Materials:

- Halobacterium sp. NRC-1 from Carolina
- Submersible water heater from Amazon
- 2x 1 L Media Bottle
- 500 g NaCl from WISRD
- 40 g MgSO4(magnesium sulfate) from Wildwood Chemistry Department
- 4 g KCl from Wildwood Chemistry Department
- 6 g NaH2C6H5O7(Sodium Citrate dihydrate) from Lab Alley
- 20 g Oxoid Peptone from Lab Mal
- 20 g Hoosier Hill Farm Agar Agar powder from Wildwood Biology Department
- Stir Plate & Bar (or a Stir Stick if not available)
- 2 L Distilled Water from WISRD(Durastill)
- .01 g – 300 g capable Scale
- Inoculation Loops
- Weigh boats

Procedure adapted from:

- **https://baliga.systemsbiology.net/highschool/intern2011/halo.htm**
- **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10202317/**
- **https://medicine.buffalo.edu/offices/facilities/services/autoclave/operating-procedure.**

WISRD Procedure for growing Halobacterium Salinarum:

Our goal is to have a stable, self-replenishing population of Halobacterium growing in an aerated tank in WISRD, with the concern that if cells are added directly into the tank might be too much of a stress and result in low survivorship. To address this issue, we will first be growing a culture of halobacterium both on agar plates as well as in smaller tubes of growth medium, with the hopes that some cells will remain for future work.

First, use weigh boats and an appropriately precise scale to measure the following chemicals:

250 g of NaCl

20 g $MgSO_4$

2 g KCl

3 g $NaH_2C_6H_5O_7$

10 g Oxoid Peptone.

Add the ingredients to 1 liter of distilled and mix into the solution with a stir bar until fully dissolved. Repeat this process an additional time to make a second 1-liter bottle of media solution.

Add 20 grams of agar powder to ONE of the media bottles and stir until all the ingredients have dissolved. Sterilize both bottles in an autoclave at 121℃ and 15psi for 30 minutes. Once it has been sterilized, pour the media with agar onto an empty petri dish until it is 1/3 full, then leave it to cool.

Use an inoculation loop to scope Halobacterium cells from the purchased sample and transfer them to the agar plate. Make sure to gently and evenly spread the cells across the plate in a zig-zag pattern. Place the plate in an incubator at 37℃, and check in on the culture once per day until the halobacterium starts forming pinkish-red colonies. Now, we are sure our media can sustain the cells.

Now, we can add the heating and agitation elements to the tank. To agitate the media, use a shaker on the medium. Halobacterium grows optimally in temperatures from 37 to

42 degrees Celsius. We are using an immersion water heater with an adjustable thermostat to control the heat of the liquid solution.

Finally, we add the halobacterium. To do this, take a 5mL sample of the media in a test tube and inoculate it with the halobacterium grown on the petri dish. Place the test tube in an incubator at 37 degrees for 24 hours. Once this larger population of bacteria has grown, once the flask appears pinkish red we can then pour the sample into the tank. Now that the halobacterium has been introduced to the tank check on the tank once per day. Once the media has grown red, the procedure has succeeded. If no halobacterium grows within a week, repeat this section of the procedure.