Protocols for the quantification and characterization of dissolved organic carbon from seaweed and its sequestration potential

ARPA-E Seaweed CDR Project (UCSB, WHOI, UCLA)

Chance J. English[1], Craig A. Carlson[1]

[1]Marine Science Institute/Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California

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Abstract

Seaweed cultivation for atmospheric CO_2 sequestration requires full accounting of net primary production (NPP). In addition to large amounts of observable biomass produced, a fraction of NPP is released as dissolved organic carbon (DOC), which can fuel heterotrophic metabolisms or resist remineralization and contribute to carbon export and sequestration. We currently lack the ability to predict the amount of NPP released as DOC, and published estimates range from <1 to 62%. This white paper describes technical and experimental considerations needed to quantify and characterize DOC released by seaweeds as well as designs to determine the fraction which is recalcitrant. Additionally, we recommend key variables that should be used to parameterize DOC production for its inclusion in large-scale seaweed farm models.

Introduction

A proposed marine-based strategy to reduce atmospheric CO_2 levels is the purposeful cultivation of seaweeds which take up inorganic carbon from the surface ocean and convert it to organic carbon. Seaweed cultivation CO_2 removal (CDR) can contribute to sequestration in two ways: the conveyance of biomass to depth and the release of recalcitrant dissolved organic carbon. Recalcitrant DOC is the fraction of DOC that resists rapid microbial degradation and can contribute to long-term carbon storage in the ocean (Hansell, 2013). Interest in recalcitrant DOC production as a pathway for ocean CDR was stimulated by a synthesis by Krause-Jensen and Duarte (2016), who concluded that natural seaweed ecosystems have the potential to sequester 0.17 PgC yr^{-1} (0.6 Gt CO_2 yr^{-1}) globally, 70% of which is in the form of recalcitrant DOC. However, this estimate is based on average DOC production by phytoplankton and global marine DOC export efficiency (Baines and Pace, 1991; Hansell et al., 2009). Measurements of extracellular DOC release by seaweeds range from <1-62% of net primary production (NPP) and may be a function of light, nutrient concentrations, or physiological condition (Khailov and Burlakova, 1969; Sieburth, 1969; Abdullah and Fredriksen, 2004; Wada et al., 2007; Reed et al., 2015; Paine et al., 2021; Weigel and Pfister, 2021; Zhao et al., 2023). Also unknown are factors controlling the composition of seaweed DOC, which is thought to play an important role in its recalcitrance (Hansell, 2013; Carlson and Hansell, 2015; Dittmar, 2015; Shen and Benner, 2020).

This paper introduces suggested methods to quantify and characterize DOC released by seaweeds and their sequestration potential.

1. Controls on DOC Release by Seaweed

1.1 Mechanisms of DOC Release

An important component of ocean CDR is monitoring, reporting, and verifying (MRV) carbon transfer between different pools so that the net sequestration of atmospheric CO_2 can be calculated. For seaweed cultivation, this includes the transfer of inorganic carbon from the surface ocean into biomass and DOC. At the proposed scale of seaweed cultivation, measuring DOC released into the surface ocean will be difficult, so models will be required for MRV. While we can currently estimate the biomass of seaweed stocks with allometric and remote sensing techniques (Rassweiler et al., 2008; Reed et al., 2008; Bell et al., 2015; Bell and Siegel, 2022), no comparable method is available for DOC release. Therefore, our research program aims to develop an empirical model that predicts DOC release based on properties that can be measured at the scale of proposed seaweed farms. Such properties include nutrient concentrations, light, tissue chlorophyll, carbon and nitrogen content, tissue age, and biomass (Snyder et al., 2020; Bell and Siegel, 2022).

