Bull. Environ. Contam. Toxicol. (1996) 56:948-955

Bulletin of Environmental Contamination and Toxicology

Bacteria Associated with Disintegrating Plastic Films Under Simulated Aquatic Environments

K. K. Leonas,[1] R. W. Gorden[2]

[1]Textile Sciences, University of Georgia, Athens, Georgia 30602, USA
[2]Biotechnology Group, Westinghouse Savannah River Company, Aiken, South Carolina 29808, USA

Received: 27 March 1995/Accepted: 12 December 1995

The degradation of plastics has been investigated for more than 30 years. Much of the early research was conducted to understand and prevent biodeterioration (Johnson 1987) and photodegradation (Day and Wiles 1972a,b). More recently, because of heightened concern over the fate of plastics in the environment, research has focused on the capacity of microorganisms to degrade plastics (Klausmeir and Osmon 1976) and on the production of biodegradable (Wool and Cole 1988) and photodegradable plastics (Horsfall 1981, 1982; Bell and Pezdirtz 1983). Here we address the impact of degradation byproducts of plastics on aquatic microorganisms. Responses of aquatic microbial communities to the addition of specific degradable material can be measured. Allion et al. (1987) suggested that three patterns of response may occur: (1) rapid initial degradation, (2) mineralization, and (3) a gradual adaptation over days or weeks. Rates of these response patterns vary according to chemical and physical complexities and accessibility of structural materials to the microbes, as well as a wide variety of environmental factors.

As an entry level contaminant plastics may provide a substrate for attachment and development of a bacterial biofilm and a source of carbon and energy for bacteria capable of degradation. Primary objectives for this study were: 1.) To determine the rates of degradation of six different bio- and photodegradable plastic films in three aquatic habitats when exposed to intense ultra violet light. 2.) To determine the effect of the plastics on the bacteria attached to the films and in the water column of these habitats during the degradation process.

Data regarding the degradation rates of these films have been previously published (Leonas and Gorden 1993). The primary purpose of this paper is to describe the changes in the bacterial populations associated with the degrading plastic films.

MATERIALS AND METHODS

Six different types of bio- and photodegradable plastic films (Table 1) were placed in established aquaria as described (Leonas and Gorden 1993). Three aquatic environments were tested. In Phase I testing, plastic films (20.3 x 27.9 cm) were carefully placed on the water surface in each aquarium, and exposed to UVA light. In Phase II, plastic films were lowered into the aquarium to the depth of 50% light penetration as measured by a Protomatic photometer. In Phase III, films were fastened to plexiglass frames and placed on the water surface. Aeration tubes fastened to the

Correspondence to: K. K. Leonas

Table 1. Research design for exposure of plastic films to UVA 340 light under simulated environmental conditions.

Film type	Degradation type	Exposure
A. Low-density polyethylene	RDR	240
B. 10% CO polyethylene copolymer	RADP	16
C. CO_2 polyethylene copolymer	RADP	240
D. Polyethylene + vinyl ketone graft	RADP	240
E. Polystyrene	RDR	240
F. Polyethylene + 6% corn starch	RABP	240

[1]The same aquaria were used for each experimental condition and each plastic type.
RADP = Reported accelerated photodegradable plastic
RABP = Reported accelerated biodegradable plastic
RDR = Reported degradation resistant plastic

sides of the aquaria released air into the water, causing a simulated wave action, with aerated waves moving intermittently over the film surface.

Film B degraded rapidly, so bacterial samples were taken every 4 hr during the 16-hr degradation period; with the other 5 films bacterial samples were obtained at 0, 60, 80, 120, and 240 hr. The UVA lights remained on throughout the testing periods (i.e., for 16 hr for film B and 240 hr for the other films). When the films were in place, black shade cloths were drawn and only controlled UVA light was available. At all other times the aquaria were exposed to normal greenhouse light and temperature conditions. All experiments were conducted with a minimum of three replications, consisting of three aquaria in each set.

All aquaria were cross-inoculated with water obtained from more than 20 temporary and permanent water sources throughout southern Illinois to provide bacteria typical of those present in natural ecosystems, especially those that had been exposed to plastics (Leonas and Gorden 1003.) It was impossible to exclude extraneous bacteria, because aquaria were uncovered and open to the air. Water samples for bacterial analyses were obtained with sterile 10-ml pipets or with a hypodermic syringe with a 6-in needle attached, placed in sterile test tubes and processed immediately. With forceps and scissors, a small piece (3 x 3 cm) of film was cut from the comer and placed in sterile phosphate buffer in a sterile petri dish.

