No. A-15-9E
PJ : Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme: Analysis and Regulation of Degradation Behaviors of Biopolymers in Underwater Environments
Organization: Yamagata University, Kyushu University
Contact: Hisao MATSUNO, h-matsuno@yz.yamagata-u.ac.jp

Introduction

Eco-friendly polymer materials

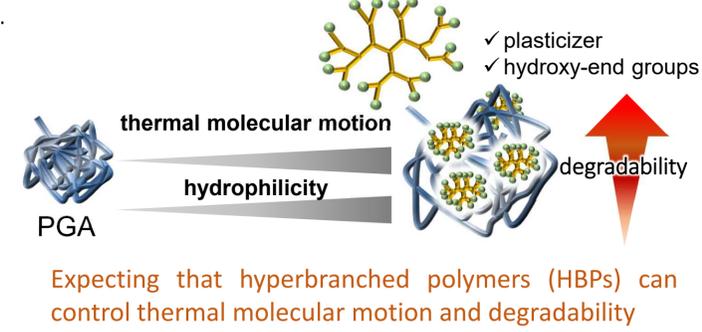- polyester → polyglycolide (PGA)
- polyamide

degradation ← water, enzyme → $CO_2 \cdot CH_4$ H_2O
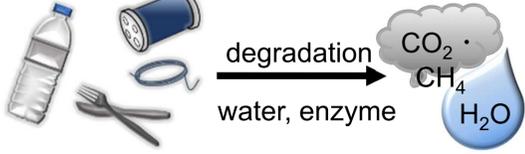
A strong need to control the degradation behavior of polymer materials

Polyester degradation

water
crystal
amorphous

degradation →

Amorphous regions preferentially degradated.

thermal molecular motion
degradability

Focusing on the relationship between thermal molecular motion and degradability

Hyperbranched polymer

✓ plasticizer
✓ hydroxy-end groups

thermal molecular motion
hydrophilicity

PGA

degradability

Expecting that hyperbranched polymers (HBPs) can control thermal molecular motion and degradability
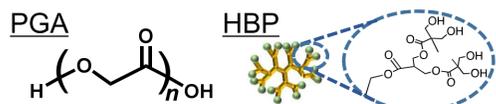
Objective

To clarify the effects of HBP on the aggregation state and thermal molecular motion of PGA, as well as on their degradation properties
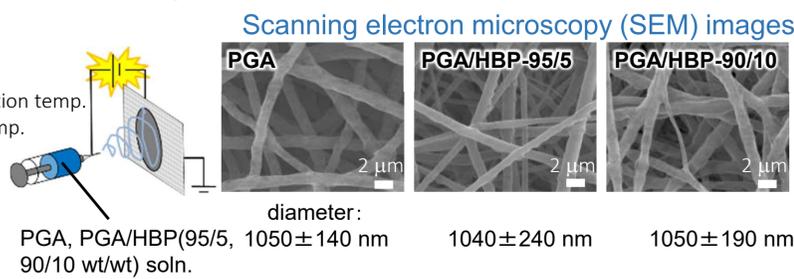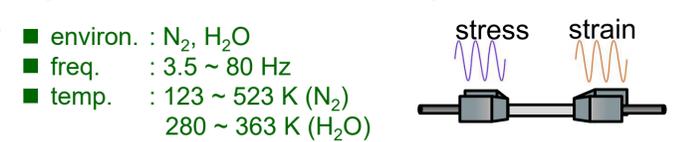
Experimental

PGA

HBP

M_n: a number-average molecular weight T_g: glass transition temp.
M_w/M_n: polydispersity index T_m: melting temp.

polymer	M_n	M_w/M_n	T_g / K	T_m / K
PGA	80k	1.7	318	481
HBP	10k	1.6	308	-

Electrospun PGA fiber-mats

Scanning electron microscopy (SEM) images

PGA   PGA/HBP-95/5   PGA/HBP-90/10
2 µm   2 µm   2 µm

diameter :
PGA, PGA/HBP(95/5), 1050±140 nm   1040±240 nm   1050±190 nm
90/10 wt/wt) soln.

Dynamic mechanical analysis (DMA)

stress   strain

- environ. : N$_2$, H$_2$O
- freq. : 3.5 ~ 80 Hz
- temp. : 123 ~ 523 K (N$_2$)
  280 ~ 363 K (H$_2$O)

Degradation test

- temp. : 310 K
- pH : 7.4
- time : 2~14 days

0.01 M phosphate buffer soln.
fiber mat

Residual weight $\%R_w = \dfrac{W_1}{W_0} \times 100$

W_0 : initial weight
W_1 : weight after degrad..
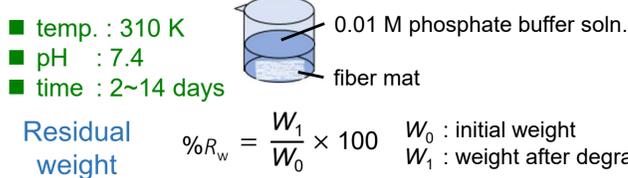
Differential scanning calorimetry (DSC)

- environ. : N$_2$
- rate. : 10 K·min^{-1}
- temp. : 430 ~ 510 K

ΔH_m : heat of fusion
ΔH_f : heat of fusion of crystal
w : weight ratio of PGA

Apparent crystallinity (%X_{ac})

$\%X_{ac} = \dfrac{\Delta H_m}{\Delta H_f \cdot w} \times 100$
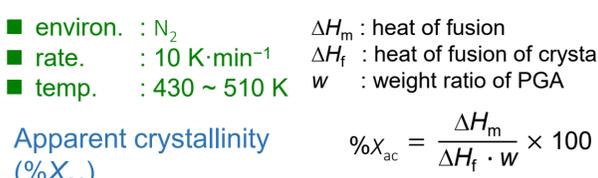
Small angle X-ray scattering (SAXS)

@SPring-8 BL03 beamline

detector
fiber mat
X-ray

- wavelength: 0.10 nm
- sample to detector length: 2,294 mm

$q = \left(\dfrac{4\pi}{\lambda}\right)\sin\theta$

q : scattering vector
λ : wavelength
θ : scattering angle
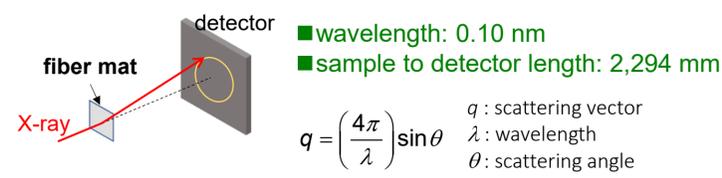
Results & discussion

Thermal molecular motion

Dynamic storage and loss moduli (E' & E'') vs temp.

under N$_2$ in water

E' / a.u.
β
α
frequency / Hz
3.5 ← → 80
100 200 300 400 500/260   300 340 380
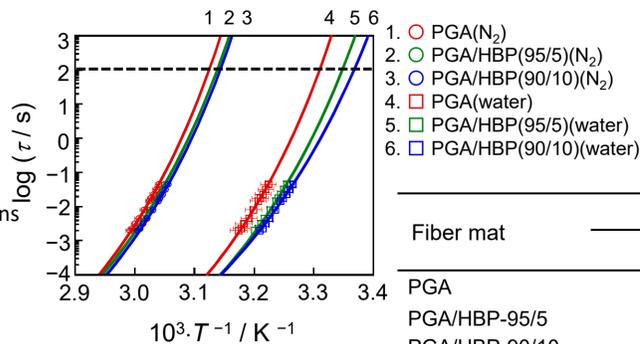Temperature / K

E' / a.u.
α
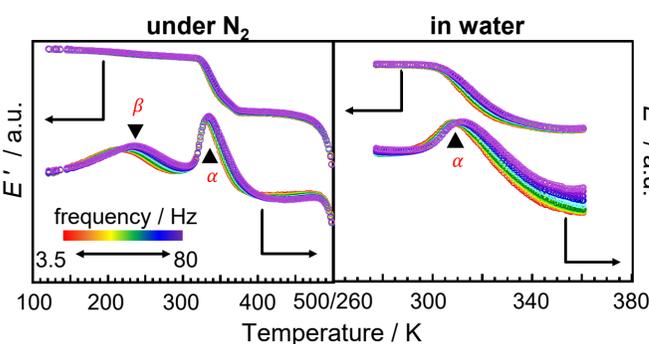
α-relaxation segmental motion

β-relaxation noncooperative local motion of main chains

Dean et al., J. Pharm. Sci., **97**, 2022 (2008).

Relaxation time (τ) of α-process vs temp.

1 2 3   4 5 6

log (τ / s)

1. ○ PGA(N$_2$)
2. ○ PGA/HBP(95/5)(N$_2$)
3. ○ PGA/HBP(90/10)(N$_2$)
4. □ PGA(water)
5. □ PGA/HBP(95/5)(water)
6. □ PGA/HBP(90/10)(water)

2.9 3.0 3.1 3.2 3.3 3.4
$10^3 \cdot T^{-1}$ / K^{-1}

Vogel-Fulcher-Tamman eq.
$\tau = \tau_0 \exp\left(\dfrac{B}{T - T_v}\right)$

τ : relaxation time
τ_0: relaxation time at a high-temp. limit
B: activation temp. T_v: Vogel temp.

$T_{g\alpha}$: temp. at τ = 100 s

Fiber mat	in N$_2$	in H$_2$O
	$T_{g\alpha}$ / K	$T_{g\alpha}$ / K
PGA	320 ± 1	302 ± 1
PGA/HBP-95/5	319 ± 1	299 ± 1
PGA/HBP-90/10	318 ± 1	297 ± 1

Degradation behaviors

%R_w vs immersion time

%R_w

1. ○ PGA
2. ○ PGA/HBP(95/5)
3. ○ PGA/HBP(90/10)

0 2 4 6 8
Immersion time / day

molecular chain scission

amorphous chain

degradation ← water →

Changes in aggregation states

T_m vs immersion time

T_m / K

0 2 4 6 8 10 12 14
Immersion time / day

%X_{ac} vs immersion time

%X_{ac}

0 2 4 6 8 10 12 14
Immersion time / day

increment in X_{ac}

degradation weight loss →

Change in long period (L)

SAXS profiles

$I(q)$

1. 0 day
2. 4 days
3. 8 days
4. 14 days

0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
q / nm^{-1}

L vs immersion time

L / nm

1. ◇ PGA
2. ◇ PGA/HBP(95/5)
3. ◇ PGA/HBP(90/10)

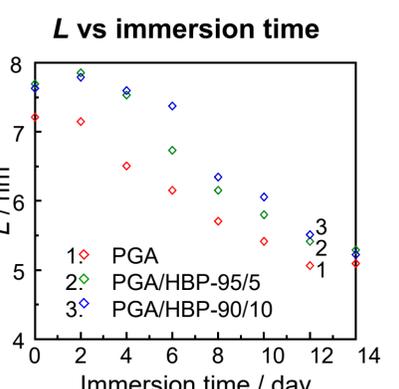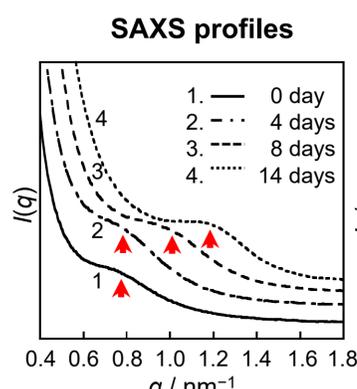0 2 4 6 8 10 12 14
Immersion time / day

T_m ⇄ σ_e, L
σ_e
L

σ_e
L

σ_e: surface free energy of folding surface, L: lamella thickness

Summary

Addition of HBP activated the segmental motion of the PGA chains and promoted the degradation of the amorphous regions. It was revealed that during the degradation process, the lamella thickness increased due to crystallization of the cleaved molecular chains, and then decreased as further molecular chains were cleaved.

