

## Chapter 13

# Biodegradation of Polymer Films in Marine and Soil Environments

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The emergence of biodegradable plastics has necessitated the development of standard methods to determine biodegradation rates in various environments. Standardized accelerated marine and soil laboratory biodegradation test systems were developed in which comparative polymer biodegradation rates could be determined by quantifying and plotting the weight loss/surface area of each sample over time and determining the maximum slopes of the curves generated. The results indicate that, in general and depending on the environment, biodegradation rates for unblended polymers were: polyhydroxybutyrate-co-valerate > cellophane > chitosan > polycaprolactone. Results from blends are more difficult to interpret since different biodegradation rates of the component polymers and leaching of plasticizers and additives can impact the data.

The assessment of biodegradability of polymer films in natural environments is a difficult problem because of the inherent variations in environmental conditions from one site to another. This is further confounded by the need to balance material performance vs. rates of biodegradation (1,2). Therefore, many laboratory approaches have been developed to simulate natural biodegradation processes, but in a more controlled setting to try and predict natural environmental susceptibility of materials to biodegradation. These methods recently have been summarized and include enzyme assays, plate tests, clearing zones or changes in optical absorbance, biological oxygen demand, changes in carbon isotope ratios, release of radioactive products from radioactively labeled polymers, automated respirometry in biometer flasks, and accelerated simulated laboratory systems or mesocosms (3-5). Many of these methods are coupled to assessments of changes in weight, molecular weight, mechanical properties, morphological appearance, or chemical functionalities of the

polymer evaluated. In addition, new practices and methods to assess biodegradability are being developed by many organizations including the American Society for Testing and Materials (ASTM). All of the above methods are potentially useful but limited when used by themselves. If the test results are properly interpreted, important data to enhance the understanding of the environmental fate of the subject polymer film can be developed. It is clear that a battery of tests is usually required to fully assess the biodegradability of a polymer coupled with environmental impact and risk assessment. Tests on individual polymers must be factored against the effects of polymer processing and blending, consideration of the disposal environment where the material may end its lifecycle, and other constraints such as avoiding entrapment and ingestion hazards to organisms in the environment.

We define biodegradation as a process carried out primarily by bacteria or fungi in which a polymer chain is cleaved or modified by hydrolytic or oxidative enzymatic activity (6). Related terms or subsets of biodegradation include biotransformation and biomineralization. Biotransformation is the biologically-mediated change in chemical structure of the target polymer. Mineralization is the biologically-mediated complete breakdown of the polymer generating simple gases like carbon dioxide, methane and nitrogen, water and biomass, so that all elements from the polymer re-enter natural geochemical and microbial cycles.

With a goal of developing biodegradable polymers useful for a range of applications, it is important to use appropriate test methods to provide data on biodegradability and environmental impact. Our primary concerns are with soil and marine environments, thus we focused on developing appropriate test methods to assess candidate materials for disposal in these environments. We have used a three tier system to characterize biodegradability and assure that environmental impact is negligible. In the first tier, susceptibility of the individual polymers to mineralization is assessed using automated respirometry (7). If the polymer is mineralizable, then in the second tier, the processed polymer blend or formulation is assessed for evidence of biodegradability in the laboratory by exposure in simulated environments (marine or soil). After exposure for varying periods of time, the materials are characterized for changes in mechanical properties or chemical structure. In the third tier, the material is exposed in natural environments to corroborate the laboratory results. In all tiers, we also assess the toxicity of the polymer, the blends after processing, and the residues subsequent to biodegradation and exposure in the various systems (8). Toxicological impact is a critical component in the assessment process since rates of biodegradation will differ significantly in different environments. For example, if no toxicity is observed, then rates of biodegradation in marine environments become less of a factor as long as the more acute effects, such as entrapment or ingestion hazards to marine animals, are addressed, or the aesthetics of litter are tolerable. This may be handled by

demonstrating rapid loss of mechanical properties in the target disposal environment, even if rates of mineralization are very low. All three tiers need to be considered for a complete environmental risk assessment before a new biodegradable polymer formulation should be considered for use. In addition, if the new formulation will be in contact with food, the U.S. Food and Drug Administration regulations also will have to be considered.

