Just a Drop in the Ocean?

A research investigation into the capacity of the marine micro-algae *Dunaliella tertiolecta* to sequester atmospheric carbon dioxide.

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1.0 Abstract

A research investigation was undertaken to estimate the capacity of micro-algae to sequester atmospheric CO_2. The algae species used, *Dunaliella tertiolecta*, is a green flagellate with a cell size of 10 – 12 µm. It was selected because it is a robust species that was likely to withstand experimental conditions in a laboratory and provide data, from which calculations could be based.

It was decided to carry out a growth experiment where flasks containing 95 mL of seawater were seeded with 5 mL of sample culture, enriched with nutrients to promote cell growth (F_2 nutrient), and left to grow for 5, 10, 15 and 20 days. When the growth periods had passed, flasks were removed from incubation and algal growth in the particular time-span, was calculated.
At the same time, a series of flasks which were carbonated with excess CO_2 were then set to incubate and were removed for analysis simultaneous with the uncarbonated samples.

There were two methods employed for the estimation of algal growth. Firstly, dry mass of algae was found by filtering the algae from the liquid medium in the growth flasks and drying them out in an oven. By subtracting the mass of each dry filter paper from its mass with dry algae, the dry mass of the algae was found and expressed in grammes
Secondly, algal growth was determined by cell count estimations using a disposable version of a haemacytometer (called a KOVA® GLASSTIC® SLIDE). When a 6.6µL portion from an incubation flask was applied to the slide, capillary action caused the fluid to be drawn into the 10 chambers, resulting in a homogenous suspension of sediment. (See: KOVA® GLASSTIC® SLIDE instructions in appendix 5). By viewing under a microscope the grids were brought into focus and the cells within one square were counted for each of the 10 grids and then averaged to get a count per grid. This average was multiplied by 90 (instruction for un-centrifuged samples), multiplied by 1000 to convert to cells per mL and multiplied by 100 to convert to cells per 100 mL (the volume of the incubation medium).

It was found that algal dry mass increased with days of incubation for both the uncarbonated and carbonated samples. However, carbonated samples achieved a greater overall gain in dry mass over 20 days (0.334 g versus 0.314 g). Algae cultures incubated without F_2 nutrient for the same time-span actually experienced a dry mass drop, indicating that nutrient enrichment of seawater is essential to promote algal growth.

A scale-up of these results demonstrates that if an algal bio-reactor were designed with a capacity of 20,000,000 L (100m x 100m x 2m), then the difference in sequestering capacity between uncarbonated and carbonated systems would amount to approximately 1.60 tonnes of atmospheric CO_2 over 20 days.

The CO_2 enrichment for this system could come from the trapping of industrial CO_2 otherwise destined to enter the atmosphere. The algae produced could be collected and used for the production of bio-diesel. Since this plant bio-mass would be created in a marine environment it would minimise the land space needed to grow crops for alternate fuel production and also eliminate the need for freshwater. Subsequently it is recommended that algal bio-reactors be employed in Australian waters as a means of curbing global warming.

2.0 Introduction

I decided to undertake an investigation into the capacity of micro-algae to sequester atmospheric CO_2 as a follow-up to a previous study I made into the capacity of Tasmanian hardwoods to fulfill the same task and therefore act as a carbon sink.

My interest in this topic stems from the high level of media attention that is being directed toward the increase in atmospheric CO_2 and its role in global warming. I have read that the global atmospheric concentration of CO_2 has increased from a pre-industrial value of about 280 ppm to 379 ppm in 2005. (IPCC Working Group1Report: Ref 7). The atmospheric concentration of CO_2 in 2005 was far greater than the natural range over the last 650,000 years (180 to 300 ppm), as has been found from ice core measurements. The report also states that the annual CO_2 concentration growth rate was larger during the last10 years (1995–2005 average: 1.9 ppm per year), than it has been since the beginning of continuous direct atmospheric measurements. The primary source of the increased atmospheric concentration of CO_2 since the pre-industrial period is stated to be the result fossil fuel usage.

A second IPCC report (Working Group 11: Ref 8) looks at the likely impacts for Australia. It suggests that, as a result of reduced precipitation and increased evaporation, water security problems are projected to intensify by 2030 in southern and eastern Australia. Significant loss of biodiversity, risks from sea-level rises and increases in the severity and frequency of storms and coastal flooding, are all predicted.

It is clear that reductions must be made in our usage of fossil fuels, but since it is thought that we have passed “peak oil” production I can see that there is a need for immediate development of clean alternative energy sources.

I recently read an article entitled “Biofuel production may raise the price of food” (New Scientist, May 2007: Ref 9) which includes a warning from the United Nations that the growth of biofuel crops could divert land, water and other resources away from food production at a time of rising population and critical pressures on the land.
Another article, “Earth suffers as we gobble up resources” (New Scientist, July 2007: Ref 2) suggests that the earth can just about cope (with food production) if we produce it more efficiently, but we are asking for trouble if we expand production of biofuels, as the only fertile land available, at this point, is tropical rainforests.

This made me wonder if it would be possible to produce plant bio-mass for conversion to bio-diesel without the use of either freshwater or land surface area. To achieve this, the crop would have to be grown off shore, in saltwater and marine micro-algae looked like an ideal choice of crop.

Another article "Biofuel made from power plant CO_2" (New Scientist, Oct 2006: Ref 3) suggested that trapping industrial emissions of CO_2 and using them to produce bio-diesel could take the CO_2 'from the smokestack to the gas tank' before it enters the atmosphere.
This prompted me to design a bio-reactor and to calculate the potential for CO_2 sequestration in both uncarbonated and (industrially sourced) CO_2 enriched seawater.

My first task was to gain some knowledge on micro-algae, so my investigation started with a trip to the CSIRO Laboratories in Hobart where I met with Cathy Johnston and Ian Jameson and we discussed suitable micro-algae species for study. We decided upon *Dunaliella tertiolecta*, a green flagellate, as it had the reputation of being a robust species which might withstand the laboratory procedures that I would need to undertake.

I started my practical work by undertaking a preliminary trial to see if the micro-algae would grow outside in direct sunlight and variable daytime temperatures instead of at constant temperature and using artificial light, as is the case at CSIRO. I grew the micro-algae for 20 days and removed samples at 5 day intervals for dry mass measurement (see Raw Data in App 1). I found that the micro-algae grew well over this time period and that this species could withstand daily temperature fluctuations from 5^{o}C to 24^{o}C. As a result of these findings I decided to proceed with the investigation.

3.0 Hypotheses

3.1 Hypothesis 1

If the dissolved oxygen content of sea water is compared with the dissolved oxygen content of carbonated sea water, then it will be found that carbonated sea water will have less dissolved oxygen.

3.2 Hypothesis 2

If both un-carbonated and carbonated sea water are incubated at 4°C for 5 days in dark conditions and the bio-chemical oxygen demand of both is estimated, then it will be found that they both have a bio-chemical oxygen demand, indicating the presence of bacteria in the water.

3.3 Hypothesis 3

If the marine micro algae, *Dunaliella Tertiolecta,* are introduced to growth flasks containing un-carbonated sea water and F2 nutrient and the dry mass estimated after 0, 5, 10, 15 and 20 days, then it will be found that the algae bio-mass increases with incubation time.

3.4 Hypothesis 4

If the marine micro algae, *Dunaliella Tertiolecta,* are introduced to growth flasks containing un-carbonated sea water and F2 nutrient and a cell count is undertaken after 0, 5, 10, 15 and 20 days, then it will be found that the cell density increases with incubation time.

3.5 Hypothesis 5

If the marine micro algae, *Dunaliella Tertiolecta,* are introduced to growth flasks containing carbonated sea water and F2 nutrient, then it will be found that both the algae bio-mass and cell density will be greater than for the flasks containing un-carbonated sea water.

3.6 Hypothesis 6

If the marine micro algae, *Dunaliella Tertiolecta,* are introduced to growth flasks with and without F2 nutrient, then it will be found that both algae bio-mass and cell density will be lower where no F2 nutrient is present.

3.7 Hypothesis 7

If the dry mass gain for algae in uncarbonated and carbonated media is calculated then it is possible to scale-up the findings and estimate the difference in sequestering capacity between uncarbonated and carbonated seawater in a bio-reactor.

4.0 Materials and Methods

4.1 Estimation of the Salt Content in Sea Water

Apparatus:

- Freshly collected Sea Water
- Permanent Marker
- Evaporating Basins
- Electronic Balance
- Drying Oven

Method:

1. Select 4 evaporating basins and label as A, B, C and D.
2. Using electronic balance, find the mass of each basin.
3. Record basin mass measurements.
4. Place basins in pre-heated oven (120 ^{o}C) and leave until all water has evaporated.
5. Measure mass of dry basin and salt residue.
6. Repeat measurements until no further drop in mass is found.
7. Subtract mass of empty basin from basin + salt residue to find mass of salt
8. Calculate percentage salt in sea water sample

Collection of sea water.

Finding Mass of Salt.

4.2 Estimation of the Dissolved Oxygen Content in Sea Water.

Apparatus:

- Freshly collected Sea Water
- 2 x 100mL Volumetric Flasks
- 3 x 250mL Conical Flasks
- 1 x 1L Volumetric Flask
- 50mL Burette
- Retort Stand with Clamp
- White Tile
- 3 x 2mL pipettes
- 250mL Measuring Cylinder
- Latex Gloves
- Protective glasses
- 2.2 mol/L Manganese Sulfate
- Alkaline Iodine Reagent
- 0.025 mol/L Sodium Thiosulfate
- 1% Starch Solution
- 40% Sulfuric Acid

Method 1: Making solutions.

1. Weigh 49.072 grams of $MnSO_4.4H_2O$ and dissolve in 100mL de-ionised water in a 100mL volumetric flask to make a 2.2 mol./L solution.
2. Dissolve 14.94 grams of KI and 70.136 grams of KOH in 100mL of de-ionised water in a 100mL volumetric flask to make alkaline iodine reagent.
3. Prepare 1L of 0.025mol./L sodium thiosulfate solution by dissolving 6.204 grams $Na_2S_2O_3$ in 1L of de-ionised water in a 1L volumetric flask.
4. Prepare 125mL of 1% starch solution by making a paste of 1.25 grams of soluble starch with a little cold water and by pouring this paste, with constant stirring, into 250mL of boiling water. After boiling for 1 min. cool the solution and add 2.5 grams of KI.

Method 2: Fixing water sample and titration to find D.O.

1. Carefully fill a 250mL conical flask (one with a stopper to fit) to nearly full with sea water. Pour the water down the side of the flask in order to minimise bubbles. Leave a space at the top that will fit about 5mL of reagents.
2. Using a 2 mL pipette, quickly add 2.0 mL manganese sulfate solution and 2.0 mL of alkaline-potassium iodide reagent.
3. Watch for a precipitate of manganese (II) hydroxide to form. Stopper the flask and invert several times to mix and bring about the fixing of dissolved oxygen.
4. Allow the precipitate to settle so that about 100 mL of clear solution is produced.
5. Add 2.0 mL of concentrated sulfuric acid with a 2 mL pipette allowing the acid to run down the neck of the flask. Stopper the flask and invert several times until all the precipitate re-dissolves.
6. Using a 250 mL measuring cylinder, take 203 mL of fixed sea water from the flask and transfer to a 250 mL conical flask. (The 203 mL allows for the addition of the other reagents and it is equivalent to 200 mL of the original water sample). It does not matter that the solution is now open to the air because the dissolved oxygen is fixed.

7. Using sodium thiosulfate, fill the 50 mL burette. Titrate with the thiosulfate to a pale straw yellow colour.
8. Add about 0.5 mL of starch indicator. (The starch reacts with iodine to give a deep blue colour). Continue the titration until the first disappearance of blue colour.
9. Record the volume of sodium thiosulfate used.
10. Repeat (steps 1-9) for three titres.
11. Use the average titre to calculate the dissolved oxygen in the sample (See App. 3)

Finding dissolved oxygen in sea water.

Titrating to find dissolved oxygen.

4.3 Estimation of the Dissolved Oxygen Content in Sea Water after carbonation.

Apparatus:

- As for Experiment 4.2, but including:
- 50mL 2M HCl
- 100 gms $CaCO_3$
- 2L freshly collected sea water
- pH meter

Method 1: Carbonation of sea water.

1. Using pH meter find and record pH of filtered sea water sample
2 Place 100 grams $CaCO_3$(s) in a buschner flask (conical flask with side arm).
3. Insert outlet tube into a container with 2L freshly collected sea water
4. Gradually add 50ml 2M HCl
5. Observe as the following reaction takes place:
   $2\ HCl_{(aq)} + CaCO_{3(s)} \rightarrow CaCl_{2\ (s)} + H_2O_{\ (aq)} + CO_{2\ (g)}$
6. Keep outlet tube inserted into sea water until $CO_{2\ (g)}$ production has ceased.
7. Remove outlet tube, cap the bottle and invert several times to distribute $CO_{2\ (g)}$ throughout the sea water.
8. Find and record pH of carbonated sea water

Method 2: Estimation of Dissolved Oxygen of the Carbonated Water.

Repeat procedure as in Exp 4.2: Method 2: Fixing water sample and titration to find D.O.

Calibrating pH meter.

Carbonating sea water.

4.4 Estimation of the Biochemical Oxygen Demand of sea water as a means of confirming the presence of bacteria in marine environments.

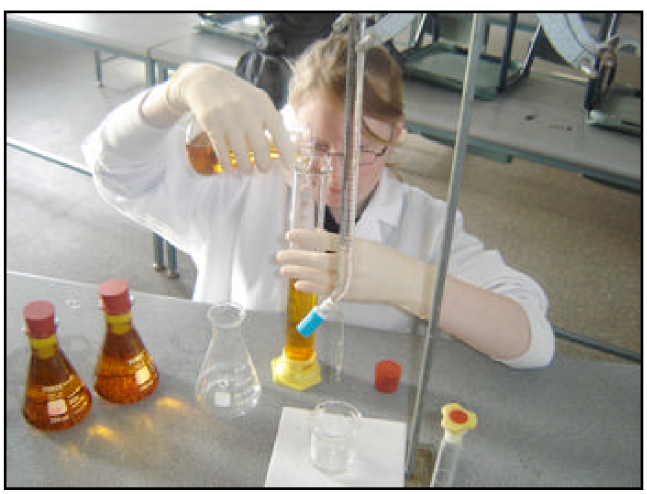
Theory: To confirm the presence of bacteria the Dissolved Oxygen (D.O.) in a water sample is found soon after sampling and then an enclosed water sample from the same site is placed in a fridge at 4-5 degrees Celcius, in a black plastic bag, for 5 days. It is assumed that any bacteria present will use up some of the dissolved oxygen, so a second D.O. analysis taken after 5 days will show a drop in oxygen level, confirming that aerobic bacteria were present. The black plastic prevents microscopic algae from photosynthesizing and increasing the D.O. (Source: Waterwatch Tasmania Field Guide)

Apparatus:

As for Experiment 4.2
Fridge (to incubate samples for 5 days)
Black plastic bag

Method:

1. Collect 2L of sea water using standard procedures (Waterwatch Tasmania Field Guide)
2. Cover the bottle with black plastic at the time of collection
3. Place the water in a fridge at 4 -5 ^{0}C for 5 days
4. When incubation period has passed remove water from fridge and carry out Dissolved Oxygen test as described in Experiment 4.2
5. Compare D.O. after 5 days with that found at time of collection, to find the Biochemical Oxygen Demand



Titrating to find Biochemical Oxygen Demand of sea water.

