

Development of Photo-Switching Ocean-Degradable Plastics with Edibility



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Implementing organizations :

Japan Advanced Institute of Science and Technology

Kobe University

Nagoya University

Kagoshima University

Tokyo University of Science

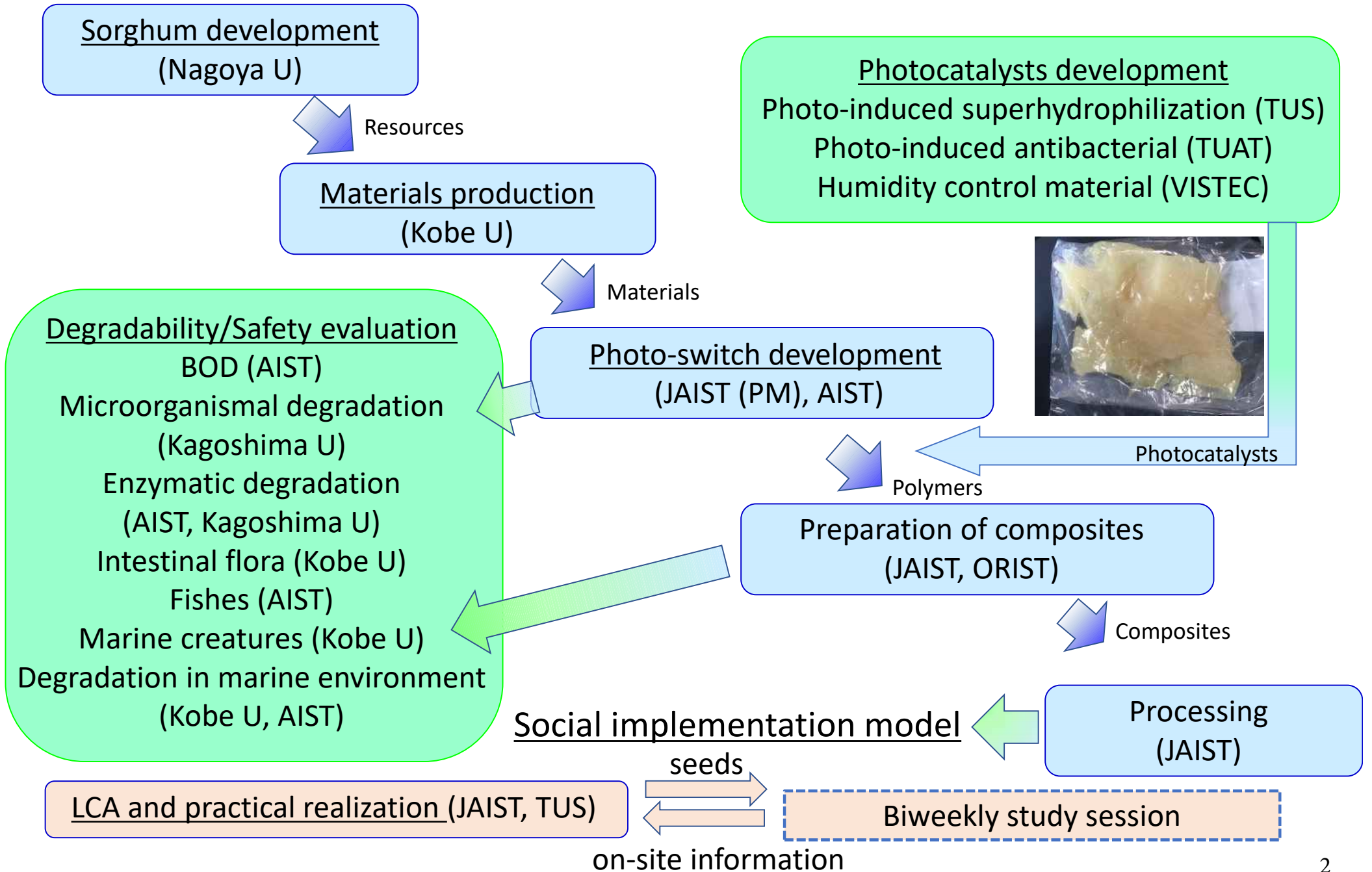
Tokyo University of Agriculture and Technology

National Institute of Advanced Industrial Science and Technology

Osaka Research Institute of Industrial Science and Technology

Research system

2020-2029



Research topics

PM management policy: Taking advantage of regional characteristics (90%: textile industry) and internationality, we manage flexibly and boldly through domestic and overseas industry-government-academia collaboration.

AIST

research item②-2 Composite of marine degradable plastic and antibacterial photocatalysts

research item③-2 Seawater biodegradation and safety evaluation by laboratory test: BOD test

research item④-1 Evaluation of degradability in digestive enzymes

research item④-3 Degradability and safety test for fishes

ORIST

research item②-3 Evaluation of antibacterial activity of OFF-type and ON/OFF-type biodegradable optical switch composites

TUS

research item①-2 New development of photocatalyst contributing to ON-type optical switch system

research item⑦-2 Strengthening and foster environment of business foundation

TUAT

research item②-1 Development of reduced antibacterial photocatalyst

JAIST

research item①-1 Development of various polymers by incorporation polymerization of nylon block from itaconic acid

research item①-3 Development of additive systems to control the degradation of biodegradable polymer with on-type switch

research item①-4 Molding process of ON-type photoswitching biodegradable plastics

research item⑦-1 LCA calculation

Kobe U

Research⑥ Fermentative production of itaconic acid from sorghum

Research③-1 Evaluation of degradability in the actual sea

Research④-2 Evaluation of degradability and safety in a simulated intestinal environment

Research④-4 Evaluation of the impact of plastic degradation products on marine ecosystems

Nagoya U

research item⑤-1 Development of cultivars with excellent root system for high biomass yield

Kagoshima U

research item④-5 Identification of nylon degrading enzyme and in vitro degradation evaluation

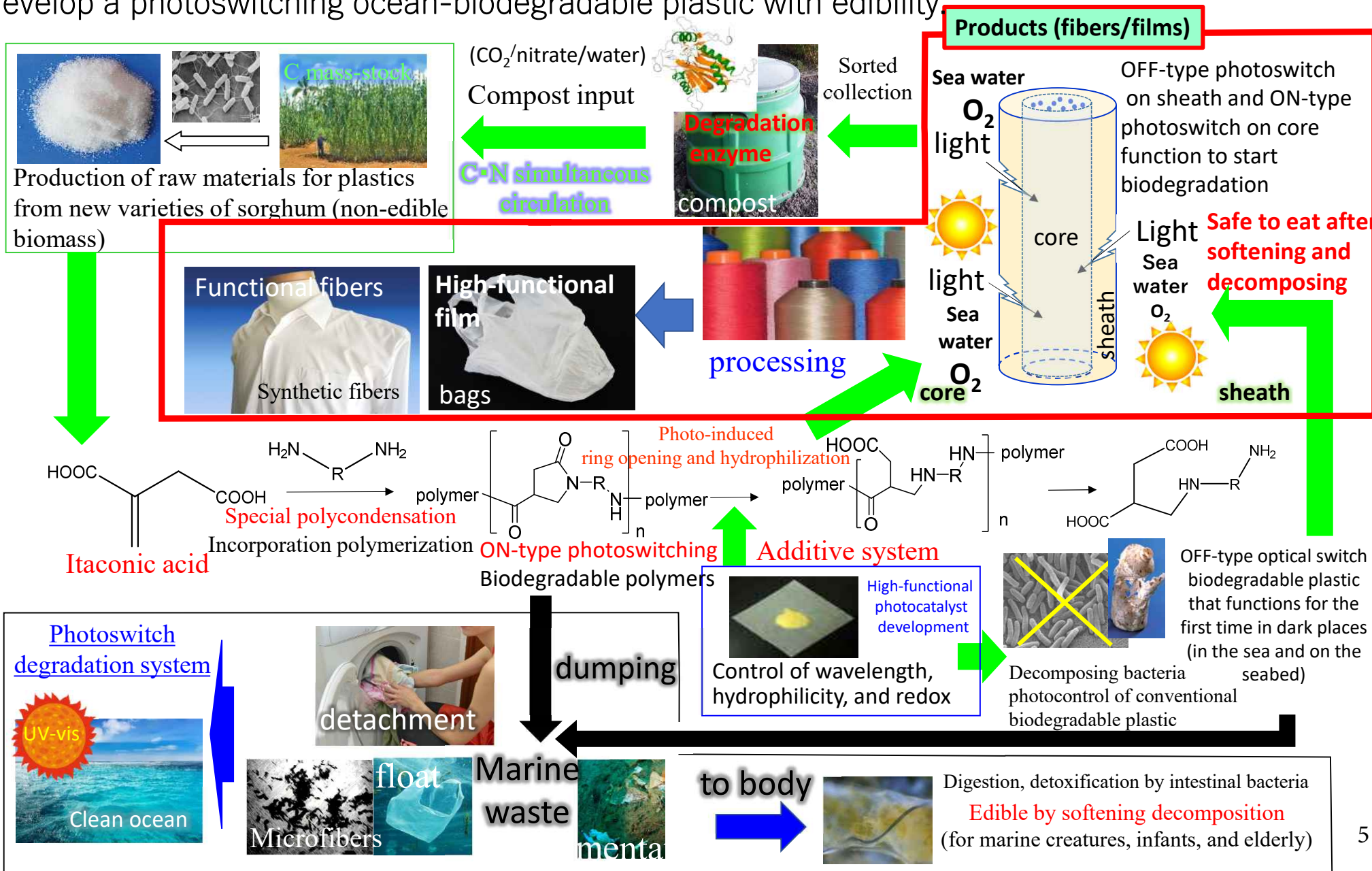
research item④-6 Composting using nylon-degrading enzyme

Schedule

Research items		2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	
Research item① Fabrication of ON-type photo-switching biodegradable plastics	①-1	ON-type block synthesis		ON-type block incorporation to polymers			Mass-production of ON-type polymers					
	①-2	Development of ON-type switch photocatalyst and establishment of synthesis method				Development of composite of ON-type switch photocatalyst and humidity control material						
	①-3	Deterioration proof of ON type switch			Stabilizer development/photocatalyst test			Completion of additive system				
	①-4	Rheological characterization		Fiberization/strengthening		Film formation/strengthening		Preparation of core-sheath fibers and multilayer films				
Research item② Development of OFF-type optical switch biodegradable plastic utilizing antibacterial function of photocatalyst	②-1	Photocatalyst syntheses			Photocatalyst modification and antibacterial activity evaluation			Photocatalyst composites				
	②-2	Establishment of switch performance evaluation method			Development and evaluation of OFF-type materials			Development and evaluation of ON/OFF type materials				
	②-3	Construction of antibacterial activity evaluation system			Optical switch performance (antibacterial) evaluation			Correlation evaluation with actual environment test				
Research item③ Evaluation of marine degradability of photoswitching biodegradable plastics	③-1	Evaluation of ON-type and OFF-type resins in actual ocean areas							Evaluation of actual ocean area with ON/OFF type			
	③-2	Stability confirmation of closed ring type ON type resin		Evaluation of OFF-type switching performance		BOD evaluation of ring-opening ON type resin		Evaluation of ON/OFF-type switching performance				
Research item④ Enzymatic degradation and safety evaluation of photoswitching biodegradable plastics	④-1	Degradability evaluation of ON/OFF-type switching resins			Analysis of decomposition mechanism		Impact assessment of polymer structure					
	④-2	Construction of a degradability evaluation system using a human intestinal microflora model			Construction of a safety evaluation system		Construction of a degradability evaluation system using intestinal flora model for a marine animal			Safety evaluation system construction		
	④-3	Evaluation of oral intake/excretion behavior of ON/OFF type resin				Evaluation of water-soluble resin (ring-opening ON type)		Evaluation of ON/OFF type				
	④-4	Evaluation of oligonylon 6i derivatives and OFF type resins				Evaluation of oligonylon 6i derivatives and OFF type		Evaluation of ON/OFF type				
	④-5	Microbial screening			Characterization and enzyme gene isolation			Optimization of digestion conditions and enhancement of enzyme functions				
	④-6	Examination of microbial function enhancement, molecular breeding, and composting						Compost system development		pilot test		
Research item⑤ Development of sorghum cultivars optimized as raw materials for photo-switching ocean degradable plastics	⑤-1	Genome-wide association analysis							Core collection trait assessment			
	⑤-1	Core collection trait assessment				QTL enrichment breeding						
Research item⑥ Fermentative production of biodegradable plastic raw materials from sorghum	⑥-1	Development of saccharification process and analysis of influence of fermentation inhibition					Development of saccharification process of designed sorghum					
	⑥-2	Construction of itaconic acid production models and strains				Development of OFF-type plastic raw material-producing bacteria						
	⑥-3	Development of hypoxia-compatible producing bacteria					Material assets from sorghum biomass through low-carbon processes					
Research item⑦ Strengthening of business foundation and management of optical switch type marine degradable plastic production	⑦-1	Clarification of LCA issues using laboratory data			Construction of mass production scale LCA calculation model			Calculation of global GHG reduction effect				
	⑦-2	Understanding the overview of the marine plastic problem			Application search and concept verification (PoC)			Application decision/PoC completion				
	⑦-2	Preparation for establishment of the study group			Promotion of study group activities and construction of a foundation for collaborative creation			Consortium formation				

