Do Photochemical Transformations of Dissolved Organic Matter Produce Biorefractory as well as Bioreactive Substrates?

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ABSTRACT

Photochemical processes appear to have an important impact on the cycling of dissolved organic matter (DOM) in a wide variety of aquatic environments. Numerous photoproducts of DOM, including dissolved inorganic carbon and low-molecular-weight organic compounds, have been identified, and the types of photoproducts and extent of phototransformation of DOM is dependent upon its chemical composition. Heterotrophic bacteria are the primary consumers of DOM, and photochemical transformations of DOM can affect its bacterial utilization in a variety of ways. Numerous studies spanning fresh and marine waters have demonstrated enhanced bacterial growth following exposure of DOM to solar radiation. These studies indicate that photochemical processes can enhance the microbial utilization of biorefractory DOM. Other studies in fresh and marine waters, however, have demonstrated that exposure of DOM to solar radiation can result in a reduction in bacterial growth rates, suggesting that photochemical and microbial processes "compete" for the same substrates. It appears that some photoproducts of DOM are resistant to microbial degradation, suggesting that photochemical processes could be involved in the production as well as destruction of biorefractory DOM.

Introduction

The last decade has seen increasing interest in the effects of ultraviolet (UV) radiation on ecosystem structure and function. This interest stems from observations of stratospheric ozone depletion [14] and uncertainty about the roles of UV radiation in biological processes and the cycling of bioactive elements. Recent studies have demonstrated inhibitory effects of UV radiation on organisms and biological processes [11, 25], and there are a multitude of indirect effects which could affect global biogeochemical cycles. This paper will summarize recent studies of the effects of UV radiation on the cycling of dissolved organic matter (DOM) in freshwater and marine environments. Interactions between UV radiation and DOM cycling are complex, and it is the authors' intention to investigate this topic primarily from the perspective of the impact of solar radiation on the microbial utilization of DOM.

Dissolved organic carbon (DOC) is the most abundant form of reduced carbon in most aquatic ecosystems, and it is one of the largest active reservoirs of reduced carbon on Earth [9]. As such, the cycling of DOC is a major component of the global carbon cycle, and understanding the factors regulating DOC cycling is critical for assessing the potential impact of environmental change on the global carbon cycle. Heterotrophic

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microorganisms, primarily bacteria, are the major consumers of DOM [23], and the decomposition of DOM is largely an enzymatically-mediated process. The production and consumption of DOM are considered predominant pathways of carbon flow in most aquatic environments. The process of microbial mineralization of DOM is very efficient, but some components of DOM are resistant to microbial degradation, and the potential role of UV radiation in the cycling of this biorefractory DOM is just beginning to emerge. Photochemical processes also appear to be involved in the production of biorefractory DOM, and this less studied aspect of DOM cycling is explored further in this paper.

The UV portion of the solar spectrum is considered responsible for most photochemical transformations of DOM [21], and DOM is responsible for most of the absorption of UV radiation in seawater [32] and lakes [22]. The penetration depths of UV radiation in natural waters are highly variable, with typical penetration in clear ocean water of ~20 m for UV-B (280-320 nm) radiation and ~50 m for UV-A (320-400 nm) radiation [25]. Photochemical transformations of DOM can occur through the direct absorption of light or through indirect, photosensitized, reactions. A variety of photoproducts have been identified following exposure of DOM to natural or simulated levels of solar radiation, including carbon dioxide, carbon monoxide, ammonium, phosphate, and numerous low-molecular-weight organic compounds [4, 6, 19, 20].

Experimental Considerations

A variety of experimental approaches has been used to investigate the effects of solar radiation on the microbial utilization of DOM in natural waters. The specific approach used will determine, to a large extent, the possible interpretations of the data. Numerous studies have used isolated fractions of DOM, such as humic substances. These studies can provide valuable information about the photoreactivity of specific substrates, but alteration of the natural milieu of water samples can have a large impact on experimental results. Light quality and quantity are extremely important variables that are influenced by the light source as well as the type of incubation vessel and depth of incubation. These and many other experimental and environmental conditions need to be considered in an evaluation of the influences of photochemistry on the cycling of DOM.

