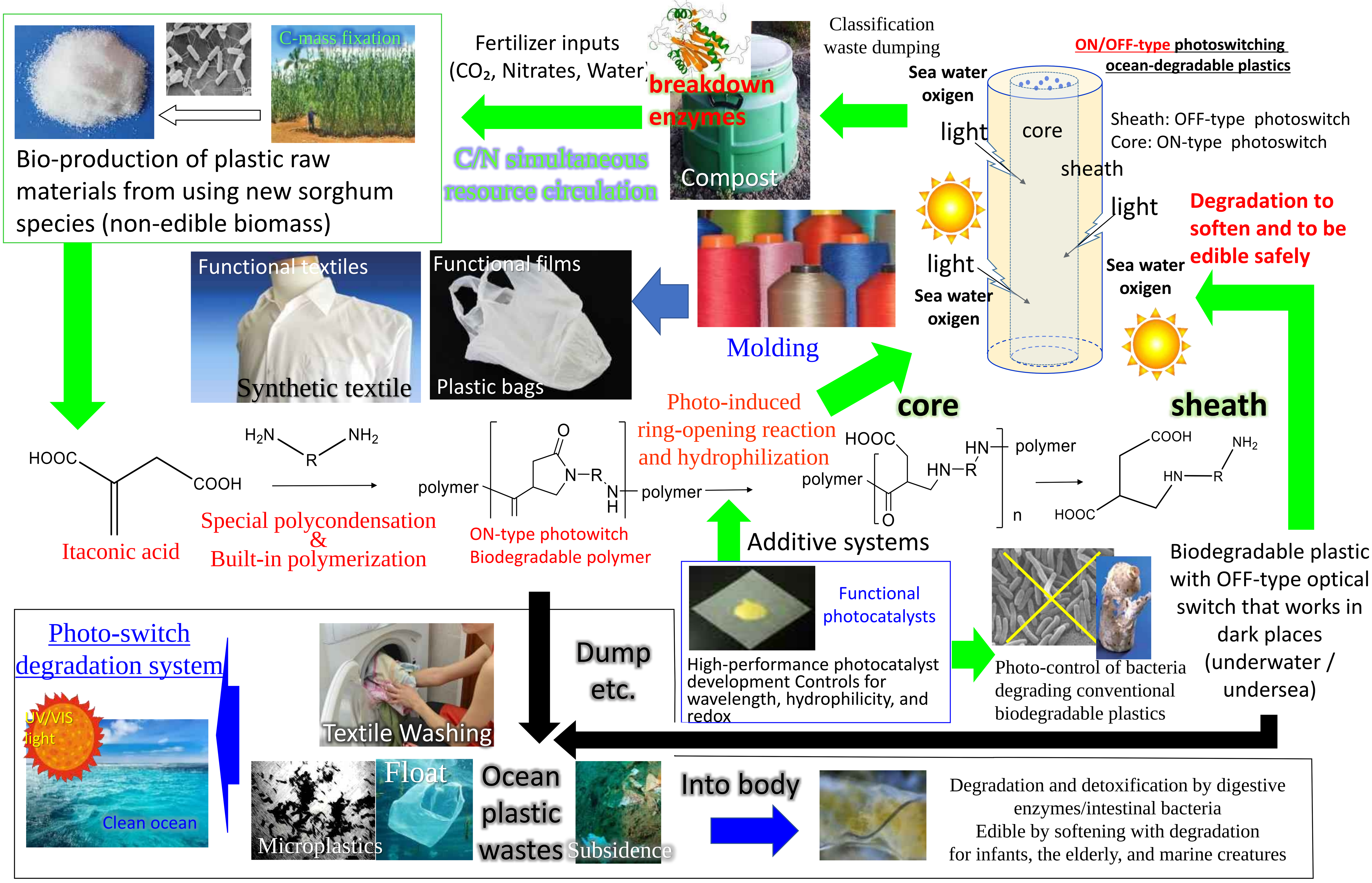
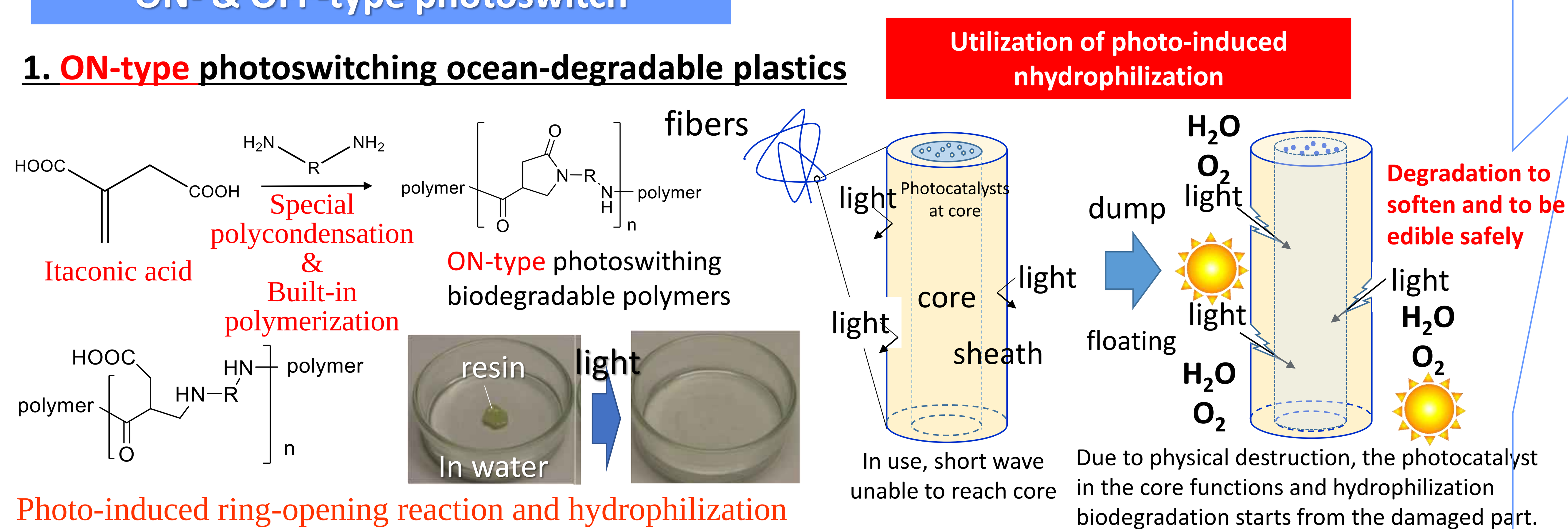
No. A-13-1E
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility
Theme: General Overview: ON/OFF-type Photoswitching Degradation
Organization: All participants
Contact: kaneko@jaist.ac.jp

[Implementation period] 2020-2029
[Implementing organization]: JAIST, Kobe U, Nagoya U, Kagoshima U, TUS, TUAT, AIST, ORIST
[Final goal (FY2029)] Developing edible plastic composites having optically-switching biodegradability in ocean, using itaconic acid and biodegradable polymers produced by fermentation from new sorghum varieties, with high-performance photocatalysts.

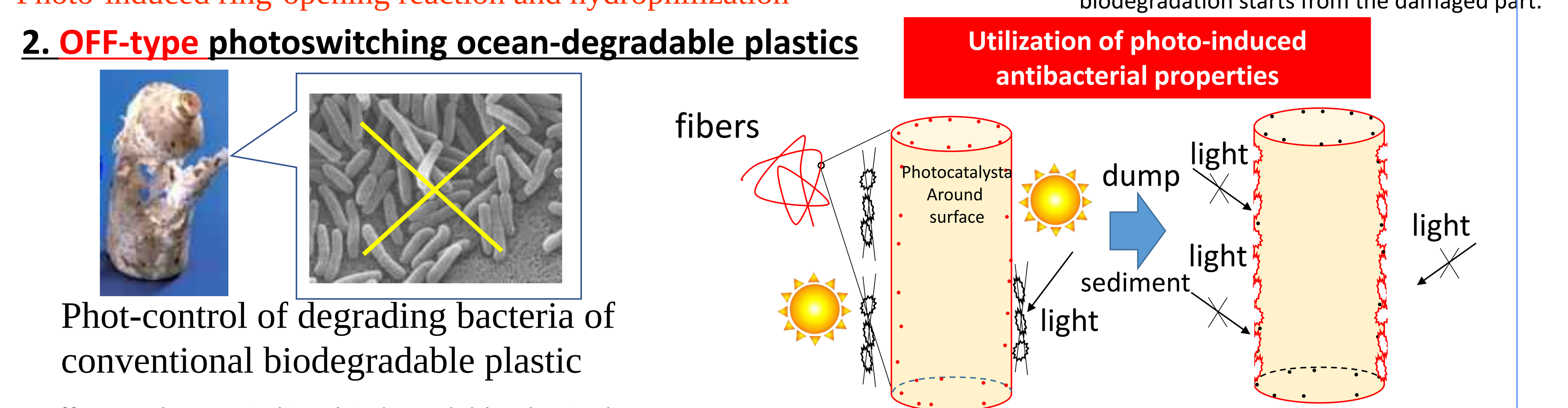


ON- & OFF-type photoswitch

1. ON-type photoswitching ocean-degradable plastics



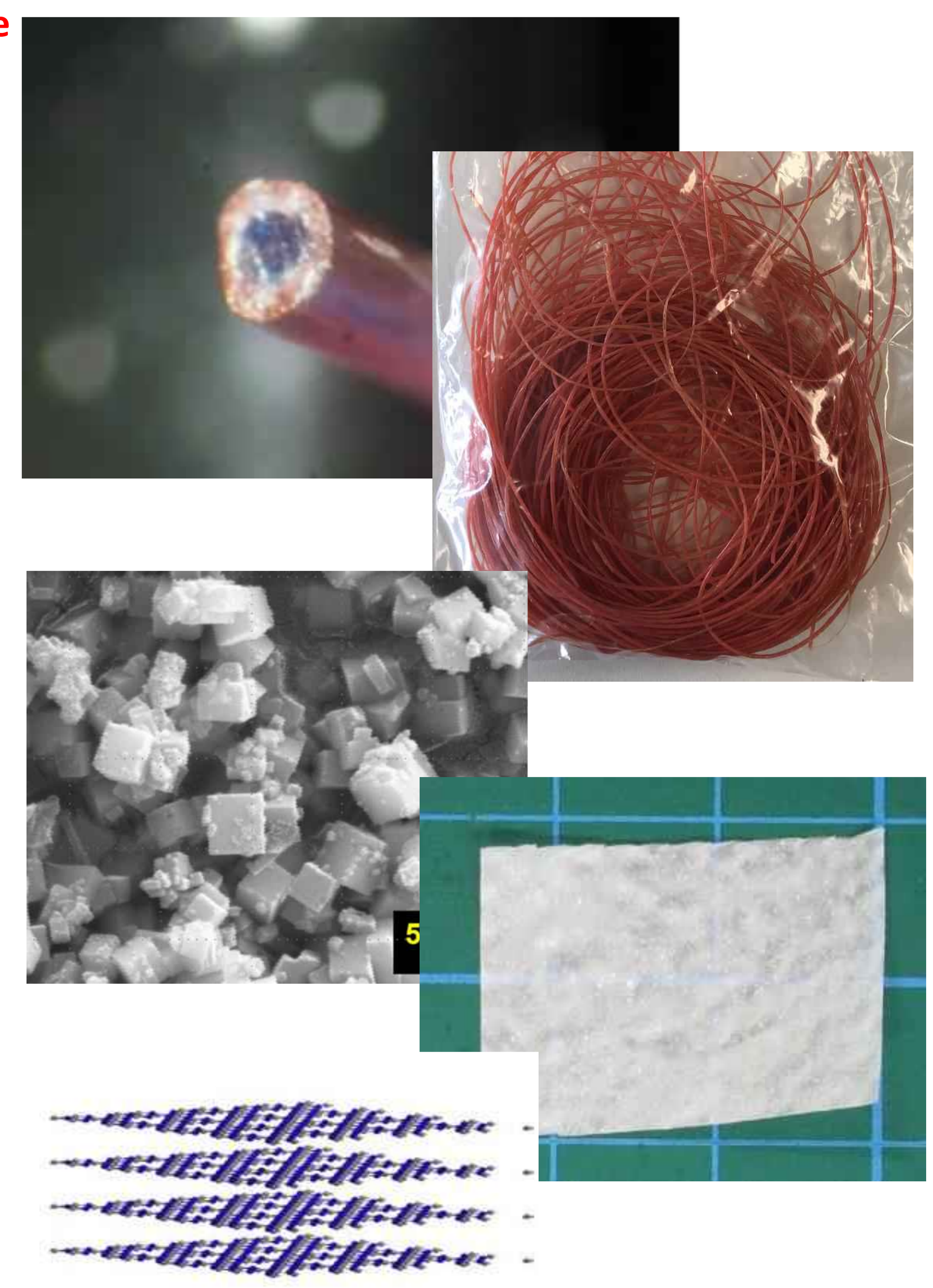
2. OFF-type photoswitching ocean-degradable plastics



An off-type photoswitching biodegradable plastic that suppresses biodegradation by light exposure and functions in dark places (under the sea and on the seabed)

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

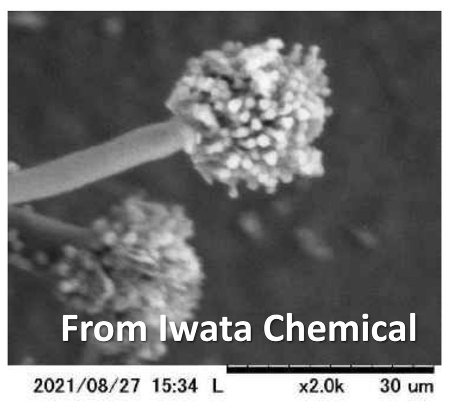
An ideal resin structurally designed to bring out the advantages of both photoswitches.



No. A-13-2E
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility
Theme: ON-type Resin Design
Organization: JAIST
Contact: kaneko@jaist.ac.jp

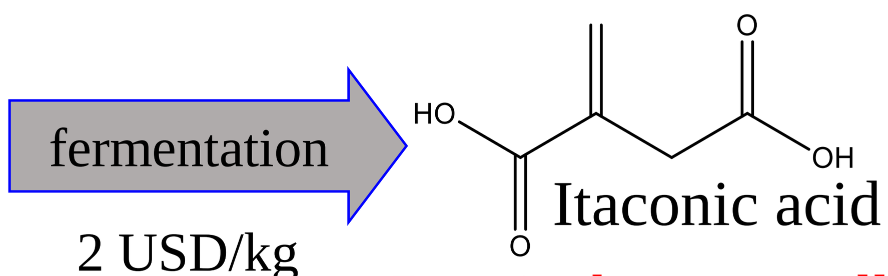
Plastic waste causes enormous economic losses. For example, the annual Economical loss by Plastic Trash in Asia-Pacific region is estimated to be 0.62 billion USD / y for tourism and 0.36 billion USD / y for fisheries and aquaculture (APEC Marine Resources). Conservation Working Group Report (2009)).

Itaconic acid



Aspergillus terreus

Annual global production: 70,000 tons
Market size: 100 million USD



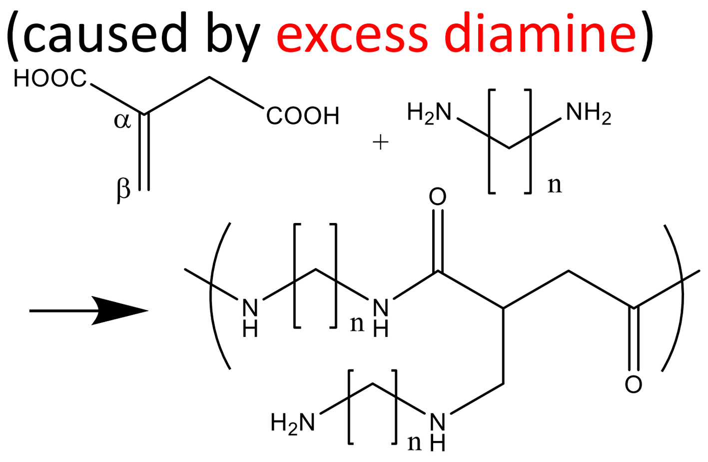
2 USD/kg

Mass-produced in China

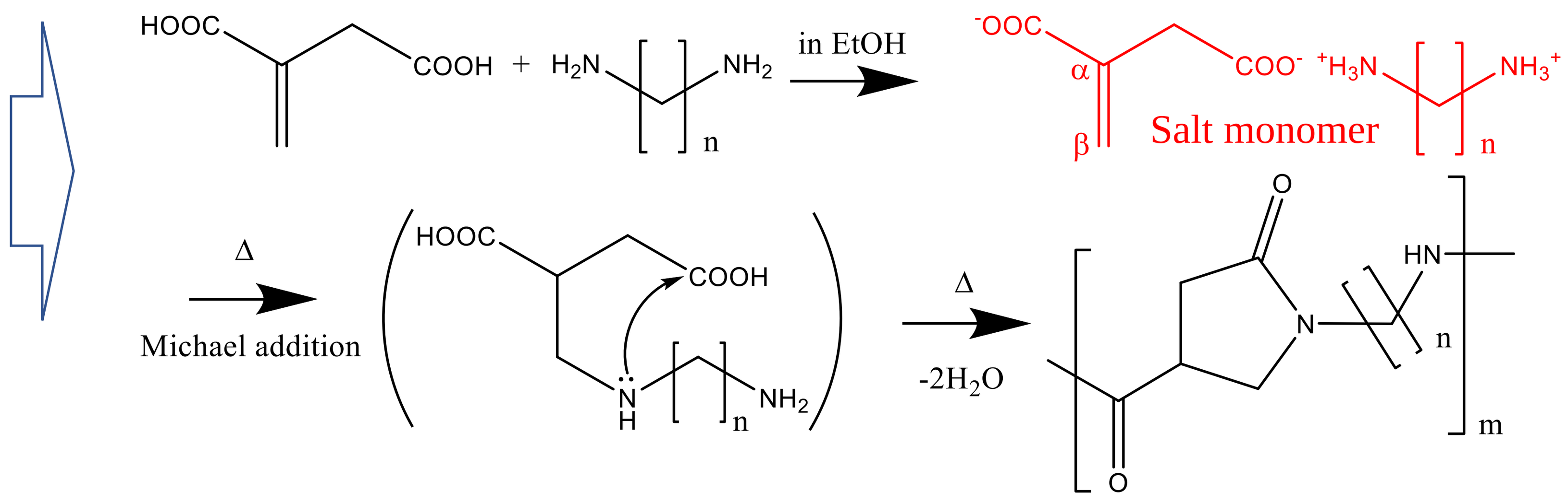
It has been used as an additive for fibers, resins, rubbers, surfactants, adhesives, etc. (expanding year by year).

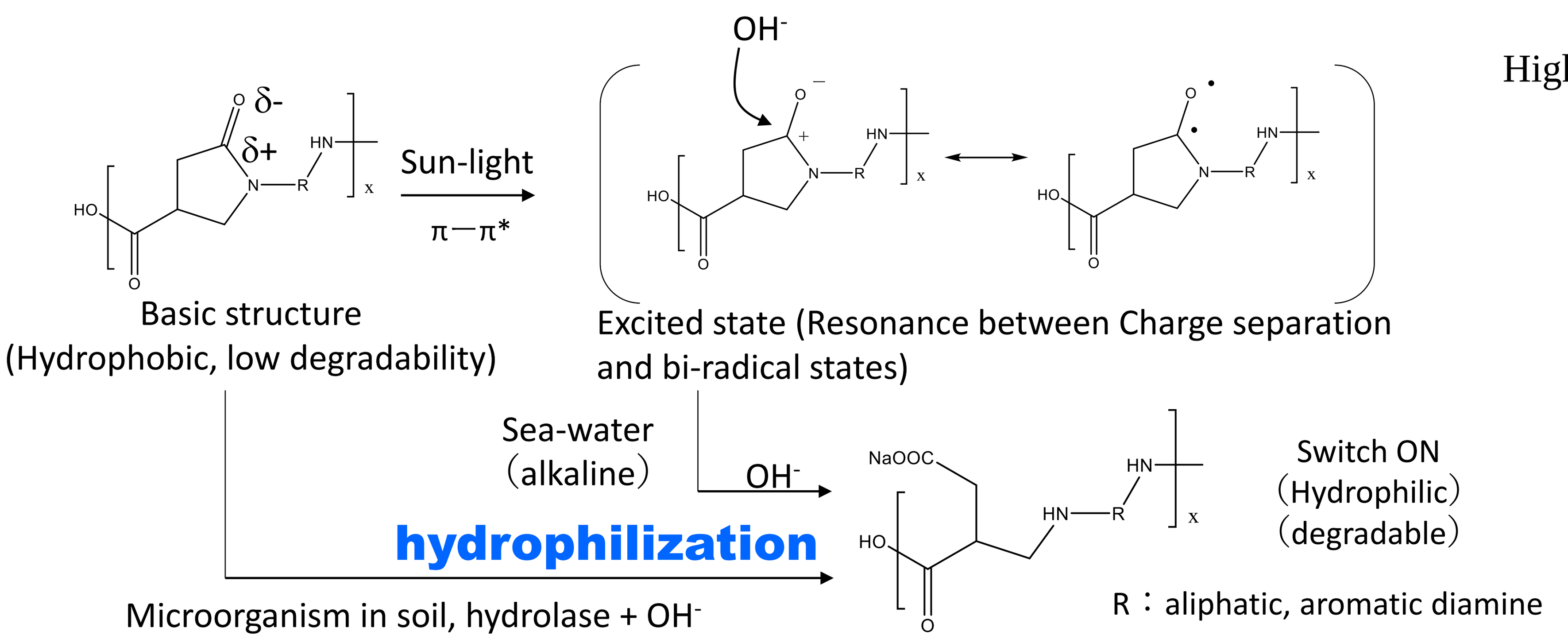
Despite being a well-known bio-derived dicarboxylic acid, synthesis of itaconic acid-derived nylon is difficult.