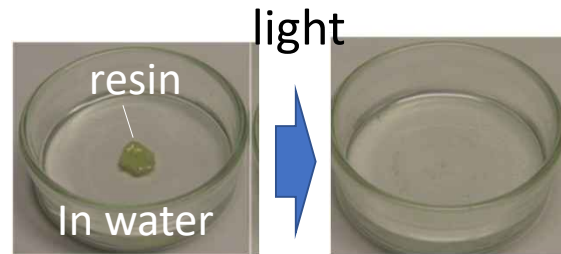
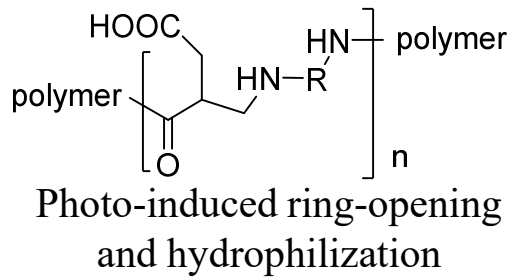
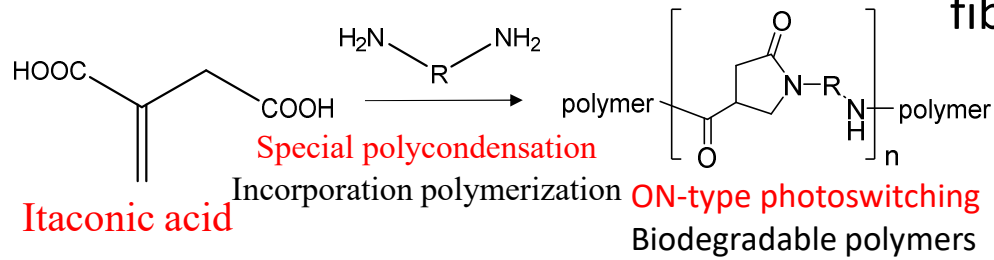
Final goal (2029) and Image of social implementation

【Final goal (2029)】 Using itaconic acid produced from a new cultivar of sorghum and a biodegradable polymer, a newly developed high-performance photocatalyst is composited to develop a photoswitching ocean-biodegradable plastic with edibility.



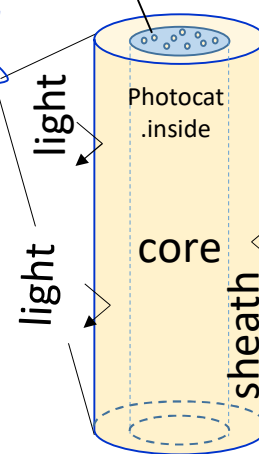
Research/content (ON-type and OFF-type photoswitch)

1. ON-type photoswitching ocean-biodegradable plastics



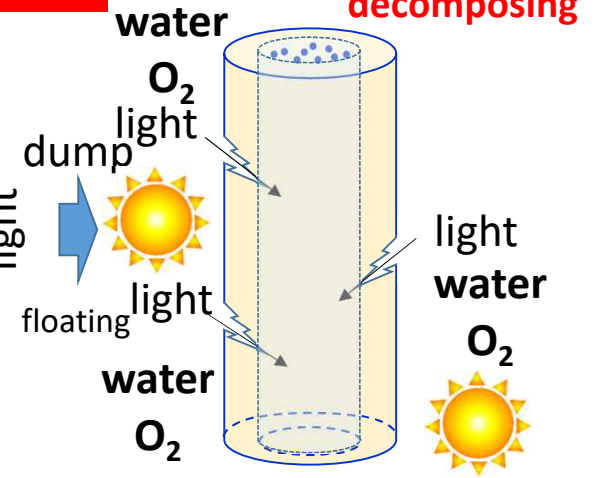
fibers

Photo-induced superhydrophilization



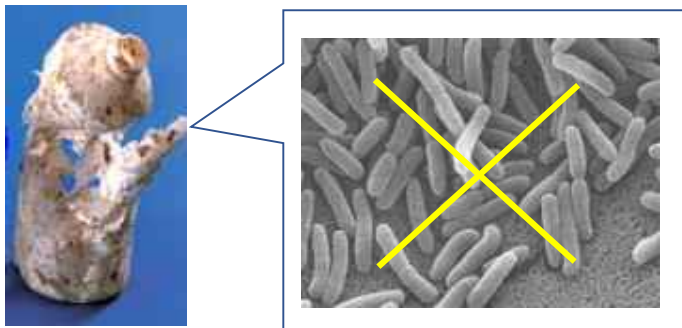
Short-wave visible light does not reach the core during use

Safe to eat after softening and decomposing



Photocatalyst in core functions due to physical destruction, and hydrophilization biodegradation starts from the damaged part.

2. OFF-type photoswitching ocean-biodegradable plastics

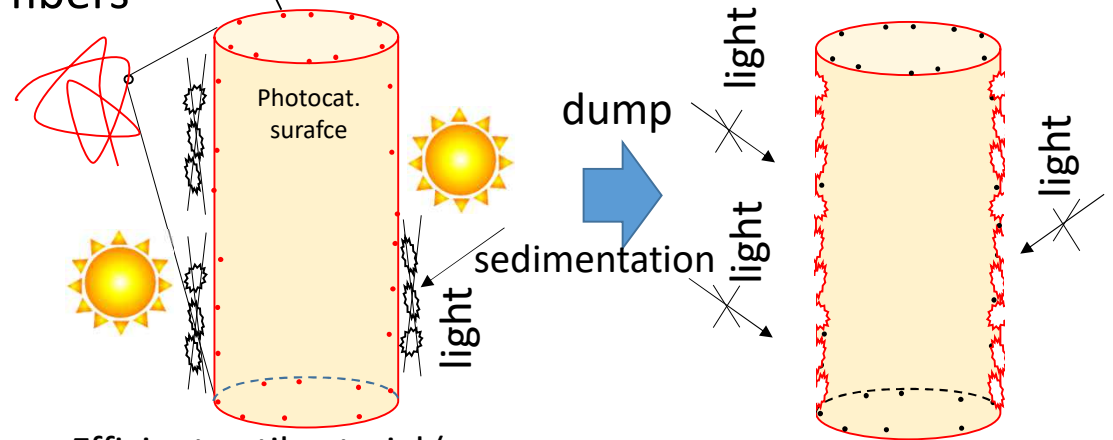


Photocontrol of degrading bacteria of conventional biodegradable plastic

Off-type photoswitch biodegradable plastics that suppress biodegradation by exposure to light in living spaces and functions for the first time in dark places (under the sea and on the seabed)

Photo-induced antibacteria

fibers

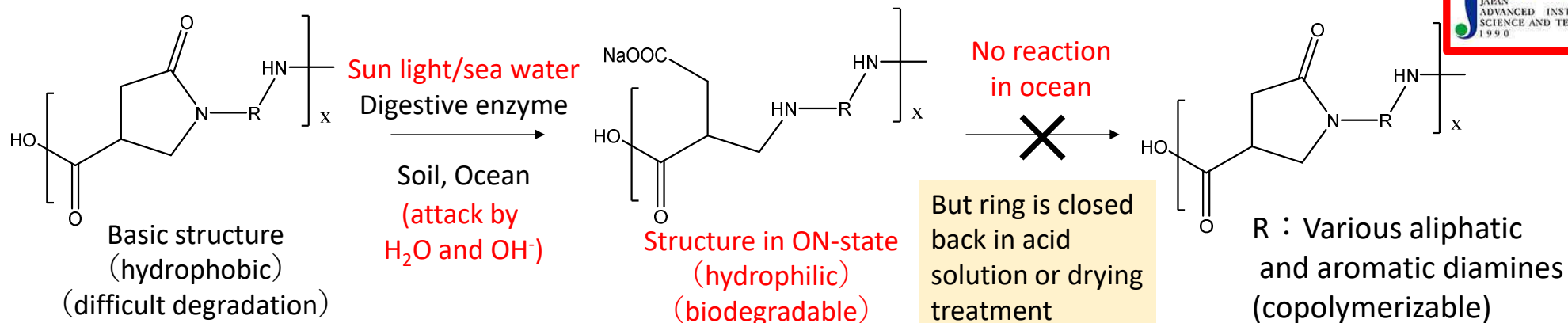


Efficient antibacterial (non-decomposing) over a wide wavelength range during use

Photocatalyst does not work in the dark and biodegradation starts ⁶

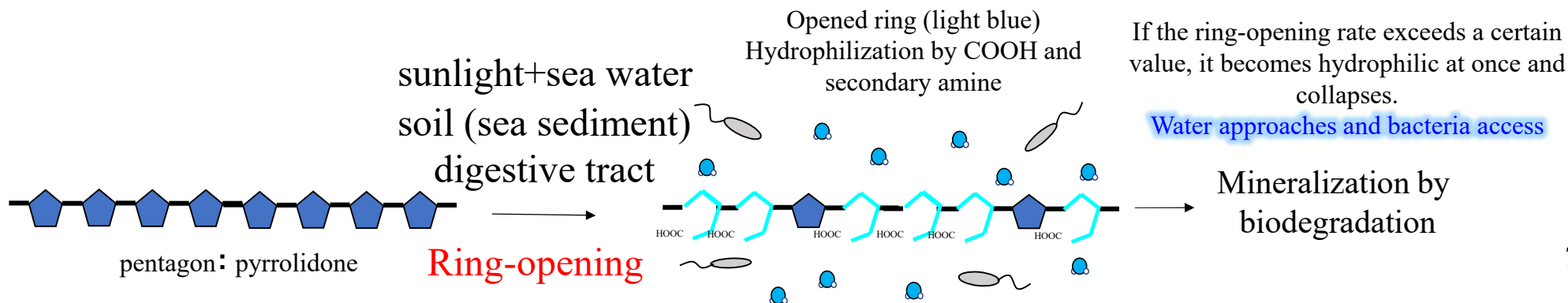
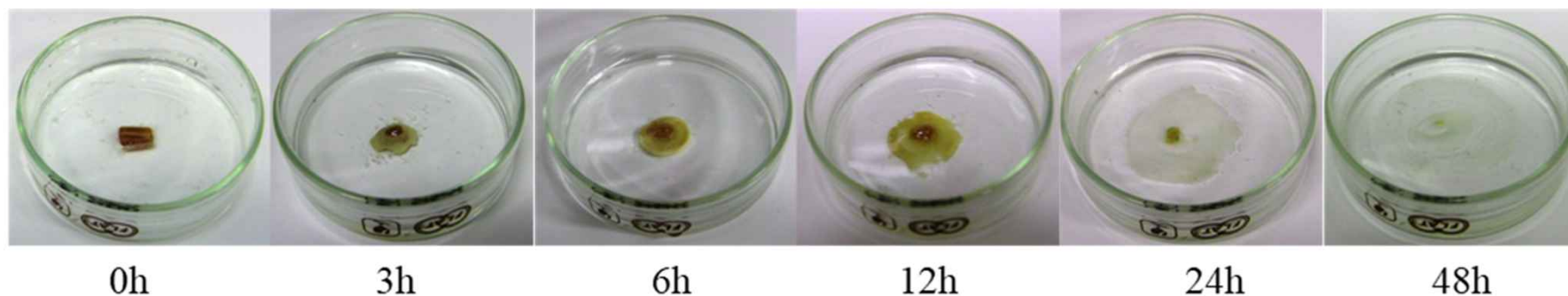
3. ON/OFF-type photoswitching ocean-biodegradable plastics

