

SANTA FE COMMUNITY COLLEGE

Biofuel Center for Excellence

TEACHER MANUAL
Introduction to Algal Biofuel:
Training & Methods

BIOFUEL CENTER FOR EXCELLENCE

Introduction to Algal Biofuel: Training & Methods

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Algal Biofuel Overview

Lecture hours required: 1.5 hours

Lab hours required: 3.5 hours

Lecture

This lecture and the exercises introduce students to concepts and ideas that provide a framework for understanding algae biofuel and how it is developing as an industry. We start with an introduction of what are algae and what is biofuel. Students should understand that algae are one of the best candidates for renewable biofuel. We will also cover a short history of algae biofuel research in the United States. The context here is examination of the demand for fuel and how that demand relates to interest in alternative fuels. Students should become familiar with components of the Aquatic Species Program as this was the program that demonstrated that algae can be used a replacement for petroleum. Also important is the current state of algae research and commercialization developments. Currently, funding is being provided for research and development intended to achieve the goals of the National Algal Biofuels Technology Roadmap. As of 2012 a major effort is underway to make biofuel from algae a reality. Make a point to students that this process is not easy, or it would already have been done. There are many challenges to making algae biofuel a successful commercial reality, both technological and economic.

Laboratory Exercises

Today's lab exercises will consist of a lab tour, economic exercise and initiating or viewing algal cultures.

Lab Tour

Students should get a general introduction to the lab. Point out important pieces of equipment and explain their uses. Identify areas where they will be working on specific tasks. Identify Safety equipment.

Economic Exercise

The goal of this exercise is to introduce students to important economic factors that affect algae businesses. Suggested answers for the exercises are as follows:

R: Amount of biomass produced, lipid content, presence of co-products, market value of these products, bioremediation, water treatment, carbon credits etc.

O_c: Labor, utilities, electricity, water, gas, maintenance, nutrients, lab supplies, transportation etc.

C_c: Growing system technology, land, legal, consultants, land surveys, groundwater surveys etc.

Starting Algae Cultures

This exercise is performed to introduce students to algae cultures. It will be mostly a demonstration, using prepared media. Start cultures of different algal strains to be used throughout the semester for class exercises. Explain some of the differences between the strains; marine, freshwater, unicellular, filamentous, lipid producer, food production, temperature preferences etc. This will also give the students an introduction to some of the growing systems used in the laboratory and how they are used to produce algae cultures of varying volumes.

Lecture Outline

- Review Course Syllabus
 - Goals of the course, material to be covered and timeline
- Introductions
 - Teacher and Student Introductions – Have students say something about themselves and their interest in algae
- Biofuel from Algae an Introduction
 - What are algae?
 - Plant-like organisms
 - What is biofuel?
 - Fuel derived from biological carbon fixation
 - Types of biofuel
 - Comparison of biofuel crops
 - Why are algae good candidates for biofuel production?
 - Fast growing, high lipid production, can use waste water and agricultural wastes to grow, co-products, CO₂ neutral?, not a food crop, drop in fuel
 - Types of biofuel from Algae
 - Diesel
 - Gasoline
 - Jet fuel
 - Ethanol
 - Hydrogen
 - Veggie Oil
 -
- History of Algae as Biofuel in the USA
 - US Oil Embargo 1973
 - Organization of Arab Petroleum Exporting Countries (OAPEC)
 - Aquatic Species Program (ASP) 1978-1996 ;18 yrs
 - Jimmy Carter
 - Department of Energy (DOE)
 - Solar Energy Research Institute (SERI)

INTRODUCTION TO ALGAL BIOFUEL

- Nation Renewable Energy Laboratory (NREL)
 - Final Report 1998
 - Hydrogen- Biodiesel
 - Culture Collection
 - Find high lipid, high production strains
 - Roswell New Mexico
 - Open Pond Research
 - Biomass production
 - CO2 utilization
 - 50 grams algae / square meter / day
 - Cold temperatures hindered growth
 - Projected biodiesel 2X current (1998 \$1.12) costs for petroleum diesel
 - Showed the potential for the use of algae as a renewable energy resource
- Current State of Algae for Biofuel
 - Renewed Department of Energy interest
 - American Recovery and Reinvestment Act 2009
 - National Algal Biofuels Technology Roadmap
 - Lower greenhouse gas emissions
 - Affordable, reliable energy
 - Many technological paths
 - “Developing the next generation of biofuels is key to our effort to end our dependence on foreign oil and address the climate crisis – while creating millions of new jobs that can’t be outsourced.” – Steven Chu – Secretary of Energy 2009
- Challenges to Biofuel production from algae
 - New Commercial Industry Parallel
 - Technological Challenges
 - Regulations and Standards

INTRODUCTION TO ALGAL BIOFUEL

- Public –Private Partnerships
- Technological Challenges
 - Biomass production
 - Growing system
 - System optimization – Algal biology
 - Harvesting
 - Conversion or Harvest of Products
 - Biomass to fuel
 - Co-products
 - Infrastructure
- Regulations and Standards
 - None exist
- Public – Private Partnerships
 - Industry – Academia
 - Partnerships challenging
 - Differing goals
 - Intellectual property vs. education
 - Both beneficial to progress
- Economics of Biofuel production from Algae
 - Cost of Production < Value of Products
 - Cost of Production
 - Capital Expenditure
 - Land
 - Infrastructure
 - Growing System
 - Laboratory
 - Equipment
 - Research and Development
 - Salaries - Staff
 - Operational Expenditure
 - Equipment operation

INTRODUCTION TO ALGAL BIOFUEL

- Utilities
 - Water
 - Electricity
 - Nutrients
 - CO₂ source
 - Transportation
 - Maintenance
 - Product processing
 - Product transport
 - Salaries
- Value of Product
 - Amount of product
 - Multiple products
 - Market value
- Tour of laboratory
 - Safety Equipment
 - Equipment
 - Algae
- Lab Exercises
 - Economic Analysis
 - Start Algae Cultures for Class Use
- Review Material Covered
 - Emphasize major points
- Optional

Have students search for an article on the economics of algae production and bring it to the next class

Lab Procedure I
Techno-Economic Analysis

Introduction:

In order for the algae biofuel industry to be successful it must be economically viable. One of the major hurdles here is acquiring affordable technology to grow algae. The technology of growing algae consists of all of the research and development that goes into selecting and optimizing strains, and designing and developing the growing, harvesting and extraction systems. The successful cultivation and use of algae will rest on developing successful growing protocols for algae strains and commercial growing systems. The costs associated with starting an algae farming business are called capital costs. The costs associated with operating an algae farm are the operating costs. Revenue is the amount earned from the algae products produced.

