*Short Communication*

# Effects of Biodegradable Plastic Components on Metabolism of an Estuarine Benthos*

**Peter H. Doering,**[1,2] **Barbara K. Sullivan,**[1] **and Heeseon Jeon**[1]

We examined the metabolic response of an estuarine benthic community to additions of three materials being considered for use in manufacture of biodegradable substitutes for plastics. Diver-collected cores containing benthos were dosed with 59 $g/m^2$ of three test materials, cornstarch, a bacterial polyester (PHBV), and ethylene vinyl alcohol (EVOH), or left undisturbed as controls. Fluxes of dissolved nutrients (ammonia, nitrate + nitrite, phosphate, silica) and dissolved inorganic carbon (DIC) were similar in control cores and cores dosed with EVOH during a 1-month test period at 20°C. Fluxes in cores dosed with starch and PHBV differed significantly from controls but not from each other. After 2 weeks of incubation, production of DIC was higher in cores containing starch and PHBV, while efflux of ammonia, nitrate, and nitrite was reduced. After 4 weeks of incubation, production of DIC was similar among all treatments and controls, while efflux of ammonia was high in the starch- and PHBV-containing cores compared to controls and cores with EVOH. Fluxes of silica and phosphate were similar in all cores during the experiment. These results indicate that both starch and PHBV are carbon-rich substrates readily metabolized by the benthic community but that their presence significantly alters normal nutrient exchange patterns. This response is expected because of the high carbon-to-nitrogen ratio of starch and PHBV and indicates that impacts of these two materials would be similar. However, the high biological oxygen demand of such materials and resulting disturbance of normal nutrient regeneration patterns of the benthos (delayed ammonia efflux and potential stimulation of denitrification) must be considered in developing strategies for their disposal.

**KEY WORDS:** Biodegradable; plastics; marine; effects; benthic; metabolism.

## INTRODUCTION

New materials are currently being evaluated for their utility as biodegradable substitutes for petroleum-based plastics and for their suitability for disposal at sea. Products manufactured from these new formulations, such as food wrap and disposable utensils, do not float as their plastic counterparts do but, instead, are likely to reach the sea floor before significant degradation. It is desirable to develop an understanding of their fate and effects in the marine benthic environment to help select materials most suitable for product development, with minimal impacts on the marine environment, and to help plan appropriate strategies for their disposal.

Most of the materials being considered for use in manufacture of biodegradable plastic substitutes represent a rich carbon source to the benthos and it is expected that bacteria and potentially multicellular organisms in the sediments will utilize this material as a substrate for growth. In fact, they may be utilized in a way similar to some natural sources of carbon to the sea floor, such as enter from forests, fields, and macroalgal beds [1]. However, it is difficult to predict the exact response to these materials, which are typically lower in nitrogen

content than the main marine source of carbon to the benthos, the phytoplankton. They will also arrive at the sea floor in a different form than natural materials and also may contain novel materials whose impact on the benthos is less predictable.

In this study we examined the metabolic response of an estuarine benthic community to the addition of three materials, cornstarch, a bacterial polyester (PHBV), and ethylene vinyl alcohol (EVOH), all delivered in powdered form. While marine disposal of biodegradables will occur, by law, mainly in deeper waters, the estuarine benthos was used because of its accessibility and more rapid response to perturbations due to higher temperatures, lower pressure, and larger biomass of organisms present than in the deep sea. Moreover, if these materials become commonly used on both land and sea, they are very likely to be transported to nearshore areas. The benthic communities used in this study should have a response representative of benthos in a wide variety of coastal areas.

Based on preliminary studies with similar materials [2] the dose (10 mg $L^{-1}$ or 59 g $m^{-2}$) chosen was the highest that could be used without anoxia becoming a problem. Assuming a carbon content of 40% by weight for starch, this dose represented about 25 g C $m^{-2}$, which is comparable to the amount of carbon deposited on the benthos by the spring phytoplankton bloom [3]. This is a large dose relative to background levels. Unlike phytoplankton this carbon source is low in nitrogen. Like terrestrial plant material, which contains a significant amount of carbonaceous structural material such as cellulose and lignin, its decomposition might require an exogenous nitrogen source [1]. Therefore we hypothesized that the addition of biodegradable plastics to the marine benthos would reduce the efflux of dissolved inorganic nitrogen back to the overlying water. Since almost all the dissolved inorganic nitrogen released by the Narragansett Bay benthos is in the form of ammonia [4], we expected ammonia effluxes to be most affected.

