Release of Dissolved Organic Carbon from the Estuarine Intertidal Macroalga *Enteromorpha prolifera*

A. M. Pregnall

Oregon Institute of Marine Biology; Charleston, Oregon 97420, USA

Abstract

The estuarine macroalga *Enteromorpha prolifera* was collected from Coos Bay, Oregon, USA during 1981, and its release of photosynthate as dissolved organic carbon (DOC) was studied using $^{14}$C as a tracer. During photosynthesis in 30% S sea water, with a fixation rate averaging 7.37 mg C g$^{-1}$ dry wt h$^{-1}$, release ranged from 0.13 to 0.57 mg C g$^{-1}$ dry wt h$^{-1}$ and from 1.65 to 6.23% of total fixed carbon. Release of DOC appears to be linear with time over 3 h. As exposed algae become increasingly desiccated, their photosynthetic rates decline dramatically, but upon reimmersion the highly desiccated algae lose a larger fraction of their fixed carbon than the slightly desiccated algae. This loss comes in a pulse release of DOC over the initial 15 min, followed by declining release rates. The pulse loss due to rainfall is 5 times greater than that due to tidal resubmergence, and may briefly exceed the prior photosynthetic rate. Although lowering the salinity from 30 to 5% does not substantially alter photosynthetic rates, it does increase the DOC release range up to 1.02 mg C g$^{-1}$ dry wt h$^{-1}$ and 16.10% of fixed carbon. Heterotrophic microbes from the algal habitat readily use the available DOC at about 15% h$^{-1}$.

Introduction

Marine macrophytes photosynthetically fix large quantities of carbon into organic substances. While it is assumed that most of this material becomes available to the aquatic community as particulate detritus some time after synthesis (Odum and de la Cruz, 1967), much of it enters the environment as dissolved organic carbon (DOC) both during photosynthesis and following senescence (Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974; Brylinsky, 1977).

Most of the DOC released during photosynthesis appears to consist of relatively small organic molecules, which are likely to be metabolic intermediates (Wetzel and Manny, 1972; Sondergaard, 1981). The abundant heterotrophic microbes in natural habitats readily utilize these substances by assimilation and respiration (Nalewajko and Lean, 1972; Bauld and Brock, 1974; Williams and Yentsch, 1976; Brylinsky, 1977).

The macrophyte assemblages of major importance in estuaries are usually the salt marshes and seagrass beds (Correll, 1978), both of which have been found to release DOC (Gallagher et al., 1976; Penhale and Smith, 1977). The estuary of Coos Bay, Oregon, USA has very little salt marsh remaining (Hoffnagle and Olson, 1974), but does have many large eelgrass beds. However, from 10 to 70% of the standing crop of these eelgrass beds consist of the associated green algae (Gonor et al., 1979). Additionally, the numerous intertidal flats support seasonally abundant populations of macroalgae, primarily of *Enteromorpha* spp. (Chlorophycophyta: Ulvales). The two major species are *E. prolifera*, a long, profusely branching, filamentous form, and *E. linza*, a flat, sheet-like form. Both species maintain high photosynthetic rates while submerged (King and Schramm, 1976; Littler and Littler, 1980).

The intertidal habitat of *Enteromorpha* spp. mats in Coos Bay subjects them to the typical fluctuations of repeated exposure and resubmergence, potential desiccation and rainfall stress, and variable estuarine salinity. These factors affect photosynthetic rates and DOC release in some marine macrophytes (Sieburth, 1969; Johnson et al., 1974; Moebus et al., 1974; Penhale and Smith, 1977; Quadir et al., 1979; Gordon et al., 1980). The purposes of the present study were to quantify the release of DOC from actively photosynthesizing thalli of *E. prolifera*, with particular attention to the above-mentioned environmental fluctuations, and to investigate the potential for use of this DOC by heterotrophic microbes.

Materials and Methods

Samples of *Enteromorpha prolifera* were collected from mudflats in the South Slough arm of Coos Bay, Oregon,
USA at +2.0 ft (0.6 m above mean lower low water, MLLW) during August–October, 1981. Thalli were gently rinsed in sea water to remove sediments, small grazers, and epiphytes, and then held overnight in aerated, ambient-temperature sea-water aquaria.

For determinations of photosynthetic rates and DOC release rates, algae were incubated in either 300 ml bottles or 1.5-liter Plexiglas chambers with magnetic stir bars. Each Plexiglas chamber had two stoppers through which fluids could be injected or withdrawn by syringe and an insert grid to which the algae were attached.