No. A–15–10E
PJ: Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme: Development of Degradable Polymers Based on Plant-Derived Renewable Resources
Organization: Nagoya University, Graduate School of Engineering (E3-1)
Contact: Masami KAMIGAITO kamigait@chembio.nagoya-u.ac.jp

Ring-Opening Polymerization of Novel Lactones with Protected Hydroxy Group Derived from Biomass and Deprotection-Induced Polymer Degradation

Introduction

Renewable Resources

Glycerol / Glycolic Acid

M1 R = TBS
M2 R = oNBn

Ring-Opening Copolymerization of M1 or M2 and Various Lactone Monomers

Lactone Monomers
δ-VL, ε-CL, LA

BnOH Catalyst
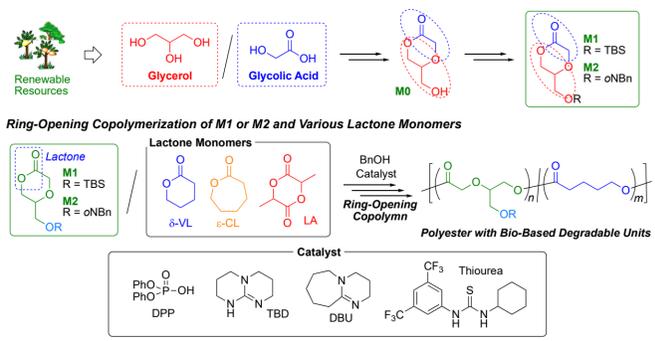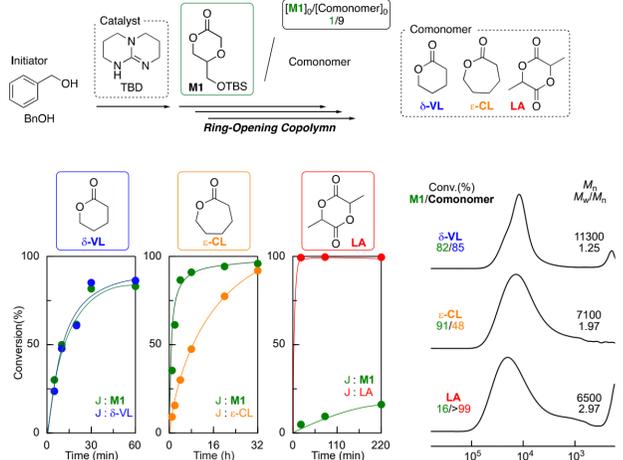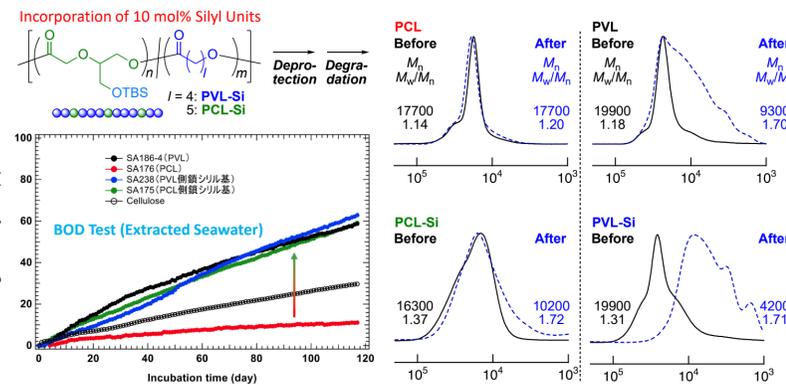Ring-Opening Copolymn

Polyester with Bio-Based Degradable Units

Catalyst: DPP, TBD, DBU, Thiourea

Degradation Triggered by Deprotection (M1)

OTBS → TBAF → Lactonization

Ring-Opening Copolymerization of Silyl-Protected Lactone

Initiator BnOH, Catalyst TBD → M1 OTBS

$[M1]_0/[Comonomer]_0$ 1/9

Comonomer: δ-VL, ε-CL, LA

Ring-Opening Copolymn

	Conv.(%) M1/Comonomer	M_n	M_w/M_n
δ-VL	82/85	11300	1.25
ε-CL	91/48	7100	1.97
LA	16/>99	6500	2.97

δ-VL: J : M1, J : δ-VL
ε-CL: J : M1, J : ε-CL
LA: J : M1, J : LA

Deprotection-Induced Polymer Degradation

Incorporation of 10 mol% Silyl Units

l = 4: PVL-Si
5: PCL-Si

Deprotection → Degradation

BOD Test (Extracted Seawater)
SA186-4 (PVL), SA176 (PCL), SA238 (PVL 保護シリル基), SA175 (PCL 保護シリル基), Cellulose

	Before M_n / M_w/M_n	After M_n / M_w/M_n
PCL	17700 / 1.14	17700 / 1.20
PVL	19900 / 1.18	9300 / 1.70
PCL-Si	16300 / 1.37	10200 / 1.72
PVL-Si	19900 / 1.31	4200 / 1.71

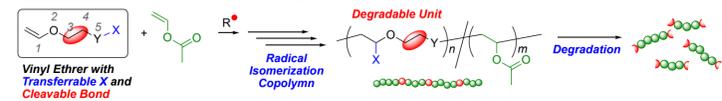## Synthesis of Degradable Polymers via 1,5-Shift Radical Isomerization Polymerization

Introduction

Synthesis of Degradable Polymers by 1,5-Shift Radical Isomerization Polymerization
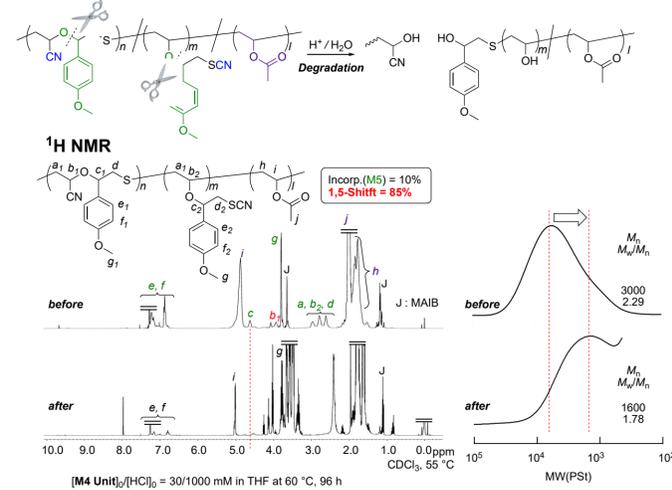
Vinyl Ethers with Transferrable X and Cleavable Bond

1,5-Shift → Radical Isomerization Polymn → Degradable Polymer → Degradation

Vinyl Monomer with Degradable Unit via Possible 1,5-Shift Radical Polymerization

M1 (H-Transfer), M2 (H-Transfer), M3 (H-Transfer), M4 (CN-Transfer)
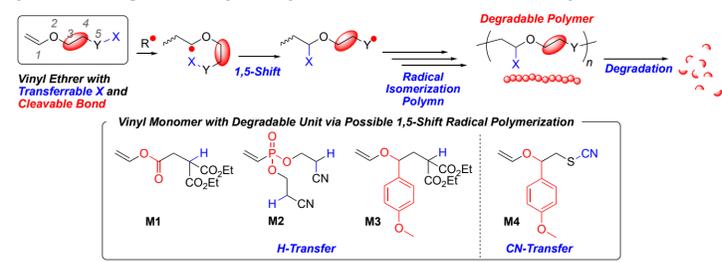
Radical Copolymerization with Vinyl Monomer

Vinyl Ethers with Transferrable X and Cleavable Bond → Radical Isomerization Copolymn → Degradable Unit → Degradation

Monomer and Polymer Obtained by Radical Homopolymerization

^1H NMR / ^{13}C NMR

Monomer

Polymer
T_g ~ 20 °C
T_{d5} ~ 190 °C
M_n = 2100
M_w/M_n = 1.64
1,5-Shift = 85%
(NMR-e, f, b_1)

Y : X = 92 : 8
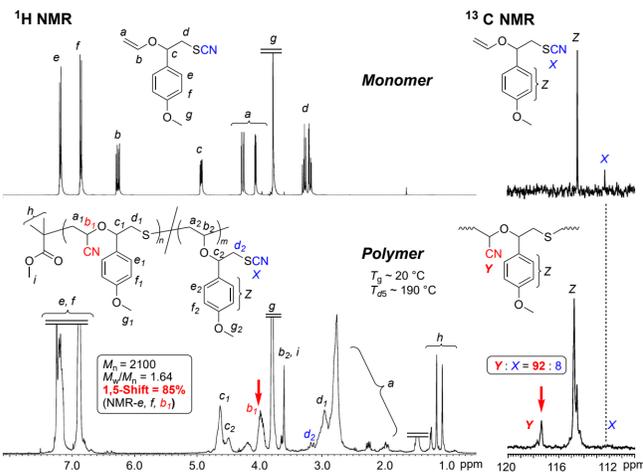
Degradable Poly(vinyl Acetate) by Radical Copolymerization

H^+/H_2O Degradation

^1H NMR
Incorp.(M5) = 10%
1,5-Shift = 85%

before / after
J : MAIB

M_n / M_w/M_n
before 3000 / 2.29
after 1600 / 1.78

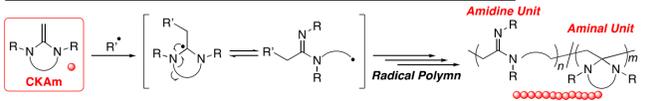[M4 Unit]$_0$/[HCl]$_0$ = 30/1000 mM in THF at 60 °C, 96 h

Synthesis and Radical Polymerization of Cyclic Ketene Aminals for Degradable Vinyl Polymers

Introduction

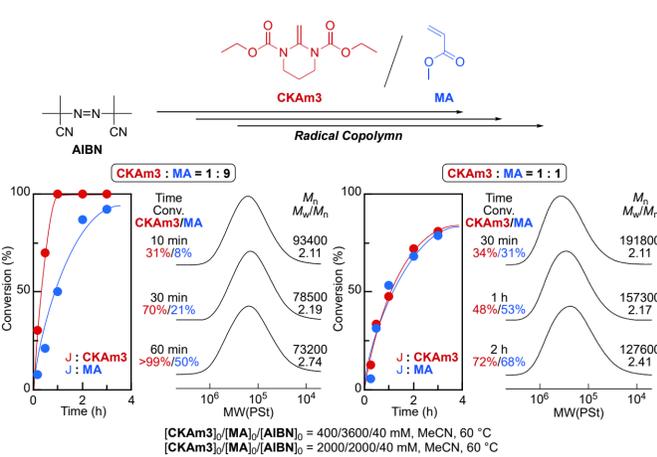Synthesis of Cyclic Ketene Aminal (CKAm)