Several studies observe a wide range in seaweed DOC exudation, yet the controls on this release are poorly constrained (Khailov and Burlakova, 1969; Brylinsky, 1977; Wada et al., 2007; Reed et al., 2015; Watanabe et al., 2020; Paine et al., 2021; Weigel and Pfister, 2021). It is thought that seaweeds release DOC via the same mechanisms as phytoplankton: active exudation (overflow model), passive release (passive diffusion model), or leakage from senescence and grazing (Carlson and Hansell, 2015; Paine et al., 2021; Thornton, 2014; Weigel & Pfister, 2021). Understanding which mechanism is active in DOC production is important as it may determine the composition and bioavailability of the photosynthate (Barber 1968; Dittmar, 2015; Shen and Benner, 2020). Under the overflow model, autotrophs actively release recently fixed organic carbon to dissipate excess energy when nutrients and light become uncoupled (Fogg, 1963). According to this model, carbon-rich DOM production should increase with light and photosynthetic rate and be carbon-rich (Baines and Pace, 1991; Sambrotto et al., 1993). Alternatively, passive release results from the accumulation of DOM within cells which then leak out across a concentration gradient. DOM released by this mechanism would be composed of low molecular weight compounds such as dissolved free neutral sugars or amino acids, be correlated with biomass and surface area, and continue at night (Bjørrisen, 1988). Lastly, the leakage of intracellular material by cell-lysis can result in the release of nitrogen- and phosphorus-rich DOM, such as proteins and nucleic acids, as well as storage carbohydrates such as laminarin (Weinbauer and Peduzzi, 1995; Chen and Wangersky, 1996; Middelboe and Jørgensen, 2006). This mechanism may be especially important in seaweeds like *Macrocystis pyrifera*, which undergo programmed senescence because of declining physiological conditions with age (Rodriguez et al., 2013; Bell and Siegel, 2022). These DOC release mechanisms are poorly constrained, and aspects of both passive and active release have been observed for phytoplankton and seaweed (Mague et al., 1980; Reed et al., 2015; Xu et al., 2021; Weigel and Pfister, 2021). Both DOC release mechanisms may occur with one becoming more important as environmental and physiological conditions of the organism change. Therefore, estimates of DOC release rates by seaweed should be conducted in tandem with measures of photosynthetic

rates, nutrient concentrations, light intensity, and tissue physiology (age, stoichiometry, pigment concentrations, surface area to volume, size, and dry mass).

1.2 Seaweed Incubation Design,

The incubation design for seaweed DOC release will depend on the organism of interest. Some species, such as *Macrocystis pyrifera,* are large. Typically a blade or blade punch has been used to measure DOC release and photosynthetic activity, whereas smaller seaweeds such as *Sargassum natans* or *Ecklonia cava* may be incubated as a whole organism (Wheeler, 1980a; Wada et al., 2007; Reed et al., 2015; Powers et al., 2019). Regardless of the seaweed, care should be taken to avoid damage to the tissue and desiccation before incubation, which may stimulate excess DOC release (Carlson and Carlson, 1984; Zhao et al., 2023). Additionally, incubations require water flow to prevent the formation of a turbulent boundary layer which can depress photosynthetic activity and DOC release (Wheeler, 1980b; Hurd, 2000; Mueller et al., 2016; Zhao et al., 2023). This can be achieved through the use of flexible chambers that transmit wave energy into the incubation (Miller et al., 2011), magnetic stir bars (Wheeler, 1980b; Mueller et al., 2016), or enclosed incubation chambers with a water pump (Zhao et al., 2023). Lastly, *in situ* temperature and salinity should be maintained throughout the incubation to prevent rapid changes in DOC exudation (Zhao et al., 2023).

Our group is currently assessing the potential of *M. pyrifera*, hereafter giant kelp, for seaweed-based CDR. Giant kelp is too large to be entirely enclosed for incubation; therefore, single-blade incubations are used to measure DOC release. Blades are clipped where the pneumatocyst meets the stipe to prevent damage to the lamina. Work with other laminarin seaweeds demonstrates no significant difference in DOC release rates when using clipped blades versus whole organisms (Weigel and Pfister, 2021). Our preliminary work with mature, clipped giant kelp blades show DOC release rates from 1.7 – 14 μmolC g_{DW} hr^{-1}. This is similar to rates observed by Reed et al. 2015 (0-12.5 μmolC g_{DW} hr^{-1}), who used polyethylene sleeves to incubate giant kelp blades without clipping. Mature blades (2m from the growing tip of a frond) are collected from Mohawk Reef in Santa Barbara, California (34° 23.660’ N, 119° 43.800’ W) and transported back to a nearshore laboratory in surface seawater and placed in 10L acrylic incubation tanks with 0.2 μm filtered seawater and magnetic stir bars to maintain flow. Incubations are conducted in triplicate at six different photosynthetically active radiation (PAR) levels (0-1350 μmol m^{-2} s^{-1}) for two hours. Blades are acclimated at incubation conditions for 20 minutes to prevent sampling of exudation driven by rapid changes to light, temperature, or salinity (Carlson and Carlson, 1984; Zhao et al., 2023).