Gently blotted algae of known fresh weight were placed in the incubation vessels, and sterilized synthetic sea water (Rila Sea Salts) of the required salinity was then added. The dissolved carbon dioxide, bicarbonate, and carbonate concentrations of the water were determined beforehand using the techniques of Strickland and Parsons (1968). The average algal density over all experiments was 0.29 g dry wt l\(^{-1}\). Bottles and chambers were maintained at 16°C in a water bath. 20 \(\mu\)Ci of NaH\(^{14}\)CO\(_3\) (New England Nuclear) in 1 ml was added to the 1.5-liter chambers, and 4 \(\mu\)Ci to the 300 ml bottles, with a final specific activity of about 0.82 \(\mu\)Ci mg\(^{-1}\) dissolved inorganic carbon. All incubations were performed outside between 11:00 and 15:00 hrs on clear, sunny days in early fall to ensure that light intensities would be above saturation (King and Schramm, 1976). Dark controls were wrapped in foil, and samples were taken just after the introduction of label to establish initial background activities.

At the end of the 3 h incubation, algae were fixed for 1 h in 100 ml of 5% formalin in 30% sea water adjusted to pH 2.0 with HCl. Following dry weight determination, the algae were ground to a fine powder, and the activities of aliquots of 10 to 30 mg were counted by liquid scintillation. Aliquots of the algal fixative were also counted, for some activity leached from the algae. Each sample was counted 3 times for either 15 min or to 1.5% accuracy on a Beckman LS 150 Scintillation Counter. Corrections for quench were made using standard curves of percent counting efficiency versus external standards ratio.

At specified intervals during the incubations, 3 ml water samples were removed from the vessels and acidified to pH 2 in scintillation vials with 0.5 ml of the algal fixative described above. These were then flushed for 10 min with CO\(_2\)-free air. Controls indicated that less than 0.1% of inorganic label remained after flushing. The resulting acid-stable \(^{14}\)C activity is the experimental measure of DOC.

Desiccation of exposed algae was determined by placing variable amounts of fresh algae in 100 \(\times\) 15 mm petri dishes with a known weight of water-saturated sediment covering the bottom. The uncovered dishes were positioned in a random grid and placed outside in the late morning. At time intervals up to a total desiccation duration of 3 h, the total weights of algae, sediment, and dish, and of just sediment and dish were measured. Dry weights of algae and sediments were determined after overnight drying in a 90°C oven.

For measurements of photosynthesis in air, a modification of the procedure of Darley et al. (1976) was used. Desiccated algal sections were placed in the 1.5-liter chambers with 20 \(\mu\)Ci of tracer in 1 ml in a cup, after which the chambers were equilibrated in the water bath, sealed, and \(^{14}\)CO\(_3\) liberated by the addition of 1 ml of 85% lactic acid to the cup. After 20 min, the algal pieces were removed and subdivided. One half of each section was fixed, and carbon uptake was determined as before. The other half was immersed in 100 ml synthetic sea water for 1 h, and DOC release was measured.

**Results**

In seven 3 h incubations of *Enteromorpha prolifera* samples in 30% S sea water, net carbon fixation in the light averaged 7.37 mg C g\(^{-1}\) dry wt h\(^{-1}\), and DOC release averaged 0.26 mg C g\(^{-1}\) dry wt h\(^{-1}\), giving a mean of 3.5% of recently fixed carbon lost (Table 1). Dark fixation and release rates were both less than 1% of the light rates. Fig. 1 shows the time course of the accumulation of DOC for one of the incubations. Over the 3 h of the experiment, DOC accumulation appeared to be linear, with a constant release rate.

The algal mats on the mudflats in Coos Bay occur between MLLW and +5.0 ft (1.5 m), and are thus uncovered by many or all outgoing tides. As tidal patterns and daylength change, the amount of time that these algae spend exposed varies. The degree to which algae become desiccated depends highly upon the duration of exposure and upon the amount of algae present. Fig. 2 indicates the moderating effect that increased standing crop has upon desiccation. If the algal mat is less than about 1 cm thick, the algae may quickly lose internal water, while above 1 cm thickness, only the surface elements of the mat

![Graph](image-url)
Table 1. Enteromorpha prolifera. Carbon fixation and dissolved organic carbon (DOC) release rates for algae in 30%o S sea water

<table>
<thead>
<tr>
<th>Carbon fixation rate (mg C g⁻¹ dry wt h⁻¹)</th>
<th>DOC release rate (mg C g⁻¹ dry wt h⁻¹)</th>
<th>% DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light incubation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.05</td>
<td>0.13</td>
<td>2.1</td>
</tr>
<tr>
<td>6.28</td>
<td>0.21</td>
<td>3.3</td>
</tr>
<tr>
<td>6.27</td>
<td>0.31</td>
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<tr>
<td>6.86</td>
<td>0.20</td>
<td>2.9</td>
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<tr>
<td>7.89</td>
<td>0.27</td>
<td>3.4</td>
</tr>
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<td>9.05</td>
<td>0.15</td>
<td>1.7</td>
</tr>
<tr>
<td>9.18</td>
<td>0.57</td>
<td>6.2</td>
</tr>
<tr>
<td>mean: 7.37 ± 1.34</td>
<td>0.26 ± 0.15</td>
<td>3.5 ± 1.6</td>
</tr>
<tr>
<td>Dark incubation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.020</td>
<td>0.003</td>
<td>15.0</td>
</tr>
<tr>
<td>0.010</td>
<td>0.001</td>
<td>10.0</td>
</tr>
<tr>
<td>0.008</td>
<td>0.003</td>
<td>37.5</td>
</tr>
<tr>
<td>mean: 0.013 ± 0.006</td>
<td>0.002 ± 0.001</td>
<td>20.8 ± 14.6</td>
</tr>
</tbody>
</table>

