Halobacterium Salinity Lab

Introduction:

Halobacterium is an Archaean extremophile that survives in very salty environments. In classroom instruction, students will learn how Halobacterium react to the changes in the salinity of their natural habitats, like the Great Salt Lake. They will test different salinities of growth medium to find which one is the most ideal. In a typical lab setting, Halobacterium can be grown in an agitating incubator. The doubling time for Halobacterium in 4.3M media, shaking at 220 rpm and at 37 degrees Celsius is around six hours. However, most high school classrooms do not have access to an agitating incubator. The purpose of this lab is to determine the amount of time it takes for significant Halobacterium growth to occur if the cultures are grown on stir plates in room temperature instead of agitating incubators.

Procedure:

1. Tilt and gently place a magnetic stir bar into each flask.
2. Add 20mL of 2.3M complete media into four of the flasks.
3. Add 400µL of Halobacterium cells to three of the four flasks (starting OD of Halobacterium should be around 0.6-0.8) excluding one for the control. Dispose of the micropipette tips.
4. Cover the tops of the flasks with aluminum foil. Cardboard holders can be made by cutting 4 holes in a piece of cardboard and putting over the top of the flasks, make sure that the piece of cardboard and the space between the holes are close enough and the right size to fit on the stir plate. Put them on the stir plates and secure them with tape. ADD PICTURE
5. Turn the stir plates to “4” and make sure the stir bars are rotating correctly. Add a stirbar in the center of the 4 flasks if they are not stirring properly, this should help to keep them stirring evenly.
6. Record the date and the time that the samples began rotating.
7. Repeat steps 2-6 using the various concentrations of media (2.8M, 3.3M, 3.8M, 4.3M)
8. Check and record the OD of the samples at varying times, starting about eighteen hours from when the flasks were started.

Notes:

Trial 1: *Pre-lab*: The Halobacterium was fresh from colony; 0.9677 OD. We had to dilute the original sample because it was ~1.8 OD. We estimated that the actual OD would be 1.2 because the Halobacterium started from colony very recently; we assumed that the reason for the high original OD (1.8) was because of the large number of gas vesicles. We diluted based on the 1.2 OD estimation to create 0.6 OD. The original OD turned out to be ~1.8 OD, because our dilutions came out to be 0.9677 OD.
Lab: Start time: 16:06 July 20th, 2011.The stir plate for the 3.8M CM work differently that the others; the stir bars spin around the entire flask instead of spinning in place. There was no cloudiness in any of the flasks, no sign of any outside growth. At 10:18 July 21st, 2011, we added a stir bar to the middle of the stir plates so that they would all spin better.