Features of bio-nylon equipped with ON-type photoswitch

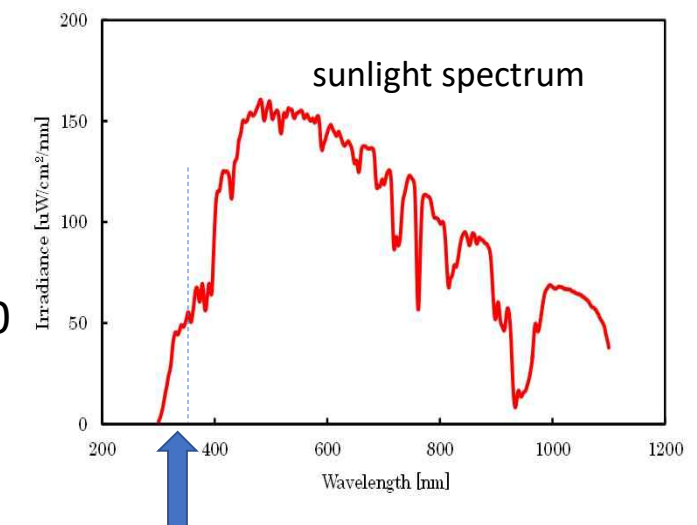
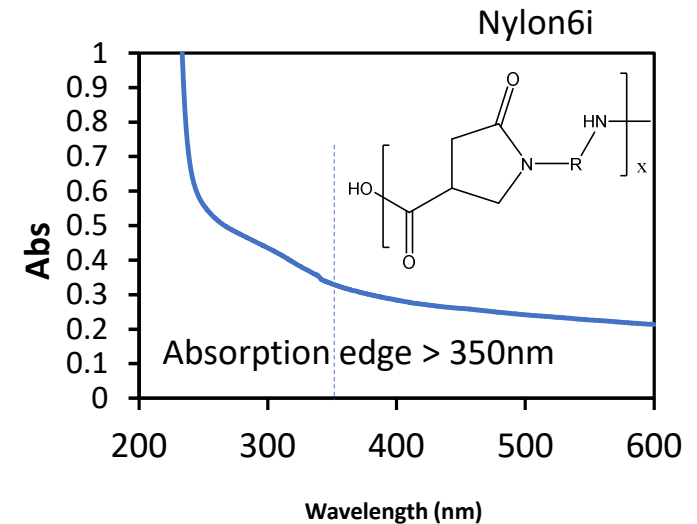
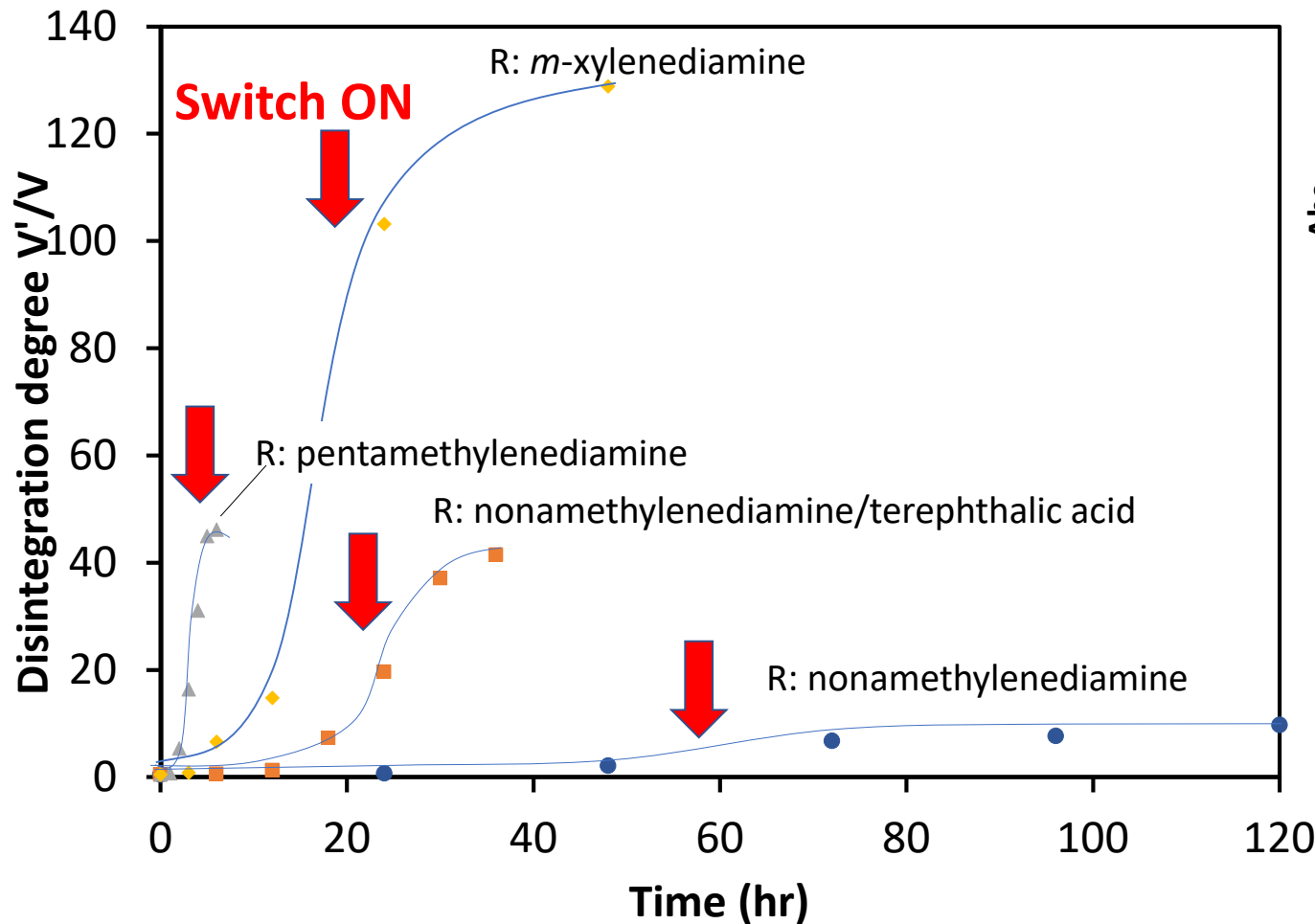


State of decay by light and water stimulation (carbonyl excitation, action of OH⁻, etc.)

R: *m*-xylenediamine



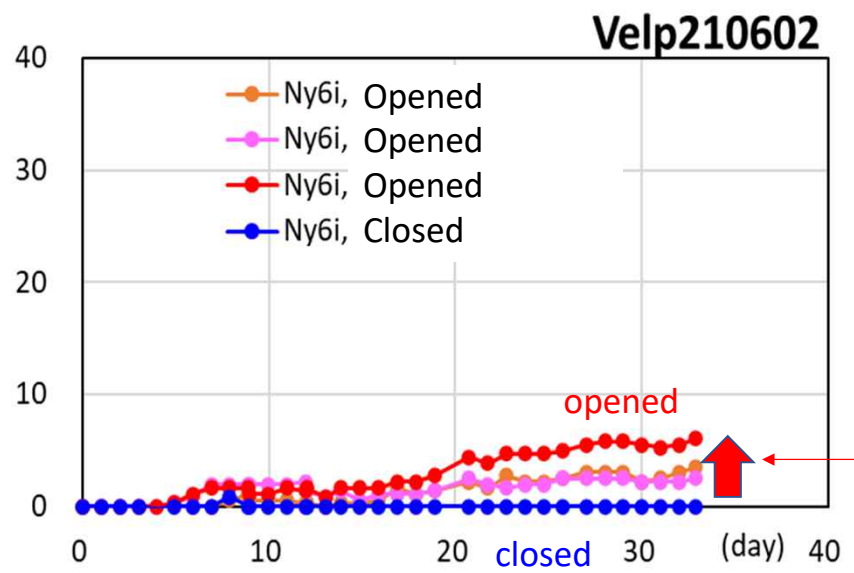
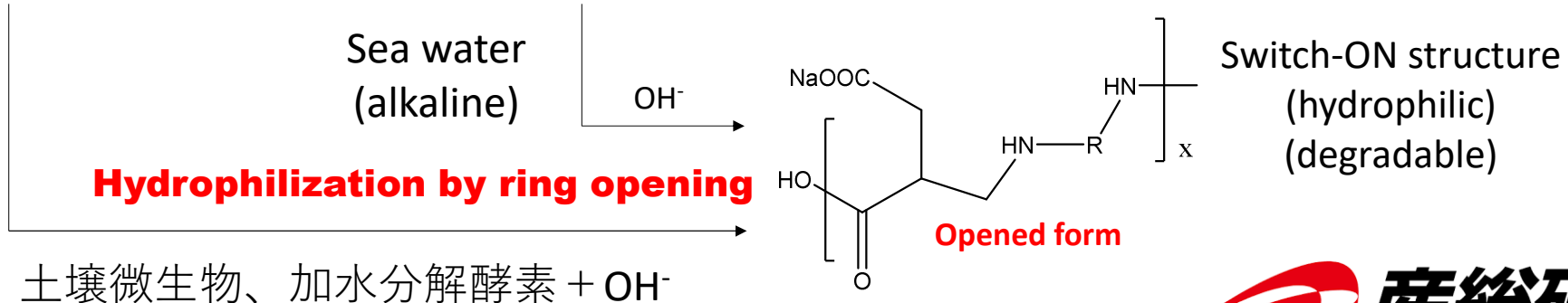
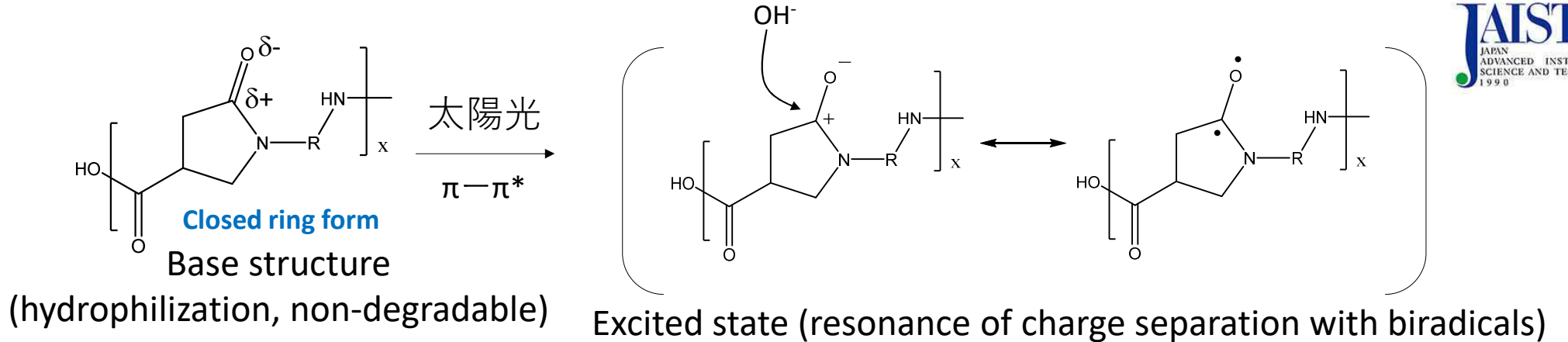
High-pressure Hg-lamp (wavelength: 250–450 nm, intensity 150 mW/cm²)
 (One hour of irradiation is equivalent to one month of sunlight irradiation)



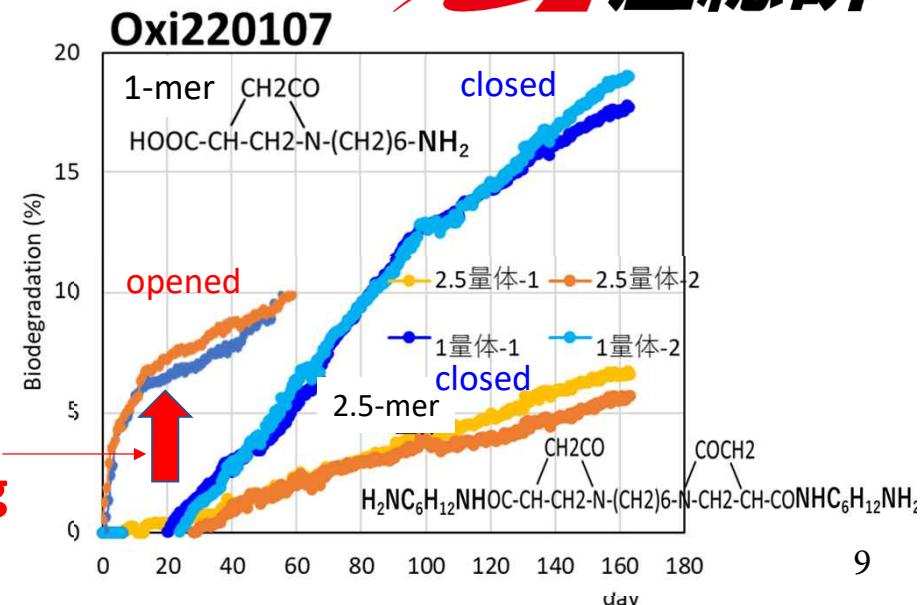
- The longer the fatty chain (more hydrophobic), the slower the decay rate
- When aromatics are added, the decay speed is accelerated (light absorption is important)

spectral overlap

ON-type photoswitch mechanism and BOD results (Research items ①-1、③-2)



Effects of ring-opening

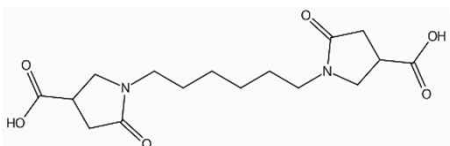


Predicted No-observed Effect Concentrations (PNECs) of water-soluble degradation products derived from ON-type resins were calculated from the acute toxicity values* on aquatic species of Table 1, divided with assessment factor 1,000.

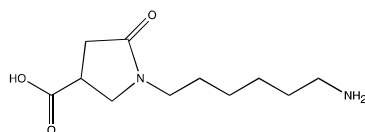
- Closed-ring dicarboxylic acid type 1.5-mer: 370 µg/l
- Closed-ring amino acid type monomer: 3,800 µg/l
- Open-ring amino acid type monomer: 4,400 µg/l

If the products are present in the aquatic environments at concentrations higher than the PNEC above, they are judged to be ecotoxic.

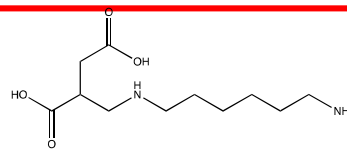
*Evaluated based on the Environmental Risk Assessment Law for Chemical Substances of the Ministry of the Environment, Japan.