Heterotrophic bacterial growth is the most commonly measured biological response to photoalteration of DOM. Bacterial growth is measured as increases in cell abundance or as increases in protein and DNA production. Few studies include measurements of bacterial respiration as an indicator of changes in the bioreactivity of DOM after exposure to sunlight. It is important to recognize that bacterial growth efficiencies are variable in nature, and bacterial metabolism responds rapidly to changes in the quality as well as quantity of utilizable substrates. It is quite possible to observe an increase in bacterial growth while respiration rates remain constant or decrease. Thus, rates of bacterial production may not be indicative of rates of microbial consumption of DOC.

All commonly used experimental approaches for investigating the effects of irradiation on microbial activity in natural waters require some manipulations. The most common approach to investigate the effects of photochemical transformations on DOM bioavailability is to remove all organisms from water samples prior to incubation under light and dark conditions. Water samples are "filter-sterilized" (< 0.2 μm pore size), incubated under light and dark conditions, inoculated with a natural microbial population, and bacterial growth is measured during a dark incubation. Differences between the rates of bacterial growth in the light and dark incubated water samples are interpreted to result

Table 1.

Environment and Source of DOM	**Bacterial Activity Indicator**	**Effect of Exposure**	Reference
Macrophyte-dominated environments:			
Mesoeutrophic lake bulk DOM	Change in [DOC]	50% increase	[7]
Whole plant leachate (*Typha* & *Juncus*)	Bacterial production	50% increase	[28]
Blackwater river bulk DOM	Bacterial production	34 – 81% decrease	[1]
Boreal pond humic substances*	Bacterial production	100% increase	[4]
Humic lakes bulk DOM	Bacterial biomass	184 – 276% increase	[16]
Saltmarsh Humic substances*	Bacterial production	200% increase	[18]
Humic lake bulk DOM	Bacterial Growth	71% increase	[24]
Stratified lake bulk DOM	Bacterial production	35 - 80% increase	[12]
Humic pond humic substances* ammended with algal derived DOM	Bacterial respiration	15-20% decrease	[27]
Seagrass-dominated lagoon bulk DOM	Bacterial production	no effect	Ziegler & Benner (unpublished).
Phytoplankton-dominated environments:			
Coastal and open ocean bulk DOM	Uptake of pyruvate	increased uptake	[15]
Estuary bulk DOM amended with specific protein	Bacterial protein incorporation	up to 40% decrease	[13]
Surface ocean, (15-115 m) bulk DOM	bacterial production	77% decrease	[2]
Deep ocean, (150-1000 m) bulk DOM	bacterial production	41% increase	[2]

*Substances isolated using Amberlite XAD-8 resin.

from photochemical transformations of DOM. Studies using this and similar approaches have reported enhanced bacterial growth, no change in bacterial growth, and reduced bacterial growth following sunlight exposure (Table 1). Clearly a variety of environmental factors, such as the source and chemical composition of DOM, influence the results of such studies.

Comparison of Microbial Responses to Photoaltered DOM in Various Aquatic Environments

Results from selected studies of the effects of sunlight exposure on the microbial utilization of DOM in various aquatic environments are summarized in Table 1. Most of the cited studies investigated photochemical-microbial interactions in freshwater and estuarine environments that have relatively high concentrations of dissolved humic substances, which are known to be very photoreactive [30]. Most of the DOM in these systems is derived from macrophytes through the leaching of soils and plant tissues. This DOM is relatively rich in aromatic structures and is resistant to microbial degradation. Numerous studies have found that exposure of this DOM to sunlight typically enhances its bioavailability, as measured by increases in bacterial growth following irradiation (Table 1). Photoalterations of DOM enhanced bacterial growth in these environments by up to 200%. There were a few exceptions to this trend, and they are discussed below. Dissolved inorganic carbon is the major identified photoproduct of DOM [8, 19], but a wide variety of bioavailable low-molecular-weight photoproducts has been observed and it is presumed that these photoproducts are responsible for enhancing bacterial growth following exposure of DOM to sunlight [21].