4.5 Growth of the micro-algae *Dunaliella tertiolecta* in sea water media

Apparatus:

- 250 mL conical flasks
- 100 mL measuring cylinder
- Filtered sea water
- *Dunaliella tertiolecta* culture
- F_2 nutrient media
- 5 mL and 1 mL pipettes
- Cotton wool
- Aluminium foil

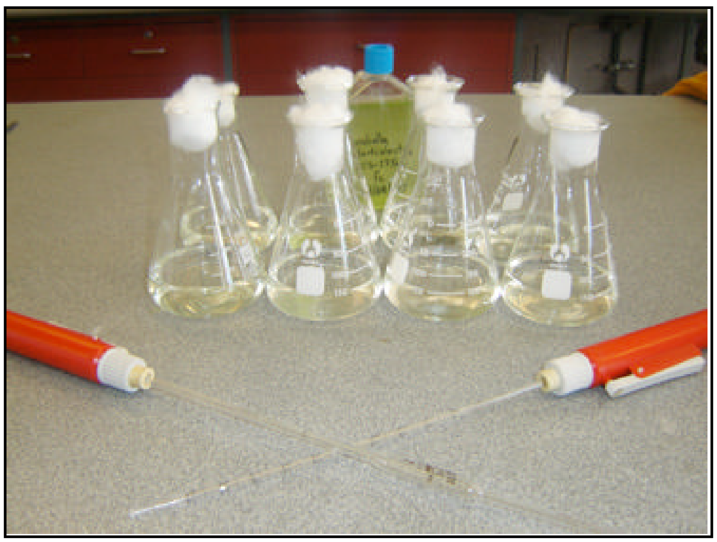
Method:

1. Thoroughly wash and sterilise conical flasks, pipettes and measuring cylinders.
2. Place 95 mL of filtered sea water in each conical flask
3. Add 5 mL of *Dunaliella tertiolecta* culture to each flask
4. Add 1 mL of F_2 nutrient medium to each flask
5. Place a loose plug of cotton wool in the neck of each flask
6. Cover each flask with a small square of aluminium foil and perforate the foil with small air-holes
7. Leave flasks out-of-doors in open sunlight for required incubation period (5, 10, 15, 20 days). Place in clear Perspex trays containing water to a depth of 2 cm (to minimize overheating/overcooling) and place a thermometer in each tray to monitor daytime fluctuations in temperature.
8. When required growth period has passed, remove flasks from incubation trays and test for:
   (a) Algae growth (by dry mass measurement)
   (b) Algae density (by cell count, using haemocytometer).

Filtering sea water.

Adding algae to flasks.

Stock solution and F2 nutrients.



Flasks seeded with algae and F2 nutrients.

Measuring F2 nutrients.

4.6 Estimation of growth rates in *Dunaliella tertiolecta* by dry mass measurements

Theory:

When the required number of days of algae growth has passed the flasks from the incubation trays need to be filtered and the dry mass of algae cells found. Selection of appropriate filter paper is critical, as, if the paper is not of fine enough gauge, the cells can pass through and escape into the filtrate. Although the approximate size of *Dunaliella tertiolecta* (L x D) is given as 10 x 6.5 micro metres (O'Meley and Daintith, 1992) it is expected that the cell size will vary with the growth phase, the physiological state of the culture and whether the cells are prior to or after cell division.
So, it was thought best to filter each flask 3 times: firstly with coarse grade filter paper (to remove the bigger cells) and then twice more with fine grade filter paper. Seeing as the filtrate appeared clear after 2 fine filtrations, it was considered that all algae cells had been trapped by the paper and could undergo the drying process.

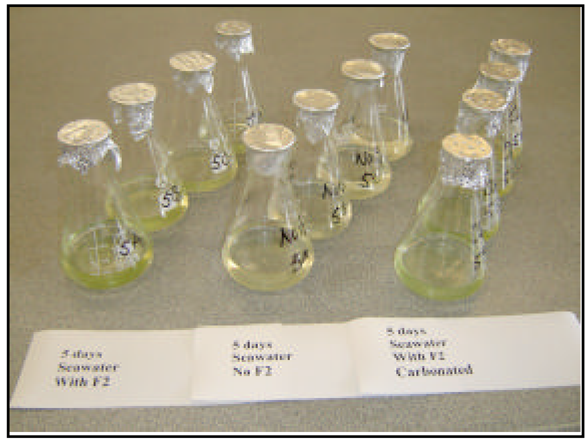
Apparatus:

Growing samples of algae after 5, 10, 15, 20 days
Filter funnels
Conical flasks
Whatmann paper (grades #2 (crude) and #42 (fine, ashless))
Drying oven
Electronic balance (to 3 d.p. accuracy)

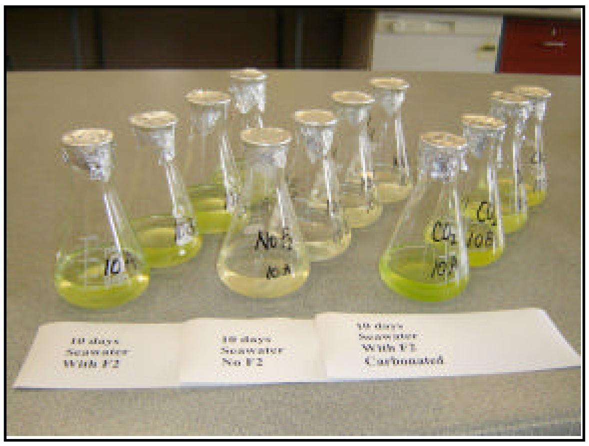
Method:

1. When the required number of days of algae growth has passed, remove the flasks from incubation trays and filter
2. Clearly mark (in pencil) on the filter papers the identity of each algae sample (days of growth, nutrient added and carbonation status)
3. Place filter papers in a pre-heated oven (105 ^{0}C) to remove any moisture. Record the actual dry mass of each paper as that found when no further drop in mass occurred
3. Filter each flask firstly with the large, coarse grade filter paper and then twice more with small, fine grade paper. At this point the filtrate should be clear
4. Place all filter papers in a pre-heated oven (105 ^{0}C) to dry
5. Weigh and record dry mass until no further drop in mass is found
6. Subtract the mass of the dry filter paper from that of the filter paper + algae to find the algae dry mass for all samples

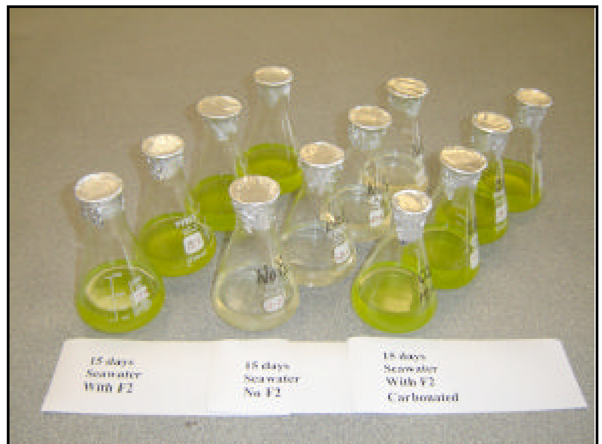
Algae flasks at 0 days.



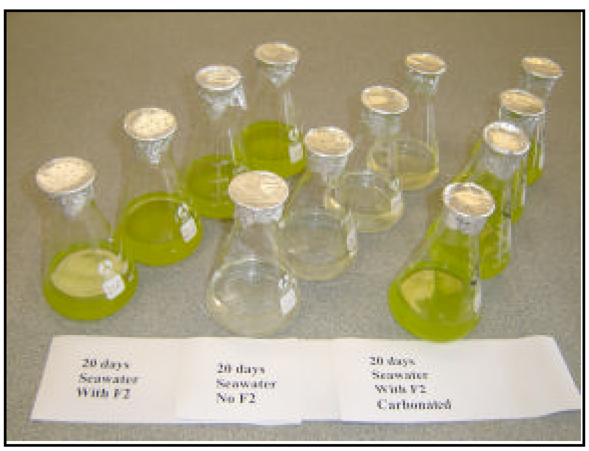
Algae flasks at 5 days.



Algae flasks at 10 days.



Algae flasks at 15 days.



Algae flasks at 20 days.

Filter papers drying in oven.

4.7 Estimation of growth rates in *Dunaliella tertiolecta* by cell count measurements

Theory:

Micro-algae cultures are known to go through four growth phases (O'Meley and Daintith, 1992). These are: Lag Phase (slow growth while acclimatising to new growth medium), Log Phase (exponential growth as cells divide and take up nutrients rapidly), Stationary Phase (rate of growth equals rate of death as nutrients, or some other factor, becomes limiting) and Decline Phase (culture has "crashed" and dead cells accumulate at the bottom of flasks).
One means of estimating the rate of growth of algae is to count the number of cells that are present in a micro-liter of growth medium and then multiply up to estimate those present per 100mL of medium in each flask.
The apparatus used for cell count was a disposable haemacytometer, known as a KOVA® GLASSTIC® SLIDE (see APP 4). This plastic slide contains a quantitative grid and is designed to be used with a microscope to determine bodily cell counts (urinalysis, haematology and seminal (sperm) count, in pathology laboratories. Since micro-algae are of an appropriate size they can also be counted in this way.

Apparatus:

Growing samples of algae after 5, 10, 15, 20 days
Very fine capillary dropper
KOVA® GLASSTIC® SLIDE
Light microscope with lamp

Method:

1. When the required number of days of algae growth has passed, remove a flasks from the incubation tray. Swirl the flask to ensure even distribution of algae throughout and then lower the capillary dropper into the liquid medium, lifting approx. 0.10 mL of fluid
2. Transfer this fluid to the filling notch on the KOVA® slide chamber and watch as capillary action causes the sample to be drawn into each of the 10 chambers, resulting in a homogenous suspension of sediment in each
3. Examine under high power (10 x 40 MAG)
4. Count the cells within the lines of the small 0.33 mm^2 grid
5. Record the cell count / square and refer to the value table (APP 5) for the cell count per micro-liter of sample (note: for un-centrifuged (neat) samples, multiply the average cells per grid by 90
6. Multiply cells per micro-liter by 1000 to convert to cells/mL
7. Multiply cells per mL by 100 to convert to cells / 100mL flask
8. Record the cell count for each of the 100 mL flasks using appropriate headings

Undertaking a cell count

Haemacytometer slide under microscope.

Filling haemacytometer slide with algae sample.

5.0 Results

5.1 Table 1: Dissolved Oxygen Content in Sea Water.

Flask	mL 0.025M $Na_2S_2O_3$ (aq) used	D.O. in ppm (* See App 2)
1	[8.40]	
2	7.30	
3	7.10	
Ave	7.20	7.20 ppm

5.2 Table 2: Dissolved Oxygen Content in Carbonated Sea Water.

Flask	mL 0.025M $Na_2S_2O_3$ (aq) used	D.O. in ppm (* See App 2)
1	7.60	
2	7.60	
3	[7.20]	
Ave	7.60	7.60 ppm

5.3 Table 3: Dissolved Oxygen Content in Sea Water after 5 Days.

Flask	mL 0.025M $Na_2S_2O_3$ (aq) used	D.O. in ppm (* See App 2)
1	[2.90]	
2	3.60	
3	3.50	
Ave	3.55	3.55 ppm

5.4 Table 4: Dissolved Oxygen Content in Carbonated Sea Water after 5 Days.

Flask	mL 0.025M $Na_2S_2O_3$ (aq) used	D.O. in ppm (* See App 2)
1	[2.90]	
2	4.60	
3	4.40	
Ave	4.50	4.50 ppm

5.5 Table 5

Weight of Algae after 0 Days

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
17/9/07	17/9/07	0A1	2.049	2.214	0.165
17/9/07	17/9/07	0A2	0.569	0.622	0.053
17/9/07	17/9/07	0A3	0.566	0.614	0.048
				Total	**0.266**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
17/9/07	17/9/07	0B1	2.102	2.279	0.177
17/9/07	17/9/07	0B2	0.554	0.611	0.057
17/9/07	17/9/07	0B3	0.561	0.610	0.049
				Total	**0.283**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
17/9/07	17/9/07	0C1	2.124	2.308	0.184
17/9/07	17/9/07	0C2	0.548	0.606	0.058
17/9/07	17/9/07	0C3	0.566	0.612	0.046
				Total	**0.288**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
17/9/07	17/9/07	0D1	2.085	2.248	0.163
17/9/07	17/9/07	0D2	0.569	0.619	0.050
17/9/07	17/9/07	0D3	0.554	0.595	0.041
				Total	**0.254**

Flask	Total Algae Mass: 0 Days (g)
A	0.266
B	0.283
C	0.288
D	0.254
Total	**1.091**

5.6 Table 6

Weight of Algae after 5 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5A1	2.047	2.220	0.173
15/9/07	20/9/07	5A2	0.576	0.631	0.055
15/9/07	20/9/07	5A3	0.581	0.638	0.057
				Total	**0.285**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5B1	2.096	2.264	0.168
15/9/07	20/9/07	5B2	0.562	0.628	0.066
15/9/07	20/9/07	5B3	0.578	0.633	0.055
				Total	**0.289**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5C1	2.095	2.275	0.180
15/9/07	20/9/07	5C2	0.578	0.638	0.060
15/9/07	20/9/07	5C3	0.593	0.647	0.054
				Total	**0.294**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5D1	2.071	2.254	0.183
15/9/07	20/9/07	5D2	0.547	0.631	0.084
15/9/07	20/9/07	5D3	0.584	0.646	0.062
				Total	**0.329**

Flask	Total Algae Mass: 5 Days (g)
A	0.285
B	0.289
C	0.294
D	0.329
Total	**1.197**

5.7 Table 7

Weight of Algae after 5 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5A1	2.032	2.224	0.192
15/9/07	20/9/07	5A2	0.561	0.622	0.061
15/9/07	20/9/07	5A3	0.587	0.646	0.059
				Total	**0.312**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5B1	2.116	2.290	0.174
15/9/07	20/9/07	5B2	0.568	0.644	0.076
15/9/07	20/9/07	5B3	0.590	0.649	0.059
				Total	**0.309**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5C1	2.102	2.295	0.193
15/9/07	20/9/07	5C2	0.586	0.644	0.058
15/9/07	20/9/07	5C3	0.584	0.648	0.064
				Total	**0.315**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	5D1	2.022	2.225	0.203
15/9/07	20/9/07	5D2	0.581	0.655	0.074
15/9/07	20/9/07	5D3	0.569	0.633	0.064
				Total	**0.341**

Flask	Total Algae Mass: 5 Days (CO_2)
A	0.312
B	0.309
C	0.315
D	0.341
Total	**1.277**

5.8 Table 8

Weight of Algae after 10 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10A1	2.131	2.288	0.157
15/9/07	20/9/07	10A2	0.590	0.678	0.088
15/9/07	20/9/07	10A3	0.607	0.659	0.052
				Total	**0.297**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10B1	2.174	2.336	0.162
15/9/07	20/9/07	10B2	0.602	0.682	0.080
15/9/07	20/9/07	10B3	0.617	0.665	0.048
				Total	**0.290**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10C1	2.147	2.308	0.161
15/9/07	20/9/07	10C2	0.603	0.679	0.076
15/9/07	20/9/07	10C3	0.606	0.663	0.057
				Total	**0.294**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10D1	2.081	2.234	0.154
15/9/07	20/9/07	10D2	0.594	0.665	0.071
15/9/07	20/9/07	10D3	0.600	0.660	0.060
				Total	**0.285**

Flask	Total Algae Mass: 10 Days (g)
A	0.297
B	0.290
C	0.294
D	0.285
Total	**1.166**

5.9 Table 9

Weight of Algae after 10 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10A1	2.160	2.312	0.152
15/9/07	20/9/07	10A2	0.580	0.655	0.075
15/9/07	20/9/07	10A3	0.599	0.654	0.055
				Total	**0.282**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10B1	2.083	2.238	0.155
15/9/07	20/9/07	10B2	0.599	0.676	0.077
15/9/07	20/9/07	10B3	0.600	0.666	0.066
				Total	**0.298**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10C1	2.113	2.281	0.168
15/9/07	20/9/07	10C2	0.598	0.693	0.095
15/9/07	20/9/07	10C3	0.611	0.668	0.057
				Total	**0.320**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	10D1	2.176	2.336	0.160
15/9/07	20/9/07	10D2	0.597	0.699	0.102
15/9/07	20/9/07	10D3	0.606	0.667	0.061
				Total	**0.323**

Flask	Total Algae Mass: 10 Days (CO_2) (g)
A	0.282
B	0.298
C	0.320
D	0.323
Total	**1.223**

5.10 Table 10

Weight of Algae after 15 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15A1	2.075	2.279	0.204
15/9/07	20/9/07	15A2	0.591	0.651	0.060
15/9/07	20/9/07	15A3	0.594	0.645	0.051
				Total	**0.315**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15B1	2.104	2.272	0.168
15/9/07	20/9/07	15B2	0.585	0.658	0.073
15/9/07	20/9/07	15B3	0.595	0.664	0.069
				Total	**0.310**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15C1	2.021	2.202	0.181
15/9/07	20/9/07	15C2	0.585	0.655	0.070
15/9/07	20/9/07	15C3	0.606	0.669	0.063
				Total	**0.314**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15D1	2.075	2.269	0.194
15/9/07	20/9/07	15D2	0.589	0.638	0.049
15/9/07	20/9/07	15D3	0.601	0.686	0.086
				Total	**0.329**

