

A Laboratory Experiment to Demonstrate the Principles of Sedimentation in a Centrifuge: ESTIMATION OF RADIUS AND SETTLING VELOCITY OF BACTERIA

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Sedimentation refers to the movement of particles in a fluid under the influence of an external force. Centrifugation is a subset of sedimentation where particles or macromolecules move through a liquid under the influence of a centrifugal force.^[1] Centrifugation is a ubiquitous unit operation in biotechnology, food, mineral processing, and many other industries. In the biotechnology industry centrifugation is used for rapid removal of cell particles from fermentation or cell culture media, removal of cell debris, and separation of protein precipitates.^[2] Commercial bioprocesses for the manufacture of penicillin, recombinant human insulin, monoclonal antibodies, therapeutic proteins, viral vaccines, bioethanol, and 1,3-propane diol use centrifugation for cell removal.^[3] Biological particles are relatively small (sub-micron to a few microns in diameter), and hence high rotational speeds are necessary for their separation. However, higher rotational speeds lead to greater shear stress. Particles separated using centrifugation in biotechnology are usually shear sensitive and hence require greater care during separation.^[4] Therefore, centrifuge operation in biotechnology is a tradeoff between separation efficiency and shear damage.

The laboratory exercise presented in this paper demonstrates the application of sedimentation principles to centrifugation, and allows students to contemplate the effect of shear on biological particles during centrifugation. *Escherichia coli* (*E. coli*) cells suspended in Luria-Bertani (LB) medium were separated using a lab-scale batch centrifuge. This experiment provides an opportunity for students to develop experimen-

tal protocols, teaches a variety of hands-on experimental skills, and uses cognitive conflict to stimulate learning. This centrifugation experiment is part of the bioprocess engineering laboratory course offered to students in the Master of Biotechnology Program at Northwestern University. Most students in this course have biology or chemical engineering undergraduate majors while a minority have chemistry-related majors. In this lab, students were divided into diverse teams based on their personality type (as determined thorough Myers-Briggs Type Indicator), their educational background, gender, and ethnicity.

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Learning outcomes from this experiment:

1. Understand the principles of centrifugation, and the shear effects of centrifugation on bacterial cells.
2. Operate a benchtop centrifuge, handle *E. coli* cells, and measure optical density of suspensions.
3. Perform calculations to estimate particle radius and settling time.
4. Critically examine and interpret results from centrifugation data.
5. Reconcile cognitive conflicts in centrifugation data.
6. Exposure to effective team skills and electronic lab notebook technology.

LITERATURE REVIEW

Motion of particles in a centrifuge is influenced by particle diameter, particle density, liquid density, and liquid viscosity.^[5] Centrifugation can be used for the separation of solids from liquid, differential separation of immiscible liquids, and fractionation of macromolecules.^[6] Benchtop centrifuges, common in biotech laboratories, can reach acceleration up to 10,000g, while industrial tubular and disk centrifuges can reach accelerations of 61,000g and 14,000g, respectively. Ultracentrifuges used to separate macromolecules such as proteins and radioisotopes can reach acceleration above 100,000g.^[7]

Laboratory-scale centrifuges typically operate in batch mode and are capable of processing submilliliter to about one liter of sample per batch.

Industry-scale centrifuges operate in either semibatch or continuous modes and can handle capacities of several hundred liters per hour.^[8,9] Commercial applications of centrifugation include cell separation for bioprocessing, fat isolation in milk processing, blood plasma isolation in clinical laboratories, and DNA and RNA separation in biology. Centrifugation also has applications in waste treatment for fine solids removal, and in mineral processing for ore separation.^[10] In all centrifugation equipment the underlying principle remains the same—solids are separated based on their density difference and particle size.

A limited number of studies on laboratory experiments that use centrifugation in a teaching context have been reported in the literature. Lefebvre (2006) reported a microcentrifuge experiment for the separation of inorganic salts and polymeric powders in organic solvents, and used it to demonstrate the

constructivist theory of learning.^[11] This short laboratory exercise was used to supplement a lecture, but did not exhaustively demonstrate the principles of sedimentation. Small-scale centrifugation experiments in a classroom learning environment have been used to demonstrate the Meselson-Stahl experiment for separation of DNA, and for differential separation of yeast cells.^[12,13] Centrifugation experiments have been used in a classroom setting for extraction of juice from mash^[14] and for separation of biodiesel.^[15] However, literature reports do not focus on or demonstrate the principles of sedimentation.

