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Comparative Study of PHA Degradation in Natural Reservoirs Having Various Types of Ecosystems

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Summary: The kinetics of polyhydroxyalkanoate (PHA) degradation in natural environment in two water reservoirs having various ecological characteristics was studied. It was shown that biodegradation of polymer essentially depends on the environment temperature and inorganic composition of water. The processes of polymer degradation under aerobic and anaerobic conditions were compared. The polymer degradation was slower under anaerobic conditions.

Keywords: biodegradable polyesters; ecosystem; natural environment; PHA; water reservoir

Introduction

The 20th century was the time when synthetic polymers not degradable in the natural environment made a revolution in human lifestyle; however, the use of synthetic polymers raised a global ecological problem. To solve the problem of "polymeric waste," a changeover to the polymers capable of degrading into components harmless for both animate and inanimate nature is the most radical approach. Aliphatic polyesters, polyamides, segmented polyesterurethane, polylactides and polyglycolides, silicone, polyethyleneterephthalate, and, since recently, polymers of fatty acid hydroxy derivatives or so-called polyhydroxyalkanoates (PHAs), are the most intensely developed biodegradable polymers.[1–3] Degradation under the conditions of the biological environment is one of the most valuable PHA properties. Specific depolymerases produced by microorganisms, as well as enzymes of animal blood and tissues and by polynuclear macrophages, decompose PHAs.[4–6] Bacteria and actinomycetes are capable of PHA degradation in soil, compost, activated silt, fresh and sea water.[7,8] Microorganisms decompose PHAs under both aerobic and anaerobic conditions and utilize the products of degradation as energy and carbon source. Under aerobic conditions, PHAs are degraded to the final products, carbon dioxide and water; under anaerobic conditions, to water and methane.[9] As the application of PHA becomes more promising, analysis of the mechanisms of PHA degradation in a natural environment is an urgent task. Degradation of polyhydroxybutyrate polymeric films in fresh and marine water has been described.[8,10] The degradation dynamics of two types of polymers in the Bugach reservoir near the city of Krasnoyarsk (Siberia, Russia) were recently studied and it was found that this process depends on the state of the ecosystem and weather, as well as on the chemical structure of the PHAs (copolymers of hydroxybutyrate (PHB) and hydroxyvalerate (PHV) were degraded more rapidly than homogeneous polyhydroxybutyrate).[11] In this study, we describe PHA degradation in natural reservoirs with different ecological parameters.

Materials and Methods

The ecological systems of the small reservoirs Bugach and Lesnoi, where we conducted our experiments, have been already

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described in detail.[12,13] These two reservoirs are 6 km away from each other and have almost the same water pH, oxygen regimen, and temperature. However, the inorganic phosphorus concentration in the Bugach is essentially higher than in the Lesnoi (0.01–0.018 and 0.0021–0.0088 mg/L, respectively); the bacterio- and phytoplankton biomasses are much more abundant in the former reservoir. Moreover, Bugach reservoir is subject to cyanobacterial blooming in summer. The subject of this study was a PHB/PHV copolymer (molecular weight, 340 kDa; hydroxyvalerate content, 8.1 mol-%) in the form of discs (30 mm in diameter, 0.08–0.1 mm in thickness). Previously weighed samples were put into nylon pouches (three disks in one pouch). The pouches were carefully sewn after placing the disks in. Then the pouches were fixed to special buoys which were placed in selected sites of natural reservoirs, for the stable location special anchors were used. In the Lesnoi reservoir, the samples were exposed in the littoral zone, where the oxygen concentration was near saturation. In the Bugach reservoir, the samples were also exposed in the littoral zone, as well as in black silt at the bottom center at a depth of 4 m (under anaerobic conditions). Each week, one nylon pouch was retrieved from the water at each sampling point to determine the weight loss. The first experimental series was conducted from May 17 to June 21, 2006, at an initial water temperature of 5.5 °C. The second series was performed from August 9 to September 27 at an initial temperature of 19.5 °C.

To perform theoretical analysis of data on the dynamics of biopolymer degradation in water reservoirs, a model was used in which the substrate studied (test substrate) was actively utilized by the bacteria that grew on another substrate. Note that the bacterial growth was exponential and the test substrate concentration (substrate availability) was low:

$$x(t) = x(0) \cdot e^{-(k_1/R)\cdot[exp(R\cdot t)-1]} \qquad (1)$$

in which $x(t)$ is polymer concentration at time t; R is maximal specific growth rate under the given conditions; $k_1 = V_{\max} \cdot B(0)/K_m$; $V_{\max}$ is maximal specific reaction rate under the given conditions; $B(0)$ is initial population; K_m is half-saturation constant.[11,14]

Results and Discussion

The degradation kinetics is presented in Figure 1. The approximation parameters for the kinetics of polymer biodegradation are shown in the Table 1. In all the experiments, the approximation proved to be statistically significant as determined using Fisher criterion test.

Comparative analysis of PHA degradation in the near-bottom anaerobic layer (black silt) of the Bugach reservoir and in the littoral aerobic layer at a depth of about 1 m showed that the polymer degradation was less intense in the anaerobic layer. At the first stage, the half-life of PHB/PHV was 55 days, and the weight of polymer discs was reduced by 21% within 35 days (Figure 1a). During the second period of observation, the polymer degradation was accelerated, due to water temperature rising, so that the half-life of polymer was reduced to 32 days (Figure 1d).

