Primary Production and Energy Conversion to Biomass

Introduction

One of the ways that solar energy can be utilized is by conversion into biomass. The group of organisms capable of conducting this conversion are called **primary producers** and the process that is utilized to conduct this conversion is **photosynthesis**. Primary producers include all plants and algae and some bacteria. The generalized chemical equation for photosynthesis is:

$$CO_2 + H_2O \xrightarrow{\text{sunlight}} CH_2O + O_2$$

This is the way solar energy is made available to food webs in nature. It is also possible to utilize this process to create **biomass** which can be used for a variety of purposes by human society. For example, all of our food is composed of energy originally captured from sunlight by the process of photosynthesis. Either plants are consumed directly by humans or we feed them to animals who, in turn, provide us with food.

In this lab we will look at the creation of biomass by single-celled primary producers called **algae** as a function of light intensity. Since the light intensity is an indication of the total amount of solar radiation reaching the algae and the final biomass gives us a measure of the total energy incorporated into biomass by photosynthesis, we can calculate the efficiency of energy capture by photosynthesis.

This lab will also give us a first look at the base of the food web in ecosystems which will be explored more thoroughly later in the course. We will allow the microalgal populations to develop over a period of one week and then measure the algal growth which has resulted.

Objectives

- Understand the process of photosynthesis and its role in primary production
- Understand the role of light intensity in primary productivity

Hypothesis

Primary productivity, as indicated by algal growth, will vary with light intensity.

Materials

- 125mL flasks
- cotton
- gauze
- permanent markers

- culture medium
- algae innoculum
- centrifuge
- centrifuge tubes
- Spec 20 spectrophotometer
- Cuvettes
- Glass fiber filter
- Filtration apparatus
- Funnel
- Hand pump
- Mortar and pestle
- Methanol
- Balance
- 10mL graduated cylinders
- acetone
- glass pipettes
- distilled water
- tissue wipes
- tube racks
- plastic bag
- beaker
- wash bottle

Procedure

Week One

1. Each lab table will work as a group.
2. Obtain two 125mL flasks and use the permanent markers to label them as follows:
   a. mark all flasks with your lab day and time and your group
   b. each of the two flasks will receive a different treatment and should be labeled as follows:
      i. "Con-Low" (control with no algae added, low light intensity) (lab table 1 & 3)
      ii. "Con-Med" (control with no algae added, medium light intensity) (lab table 2 & 4)
      iii. "Con-High" (control with no algae added, high light intensity) (lab table 5 and 6)
      iv. "Alg-Low" (algae innoculum added, low light intensity) (lab table 1 & 3)
      v. "Alg-Med" (algae innoculum added, medium light intensity) (lab table 2 & 4)
      vi. "Alg-High" (algae innoculum added, high light intensity) (lab table 5 & 6)
3. Add 40mL of culture medium to each flask.
4. Place 5 drops of algal innoculum into each treatment flask (Alg-Low, Alg-Med, Alg-High). **Do not add algal innoculum to the control flasks** (Con-Low, Con-Med, Con-High).
5. Insert into the mouth of each flask a plug made by wrapping a cotton ball in a square piece of gauze (app. 8cm x 8cm).

6. Place the appropriate flasks in the appropriate location based on light intensity as directed by your instructor. The flasks will remain in these locations over the course of next week.

Week Two

1. Determine **turbidity** of each flask as follows:
   a. Calibrate and zero the Spec 20 using distilled water
      i. fill a cuvette ¾ full with distilled water
      ii. wipe exterior of cuvette dry using a tissue wipe
      iii. place the cuvette in the Spec 20
      iv. set the wavelength at 450nm
      v. read the absorbance of the distilled water from the absorbance scale (which reads from right to left)
      vi. calibrate to zero
   b. determine the turbidity of each treatment flask as follows:
      i. thoroughly mix the contents of the flask by swirling the flask for approximately 10 seconds
      ii. pour the contents of the flask into a clean cuvette until it is ¾ full with the sample
      iii. wipe exterior of cuvette dry using a tissue wipe
      iv. place the cuvette in the Spec 20
      v. set the wavelength at 450nm
      vi. read the absorbance of the sample from the absorbance scale (which reads from right to left)
      vii. record data in Table 1
      viii. **important** - return the contents of the cuvette back to the sample flask from which it came
      ix. rinse the cuvette with distilled water
      x. repeat steps I through vii until the turbidity of all samples has been determined
2. Determine the **chlorophyll concentration** of each flask as follows:
   a. Place a clean glass fiber filter on the center filtration apparatus and secure the funnel.