Two studies listed in Table 1 under macrophyte-dominated environments noted a decrease in DOM bioavailability following sunlight exposure. Amon and Benner [1] studied the photochemical and microbial transformations of DOM in the Rio Negro, a large blackwater tributary of the Amazon River. DOM in this river was highly photoreactive, as indicated by rapid rates of DOC loss during exposure to sunlight (i.e. photomineralization). However, rates of bacterial growth in sunlight-exposed Rio Negro water were typically lower than those in dark controls. Apparently, bioreactive substrates can also be photoreactive, and microbial and photochemical processes can "compete" with each other for the same substrates. Bioreactive substrates could be photomineralized or phototransformed to biorefractory substrates resulting in reduced rates of bacterial growth in waters exposed to sunlight.

There is good evidence that photochemical transformations produce biorefractory as well as bioreactive substrates. Studies by Keil and Kirchman [13] and Tranvik and Kokalj [27] found that bacterial growth on relatively labile substrates (protein and algal extracts) was reduced after these labile substrates were added to waters with natural DOM and irradiated. Bacterial growth in similarly treated dark controls was considerably higher, indicating that photochemical transformations of the labile substrates reduced their bioreactivity. Both of these studies found that the presence of natural DOM was essential to induce the reduction in bioreactivity of labile substrates during irradiation, suggesting that natural DOM acted as a photosensitizer or became complexed with the labile substrates.

Sunlight exposure was found to have no measurable effect on the bioavailability of DOM in a shallow, seagrass-dominated lagoon on the Texas coast of the Gulf of Mexico [Ziegler and Benner, unpublished]. Bacterial growth rates are very high in this system, and

previous investigators noted diel patterns in bacterial growth with the highest rates occurring in the early afternoon [5]. The high rates of bacterial growth and water column respiration during daylight hours were found to be strongly correlated with the light-mediated release of DOM from seagrasses [31]. The DOM released by seagrasses is dominated by carbohydrates, which are rapidly utilized by bacteria in the water column (unpublished data). Some photobleaching of DOM was observed, but no photomineralization was measured indicating the DOM was not very photoreactive. It appears that photochemical transformations play a minor role in the cycling of DOM in this environment.

Much less work on photochemical-microbial interactions has been conducted in phytoplankton-dominated systems, particularly the oceans. The concentrations of DOM are much lower in seawater than in the macrophyte-dominated systems discussed earlier, and seawater DOM has less of an aromatic nature and lower concentrations of humic substances [10] suggesting rates of photochemical processes are considerably lower in oceanic environments. Pioneering studies by Kieber et al. [15] and Mopper et al. [20] demonstrated the photoproduction and consequent microbial utilization of specific low molecular weight carbonyl compounds in seawater. Higher rates of photoproduction of bioreactive substrates were found in deep ocean water than in surface water when exposed to surface levels of irradiation. The DOM in deep ocean water has a higher relative percentage of aromatic carbon and a much lower percentage of carbohydrate carbon than surface water DOM [3], and these differences in composition appear to affect photoreactivity. These studies clearly demonstrated the photoproduction of bioreactive substrates from marine DOM, but they did not investigate the overall effect of photochemical transformations on DOM bioavailability.