## METHODS

### Core Collection and Dosing

A total of 40 cores of benthic material was collected on December 12, 1992, from a site north of Conanicut Island in Narragansett Bay. Benthic macrofauna from this site have been well studied and is characterized by two deposit feeders, the polychaete *Mediomastus ambiseta* and the bivalve *Nucula annulata*, dwelling in a sediment consisting of 18% sand, 60% silt, and 22% clay [5, 6].

Cores were collected by divers to ensure as little disturbance to the community as possible. Core tubes made of Lexan, 5 cm in diameter and 30 cm long, were inserted into the sediment to a depth of 20 cm, removed, and capped on both ends with rubber stoppers. The cores were immediately transported to the laboratory, and 35 were selected for incubation in a 5 × 7 array in the dark in a controlled-temperature water bath. Each tube was filled to the top with 0.45 $\mu$m filtered seawater to remove phytoplankon. The water was replaced daily and aerated with a gently bubbling airstone. The cores were held at ambient temperature for 1 week, after which time the temperature in the water bath was raised 1.5°C per day until 20°C was reached.

Test materials were assigned to cores randomly and added to the top of the core tube at a dose of 59 g/$m^2$. There were eight replicate control cores and nine replicate cores for each test material treatment. Aeration was suspended for 8 h, while the test materials settled to the sediment surface. A complete description of materials added is given in Table I.

### Flux Measurements

Benthic nutrient and carbon fluxes were measured by following concentration changes over time in the water column above the benthic cores. Measurements were made twice, at 2 and 4 weeks after the materials were added. At the time of the measurement, water in the core tubes was flushed twice and replaced with freshly filtered seawater, which was sampled for initial values. Dissolved inorganic carbon (DIC), dissolved inorganic nitrogen (DIN), nitrate + nitrite ($NO_2$ + $NO_3$), ammonia ($NH_4$), phosphate ($PO_4$), and silicate ($SIO_4$) were determined from six replicate measurements. The core tubes were then capped with a rubber stopper, incubated in the dark for 5 h, mixed gently with a stirring rod, and resampled for carbon and nutrient concentra-

Table I. Description of Materials Introduced to Benthic Cores

| Name | Chemical composition | Source | Size ($\mu$m) Mean | Size ($\mu$m) Range |
|------|---------------------|--------|------|-------|
| Starch | Cornstarch | ARGO | 7 | |
| PHBV | 3-Hydroxybutyrate, 3-hydroxyvalerate PHB/5%PHV | ICI | 40 | 10–80 |
| EVOH | Ethylene vinyl alcohol | U.S. Army Natick Research and Development Center | 184 | 40–800 |

tions (two replicates per core). Water was stirred to break down concentrated gradients that might inhibit the flux of materials across the sediment–water interface and to ensure a homogeneous sample from the water column. DIC samples were obtained by siphoning water from the cores into 40-ml glass vials and letting these overflow for 3 vial vol. Samples were preserved with $HgCl_2$, capped with Teflon-lined septa leaving no head-space, and immediately refrigerated at 4°C. Samples were analyzed for DIC on an O.I. Corporation Total Carbon Analyzer. Samples for nutrients were removed with a syringe and filtered through a 0.4-$\mu$m filter, preserved with chloroform, frozen in 100-ml polyethylene bottles, and stored for later analysis on a Technicon Autoanalyzer [7]. Fluxes were calculated from the difference between initial and final concentrations following Nixon et al. [8].