Direct microscopic counts of water and film were made using acridine orange (AODC) staining techniques. Duplicate water samples were filtered, and replicate counts of 10 fields per slide were made using a Zeiss epifluorescent microscope. Film samples were placed on slides, and direct counts of rods, cocci, and algal cells of water column samples were made using epifluorescent methods (Hobbie et al. 1977.) Replicate plate counts of water column samples were made using one half-strength nutrient agar and serial dilution plates. All plates were incubated at 25°C

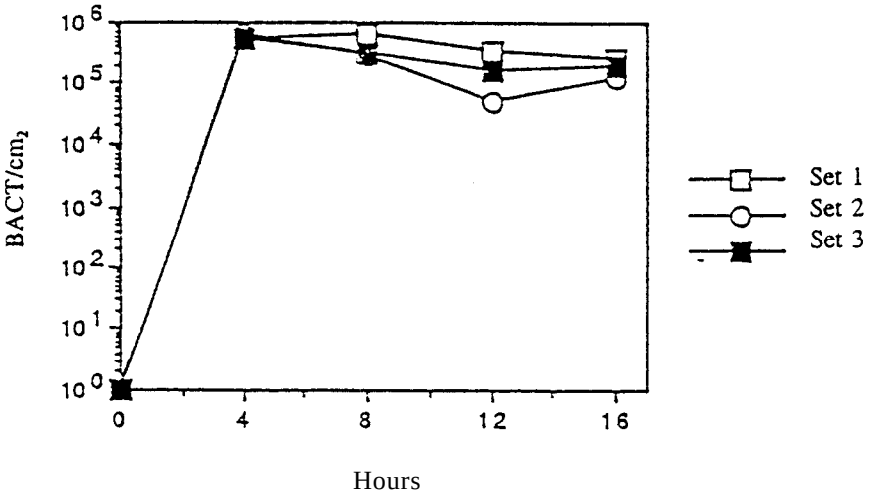

Figure 1. Direct epifluorescent microscopic counts of bacteria attached to film B under wave action, Phase III.

for 7-10 d before counting using a stereo-dissecting microscope. Bacterial colony forming units (CFU) were separated according to colony characteristics as dominant, abundant, or present; thus, pour plates were used to observe both populationnumbers and succession.

All AODC and plate counts were statistically analyzed using the Fisher PLSD and Scheffe F tests.

Identification methods for bacterial isolates included gram stains and growth on lactose, dextrose and mannitol broths, SIM media, gelatin, and litmus milk. In addition, all isolates were inoculated on duplicate Biolog® GN multi-well plates, and Biolog® patterns were recorded after 24-48 hr of incubation.

RESULTS AND DISCUSSION

Bacterial populations in the water column quickly colonized the plastic films within the first sampling period (4 hr for film B; 60 hr for films ACDEF) (Figs 1,2), and populations remained at relatively high levels (10^{-5} to 10^{-6} cells/cm^{-2}) throughout all phases of the experiment. Direct counts of bacterial cells in the water column were generally near 1 x 10^{-5} cells/ml during Phases I and II and an order of magnitude higher during Phase III (Fig. 3). Plate counts of bacterial cells in the water column fluctuated over a wider range (10^{-3} to 10^{-5} cells/ml) than did direct counts.

Bacterial populations in the water column passed through successional stages during each phase of the experiment, but the dominant and abundant CFU were not predictable on the basis of plastic film type, aquarium number, or phase and did not remain dominant or abundant during the next replicated experiment (Table 2). If bacteria

