High Quality Sterile Media Lab for Growing Salt Water Algae

Introduction:

The purpose of this lab is to give your students the opportunity to apply their knowledge of molar solutions by supplying the biology classes with a media that is ideal for the growth of algae in a sterile environment. The algae that the biology classes grow will be used to teach the reasons behind why plants make oils and how those oils could be used to create a biofuel that could eventually replace diesel as our primary fuel choice. Your chemistry students will learn sterile technique using items that are normally found in a science classroom, and their final product can be assessed by the solution's ability to act as a medium for algae to successfully grow in.

Learning Outcomes:

- Students will learn why certain chemicals are required to grow algae
- Students will learn introductory sterile techniques required in most professional biochemical laboratories.
- Students will learn about molar solutions through a medium that will be used to assist with vertically aligning science classes.

Common Core Standards Addressed for Chemistry:

Chm.2.2-Analyze chemical reactions in terms of quantities, product formation, and energy.

- Chm.2.2.4-Analyze the stoichiometric relationships inherent in a chemical reaction.
- Chm.2.2.5-Analyze quantitatively the composition of a substance (empirical formula, molecular formula, percent composition, and hydrates).

Chm.3.2-Understand solutions and the solution process.

- Chm.3.2.1-Classify substances using the hydronium and hydroxide ion concentrations.
- Chm.3.2.2-Summarize the properties of acids and bases.
- Chm.3.2.3-Infer the quantitative nature of a solution (molarity, dilution, and titration with a 1:1 molar ratio).
- Chm.3.2.4-Summarize the properties of solutions.
- Chm.3.2.6-Explain the solution process.

Time Required:

This lab could be completed in one Block schedule class if the teacher makes the metal stock solution beforehand. If the teacher would like for the students to make the stock solution, then it will take two days.

Pre-Activities:

Students should have a basic understanding of moles, molecular weight, and simple stoichiometry. If you have more than one analytical balance, then you could have the students make the entire media. If you only have one, then the teacher should create the metal stock solution and the EDTA/$FeCl_3$ stock solution beforehand and let the students add the appropriate amounts to save adding an extra day. The stock solutions you create should last until the expiration date of the chemicals listed on the charts below.

Materials per group of three or four:

Water bottles (16oz or 500mL)
Chemical list (see attached media creation worksheet)
Gloves
Goggles
Digital balance
Goggle Sterilizing UV cabinet
Bunsen Burner and gas source
Distilled Water
Ethanol (70%)
5mL and 10mL pipets (sterile)
Micropipette (1000-100µL)
Hand Crank Pump for pipettes
Magnetic stirring rod options with stirring plates
pH tester
4 1000mL jars
Graduated Cylinders (1000mL) per group

Pre-Lab:

1.) Look at the media preparation page. Show your knowledge of molar solutions by filling in the blanks on the worksheet.
2.) Have your teacher check off your answers before creating the medium.
3.) Go over classroom safety procedures with using Ethanol, Bunsen Burners, and various chemicals.

Post-Lab:

1.) Name three reasons why you think it is important that professional biochemistry labs use sterile technique?
2.) Why do algae (and plants in general) need the metals magnesium, potassium, manganese, copper, zinc, and molybdenum? Make a chart that lists the elements and their usefulness.

Metal	Usefulness

3.) Why do you think nitrate and chloride compounds are so commonly used in a solution?
4.) If you found out that Mg helped grow algae more quickly, how would you adjust the media to incorporate more Mg?

Vocabulary:

See Google Definitions, www.oilgae.com, or medical-dictionary.thefreedictionary.com for best examples

Flocculation-

Sterile-

Mole-

Molecular weight-

Molar Solution-

Alternative Assessment:

Was the Biology class successful growing alga in your media? Discuss some other ways that a Chemist might be needed in a life science field.

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I am a 14 year National Board Certified Teacher and Kenan Fellow working as an AP Environmental/Earth Science teacher at Holly Springs High School in Wake County. I have a degree in Geology from North Carolina State University and am General Science Certified. In 2009, I was a finalist for the Wake County Teacher of the Year, and was named as the N.C. Outstanding Science Teacher of the Year for District 3 by the N.C. Science Teacher Association in 2011. I have assisted with the development and implementation of multiple state-wide curricula including two from the N.C. Environmental Education Fund titled “It’s Our Water!” and “It’s Our Air!,” and was an original member of the NSF funded EarthView program back in 2000 when Earth Science became a graduation requirement.

