

Efficiency of biological motors

1 Background information

Escherichia coli are bacteria that can move around and explore the environment by using a flagellar motor to propel themselves. In this lab, we will measure the power generated by the motor by analyzing the trajectories of the *E. coli* and measuring their speed as they swim around in a nutrient solution. The energy used to power the *E. coli* can then be compared to the energy it needs to reproduce. This comparison turns out to be important to modern analysis of *E. coli*.

E. coli are rod-shaped bacteria that are approximately 2 μm in length and 0.8 μm in diameter, and they have an estimated velocity of 35 diameters per second (see figure ??). Each *E. coli* has between 3–6 flagella that rotate CCW and propel it forward at a rate of about 270 revolutions per second. *E. coli* can measure the concentration of certain chemicals in its surrounding environment. If it is favorable, the *E. coli* will continue swimming forward in what is called a run. If the concentration of the nutrients it needs is too low, the flagella will rotate CW, which causes the *E. coli* to tumble and lose track of the direction it came from. When it starts swimming again, it will go randomly in a different direction. These runs and tumbles form a random walk that is similar to Brownian motion (see figure ??).

As the *E. coli* swim in the nutrient solution, the main resistance force that they have to counteract comes from the viscosity of the fluid. We can assume that the front of the rod-

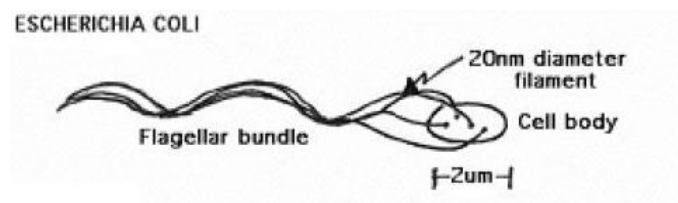


Figure 1: *Escherichia coli*.

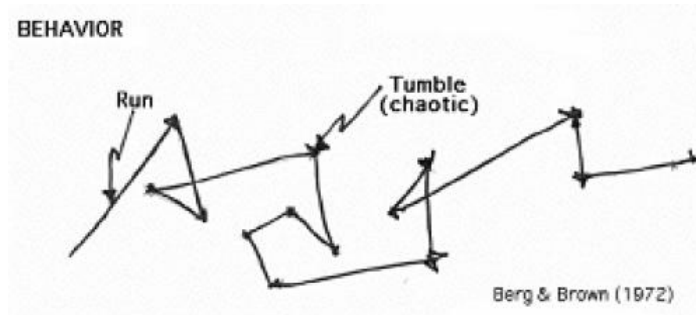


Figure 2: The random-walk trajectory of an *E. coli*.

shaped *E. coli* is similar to a sphere, so the drag force on the bacterium is not too different from the drag force on a sphere of the same diameter. The expression is given by Stoke's law

$$F_d = 6\pi\eta r v , \quad (1)$$

where r is the radius of the sphere, v is its velocity, and η is the viscosity of the fluid (which, in our case, can be approximated to the viscosity of water). By making all these approximations, you will be able to compute the average power of the *E. coli* motor provided that you measure their sizes and velocities. Once you find this power, compare it to the energy needed for reproduction. Do you expect the ratio to be smaller or greater than one?

2 Experimental procedure

In order to grow the *E. coli*, you first need to prepare the solutions in which they will swim. You will find all the recipes you need at the end of this manual. After you've prepared the solutions, you'll have to grow the bacteria, prepare slides and analyze them by using a phase contrast microscope together with the data collection software. All these steps are outlined below.

Solution preparation

You will need to prepare the LB solution and Terrific broth in separate beakers. Combine 5 grams of the LB powder with the provided distilled water to create a 200 ml solution. To prepare the TB solution, combine 2 grams of Tryptone, 1 gram of NaCl and distilled water to create a 200 ml solution. You will be given a prepared motility medium. Put 200 ml of the motility medium in a third beaker. All three solutions will need to be autoclaved.

Biological specimen preparation

Always wear goggles, gloves, and lab coat when handling the *E. coli*. Take a sample of *E. coli* from the cryo vial located in Professor Austin's area (room 112, middle freezer). Use gloves because the cryo vial is at -80°C . Swab a little with a sterile stick and dip it in about 20 ml of LB solution in an Erlenmeyer flask. Let this grow overnight to saturation on the orbital shaker at 140 rpm in the incubator at about 34°C . This overnight sample can be kept in the minifridge with a cap and will stay good for a few weeks. Next, combine about 6 drops of the overnight sample with 20 ml of TB solution in a new Erlenmeyer flask. Let this *E. coli* sample culture for 3–4 hours on the orbital shaker in the incubator at 30°C . Do not let them culture much longer than 4 hours because they will grow exponentially and saturate the solution, at which point they will stop swimming and you will not be able to record their velocity. When the *E. coli* is ready, put some of the solution in a microtube and place it in the centrifuge. Be sure to always balance the centrifuge by placing another microtube of distilled water directly across from your tube. When it is spun down, there should be a whitish pellet of *E. coli* near the bottom of the microtube. This may take a minute. Dump the excess solution out in the Hazmat container and pour in motility medium. Mix the contents using the test tube shaker. Spin this down again, dump out excess solution and add more motility medium. Add a few drops of BSA (bovine serum albumin) to prevent the bacteria from sticking to the slides (this is very important!). Now you are ready to view the *E. coli*.