Flask	Total Algae Mass: 15 Days (g)
A	0.315
B	0.310
C	0.314
D	0.329
Total	**1.268**

5.11 Table 11

Weight of Algae after 15 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15A1	2.075	2.245	0.170
15/9/07	20/9/07	15A2	0.580	0.637	0.057
15/9/07	20/9/07	15A3	0.600	0.724	0.124
				Total	**0.351**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15B1	2.086	2.255	0.169
15/9/07	20/9/07	15B2	0.598	0.691	0.093
15/9/07	20/9/07	15B3	0.608	0.676	0.068
				Total	**0.330**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15C1	2.165	2.325	0.160
15/9/07	20/9/07	15C2	0.601	0.671	0.070
15/9/07	20/9/07	15C3	0.604	0.698	0.094
				Total	**0.324**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	15D1	2.111	2.267	0.156
15/9/07	20/9/07	15D2	0.608	0.672	0.064
15/9/07	20/9/07	15D3	0.615	0.678	0.063
				Total	**0.283**

Flask	Total Algae Mass: 15 Days (CO_2) (g)
A	0.351
B	0.330
C	0.324
D	0.283
Total	**1.288**

5.12 Table 12

Weight of Algae after 20 Days (Not Carbonated)

Date Set to Grow	**Date Weighed**	**Paper**	**Dry Mass of Paper (g)**	**Dry Mass of Paper and Algae (g)**	**Dry Mass of Algae (g)**
15/9/07	20/9/07	20A1	2.049	2.220	0.171
15/9/07	20/9/07	20A2	0.607	0.684	0.077
15/9/07	20/9/07	20A3	0.612	0.783	0.171
				Total	**0.419**

Date Set to Grow	**Date Weighed**	**Paper**	**Dry Mass of Paper (g)**	**Dry Mass of Paper and Algae (g)**	**Dry Mass of Algae (g)**
15/9/07	20/9/07	20B1	2.106	2.248	0.142
15/9/07	20/9/07	20B2	0.603	0.669	0.066
15/9/07	20/9/07	20B3	0.599	0.739	0.140
				Total	**0.348**

Date Set to Grow	**Date Weighed**	**Paper**	**Dry Mass of Paper (g)**	**Dry Mass of Paper and Algae (g)**	**Dry Mass of Algae (g)**
15/9/07	20/9/07	20C1	2.058	2.226	0.168
15/9/07	20/9/07	20C2	0.603	0.678	0.075
15/9/07	20/9/07	20C3	0.614	0.706	0.092
				Total	**0.335**

Date Set to Grow	**Date Weighed**	**Paper**	**Dry Mass of Paper (g)**	**Dry Mass of Paper and Algae (g)**	**Dry Mass of Algae (g)**
15/9/07	20/9/07	20D1	2.095	2.248	**0.153**
15/9/07	20/9/07	20D2	0.611	0.681	**0.070**
15/9/07	20/9/07	20D3	0.611	0.694	**0.083**
				Total	**0.306**

Flask	**Total Algae Mass: 20 Days (g)**
A	0.419
B	0.348
C	0.335
D	0.306
Total	**1.408**

5.13 Table 13

Weight of Algae after 20 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20A1	2.160	2.362	0.202
15/9/07	20/9/07	20A2	0.598	0.698	0.100
15/9/07	20/9/07	20A3	0.596	0.710	0.114
				Total	**0.416**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20B1	2.076	2.239	0.163
15/9/07	20/9/07	20B2	0.601	0.689	0.088
15/9/07	20/9/07	20B3	0.608	0.704	0.096
				Total	**0.347**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20C1	2.035	2.233	0.198
15/9/07	20/9/07	20C2	0.610	0.699	0.089
15/9/07	20/9/07	20C3	0.611	0.665	0.054
				Total	**0.341**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20D1	2.121	2.332	0.211
15/9/07	20/9/07	20D2	0.591	0.636	0.045
15/9/07	20/9/07	20D3	0.608	0.673	0.065
				Total	**0.321**

Flask	Total Algae Mass: 20 Days (CO_2) (g)
A	0.416
B	0.347
C	0.341
D	0.321
Total	**1.425**

5.14 Table 14

Weight of Algae after 20 Days (No F2 Nutrient)

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20A1	2.065	2.220	0.155
15/9/07	20/9/07	20A2	0.598	0.645	0.047
15/9/07	20/9/07	20A3	0.606	0.641	0.035
				Total	**0.237**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20B1	2.106	2.206	0.100
15/9/07	20/9/07	20B2	0.594	0.638	0.044
15/9/07	20/9/07	20B3	0.604	0.640	0.036
				Total	**0.180**

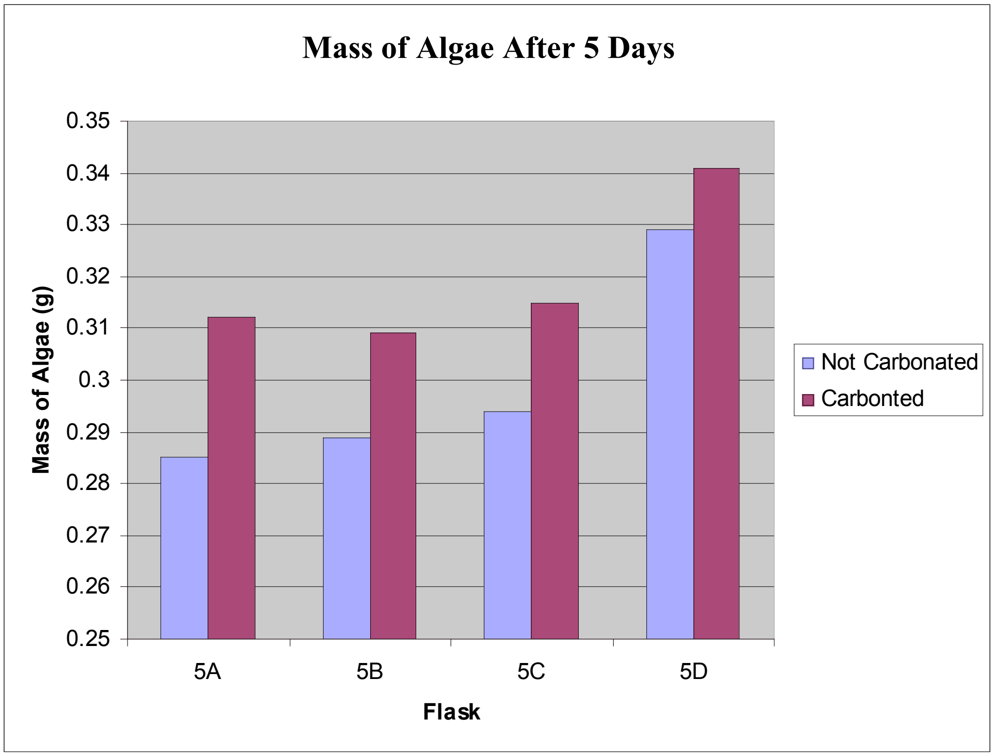
Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20C1	2.125	2.304	0.179
15/9/07	20/9/07	20C2	0.597	0.641	0.044
15/9/07	20/9/07	20C3	0.616	0.646	0.030
				Total	**0.253**

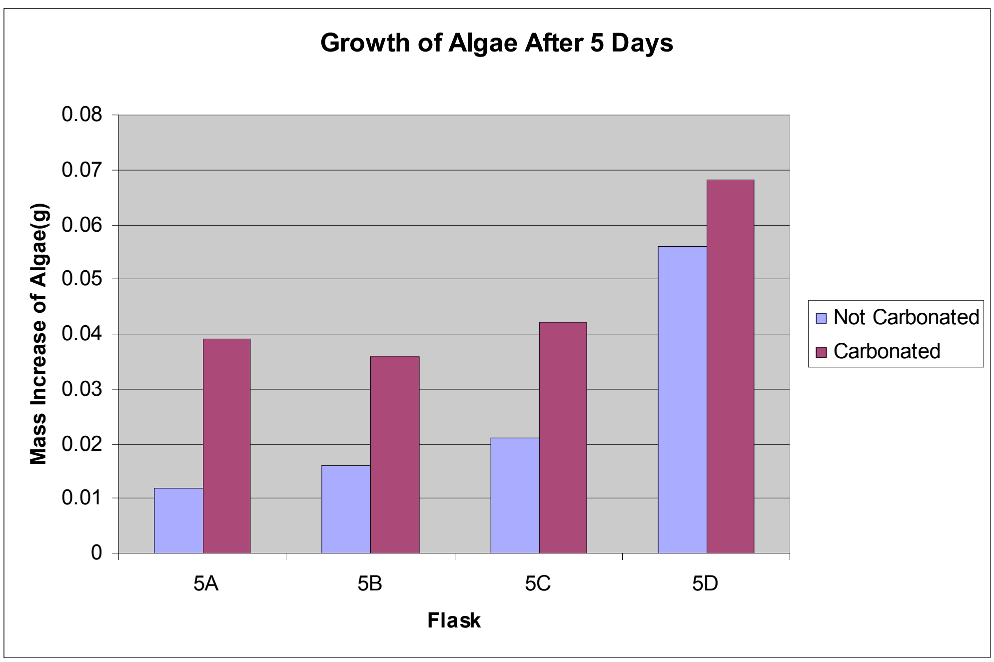
Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
15/9/07	20/9/07	20D1	2.098	2.199	0.087
15/9/07	20/9/07	20D2	0.598	0.632	0.034
15/9/07	20/9/07	20D3	0.614	0.639	0.025
				Total	**0.146**

Flask	Total Algae Mass: 20 Days (No F_2) (g)
A	0.237
B	0.180
C	0.253
D	0.146
Total	**0.816**

5.15 Graph 1 and 2

Mass and Growth of Algae After 5 Days: Uncarbonated vs. Carbonated

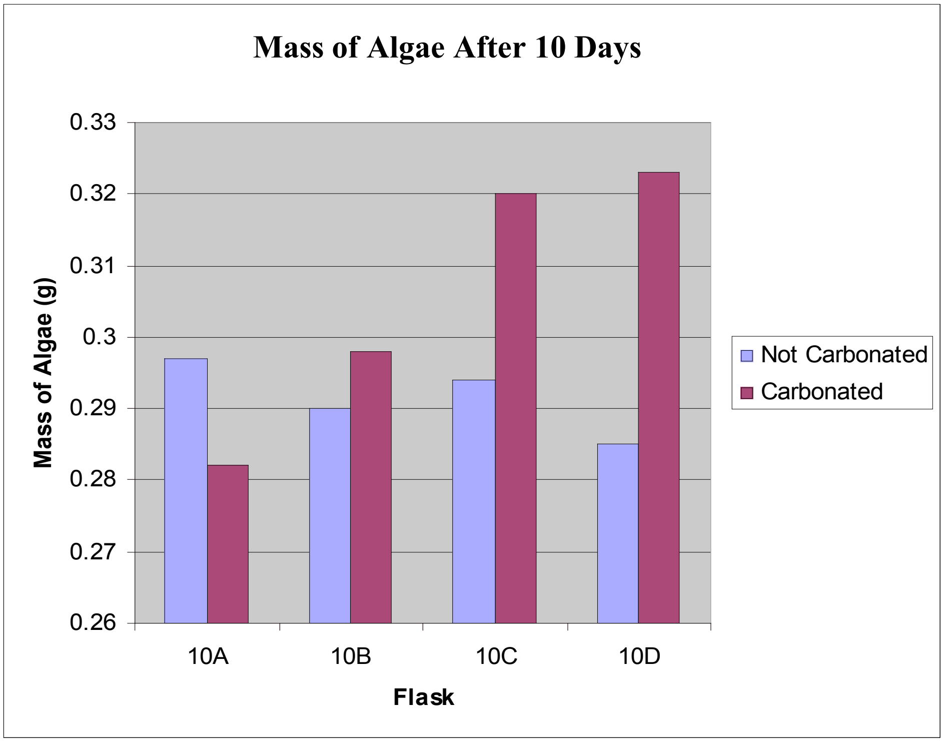


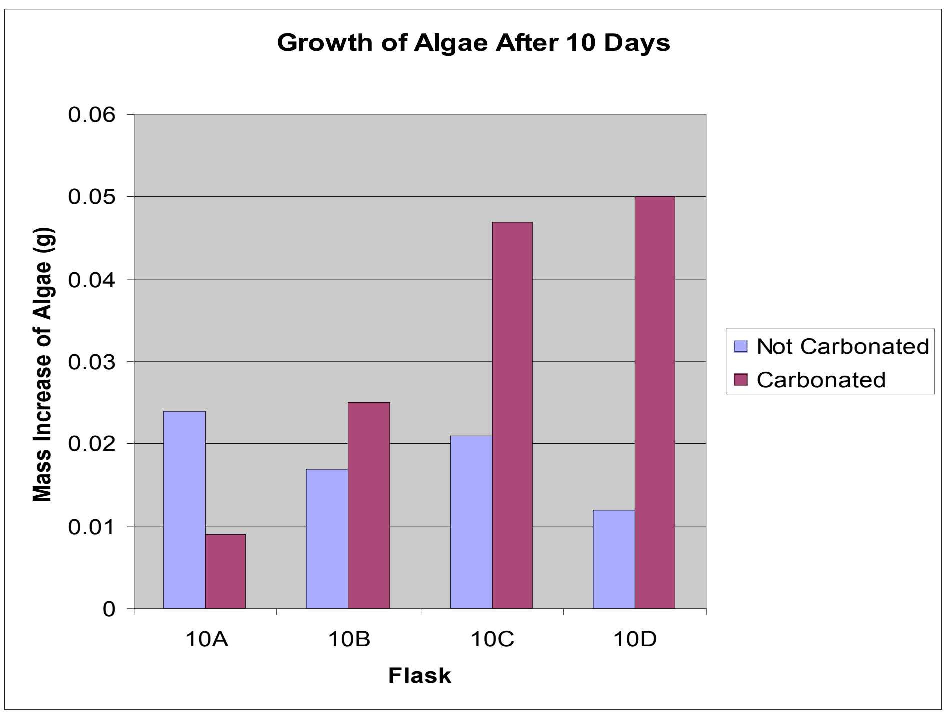


	Not Carbonated (g)	Carbonated (g)
5A	0.012	0.039
5B	0.016	0.036
5C	0.021	0.042
5D	0.056	0.068

