AD-A254 051

(2)

David Taylor Research Center

Bethesda, MD 20084-5000

DTRC-SME-CR-19-90 March 1992

Ship Materials Engineering Department

Research and Development Report

Research and Development of Two Marine-Degradable Biopolymers

by

Anthony L. Andrady, Ph.D.

Jan E. Pegram, Ph.D.

Research Triangle Institute

and

Todd M. Olson

David Taylor Research Center

DTIC
ELECTE
AUG 19 1992
S A D

Approved for public release; distribution unlimited.

92-22925

92 8 17 009

DTRC-SME-CR-19-90 Research and Development of Two Marine-Degradable Biopolymers

MAJOR DTRC TECHNICAL COMPONENTS

CODE 011	DIRECTOR OF TECHNOLOGY, PLANS AND ASSESSMENT
12	SHIP SYSTEMS INTEGRATION DEPARTMENT
14	SHIP ELECTROMAGNETIC SIGNATURES DEPARTMENT
15	SHIP HYDROMECHANICS DEPARTMENT
16	AVIATION DEPARTMENT
17	SHIP STRUCTURES AND PROTECTION DEPARTMENT
18	COMPUTATION, MATHEMATICS & LOGISTICS DEPARTMENT
19	SHIP ACOUSTICS DEPARTMENT
27	PROPULSION AND AUXILIARY SYSTEMS DEPARTMENT
28	SHIP MATERIALS ENGINEERING DEPARTMENT

DTRC ISSUES THREE TYPES OF REPORTS:

1. **DTRC reports, a formal series,** contain information of permanent technical value. They carry a consecutive numerical identification regardless of their classification or the originating department.

2. **Departmental reports, a semiformal series,** contain information of a preliminary, temporary, or proprietary nature or of limited interest or significance. They carry a departmental alphanumerical identification.

3. **Technical memoranda, an informal series,** contain technical documentation of limited use and interest. They are primarily working papers intended for internal use. They carry an identifying number which indicates their type and the numerical code of the originating department. Any distribution outside DTRC must be approved by the head of the originating department on a case-by-case basis.

NDW DTNSRDC 5602/51 (Rev. 2-88)

David Taylor Research Center
Bethesda, MD 20084–5000

DTRC–SME–CR–19–90 March 1992
Ship Materials Engineering Department
Research and Development Report

Research and Development of Two Marine-Degradable Biopolymers

by
Anthony L. Andrady, Ph.D.
Jan E. Pegram, Ph.D.
Research Triangle Institute
and
Todd M. Olson
David Taylor Research Center

DTIC QUALITY INSPECTED 5

Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

CONTENTS

	Page
Abbreviations	v
Abstract	1
Introduction	1
Background	2
Administrative Information	2
Approach	2
Progress: October 1989 to September 1990	3
Developing Definitions and Test Protocols	3
Definition of Degradability	3
Clarification of the Definition of Plastics for the Coast Guard	3
Justification for Chitosan/Regenerated Cellulose Approach	3
Establishing the Degradation of Chitosan and Cellulose Materials	6
Exposures of Chitosan and Regenerated Cellulose Under Marine Conditions	6
Gas Evolution Studies Under Anaerobic Exposure	6
Large-Scale Production of Chitosan and Regenerated Cellulose	10
Chitosan	10
Regenerated Cellulose	13
Plasticization of Chitosan and Cellulose Materials	13
Initial Screening Experiments With Chitosan	13
Preliminary Studies on Plasticizer Performance in Chitosan	14
Further Studies on Plasticization of Chitosan	15
Plasticization of Regenerated Cellulose by Lithium Salts	17
Additives for Improving Tensile Properties and Degradability	20
Effect of Additive on the Rate of Change in Gas Evolution on Exposure	20
Effect of Additive on the Rate of Change in Strength on Exposure	20
Study of Adhesives	21
Conclusions and Future Work	22
Appendix A—Progress During the Period Oct 1988 Through Sept 1989	25
Reference	39

FIGURES

1. Tear resistance of candidate films. 5
2. Marine exposure of chitosan at Beaufort, N.C. 7
3. Marine exposure of regenerated cellulose at Beaufort, N.C. 8

4. Total degradation over time in marine sediment (25 °C). 10
5. Break stress vs. strain for final candidates. 17
6. Tensile properties of regenerated cellulose films treated with lithium salts. 19
7. Effect of ammonium phosphate on degradation rate of regenerated cellulose film under soil burial. 21
A.1. Rapid biodegradation of chitosan film by soil microorganisms. 29
A.2. Accelerated biodegradation apparatus. 31
A.3. Instron machine with tensile specimen. 33
A.4. Stress relaxation test apparatus. 34
A.5. Force-time relationships for a chitosan film. 35
A.6. Biometer flask for mineralization measurements. 36
A.7. Total biodegradation (mineralization) measured by CO_2 evolution in biometer tasks. 37
A.8. Flow chart for evaluation of biopolymer films. 38

TABLES

1. Results for ASTM D1004. 4
2. Anaerobic degradation of chitosan and cellulose at 25 °C. 9
3. Reproducibility of anaerobic degradation of chitosan and regenerated cellulose in garden soil (25 °C). 11
4. Tensile properties of plasticized chitosan films—effect of immersion of plasticized film in water. 15
5. Tear resistance of unrinsed plasticized chitosan films (ASTM D1004). 16
6. Tensile properties and tear resistance of selected plasticizer candidates in chitosan films. 18
7. Effect of urea/phosphate on aerobic degradation of cellulose samples in garden soil. 20
8. Data for the strength of adhesive bonds on regenerated cellulose. 23
A.1. Tensile data for unexposed films of various compositions 28
A.2. Effect of soil burial on tensile strength of chitosan. 30
A.3. Effect of Miami marine exposure on tensile strength and viscosity of chitosan. 30
A.4. Effect of Beaufort marine exposure on the strength of chitosan 30
A.5. Effect of accelerated laboratory exposure on the strength of chitosan 32

ABBREVIATIONS

ASTM	American Society for Testing and Materials
DAS	dialdehyde starch
ICI	Imperial Chemical Industries
LDPE	low density polyethylene
MARPOL	International Convention for the Prevention of Pollution from Ships (73/78)
mmole	millimole
ONT	Office of Naval Technology
PEG	polyethylene glycol
PHBV	polyhydroxy butyrate valerate
P.L.	Public Law
RTI	Research Triangle Institute
TEG	tetraethylene glycol
w/w	weight/weight

ABSTRACT

The Navy is developing a biopolymeric film material suitable for fabrication into marine-disposable trash bags so that it can comply with impending national and international requirements which will prohibit the discharge of plastics into the sea. Two biopolymers, chitosan and regenerated cellulose, were selected and tested to meet this need.

After 6 weeks of marine exposure, regenerated cellulose samples disappeared; after 10 weeks, chitosan samples became brittle and separated. While chitosan showed greater anaerobic degradation than regenerated cellulose in soil studies, the opposite occurred in the marine sediment environment. Aerobic degradation was much higher than anaerobic degradation for both biopolymers.

To improve flexibility, 50 plasticizers were tested in chitosan. Ten percent lithium bromide and 5% lithium acetate/10% PEG 400 in chitosan were the most effective plasticizers. Regenerated cellulose films treated with lithium salt solutions also showed improved flexibility.

Incorporating urea and potassium phosphate into cellulose showed that degradation could be increased in soil. Tests are ongoing to further accelerate the rate of biodegradation by increasing the availability of nitrogen and phosphorus.

Fabricating trash bags will require adhesive bonding. Five adhesives were evaluated with regenerated cellulose. Covinax 220, JW 2–47, and Adcote 333T proved acceptable.

Chitosan requires further development to be produced and processed into bags efficiently. With minor adjustments, regenerated cellulose presently meets this requirement; thus, it is the more promising film.

Progress towards our goal of developing a biopolymeric film material meeting the Navy's requirements is continuing. Future work will focus on increasing strength through lamination; improving tear strength, flexibility, and degradability; selecting the optimum adhesive; and adapting a composite film to a mechanized bag-making process.

INTRODUCTION

The Marine Plastic Pollution Research and Control Act of 1987 (Public Law [P.L.] 100–220) implements the provisions of the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78 Annex V), which prohibits the discharge of plastics into the ocean. The Navy must comply with P.L. 100–220 by January 1, 1994. In order to comply, Navy ships will have to destroy plastics at sea, store waste plastics onboard until they can be off-loaded, or develop a new class of degradable material suitable for processing and disposing at sea. In January 1989, Navy crews were instructed to segregate plastics from nonplastics and store the plastic waste onboard for offloading ashore. Ships are required to store nonfood-contaminated plastic wastes for a minimum of 20 days and food-contaminated plastic wastes for the final 3 days at sea.* Presently, ships continue to discard nonplastic waste at sea in accordance with other provisions of Annex V. For handling and safety reasons, this nonplastic waste must be contained when it is

*The Navy's strategy on waste management relies on a broad spectrum of technologies, including Navy-developed vertical trash compactors, solid waste pulpers, and plastic waste processors.

thrown overboard. An environmentally safe nonplastic trash bag is a suitable container to attain this unique requirement.

BACKGROUND

In 1987, the Research Triangle Institute (RTI) was contracted by the Environmental Protection Branch of the David Taylor Research Center (DTRC) to develop a degradable plastic trash bag suitable for waste disposal at sea. After reviewing the various technologies available then for the production of a degradable plastic bag for marine use, we selected a single approach. This approach blended natural, readily available biodegradable polymers such as starch, cotton, cellulose, etc., with synthetic plastics such as polypropylene and polyethylene to obtain a rapidly biodegradable product.

Starch-plastic films and also blends of polyethylene with polycaprolactone were good candidates for a film material that would biodegrade within several years. We evaluated commercially available film samples or other promising samples which industry claimed to be biodegradable. Both the commercially available films and those films fabricated at RTI were tested for their biodegradability in both the marine environment and in an accelerated biodegradation apparatus. We found that the time to embrittlement for these films, when exposed in the marine environment, would be longer than 1 year.[1]

After the passage of MARPOL Annex V and P.L. 100–220, perception of the desirable lifetimes of degradable plastics changed; these changes were reflected in our research. Our new goal is to produce a film that is composed entirely of natural materials which degrades within 4 weeks.

Chitosan, the second most abundant biopolymer (obtained from shellfish, crabs, lobster, etc.) was regarded as a good candidate. It occurs naturally and is known to biodegrade in soil. We also considered amorphous regenerated cellulose films (a cellulose derivative processed to produce a desired shape and then treated to remove the modifying groups to regenerate unmodified cellulose) as a suitable material in fabricating a marine-degradable bag.

ADMINISTRATIVE INFORMATION

The Exploratory Development program is being conducted for the Office of Naval Technology (ONT) under program element 62233N and DTRC work unit 2830–102. The program was coordinated by CDR A. Baivier (ONT Code 226) and Mr. N. Albertsen, CE2A block manager (Naval Civil Engineering Laboratory, Code L03BPM).

APPROACH

The approach to develop a degradable biopolymer film suitable for fabrication into waste disposal bags consists of four subtasks:

1. Develop definitions and test protocols;
2. Test candidate biopolymer materials for degradability under soil, marine, and accelerated biodegradation conditions;
3. Fabricate prototype films with emphasis on large-scale commercial production; and

4. Investigate additives to improve physical properties

PROGRESS: OCTOBER 1989 TO SEPTEMBER 1990

DEVELOPING DEFINITIONS AND TEST PROTOCOLS

Definition of Degradability

The Navy and RTI participate in and monitor the American Society for Testing and Materials' (ASTM) efforts at arriving at suitable definitions, test protocols, and standards for degradable plastics. ASTM, the foremost standards-writing organization in the world, is in an excellent position to lead such an effort. ASTM's interpretation of degradability will impact the activities of the Navy within this task.

The standard-making process has been progressing slowly within ASTM because it is a consensus organization and because of the difficulty inherent in defining "degradability." In the absence of universally acceptable definitions, a set of working definitions has been used until ASTM definitions are available. According to these, a film material is said to be degradable on the basis of meeting the following two criteria:

1. It must disintegrate upon exposure to the environment. This is measured in terms of a relevant mechanical property such as tensile strength or ultimate elongation.
2. Such disintegration must be due to a chemical or biological process (as opposed to physical factors). The occurrence of the process can be established by demonstrating carbon dioxide and/or methane evolution when the plastic-like material is exposed to the environment.

These criteria were used in addressing the degradability of plastic-like materials within the scope of this program.

Clarification of the Definition of Plastics for the Coast Guard

Acceptable film materials for shipboard use center around the use of biopolymers. There should be no ecological basis for restricting their use because they are biodegradable at a rate determined by nature's carbon cycle.

The Coast Guard, like the Navy, is concerned with marine pollution. However, the Coast Guard has its own definition of plastics, which does not include biopolymers. Recent attempts to interpret this definition involved the inclusion of biopolymers "harvested and adapted for use by man"* in the category of synthetic polymers. Such a ruling would include chitin, chitosan, cellulose, polyhydroxy butyrate valerate (PHBV), paper, and cotton textiles under the definition of plastics for the purposes of MARPOL Annex V. We have responded to the invitation for public comments on Annex V and definitions by the Coast Guard, stating our objection to their interpretation of plastic.