Slide preparation

Take a specimen slide and put one layer of double-sided tape on it to create a slot between the slide and the cover slip for the *E. coli* to rest in as seen in figure 1. Leave two sides open for the *E. coli* to enter. Tape the cover slip down in place. Using an eye-dropper, squeeze some of the *E. coli* near the opening of the slot and it will wick underneath. To keep the slide fresh for later viewing, you can apply nail polish to the open edges to seal them.

Phase contrast microscope and LabVIEW data collection

You will look at your slide by using a phase contrast microscope, which is nothing but brightfield with two additional components. The first is the annulus which is combined with the condenser lens to shine a cone of light on the specimen slide. The second piece is the phase plate which is combined with the objective lens and specifically matched to

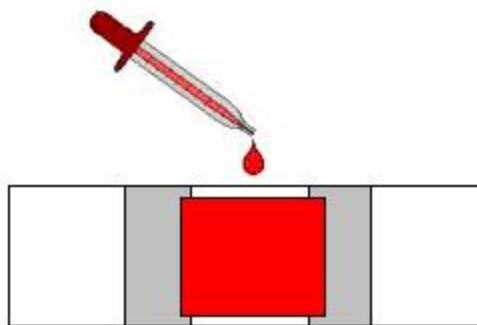


Figure 3: Specimen slide.

the condenser annulus. The phase plate alters the phase of the surrounding light so that when it interferes with the phase shifted light from the specimen, constructive or destructive interference occurs. The interference either increases or decreases the amplitude of the light, producing a high-contrast image of the specimen. One advantage of this technique is that it allows for biological specimens to be viewed while they are alive, because no staining or fixing is necessary.

Now open *VidCap4* on the desktop and go to *Operate* on the tool bar. Click on *Run*. A window should appear asking for a file name. Create a file and name it something you will remember. Save it in a folder on the desktop. The *VidCap4* screen should have the camera image on it. Focus the image using the 40x objective lens and the Ph2 phase plate. This will give the best image size for the video capture. You will most likely have to adjust the brightness, gain, and shutter speed sliders in order to see the picture. Also notice that the region captured by the camera is much smaller than the region you can see through the eyepiece. Set the number of frames per second to 2. Click *Capture* and wait until the status bar reads “finished.” You may wish to make additional videos at this time, to do so simply click operate again and repeat the procedure outlined above. After you are done making videos you can close *VidCap4*. Open the *ImageJ* icon on the desktop. Go to *File - Import - Image Sequence*. Find your file of images and select the first image in sequence. You may have to click ‘Ok’ several times. Note that if you have viewed the images using windows explorer a thumbs.db file will be created. This file will cause errors in *ImageJ* but they can be safely ignored. Find the *Manual Tracking Plugin* from the toolbar. Enter the time interval and distance calibration and click the *Add Track* button and the *Overlay Dots & Lines* button. Wait for the new image screen to appear, this may take a few moments

so be patient. When the new window appears it should say *Overlay Dots & Lines*. The other image screen is no longer needed. Click *Add Track* again. Now you can begin to track the *E. coli* as it moves across the screen. Choose an *E. coli* that's swimming as opposed to tumbling and click on it. The software will advance the screen to the next slide. Find the same *E. coli* and click again. You will be able to see the path your *E. coli* is moving along. When you are finished, click *End Track*. Repeat the above steps to trace different *E. coli*. When you're done with this image sequence, save the results from your tracking in the folder with your images. Open your data file in *Excel*. When the *Text Import Wizard* comes up, click *Finish*.

3 Data analysis

From the data you collected, determine the average velocity of your *E. coli* and use Stoke's law to calculate the viscous drag force and to find the work done by the motor.

What are other factors that could have affected the velocity or movement of the *E. coli*? Are they significant? How would they influence the results of this experiment?

Recipes for bacteria

STOCK Solutions:

1M K₂HPO₄

1M KH₂PO₄

10mM EDTA

3.3M NaCl

1mM L-methionine

1. LB Broth:

10 g/L Bacto Tryptone

5 g/L Yeast Extract

5 g/L NaCl

Fill to 1 liter with DI water and autoclave.

2. TB Broth:

10 g/L Bacto Tryptone

5 g/L NaCl

Fill to 1 liter with DI water and autoclave.

3. LB Plates:

10 g/L Bacto Tryptone

5 g/L Yeast Extract

5 g/L NaCl

Fill to 1 liter with DI water.

Add

20 g/L Bacto Agar

Autoclave. Pour 25 ml into 9 cm plates.

4. Motility Media:

10mM KPO_4 =
 6.2mL/L of 1M K_2HPO_4
 3.8mL/L of 1M KH_2PO_4
0.1mM EDTA =
 10mL/L of 10mM EDTA
0.067M NaCl =
 20.3mL/L of 3.3M NaCl

Fill to 1 liter with DI water and autoclave.

5. Tether Buffer:

Make motility media above:

10mM KPO_4 =
 6.2mL/L of 1M K_2HPO_4
 3.8mL/L of 1M KH_2PO_4
0.1mM EDTA =
 10mL/L of 10mM EDTA
0.067M NaCl =
 20.3mL/L of 3.3M NaCl

And add:

1 μ M L-methionine =
 1mL/L of 1mM L-methionine
5g/L glycerol

Fill to 1 liter with DI water and autoclave.