b. Pour the contents of a flask through the filter and apply suction with the hand pump to pull all of the water through the filter.

c. Remove the filter from the filter funnel and place it in a mortar and pestle.

d. Add 10mL of methanol to the filter in the mortar and grind the filter by rubbing it between the mortar and pestle.

e. Pour the ground suspension out of the mortar into a centrifuge tube labeled with the appropriate sample information. Sparingly rinse any remaining content of the mortar into the centrifuge tube using a wash bottle containing methanol. Place the centrifuge tube into a rack to await centrifuging.

f. After all flasks have been processed, centrifuge the tubes at setting #4 for a period of 5 minutes to separate the filter material from the methanol extract. Your instructor will demonstrate and help with this step as care needs to be taken to ensure that the tubes in the centrifuge are properly balanced.

g. Calibrate and zero the Spec 20 using methanol as follows:
   i. fill a cuvette ¾ full with methanol
   ii. wipe exterior of cuvette dry using a tissue wipe
   iii. place the cuvette in the Spec 20
   iv. set the wavelength at **665nm**
   v. read the absorbance of the methanol from the absorbance scale (which reads from right to left)
   vii. calibrate to zero

h. Determine the chlorophyll content of each sample as follows:
   i. Decant the green solution from the centrifuge tube into a clean, properly labeled 10mL graduated cylinder. Use a disposable pipette to add methanol to bring volume in cylinder up to 10mL.
   ii. Use the same disposable pipette used in step h, above, to agitate the contents of the graduated cylinder and then immediately

pour the contents of the cylinder into a clean cuvette until the cuvette is ¾ full.

iii. wipe exterior of cuvette dry using a tissue wipe

iv. place the cuvette in the Spec 20

v. set the wavelength at **665nm**

vi. read the absorbance of the sample from the absorbance scale (which reads from right to left)

viii. record data in Table 2

ix. repeat steps 2.h.i – viii until all samples have been processed.

Data Analysis

1. Record your group's data in the appropriate column of Table 1 and 2 and on the overhead transparency (or blackboard).
2. Copy all other groups' data from the overhead transparency or blackboard to your Table 1 and Table 2.
3. Prepare (using Excel or by hand) and attach the following bar graphs:
   a. Graph 1 will compare the mean (across all groups) turbidity (based on absorbance at 450nm) for the control versus the algal treatment at low, medium and high light intensity
   b. Graph 2 will compare the mean (across all groups) chlorophyll content (based on absorbance at 665nm) for the control versus the algal treatment at low, medium and high light intensity

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Primary Production LAB WRITE-UP: Submit pages 7-10

Student Name: _____ Lab Date: _____

Lab Instructor: _____ Lab Section: _____

Results (Data)

Table 1. Turbidity (absorbance at 450nm) of control versus algal treatment incubated at low, medium and high light intensity for all groups.

	Turbidity (Absorbance at 450nm)					
Group	Con-Low	Con-Med	Con-High	Alg-Low	Alg-Med	Alg-Med
1						
2						
3						
4						
5						
6						
Mean						

Table 2. Chlorophyll content (absorbance at 665nm) of control versus algal treatment incubated at low, medium and high light intensity for all groups.

	Chlorophyll Content (Absorbance at 665nm)					
Group	Con-Low	Con-Med	Con-High	Alg-Low	Alg-Med	Alg-Med
1						
2						
3						
4						
5						
6						
Mean						

Graph 1. Mean turbidity (absorbance at 450nm) across all groups for control versus algal treatment incubated at low, medium and high light intensity.

Attach graph

Graph 2. Mean chlorophyll content (absorbance at 665nm) across all groups for control versus algal treatment incubated at low, medium and high light intensity.

Attach graph

Conclusions (Questions): *For full credit, these questions should be answered thoroughly, in complete sentences, in legible handwriting*.

1. Describe the results of the turbidity analysis. How did turbidity vary between the control and the algal treatment and between the various light intensities? Were these results consistent with your expectations? If not, provide a possible reason for the inconsistency.

2. Were the results of the turbidity analysis consistent among the groups? If not, provide an explanation for any observed differences.

3. Describe the results of the chlorophyll content analysis. How did chlorophyll content vary between the control and the algal treatment and between the various light intensities? Were these results consistent with your expectations? If not, provide a possible reason for the inconsistency.

4. Were the results of the chlorophyll content analysis consistent among the groups? If not, provide an explanation for any observed differences.