Quantifying the sorting efficiency of self-propelled run-and-tumble swimmers by geometrical ratchets

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It has been recently shown that suitable asymmetric microstructures can be used to achieve microorganism separation. Here we study numerically how the separation process depends on the specific motility strategies of the microorganisms involved. Crucial properties such as the separation efficiency and the separation time for two bacterial strains are precisely defined and evaluated. In particular, the sorting of two bacterial populations inoculated in a box consisting of a series of chambers separated by columns of asymmetric obstacles is investigated and compared to that occurring in a box free of geometrical constraints.

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I. INTRODUCTION

Self-propelled objects moving in confining environments at low Reynolds numbers exhibit interesting physical properties, some of which are not yet well understood and deserve to be studied in view of their technological applications. These objects range from artificial microswimmers that can be actuated upon by using applied magnetic fields \[1\] to motile cancer \[2, 3\] and stem \[4\] cells, to motile bacteria \[5, 6\] and spermatozoa \[7, 8\]. The study of their properties in confined regions has been made possible by recent advances in microfabrication \[9, 10\] and observation \[11, 12\] techniques.

Aside from the intrinsic problems posed by the motion of confined, self-propelled, run-and-tumble microorganisms, there is strong interest in the biomedical and engineering communities in controlling efficiently microorganism motion in view of their promising technological applications. A physical, nondestructive method of achieving this control is suggested by the geometrical-induced guidance caused by walls of asymmetric openings (funnels), so called ratchet effect, which was first used by Galajda and coworkers to generate bacterial sorting and geometrical ratchets \[1\] for the case of the paradigmatic \textit{Escherichia coli}, during the run the flagella rotate counterclockwise and the microorganism moves in a forward, relatively straight direction, whereas during the tumble mode, one or more flagella rotate clockwise and the bacterium is reoriented in a new direction \[19\]. As shown forty years ago by Berg and Brown for \textit{E. coli} \[20\]: (A) runs are not strictly straight due to rotational diffusion, (B) run lengths are exponentially distributed, and (C) bacterial heading changes at tumbles by less than 90°, preserving some memory of the previous run, a fact that is often neglected in theoretical treatments. It is worth noting that some marine bacteria show anti-persistency, in what is called a run-and-reverse strategy \[21, 22\].

Various aspects of the mathematical modeling of microorganism motion have been the subject of recent work \[23, 24\]. The case of bacterial directed motion under asymmetrical geometrical confinement, first observed and explained in Ref. \[5\], was modeled phenomenologically in Ref. \[25\]. The authors considered point-like swimming bacteria following run-and-tumble dynamics with a constant motor force magnitude and thermal fluctuations. Although this model neglects the details of the swimmer dynamics, it reproduces the most important ex-
perimental findings and has been an inspiration for further theoretical work. In Ref. [30] the relation between the ratchet effect and symmetry breaking by the funnel array geometry was clarified. Later, the influence of the specific dynamical properties, from (A) to (C), described by Berg and Brown was studied in details in Ref. [31] using a more realistic phenomenological model and including the motility parameters obtained by measurements. It was found that different swimming strategies may yield very different microorganism rectification efficiencies, being measured as the device capacity to concentrate cells (number of concentrated cells/number of inoculated same cells). This effect may be used to separate and purify particular swimmer types. We summarize the main results of that work:

1. In unbounded environments there are two processes that degrade the orientational correlation: tumbling and rotational diffusion. The first is much more important for systems with short runs, while rotational diffusion gives the dominant contribution to memory loss in systems characterized by long runs. These effects can be quantified by the velocity correlation function.

2. A study of the mean square displacement in unbounded environments reveals that the translational diffusion coefficient $D_T$ decreases strongly as the change-of-heading angle at a tumble increases. Unless typical runs are very long, $D_T$ is approximately given by its value in the absence of rotational diffusion. These results indicate that, to make accurate predictions about swimmer sorting, it is necessary to consider the specific motility properties of the microorganisms involved.