So, the revenue produced must be greater than or equal to the sum of capital and operational costs.

Revenue = R; Income

Operational Cost = O_c ; Cost associated with running business

Capital Cost = C_c ; Cost of investment

$$R \geq O_c + C_c$$

Exercise 1

In the space provided, list factors that you think will affect R, O_c and C_c .

R:

O_c :

C_c :

INTRODUCTION TO ALGAL BIOFUEL

Accurate economic models for algal biomass production are difficult to create. For instance, it is difficult put an accurate number on the value for services like wastewater treatment, labor and product processing since there is not a directly comparable industry. Still, estimates can be made to help guide where costs must be cut or changed to make projects successful. Many algae business have and will fail due to inefficient or incorrect use of funds.

Exercise 2

From your list made above for R , O_c and C_c choose important factors and list how your can either maximize revenue or reduce cost for each variable.

R :

O_c :

C_c :

Powerpoint Presentation Slides:

Algal Biofuel Overview

What are Algae?

- Plant-like organisms
- Photosynthesis
- Ancient precursors to plants
- Very important in global environment



What is Biofuel?

- Fuel derived from biological carbon fixation
- Fossil fuels
- Corn
- Vegetable oil
- Food
-



Why Algae for Biofuel?

- Fast growing
- High lipid content
- Grow on waste water
- Grow on agricultural waste



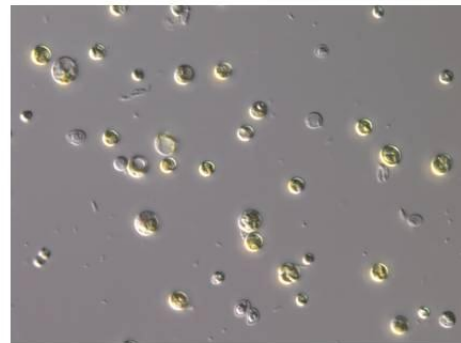
Why Algae for Biofuel?

- Co-products
- CO₂ neutral (?)
- Not competing with agriculture
- Replacement for fuels already in use



Types of Biofuel from Algae

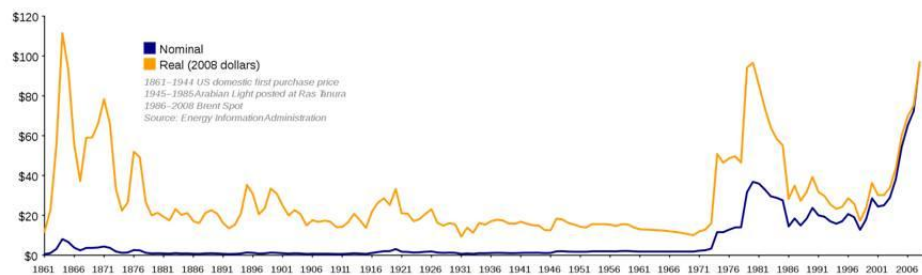
- Diesel
- Gasoline
- Jet fuel
- Ethanol
- Hydrogen
- Veggie Oil
-



History of Algae Biofuel in USA

- 1973 – US Oil Embargo
- Organization of Arab Petroleum Exporting Countries (OAPEC)
- Sharp oil price increase

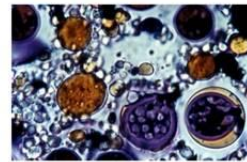
Oil Prices



Aquatic Species Program (ASP)

- 1978-1996
- Jimmy Carter
- Funded by Department of Energy
- Develop renewable transportation fuel

A Look Back at the U.S.
Department of Energy's
Aquatic Species Program:



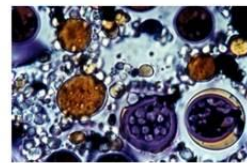
Biodiesel from Algae

Part I:
Program Summary

Aquatic Species Program

- Initial focus – Hydrogen
- Focus shift – Biodiesel
- Culture Collection
 - High lipid
 - High production
 - 3,000 isolates
- Final Report 1998

A Look Back at the U.S.
Department of Energy's
Aquatic Species Program:

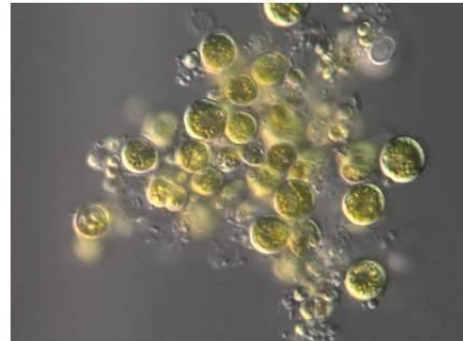


Biodiesel from Algae

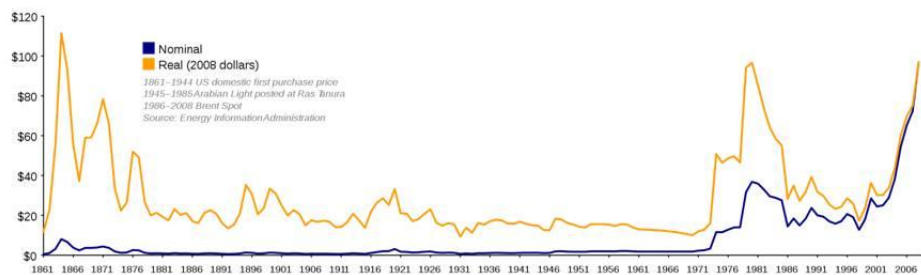
Part I:
Program Summary

ASP: Roswell New Mexico

- Biomass production:
Open Ponds - CO₂
Utilization
- 50 g/m²/d
- Projected cost of
biodiesel 2x cost of
diesel (\$1.12) in 1998
- Showed potential of
algae as renewable
energy