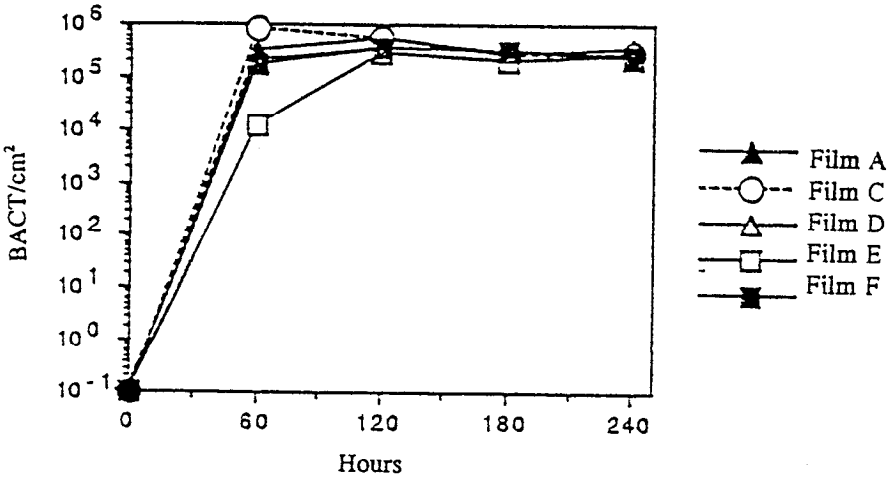

Figure 2. Direct epifluorescent microscopic counts of bacteria attached to films placed on the surface of the water during Phase I.

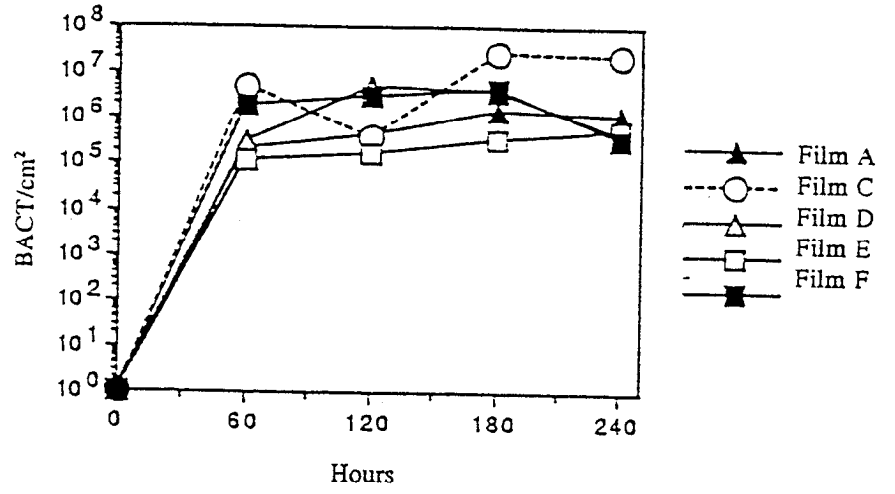

Figure 3. Direct epifluorescent microscopic counts of bacteria attached to films during wave action, Phase III.

introduced by technicians had been consistently dominant or abundant the same organisms would have been dominant in all or several of the aquaria.

Dominant and abundant isolates were primarily gram-negative short rods and gram-positive cocci. No isolates conformed to metabolic patterns of known organisms recorded in the available Biolog® library. Dominant organisms were usually in the Sarcina-Gaffkya groups (gram + cocci) or were Pseudomonas-like, non-fermentative, gram-negative rods. Bacteria attached to films were similar in size and shape to those

Table 2. Dominant and abundant bacterial colony forming units in the water column of replicate aquaria containing films A,C,D,E,F on the water surface.

Aquarium	Film	Replicate	0 hr	60 hr	80 hr	120 hr	240 hr
5	A	1	Y	Cr	Y/O/P	W	W/LY
		2	W/LY	W/B	B/W	B/Cr	B/Y/W
		3	B/Y/W	B/W	Y	P/W/Y	W/Y
10	A	1	P	P	Cr	----	W
		2	W	LY/W	LY/W	O/W/LY	W/O/P
		3	W/O/P	B/W	Y/P	W/O/Y	W/Cr/Y
4	C	1	Y	P	W	YO	W/LY
		2	W/LY	W/LY	P/W	W/P	W/LY
		3	W/LY	Cr/W	Y/W	P/Cr/W/Y	W/Y
9	C	1	W	Sm W	R/Y	W	Y/P
		2	Y/P	O/LY	W/LY	W/Y/O	W/P/O
		3	W/P/O	W/Y	W/Y	W/O/Y	Y
8	D	1	P	P/W	W	W	W
		2	W	O/W	W/LY	W/Y	P/O/B
		3	P/O/B	W/B	Y	W/Y	O/Y
13	D	1	Y/O	W	W/P	Y/W	W/LY
		2	W/LY	W	B/W/P	B/W	O/B/W
		3	O/B/W	W/Y	LY	W/O/Y	Y/W
3	E	1	Y	Cr/P	Y/W/P	W	W/LY
		2	W/LY	W/Y/LY	W/Y	W/LY	W/Y
		3	W/Y	W/Cr	Y/W	Y/Cr/W	Y
7	E	1	Y	W	W	Cr	P/W
		2	P/W	W/Y/B	F/W	W	P/B/W
		3	P/B/W	W/B	Y	W/Y	Y
1	F	1	Cr/Cm/W	Y/Sm W	Y/W	Cr	Cr/W
		2	Cr/W	LY/W	W/Y	W	W/Y/Cr
		3	W/Y/Cr	Cr/W	W/Cr	W/Y	Y
6	F	1	Y	Y/O	Y	W	W/Y/Cr
		2	W/Y/Cr	P/W	W/F	B/Cr	B/W
		3	B/W/Y	Y	Y/W	Y/W	LY/W