Justification for Chitosan/Regenerated Cellulose Approach

Annex V and its associated definition of plastics do not distinguish between biodegradable and nonbiodegradable plastics. The marine disposal restriction uniformly applies to all high polymers of synthetic origin. The use of biopolymers would present an

*Quote from the *Federal Register* (Vol. 55, No. 171).

optimum solution to the need for a film material that is disposable at sea. These materials are derived from renewable resources, are satisfactorily biodegradable, and are not associated with toxic degradation products. The only drawback to their use is the need for a greater developmental effort (compared to synthetic polymers) before the material can be used.

In addition to cellulose and chitin/chitosan (the first and second most abundant biopolymers, respectively), there is also current interest in bacterial polyesters such as PHBV. These polymers, which are produced by bacteria, are biodegradable under both soil and marine conditions.

For Navy applications, the key properties are tensile and tear properties. The strength of PHBV is comparable to that of chitosan and cellulose. However, PHBV is much more expensive than chitosan and regenerated cellulose. Its selection over cellulose/chitosan as a candidate for the present application can only be justified if PHBV displays superior tear resistance.

Figure 1 compares the tear properties for the different materials. (Only the data in the machine direction are shown.) Figure 1a shows data from the ASTM D1938 ("Standard Test Method for Tear Propagation Resistance of Plastic Film and Thin Sheeting by a Single-Tear Method") test, which is a measure of the ease with which a tear will propagate once a cut is initiated. The data are displayed as a function of thickness for films of different thicknesses. Figure 1b shows the data from ASTM D1004, "Tear Resistance of Plastic Film and Sheeting," which measures the load required to initiate a tear in a specially shaped test piece made from the film.

The data show that resistance to tear propagation is approximately the same for all three biopolymers (an order of magnitude lower than low density polyethylene). Interestingly, the resistance to tear initiation is higher for chitosan and cellulose compared to PHBV. (PHBV films supplied by ICI were used for this test.) The data for cross direction showed a similar trend. Table 1 shows the data for the tear initiation test.

Table 1. Results for ASTM D1004.

Material	Tear Direction	Thickness (mm)	Peak Load (kg)	Load Standard Deviation (kg)	Number of Specimens
Low Density Polyethylene	Machine	0.075	0.532	0.008	10
	Transverse	0.075	0.457	0.05	9
Low Density Polyethylene	Machine	0.052	0.384	0.033	10
	Transverse	0.052	0.371	0.036	10
PHBV	Machine	0.016	0.212	0.047	10
	Transverse	0.016	0.235	0.020	10
Chitosan	Isotropic	0.050	1.30	0.24	8
Nonplasticized Cellulose	Machine	0.039	1.65	0.12	10
	Transverse	0.039	1.33	0.14	10
Plasticized Cellulose	Machine	0.039	1.04	0.09	10
	Transverse	0.039	0.832	0.05	10

Tested at a crosshead speed of 50 mm/min and a gauge length of 25 mm. All samples were conditioned at 50% relative humidity.

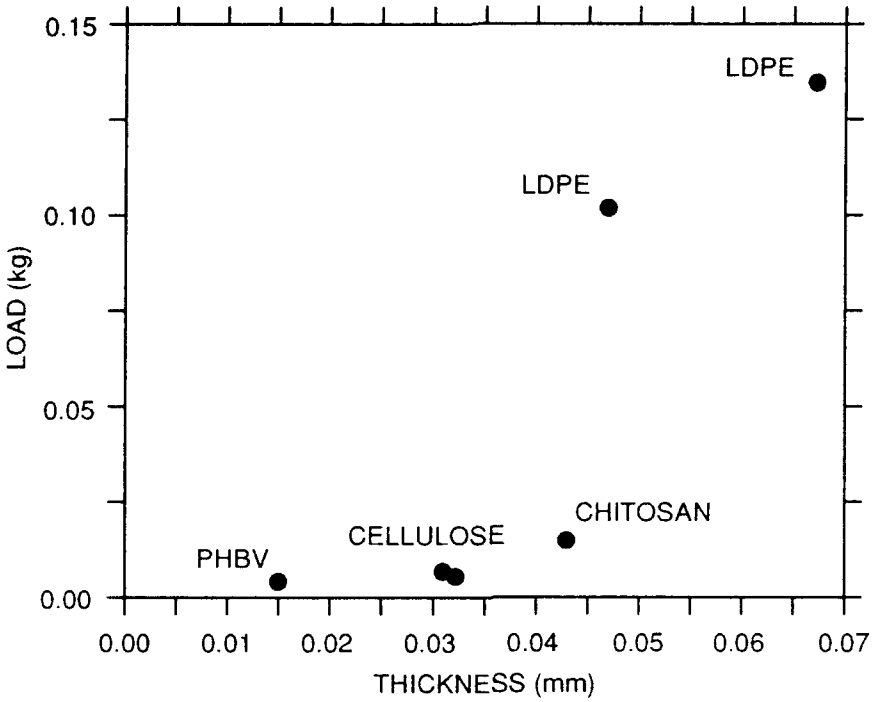


Figure 1a. ASTM D1938 data.

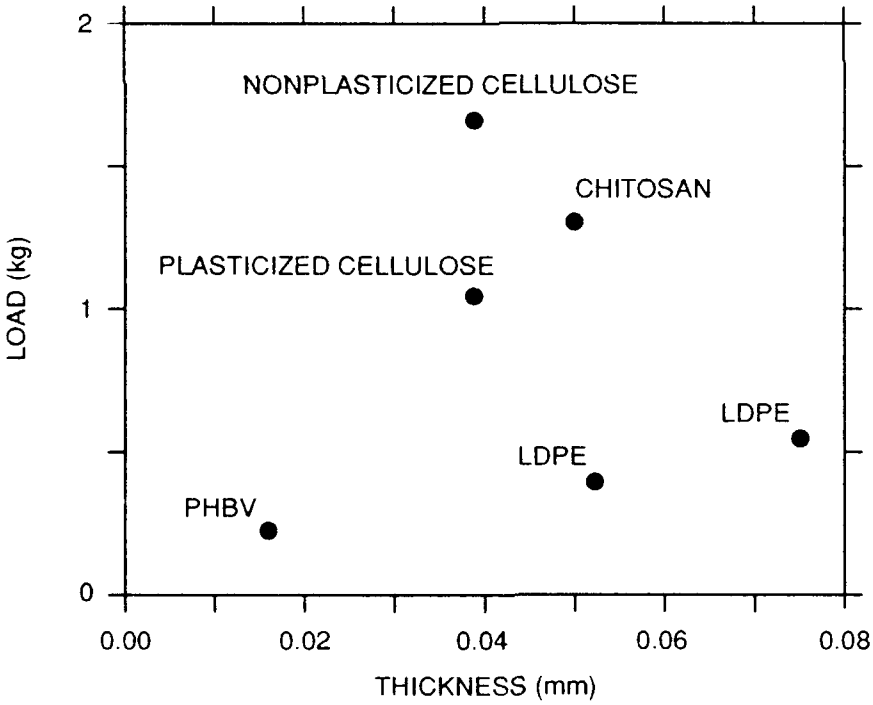


Figure 1b. ASTM D1004 data.

Figure 1. Tear resistance of candidate films.

ESTABLISHING THE DEGRADATION OF CHITOSAN AND CELLULOSE MATERIALS

Extensive film testing of chitosan and chitosan-starch blends was undertaken and previously reported (see appendix A).

More complete data relating to the test program are now available; however, less data are available on the regenerated cellulose films than on chitosan. The information we have basically covers two areas: tensile property and gas evolution data on exposure to microbial environments.

Exposures of Chitosan and Regenerated Cellulose Under Marine Conditions

This work was carried out at Beaufort, N.C. Samples of both types of biopolymer films were mounted in frames and exposed simultaneously at the bottom of Bogue Sound, located in Beaufort Inlet. Two-week sampling intervals were used, with a total exposure time of 10 weeks. The testing took place in the winter of 1989.

Figures 2 and 3 show the appearance of samples at each sampling interval. We did not tensile-test the exposed films because of the difficulty in obtaining minimum size pieces for specimens. Cellulose samples were completely dissolved within 6 weeks of exposure, whereas chitosan endured the full 10 weeks. The chitosan samples were very brittle and could easily be broken into pieces. Previously reported results have shown a significant drop in breaking strength, elongation at break, and viscosity of chitosan after only 3 days exposure under marine conditions in Miami, Fla.

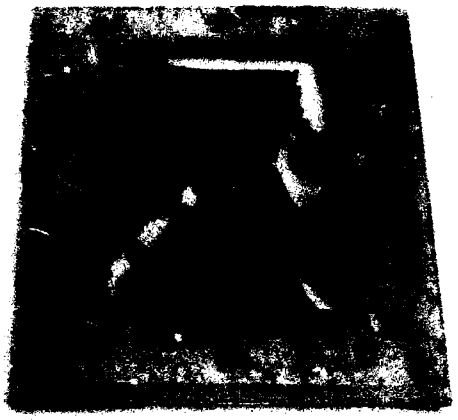
Additional marine exposures of both types of biopolymer films aimed at generating samples exposed for a shorter period, which can be tested (using tensile and tear test procedures), are planned. A few samples are being tested at the Army's Research, Development, and Engineering Center in Natick, Mass. using an accelerated exposure protocol they developed.

Chitosan and cellulose films have a degree of crystallinity associated with them. In semicrystalline polymers, the biodegradation is expected to occur at least initially in the amorphous fraction of the system. As a result, the relative degree of crystallinity of the sample increases with the time of exposure. An x-ray diffraction study of the chitosan films showed the material has a crystalline index of approximately 22%. Samples exposed rapidly increased in crystallinity up to about 3 weeks and thereafter maintained a crystallinity of approximately 37%.

Gas Evolution Studies Under Anaerobic Exposure

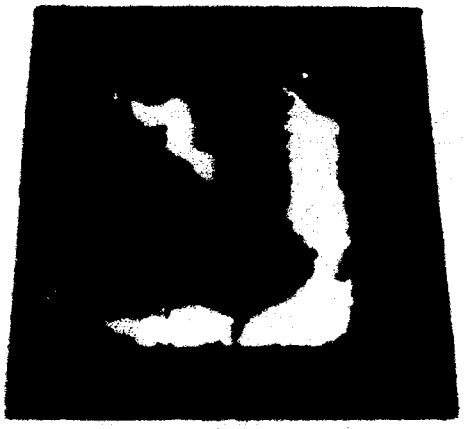
Anaerobic degradation of chitosan and cellulose is measured by the CO_2 and CH_4 evolved. Samples are prepared under nitrogen atmosphere in 40 mL amber screw-cap vials fitted with Mininert® valves. Each bottle contains 5 g of either garden soil or marine sediment; 0.5 g of the film to be degraded; 0.1% urea; 0.05% K_2HPO_4, based on the weight of the sample; and 5 mL of anaerobic municipal waste sludge. Head space samples are taken and analyzed for carbon dioxide and methane using gas chromotography.

The earlier progress report (appendix A) included partial data for CO_2 and CH_4 release for chitosan and cellulose under anaerobic exposure conditions, both in garden soil and marine sediment. These experiments have since been completed.



CHITOSAN
MARINE EXPOSURE AT
BEAUFORT, NC
2 WEEKS: 11/6/89-11/20/89

CHITOSAN
MARINE EXPOSURE AT
BEAUFORT, NC
4 WEEKS: 11/6/89-12/4/89



CHITOSAN
MARINE EXPOSURE AT
BEAUFORT, NC
8 WEEKS: 11/6/89-1/1/90

CHITOSAN
MARINE EXPOSURE AT
BEAUFORT, NC
10 WEEKS: 11/6/89-1/15/90

Figure 2. Marine exposure of chitosan at Beaufort, N.C.