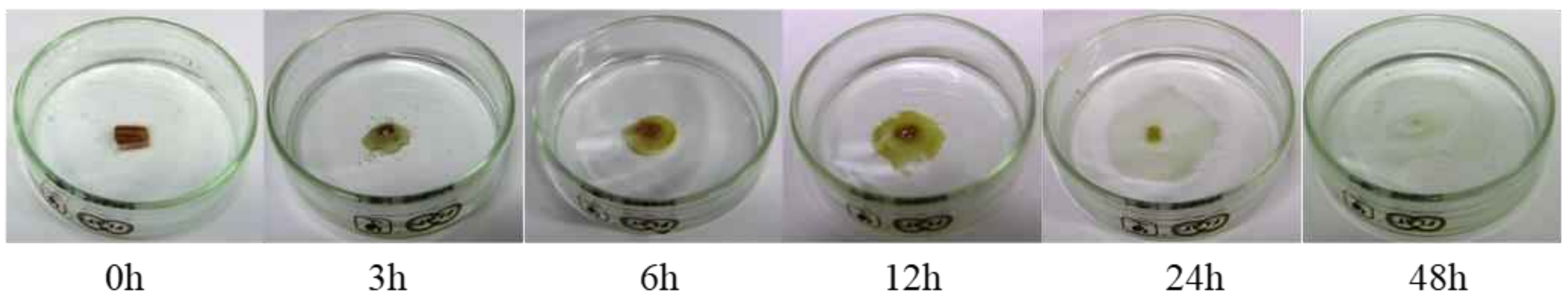
Side reactions (caused by excess diamine)



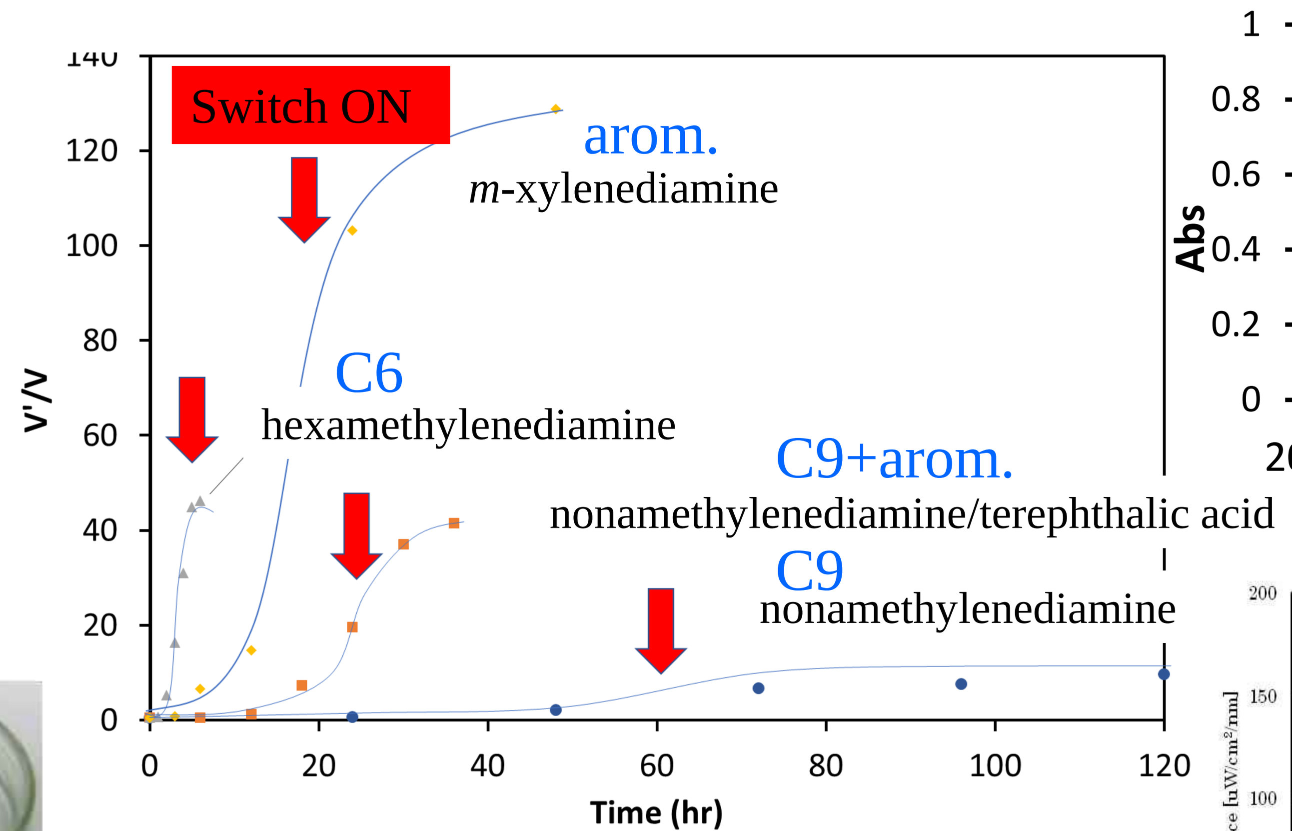
Salt monomer method



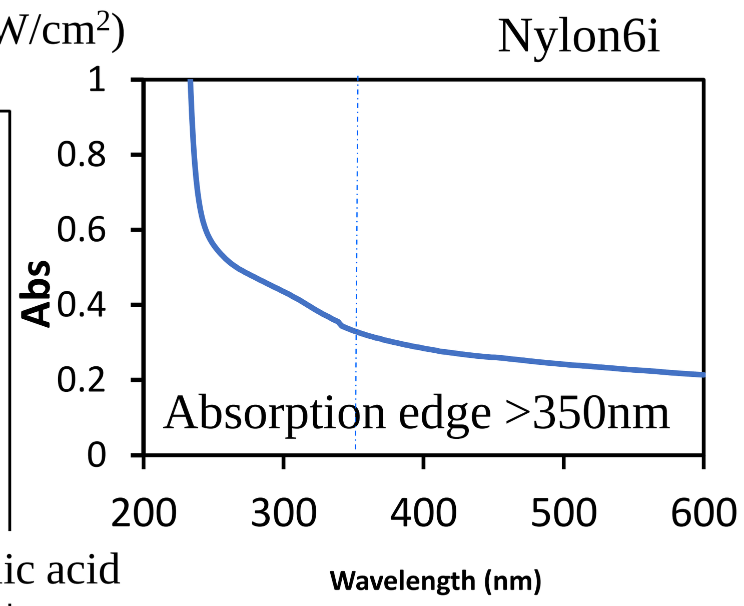




High-pressure Hg-lamp (wavelength: 250-450 nm, intensity: 150 mW/cm^2)



/Longer aliphatic chain (more hydrophobic), slower disintegration
/Aromatic component raised disintegration speed.



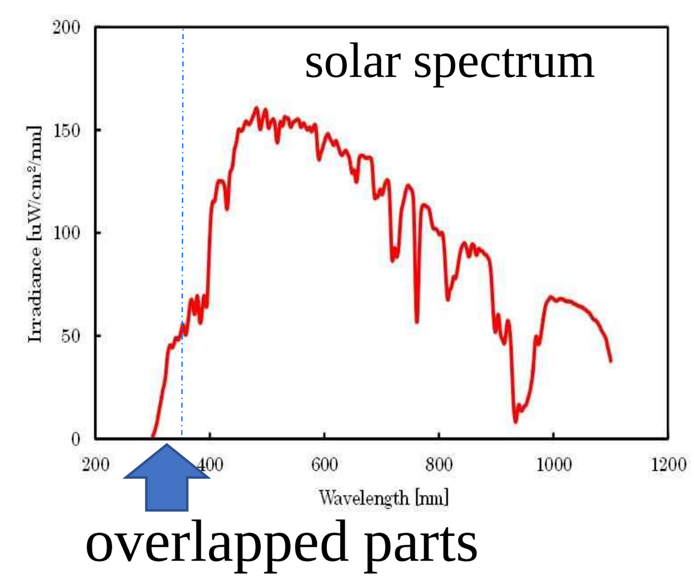
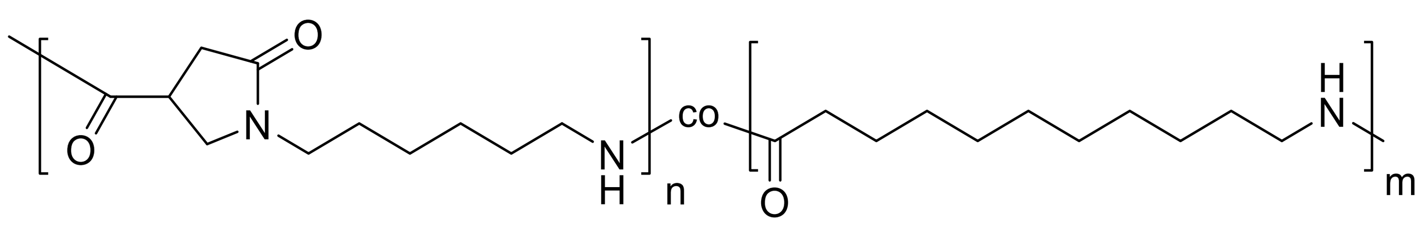
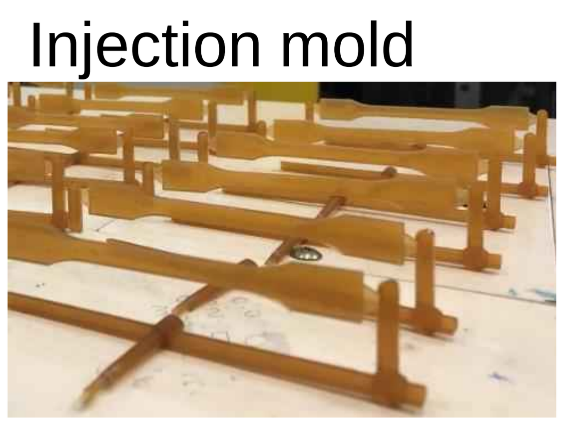
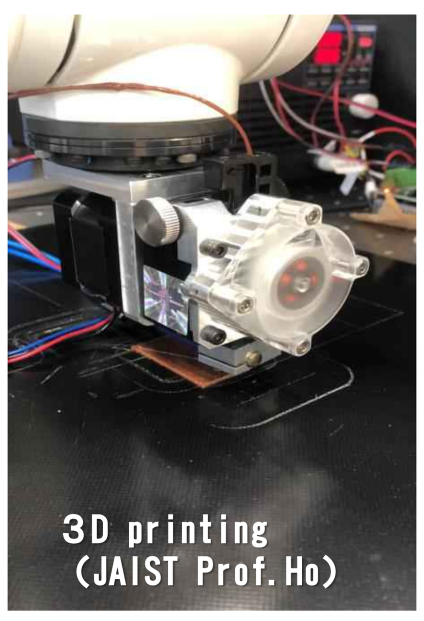


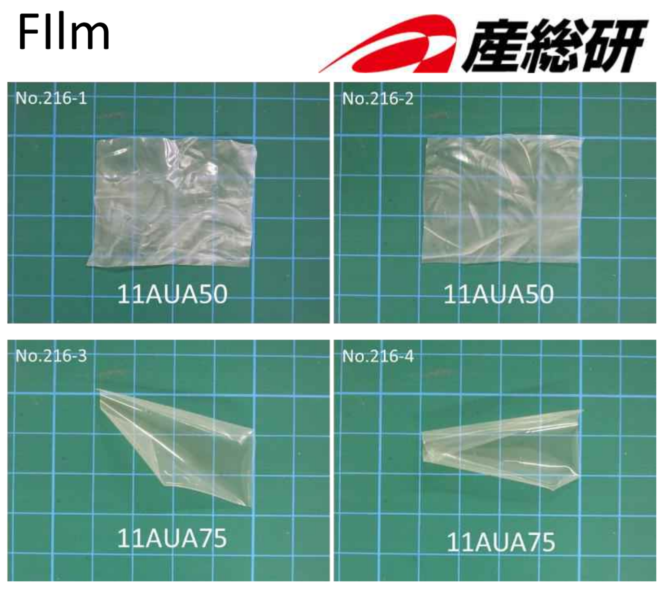
Photo-switch incorporation into Nylon11



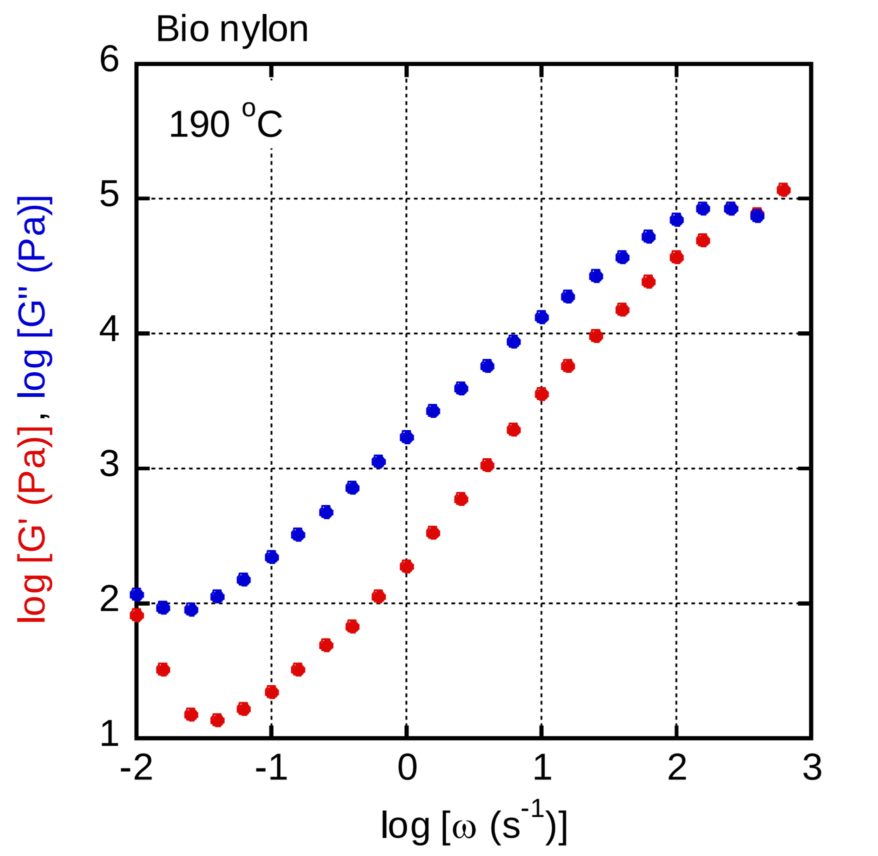


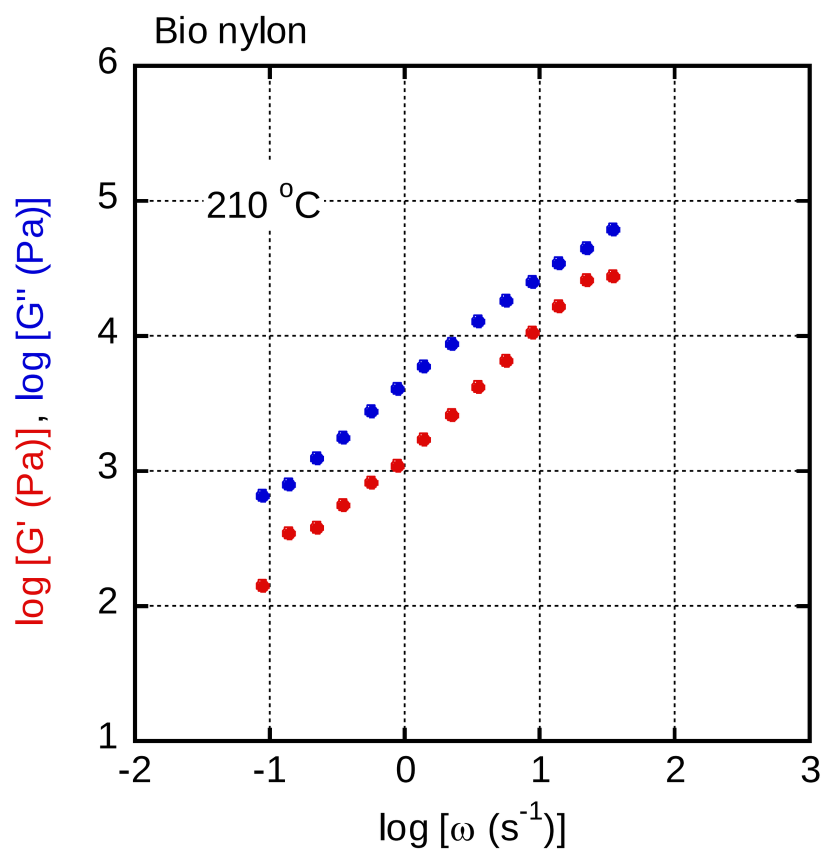


FIlm



Cone-plate rheometer AR2000ex, TA Instruments
Condition: At 190 and 210˚C under a nitrogen atmosphere.





1) **Almost no cross-linking occurred during synthesis.**
2) **Crosslinking gradually at 190 °C under nitrogen for about 30 minutes.**
3) **Crosslinking progresses in a short time at 210 °C (about 10 minutes), to make molding.**
4) **We were able to obtain fine fibers (pict)**

Disintegration of films induced by photoirradiation

Conclusions

1) Pyrrolidone-containing nylons derived from itaconic acid showed hydrolysis with ring-opening in the excited state, accompanied by photo-induced disintegration.
2) By incorporating a pyrrolidone ring-containing nylon into nylon 11, it became possible to impart photoinduced disintegration to nylon 11.
3) It was clarified that the modified nylon 11 exhibited excellent moldability to the extent that fine fibers were obtained.