Closed ring dicarboxylic acid



Closed ring amino acid



Open ring amino acid

Table 1 Acute toxicity of degradation products from bionylon on aquatic (EC50, LC50 in mg/l, initial pH adjusted)

test organisms	Closed ring		Open ring
	Dicarboxylic type 1.5 dimer	Amino acid type monomer*	amino acid type monomer*
Marine luminescent bacteria	> 1,000	>10,000	>10,000
Marine microalgae	> 1,000	7,200	7,100
Brine shrimp	> 1,000	>10,000	>10,000
Marine rotifer	> 1,000	>10,000	>10,000
Freshwater microalgae	> 1,000	<u>3,800</u>	<u>4,400</u>
Freshwater crustacean	820	>10,000	7,600
Freshwater rotifer	<u>370</u>	>10,000	6,300

*including salt

Environmental risk =

$$\frac{PEC}{PNEC} > 1 \quad \text{positive}$$

$$\frac{PEC}{PNEC} < 1 \quad \text{negative}$$

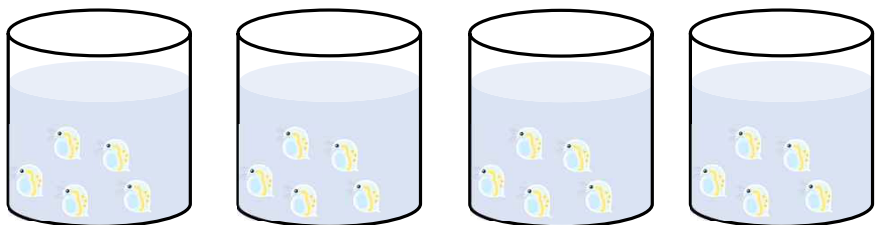
PEC: Predicted Environmental Concentration
PNEC: Predicted No-Effect Concentration

Daphnia magna NIES-R strain



Acute toxicity assay (base on OECD TG 202)

- 5 larvae/100-mL glass vessel, four replicates, (total of 20 larvae per treatment)
- Nominal concentration; Control, 7.5 mg/L
- Exposure period; 48 hours
- Endpoint; Swimming behavior, Survival rate

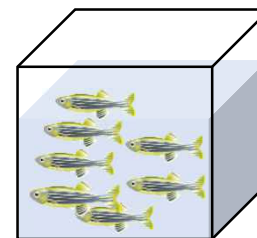


Danio rerio NIES-R strain



Acute toxicity assay (base on OECD TG 203)

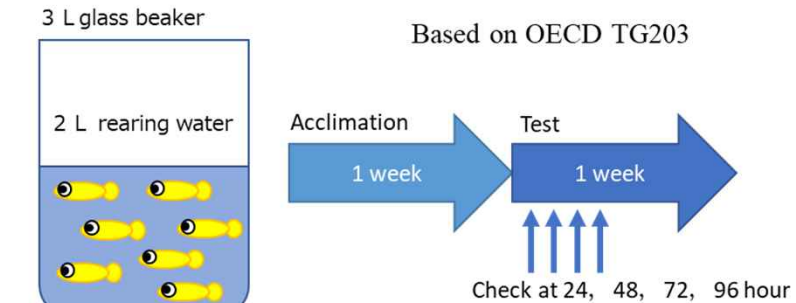
- 7 fishes/5-L aquarium tank
- Nominal concentration; Control, Exposure group
- Exposure period; 96 hours
- Exposure; 7mg/one time, two times per day
- Endpoint; Survival rate



Outcome

1. Particle size analysis of pulverized material of existing plastic
2. Established fluorescent dyeing method for existing plastic powder
3. Establishment of method for efficient oral intake of plastic powder
4. Imaging of pharmacokinetics of ingested plastic powder
PS, Ny6-L, Ny6i(0.5%TiO₂), Ny6i(1%TiO₂), Ny6i (1.5 mer), Ny6i-11
5. Acute toxicity evaluation of Ny6i-11 containing NaNbO₃
➡ No toxicity was observed
6. Gene expression analysis when ON-type resin is orally ingested

Acute toxicity test by medaka

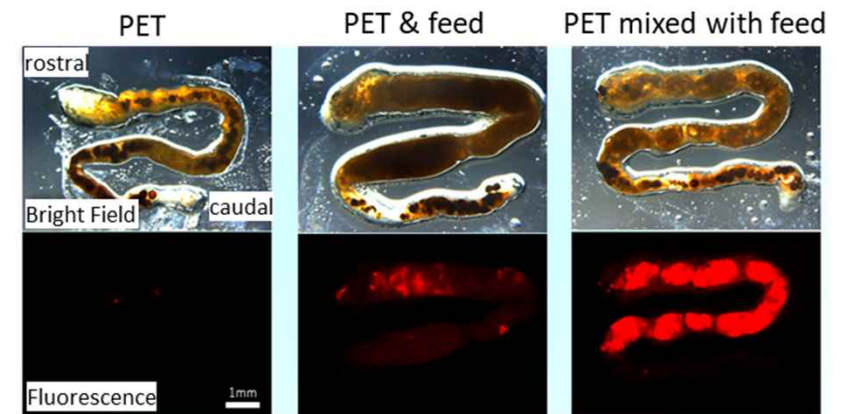
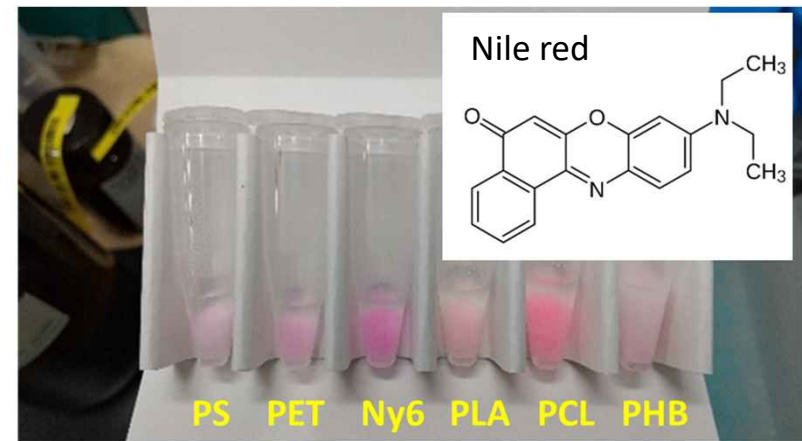


Appropriate feeding amount:
2~3% of Body weight (250mg)
⇔ 7.3mg/medaka/day → 360mg/week

Feed : Plastic = 360mg : 180mg
→ Plastic : 3.7mg/medaka/day

Plastic Types	Acute toxicity
Ny6	No
Ny6-L	No
Ny6i(0.5%TiO ₂)	No
Ny6i(1%TiO ₂)	No
Ny6i(1.5-mer)	No
Ny6i 75%	No
Ny6i 11 50%	No
Ny6i 11 50% CuI NaNbO ₃	No

Fluorescent staining and imaging



Construction of the colonic microbiota model of marine mammals (Research item ④-2)

The knowledge obtained from the human colonic microbiota model will be extended to marine mammals.



Pacific white-sided dolphin × 3

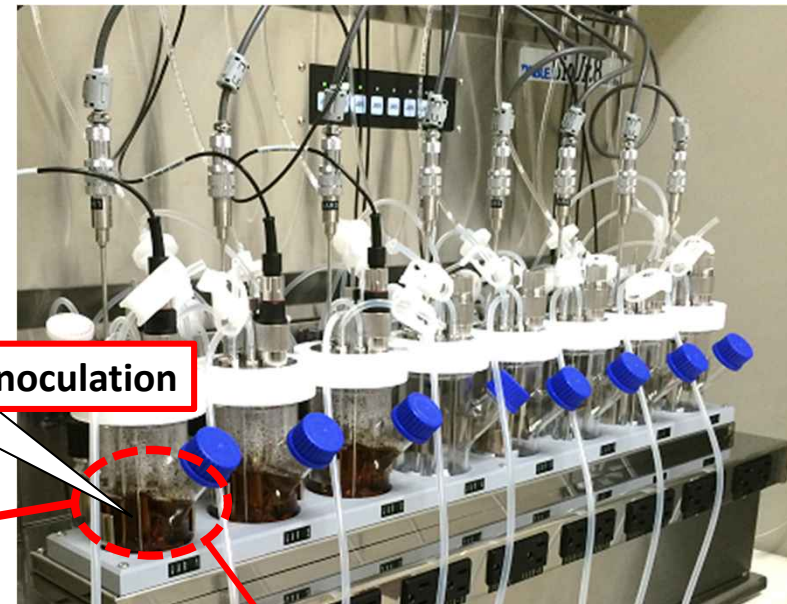


California sea lion × 3

Collection and inoculation of fecal samples

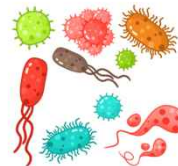


• The fecal samples can be stored at room temperature for 48 h while maintaining anaerobic conditions.



inoculation

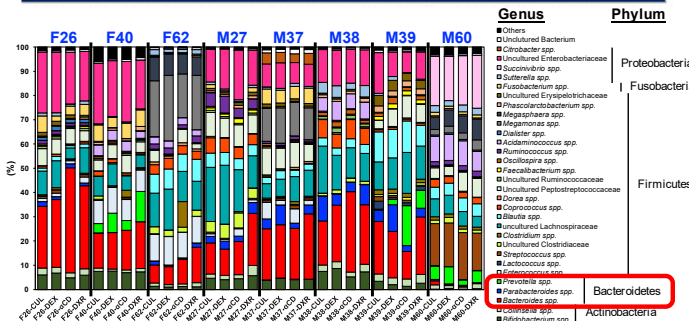
Bacterial DNA extraction



Microbiota analysis (Structure of colonic microbiota)

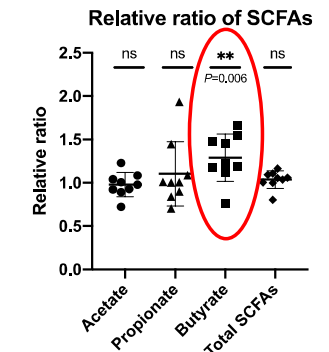


Genus level compositional view of individual 16S rRNA genes data.



Culture supernatant

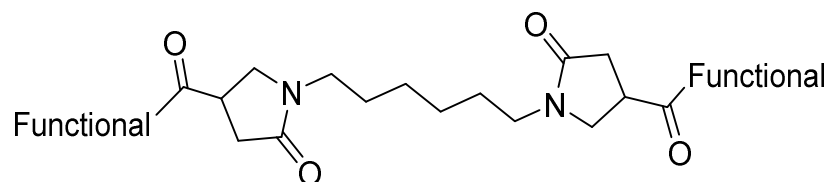
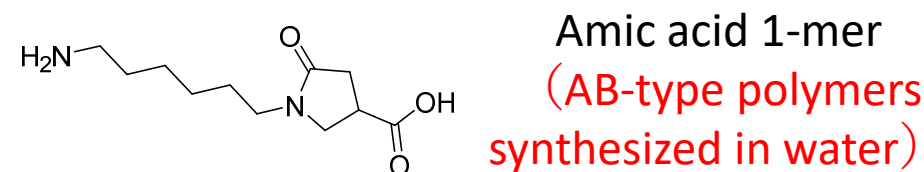
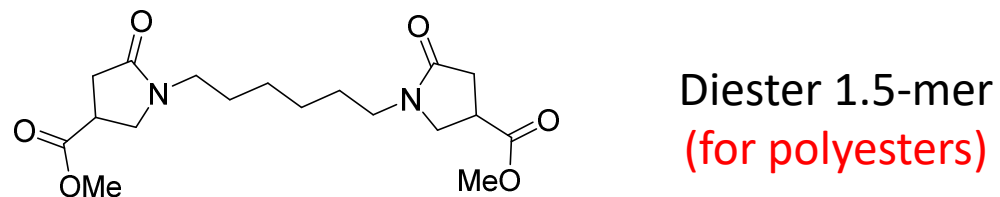
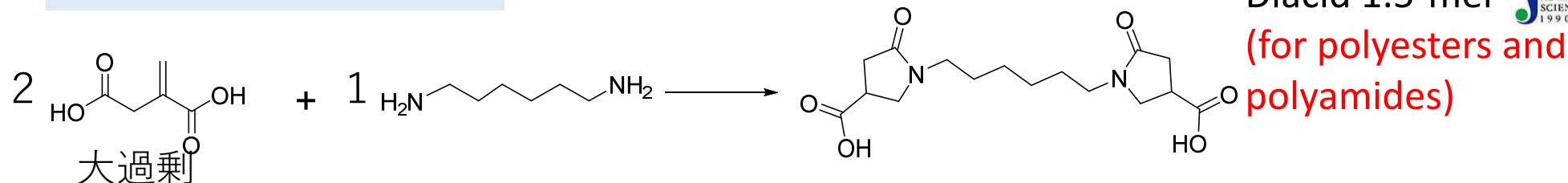
Metabolite Analysis



Future direction

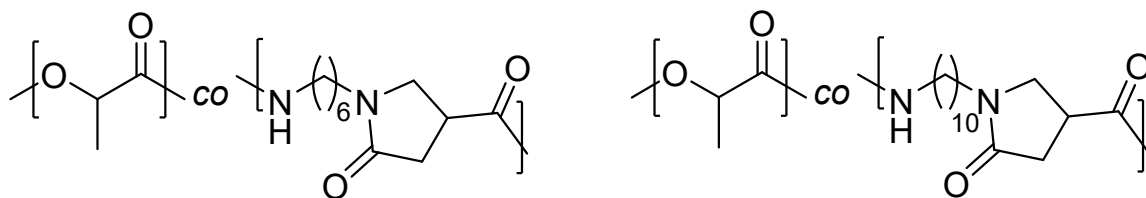
- An MTA was signed with an aquarium for the provision of fresh fecal samples of two species of marine mammals.
- Cultivation of the fecal samples will be conducted to confirm reproducibility of their microbiota.