THEORETICAL BACKGROUND AND EQUATIONS OF PARTICLE MOTION

Motion of particles through liquid during centrifugation is influenced by three forces; viz., centrifugal force acting downward, and buoyancy and drag forces acting upward (Figure 1). The sum of these three forces will determine the net velocity of particles in a centrifuge.^[5] The particle will sink or settle if the downward force is more than the upward force, and the particle will float if the downward force is less than the upward force. Particles are assumed to be stationary and homogeneously suspended in the liquid before the application of centrifugal force. We used a centrifuge with a swing bucket rotor in this experiment. When the centrifuge is stationary, the centrifuge tubes are vertical, *i.e.*, they are parallel to the axis of rotation (Figure 2A, next page). Sedimentation here happens through gravitational settling. But when the centrifuge starts rotating, the tube holder and the centrifuge tube "swing" to a position that

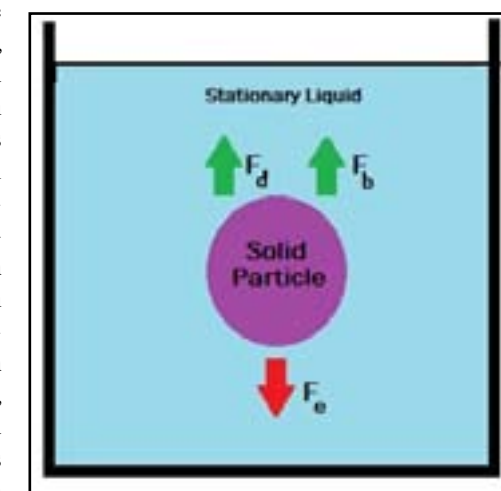


Figure 1. Forces acting on a particle during settling. F_g is the external force (either gravitational or centrifugal), F_b is the buoyant force, and F_d is the drag force.

is perpendicular to the axis of rotation (Figure 2B, next page). The applied centrifugal force moves the particles away from the axis of rotation and towards the bottom of the tube. The effect of gravitational forces, which pull the particles down in the direction parallel to the axis of rotation, becomes negligible. Hence, particles move in a horizontal plane towards the bottom without many collisions with the centrifuge tube surface. However, collisions between particles might exist.

During operation of the centrifuge, after a brief acceleration period velocity of particles reaches a constant value, called the terminal velocity, v_t . After the particle reaches v_t , there is no acceleration, *i.e.*, $dv/dt = 0$. A force balance can be applied to derive an expression for velocity of a particle moving through the liquid in a centrifuge. Applying the condition $dv/dt = 0$ to the force balance equation and further simplification results in an equation for terminal velocity given by Reference 5,

Figure 2. Illustration of particle motion in a centrifuge with swing bucket rotor. (A) particle motion in a centrifuge at rest, and (B) particle motion in a centrifuge during rotation.

$$v_t = \frac{2a_p^2(\rho - \rho_0)\omega^2 R}{9\mu} \quad (1)$$

Where,

- a_p - radius of particles, m
- R - distance between axis of rotation and a specific location in the centrifuge tube, m. Refer to Figure 2B for a graphic indicating distances.
- v_t - terminal settling velocity, m/s
- μ - viscosity of liquid, kg/m·s
- ρ - density of particles, kg/m³
- ρ_0 - density of liquid, kg/m³
- ω - angular velocity of centrifuge, rads/s

The following assumptions are inherent in the above expression: (i) Particles are spherical with a circular projected area; (ii) particles settle without the influence of other particles or the surface of the container (unhindered settling); (iii) Particle motion is in the Stoke's law regime, *i.e.*, $Re_p = D_p v_t \rho / \mu < 1.0$, (iv) particle motion due to gravitational force is negligible, and (v) distance required to reach v_t is negligible.

When the particle is moving horizontally away from the center of rotation, its velocity is given by the rate of particle displacement in the radial direction, *i.e.*, $v_t = dR/dt$, which on substitution from Eq. (1) gives,^[5]

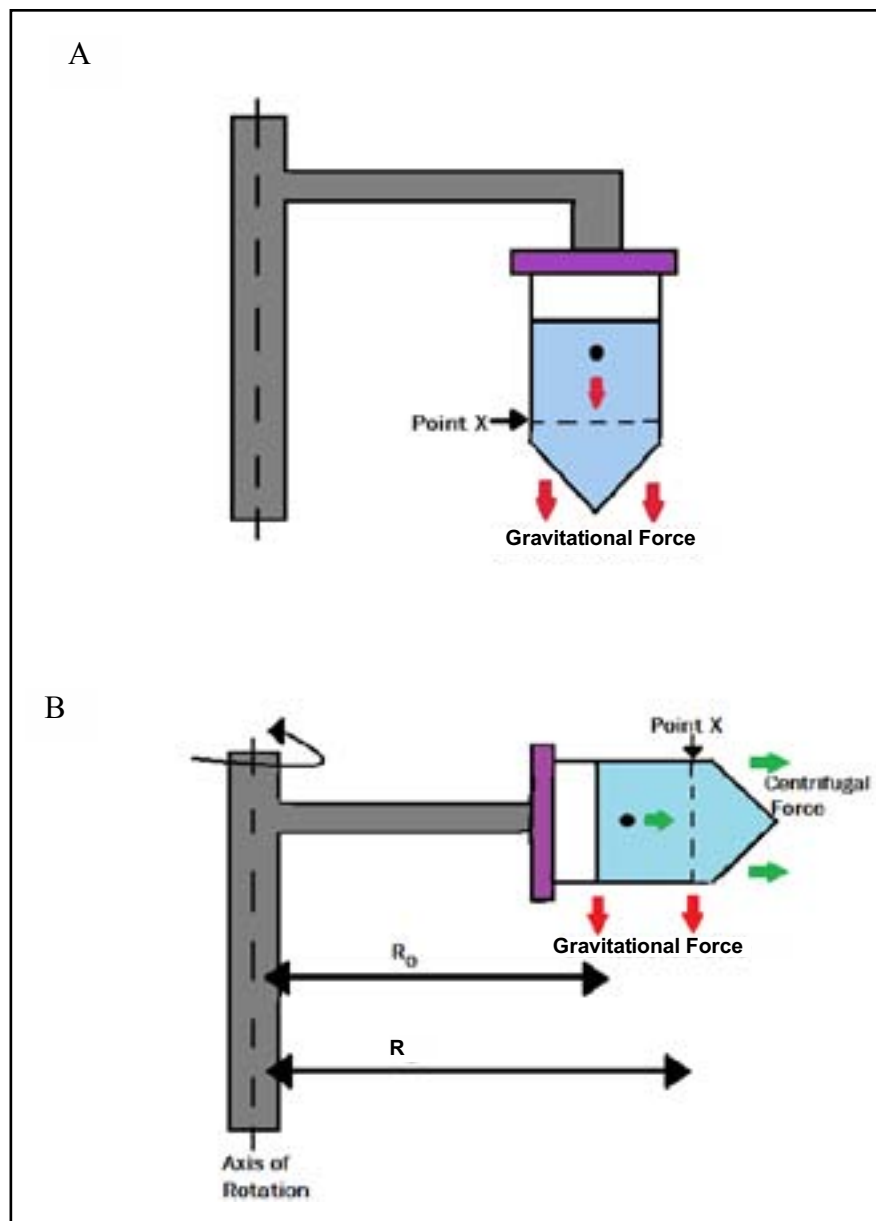
$$\frac{dR}{R} = \left[\frac{2a_p^2(\rho - \rho_0)\omega^2}{9\mu} \right] dt \quad (2)$$