Oil Prices



Recent Policy Towards Biofuel

- Energy Independence and Security Act 2007
 - Renewable Fuel Standard
 - 36 billion gallons of renewable biofuel by 2022
- American Recovery and Renewal Act
 - \$800M
 - DOE – Biomass program

National Algal Biofuels Technology Roadmap

- Lower greenhouse gas emissions
- Reduce dependence on foreign oil
- Affordable, reliable energy
- Many technology paths



National Algal Biofuels Technology Roadmap

- “Developing the next generation of biofuel is key to our effort to end our dependence on foreign oil and address the climate crisis – while creating millions of new jobs that can’t be outsourced.” – Steven Chu – Secretary of Energy 2009

Challenges to Biofuel production from Algae

- Technological
- Regulations and Standards
- Public – Private Partnerships
- Economic



Technological Challenges

- Biomass Production
 - Growing System
 - System optimization – Algal Biology
 - Harvesting – Extraction
- Conversion or Harvest of Products - Market
 - Biomass to fuel
 - Co-products
 - Infrastructure

Regulation and Standards

- None exist
- GMO?
- Product quality
- Fuel Standards
- USDA
- EPA

Public – Private Partnership Industry – Academia

- Partnership challenging
- Differing goals
- Intellectual property vs education
- Both beneficial to progress of industry



Economics of Biofuel Production from Algae

- Cost of Production
 - Operational Costs
 - Utilities
 - Labor
 - Nutrients
 - Transportation
 - etc
 - Capital Costs
 - Land
 - Growing System
 - etc
- Revenue
 - Value of Products
 - Carbon sequestration
 - Bioremediation
 - Water treatment

Algal Biology – Diversity and Ecology; Microscopy

Lecture hours required: 1 hour

Lab hours required: 4 hours

Lecture

Today's lecture aims at providing a general understanding of algal biology and its relationship with species diversity and ecology. We will also investigate microscopy. Goals for this lecture are to explore the incredible variety of algal species that occur in varying habitats on the earth. Explain how these unique organisms are important to global nutrient cycles, primary production and oxygen evolution. This understanding will help students create an understanding and contextual reference of how this diversity is important in selecting strains of algae for large scale production under varying environments.

Laboratory Exercises

There is a significant amount of material to cover in this class. Timing is important especially in transitioning from one activity to the next. It will be important that students are introduced thoughtfully to the material and thus gain a good understanding of lab methodology. They will have further chances to master lab skills. Break students into groups based on availability of equipment. Have students leave equipment cleaned and ready for next person.

Lab Etiquette and Safety

Review sheet of guidelines. Make sure students understand how to be safe in the lab.

Microscopy – Wet mount viewing

Assign microscopes by number. Students will use this scope for the remainder of the course. Review procedure document. Learn parts of microscope and use. Learn to make a wet mount.

Cell Counts using a Hemocytometer

Students will perform cell counts using a hemocytometer. Review procedure for use of hemocytometer. Emphasize proper use.

Algal Dry Weight Determination

Review procedure for dry weight. Identify equipment to be used and setting up proper workspace.

Optical Density Measurement

Students will use a **spectrophotometer** to measure optical density at A680 and A750. They will later use this method in relation to other biomass measures. Review procedure for determining OD and operation of the spectrophotometer.

Lecture Outline

- Algae Diversity
 - Plant-like organisms
 - Unicellular – Microalgae
 - Many types
 - Multicellular – Macroalgae
 - Many types
 - Thousands of species
 - Live in diverse habitats
 - Deserts
 - Ocean
 - Lakes
 - Rivers
 - Hypersaline
 - Leads to different ways of surviving
 - Diverse physiologies
 - Light requirements
 - Nutrient requirements
 - Temperature preference
 - pH preference
 - Storage products
 - Algae in the Environment
 - Typically not abundant
 - Factors limit success of single species
 - Lack of nutrients – Natural levels
 - Many species co-occur – share resources
 - Competition
 - Herbivory

INTRODUCTION TO ALGAL BIOFUEL

- Algal Blooms
 - Usually single species
 - High density of algal cells
 - Result of Nutrient enrichment (Pollution)
 - Harmful Algal Blooms (HAB's)
 - Toxic
- Isolating Algae from the Environment
 - Different methods
- Economic Uses of Algae
 - Food
 - Fertilizer
 - Agar
 - Bioremediation
 - Fuel
 - Plastics
- Lab Exercises
 - Working in the Lab
 - Lab etiquette
 - Keeping the lab clean
 - Workspace
 - Review Procedures
 - Microscopy
 - Handling a microscope
 - Assigning microscopes
 - Making wet mounts
 - Observing algae
 - Performing cell counts using **hemocytometer**
 - Algae Dry Weight Determination
 - Sample collection
 - Sample preparation and handling
 - Dry weight procedure

Lab Procedure II
Lab Etiquette and Safety

- Keep your workspace clean.
- No eating or drinking in laboratory space.
- Keep aisles clear of chairs, equipment and personal items.
- Always leave workspace and equipment clean and stored properly for the next person's use.
- Do not put chemicals or trash down the sinks.
- Follow instructions and procedures when using equipment.
- Do not interfere with other's work or experiments.
- Take your time when working.
- Follow safety standards for fire safety, chemical safety and equipment safety.
- Place broken glass or sharp wastes in designated waste container.
- Know where safety equipment is located and how to use it.

Observe these guidelines to help ensure that the lab is a safe and enjoyable environment to work in.