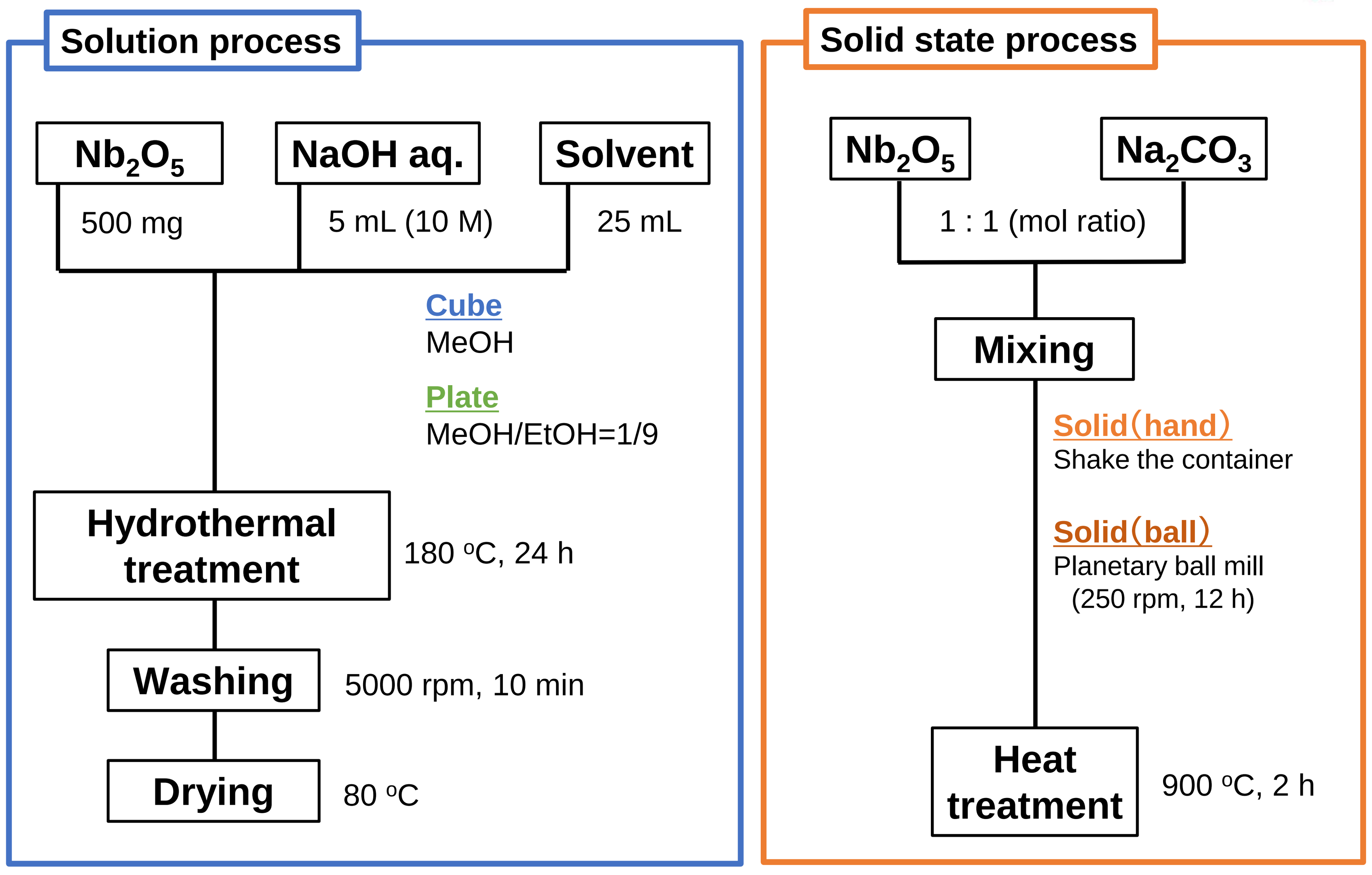
No. A-13-3E

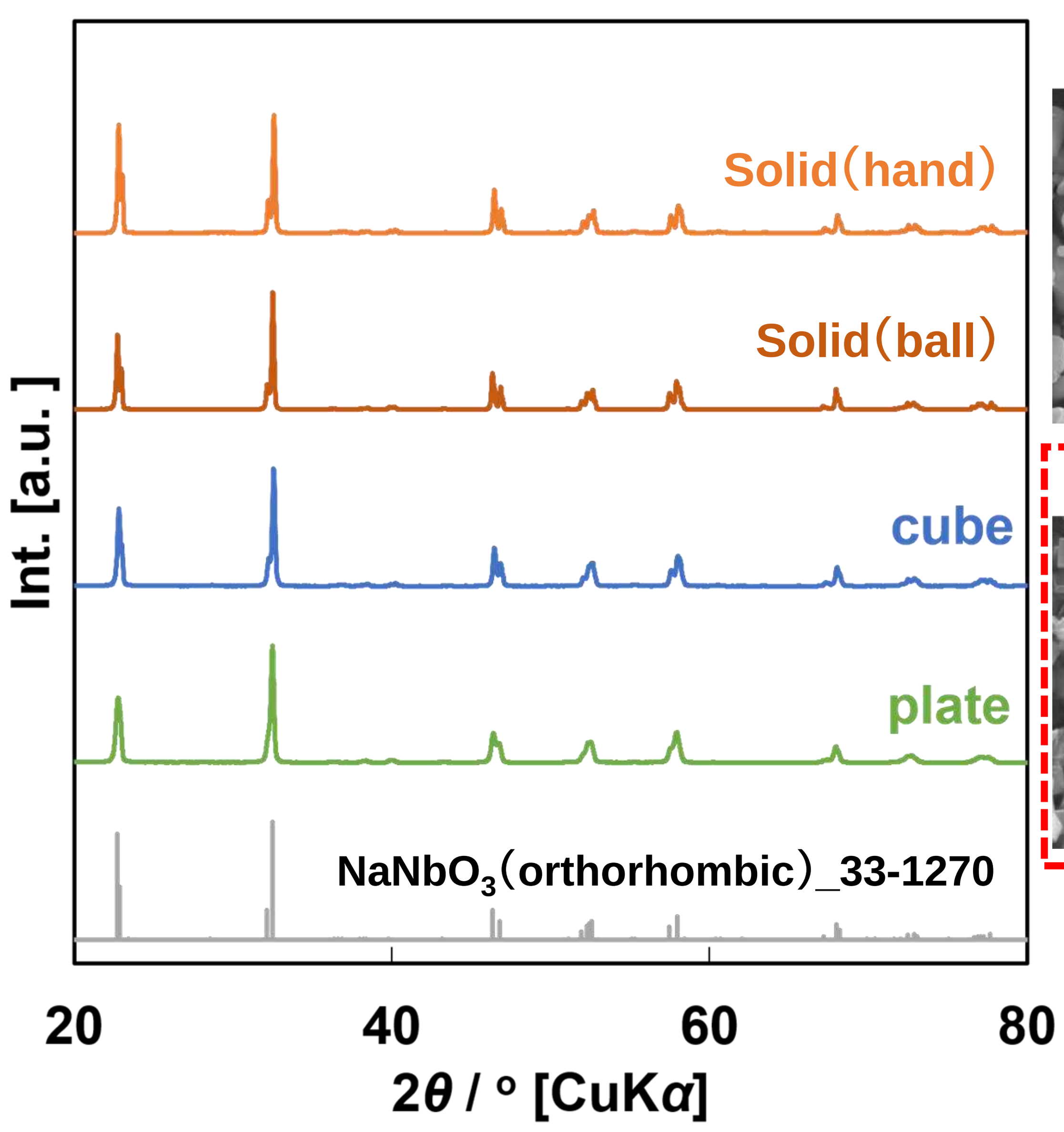
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility

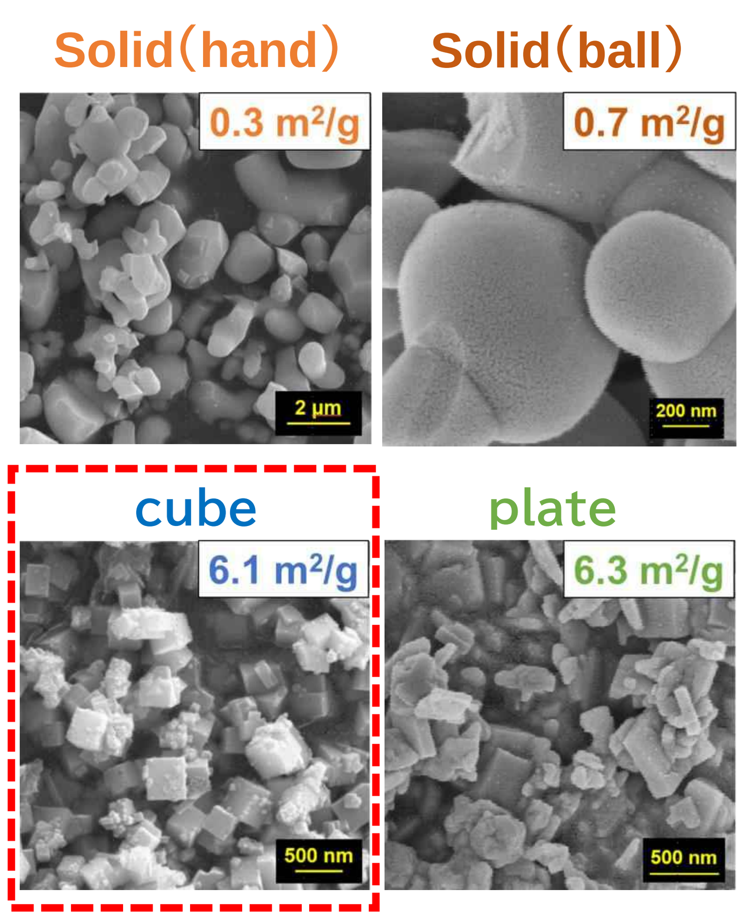
Theme: Nanocomposites with Photo-catalytic Switch

Organization: TUS, JAIST
Contact: k.katsumata@rs.tus.ac.jp, taniike@jaist.ac.jp

1.1. Synthesis of ON-type photocatalyst



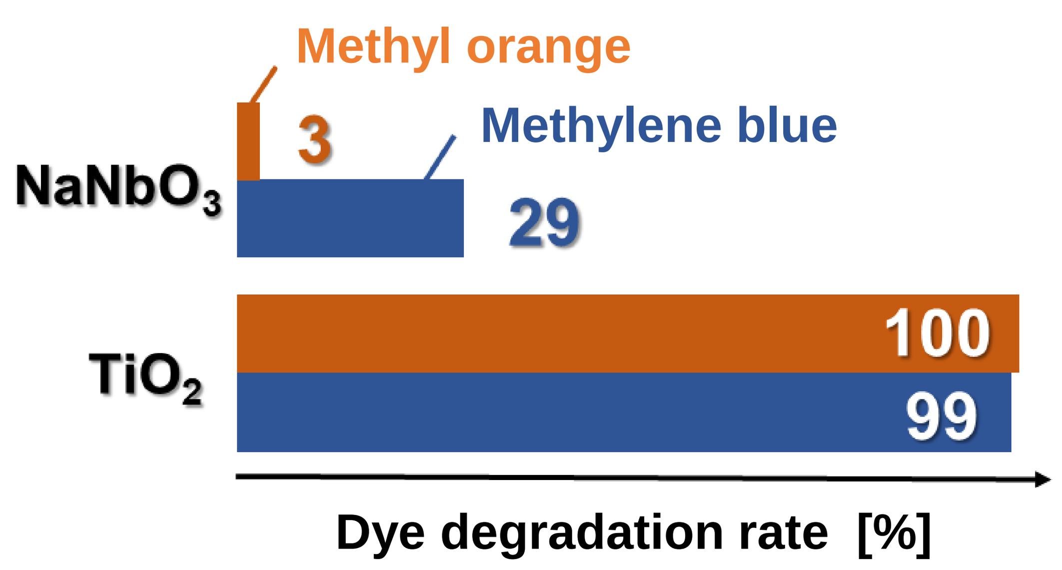


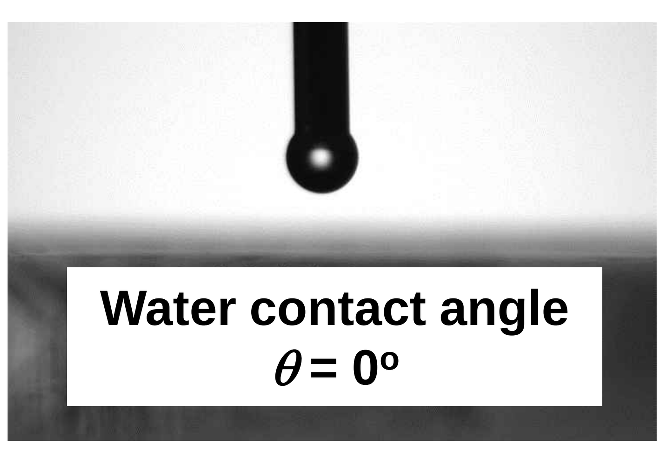


【1a】The same $NaNbO_3$ can be prepared.

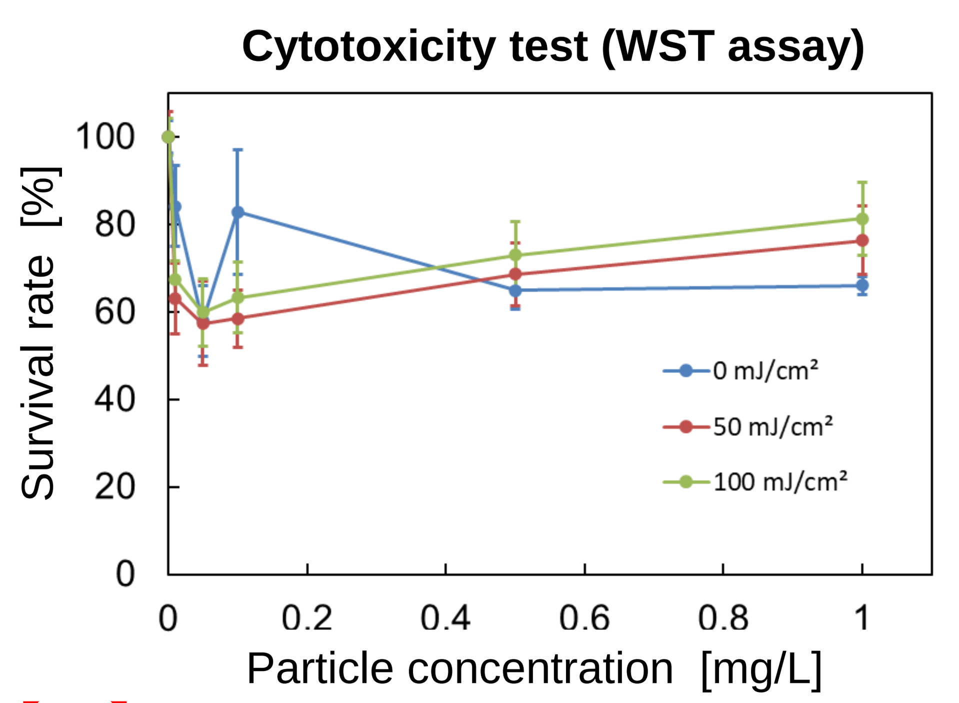
Characterization
- Photocatalytic activity
  → Photodegradation of dye
  → Photoinduced hydrophilicity
- Cytotoxicity test
  → WST assay
- Antimicrobial performance
  → *Escherichia coli (E. coli)*

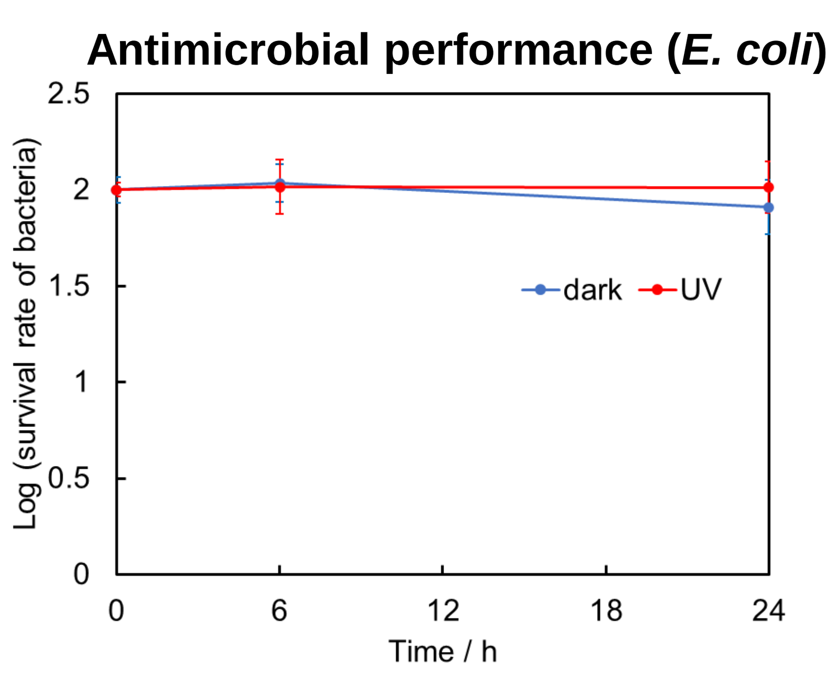
1.2. Photocatalytic activity





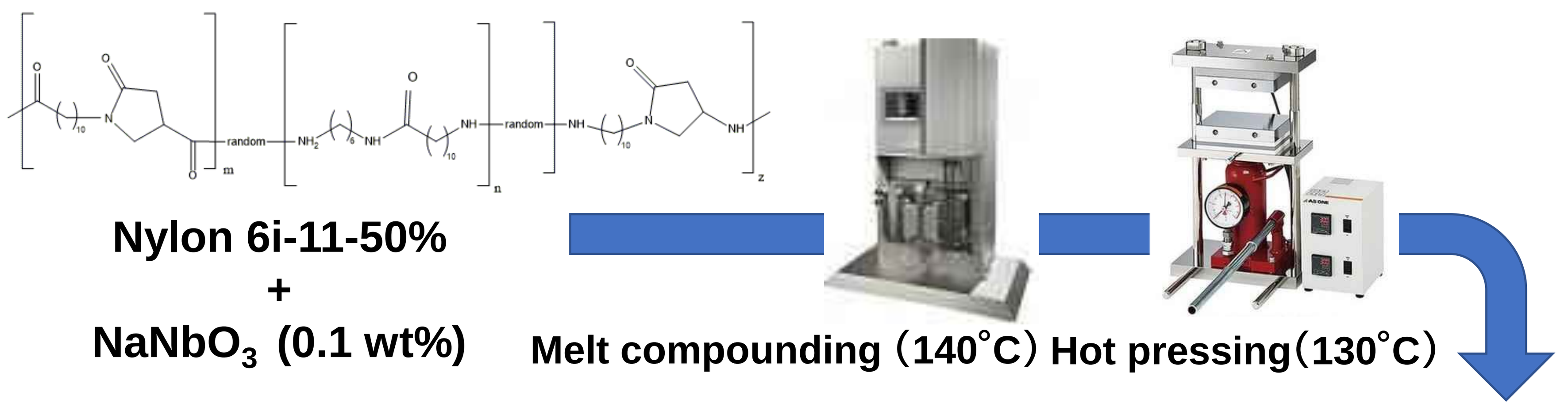
【1b】$NaNbO_3$ has lower degradation activity than TiO_2.
【1c】$NaNbO_3$ exhibits photoinduced superhydrophilicity.

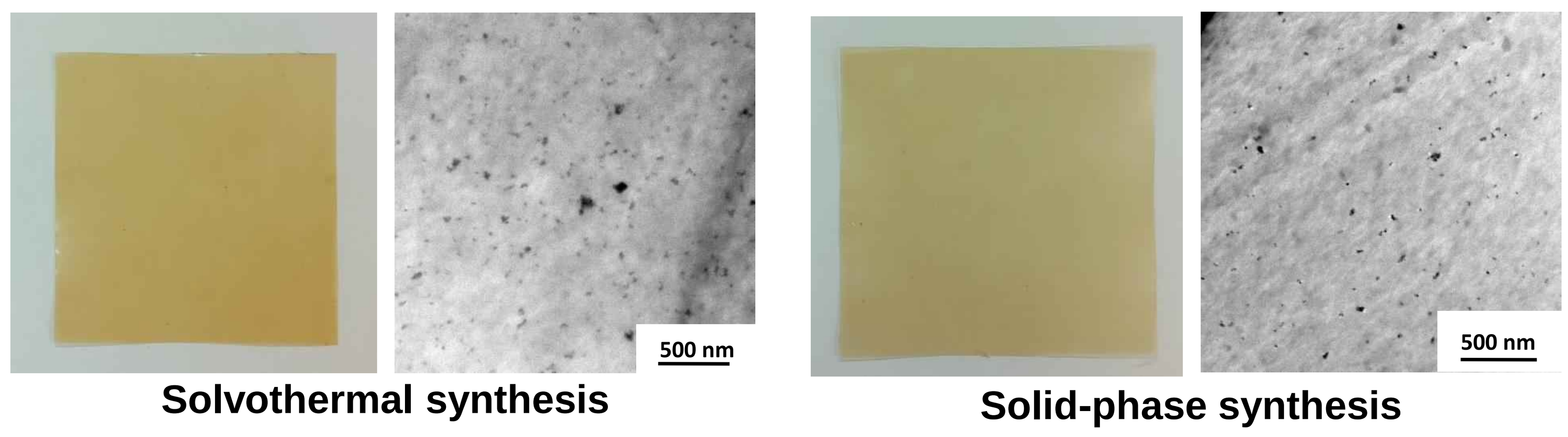




【1d】$NaNbO_3$ has little or no cytotoxic under light irradiation.
※Ack. Prof. C. Ogino (Kobe-U)
【1e】$NaNbO_3$ has little or no antimicrobial performance under light irradiation.
※Ack. Prof. K. Nakata and Dr. S. Usuki (TUAT)

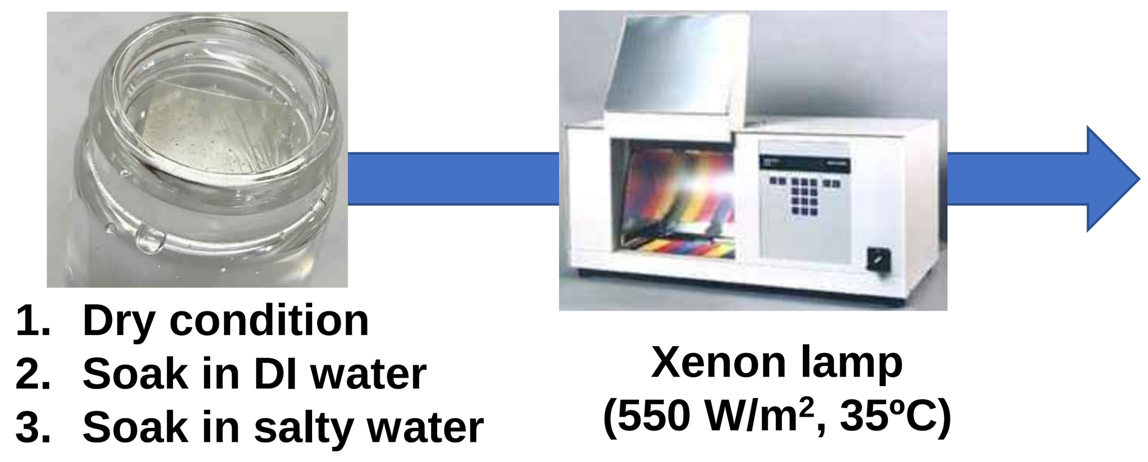
2.1. Nanocomposite films



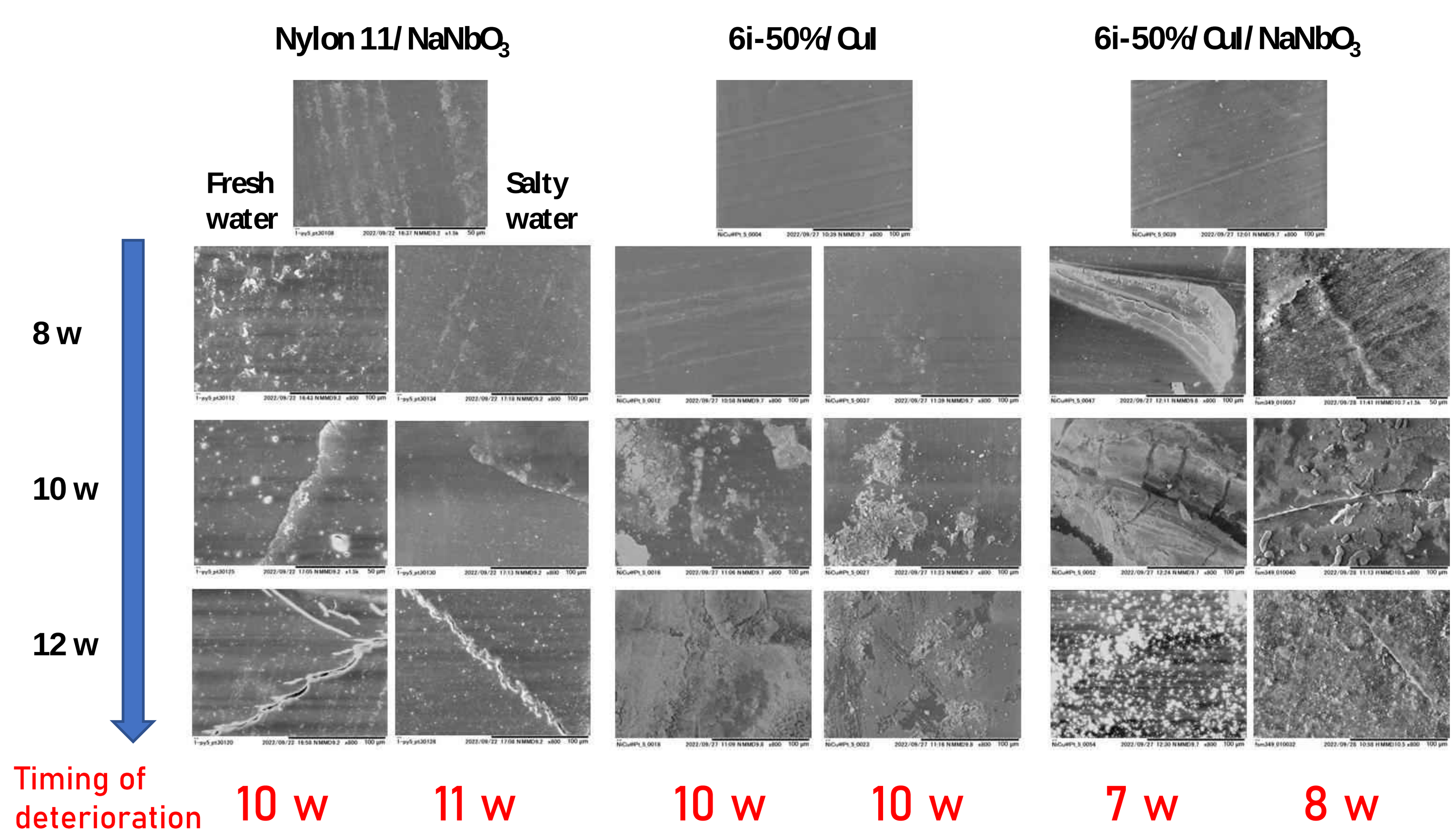


【2a】Nanocomposites with uniform dispersion were obtained.

