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# Determining inorganic and organic carbon

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Abstract

Carbon is the element which makes up the major fraction of lipids and carbohydrates, which could be used for making biofuel. It is therefore important to provide enough carbon and also follow the flow into particulate organic and potential loss to dissolved organic forms of carbon. Here we present methods for determining dissolved inorganic carbon, dissolved organic and particulate organic carbon.

Key words: dissolved inorganic carbon, dissolved organic carbon, particulate organic carbon, pH

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# 1 Introduction

Carbon (C) is a fundamental component for all life. It is fixed from carbon dioxide ($CO_2$) into biomass during photosynthesis, and microalgae account for approximately half the carbon fixation globally [1]. In culture, being able to measure different carbon pools is useful for understanding the conditions the algae have for carbon fixation (e.g., ensuring that they are not carbon limited) and follow the flow into particulate organic carbon and potential loss to dissolved organic forms of carbon.

Particulate organic carbon is fundamental for understanding biological relevant stoichiometric relationships, most notably the carbon:nitrogen:phosphorus (C:N:P) ratio, but other ratios can be useful, such as carbon to lipids ratio, which reveal information about carbon allocation of the algae. The Redfield ratio is often used as an indicator of algal growth requirements [2]. This ratio is originally derived from the average nutrient need for marine microalgae and the availability of these nutrients in the open ocean. The Redfield ratio is 106 carbon atoms to 16 nitrogen atoms to 1 phosphorus atom, but there is large variability of this ratio depending on the species cultivated and available resources (C, N, P). For example, increasing light leads normally to higher C fixation and an increase in the C:N:P ratio; in addition temperature and pH might also affect the stoichiometric ratio of the harvested biomass [2, 3]. For biofuel as the end product of microalgal cultivation, getting the C:N:P ratio as high as possible is beneficial as it is the carbon which forms the main component of lipids and carbohydrates.

In this chapter we present methods for determining dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC). DIC is a direct

measure of carbon availability for photosynthesis, but how much of the DIC is in the form of $CO_2$ is depending on the pH [4, 5]. As such, pH is a good indicator of carbon availability as removal of $CO_2$ during photosynthesis increases pH (dissolved $CO_2$ is a weak acid). Measurements of DIC are needed for most methods used to determine carbon fixation (e.g., converting measurements of radiolabeled $CO_2$ fixation into total carbon fixation). Some of the carbon fixed during photosynthesis may be released into the water as DOC. High concentrations of DOC can inhibit growth and would in most cases increase bacterial production and biomass.

During DIC measurements, the carbonates within the sample are converted with an acid to $CO_2$ which is detected with an infrared gas analyser [5]. The DOC method is based on high temperature catalytic oxidation (> 700 °C) of a sample from which particulate carbon has been removed by filtration and all inorganic carbon has been removed by acidification and sparging [4]. DOC can be determined together with total dissolved nitrogen (TDN) and POC with particulate organic nitrogen (PON), which is presented in a separate chapter. The method for determining POC is based on filtration and is consequently a measure of all particulate carbon in the culture, but unless there are other particles in the cultivation water, it would be equal to the carbon found in the algal biomass.

# 2 Materials

Use only analytical grade reagents.

### 2.1. Dissolved inorganic carbon

1. 25-50 ml glass BOD bottles with stoppers.
2. 100 ml volumetric flasks.

3. Sodium bicarbonate ($NaHCO_3$).
4. Spatulas.
5. Analytical balance.
6. Small aluminum cups (approximately 5 mm Ø, 5 mm high).
7. Ultrapure water (e.g., Milli-Q).
8. 1 ml glass syringe.
9. Dissolved inorganic carbon analyzer with instrument-specific carrier gas and oxidizing agents.
10. Concentrated nitric acid (65% $HNO_3$).
11. Refrigerator.

**2.2 Dissolved organic carbon**

1. 24 ml glass autosampler vials with screw caps.
2. Oven (450 °C).
3. Concentrated hydrochloric acid (37% HCl).
4. Ultrapure water (e.g., Milli-Q).
5. 0.7 µm glass fiber filters (25 mm Ø) and swinnex filter holders.
6. Disposable 20 ml sterile syringes.
7. Potassium hydrogen phthalate ($C_8H_5KO_4$).
8. Spatulas.
9. Analytical balance.
10. 100-1000 ml volumetric flasks.
11. Adjustable pipettes and pipette tips.
12. Ultrasonicator bath.
13. Plastic film (e.g., Parafilm).

14. Refrigerator and freezer.
15. Carbon analyzer with autosampler and instrument-specific carrier gas and oxidizing agents.