Eq. (2) can be integrated with limits $R=R_0$ at $t = 0$ and $R = R$ at $t = t$ to give,

$$\ln\left(\frac{R}{R_0}\right) = \left[\frac{2a_p^2(\rho - \rho_0)\omega^2 t}{9\mu} \right] \quad (3)$$

Where

- R_0 - distance between axis of rotation and top of fluid in centrifuge tube, m
- R - distance between axis of rotation and a specific location in the centrifuge tube, m



t - time taken by the particle to travel from R_0 to R , s

Eq. (3) can be rearranged to estimate the particle radius given by,

$$a_p = \left[\frac{9}{2} \frac{\ln\left(\frac{R}{R_0}\right)\mu}{(\rho - \rho_0)\omega^2 t} \right]^{1/2} \quad (4)$$

If time taken for the particle to reach (or settle) a certain distance in the centrifuge is experimentally determined, then Eq. (4) can be used to estimate the particle radius. Densities of particle and liquid, and viscosity of the liquid, should be obtained from literature. After estimating particle radius,

Eq. (1) can be used to estimate terminal velocity. This is the unhindered settling velocity.

In practice, *E. coli* particles settle under the influence of other *E. coli* particles. This is called hindered settling. For a uniform suspension, hindered settling velocity (v_{hs}) is given as,^[5]

$$v_{hs} = v_t(1 - \phi)^n \quad (5)$$

where,

- v_{hs} - hindered settling velocity, m/s
- ϕ - volume fraction of particles, dimensionless
- n - exponent dependent on particle Reynolds number

Values of n can be determined from Re_p vs. n charts available in the literature.^[16] The exponent n accounts for: (i) increase in relative velocity of solid above the unhindered settling velocity due to upward motion of the liquid displaced by the solid; and (ii) redistribution of flow around a particle caused by the presence of other particles, subsequently affecting the drag coefficient.^[17] But for *E. coli* cells, convenient empirically developed v_{hs}/v_t vs. ϕ data can be used.^[5] Linear interpolation of v_{hs}/v_t vs. ϕ data is acceptable when estimating v_{hs}/v_t from ϕ values. Estimation of volume fraction of cells is discussed in the Experimental Methods section, and linear interpolation of v_{hs}/v_t vs. ϕ data is discussed in the Results and Data Analysis section.

Estimation of hindered settling velocity is important in centrifugation to determine the time required for centrifugation when the centrifuge tube length is known or vice-versa. It is also useful in determining the relative position of particles in a centrifuge when it is used for fractional separation based on particle size and density differences. Terminal velocity values can be used to obtain quick estimates of particle radius using centrifugation data.

EQUIPMENT, SUPPLIES, AND PREPARATION OF REAGENTS

A benchtop centrifuge with a swing bucket rotor that can hold 15 mL centrifuge tubes was used in this experiment (Fisher Scientific AccuSpin 3R). Other required supplies are graduated 15 mL centrifuge tubes, *E. coli* cell suspension, Luria-Bertani (LB) media powder, 250-ml shaker flasks, autoclave, temperature-controlled shaker, spectrophotometer to measure absorbance at 600 nm, cuvettes for absorbance measurements, and pipette with capacity of 200 μ L – 1 mL. LB media was prepared by adding 0.4 L of deionized water to 10.0 g of LB media powder and mixing it on a magnetic stirrer until a clear yellowish solution was obtained. LB media was then distributed into four 250-ml shake flasks, each containing 100 mL of LB media. The shake flasks with LB media were covered with aluminum foil and autoclaved at 121°C for 20 minutes to sterilize the contents. Cooled, sterile LB media was inoculated with 20 μ L of concentrated *E. coli* cell

stock solution (OD_{600} of approximately 0.75 units at a dilution 1:10) in a biological safety cabinet. Inoculated LB media was incubated in a shaker at 250 rpm and 37 °C for about 16 hours (overnight). Our reference growth curve experiments have shown that *E. coli* cells were in stationary phase at 16 hours with OD_{600} of approximately 1.6 ± 0.2 units measured against LB blank. We verified that the *E. coli* cell suspension used in centrifugation experiment had OD_{600} within the range mentioned above after 16h of incubation.