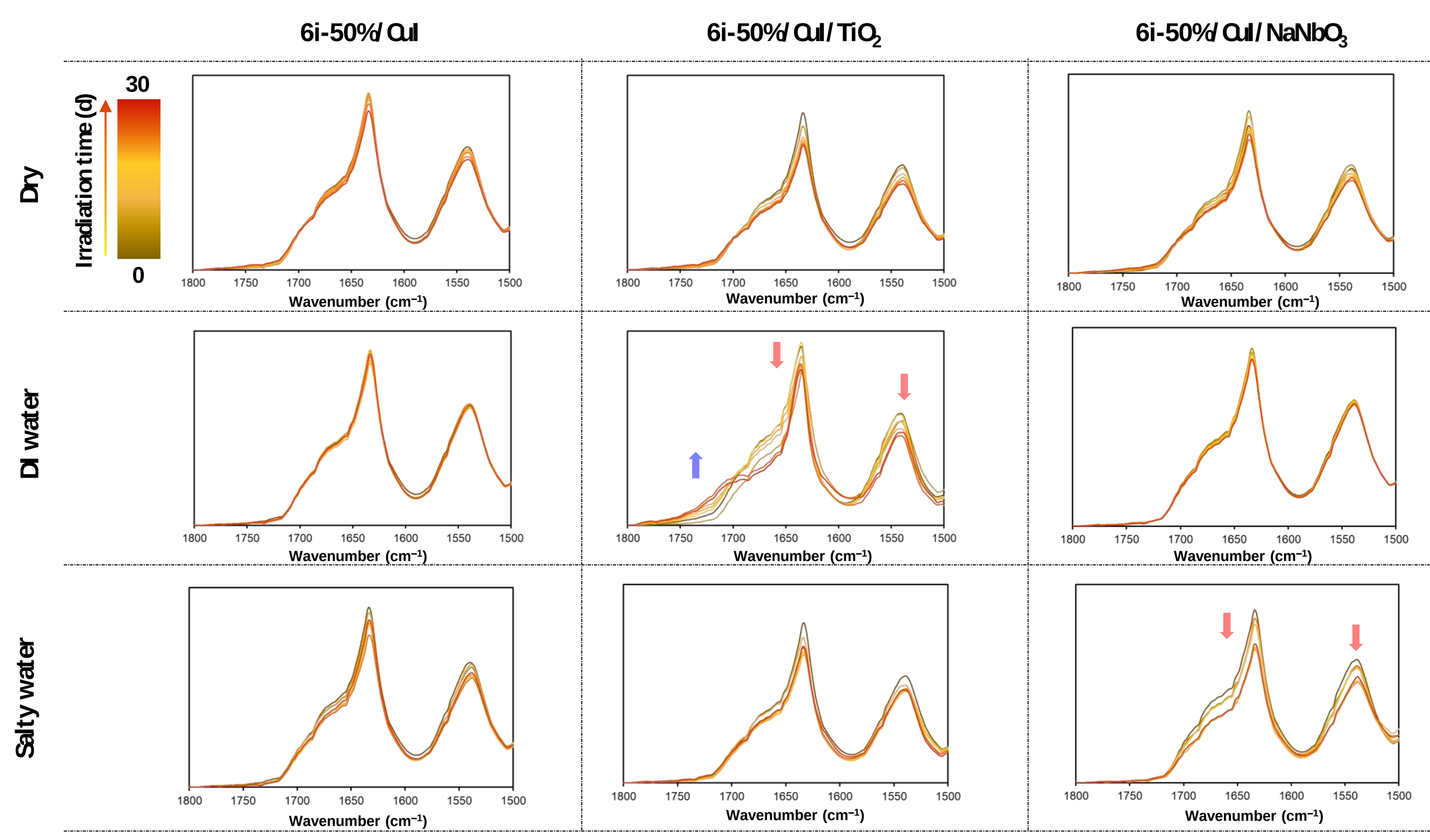
2.2. Photodegradation



Characterization
a. SEM
b. ATR-IR
c. Water contact angle
d. Weight loss
e. NMR
f. LC-MS



【2b】The bio-nylon degrades faster than nylon 11.
【2c】$NaNbO_3$ accelerates the degradation of the bio-nylon.



【2d】The addition of CuI stabilizes the bio-nylon.
【2e】TiO_2 accelerates oxidative degradation in DI water.
【2f】$NaNbO_3$ accelerates hydrolysis under irradiation.
【2g】The $-CH_2-$ groups adjacent to N (amide) of the pyrrolidine ring is first oxidized, followed by hydrolysis.

Ack. Dr. D. Kato (Kagoshima-U)

No. A-13-4E
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility
Theme: ON type: Biodegradation and Safety Assessment

Organization: JAIST, Kobe U, AIST

Contact: kaneko@jaist.ac.jp, okamurah@maritime.kobe-u.ac.jp, a.nakayama@aist.go.jp, daisuke@port.kobe-u.ac.jp

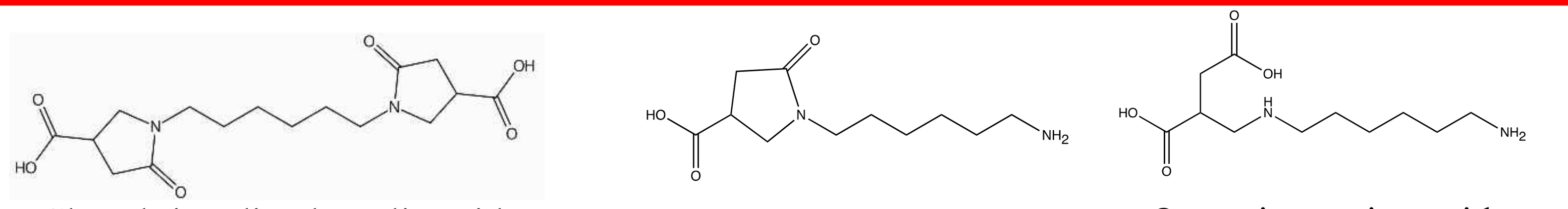
Safety assessment for the life below water

Predicted no-observed-effect concentrations (PNECs) of water-soluble degradation products from ON-type resins were derived from the acute toxicity values* on aquatic species (Table 1).

・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.



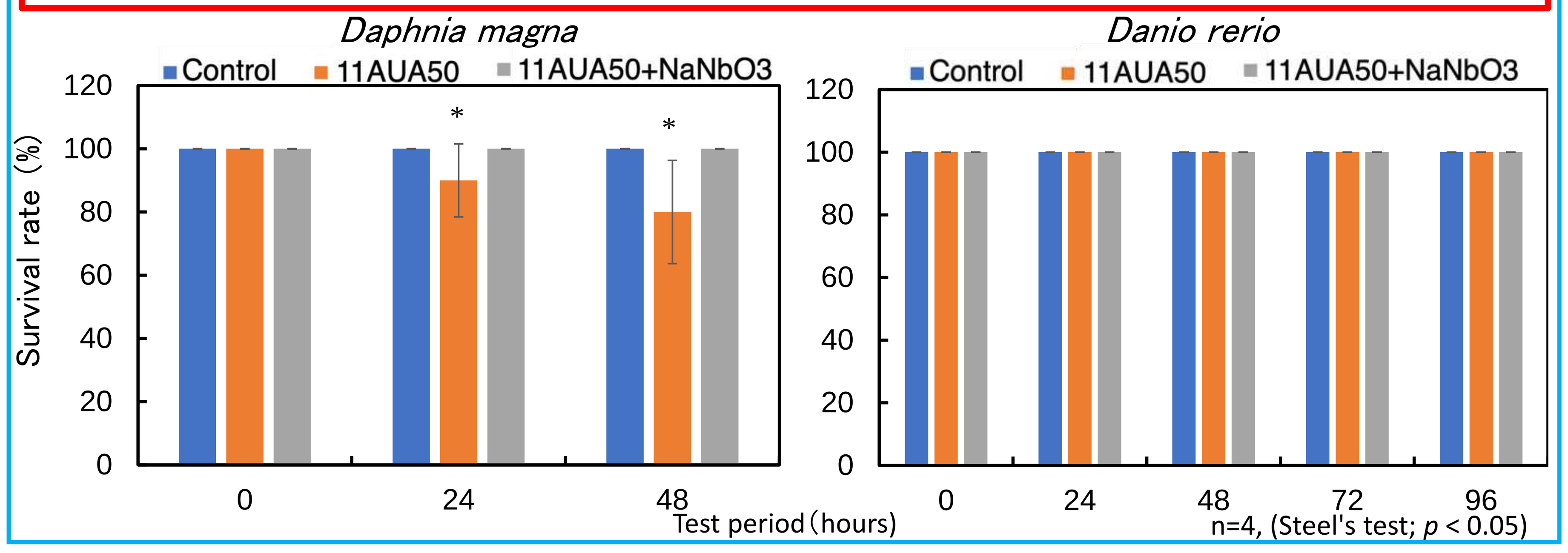
Closed ring dicarboxylic acid　　Closed ring amino acid　　Open ring amino acid

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

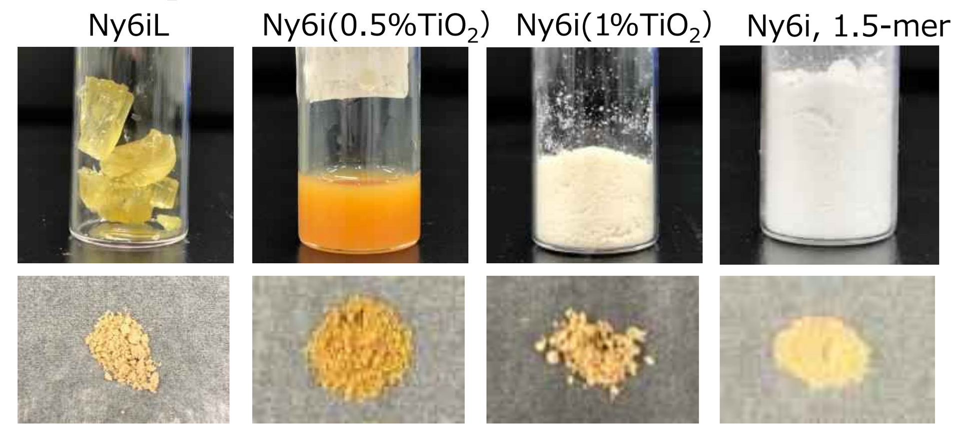
test organisms	Closed ring: Dicarboxilic type 1.5 dimer	Closed ring: Amino acid type monomer*	Open ring: amino acid type monomer*
Marine luminescent bacteria	> 1,000	>10,000	>10,000
Marine microalgae	> 1,000	7,200	7,100
Brine shrimp	> 1,000	>10,000	>10,000
Marine rotifer	> 1,000	>10,000	>10,000
Freshwater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

*including salt

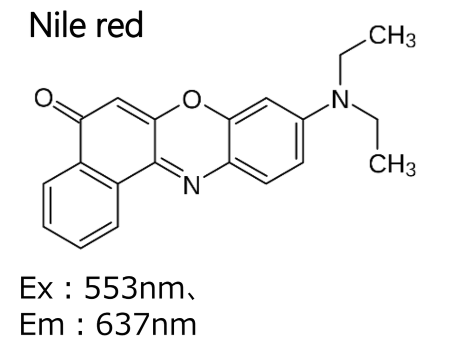
Estimated acute toxicity effects of 11AUA50 and 11AUA50+NaNbO3 on *Daphnia magna* and *Danio rerio*. The acute toxicity assay were based on OECD test 202 (for *Daphnia magna*) and test 203 (for *Danio rerio*) with minor modifications.

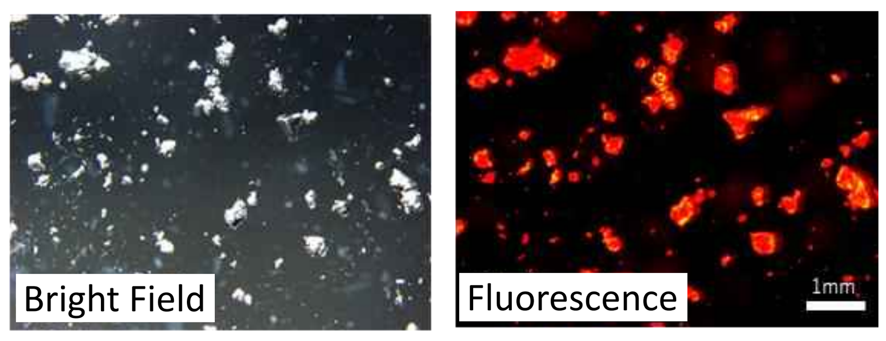


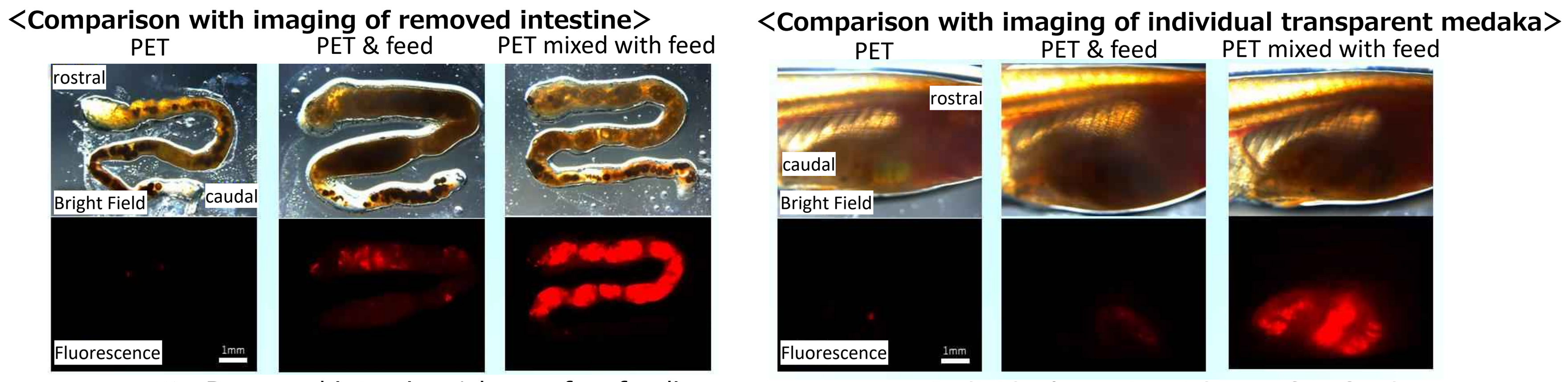
Conversion of plastic to feed for medaka



Monitoring oral feeding of medaka by fluorescent staining of plastics

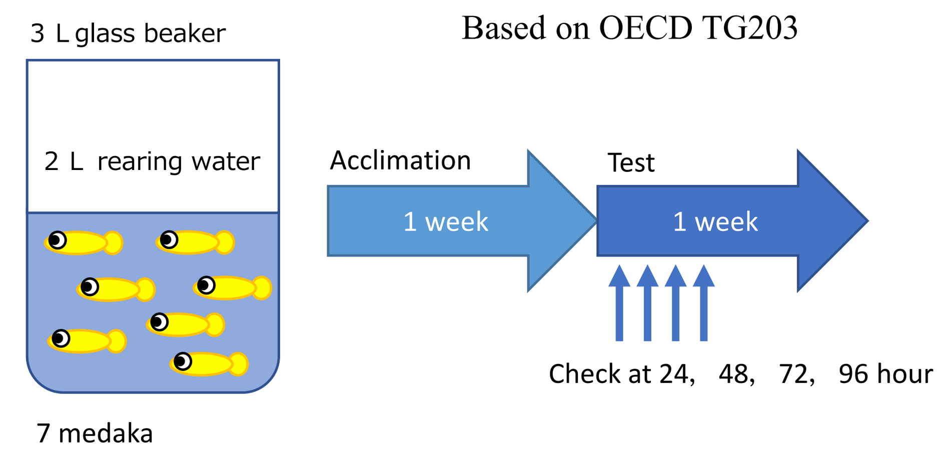






※ Removed intestine 1 hour after feeding　　※ Individual imaging 1 hour after feeding

Acute toxicity test by medaka



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

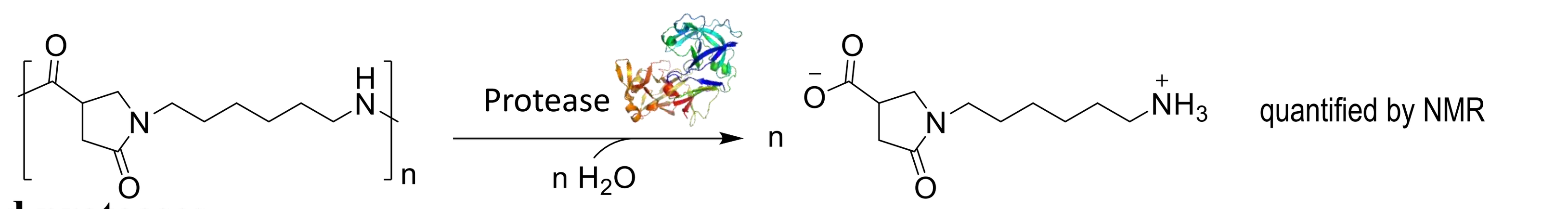
Appropriate feeding amount: 2~3% of Body weight (250mg)
≒7.3mg/medaka/day→360mg/week
Feed : Plastic = 360mg : 180mg
→ Plastic : 3.7mg/medaka/day

To examine the effects in more detail, we are analyzing changes in gene expression in the intestine after plastic feeding.

In vivo enzymatic degradation

1. NMR quantitative method

An evaluation method for the enzymatic degradation of synthetic polymers possessing amide bonds was developed. Proteases, which digest proteins and peptides in living organisms, were chosen for hydrolysis of the peptide bonds within the targeted polymer. The generated monomers could be detected by NMR spectroscopy.



Tested proteases

1. Pepsin (aspartic protease), optimum pH1-3, cleave the bond neighboring acidic or aromatic amino acids
2. Papain (cysteine protease), optimum pH7-8, cleave the bond neighboring basic or Glycine or Leucine
3. Trypsin (serine protease), optimum pH7-8, cleave the bond neighboring basic amino acids
4. Chymotrypsin (serine protease), optimum pH8-9, cleave the bond neighboring aromatic amino acids

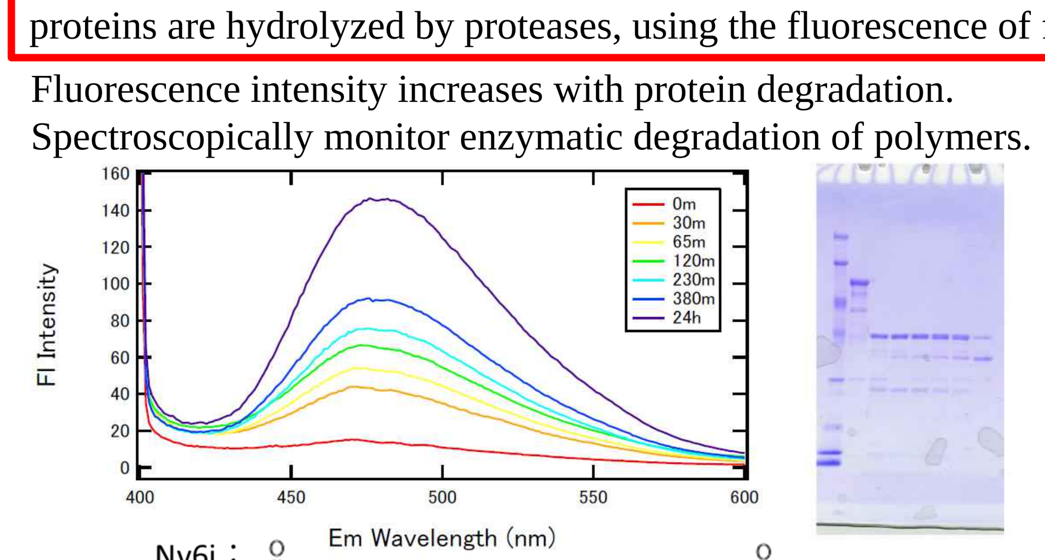
Method

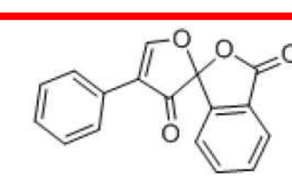
A protease and the polymer were mixed in a buffer with the optimum pH. After the reaction, the solid was filtered off, and the resultant solution was analyzed by NMR spectroscopy to detect soluble monomers.