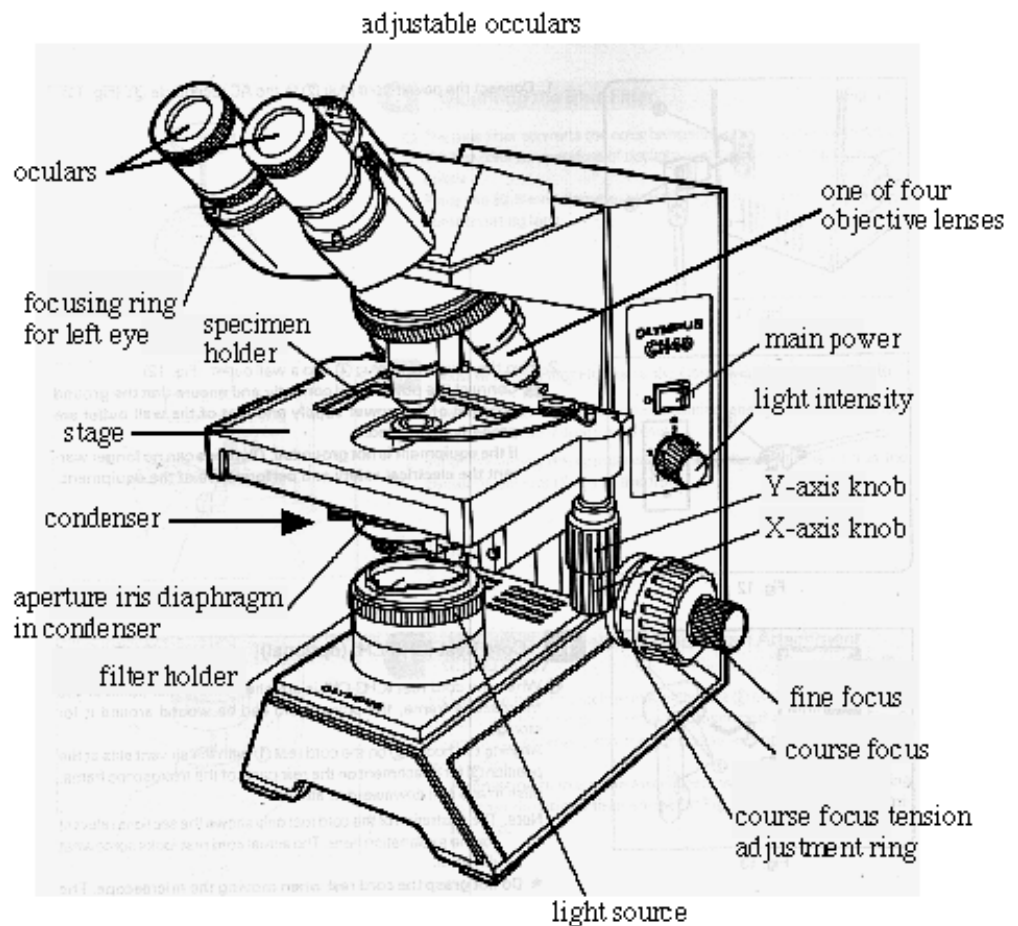
Lab Procedure III Microscopy

Introduction

We will be using the microscope to view algae that are invisible to the unaided eye. The microscope is a tool that will help us to assess the health of algae cultures, identify species present and determine the density of algae in a sample. We will start by learning about the various parts of a microscope and how to operate it successfully. We will then learn how to make a wet mount of an algae sample and view it on the microscope, making drawing of what we see.

Exercise 1

Parts of a Microscope



INTRODUCTION TO ALGAL BIOFUEL

Adjustable Oculars – can be adjusted to fit distance between eyes. Move closer together or further apart until both oculars create single image.

Oculars – eyepieces through which you will view a specimen.

Focusing ring for left eye – to adjust, close left eye and bring specimen into focus using the fine focus knob. Then close right eye and adjust focusing ring for the left eye. This compensates for any differences in vision between eyes.

Stage – Platform for placing microscope slide.

Specimen holder – Holds microscope slide on stage and moves slide.

Y-axis knob – moves slide in the y-axis on the stage. From top of stage to bottom.

X-axis knob – moves slide in the x-axis on the stage. From left to right.

Fine focus – moves the stage up and down in very small increments in order to focus the specimen. Use at high power objectives.

Coarse focus – moves the stage up and down in large increments in order to focus the specimen. Use at low power magnification. **DO NOT USE at 100x OBJECTIVE.**

Main power – provides power to the light source.

Light intensity – changes voltage supply to light source, dims or brightens light.

Condenser – focuses light into the objectives from the light source. Proper adjustment increases resolution of specimen.

Iris diaphragm – adjusts the amount of light and size of light cone sent to the objective.

Objective lens – lenses that magnify the specimen. Usually 4x, 10x, 40x, and 100x.

Exercise 2

Preparing a wet mount

1. Place one or two drops of sample containing living algae onto the center of a microscope slide
2. For quick viewing, angle a single coverslip at a 45° toward the drops of sample. Move the bottom of the coverslip along the slide until it makes contact with the sample. Ease the coverslip onto slide.
3. For longer viewing, place a small bead of Vaseline around the bottom edges of the coverslip. Gently lower the coverslip over sample. Press to seal Vaseline to slide.

Exercise 3

Viewing a wet mount

1. Make sure microscope is set to the lowest power objective and that the stage is at its lowest position.
2. Place slide with prepared wet mount onto the stage of the microscope and secure in the specimen holder.
3. Using the X-Y- axis knobs, move the specimen directly under the low power objective.
4. Turn on the main power.
5. Adjust oculars to fit eye distance.
6. Looking through the oculars, begin raising the stage using the course focus knob.
7. When specimen comes into view, begin to use the fine focus knob to bring into focus.
8. Change to the next power objective, up to 40x for viewing live algae. Do not use the 100x objective at this point.

Exercise 4

Illustrate living algae

Make several drawings of algae that you see. Use texts to tentatively identify the species found.

Drawings

Lab Procedure IV
Cell Counts using a Neubauer Hemocytometer

Introduction

Algal cell density is a useful measure to determine biomass. Cell density can be correlated with other measures of biomass such as optical density and dry weight in order to better understand biomass production rates. This procedure describes how to perform algal cell counts using a **hemocytometer**.