1.3 DOC and DON Analyses

Detailed information on the best practices for DOC and DON sample collection, storage, and analyses can be found in Halewood et al. (2022). Briefly, duplicate samples for DOC and DON are taken from the beginning and end of each incubation. Samples are filtered through pre-combusted 25 mm GF-75 (nominal pore size of 0.3 μm) into pre-combusted 40 ml EPA vials with PTFE lined caps and acidified to pH < 3 with the addition of 60 μl of DOC clean 4N HCl. At least 20 ml of the sample are flushed through the GF-75 filter, and vials should be rinsed three times with ~10 ml of the sample before being filled ~¾ full. DOC concentrations are determined by the high-temperature combustion method using a TOC-V or TOC-L analyzer (Shimadzu,

Kyoto, Japan). Total dissolved nitrogen (TDN) is determined on the same instruments but with a detector for NO by chemiluminescence (Walsh, 1989). DON is calculated as the difference between TDN and dissolved inorganic nitrogen, so measurements for nitrate and nitrite are required.

DOC & DON exudation rates are calculated as follows:

$$\mu molC/N\ g_{DW}\ hr^{-1} = \frac{[DOC/N]_t - [DOC/N]_0 * V}{T * M} \qquad (1)$$

where $[DOC/N]_t$ and $[DOC/N]_0$ are the DOC or DON concentrations in µmol C or N L^{-1} at the end and beginning of each incubation, respectively. V is the volume (liters) of seawater during the incubation, T is the incubation time (hours), and M is the dry mass (grams) of the seaweed tissue used in the incubation.

1.4 Photosynthetic Rate

Constraining the fraction of fixed carbon released as DOC (i.e., percent extracellular release) requires simultaneous quantifying primary production and DOC exudation. Seaweed primary production can be measured in laboratory and field incubations through changes in oxygen and dissolved inorganic carbon (DIC) or the labeling of tissue with stable (^{13}C) or radio-isotopically (^{14}C) labeled bicarbonate (Wheeler, 1980b; Dunton and Jodwalis, 1988; Miller and Dunton, 2007; Miller et al., 2011; Weigel and Pfister, 2021). We chose to use changes in DIC as a measure of primary production as it does not require a photosynthetic quotient, can be sampled easily, and does not require costly labeled bicarbonate. Additionally, changes in DIC measures the simultaneous uptake of CO_2 and/or HCO_3^- during photosynthesis and the production of CO_2 from respiration and so is a measure of net primary production.

Samples are obtained simultaneously with DOC during incubations by overflowing a 125 ml glass serum bottle with seawater at least twice using silicon tubing placed at the bottom of the bottle. Samples are preserved with 100µl of saturated $HgCl_2$ (1g $HgCl_2$:10ml deionized water). The sample is sealed with a small headspace (<1% total sample volume) with a rubber stopper and aluminum crimp seal and stored at room temperature until analysis. DIC samples are analyzed using an automated infrared inorganic carbon analyzer (AIRICA; Marine Analytics and Data, Kiel, Germany). Briefly, 1.75 ml of the sample is injected into a gas stripper, where it is acidified with 10% H_3PO_4. Liberated CO_2 is carried by an inert reference gas (N_2) and measured with a non-dispersive infrared detector (LI-COR 7000, Illinois, USA). A condenser, Nafion drying tube, and magnesium perchlorate trap are used to remove water from the sample before it enters the LICOR cell. Three sample injections are used to determine the average pCO_2 peak area and converted to µmol C L^{-1} using a coefficient calculated from certified reference material (as specified in Dickson et al., 2007). Reference materials are run every 12 samples to check for analytical stability throughout a run. Photosynthetic rates are determined as follows:

$$\mu molC\ g_{DW}\ hr^{-1} = \frac{[DIC]_0 - [DIC]_t * V}{T * M} \qquad (2)$$

where $[DIC]_0$ and $[DIC]_t$ are the DIC concentrations (µmolC L^{-1}) at the beginning and end of each incubation, respectively. V is the volume (liters) of seawater during the incubation, T is the incubation time (hours), and M is the dry mass (grams) of the seaweed tissue in the incubation.