### 2.3 Particulate organic carbon

1. 0.7 µm glass fiber filters (e.g., 25 mm Ø).
2. Concentrated hydrochloric acid (37% HCl).
3. Forceps.
4. Ultrapure water (e.g., Milli-Q).
5. Oven (450 °C).
6. Vacuum filtration device with a vacuum pump.
7. Small (30 mm Ø) disposable petri dishes.
8. Aluminum foil circles (30 mm).
9. Handheld plier suitable for making small balls out of aluminum.
10. 96-well plates.
11. Bunsen burner.
12. Ethanol (96%).
13. Glycine ($C_2H_5NO_2$).
14. Spatulas.
15. Analytical balance.
16. 100 ml volumetric flask.
17. Adjustable pipettes and pipette tips.
18. Refrigerator.
19. CN elemental analyzer with autosampler and instrument-specific carrier gas and oxidizing agents.

# 3 Methods

Use acid-washed glassware (soaked for 4 h in 6% HCl and rinsed with deionized water).

### 3.1 Dissolved inorganic carbon

1. Combust the sample bottles and aluminum cups for 4 h at 450 °C, and cool them to room temperature before use.
2. Prepare standards by weighing sodium bicarbonate (0-100 mg) in small aluminum cups (see Note 1). Put the cup into a 100 ml volumetric flask and fill up with ultrapure water.
3. Prepare acid solution for DIC analyzer (see Note 2).
4. Take samples and put it in BOD bottle. The samples can be stored in a refrigerator for a short time (1-2 h) (see Note 3).
5. Prepare the carbon analyzer for analyses according to the operation manual (see Note 4).
6. Place standards in the autosampler of an analyzer for a calibration curve and linearity test (see Note 5) or measure the standards manually (see Note 6).
7. Prepare the samples for analyses by filtering (see Note 7). The sample can be stored in a refrigerator for a short time (1-2 hours).
8. The samples might need to be prepared before analyses by filtering (see Note 7).
9. Run the samples with the analyzer or manually by measuring 3-5 replicates per sample. Verify proper operation of the instrument with reference material, and include ultrapure water blanks in the run (see Note 8).
10. In manual analyses, calculate DIC concentration by the calibration curve (see Note 9).

3.2. **Dissolved organic carbon**

1. Combust glass vials and filters for 4 h at 450 °C, and cool them to room temperature before use.
2. Prepare 2 mol/l HCl by dissolving 33.2 ml concentrated hydrochloric acid in ultrapure water, and fill up to a volume of 200 ml. Store in a glass bottle.
3. Filter the sample with the syringe and syringe filter. Rinse the syringe with the sample before filtering. Discard first 5 ml of the filtrate and collect 20 ml into the glass vial. Prepare filter blank by filtering ultrapure water.
4. Add 80 µl 2 mol/l HCl to the glass vial.
5. Put on the lid, and place the glass vial in a refrigerator or freezer until it can be run in the carbon analyzer (see Note 10).
6. Prepare a stock standard solution containing 1000 mg C/l by dissolving 2.125 g potassium hydrogen phthalate (pre-dried at 105 ºC for 1 h) in ultrapure water, and then add ultrapure water to a final volume of 1 l. Store the stock solution in a glass flask in the refrigerator (stable for 1 month).
7. At the time of measurements, prepare suitable working standards (e.g., 0-10 mg/l) for the calibration of the analyzer (see Note 11). Pipette potassium hydrogen phthalate stock solution and 2 mol/l HCl (200 µl/100 ml) into volumetric flasks, and fill with ultrapure water (for blanks only ultrapure water and HCl). Calibration standards should be made weekly.
8. Fill each calibration vial about 1/2 to 2/3 full, put on a plastic film (see Note 12), and make holes on the film with a clean syringe needle.

9. Prepare the carbon analyzer for analyses according to the operation manual (see Note 4).
10. Place standards in the autosampler of an analyzer and run the standards for a calibration curve and linearity test (see Note 5).
11. Take the samples out of refrigerator/freezer, and let them come to room temperature.
12. Place the samples in an ultrasonication bath for 10 min.
13. Remove the lids, and put on a plastic film (see Note 12), and make holes on the film with a clean syringe needle.
14. Run samples with the analyzer (see Note 13). Verify proper operation of the instrument with reference material (Consensus Reference Water or equivalent), and include ultrapure water blanks in the run (see Note 8).