Photoswitch syntheses

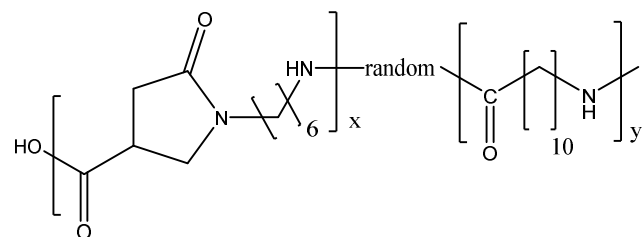


Terminally aminated or hydroxylated nylon blocks
(polyester, polyamide, polyurethane)

Photoswitch incorporation



It was found that the 10i monomer can be efficiently incorporated into PET and PLA into polylactic acid.



Successful incorporation of amino acid-type monomers into Nylon 11

[Patent applicant] Japan Advanced Institute of Science and Technology
[Application date] October 29, 2020
[Application number] Patent application 2020-181536
[Inventors] Tatsuo Kaneko, Huaiyu Wang, Singh Maninder
[Title of Invention] Polyamide-based polymer

Strand



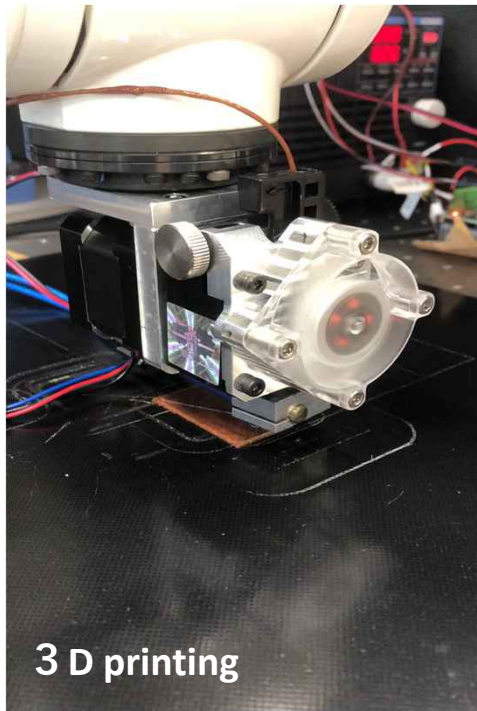
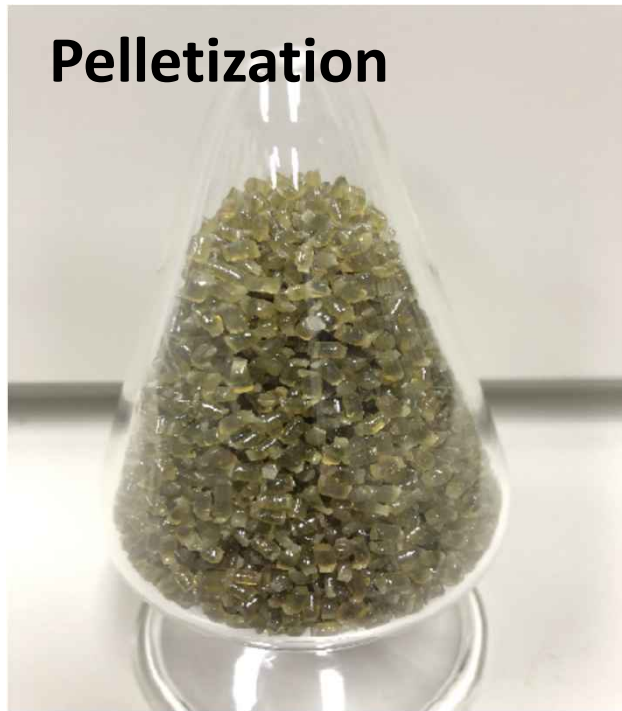
Fibers



Injection molding

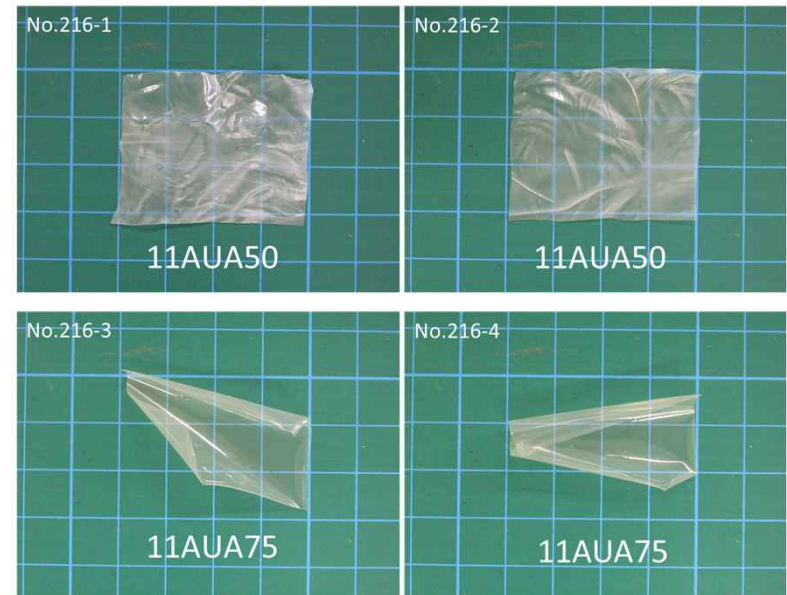


Pelletization



3 D printing

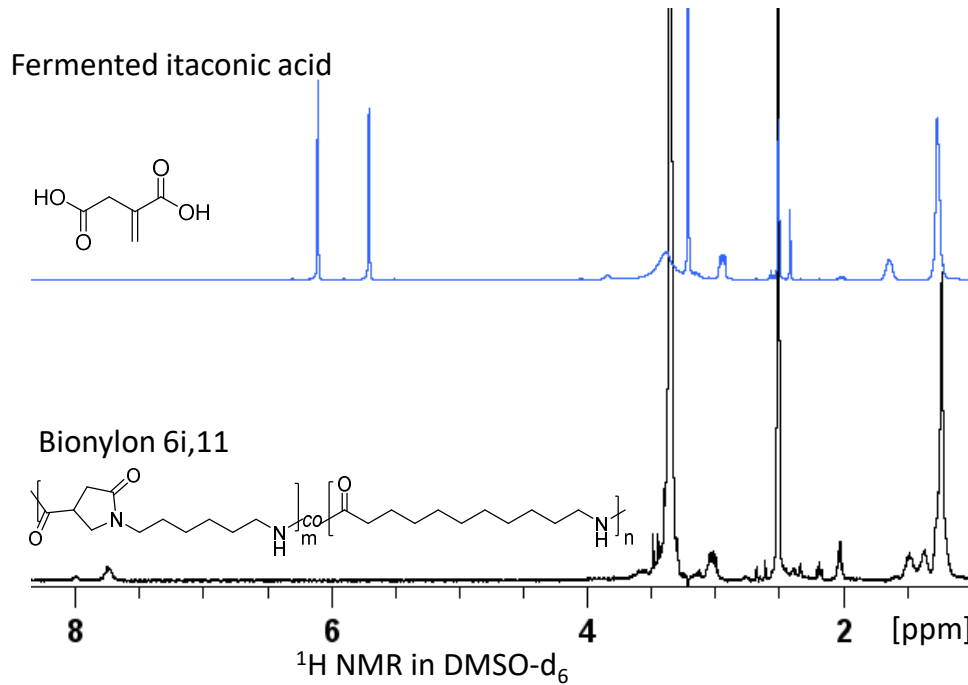
Cast films



Good processability

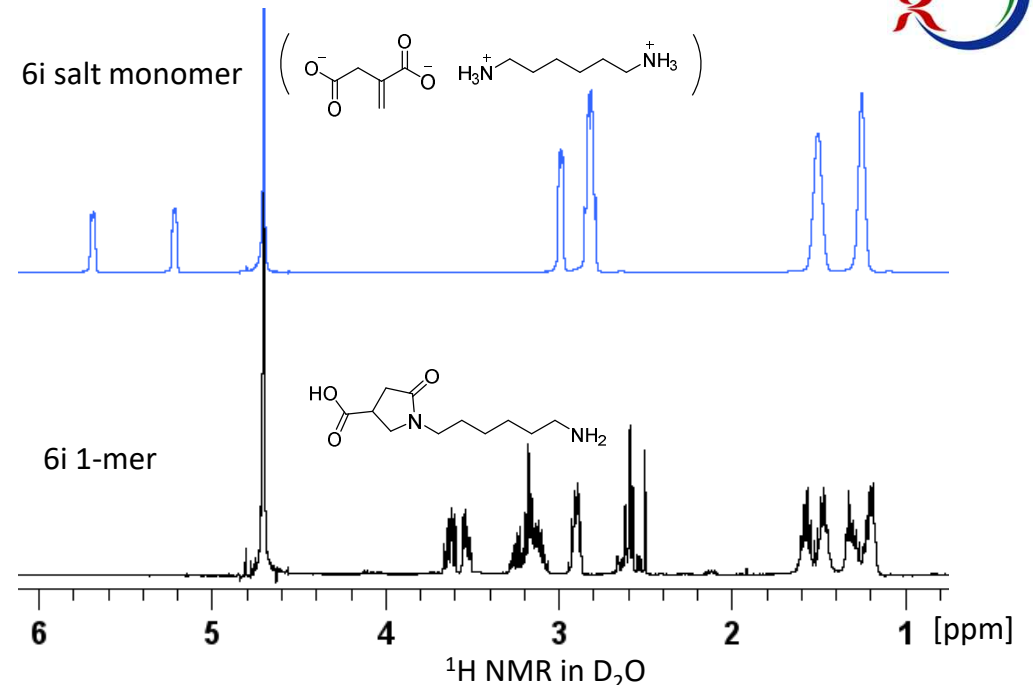
Preparation of bio-nylon fibers from fermented itaconic acid (Research items ①、⑤、⑥)

Itaconic acid produced by fermentation from sorghum molasses was reacted with hexamethylenediamine to synthesize 6i salt monomer and 6i monomer. Further, it was copolymerized with 11-aminoundecanoic acid to form nylon 6i,11 to obtain a fiber.



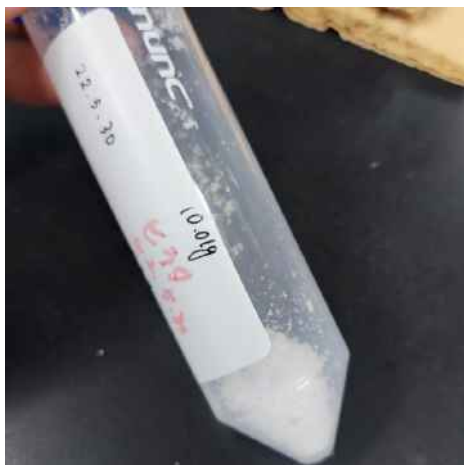
Fermented itaconic acid

Nylon 6i,11



Nylon fiber

The itaconic acid produced by fermentation contains impurities such as salt and extraction solvent, but the impurities can be removed during the synthesis process of salt, and the synthesis of nylon will not be affected.



Future: Research and development by the biomass team and LCA team aiming for cost reduction of raw materials and low carbonization

Degradability of ON type polymer (Ny6i,11)

(Research items ①-1,③-1)



0 hour

16 hours

72 hours

>100 hours

Photo-disintegration was confirmed in water.