LABORATORY DESCRIPTION

Parameters that need to be experimentally determined are the settling time and volume fraction of particles. Settling time will be substituted in Eq. (4) to determine the particle radius, which is then used in Eq. (1) to determine terminal velocity. Volume fraction of particles and terminal velocity are used in Eq. (5) to determine hindered settling velocity. The general work flow of this experiment is shown in Figure 3, next page.

Determination of settling time

Settling time is defined as the time required for particles to travel a certain distance in the fluid. In this experiment the particles were monitored as they traveled from the top of the fluid (which in this experiment was at the 12 mL mark in the graduated 15 mL centrifuge tube) to some point X (which in this experiment was at the 6 mL mark in the graduated 15 mL centrifuge tube). Figure 2B shows the location and distances of measurement points. Theoretically, settling is considered to be complete when all particles have crossed the 6 mL mark. Cells are assumed to exist as a homogenous suspension at the start of the experiment. Chronological steps in this experiment are:

- Pool and mix *E. coli* suspension from four shake flasks.
- Aliquot 12 mL of *E. coli* suspension each into 12 separate 15 mL centrifuge tubes. Set aside two tubes for particle volume fraction estimation.
- Load the centrifuge with the remaining 10 aliquots and run it at the required speed (such as 1500 rpm). Pipette about 1 mL of sample every five minutes from point X without disturbing the liquid. Measure the optical density of samples taken from point X at 600 nm (OD_{600}) using fresh Luria Bertani medium as blank. OD_{600} values give an indirect measurement of *E. coli* concentration in the sample!^[18]
- Return the sample to the tube from which it was taken. Do not reuse this tube for further measurements, but keep the centrifuge balanced.
- Continue centrifugation and sampling until OD_{600} at point X reaches a constant value, at which point complete settling has been achieved. The time at which complete settling happens is called settling time, t . It is good practice to narrow the time intervals for sampling as the particles get closer to complete settling, which can be predicted

by plotting OD_{600} vs. time and following the curve as it begins to level off. Use this t value in Eq. (4) to estimate particle radius.

- Repeat the experiment at different centrifuge speeds to check for consistency in particle radius estimations. Use the particle radius value in Eq. (1) to estimate terminal settling velocity.

Determination of particle volume fraction

Centrifuge 12 mL of *E. coli* suspension at 3000 rpm for 20 minutes to sediment the cells. Remove all supernatant by careful pipetting without disturbing the cell sediment. Measure the volume of supernatant that was removed (V_{sup}). The

volume fraction of cells is given by

$$\phi = (12 - V_{sup})/12 \quad (6)$$

Use this value of ϕ to determine n , and then use v_t and n in Eq. (5) to estimate hindered settling velocity, or use the convenient v_{hs}/v_t vs. ϕ data available for *E. coli* cells.^[5]

RESULTS AND DATA ANALYSIS

Goals of the centrifugation experiment are to determine: (i) the radius of *E. coli* particles; and (ii) unhindered and hindered settling velocities of *E. coli* particles. This experiment was done by two different teams each week, and each team did

the experiment at two different centrifugation speeds. Student teams were required to propose a written, step-by-step protocol to accomplish the goals of this experiment. The protocol should include: (i) data that needs to be collected; (ii) templates to record and tabulate data (these will be filled in during the experiment); (iii) step-by-step description of experimental methods; (iv) list of equations that will be used and their purpose; and (v) the list of graphs that need to be generated to estimate settling time. The protocol should also include the assumptions inherent in the equations, possibilities for error, and the approximations necessary to do the calculations. The instructor or the teaching assistant reviewed student team protocols, and interactively worked with them