3. When the dynamics of free swimmers is incorporated into a spatially constrained environment (asymmetric geometry) long run lengths and small tumble emergence angles lead to high wall accumulation and, consequently, to fast net displacement in the easy ratchet direction. In general, long permanence near the walls and suitable wall of funnels architecture, i.e., funnel walls at least as long as the run length and funnel openings of the order of the cell size, favor rectification or cell concentration.

4. Increasing the average run duration, $\tau$, increases the time of permanence close to the walls, leading to enhanced rectification. If $\tau$ is negligible, the swimmers cannot take advantage of swimming along the funnel walls and directed motion does not ensue.

5. Increasing the average speed during the run and decreasing the average change-of-heading angle at a tumble, i.e. increasing persistence, enhance wall accumulation and rectification.

6. Good agreement was obtained with available experimental data, specifically regarding the time of rectification and the efficiency of a microfabricated wall of funnel-shaped openings as the one used in Ref. [5].

In this work, we use the improved phenomenological model introduced by Berdakin et. al. in Ref. [31] to investigate the efficiency of asymmetric microarrays used as sorting devices, and their dependance on the swimming strategies of the microorganisms involved. Our aim is to help to design good sorters, using a model which incorporate real motility parameters. In Section 2 we review the computational model and define the quantities to be calculated, such as the extraction time and the sorting efficiency. In Section 3 we present our numerical results, which are briefly discussed in the concluding section.

### II. METHODS

**The model.** We study numerically a dilute system of $2N_s$ microscopic self-propelled particles, the swimmers, moving under low Reynolds number conditions and confined to a micro-patterned two-dimensional box of size $L_x \times L_y$. The box contains $M$ identical, equidistant columns of obstacles, each consisting of $N_f$ openings (asymmetric funnels), of gap size $l_g$ and wall length $l_f$ (see Fig. 1(a)). We choose $N_f = 3$ and $M$ from 10 to 20. The $M-1$ inner chambers and the last chamber have all the same length $l_D = 150 \mu m$. The length of the inoculation (leftmost) chamber is kept constant, $l_I = 450 \mu m$ in order to have a fixed fractional occupied area as a initial diluted condition at $t = 0$. The relevant geometrical parameters are illustrated in Fig. 1(a) for the array configuration and (b) for the single funnel shape.

Each swimmer, whose location is given by a vector $\vec{r}_i$, is represented by a soft disk of radius $r_s = 0.5 \mu m$, moving in two dimensions with speed $\vec{v}_i$ and heading in the direction of the unit vector $\vec{v}(\Phi_i) = \cos(\Phi_i)\hat{i} + \sin(\Phi_i)\hat{j}$. In a confined space, the swimmer motion is determined by:

1. **Self propulsion.** When starting to move under low Reynolds number conditions, swimmers get almost instantly to a constant final speed. In our model this constant speed is given by $F^m/\gamma$ where $\gamma$ is the medium damping constant and $F^m$ the modulus of the propelling force. The initial condition for the swimmer population speed is chosen as a normal speed distribution with mean $\bar{v}$ and standard deviation $\sigma_v$. 

| Swimmer $\bar{v} [\mu m/s]$ | $\sigma_v [\mu m/s]$ | $\phi_0 [\circ]$ | $\sigma_\phi [\circ]$ | $\tau [s]$ | $D_R [\text{rad}^2/s]$ |
|-------------------------------|---------------------|-------------------|---------------------|-----------|---------------------|
| $s_1$                         | 14.2                | 3.4               | 68                  | 36        | 0.86                | 0.18 |
| $s_2$                         | 20.0                | 4.9               | 33                  | 15        | 6.30                | 0.06 |
2. **Changes in direction.** The heading of the swimmer is altered only by tumbling or rotational diffusion. Tumbles are assumed to be instantaneous (real tumble lasts 0.1 second in mean) and give place to a rotation, $\phi$, from the previous direction of motion, which we consider Gaussian distributed and centered at $\phi$ with a width $\sigma_\phi$. (see Table I and Ref. [31]). Successive tumbles are spaced by almost straight runs exponentially distributed with mean duration $\tau$. During a run, asymmetries in the self propulsion system and environmental fluctuations produce deviations from a perfectly straight path. These deviations are measured by the rotational diffusion coefficient, $D_r$, and included in our model via changes on the swimmer heading [32]:

$$\Delta \Phi = \nu \sqrt{2D_R \Delta t}$$

where $\nu$ is a gaussian distributed random number and $\Delta t$ the numerical integration time step.

3. **Interaction with the walls.** It is modeled by a steric repulsive force $F_{sw}^i$ normal to the walls,

$$F_{sw}^i = F_{sw}(1 - r_{ik}/a) \Theta(1 - r_{ik}/a) \hat{n}_k,$$

where $\Theta$ is the step function, $\hat{n}_k$ is a versor normal to the k-th wall, $r_{ik}$ is the distance between the i-th particle and the center of the k-th wall, $a = r_s + w/2$, and $w$ is the wall width. Since the swimming direction is unchanged during the collision, the swimmer keeps slightly bouncing against the wall and advances parallel to the wall with a reduced speed that is proportional to the sine of the
angle formed by the incidence direction and \( \hat{n}_k \). Either a tumble or rotational diffusion may allow the swimmer to move away from the wall. This interaction is responsible for the observed accumulation at the walls [32], and for the bacteria directed motion and sorting [5]. As remarked with measurements of wall-accumulation for bacteria with different swimming strategies in Ref. [34], the wild-type \( E. coli \) was significantly less attracted to the surfaces than a mutant strain that does not display tumbling.

4. A purely steric swimmer-swimmer repulsion of maximum intensity \( F_s \),

\[
\tilde{F}_{ij}^s = F_s(1 - |\vec{r}_{ij}/2r_s|)\Theta(1 - |\vec{r}_{ik}/2r_s|)\vec{r}_{ij}, \tag{2}
\]

with \( \vec{r}_{ij} = \vec{r}_i - \vec{r}_j \). The hydrodynamic interaction between microswimmers is not important at very low swimmer concentrations [32], and we disregard it here. Our approximations are buttressed by recent measurements of cell-cell and cell-wall interactions using \( E. coli \), that show that thermal and intrinsic stochasticity wash out the effects of long-range fluid dynamics [33]. These experimental results imply that physical interactions between bacteria are mainly determined by steric collisions and near-field lubrication forces.

Taking into account all the interactions described above we arrive to our set of dynamical equations to be solved numerically [31] for the \( 2N_s \) run-and-tumble microswimmers. Using a Runge-Kutta algorithm we integrate the dynamical equations of motion and we obtain the time evolution (trajectories) for each confined swimmer. The corresponding realization averages are performed later. We assume that all swimmers, the mixed population, are initially randomly distributed in the inoculation chamber. For simplicity we will always compare only two swimmer types, a situation that is the easiest to implement in the laboratory, using two different fluorescent markers. Table I specifies the motility parameters of the swimmers simulated in this work, the tumbling \( s_1 \) and non-tumbling \( s_2 \) strains. The optimal single funnel geometry for an efficient rectification of wild-type \( E. coli, s_1 \), where found in Ref. [31] to be \( b_g = 2 \mu m, l_f = 30 \mu m, \) and \( \theta = 68^\circ \), so we keep this parameters fixed here for all simulations. The box height used is \( L_y = 80 \mu m \) and the wall width is taken to be \( w = 2 \mu m \) for both, the box walls and the funnel walls. The width of the inoculation chamber, \( l_f = 450 \mu m \), has been chosen to keep a initial low swimmer density. The number of swimmers, \( N_s \), of each strain, is adapted to maintain for all array geometries used an initial area fraction occupied of 0.05 at the inoculation chamber. The magnitudes of the forces acting upon the swimmers are \( F_s = 200 \) and \( F_{sw} = 300 \) in units of \( \gamma \).