In the process of carrying out our program goals as described above, we found it necessary to develop new test systems and methods for some of the evaluations we needed to perform on biodegradable materials. We describe our efforts to develop laboratory-scale simulated soil and marine biodegradation test systems and some of the biodegradation kinetics observed for candidate materials. Some aspects of the development of these methods have been previously published (6,9). Our test methods for automated respirometric analysis of the polymers have already been described (7) and the toxicological studies have also, in part, been published (8).

### Materials and Methods

**Polymer Films** Polyhydroxybutyrate-co-valerate (PHBV), containing 8, 16 and 24% valerate, were obtained from Imperial Chemical Industries (Zeneca), Billingham, UK. Uncoated- and nitrocellulose-coated cellophane films were supplied by DuPont, Wilmington, DE. Crosslinked chitosan (Protan Laboratories, Redmond, WA) films were produced by reaction with epichlorohydrin (10). Starch/ethylene vinyl alcohol (St/EVOH) blend films and pure EVOH film (38 mole percent ethylene) were obtained from Novamont (Novara, Italy) and EVALCo (Lisle, IL), respectively. Polycaprolactone (PCL), molecular weight 80,000 Daltons, in film form, was received from Union Carbide (Bound Brook, NJ).

**Cellulose Acetate/Starch (CA/St) Blends for Tensile Bars** CA/St blends were produced by mixing cellulose acetate (degree of substitution 2.1) (Eastman Chemical, Kingsport, TN) with 30% amylose starch (Melogel, National Starch, Inc., Bridgewater, NJ) and propylene glycol (Dow Chemical, Midland, MI) at a ratio of 59:19:22 (weight percent) (11). Mixing was in a high intensity Henschel mixer (Purnell International, Houston, TX) at 85°C, 3000 rpm for approximately two minutes. Pellets were then produced using a Brabender (Hackensack, NJ) single screw extruder (1.9 cm diameter X 47.6 cm length) operating at 60 rpm with the following temperature profile: Zone 1 = 120°C, Zone 2 = 140°C, Zone 3 = 160°C, and die (4 hole, 4 mm) = 170°C. Pellets were converted into dogbone shaped tensile bars (3.7 cm X 0.3 cm) using a bench top miniature injection molder (Custom Scientific Instruments, Inc., Cedar Knolls, NJ) heated to 185°C.

**Marine Simulator** Sample exposures were conducted in 76 liter aquaria maintained at 30°C as previously described (9). Aquaria were filled with 50 mm thick sediment (natural or defined - see later) topped with standardized simulated seawater (Aquarium Systems, Mentor, OH). The components (weight percent as provided by the manufacturer) included: chlorine, 54.2; sodium, 31.5; sulfate, 8.1; magnesium, 3.9; calcium, 1.1; potassium, 1.1; in mg/L, included: boron, 32.1; strontium, 10.0; phosphorus, 2.4; lithium, 2.24; tin, 2.22; aluminum, 0.87; vanadium, 0.57; molybdenum, 0.42; silicon, 0.42; iron, 0.21; barium, 0.13; chromium, 0.13; nickel, 0.1; cobalt, 0.06; manganese, 0.03; zinc, 0.02. This composition is designed to mimic natural seawater (12). The marine water was aerated by aquaria air pumps (Willinger Bros., Oakland, NJ) at approximately one liter per minute. The water in each aquarium was continuously replaced by a peristaltic pump (Rainin Instrument Co., Woburn, MA) with fresh simulated sea water at a weekly exchange rate of 15% of the total aquarium volume to minimize accumulation of potentially inhibitory biodegradation metabolites. Twenty watt fluorescent lights were used at 12 hour on/off cycles to simulate light effects on marine organisms. Triplicate samples of films (72 mm X 25 mm) or tensile bars (3.7 cm X 0.3 cm) were placed in fiberglass screening (18 X 16 mesh, opening size of approximately 1 mm). Screens were placed both vertically in the water phase and horizontally in the sediment, 25 mm below the surface. Total maximum loading of polymer in the system was approximately 1.3 g/L.