5.16 Graph 3 and 4

Mass and Growth of Algae After 10 Days: Uncarbonated vs. Carbonated

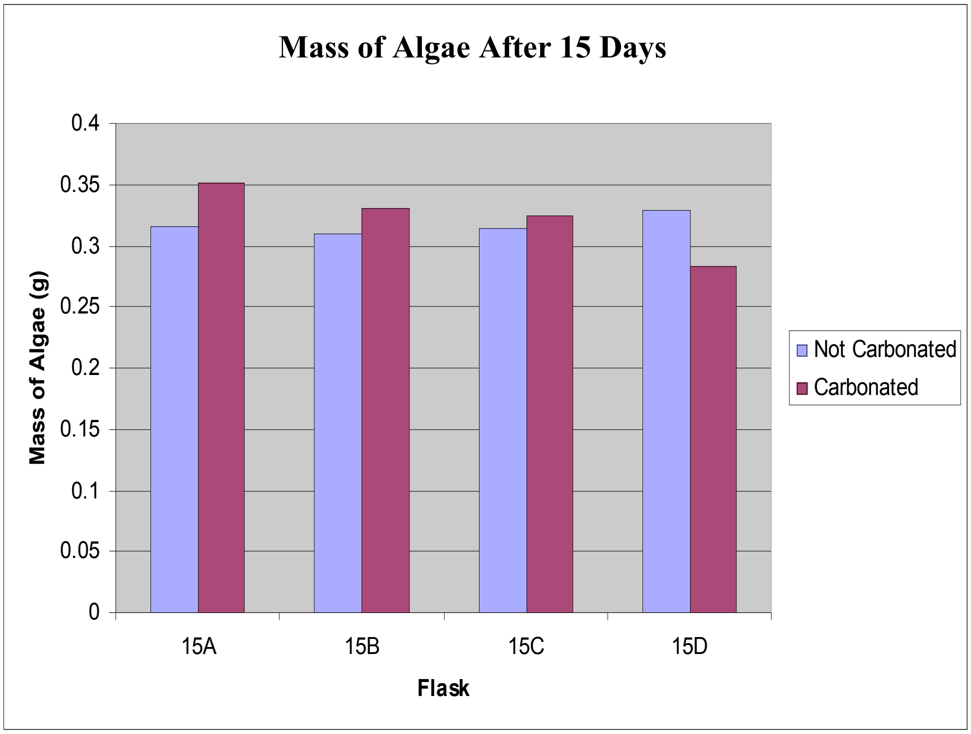


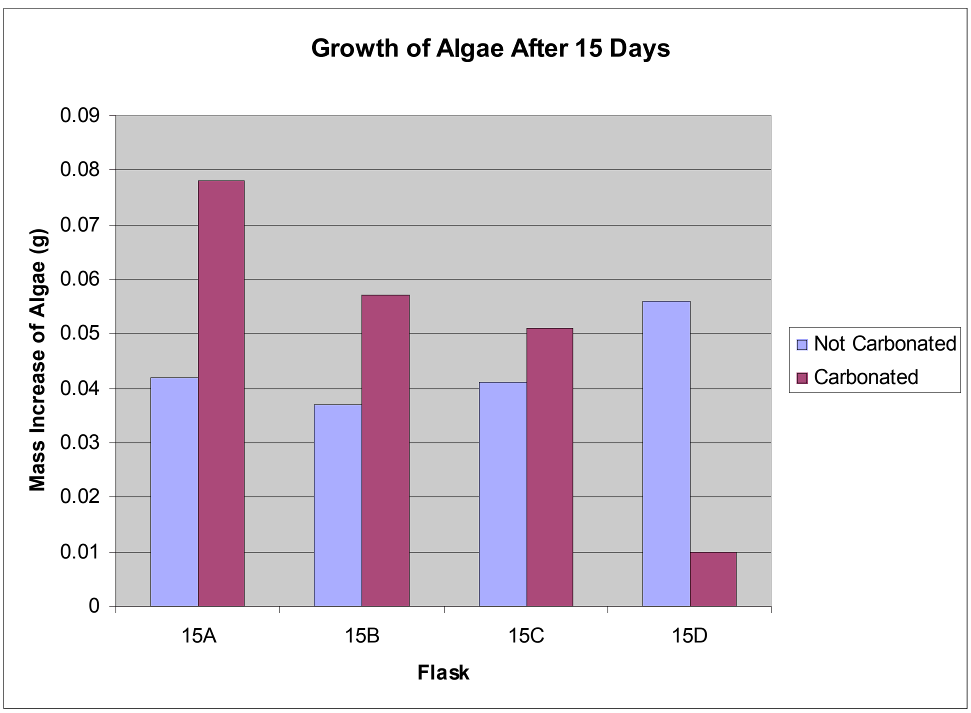


	Not Carbonated (g)	Carbonated (g)
10A	0.024	0.009
10B	0.017	0.025
10C	0.021	0.047
10D	0.012	0.050

5.17 Graph 5 and 6

Mass and Growth of Algae After 15 Days: Uncarbonated vs. Carbonated

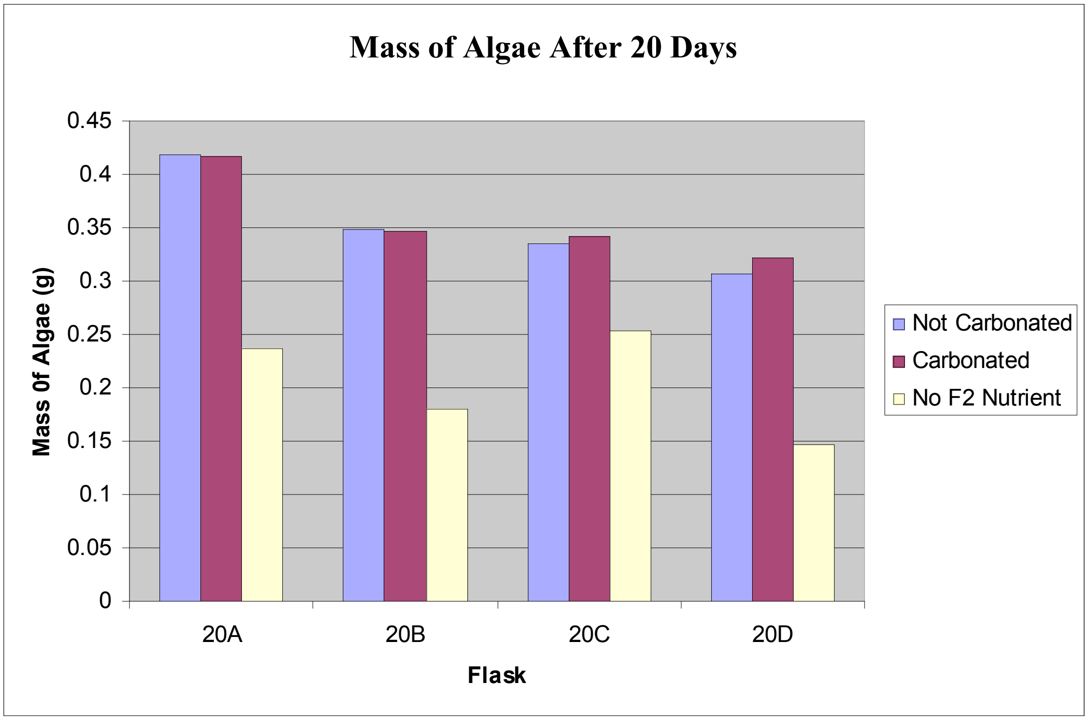


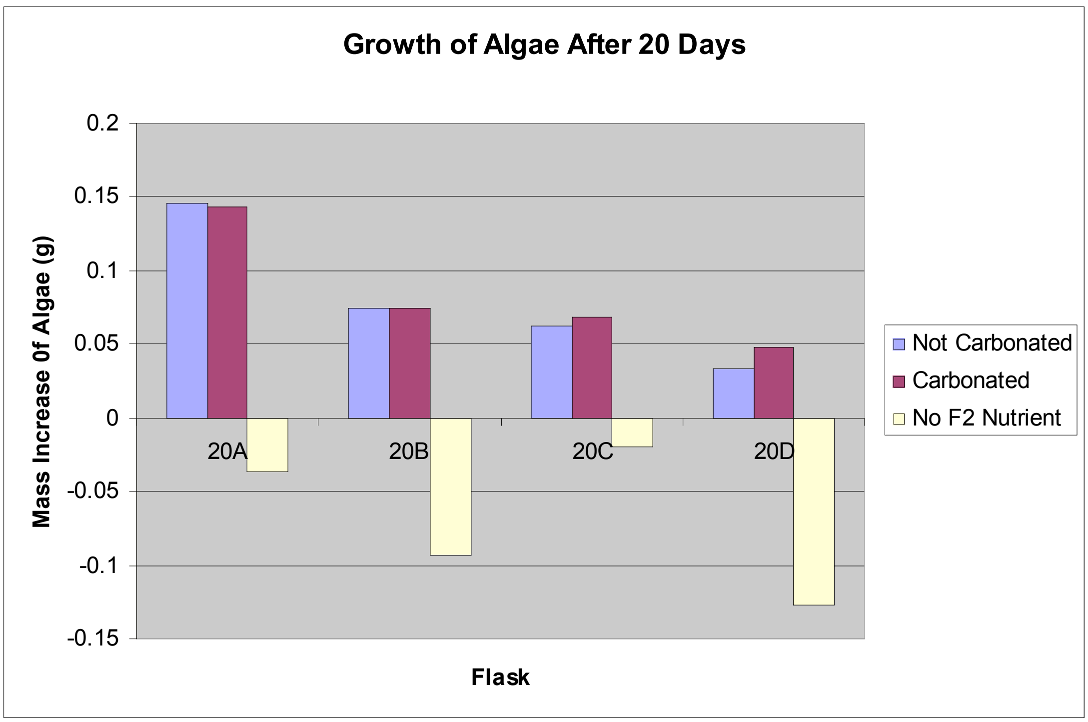


	Not Carbonated (g)	Carbonated (g)
15A	0.042	0.078
15B	0.037	0.057
15C	0.041	0.051
15D	0.056	0.010

5.18 Graph 7 and 8

Mass and Growth of Algae After 20 Days: Uncarbonated vs. Carbonated vs. No F2





	Not Carbonated (g)	Carbonated (g)	No F2 Nutrient (g)
20A	0.146	0.143	-0.036
20B	0.075	0.074	-0.093
20C	0.062	0.068	-0.020
20D	0.033	0.048	-0.127

5.19 Table 15

Summary Table: Algae Mass versus Days of Incubation

Uncarbonated

Mass of Algae at Start (g)	Mass of Algae 5 Days (g)	Mass of Algae 10 Days (g)	Mass of Algae 15 Days (g)	Mass of Algae 20 Days (g)
1.091	1.197	1.116	1.268	1.408

Carbonated

Mass of Algae at Start (g)	Mass of Algae 5 Days (g)	Mass of Algae 10 Days (g)	Mass of Algae 15 Days (g)	Mass of Algae 20 Days (g)
1.091	1.277	1.223	1.288	1.425

5.20 Table 16

Summary Table: Gain in Algae Mass versus Days of Incubation

Uncarbonated

Gain in Mass of Algae 5 Days (g)	Gain in Mass of Algae 10 Days (g)	Gain in Mass of Algae 15 Days (g)	Gain in Mass of Algae 20 Days (g)
0.106	0.075	0.177	0.317

Carbonated

Gain in Mass of Algae 5 Days (g)	Gain in Mass of Algae 10 Days (g)	Gain in Mass of Algae 15 Days (g)	Gain in Mass of Algae 20 Days (g)
0.186	0.132	0.197	0.334

5.21 Table 17

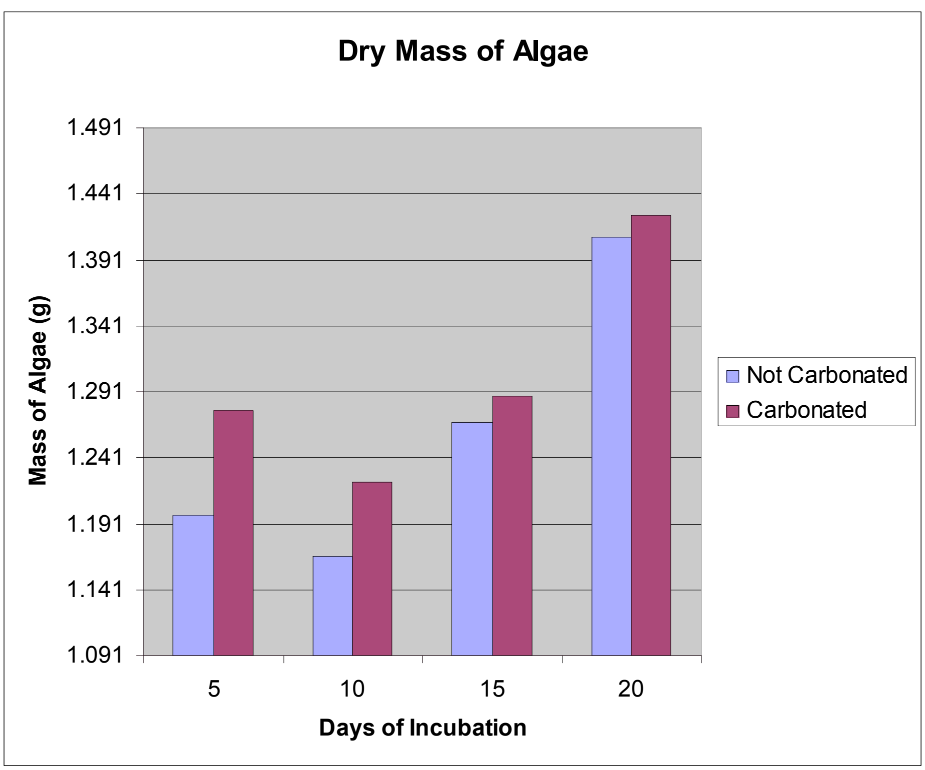
Summary Table: Comparison of Growth in Uncarbonated, Carbonated and Nutrient Deficient Algae after 20 Days

Mass of Algae 20 Days Uncarbonated with F2 Nutrient (g)	Mass of Algae 20 Days Carbonated with F2 Nutrient (g)	Mass of Algae 20 Days Uncarbonated with No F2 Nutrient (g)
1.408	1.425	0.816

Gain in Mass of Algae 20 Days Uncarbonated with F2 Nutrient (g)	Gain in Mass of Algae 20 Days Carbonated with F2 Nutrient (g)	Gain in Mass of Algae 20 Days Uncarbonated with No F2 Nutrient (g)
0.317	0.334	-0.275

5.22 Graph 9

Summary Graph: Mass Increase of Algae over 20 Days



5.23 Table 18

Cell Count: Not Carbonated

Grid	0 Days	5 Days	10 Days	15 Days	20 Days
1	1	1	4	15	18
2	2	2	6	14	12
3	1	2	4	16	14
4	1	1	4	14	23
5	1	3	7	10	32
6	0	4	10	9	20
7	1	5	10	8	20
8	1	4	6	15	38
9	1	3	9	9	28
10	0	3	7	30	40
Average	0.9	2.8	6.7	14	25.5
x 90	81	252	603	1260	2295
µL to mL x 1000	81,000	252,000	603,000	1,260,000	2,295,000
mL to 100mL x 100 (per flask)	8,100,000	25,200,000	60,300,000	126,000,000	229,500,000

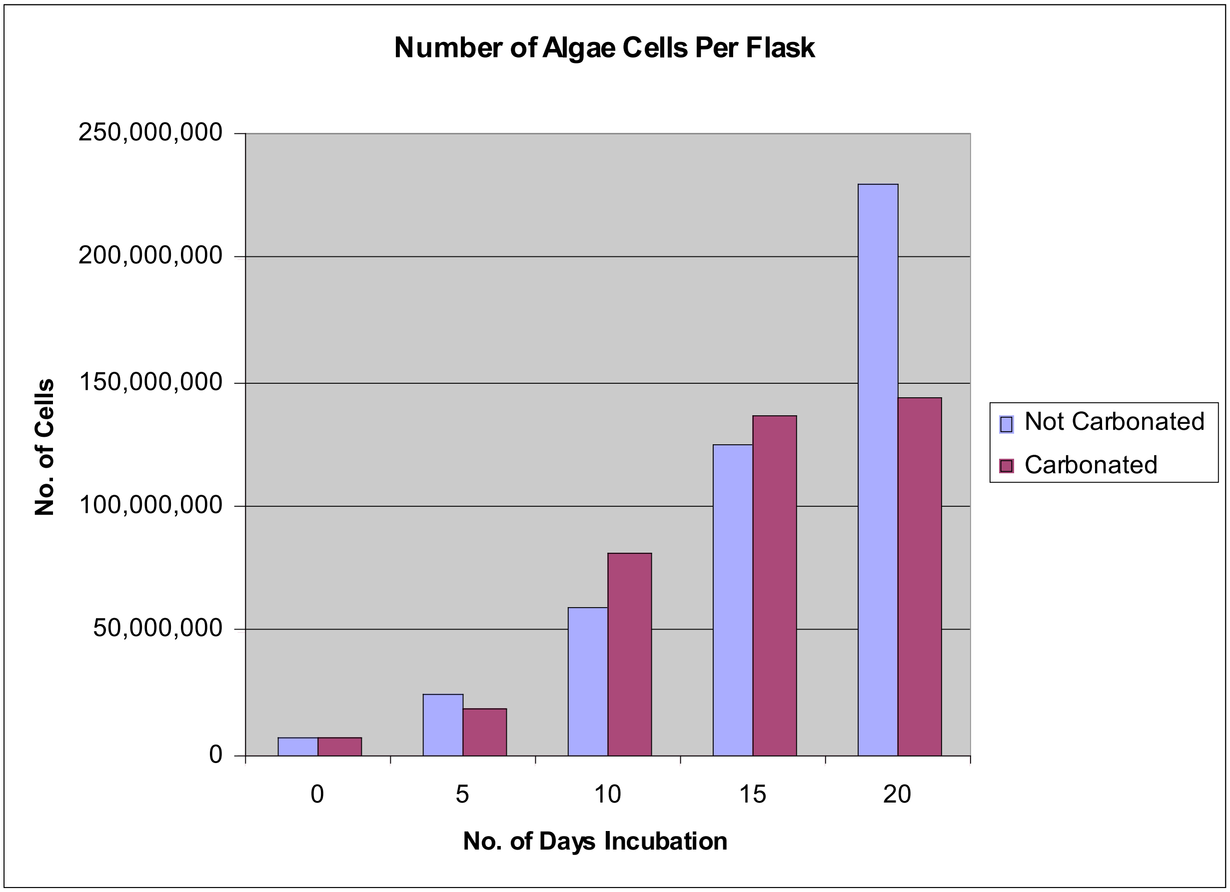
5.24 Table 19

Cell Count: Carbonated

Grid	0 Days	5 Days	10 Days	15 Days	20 Days
1	1	3	10	24	14
2	2	1	9	13	19
3	1	2	7	14	18
4	1	2	7	9	24
5	1	3	7	18	20
6	0	1	9	17	12
7	1	3	9	19	10
8	1	1	11	14	16
9	1	2	12	8	15
10	0	3	10	16	12
Average	0.9	2.1	9.1	15.2	16
x 90	81	189	819	1368	1440
µL to mL x 1000	81,000	189,000	819,000	1,368,000	1,440,000
mL to 100mL x 100 (per flask)	8,100,000	18,900,000	81,900,000	136,800,000	144,000,000

5.25 Graph 10

Cell Count: Uncarbonated vs. Carbonated



6.0 Scale up of Sequestering Capacity in Bio-reactor

Scale up of sequestering capacity of proposed bio-reactor over 20 days, when seeded to the same extent as was 100 mL of seawater in the test flasks.