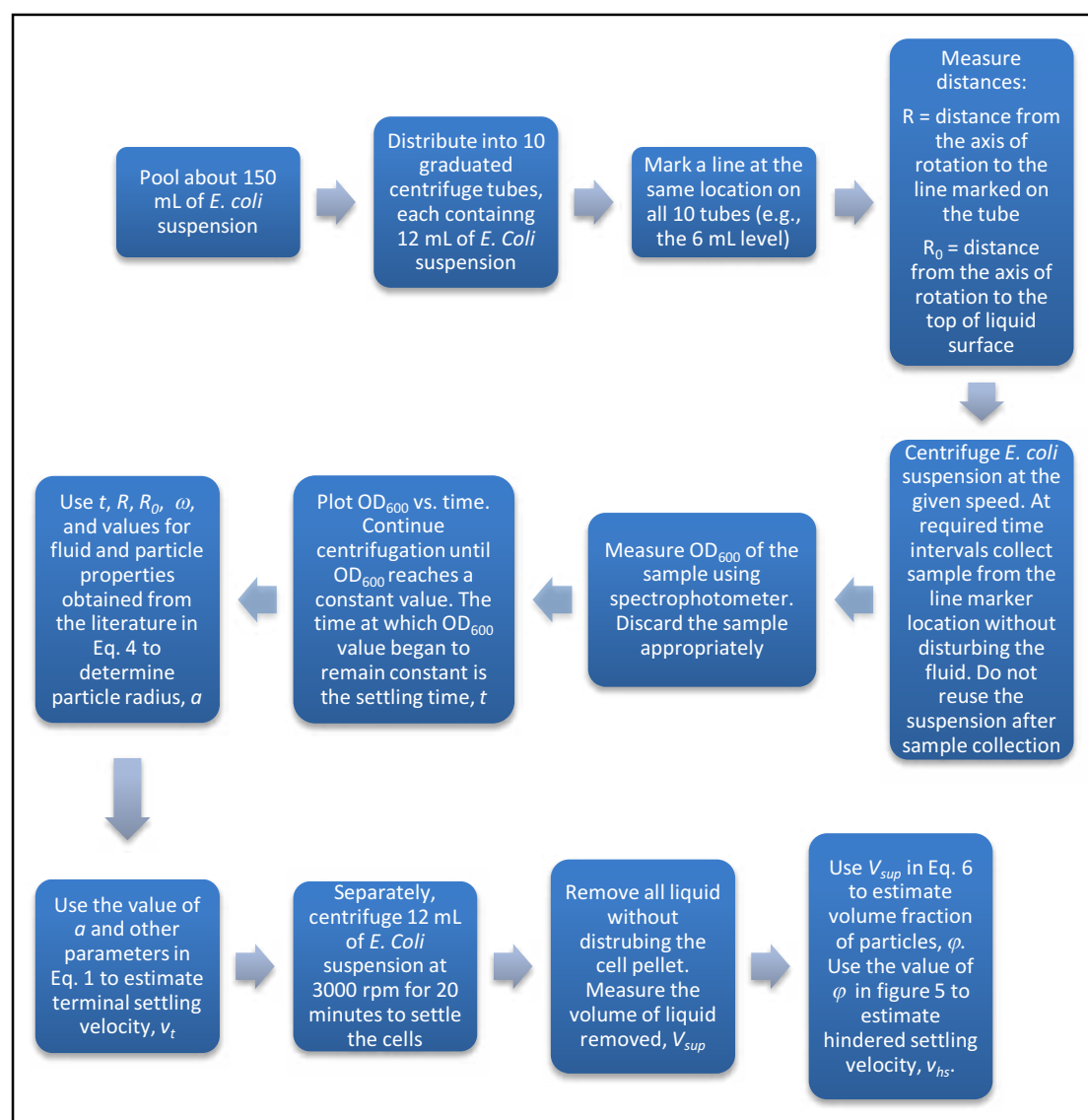


Figure 3. General work flow for the centrifugation experiment. Overall goals of the experiment: (i) Estimate particle radius; (ii) estimate terminal settling velocity; and (iii) estimate hindered settling velocity.

