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Biodegradation of Poly-β-Hydroxyalkanoates in a Lake Sediment Sample Increases Bacterial Sulfate Reduction

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The addition of a poly-β-hydroxyalkanoate (PHA) to incubation bottles containing anaerobic sediment from Lake Cisó (Banyoles, Catalonia, northeast Spain) was correlated with an increase in sulfide production. The concentration of PHA diminished to 51 to 99% of the initial amount. Sodium molybdate (1 mM) decreased the rate of PHA degradation and inhibited sulfide production, thus indicating that PHAs serve as carbon and electron sources for sulfate reduction.

Although anaerobic degradation of poly-β-hydroxyalkanoates (PHAs) has been previously reported (1, 4, 5), the use of PHAs as a substrate for sulfate reduction has not been investigated. Only a few small carbon fermentation products (C-2 and C-3) are usually used as substrates by sulfate-reducing bacteria (SRB) (7). However, Widdel (17, 18) has reported the oxidation of fatty acids, amino acids, alcohols, aromatic compounds, and hydrocarbons.

Gypsum-based karstic lakes are among the most permanent habitats of SRB. The sediment of Lake Cisó, a small freshwater body located in the karstic area of Banyoles, northeast Spain, is populated by heterotrophic fermentative bacteria, as well as *Desulfovibrio* spp. and other genera of SRB (12). In the water column, *Chromatium* spp., together with a small aggregate-forming species, *Amoebobacter*, dominate the phototrophic bacterial community (3). *Chromatium* cells accumulate PHAs in natural conditions, as has been shown both ultrastructurally (2) and quantitatively (3). These purple sulfur bacteria also accumulate intracellular granules of PHAs in the laboratory under several growth conditions (8).

PHAs reach the bottom of the lake both by whole-cell settling and by polymer granule sedimentation. In the first case, cell lysis occurs in the sediment; in the second, lysis occurs in the water column. Cells that are rich in PHAs tend to settle faster than cells that are not (14). The sinking speeds of *Chromatium* organisms in the stratified lake in summer have been investigated (9, 13, 14).

Lake Cisó samples from a given site were taken in October 1991 and October 1992. After the samples were taken from the uppermost sediment with a grabbing device (2- to 3-cm depth), they were placed in a sterile bottle, maintained at 4°C, and transported to the laboratory. A sediment slurry was prepared by thoroughly mixing 500 ml of compact sediment with 100 ml of lake water under anoxic conditions. Samples (25 ml) of the slurry were placed in 125-ml glass bottles. A PHA (Aldrich, Alcobendas, Spain) was added to each sample bottle, which was then filled with anoxic water from the lake. The surface area of the water-sediment interface in the incubation bottles was 21.5 cm^2. The PHA used was poly-β-hydroxybutyrate-co-poly-β-hydroxyvalerate at a 93:7 molar ratio. Three controls were carried out. (i) Sediment bottles were sterilized at 121°C for 20 min to assess possible chemical PHA degradation, (ii) sediment samples lacking PHA were used to estimate natural sulfide production, and (iii) one bottle was filled with only anoxic water to evaluate the activity of the bacteria present in the water column.

SRB activity was inhibited by the addition of sodium molybdate (Na_2MoO_7) from Merck (Darmstadt, Germany) to the bottles to a final concentration of 1 mM. To prevent any interference by sulfide reacting with molybdate ions, which forms an orange-brownish chemical precipitate, we used the inhibitor at its lowest inhibitory concentration. We decided to do this on the basis of the results of previous experiments that used either higher (20 mM [16]) or lower (0.1 mM [6]) concentrations of inhibitor.

Two replicates were used for each treatment. Bottles were incubated in the dark at 15°C in a water bath for 42 days. The sulfide concentration was monitored by taking 0.5- to 1-ml samples of overlying water at stipulated intervals and were analyzed by colorimetry (11). At the end of the incubation period, the remaining polymer content in the sediment was measured as described previously (10). The polymer was extracted from the sediment with chloroform in a Soxhlet extractor at 80°C recirculating for 4 h. The extracted residue was analyzed by high-performance liquid chromatography (HPLC) with an HPAH 125-0100 fast-acid column (100 by 7.8 mm) from Bio-Rad (Richmond, Calif.) in a Hewlett-Packard series 1050 apparatus. Prior to HPLC analysis, the dried residues were digested with 1 ml of concentrated sulfuric acid at 100°C for 1 h. Sulfuric acid (1 mM) was used as the mobile phase at a flow rate of 1 ml min^{-1}. The detection of the peaks of crotonic acid generated in the acidic digestion of the polymer was performed at 210 nm in a Hewlett-Packard series II-1040-M detector. A calibration curve was made by using crotonic acid ($CH_3CH{=}CHCOOH$) from Merck at concentrations from 0 to 200 mg $liter^{-1}$.