Procedure

1. Sample algae from a well mixed system so that it accurately represents cell density of the entire volume of the system.
2. Always carefully handle the hemocytometer. Use lens paper to clean the counting surfaces and thick coverslip with lab grade water. Make sure you are using the coverslip provided with the hemocytometer.
3. Place the coverslip over the counting chambers.
4. Mix algae sample by vortexing or shaking.
5. Load both counting chambers by placing the algae suspension at the edge of the coverslip with a pipette. Capillary action will fill the void of the counting chamber.
6. Let hemocytometer settle for 10 minutes.
7. Start at the low power magnification and bring specimen into focus. Move up to 40x for counting.
8. Count all algae cells within the inner grid for both counting chambers. Move the slide carefully so not to disturb the settled cells.
9. Use formula provided with hemocytometer to calculate cell density per milliliter from counts.

Lab Procedure V
Algal Dry Weight Determination

Equipment and Supplies

- Whatman GFF filters
- Filter apparatus and holder
- Filter flask
- Vacuum tubing
- Vacuum
- 10-20 ml pipette
- Aluminum Trays to hold filters
- Dessicator
- Drying oven
- Balance capable of 0.000 g precision
- DI water in squirt bottle
- Vortex
- Algae Sample

Procedure

1. Sample algae from a well mixed system so that it accurately represents dry weight of the entire volume of the system.
2. Number aluminum trays by marking tab. Prepare enough trays to process the number of samples you have with three replicates. Record tray number in datasheet. Avoid touching aluminum with bare hands.
3. Using forceps, place a single filter smooth side down in each tray. Record weight of each tray + filter in datasheet.
4. Assemble filter apparatus and connect to vacuum with hose.
5. Turn on vacuum and rinse several times with DI water.
6. Using forceps, place filter in filter apparatus.
7. Rinse filter with DI water.
8. Mix algae sample well by vortexing and shaking.
9. Depending on the density of the algae sample add known volume of mixed sample to the filter apparatus. Dense cultures may require 5-10 ml. Less dense up to 50 ml.
10. If the algae sample is grown in seawater rinse three times with 5-10 ml DI water.

11. Wait until all visible moisture is removed from the surface of the filter.
12. Disassemble filter apparatus and carefully remove filter with forceps.
Place filter in its numbered aluminum tray.
13. Process three replicates for each sample and place all samples on metal tray.
14. Place tray of samples in drying oven at 100°C for at least 8 hours.
15. Weigh samples. Record weight in datasheet.
16. Place samples back in drying oven at 100°C. Weigh again and record weight.
17. Repeat drying and weighting until you have the same weight three times in a row in order to determine sufficient drying time.
18. Samples can be stored in desiccator after complete drying in oven.

Lab Procedure VI
Measuring Optical Density using a Spectrophotometer

Equipment and Supplies

- Kimwipes
- Thermo Genesis 20 Spectrophotometer
- Cuvettes
- DI water
- Algae Sample
- Pipettes
- Datasheets

Procedure

1. Power on **spectrophotometer** by turning on power switch on the back of the machine. Let the lamp warm up for 10 minutes.
2. Select the wavelength 680nm by pushing the wavelength arrows up or down.
3. Run a blank of DI water by placing a clean **cuvette** in the cuvette holder, closing the door and pressing the blank key.
4. Measure all samples for this wavelength. Mix samples well in cuvette using a pipette before reading. Make sure the cuvette is clean and that there are no air bubbles or debris in the sample.
5. Record data in datasheet.
6. Verify that the machine is not drifting by running the DI water as a sample.
7. If the DI water does not read + or - 0.001, re-blank the machine.
8. After all samples have been run for 680nm, change the wavelength to 750nm and start at step 3 again. Be sure to re-blank the machine when changing wavelengths.

Powerpoint Presentation Slides:

Class II: Algal Biology – Diversity & Ecology; Microscopy

Introduction to Algal Biofuel

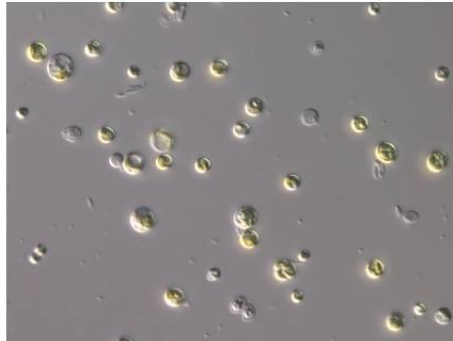
Class Outline

- Lecture
 - Algae Diversity
- Lab Exercises
 - Lab Etiquette and Safety
 - Microscopy
 - Cell Counts using a Hemocytometer
 - Algal Dry Weight Determination
 - Optical Density Measurements
 - Algal isolation

Algae Diversity

- Plant-like organisms

Unicellular (Microalgae)



Multicellular (Macroalgae)



Algae Diversity



- Diverse Habitats
 - Deserts
 - Oceans
 - Lakes
 - Rivers
 - Hypersaline

Algae Diversity



- Diverse Physiologies
 - Light
 - Nutrients
 - Temperature
 - pH
 - Storage Products

Algae in the Environment

- Typically not abundant
 - Factors limit success of single species
 - Lack of Nutrients – Natural levels
 - Many species co-occur
 - Share resources
 - Competition
 - Herbivory



Isolating Algae in the Environment



Economic Uses of Algae

- Food
- Fertilizer
- Agar
- Bioremediation
- Fuel
- Plastics



Lab Exercises

- Lab Etiquette
- Keeping the lab clean
- Workspace



Lab Exercises

- Microscopy
 - Handling a microscope
 - Assigning microscopes
 - Making wet mounts
 - Observing algae



Lab Exercises

- Performing cell counts using Hemocytometer



Lab Exercises

- Algae Dry Weight Determination
 - Sample collection
 - Sample preparation and handling
 - Dry weight procedure



Lab Exercises

- Optical Density Measurements
 - Spectrophotometer
 - A680nm
 - A750nm



Algal Biology – Physiology, Culturing, and Measuring the Environment

Lecture hours required: 1 hour

Lab hours required: 4 hours

Lecture

This lecture is the continuation of Algal biology, focusing on algal physiology, culturing methods and environmental monitoring. These topics will help students understand how well-designed algae production operations can maximize the growth of a particular species of algae. The training in this course will emphasize developing hands-on skills in order to better prepare participants for employment in the developing algae industry in New Mexico. A most important skill is the ability measure and quantify algal biomass.