1.5 Environmental & Physiological Variables

Light, nutrients, and intrinsic biotic factors (age, tissue stoichiometry, and pigment concentrations) control seaweed growth and may also control the magnitude of DOC release (Wheeler, 1980a; Reed et al., 2015; Mueller et al., 2016; Bell et al., 2018; Powers et al., 2019; Paine et al., 2021; Weigel and Pfister, 2021; Xu et al., 2021). Studies of seaweed DOC release show higher DOC release rates during the day than at night (Abdullah and Fredriksen, 2004; Reed et al., 2015; Powers et al., 2019; Buck-Wiese et al., 2023). However, daytime DOC release rates of single species show conflicting responses to changes in PAR (Reed et al., 2015; Weigel and Pfister 2021; Zhao et al. 2023), possibly because these studies do not control for blade physiological state or nutrient concentrations. Mueller et al. 2016 observed DOC release increased with light availability, but this relationship disappeared when excess nutrients were added. Weigel and Pfister 2021 found DOC release rates increased under a combination of high tissue C:N and low nutrient uptake rates. This suggests that nutrient availability and intrinsic biotic factors modulate the relationship between DOC exudation and light as observed for seaweed carbon fixation rates (Wheeler, 1980a; Rodriguez et al., 2016; Dean and Jacobsen, 1984). Age may be especially important for seaweeds such as giant kelp that undergo progressive senescence. Incubations with seaweed should evaluate DOC exudation and primary production across light levels using seaweed cohorts with similar ages and tissue stoichiometry.

For our incubations, light levels are controlled using a dimmable full spectrum LED light source (VIPARSPECTRA XS4000) and measured with a handheld quantum PAR meter (Phantom PHOTOBIO). DOC exudation and photosynthesis are measured from six mature giant kelp blades collected 2m from the tip of a growing frond under six different PAR levels from 0-1350 μmol m^{-2} s^{-1}. Nutrients (nitrate + nitrite) are measured using flow injection analysis with a QuikChem 8500 Series 2 (Lachat Instruments, Wisconsin, USA). Nutrient samples are collected in 20ml scintillation vials alongside DOC samples using the same GF-75 filter and stored at -20°C until analysis. Intrinsic biotic factors are determined by known methods (Seely et al., 1972; Rodriguez et al., 2016; Bell et al., 2018). Briefly, tissue age is measured by tagging up to 100 fronds 2 m back from the growing tip and sampling one blade at the tagged site from six different fronds. Tagged fronds are sampled at the tag site every 2-3 weeks over 100 days (Rodriguez et al., 2016; Bell and Siegel, 2022). Tissue stoichiometry is measured as the C:N ratio determined using a CE-440 CHN/O/S elemental analyzer (Exeter Analytical, Massachusetts, USA). Following incubations, tissue is rinsed with 10% HCl followed by deionized water to remove any $CaCO_3$ from epibionts. Blades are dried at 60 °C for three days, after which dry mass is recorded and then ground to a fine powder and analyzed for carbon and nitrogen content. To measure pigment concentrations (Chlorophyll a, fucoxanthin, chlorophyll c), a 0.8 cm^2 disk is excised from the tissue before drying, cleaned of any epibionts, and weighed. Pigment samples are placed in 4 ml of dimethyl sulfoxide for 45 minutes at room temperature in the dark. The disk is removed, rinsed with 1 ml of ultrapure water, and placed in a 5ml mixture of acetone, methanol, and ultrapure water (3:1:1) for 2 hours at 4°C in the dark (Seely et al. 1972; Wheeler 1980a; Bell et al. 2018). The dimethyl sulfoxide and acetone:methanol:water extracts are then placed in 1 cm^3 quartz cuvettes and absorbance is measured from 350 nm to 800 nm using a UV 2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Concentrations of Chl a, Chl c, and fucoxanthin are calculated from absorbance following Seely et al. (1972).

