## **Essential Materials**

Essential Solvents:

MTBE, Acetate, Distilled water, Ethyl Ether

Essential Equipment:

Safety Goggles

Fisherbrand disposable Pasteur pipettes

Sink

Gloves (vinyl examination gloves)

Lab Coats

Graduated Cylinder

Fume Hood

Experimental Procedure:

## **Preparation of Hoagland’s Hydroponics Solution**

## **Growing and Raising Plants for Experiment**

## **Preparation of Apparatus for Closed Root System: AKA THP Bottle**

## **Preparation of closed plant system for experimentation**

## **Devising the Standard Curve**

## **Sampling Procedure**

**Must wear safety goggles, gloves, lab coat at all times. Must work under a fume hood if conducting 1V) through V1).**

**I) Preparation of Hoagland’s Hydroponics Solution:**

Production of 1 liter of solution:

Component parts of mixture:

**Macro-element stocks** (mix each compound separately – all aqueous solutions ):

1M KH2PO4

1M KNO3

1M Ca(NO3)2

1M MgSO4

0.5% Fe(NO3)2; can also use Fe tartrate

**Micro-element stock(**combine all compounds TOGETHER):

0.286% H3B03

0.022% MmCl2\*7H20; can also use MnCl2\*4H20

0.002%H2MoO4\*H2O; can also use sodium or ammonium molybdate

1) Measure out proper amounts of solutions below with the aid of the volumetric pipettes, and add to a one liter container

1mL 1M KH2PO4

5mL 1M KNO3

5mL 1M Ca(NO3)2

2ml 1M MgSO4

1mL 1M Fe tartrate or Fe(NO3)2

1mL MIcroelement stock

2) Fill container with distilled water to the one-liter mark.

3) Shake the bottle vigorously to make sure the solution is properly mixed

4) Use pH paper to test the acidity of solution. Use NaOH to adjust the pH of the solution

accordingly to bring it to the optimum range of 6-7.

**II) Growing and Raising Plants for Experiment:**

Supplies: soy bean, broad bean, barley, humidity chamber, starter pot(s), metal pan, Hoagland’s hydroponics solution, soil, aluminum foil, tap water, paper towel(s), 400 ml beaker(s)

Three types of plants were used in this experiment. The plants used were broad bean, soybean, and barely. However, barely was later discarded in our experiment because of its difficulty handling; the small stems would break easily when handling occurred, and their size was not feasible for our experimental apparatus.

The plants for our experiment were planted on February 15th, and actual use in our experiment began March 19th.

1) Germinate seeds in a metal pan by placing a moist paper towel and letting them soak overnight.

2) Remove seeds from towel and transfer them into starter pots with basic potting soil

3) Place the plants into a humidity chamber for germination. Water when needed for moist soil.

Note: Temperature of chamber:

Night: 22 degrees Celsius

Day: 27 degrees Celsius

4) After the plants have a well-established root system, remove the plants from the soil to a 400 ml beaker filled with Hoagland’s hydroponics solution (use approximately 300 ml of solution, or just enough so that the root system is completely submerged.)

Note: Rinse the plant’s roots of dirt before adding to solution.

5) Place aluminum foil around beakers and cover the top of beaker as best as possible to prevent roots from being exposed to light.

Note: Roots will become damaged and/or new shoots will sprout when exposed to light.

6) Return plants to chamber.

7) Refill the solution when needed.

8) Note that before experimentation, allow at least a week for the plants to become adjusted to the new hydroponics environment.

**III) Preparation of Apparatus for Closed Root System: AKA THP Bottle:**

Supplies: 500 cc bottles, acetate, GC/MS sample vial, electric drill, 3/4 inch drill bit, 0.5cm drill bit, super glue, disposable Pasteur pipettes

The development of this apparatus was through the combined efforts of Maulin Patel, Ryan Hoshi, and Christian Thomas, hence the name: T(Thomas)H(Hoshi)P(Patel) bottle. This unique apparatus was designed in order to produce an airtight system to prevent leakage of MTBE through volatilization. Also, an efficient design was needed for easy sampling of the solution with the least amount of human error. The development of which was through trial and error experienced in the first trial of our experiment. (Further discussed in conclusion ).

1. Remove lid from the 500cc amber bottle.
2. Carefully anchor the lid to limit its movement.
3. Drill two holes on top of the lid. Drill the first hole with a 3/4inch bit directly in center of the lid. The second hole will be drilled using a 0.5cm bit and will be placed off to the side of the lid, roughly between the first hole and the edge of the lid.

*Note: We initially painted the lids with acrylic latex paint, with the logic that this paint membrane would prevent the plastic lid’s exposure to MTBE, which may degrade the plastic. However, we devised an even better solution by placing an aluminum foil barrier over the lid to prevent exposure to MTBE.*

1. Obtain GC/MS sample vial, and separate its membrane from cap (lid). (set aside)
2. Prepare super glue, and obtain disposable Pasteur pipettes for use in mixing and applying glue. (or use any long thin disposable object to work with glue)
3. Add glue to the top of the lid and place the cap on the “second hole” (0.5 cm hole) as quickly as possible.

Note: The super glue may react if exposed to MTBE. To avoid this, make sure glue is only placed where the GC/MS lid meets the lid on the amber bottle.

1. Add glue to the side of the lid to increase the bond strength of the GCMS lid and the bottle’s lid. Place the septum back into to center of the GCMS sample vial lid, while making sure that the septum is placed right side up.
2. Once glue has dried screw back the GCMS sample vial bottle to its lid – this allows for an air-tight seal to prevent leakage from the closed system.
3. Leave the bottle lid unscrewed for later use in the experiment.