**Marine Sediments** Natural marine sediment was collected in the tidal zone along the beach at Gloucester, MA. In the defined marine sediment, commercial grade sand, 0.45 - 0.55 mm particle size, served as the matrix. Marine agar 2216 (Difco Laboratories, Detroit, MI) was mixed with the sand at 0.2, 0.4 and 0.6 percent (wt/wt) to determine the levels of the agar required to support equivalent numbers of marine microorganisms in comparison to the levels present in the natural sediments. Organic carbon contents of natural sediment and the commercial sand were determined by heating samples at 560°C for 18-24 hours in a furnace (Thermolyne, Dubuque, IA).

**Marine Inoculum and Counts** For the natural sediment marine test systems, water containing local flora were obtained from marine waters off a beach in Gloucester, MA. For the defined sediment marine systems, the inoculum contained nine marine microorganisms that we had previously isolated based on their ability to utilize a series of polymer substrates as carbon sources (9). Some of the organisms were identified using the Biolog Microstation System, Release 3.5 (Biolog, Inc., Hayward, CA). Table I identifies the marine isolates used to inoculate the defined

marine systems and the substrates they were able to utilize as sole carbon sources in liquid minimal media. During the exposures of polymer samples in the marine test systems, microbial counts in the marine water and sediment were determined by serial dilutions in sterile simulated seawater and using spread plates with 0.1 mL of each dilution bottle onto marine agar. Plates were incubated 1-2 days at 30°C.

**Soil Simulators** A water retentive, aerated soil was produced by mixing equal parts by weight topsoil (Earthgrow, Inc., Lebanon, CT), composted cow manure (1881 brand, Earthgrow, Inc., Lebanon, CT) and sand (0.45 - 0.55 mm particle size) with 30% (wt/wt) water. Moisture content and pH were 30% and 7.1, respectively. The natural flora in the soil served as the inoculum. The counts of microorganisms in the sand, topsoil and composted cow manure were determined by placing one gram of each medium into phosphate buffer, pH 7.0, diluting, spread plating 0.1 ml onto nutrient agar, and incubating the plates at 30°C for 1-2 days. The soil mixture was placed in soil boxes (33 cm long X 22 cm wide X 8 cm high) covered with sliding plexiglass sheets and turned periodically. Film and tensile bar samples were placed into the soil boxes without fiberglass screens and incubated at 30°C. The soil and composted cow manure were analyzed for pH, nitrate and ammonia.

**Sample Retrieval and Analysis.** Samples were retrieved from marine and soil simulators at selected weekly intervals, washed with distilled water to remove debris, dried to constant weight at 70°C and weighed. Weight loss data are presented as weight loss/surface area ( $\mu\text{g}/\text{mm}^2$ ) and plotted vs. time. Maximum rates of biodegradation for each polymer or blend were determined using the Origin software program (Version 2.67, Microcal Software, Inc, Northampton, MA) to calculate the maximum slope for each weight loss/surface area vs. time curve.

### Results and Discussion

Weight loss/surface area is equated here with biodegradation. We recognize this is an extrapolation due to dissolution and hydrolytic effects being confounded with biodegradation as defined earlier. However, in referring back to the context of our three tier system, we will be discussing polymers that we have already studied for mineralizability, and therefore those polymers lost from the samples, by biodegradation, solubilization or hydrolysis, will be mineralized over time, and thus we consider them part of the biodegradable fraction. In addition, we have examined several of these polymers in sterile control systems and found negligible effects of hydrolysis alone on weight loss at 30°C. Finally, there are many reports in the literature detailing rates of hydrolytic degradation or rates of solubilization of these

polymers. In many cases these rates of hydrolysis are very low (*see later 12*), unless high temperatures or pH extremes were used in the studies.

**Table I. Substrates Utilized by Microorganisms in the Defined Marine Systems.**

<u>Organism</u>	<u>Polymer Substrate</u>				
	<u>Starch</u>	<u>EVOH</u>	<u>PHBV(16%V)</u>	<u>PCL</u>	<u>Cellulose</u>
<i>Vibrio halophlanktis</i>	X <sup>1</sup>	--- <sup>2</sup>	---	---	X
<i>Vibrio proteolyticus</i>	X	---	---	---	---
<i>Bacillus megaterium</i>	X	---	X	X	X
<i>Pseudomonas</i> sp. 1	X	---	---	---	X
<i>Zoogloea</i>	X	---	X	---	---
<i>Pseudomonas</i> sp. 2	X	---	---	---	X
<i>Actinomyce</i> te sp.	---	X	X	X	X
<i>Bacillus</i> sp.	X	---	---	---	X
Unknown	X	---	---	---	X