The variation in sulfide concentration (millimolar) with time in the overlying water from the incubation bottles is shown in Fig. 1. Sediment without added polymer showed a slight increase in sulfide concentration due to the remaining organic matter in the sediment. Sulfate is not a limiting factor in this process because of the high gypsum content of the sediment. Sediments with PHA added showed an increase in sulfide production. The amount of polymer added correlated with sulfide production. Sterilized sediment was also used to prevent any chemical production of sulfide that may have evolved in the sediment and any chemical degradation of the polymer. The initial sulfide concentration in the sterilized bottles was very low (0.17 mM) because sterilization causes the gases to

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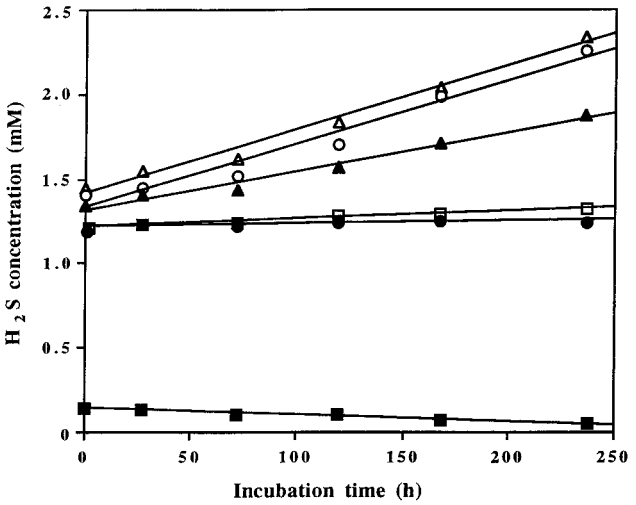

FIG. 1. Sulfide concentrations generated by different quantities of added PHA. ●, water without sediment plus 50 mg of PHA (r = 0.921); △, sediment plus 25 mg of PHA cm^{-3} (r = 0.993); ○, sediment plus 5 mg of PHA cm^{-3} (r = 0.983); ▲, sediment plus 2.5 mg of PHA cm^{-3} (r = 0.989); □, sediment alone (r = 0.958); ■, sterilized sediment plus 5 mg of PHA cm^{-3} (r = 0.957).

strip out from the liquid phase. In this case, the sulfide concentration in the overlying water decreased because of loss during manipulation of the bottles. Hardly any sulfide production was observed in bottles which contained lake water lacking sediment.

The rate of sulfide production (millimoles per square meter per day) was directly correlated with the quantity of PHA added (Fig. 2). This relation was linear up to 5 mg of PHA added per cm^3 of sediment. After this value was reached (data not shown), the rate of sulfide production did not further increase and production reached saturation. Natural sulfide production rates of Lake Cisó sediment, obtained in experiments with incubation bottles, ranged from 0.68 to 4.05 mmol of S^{2-} m^{-2} day^{-1}, depending on the sediment sample. Sulfide production increased as PHA was added to the sediment at a

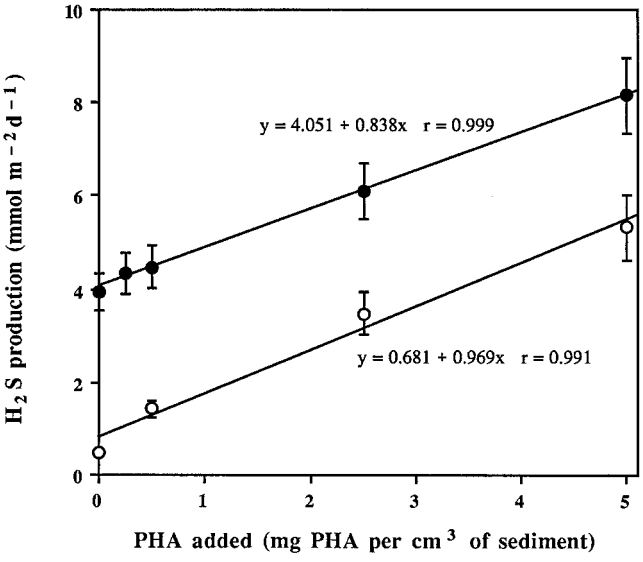

FIG. 2. Sulfide production in sediment in relation to PHA added to incubation bottles on two sampling occasions, October 1991 (○) and October 1992 (●).

