APP N13-1

Engineering 1282H

Spring 2020

Team Y4

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Methodology and Purpose:

The purpose of this experiment is to see how well yeast cells adhere at varying salinities. Testing the effects of salinity on growth of yeast can provide important data on how to set up future experiments, especially ones involving yeast incubation.

The height of the wash will be kept constant while the concentration of salt solution will serve as the independent variable. The dependent variable is the number of yeast cells that can be seen in the channel. In this experiment, flow time and syringe height remain constant, while the flow volume is controlled by the design of the channel. The null hypothesis is that adhesion of yeast cells will not be affected by the salinity of the solution, so the number of yeast cells seen after the wash should be the same across all concentrations. The alternative hypothesis is that the number of adhered yeast cells will be affected by the salinity of the solution, leading to a varying presence of yeast cells in the channel based its salinity. A one tailed t-test will be used to compare the number of observed yeast cells at each data point. The chip has been designed with several channels and adequate space to encourage laminar flow, so cell adhesion can be properly observed. The syringe will be precisely aligned at the appropriate heights to mitigate any variation. Finally, the chip will be cleaned between each trial to ensure that every trial starts the same way.

Experimental Procedure:

1. Add 2.0 grams of yeast and10 mL of hot water together in a beaker
2. Let it incubate for 20 minutes and stir occasionally
3. Obtain chip and punch entrance and exit ports into the wells using a hole punch specific for a nanoscale
4. Screw chip holder top and bottom onto chip, ensuring that screws are finger tight- liquid should remain in channel without restricting flow through channel.
5. Using a syringe and swirling the yeast in the beaker, obtain about 4 ml of yeast in the syringe. Put a plastic tip on the syringe and insert yeast into channel of chip slowly
6. Incubate yeast for 15 minutes to allow yeast to adhere to channel
7. Set up low pressure wash by attaching a clean syringe to a platform at 25 cm tall
8. Mass a plastic cup
9. Insert plastic tip on tube attached to the syringe into the entrance port
10. Insert other plastic tip into the exit port with the attached tube going into a plastic cup. Make sure the base of the stand and the plastic cup are at the same height to ensure the same pressure difference
11. Allow water to flow from the syringe, through the channel, and into the cup for 3 minutes. This is the low-pressure flush.
12. After time has completed, mass the cup with the water
13. Clean all parts used in the experiment
14. Observe chip under a microscope to see if yeast cells have adhered to the walls of the channel. Flushing is complete when no unadhered yeast cells are flowing through the channel.

15. Mark a small square on the chip holder where yeast cells are most apparent under the microscope
16. Count how many yeast cells are present in the square. This will be the initial amount of yeast cells in the square
17. Repeat steps 5-13 with the syringe at 30 cm of height on the stand and use 25% salt water instead of plain water
18. Observe the chip under the microscope and mark a small square where yeast cells are most apparent
19. Count how many cells are present in this region, this will be the final amount of yeast cells. If at least 50% of the yeast cells are sheared, then it can be considered as a data point.
20. Repeat step 17-19, 3 times
21. Repeat steps 5-13 with the syringe at 30 cm of height on the stand and use 50% salt water instead of plain water
22. Observe the chip under the microscope and mark a small square where yeast cells are most apparent
23. Count how many cells are present in this region, this will be the final amount of yeast cells. If at least 50% of the yeast cells are sheared, then it can be considered as a data point.
24. Repeat steps 21-23, 3 times
25. Repeat steps 5-13 with the syringe at 30 cm of height on the stand and use 75% salt water instead of plain water
26. Observe the chip under the microscope and mark a small square where yeast cells are most apparent
27. Count how many cells are present in this region, this will be the final amount of yeast cells. If at least 50% of the yeast cells are sheared, then it can be considered as a data point.
28. Repeat steps 25-27, 3 times
29. Repeat steps 5-13 with the syringe at 30cm and use plain water. This will be the control treatment
30. Observe the chip under microscope and mark a small square where the yeast cells are most apparent
31. Count how many cells are present in this region, this will be the final amount of yeast cells. If at least 50 % of the yeast cells are sheared, then it can be considered as a data point.
32. Repeat steps 29-31, 3 times
33. Once all trials are finished, pour all liquids down the drain and clean up already and equipment

Experiment Schedule:

The goal for each day is to get one data point. If the team is behind, the team must attend open lab to ensure that the weekly goal of three points a week is achieved. If the data point is not able

to be collected in class, the team must go to open lab to achieve that data point the day of or the day after, as long as it is before the next class period.

3/23- Control data point 1
3/24- Attend open lab if not able to complete
3/25- Control data point 2
3/26- Attend open lab if not able to complete
3/27- Control data point 3

3/30- Control data point 4
3/31- Attend open lab if not able to complete
4/1- Control data point 5
4/2- Attend open lab if not able to complete
4/3- 25% Concentration data point 1

4/6-25% Concentration data point 2
4/7- Attend open lab if not able to complete
4/8- 50% Concentration data point 1
4/9- Attend open lab if not able to complete
4/10- 50% Concentration data point 2

4/13- 75% Concentration data point 1
4/14- Attend open lab if not able to complete
4/15- 75% Concentration data point 2
4/16- Attend open lab if not able to complete