Microgravity Science and Technology (2022) 34:15
https://doi.org/10.1007/s12217-022-09933-8

ORIGINAL ARTICLE

Effects of Microorganisms on Drop Formation in Microgravity During a Parabolic Flight with Residual Gravity and Jitter

Patrick M. McMackin[1] · Joe A. Adam[1,2,5] · Shannon R. Griffin[1] · Richard P. Bonocora[2] · Kenneth A. Brakke[3] · Juan M. Lopez[4] · Amir H. Hirsa[1,5]

Received: 4 November 2021 / Accepted: 12 February 2022

Abstract

Wetting and contact-line dynamics, as well as growth (interface creation) and stability of aqueous drops with microorganisms in microgravity is important for understanding and controlling complex fluids in space. The study of biofluid drops in microgravity has applications in biological 3D printing, pharmaceutical production, and bioremediation. Here, liquid cultures of the microorganisms *E. coli*, *S. cerevisiae* (baker's yeast), and *D. radiodurans* were deployed in centimeter-scale drops using a simple tube during a parabolic flight. Residual gravity and *g*-jitter inherent in parabolic flights allowed for the study of how these forces affect the growth of biofluid drops in microgravity. The growth of drops with microorganisms was compared to sterile growth media. Quasi-static simulations were used to assess whether each solution produced measurable changes in the growing droplet. Images of growing drops were analyzed in terms of drop aspect-ratio, contact angles, and the differences in contact angles due to variations in gravity. Results demonstrate that the presence of microorganisms has minimal influence on the behavior of centimeter-scale drops. The small impact of microorganisms on growing drops bodes well for the adaptation of existing Earth-based drop technologies for working with biofluids in reduced gravity.

Keywords Drop dynamics · Biofluids · Microorganisms · Air-liquid interfaces · Parabolic flight

Introduction

Developing technologies that leverage the microgravity environment is necessary to prepare for long-term deep-space missions. In microgravity, drop dynamics are important for fluid control (Noori et al. 2020), wetting phenomena (Guo and Liu 2022), contact line dynamics (Karchevsky et al. 2021), and deposition (Li et al. 2020; Reitz et al. 2021). One advantage of microgravity is the ability to utilize large-scale drops. In many space-based technologies, such as 3D printing, tissue engineering, and biotechnology (National Research Council 2000, 2003, 2011, 2013), the fluids involved are complex

✉ Amir H. Hirsa
hirsaa@rpi.edu

Patrick M. McMackin
mcmacp@rpi.edu

Joe A. Adam
adamj2@rpi.edu

Shannon R. Griffin
griffs3@rpi.edu

Richard P. Bonocora
bonocr@rpi.edu

Kenneth A. Brakke
brakke@susqu.edu

Juan M. Lopez
jmlopez@asu.edu

[1] Mechanical, Aerospace, and Nuclear Engineering, Rensselaer Polytechnic Institute, Troy 12180-3590, NY, USA

[2] Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy 12180-3590, NY, USA

[3] Department of Mathematics and Computer Science, Susquehanna University, Selinsgrove 17870-1164, PA, USA

[4] School of Mathematical and Statistical Sciences, Arizona State University, Tempe 85287, AZ, USA

[5] Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy 12180-3590, NY, USA

Published online: 22 February 2022

(non-Newtonian) (Rabani et al. 2019; Vailati et al. 2020). The dynamics of drops in microgravity are particularly relevant to biophysics, biotechnology, biochemical reactors, bioremediation, and pharmaceutical production when such systems include microorganism cultures or liquid suspensions (Walther 2002; Horneck et al. 2010; Barzegari and Saei 2012; Menezes et al. 2015; Blue et al. 2019). Such liquid cultures of microorganisms can be classified as active complex fluids (Morrison 2001; Saintillan 2018). The response of active complex biofluids to microgravity conditions, including residual gravity and *g*-jitter, is essential for understanding how these fluids may alter drop dynamics in technologies adapted for reduced gravity.