Media Creation Lab

Lab Activity:

Consider the amount of biology groups and make enough for each group as well as some for each teacher. Each group will need 500 mL (two water bottles filled with 250mL each) and each teacher will need 250mL.

To create the medium:

1.) Start with 750mL of distilled water in a beaker.
2.) Mix the components in order of listing on the Media Preparation Worksheet using the stirring rod option on a hot plate during the addition of each chemical to the specified amounts.
3.) Check the pH. The pH of the medium should hopefully be around 7.5. Adjust as needed with NaOH to raise pH or HCl to lower pH (preferably not both-add one or the other in very small increments.)
4.) Pour the medium into a 1000mL graduated cylinder in order to insure amount is accurate. Add distilled water until the total amount is 1L.
5.) Gather some jars small enough to fit into the ultraviolet goggle sterilizing cabinet and sterilize the inside of the jars using UV light for 20+ minutes. Sterilize the outside of the jars by spraying the 70% ethanol.
6.) Pour the medium into the jars and place the jars in the goggle cabinet. Sterilize medium once again with the UV light for 20+ minutes.
7.) Keep the medium in a refrigerator until ready to grow algae. Warm the medium in warm water or leave overnight in room temperature before adding algae.

Procedure for adding medium to a 500mL water bottle with sterile technique*: Use video for demonstration of steps 3-7.*

1.) Wash hands thoroughly with proper technique as shown in attached diagram.
2.) Sterilize the water bottle and a plastic wrap covered 250mL graduated cylinder by placing them in the goggle sterilizing cabinet and turning the UV light on for four 5min cycles (20 minutes).
3.) **See Video for a demonstration of the next 5 steps:** Spray the water bottle with ethanol and place the water bottle and graduated cylinder in the sterile area.
CAUTION! CREATE AN ETHANOL SPRAYING AREA THAT IS FAR AWAY FROM THE BUNSEN BURNERS.
4.) Light a bunsen burner to create a 10cm diameter sterile zone, spray the water bottle with ethanol, and place the water bottle and the graduated cylinder in the sterile area. Once they are in the 10cm sterile field you can remove the plastic wrap from the graduated cylinder.
5.) Spray the outside of the medium container with ethanol and place the medium inside the sterile area.
6.) Loosen (but do not remove) the cap on the water bottle and the cap on the medium without removing your hands from the sterile field. Remove and hold the medium cap in one hand without touching the inside of the cap and pour 250mL of the medium into a sterile 250mL graduated cylinder. Tighten the medium cap back on and remove and hold the water bottle cap without touching the inside of the cap. Pour the medium into the water bottle and seal the cap on the water bottle. Leave the other 250mL of volume in the water bottle free to allow for gas exchange.
7.) Close cap tightly and place in the UV goggle cabinet to sterilize for another 20 minutes. The water bottle can now be handled safely without compromising the sterility as long as the cap is not loosened before being placed in a sterile field once again.

Optional Procedure for adding algae with sterile technique to the water bottle photo bioreactor: See Video for demonstration.

1.) Spray closed container of algae, water bottles, and all necessary equipment (including hands) with ethanol and place in sterile zone.
2.) Place all the water bottles and equipment nearby a Bunsen burner and then light the Bunsen burner to create a 10cm diameter sterile zone.
3.) Loosen cap of water bottle but do not remove.
4.) Swirl the algae container so that each group is getting the same approximate amount of algae. Loosen the top of the algae without removing it.
5.) Open the top of the sterile pipette packaging where you will be attaching the pipette to the hand crank pump. DO NOT TOUCH THE PIPETTE OR LAY IT DOWN. Keep the pipette in the sterile field.
6.) Open algae container in the sterile field and use 10mL sterilized pipette and a hand crank pump pipette to withdraw 13mL of algae (max that 10mL pipette will hold).
7.) Take the cap off of the water bottle and release the algae into the bottle from the pipette. Hold the cap near the bottle in the sterile field with one hand while you release the algae into the bottle.
8.) Seal the bottle without touching inside the cap and gently swirl the algae to spread them throughout the medium.
9.) Place in aquarium and loosen the cap WITHOUT REMOVING to allow for venting of gas exchange.

