [18]. This discrepancy is likely due to the difference in experimental geometry; while our experiments are performed in free-standing films, previous studies were conducted in microfluidic channels with no-slip boundary conditions [18, 19].

We further examine the spatial fluxes of energy and enstrophy using a recently developed filtering technique [47–49]. Central to this technique is to obtain a low-pass filtered velocity field, \( u^{(k)} \), containing the information only at wavenumbers smaller than \( k \). Here, the low-pass filter is chosen to be a Gaussian function [50]. The local energy flux is defined as: \( \Pi_E^{(k)} = -\tau^{(k)}_{ij}(\partial u^{(k)}_i/\partial x_j) \), where \( \tau^{(k)}_{ij} = (u_i u_j)^{(k)} - u^{(k)}_i u^{(k)}_j \), is a stress tensor arising from the coupling between the resolved large scales and the filtered small scales [51]. By definition, the local energy flux is positive for energy flow to smaller scales (\( > k \)) and negative for energy flow to larger scales (\( < k \)). The local enstrophy flux is defined in a similar manner, with the same sign convention: \( \Pi_\Omega^{(k)} = -\sigma^{(k)}_{ij}(\partial \omega^{(k)}_i/\partial x_j) \), where \( \sigma^{(k)}_{ij} = (\omega u_j)^{(k)} - \omega^{(k)} u^{(k)}_j \), is a vector describing the spatial transport of vorticity due to the elimination of smaller scale vortices [48].

The mean energy flux, \( \langle \Pi_E^{(k)} \rangle \), is shown in Fig. 5(a), for 0 (buffer) and 20 ppm CMC cases. To compensate for the increase in kinetic energy and enstrophy with polymers, \( \langle \Pi_E^{(k)} \rangle \) is normalized by the factor \( \bar{\omega}^2 \Omega^{1/2} \) to be dimensionless. Results show that in the buffer (0 ppm) case the net flow of energy is upscale (inverse) at all wavenumbers, consistent with the \( k^{-5/3} \) inverse energy cascade scaling. In the polymeric liquid (20 ppm), however, energy moves primarily towards larger scales, with a reverse of direction towards smaller scales at low \( k \). This polymer-induced forward energy transfer is likely due to polymers extracting energy from large-scale flows by getting stretched, and releasing it at smaller scales through relaxation. The result seems unexpected, since the downscale transfer of energy should aid the breakdown of large-scale flow structures, while an increase in vortex size with polymers is observed. This is explained below by the changes in the mean enstrophy flux.

The mean enstrophy flux, \( \langle \Pi_\Omega^{(k)} \rangle \), normalized by \( \bar{\Omega}^{3/2} \) to be dimensionless, is shown in Fig. 5(b). We find that the transfer of enstrophy is primarily upscale with slight downscale flux at low \( k \) values for both 0 and 20 ppm CMC cases. This inverse transfer of enstrophy in bacterial turbulence may have been expected (it has been suggested for sperm cells [52]) since both energy and enstrophy are injected at the smallest length scale. Remarkably, the addition of polymers to the media enhances the inverse transfer of enstrophy towards larger scales, which may explain the increase in vortex size. In other words, the origin of larger coherent structures is not the accumulation of kinetic energy at large scales; rather, it is due to a enhanced transfer of vorticity or enstrophy in the presence of polymers.

In summary, we investigate the effects of polymers on 2D bacterial turbulence. Our experiments show that polymers can significantly affect bacterial collective motion by suppressing velocity fluctuations and increasing the size and lifetime of coherent flow structures. Further analysis on the energy and enstrophy fluxes reveals that polymers enhance the transfer of enstrophy, which can be responsible for the large-scale coherence. Our work extends the studies of active turbulence in Newtonian fluids to a more general case of viscoelastic fluids. These results can be helpful in understanding the collective dynamics of microswimmers in more common viscoelastic fluid environments, such as spermatozoa in cervical mucus and bacteria in biofilms.

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