The air-liquid interface and contact-line dynamics with solid surfaces are of key importance when considering microorganism drop dynamics. The air-liquid interface, which can provide drop containment and stability, may be affected by the presence of microorganisms and their potential affinity for the interface (Marshall 1980; Loosdrecht et al. 1990; Brune et al. 2000; Persat et al. 2015; Kaminski et al. 2016; Sokolov et al. 2018; Madigan et al. 2019). In addition to the presence of microorganism cells, the alteration of the medium through metabolic activity and the production of biological compounds, including biosurfactants, can also affect interface behavior (Healy et al. 1996; Desai and Banat 1997; Bodour and Miller-Maier 1998; Tugrul and Cansunar 2005; Rodrigues et al. 2006; Das 2014; Ariech and Guechi 2015; Kurata et al. 2016; Naughton et al. 2019). As such, the effects of these potential alterations in fluid interface behavior can be studied to determine the impact biofluids may have on drop growth in space. This investigation studied the impact of spaceflight-relevant model microorganisms on centimeter-scale drop growth and stability in microgravity, subject to residual gravity and accelerative perturbations (jitter).

Three model microorganisms were selected for microgravity drop dynamics. First, the model bacterium *Escherichia coli* (*E. coli*) was chosen for the organism's genetic and phenotypic study in spaceflight (Bouloc and D'Ari 1991; Kim et al. 2014; Zea et al. 2017; Aunins et al. 2018; Padgen et al. 2020) and activity at air-liquid interfaces (Sinibaldi et al. 2017; Mathijssen et al. 2019; Martinez et al. 2020; Zhao et al. 2020). Second, baker's yeast (a unicellular fungus) *Saccharomyces cerevisiae* (*S. cerevisiae*) was selected in relation to studies of the organism's survival in microgravity (Kennedy and Volz 1983; Walther et al. 1996; Nislow et al. 2015; Hammond et al. 2018) and biosurfactant production relevant to alterations in surface properties (Alcantara et al. 2010; Atis et al. 2019; Ali and Al 2019; Ribeiro et al. 2020). The third model organism, the extremophile bacterium *Deinococcus radiodurans* (*D. radiodurans*), was chosen for its high resistance to radiation, a desirable trait for space missions (Dose et al. 1996; Kobayashi et al. 1996; Saffary et al. 2002; Ott et al. 2020), and notable hydrophilicity in liquid or multiphase cultures (Gargiulo et al. 2007; Gargiulo et al. 2008). Each of the microorganisms selected provided relevance to unique biological fields of study, including applications in virulence, genetics, pharmaceutical production, gas transfer, radiation resistance, and bioremediation.

In this study, parabolic flight experiments subjected growing centimeter-scale drops to microgravity conditions, with drops containing stationary phase liquid cultures of *E. coli*, *S. cerevisiae*, and *D. radiodurans*, along with their supporting media (LB, YPD and TGY, respectively). This study reports on the effects of microorganisms on the formation of droplets as measured through the response of these growing drops to *g*-jitter and residual gravity present in parabolic flights. The goal of these experiments was to determine if and how microorganisms affect drop growth in microgravity. Drop deployment and pinning are critical stages in surface-tension contained experiments. How complex fluids behave in these critical stages is important for the success of such experimental apparatuses. Analysis of drop behavior while subject to perturbations during drop formation alludes to a biofluid's stability and feasibility for use during long term space research. This investigation details early stage short timescale microorganism drop behavior (parabolic flights provide approximately 20s of microgravity), providing a necessary foundation for more long term space missions (e.g. International Space Station). Specifically, an aim of this study is to provide data for space-based biochemical reactors and processing methods, which may use various aspects of drop growth and interfacial dynamics (Walther 2002; National Research Council 2003; Gulati et al. 2017; McMackin et al. 2020; Riley et al. 2021). For applicability to current and future space technologies, this study focuses on drops of a scale not attainable at 1-*g*.

Methods

Strains and Media

Three types of growth media were used, each supporting the growth of a specific microorganism, LB for *E. coli*, YPD for *S. cerevisiae*, and TGY for *D. radiodurans*. These liquid media consisted of: Luria-Bertani (LB; 5g/L yeast extract, 10g/L NaCl, 10g/L tryptone), yeast extract peptone dextrose (YPD; 10g/L yeast extract, 20g/L peptone, 20g/L dextrose), and tryptone glucose yeast extract (TGY; 10g/L tryptone, 1g/L glucose, 5g/L yeast extract). All media solutions were autoclaved at 121 °C for 15min to ensure sterility. The sterile media was used as a control for each microorganism.

The three microorganism species used were *E. coli* strain BL21, *S. cerevisiae* strain ATCC 9763, and *D. radiodurans* strain ATCC 13939. Microorganism cultures were grown in

an orbital shaker at 37 °C and 200RPM to the late stationary phase (optical density of 4.0 at 600nm). These liquid microorganism cultures were stored at 2 °C for 6 days during transportation and flight preparation. Microorganism cultures were allowed to reach room temperature of nominally 20 °C prior to experimental tests and all experiments were conducted at this temperature.