Analytical and lab methodology skills will be emphasized for the remainder of the course.. Encourage discussion.



Laboratory Exercises

Provide plenty of time for students to work through these exercises. However do not allow them to finish too quickly. These methods are relatively easy, but for the untrained person it is easy to make mistakes. Being able to reliably perform these methods is key to successful experimentation, data collection and project management. Watch students and make suggestions where necessary.

Algal Biomass Monitoring Exercise

Students will perform biomass measurements on three different density cultures, representing three periods in a growth phase. They will collect their own data using methods learned last week and compare with other students.

Environmental Monitoring Exercise

Students will use environmental monitoring probes to measure variables of algal cultures. They will become familiar with the operation of instruments and how to collect reliable data.

Algal Culturing

Students will learn how to make algae media and transfer cultures for experiments. It will be key to future experiments.

Lecture Outline

- Algal Physiology
 - How algal cells carry out the biological functions that keep them alive
 - Algae have a diversity of biological functions
 - Salinity
 - Temperature
 - pH
 - Nutrient conditions
 - Light
 - Lipid production
 - Toxin production
 - Growth rate
 - Measuring Algal Growth
 - Growth Response to measured variable
 - Cell count
 - Optical Density
 - Dry weight
 - Fluorescence
 - Chlorophyll content
- Culturing Algae
 - Types of Media
 - Defined
 - Known components + pure water
 - Can determine growth response to specific changes
 - Many recipes formulated for specific algae species
 - Undefined
 - Custom media

INTRODUCTION TO ALGAL BIOFUEL

- Unknown components + (pure water)
 - Wastewater, soil extract, groundwater – can change
 - Grows “picky” species
- Mixing Media from Stock Solutions
 - Calculating Concentrations and Volumes
- Measuring the Environment
 - Variable Environment
 - In nature, leads to diversity
 - Don’t want growing systems to vary from optimized conditions
 - pH shifts
 - CO₂ gradients
 - O₂ gradients
 - Nutrient depletion
 - Contamination of non-target species
 - Monitoring and Control in Growing Systems
 - Monitor variables important to growth
 - Control variables within range
 - Controlling environment
 - Easier in Bioreactors
 - Hard in Open ponds
 - Optimal Growth vs. Compound production
 - Optimal growth favored by optimal conditions
 - Optimal compound production often triggered by stress
- Lab Exercises
 - Using a Spectrophotometer
 - Measuring Optical Density
 - Algae Culturing
 - Sterile / Unialgal conditions
 - Making media
 - Transferring cultures

Lab Procedure VII

Algal Biomass and Environmental Monitoring

Introduction

Today we will be practicing the methods of measuring biomass learned in the previous class. Several samples of algae at varying concentrations and varying environmental conditions will be provided for this exercise. Students will be performing cell counts, dry weights and optical density measures in order to determine algal biomass. In order to become familiar with collecting environmental data students will also use sensors to measure salinity, pH and dissolved oxygen on the same samples. Students should take plenty of time and focus on gaining an understanding of these procedures.

Exercise 1: Algal biomass Measurements

Students will be provided with three samples of varying cell densities and environmental conditions. Measure three replicates for each sample for each method. Using the methods in this manual, collect the following data:

Cell Count

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

Optical Density 680nm

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

INTRODUCTION TO ALGAL BIOFUEL

Optical Density 750nm

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

Dry Weight

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

Exercise 2 Environmental Monitoring

Use the Vernier sensors to gather the following data:

Salinity

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

pH

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

Dissolved Oxygen

	Rep. 1	Rep. 2	Rep. 3
Sample 1	_____	_____	_____
Sample 2	_____	_____	_____
Sample 3	_____	_____	_____

Use data to compare with classmates. The instructor will compile all student data and demonstrate how to view using graphs.

Lab Procedure VIII

Algal Culturing

Introduction

Successfully growing algae in the laboratory is a key step in mass cultivation of algae. In order to grow algae students must be presented with conditions that are similar to the natural algae environment. Key variables in growing algae include light level, temperature and important elements of water chemistry. Optimizing these variables for each strain of algae will help algae samples to grow as fast as possible. In this procedure students will learn how to make media to grow algae in the lab. There are many algae media recipes available, but we will focus on two of the most commonly used. The first recipe will be for f/2, a medium used to simulate high nutrient seawater. Second we will look at Bolds Basal Medium (BBM) a medium that grows many species of freshwater algae.

Procedures

The following tables shows the solutions used to make 1 liter of f/2 and BBM media. For f/2, solution 3 is only used if growing diatoms, otherwise it is omitted.

Mixing 1 liter of f/2

		Volume Used
Solution 1	NaNO ₃	1 ml
Solution 2	NaH ₂ PO ₄	1 ml
Solution 3	Na ₂ SiO ₃ * 9H ₂ O	1 ml
Solution 4	Trace metals	1 ml
Solution 5	Vitamins	0.5 ml

Equipment and supplies

- Autoclave
 - f/2 stock solutions
 - 1 ml pipette
 - 1 liter volumetric flask
 - 1 liter media bottle
 - Instant Ocean seawater
 - Autoclave tape
 - Labeling tape
1. Using a 1 liter volumetric flask, add around 500 ml of instant ocean seawater.
 2. Add the specified volumes of the f/2 stock solutions.
 3. Bring the volume up to 1 liter using instant ocean seawater
 4. Mix well. Make sure there is no precipitate.
 5. Transfer f/2 to 1 liter media bottle. Label bottle and apply autoclave tape. Keep cap loose.
 6. Autoclave.
 7. After autoclaving allow f/2 to cool for 24 hrs before use.