A recent investigation [2] of photochemical-microbial interactions in seawater was conducted using the same experimental approach as most studies in the macrophyte-dominated environments discussed earlier. Filtered (< 0.2 μm pore size) seawater collected from surface and deep waters was exposed to sunlight in a deck incubator, and rates of bacterial growth were measured in the dark following irradiation. A profile of the concentrations of DOC at the study site is presented in Figure 1-A. Concentrations of DOC were 70-80 μM above the chlorophyll maximum at 115 m and 48-61 μM from 150 to 1000 m depth. Rates of bacterial production in seawater that was exposed to sunlight are expressed as a percentage of the rates measured in parallel incubations kept in the dark (Fig. 1-B). There was a dramatic depth-dependent difference in the bacterial response to the exposure of seawater DOM to sunlight. Bacterial growth decreased by 68-84% in incubations with surface water that was irradiated, whereas bacterial growth increased by 22-79% in incubations with deep water that was irradiated (Fig. 1-B).

These results indicate the net photoproduction of biorefractory substrates in surface ocean water and the net photoproduction of bioavailable substrates in deep ocean water. It is important to recognize that this experimental approach provides a measure of the net bacterial response to the photochemical reactions that occur in irradiated water samples. We can not conclude that bioavailable substrates were not photoproduced in surface water or that biorefractory substrates were not photoproduced in deep water.