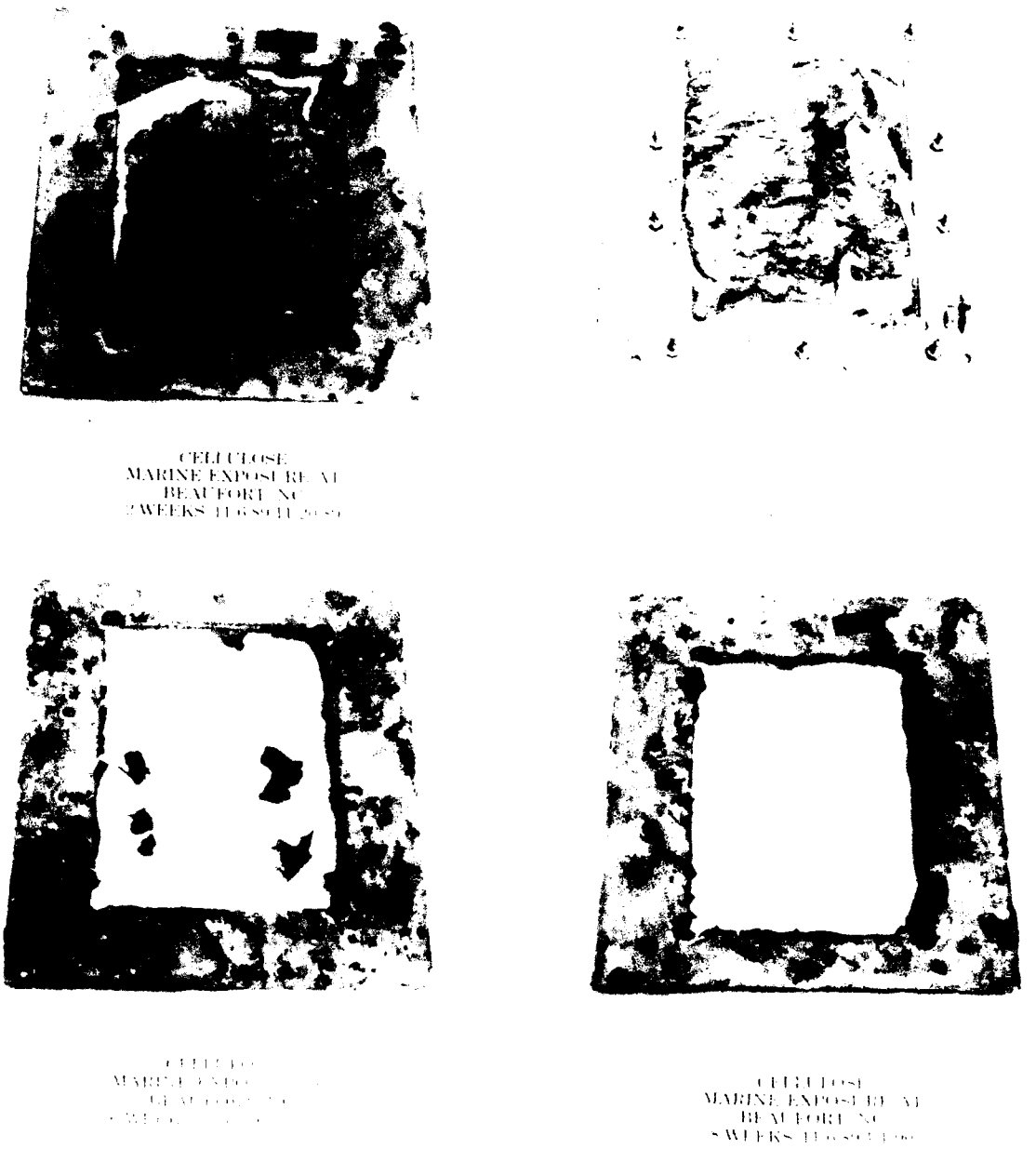


Figure 3. Marine exposure of regenerated cellulose at Beaufort, N.C.

The laboratory anaerobic degradation study was repeated for a longer period (6 weeks) to determine if levels of gas release comparable to aerobic degradation could be achieved (Table 2). The data were in agreement with trends suggested by the short-term studies, i.e., that chitosan shows greater anaerobic degradation in garden soil than cellulose, whereas cellulose shows more rapid degradation than chitosan in a marine sediment environment.

Data from the long-term study were converted to values for the percent of total degradation based on the amount of carbon consumed. Total degradation, in terms of the percent of carbon released, is much higher for aerobic degradation than anaerobic degradation for a comparable time scale. In aerobic marine sediment, both chitosan and cellulose release 30 to 50% of the carbon as gas within 30 days following exposure. Since some of the carbon from the substrate is generally utilized by the microorganisms for growth, this number underestimates the actual degradation.

Table 2. Anaerobic degradation of chitosan and cellulose at 25 °C.

Table 2a. Garden soil exposure.

	CO_2 Release (mmole)			CH_4 Release (mmole)			Percent of Total Degradation*	
Day	Control	Chitosan	Cellulose	Control	Chitosan	Cellulose	Chitosan	Cellulose
7	0.0730	0.0278	0.1572	0.0003	0.0017	0.0014	0.01	0.46
14	0.1256	0.2140	0.2918	0.0000	0.0128	0.0259	0.54	1.04
21	0.1486	0.6915	0.2592	0.0000	0.0302	0.0354	3.08	
28	0.1756	0.9248	0.3820	0.0000	0.0480	0.0737	4.29	1.52
35	0.1879	1.0096	0.3206	0.0000	0.0556	0.0765	4.72	
43	0.1351	0.7467	0.1147	0.0000	0.0800	0.0472		

*Based on theoretical carbon in substrate.

Table 2b. Marine sediment exposure.

	CO_2 Release (mmole)			CH_4 Release (mmole)			Percent of Total Degradation*	
Day	Control	Chitosan	Cellulose	Control	Chitosan	Cellulose	Chitosan	Cellulose
7	0.0101	0.0182	0.0555	0.0004	0.0026	0.0053	0.06	0.27
14	0.0395	0.0190	0.2009	0.0016	0.0237	0.0497	0.12	1.13
21	0.0549	0.0689	0.3267	0.0113	0.0790	0.1411	0.44	2.17
28	0.0835	0.0800	0.4348	0.0204	0.2493	0.3304	1.23	3.57
35	0.0865	0.0582	0.4655	0.0211	0.3390	0.3783	1.71	3.98
43	0.0606	0.0612	0.4986	0.0071	0.3783	0.4360	2.00	4.68

*Based on theoretical carbon in substrate.

Figure 4 compares aerobic to anaerobic degradation for marine sediment exposure. (Data for soil exposure are not shown.) Degradation under totally anaerobic conditions is much slower than aerobic degradation. Both chitosan and cellulose would, therefore, be

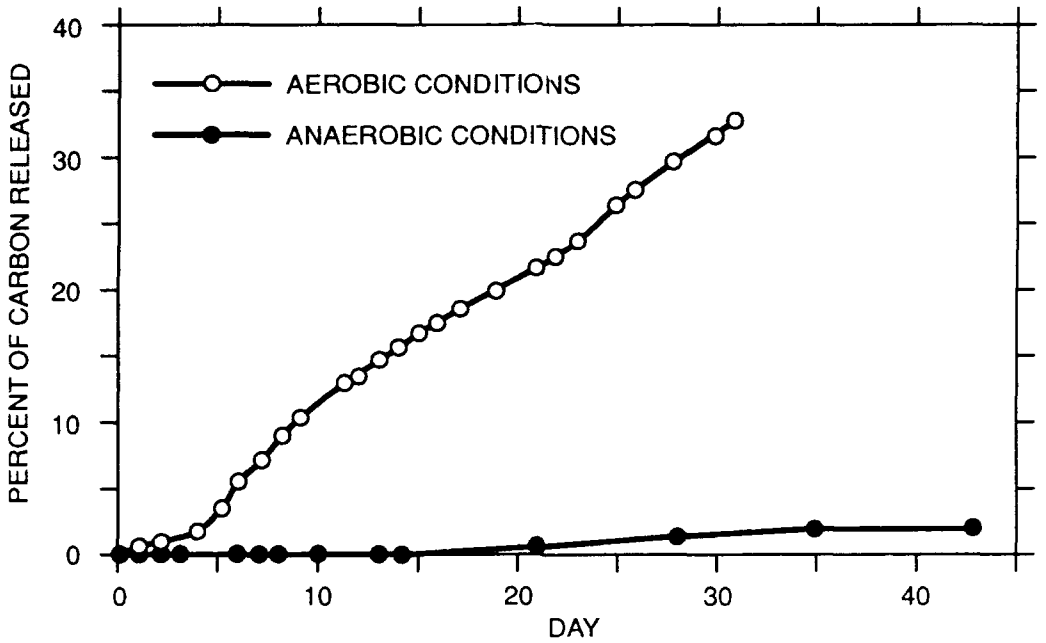


Figure 4a. Chitosan.

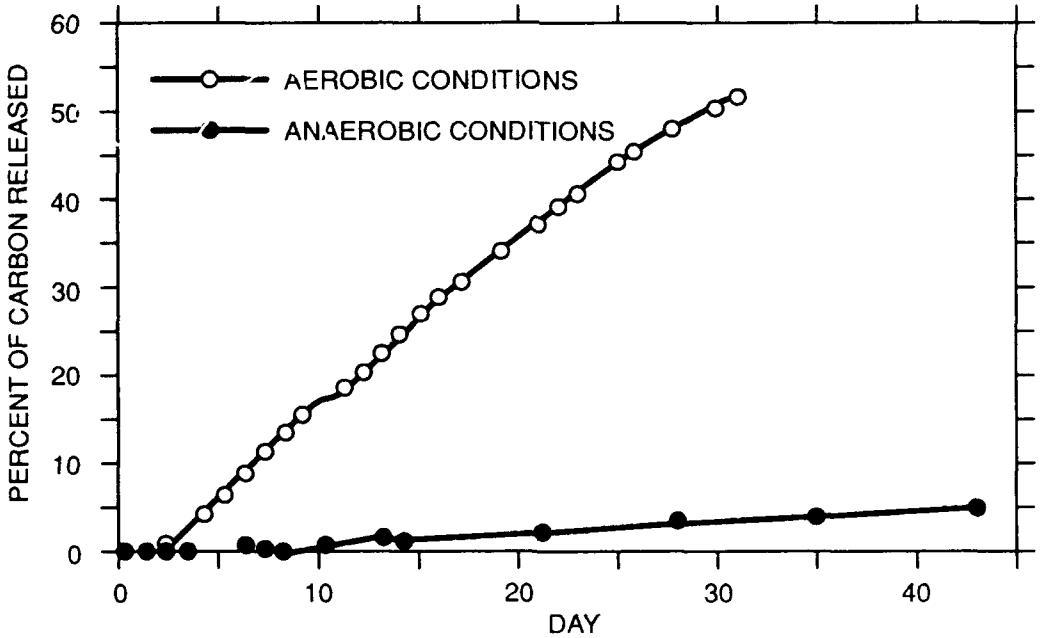


Figure 4b. Cellulose.

Figure 4. Total degradation over time in marine sediment (25 °C).

expected to degrade more slowly under deep ocean conditions than surface, mid-water, or moderate depth conditions at sea. Some experiments are being conducted to determine if the observed slower rates might be due to limiting nutrient factors.

Additional studies were conducted to establish the reproducibility of data associated with the anaerobic procedure using triplicate samples of chitosan and cellulose in garden soil (Table 3). The method shows good reproducibility, except for some methane release data points. The level of methane release is so slight, however, that a variation low in magnitude results in a comparatively high standard error.

Table 3. Reproducibility of anaerobic degradation of chitosan and regenerated cellulose in garden soil (25 °C).

Table 3a. Chitosan (CO_2 release [mmole]).

Day of Study	Control	1	2	3	Average	Standard Deviation
1	0.0324	0.0103	0.0111	0.0138	0.0117	0.0018
3	0.0395	0.0196	0.0155	0.0265	0.0205	0.0056
7	0.0634	0.0292	0.0169	0.0295	0.0252	0.0072
9	0.0737	0.0386	0.0246	0.0359	0.0330	0.0074
11	0.0666	0.0607	0.1847	0.0670	0.1041	0.0698
14	0.0869	0.5271	0.6597	0.5553	0.5807	0.0699

Table 3b. Chitosan (CH_4 release [mmole]).

Day of Study	Control	1	2	3	Average	Standard Deviation
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0002	0.0002	0.0001	0.0001
7	0.0002	0.0003	0.0009	0.0012	0.0008	0.0005
9	0.0002	0.0011	0.0026	0.0018	0.0018	0.0008
11	0.0003	0.0048	0.0137	0.0093	0.0092	0.0044
14	0.0006	0.0502	0.0167	0.0249	0.0306	0.0175

Table 3c. Cellulose (CO_2 release [mmole]).

Day of Study	Control	1	2	3	Average	Standard Deviation
1	0.0324	0.0359	0.0427	0.0378	0.0388	0.0035
3	0.0395	0.0528	0.0788	0.0796	0.0704	0.0152
7	0.0634	0.1481	0.1704	0.1530	0.1572	0.0117
9	0.0737	0.1491	0.2166	0.1781	0.1813	0.0339
11	0.0666	0.1884	0.1984	0.1680	0.1849	0.0155
14	0.0869	0.1849	0.2171	0.2230	0.2084	0.0205

Table 3d. Cellulose (CH_4 release [mmole]).

Day of Study	Control	1	2	3	Average	Standard Deviation
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0003	0.0003	0.0002	0.0002
7	0.0002	0.0007	0.0012	0.0010	0.0010	0.0002
9	0.0002	0.0000	0.0038	0.0022	0.0020	0.0019
11	0.0003	0.0038	0.0113	0.0049	0.0067	0.0041
14	0.0006	0.0095	0.0195	0.0150	0.0147	0.0050

Data relating to biodegradation under aerobic conditions were discussed in a previous progress report (see appendix A).

LARGE-SCALE PRODUCTION OF CHITOSAN AND REGENERATED CELLULOSE

A successful, acceptable MARPOL film material to be used in bag fabrication and other applications must be readily processed using existing industrial machinery. The cost will be prohibitive and the developmental effort will exceed the projected time scale if specialized processing machinery has to be fabricated for this purpose.

Chitosan

We considered the possibility of using available solvent-casting equipment to produce chitosan films in quantity. Three suspected difficulties, which were anticipated on the basis of laboratory casting experiments, were as follows:

1. Chitosan must be cast from solutions of low solids content (often less than 3%). A higher concentration of chitosan yields too viscous a solution to handle. Therefore, a long drying tunnel and slow speeds on production lines will be needed to cast film successfully. Most solvent-casting operations use volatile organic solvents and solids contents in excess of 20% and, therefore, require shorter drying ovens. While we tried to reduce the molecular weight of chitosan by reaction with sodium nitrite in an attempt to decrease viscosity and therefore increase the solids content, the resulting lower molecular weight chitosan did not yield a film of consistent quality.
2. Commercial chitosan contains debris, generally small fractions of crab shells. The coating process is sensitive to the presence of such debris, especially under large-scale casting conditions. Prefiltering of the viscous solution under pressure would be required to remove the debris.
3. In solvent casting, the "take-up" equipment imposes tension on the drying film coming off the stainless steel casting belt. Films should have sufficient tear strength to withstand this tension. Unless the casting line is performed at very low speeds, the process may encounter film-tearing difficulties at the "take-up" end. Speciality Products Co., which routinely produces solvent-cast polymers such as polyvinyl alcohol, collaborated in testing the chitosan material as a candidate for large-scale casting.