In nutrient enriched (F_2) seawater (not carbonated)

1. Gain in dry mass of algae after 20 days (4 x 100 mL) flasks = **0.3170 g**
2. Gain in dry mass per litre $\frac{(0.317 \times 1000)}{400}$ = **0.7925 g**
3. Volume of bio-reactor (100 x 100 x 2 m^3) = **20,000 m^3**
4. Volume of water in bioreactor (litres)
   (note: 1 m^3 = 100 x 100 x 100 cm^3 = 1,000,000 cm^3 or 1,000,000 mL)
   (So, 1 m^3 = 1,000,000 mL = 1,000 Litres)
   Therefore, 20,000 m^3 bio-reactor volume holds 20,000 x 1,000 L of seawater.
   Volume of water in bioreactor (litres) = **20,000,000 L**
5. Gain in dry mass by the algal content of bio-reactor (g)
   (note: 0.7925 g/L x 20,000,000 L of seawater) = **15,850,000 g**
6. Gain in dry mass of algae (Kilograms) **= 15,850 kg**
7. Carbon content in algae dry mass (50% of dry mass) = **7925 kg**
8. CO_2 sequestered by algae dry mass
   (note: $\frac{7925 \text{ kg} \times 44)}{12}$ = **29,058.30 kg** = **29.06 tonnes**

In nutrient enriched (F_2) seawater (carbonated)

1. Gain in dry mass of algae after 20 days (4 x 100 mL) flasks = **0.3340 g**
2. Gain in dry mass per litre $\frac{(0.334 \times 1000)}{400}$ = **0.8350 g**
3. Volume of bio-reactor (100 x 100 x 2 m^3) = **20,000 m^3**
4. Volume of water in bioreactor (litres)
   (note: 1 m^3 = 100 x 100 x 100 cm^3 = 1,000,000 cm^3 or 1,000,000 mL)
   (So, 1 m^3 = 1,000,000 mL = 1,000 Litres)
   Therefore, 20,000 m^3 bio-reactor volume holds 20,000 x 1,000 L of seawater.
   Volume of water in bioreactor (litres) = **20,000,000 L**
5. Gain in dry mass by the algal content of bio-reactor (g)
   (note: 0.8350 g/L x 20,000,000 L of seawater) = **16,700,000 g**
6. Gain in dry mass of algae (Kilograms) **= 16,700 kg**
7. Carbon content in algae dry mass (50% of dry mass) = **8350 kg**
8. CO_2 sequestered by algae dry mass
   (note: $\frac{8350 \text{ kg} \times 44)}{12}$ = **30,616.70 kg** = **30.6 tonnes**

Difference between uncarbonated and carbonated bio-reactor systems:
30.617 tonnes - 29.058 tonnes = 1.56 tonnes more CO_2 sequestered, over 20 days, when carbonated.

7.0 Discussion

7.1 Discussion of Dissolved Oxygen Content in Sea Water

The Winkler Method was used to find the dissolved oxygen (D.O.) content in sea water samples. The object of this investigation was to see if there was sufficient oxygen in sea water to support plant respiration. Secondly, it was necessary to find if carbonating the sea water would diminish the dissolved oxygen levels.
The Biochemical Oxygen Demand (B.O.D.) test on both uncarbonated and carbonated sea water was undertaken to see if these samples contained aerobic bacteria which would consume some of the oxygen over five days of storage time (as per Waterwatch Tasmania Draft Fieldguide, 1996).

The results show that uncarbonated and carbonated sea water contained very similar levels of dissolved oxygen (7.20 ppm and 7.60 ppm respectively).
The carbonated sample, as can be seen, had 0.40 ppm more D.O. This can probably be explained by the fact that the carbonated sample had to be exposed to the atmosphere and agitated in order to incorporate the carbon dioxide.

The results for the B.O.D. tests showed lower D.O. in both uncarbonated and carbonated water samples after five days of incubation at 4°C (3.55 ppm and 4.50 ppm respectively). This means that bacteria are present in both uncarbonated and carbonated sea water and they consume some of the dissolved oxygen.
I found it interesting that the B.O.D. of carbonated sea water is less than that of the uncarbonated sea water. Although I cannot prove this, I speculate that bacteria are not as active in acidic carbonated sea water, and so do not have as high a B.O.D.

7.2 Discussion of Algae Growth during Incubation (By Dry Mass)

The first method that was used to determine algae growth was dry mass measurements by filtration. All incubation flasks were first filtered with crude, qualitative (no.2), 150mm filter paper and then with two smaller, fine grade ashless (no.42) 90mm filter papers. This triple filtration method was developed in an attempt to trap all algal cells, irrespective of size. As an extra attempt to trap all cells, the filtrate from the third filtration was passed through the paper a second time.

As algal density increased (especially 15 and 20 days) it was evident that some algal cells were passing through the final filtration, as the final filtrate still appeared a little green. However they were not filtered further as the standard test I employed for all flasks was to filter with three papers and pass the final filtrate through twice.

As can be seen from the raw data (appendix 2), the results tables (5.5-5.14) and the summary table (5.15), when placed in nutrients (F2), both uncarbonated and carbonated algae increased in mass over the 20 days of incubation.
The uncarbonated samples increased in mass from 1.091g to 1.408g in this time. This represents approximately a 29% increase in dry mass measurement. In the same time-span (20 days) the carbonated samples increased in mass from 1.091g to 1.425g, representing approximately a 31% increase in dry mass measurement.

Whilst the trend in both uncarbonated and carbonated samples showed a general increase in mass, the ten day samples showed a slight decline. If this had happened only in one or other of the samples (uncarbonated or carbonated) I would have considered this an anomaly. But since it happened in both media, I speculated that there must be an explainable reason for the apparent drop in dry mass after 10 days.
My reasoning was that cell division may be occurring in both media after approximately 1 week of 'settling-in' to the new growth media and as a result the cells may have been very small and more able to penetrate the filter papers after 10 days. Cell counts, using a haemacytometer (to be discussed later) showed great variation in cell size and it is possible that the smaller cells were a more recent product of cell division.

It was interesting to note that the algae placed in media without F2 nutrient actually dropped in dry mass when compared with that of the starter solution (0.816g vs.1.091g respectively). This could mean that some of the algae died in the absence of nutrient.

7.3 Discussion of Algae Growth during Incubation (By Cell Count)

The second method used to determine algal growth was cell count estimations. For this process a disposable version of a haemacytometer called a KOVA® GLASSTIC® SLIDE was used. When a 6.6µL portion from an incubation flask (at 0, 5, 10, 15, 20 days) was applied to the slide, capillary action caused the fluid to be drawn into the 10 chambers, resulting in a homogenous suspension of sediment. (See: KOVA® GLASSTIC® SLIDE instructions in appendix 3). By viewing under medium power (10 x 10 = 100 mag.) the grids were brought into focus and the cells within one square were counted for each of the 10 grids. The counts over 10 grids were averaged to get a count per grid. This average was multiplied by 90 (instruction for uncentrifuged samples), multiplied by 1000 to convert to cells per mL and multiplied by 100 to convert to cells per 100 mL (the volume of the incubation medium).

As the days of incubation increased, there was a corresponding increase in the number of cells found inside each square. Initially the flasks were seeded with algae culture which was found to contain 8.1 million cells in the 100 mL incubation volume.

For the uncarbonated samples, over 5, 10, 15 and 20 days, there was an increase in cell density up to 25.2, 60.3, 126.0 and 229.5 millions, respectively.
For the carbonated samples, the increases were up to 18.9, 81.9, 136.8 and 144.0 millions, respectively.

The carbonated samples showed an increase in cell density over the uncarbonated samples for 10 and 15 days. But the cell count for 5 and 20 days seemed inconsistent.
I felt that cell count was not a very accurate way of measuring or comparing algal growth. To be consistent, the cells needed to uniformly divide themselves between grids, but in actual fact they gravitated to the outside of the slides (an area not covered by the grids). They also banked up at the liquid's edge or, if any air bubbles were found, they congregated at the edges of the bubbles.

8.0 Hypotheses Outcomes.

8.1 Hypothesis 1

"If the dissolved oxygen content of sea water is compared with the dissolved oxygen content of carbonated sea water, then it will be found that carbonated sea water will have less dissolved oxygen."

Outcome: Hypothesis not supported

As can be seen from Results Tables 5.1 and 5.2, the D.O. content of seawater was initially 7.20 ppm and when the seawater was carbonated the D.O. increased slightly to 7.60 ppm. It is my opinion that exposure to the atmosphere and agitation of the seawater to incorporate the CO_2 may have accounted for this slight increase in D.O.

8.2 Hypothesis 2

"If both un-carbonated and carbonated sea water are incubated at 4°C for 5 days in dark conditions and the bio-chemical oxygen demand of both is estimated, then it will be found that they both have a bio-chemical oxygen demand, indicating the presence of bacteria in the water."

Outcome: Hypothesis supported

It is evident from Tables 5.3 and 5.4 that the D.O in both uncarbonated and carbonated seawater decreased (to 3.55 ppm and 4.50 ppm, respectively) when incubated in darkness for 5 days at 4 ^{o}C. This diminishing of the D.O indicates a bio-chemical oxygen demand on the water due to the presence of aerobic bacteria. The reduction in D.O. was less pronounced in carbonated seawater, perhaps indicating that bacteria are less active in the slightly acidic carbonated seawater.

8.3 Hypothesis 3

"If the marine micro algae, Dunaliella tertiolecta, is introduced to growth flasks containing un-carbonated sea water and F2 nutrient and the dry mass estimated after 0, 5, 10, 15 and 20 days, then it will be found that the algae bio-mass increases with incubation time."

Outcome: Hypothesis supported

It can be seen from Result 5.5: Table 5 that the total mass of algae culture used to seed growth flasks was 1.091 g. From Tables 6, 8, 10 and 12 it can be seen that the mass of algae after 5, 10 15 and 20 days was 1.197, 1.116, 1.268 and 1.408 g respectively. This shows a gradual increase in mass with days of incubation, eventually showing a 29% increase in dry mass over 20 days.

8.4 Hypothesis 4

"If the marine micro algae, Dunaliella tertiolecta, is introduced to growth flasks containing un-carbonated sea water and F2 nutrient and a cell count is undertaken after 0, 5, 10, 15 and 20 days, then it will be found that the cell density increases with incubation time."

Outcome: Hypothesis supported

It can be seen in Results 5.22: Table 18 that the number of algal cells in the seeding stock for flasks, was found to be 8.1 million. This increased with incubation time of 5, 10, 15, 20 days to yield 25.2, 60.3, 126 and 229.5 million cells per flask, respectively. This represents a 28 fold increase in algal cell density over a 20 day time-span.

8.5 Hypothesis 5

"If the marine micro algae, Dunaliella tertiolecta, is introduced to growth flasks containing carbonated sea water and F_2 nutrient, then it will be found that both the algae bio-mass and cell density will be greater than for the flasks containing un-carbonated sea water."

Outcome: Hypothesis supported

It can be seen from Results 5.23: Table 19 that the algal cell count was generally higher for carbonated seawater than for uncarbonated over the first 15 days. At 15 days the carbonated cell count was found to be 136.8 million, compared with 126 million for uncarbonated cells. This represents almost a 17% increase in cell density over 15 days for carbonated samples, compared with 15.5% increase over the same time period in uncarbonated samples.
A slowing down of this increasing cell density was found with the cell count of 144 million cells over 20 days. This may be evidence that carbonated samples experience an early surge in growth and then slow down as CO_2 becomes used up.

8.7 Hypothesis 7

"If the marine micro algae, Dunaliella tertiolecta, are introduced to growth flasks with and without F2 nutrient, then it will be found that both algae bio-mass and cell density will be higher where F2 is present."

Outcome: Hypothesis supported

It can be seen from Results 5.15: Summary Table 3 that if no F_2 nutrient was added to the incubation flasks, there was an actual drop in the dry mass of the algae over 20 days, when compared with the mass of algae used to seed the flasks (0.816 g versus 1.091 g). When a sample from a flask lacking F_2 was used for cell count analysis, cells were found to be practically non-existent. This outcome was seen to provide evidence for the critical need there is to add nutrients to the algal growth media.

8.7 Hypothesis 7

"If the dry mass gain for algae in uncarbonated and carbonated media is calculated then it is possible to scale-up the findings and estimate the difference in sequestering capacity between uncarbonated and carbonated seawater in a bio-reactor".

Outcome: Hypothesis supported

It was found that the difference between uncarbonated and carbonated bio-reactor systems would be as much as 1.56 tonnes of extra atmospheric CO_2 sequestered when the system is enriched with CO_2.
This assumes that the bio-reactor (as seen in appendix 6) is seeded with algae culture at the same rate as the growth flasks in the laboratory.

9.0 Conclusions

9.1 Conclusions to Dissolved Oxygen (D.O.) and Biochemical Oxygen Demand (B.O.D.) in Sea Water

The D.O. in sea water was found to be 7.20 ppm and, when carbonated, it increased slightly to 7.60 ppm. It is expected that agitation of the sea water to incorporate the carbon dioxide was the cause of this increase.
The critical conclusion from this test is that **carbonating sea water was not found to reduce the dissolved oxygen content.**

The B.O.D. tests on both uncarbonated and carbonated sea water showed that there is an oxygen demand on both water types (due to the presence of aerobic bacteria). A lower B.O.D. in the carbonated sample could suggest that bacteria are less active, perhaps because of the reduction in pH, associated with carbonation (Pre-carbonation pH of 7.82 reducing to pH of 6.17 after carbonation).

9.2 Conclusions to Algae Growth during Incubation (By Dry Mass)

Dry mass measurements on algae showed that uncarbonated samples in an F2 nutrient-rich medium increased in mass by 29% over 20 days of incubation. When carbonated, the increase in mass was 31% over the same time span. This shows that carbonation of the growth media causes an increase in dry mass production of 2% when compared with uncarbonated samples.

Examination of the graphs comparing growth rates (uncarbonated vs. carbonated: see results) indicates a greater difference between uncarbonated and carbonated samples over the first ten days, **with carbonated samples showing superior growth rates. However, between ten and twenty days the growth rates seem to even out, possibly indicating that the carbon dioxide had been used up in the carbonated samples.**

It might be concluded that if carbonated flasks were re-carbonated after ten days, they might maintain a higher rate of growth.

It was also concluded that **incubation without F2 nutrients caused a decline in algae dry mass** when compared with the mass of the starting culture (0.816g vs. 1.091g, respectively). This shows that nutrient enrichment of sea water is essential to sustain algal growth.

9.3 Conclusions to Algae Growth during Incubation (By Cell Count)

For both uncarbonated and carbonated samples there was an increase in cell count over the 20 days of incubation. The greatest jumps in cell density were seen between 10 and 15 days for both samples: **60 to 126 million cells for uncarbonated versus 82 to 137 million cells** for carbonated. This represents **a doubling of cells in the uncarbonated samples and approximately a 67% increase for the carbonated samples in the same time-span.** Considering the fact that the carbonated samples experienced a slowing-down in the rate of cell multiplication for 20 days, it might be concluded that the carbonated samples had utilized most of the CO_2 by this stage. It might also be concluded that re-carbonation after 10-15 days might promote further cell multiplication.

