Author: Sunyoung Hong

Proposal

Analysis of crack patterns of active matters

Introduction

Two particles in suspensions create a meniscus between them, which exerts a capillary force, attracting each other. While being dried the particles are attracted, and the friction from the substrate allows them paired up creating cracks instead of all the particles aggregating at one point. However, the varying concentration of the bacteria can interplay with the crack formation mechanism, and the purpose of this study is to determine the crack pattern changes of Escherichia coli suspensions with respect to its concentration. Its crack patterns after becoming sessile are analyzed, and the results can help characterize any suspensions which form cracks better.

Procedures

The active particle was prepared by using Escherichia coli as bacteria (E. coli), ampicillin (Ap) as anti-bacteria, and terrific broth (TB) as a medium in a two-day procedure. 3mL of the 2M TB medium was transferred into an autoclaved tube and mixed with 3 μL of the antibiotic solution and a small chunk of the frozen bacteria using a pipette tip. The tube was covered with its sterile cap and placed in an incubator shaker set at 37 ℃ for approximately half a day. The overnight culture was diluted into four different test tubes. 30 μL of the bacteria were mixed

with 3mL of the liquid TB and 3 µL of the antibiotic solution. The test tubes were incubated for 6-6.5 hours at 30 ℃ and 220 rpm in a shaking incubator to prepare motile bacteria.

To better increase motility and concentration of the bacterial sample, the obtained motile bacteria were centrifuged. The solution in one test tube was split equally in centrifuge tubes and centrifuged for 5 minutes at 800 rcf. The supernatant was poured out as soon as the centrifuge was complete, and the tubes were allowed to be open to the air for a minute. The left-over liquids were transferred into a new tube and filled up to two-thirds of the tube volume with a buffer solution (MB); The MB solution was made of 0.01 M potassium phosphate, 10-4 M EDTA, pH 7.0, and 0.002% Tween 20. The centrifuge and separation procedure was repeated. Here instead, all the left-over liquids were combined into one centrifuge tube. Another centrifuge was conducted, and all the left-over liquids were collected in one tube. 10 µL of the suspension was extracted into a cuvette, and 990 µL of deionized water was added. The solution was tested for its concentration in a spectrophotometer in 251 using the OD600 program. The resulting number times 100 is the number density of the tested suspension in the unit of n_0 (8 x 10^8 cells/ml). The successful samples showed absorbance above 0.8 A, which is equivalent to 80 n_0.

The harvested bacteria were pipetted for a volume of 2.5 µL. A drop of it was placed on a glass slide and allowed to evaporate. As soon as the first crack had been observed in a bright-field microscope with a 4x objective lens, a 3-minutes video was recorded with a captured image every 20 milliseconds. A final image of the crack pattern was captured after the video, as well.

Results and Measurements

With the restrictions due to COVID-19, the obtained data and experimental images could not be included, and a general trend was instead explained in this report. The successful concentration results ranged from approximately 1.0 A to 1.65 A. When a drop suspension was placed on the glass plate, the suspension contained active visible swimming bacteria. As the solvent continued to diffuse into the air, the contact line shrank toward the center of the droplet, and the active bacteria became sessile. Figure 1 shows the captured images of two samples prepared at different conditions.

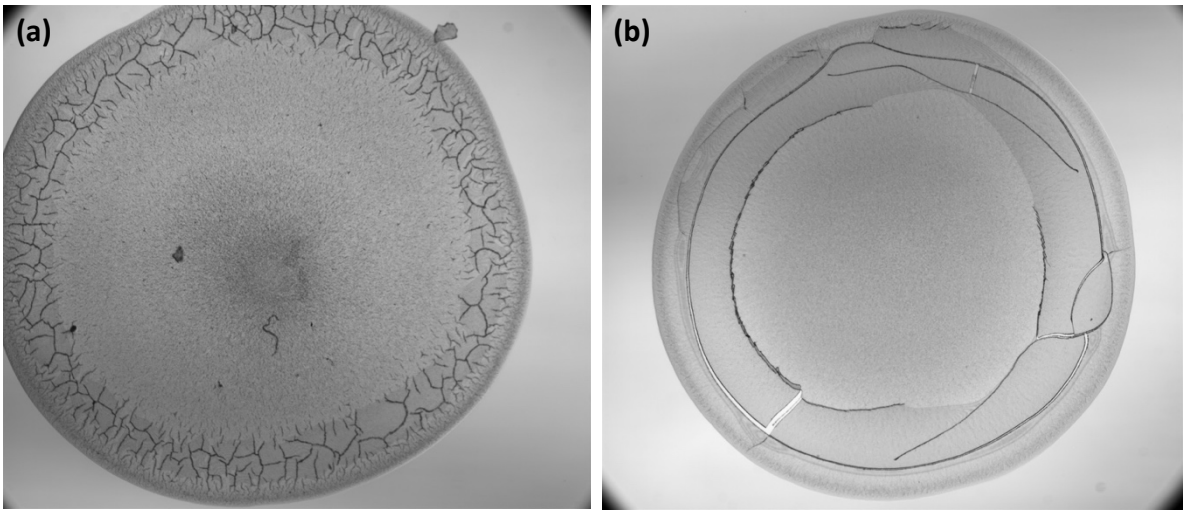

Figure 1. Pattern formation of RP* in water, $128n_0$, 2.5 ul on the glass substrate (a) and of PR* in TW5*, $95n_0$ on the glass substrate (b). PR*: light-controlled E. coli; RP*: tumbler E. coli; TW5*: tween 20, 5e-6 v/v

The figures show that the crack patterns from swimmers and tumblers are different. Also, even though the supporting image was not attached, it was observed that the more circular the outer edge of the drop was the more continuous and circular the crack pattern it had. All tested cracks showed concentric circles. The distance between two concentric circles decreased with a higher concentration suspension. Most of the tested suspensions showed a pattern of cracks in

concentric circles near to the center of the droplet and cracks along the radial direction near to the exterior edge of the droplet. The inner cracks were observed to be more continuous than the outer edge. More tests need to be conducted and analyzed to determine what the dependent variables of the crack patterns are.

Conclusion

The current data found that the proposed method was able to produce the desired sample of bacteria for the experiment. The experimental data showed that the crack patterns are more continuous with the more circular shape of the droplet and that a higher concentration of the suspension gave a more concentric circle for its crack pattern. Further data acquisition, as well as data analysis, need to be conducted to better understand the crack formation process. From this UROP opportunity, I was able to learn more about the bacteria culture and its properties with crack patterns, which are not discussed in my college curriculum in the chemical engineering department. Also, it helped train me on how to conduct experiments effectively and plan accordingly.