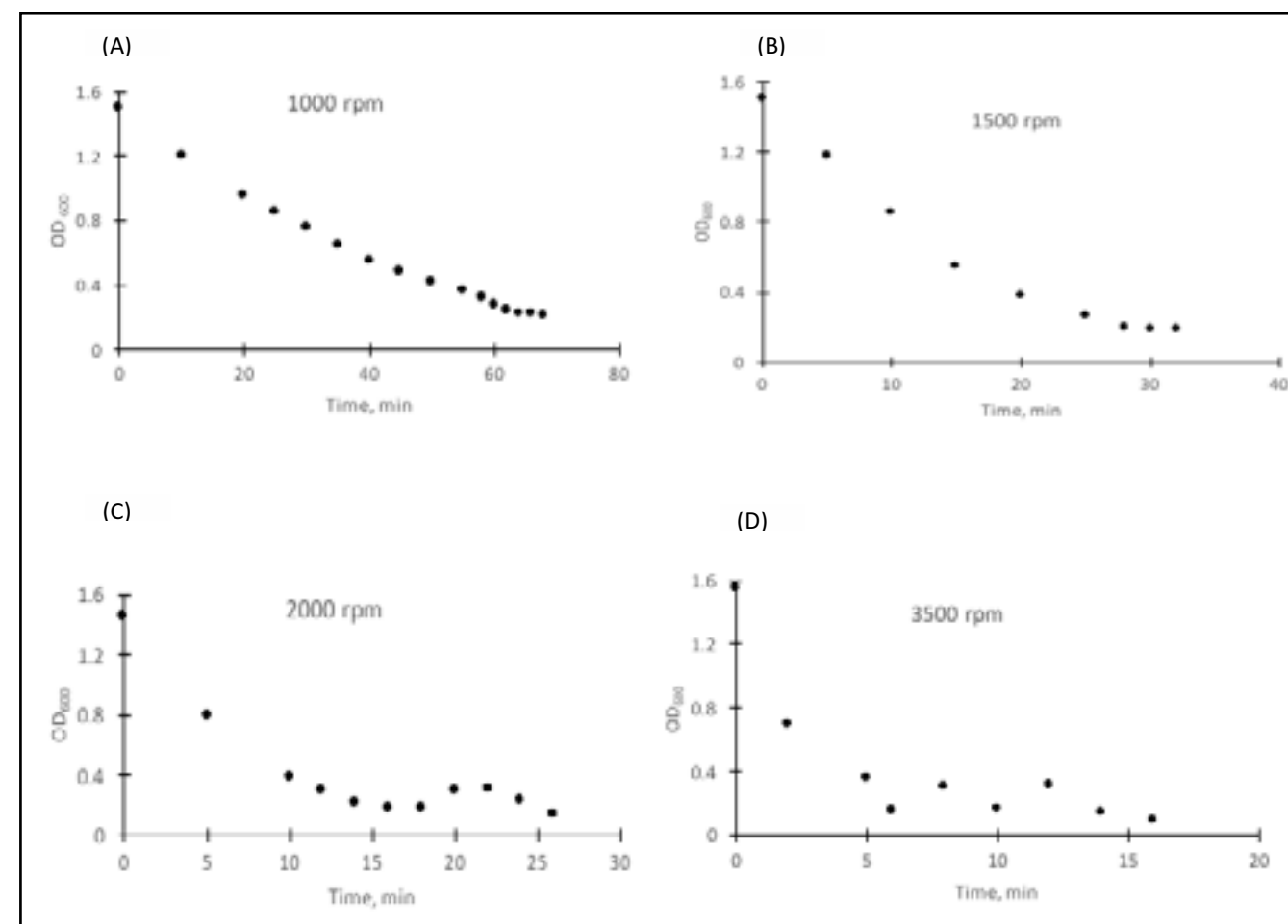


Figure 4. Representative OD_{600} vs. time data. (A & B) at low rotational speeds where shear forces are expected to be low, (C) at intermediate rotational speeds where some shear forces capable of disrupting the cells are expected, and (D) at high rotational speeds where significantly high shear forces are expected. OD_{600} values shown are from multiple sets of student data ($n \approx 85$) normalized to account for differences in starting cell concentrations and for varying spectrophotometer blanking methods. Erroneous data sets ($n \approx 8$) that were a result of equipment failure or significant human error are not included in the normalization.

to improve it. The intent of this exercise is to nudge students to learn more about the experiment in advance and to challenge them to apply their theoretical knowledge to the centrifugation experiment. There was a grade penalty for coming to the lab without the protocol but the protocol itself was not graded. Protocol writing was an opportunity for students to learn through interaction and iteration. The protocol review typically took about 30 minutes.

Each student team performed the centrifugation experiment at two different pre-assigned rotational speeds. At each rotational speed, time vs. OD_{600} data (of samples taken from point X) was obtained. Initial optical density measurements were made at 5 minute intervals, followed by shorter intervals as the settling progressed. Example plots of time vs. OD_{600} profiles at different rotational speeds are given in Figure 4. The profiles are characterized by a rapid initial decrease in OD_{600} followed by

a slow decrease and then a constant OD_{600} . At higher rotational speeds more than one plateau was observed (Figure 4C&D). The time at which the OD_{600} begins to remain constant is the settling time for *E. coli* cells. This value of settling time was substituted in Eq. (4) to estimate *E. coli* particle radius which was then used in Eq. (1) to estimate terminal velocity. Students were required to find values for ρ , ρ_0 , and μ from the literature.

In this example V_{sup} was experimentally determined to be 11.6 mL, and using this value in Eq. (6), ϕ was estimated to be 0.033. Values for v_{hs} were determined based on linear interpolation of v_{hs}/v_t vs. ϕ data available in the literature^[5] and plotted in Figure 5 (next page). Parameter values determined through experimentation and calculations for this example are given in Table 1 (next page). Hands-on experimentation took 3-4 hours. Student teams generated the data and recorded it in their electronic lab notebook.

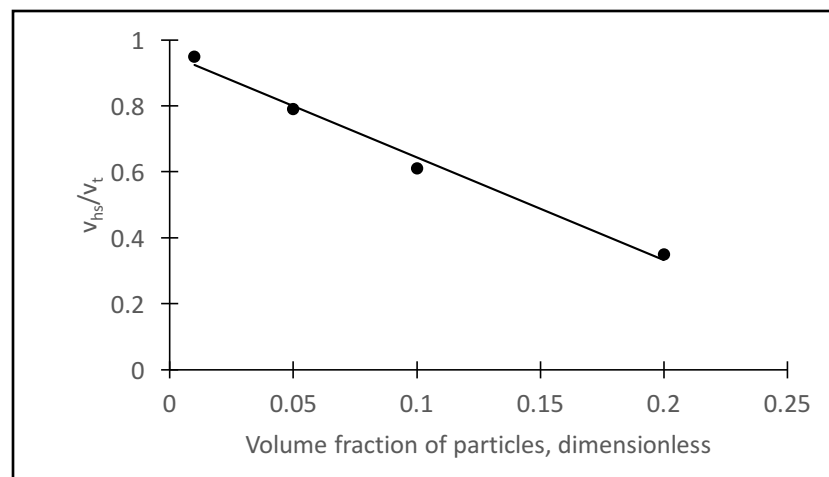


Figure 5. v_{hs}/v_t vs. ϕ data for estimation of hindered settling velocity. Data are adapted from Reference 5. Trend line used for interpolation of v_{hs}/v_t vs. ϕ data is shown.