Preliminary efforts to cast the material revealed that the first suspected problem was, in fact, not a difficulty; it could be cast with a hot solution of chitosan, and the available drying oven was able to dry the film without difficulty. The other two concerns were valid. The casting solution had to be prefiltered under pressure, and the casting-line personnel at Specialty Products Co. confirmed that there were difficulties in removing the cast film without tears interrupting the process.

Regenerated Cellulose

Flexel, Inc. of Covington, Ind. is the only company in the United States known to manufacture regenerated cellulose. The films are made by a wet process, which somewhat limits the thickness of the available film to less than 1.3 mils.

With some adjustment of process parameters, however, the process should be able to produce films of 1.5 mils with little difficulty. Two such laminates joined with an adhesive layer would yield a film thickness more than adequate for our applications.

Success in using regenerated cellulose as a MARPOL-acceptable film useful for bag applications will depend upon the following:

1. Fabrication of 3-mil films of regenerated cellulose.
2. Design of a multilayer system that will render the films water resistant, somewhat flexible, and less prone to tear, without affecting the biodegradability or the MARPOL-acceptability of the product.
3. Assurance that the previous design of the film material is compatible with commercial bag fabrication techniques. Most bags are heat-sealed. Cellulose film uncoated with a synthetic polymer is not heat-sealable. Either adhesive sealing must be resorted to or a MARPOL-acceptable, heat-sealable coating must be developed.
4. A successful demonstration that the bags are degradable.

PLASTICIZATION OF CHITOSAN AND CELLULOSE MATERIALS

Both chitosan and regenerated cellulose films are not as flexible as commodity thermoplastic films such as polyethylene. Lack of flexibility imparts a more rigid "feel" to the film and makes it more prone to tear. Improvement of flexibility in plastics is generally achieved using a suitable plasticizer.

An attempt was made to identify a plasticizer for chitosan (and to a limited extent for cellulose), which could be used in large-scale production of these films.

Initial Screening Experiments With Chitosan

Initially, approximately 50 compounds were chosen as possible plasticizers for chitosan. In general, these compounds contain hydroxyl or amine groups for hydrogen bonding with chitosan and a long chain hydrocarbon to provide flexibility. In addition, several common industrial plasticizers were included in the study.

Small samples of chitosan film containing up to 25% by weight of each plasticizer were prepared. Plasticizer candidates were eliminated based on the application of a twist test to each film. The films that twisted and cracked were eliminated. Also eliminated were plasticizer compounds that were insoluble in water and could not be dispersed effec-

tively in the chitosan solution. The remaining films were tested on an Instron® testing machine to determine preliminary tensile properties. These samples were not conditioned prior to testing, so these results could only be compared to each other for the purpose of eliminating plasticizer candidates. Test results for the preliminary screening test are not provided herein.

Based on these results, the following compounds were selected for further study:

1. Lithium chloride (10% w/w solution)
2. Urea (10% w/w solution)
3. Stilwet® Surfactant L–7614 (polydimethyl siloxane/ethylene oxide copolymer)
4. Tetraethylene glycol
5. Glycerol
6. Polyethylene glycol 400 (PEG 400)
7. Diethyl succinate
8. Dextrin-based adhesive (Swift 37189)
9. 1,2,6-trihydroxyhexane

Chitosan films containing these selected plasticizers were prepared by mixing the plasticizer (25% by weight of chitosan) with the 2.5% aqueous solution of chitosan in acetic acid before casting the film. Dried films were rinsed in a 5% solution of sodium methoxide in methanol for 5 min to neutralize residual acetic acid.

Table 4a shows the tensile properties of the films tested after conditioning at 50% relative humidity for 24 h. Based on these results, Surfactant L–7614, diethyl succinate, and the Swift Adhesive were eliminated as plasticizer candidates. The remaining films were immersed in water for 5 min, dried, conditioned, and retested; the results are shown in Table 4b. The samples were not dried under tension, and shrinkage occurred, as evidenced by the increased thickness values. In most cases, some plasticizer appeared to have leached out during immersion in water, but the plasticized films were still more extensible than the control after immersion.

Preliminary Studies on Plasticizer Performance in Chitosan

Further investigation included the determination of the effect of plasticizer upon the tear resistance and tensile properties of as-cast chitosan films.

Table 5 shows values for tear resistance for unrinsed plasticized films tested according to ASTM D1004. Lithium chloride and urea, both effective plasticizers, adversely affect the material's tear resistance. However, a combination of urea and PEG 400 does not greatly reduce the tear resistance.

Unrinsed, conditioned films also were tested for tensile properties. The tensile property data are not provided herein. Those films with breaking elongations of less than 30% were eliminated. Minimization of strength loss was also desirable; lithium chloride, which greatly increases extensibility, caused a significant decrease in breaking strength. This loss was somewhat offset by lowering the LiCl level or by using LiCl in combination with PEG 400.

Films were made using lithium chloride, lithium bromide, lithium acetate, and urea at levels of 5, 10, 15, and 20% by weight to determine optimum plasticizer levels. A level of 10 to 15% plasticizer appeared to be the best compromise between the desired increase in breaking elongation and the accompanying loss in breaking strength.

Further Studies on Plasticization of Chitosan

Several plasticizer candidates at various levels and combinations were chosen for further study. The candidate systems of plasticizers were further narrowed on the basis of tensile properties. Selections were based on the ability of the plasticizing additive at low concentrations to provide a significant increase in elongation at break with a minimal decrease in the tensile and tear strength. Detailed data on the tensile and tear resistance properties of individual compositions have been determined but are not tabulated herein.

Table 4. Tensile properties of plasticized chitosan films—effect of immersion of plasticized film in water.*

Table 4a. Before immersion.

Plasticizer	Average Thickness (mm)	Break Stress (kg/cm^2)	Stress Standard Deviation (kg/cm^2)	Break Strain (%)	Standard Deviation (%)	Strain Number of Specimens
None (control)	0.055	770	145	25.4	8.6	5
Lithium Chloride	0.066	512	57	68.1	8.2	4
Urea	0.059	420	81	60.3	9.1	6
Surfactant L–7614	0.048	424	57	12.6	2.8	4
Tetraethylene Glycol	0.056	635	80	54.7	8.8	6
Glycerol	0.055	657	87	63.8	8.0	5
Polyethylene Glycol 400	0.042	902	33	68.8	12.3	5
Diethyl Succinate	0.050	795	126	30.0	8.8	5
Swift Adhesive 37189	0.048	613	75	28.0	10.0	5
1,2,6-Trihydroxyhexane	0.053	723	73	66.8	5.0	4

Table 4b. After immersion.

Plasticizer	Average Thickness (mm)	Break Stress (kg/cm^2)	Stress Standard Deviation (kg/cm^2)	Break Strain (%)	Standard Deviation (%)	Strain Number of Specimens
None (control)	0.085	683	52	17.7	16.2	3
Lithium Chloride	0.083	738	17	47.2	5.3	4
Urea	0.064	744	22	29.4	5.6	3
Tetraethylene Glycol	0.088	643	67	33.0	9.8	5
Glycerol	0.079	778	132	61.3	8.5	4
Polyethylene Glycol 400	0.080	639	–	44.9	–	1
1,2,6-Trihydroxyhexane	0.103	630	36	40.4	0.7	3

*Tested at a crosshead speed of 20 mm/min and a gauge length of 30 mm. All samples contained 25% by weight plasticizer. Films were rinsed in $NaOCH_3$/MeOH and conditioned at 50% relative humidity.

Table 5. Tear resistance of unrinsed plasticized chitosan films (ASTM D1004).

Plasticizer	Thickness (mm)	Peak Load (kg)	Load Standard Deviation (kg)	Peak Stress (kg/mm)	Number of Specimens
None	0.060	0.954	0.035	15.90	4
25% LiCl	0.071	0.365	0.056	5.14	4
25% Urea	0.061	0.342	0.060	5.61	7
25% TEG	0.054	1.112	0.166	20.59	4
25% Glycerol	0.050	0.868	0.131	17.36	7
25% PEG 400	0.053	1.123	0.199	21.19	5
25% 1,2,6-Trihydroxyhexane	0.086	0.886	0.331	10.30	5
12.5% Urea/12.5% Glycerol	0.041	0.432	–	10.54	1
12.5% Urea/12.5% PEG 400	0.089	1.259	0.106	14.15	6

*Tested at a crosshead speed of 50 mm/min and a gauge length of 25 mm. All samples were conditioned at 50% relative humidity.

On the basis of the test results, three combinations of plasticizers, as well as the single plasticizers urea and lithium bromide, were chosen for final studies. Films measuring 6 in. by 9 in. containing the selected plasticizers were cast in duplicate. The unrinsed films were conditioned and tested for both tensile properties and tear resistance (see Table 6). Tear resistance was lowered in each case by the addition of plasticizer, but even the values for plasticized films exceeded those for low density polyethylene (LDPE). Figure 5 shows the breaking stress versus breaking strain relationship for the films. On the basis

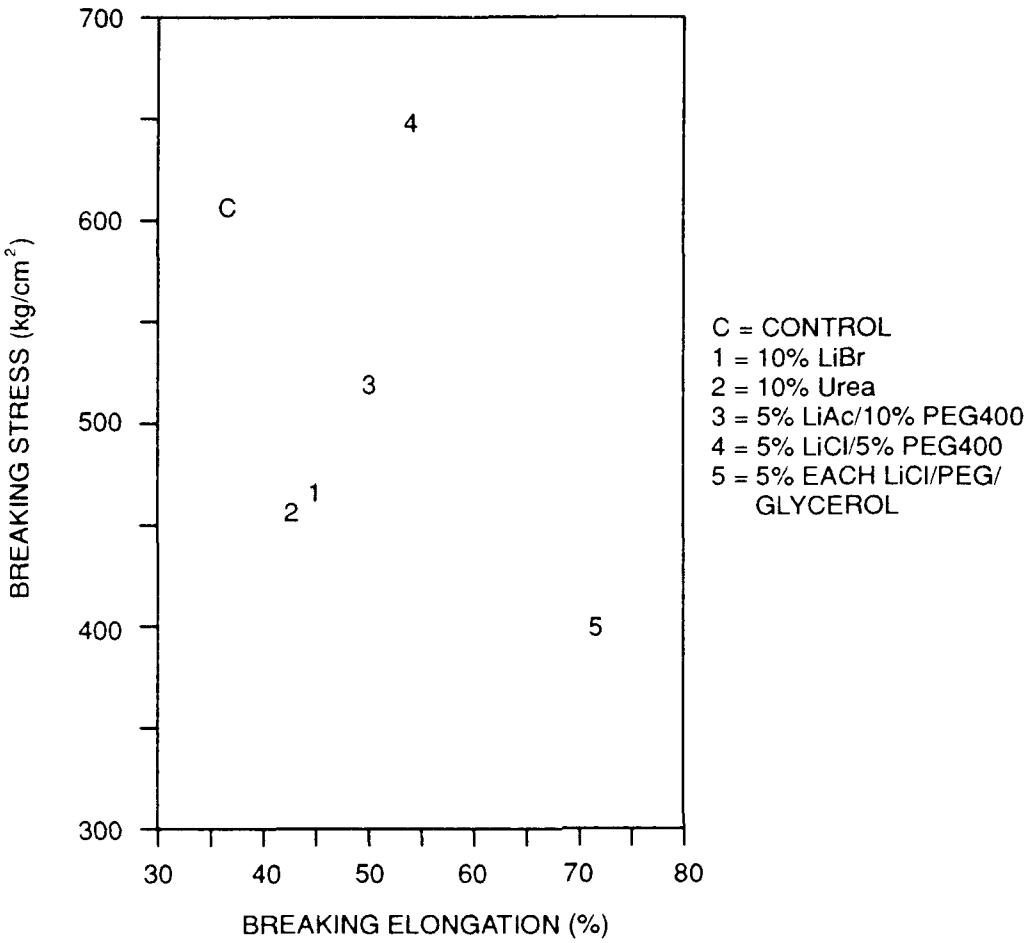


Figure 5. Break stress vs. strain for final candidates.

of the performance considerations previously discussed, the compounds chosen as effective plasticizers were 10% lithium bromide or 5% lithium acetate/10% PEG 400.

Plasticization of Regenerated Cellulose by Lithium Salts

Certain lithium salts at low concentrations acted as good plasticizers of chitosan film material. Since the molecular structure of chitosan and cellulose are similar, the same mechanism is likely to operate in the latter system as well. The following investigation was designed to test this assumption.

Table 6. Tensile properties and tear resistance of selected plasticizer candidates in chitosan films.