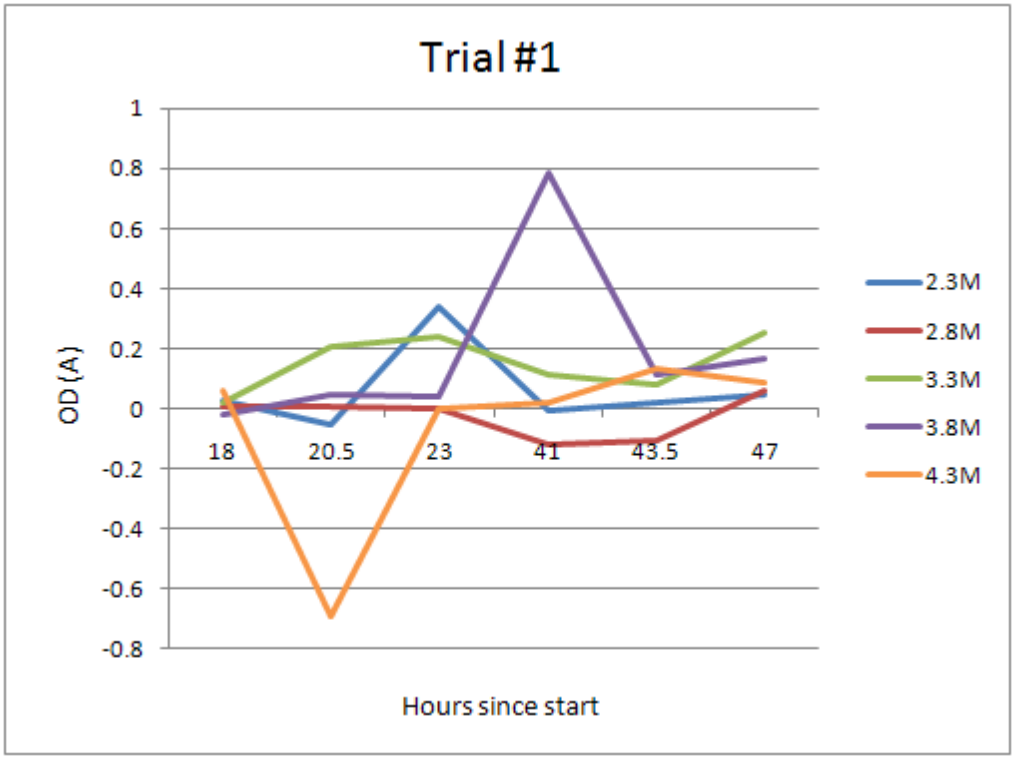
Trial 2: *Pre-lab*: Starting OD ~.7934.
Lab: Start time: 3:16pm 8/1/11. In the second set, the 4.3M media had Halobacterium in it, so we started it over at 11:14 on 08/02/2011. Also in the second set, the 3.3M #3 flask broke and was started again at 9:40 on 08/03/2011.

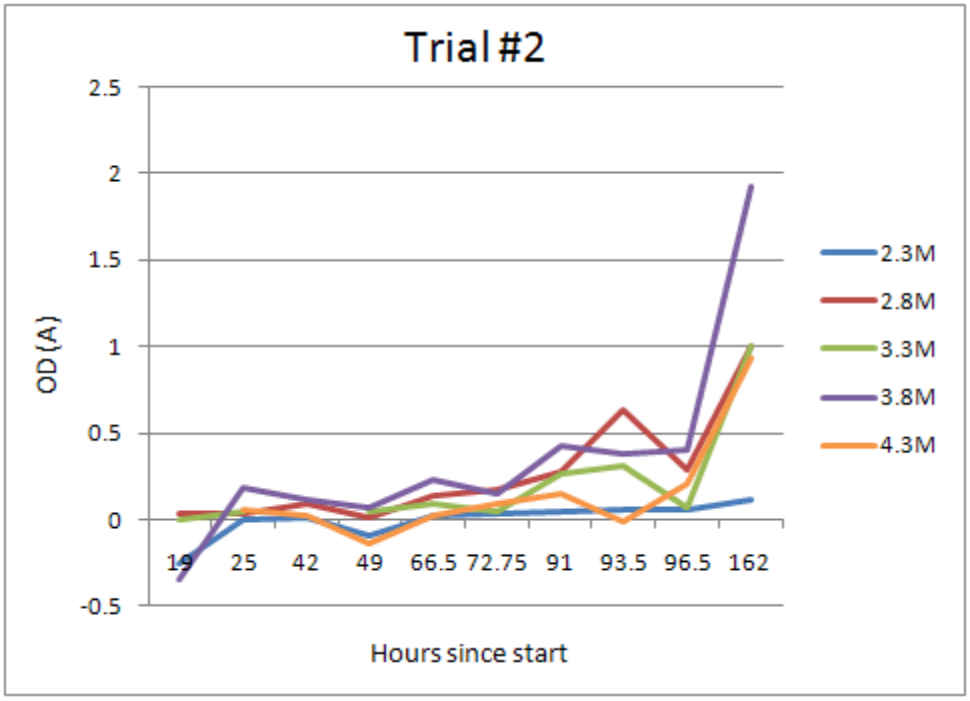
Trial 3: *Pre-lab*: Only the eight flasks for 2.3M and 2.8M were autoclaved. The other flasks were rinsed thrice with distilled water. Starting OD:
Lab: Started at 12:37 on 08/08/2011. The 4.3M control looked contaminated so we started it over at 14:00 08/09/11. We assumed the media was contaminated so we used our own media, made here at ISB. We needed to take an extra 50uL out of 3.3M #1 and 3.8M #3.