INTRODUCTION TO ALGAL BIOFUEL

Mixing 1 liter of BBM

Used		Volume
Solution 1	NaNO_3	10 ml
Solution 2	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10 ml
Solution 3	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10 ml
Solution 4	K_2HPO_4	10 ml
Solution 5	KH_2PO_4	10 ml
Solution 6	NaCl	10 ml
Solution 7	Alkaline EDTA	1 ml
Solution 8	Acidified Iron Solution	1 ml
Solution 9	Boron Solution	1 ml
Solution 10	Trace Metals Solution	1 ml

Equipment and supplies

- Autoclave
- BBM stock solutions
- 1 ml pipette
- 1 liter volumetric flask
- 1 liter media bottle
- DI water
- Autoclave tape
- Labeling tape

1. Using a 1 liter volumetric flask add around 500 ml of DI water.
2. Add the specified volumes of the BBM stock solutions.
3. Bring the volume up to 1 liter using DI water
4. Mix well. Make sure there is no precipitate.
5. Transfer BBM to 1 liter media bottle. Label bottle and apply autoclave tape. Keep cap loose.
6. Autoclave.
7. After autoclaving allow BBM to cool for 24 hrs before use.

Transferring Algal Cultures

Equipment and supplies

- Stock algae culture
- Sterile culture flasks with 50 ml growth medium and cotton plugs
- Labels
- Laminar flow hood or sterile environment
- Alcohol lamp
- Sterile pipettes
- Spray bottle with alcohol

1. Spray working space with 70% alcohol and wipe clean.
2. Place equipment and supplies in working area and spray lightly with alcohol.
3. Light alcohol lamp and place near working area.
4. Remove cotton plug from sterile culture flask, holding by the top, keep it between two fingers.
5. With a sterile pipette transfer around 2 ml from a healthy culture to the fresh sterile medium.
6. Replace cotton plug.
7. Place new culture on growing rack.

Powerpoint Presentation Slides:

Class III: Algal Biology – Physiology, Culturing, and Measuring the Environment

Introduction to Algal Biofuel

Class Outline

- Lecture
 - Algal physiology, culturing method, and environmental monitoring
- Lab Exercises
 - Algal Biomass Monitoring
 - Algal culturing
 - Environmental Monitoring

Algal Physiology

- How do algal cells carry out the biological functions that keep them alive?



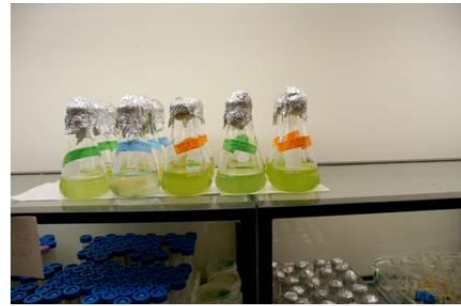
Algal Physiology



- Algae have diverse biological functions
 - Salinity
 - Temperature
 - pH
 - Nutrient conditions
 - Light
 - Lipid Production
 - Toxin Production
 - Growth Rate

Algal Physiology

- Measuring Algal Growth
 - Growth response to measured variable
 - Cell count
 - Optical density
 - Dry weight
 - Fluorescence
 - Chlorophyll content



Culturing Algae

Types of Media:

Defined

- Known components + pure water
- Can determine growth response to specific changes
- Many recipes formulated for specific algae species

Undefined

- Custom media
- Unknown components + pure water
- Wastewater, soil extract, groundwater – can change
- Grows “picky” species

Culturing Algae

- Mixing Media from Stock Solutions
 - Calculating concentrations and volumes



Measuring the Environment

- Variable Environment
 - In nature, leads to diversity
 - Don't want growing systems to vary from optimized conditions
 - pH shifts
 - CO₂ gradients
 - O₂ gradients
 - Nutrient depletion
 - Contamination of non-target species



Measuring the Environment

- Monitoring and Control in Growing Systems
 - Monitor variables important to growth
 - Control variables within range



Measuring the Environment

- Controlling environment
 - Easier in Bioreactors
 - Hard in Open Ponds



Measuring the Environment

Optimal Growth

- Provide optimal growing condition
 - pH
 - Salinity
 - Nutrients
 - temperature

Compound Production

- Stressful condition
 - Limited nutrient
 - High salt
 - Etc

Lab Exercises

- Using a Spectrophotometer
 - Measuring optical density

Lab Exercises

- Algae Culturing
 - Sterile/Unialgal conditions
 - Making media
 - Transferring cultures



Algal Biology – Experimentation

Lecture hours required: 0.75 hours

Lab hours required: 4.25 hours

Lecture

The scientific method is key to developing and answering questions in a reliable way. In this lab our goal is to give students an introduction to the scientific method and hypothesis testing via experimentation. It is difficult to know at what level the students are working so be prepared to work on many different levels. This lecture is short to allow time for students to develop and set up an experiment in the lab.

Laboratory Exercises

The main goals of today's lab are to use skills previously learned in the lab to design and carry out an experiment. Students will keep detailed notes on experimental design, experimental setup, data collection and data analysis. It is most important that students develop a strong familiarity with the process. It is likely this is something new for them. Students can choose from suggested experiments or design one of their own. The experiment they set up today will be used in the next class to collect data. The data will be presented to the class in the form of graphs.

Lecture Outline

- Experimentation
 - What is an experiment?
 - Method of testing a hypothesis
 - Compare the effects of treatments
 - Repeatable
- Scientific Method
 - Make Observations
 - Form Hypothesis
 - Test Hypothesis – Experiments
 - Expand Idea
 - More Experiments
 - Interpret results
 - Conclusions
- Testing a Hypothesis
 - Statistical significance
 - Replication of treatments
- Lab Exercises
 - Design and setup an experiment

Lab Procedure IX

Design and Setup of Experiment and Data Collection

Introduction

Today's lab exercise will deal with designing and setting up an experiment using algae. So far we have covered factors that affect algal growth and how we can quantify both growth and the environmental factors that may be controlling it. Below are some examples of how students might conduct their experiment. Students should keep detailed records in their lab notebook about their ideas, how they are carrying out their experiment, how to operate equipment and other important details of their work. If they have time they can set up more than one experiment. After designing the experiment, they should set it up and begin collecting data. The instructor will work with them.