Table 6a. Tensile properties.*

Plasticizer	Thickness (mm)	Break Stress (kg/cm^2)	Stress Standard Deviation (kg/cm^2)	Break Strain (%)	Standard Deviation (%)	Number of Specimens
None	0.051	604	65	34.74	6.63	10
10% LiBr	0.066	464	75	44.46	12.17	10
10% Urea	0.058	457	8	42.63	8.03	10
5% LiAc/10% PEG 400	0.059	515	128	49.91	11.87	6
5% LiCl/5% PEG 400	0.050	650	106	53.42	6.79	8
5% each LiCl/ PEG/Glycerol	0.068	396	43	71.19	4.12	8

*Tested according to ASTM D882 ("Standard Test Method for Tensile Properties of Thin Plastic Sheeting") at a crosshead speed of 20 mm/min and a gauge length of 30 mm. All samples were conditioned at 50% relative humidity.

Table 6b. Tear resistance.*

Plasticizer	Thickness (mm)	Peak Load (kg)	Load Standard Deviation (kg)	Peak Stress (kg/min)	Number of Specimens
None	0.058	1.326	0.118	22.86	4
10% LiBr	0.059	0.892	0.232	15.12	5
10% Urea	0.056	0.957	0.126	17.09	5
5% LiAc/10% PEG 400	0.053	0.936	0.147	17.66	5
5% LiCl/5% PEG 400	0.053	0.796	0.160	15.02	4
5% each LiCl/ PEG/Glycerol	0.060	0.824	0.057	13.73	5

*Tested according to ASTM D1004 at a crosshead speed of 50 mm/min and a gauge length of 25 mm. All samples were conditioned at 50% relative humidity.

Uncoated, nonplasticized, regenerated cellulose films were dipped in lithium salt solutions for 30 s, then dried at room temperature for 6 h. The salts used were lithium chloride (LiCl), lithium bromide (LiBr), and lithium acetate (LiAc). A linear relationship existed between the concentration (weight percent) of lithium salt absorbed by the film and the concentration (weight percent) of the solution. At equal solution concentrations, LiCl was more highly absorbed by the cellulose, followed by LiBr and LiAc.

Films treated with lithium salt solutions became softer and more flexible. As expected, the results of the tests showed a decrease in tensile and tear strength and an increase in breaking extension with increasing salt concentrations. The test results are shown in figure 6. It appears that lithium salts can be used to modify the properties of regenerated cellulose film.

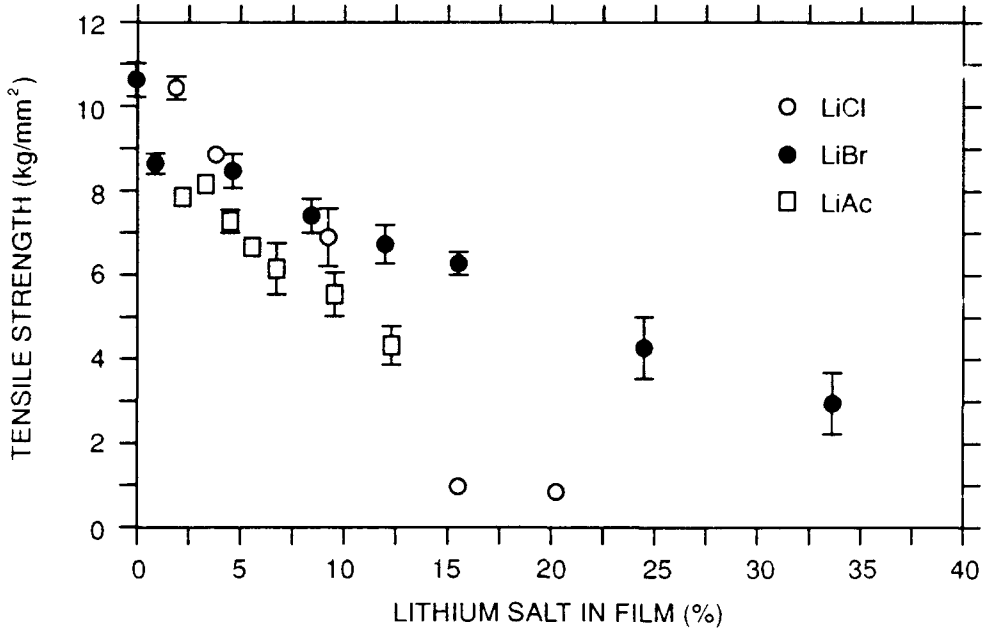


Figure 6a. Breaking strength.

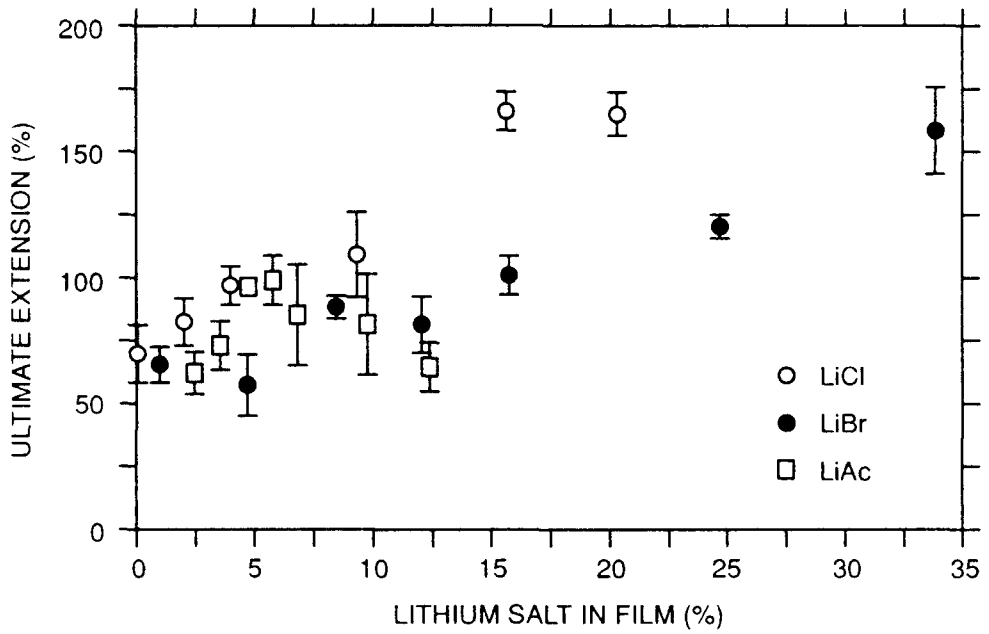


Figure 6b. Breaking elongation.

Figure 6. Tensile properties of regenerated cellulose films treated with lithium salts.

ADDITIVES FOR IMPROVING TENSILE PROPERTIES AND DEGRADABILITY

While regenerated cellulose does biodegrade effectively under marine conditions, it is desirable to further accelerate the process wherever possible. In some instances, the biodegradation of a substrate might be limited by the availability of nutrients, particularly N and P. The presence of these in the regenerated cellulose film may enhance the rate of biodegradation. This effect was investigated using a gas evolution study and a tensile property study.

Effect of Additive on the Rate of Change in Gas Evolution on Exposure

We carried out several experiments to determine if the biodegradation rate of cellulose could be increased by incorporating urea and phosphate into the film. The nutrients were incorporated in two ways: first, by mixing urea and K_2HPO_4 into an adhesive and laminating unplasticized cellulose sheets together and, second, by dipping the cellulose sheets into aqueous solutions of urea and K_2HPO_4. In preparing the laminated sheets, it was necessary to use a water-based adhesive since the urea and phosphate could not be incorporated into Adcote 333T without initiating the curing reaction. Carbon dioxide evolution of the cellulose samples was measured by the biometer flask method; the results are shown in Table 7. The data show a higher CO_2 release for the urea/phosphate-containing cellulose film samples, showing that the provision of these nutrients does increase degradation under soil exposure conditions.

Table 7. Effect of urea/phosphate on aerobic degradation of cellulose samples in garden soil.

	Cumulative CO_2 Release (mmole)					
Day	Control (Soil Only)	Control (Soil and Urea/ Phosphate)	Cellulose Film	Cellulose Film Dipped in Urea/ Phosphate	Cellulose Laminated With Adhesive	Cellulose Laminated With Adhesive and Urea/ Phosphate
0.958	0.055	0.085	0.115	0.110	0.085	0.090
1.958	0.105	0.180	0.245	0.240	0.185	0.185
3.000	0.155	0.265	0.355	0.345	0.260	0.260
5.938	0.245	0.410	0.590	0.600	0.470	0.470
7.958	0.315	0.525	0.840	0.890	0.675	0.715
10.000	0.385	0.640	1.140	1.245	0.920	0.985
13.000	0.490	0.810	1.595	1.825	1.305	1.475
15.000	0.565	0.920	1.910	2.235	1.575	1.780

This result is only preliminary, and the experiment has not been optimized. The same effect has not been shown for biodegradation under marine exposure where rapid diffusion of the water-soluble salts away from the cellulose matrix is a possibility.

Effect of Additive on the Rate of Change in Strength on Exposure

Sheets of plasticized cellulose were laminated with Adcote 333T containing 2% (based on the weight of the adhesive) ammonium phosphate. This method is of limited practical use because the aqueous solution of $(NH_4)_2HPO_4$ cured after only approximately 10 min. Samples were prepared, however, and were exposed under outdoor soil burial

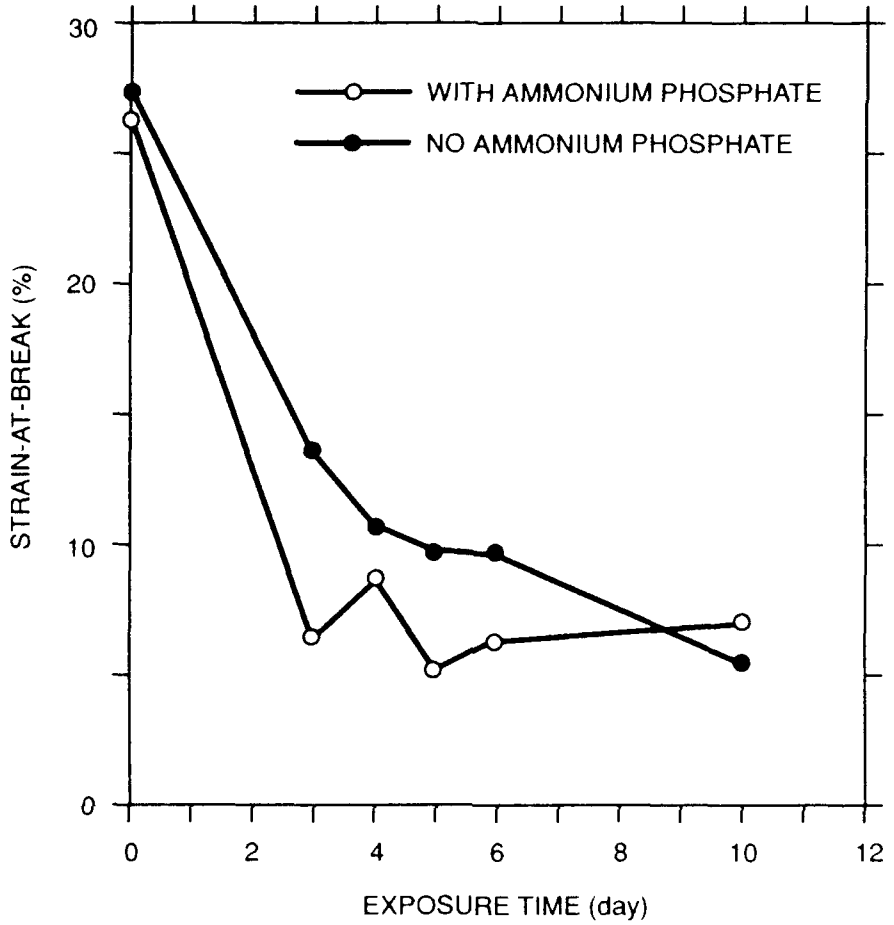


Figure 7. Effect of ammonium phosphate on degradation rate of regenerated cellulose film under soil burial.

conditions. Laminated sheets with no ammonium phosphate were buried as controls. The samples containing ammonium phosphate degraded at a slighter faster rate, but the extent of degradation was unaffected (figure 7).

STUDY OF ADHESIVES

The use of chitosan and/or cellulose films is likely to require adhesive bonding (as opposed to thermal bonding) in bag fabrication. We screened some adhesives to determine if this approach is viable. A functionally good adhesive bond should not fail under testing; the bond usually is stronger than the material itself, and the failure occurs in the film.

The adhesive candidates chosen for study are identified in Table 8. The adhesives were applied to plasticized regenerated cellulose for two types of testing: tensile and peel strength (ASTM D903, "Peel or Slipping Strength of Adhesive Bonds"). For the tensile tests, 1/2-in. strips were tested with a 3-cm gauge length in the same way as all previous chitosan and cellulose samples (unbonded) were tested, with the exception that the adhe-

sive bond (a 1/2-in.-by-1/2-in. area) was located in the center of the strip. During application of the adhesives, the water-based adhesives caused the cellulose to swell and wrinkle, whereas the urethane adhesive (Adcote 333T) resulted in the smoothest application.

Tensile test results for the adhesives are also provided in Table 8. All adhesives except the dextrin-based adhesive had bond strengths that equaled or exceeded the tensile strength of the cellulose.

Peel strength was determined according to ASTM D903. All the water-based adhesives resulted in very uneven coverage of the film, so the peel strength values were somewhat erratic. For Covinax 220, JW 2–47, and Adcote 333T the bond strength was in most cases greater than the tear resistance of the cellulose. These three would all be suitable adhesives, with Adcote 333T having the smoothest and most even coverage because it is not water-based.