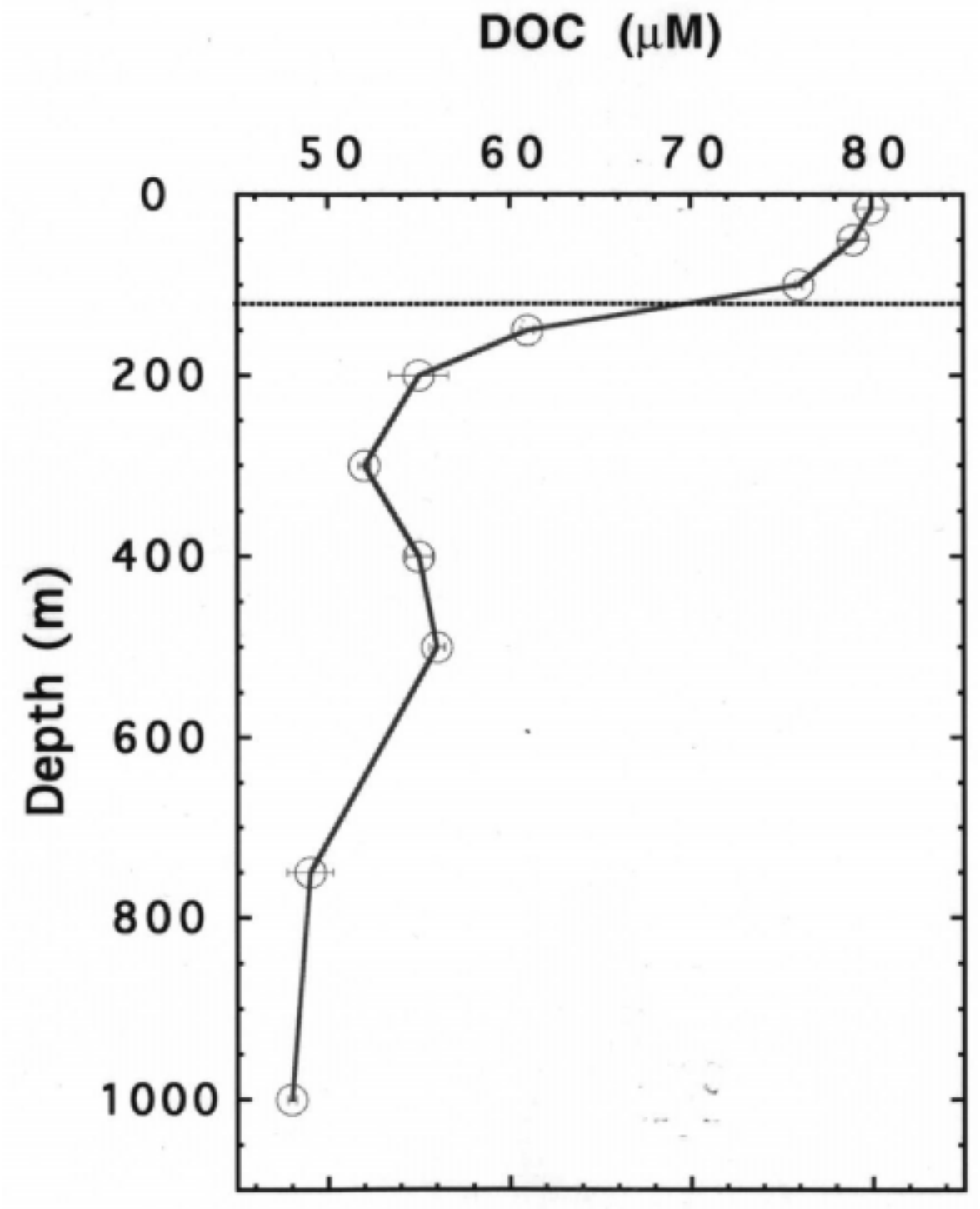