9.4 Conclusions to scale up of sequestering capacity of proposed bio-reactor over 20 days

It can be seen from 6.0: "Scale-up Calculations" that the difference between uncarbonated and carbonated bio-reactor systems is an ability to sequester **1.56 tonnes more atmospheric CO_2, when the system is enriched with CO_2**.
(30.617 tonnes when carbonated - 29.058 tonnes when uncarbonated = 1.56 tonnes more sequestered over 20 days, when carbonated).

This assumes that the bio-reactor (as seen in appendix 5) is seeded with algae culture at the same rate as the growth flasks in the laboratory. This would be an enormous amount of algae culture (5 mL of culture in 95 mL of growth medium corresponds to approx. 1,000,000 litres of culture in 20,000,000 litres of seawater in the bio-reactor).

In reality a lot less algae could be used in seeding the system, but it would take longer for the medium to reach the same algal density as in my findings in the laboratory.

10.0 Recommendations

Summary of Findings

From my research it can be seen that the yield of algae that can be achieved in a carbonated medium is approximately 2% higher than that from uncarbonated media over 20 days of growth. The growth gain in carbonated media was 0.334 grams of dry mass in 4 x 100 mL flasks. This represents a dry mass gain of 0.835 g per litre of seawater.
It was also found that carbonation reduces the pH of the seawater slightly (from pH of 7.82 for uncarbonated, to pH 6.17 for carbonated) but this acidity did not seem to hinder algal growth. Carbon dioxide enrichment was also seen to have no impact on the dissolved oxygen content in the water. However, it was found that nutrient enrichment (F_2 preparation, in the case of my tests) was essential to sustain algal growth.

Implications of Findings

From these findings I think it is clear that bio-reactors for the controlled growth of algae could be established in Australia as a means of sequestering atmospheric CO_2 and converting it into usable plant bio-mass.

If we consider that almost 50% of the dry mass of algae is carbon (Ref 11) then each litre of seawater is capable of trapping (into plant bio-mass) 0.334 g / 2 = 0.167 g of carbon. This corresponds to (0.167 g / 12) x 44 = 0.612 g of atmospheric CO_2 that is sequestered in the process.

Recommendations

I recommend that, in the world of the 21^{st} Century:

- Bio-reactors should replace oil-rigs as the fuel providers for the future. I suggest that industrial plants be fitted, not with smoke stacks for the release of CO_2 (and other waste gases), but with collection pipes for trapping and conducting of CO_2 to bio-reactor sites. (see artist's impression in Appendix 6)
- That the dimensions of bio-reactors be approximately 100m x 100m x 2m and that they be located offshore to minimise land use at a time of great pressure on land for food production
- That they are made of a strong, UV resistant, transparent material
- That they have some buoyancy and are only partially immersed in sea water (for maximum absorption of sunlight
- That they are a closed system and contain nutrient enriched seawater (F_2 nutrients would be suitable) with nutrient top-up as necessary
- That they have a controlled supply of industrial CO_2 (controlled to match maximum solubility of CO_2 in water (90 mg CO_2 per 1000 g of seawater: Ref 12))
- That they are aerated continuously with air extracted from the atmosphere to maintain high levels of dissolved oxygen (mindful of the fact that photosynthesis will produce O_2 but, at the high growth rates in micro-algae, respiration will soon deplete dissolved oxygen). Wind generation might be suitable for operation of these pumps.

- That they are seeded at the start with a species of micro-algae that is suited to conversion to bio-diesel. This, most likely, will not be *Dunaliella tertiolecta*, as my research suggests that while it was a good, robust alga for my study, it does not have the HUFA (highly unsaturated fatty acids) levels to make it valuable for bio-diesel production. A better choice (O'Meley and Daintith) might be the golden brown flagellate, *Pavlova lutheri* (19.7% HUFA) or the centric diatom, *Thalassiosira pseudonana* (19.3% HUFA). As continuous nutrient and CO_2 enrichment would promote continuous cell division and growth, re-seeding may not be necessary
- That the system have a settling tank on the bottom onto which dead algal cells will drop and build up (as evidenced in my post growth observations) and the system should include an extraction port for the removal of this build-up
- That the site be serviced continually and algae bio-mass be removed as necessary to an adjacent plant for conversion to bio-diesel

The sustainable aspects of my design

- This system does not rely on freshwater. It is capable of producing plant bio-mass, suitable for conversion to bio-diesel using unprocessed saltwater. Considering the pressure that is on our limited freshwater supplies and the cost of de-salination, the fact that this system can use seawater is a real bonus
- The system sequesters, into plant bio-mass, CO_2 which would otherwise be released to the atmosphere, further increasing global warming
- The system allows the ocean to be nutrient-enriched in a controlled manner, without the possibility of escape of nutrients that may have hazardous effects elsewhere in the oceans. (Some of the articles that I have read on this topic suggest adding nutrients to the open ocean to promote sequestration of atmospheric CO_2 into plant biomass. I am very much against this proposal).
- Being located in the ocean, this system does not put any pressure on land for the production of bio-mass to be used for alternate fuels
- Aeration for this system could be achieved using wind power, thus mimimising the energy needs.

Engineering Features that need to be considered

Some thought would have to go into the anchoring of the bio-reactor to maintain its location (so it would not drift and crash against rocks, etc) and that it be made of a material flexible enough to withstand wave action.

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13.0 Appendices

13.1 Preliminary Trial: Raw Data and Discussion

13.2 Second Trials: Raw Data

13.3 F_2 nutrient composition

13.4 Dissolved Oxygen Calculation from First Principles

13.5 KOVA® GLASSTIC® SLIDE instructions

13.6 Artist's impression of algae bio-reactor

13.7 Range of temperature variations in algal growth flasks.

13.1 Preliminary Trial: Raw Data and Discussion

Weight of Algae after 5 Days

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
9/9/07	14/9/07	5A1	2.159	2.129	2.105	5A1	2.292	2.280	2.279	**0.174**
9/9/07	14/9/07	5B1	2.224	2.191	2.171	5B1	2.357	2.340	2.336	**0.165**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
9/9/07	14/9/07	5A2	0.556	0.550	0.554	5A2	0.593	0.594	0.592	**0.038**
9/9/07	14/9/07	5B2	0.549	0.546	0.542	5B2	0.594	0.583	0.591	**0.049**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
9/9/07	14/9/07	5A3	0.575	0.574	0.574	5A3	0.627	0.621	0.622	**0.048**
9/9/07	14/9/07	5B3	0.547	0.541	0.545	5B3	0.596	0.596	0.596	**0.051**

Weight of Algae after 10 Days

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
1/9/07	11/9/07	10A1	2.168	2.102	2.087	10A1	2.235	2.220	2.214	**0.127**
1/9/07	11/9/07	10B1	2.218	2.149	2.133	10B1	2.266	2.260	2.258	**0.125**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
1/9/07	11/9/07	10A2	0.541	0.540	0.540	10A2	0.585	0.572	0.584	**0.044**
1/9/07	11/9/07	10B2	0.558	0.553	0.560	10B2	o.612	0.609	0.609	**0.049**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
1/9/07	11/9/07	10A3	0.545	0.543	0.545	10A3	0.606	0.595	0.591	**0.049**
1/9/07	11/9/07	10B3	0.593	0.538	0.537	10B3	0.623	0.610	0.605	**0.068**

Weight of Algae after 15 Days

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
25/8/07	9/9/07	15A1	2.128	2.121	2.122	15A1	2.334	2.268	2.264	**0.142**
25/8/07	9/9/07	15B1	2.080	2.072	2.073	105B1	2.225	2.224	2.225	**0.152**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
25/8/07	9/9/07	15A2	0.562	0.549	0.545	15A2	0.588	0.587	0.586	**0.041**
25/8/07	9/9/07	15B2	0.530	0.531	0.532	105B2	0.573	0.570	0.570	**0.038**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
25/8/07	9/9/07	15A3	0.539	0.539	0.538	15A3	0.602	0.597	0.600	**0.062**
25/8/07	9/9/07	15B3	0.526	0.527	0.527	105B3	0.578	0.573	0.570	**0.043**

Weight of Algae after 20 Days

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
18/8/07	7/9/07	20A1	2.218	2.110	2.108	20A1	2.308	2.301	2.297	**0.189**
18/8/07	7/9/07	20B1	2.158	2.041	2.040	20B1	2.247	2.241	2.239	**0.199**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
18/8/07	7/9/07	20A2	0.664	0.618	0.617	20A2	0.686	0.681	0.674	**0.057**
18/8/07	7/9/07	20B2	0.648	0.608	0.607	20B2	0.683	0.679	0.676	**0.069**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
18/8/07	7/9/07	20A3	0.628	0.608	0.608	20A3	0.657	0.652	0.649	**0.041**
18/8/07	7/9/07	20B3	0.654	0.620	0.620	20B3	0.689	0.679	0.677	**0.057**

Weight of Algae after 5 Days

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
9/9/07	14/9/07	5A1	2.105	2.279	0.174
9/9/07	14/9/07	5A2	0.554	0.592	0.038
9/9/07	14/9/07	5A3	0.574	0.622	0.048
				Total	**0.260**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
9/9/07	14/9/07	5B1	2.171	2.336	0.165
9/9/07	14/9/07	5B2	0.542	0.591	0.049
9/9/07	14/9/07	5B3	0.545	0.596	0.051
				Total	**0.265**

Flask	Total Algae Mass: 0 Days (g)
A	0.260
B	0.265
Total	**0.525**

Weight of Algae after 10 Days

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
1/9/07	11/9/07	10A1	2.087	2.214	0.127
1/9/07	11/9/07	10A2	0.540	0.584	0.044
1/9/07	11/9/07	10A3	0.545	0.591	0.049
				Total	**0.220**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
1/9/07	11/9/07	10B1	2.133	2.258	0.125
1/9/07	11/9/07	10B2	0.560	0.609	0.049
1/9/07	11/9/07	10B3	0.537	0.605	0.068
				Total	**0.242**

Flask	Total Algae Mass: 0 Days (g)
A	0.220
B	0.242
Total	**0.462**

Weight of Algae after 15 Days

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
25/8/07	9/9/07	15A1	2.122	2.264	0.142
25/8/07	9/9/07	15A2	0.545	0.586	0.041
25/8/07	9/9/07	15A3	0.538	0.600	0.062
				Total	**0.245**

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
25/8/07	9/9/07	15B1	2.073	2.225	0.152
25/8/07	9/9/07	15B2	0.532	0.570	0.038
25/8/07	9/9/07	15B3	0.527	0.570	0.043
				Total	**0.233**

Flask	Total Algae Mass: 0 Days (g)
A	0.245
B	0.233
Total	**0.478**

Weight of Algae after 20 Days

Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
18/8/07	7/9/07	20A1	2.108	2.297	0.189
18/8/07	7/9/07	20A2	0.617	0.674	0.057
18/8/07	7/9/07	20A3	0.608	0.649	0.041
				Total	**0.287**

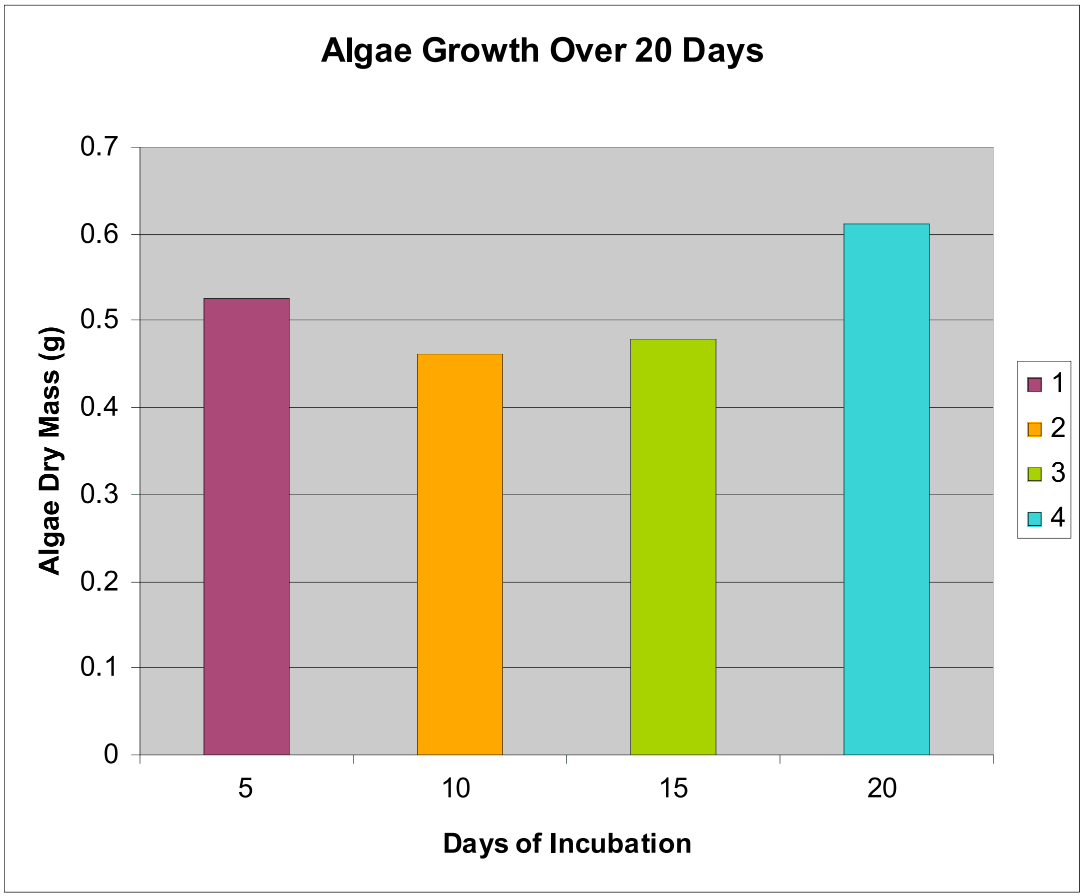
Date Set to Grow	Date Weighed	Paper	Dry Mass of Paper (g)	Dry Mass of Paper and Algae (g)	Dry Mass of Algae (g)
18/8/07	7/9/07	20B1	2.040	2.239	0.199
18/8/07	7/9/07	20B2	0.607	0.676	0.069
18/8/07	7/9/07	20B3	0.620	0.677	0.057
				Total	**0.325**

Flask	Total Algae Mass: 0 Days (g)
A	0.287
B	0.325
Total	**0.612**

Preliminary Trials: **TABLE A: Table of Growth Dates for Algae**

August '07														September '07													
18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14

GRAPH A: Mass of Algae after 5, 10, 15 and 20 Day



Discussion on Preliminary Trials

In the preliminary trials, algae cultures were set to grow at weekly intervals starting on 18th August. The objective of the first trial was to develop standard procedures to be used in the second trial, which would then be used for analysis.

As can be seen from GRAPH A, algae dry masses (5-20 days), growth rates are inconsistent, with growth for 10 and 15 days appearing lower than expected. However, the algae still showed a mass increase over 20 days of growth. A number of factors could explain these inconsistencies:

- Algae cultures were not all set to grow on the same date (see TABLE A). This meant that, as well as number of days of growth being a variable factor, the length of day varied as days were getting longer.
- The sterile cultures (from CSIRO, Hobart) were longer sitting (and deprived of nutrients) for the later samples. It was a possibility that cultures may be less (or more) active after longer storage.
- It was also possible that growth flasks located on the outside of the trays (while incubating for 5, 10, 15, 20 days) might be getting a higher intensity of sunlight than those on the inside of a row.

As a result of observations made in the first trials, some standard procedures were set in place for the second trials:

1. All flasks would be set to grow with new culture on the same date to eliminate the possibility of culture deterioration, while in storage
2. Flasks would be rotated in the incubation trays on a daily basis, so that all would receive approximately the same sunlight intensity during growth
3. The dry mass of algae used as starter cultures would be found (by filtering, drying and weighing), so that the "actual growth" of algae over 5, 10, 15 and 20 days could be calculated, by subtraction from starting mass. (note: those given in GRAPH A represent the final dry mass, not the mass increase over the relevant number of days)
4. It was planned to grow some algae without the F_2 nutrient, as this would represent the ability of the algae to grow in ordinary sea water, without nutrient enrichment
5. Instead of growing 8 flasks of algae at each of the time variables (5, 10, 15, 20 days) and measuring the dry mass of 2 samples from each batch, as in the first trials, it was decided to grow 4 uncarbonated and 4 carbonated flasks for each time-span and to find the collective dry mass of algae from all flasks
6. It was planned to carbonate the samples with CO_2 generated as a reaction by-product, to model the use of industrial CO_2 as a means of promoting algae growth
7. It was planned to use cell counts as a second method of estimating algae growth. A disposable form of haemacytometer, known as a KOVA® GLASSTIC® SLIDE was chosen for this task.