**3.3 Particulate organic carbon**

1. Prepare glass fiber filters by placing them in an acid bath (6% HCl) for 4 h and rinse then thoroughly with plenty of ultrapure water (see Note 14).
2. Combust the filters and aluminum circles for 4 h at 450 °C, and cool to room temperature.
3. Place a filter in the filtration stand, and apply suction with the vacuum pump.
4. Pipette a known volume of culture through the filter (see Note 15). Rinse with 5 ml ultrapure water to make sure all POC is on the filter. For blanks use ultrapure water.
5. Remove the filter with forceps while the suction is on (see Note 16). Rinse the filtration device with ultrapure water between samples.
6. Place the filter in the small Petri dish, and dry it (see Note 17). For blanks, place pure glass fiber filters in the Petri dishes.

7. Prepare a stock standard solution for POC by dissolving 1.340 g glycine in ultrapure water, and add ultrapure water to a final volume of 100 ml. Store the stock solution in a glass flask in a refrigerator (stable for 1 month).
8. Prepare standards for the calibration of the analyzer by adding a known volume (e.g., 40 µl) glycine stock solution onto glass fiber filters, and dry the (see Note 17).
9. Place dried filters on the aluminum circles, one filter per circle.
10. Fold with two clean forceps (cleaned with ethanol and burned).
11. Make into a small ball with a handheld plier.
12. Put the small balls into a 96-well plate (see Note 18).
13. Prepare the analyzer for analyses according to the operation manual (see Note 4).
14. Place the aluminum balls in the autosampler of the analyzer (see Note 19). Verify proper operation of the instrument with reference material, and include blanks in the run (see Note 8).

# 4 Notes

1. Prepare 3-5 standards according to the level of DIC in the water you are measuring.
2. Specifics of acid and carrier gas vary between instruments. We have used 1 % $HNO_3$ for Elektro-Dynamo URAS 3-E DIC instrument (dissolve 11 ml concentrated nitric acid in ultrapure water, fill up to a volume of 1000 ml, and store in a glass bottle).
3. The point is that no head spaced exists so that there is no gas exchange. The bottles should preferably be kept a refrigerator as temperature determines gas solubility, i.e., keeping them warm would create bubbles inside, which would lead to underestimation of the concentration of inorganic carbon.

4. It is critical to have proper training before running the analyzer. This will depend on the system that you are using, and it is beyond the scope of this chapter to go into the details of the operation of the instrument.
5. Calibration curve is prepared by plotting measured area of standards versus standard concentrations (linear regression).
6. In manual injection it is important that there is no air getting into the sample chamber, so make sure that all air bubbles get loose by tapping on the syringe, and push them out before adding the last 300 µl into the sample chamber.
7. Samples must be pre-filtered in cases where calcium-containing organisms are cultivated in order to measure only the dissolved inorganic carbon.
8. Start each sample run with blank(s) and reference material to verify proper operation of the instrument. Analyze samples in sequences of max 20 samples followed by a blank and reference material. Insert also standards in the sequence to test accuracy and drift during the analytical runs.
9. Determine concentration in the sample by the slope of the calibration curve.
10. These can be stored in the refrigerator for up to 3 weeks, in the freezer for up to 6 months.
11. We have used ca. 5-10 different dilution steps for calibration depending on the concentration span of the samples.
12. This is to prevent contamination of other samples in case of bubbles forming during measurements.
13. We have for Shimadzu TOC-$V_{CPH}$ with ASI-V auto sampler. The sample is first purged with $CO_2$ free air to remove inorganic carbon, and then an aliquot is injected onto a combustion tube filled with platinum-coated alumina beads. The sample is purged through the combustion column where non-purgeable organic carbon

compounds are converted to CO2, which is detected by a non-dispersive infrared detector.

14. Using a large filtration device (e.g., 50 mm Ø) you may place several filters on top of each other, and suck ultrapure water through the filters.
15. There should be a clear color on the filter but not forming a ‘cake’ of biomass. The detection limits of the instrument used to determine the POC should be considered when making the filtration, and measuring the dry weight first will give you some idea of how much water you need to filter.
16. This is to minimize the water content as much as possible.
17. Make sure to use lids not closing the Petri dish completely, or alternatively use some aluminum foil, to let the filter dry completely. The filter can be stored dry at room temperature in a desiccator.
18. Marking the sample name with well plate name makes it easy to follow the samples.
19. We have used Europa Scientific ANCA 20-20 Stable Isotope Analyser with a Roboprep-CN Biological Sample Converter. The combustion tube is filled with $Cr_2O_3$ (oxidation catalyst), CuO wire and Ag wool. The balls are combusted at 1000 °C to oxidase hydrocarbons, and the gases produced ($CO_2$, etc.) are transferred through a reduction tube for measurement in the detector of the analyzer.

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