CONCLUSIONS AND FUTURE WORK

We studied the biodegradability of two biopolymers—chitosan and cellulose—and both were found to be adequately biodegradable under marine exposure conditions. The time of degradation in field experiments was several weeks for both films.

Despite production limitations, both chitosan and regenerated cellulose have the potential to be developed for our particular application. However, given the short time frame within which a biodegradable system has to be developed and in view of the high developmental costs associated with chitosan, regenerated cellulose is suggested as the more promising candidate material. While some developmental work might be needed for cellulose films as well, the effort required will be relatively small.

In continuing work, emphasis will be placed on regenerated cellulose, particularly the following aspects:

1. Lamination approach to obtain 3.0-mil sheets;
2. Multilayer sheets with good biodegradability, improved tear resistance, improved flexibility, and some hydrophobicity;
3. Selection of adhesives;
4. Adaptation of a composite film to a mechanized bag-making operation; and
5. Degradability, shelf-life, and toxicity studies of the final product.

Table 8. Data for the strength of adhesive bonds on regenerated cellulose.

Table 8a. Tensile properties.*

Adhesive	Thickness (mm)	Break Stress (kg/cm^2)	Stress Standard Deviation (kg/cm^2)	Break Strain (%)	Strain Standard Deviation (%)	Number of Specimens	Comments
Covinax 220	0.032	956	46	21.1	2.7	10	All samples broke in film, not at bond.
JW 2–47	0.032	989	45	23.3	2.8	10	All samples broke in film, not at bond.
Adcote 333T	0.032	1034	61	25.5	3.7	10	Six samples broke in film; four broke at bond.
Casco S–97 (Casein)	0.032	935	123	23.7	7.5	10	Seven samples broke in film; three broke at bond.
Swift 37189 (Dextrin)	0.032	593	79	10.4	4.6	10	All samples broke at bond.

*Tested at a crosshead speed of 20 mm/min and a gauge length of 30 mm. All samples were conditioned at 50% relative humidity.

Table 8b. Peel strength data.*

Adhesive	Thickness (mm)	Width (mm)	Average Load (kg)	Load Standard Deviation (kg)	Number of Specimens	Comments
Covinax 220	0.032	25.4	0.337	0.094	7	Five adhesive failures; two cohesive failures. Uneven coverage of adhesive.
JW2–47	0.032	25.4	0.184	0.088	3	All cohesive failure; tear in cellulose. Most of these samples could not be tested; cellulose tore as sample was being prepared for testing. Adhesive bond stronger than cellulose tear strength.
Adcote 333T	0.032	25.4	0.619	0.088	5	Three adhesive failures; two cohesive failures. Uneven coverage of adhesive. Three of these samples could not be tested; cellulose tore as sample was being prepared for testing. Adhesive bond stronger than cellulose tear strength.
Casco S–97 (Casein)	0.032	25.4	0.215	0.125	8	Seven adhesive failures; one cohesive failure. Uneven coverage of adhesive.

Table 8b. (Continued).

Adhesive	Thickness (mm)	Width (mm)	Average Load (kg)	Load Standard Deviation (kg)	Number of Specimens	Comments
Swift 37189 (Dextrin)	0.032	25.4	0.007	–	2	Adhesive failure. Adhesive very weak; most samples could not be tested.

*Tested according to ASTM D903 at a crosshead speed of 200 mm/min and a gauge length of 200 mm. All samples were conditioned at 50% relative humidity.

Table 8c. Identification of adhesives used.

Name	Manufacturer	Adhesive Type	Solvent	Coverage (gal/m^2)*	Cure Temperature (°C)
Covinax 220	Franklin International	Vinyl Acrylic Copolymer	Water	3	50
JW 2–47	Franklin International	Vinyl Acrylic Copolymer	Water	3	50
Adcote 333T	Morton-Thiokol	Polyester Urethane	Tetrahydrofuran	3	80
Casco S–97	Borden	Casein-based	Water	3	Ambient
Swift 37189	Swift Adhesive	Dextrin-based	Water	3	Ambient

*As recommended by the manufacturer.

APPENDIX A
PROGRESS DURING THE PERIOD OCT 1988 THROUGH SEPT 1989

DEVELOPING DEFINITIONS AND TEST PROTOCOLS

One of the most challenging problems facing us in the area of marine waste disposal is that of defining degradability and establishing standard marine test procedures to determine the degradability of a material. How much degradation of a material renders it environmentally acceptable? Should a distinction be made between degradation (a change in the chemistry of the molecule which results in a decrease in the molecular weight of the material) and deterioration (a change in the size of the material due to physical processes)? It is important to have standardized testing methodology to assess degradability under marine conditions and compare results of the performance of different types of plastics. The evolution of consensus definitions is a prerequisite for such standards.

The American Society for Testing and Materials (ASTM), the foremost standards-making organization in the world, recognized the need for definitions and standard test procedures by formally establishing the Environmentally Degradable Plastics Subcommittee (D20.96) in March 1989. The members of the subcommittee approved the following scope: "The promotion of knowledge and the development of standards (classifications, guides, practices, specifications, terminology, and test methods) for plastics that are intended to degrade." Five task groups have been formed: terminology, biodegradability, photodegradability, chemical degradability, and environmental fate. The Navy is working actively with the members of the first two groups. Several definitions and test methods have been formulated and enthusiastically debated since the activity began. The following six definitions, as well as four test methods, were distributed to the members for ballot in September 1989.

- Degradable Plastic (Definition 1) — Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time.
- Degradable Plastic (Definition 2) — Plastic materials that undergo chemical bond scission in the backbone of a polymer through chemical, biological, and/or physical forces in the environment at a rate which is reasonably accelerated, as compared to a control, and which leads to fragmentation or disintegration of the plastic.
- Photodegradable Plastic (Definition 1) — Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time, where the primary mechanism is through the action of sunlight.
- Photodegradable Plastic (Definition 2) — Degradable plastics (as defined in Degradable Plastics Definition 2) where the primary mechanism of degradation is the action of sunlight.
- Biodegradable Plastic (Definition 1) — Plastic materials that disintegrate under environmental conditions in a reasonable and demonstrable period of time, where the primary mechanism is through the action of microorganisms such as bacteria, yeast, fungi, or algae.

- Biodegradable Plastic (Definition 2) — Degradable plastics (as defined in Degradable Plastic Definition 2) where the primary mechanism of degradation is through the action of microorganisms such as bacteria, yeast, fungi, or algae.

The purpose of these definitions is to establish order and understanding leading to effective compliance with Public Law 100–220. To date, the ASTM subcommittee has not reached a consensus on either the definitions or the test methods. However, the effort to reach a consensus is continuing. The Navy must stay current in world-wide activities related to degradability standards. It is likely that the adopted ASTM consensus definitions and test methods will be used in the interpretation of Annex V of MARPOL.

FABRICATING PROTOTYPE FILMS

Pure Chitosan Films. Chitosan is the partially deacetylated form of chitin, a natural polymer available in large quantities from the waste of consumed shellfish. Pure chitosan films have been solvent-cast at RTI with excellent results from tests on initial strength and rapid degradability. Preliminary large-scale casting was attempted by Capfilm Company in Lee, MA, using a 2.5% chitosan solution in 0.75% aqueous acetic acid. Several attempts to apply the film-making process to industrial scale machinery were unsuccessful. The basic problem in the solvent casting of chitosan films is the low percentage of solids in the solution. A thick initial layer of the viscous solution and, consequently, a long drying time, are required to obtain a film of workable thickness. Drying ovens available on casting lines generally are suited for organic solvents, which evaporate more quickly. The residence time of the industrial ovens was too short for the continuous casting of the chitosan films.

The solubility limits of chitosan in water and the high viscosity of the solution preclude the use of higher concentrations of chitosan in the casting solutions. Attempts were made to lower the solution viscosity by adding sodium nitrite to decrease the molecular weight. However, it was still not possible to increase the percentage of solids in the solution, despite the decreased molecular weight.

A $40 million chitosan research initiative was implemented recently by the Japanese government. Contacts are being made with Japanese researchers, and we will monitor these developments closely in a search for a solution to this problem.

Regenerated Cellulose. Cellulose is a carbohydrate with the molecular formula $(C_6H_{10}O_5)_n$, where n is in thousands. It is the most abundant biopolymer on earth (chitin is second). A solution of derivatized cellulose can be processed by extrusion to produce a desired shape and then treated to remove the modifying groups to reform or regenerate unmodified cellulose; this material is known as regenerated cellulose. While there has not been a high demand for regenerated cellulose since the advent of thermoplastics, regenerated cellulose sheets are available commercially from a single manufacturerin the United States. Two products are available: one is coated on both sides with polyethylene and the other is uncoated. The uncoated regenerated cellulose sheets can be cast into a strong film using existing large-scale manufacturing equipment.

Results of preliminary field exposure on regenerated cellulose show degradation to be as rapid as chitosan. The uncoated sheets, however, are not heat-sealable or flexible enough for bag-making. We are studying possible "MARPOL acceptable" adhesives and

additives to allow for heat-sealability and improved flexibility. We are confident that both problems can be solved.

CHITOSAN/STARCH BLENDS

Incorporating corn starch into chitosan film may lower the cost of the material without compromising biodegradability. It may also increase the solids content to facilitate the film-casting process. Experiments have been conducted to improve the bonding between the chitosan and starch interface. Various compositions and percentages of chitosan / starch-blended films were made using straight corn starch, dialdehyde starch (DAS), and blocked modified reactive group starch (BRG). After each blended chitosan/starch film underwent a conditioning step, they were tested for stress-at-break (tensile strength) and strain-at-break (elongation). Tensile data appear in table A.1.

Table A.1. Tensile data for unexposed films of various compositions.

Sample Description	Stress at Break (kg/cm^2)		Strain at Break (%)		No. of Specimens
	Avg	Std Dev	Avg	Std Dev	
Chitosan	650	80	39.5	21.0	20
Regenerated Cellulose	1200	60	62.1	7.8	18
Chitosan/10% DAS (0.67% Conversion)	754	55	44.3	14.1	4
Chitosan/10% DAS (3.29% Conversion)	839	137	36.9	3.7	4
Chitosan/10% DAS (7.12% Conversion)	1035	113	45.4	9.2	4
Chitosan/10% DAS (14.1% Conversion)	476	51	6.5	2.3	5
Chitosan/40% Corn Starch	640	80	60.6	13.5	6
Chitosan/80% Corn Starch	410	50	65.5	26.7	7
Chitosan/120% Corn Starch	390	30	42.6	18.1	6
Chitosan/40% BRG Starch	470	50	60.6	10.9	6
Chitosan/80% BRG Starch	390	50	20.6	8.4	10
Chitosan/120% BRG Starch	510	70	70.1	13.4	7

Analysis of the tensile data shows that:

- Unexposed regenerated cellulose is stronger and more flexible than unexposed chitosan (both good qualities).
- 40% each of straight corn starch and BRG starch added to chitosan did not significantly lower the strength of the film, while flexibility increased.
- Chitosan plus 10% DAS with 0.67%, 3.29%, and 7.12% conversions each showed increased strength and had little effect on flexibility; chitosan plus 10% DAS with 14.1% conversion showed a significant decrease in both properties.

The DAS conversion methodology is difficult and time-consuming. These results do not warrant additional effort; therefore, degradability studies are being conducted on sample films of 20%, 40%, and 60% corn starch and on BRG starch added to chitosan.

Sample Exposure Procedure and Results

Soil Burial Exposure. A preliminary experiment was performed on pure chitosan films at the soil burial site at RTI. Films were sampled daily, dried, conditioned, and tested for tensile strength and elongation. The size and frequency of the holes in the film

increases as exposure time increases, which illustrates the deterioration due to soil microbes, as shown in figure A.1. This **preliminary** experiment was performed in soil because it is analogous to marine sediment in that it contains moisture, nutrients, and a high concentration of microorganisms (see Degradability Tests below). These biopolymers will sink to the bottom and degrade; in the water alone, they will dissolve and degrade. Tensile testing could be performed for up to 4 days of exposure; only small pieces of the original film could be recovered after that time.

Table A.2 shows the resulting tensile data through 4 days for these samples. Apparently, soil burial conditions have a greater effect on the strength of the film than on its extensibility. Soil burial tests will be repeated with concurrent burial of samples of chitosan, regenerated cellulose, chitosan/corn starch (20%, 40%, and 60%), and chitosan/BRG starch (20%, 40%, and 60%) to show relative degradation rates of the different materials.

Marine Exposure in Miami, Florida

In 1987, we began our program to obtain base line scientific data on the degradability of plastics in the marine benthic environment. We are still using the original test site off the coast of Miami, Florida. Samples are mounted on a frame which allows an 8- × 5-in. (20.3- × 12.7-cm) area of film to be exposed. Securing ropes are tied to the frame assembly, and the samples are lowered to the bottom under 8 to 10 ft (2.4 to 3.1 meters) of water. Exposed samples are removed from the water, dried, and tested for tensile strength, elongation, and viscosity.

Figure A.1. Rapid biodegradation of chitosan film by soil microorganisms.

Results of tensile and viscosity measurements on chitosan samples are shown in table A.3. The samples are severely degraded by the tenth day of exposure. Tensile specimens could not be obtained after 14 days, and viscosity samples could not be obtained after 24 days. Preliminary tests of regenerated cellulose films in Miami resulted in all samples being lost due to biodegradation after 1 month of exposure. The experiment will be repeated using more frequent sampling intervals.