★ New exo-Methylene-Type Vinyl Monomer
★ Tunable Reactivity
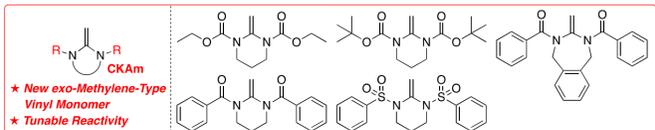
Radical Ring-Retaining and Ring-Opening Polymerization of CKAm

Radical Polymn → Amidine Unit, Aminal Unit → Novel Degradable Polymer

Radical Copolymerization of Vinyl Monomers and CKAm

CKAm + Vinyl Monomer → Radical Copolymn → Amidine Unit, Aminal Unit → Novel Degradable Copolymer

Radical Copolymerization of CKAm and Methyl Acrylate

CKAm3 / MA, AIBN, Radical Copolymn

CKAm3 : MA = 1 : 9

Time	Conv.(%) CKAm3/MA	M_n	M_w/M_n
10 min	31%/8%	93400	2.11
30 min	70%/21%	78500	2.19
60 min	>99%/50%	73200	2.74

J : CKAm3, J : MA

CKAm3 : MA = 1 : 1

Time	Conv.(%) CKAm3/MA	M_n	M_w/M_n
30 min	34%/31%	191800	2.11
1 h	48%/53%	157300	2.17
2 h	72%/68%	127600	2.41

J : CKAm3, J : MA

[CKAm3]$_0$/[MA]$_0$/[AIBN]$_0$ = 400/3600/40 mM, MeCN, 60 °C
[CKAm3]$_0$/[MA]$_0$/[AIBN]$_0$ = 2000/2000/40 mM, MeCN, 60 °C

Degradation of Copolymers of CKAm and Methyl Acrylate

HCl Degradation
Incorp.(CKAm3) = 51%

1N HCl$_{aq}$, 60 °C, 10 min
1N HCl$_{aq}$, 25 °C, 10 min
H_2O, 60 °C, 96 h
1N NaOH$_{aq}$, 60 °C, 96 h

HCl (60 °C): before M_n 157900 M_w/M_n 2.56 → after 2700 / 2.04
HCl (25 °C): before 157900 / 2.56 → after 2200 / 2.51
H_2O: before 157900 / 2.56 → after 48700 / 2.07
NaOH: before 157900 / 2.56 → after 84700 / 2.56

[CKAm Unit]$_0$/[HCl]$_0$ = 5.0/50 mM, THF/H_2O = 19/1, 60 °C
[CKAm Unit]$_0$/[HCl]$_0$ = 5.0/50 mM, THF/H_2O = 19/1, 25 °C
[CKAm Unit]$_0$ = 5.0, THF/H_2O = 19/1, 60 °C
[CKAm Unit]$_0$/[NaOH]$_0$ = 5.0/50 mM, THF/H_2O = 19/1, 60 °C

Direct Radical Polymerization of Carbon-Heteroatom Double Bonds for Degradable Vinyl Polymers

Introduction

Carbon-heteroatom double bond (C=X):
Application for radical polymerization via appropriate design

C=X Monomers (Main-chain functionalization) + Various Vinyl Monomers (Side-chain functionalization)

Design of both main chain and side chain to generate novel degradable polymers

Degradation of Various Vinyl Copolymers with Thioamide

Before / After, Oxidation with Metal Ions

	Before M_n / M_w/M_n	After M_n / M_w/M_n
AcMeThiFA/MA = 16/84	23700 / 3.97	1000 / 1.67
AcMeThiFA/DMAA = 22/78	23700 / 3.97	160 / 1.67
AcMeThiFA/VAc = 53/47	77000 / 2.91	–
AcMeThiFA/St = 10/90	22200 / 2.04	1600 / 2.02

Imines for Degradable Vinyl Copolymers with C–N Backbones

NaOH, THF/EtOH = 1/1, r.t. → Degradation

^1H NMR
before: M_p = 9200; M_n 6100, M_w/M_n 1.69
after: M_p = 3000; M_n 700, M_w/M_n 4.82

Conv.(%) OE1/St = 7/32
n/m = 6/94
* : THF

Possible Degradation Mechanism

Hydrolysis → Degradation

1wt% Polymer in 500 mM NaOH solution (THF/EtOH = 1/1 (v/v)) at r.t.

No. A-15-11E
PJ : Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme: Precision Polymerization of Plant-Derived Monomers for Multi-Locked Degradable Biopolymers
Organization: Tokyo Institute of Technology
Contact: Kotaro Satoh (satoh@cap.mac.titech.ac.jp)

For developing multi-locked degradable polymers from non-edible biomass, we will develop a multi-lock technology by utilizing the technology of precision polymerization, which we had cultivated in the petroleum chemicals, to biomass-based and multi-locked degradable polymers. By the polymerization of non-edible biomass as a raw material, we propose the concept of a manufacturing method for multi-lock biopolymers that can be degraded in the ocean collaborating with industry.

Switch Degradable Polymers From Well-Defined Oligo(Butylene Succinate)

Discrete Oligomer of PBS (E5) @Tokyo Tech

脂溶性の保護基 (*tert*-BuMe₂Si-)

過剰

選択的抽出

EDC, DMAP DCM
縮合剤によるエステル化

選択的抽出

DMAP DCM → EDC, DMAP DCM

DMAP DCM → EDC, DMAP DCM

脱保護
TBAF AcOH THF
DMAP DCM

ターゲット 分子: PBS4 量体

	M_n / M_w/M_n
0.5量体	230 / 1.02
1.5量体	410 / 1.04
2.5量体	600 / 1.04
3.5量体	840 / 1.04

Well-Defined Discrete Oligomer

+

Nagoya Univ./Tokyo Tech

+

Nagoya Univ.

Cationic Polyaddition

Lactionization

Collaboration

Biomonomers @RITE

Hydrolysis (pH Switch)   Acetal Linkage

Hemiacetal Eater Linkage

pH Switch
Pendent Functionality →
Triggerd Degradation

Degradability@U Tokyo/Ehime U   Toxicity@CERI

Bio-Degradability of Oligomer @CERI
PBS 4量体
41 days ave.79 %
相対生分解度:94%

Degradability of Commercial Polymer
Commercial PBS
Ave. : 2.5 % (40d)

Telechelic OH PBS Oligomer
Non-Toxic Oligomer   Acetal Linkage (Hydrolysis)
Polymer with $M_n > 10^5$

DSC analysis
T_c: 45 °C
T_{cc}: 76 °C
T_g: -43 °C
T_m: 92 °C
5 °C/min

	M_n / M_w/M_n
PBS 4.5mer 0 h	836 / 1.04
Polymer 6 h	31500 / 2.44
After HCl Treatment 100	816 / 1.05

Not Degraded in Sea Water

Telechelic COOH PBS Oligomer
Acid   Acid
No catalyst
Non-Toxic Oligomer   Hemiacetal Eater Linkage (Hydrolysis)

DSC
T_{cc}: 18 °C
T_g: -38 °C
T_m: 52 °C

Sea water (pH ~8)

Degraded in Sea Water (41℃, 1 week)

Addition–Fragmentation Ring-Opening Polymerization of Bio-Based Thiocarbonyl L-Lactide for Dual Degradable Vinyl Copolymers

L-Lactide Bio-Based → Thiocarbonyl L-Lactide (SLA)

Addition → β-Scission Fragmentation → Acrylic Radical

Ring-Retained Form   Ring-Opened Form

(Vinyl Monomer) + SLA

Radical Copolymn

AgNO₃   n-BuNH₂

Main Units   Degradable Units

Degradation

(A) n-BuNH₂
Reaction Time	M_n / M_w/M_n
0 h	19300 / 1.87
1 h	12400 / 1.91
70 h	7200 / 1.77

(B) AgNO₃
Reaction Time	M_n / M_w/M_n
0 h	19300 / 1.87
1 h	4900 / 1.34
70 h	5400 / 1.30

(C) $M_{n,after}/M_{n,before}$ vs Number of SLA derived units per chain
● : n-BuNH₂
● : AgNO₃
Calcd

Japanese Patent Application 2021-131293. 2023-025873.
Macromol. Rapid Commun. **2023**, *44*, 2200537.

Hydrophilic Bio-Based Polymers by Radical Copolymerization of Cyclic Vinyl Ethers Derived from Glycerol

From Renewable Resources

HO–OH (Glycerol) → BuMDO   CyMDO   PhMDO
Glycerol-Derived Cyclic Vinyl Ethers

RAFT (Block) Copolymn → No Ring-Opening → Acetal Deprotection → *Hydrophilic Polymer*

CPBD + AIBN → RAFT Radical Copolymn

▲,▲ : CyMDO
●,▲ : PhMDO
■,▲ : BuMDO
calcd

$M_n \times 10^{-3}$ vs Conversion (%)

[MA]₀/[VE]₀/[CPBD]₀/[AIBN]₀ = 1500/1500/20/5.0 mM in toluene, 60 ℃.

-R:	Conv. (%)	M_n / M_w/M_n
BuMDO	60/87	9200 / 1.29
CyMDO	58/94	10400 / 1.30
PhMDO	55/83	8600 / 1.26

Chem. Commun. **2022**, *58*, 8766.

ChemComm

PJ : Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses

Theme: Biomonomer production from non-food biomass and development of polymer degrading enzymes

Organization: Research Institute of Innovative Technology for the Earth (RITE)

Contact: Masayuki Inui inui@rite.or.jp (T. Shimizu, D. Grinanda, M. Suda, Y. Tanaka, K. Hiraga)

【Purpose】To solve marine plastic pollution, we are working on research and development of a **"multilock biopolymer"** that shows high strength during uses, whereas immediately degrade to CO_2 and H_2O when it leaked to marine environment. We challenge development of an unprecedented polymer that exhibits both toughness and biodegradability for implementation of sustainable resource circularization.