1.6 Results

Our incubation design captures a typical relationship between NPP and irradiance and shows that DOC release has a similar relationship to light (Figure 1a). DOC production increased linearly with NPP and was a constant fraction of NPP (3.7 ± 0.8%) across light levels greater than zero (Figure 1b). When PAR was zero, DOC production continued, which has been observed in other studies (Abdullah and Fredriksen, 2004; Weigel and Pfister, 2021; Buck-Wiese et al., 2023), and may be a substantial loss of NPP at night. Percent extracellular release was constant; however, this could change along with physiological (i.e. age, tissue stoichiometry) and environmental conditions (i.e. temperature, nutrients).

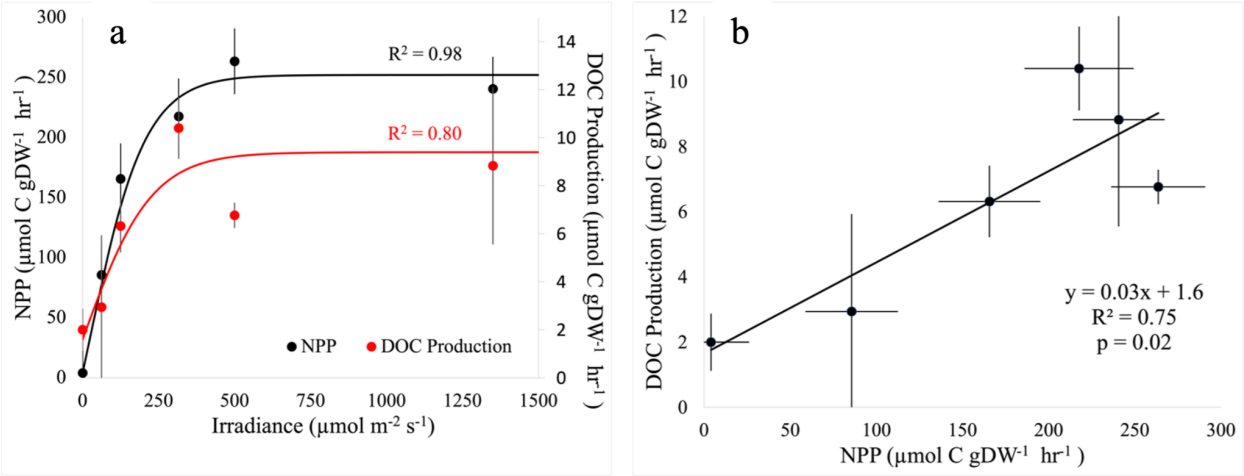

Figure 1. (a) Observed (circles) and modeled (lines) DOC production and NPP response to irradiance. Modeled rates follow a hyperbolic tangent function described by Jassby and Platt 1976. R^2 values (Model I linear regression) compare observed and modeled rates. (b) DOC release increases linearly with NPP. Inset are the results of model II linear regression.

2. Seaweed DOM Composition

2.1 Total Carbohydrates and Sugar Monomers

Carbohydrates are a large fraction of marine particulate and dissolved organic matter (Borch and Kirchman, 1997; Kaiser and Benner, 2009; Goldberg et al., 2009). Brown seaweed exudates are enriched in the sugars fucose, galactose, and glucuronic acid (Wada et al., 2007; Haas and Wild, 2010; Nelson et al., 2013), which has been linked to the exudation of fucoidan (Buck-Wiese et al., 2023). Large sulfated polysaccharides such as fucoidan require hundreds of enzymes to be degraded by marine bacteria, which may enhance their sequestration potential (Sichert et al., 2020). Additionally, these fucose-containing sulfated polysaccharides precipitate DOC and form marine snow aggregates, which may enhance their export out of the surface ocean (Huang et al. 2021; Vidal-Melgosa et al. 2021). Lastly, the specific sugar composition of carbohydrates select for different bacteria (Nelson et al., 2013) and should be evaluated to constrain potential impacts to microbial communities.