Experimental Apparatus and Procedure

A two-day parabolic flight campaign was carried out (April 28 and 29, 2021) onboard a modified Boeing 727 (Zero Gravity Corporation). The aircraft executed a series of parabolic maneuvers which involved a brief period of freefall, allowing for the simulation of a microgravity environment in the cabin. All data reported here was obtained on the second day, when 6 sets of 5 parabolas were executed, with each parabola providing nominally 20s of microgravity. In the experimental setup (Fig. 1), the test cell was secured to a base plate, consisting of a $61 \times 61 \times 1.27$ cm optical breadboard. The base plate was bolted to the padded floor of the aircraft, which transferred the accelerations of the aircraft to the test cell. The test cell consisted of 1.27cm thick aluminum (the bird-house shaped structure in Fig. 1a), with a magnetically-locked hinged roof which allowed for rapid cleaning of the dispensing tube (D-tube) between parabolas. Inside the aluminum housing, a piece of machined Delrin held the 10-gauge stainless steel dispensing tube (Hamilton 9-91010-PK) in place. From the D-tube, liquid drops were grown up to 1.5cm in diameter. Each D-tube was machined to ensure a flat end with macroscopically sharp corners. A hydrophobic coating (Scotchgard) was applied to the outer surface of the D-tube to minimize wetting outside the tube.

A 10mL syringe containing a fluid sample was connected to the D-tube and dispensed at a flow rate of 14mL/min using a syringe pump. For each set of parabolas, a different fluid sample (*E. coli.*, *D. radiodurans*, *S. cerevisiae.* or their respective growth medium) was utilized. After the period of microgravity during each parabola, the drop would fall, and the liquid was absorbed by a sponge at the bottom of the test cell. Additionally, the D-tube was wiped dry between each parabola with Kimwipes to minimize wetting the outside of the D-tube.

Two sets of LED lights were used for illumination. A series of blue LED lights were mounted around a circle on the side of the test-cell where the D-tube entered and another