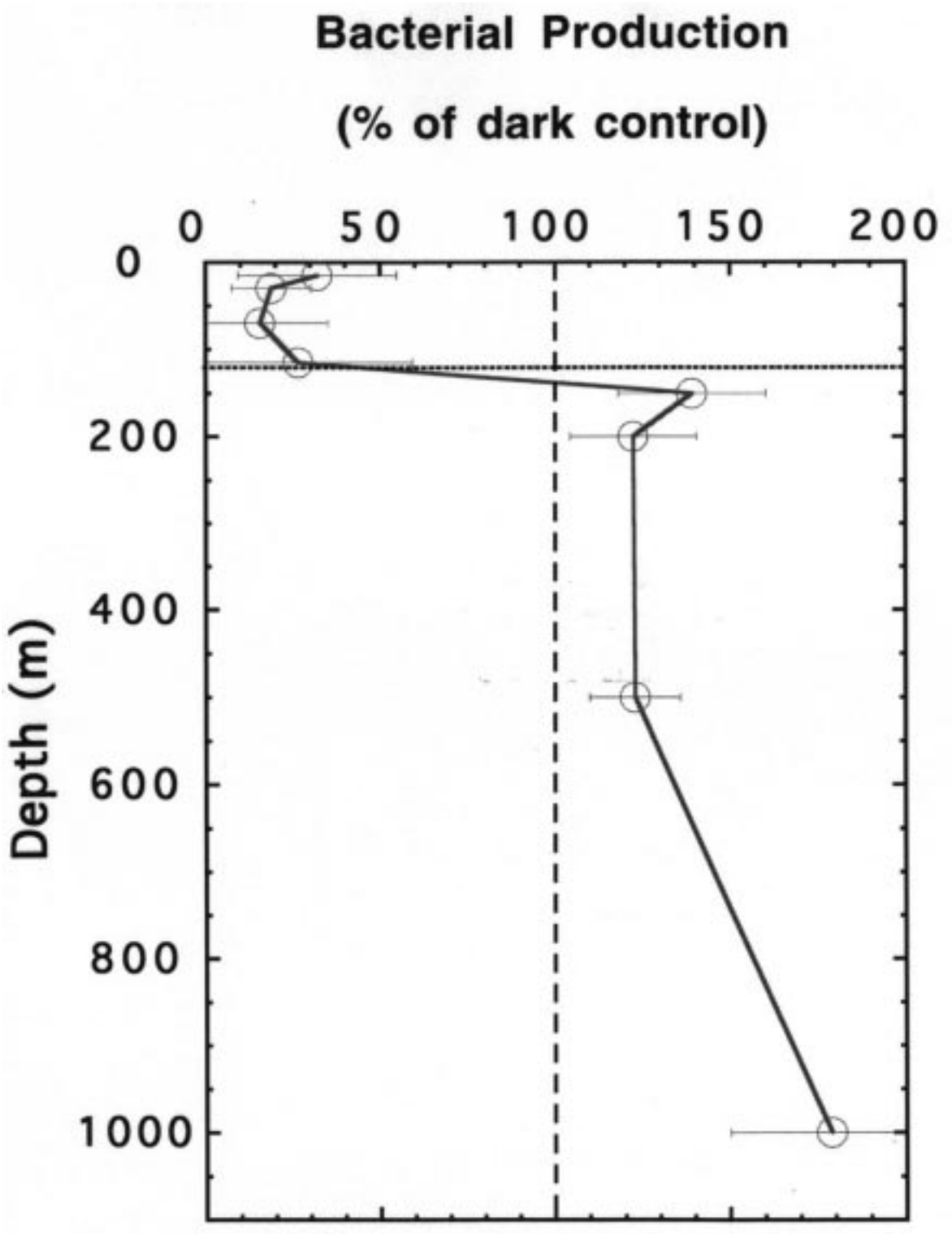