become dried to any great extent, and the lower layers remain quite moist and capable of photosynthesis if they are not too greatly shaded.

As the algae become increasingly desiccated, their photosynthesis drops quickly (Fig. 3) until they are barely fixing carbon after 50% fresh weight loss. However, when these desiccated algae are reimmersed by the incoming tide, they lose some of their recently fixed carbon. The greater the fresh weight loss during drying, the greater the fraction of their fixed carbon lost becomes (Fig. 4).

Exposed algae may either be submerged by the incoming tide or be subjected to rainfall. Previously labelled algae were desiccated to 65% fresh weight, and carbon fixation was determined from a subsample. A portion of the algae was reimmersed in 100 ml of 30%oS synthetic sea water, and another was subjected to simulated rainfall: 100 ml fresh water was sprinkled through an inverted Buchner funnel, and the algae allowed to remain covered by the accumulated water. Water samples were removed at 0, 15, 30, 60, and 180 min after initiating reimmersion or rainfall and analyzed for DOC. Release rates were calculated for each time interval between samplings. These slightly desiccated algae release DOC upon reimmersion in a rapid pulse over the first 15 to 30 min (Fig. 5). If the reimmersion is due to steady rainfall, the loss of DOC is greater in both magnitude and duration; initially, the rate of release may be greater than the prior photosynthetic rate.

While the carbon fixation rate of Enteromorpha prolifera is slightly reduced to an average of 5.6 mg C g⁻¹ dry wt h⁻¹ at lower salinities, it does not vary consistently or greatly over the range tested here (30 to 5%), with a standard deviation of only 0.66 mg C g⁻¹ dry wt h⁻¹ (N = 10). However, the fraction of fixed carbon that is lost as DOC increases to about 15% at 5%, from the more typical 5% at 30% (Fig. 6). It is not known whether the DOC release is reduced at higher salinities.
The labelled material that Enteromorpha prolifera releases during short-term photosynthetic incubations probably consists of intermediate metabolites. The heterotrophic microbes from the algal habitat, present on the algal thalli as epiphytes and in the sediments, should prove capable of taking up labelled DOC from sea water. To test this, 150 ml of water in which algae had been incubating and releasing labelled DOC for 3 h were added to replicate foil-wrapped bottles containing either 20 g of mudflat surface sediment or 3.5 g fresh weight of heavily epiphytized E. prolifera. The bottles were held at 16°C for 3 h and hand-swirled every 30 min to circulate the water without stirring up the sediments. The initial and final DOC concentrations were determined as above. Controls were autoclaved sediments and algae. Table 2 shows the decrease in DOC activity in the presence of these microbes. The control reductions are likely to be due to adhesion of the organic molecules to particulates. Thus, over a 3 h period, about 40% of the available DOC was utilized by sediment-associated microbes, and about 47% was utilized by epiphytic microbes.

In a separate experiment, 300 ml of DOC-containing water from incubations with Enteromorpha prolifera and from E. linza was incubated for 5 h with sediments. There was a control-corrected decrease of 75.4±5.8% of the DOC from E. prolifera, and a decrease of 71.5±4.1% of the DOC from E. linza. The rate of utilization appears to increase directly with the amount of DOC available, and there is no apparent difference in the use of DOC by the two different species (Fig. 7).
Discussion

Release of dissolved organic carbon from Enteromorpha prolifera falls among the higher values reported in the literature, particularly for studies using 14C incorporation and release techniques (Khailov and Burlakova, 1969; Sieburth, 1969; Moebus and Johnson, 1974; Brylinsky, 1977; Penhale and Smith, 1977); this is largely due to the high photosynthetic rate of this alga. The DOC activity of E. prolifera is composed of recently labelled materials, so there may well be other substances being released which have not been detected. Thus, all values reported here are conservative. However, because of the intertidal location of the alga, the duration of submergence in daylight and the period of such photosynthesis may be only a few hours at a time, so the values measured here should be realistic.