2. Terminal amino group determination method by spectroscopic method

Developed a method to detect amino groups (newly derived from the N-terminus), which increase as proteins are hydrolyzed by proteases, using the fluorescence of fluorescamine.

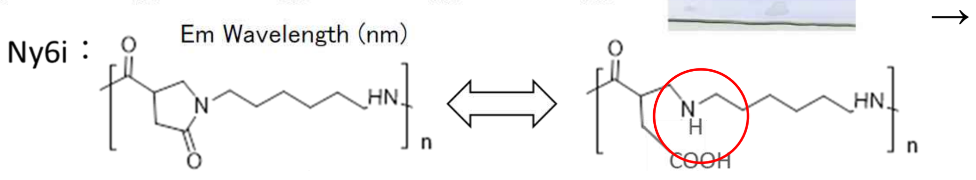
Fluorescence intensity increases with protein degradation.
Spectroscopically monitor enzymatic degradation of polymers.





Distinguish between primary amino groups resulting from hydrolysis of the main chain amide bond and secondary amino groups resulting from cleavage of the main chain lactam structure.

→Analysis of switch function vs photolysis

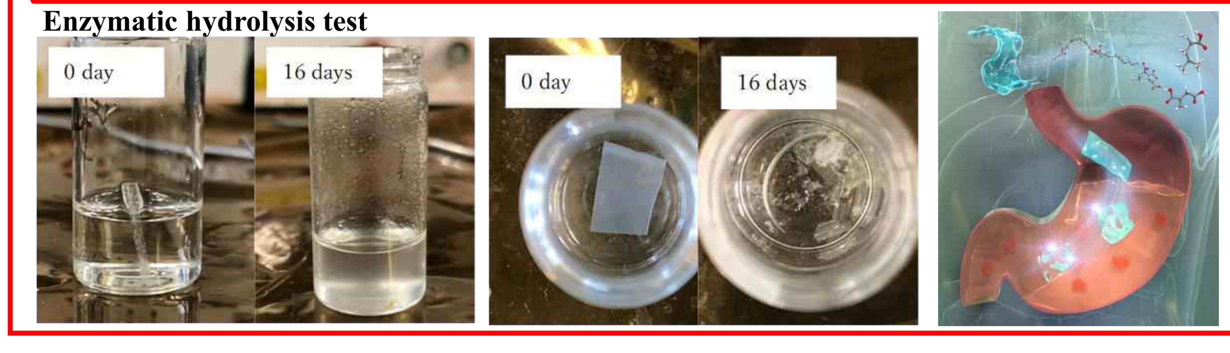


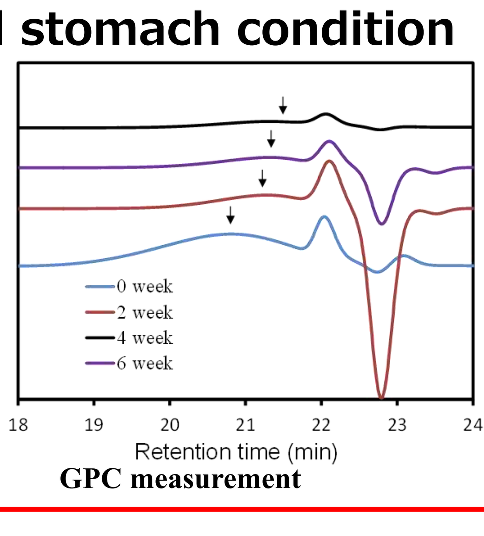
CH2CO
Polymer-OC-CH-CH2-N-(CH2)6-NH_2

3. Degradation test of ON-type nylon under artificial stomach condition

A decrease in the weight and molecular weight of Ny6i11 with pepsin under simulated gastric juice conditions was confirmed.

Enzymatic hydrolysis test



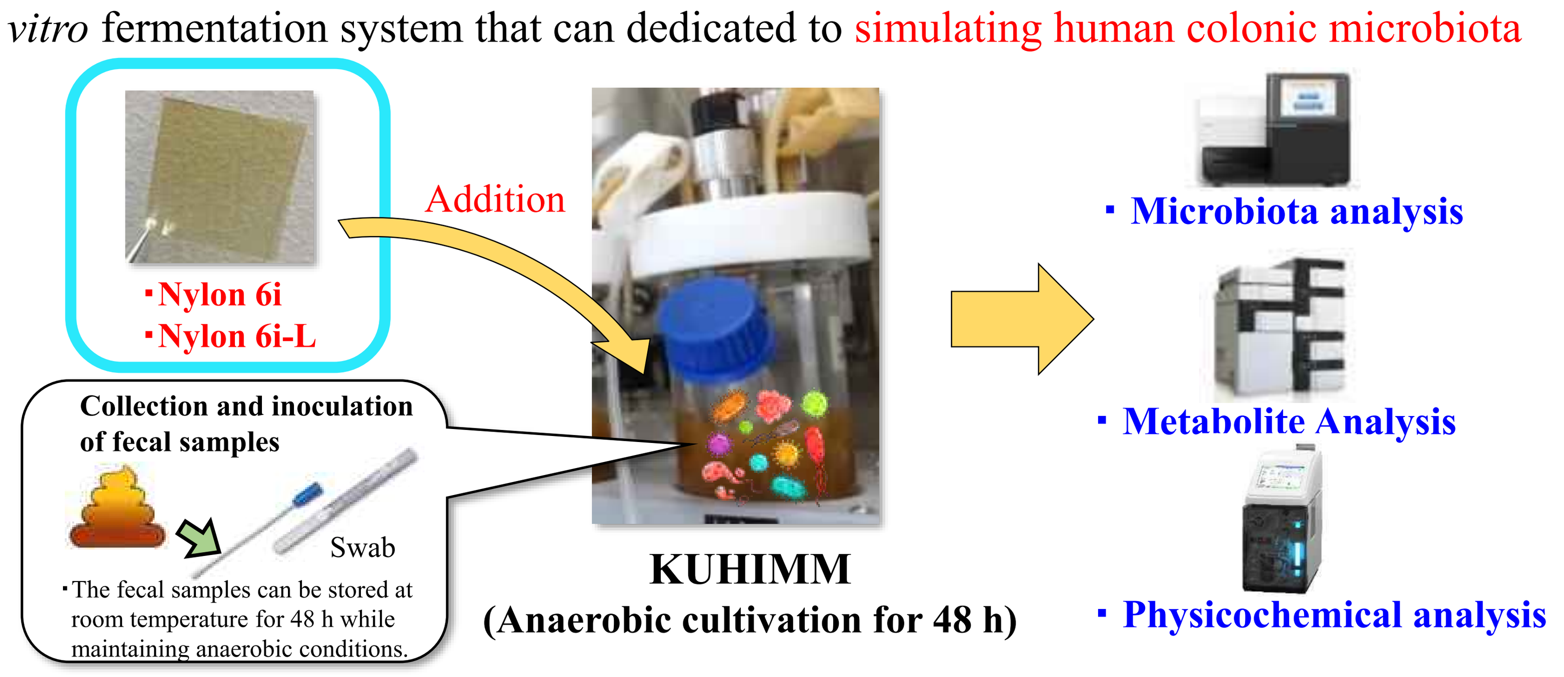


GPC measurement

Evaluation of degradability and safety in mimicked intestinal environment

KUHIMM (Kobe University Human Intestinal Microbiota Model)

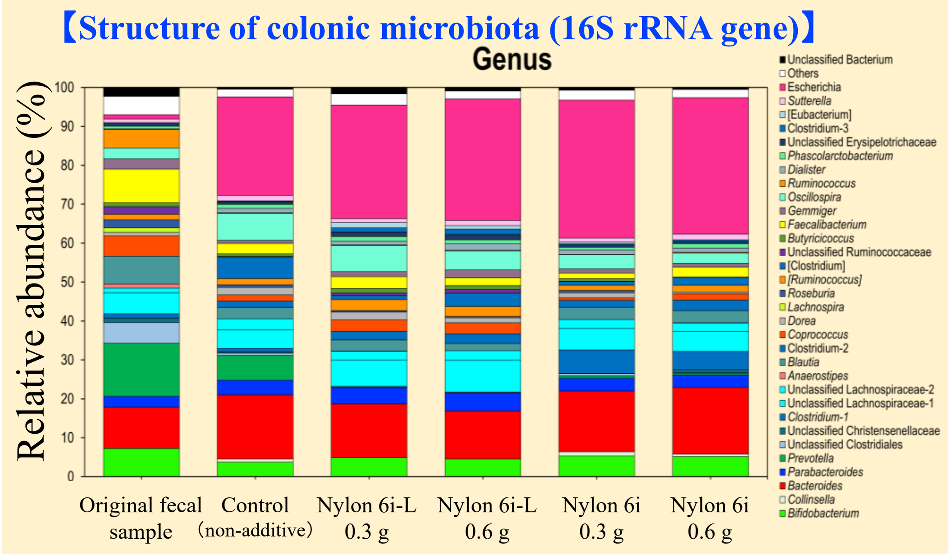
・*in vitro* fermentation system that can dedicated to simulating human colonic microbiota



→ Evaluation of degradability and safety when biodegradable plastics are ingested by humans and marine mammals using KUHIMM

Evaluation of effects on the human intestinal environment

【Structure of colonic microbiota (16S rRNA gene)】



【Metabolites (Short-chain fatty acids)】

	Control (non-additive)	Nylon 6i-L 0.3 g	Nylon 6i-L 0.6 g	Nylon 6i 0.3 g	Nylon 6i 0.6 g
Acetate (mM)	97.5	99.3	105.6	87.8	94.8
Propionate (mM)	28.1	24.6	26.7	25.2	29.6
Butyrate (mM)	20.7	18.2	16.0	33.6	27.6

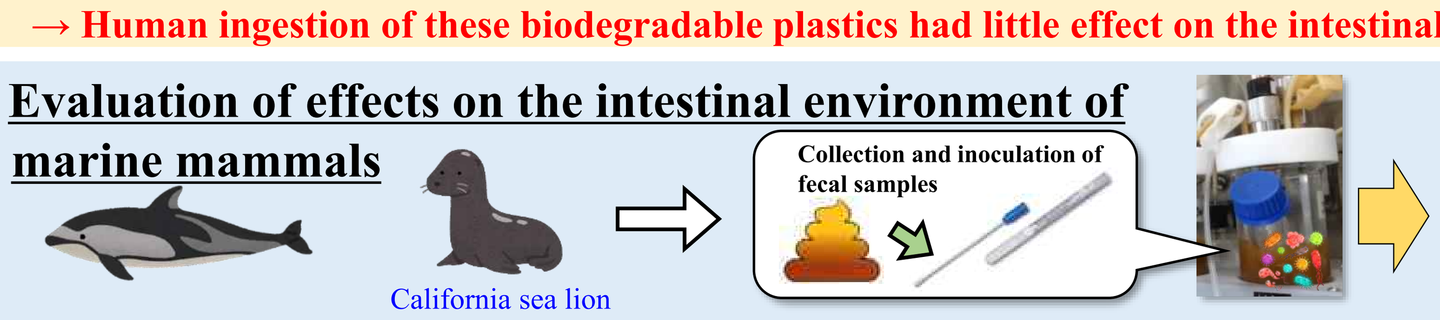
【Total organic carbon (TOC) of culture supernatant 】

	Control (non-additive)	Nylon 6i-L 0.3 g	Nylon 6i-L 0.6 g	Nylon 6i 0.3 g	Nylon 6i 0.6 g
Before cultivation (mg/L)	16,000	16,500	19,500	15,500	16,500
After cultivation (mg/L)	13,500	15,000	17,000	12,500	14,179
Reduction (mg/L)	2,500	1,500	2,500	3,000	2,321

・Addition of the test samples did not significantly change the structure and diversity of the human colonic microbiota and its metabolites.
・Addition of the test samples did not significantly change the total carbon loss before and after cultivation, suggesting little potential for microbial utilization of the sample-derived components.

→ Human ingestion of these biodegradable plastics had little effect on the intestinal environment

Evaluation of effects on the intestinal environment of marine mammals



・In order to construct the world's first *in vitro* culture model of the colonic microbiota of marine mammals, an MTA was signed with an aquarium for the provision of fresh fecal samples of two species of marine mammals.

→ Fecal samples will be collected soon, and then construction of the evaluation system will begin.

No. A-13-5E

PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility

Theme: ON type: Evaluation of Marine Biodegradation

Organization: Kagoshima U, Kobe U, AIST, ORIST
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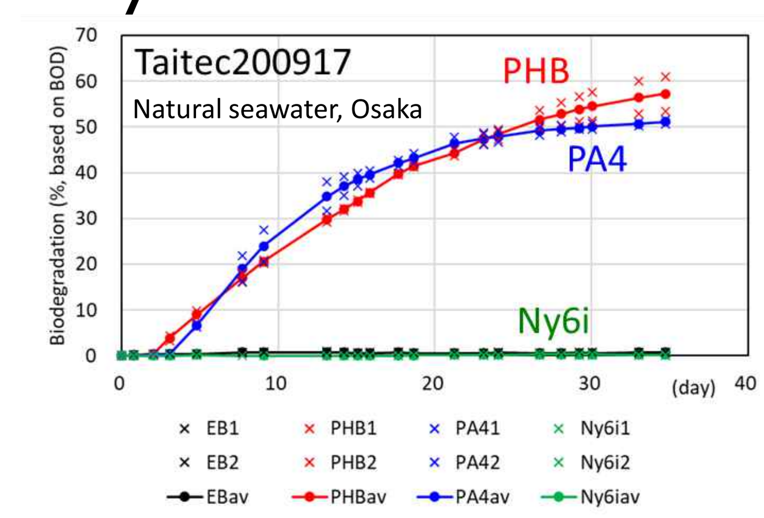
Biodegradation of ON type Polymer

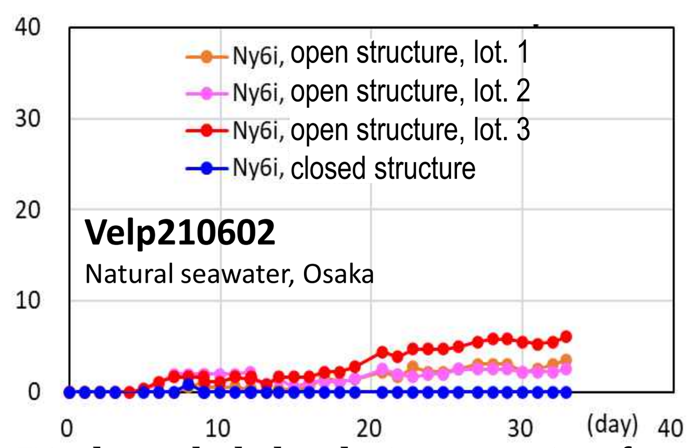
Ny6i :

Biodegradation BOD test

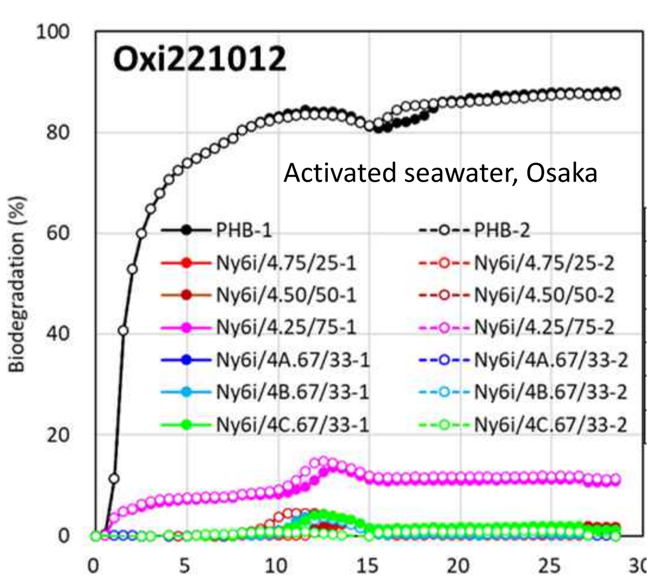
Polymer



Stable in the OFF state (closed ring form)

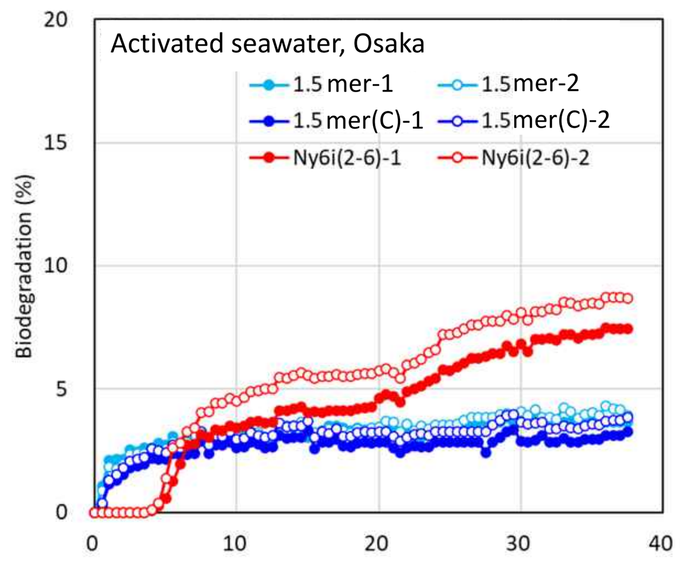


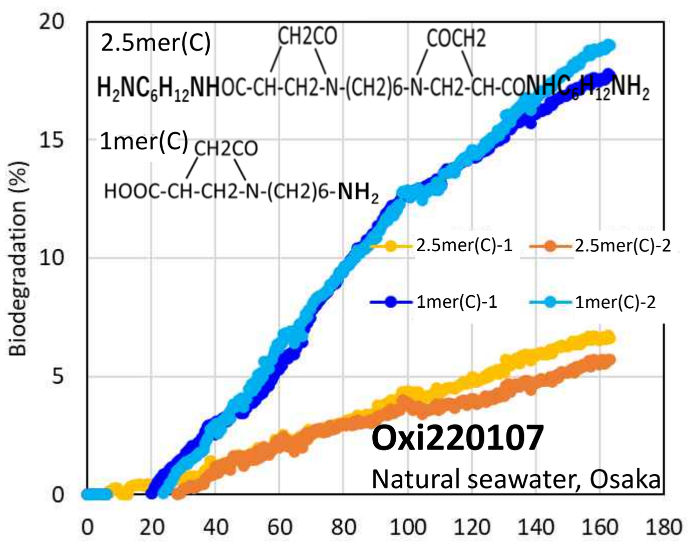
Biodegraded slowly in open-ring form

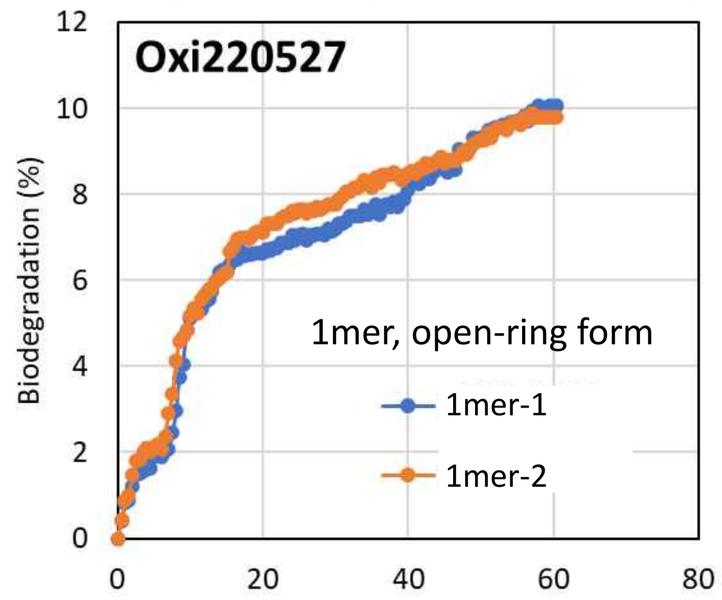


Copolymers were biodegraded even in the OFF state (closed ring form)