y= yellow, LY = light yellow, W = white, P = pink, O = orange, LO = light orange, R = red, B = brown, Cr = cream, Ch = chocolate, and F = fuzzy

isolated from CFU on agar plate. Few algae (< 5 %) were observed on films or pour plates.

Bacteria are commonly sensitive to UVA light which may cause mutation, injury or death to the cells. UVA does not penetrate far into the water column nor through

barriers such as glass or plastic film, and it was not expected that the UVA light would cause any general decrease in the microbial community. In contrast, the degradation products of the films might be either inhibitory or stimulatory to selected bacterial species. The addition of plastic films to the aquaria resulted in increases in numbers and diversity of the CFU of bacterial populations. From 10 to 15 colony types representing both gram-positive and gram-negative bacteria were present, dominant or abundant during any 240-hr experiment, demonstrating that films and degradation products did not enhance the growth of any select group of bacteria.

Imam and Gould (1990) demonstrated that KB-1 (Arthrobacter--like) adhered to starch-polymethylacrylate film but not to other studied films, including some that contained starch. In our study, mixed populations of bacteria adhered quickly (i.e., in less than 4 hr) to film B and in less than 60 hr to all other films. Although adherence to films D and E was delayed, there was no evidence that the films inhibited bacterial attachment.

Film B reached the brittle point in all experiments within 12 hr. The film was colonized by bacteria within 4 hr of being placed on or in water (Fig. 1). Direct film counts were: 50 % light > wave > surface. Populations were approximately 10^{-5} cells/cm^2 for surface and wave treatments and 10^{-6} cells/cm, for 50% light treatment. Rods were equal to or greater in number than cocci in virtually all samples, and bacteria were always more abundant than algae on all films. Direct counts of bacteria in the water column were ranked as follows: wave > 50 % light > surface. Bacteria in the water column were 1.3-3.6 x 10^{-6} cells/ml for wave treatment, 1.3-6.4 x 10^{-5}/ml for 50% light treatment, and 5.9 x 10^{-4} to 4.3 x 10^{-5}/ml for surface treatment. Greater variation in numbers was observed in plate counts than in direct counts. Significant differences ($P \leq 0.05$) in population numbers were observed within sets at 4 and 12 hr but not between sets at the same sampling intervals (Fig. 1). Few bacteria were attached to film B at time zero.

Bacteria were not attached to films A,C,D,E, and F at zero time but colonized most films during all phases within 60 hr. Bacteria adhering to films during Phases I and II were near 10^{-5} cells/cm^2 (Fig. 1-2). In Phase III, fewer bacteria were attached to films A and E (10^{-5} cells/cm^2 than D and F (10^{-6} cells/cm^2) and C 10^{-7} cells/cm^2) (Fig. 3). A wider range of bacterial numbers were observed during the wave treatment than during surface or 50% light treatments. Bacterial cells attached to film C were significantly higher ($P = <0.05$) than those attached to film E at several sampling times during Phases I and III but were not significantly different from those attached to other films.

Direct (AODC) counts of bacteria in the water column were generally within a range of 6.0 x 10^4 to 5.0 x 10^5 CFU/ml, except during Phase III when populations were approximately an order of magnitude higher, probably due to increased aeration during simulated wave action. Bacterial population numbers were relatively constant during each of the three experimental phases, although significant differences in numbers were observed between films C and E and between films C and F.