**Preparation of closed plant system for experimentation:**

Supplies: plastic wrap, hydroponics solution, THP bottles, super glue, plants, caulking, acids needed for acid wash, DI water, MTBE, analytical balance, aluminum foil, acetate, graduated cylinder

1. Remove plant from hydroponics solution
2. Wrap plant’s roots in plastic wrap by rolling the roots into a thin narrow tubular shape. (this will allow for easy insertion through the hole of the bottle’s lid)
3. Carefully insert the plant’s covered roots through the hole in the jar’s lid. (make sure not to forcefully jam the roots, this may cause severe damage)
4. Unwrap the plastic wrap from the roots
5. Place a small amount of super glue onto the plant’s base. (where the stem meets the roots) and fix it to the side of the hole in the jar’s lid. (this allows for the plant to remain fixed to the lid)
6. Once super glue is dry use caulking to spread it around the plant’s base. (apply a generous amount of caulking to create an air-tight seal around the lid’s hole) Allow for caulking to dry before going on to preparing the MTBE solution.
7. After caulking has dried, set plant aside in hydroponics solution temporarily while preparing MTBE solution. (set aside in separate bottle, not using the amber bottles which will be used later to prepare the MTBE solution
8. Clean 500 ml amber bottles with acid wash, and then thoroughly rinsing with DI water.

9) Pour 50 ml of Hoagland’s hydroponics solution in 500 ml amber chemical bottle.

Note: *If this step is not conducted then when you add MTBE, the level of MTBE*

*vaporization will greatly increase. The solution decreases the rate of MTBE*

*vaporization because of its high solubility in the solution.*

10) Place the bottle on analytical balance and tare the balance.

1. Once the balance has been set to 0, add approximately 0.5 grams of MTBE using the micropipette. (this amount gives an initial concentration of 1000ppm)
2. Once MTBE has been successfully measured and weighed, immediately place plant into solution.
3. Create an aluminum foil barrier between the plastic cap and cardboard layer to prevent reaction of chemical with the plastic.
4. Once the aluminum foil barrier is properly in place, immediately cap the bottle – making sure it is tightly secure.
5. Place caulking around the seal to make it air-tight. Add any additional caulking around the plant’s base where it meets the lid.

# **Devising the Standard Curve:**

Supplies: Hoagland’s solution, graduated cylinder, MTBE, analytical balance, GCMS sample vials, glass vials, disposable Pasteur pipettes, refrigerator, micropipettes, GCMS

The GC/MS chromatogram plot calculates the specific peaks of chemical elements in solution. In order to calculate the concentration of the chemical elements relative to their chromatogram peaks, a standard curve must be devised. The standard curve is created by sampling known concentrations of chemical elements (in our case, MTBE) and then comparing it to the area of the chromatogram peak. A statistical plot of the data yields a least-squares regression line for the relationship between peak area of a chromatogram plot and its concentration.

1. Measure out 10 ml of Hoagland’s solution in a graduated cylinder and pour into a glass vial.
2. Produce different solution concentrations by varying amount of MTBE used in 10ml of Hoagland’s solution. The following is a example of how to produce/calculate a solution with a concentration of 250 ppm:

1 part per million=1 milligram/1 liter

250mg/1 liter=250 ppm

250mg/1liter \*1 liter/1,000 ml= 2.5 mg/10 m

2.5 mg/1 ml\*1 g/1000mg= 0.0025g/10 ml

* 1. First place the glass vial with Hoalgland’s solution on an analytical balance and tare the balance.
  2. Measure out the required grams of MTBE with the aid of a disposable Pasteur pipette.
  3. Once you have the correct number of grams, close the vial as quickly as possible to prevent MTBE loss from solution.
  4. Let the solution equilibrate in a refrigerator for approximately 30 minutes.
  5. Take out GCMS sample vial.
  6. Remove the refrigerated vial for transfer into GCMS sample vial. Remove the lid of both vials. Use a micropipette to remove 300 micro liters from the refrigerated vial and transfer it to the GCMS sample vial (work under a hood). Once transfer is complete, cap the GCMS sample lid and refrigerate the sample for another 30 minutes as to allow the sample to equilibrate.

1. Place sample in GCMS sample holding rack and record the locations of each vial. Run the GCMS on auto-sampling mode to run all of the various concentrations on a 15-minute time interval.
2. Graph the areas obtained by integrating the peaks of each individual sample run (GC/MS software calculates the area under the curve) and plot these values vs. the concentrations to have a visual representation of what area vs. know concentrations should look like. Area vs. concentration should have a linear relationship over an interval of 100 ppm to 2000 ppm.

# **Sampling Procedure:**

Supplies: test subjects, GCMS sample vials, Ethyl Ether, micropipettes, GCMS

The sampling was conducted around 4:00-5:00 every evening for a period of five days. Sampling is done in the same room at the same temperature and samples are given the same amount of time to equelibrate.

1. Remove test subjects form humidity camber.

Note: *Take out plant samples very carefully, quick or exaggerated movements can*

*shift the equilibrium and lead to errors.*

1. Acquire GCMS sample vials and cap them (as many as the number of subjects).
2. Clean out siring with the aid of ethyl ether.
3. Remove GCMS vial from the lid of the THP bottles by a twisting action.
4. Draw 300 micro liters of solution from the center of the THP bottles by injecting siring through the septum of the GCMS vial that was glued on earlier.
5. Inject the solution through the septum’s of the GCMS sample vials(empty vials) and empty contents of siring.
6. If you have more than one sample follow steps 1 through 6 accordingly.
7. Allow sample vials to equilibrate for approximately ten minutes.
8. Run samples through the GCMS and integrate MTBE peaks to obtain area under graph.

**How we came up with our Apparatus**