## Analysis of Flux Data

One-way analysis of variance with unequal cell size ($n = 8$ for control and 9 for treatments) was used to determine the statistical significance of treatment effects on each sampling date. Analyses were conducted using the SAS General Linear Models Procedure [9]. When the main effect of treatment was statistically significant ($P < 0.05$) as determined by the $F$ test, specific differences between treatment means were evaluated using Duncan's multiple-range test.

## RESULTS AND DISCUSSION

Measurements made 2 weeks following the addition of the materials clearly indicated metabolic differences among the treatments (Table IIa). While fluxes of dissolved inorganic carbon from cores dosed with EVOH were not significantly different from controls, both the starch and the PHBV cores had significantly higher fluxes of DIC. Rates in starch- and PHBV-treated cores were similar and about two times higher than in control and EVOH-treated cores (Fig. 1). This indicated that both starch and PHBV are similarly available for decomposition by the marine benthos. However, while the difference was not significant, there was a trend for a slightly lower flux of DIC in the PHBV- than in the starch-treated cores, indicating that PHBV may biodegrade at slightly slower rates.

Nitrogen fluxes also differed significantly in the cores with PHBV and starch versus the control and EVOH-treated cores (Fig. 1, Table IIa). The flux of ammonia was directed out of control and EVOH cores, while PHBV and starch exhibited uptake. This pattern, at least for PHBV and starch, is consistent with the initial hypothesis. The decomposition of biodegradable plastic required an additional source of nitrogen supplied by the benthos. This nitrogen presumably fueled the growth of microbes decomposing the plastic, a situation analogous to the decomposition of vascular plant detritus [3].

Table II. Fluxes (Mean Values) of Dissolved Carbon, Nitrogen, Phosphorous, and Silica from Sediment Cores Following the Addition of Test Materials[a]

| Flux | Control | EVOH | PHBV | Starch | $P$ | D |
|---|---|---|---|---|---|---|
| (a) Two weeks after addition | | | | | | |
| DIC | 14.48 | 11.83 | 24.75 | 27.24 | 0.0004 | PS > CE |
| $NO_2$ | 1.73 | 0.97 | −0.91 | −2.45 | 0.0001 | CE > P > S |
| $NO_2 + NO_3$ | −6.31 | −13.41 | −33.62 | −67.33 | 0.0003 | CEP > S |
| $NH_4$ | 34.58 | 34.09 | −2.04 | −19.32 | 0.0001 | CE > PS |
| $PO_4$ | −0.13 | −0.41 | −1.09 | −2.01 | 0.8733 | No diff. |
| $SIO_4$ | 146.56 | 119.23 | 155.28 | 142.18 | 0.8969 | No diff. |
| (b) Four weeks after addition | | | | | | |
| DIC | 10.34 | 9.80 | 11.49 | 11.21 | 0.6949 | No diff. |
| $NO_2$ | 1.88 | 1.51 | 1.91 | 1.65 | 0.7292 | No diff. |
| $NO_2 + NO_3$ | −37.22 | −22.86 | −36.71 | −28.26 | 0.5335 | No diff. |
| $NH_4$ | 51.71 | 51.77 | 100.65 | 110.75 | 0.0137 | SP > EC |
| $PO_4$ | 1.21 | 0.89 | 3.28 | 2.07 | 0.5903 | No diff. |
| $SIO_4$ | 103.76 | 193.48 | 180.28 | 199.18 | 0.0905 | No diff. |