【R&D tasks of RITE】
① High production of biomonomer
② High functionalization of polymer-degrading enzymes

Uncollectable ocean plastic litters
- Fishing tool litter
- Fiber litter
- Plastic litter

Leak by accident

"Multilock polymer"

Multiple environmental stimuli to unlock

Light, Ion etc

On demand decomposition

Toughness during uses

Monomer — RITE — Bioprocess — Non-food biomass

Multilock biopolymer

Polymerization Processing

Enzyme — RITE
② High-functionalization of polymer-degrading enzymes

Photosynthesis

CO_2
H_2O

① High production of biomonomer

E3c-1 Development of biomonomer production process from non-food biomass

E3c-2 High-functionalization of polymer degrading enzymes

Focus on development of high-performance polymer degrading enzymes in FY2023

Thermostable esterase degrading aliphatic polyesters

Degradation of aliphatic polyesters

PLA	Poly(lactate)
PCL	Poly(caprolctone)
PES	Poly(ethylene succinate)
PBS	Poly(butylene succinate)

Reaction conditions
10 µg ml^{-1} enzyme, 37°C, pH8.0, 24 h

Weight loss(%) — 0, 20, 40, 60, 80, 100, 120

Thermostable esterase

Before treatment — After treatment
PBS was completely degraded after 24 h
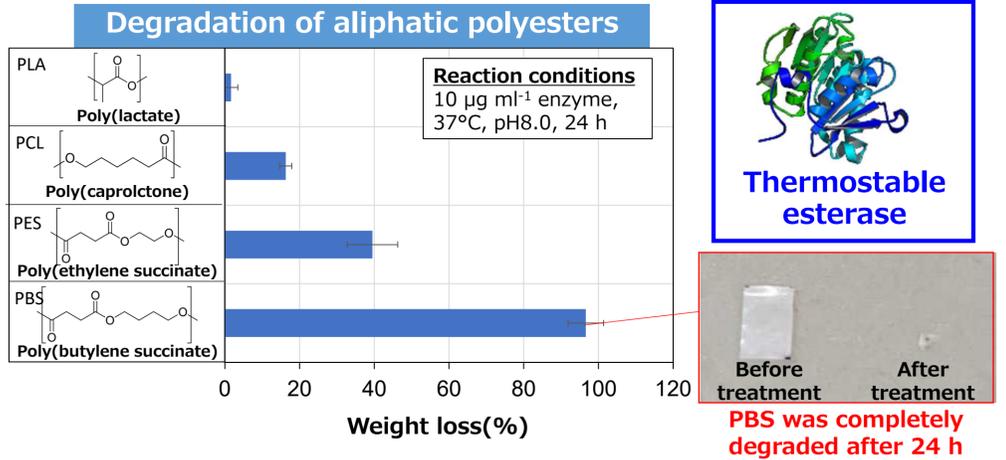
We discovered a thermostable esterase that degrades various aliphatic polyesters

Demonstration of switching in seawater

Immobilized enzyme
- Thermostable esterase
- Carrier matrix

Without salt
Enzyme is captured by carrier matrix to prevent degradation

Degradation ✕

Polymer

With salt (seawater)
Enzyme is released to degrade polymer = switching by salt

Degradation

Polymer

Water (without salt) — Seawater (with salt)

PBS film soaked in water or seawater with immobilized enzyme

37°C, 48 h

Degraded PBS film

Switching was confirmed in seawater

Immobilized enzyme-embedded PBS film

GreenMAP Institute Yamagata University

https://greenmap.yz.yamagata-u.ac.jp/

Films were prepared with direction by Prof. Ito lab in Research center for GREEN materials and Advanced processing at Yamagata university

Preparation of immobilized enzyme-embedded PBS film
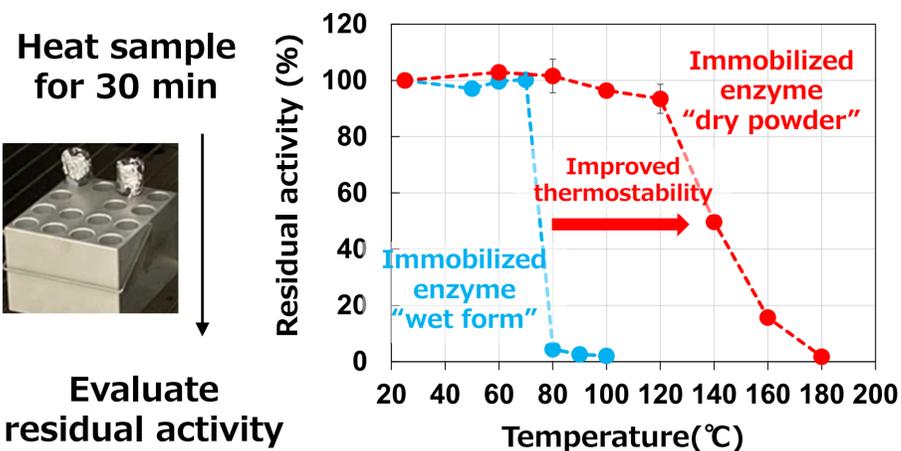
PBS film embedded with carrier matrix — Enzyme-embedded PBS film

Degradation of enzyme-embedded films in seawater

Film soaking experiment
Films (5~6 mg) were incubated in sea water at 37°C for 5 days

PBS film embedded with carrier matrix (control) — Enzyme-embedded PBS film

Sea water — film

×500 — ×500
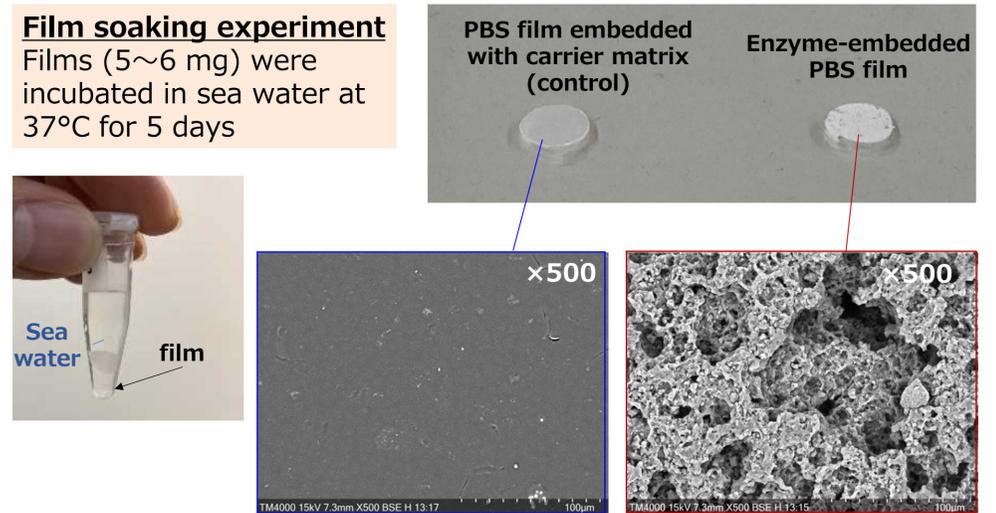
Immobilized enzyme-embedded PBS film degraded in sea water →enzyme was still active after molding into the film

Improvement of thermostability of enzyme by immobilization and freeze drying

Purified enzyme solution

Immobilzed to Carrier matrix

Immobilized enzyme "wet form"

Freeze dry

Immobilized enzyme "dry powder"

Thermostability of enzyme was dramatically improved by immobilization and freeze drying

Heat sample for 30 min

Evaluate residual activity

Residual activity (%) vs Temperature(°C)

Immobilized enzyme "dry powder"

Improved thermostability

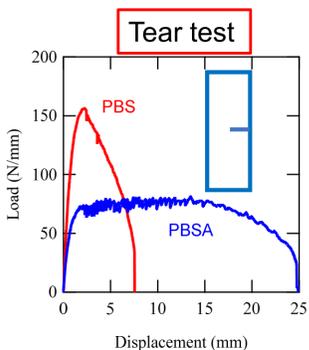Immobilized enzyme "wet form"

■ Future plans
- Screening of polymer degrading enzymes
- Investigation of carrier matrix
- Mutation of enzymes to improve thermostability
- Optimized preparation for immobilized enzyme-embedded PBS film (temperature、time、shear force、enzyme amount)
- Collaboration with companies

No. A-15-13E
PJ : Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme : Control of Higher-Order Structure and Toughness of Marine Bio-degradable Polymers through Polymer Processing
Organization : Yamagata University
Contact : ihiroshi@yz.yamagata-u.ac.jp

Analysis of the mechanism of low tearing property of PBS film and enhancement of strength

Introduction

BioPBS(Polybutylene Succinate)
- ✓ Biodegradable
- ✓ Biomass-based
- ✓ High heat resistance
- ✓ Flexiblity

→ Ustainable Circular economy
→ Used as film application

Tear test ↔ Crystalline Structure

Crystal lattice Lamellae Branch Spherulite

1 nm   10 nm   100 nm   1 μm   10 μm
WAXS   SAXS   USAXS   OM
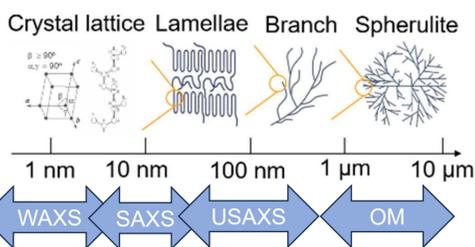
Controlling of crystalline structure

PBS and PBSA has poor tear strength

Analysis of the mechanism of low tearing property
Enhancement of the tear strength by controlling crystalline structure

Experimental

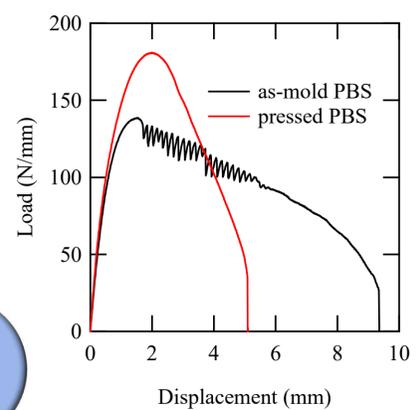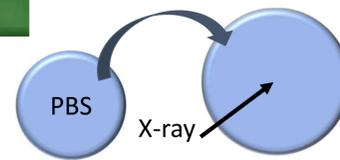**Time-resolved X-ray scattering method using SPing-8 BL38B1**

Wavelength : 0.8 Å
Sample to detector length : 27.3 cm
Scattering vector : $q = \frac{4\pi}{\lambda} sin\theta$

Detector
X-ray
Double-sided drive type tensile testing machine

Ligament length
Test speed : 1 mm/min
X-ray

Sample size
width 10 mm
Thickness: 100 um
Ligament length 5 mm
Chuck distance 14 mm

Crystalline structure

1.6 mm
4.0 mm
5.3 mm
(103)β
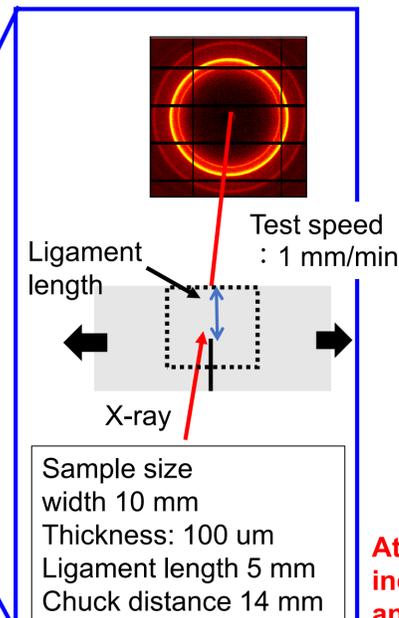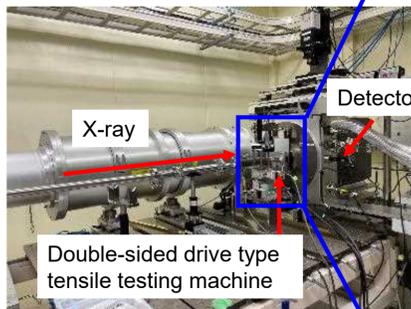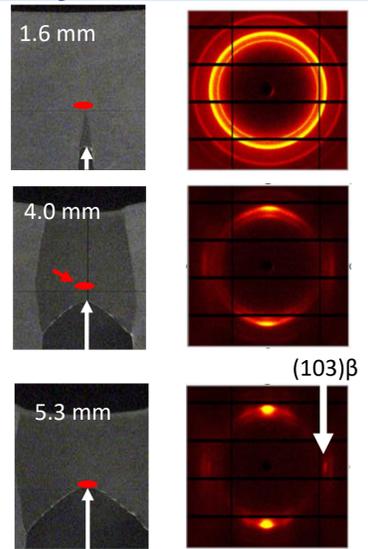
Appearance of the peak derived from β–crystal at notch tip

At the tearing tip, the crystallinity increases due to recrystallization, and a crystal transition from α to β-crystal occurs.