<sup>1</sup>substrate utilized, <sup>2</sup>substrate not utilized

In developing the defined marine simulators, we assessed the effect of adding various amounts of marine agar to commercial sand on the biodegradation rate of PHBV(8%V) in comparison to the natural marine sediment (Figure 1). The results with 0.2% marine agar most closely mimicked the rates found in natural sediment, based on comparisons of rates of weight loss/surface area. The organic analysis of the natural sediment and the commercial sand showed 0.18% and 0.04% organic content, respectively. These data correlate well with the degradation results in that approximately 0.2% organic content correlates to maximum activity in both the defined and native marine simulators.

The counts of microorganisms from marine and soil test simulators are presented in Table II. The marine counts varied by approximately two logs in magnitude in the natural population due to seasonal variations. Topsoil contained the highest microbial counts, followed by the composted cow manure and then the sand. Based on morphology of colony types, the topsoil appeared to contain the most diverse populations.

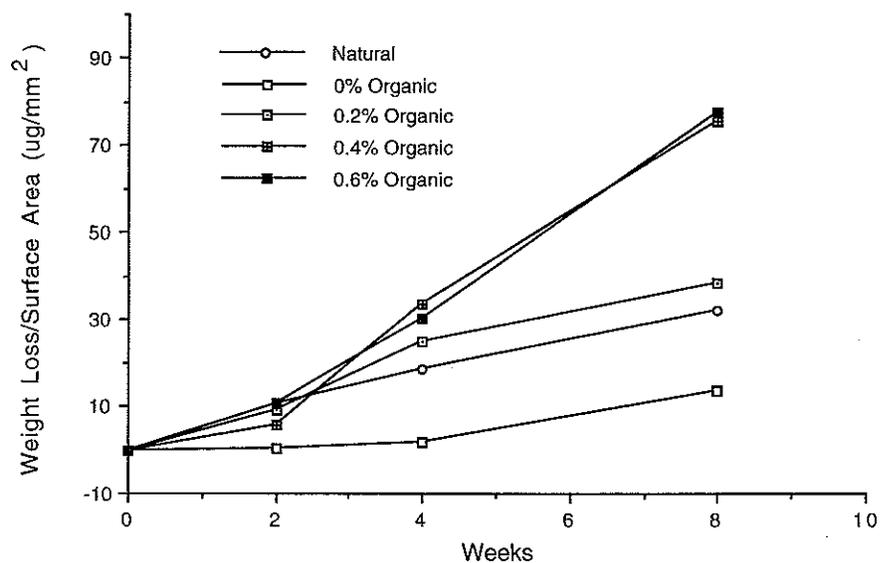


Figure 1. Influence of marine agar on rates of biodegradation of PHBV(8% V).

Table II. Counts of Microorganisms in the Simulator Components

<u>Source</u>	<u>Microbial Counts/ml or gram</u>
Natural Marine Sediment	$10^3$ - $10^5$
Natural Marine Water	$10^3$ - $10^5$
Defined Marine Sediment (0.2% marine agar)	$10^6$
Topsoil	$10^6$
Composted Cow Manure	$10^5$
Sand	$10^4$

The analysis of the nutrient content of some of the components used in the soil simulators indicates that the composted cow manure contributes a large amount of ammonia nitrogen which should facilitate the biodegradation of the carbon-rich, nitrogen-poor polymers. The pH, nitrate (mg/L), ammonia (mg/L) and total kjeldahl nitrogen contents of topsoil were 6.3, 110, 167, and 815, respectively, while the corresponding numbers for the composted cow manure were 8.6, 0.8, 2570 and 6130.