Table A.2. Effect of soil burial on tensile strength of chitosan.

Exposure Time (days)	Stress at Break (kg/cm^2)		Strain at Break (%)		No. of Specimens
	Avg	Std Dev	Avg	Std Dev	
0	650	80	39.5	21.0	20
1	220	30	22.6	5.4	8
2	190	30	24.4	3.4	7
3	150	50	19.7	6.5	7
4	100	20	23.4	4.4	6

Table A.3. Effect of Miami marine exposure on tensile strength and viscosity of chitosan.

Exposure Time (days)	Stress at Break (kg/cm^2)		Strain at Break (%)		No. of Specimens	Viscosity (dL/g)
	Avg	Std Dev	Avg	Std Dev		
5	650	80	39.5	21.0	20	6.23
2	490	40	50.0	0.4	2	4.14
10	100	20	2.8	0.4	3	–
14	260	30	4.5	0.3	2	1.89
24	–	–	–	–	–	0.22

In 1989, we established a test site off the coast of Beaufort, North Carolina, where chitosan samples were exposed during the month of August; table A.4 shows the resulting tensile testing data. Tensile specimens could not be obtained after 8 days of exposure, and the viscosity measurements could not be obtained after 24 days. Concurrent exposure of chitosan and regenerated cellulose is underway in Beaufort.

Table A.4. Effect of Beaufort marine exposure on the strength of chitosan.

Exposure Time (days)	Stress at Break (kg/cm^2)		Strain at Break (%)		No. of Specimens
	Avg	Std Dev	Avg	Std Dev	
0	650	80	39.5	21.0	20
4	510	80	6.6	1.3	6
8	210	70	4.9	2.0	4

Marine Exposure in Beaufort, North Carolina

Compared to the unexposed chitosan samples (control), the samples exposed to seawater lost elongation (strain-at-break) before losing strength (stress-at-break). Conversely, the samples exposed to soil burial lost strength but retained elongation, probably due to the initial swelling and subsequent embrittlement when the seawater-exposed samples were dried.

Accelerated Biodegradation Exposure in the Laboratory

Field testing is a slow and expensive means to screen materials for degradability. Fluctuations and extremes in water quality, ambient temperature, and microbial growth cycles are not readily reproducible. While marine environment testing will always be required to fully demonstrate degradability of the most promising formulations, an accelerated degradation procedure is an attractive alternative for rapid assessment of candidate materials under well-defined conditions. This process will allow us to accelerate the degradation process by intensifying one or more of the following basic conditions involved in biodegradation: availability of nutrients, microbial population density, oxygen availability, temperature, and pH of the medium.

The accelerated biodegradation apparatus, shown in figure A.2, was designed and built with these conditions in mind. It consists of three glass biodegradation chambers placed in a constant temperature water bath. Each chamber has several outlets, inlets, and stirring devices. Sensors to measure temperature, pH, and airflow are fitted into each

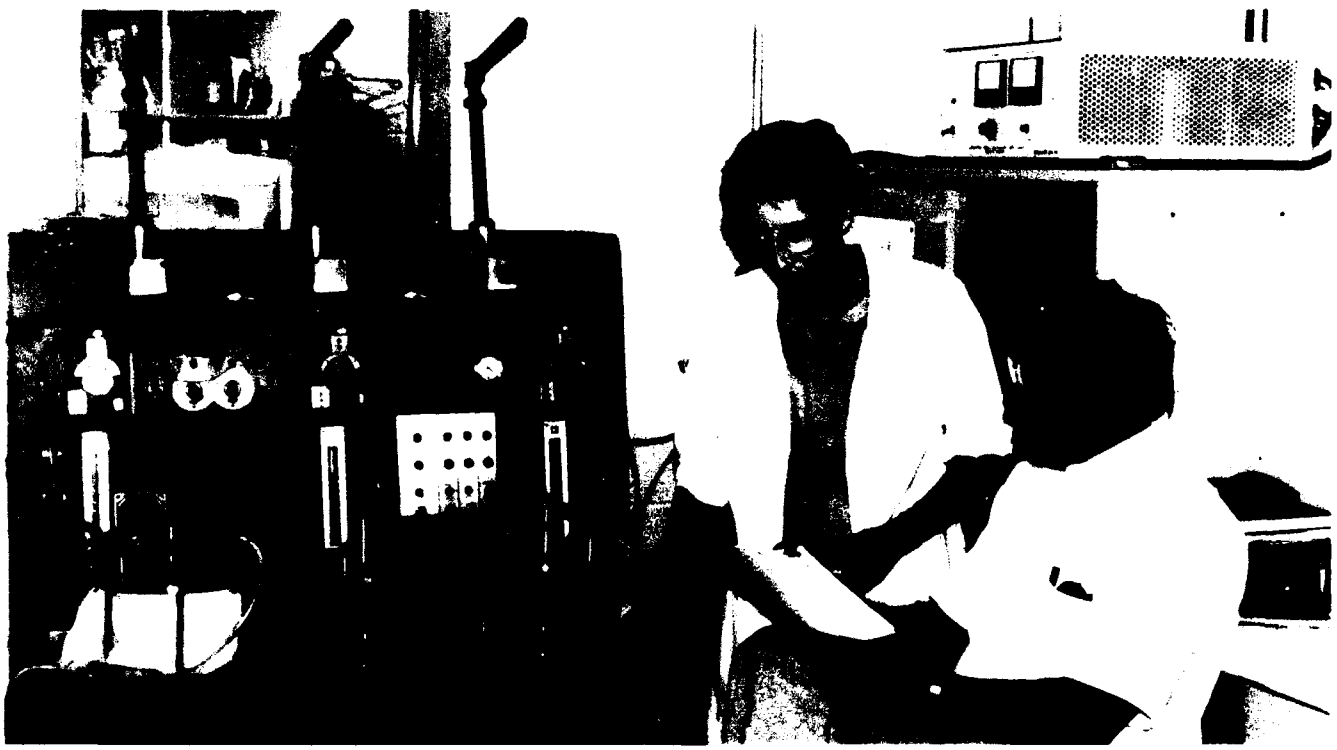


Figure A.2. Accelerated biodegradation apparatus.

chamber, and signals from the sensors are recorded via a dedicated computer interface system.

A glass chamber containing marine sediment from the Beaufort test site was set up in the apparatus. Five chitosan samples were placed in the chamber, which was maintained at 37°C with a constant airflow. One sample was removed each day and measured for tensile strength and viscosity. Table A.5 shows the tensile data for chitosan. Accelerated biodegradation tensile and viscosity data can be correlated with actual marine exposure conditions in Beaufort and Miami. Regenerated cellulose and chitosan/starch blends will be evaluated with this apparatus also.

Table A.5. Effect of accelerated laboratory exposure on the strength of chitosan.

Exposure Time (days)	Stress at Break (kg/cm^2)		Strain at Break (%)		No. of Specimens
	Avg	Std Dev	Avg	Std Dev	
0	650	80	39.5	21.0	20
1	460	20	17.3	7.4	5
2	320	110	8.5	1.1	5
3	300	80	4.5	1.5	4
4	90	20	7.7	2.4	2
6	170	10	3.9	0.9	4

Measures of Degradability

Strength tests. Data obtained from stress-at-break (strength) and strain-at-break (elongation) are the most reliable measures of partial degradation of films exposed to soil and the marine environment. Figure A.3 shows an Instron machine used to generate the data. Tensile strength may be only a reflection of the physical deterioration of the film (i.e., reduced cross section from hole formation by microbes); therefore, we also should measure the viscosity of the film to determine the effect of biodegradation. This measures reduced molecular weight of the biopolymer and relates to Definition 2 for degradable plastic, which involves chemical bond scission. Viscosity measurements are made with an Ubbelohde viscometer.

Water-soluble materials that degrade rapidly require a repeatable method to detect pH-dependent solubility of films based on changes in tensile strength with time. We devised an apparatus and procedure to measure the load-bearing (strength) characteristics of films under uniaxial tension while immersed in water. The procedure can be applied to chitosan, chitosan-starch blends, and other water-soluble films. A strip of film is clamped at an initial gage length and thickness and lowered vertically into water, as shown in figure A.4. The clamp separation is increased to obtain a given load, which is measured by a force meter connected to a chart recorder. The percent elongation then remains constant, and the force required to maintain this extension is measured so that a force curve can be plotted. The force decreases with time as the film relaxes and/or dissolves. The magnitude of the decrease depends on the solubility of the film in the liquid. An example of such measurements is presented in figure A.5.

Degradability Tests

Total biodegradation or mineralization is measured by carbon dioxide (CO_2) evolution, and is accomplished easily through the use of a biometer flask and a piece of

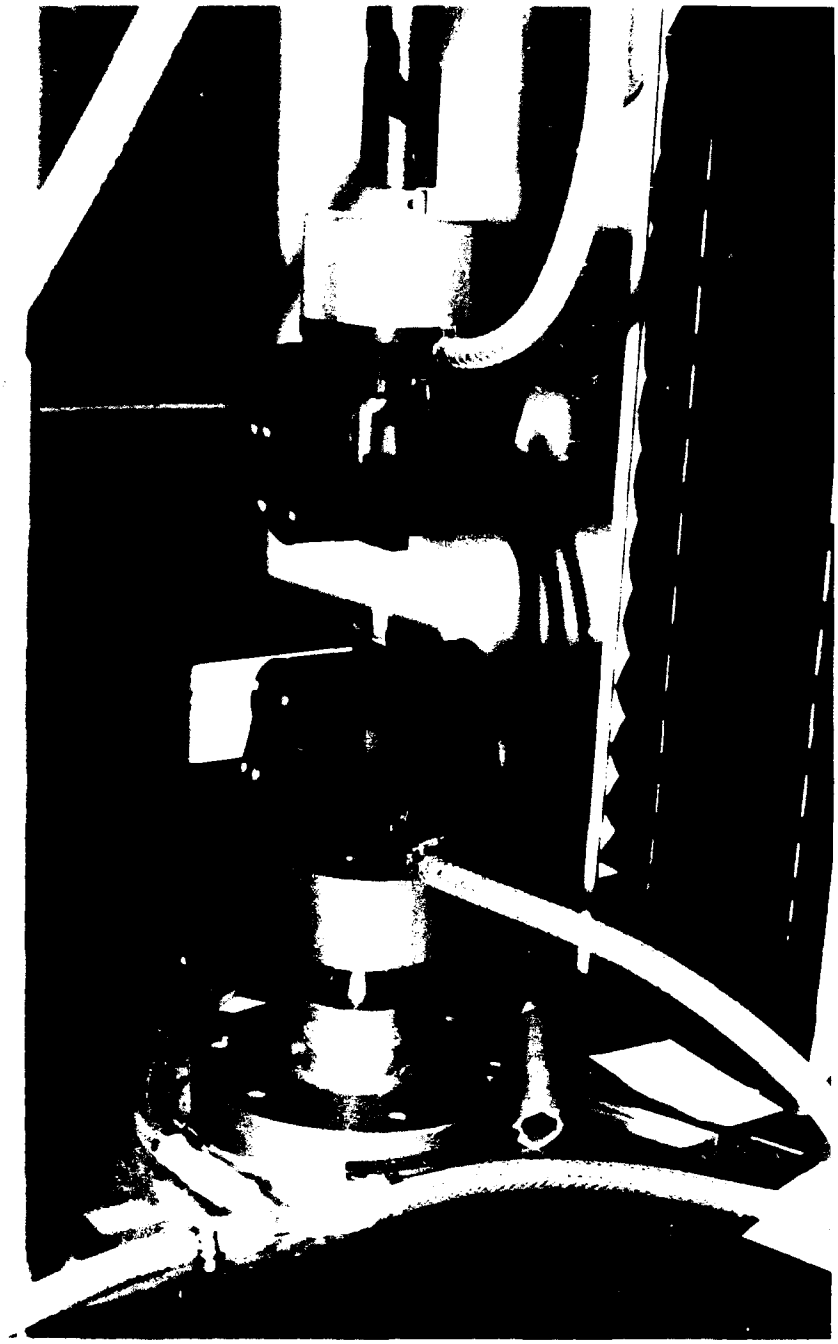


Figure A.3. Instron machine with tensile specimen.

Figure A.4. Stress relaxation test apparatus.