Total carbohydrates can be measured colorimetrically using 2,4,6-tripyridyl-s-triazine (TPTZ) following Myklestad et al. 1997. Briefly, 1 ml of the sample is hydrolyzed for 20 hours

at 100°C. An acid strength of 0.4 M HCl yielded the highest concentration of sugars compared to 0.1 and 0.8 M HCl, consistent with the dominance of fucose in macroalgal exudates (Engel and Händel, 2011). Following hydrolysis, samples are neutralized under N_2 at 50°C. After the first evaporation, samples are resuspended in 0.5 ml of ultrapure water and evaporated under N_2 to fully neutralize the sample. Once neutralized, samples are resuspended in 1 ml of ultrapure water and reacted with the following reagents:

A: 150 ml ultrapure water + 79.4 µl 50/50 Sodium Hydroxide + 3g Sodium Carbonate + 34.5mg Potassium Ferricyanide

B: 150 ml 5M Acetic Acid + 24.6 g Sodium Acetate + 6.3 g Citric Acid + 48.6 mg Ferric Chloride

C: 250 ml 3M Acetic Acid + 195 mg TPTZ

One ml of reagent A is added and reacted at 100°C for 10 minutes, followed by one ml of reagent B and two ml of reagent C. Samples are then vortexed, allowed to cool and their absorbance is measured in a $1cm^3$ cuvette at 595 nm. Samples are calibrated against a standard curve (1-100 µMC) of a known reducing sugar, reacted in the same manner as samples. We recommend using L-fucose as the standard sugar instead of glucose as it is the main sugar in seaweed carbohydrates (Wada et al., 2007; Nelson et al., 2013; Buck-Wiese et al., 2023). Fucose has a lower relative absorbance response than glucose using the TPTZ method, so total carbohydrate concentrations would be underestimated if glucose was used as the standard (Myklestad et al., 1997). A limitation of the TPTZ method and other colorimetric carbohydrate assays is they fail to measure mannitol, a low molecular weight sugar alcohol that functions as an osmolyte in brown macroalgae (Reed et al., 1985; Reed and Wright, 1986). However, mannitol is degraded by bacteria quickly and preferentially to larger polysaccharides such as alginate (Moen et al., 1997) and so likely does not contribute to the recalcitrant DOC pool. If desired, mannitol can be measured with established protocols using Gas Chromatography/Mass Spectrometry (Sogin et al., 2019).

Specific sugar components of seaweed carbohydrates can be measured using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). We follow dialysis and gradient protocols specified in Engel and Händel 2011. Briefly, samples are dialyzed overnight using Spectra/Por 7 tubing (1 kDa membrane cutoff) against ultrapure water and hydrolyzed for 20 hours at 100°C in 0.4 M HCl and neutralized under N_2. Samples are analyzed using a DIONEX ICS5000+ (ThermoFisher, Massachusetts, USA) and separated using a Carbopac PA10 column (4x250mm) with a Carbopac PA10 guard column (4x50mm). Neutral and amino sugars are eluted isocratically with 18mM NaOH followed by 100mM NaOH/200mM Na-Acetate to elute acidic sugars. The system is calibrated with a standard sugar mix containing fucose, rhamnose, arabinose, galactosamine, glucosamine, galactose, glucose, mannose+xylose, galacturonic acid, and glucuronic acid. Linearity of the calibration curves was observed for concentrations ranging from 10nM-10µM. Dialyzed samples, which include only carbohydrates larger than 1 kDa can be further analyzed using the TPTZ method to determine the fraction of carbohydrates that are high (>1 kDa) and low (<1 kDa) molecular weight. The amount of low molecular weight carbohydrates is calculated as the difference between total carbohydrates and high molecular weight carbohydrates.