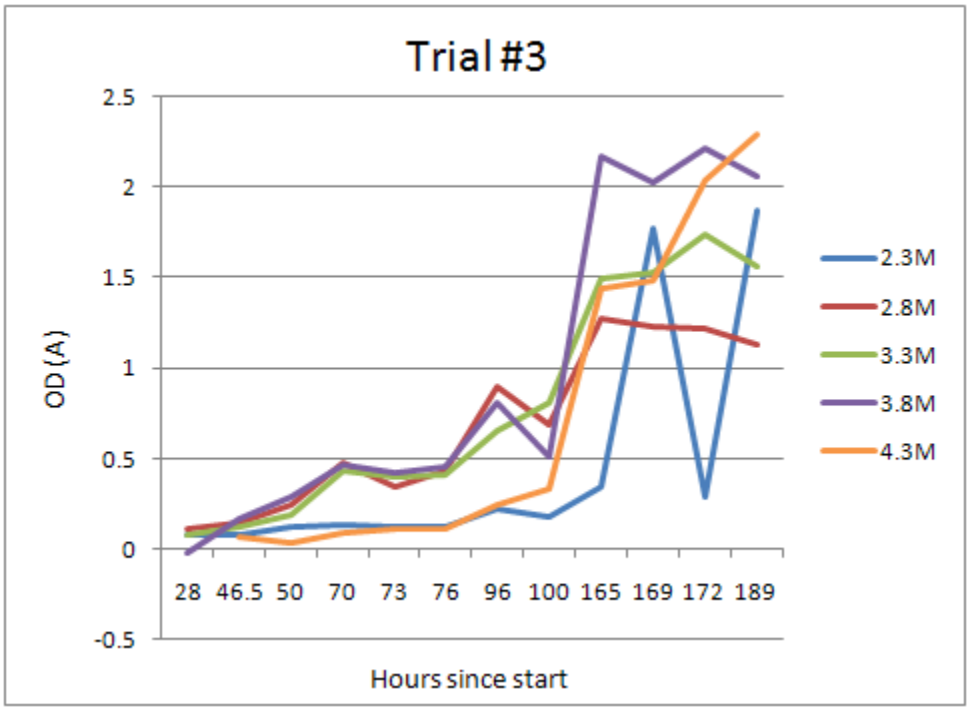
Trial 4: Pre-lab: Start OD:
Lab: Started at 10:00 on 08/09/2011The 4.3M got contaminated because we did not use our own media; we had to throw the 4.3M flasks out. The data for 3.8M looked strange so we averaged the three flasks and determined that 3.8M #3 was a fluke.