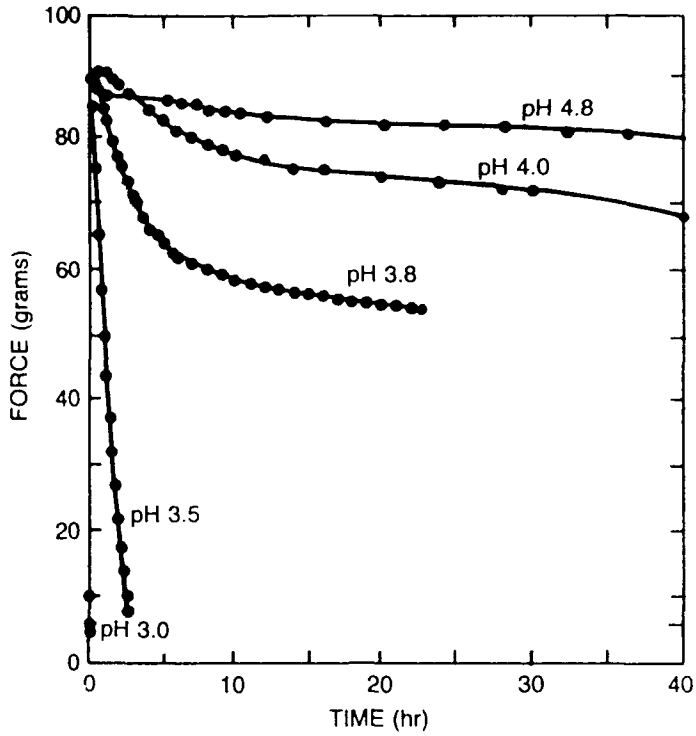


Figure A.5. Force-time relationships for a chitosan film.

glassware consisting of a 250-mL Erlenmeyer flask connected to a glass "side arm" tube, as shown in figure A.6. The flask contains the desired environment for degradation (soil, marine sediment, seawater, etc) and shredded pieces of sample film. The carbon dioxide released by the degradation reaction is absorbed by a potassium hydroxide (KOH) solution contained in the side arm. The KOH solution is removed daily for titration and replaced by fresh KOH. The amount of carbon dioxide evolved is calculated from the titration. Figure A.7 shows a graphic comparison of total degradation as measured by CO_2 evolution in biometer flasks for chitosan, chitosan-starch, and regenerated cellulose in garden soil and marine sediment at 25°C. This procedure will be repeated for other temperatures and conditions.

In view of the time constraints placed on the Navy to comply with Public Law 100–220, we will pursue our research aggressively in accordance with the plans shown in figure A.8.

Figure A.6. Biometer flask for mineralization measurements.

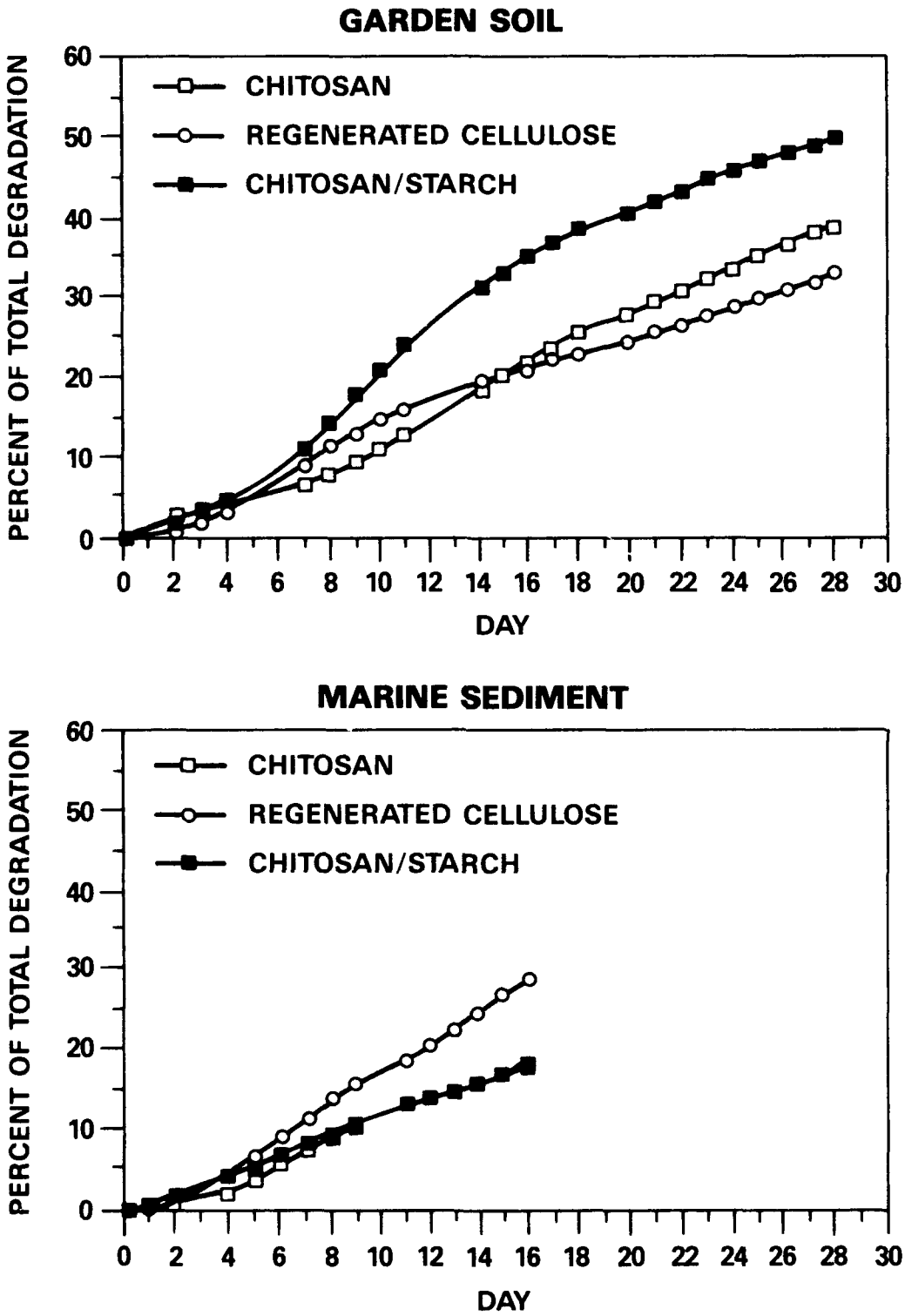


Figure A.7. Total biodegradation (mineralization) measured by CO_2 evolution in biometer tasks.

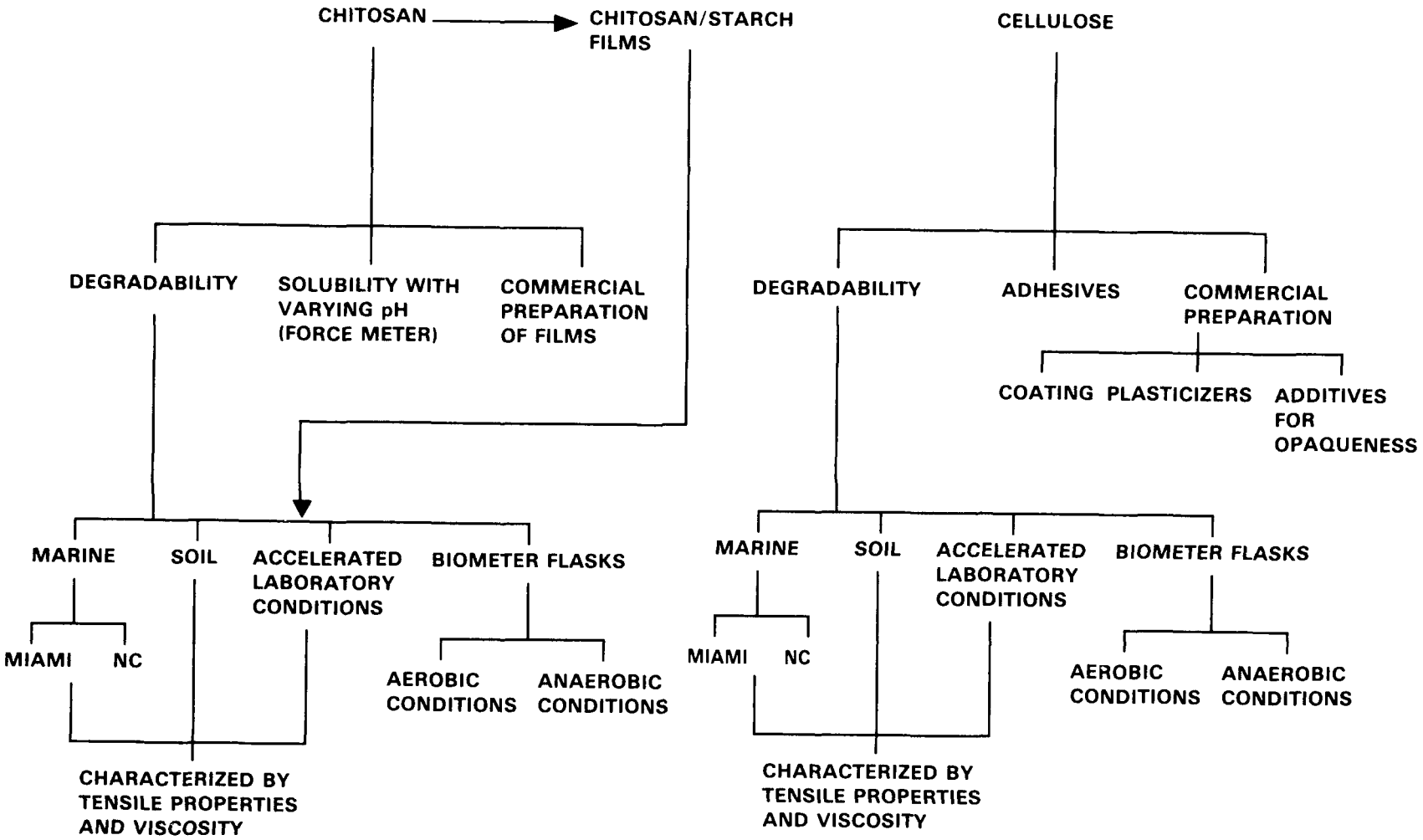


Figure A.8. Flow chart for evaluation of biopolymer films.

REFERENCE

1. Andrady, A. and T. Olson, "Research and Development on Enhanced Degradable Plastics," DTRC–SME–CR–08–89 (Jan 1989).

INITIAL DISTRIBUTION

Copies

2 CNO (OP–45) (Larry Koss)

2 ONT (Code 226)

5 NAVSEA
- 1 SEA 51126 (A. Smookler)
- 4 SEA 56YP (T. Scarano)

1 NAVSUP (Code 0321)
(J. Ballard)

3 U.S. Army Natick Research, Development, and Engineering Center
(J. McCassie)
(D. Kaplan)
(J. Mayer)

1 NCEL (Code L03BPM)

12 DTIC

2 COMNAVAIRLANT (Code 0213)
(LCDR P. Gillooly)

1 NOAA (Jim Coe)

1 CINCLANTFLT

1 CINCPACFLT

1 CNSL

1 CNSP

1 CNAL

1 CNAP

CENTER DISTRIBUTION

Copies	Code	Name
1	0112	B. Douglas
1	28	G. Wacker
1	2801	D. Ventriglio
1	2802	A. Morton
1	2803	J. Cavallaro
1	2809	A. Malec
1	281	P. Holsberg
3	283	H. Singerman
3	283S	M. Weibe
5	2830	P. Schatzberg
10	2834	C. Alig
30	2834	T. Olson
1	2834	D. Jackson
1	2834	L. Copeland
1	2834	S. Gill
1	284	E. Fischer
1	3411	W. Rice
1	3422	Unclass Lib (A)

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
March 1992

3. REPORT TYPE AND DATES COVERED
10/1/89 – 9/30/90

4. TITLE AND SUBTITLE
Research and Development of Two Marine-Degradable Biopolymers

5. FUNDING NUMBERS
Program Element 62233N
Work Unit 2830–102

6. AUTHOR(S)
Anthony L. Andrady, Ph.D.*
Jan E. Pegram, Ph.D.*
Todd M. Olson**

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
*Research Triangle Institute
P.O. Box 12194
Research Triangle Park, N.C. 27709

**David Taylor Research Center
Bethesda, Md. 20084–5000

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Technology (ONT)
Washington, D.C. 20362

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
DTRC–SME–CR–19–90

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The Navy is developing a biopolymeric film material suitable for fabrication into marine-disposable trash bags so that it can comply with impending national and international requirements which will prohibit the discharge of plastics into the sea. Two biopolymers, chitosan and regenerated cellulose, were selected and tested to meet this need.

After 6 weeks of marine exposure, regenerated cellulose samples disappeared; after 10 weeks, chitosan samples became brittle and separated. While chitosan showed greater anaerobic degradation than regenerated cellulose in soil studies, the opposite occurred in the marine sediment environment. Aerobic degradation was much higher than anaerobic degradation for both biopolymers.

To improve flexibility, 50 plasticizers were tested in chitosan. Ten percent lithium bromide and 5% lithium acetate/10% PEG 400 in chitosan were the most effective plasticizers. Regenerated cellulose films treated with lithium salt solutions also showed improved flexibility.

Incorporating urea and potassium phosphate into cellulose showed that degradation could be increased in soil. Tests are ongoing to further accelerate the rate of biodegradation by increasing the availability of nitrogen and phosphorus.

Fabricating trash bags will require adhesive bonding. Five adhesives were evaluated with regenerated cellulose. Covinax 220, JW 2–47, and Adcote 333T proved acceptable.

Chitosan requires further development to be produced and processed into bags efficiently. With minor adjustments, regenerated cellulose presently meets this requirement; thus, it is the more promising film.

Progress towards our goal of developing a biopolymeric film material meeting the Navy's requirements is continuing. Future work will focus on increasing strength through lamination; improving tear strength, flexibility, and degradability; selecting the optimum adhesive; and adapting a composite film to a mechanized bag-making process.

14. SUBJECT TERMS
Biopolymers, Chitosan, Regenerated cellulose, Biodegradation, Plastics

15. NUMBER OF PAGES
49

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT
Unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT
Unclassified

20. LIMITATION OF ABSTRACT
Same as report