Monomers



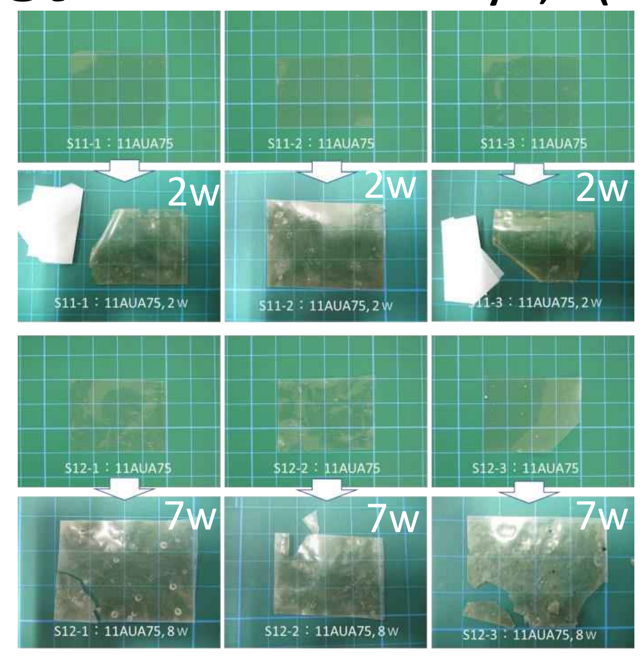




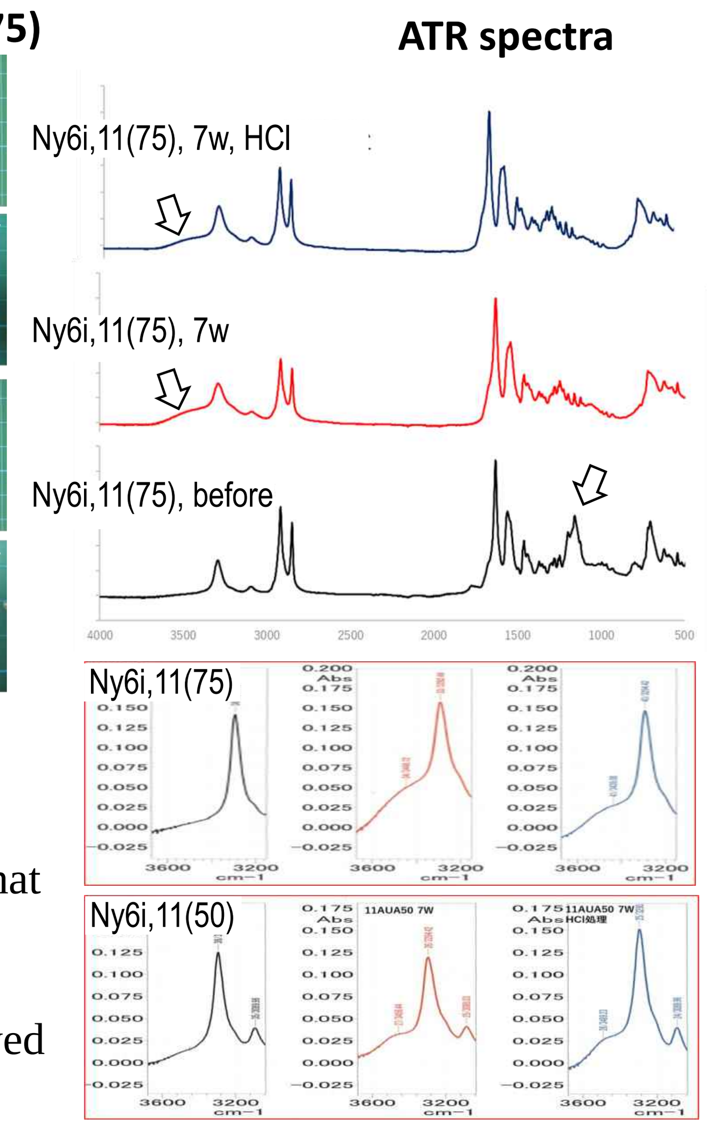
Biodegraded regardless of whether monomers are ring-closed or -opened

Marine immersion test

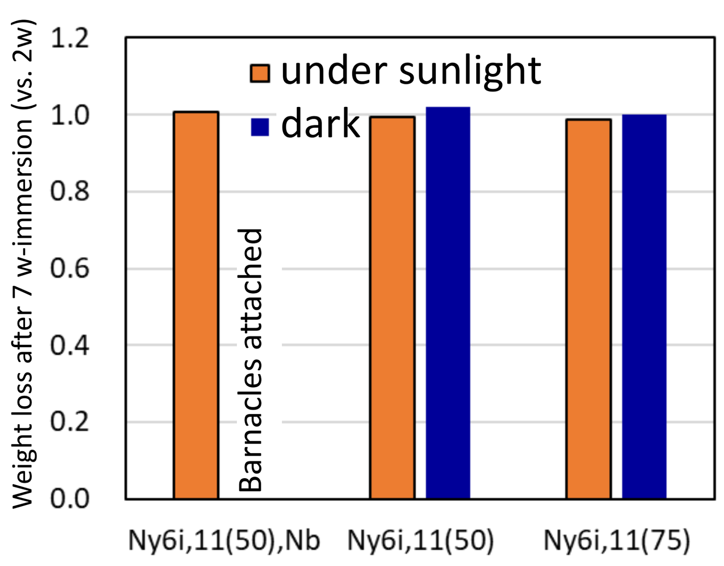
before and after of Ny6i,11(75)

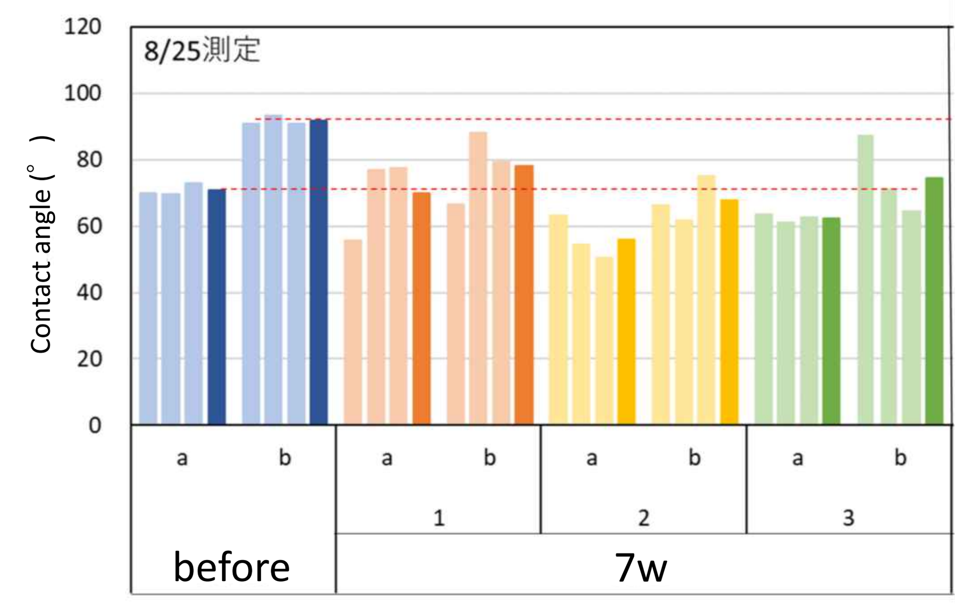


ATR spectra



Almost no change in weight and appearance after immersion. However, ATR analysis showed that hydrolysis of the amide bond had proceeded, and contact angle measurements showed increased surface hydrophilicity.



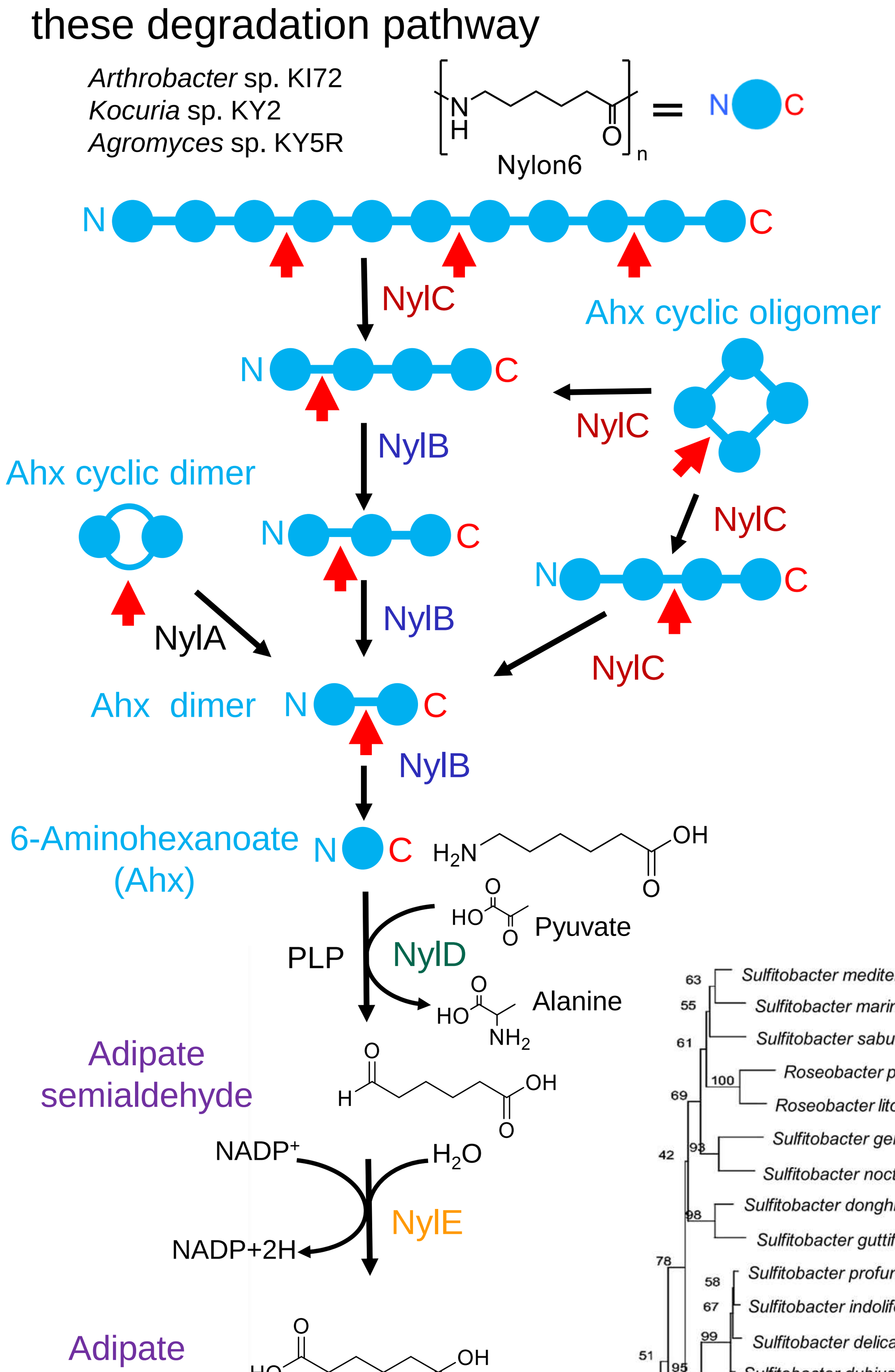


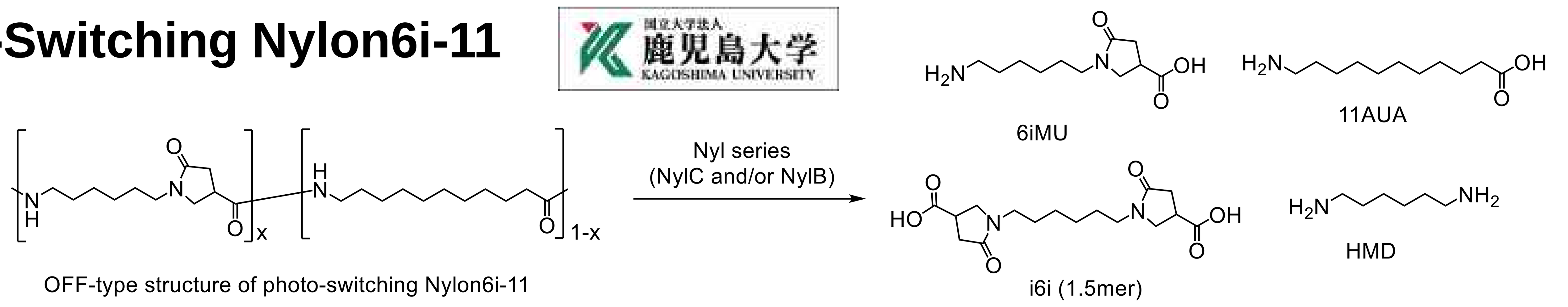
Contact angle

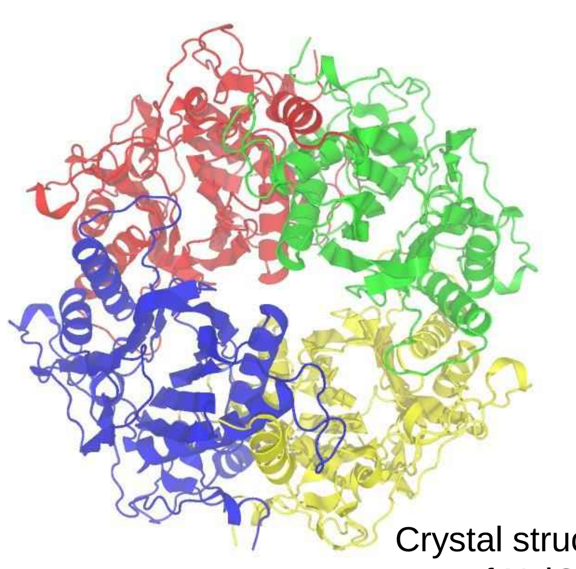
A and b show the front and back sides of the film.

Ocean Degradability of Photo-Switching Nylon6i-11

Nylon degrading microorganisms and these degradation pathway





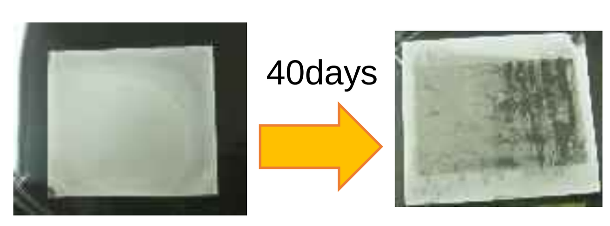


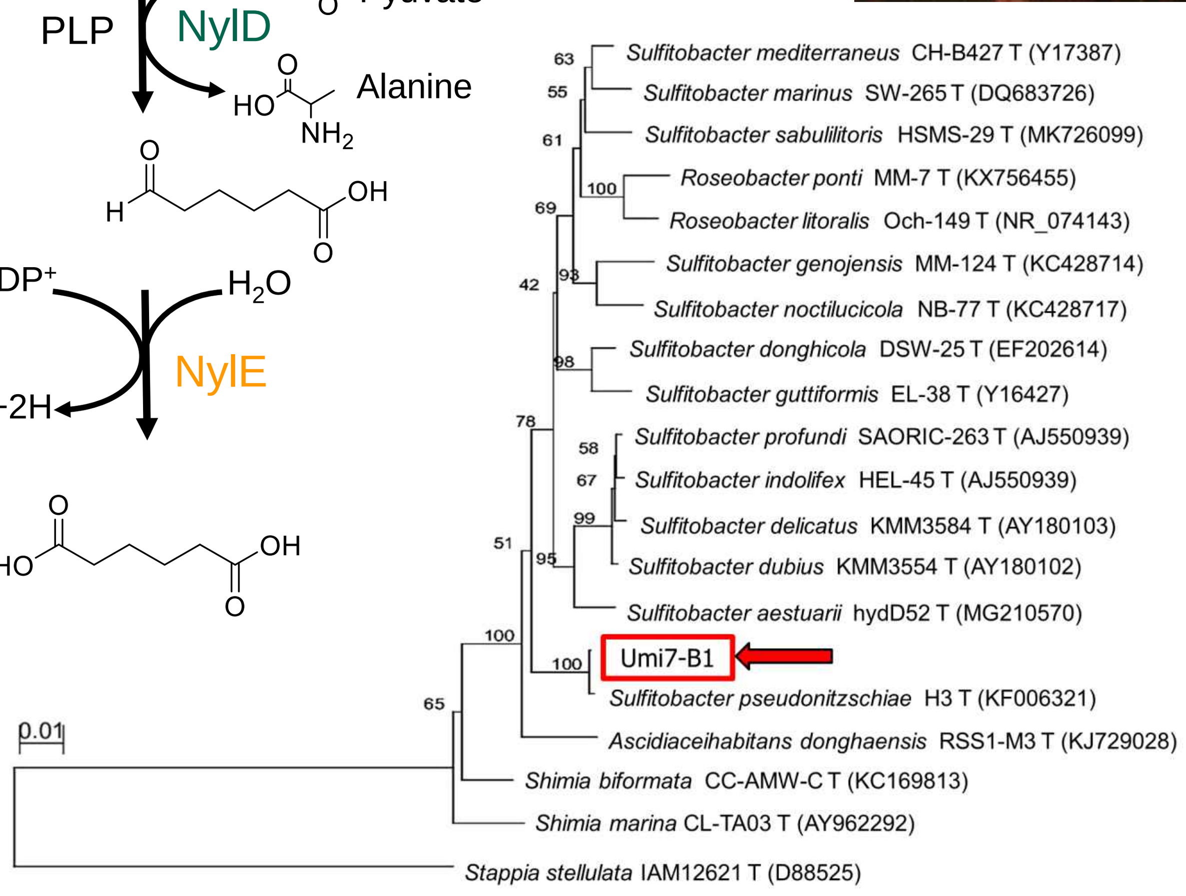
Crystal structure of NylC (tetramer)

Photo-switching Nylon		x	M_w	Pretreat.[1]	Monomerization (%)
H-Nylon6i		1	67,000	−	50
				+	−
Nylon6i-11(50%)		0.5	61,100	−	3
				+	64
Nylon6i-11(75%)		0.25	120,800	−	0.1
				+	26

1: Dai-Ichiro Kato, Yuki Shiraishi, JP2022-150274.

The biodegradability by the Nyl series enzymes was confirmed.

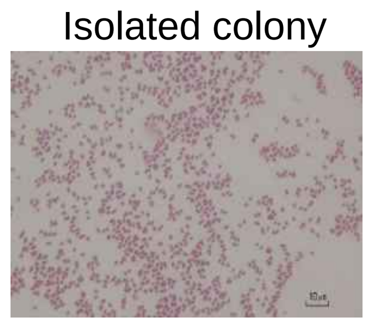




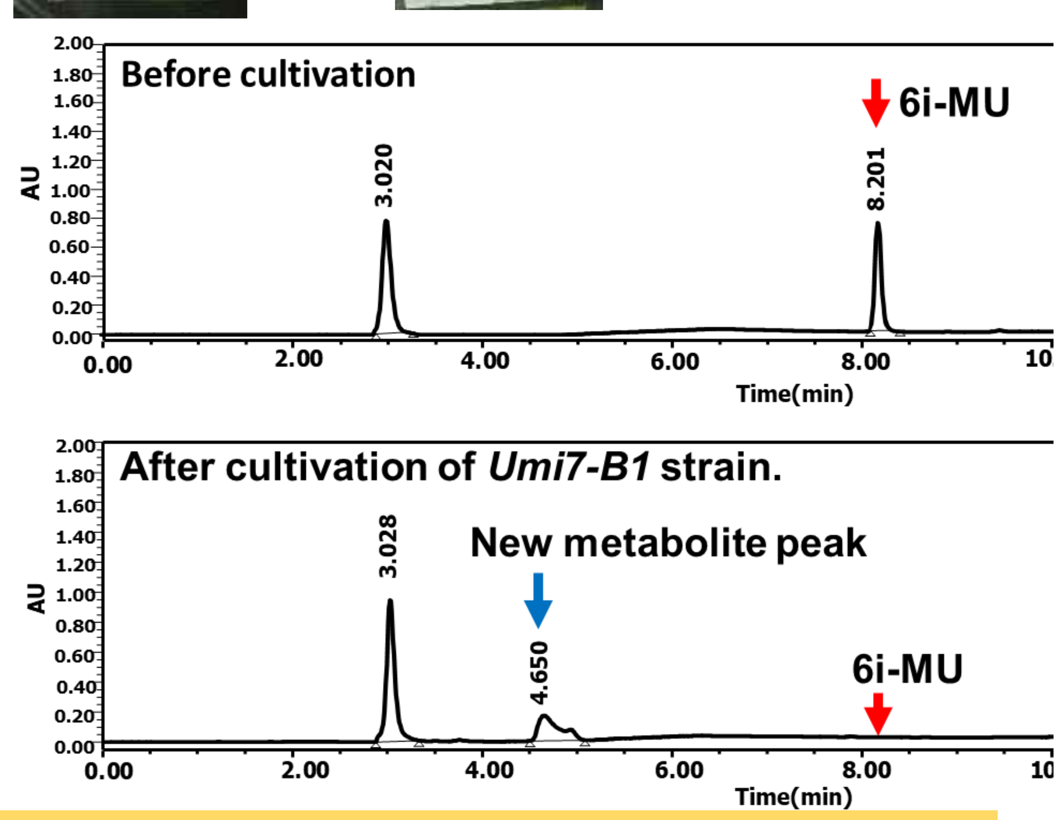
Sulfitobacter gulosus Umi7-B1

protein	Identities toward KI72 enzymes
NylA	28%(1)
NylB	35%(1)
NylC	−
NylD	33%(1)
NylE	41%(1)

Isolated colony



Gram-staining



A new marine bacterium degrading 6iMU was successfully isolated.[2]

2: Dai-Ichiro Kato, Yoko Furuno, Risa Yokoyama, JP2022-034081.