Graphs of weight loss/surface area vs. time of PHBV(8%V) following marine and soil exposures are presented in Figure 2, and a graph for the different polymers in soil is shown in Figure 3. It is often difficult to extrapolate relative biodegradation rates by comparison of the lines on the graphs, therefore, we used maximum slope calculated by the Origin software. Table III presents the data for maximum biodegradation rates in  $\mu\text{g}/\text{mm}^2/\text{week}$  for several polymers or blends in the soil simulators, the defined marine water and sediment simulators, and the natural marine water and sediment simulators. The results indicate, in general, that biodegradation in soil is more rapid

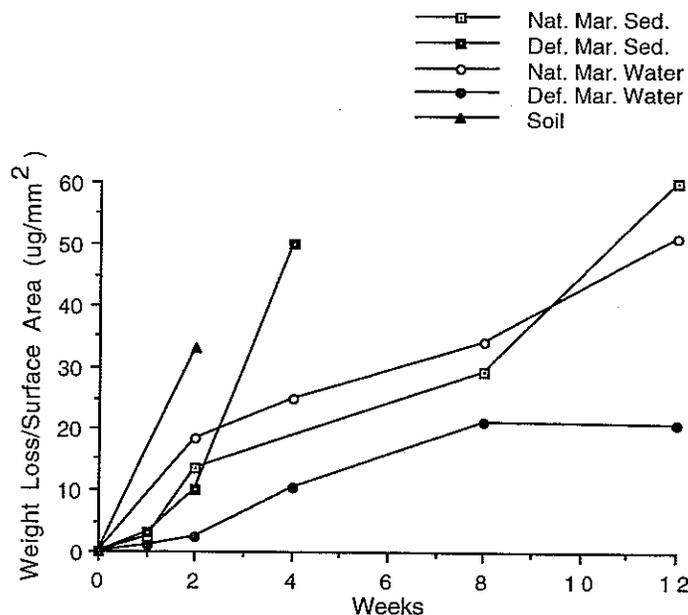


Figure 2. Weight loss/surface area vs. time of PHBV(8%V) in marine and soil simulators.

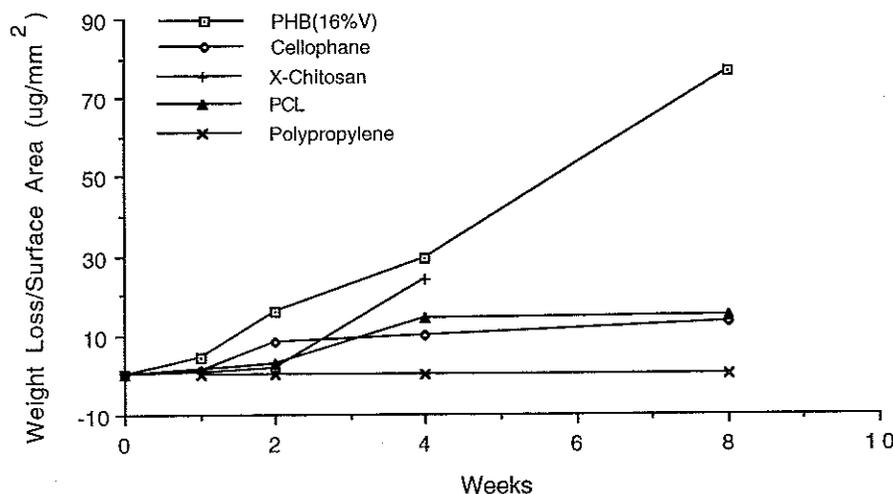


Figure 3. Weight loss/surface area vs. time of the different polymers in soil simulators.

than in marine water and sediment, except for the PCL which appears to biodegrade at about the same rate in all environments (all of which are at 30°C). PCL exhibits slow initial weight loss in all the test environments, followed by rapid weight loss after approximately eight weeks incubation time. This effect could be due to a requirement for initial nonbiological hydrolysis of the amorphous domains to lower molecular weight to a more suitable range for microbial degradation. Therefore, rates of biodegradation of PCL and related polyesters may be greatly influenced by temperature based on the correlation between temperature and hydrolytic attack and dissolution. This may be a critical factor to consider in designing biodegradable materials that may be disposed in a marine environment since the temperature on the deep ocean floor often ranges between 5 - 15°C (personal communication - C. Wirsén).

Of the homopolymers evaluated, PHBV, uncoated cellophane and crosslinked chitosan were readily biodegraded based on rates generally above around 5  $\mu\text{g}/\text{mm}^2/\text{week}$  (Table III). PCL was moderately biodegradable at rates around 3 to 4  $\mu\text{g}/\text{mm}^2/\text{week}$ , and polypropylene and EVOH were recalcitrant under the simulator test conditions. A marine actinomycete was isolated that appeared to grow well on EVOH as the sole carbon source in preliminary experiments; however, rates appear to be too low to detect in the simulators or in respirometry. St/EVOH blends show some evidence they are susceptible to biological activity, but total degradation does not exceed that expected from the starch alone. The data for the maximum rates of biodegradation for many of the polymers are higher than the rate observed for bond paper. This is a particularly important consideration when designing materials to degrade in a municipal compost environment with well prescribed cycle times.