Mechanism of low tearing property

PBS
(103)β

PBSA

Crystallinity and amount of β-crystal change more drastically with PBS than with PBSA

The position of X-ray
α-crystal acute β-crystal A lot
Lamella

The position of X-ray
α-crystal obtuse β-crystal few
α-crystal
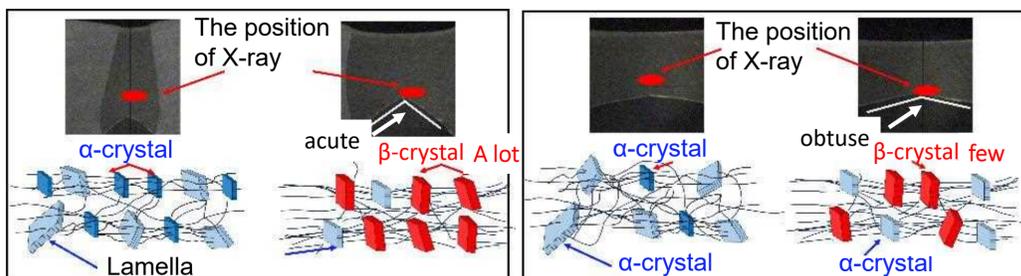
β-crystal make the sample hard and brittle

Enhancement of tear strength

Load
Pressing machine
Iron plate
PBS

High-pressure press under Tm

PBS   X-ray

as-mold PBS
pressed PBS

SAXS   WAXS

	As-mold PBS	Pressed PBS
Yield strength (N/mm)	138	181
Displacement at break (mm)	9.34	5.09
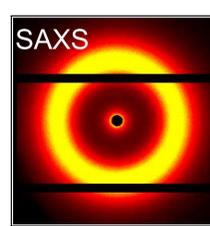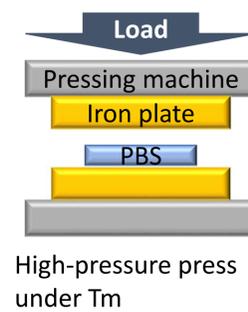
Isotropically spread → plane oriented   **Improved tear strength**

Study on jellyfish protein as a marine-degradable fillers

Tear test of PCL/protein films

Length 30 mm, Width 10 mm, Thickness ≒0.25 mm
Chuck distance 15 mm
5 mm

Test speed 5 mm/min
Temperature 23 ℃
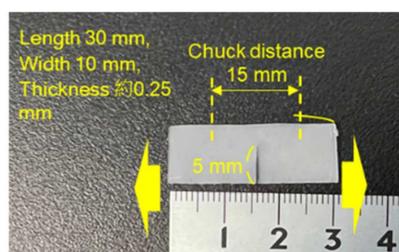
Sample (n=5)
PCL
PCL/J1
PCL/J5
PCL/J10

PCL
PCL/J1
PCL/J5
PCL/J10

Results of tear test

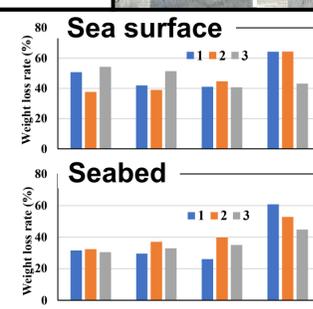| Sample	Tearing force (N)	Tearing strength (N/mm)	Displacement (mm)
PCL	21.4	76.5	20.3
PCL/J1	22.1	82.9	19.1
PCL/J5	21.7	75.0	18.4
PCL/J10	19.6	72.0	18.8
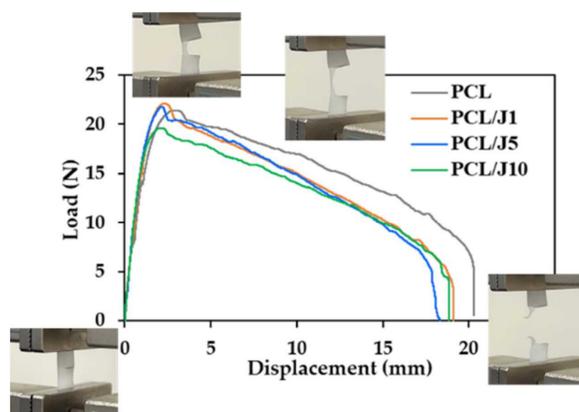
When 1wt% of jellyfish was added, tear strength improved. However, when the jellyfish content was increased to 5wt% and 10wt%, the tear strength decreased.

The tear displacement was slightly reduced due to the jellyfish content.

Marine field test After 6 months Washed with pure water

	PCL	PCL/J1	PCL/J5	PCL/J10
Jelyfish	0 wt%	1 wt%	5 wt%	10 wt%
Sea surface				
Seabed				
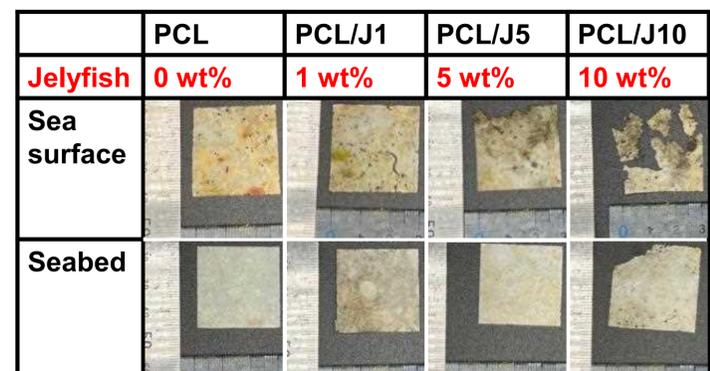
Weight loss rate n=3

Sea surface
Seabed

Films with higher jellyfish content have higher weight loss rates.

PCL/J10 showed a high weight loss rate of approximately 60% in both surface and seabed.
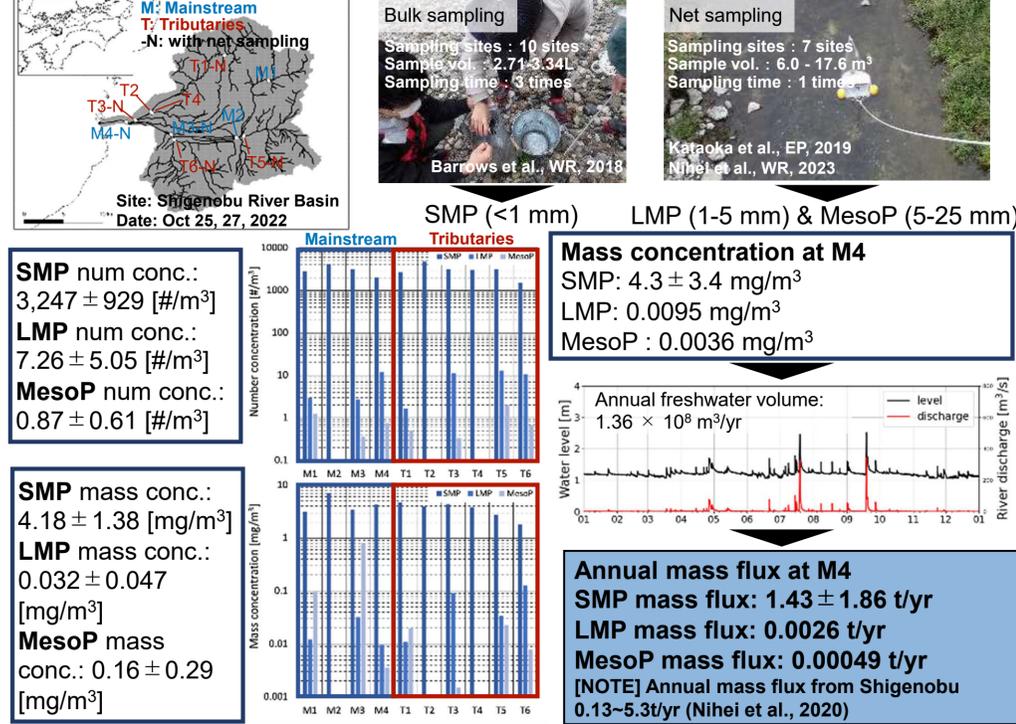
No. A-15-14E
PJ : Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme: Development of a prediction model for long-term impacts of multilocked new polymers on the marine environment
Organization: Ehime University
Contact: Graduate School of Science and Engineering, Hirofumi Hinata (hinata.hirofumi.dv@ehime-u.ac.jp)

■ Outline of our research

1) To develop a prediction model for the long-term impacts of new polymers on the marine environment consisting of physical, chemical, and biological models.
2) To understand the standing stocks in the marine reservoirs, such as water columns, beaches, bottom sediments, marine biota, and fluxes between them with the integrated model.
3) To comprehend the polymer behaviors in the marine environment and assess the impacts based on an input-output system approach.
Researches start with the Seto Inland Sea and then extends to the North Pacific.
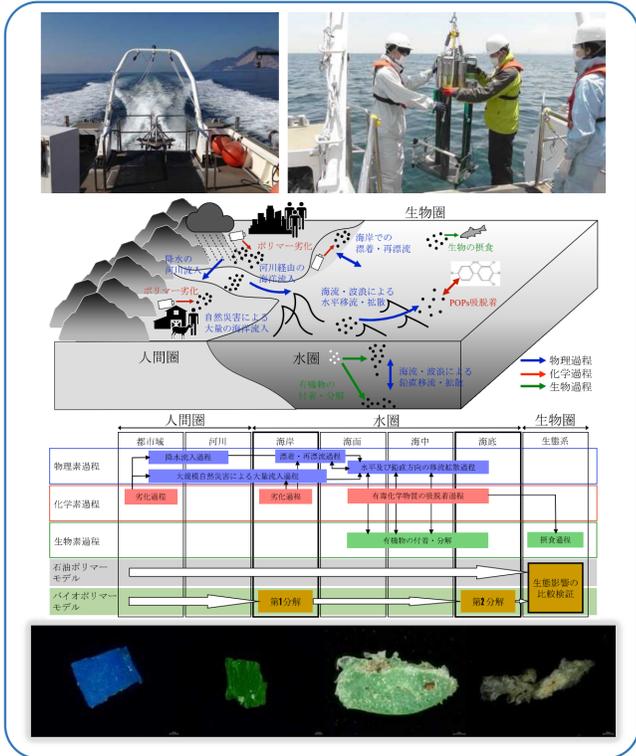
■ Estimation of land-based plastic transport

We estimated the annual mass flux of plastic particles from the Shigenobu River based on field surveys

M: Mainstream
T: Tributaries
-N: with net sampling

Bulk sampling
Sampling sites : 10 sites
Sample vol. : 2.71-3.34L
Sampling time : 3 times
Barrows et al., WR, 2018

Net sampling
Sampling sites : 7 sites
Sample vol. : 6.0 - 17.6 m³
Sampling time : 1 time
Kataoka et al., EP, 2019
Nihei et al., WR, 2023

Site: Shigenobu River Basin
Date: Oct 25, 27, 2022

SMP (<1 mm)   LMP (1-5 mm) & MesoP (5-25 mm)