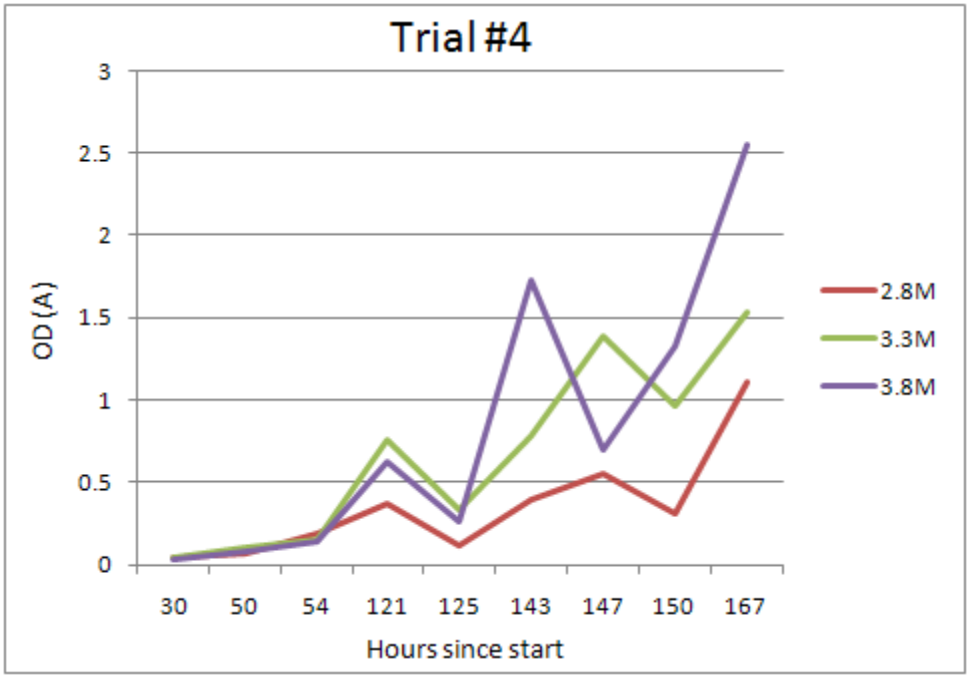
Data:

	2.3M	2.8M	3.3M	3.8M	4.3M
Batch #	JRH:1 11/23/09	JRH:1 11/02/09	JRH: 2/22/10	JRH: 12/30/09	JRH:1 11/02/09
Temperature ˚C	24.9	24.8	24.6	25.1	24.6




Trial #2
2.5
2
1.5
1
0.5
0
-0.5
OD (A)
19
25
42
49
66.5
72.75
91
93.5
96.5
162
Hours since start
2.3M
2.8M
3.3M
3.8M
4.3M


Trial #3
2.5
2
1.5
1
0.5
0
-0.5
OD (A)
28
46.5
50
70
73
76
96
100
165
169
172
189
Hours since start
2.3M
2.8M
3.3M
3.8M
4.3M



Discussion/Conclusion:

In a standard agitating incubator, the doubling time for Halobacterium in 4.3M complete media is ~6 hours. With the data we found, we cannot find a conclusive answer to the question at hand, but we did see that there was significant Halobacterium growth after 48 hours consistently. This is may mean that teachers can stop their experiments after 48 hours. We ran into a few problems while running the experiment; the first trial ran well except that the stir bars were either stopping or not running smoothly, and this causes oxygen to be unevenly circulated or not circulated at all. When we took the optical density we found some numbers that did not make sense, even when we re-took the optical density, two of the outlying numbers we found were: 0.691 (4.3M) after 20.5 hours (the normal OD for this time should have been around 0.05-0.06 range.), and 0.7558 (3.8M) after 41 hours (the normal OD for this time should have been around 0.1). Even if these points were thrown out, out data still have various spikes or dips that make the data inconclusive. These random spikes and dips are true for every trial except trial 2; this trial had a few lows and one spike from 2.8M after 93.5 hours but otherwise the data showed a gradual increase in growth, unfortunately the ups and downs make it impossible to even get a rough conclusion from this single trial. The second trial had a contamination in the 4.3M control flask so we had to restart it at 11:14 on 08/02/2011; the 3.3M #3 flask also broke and that was restarted at 9:40 on 08/03/2011. These setbacks made it difficult to take data because we had two sets running on separate times. Our 4.3M control got contaminated in the 3rd trial as well and this led us to think that the growth media was contaminated with Halobacterium so we restarted the 4.3M and using media that we made. The 4th trial of Halobacterium was contaminated, but the 4th trial was more of a test to see if we could get accurate data for the sets we were having more trouble with. Using the growth media made in the lab instead of the

packaged media, putting a stir bar in the middle of the plates from the beginning, and taking the OD of all the flasks at the intervals instead of rotating from #1 to #2 to #3 would all contribute to better results. Also, because the data was inconclusive, doing 5-6 trials rather than 3-4 would help in drawing a firm conclusion.