2.2 Sulfate Content

Fucoidan contributes up to 50% of brown macroalgal DOC (Buck-Wiese et al., 2023). These large polysaccharides are highly sulfated and difficult to degrade due to the diversity of enzymes required to remove sulfate groups, hydrolyze glycosidic linkages, and metabolize fucose (Sichert et al., 2020). These fucoidans have a range in sulfate content (Sichert et al., 2021), which may change their bioavailability. The sulfate content of macroalgal exudates can be determined following the procedure to measure sugar monomers by HPAEC-PAD (Section 2.1). Following dialysis, hydrolysis, and neutralization, liberated sulfate from the hydrolysis of sulfate ester groups can be measured by ion chromatography. Sulfate is measured on a Metrohm 930 equipped with a Metrosep A Supp 5 column. Samples are eluted isocratically with a 0.32M/0.1M sodium carbonate/bicarbonate buffer with a 20-minute run. Sulfate is eluted between 11 and 12 minutes and calibrated against a standard range from 0.1 to 100 ppm.

2.3 Total Phenolic Content & Colored DOM

Brown seaweeds are a large source of polyphenolic compounds (Sieburth and Conover, 1965; Carlson and Mayer, 1983; Powers et al., 2019). These aromatic compounds have an absorption peak at 270 nm and may function as UV absorbents and antimicrobial agents (Wada et al., 2007; Le Lann et al., 2016; Powers, 2020). Polyphenolic compounds, such as lignin and condensed tannins are important recalcitrant components of soil organic matter (Freeman et al., 2001; Zak et al., 2019; McGivern et al., 2021), but the fate of algal polyphenols in the ocean is unknown. Polyphenols are 2-20% of DOC exudates in brown seaweeds (Sieburth and Conover, 1965; Carlson and Carlson, 1984; Abdullah and Fredriksen, 2004; Powers et al., 2019) and therefore, could be an important component of seaweed sequestration.

Total phenolic content (TPC) is measured using the Folin-Ciocâlteu method (Box, 1983; Takeda et al., 2013; Powers et al., 2019). Salt interferes with the measurement, so samples must be desalted before analysis. Samples can be desalted using Spectra/Por 7 dialysis membranes or by solid-phase extraction (Takeda et al., 2013; Powers et al., 2019). Desalting with a 1 kDa cutoff also removes small reducing sugars, disaccharides, and free amino acids, which can react with the Folin-Ciocâlteu reagent and give erroneously high TPC values (Sánchez-Rangel et al., 2013). After desalting, 3.6 ml of sample is reacted with the following:

A: 400µl 1M Sodium Hydroxide and 200 µl Folin-Ciocâlteu reagent

B: 4ml of 2M Sodium Carbonate and 1.8 ml of ultrapure water to bring the final volume to 10ml.

The sample is reacted with **A** at room temperature for 30 minutes in the dark, followed by **B** for 30 minutes at 40°C in the dark. Absorbance is measured on a Thermo Scientific Genesys Spectrophotometer (Cole-Palmer, Illinois, USA) with a 10 cm pathlength quartz cuvette at 720 nm and calibrated against a standard curve of phloroglucinol ranging from 1-100 µMC phloroglucinol equivalents.

Phenolic compounds derived from seaweeds have an absorption peak at ~ 270 nm (Wada et al., 2007; Hulatt et al., 2009; Powers, 2020) and can be monitored using UV-Vis spectroscopy. CDOM samples are 0.2 µm filtered and collected simultaneously with DOC samples and stored

at 4°C in pre-combusted 60 ml amber vials with PTFE-lined caps. Absorption spectra are obtained using an UltraPath™ liquid core waveguide scanning spectrophotometer (World Precision Instruments, Florida, USA) with a 1.943 m path length. Samples are corrected for baseline drift and salinity following Nelson et al., 2007. Samples with high contributions of seaweed DOC (>50 μM Carbon) should be diluted to prevent saturation when using a long path length.

2.3 Results

Using the incubations described above (Section 1.6), accumulated DOC are collected and characterized by HPAEC-PAD and the Folin-Ciocâlteu colorimetric method for neutral sugars and polyphenols, respectively. From the incubations, neutral sugars and polyphenols accounted for 30 ± 21% and 24 ± 16% of the accumulated DOC. Carbohydrates are enriched in fucose, galactose and glucuronic acid, consistent with the exudation of fucoidan (Buck-Wiese et al., 2023).