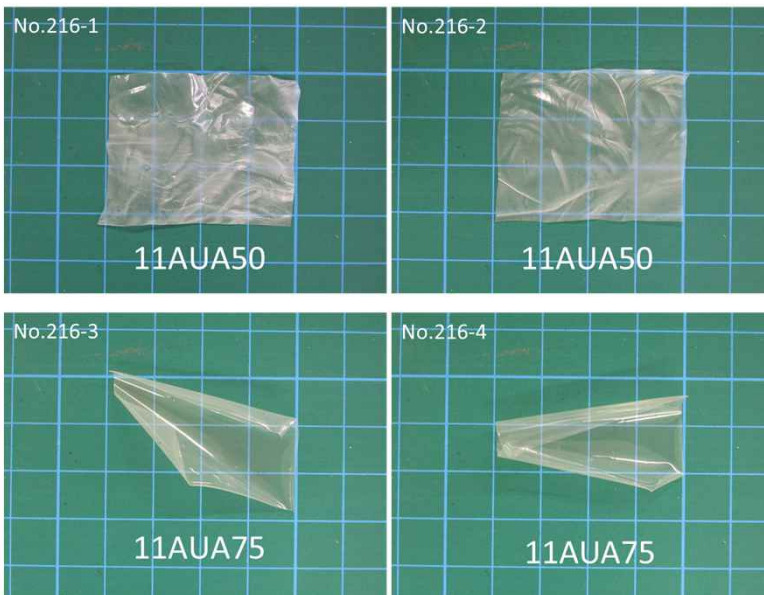
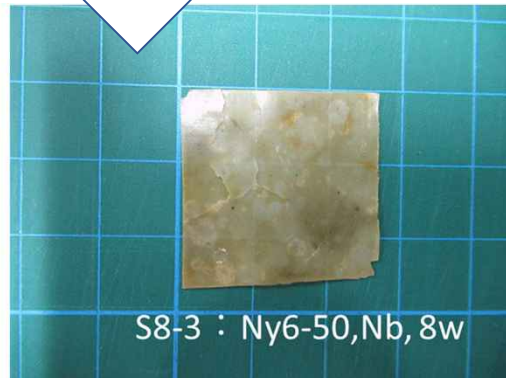
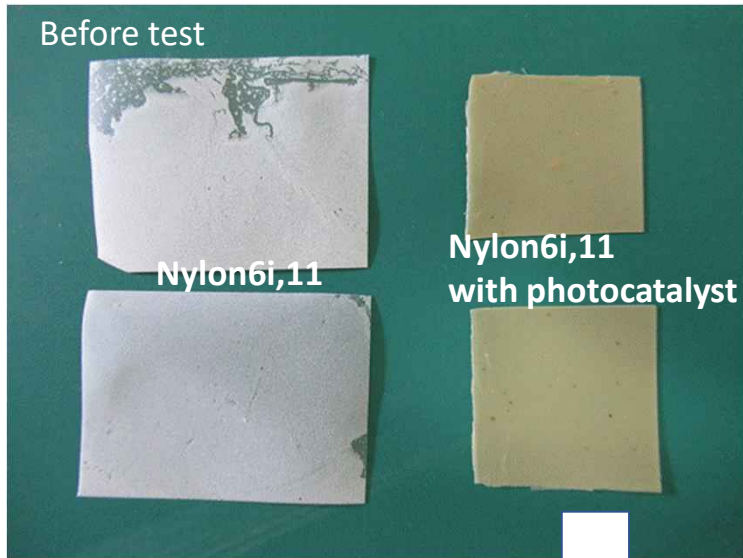


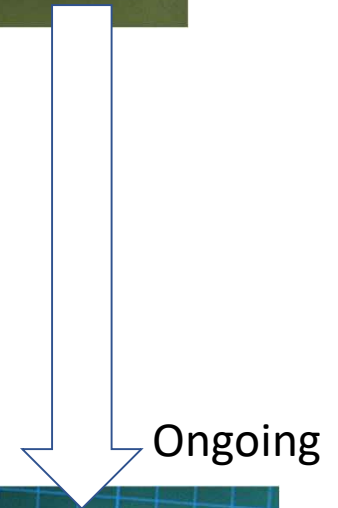
Photo-disintegration test in the sea

Marine Disintegration Test of ON type Nylons (Research item ③-1)

6/9~



8/1~



Ongoing



Bacterial Flora analysis on Photo-switching Nylon in Ocean Environment

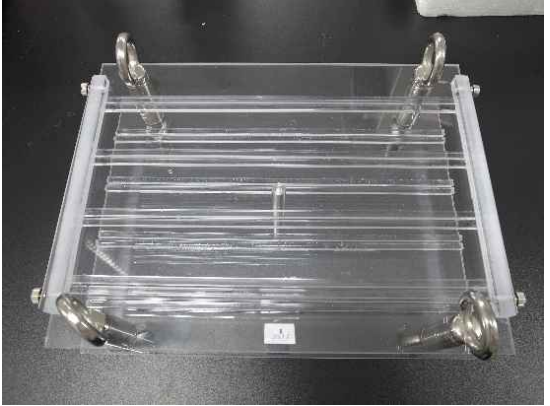


The experimental method was based on "最新の海洋生分解性プラスチックの研究動向", published by テクノシステム社.



Nylon 6i film was provided by Prof. Kaneko, JAIST
Bacterial flora analysis was joint research with Dr. Wakai, JAMSTEC.

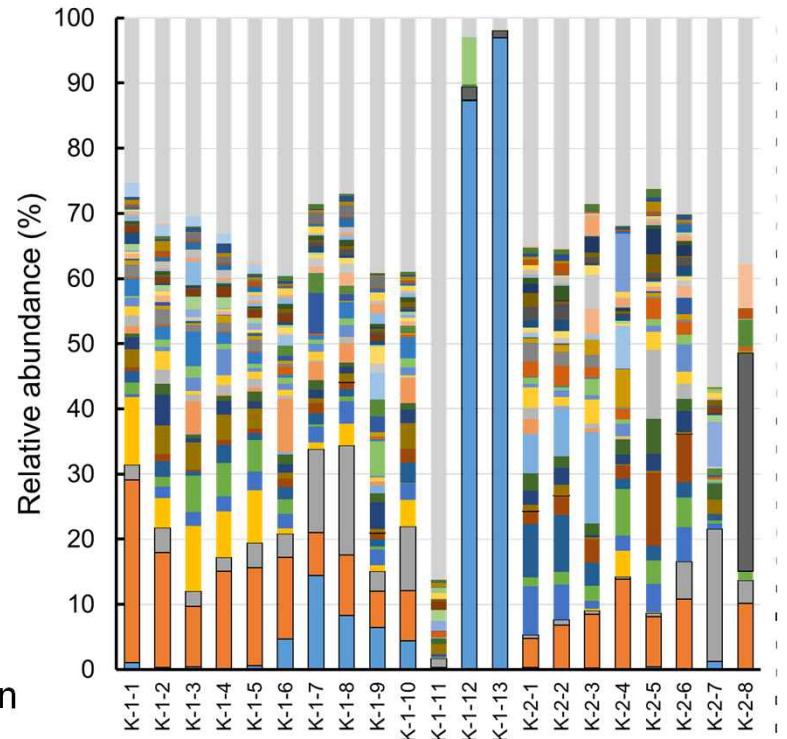
Immersion jig



Film mount



Test period : 2021.August - December



40 days
→

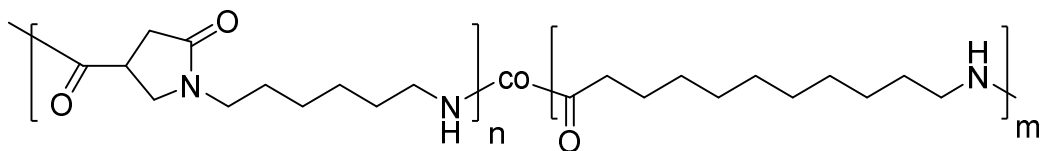


Gathering information on bacterial flora on film surfaces and Comparing differences in flora by depth and season

Bacterial flora on Nylon were different from those of control samples such as PET.

"BOD biodegradation test" and "Enzymatic hydrolytic test by nylon hydrolase" toward ring-opened Nylon sample are also in progress.

ON-type photoswitch incorporation to Nylon11



We found the conditions for introducing 11-aminoundecanoic acid and polymerizing it. (Establishment of 10L bench-scale synthesis conditions: front-loaded task)



Films and injection molded products
(regarding raw material production)



- Coating by spray method
- 1: Coatability
 - 2: Court stability
 - 3: Post-use degradability (disassembly switching)

Table 1 Thermomechanical properties of bionylon with long fatty chains

Diamine, diacid	Tensile Strength (MPa)	Elongation (%)	Young's Moduli (MPa)	T_{d5} (°C)	T_g (°C)	T_m (°C)
10,i	91	10	242	402	57	ND
12,i	96	12	255	399	55	ND
10,i12	120	70	200	427	50	155
12,i12	115	65	180	423	45	155
6,i11	120	60	214	428	40	170
6,i14	ND	ND	ND	392	48	160
6,i16	98	9	200	389	50	161
6,i18	101	10	215	421	48	166
6,i20	96	10	189	412	44	158

Expanding the structural diversity of itaconic acid-derived Nylons

[Patent applicant] Japan Advanced Institute of Science and Technology
 [Application date] December 28, 2021
 [Application number] Japanese Patent Application 2021-215456
 [Inventors] Tatsuo Kaneko, Munehiro Tamiya, Singh Manninder
 [Title of Invention] Polyamide-based polymer

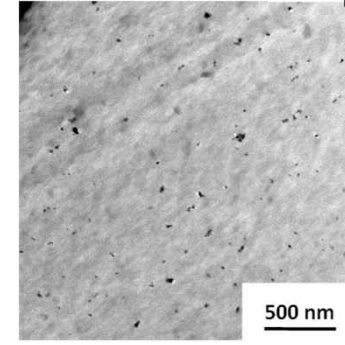
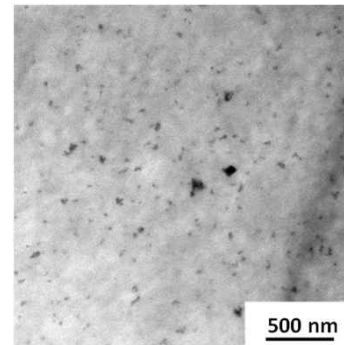
1. Preparation of composite films via melt processing



+CuI (1.0 wt%)
Stabilizer only



+CuI (1 wt%)+NaNbO₃ (0.5 wt%)
Photoinduced hydrophilization agent



NaNbO₃ samples synthesized by a solvothermal method and a solid-phase method were nicely dispersed.

- Bio-nylon is more stable than nylon 11 in air, but hydrolyzes in water under photo irradiation.
- CuI inhibits the photo-degradation, while NaNbO₃ promotes photo-hydrolysis.

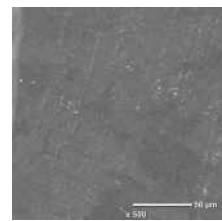
2. Degradation mechanism (35°C, Xenon lamp)

Extract after 7-week photo irradiation

+CuI (1.0 wt%)



Film Appearance

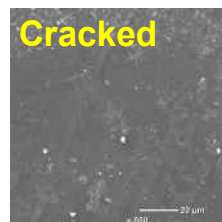


Film surface

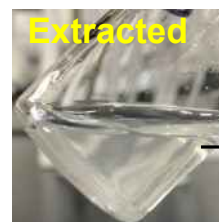


Extract in salty water

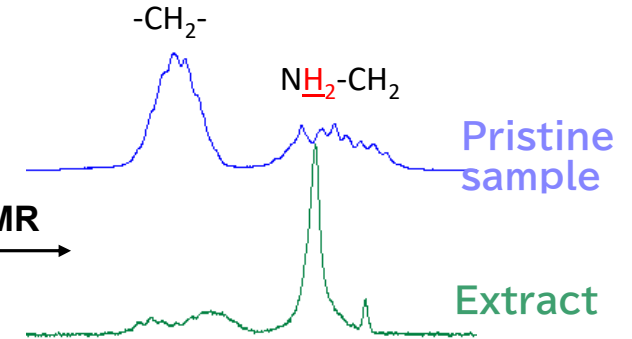
+CuI (1 wt%)
+NaNbO₃ (0.5 wt%)



Cracked



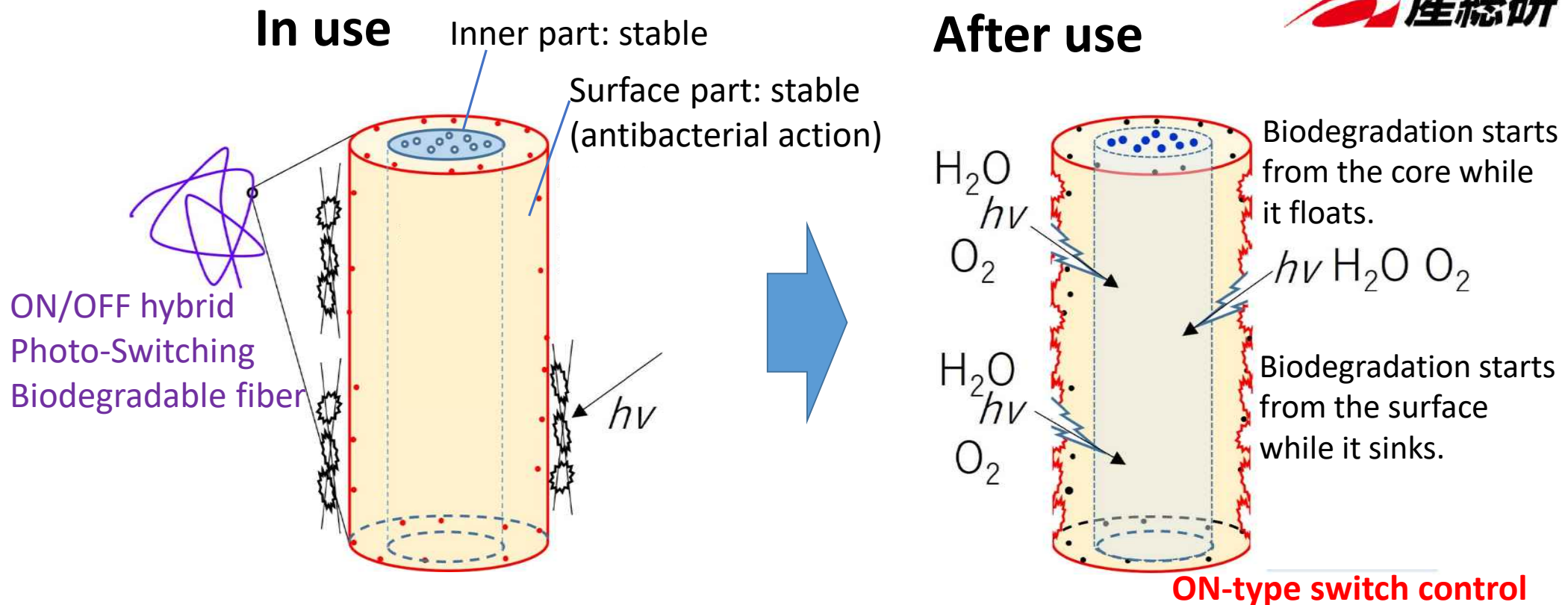
Extracted



The extract contains a large amount of terminal amines, supporting hydrolysis.