**Calculated quantities.** With our aim to quantify the sorters efficiency we propose two parameters as convenient indicators of the separation process: (a) the separation time, \( t^* \), defined as the time elapsed between the arrival of the first swimmer in the fast class and that of the first swimmer in the slow class to the last chamber (chamber from where a pure population of cell could be extracted or concentrated), and (b) the separation efficiency, \( \epsilon_s \), which we define as the fraction of the fastest type that has arrived to the last chamber by the time \( t^* \). It is convenient to define the percent extraction efficiency as follows,

\[
\epsilon_s = 100 \frac{N_F(t^*)}{N_s}, \tag{3}
\]

being \( N_F(t^*) \) the number of swimmers in the fast species that is present in the last chamber at \( t^* \), when its purity is still 100%. 

![](image.png)
III. RESULTS

We first consider a sample with \( M = 20 \) funnel columns and two homogeneous bacterial distributions initially inoculated in the first chamber. These bacteria are wild-type \( E. \) coli and a mutant studied by Berg and Brown \cite{20}; their characteristic dynamical parameters are specified in Table I. We compute the variation of the total bacterial populations of each mutant in the first and the last chambers, Fig. 1(c). After 30 independent realizations, the average separation time for this system is \( t^* = 16 \) min and the high average separation efficiency for the mutant \( s_2 \) is \( \epsilon_{s_2} = 67 \pm 19\% \). Three factors contribute to the high extraction efficiency for this mutant: its higher average speed, its higher persistence, i.e. low \( \phi \), and, mainly, the longer duration of the runs, which increases both \( D_T \) and the contact time with the rectifying walls. The advance of both populations through the various chambers is shown in Fig. 1(d), where we see that the purification process improves with successive chambers. From chamber 12 onwards, we also observe that the time evolution of the \( s_2 \) pulse (blue) is almost position-independent until it reaches the last column. Instantaneous snapshots of the bacteria populations considered in Fig. 1 is shown, as a function of time in Fig. 2(a), where they are seen to start from a uniform distribution in the inoculation chamber and advance at different rates in the easy ratchet direction. Comparison between upper panels in Fig. 2(b-e) and bottom panels in Fig. 2(f-i) show clearly how these rates are enhanced by the ratchet geometry of the column array, giving an estimated 5 \( \mu \)m/s drift velocity for the \( s_2 \) population, five times larger than that found for \( s_1 \). As a result of this different velocities inside the box, both populations are soon largely separated and can be readily sorted out.

It is interesting to compare what happens in the specially designed box, an array of funnel columns, with the result obtained in a single channel with the same area and clean of obstacles, when the bacterial populations are subject to the same initial conditions. In the clean box, as the histograms in Fig. 2(b-e) show, the fast type also moves forward first, in part taking advantage of its longer runs along the side walls, but the separation is much less efficient than for the funnel-containing box, for which at \( t = 20 \) min there is no \( s_1 \) swimmer from chamber 16 to 21. Purification is complete there. For the particular realization represented in Fig. 2(b) the extraction time and extraction efficiency are, respectively, \( t^* = 24.1 \) min and \( \epsilon_{s_1} = 85\% \). At very long times, a uniform distribution is expected for the single-chamber configuration, while an exponentially increasing population of each bacterial type is expected in the specially designed box. This exponential increase is responsible for the high concentrations near the end of the array of columns, which permit the extraction of a high fraction of the first bacterial type arriving there. This situation is clearly shown in Fig. 2 from (f) to (i), where it is also possible to observe \( s_2 \) concentration spikes where the obstacle columns are located. The stronger tendency of \( s_2 \) to concentrate near the walls, as compared to \( s_1 \), was recently studied in detail \cite{31}.