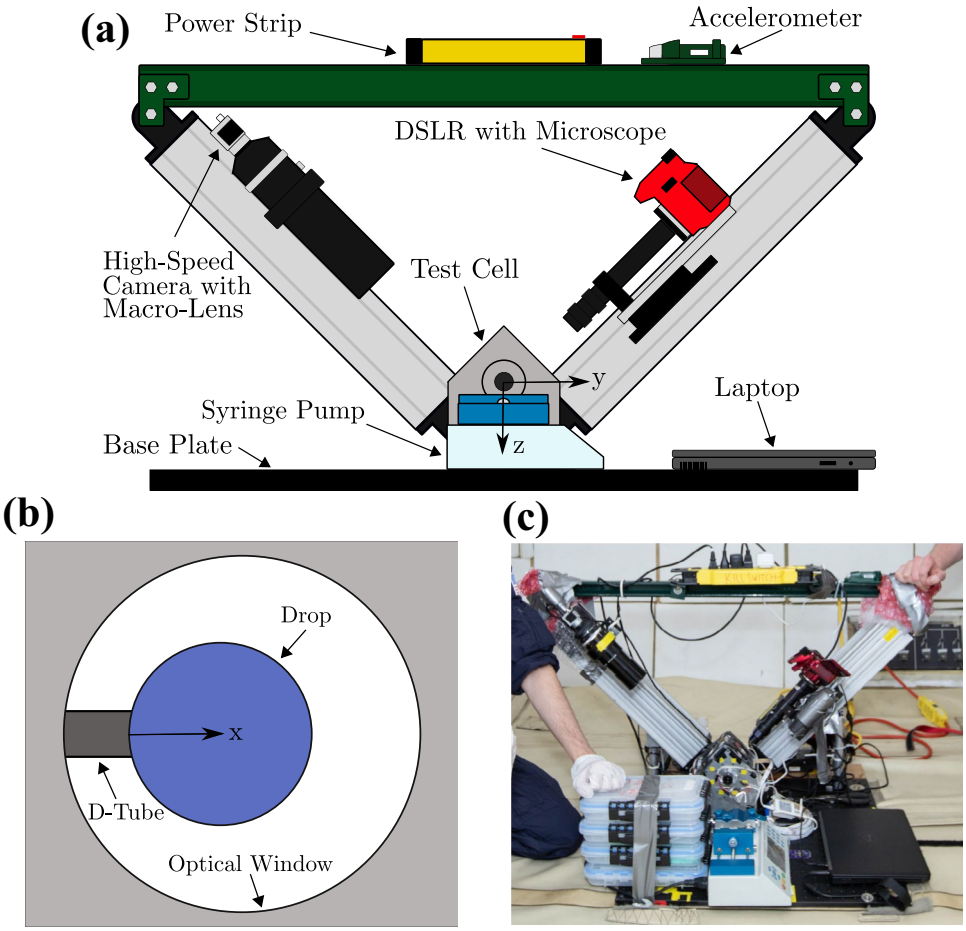

Fig. 1 (**a**) Scaled hardware schematic, (**b**) camera view test cell schematic, and (**c**) hardware photograph onboard the aircraft. Growing drops within the test cell were imaged by the two orthogonal cameras through optical windows. All components were rigidly attached to the floor of the aircraft

set on the opposite side. Additional illumination was provided by a strip of white LEDs with approximately 3cm spacing between each LED, which was wrapped several times inside the test cell. An example image of a drop of each fluid is shown in Fig. 2. Here, the LED lighting can be seen reflecting off the surface of the drops with microorganisms producing turbidity, particularly in the case of *E. coli* and *S. cerevisiae*.

A high-speed black-and-white camera with 2048×1088 pixels (Basler acA2000-165um) was used with a macro-lens (Nikkor 105mm, f/4) to capture uncompressed images of drop formation at 90 frames per second (see Fig. 2). Recordings were controlled by PYLON VIEWER 6.1.1 software in intervals of 25s in order to allow the memory buffer (9,999 frames) to write to the flight computer's storage between periods of microgravity. A second DSLR camera (Nikon D5500) was connected to a microscope zoom lens (Optem XL-70) and used to make color 1920×1080 pixels 30fps video at high magnification (see Fig. 3). This DSLR camera was controlled using CAMERA CONTROL PRO

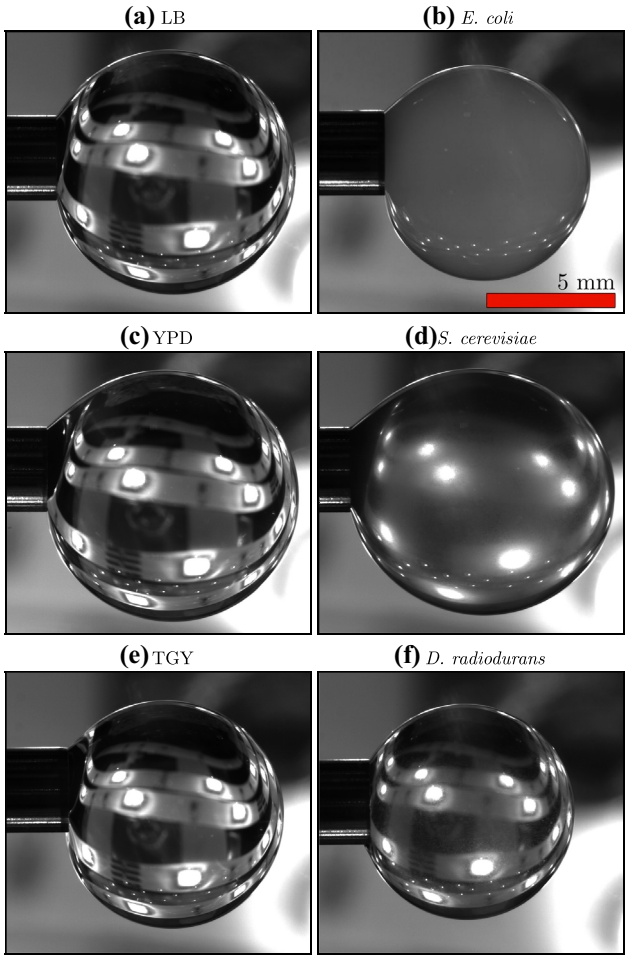

Fig. 2 High-speed camera photographs of each fluid at various stages of deployment