In both the first and second experimental series, the polymer half-life in the aerobic littoral layer of the Lesnoi reservoir was several times longer than in the littoral zone of the Bugach reservoir (Table 1). In the first experiment, the copolymer half-life in the littoral aerobic zone of the Bugach reservoir was 41 days. In the Lesnoi reservoir, polymer degradation lasted almost 2.5 times longer, and the polymer half-life was 109 days (Figure 1b, 1c).

During the second experimental series, which started at a higher temperature (19 °C versus 5 °C during the first experiment), polymer degradation was more active in the littoral zone of the Bugach reservoir: the polymer half-life was 21 day, i.e., almost twice shorter than during the first experimental series. By the end of the observation (after 49 days), the polymer

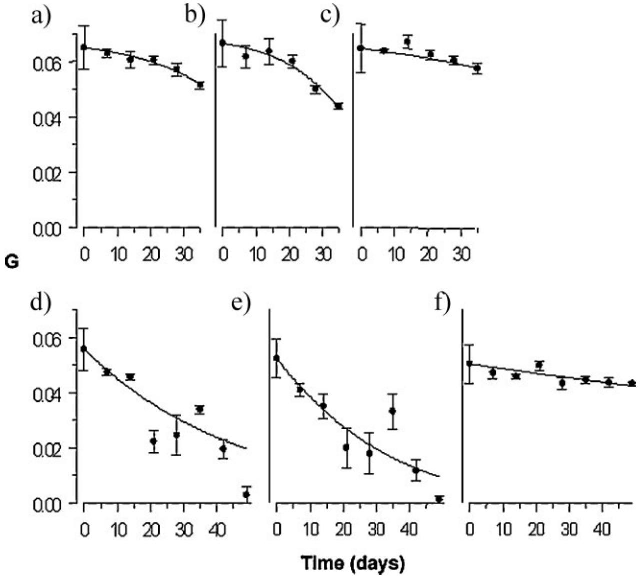

Figure 1.
Degradation of PHB/PHV films in the Bugach and Lesnoi reservoirs and degradation approximation using equation (1) (the solid line): (a) May 17 to June 21, 2006; Bugach, bottom; (b) May 17 to June 21, 2006; Bugach, littoral zone; (c) May 17 to June 21, 2006; Lesnoi, littoral zone; (d) August 9 to September 27, 2006; Bugach, bottom; (e) August 9 to September 27, 2006; Bugach, littoral zone; (f) August 9 to September 27, 2006; Lesnoi, littoral zone. (G: Weight).

films degraded almost completely, and their residual weight was 2.3–5.2% of the initial value. In the Lesnoi reservoir, the weight loss of the sample was smaller, and the remaining weight of the polymer film was 14% of the initial one. In the second experimental series, the rate of polymer degradation in the Lesnoi reservoir was lower despite the water temperature was higher than it was during the first experiment: the polymer half-life was 194 days, i.e., at least 1.5 times longer than during the first observation period.

It was shown earlier that the effect of aquatic bacteria degrading elementally simple substrates was limited as a rule by the concentration of inorganic phosphorus.[15] Indeed, the average seasonal concentration of the inorganic phosphorus in Bugach was 14 μg/L, whereas in the Lesnoi reservoir, the phosphorus concentration was 6 μg/L. On the basis of the long-term study the phytoplankton biomass and phosphorus concentration in the Lesnoi and Bugach reservoirs [12–13], it could be assumed that polymer biodegradation in the Lesnoi

Table 1.
Approximation parameters of PHA biodegradation kinetics in the Bugach and Lesnoi reservoirs.

Reservoir, station	Date	*n*	*RSS*	*F*	k_1 (day^{-1})	*R* (day^{-1})	*HL*, day
Bugach, bottom	May 17 to June 21, 2006	6	$4.368 \cdot 10^{-6}$	101.7	0.00237	0.0500	54.0
Bugach, littoral zone		6	$2.060 \cdot 10^{-5}$	71.7	0.00282	0.0705	41.2
Lesnoi, littoral zone		6	$3.907 \cdot 10^{-5}$	204.8	0.00250	0.0152	108.6
Bugach, bottom	August 9 to September 27, 2006	8	$3.916 \cdot 10^{-4}$	13.1	0.02130	0.0001	32.5
Bugach, littoral zone		8	$5.401 \cdot 10^{-4}$	22.5	0.03010	0.0058	21.6
Lesnoi, littoral zone		8	$3.145 \cdot 10^{-5}$	8.4	0.00353	0.0001	194.5

n - the number of experimental points; *RSS* - residual sum of squares; *F* – Fisher criterion; k_1 and *R* – constants of the equation (1); *HL* - half-life time.

reservoir was limited precisely by the lack of inorganic phosphorus in water, which prevented the growth of the phytoplankton capable of PHA decomposition.

Conclusion

The degradation dynamics of a PHB/PHV copolymer was studied in two natural reservoirs, and the rate of this process proved to depend significantly on the ambient temperature and the mineral component of water. It was assumed that PHB/PHV degradation was limited by the lack of inorganic phosphorus in water. In the Bugach reservoir, the polymer biodegradation was studied under both aerobic and anaerobic conditions. The rate of polymer biodegradation proved to be lower under anaerobic conditions. In the anaerobic zone, the polymer half-life exceeded the same parameter in the aerobic zone by a factor of 1.5.

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