BOD and toxicity testing, LC-MS

Concept of OFF Type Photo-Switching Biodegradable Plastics

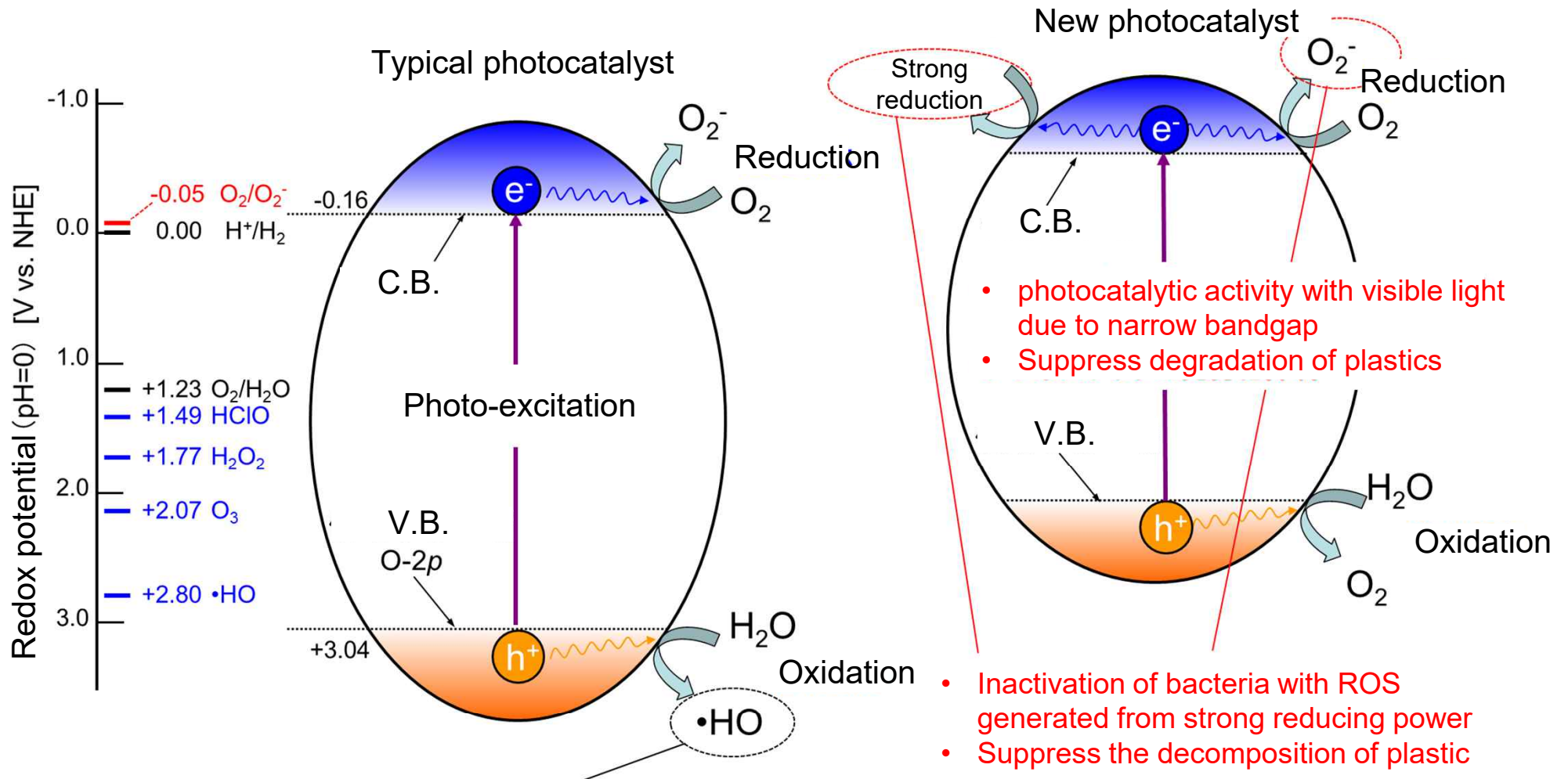


Development of antibacterial photocatalyst for ON/OFF-type photo-switching marine degradable plastic applications

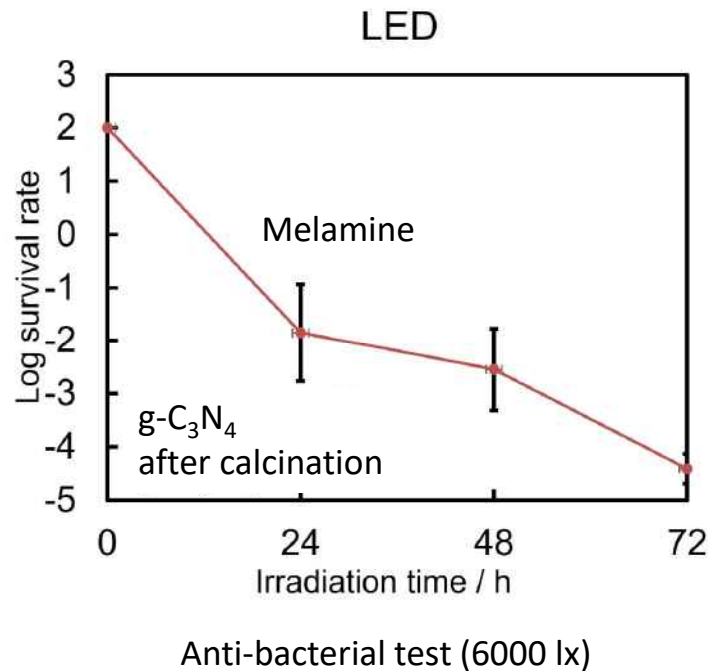
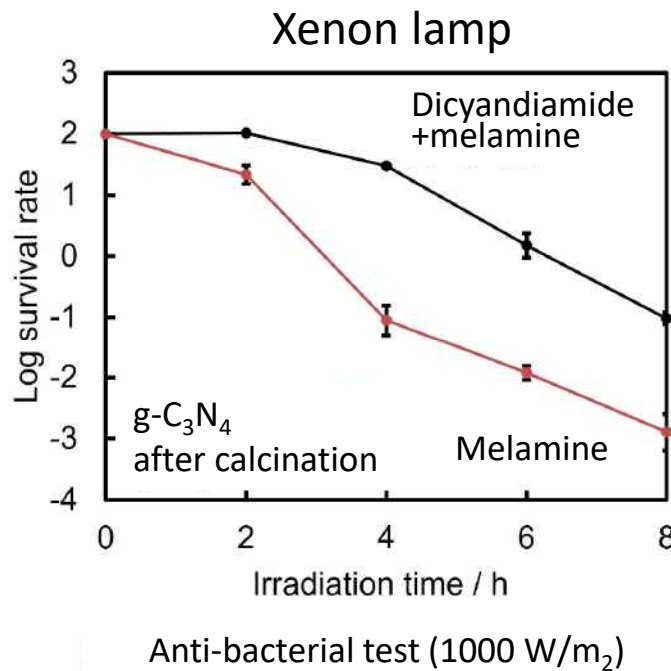
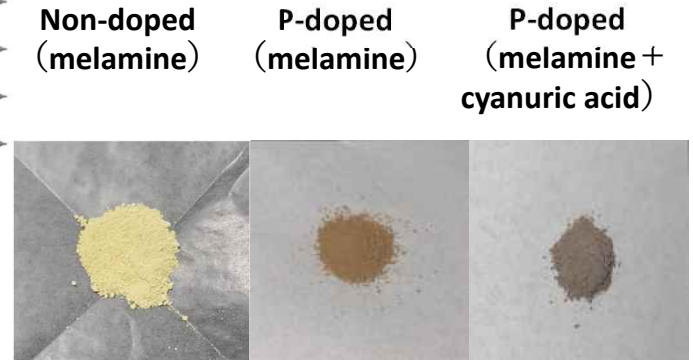
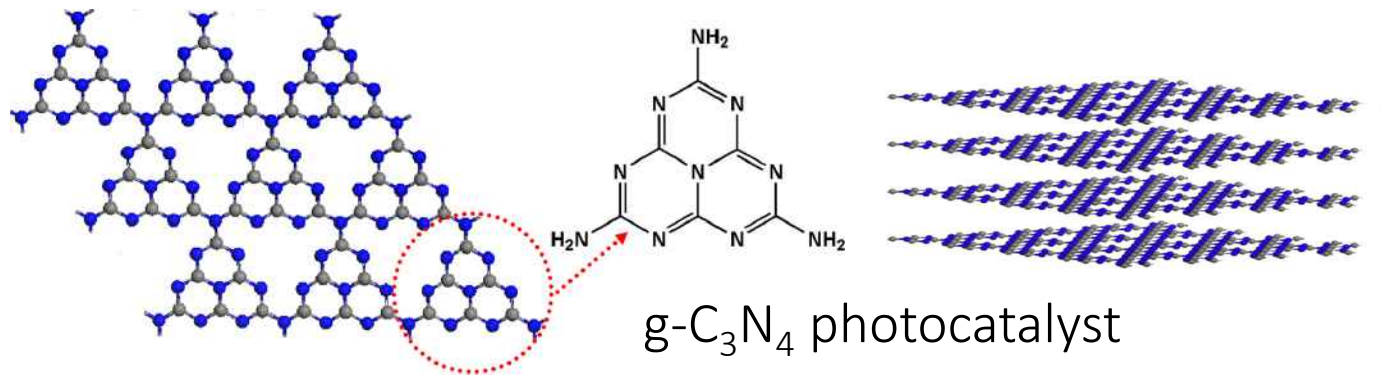
Development of OFF-type biodegradable polymer

- 1) Development of a novel photocatalyst that does not decompose resin but can perform antibacterial
- 2) Establishment of evaluation method for degradability in laboratory and real environment

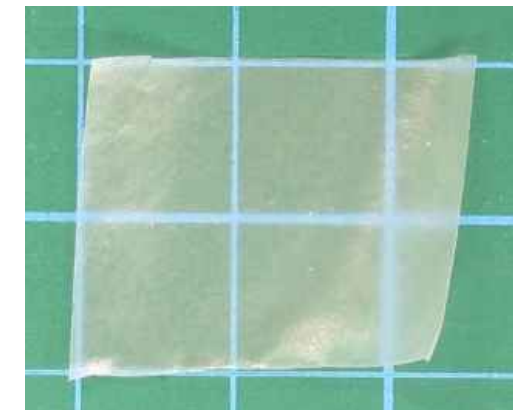
Photocatalyst that can be sterilized under visible light without decomposing polymer



Most organic substances including polymer and bacteria can be decomposed by the strong oxidizing power of reactive oxygen species.



Phosphorus doping of carbon nitride absorbs long wavelength light



Composite of g-C₃N₄ into PCL has been succeeded

- Antibacterial activity is improved by changing starting materials
- Exhibits antibacterial properties even under LED

Photo stability evaluation

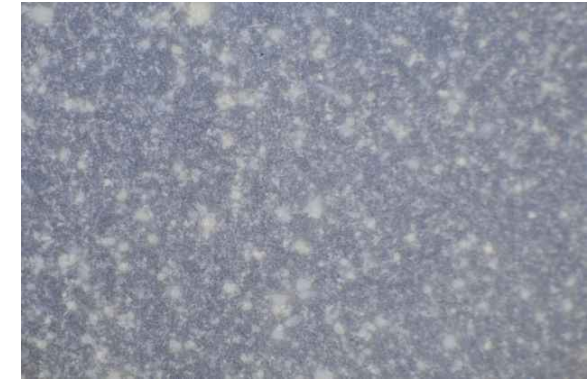
LED lamp irradiation : 5,000 Lx
Composite film : PCL + photocatalyst



Microphotographs of composite film
after 600 h irradiation



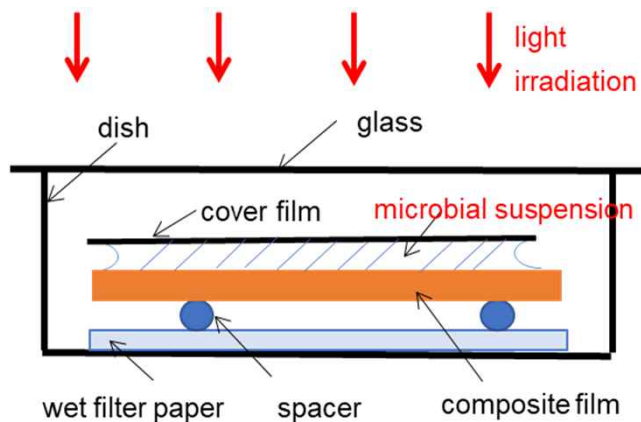
PCL + g-CN 5%



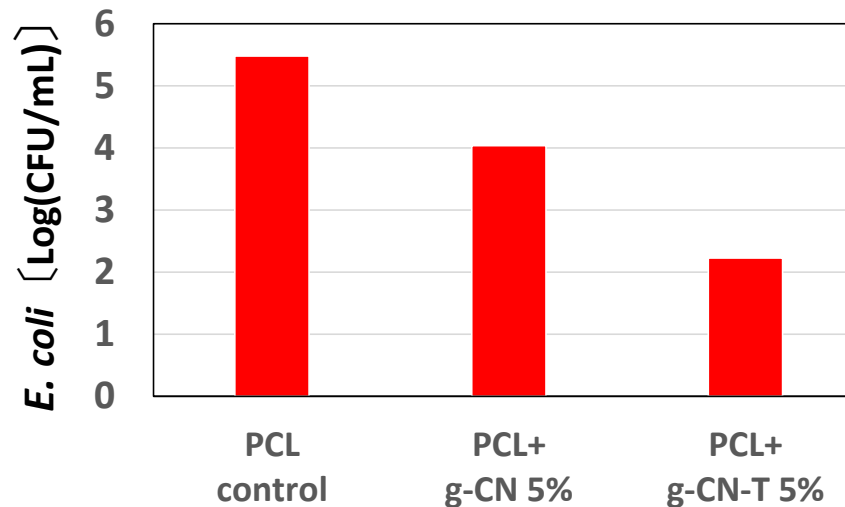
PCL + g-CN-T 5%

→ No cracks observed.