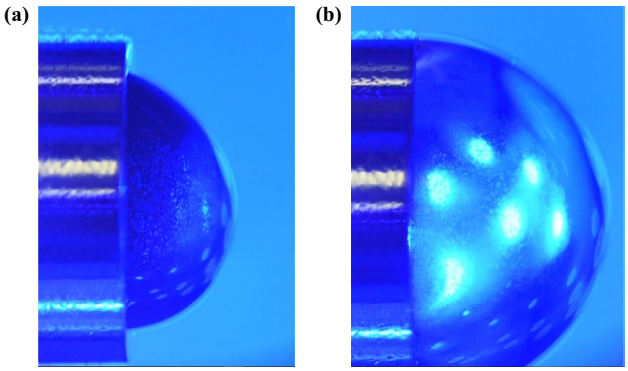

Fig. 3 Microscope snapshots of a *D. radiodurans* drop showing early stages of drop growth: (**a**) pinning at the inner radius and (**b**) subsequent pinning at the outer radius of the D-tube

with an ISO of 1250 and an exposure time of 16.7ms. The microscopic videos provide essential data of the early stages of drop formation, during which the contact-line moves from the inner diameter of the D-tube rim to the outer edge. An example of this phenomena is presented in Fig. 3.

The instantaneous acceleration vector was measured at 10Hz for the duration of the flight using a 3-axis accelerometer (EXTECH G-Force Datalogger VB300) mounted on the flight hardware. This sensor was controlled using EXTECH VB300 DATALOGGER 2.6 and operated continuously throughout the series of parabolic maneuvers. Using a series of 8mm free-fall calibration tests, the sensor was found to read $0.043 \pm 0.025 g_0$, with an estimated $0.027 g_0$ of that being the result of air resistance. In stationary 1-g testing, the sensor read $1.02 \pm 0.01 g_0$. Based on these findings, $0.02 g_0$ was used as the uncertainty of the acceleration data presented in this study.

Static Drop Simulations

In order to quantify the behavior of growing drops, two features of the drops' shapes were optically tracked: contact angles in the image plane, θ_1 and θ_2, and drop aspect-ratio, $\mathcal{AR}$. A MATLAB image analysis script extracted these features from raw experimental data. The air-liquid interface was identified for each video frame of the high-speed camera using an edge-detection method via background removal with MATLAB's `edge` function for error-correcting (McMackin et al. 2020; Riley et al. 2021).

The drop aspect-ratio was obtained by taking the quotient of the maximum width and height of the interface for each frame. The shape of the interface at the D-tube was analyzed to determine the instantaneous contact angles of the drop in the image plane. This method of quantitative analysis, depicted in Fig. 4, allowed the drop's behavior to be quantified for all trials of the experiment.

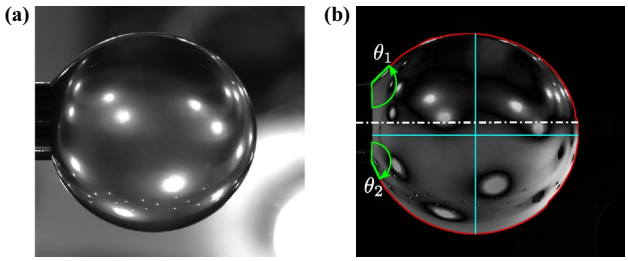

Fig. 4 (**a**) Example frame from the high-speed camera of *S. cerevisiae* and (**b**) the same frame after undergoing image analysis. The red curve is the detected interface, the cyan cross displays the drop's maximum height and width for determining aspect-ratio $\mathcal{AR}$, and the green line segments with arrows represent the measured contact angles of the liquid on the D-tube. The axis of the D-tube is shown by the white centerline

Simulations of drop growth were performed using SURFACE EVOLVER (Brakke 1992), which models the static behavior of capillary surfaces. To simulate the experiments, discrete volume steps were used to increase drop volume, simulating drop growth. At each volume, the program performed an energy balance to minimize the system's surface and gravitational energy and iterated to determine the equilibrium state. In order to simulate the experimentally measured residual gravity and g-jitter, measured accelerations in all three axes were input into SURFACE EVOLVER. Values of the acceleration components were determined by averaging accelerometer data over the period of drop growth for the corresponding experimental trial. In this model, surface tension remained constant, using the value for water of $\sigma = 72$ dynes/cm. The data being analyzed is taken over about 2 seconds during the initial creation of the drop interface. Over this short time, the interfacial tension is not expected to depart significantly from that of air-water. The contact angle on the flat end of the stainless steel D-tube was taken as 60° (Martinez-Urrutia et al. 2018), and the contact angle on the outside of the hydrophobically coated D-tube was estimated to be 150°.