Now we study the sorter efficiency of two swimmers (one real and the second real or artificial) as a function of the specific dynamical parameters characterizing the microswimmers. In Fig. 3 we show the extraction times and sorting efficiencies for two swimmers, one of which is wild-type \( E. \) coli, \( s_1 \), and the other \( s_x \), for which only the average run speed is changed. (c) and (d): the same quantities when only the average tumbling angle \( \phi_x \) of the mutant is changed. Inset: sorting efficiency when only the mean run duration, \( \tau_x \), of the second swimmer is changed. Note the different vertical scales between (b) and (d). Here we use a smaller array with \( M = 10 \).

![FIG. 3. Color online. (a) Extraction or pick up time and (b) sorting efficiency for the fastest swimmer to reach the last chamber when we simultaneously simulate wild-type \( E. \) coli, \( s_1 \), and a mutant, \( s_x \), for which only the average run speed is changed. (c) and (d): the same quantities when only the average tumbling angle \( \phi_x \) of the mutant is changed. Inset: sorting efficiency when only the mean run duration, \( \tau_x \), of the second swimmer is changed. Note the different vertical scales between (b) and (d). Here we use a smaller array with \( M = 10 \).](image-url)
order as changing the mean speed, the corresponding sorting efficiencies, panel (d), are markedly lower. In this case, we compared a hypothetical swimmer $s_x$, whose average tumbling angle $\phi_x$ is modified, with the wild type, for which $\phi_x = 68^\circ$. The easiest to separate are the persistent-walk bacteria, for which $\phi_x = 0^\circ$ and the run-and reverse bacteria, for which $\phi_x = 180^\circ$. It is worth noting that large $t^*$ does not necessarily mean large $\epsilon_{\phi x}$. For example, if $\phi_x = 180^\circ$, $t^* = 16$ min, but $\epsilon_{\phi x}$ is only 5%, a relatively low value when compared with $\epsilon_{\phi x} = 20\%$ that results for $\phi_x = 0^\circ$, for which $t^*$ is only 7 min.

**IV. DISCUSSION**

We have investigated arrays of asymmetric-funnel columns built for the purpose of concentrating or sorting out one type of self-propelled swimmer in a run-and-tumble microorganism mixture. As characteristic parameters to measure the suitability of a given architecture, we introduced the extraction time and the sorting efficiency. The first is important because it gives us the length of the temporal window available to the experimentalist to pick up the chosen strain, but does not tell us anything about the number of swimmers able to be extracted. This is given by the separation or sorting efficiency.

The separation efficiency depends both on the motility parameters of the swimmers and on the geometrical dimensions of the device, which we can modify according to the swimmer types we are dealing with. Here we have considered the competition between swimmers having different intrinsic dynamical properties; and we are working out in detail the effect of modifications in the geometrical array parameters that define the asymmetric confining system. The following are some predictions from our study:

- Asymmetric funnel arrays are capable of sorting diluted distributions of run-and-tumble swimmers, enhancing the efficiency obtained by a box free of geometrical constraints.
- A sizable fraction of the chosen swimmer can be purified even if the original mixture is composed of swimmers that are dynamically not too different.
- In general, unless the motility properties of the swimmers are very similar, for $M$ of the order of 10 the extraction time should be long enough to allow the experimentalist to purify the sample.

Although run-and-tumble strategies are common in the bacterial world, this type of motion is not restricted to bacteria. The locomotion of the unicellular alga *chlamydomonas* exhibits, in the dark, nearly straight swimming runs interrupted by abrupt changes in direction. The run distributions are exponentially distributed, with $\bar{\tau} = 11.2 s$. Consequently, the dynamics of this eukaryote are likely to be describable by the model discussed in this paper as well. Our numerical calculations can be easily generalized to include the possibility of bacterial death during the experiment, work we have in progress. Further work should also be done to investigate microorganism sorting at high bacterial concentrations. Phenomena such as correlated motion, wall screening and funnel clogging could be expected in this case, but this calls for a far more complete model.

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