Teacher Answer Sheet for Pre and Post Lab Questions plus Vocabulary

Pre Lab: See Teacher Answer Key for Media Worksheet.

Post Lab:

1.) Name three reasons why you think it is important that professional biochemistry labs use sterile technique? ***Multiple answers are possible. Professional labs want to make sure that the products they make are safe, consistent and reproducible, and uncontaminated.***

2.) Why do algae (and plants in general) need the metals magnesium, potassium, manganese, copper, zinc, and molybdenum? Make a chart that lists the elements and their usefulness.

Metal	Usefulness
Magnesium	**Used in photosynthesis and in the uptake of other essential nutrients. Also used to make seeds.**
Potassium	**Nutrient used to build cells and tissue. Contributes to overall hardiness of the plant.**
Manganese	**Necessary for chlorophyll formation and essential cellular functions.**
Copper	**Contributes to plant metabolism and reproduction.**
Zinc	**Multiple answers possible. Combines with other elements to carry out numerous natural processes including the formation of chlorophyll.**
Molybdenum	**Needed to produce essential proteins.**

3.) Why do you think nitrate and chloride compounds are so commonly used in a solution? ***These two compounds are both easily soluble in water, so they are great for incorporating various desirable elements into a growth media.***

4.) If you found out that Mg helped grow algae more quickly, how would you adjust the media to incorporate more Mg? **Run multiple trials in which they increase the molarity of $MgSO_4$ by adding more $MgSO_4$ crystals, or you could keep the molarity of all the solutions the same, but increase the volume of the solution added to the sample. A higher level answer would be if you left $MgSO_4$ at .5M but increase the volume to 150mL, you have also added more Mg ions without increasing the concentration, meaning no possible increase in the toxicity of the media.**

Critical Vocabulary:

Flocculation- The gathering together of fine particles in water by gentle mixing after the addition of coagulant chemicals to form larger particles

Sterile- Free from all live bacteria or other microorganisms and their spores.

Mole-The SI unit of amount of substance, equal to the quantity containing as many elementary units as there are atoms in 0.012 kg of carbon-12.

Molecular weight-The ratio of the average mass of one molecule of an element or compound to one twelfth of the mass of an atom of carbon-12.

Molar Solution- a homogeneous mixture of one or more substances (solutes) dispersed molecularly in a sufficient quantity of dissolving medium (solvent).

Alternative Assessment:

Was the Biology class successful growing alga in your media? Discuss some other ways that a Chemist might be needed in a life science field. **Partnering a Biology class with your Chemistry class can show your students how the molar solution they created is actually being used to grow a living organism. This gives relevance and a greater sense of accomplishment which will assist with deepening their understanding and could provide an opportunity for you to discuss career opportunities for chemistry.**

Media Preparation Worksheet

Name: ___________________________ Date: _________________________________

Directions: Use your knowledge of molar solutions to fill in the blanks below. Do not start to create the media until your teacher has approved your answers.

The following solutions need to be created beforehand and then added in order to the media below

Compound	Stock Concentration	Grams per 1 Liter	Molecular weight (g/mole)	Stock molarity (M)	Final concentration in Wang 2007 (M)	Amount to put in 1 Liter of Wang 2007 from stock solution (mL)
MgSO4 7H2O (mL)	12g/100mL	120	____________	0.500	.005	10.0
CaCl2 2H2O (mL)	3g/100mL	30	147.0154	0.200	______________	0.600
KH2PO4 (mL)	5g/100mL	50	136.0852	0.400	0.00012	____________

2007 Wang Media for algae growth

Chemical Name	Final Concentration	Molecular Weight	1 Liter of media pH buffered to 7.5
NaCl	**1 M**	____________________	58.448 g
NaHCO3	__________________________	84.007g/mol	4.2 g
KNO3	__________________________	101.103g/mol	0.5055 g
MgSO4 (mL) From stock	0.005 M	246.47g/mol	10mL of student created stock
CaCl2 2H2O (mL) From stock	0.00012M	147.0154g/mol	0.600mL of student created stock
KH2PO4 (mL) From stock	0.00012M	136.0852g/mol	0.300mL of student created stock
EDTA	0.000006 M	292.24g/mol	10 mL of the teacher created EDTA and FeCl3 stock
FeCl3	0.000002 M	270.3g/mol	
MnCl2	0.000007 M	____________________	1 mL of the 1000X teacher created metal stock solution
ZnSO4	0.000001 M	179.461g/mol	
Co(NO3)2	0.000001 M	182.943g/mol	
CuSO4	0.000001 M	159.610g/mol	
(NH4)6Mo7O24	0.000001 M	____________________	
**Tris	0.010 M (only in pH 7.5)	121.14g/mol	10 mL of 1M stock at pH 7.5