In order to quantitatively compare experimental results to SURFACE EVOLVER simulations, a normalized, averaged deviation was calculated using:

$$\langle x \rangle = \frac{1}{n} \sum_{i=1}^{n} \frac{|x_i^{\mathrm{e}} - x_i^{\mathrm{s}}|}{x_i^{\mathrm{s}}}, \tag{1}$$

where x is some vector of length n, x_i^{e} and x_i^{s} are, respectively, the values of experimentally measured data and predictions from SURFACE EVOLVER at the same index in time. This allows for direct comparison of drop growth with varying levels of residual gravity and jitter. These deviations of drop features were calculated for three parabolas for each of the fluids (three technical replicates). The averaged results of these calculations are presented in Table 1.

Results

The shapes of the centimeter-scale drops were found to be sensitive to the presence of small acceleration forces. While the aim of a zero-g parabola is to simulate the complete absence of gravitational acceleration, small but measurable accelerations in all three coordinate axes are present and vary between parabolas. These accelerations, which can be caused by a combination of aeroelastic vibrations, turbulence and pilot technique, result in unique behavior for each parabola. In order to make a comparison between different fluids over the course of a number of parabolas with unique acceleration vectors, a simulated control case with the same acceleration vector was produced to act as a baseline reference. This reference was produced using SURFACE EVOLVER simulations (see the Methods section for details) with the fluid properties of water and the same acceleration environment as measured for each of the experimental runs.

Examples of experimental data for all fluids are shown in Fig. 5, along with their respective SURFACE EVOLVER

Table 1 Average measured acceleration normalized by Earth gravity, $|\vec{g}|/g_0$, and averaged differences between experiments and simulations of $\mathcal{AR}$ and contact angles for three runs of each microorganism and growth medium. The error represents averaged standard deviation from three technical replicates

	LB	*E. coli*	YPD	*S. cerevisiae*	TGY	*D. radiodurans*
$\lvert\vec{g}\rvert/g_0$	0.11 ± 0.03	0.11 ± 0.03	0.17 ± 0.02	0.11 ± 0.02	0.09 ± 0.04	0.09 ± 0.03
$\langle \mathcal{AR} \rangle$	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.10 ± 0.07	0.05 ± 0.02
$\langle \theta_1 \rangle$	0.23 ± 0.43	0.26 ± 0.06	0.09 ± 0.29	0.23 ± 0.17	0.13 ± 0.06	0.01 ± 0.89
$\langle \theta_2 \rangle$	0.32 ± 0.13	0.22 ± 0.21	0.21 ± 0.17	0.25 ± 0.10	0.27 ± 0.07	0.29 ± 0.45
$\langle \lvert\Delta\theta\rvert \rangle$	0.60 ± 0.10	1.80 ± 0.90	0.31 ± 0.09	0.45 ± 0.12	0.58 ± 0.14	0.64 ± 0.35