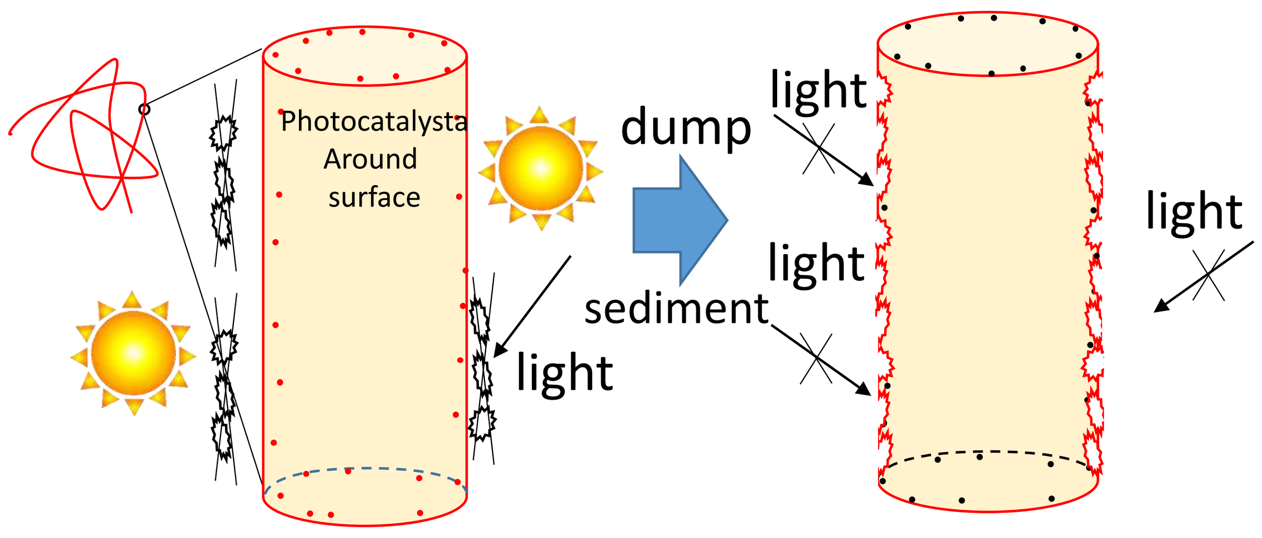
No. A-13-6E
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility
Theme: Design of OFF-type composites with photocatalysts

Organization: TUAT, AIST, ORIST

Contact: nakata@go.tuat.ac.jp

Introduction

OFF-type photoswitching ocean-degradable plastics

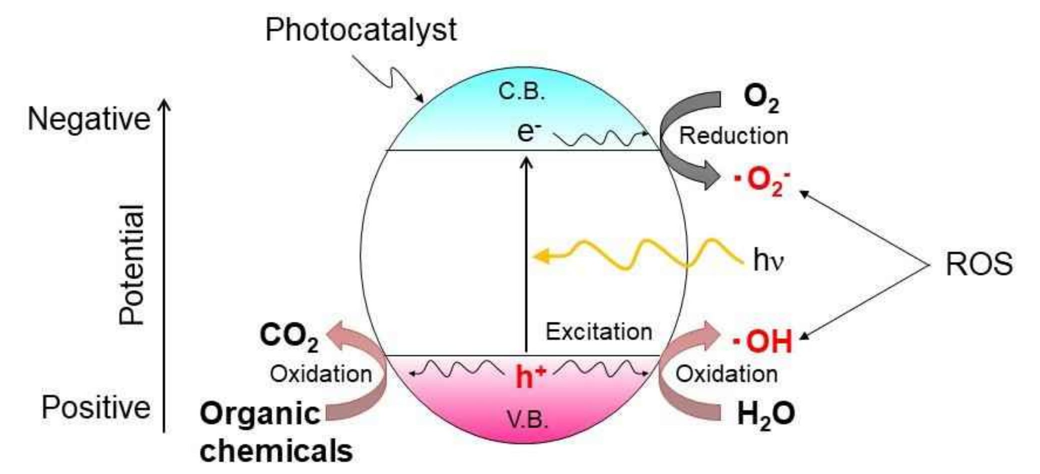


Efficient antibacterial (non-decomposing) over a wide wavelength range during use

Photocatalyst does not work in the dark and biodegradation starts

What kind of photocatalyst is required?

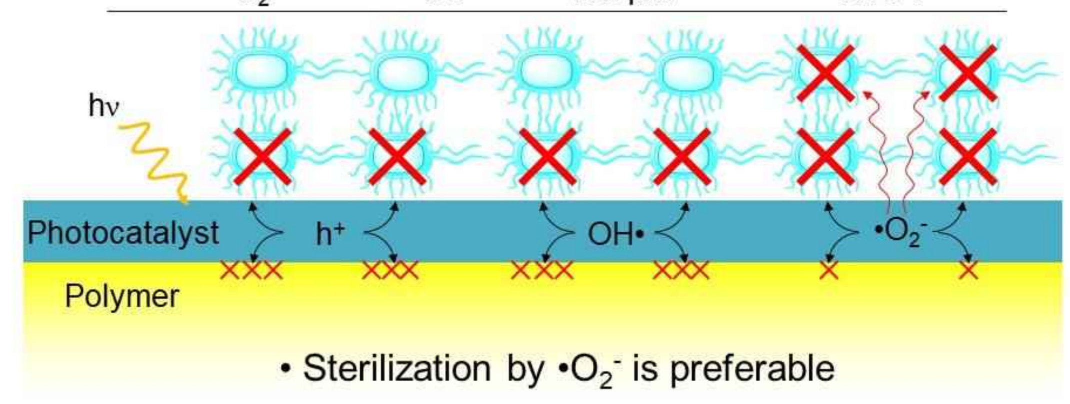
(1) Under visible light, (2) without decomposing polymer, (3) sterilizable photocatalyst



Three types of h^+, OH•, and O_2^-• affect for sterilization and polymer decomposition.

Comparison of ROS

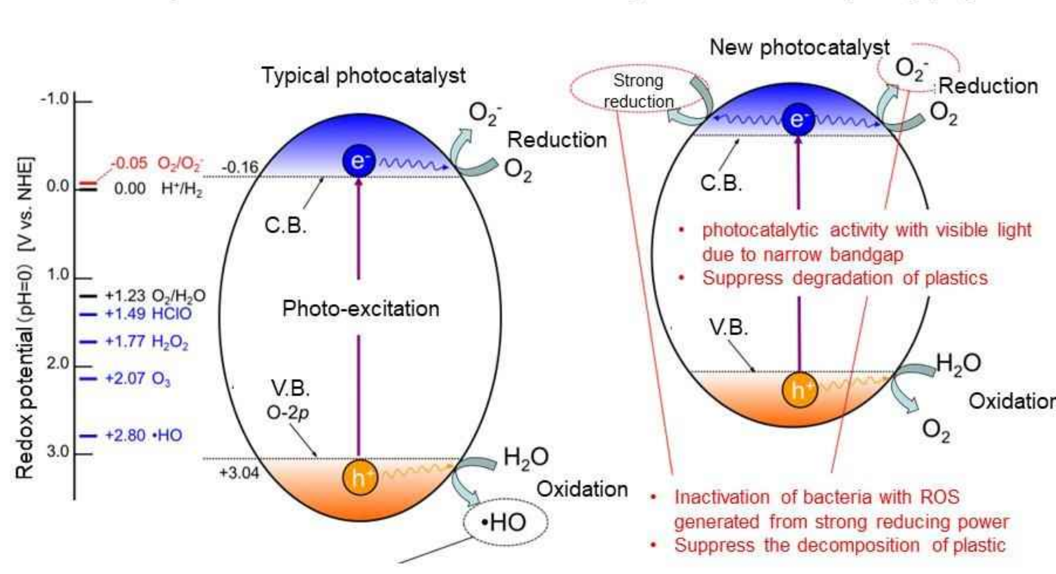
ROS	Life time	Diffusion length	Redox potential (vs. NHE)
h^+	<1 ns	In photocatalyst	Depends on photocatalyst
OH•	70 ns	20 nm	+2.8 V
•O_2^-	5 s	100 μm	+0.16 V



• Sterilization by •O_2^- is preferable

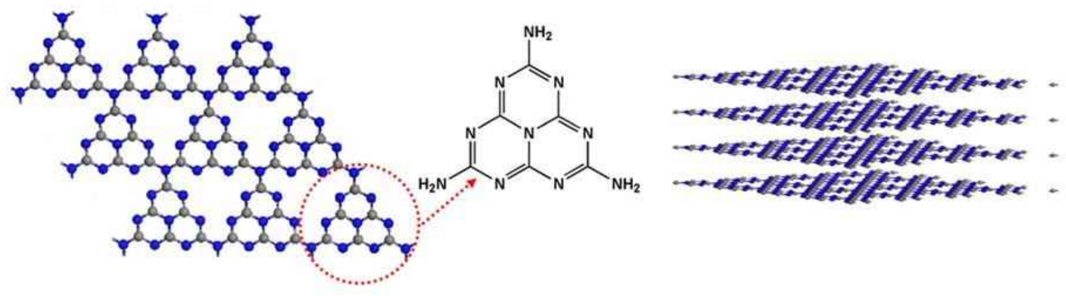
Development of antibacterial photocatalyst

Photocatalyst that can be sterilized under visible light without decomposing polymer



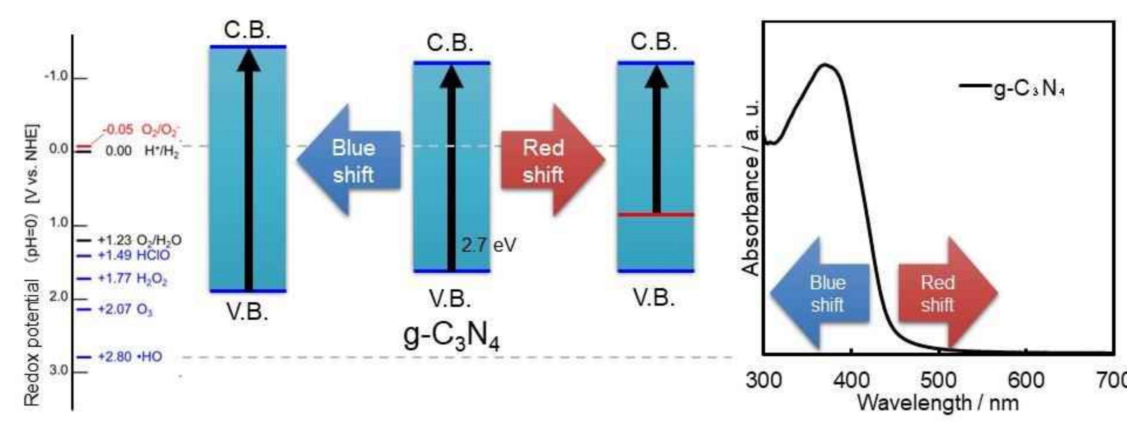
Most organic substances including polymer and bacteria can decomposed by the strong oxidizing power of reactive oxygen species.

g-C_3N_4 photocatalyst



- Visible-light-responsive photocatalyst composed of carbon and nitrogen
- Metal-free and low toxicity
- Layered structure stacking 2D sheets
- 2D sheet can be peeled off by various treatments
- It is possible to dope elements, allowing control of the electronic structure
- Easy to generate O_2•- than OH radicals

Improvement of g-C_3N_4

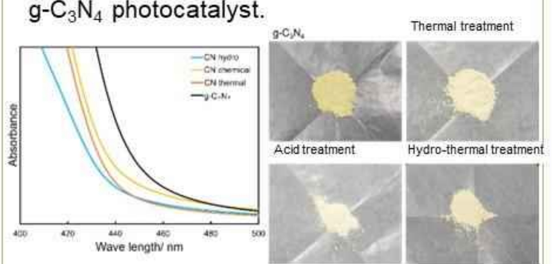


	Blue shift	Red shift
Adv.	High activation by improving oxidization ability (less amount of sample necessary)	Works in environments where short wavelength light is not present (Can be used in a wide range of environments)
Disadv.	Inactivated in the absence of short wavelength light	Low activation due to decreased oxidation ability
Method	Delamination by thermal oxidation treatment	Doping

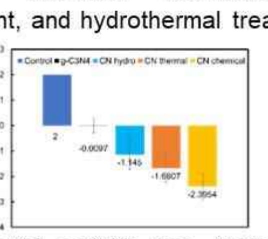
Development of blue-shift type photocatalyst

Effect of treatments

Various treatments were performed on the g-C_3N_4 photocatalyst.

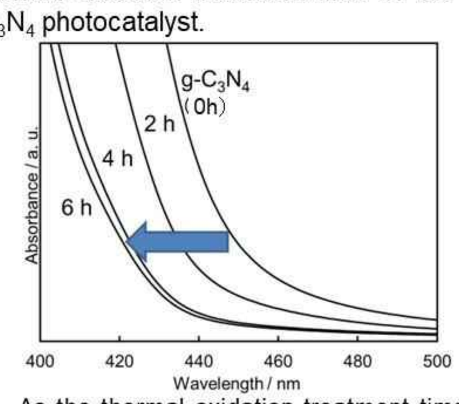


- All of g-C_3N_4 were blue-shifted by thermal oxidation treatment, acid treatment, and hydrothermal treatment.



- Antibacterial activity was improved by thermal oxidation treatment, acid treatment, and hydrothermal treatment of g-C_3N_4.

We investigated the optimization of the thermal oxidation treatment time of the g-C_3N_4 photocatalyst.



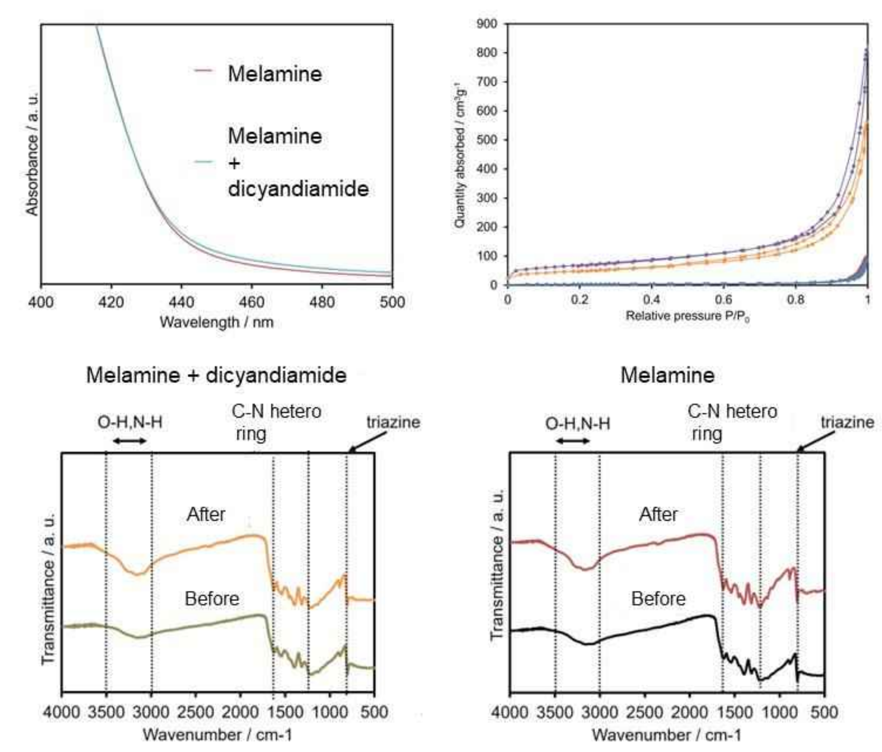
- As the thermal oxidation treatment time became longer, the absorption edge was blue-shifted.
- Since the sample having a long thermal oxidation treatment time is lighter in color than the sample having a short thermal oxidation treatment time, it is expected that it will be easier to apply to white fibers in the future.

Effect of starting material

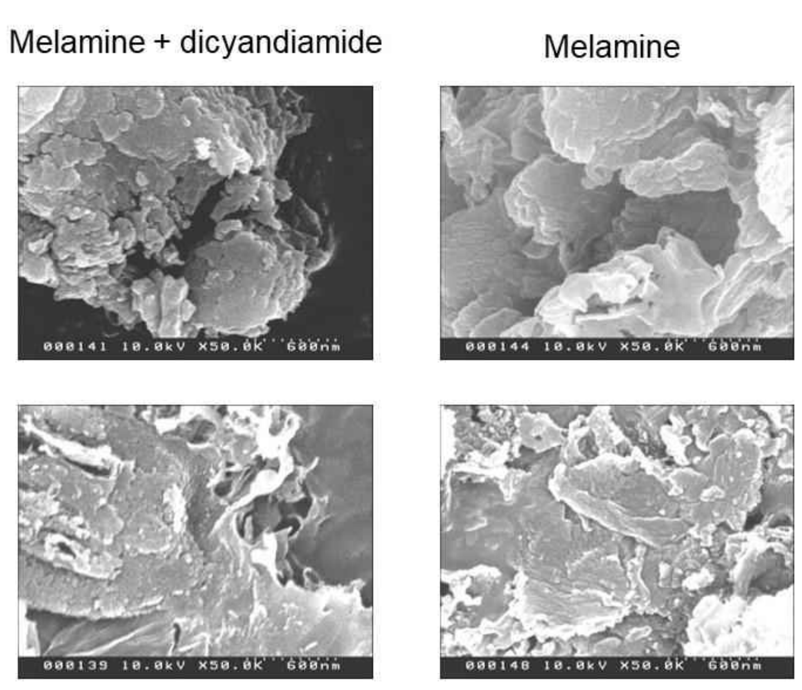
Dicyan diamide + Melamine
↓ Mixed (15 min)
↓ Air flow
Calcination (550ºC, 4 h, speed 2.3ºC/min)
↓
g-C_3N_4
↓ Air flow
Calcination (500ºC, speed 5ºC/min)
↓
Thermal treated C_3N_4

Melamine
↓ Air flow
Calcination (550ºC, 4 h, speed 2.3ºC/min)
↓
g-C_3N_4
↓ Air flow
Calcination (500ºC, speed 5ºC/min)
↓
Thermal treated C_3N_4

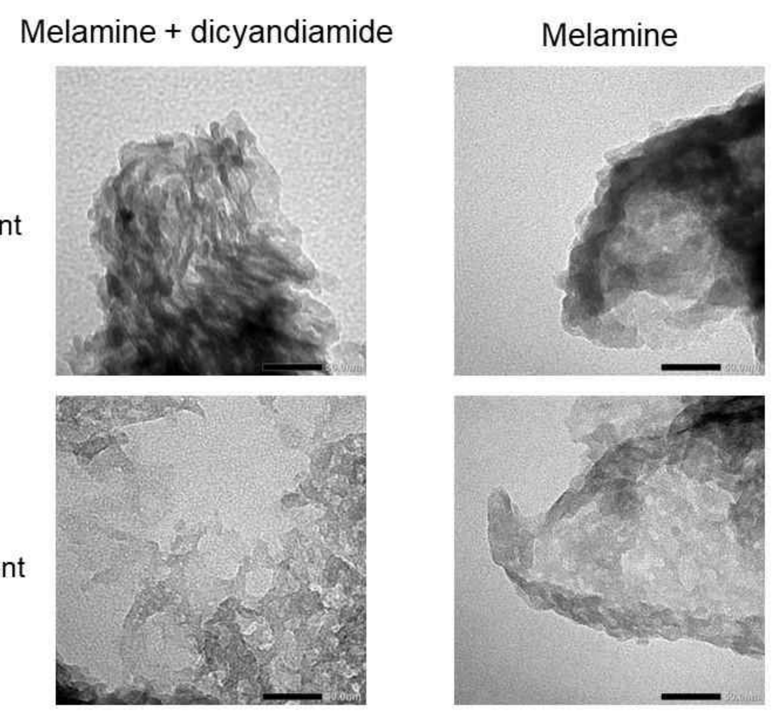
Charcterization



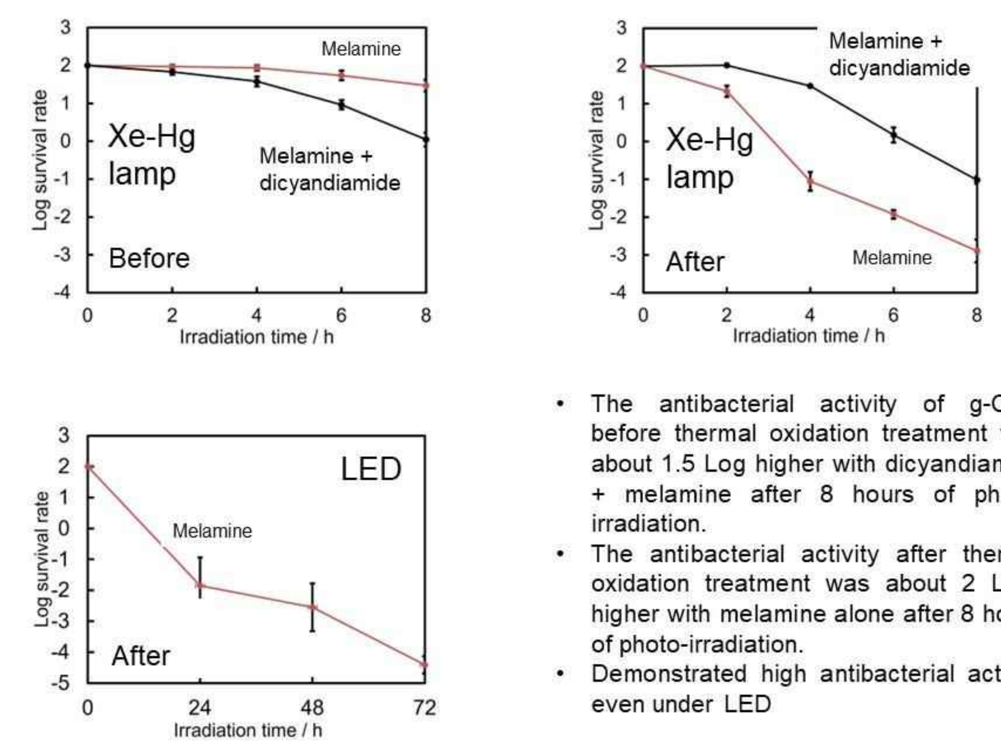
SEM



TEM



Antibacterial activity

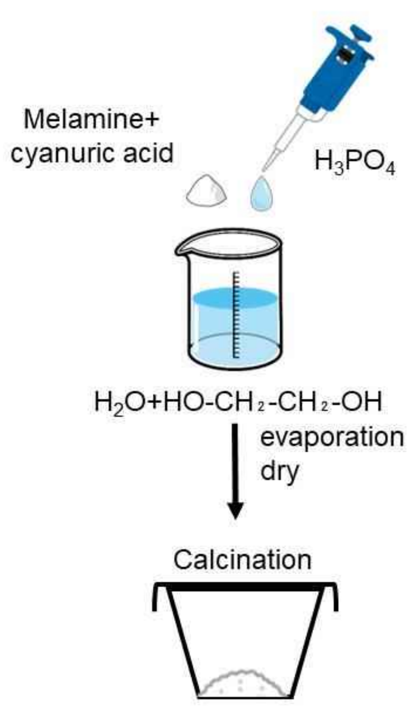


- The antibacterial activity of g-C_3N_4 before thermal oxidation treatment was about 1.5 Log higher with dicyandiamide + melamine after 8 hours of photo-irradiation.
- The antibacterial activity after thermal oxidation treatment was about 2 Logs higher with melamine alone after 8 hours of photo-irradiation.
- Demonstrated high antibacterial activity even under LED