Table III. Maximum biodegradation rates ( $\mu\text{g}/\text{mm}^2/\text{week}$ ) for polymers and blends

Polymer/Blend	Soil	Defined Marine Sediment	Natural Marine Sediment	Defined Marine Water	Natural Marine Water
PHB(8%V)	17.0 (n=3)	15.6 (n=27)	6.2 (n=6)	3.7 (n=18)	11.4 (n=6)
PHB(16%V)	12.0 (n=6)	ND <sup>1</sup>	6.8 (n=12)	ND	22.2 (n=9)
PHB(24%V)	25.5 (n=6)	12.6 (n=12)	5.0 (n=12)	11.2 (n=12)	10.5 (n=15)
PCL	3.5 (n=3)	3.0 (n=18)	3.2 (n=6)	3.2 (n=12)	3.9 (n=4)
St/EVOH	2.0 (n=6)	3.0 (n=9)	4.2 (n=6)	1.2 (n=3)	2.8 (n=6)
EVOH	0 (n=3)	0 (n=3)	0 (n=3)	0 (n=3)	0 (n=3)
PP <sup>2</sup>	0 (n=3)	0 (n=3)	0 (n=3)	0 (n=3)	0 (n=3)
Uncoated	12.0	2.0	3.1	9.4	4.7
Cellophane	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)
Coated	4.0	ND	1.6	ND	1.5
Cellophane	(n=3)		(n=9)		(n=9)
CA <sup>2</sup> /St/PG <sup>2</sup>	ND	43.7 (n=9)	42.5 (n=9)	47.3 (n=9)	39.0 (n=9)
PE <sup>2</sup> /Paper	12.7 (n=3)	ND	2.0 (n=3)	ND	7.8 (n=3)
Bond Paper	ND	8.0 (n=3)	4.7 (n=3)	10.8 (n=3)	4.5 (n=3)
X-linked	10.1	ND	11.6	ND	7.5
Chitosan	(n=3)		(n=3)		(n=3)

<sup>1</sup>ND = no data<sup>2</sup>PP = polypropylene; CA = cellulose acetate; PG = propylene glycol; PE = polyethylene

Mergaert *et al.* (13) reported 0.03 to 0.64% weight loss/day upon exposure of PHB or PHBV (10%V) in soils. Rates increased from 15°C up to 40°C. They also found no loss in weight in sterile buffers up to 55°C over 98 days, although some loss in molecular weight was attributed to abiotic hydrolysis. Doi *et al.* (14) reported the biodegradation of various copolymers of PHBV in film and fiber forms in natural marine environments over one year of exposure. Surface erosion was the primary effect observed and this was found to correlate directly with sea water temperature and not polymer composition. Changes in gravimetric weight were more pronounced than polymer molecular weight due to the surface effects. Two actinomycetes were also isolated and found capable of using PHB as a sole carbon source.

The three tier system we have described (only a part of these assessments was included in this paper), provides for a reasonable assessment of new biodegradable polymers. Many other methods to assess biodegradation are being developed which can be integrated into this tier system as they develop. For example, Yabannavar and Bartha (15) recently presented soil degradation data for polymer films and found GPC-coupled with carbon dioxide evolution useful to distinguish between biodegradation due to plasticizer and additives vs. the polymers themselves. However, the author's recognized that even this approach can lead to problems in interpretations when polymer cross-linking occurs or in cases where there are surface erosion effects.

The rationale for using a limited set of previously isolated microorganisms in the defined marine simulators was to overcome the high degree of variability observed when using natural populations collected at different sites at different times. The goal was to 'standardize' everything in the simulators, the water, organisms and sediment, so that reproducibility would be optimal. The use of a set of defined microorganisms is inherently prone to possible omissions of key microorganisms; however, this is addressed with the inclusion of new isolates to the mix that are selected for their ability to biodegrade new types of polymers.

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