As compared to my data, the release rates reported by Khailov and Burlakova (1969) and by Sieburth (1969) are higher, presumably due to (1) differences in their methods of measurement, which would detect release of materials synthesized far prior to the time of release, and (2) the potential injury or senescence of their algal material, as they suggest. Moebus and Johnson (1974) found substantial loss of DOC from injured holdfasts of fucoid algae. The potential for overestimation of normal DOC release due to injury of Enteromorpha prolifera in the present study is small, for only the cells at the very ends of the thin filaments would be broken. The carbon-fixing mechanisms of the broken cells would be disrupted, which should reduce the incorporation of label into stable products. The slightly higher release rates for submerged photosynthesis reported by Sieburth (1969) coupled with the lower photosynthetic rates of his brown algae, relative to E. prolifera, result in his much higher percent release values of 25% compared with about 3.5% in the present study.

The materials most likely to be released during photosynthesis would be relatively small, labile molecules such as amino acids, sugars, organic acids, and sugar phosphates. Sondergaard (1981) determined that most of the DOC released by the freshwater macrophyte Littorella uniflora has a molecular weight of approximately 200. Wetzel and Manny (1972) determined that about 90% of the organic matter excreted by another freshwater macrophyte, Najas flexilis, are sugars and other labile, low-molecular-weight compounds. Fogg (1976) found measurable release of glycollate from some tropical marine macrophytes. Patil and Joshi (1970) found a high intracellular turnover of such metabolites in Ulva lactuca, with the ethanol-soluble activity remaining fairly constant over several hours, while the ethanol-insoluble components continued to increase in activity. If the pool of potentially releasable molecules is fairly constant in size, one would expect a constant release rate, with a linear accumulation of DOC in the incubation medium.

While some algae, particularly the high-intertidal fucoids, increase their photosynthetic rates in air after some desiccation (Johnson et al., 1974; Quadir et al., 1979), Enteromorpha prolifera shows much reduced carbon fixation rates after as little as 30% fresh weight loss. Such a reduction in photosynthesis is typical for algae with very thin thalli (Imada et al., 1970; Johnson et al., 1974; Wiltens et al., 1978; Quadir et al., 1979).

The observations that increased desiccation of algae results in an increased fraction of fixed carbon lost upon reimmersion and that the release comes in a pulse over the first 15 min suggest that moderate desiccation can be quite stressful to the cellular stability of Enteromorpha prolifera. The increased magnitude and duration of DOC release indicate that rainfall on exposed algae is another severe stress. These increases are likely to be due to the influence of reduced salinities as well as the shock of resubmergence.

It is possible that the organics released following reimmersion under stressful conditions include not only the smaller metabolites presumed for typical submerged release, but also larger, more complex substances such as polypeptides and structural carbohydrates released by cell-wall damage. Such damage could well result in a large but brief pulse release after reimmersion.

Sieburth (1969) found reduced DOC release with lower salinities in fucoid algae and suggested that it was due to lowered photosynthetic rates. The total carbon fixation rate of Enteromorpha prolifera was not reduced with decreased salinity in the present study, and DOC release increased at lower salinities; thus the release is probably passive and may be affected by the osmotic relation between the cells and the surrounding medium.

All of the typical estuarine fluctuations in this study of Enteromorpha prolifera increased the rate of DOC release and/or the fraction of fixed carbon released. This indicates that field populations of the algae are repeatedly confronted with a variety of stresses, and that the fragile structure of these green algae (relative to that of the fucoids or laminarians, for example) provides little protection. They are thus able to achieve standing crop levels of 100 to 350 g dry wt m⁻² over a period of weeks only by virtue of their substantial carbon fixation rates.

The heterotrophic microbes from the algal habitat removed much of the available labelled DOC from the incubation medium over the course of a few hours. If indeed the DOC consists primarily of useful metabolic intermediates, the microbes might scavenge them from the water by some active transport mechanism against a concentration gradient. Additionally, if the microbes were previously adapted to the availability of such substrates in their habitat, such transport would occur without a lag time for the potential induction of necessary permeases. The observation that the DOC removal rate increases linearly with DOC availability over the range of concentrations measured suggests that such a presumed active transport has not yet reached saturation. However, the same result is also consistent with the possibility that DOC removal is due to a passive process such as diffusion of labile molecules into microbial cells, if the molecules are quickly metabolized in some way to maintain the necessary concentration gradient.
The populations of *Enteromorpha* spp. which dominate the intertidal flats of Coos Bay in summer and fall, and which comprise a substantial part of the eelgrass communities, contribute large quantities of organic matter to the estuary. Much of this photosynthetically fixed material enters the environment as DOC. All of the typical estuarine fluctuations tested here increased DOC release. Most of this organic matter probably consists of labile metabolic intermediates, which the heterotrophic microbes readily utilize. This estuarine carbon pathway is characterized by short residence times in any of the components; thus, such a material and energy flux would presumably be underestimated by static measurements of any single component.

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**Literature Cited**


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