TABLE 1. PHA biodegradation in incubation bottles after 42 days

Amt of PHA added in incubation bottle (mg)[a]	Amt of PHA added (mg of PHA cm of sediment^{-3})	Amt of PHA degraded			
		mg of PHA cm of sediment^{-3}		%	
		Non-inhibited	Inhibited	Non-inhibited	Inhibited
5	0.25	0.25	0.14	99.4	55.4
10	0.5	0.48	0.27	96.7	53.8
100	5	3.99	2.13	79.8	42.6
200	10	6.47	3.46	64.7	34.6
300	15	7.75	2.13	51.7	14.2
50[b]				<1	NT[c]

[a] Incubation bottles contained anaerobic sediment, lake water, and the amount of PHA indicated (see text) except as noted.
[b] Incubation bottle with water only.
[c] NT, not tested.

rate ranging from 0.84 to 0.97 mmol of S^{2-} m^{-2} day^{-1} mg of PHA added cm^{-1} of $sediment^{-3}$. The slopes of the graph represent the rate of sulfide production in relation to the amount of PHA added. The two lines in Fig. 2 represent two replicas at different sampling times. Different concentrations of biomass in the sediments might account for the observation that there was more activity in the samples taken in October 1992.

Sulfide production in the sediment was accompanied by polymer degradation. The percentage of added polymer degraded in bottles with molybdate was compared with that of controls (Table 1). Samples lacking SRB inhibition showed higher percentages of degradation than the inhibited ones. Degradation was affected by the amount of polymer added. Small quantities of PHA (0.25 and 0.5 mg of PHA per cm^3 of sediment) were almost completely degraded after 42 days. Large quantities of PHA were degraded to a lesser extent (from only 65 to 70%). Pedrós-Alió et al. (15) suggested that polymer degradation in the lake was carried out in the water column either by purple phototrophic bacteria or by heterotrophic bacteria decomposing lysed cells. However, in the current study, no PHA degradation was observed in bottles which contained lake water lacking sediment.

The PHA degradation rates with different amounts of PHA added are shown in Fig. 3. The slopes of the graph indicate micrograms of PHA degraded per day per milligram of PHA added per cubic centimeter of sediment. Inhibition of the activity of SRB decreased PHA biodegradation. The specific rate of degradation is three times lower in the inhibited samples than in the noninhibited ones.

The *Chromatium* layer in Lake Cisó over the summer stratification period has a volume of 75 m^3 (area, 500 m^2 by 0.15 m thick). If we consider cell concentration (10^6 cells ml^{-1} [12]), loss factors (sedimentation and decomposition of −0.015 and −0.016 day^{-1}, respectively [9]), and average specific content of 30 pg of PHA $cell^{-1}$ (10), we can calculate that the amount of PHA reaching the lake sediment is about 139.5 mg of PHA m^{-2} day^{-1}.

Intracellular PHAs produced by the phototrophic community (purple and green sulfur planktonic bacteria) represent a major source of organic carbon in the sediment of karstic lakes dominated by those populations. The continuous supply of PHAs supports the sulfate-reducing activity. Therefore, SRB could play a very significant role in the breakdown of PHAs in those ecosystems.

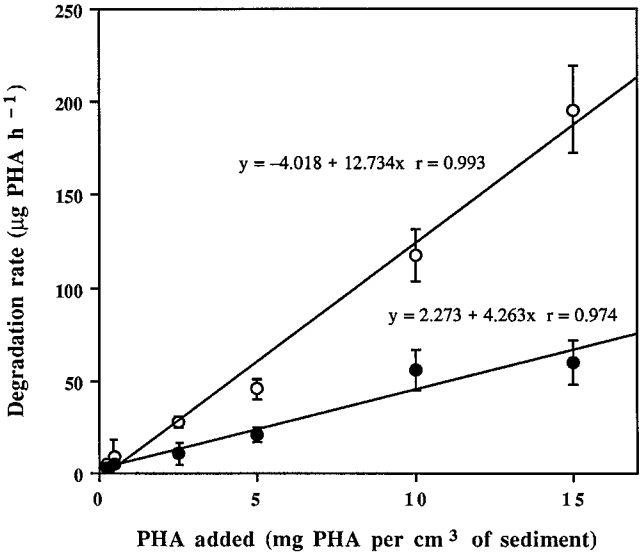

FIG. 3. PHA degradation rates correlated with the quantity of PHA added to incubation bottles. ○, controls; ●, molybdate-inhibited SRB.

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