Procedures

Potential factors for experimentation

Light

We know that most algae require light to survive. Some algae may be adapted to high light and some to low light. Design an experiment that tests an algae's growth response to varied light conditions.

pH

pH may well be one of the most important environmental factors affecting algal growth. pH can affect many components of water chemistry including the availability of nutrients to algal cells. Design an experiment that shows how algae respond to changes in pH.

Temperature

Many algae exhibit a seasonal preference. While some of this response may be attributed to day length, temperature can have a significant effect. Temperature can increase metabolism and hence growth. However temperatures that are too high or too low can be inhibitory. Students should design an experiment that demonstrates a response to temperature by a strain of algae.

Nutrients

In natural environments, key nutrients are often limiting for algae. In particular, key limiting nutrients are Nitrogen and Phosphorus. In areas that are polluted, algal blooms often occur in response to *increased* nutrients. However, different species will respond to changes in nutrient levels in specific ways. Increasing nutrients is not always good for growth. Students should design an experiment that shows how changes in nutrient levels can affect the growth of a strain of algae.

Powerpoint Presentation Slides:

Class IV: Algal Biology – Experimentation

Introduction to Algal Biofuel

Class Outline

- Lecture
 - Experimentation
 - Scientific Method
 - Testing a Hypothesis
- Lab Exercises
 - Experimental Design
 - Experimental Implementation

Experimentation



- What is an experiment?
 - Method of testing a hypothesis
 - Compare the effects of treatments
 - Repeatable

Experimentation



- Scientific Method
 - Make Observations
 - Form Hypothesis
 - Test Hypothesis – Experiments
 - Expand Idea
 - More Experiments
 - Interpret Results
 - Conclusions

Experimentation

- Testing a Hypothesis
 - Statistical significance
 - Replication of treatments



Lab Exercises

- Experimental Design
- Experimental Implementation





Large Scale Algal Biomass Production

Lecture hours required: 0.75 hours

Lab hours required: 4.25 hours

Lecture

Today's lecture will cover large scale production systems: open pond vs. bioreactors. We will compare and contrast these two growing systems. Students should understand that both have their advantages. One may work better than the other in certain situations. Both systems or a combination of systems (hybrid) will certainly be used in the future for algal biomass production.



Laboratory Exercises

Students will continue work on their experiments, practicing methods and collecting data. We will also provide two demonstrations: the operation of the the **New Solutions Energy bioreactor**, and the harvest of algal biomass using a **centrifuge**.

Lecture Outline

- Mass Production of Algae
 - Comparable Systems
 - Spirulina farming
 - Agriculture
 - None identical
 - Considerations
 - Cost of system / operation
 - Type of algae grown
 - Ease of operation / maintenance
- Production Systems
 - Closed
 - Advantages
 - High Production
 - Little water / CO₂ loss
 - Less contamination
 - More controlled conditions
 - Disadvantages
 - Less gas exchange
 - Biofilms
 - Cost
 - Open
 - Advantages
 - Cost
 - Simple / easy to operate
 - Disadvantages
 - Varying conditions
 - Contamination
 - Water loss
 - Light limitation
- Laboratory Exercises
 - Demonstration of NSE Bioreactor
 - Describe
 - Biomass Harvest Demonstration
 - Centrifugation
 - Continued Experimentation
 - Collect data
 - Analyze data

Powerpoint Presentation Slides:

Class IV: Large-Scale Algal Biomass Production

Introduction to Algal Biofuel

Class Outline

- Lecture
 - Mass production of algae
 - Production Systems
- Lab Exercises
 - Demonstration of NSE Bioreactor
 - Biomass Harvest Demonstration
 - Continued Experimentation

Algae Production

- Mass Production
 - Comparable Systems
 - Spirulina farming
 - Agriculture
 - None identical



Algae Production

- Considerations
 - Cost of system/operation
 - Type of algae grown
 - Ease of operation/maintenance



Algae Production

- Production Systems

- Closed

- Advantages
 - High production
 - Little Water/CO₂ loss
 - Less contamination
 - More controlled conditions
 - Disadvantages
 - Less gas exchange
 - Biofilms
 - Cost



Algae Production

- Production Systems

- Open

- Advantages
 - Cost
 - Simple/easy to operate
 - Disadvantages
 - Varying conditions
 - Contamination
 - Water loss
 - Light limitation

Lab Exercises

- Demonstration of NSE Bioreactor
 - describe



Lab Exercises



- Biomass Harvest Demonstration
 - centrifugation

Lab Exercises

- Continued Experimentation
 - Collect Data
 - Analyze Data



Data Analysis – Presentation of Findings – Course Conclusions

Lecture hours required: 0 hours

Lab hours required: 5 hours

Laboratory Exercise

Today's class has two goals; helping students compile their data and perform appropriate analyses, and helping students synthesize conclusions about their experiments so that they can present findings to the class.

Spend no more than half of the period on preparing the student reports. This most likely will require using a computer lab for students to work with their data. Most students will not have experience using Excel, but for the purposes of this class and the data collected, the methods can easily be learned.

Student presentations need to consist of the following elements:

- Description of experiment
 - Goals
 - Design
 - Data Collection
- Presentation of findings
 - Graphs
 - Tables
 - Interpretations
 - Recommendations

Class time will also be needed for cleaning up experiments and putting away equipment.

End the class with a summary of the course and discussion with the students about things they enjoyed and how they might like the class to change.

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This workforce solution was funded by a grant awarded by the U.S. Department of Labor's Employment and Training Administration. The solution was created by the grantee and does not necessarily reflect the official position of the U.S. Department of Labor. The Department of Labor makes no guarantees, warranties, or assurances of any kind, express or implied, with respect to such information, including any information on linked sites and including, but not limited to, accuracy of the information or its completeness, timeliness, usefulness, adequacy, continued availability, or ownership. This solution is copyrighted by the institution that created it. Internal use by an organization and/or personal use by an individual for non-commercial purposes is permissible. All other uses require the prior authorization of the copyright owner.