13.2 Appendix 2: Raw Data from Investigation

Weight of Algae after 0 Days

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	17/9/07	0A1	2.063	2.054	**2.049**	0A1	2.283	2.222	**2.214**	**0.165**
17/9/07	17/9/07	0B1	2.115	2.102	**2.102**	0B1	2.308	2.281	**2.279**	**0.177**
17/9/07	17/9/07	0C1	2.141	2.125	**2.124**	0C1	2.316	2.304	**2.308**	**0.184**
17/9/07	17/9/07	0D1	2.097	2.085	**2.085**	0D1	2.297	2.268	**2.248**	**0.163**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	17/9/07	0A2	0.568	0.565	**0.569**	0A2	0.624	0.623	**0.622**	**0.053**
17/9/07	17/9/07	0B2	0.555	0.553	**0.554**	0B2	0.608	0.604	**0.611**	**0.057**
17/9/07	17/9/07	0C2	0.554	0.555	**0.548**	0C2	0.605	0.600	**0.606**	**0.058**
17/9/07	17/9/07	0D2	0.569	0.569	**0.569**	0D2	0.630	0.621	**0.619**	**0.050**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	17/9/07	0A3	0.567	0.569	**0.566**	0A3	0.615	0.615	**0.614**	**0.048**
17/9/07	17/9/07	0B3	0.564	0.564	**0.561**	0B3	0.613	0.609	**0.610**	**0.049**
17/9/07	17/9/07	0C3	0.569	0.571	**0.566**	0C3	0.619	0.618	**0.612**	**0.046**
17/9/07	17/9/07	0D3	0.560	0.557	**0.554**	0D3	0.593	0.598	**0.595**	**0.041**

Weight of Algae after 5 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A1	2.155	2.070	**2.047**	5A1	2.228	2.224	**2.220**	**0.173**
15/9/07	20/9/07	5B1	2.179	2.117	**2.096**	5B1	2.264	2.65	**2.264**	**0.168**
15/9/07	20/9/07	5C1	2.171	2.125	**2.095**	5C1	2.285	2.281	**2.275**	**0.180**
15/9/07	20/9/07	5D1	2.152	2.096	**2.071**	5D1	2.256	2.257	**2.254**	**0.183**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A2	0.576	0.576	**0.576**	5A1	0.637	0.630	**0.631**	**0.055**
15/9/07	20/9/07	5B2	0.564	0.563	**0.562**	5B1	0.628	0.629	**0.628**	**0.066**
15/9/07	20/9/07	5C2	0.576	0.578	**0.578**	5C1	0.640	0.637	**0.638**	**0.060**
15/9/07	20/9/07	5D2	0.567	0.558	**0.547**	5D1	0.632	0.632	**0.631**	**0.084**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A3	0.587	0.583	**0.581**	5A1	0.643	0.639	**0.638**	**0.057**
15/9/07	20/9/07	5B3	0.584	0.582	**0.578**	5B1	0.640	0.634	**0.633**	**0.055**
15/9/07	20/9/07	5C3	0.596	0.592	**0.593**	5C1	0.653	0.649	**0.647**	**0.054**
15/9/07	20/9/07	5D3	0.584	0.585	**0.584**	5D1	0.656	0.648	**0.646**	**0.062**

Weight of Algae after 5 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A1	2.065	2.042	**2.032**	5A1	2.231	2.223	**2.224**	**0.192**
15/9/07	20/9/07	5B1	2.130	2.122	**2.116**	5B1	2.293	2.290	**2.290**	**0.174**
15/9/07	20/9/07	5C1	2.134	2.112	**2.102**	5C1	2.296	2.296	**2.295**	**0.193**
15/9/07	20/9/07	5D1	2.050	2.034	**2.022**	5D1	2.221	2.223	**2.225**	**0.203**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A2	0.567	0.562	**0.561**	5A1	0.623	0.620	**0.622**	**0.061**
15/9/07	20/9/07	5B2	0.578	0.574	**0.568**	5B1	0.635	0.638	**0.644**	**0.076**
15/9/07	20/9/07	5C2	0.584	0.586	**0.586**	5C1	0.646	0.643	**0.644**	**0.058**
15/9/07	20/9/07	5D2	0.585	0.586	**0.581**	5D1	0.652	0.654	**0.655**	**0.074**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	20/9/07	5A3	0.586	0.586	**0.587**	5A1	0.653	0.649	**0.646**	**0.059**
15/9/07	20/9/07	5B3	0.592	0.590	**0.590**	5B1	0.652	0.650	**0.649**	**0.059**
15/9/07	20/9/07	5C3	0.588	0.584	**0.584**	5C1	0.648	0.651	**0.648**	**0.064**
15/9/07	20/9/07	5D3	0.571	0.567	**0.569**	5D1	0.637	0.634	**0.633**	**0.064**

Weight of Algae after 10 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A1	2.152	2.133	**2.131**	10A1	2.291	2.289	**2.288**	**0.157**
15/9/07	25/9/07	10B1	2.184	2.180	**2.174**	10B1	2.344	2.341	**2.336**	**0.162**
15/9/07	25/9/07	10C1	2.151	2.148	**2.147**	10C1	2.321	2.311	**2.308**	**0.161**
15/9/07	25/9/07	10D1	2.079	2.080	**2.081**	10D1	2.241	2.236	**2.234**	**0.154**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A2	0.595	0.594	**0.590**	10A1	0.685	0.679	**0.678**	**0.088**
15/9/07	25/9/07	10B2	0.606	0.602	**0.602**	10B1	0.695	0.688	**0.682**	**0.080**
15/9/07	25/9/07	10C2	0.607	0.601	**0.603**	10C1	0.686	0.681	**0.679**	**0.076**
15/9/07	25/9/07	10D2	0.593	0.591	**0.594**	10D1	0.675	0.669	**0.665**	**0.071**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A3	0.606	0.612	**0.607**	10A1	0.631	0.630	**0.659**	**0.052**
15/9/07	25/9/07	10B3	0.618	0.618	**0.617**	10B1	0.669	0.665	**0.665**	**0.048**
15/9/07	25/9/07	10C3	0.608	0.606	**0.606**	10C1	0.668	0.664	**0.663**	**0.057**
15/9/07	25/9/07	10D3	0.603	0.601	**0.600**	10D1	0.670	0.663	**0.660**	**0.060**

Weight of Algae after 10 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A1	2.162	2.158	**2.160**	10A1	2.330	2.321	**2.312**	**0.152**
15/9/07	25/9/07	10B1	2.091	2.084	**2.083**	10B1	2.257	2.241	**2.238**	**0.155**
15/9/07	25/9/07	10C1	2.118	2.115	**2.113**	10C1	2.301	2.281	**2.281**	**0.168**
15/9/07	25/9/07	10D1	2.184	2.180	**2.176**	10D1	2.340	2.338	**2.336**	**0.160**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A2	0.576	0.580	**0.580**	10A1	0.666	0.661	**0.655**	**0.075**
15/9/07	25/9/07	10B2	0.601	0.596	**0.599**	10B1	0.685	0.676	**0.676**	**0.077**
15/9/07	25/9/07	10C2	0.599	0.598	**0.598**	10C1	0.710	0.698	**0.693**	**0.095**
15/9/07	25/9/07	10D2	0.597	0.598	**0.597**	10D1	0.703	0.699	**0.699**	**0.102**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	25/9/07	10A3	0.599	0.594	**0.599**	10A1	0.661	0.659	**0.654**	**0.055**
15/9/07	25/9/07	10B3	0.598	0.607	**0.600**	10B1	0.670	0.668	**0.666**	**0.066**
15/9/07	25/9/07	10C3	0.613	0.615	**0.611**	10C1	0.669	0.669	**0.668**	**0.057**
15/9/07	25/9/07	10D3	0.609	0.609	**0.606**	10D1	0.671	0.669	**0.667**	**0.061**

Weight of Algae after 15 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A1	2.109	2.075	**2.075**	15A1	2.274	2.274	**2.279**	**0.204**
15/9/07	30/9/07	15B1	2.111	2.110	**2.104**	15B1	2.276	2.276	**2.272**	**0.168**
15/9/07	30/9/07	15C1	2.025	2.022	**2.021**	15C1	2.210	2.202	**2.202**	**0.181**
15/9/07	30/9/07	15D1	2.080	2.080	**2.075**	15D1	2.269	2.261	**2.269**	**0.194**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A2	0.599	0.595	**0.591**	15A1	0.656	0.653	**0.651**	**0.060**
15/9/07	30/9/07	15B2	0.589	0.580	**0.585**	15B1	0.661	0.660	**0.658**	**0.073**
15/9/07	30/9/07	15C2	0.590	0.584	**0.585**	15C1	0.663	0.660	**0.655**	**0.070**
15/9/07	30/9/07	15D2	0.592	0.591	**0.589**	15D1	0.642	0.640	**0.638**	**0.049**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A3	0.597	0.599	**0.594**	15A1	0.651	0.646	**0.645**	**0.051**
15/9/07	30/9/07	15B3	0.598	0.598	**0.595**	15B1	0.666	0.655	**0.664**	**0.069**
15/9/07	30/9/07	15C3	0.620	0.610	**0.606**	15C1	0.665	0.666	**0.669**	**0.063**
15/9/07	30/9/07	15D3	0.610	0.599	**0.601**	15D1	0.683	0.687	**0.686**	**0.086**

Weight of Algae after 15 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A1	2.097	2.086	**2.075**	15A1	2.252	2.249	**2.245**	**0.170**
15/9/07	30/9/07	15B1	2.100	2.097	**2.086**	15B1	2.264	2.257	**2.255**	**0.169**
15/9/07	30/9/07	15C1	2.167	2.165	**2.165**	15C1	2.329	2.317	**2.325**	**0.160**
15/9/07	30/9/07	15D1	2.111	2.110	**2.111**	15D1	2.286	2.272	**2.267**	**0.156**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A2	0.580	0.583	**0.580**	15A1	0.641	0.639	**0.637**	**0.057**
15/9/07	30/9/07	15B2	0.602	0. 602	**0.598**	15B1	0.699	0.699	**0.691**	**0.093**
15/9/07	30/9/07	15C2	0.607	0.604	**0.601**	15C1	0.675	0.674	**0.671**	**0.070**
15/9/07	30/9/07	15D2	0.620	0.617	**0.608**	15D1	0.682	0.679	**0.672**	**0.064**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	30/9/07	15A3	0.604	0.602	**0.600**	15A1	0.729	0.712	**0.724**	**0.124**
15/9/07	30/9/07	15B3	0.620	0.610	**0.608**	15B1	0.672	0.671	**0.676**	**0.068**
15/9/07	30/9/07	15C3	0.614	0.604	**0.604**	15C1	0.712	0.699	**0.698**	**0.094**
15/9/07	30/9/07	15C3	0.620	0.617	**0.615**	15D1	0.677	0.679	**0.678**	**0.063**

Weight of Algae after 20 Days (Not Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	5/10/07	20A1	2.062	2.059	**2.049**	20A1	2.192	2.220	**2.220**	**0.171**
15/9/07	5/10/07	20B1	2.118	2.113	**2.106**	20B1	2.251	2.250	**2.248**	**0.142**
15/9/07	5/10/07	20C1	2.062	2.061	**2.058**	20C1	2.231	2.228	**2.226**	**0.168**
15/9/07	5/10/07	20D1	2.102	2.100	**2.095**	20D1	2.249	2.249	**2.248**	**0.153**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	5/10/07	20A2	0.611	0.607	**0.607**	20A1	0.689	0.686	**0.684**	**0.077**
15/9/07	5/10/07	20B2	0.607	0.605	**0.603**	20B1	0.675	0.672	**0.669**	**0.066**
15/9/07	5/10/07	20C2	0.608	0.609	**0.603**	20C1	0.683	0.679	**0.678**	**0.075**
15/9/07	5/10/07	20D2	0.610	0.607	**0.611**	20D1	0.691	0.686	**0.681**	**0.070**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
15/9/07	5/10/07	20A3	0.623	0.620	**0.612**	20A1	0.790	0.783	**0.783**	**0.171**
15/9/07	5/10/07	20B3	0.611	0.600	**0.599**	20B1	0.741	0.738	**0.739**	**0.140**
15/9/07	5/10/07	20C3	0.621	0.618	**0.614**	20C1	0.711	0.707	**0.706**	**0.092**
15/9/07	5/10/07	20D3	0.623	0.615	**0.611**	20D1	0.704	0.699	**0.694**	**0.083**

Weight of Algae after 20 Days (Carbonated)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A1	2.171	2.167	**2.160**	20A1	2.379	2.370	**2.362**	**0.202**
17/9/07	5/10/07	20B1	2.083	2.078	**2.076**	20B1	2.242	2.240	**2.239**	**0.163**
17/9/07	5/10/07	20C1	0.2054	2.045	**2.035**	20C1	2.238	2.234	**2.233**	**0.198**
17/9/07	5/10/07	20D1	2.137	2.132	**2.121**	20D1	2.341	2.337	**2.332**	**0.211**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A2	0.605	0.605	**0.598**	20A1	0.702	0.699	**0.698**	**0.100**
17/9/07	5/10/07	20B2	0.608	0.601	**0.601**	20B1	0.693	0.692	**0.689**	**0.088**
17/9/07	5/10/07	20C2	0.614	0.611	**0.610**	20C1	0.708	0.701	**0.699**	**0.089**
17/9/07	5/10/07	20D2	0.590	0.592	**0.591**	20D1	0.638	0.636	**0.636**	**0.045**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A3	0.598	0.596	**0.596**	20A1	0.716	0.711	**0.710**	**0.114**
17/9/07	5/10/07	20B3	0.607	0.608	**0.608**	20B1	0.710	0.706	**0.704**	**0.096**
17/9/07	5/10/07	20C3	0.613	0.613	**0.611**	20C1	0.678	0.670	**0.665**	**0.054**
17/9/07	5/10/07	20D3	0.611	0.609	**0.608**	20D1	0.676	0.672	**0.673**	**0.065**

Weight of Algae after 20 Days (No F2 Nutrient)

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A1	2.085	2.068	**2.065**	20A1	2.233	2.231	**2.220**	**0.155**
17/9/07	5/10/07	20B1	2.113	2.108	**2.106**	20B1	2.220	2.218	**2.206**	**0.100**
17/9/07	5/10/07	20C1	2.154	2.138	**2.125**	20C1	2.315	2.311	**2.304**	**0.179**
17/9/07	5/10/07	20D1	2.116	2.112	**2.098**	20D1	2.213	2.201	**2.199**	**0.087**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A2	0.599	0.598	**0.598**	20A1	0.653	0.649	**0.645**	**0.047**
17/9/07	5/10/07	20B2	0.602	0.599	**0.594**	20B1	0.644	0.641	**0.638**	**0.044**
17/9/07	5/10/07	20C2	0.599	0.597	**0.597**	20C1	0.653	0.645	**0.641**	**0.044**
17/9/07	5/10/07	20D2	0.598	0.598	**0.598**	20D1	0.641	0.630	**0.632**	**0.034**

Date Set to Grow	Date Weighed	Paper	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Paper + Algae Culture	1st Dry Mass (g)	2nd Dry Mass (g)	3rd Dry Mass (g)	Algae Mass (g)
17/9/07	5/10/07	20A3	0.615	0.607	**0.606**	20A1	0.655	0.650	**0.641**	**0.035**
17/9/07	5/10/07	20B3	0.611	0.603	**0.604**	20B1	0.647	0.642	**0.640**	**0.036**
17/9/07	5/10/07	20C3	0.621	0.614	**0.616**	20C1	0.651	0.643	**0.646**	**0.030**
17/9/07	5/10/07	20D3	0.620	0.618	**0.614**	20D1	0.647	0.641	**0.639**	**0.025**

Appendix 3: Nutrients for Algae Growth

O'Meley and Daintith – Algae Cultures for Marine Hatcheries

Medium f_2

The f_2 medium originally developed by Guillard, 1962, is a balanced mixture of nutrients that has been found to support the growth of most marine species used in aquaculture. The medium is relatively straight forward to make with easily obtainable chemicals.