SMP num conc.: $3{,}247 \pm 929$ [#/m³]
LMP num conc.: 7.26 ± 5.05 [#/m³]
MesoP num conc.: 0.87 ± 0.61 [#/m³]

SMP mass conc.: 4.18 ± 1.38 [mg/m³]
LMP mass conc.: 0.032 ± 0.047 [mg/m³]
MesoP mass conc.: 0.16 ± 0.29 [mg/m³]

Mass concentration at M4
SMP: 4.3 ± 3.4 mg/m³
LMP: 0.0095 mg/m³
MesoP : 0.0036 mg/m³

Annual freshwater volume: 1.36×10^8 m³/yr

Annual mass flux at M4
SMP mass flux: 1.43 ± 1.86 t/yr
LMP mass flux: 0.0026 t/yr
MesoP mass flux: 0.00049 t/yr
[NOTE] Annual mass flux from Shigenobu 0.13~5.3t/yr (Nihei et al., 2020)

■ Modeling for fragmentation process

Fragmentation model of foamed polystyrene (FPS) in Hiroshima Bay
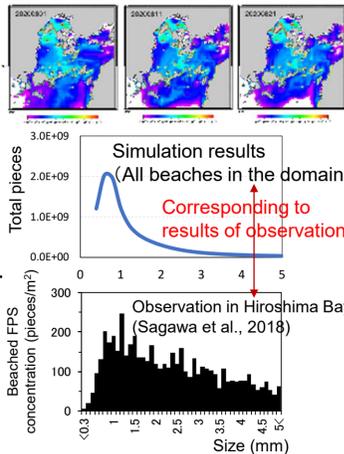
1. Governing Equation
$$\frac{\partial C}{\partial t} = -\left(\frac{\partial (uC)}{\partial x} + \frac{\partial (vC)}{\partial y}\right) + \frac{\partial}{\partial x}\left(K_h \frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(K_h \frac{\partial C}{\partial y}\right) + S$$
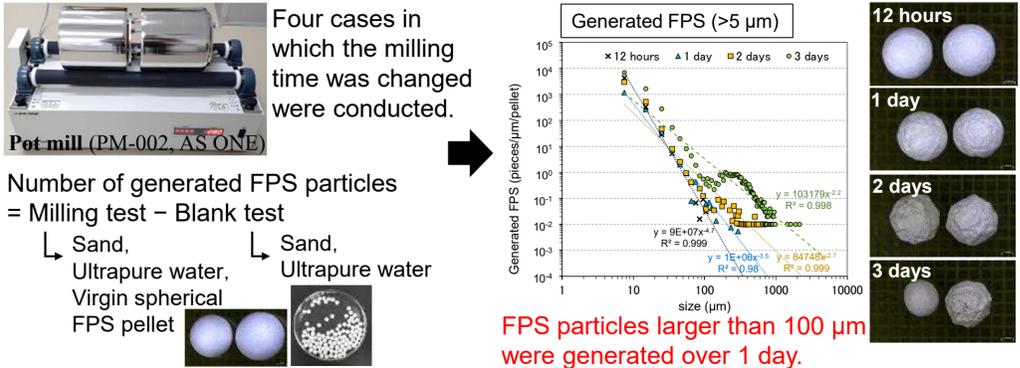
2. Beach process
$$K_h \frac{\partial C}{\partial x} + K_h \frac{\partial C}{\partial y} = \left(K_{SB}\frac{\partial C}{\partial x} + K_{BS}\frac{\partial C}{\partial x}\right) + \left(K_{SB}\frac{\partial C}{\partial y} + K_{BS}\frac{\partial C}{\partial y}\right)$$

C; Concentration (-/m²), u, v; Current velocity (m/s), K_h; Horizontal diffusion coefficient of sea (m²/s), S; Input flux (-/m² · s), K_{SB}, (K_{BS}); Backwashing (beaching) diffusion coefficient (m²/s)

3. Fragmentation process; Size division based on the normal distribution curve (σ = 0.1) was performed for each size of beached FPS after red ten days.

Simulation results (All beaches in the domain)
Corresponding to results of observation.

Observation in Hiroshima Bay (Sagawa et al., 2018)

Temporal changes in the size distribution of FPS generated from the milling test

Pot mill (PM-002, AS ONE)

Four cases in which the milling time was changed were conducted.

Number of generated FPS particles = Milling test − Blank test
Sand, Ultrapure water, Virgin spherical FPS pellet
Sand, Ultrapure water

Generated FPS (>5 μm)
× 12 hours ▲ 1 day ■ 2 days ◆ 3 days

12 hours
1 day
2 days
3 days

FPS particles larger than 100 μm were generated over 1 day.

■ Transport of degradable biopolymers in Seto Inland Sea

➤ Governing Equation:
$$\frac{\partial C}{\partial t} + \frac{\partial UC}{\partial x} + \frac{\partial VC}{\partial y} + \frac{\partial (W+W_s)C}{\partial z} = \frac{\partial}{\partial x}\left(A_H \frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(A_H \frac{\partial C}{\partial y}\right) + \frac{\partial}{\partial z}\left(K_H \frac{\partial C}{\partial z}\right) + S$$

➤ Degradation term: $S = -\gamma_w C, \quad S_{sed} = -\gamma_{sed} C$

No.	Cases	γ_w (/day)	γ_{sed} (/day)	W_s (m/s)
1	CERI (γ_{w1}, γ_{sed1})	7.352×10^{-4}	4.818×10^{-3}	$10^{-1} \sim -10^{-1}$
2	γ_{w2}[1], $\gamma_{sed2} = \gamma_{w2}$	6.187×10^{-5}	6.187×10^{-5}	$10^{-1} \sim -10^{-1}$
3	$\gamma_{w3} = \gamma_{sed3} = (\gamma_{w1}+\gamma_{w2})/2$	3.985×10^{-4}	3.985×10^{-4}	$10^{-1} \sim -10^{-1}$
4	$\gamma_{w4} = \gamma_{w3}, \quad \gamma_{sed4} = (\gamma_{sed1}+\gamma_{sed2})/2$	3.985×10^{-4}	2.44×10^{-3}	$10^{-1} \sim -10^{-1}$

Note: [1] Sudhakar, et al., 2007

—No.1 —No.2 —No.3 —No.4

Ratio of mass in the seawater to the total river input

Ratio of mass in the sediment to the total river input

Settling velocity (m/s)

■ MP sinking process model

Reproduction of secular changes in MP sedimentation flux in Beppu Bay (one-dimensional MP sedimentation model)

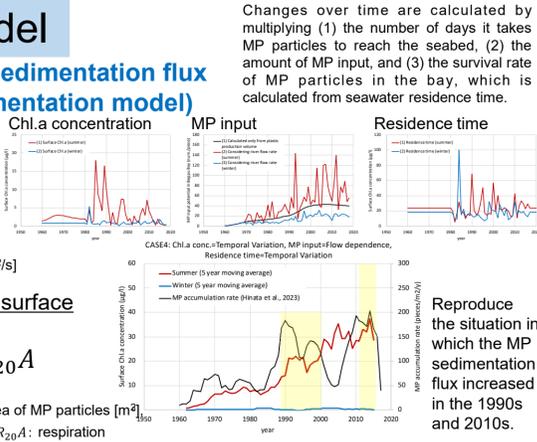① Basic equation: settling velocity of MP particles
$$V_s(z,t) = -\left(\frac{\rho_{tot} - \rho_{sw,z}}{\rho_{sw,z}} g \omega_* \nu_{sw,z}\right)^{1/3}$$

V_s: Settling velocity [m/s], z: Depth [m], ρ_{tot}: Total particle density [kg/m³], $\rho_{sw,z}$: Water density [kg/m³], g: Gravitational acceleration [m/s²], ω_*: Dimensionless settling velocity[-], $\nu_{sw,z}$: Kinematic viscosity coefficient [m²/s]
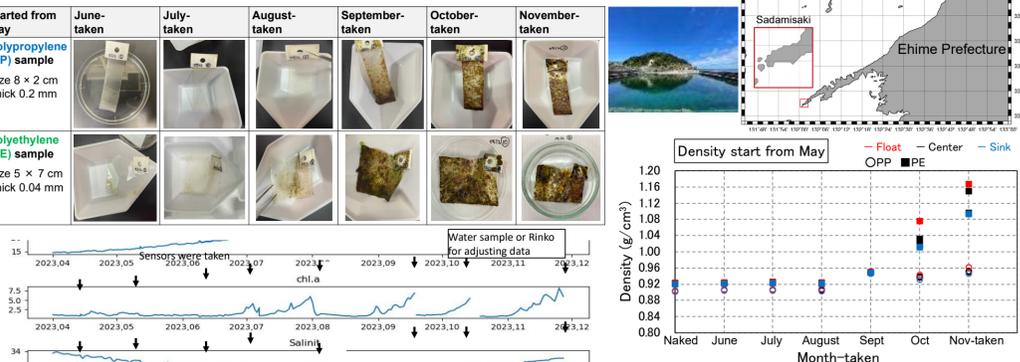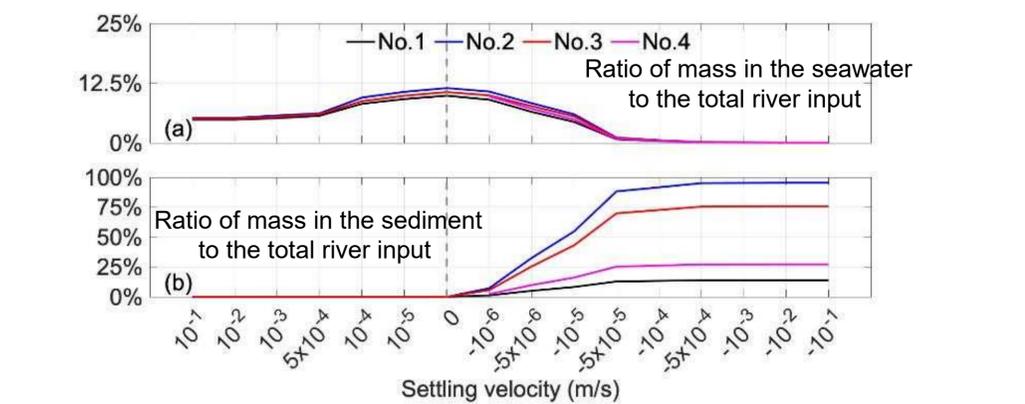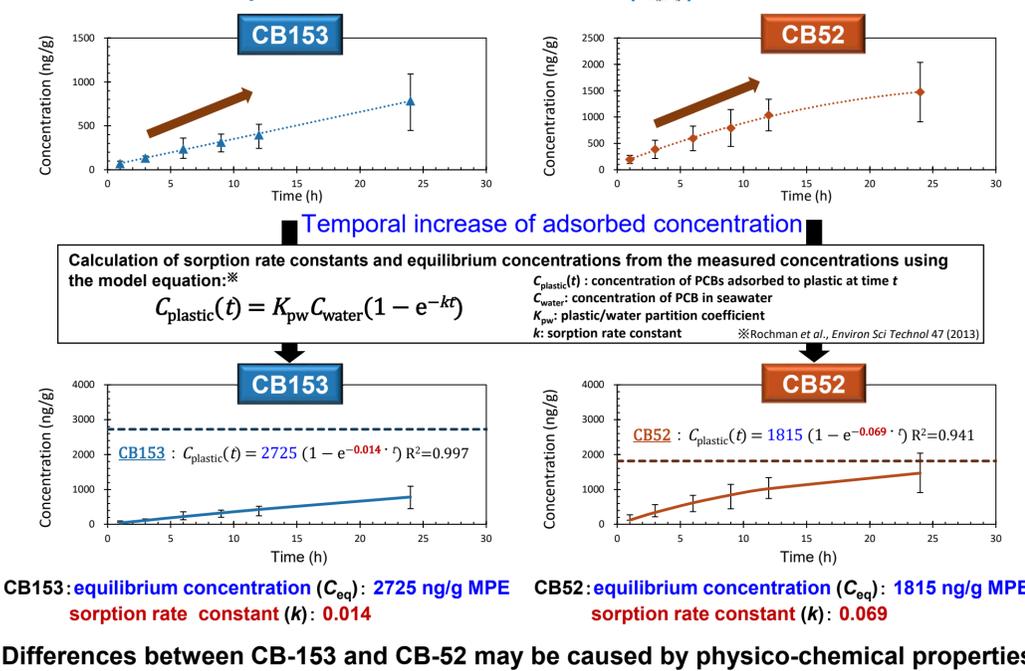
Chl.a concentration   MP input   Residence time