Ω , rpm	t, min	a, $\times 10^{-6}$ m*	v_t , $\times 10^{-6}$ m/s	v_{hs} , $\times 10^{-6}$ m/s
1000	62	0.586	1.612	1.372
1500	28	0.544	3.863	3.566
2000	16	0.460	7.053	6.966
3500	6	0.342	11.024	10.24

* Average literature reported radius of *E. coli* cells $\sim 0.5 \times 10^{-6}$ m.^[21]

COGNITIVE CONFLICT OF CENTRIFUGATION CONCEPTS TO STIMULATE LEARNING

Particle settling in a centrifuge is influenced by a multitude of parameters, and the equations derived to describe particle motion are inherent with a few assumptions. Therefore, the experimental results will contradict theoretical expectations in some cases. This cognitive conflict presents an opportunity for learning and critical thinking.

Cognitive conflict is known to promote learning by challenging preconceived notions.^[19,20] Data obtained in this experiment often contradicted common beliefs, thus providing students with an opportunity to think beyond the obvious. Three instances of cognitive conflict are presented by centrifugation data: (i) at higher rotational speeds more than one plateau is observed in the time vs. OD_{600} data, when in theory only one plateau should be observed; (ii) predicted values for cylindrical *E. coli* particles match with literature-reported values that were obtained through microscopic methods—how do the equations developed for spherical geometry correctly predict the radius of cylindrical particles?; and (iii) the predicted radius decreases with an increase in rotational speed, but particle radius is not a variable quantity. Literature-reported value for *E. coli* radius is about $0.5 \mu\text{m}$.^[21]

Student teams were asked to reconcile these cognitive conflicts in their lab reports. The first cognitive conflict can be explained through the effect of shear stress on *E. coli* cells. Higher centrifugation speeds lead to higher shear stress and subsequent cell breakage.^[22] Cells settle rapidly at higher rotational speeds and hence the plateau is reached faster leading to shorter settling time. But immediately after reaching the plateau, due to higher shear stress cells start to break, and cell debris are re-suspended in solution, which consequently leads to an increase in OD_{600} . Further centrifugation for a longer time period leads to settling of cell debris and hence OD_{600} starts to plateau again. In occasional cases with much higher rotation speeds (such as 3500 rpm, Figure 4D) multiple plateaus are observed, which indicates sequential breakage of cell debris into even smaller particles and subsequent settling. The first plateau is representative of whole cells and should be used for cell radius estimation.

The second cognitive conflict can be explained by looking closely at the shape and dimensions of *E. coli*. Typical *E. coli* cells are cylindrical particles with approximate diameter and height of $1 \mu\text{m}$ and $2 \mu\text{m}$, respectively, *i.e.*, an H/D ratio of 2.0. Sphericity of a particle with H/D ratio of 2.0 is 0.833. Thus the *E. coli* particles when moving in a liquid

might behave geometrically close to a sphere. Therefore, the value of *E. coli* radius estimated by this experiment matches with reported values. The third cognitive conflict is again related to shear effects. At higher rotational speeds, *E. coli* particles will disintegrate quickly resulting in a suspension that has a sub population of smaller-size particles. Since radius estimations represent an average value for the population, a sub population of smaller particles will result in underestimation of particle radius. Higher rotational speeds will lead to a larger sub population of smaller particles and subsequently a greater underestimation of particle radius. Thus the predicted particle size will progressively decrease with increase in rotational speed.

TEAMWORK AND DATA REPORTING EXPERIENCES

The centrifugation laboratory experiment was done by two different three-member student teams each week. Students were divided into teams based on Myers-Briggs Type Indicator (MBTI) assessment of their personality type, educational background, gender, and ethnicity. MBTI is a questionnaire-based personality assessment tool, the results of which define an individual's personality into one of the 16 possible

personality types.^[23] The intent of this team-forming exercise was to create teams that were diverse, which would stimulate problem solving from multiple perspectives. Students attended a team dynamics workshop at the beginning of the lab course followed by individual team coaching as necessary to work through any group conflicts. The workshops covered topics related to formation and management of teams, effective team communication, and conflict resolution. Strategies to leverage self-awareness through MBTI assessment were included in the workshops and coaching sessions. Team performance, assessed through peer evaluation, contributed to 10% of final grade. Overall team experience was assessed through a team dynamics feedback survey. Teams self-selected a rotating team leader for every experiment. The team leader delegated tasks and was responsible for proper conduct of the experiment. The team leader wrote the progress report and became the single point of contact for matters related to that particular experiment. A deeper focus on team coaching resulted in students leveraging the personal and technical strengths of the other team members and identifying conflicts early and finding ways to resolve them. Students appreciated the concept of rotating team leaders since it engaged individuals reluctant to take charge of an experiment.