Fig. 1. Effects of solar radiation on the bacterial utilization of marine dissolved organic matter (from [2]). (A) Vertical distribution of dissolved organic carbon (DOC) in the Gulf of Mexico at 26°15' N and 94°00' W. The dashed horizontal line at 115 m depth denotes the chlorophyl maximum. (B -- next page) Bacterial production in seawater samples following exposure to solar radiation. Bacterial production is expressed as a percentage of production in parallel dark incubations. Dark controls are represented by the vertical dashed line. (Error bars represent one standard error.)


Bacterial Production
(% of dark control)
0
50
100
150
200
0
200
400
600
800
1000
Depth (m)

Effects of Photochemical Transformations on DOM Cycling

A conceptual diagram of the effects of photochemical transformations on DOM cycling is presented in Fig. 2.

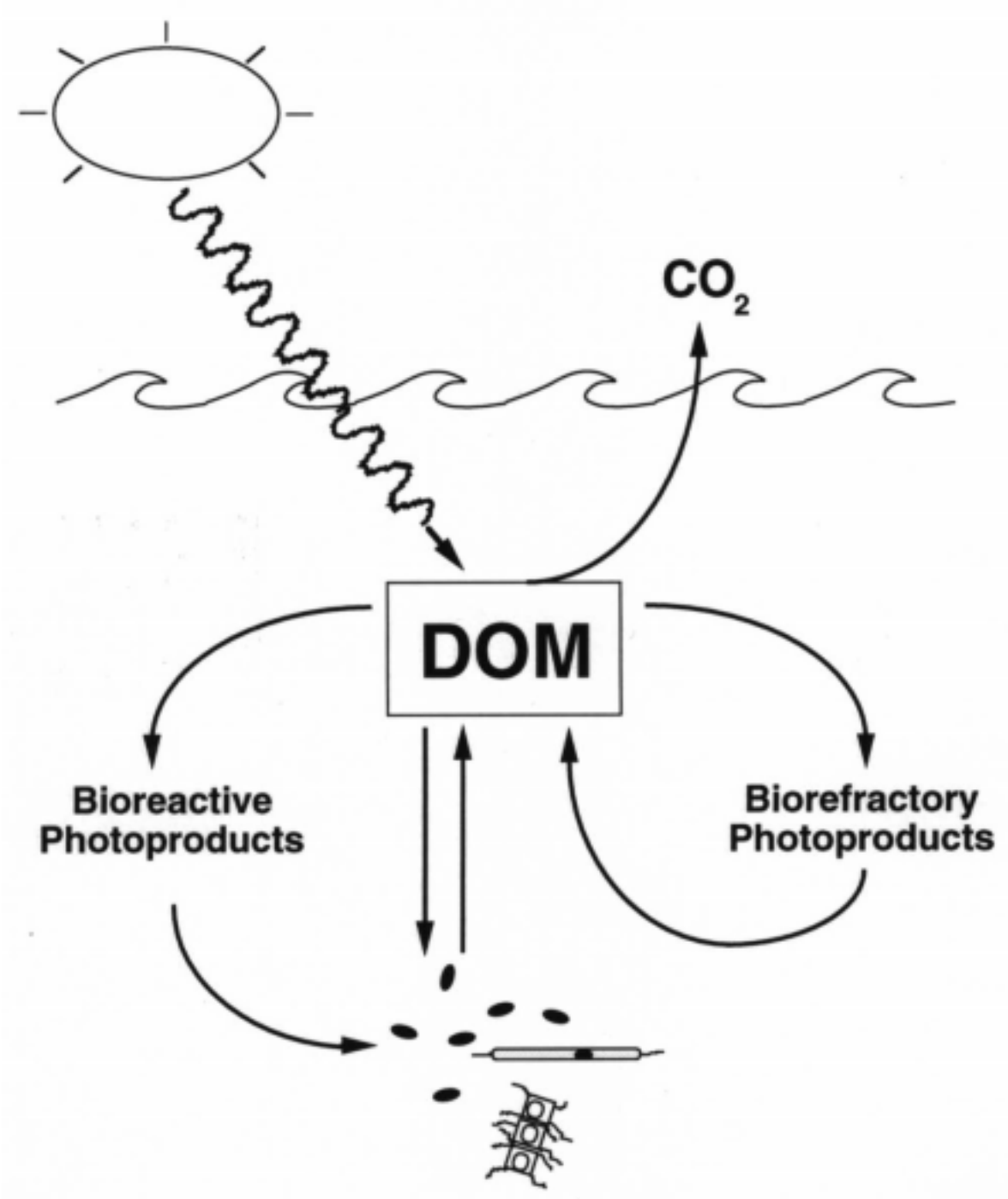


Fig. 2. Conceptual diagram of the effects of photochemical processes on dissolved organic matter (DOM) cycling in aquatic environments.

The major photoproduct of DOM is dissolved inorganic carbon (shown as carbon dioxide). Rates of photomineralization of DOM can be higher than rates of microbial mineralization

in surface waters, but when integrated throughout the water column microbial mineralization is typically a much more quantitatively important mechanism of DOM cycling than photomineralization. Photomineralization of DOM is believed to result from the removal of carboxyl groups from organic molecules [17, 19], which are abundant in DOM from fresh and marine waters [3, 10]. Further investigations are needed to determine the sources and mechanisms of photochemical decarboxylation and its effects on the bioreactivity of the remaining DOM.

A variety of bioreactive low-molecular-weight photoproducts of DOM have been identified at the molecular level, and the photochemical production of bioreactive substrates from biorefractory DOM appears to be an important mechanism for DOM cycling in a wide variety of environments. This process could have a major impact on the cycling of DOM in the ocean [2, 20]. The deep ocean is the largest reservoir of reduced carbon in the ocean. This DOM is resistant to microbial degradation, and its ^{14}C content indicates it is several thousand years old [29]. The age of this DOM exceeds the mixing time of the oceans, so this DOM is periodically exposed to sunlight in the surface ocean. Combined photochemical and microbial transformations of this DOM during its residence in surface waters could be the major mechanism for its mineralization.

Much less is known about the photochemical production of biorefractory substrates. Relatively few studies have reported evidence for biorefractory photoproducts, and no biorefractory photoproducts have been identified at the molecular level. Structural characterization of biorefractory photoproducts is exceedingly difficult, because it is unlikely these photoproducts resemble biomolecules. Given the complex nature of photochemical reactions and the common involvement of free radicals in these reactions, it seems likely that some photoproducts of DOM will no longer be recognized by the enzymes responsible for the microbial degradation of DOM or the chemical methods used for its molecular characterization. The photoproduction of biorefractory substrates provides a mechanism to explain how some components of DOM escape microbial degradation and survive for long periods of time in aquatic systems. Further photochemical transformations of this biorefractory DOM could also trigger its eventual mineralization.

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