CASE4: Chl.a conc.=Temporal Variation, MP input=Flow dependence, Residence time=Temporal Variation

② Time-varying equation of biofilm on MP particle surface
$$\frac{dA}{dt} = \frac{A_A \beta_A}{\theta_{pl}} + \mu_A A - \overset{\text{(not considered)}}{m_A A} - Q_{10}^{(T-20)/10} R_{20} A$$

A_A: Algae concentration [m⁻³], β_A: Encounter rate [m³/s], θ_{pl}: Surface area of MP particles [m²], μ_A: Growth of attached algae [s⁻¹], m_A: Falling off/withering [s⁻¹], $Q_{10}^{(T-20)/10} R_{20} A$: respiration

Reproduce the situation in which the MP sedimentation flux increased in the 1990s and 2010s.

Plastic incubation at Sadamisaki for analyzing biofilm growth

Started from May
June-taken July-taken August-taken September-taken October-taken November-taken
Polypropylene (PP) sample
Size 8 × 2 cm
Thick 0.2 mm
Polyethylene (PE) sample
Size 5 × 7 cm
Thick 0.04 mm

Sadamisaki
Ehime Prefecture

Density start from May
—Float —Center —Sink
●OPP ■PE

■ A 3D numerical model for the POPs and its interactions with planktons and polymers

Evaluation of sorption parameters of hexa-chloro CB-153 from sea-water to MicroPE, and comparison with tetra-chloro CB-52

CB153   CB52

Temporal increase of adsorbed concentration

Calculation of sorption rate constants and equilibrium concentrations from the measured concentrations using the model equation:※
$$C_{plastic}(t) = K_{pw} C_{water}(1 - e^{-kt})$$

$C_{plastic}(t)$: concentration of PCBs adsorbed to plastic at time t
C_{water}: concentration of PCB in seawater
K_{pw}: plastic/water partition coefficient
k: sorption rate constant
※Rochman et al., Environ Sci Technol 47 (2013)

CB153   CB52
CB153 : $C_{plastic}(t) = 2725 (1 - e^{-0.014 \cdot t})$ R²=0.997
CB52 : $C_{plastic}(t) = 1815 (1 - e^{-0.069 \cdot t})$ R²=0.941

CB153: equilibrium concentration (C_{eq}): **2725 ng/g MPE** sorption rate constant (k): **0.014**
CB52: equilibrium concentration (C_{eq}): **1815 ng/g MPE** sorption rate constant (k): **0.069**

Differences between CB-153 and CB-52 may be caused by physico-chemical properties

番号： A-15-15E
PJ：Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses
Theme：Development of accelerated evaluation of biodegradability in marine
Organization: Chemicals Evaluation and Research Institute, Japan
Contact: Takako KIKUCHI

NEDO
CERi
MOONSHOT

1. Background

◆ International Standard about determination of aerobic biodegradation by ISO
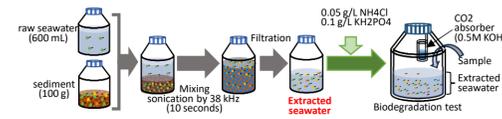(Each method is s simulation under laboratory conditions of each habitat found)
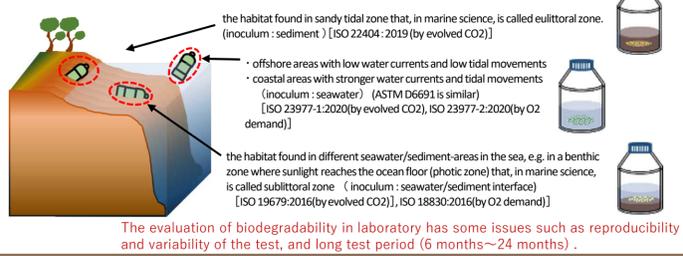
✓ For the widespread adoption and societal implementation of marine biodegradable plastics, reliable evaluation methods of biodegradability are essential.
✓ Clarifying the correlation between lab and field tests and providing feedback for material development accelerates material innovation.

the habitat found in sandy tidal zone that, in marine science, is called eulittoral zone.
(inoculum : sediment）[ISO 22404: 2019 (by evolved CO2)]

• offshore areas with low water currents and low tidal movements
• coastal areas with stronger water currents and tidal movements
(inoculum : seawater) (ASTM D6691 is similar)
[ISO 23977-1:2020(by evolved CO2), ISO 23977-2:2020(by O2 demand)]

the habitat found in different seawater/sediment-areas in the sea, e.g. in a benthic zone where sunlight reaches the ocean floor (photic zone) that, in marine science, is called sublittoral zone（ inoculum : seawater/sediment interface）
[ISO 19679:2016(by evolved CO2)], ISO 18830:2016(by O2 demand)]

The evaluation of biodegradability in laboratory has some issues such as reproducibility and variability of the test, and long test period (6 months~24 months) .

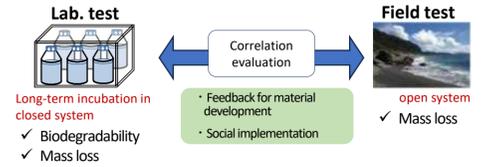**(1) Development of the accelerated test method for marine biodegradability**

Validation of the accelerated testing method

Outline of development method

(1) Increasing number and diversity of bacteria (2) Increasing number and diversity of bacteria

Simple operation and quick preparation

raw seawater (600 mL)
sediment (100 g)
Mixing sonication by 38 kHz (10 seconds)
Filtration
Extracted seawater
0.05 g/L NH4Cl 0.1 g/L KH2PO4
CO2 absorber (0.5M KOH)
Sample
Extracted seawater
Biodegradation test

(2) Comparative verification of biodegradation rates in the field and in the laboratory

Verification of field tests and comparison of the developed accelerated test methods (laboratory) with field tests

Lab. test
Correlation evaluation
Field test

Long-term incubation in closed system
✓ Biodegradability
✓ Mass loss

Feedback for material development
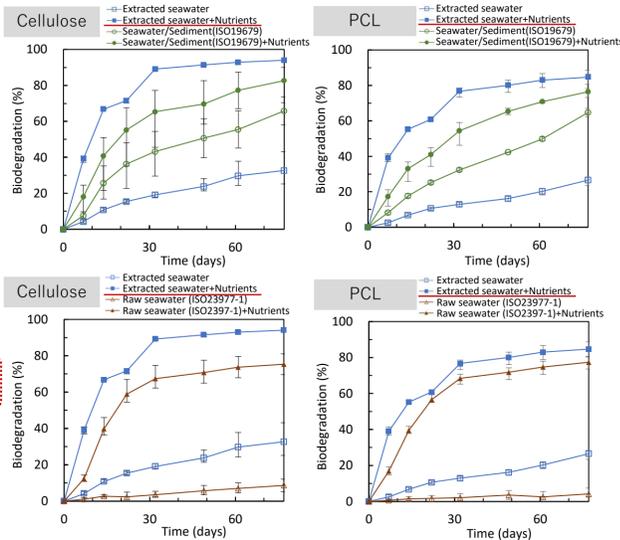Social implementation

open system
✓ Mass loss

2. Development of acceleration test method

◆ Evaluation of degradation rates of positive control (cellulose) and biodegradable polymers by the developed accelerated test method, ISO 19679 (seawater/sediment interface) and ISO 23997-1 (seawater)

◆ Test condition   Inoculum：Hayama port, Kanagawa

	Inoculum	Nutrients
ISO 19679 1	Seawater/Sediment (ISO19679)	—
2	Seawater/Sediment (ISO19679)	0.05 g/L NH4Cl 0.1 g/L KH2PO4
ISO 23977-1 3	Raw seawater (ISO23977-1)	—
4	Raw seawater (ISO2397-1)	0.05 g/L NH4Cl 0.1 g/L KH2PO4
Extracted seawater 5	Extracted seawater	—
6	Extracted seawater	0.05 g/L NH4Cl 0.1 g/L KH2PO4

Accelerated test method

◆ Sample （particle size 250～125 μm）
· Cellulose [positive control]
· Polycaprolactone (PCL)

Cellulose
- Extracted seawater
- Extracted seawater+Nutrients
- Seawater/Sediment(ISO19679)
- Seawater/Sediment(ISO19679)+Nutrients

PCL
- Extracted seawater
- Extracted seawater+Nutrients
- Seawater/Sediment(ISO19679)
- Seawater/Sediment(ISO19679)+Nutrients

Cellulose
- Extracted seawater
- Extracted seawater+Nutrients
- Raw seawater (ISO23977-1)
- Raw seawater (ISO23977-1)+Nutrients

PCL
- Extracted seawater
- Extracted seawater+Nutrients
- Raw seawater (ISO23977-1)
- Raw seawater (ISO23977-1)+Nutrients

◆ Validation of the accelerated test method
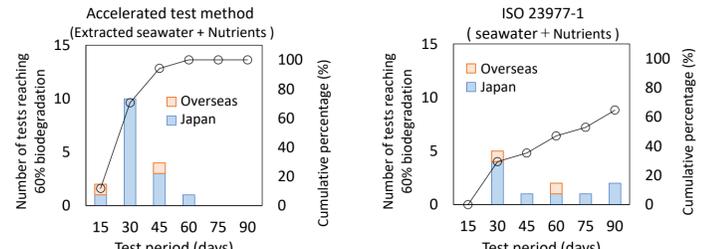
Inoculum collection location
（1） 15 marine areas in Japan
（2）Overseas
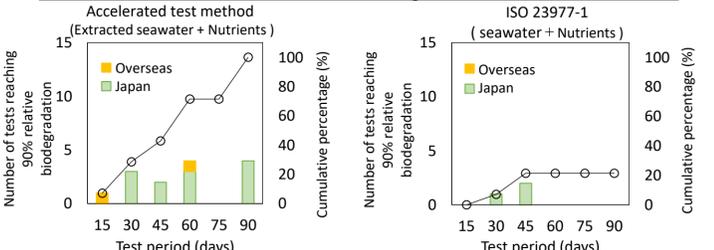（English Channel）（Gulf of Thailand）

Validity of tests in ISO
After 180 days (at the end of the test?), the biodegradability of the cellulose in the control material must be at least 60%

Requirements for marine biodegradable plastics in ISO 22403
The biodegradability of the test material within 2 years must be at least 90%, or at least 90% relative to the positive control material.