Antibacterial activity evaluation



Xenon lamp irradiation : 12,000 Lx、 0.20 mW/cm²、 4 h



→ The composite films containing g-CN and g-CN-T had improved antibacterial activity compared to the control.

Marine Immersion Test

Kobe U, Wharf

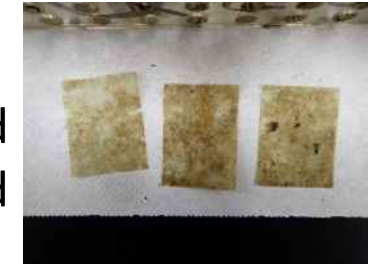


Immersion period

1. (2021.11/26-12/9) 2w
2. (2022.2/10-4/28) 8w
3. (2022.6/9-6/23) 2w
4. (2022.8/4-8/10) 1w

Film

- Simple blend
- Simple blend
- Fine mixed blend
- Fine mixed blend

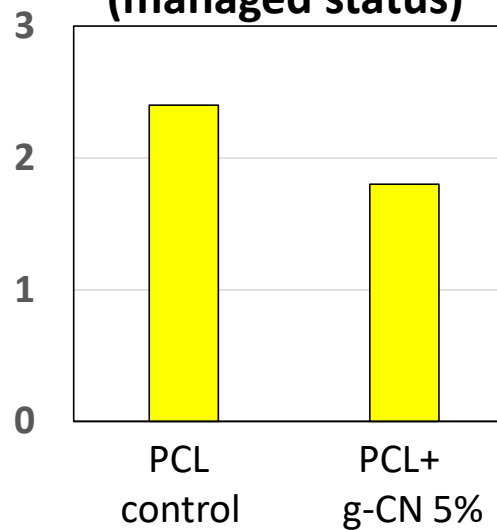


Marine immersion

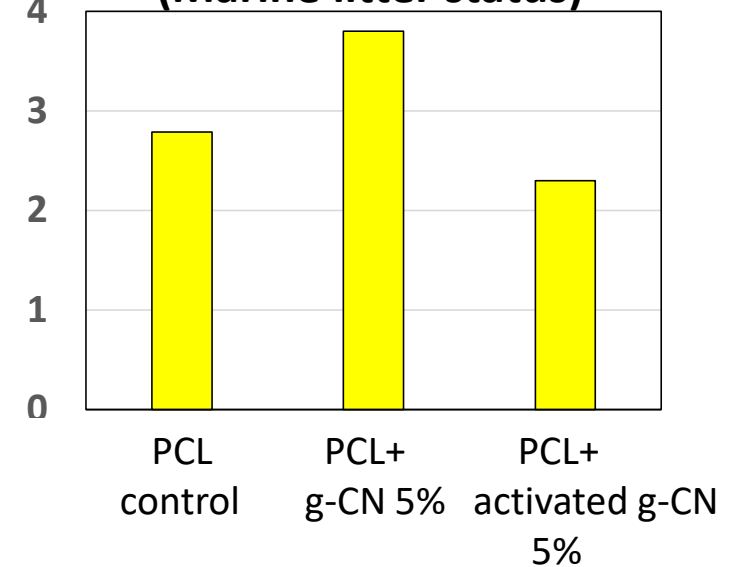


Film thickness decrease rate

Less surface dirt
(managed status)

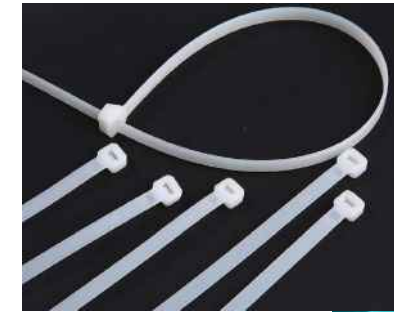


Severely slugged surface
(Marine litter status)



Biodegradation of films containing activated g-CN is greatly suppressed under sun-light exposure.

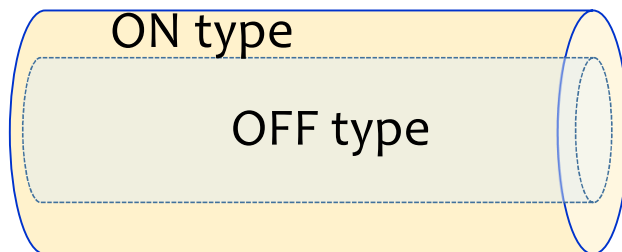
- 1) Agricultural materials
sheet, pole, rope, cable ties
- 2) Fishery materials



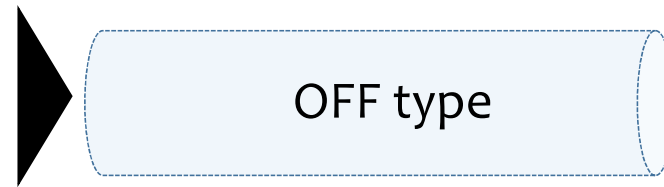
Cage of seedlings for regeneration of algae bed

→ The switch works when light is blocked by seaweed overgrowth.

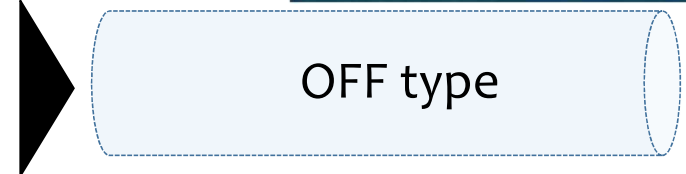
- 3) Using an ON/OFF type material for the rope of equipment for blue carbon countermeasures, it is submerged on the seabed with seaweed, contributing to global warming countermeasures while avoiding the marine plastic problem.



During the seaweed growth process, the On-type resin collapses under the action of sunlight and seawater, then biodegrades in the ocean.



The Off type resin cannot withstand the weight of the seaweed and is cut off. ⇒ Sinks on the seabed with seaweed



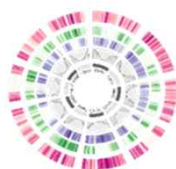
Off-type resin biodegrades due to the action of microorganisms in an environment not exposed to sunlight on the seabed.

Using 250 accessions collected around the world



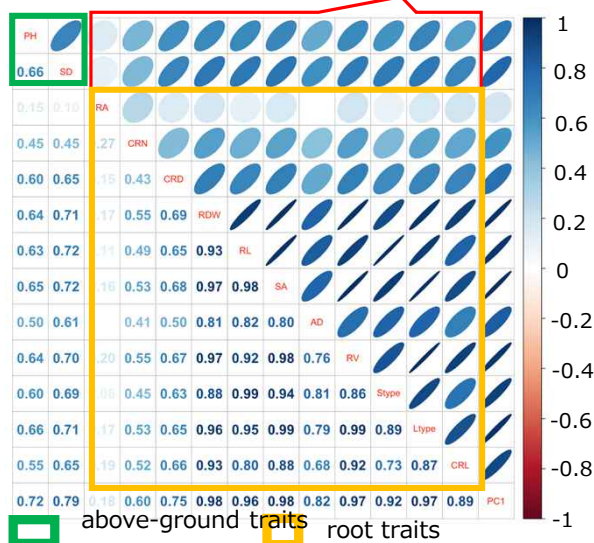
Whole-genome sequencing (247 accessions)
7.1Gb/1accession

Genome database



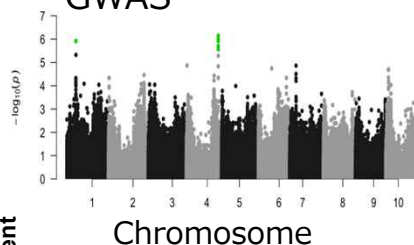
Genome-wide association study

• Correlation analysis between above-ground traits and root traits



※ The fineness and color of the ellipses represent the magnitude of the correlation coefficient.

• GWAS



• Root traits and above-ground traits were positively correlated → It is possible that improving root system lead to increasing biomass.

• In the GWAS, two QTLs were detected. → In future, they need confirmation.

Itaconic acid production from sorghum biomass

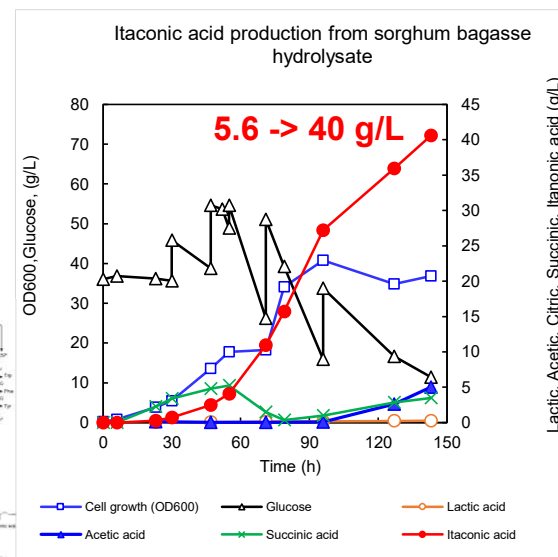
(1) Preparation of enzymatic hydrolysate of sorghum bagasse



(2) Itaconic acid production from sorghum biomass

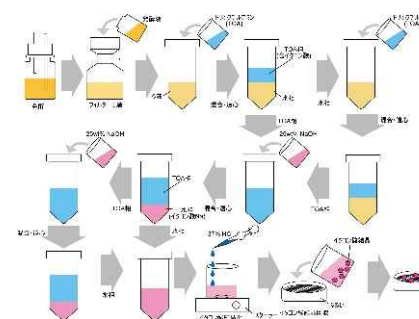


Metabolically engineered cell designed by computation model



(3) Purification of itaconic acid after fermentation

Development of purification method of itaconic acid from culture broth
Purity: ~70%



Purified itaconic acid from:

1. Regent
2. Glucose medium
3. Sorghum medium

Promote application exploration/supply chain building based on study group activities

① Study Group on the Future of Plastics

- Held twice/month at 10 institutions10

② Application exploration : Optimal use of principle

Principle : Light irradiation ⇒ Hydration ⇒ Biodegradation

- Covered fertilizers

Fertilization ⇒ Fertilizer leaching ⇒ Rice harvesting ⇒ Light irradiation
⇒ Hydrophilization ⇒ Biodegradation

- Equipment for Blue Carbon Control by Seaweed(Rope material)

Seaweed breeding/sunlight irradiation/water ⇒ Cutting the rope
⇒ Seaweed/rope deep-sea deposition (long-term CO₂ fixation) ⇒ Biodegradation

③ Supply Chain Development in R&D Phase

- Collaboration with manufacturer of photo-switch materials and photocatalysts

- Supply of high-quality materials from raw material manufacturers to application development companies

Main results

1. Integrated production of bio-nylon fibers with an ON-type photoswitching ocean-degradability in seawater, from grown plants.
2. Establishment of model compositions
 - ON-type Nylon: Company and sample work with Nylon 6i, 11
 - OFF-type polymer: Fast-biodegrading marketed resin such as PCL
 - ON-type photocatalyst: photoinduced superhydrophilization NaNbO_3
 - OFF-type photocatalyst: photo-induced antibacterial $\text{g-C}_3\text{N}_4$
3. Isolation of microorganisms for ON-type photoswitch marine biodegradation and start of test in actual marine environment, and confirmation of promotion of photo-induced hydrolysis by photocatalyst
4. Confirmation of biodegradation inhibition effects of OFF-type composite under laboratory light irradiation and immersion test in actual marine environment, establishment of research groups and proposal of application

Patents		Papers		Conference presentations	
international	domestic	reviewed	Non-reviewed	international	domestic
0	5	75	10	47	103

<https://www.jaist.ac.jp/project/moonshot/>