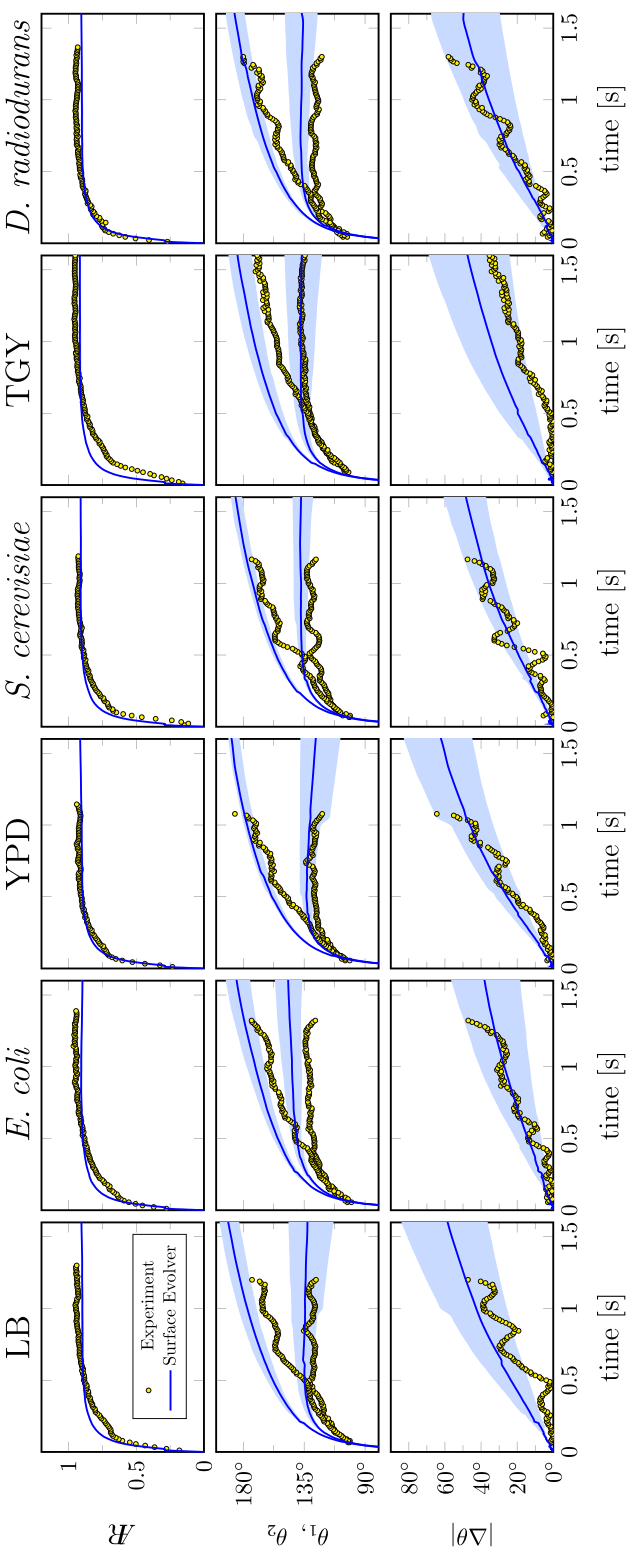

Fig. 5 Time series of drop aspect-ratio, $\mathcal{R}$, the two contact angles between the drop and the D-tube, θ_1 and θ_2, and their absolute difference $|\Delta\theta|$, determined from experimental measurements (yellow circles), compared to SURFACE EVOLVER simulations (blue lines). Light blue uncertainty regions represent a range of one standard deviation of the measured accelerations during drop deployment

simulations. As expected, $\mathcal{R}$ starts from zero and increases monotonically in the simulations and experiments. For all cases, the simulated $\mathcal{R}$ generally agree with the experimental results. Drop contact angle behavior (θ_1, θ_2, and $|\Delta\theta|$) was notably more sensitive to changes in local accelerations than drop aspect-ratio $\mathcal{R}$, which showed little change between trials. In these plots, the uncertainty regions represent one averaged standard deviation of the accelerations measured during drop deployment and serve to represent the effects of g-jitter. The magnitude of local accelerations experienced by each fluid relative to Earth gravity, $|\vec{g}|/g_0$, is provided in the first row of Table 1.

Fig. 5 shows that experimentally measured drop behavior does not remain entirely within the acceleration uncertainty of the simulations. It is important to note that SURFACE EVOLVER simulations are strictly static calculations and do not capture the complexities associated with the fluid dynamics in these experiments. Experimental accelerations fluctuate, which can result in a slight oscillation observed for most drops; these are most apparent in the time series plots of θ_1 and θ_2 (row 2 of Fig. 5). Also, as the D-tube is not changed between every drop formation trial, there is likely nonuniform wetting that is not captured by these simulations. Furthermore, as each volume step change in the simulations involves an instantaneous increase in volume, there is no influence of flow from the D-tube and in the drop. However, $\mathcal{R} \leq 1$ for all cases, which indicates that fluid inertia is not a dominant force in the system at the experimental flow rate. This is consistent with previous work (Karbaschi et al. 2012), which showed that for Reynolds number $Re = d_i\, v\, \rho/\mu \leq 70$ (where d_i is the inner diameter of the tube, v is velocity, ρ is density and μ is the dynamic viscosity of the fluid), the drop shape is negligibly affected by the flow from the D-tube. In the present experiments, $28 < Re \leq 75$, which allows the quasi-static assumptions made in SURFACE EVOLVER.

The time average of the difference between the contact angles was found to be non-zero for all cases due to small accelerations which occurred primarily in the z-axis of the test cell, and therefore imparted an observable effect in the image plane. Previous work showed that the behavior of drop contact angles due to varying gravitational acceleration can be characterized by the Bond number, $Bo = \rho g h^2/\sigma$ (Zhu et al. 2012). Here, with $g = 0.1 g_0$, we estimate $Bo \approx 1$.