Data from student feedback survey indicate a high level of satisfaction with team experiences (Figure 6). The level of satisfaction varied between “very satisfied,” “satisfied,” and “somewhat satisfied.” No one expressed dissatisfaction with their team experiences. The average satisfaction score was 5.4/6.0. To better understand the numerical scores, students were asked to provide a freestyle explanation of their score. We processed the explanations through a web-based, open source phrase frequency counter, which returned a ranked listing of most frequent phrases from student responses (<http://www.writewords.org.uk/phrase_count.asp>). The survey question was “Please explain your overall rating of team experiences.” We set the filter to identify frequency of four-letter phrases, which returned most discernible phrases. Results of the frequency count given in Table 2 indicate that student teams worked very well together, and that students learned a lot from team coaching. Next we asked the students to “Suggest one or more changes that will make your team experience better.” Processing student responses to this question through the phrase-frequency counter did not generate any discernible phrase. Hence, we manually analyzed the responses and ranked thematic phrases, the results of which are presented in Table 3. It is clear that students would like to have better communication within teams, and a greater emphasis on shared responsibilities among team members. These two themes are interconnected. Insufficient communication often



Figure 6. Overall rating of student satisfaction with team experiences. Average score = 5.4/6, $n = 31$. Rating scale: 6 - Very satisfied, 5 - Satisfied, 4 - Somewhat Satisfied, 3 - Somewhat Dissatisfied, 2 - Dissatisfied, 1 - Very Dissatisfied.

Phrase*	Rank [†]
Worked very well together	1
Satisfied with my team	2
I learned a lot	2

* Analysis based on phrase frequency count through: <http://www.writewords.org.uk/phrase_count.asp>.
[†] Phrases with similar themes are merged together for ranking.

Phrase*	Rank
Better communication among team members	1
Emphasis of individual responsibilities	2
More team coaching	3

* Analysis based on manual screening of student responses to identify thematic phrases.

led to a situation where team members were unclear of their responsibilities in completing the experiment. In the future, we plan to provide students with additional training and tools for better team communication, and coaching specific to team leaders on assigning responsibilities and establishing channels of communication with and among all team members.

Students entered raw data in LabArchives, an electronic lab notebook (ELN). They were required to complete the ELN before exiting the lab, and if needed were allowed until

end-of-day to finish entering data. Teaching assistants and instructor had access to student ELNs, which were assessed based on completeness and clarity of data presentation. ELNs allowed students to share data with other teams through the click of a button without the need to share paper lab notebook copies. It was crucial for the teams to share data immediately with the other team that did the centrifugation experiment for comparative data analysis. Student teams had six days to share the data, compare and analyze the data, and write a comparative lab report. ELNs are increasingly used in the industry and academia,^[24] and in this lab students were exposed to the nuances of ELN. From an instructor's perspective, ELN allows for real-time monitoring of experiments and to track history of data entry and changes that were made. The use of ELN allowed us to maintain superior data integrity of the centrifugation experiment.

Overall, the centrifugation experiment simulated the entire life-cycle of a scientific study, which included translating theory to designing an experiment (protocol writing), learning experimental skills, data reporting, data analysis, teamwork, and reconciling cognitive conflicts between experimental observations and theoretical expectations. Students were able to satisfy all the expectations, and qualitative feedback indicates that they enjoyed learning this experiment.

SAFETY CONSIDERATIONS

Ingredients in LB media are considered to be non-hazardous according to OSHA standards. Solid LB media is a fine powder that can result in dust during handling. Use of impervious protective wear (such as labcoats), eye protection (safety goggles), and skin protection (gloves) is required when handling LB media. Use of respiratory protection when handling large amounts of LB media powder is highly recommended.

E. coli cells used in this experiment are a class I biohazard and should be handled aseptically in a biological safety cabinet or around a flame. Lab coats, safety goggles, and gloves should be used when handling *E. coli* cells. Thermal gloves should be used during heat sterilization of media. All spent *E. coli* suspension should be decontaminated with 20% sodium hypochlorite (bleach) or should be autoclaved before disposal. Any container or supplies that come in contact with *E. coli* should be considered as a biohazard waste and disposed accordingly.

Centrifuges operate at high rotational speed, but moving components are typically contained in a closed unit. Follow safety guidelines provided in the centrifuge operating manual.

CONCLUSIONS

We have developed a simple centrifugation experiment to demonstrate the principles of sedimentation, and to estimate particle radius and settling velocities. This experiment is also an opportunity to examine the effect of shear stress and

the non-spherical geometry of *E. coli* particles during centrifugation. Students learned experimental techniques such as operating a benchtop centrifuge, measuring optical density, estimating settling time, measuring volume fraction of particles, and handling of *E. coli* cells. Following data collection, they learned to perform calculations to estimate particle radius as well as hindered and unhindered settling velocities. Students also had the opportunity to critically analyze the data, reconcile cognitive conflicts, and reflect on interpretations that are non-obvious. Students were also exposed to team skills and use of the electronic lab notebook. The centrifugation experiment is straightforward, cost-effective, and can be completed in 3-4 hours. *E. coli* cells were used in this experiment to fit with the general subject of the Bioprocess Engineering lab course. *E. coli* particles can be readily replaced with other suspension such as polymeric microspheres or fine sand to fit with the subject matter of other lab courses. This centrifugation experiment is adaptable to many lab courses in chemical and biochemical engineering.

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