Medium F_2 Chemicals

Nutrients Required by Algae	Chemical Formula	Chemical Name
Nitrogen (N)	$NaNO_3$	Sodium Nitrate
Phosphate (P)	NaH_2PO_4	Sodium Orthophosphate
Silicate (Si)	Na_2SiO_3	Sodium Meta-silicate
Trace Metals	$CuSO_4$ $ZnSO_4$ $CoCl_2$ $MnCl_2$ Na_2MoO_4	Copper Sulphate Zinc Sulphate Cobalt Chloride Manganese Chloride Sodium Molybdate
Iron (Fe) and Chelator	-	Ferric Citrate / Citric Acid
Vitamins	- - -	Thiamine HCl Vitamin B_{12} Biotin

13.4 Appendix 4: Dissolved Oxygen Calculation from First Principles

Theory

The Dissolved Oxygen (D.O.) Content in water is easy to estimate since the average titre of $Na_2S_2O_3$ (mL) corresponds exactly to the D.O. in ppm. The reason for this is outlined in the sample calculation below:

Results and Calculations

Reactions:

$Mn^{2+}_{(aq)} + 2OH^-_{(aq)} + ½O_{2\,(aq)} \rightarrow MnO_{2\,(s)} + H_2O_{(l)}$

$MnO_{2\,(s)} + 4H^+_{(aq)} + 2I^-_{(aq)} \rightarrow I_{2\,(aq)} + Mn^{2+}_{(aq)} + 2H_2O_{(aq)}$

$I_{2\,(aq)} + 2Na_2S_2O_{3\,(aq)} \rightarrow Na_2S_4O_{6\,(aq)} + 2NaI_{(aq)}$

Reaction Ratios:

2 x n ($Na_2S_2O_3$) : 1 x n (MnO_2) : ½ n (O_2)

or

n ($Na_2S_2O_3$) : ½ n (MnO_2) : ¼ n (O_2)

First titration outcome:

See Results 5.1 Table 1: Dissolved Oxygen Content in Sea Water.

Flask	mL 0.025M $Na_2S_2O_3$ (aq) used	D.O. in ppm (* See App 2)
1	[8.40]	
2	7.30	
3	7.10	
Ave	7.20	7.20 ppm

(Time of sampling: 9.30 am, Air temp: 13^0 C, Water temp: 11^0 C, pH = 7.82)

Sample Calculation for D.O. content.

Titre	**Vol of 0.025molL^{-1} $Na_2S_2O_3$ needed**
1	Disregarded
2	7.30 mL
3	7.10 mL
Average	**7.20 mL**

1. n ($Na_2S_2O_3$) in ave. titre = c x v

   = 0.025 m.L^{-1} x 7.20 x 10^{-3} L

   = 1.80 x 10^{-4} moles

2. n (MnO_2) in 200mL sample = n ($Na_2S_2O_3$)/2 = 9.0 x 10^{-5} mol

3. n (O_2) in 200mL sample (H_2O = n (MnO_2)/2 = 4.50 x 10^{-5} mol

4. mass (O_2) in 200mL sample = n x M

   = 4.50 x 10^{-5} mol x 32 gms.mol^{-1}

   = 1.44 x 10^{-3} gms

5. mass (O_2) / Litre = 5 (1.44 x 10^{-3}) gms

   = 7.20 x 10^{-3} gms

6. Dissolved Oxygen (mg/L) = (7.20 x 10^{-3} gms) x 1000

   = 7.20 mg.L^{-1} or ppm

13.5 KOVA® GLASSTIC® SLIDE instructions

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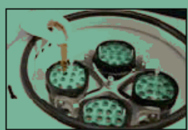
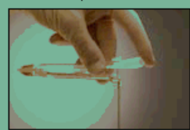
KOVA® GLASSTIC® SLIDE 10 WITH GRIDS

INSTRUCTIONS FOR USE

The KOVA Glasstic Slide 10 with quantitative grid is designed to be used with the standardized hygienic KOVA Microscopic Urinalysis System:

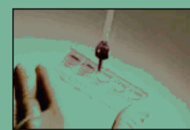


Fill the KOVA Tube to 12mL and firmly attach the KOVA Cap. Centrifuge at 400 rcf (~1500 rpm) for 5 minutes.



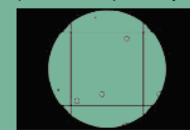
Insert the KOVA Petter firmly and decant. 1.0mL of sediment will be trapped by the KOVA Petter.

Gently resuspend using the KOVA Petter. If desired, add 1 drop of KOVA Stain prior to resuspension.



Using the KOVA Petter, transfer the sample to the notch on the slide chamber. Carefull addition of samples insures the hygienic handling properties of the KOVA System.

By capillary action 6.6 µL of the sample will be drawn into the KOVA Slide[2] 10 chamber resulting in a homogenous suspension of the sediment.



Quantitate the casts at low power (100x). Quantitate all cells at high power (400x). Count the cells within the lines of the small 0.33 mm square grid (as shown). Refer to the value table for the cell count per µL of patient sample.

KOVA Glasstic Slide 10 with Grid Chamber

CAT/REF: 87144

Chamber Volume: *6.6µL*
Chamber Depth: *0.1 mm*
Outer Grid Dimension: *3 mm x 3 mm*
Volume within Grid: *0.9µL*
Small Grid Size: *0.33 mm x 0.33 mm*
Small Grid Volume: *0.01111µL*

VALUE TABLE

Low Cell Count Samples:
Count the total cells of a specific type contained in 10 small grids within different quadrants of the counting grid.

Total Cells	Cells / µL
1	1
2	2
3	2
4	3
5	4
6	5
7	5
8	6
9	7
10	8
11	8
12	9
13	10
14	11
15	11
16	12
17	13
18	14
19	15
20	15
21	16
22	17
23	18
24	18
25	19
26	20
27	21
28	21

Higher Cell Count Samples:
Count the total cells of a specific type contained in 5 small grids within different quadrants of the counting grid.

Total Cells	Cells / µL
5	8
6	9
7	11
8	12
9	14
10	15
11	17
12	18
13	20
14	21
15	23
16	24
17	26
18	28
19	29
20	31
21	32
22	34
23	35
24	37
25	38
30	46
35	54
40	61
45	69
50	77
60	92
70	107

NOTE: For samples that are less than 12mL, reduce the centrifuged quantity to 6mL and double the results obtained before using the table (above).

Cell Type	Normal	Borderline	Pathological*
Leukocytes	0-4/µL	4-6/µL	> 6/µL
Erythrocytes	0-2/µL	2-3/µL	> 3/µL

Alternative Calculation: Determine the average number of cells per small grid and then use the following multiplication factor to calculate the cells per µL.

To calculate cells / µL using KOVA Glasstic Slide 10 with Grid:

- For uncentrifuged or neat samples, multiply the average cells obtained per grid x **90**.
- For 10mL samples concentrated to 1mL, multiply the average cells obtained per grid x **9**.
- For 10mL samples concentrated to 0.5mL, multiply the average cells obtained per grid x **4.5**.
- For 12mL samples concentrated to 1mL (KOVA System), multiply the average cells obtained per grid x **7.5**.

Calculation example (Using KOVA System 12mL to 1mL method):

Cells	Grids Counted	Total Cells	Average Cells / Grids	Multiple x Factor (7.5)	Cells per µL of Samples
Leukoytes	10	5	0.5	0.5 x 7.5	3.8
Erythrocytes	10	14	1.4	1.4 x 7.5	10.5

* Reference: Aiken, C.D. and Sokeland, J. (1983). Urologie. Thiems. Stuttgart, Ninth Edition, p.79

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KOVA® GLASSTIC® SLIDE 10 WITH GRIDS

VALUE TABLE
UNDILUTED, UNCENTRIFUGED URINE OR BODY FLUID SPECIMENS

LOW CELL COUNT SAMPLES
Count the total cells of a specific type contained in **36** small grids or 4 complete quadrants of the counting grid.

Total Cells	Cells/µL	Cells/mL
1	3	2,500
2	5	5,000
3	8	7,500
4	10	10,000
5	13	12,500
6	15	15,000
7	18	17,500
8	20	20,000
9	23	22,500
10	25	25,000
11	28	27,500
12	30	30,000
13	33	32,500
14	35	35,000
15	38	37,500
16	40	40,000
17	43	42,500
18	45	45,000
19	48	47,500
20	50	50,000
25	63	62,500
30	75	75,000
40	100	100,000
50	126	125,500

<u>Alternative Calculation</u>:
Multiply the average number of cells per small grid by 90 to obtain cells per µL; multiply by 90,000 to obtain cells per mL.

HIGH CELL COUNT SAMPLES
Count the total cells of a specific type contained in **10** small grids in different quadrants of the counting grid.

Total Cells	Cells/µL	Cells/mL
1	9	9,000
2	18	18,000
3	27	27,000
4	36	36,000
5	45	45,000
6	54	54,000
7	63	63,000
8	72	72,000
9	81	81,000
10	90	90,000
20	180	180,000
25	225	225,000
30	270	270,000
35	315	315,000
40	360	360,000
50	450	450,000
60	540	540,000
70	630	630,000
80	720	720,000
90	810	810,000
100	900	900,000
150	1350	1,350,000
200	1800	1,800,000
250	2250	1,250,000

<u>Alternative Calculation</u>:
Multiply the average number of cells per small grid by 90 to obtain cells per µL; multiply by 90,000 to obtain cells per mL.

DILUTED BODY FLUIDS CALCULATION METHOD:

Cells / µL = Average number of cells per small grid x 90 (multiplication factor) x dilution

e.g., Spinal fluid diluted 1:10; a total of 50 RBC's counted in 10 small grids

$$\text{RBC}/\mu\text{L} = \frac{50 \text{ cells}}{10 \text{ grids}} \times 90 \text{ (factor)} \times 10 \text{ (dilution)}$$

$$= 5 \times 900 = 4{,}500 \text{ RBC's}/\mu\text{L}$$

e.g., Semen diluted 1:20; a total of 150 sperm counted in 5 small grids

$$\text{Sperm}/\mu\text{L} = \frac{150}{5} \times 90 \text{ (factor)} \times 20 \text{ (dilution)}$$

$$= 30 \times 1800 = 54{,}000 \text{ sperm}/\mu\text{L}$$

TOTAL CELL COUNT NORMAL RANGES [1]

FLUID	CELL TYPE	NORMAL	ABNORMAL	FLUID	CELL TYPE	NORMAL	ABNORMAL
Urine (2)	Leukocytes Erythrocytes	0-6/µL 0-3/µL	> 6/µL > 3/µL	Synovial	Leukocytes Erythrocytes	< 200/µL < 2,000/µL	> 200/µL > 2,000/µL
CSF (Adult Range)	Leukocytes	0-5/µL	> 5/µL	Pleural	Leukocytes	< 1,000/µL	> 1,000/µL
				Pericardial	Leukocytes	< 1,000/µL	> 1,000/µL
Seminal	Sperm	40,000/µL - 160,000/µL	< 40,000/µL	Pertoneal	Leukocytes Erythrocytes	< 300/µL < 100,000/µL	> 300/µL > 100,000/µL

References: (1) Strasinger, S.K. (1985) **Urinalysis and Body Fluids**, F.A. Davis, Philadelphia • (2) Alken, C.D., and Sokeland, J. (1983) Urologie, Thiems, Stutgart, Nineth Edition, pg. 79

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1. U.S. Patent Number: 4,563,332
2. U.S. Patent Numbers: 4,997,268 – 5,128,802

PN 91064-09
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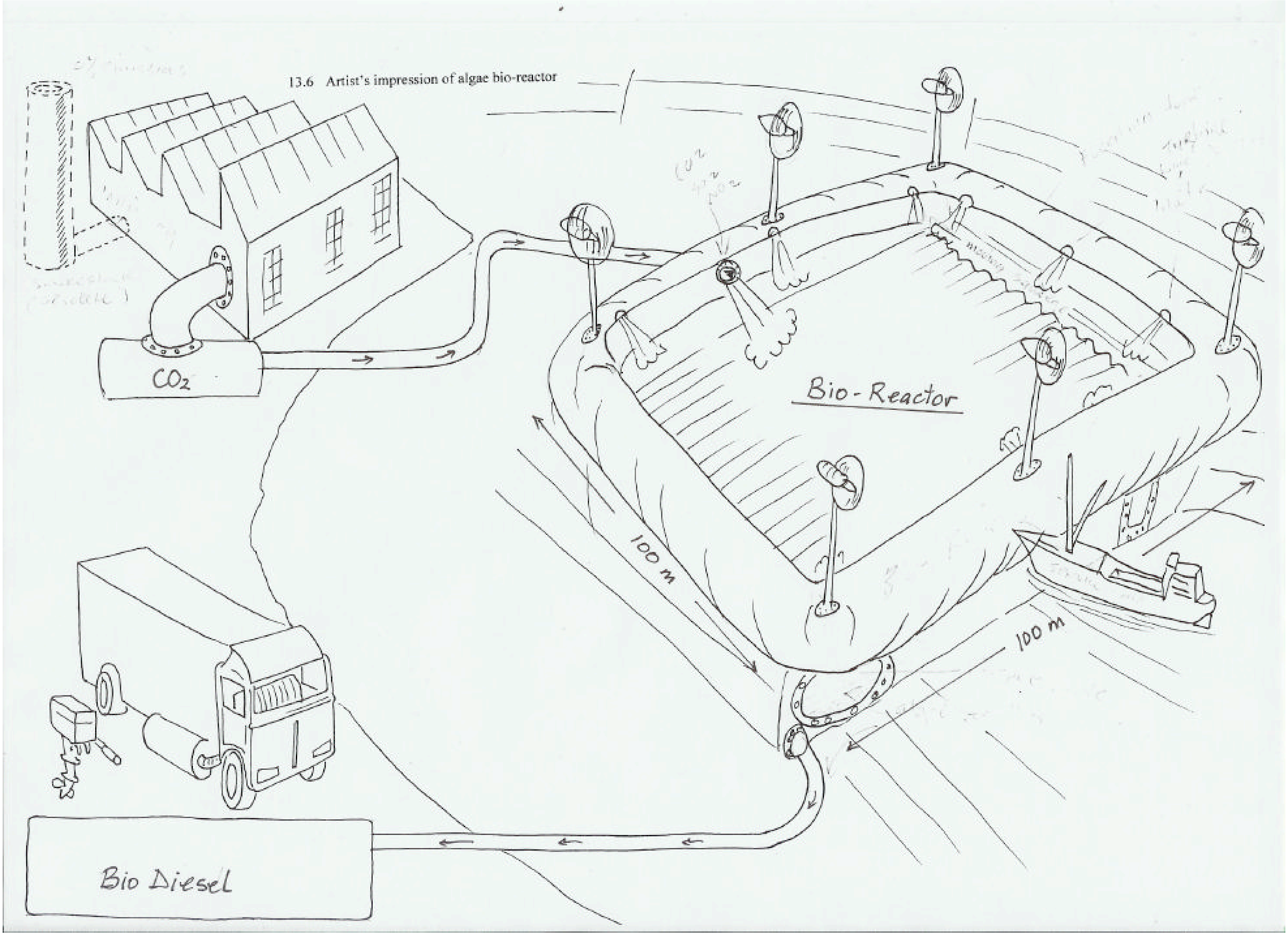
13.6 Artist's impression of algae bio-reactor



13.7 Appendix 7: Range of temperature variations in algal growth flasks.

From tome to time the temperature in the growth flasks was recorded (at 4 times over the course of the day) to get a picture of the range of temperatures that the algae were exposed to.

Preliminary trials:

Date	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**
19/8/07	5	19	19	12
26/8/07	6	18	21	13
1/9/07	6	19	22	14
8/9/07	7	19	21	13

Actual Investigation:

Date	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**	**Temp at 8.30am (^oC)**
21/9/07	10	19.5	21	14.5
22/9/07	7	23	25	16
23/9/07	9	23	19	15

To minimise heat/cold stress, the flasks were places in water to a depth of approximately 2 cm while growing (5 – 20 days). This was important because at CSIRO, Hobart, where these micro-algae are grown for use as feedstock in aquaculture, they are maintained at a constant temperature of 21^oC.