Quantification of the effects of microorganisms on drop growth was performed by comparisons between microorganism suspensions and their inoculated growth medium. Microorganisms did not produce increased departure from simulated results, with the averaged differences in $\mathcal{R}$ and contact angles being comparable between media and microbe cases (rows 2–5 of Table 1). Analyzing the time series from Fig. 5 and the values of Table 1, for each

medium the drop aspect-ratio was unaffected by the presence of microorganisms. Furthermore, contact angles and $|\Delta\theta|$ show no significant difference in the presence of microorganisms. *E. coli* did show a sizeable difference of $|\Delta\theta|$ from LB growth medium, however this difference is not statistically significant, being within the uncertainty of the cases analyzed.

An interesting auxiliary result of this investigation is that flow visualization in growing drops can be performed using microorganism aggregates as tracer particles. These aggregates displayed buoyancy-driven flow within the drop before the onset of microgravity, upon which this flow (and the tracer particle microorganism aggregates) ceased motion. An example of this flow visualization for a drop containing *S. cerevisiae* is shown in supplemental `movie01.mov`.

Discussion

The impact of microorganisms on the physical behavior of air-liquid interfaces and contact line dynamics is important for the development and adaptation of technologies for operation in reduced gravity. This investigation focused on the formation of centimeter-scale drops with and without microorganisms. The drop aspect-ratio $\mathcal{AR}$ was found to be unaffected by the presence of these microorganisms (row 1 of Fig. 5). In fact, the $\mathcal{AR}$ predicted by the quasi-static simulator SURFACE EVOLVER, for both the growth media as well as the organisms, were relatively close to those of pure water.

When examining the effects of microorganisms, the time scale of the experiments is important. During drop formation, the gas-liquid interface is continuously being created and is observed on the order of seconds. In this time frame, organisms likely do not have sufficient time to localize to the surface in high enough concentrations to effect a change in the capillary response of the interface. Specifically, the microbial processes typically responsible for alterations in interfacial properties, such as chemotaxis, swarming, bacterial turbulence, biosurfactant production, and biofilm formation, function at time scales greater than the 20s experiment duration. Likewise, drop contact angles did not significantly change in the presence of microorganisms. Once again, the time scale associated with the drop deployment process likely limited effects from the microorganisms. Furthermore, the late stage of growth of these microorganisms may also have limited their activity and further reduced effects on drop formation.

The minute effect of microorganisms measured in this investigation (see Table 1) bodes well for the adaptation of existing Earth-based drop technologies for operation with biofuids in microgravity. In the case that microorganisms severely affect fluid dynamics in microgravity, new engineering efforts would be required to support space technology. While such engineering may still be required to some extent, this investigation demonstrates that the macroscale effects of select microorganism solutions are minimal for the given experimental stage and timescale. This provides a foundation for future experiments on both more detailed effects of microorganisms and regarding long duration space operations.

Further investigations, not possible on parabolic flights, will be required to reveal the intricacies of microbial complex fluid effects and long term effects of microorganisms in microgravity and surface tension dominated systems. These studies can be grouped in two main areas: ground studies and space studies. Ground studies have the potential to parameterize the properties and behavior of microorganism solutions, including surface tension and the dependence of measured rheological properties on microorganism type and state, such as growth phase, interface aging, and long duration effects. Space studies could test the robustness of surface tension contained experimental apparatuses as well as provide insight pertaining to altered, potentially time dependent, microorganism and fluid behavior in microgravity.

Supplement Caption

Movie 1 (`movie01.mov`): Microscope video showing a drop of *S. cerevisiae* during the transition between hypergravity and microgravity. During hypergravity, aggregates of the organism can be seen undergoing a buoyancy-driven flow which stops when microgravity is achieved at time 00:57

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12217-022-09933-8.

Acknowledgements The authors thank Michelle Peters and her colleagues at ZeroG, the NASA Flight Opportunities Office including Earl Adams, and Louise Strutzenburg and her team at NASA Marshall Space Flight Center for their assistance during the parabolic flight campaign. The authors also thank Dan Speer for his contribution to the test cell's construction.

Author Contribution JA, RB and AH conceived the experiment PM, JA and AH prepared the experimental hardware PM, JA and SG conducted the parabolic flight experiments. PM performed the experimental data extraction and analysis. PM, JA, AH, JL and SG wrote the manuscript with support from RB and KB. KB wrote the base SURFACE EVOLVER data-file, which was then modified and executed by PM.

Funding This work was supported by NASA Grants 80NSSC20K1726 and 80NSSC18K1663, and NSF Grants 1929134 and 1929139.

Data Availability The data collected during this study is available from the corresponding author upon reasonable request.

Code Availability The code developed for this study is available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare that there are no competing interests.

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