*EDTA is used to hold the iron so that the iron is used by the algae and not oxidized or taken up by other reactions (not needed for this media)

**Tris works as a buffering molecule that changes structure as the pH changes. The intent is to slow the process of alkalinity that the alga naturally creates. Tris is a base, so you may have to change the pH of the Tris to 7.5 preferably using either NaOH or HCl to adjust your 1 molar solution.

Teacher Answer Key for Media Worksheet

Directions: Use your knowledge of molar solutions to fill in the blanks below. Do not start to create the media until your teacher has approved your answers.

The following solutions need to be created beforehand and then added in order to the media below

Compound	Stock Concentration	Grams per 1 Liter	Molecular weight (g/mole)	Stock molarity (M)	Final concentration in Wang 2007 (M)	Amount to put in 1 Liter of Wang 2007 from stock solution (mL)
MgSO4 7H2O (mL)	12g/100mL	120	246.47	0.500	.005	10.0
CaCl2 2H2O (mL)	3g/100mL	30	147.0154	0.200	0.00012	0.600
KH2PO4 (mL)	5g/100mL	50	136.0852	0.400	0.00012	0.300

2007 Wang Media for algae growth

Chemical Name	**Final Concentration**	**Molecular Weight**	**1 Liter of media pH buffered to 7.5**
NaCl	**1 M**	58.448g/mol	58.448 g
NaHCO3	0.050 M	84.007g/mol	4.2 g
KNO3	0.005 M	101.103g/mol	0.5055 g
MgSO4 (mL)	0.005 M	246.47g/mol	10mL of student created stock
CaCl2 2H2O (mL)	0.00012M	147.0154g/mol	0.600mL of student created stock
KH2PO4 (mL)	0.00012M	136.0852g/mol	0.300mL of students created stock
EDTA	0.000006 M	292.24g/mol	10 mL of the teacher created EDTA and FeCl3 stock
FeCl3	0.000002 M	270.3g/mol	
MnCl2	0.000007 M	125.844g/mol	1 mL of the 1000X teacher created metal stock solution
ZnSO4	0.000001 M	179.461g/mol	
Co(NO3)2	0.000001 M	182.943g/mol	
CuSO4	0.000001 M	159.610g/mol	
(NH4)6Mo7O24	0.000001 M	1235.86g/mol	
**Tris	0.010 M (only in pH 7.5)	121.14g/mol	10 mL of 1M stock at pH 7.5

*EDTA is used to hold the iron so that the iron is used by the algae and not oxidized or taken up by other reactions (not needed for this media)
**Tris works as a buffering molecule that changes structure as the pH changes. The intent is to slow the process of alkalinity that the alga naturally creates. Tris is a base, so you may have to change the pH of the Tris to 7.5 preferably using either NaOH or HCl to adjust your 1 molar solution.

Teacher Stock Solution Preparation Worksheet

2007 Wang Metal Stock Solution

A little goes a long way with this stock solution. 100mL of stock should be enough for 9 chemistry classes with eight groups of four students each.

Chemical Name	Final concentration in 1000X stock (M)	Molecular Weight (g/mole)	Amount for 1 L (g)
MnCl2 4H2O	0.007M	197.9057 g/mole	1.385 g
ZnSO4 7H2O	0.001M	287.54 g/mole	0.288 g
Co(NO3)2 6H2O	0.001M	291.03 g/mole	0.291 g
CuSO4 5H2O	0.001M	249.68 g/mole	0.250 g
(NH4)6Mo7O24 4H2O	0.001M	1235.8727 g/mole	1.236 g

2007 Wang EDTA and FeCl3 stock solution

One Liter of the following stock solution will be enough for 9 chemistry classes with eight groups of four students each.

Chemical Name	Final concentration in 100X stock (M)	Molecular Weight (g/mole)	Amount for 1 L in grams
EDTA	0.0006M	292.24 g/mole	0.175 g
FeCl3 6H2O	0.0002M	270.3 g/mole	0.054 g