3. Remineralization of Seaweed DOC

3.1 Remineralization Bioassays

Remineralization bioassays are essential to quantify the fraction of seaweed-derived DOC that is bioavailable to microbial remineralization at the surface versus how much survives degradation and is available for export below the mixed layer (Carlson et at., 1994; Baetge et al., 2020). In addition, microbial remineralization experiments with mesopelagic microbes (>100 m) simulate vertical mixing and investigates bioavailability to mesopelagic bacterioplankton which are thought to specialize in degrading recalcitrant DOM (Carlson et al. 2004; Landry et al. 2017; Saw et al. 2020; Liu et al. 2020). Remineralization culture experiments are set up as seawater dilution cultures in which a source microbial assemblage from the euphotic and mesopelagic zones (1.2 - 3.0 μm filtrate) are inoculated into a naturally occurring seawater media (0.2 μm filtrate) (Carlson et al. 2004). Accumulated DOC from seaweed incubations (Section 1.2) is then amended into these batch cultures and the concentration and composition is measured over time. The cultures are incubated at in situ temperatures in the dark for one year. Measurements include bacterioplankton abundance, DOM concentrations, total carbohydrates, sugar composition, total phenol content, and CDOM and are collected from incubations in periods from days to one year. DNA samples targeting the 16s rRNA V4 region are also collected to assess changes in microbial community composition in response to seaweed DOC (Wear et al. 2018; Liu et al. 2020). Changes in DOC composition will determine the compounds produced by seaweed that are bioavailable or contribute to long-term carbon sequestration.

3.2 Photomineralization

Some studies demonstrate that a fraction of accumulated seaweed DOC is rapidly oxidized when exposed to light (Shank et al. 2010; Wada et al. 2015). This photooxidation occurs alongside the degradation of seaweed DOC optical properties indicating polyphenols such as phlorotannins may be oxidized or degraded when exposed to light, limiting their potential to contribute to sequestration. Photooxidation experiments should be conducted to determine the fraction of DOC oxidized by light in the surface ocean. Additionally, the production of partial

oxidation products such as CO has been observed from seaweed DOC (Shank et al. 2010) and should be quantified as it can affect atmospheric redox chemistry and methane residence time (Valentine and Zepp 1993; Conte et al. 2019; Ossola et al. 2022).

Photomineraliation experiments are conducted in 15-20 ml quartz vials using an LS1000W Solar Simulator (Solar Light CO. Inc, Pennsylvania, USA). Seaweed exudate is collected and filled into quartz vials without headspace and closed with PTFE-lined caps. Experiments should be conducted at seawater salinity (~33-36 ppt) and pH (~8.1) as both can influence the rate and magnitude of DOM photo-degradation or oxidation (Minor et al. 2006; Zhang et al. 2019). Samples are placed in a circulating water bath to maintain temperature and irradiated for 48 hours. Vials are sampled sacrificially at 0, 24, and 48 hours and subsampled for DOC concentrations, optical properties (CDOM), total phenol content, dissolved inorganic carbon (DIC) and carbon monoxide (CO), and dioxygen (O_2). DOC, CDOM, TPC, and DIC are measured as described above. CO production is measured following Ossola et al. 2022 and incubated in custom quartz serum vials without headspace and closed with Teflon-lined rubber seals. CO is measured by transferring ~6 ml of irradiated sample to an N_2 flushed 20 ml serum vial using a disposable syringe. Samples are shaken for 20 minutes and allowed to come to room temperature before analysis on an 8610C gas chromatograph (SRI, California, USA) and calibrated using a CO standard balanced with N_2. Oxygen is measured using an optical oxygen probe (PreSens; Regensburg, Germany) calibrated according to manufacturer protocols. Oxygen concentrations are corrected for temperature and salinity (Garcia and Gordon, 1992; Bittig et al., 2018). Lastly, exposure to light can degrade high molecular weight DOC into smaller molecules (Helms et al., 2008) which may enhance their lability. Also, conversely, partial photo-oxidation of DOC can produce more recalcitrant DOC compounds such as carboxyl-rich alicyclic molecules (Hertkorn et al. 2006; Ward and Cory 2020). Seaweed DOC that has been exposed to light should be further assayed using remineralization experiments (Section 3.1) to determine the cumulative losses to heterotrophic and photo-oxidation.

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