Number of tests where the biodegradation of cellulose reached 60%

Accelerated test method (Extracted seawater + Nutrients)
ISO 23977-1 (seawater + Nutrients)
Overseas / Japan

Number of tests where the relative biodegradation of PCL reached 90%

Accelerated test method (Extracted seawater + Nutrients)
ISO 23977-1 (seawater + Nutrients)
Overseas / Japan

In all areas tested, the accelerated test method was found to be effective in assessing the marine biodegradability potential of the materials.
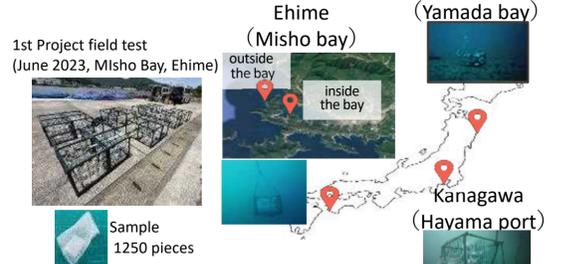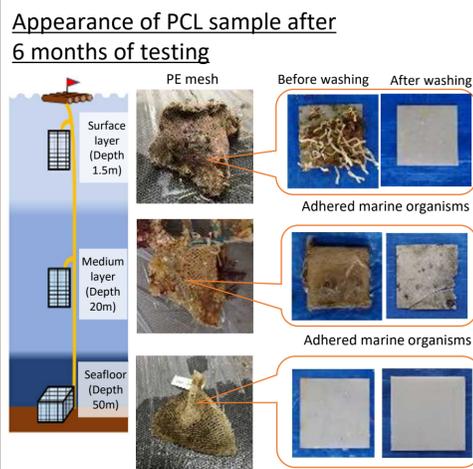
3. Field test

◆ Test procedure
Sample
· Polycaprolactone (PCL)
· Polyethylene (PE)

PE mesh bag for samples (2 mm gap)
Fixed to test jig

◆ Evaluation
· Visual observation of samples after exposure
· Degradation rate by mass loss
· 16S rRNA analysis of bacterial flora [biofilm on seawater, sediment, samples].
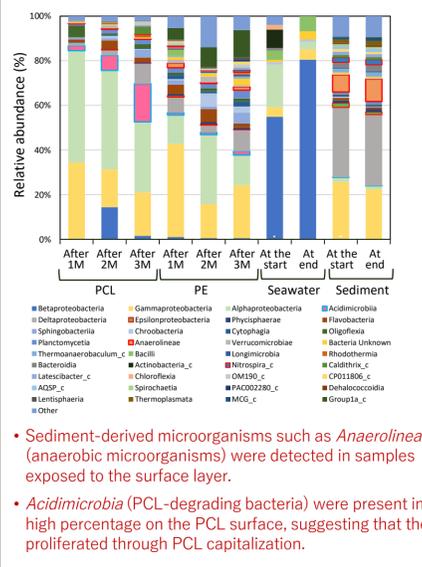
◆ Test location
1st Project field test (June 2023, MIsho Bay, Ehime)
Iwate（Yamada bay）
（Ehime（Misho bay）
outside the bay / inside the bay
Kanagawa（Hayama port）
Sample 1250 pieces

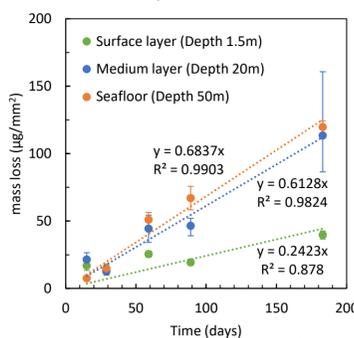|	Kanagawa Hayama bay	Iwate Yamada bay	Ehime Misho bay inside the bay	Ehime Misho bay outside the bay
Test period	2020/11 ~ 2021/2	2022/2 ~ 2022/6	2020/12 ~ 2021/3	2023/6 ~ 2023/12
Depth (m)	10	10	1.5 45	1.5 20 50
Number of microorganisms (count/mL)	1.5×10⁴	2.6×10⁴	5.1×10²	1.6×10³	1.6×10³
Total Nitrogen (μmol/L)	140	190	18	14	13
Total phosphorus (μmol/L)	4.5	4.2	4.8	1.0	1.0
Water temp. (°C) (Min-Max)	17 (14-21)	18 (15-23)	9.4 (7-14)	26 (20-28)	22 (20-25)

◆ Field test in Misho Bay (outside the bay), Ehime

Appearance of PCL sample after 6 months of testing

Surface layer (Depth 1.5m)
Medium layer (Depth 20m)
Seafloor (Depth 50m)

PE mesh
Before washing   After washing
Adhered marine organisms

Mass loss per unit area of PCL
- Surface layer (Depth 1.5m)
- Medium layer (Depth 20m)
- Seafloor (Depth 50m)

$y = 0.6837x$
$R^2 = 0.9903$
$y = 0.6128x$
$R^2 = 0.9824$
$y = 0.2423x$
$R^2 = 0.878$

• Large amounts of marine organisms adhere to the surface and middle layers
• Degradation proceeds at a constant rate on the seafloor

Bacterial flora of biofilm on PCL and PE, seawater, and sediment at the class level

After 1M / After 2M / After 3M (PCL)
After 1M / After 2M / After 3M (PE)
At the start / At end (Seawater)
At the start / At end (Sediment)

Betaproteobacteria, Deltaproteobacteria, Sphingobacteria, Planctomycetia, Thermoanaerobacum_c, Bacilli, Latescibacter_c, AQSP_c, Lentisphaeria, Other
Gammaproteobacteria, Epsilonproteobacteria, Chroobacteria, Anaerolineae, Actinobacteria_c, Chloroflexia, Spirochaetia, Thermoplasmata
Alphaproteobacteria, Phycisphaerae, Cytophagia, Verrucomicrobiae, Longimicrobia, Nitrospira_c, OM190_c, PAC002280_c, MCG_c
Acidimicrobia, Flavobacteria, Oligoflexia, Bacteria Unknown, Rhodothermia, Caldilhrix_c, CP01806_c, Dehalococcoidia, Group1a_c
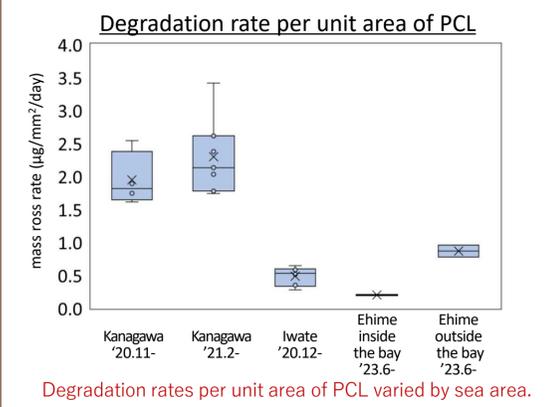
• Sediment-derived microorganisms such as Anaerolineae (anaerobic microorganisms) were detected in samples exposed to the surface layer.
• Acidimicrobia (PCL-degrading bacteria) were present in a high percentage on the PCL surface, suggesting that they proliferated through PCL capitalization.

In the lab/field Biodegradation rate of PCL
- Mass loss rate in the lab
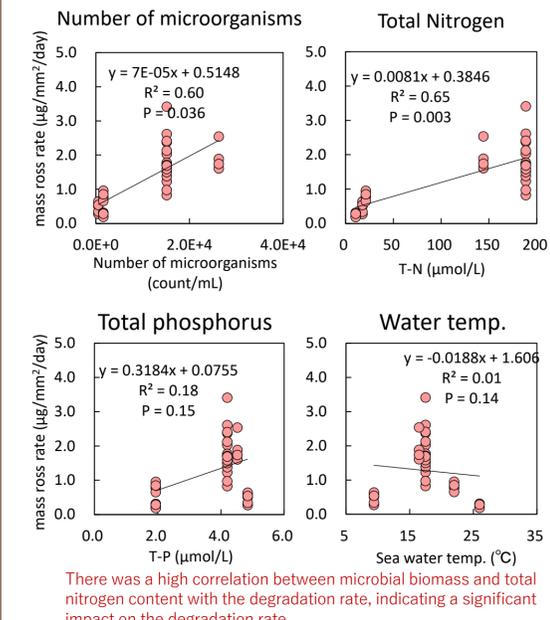- Mass loss rate in the field
- Biodegradability in the lab
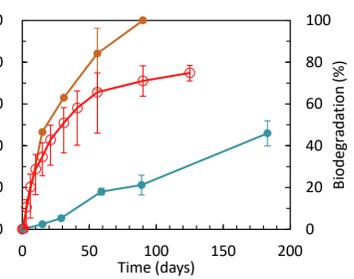
• In lab tests, there was a divergence between the mass reduction rate and biodegradability, with the mass reduction rate being higher. This result is thought to be due to weight loss from the samples' fragmentation and partial conversion into biomass.
• In the closed system of the lab test, the degradation activity decreased over time.
• In the field, the degradation activity remained stable, and the degradation rate was almost constant over 6 months.
• The microbial activity and its temporal changes differed between the lab and field.

◆ Degradation rates and influencing factors in five ocean regions

Degradation rate per unit area of PCL

Kanagawa '20.11- / Kanagawa '21.2- / Iwate '20.12- / Ehime inside the bay '23.6- / Ehime outside the bay '23.6-

Degradation rates per unit area of PCL varied by sea area.

Effects of four environmental factors on PCL degradation rates (excluding marine flora)

Number of microorganisms
$y = 7E-05x + 0.5148$
$R^2 = 0.60$
$P = 0.036$

Total Nitrogen
$y = 0.0081x + 0.3846$
$R^2 = 0.65$
$P = 0.003$

Total phosphorus
$y = 0.3184x + 0.0755$
$R^2 = 0.18$
$P = 0.15$

Water temp.
$y = -0.0188x + 1.606$
$R^2 = 0.01$
$P = 0.14$

There was a high correlation between microbial biomass and total nitrogen content with the degradation rate, indicating a significant impact on the degradation rate

4. Conclusions

➢ The accelerated test method (extracted seawater + nutrients) in the laboratory developed was able to evaluate marine biodegradability more rapidly than the existing ISO 19679 and ISO 23977-1. Its effectiveness, confirmed using plants from 15 Japanese and 2 international sites (English Channel, Gulf of Thailand), showed reduced variability, faster degradation, and reliable short-term biodegradability assessment.

➢ The results of field tests in Misho Bay, Ehime (at a depth of 20 m) showed that microorganisms present in seafloor sediments were detected in PE exposed to the surface layer.

➢ The degradation rate of PCL in field tests differed with water depth and remained constant at seafloor with few marine organisms. Results from field tests in five marine areas showed that the degradation rate of PCL varied by area. Multiple regression analysis indicated that the number of marine microorganisms and nitrogen significantly affected the degradation rate in field tests.

➢ In lab tests, the degradation rate decreased over time, suggesting that microbial activity differs between the lab and field environments.

➢ In the future, enzyme activity and genetic analysis of the biodegradation process will be carried out.