[a]Fluxes for DIC are mg C m$^{-2}$ · h$^{-1}$. Fluxes for nutrients are $\mu$mol m$^{-2}$ h$^{-1}$. Incubation was at 20°C. Differences across treatment were based on ANOVA calculated on eight replicate cores for control and nine cores for each material. The significance of $F$ values is indicated ($P$) and results of Duncan's multiple-range test (D) are indicated. C, control; E, EVOH; P, PHBV; S, starch.
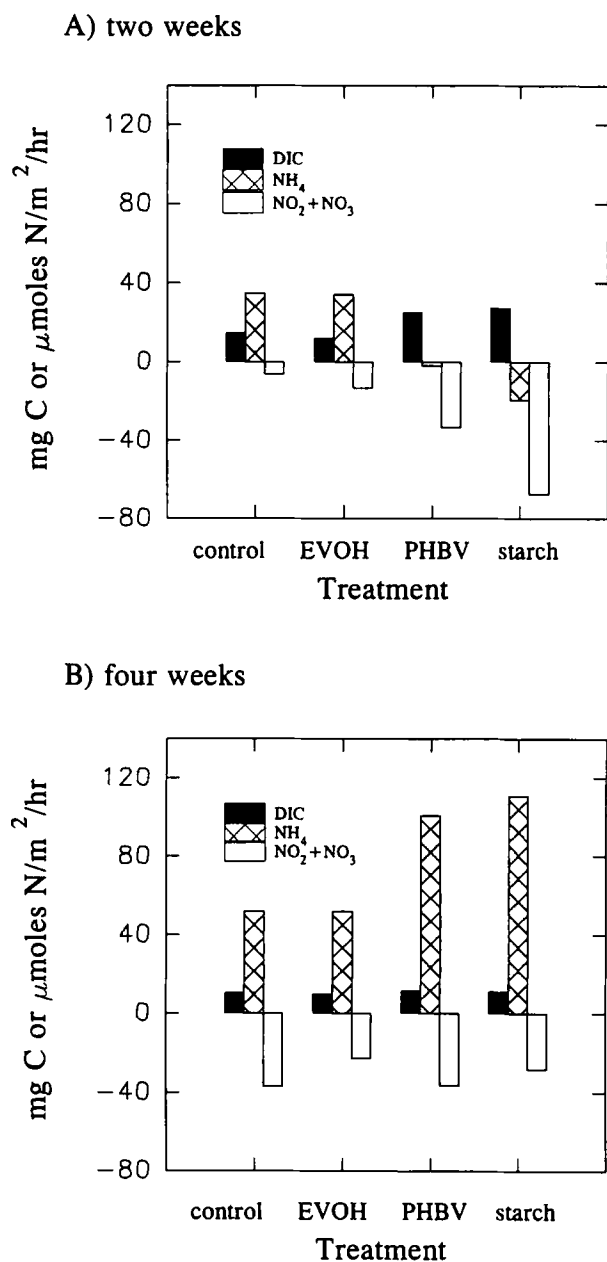
A) two weeks



B) four weeks



Fig. 1. Fluxes of dissolved inorganic carbon, ammonia, and nitrate + nitrite from control and treated cores.

Somewhat more surprising was the significant uptake of nitrate and nitrite by the PHBV- and starch-dosed cores. Both nitrate and nitrite are intermediates in the microbially mediated conversion of dissolved inorganic nitrogen to $N_2$ gas [10]. In fact the disappearance of nitrate from the water overlying sediments has been used to estimate rates of denitrification [10]. Evidence suggests that the supply of carbon limits the rate of denitrification in marine sediments [11, 12]. Our results, then,

are consistent with the hypothesis that the addition of a carbon source to the benthos not only may have required nitrogen to fuel its decomposition but also may have stimulated the conversion of nitrogen to gas.

The pattern of carbon and nitrogen flux in control and EVOH-treated cores remained relatively stable between the 2- and the 4-week sampling periods, while fluxes in PHBV and starch changed over time. Flux of dissolved inorganic carbon changed very little in control and EVOH-dosed cores, whereas it declined to control levels in the starch and PHBV cores after 4 weeks of incubation (Table IIb). This reduction indicates that decomposition of the added materials had ceased based on the fact that there was no significant treatment effect on flux of DIC. On the other hand, flux of ammonia, which increased only slightly over the 2 weeks in control and EVOH cores, was very elevated in both starch and PHBV treatments (Table IIb, Fig. 1). This enhanced ammonia flux could represent remineralization of nitrogen from microbial biomass accumulated during the decomposition of starch and PHBV.

There were no treatment differences in fluxes of phosphate or silica at either sampling period. The diagenesis of silica is primarily a physical dissolution process. Stimulation of biological processes in the sediment by the addition of carbon would not be expected to influence the flux of silica.