The tensile strength of film C (2% ECO polymer) was reduced from >200 MPa to 11 MPa (Phase I), 24 MPa (Phase II) and 45 MPa (Phase III) within 60 hr compared to a mean tensile strength of 15 MPa(dry -UVA), far surpassing the tensile strength losses of all other plastics. Elongation and TEB showed similar decreases in film C but not in other films during the 240-hr exposure to UVA light. The tensile strength (mean 35 MPa), elongation (13 MPa) and TEB (7 MPa) of film E (polystyrene) were very low compared to the values for all other films (i.e., tensile strength >200 MPa, elongation > 1200 MPa, and TEB >700 MPa). Each of these values for film E increased during exposure to UVA under both dry and wet conditions. Measurable changes were not observed in film D and F. Film A showed only slight decreases in each parameter after exposure to UVA for 180 hr under dry conditions: it showed no changes in the aquaria.

Adding plastic films under intense UVA to simulated aquatic ecosystems did not significantly decrease bacterial populations, whether free-living or attached to the films. Within 4 (film B) to 60 hr (other films) bacterial numbers increased, with populations of bacteria attached to the film surfaces equaling or surpassing the bacterial populations in the water column, per unit area. A conservative estimate of the microbial population attached to the glass of the aquaria places them equal to or greater than those adhering to the plastic films. Each aquarium also supported populations of 10^6-10^7 cells/cm^2 on the walls and floors. Bacteria in the water column may have simply attached to the film; however, no decreases in water column bacteria were observed. Because of bacterial population fluctuations during exposure to the plastic films, no definitive correlations between the degradation rates of films and bacterial populations or cell numbers were observed. Relatively high numbers and diversity of bacterial colony types were present in the water column and attached to each of the plastic films throughout the experimental period, whether or not the films were measurably degraded.

Bacterial succession occurred, and a minimum of 20 strains or species representing both gram-positive and gram-negative bacteria were present or abundant in all aquaria. Some bacteria were more likely than others to be dominant. Species richness and population numbers in all aquaria were sufficient to suggest that neither the plastic films nor their degradation products greatly enhanced, enriched or inhibited the growth of any select group of bacteria.

Acknowledgments. This work was supported by a grant from Dow Chemical Company, Freeport, TX 77541. We gratefully acknowledge the technical assistance of Margaret Gaesor, Duane Kimme, and Anique Colbert.

Reference

Allison CM and Swindoll CM and Pfaender FK (1987) Adaptation to and biodegradation of xenobiotic compounds by microbial communities from a pristine aquifer. Appl Environ Microbiol 83:2212-2217

Bell VL and Pezdirtz GF (1983) Effects of ionizing radiation on linear aromatic polyesters. J Polymer Sci 21:3083-3092

Day M and Wiles UM (1972a) Photochemical degradation of poly (ethylene terephthalate). II. Effect of wavelength and environmental on the decomposition process. J Appl Polymer Sci 16:191-202

Day M and Wiles UM (1972b) Photochemical degradation of poly(ethylene terephthalate). III. Determination of decomposition products and reaction mechanism. J Appl Polymer Sci 16:203-215

Hobbie JE and Daley RJ and Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl Envir Microbiol 33:1225-1228

Horsfall GA (1981) The daylight photodegradation of polyester and nylon fabrics. Textile Month 2:69-71

Horsfall GA (1982) Factors influencing the daylight photodegradation of nylon 6.6, nylon 6 and polyester in commercial fabrics. Textile Research J 52(3):197-205

Imam SH and Gould JM (1990) Adhesion of an amylolytic Arthrobacter sp. to starch-containing plastic films. Appl Envir Microbiol 56:872-876

Johnson R (1987) An SPI overview of degradable plastics. In "Degradable Plastics," Society of the Plastic Industry, Washington, DC, pp 6-13

Klausmeir RE Osmon JL (1976) Biodegradation of plastics by Actinomycetes. In Sharpley, J. M. and A. M. Kaplan [eds] "Proc. of the Third International Biodegradation Symposium," Applied Science, London, pp 815-881

Leonas KK and Gorden RW (1993) An accelerated laboratory study evaluating the disintegration rates of plastic films in simulated aquatic environments. J Environ Polym Degrad 1:45-51

Wool RP and Cole M (1988) Microbial degradation. Engineering Plastics 2:783-787 ASM International. Metals, Park, OH