The diagenesis and release of dissolved inorganic phosphorus from marine sediments are complex, involving both biologically mediated remineralization and geochemical reactions including surface adsorption and formation of iron oxides [13]. Depending on the balance between these processes, the release of phosphorus can be retarded or enhanced, and this explains in part the often-conflicting measurements of phosphorus flux in marine sediments. The lack of a statistical treatment effect on phosphorus flux in this study is therefore not unusual. However, despite the lack of statistical significance, the pattern of phosphorus flux among treatments exhibited the same pattern as ammonia. After 2 weeks there was greater uptake by PHBV and starch, and after 4 weeks there was greater release in these treatments.

Our results demonstrate that the addition of starch and PHBV-based types of biodegradable plastic materials to marine sediments does affect the return of dissolved inorganic nutrients to the overlying water. Because nitrogen most often limits primary productivity in the ocean [14], effects of nitrogen flux are of particular interest. In coastal waters, the sediments play a major role in supplying nitrogen for phytoplankton production [15]. The addition of biodegradable plastic to the sediments may delay the return of ammonia to the water

column. It should be noted, however, that the materials used in this study were the components of biodegradable plastic substitutes and may be more reactive than the final formulations. The disposal of actual biodegradable plastics may result in slower degradation and a lesser effect on ammonium flux from sediments.

The potential stimulation of denitrification deserves further investigation in more realistic experimental settings. In oligotrophic (nutrient-poor) waters stimulation of denitrification may further decrease an already low rate of production. In eutrophic (nutrient-rich) waters it may provide a much needed sink, ameliorating the effects of excessive nitrogen loading.

# REFERENCES

1. K. R. Tenore, L. Cammen, S. E. G. Findlay, and N. Phillips (1982) *J. Mar. Res.* **40**, 473–490.
2. B. K. Sullivan, C. A. Oviatt, and G. Klein-MacPhee (1993) in C. Ching, D. Kaplan, and E. Thomas (Eds.), *Biodegradable Polymers and Packaging*, Techonomic, Lancaster, PA, pp. 281–296.
3. D. T. Rudnick and C. A. Oviatt (1986) *J. Mar. Res.* **44**, 815–837.
4. S. W. Nixon, C. A. Oviatt, and S. S. Hale (1976) in J. M. Anderson and A. Macfadyen (Eds.), *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*, Blackwell, Oxford, pp. 269–283.
5. J. B. Frithsen (1984) *Ecological Studies of Benthic Meiofauna in Mesocosms*, Ph.D. thesis, University of Rhode Island, Kingston.
6. D. T. Rudnick (1984) *Seasonality of Community Structure and Carbon Flow in Narragansett Bay Sediments*, Ph.D. thesis, University of Rhode Island, Kingston.
7. C. E. Lambert and C. A. Oviatt (1983) Mar. Tech. Rep. 85, Graduate School of Oceanography, University of Rhode Island, Kingston.
8. S. W. Nixon, J. R. Kelly, B. N. Furnas, C. A. Oviatt, and S. S. Hale (1980) in K. R. Tenore and B. C. Coull (Eds.), *Marine Benthic Dynamics*, University of South Carolina Press, Columbia, pp. 219–242.
9. SAS Institute Inc. (1985) *SAS User's Guide: Statistics, Version 5 Edition*, SAS Institute Inc., Cary, NC.
10. S. P. Seitzinger (1988) *Limnol. Oceanogr.* **33**, 702–724.
11. S. V. Smith, J. T. Hollibaugh, S. J. Dollar, and S. Vink (1989) *Limnol. Oceanogr.* **34**, 37–52.
12. I. Brettar and G. Rheinheimer (1992) *Limnol. Oceanogr.* **37**, 1146–1163.
13. B. Sundby, G. Gobeil, N. Silverberg, and A. Mucci (1992) *Limnol. Oceanogr.* **37**, 1129–1145.
14. J. H. Ryther and W. M. Dunstan (1971) *Science* **171**, 1008–1013.
15. P. H. Doering (1989) *J. Mar. Res.* **47**, 371–383.