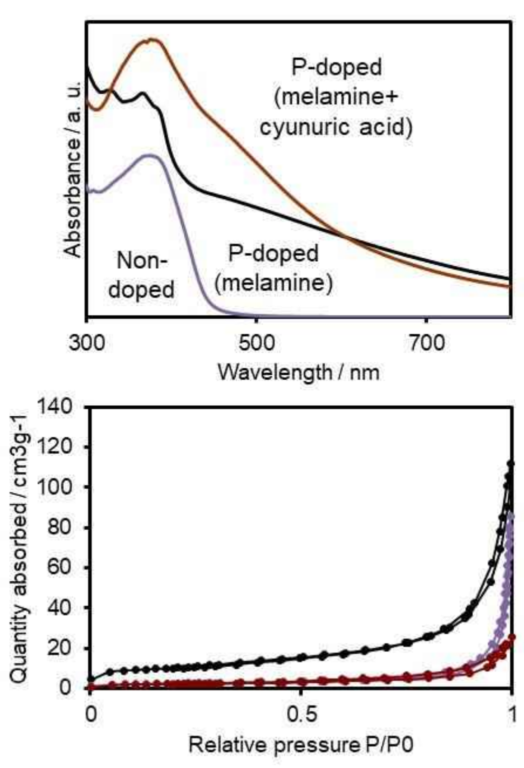
Development of red-shift type photocatalyst

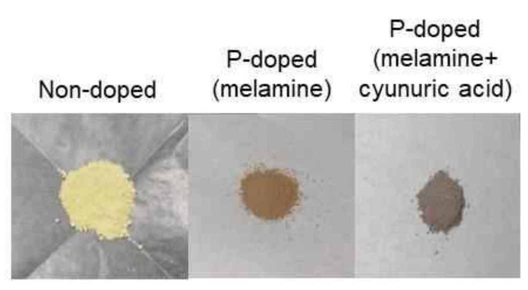
Preparation of P-doped C_3N_4

Melamine+cyanuric acid
↓ H_2O, HO-CH_2-CH_2-OH, H_3PO_4
evapolation
↓
Dry (80ºC)
↓
Calcination (550ºC, 2.3ºC/min, 4 hours)



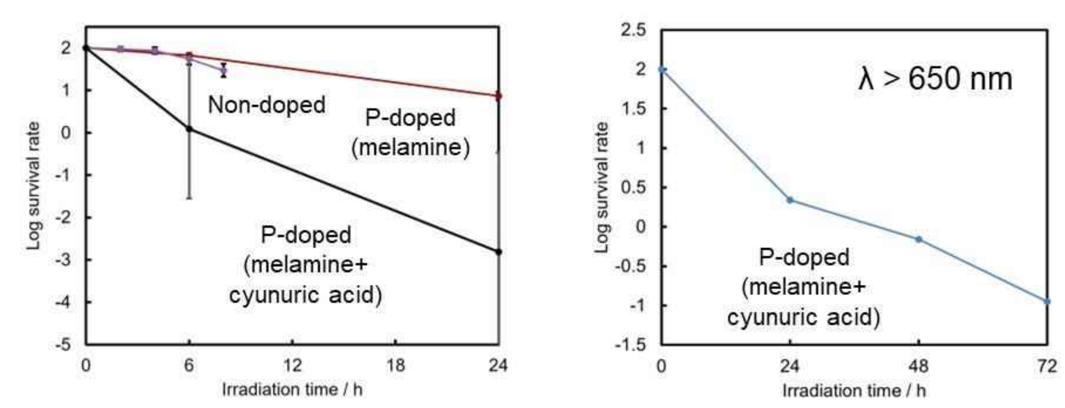
Characterization





Sample	Specific surface area (m^2/g)
P-doped (melamine+cyunuric acid)	36.0
P-doped (melamine)	7.74
Non-doped	8.92

Antibacterial activity



- Phosphorus-doped C_3N_4 with added cyanuric acid showed high antibacterial activity
- It exhibited antibacterial activity even under light irradiation with a wavelength of more than 650 nm.

No. A-13-7E

PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility

Theme: OFF type: Evaluation of Biodegradation and Safety Assessment

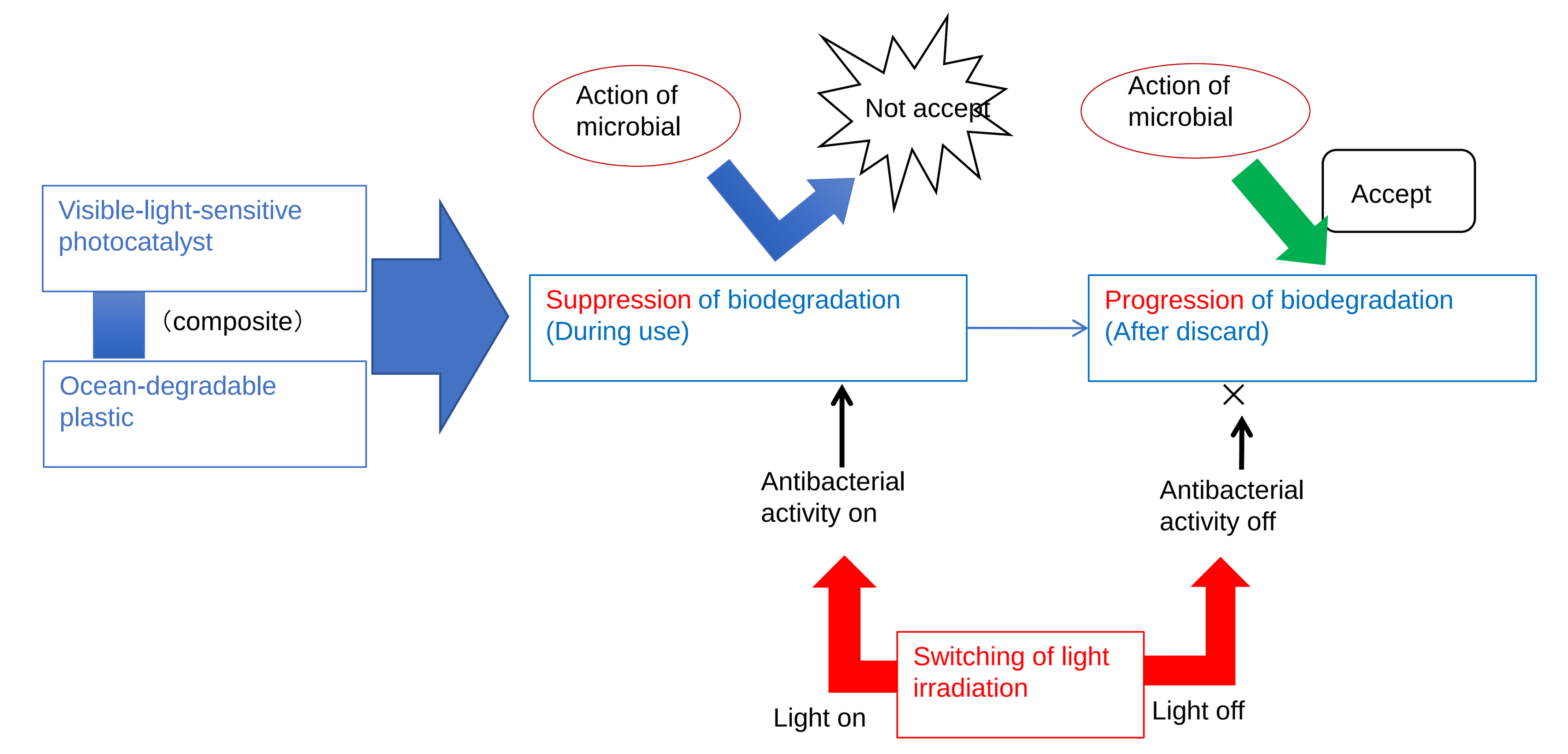
Organization: Kobe U, AIST, ORIST, TUAT

Contact: okamurah@maritime.kobe-u.ac.jp, a.nakayama@aist.go.jp, aki@orist.jp

NEDO

MOONSHOT RESEARCH & DEVELOPMENT PROGRAM

Biodegradation control model in ocean-degradable plastic composite by on/off light irradiation



Evaluation method

A photocatalyst and an organic dye were used as photoantibacterial agents. Biodegradable resin and these were composited by casting method.

Antibacterial test The antibacterial properties were evaluated by contacting the film sample with the test bacteria under light irradiation and measuring the increase or decrease in the number of viable bacteria after a certain period of time.

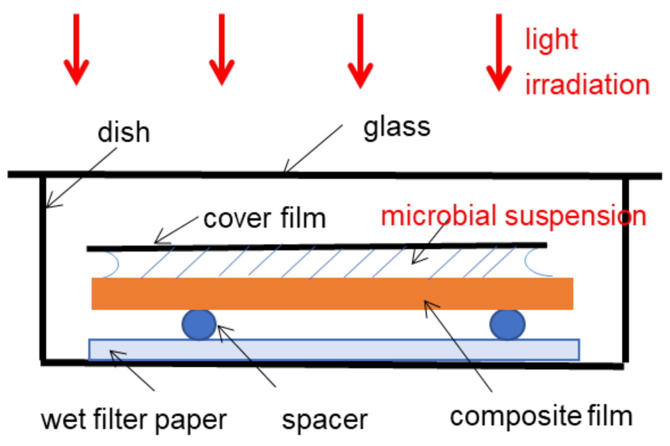
Acute toxicity test Using OECD Test Guideline 203 as a reference, we evaluated the effects on fish when exposed to OFF-type samples for 96 hours.

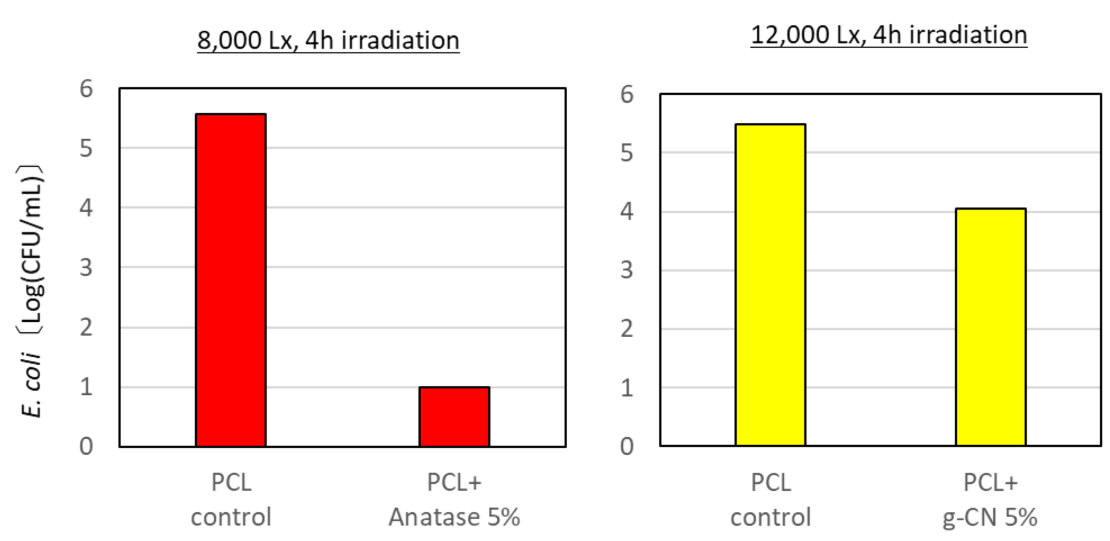
Biodegradation BOD test with Seawater Powder or film samples were placed in seawater, and BOD biodegradation tests were carried out in a constant temperature chamber equipped with 12 fluorescent lamps or LED lamps. Also, a weight change was measured in a simulated seawater immersion test in a beaker.

Marine immersion test A film sample was placed in a plastic container, immersed in the seawater at the Kobe U within a depth of 1m, recovered after a certain period of time, and weighed.

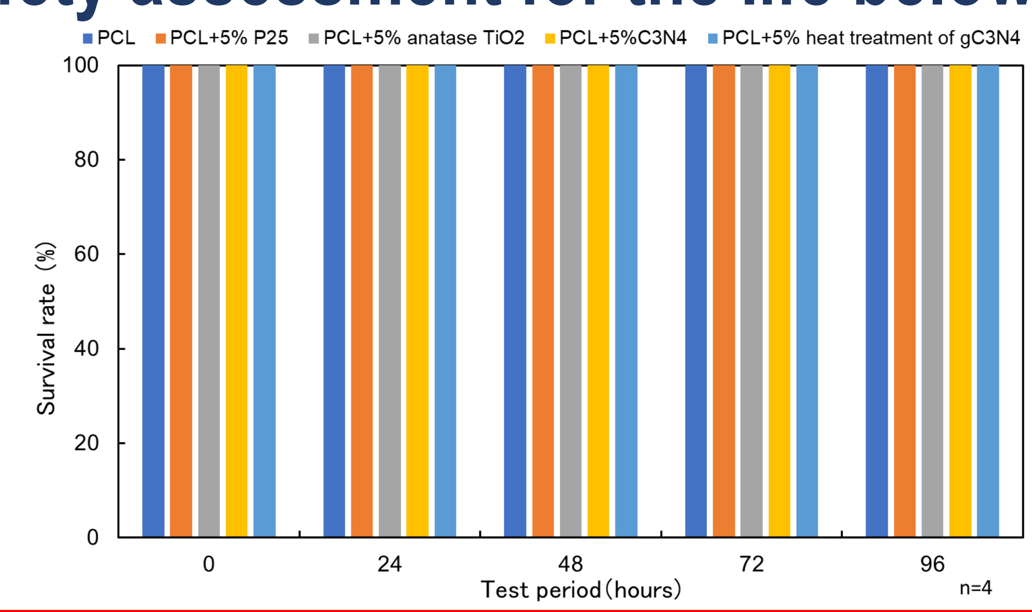
Antibacterial activity evaluation

ORIST





Safety assessment for the life below water



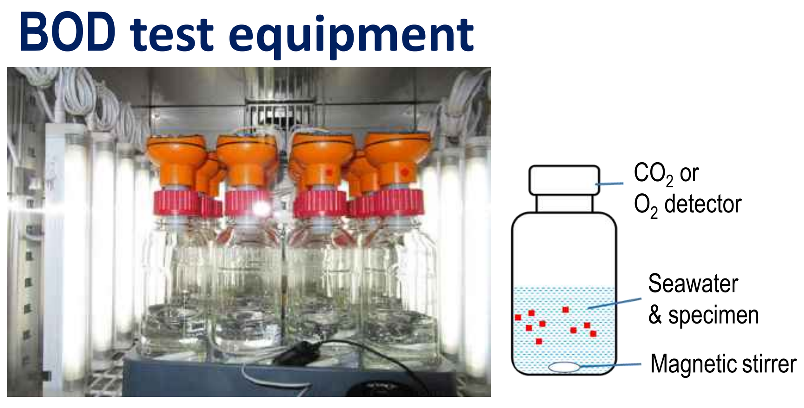
Estimated acute toxicity effects of PCL, PCL+5% P25, PCL+5% anatase TiO_2, PCL+5% g-C_3N_4, and PCL+5% heat treatment of g-C_3N_4 on *Danio rerio*. The acute toxicity assay were based on OECD 203 test with minor modifications.

Biodegradation BOD test with Seawater

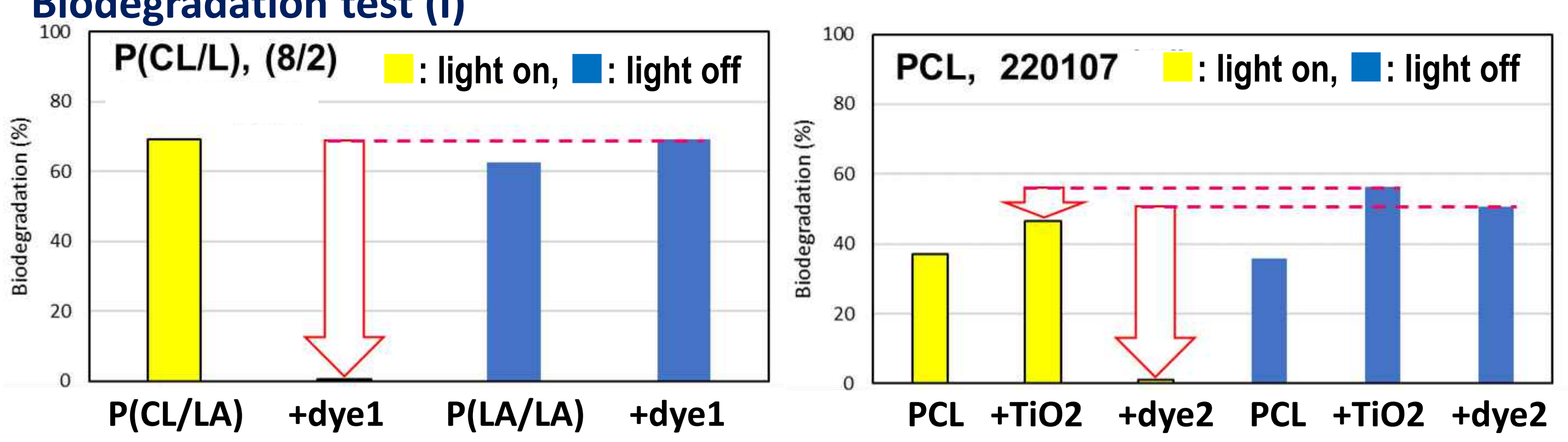
KOBE UNIVERSITY ORIST 産総研

PCL cast film

BOD test equipment

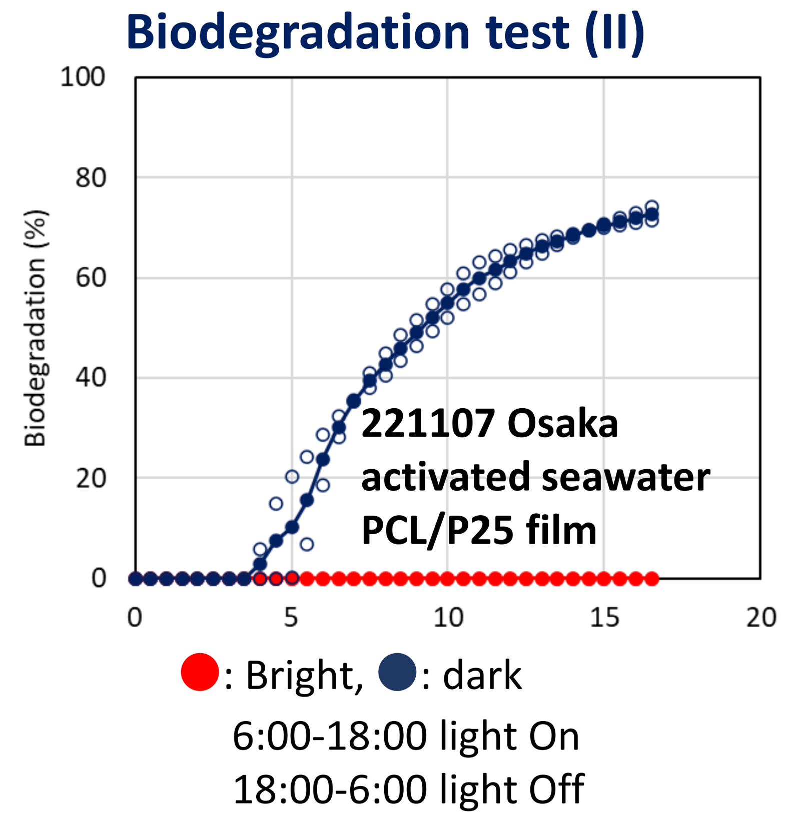


Biodegradation test (I)



In the BOD biodegradation test in natural seawater under continuous fluorescent light, the biodegradation of OFF-type resin was greatly suppressed.

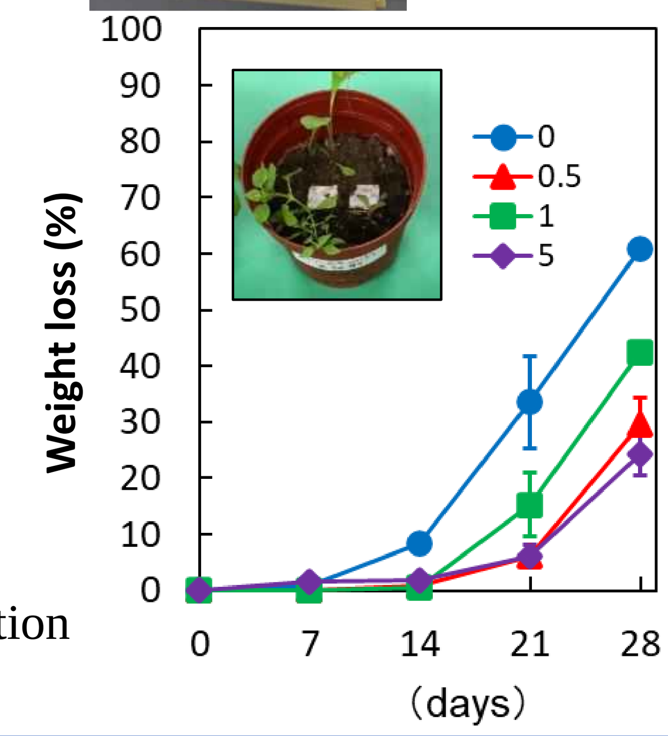
Biodegradation test (II)



Remarkable switching performance was exhibited under the light exposure condition of ON/OFF switching every 12 hours.

Field test in soil

Specimen：CopolyCL/LA, 9/1

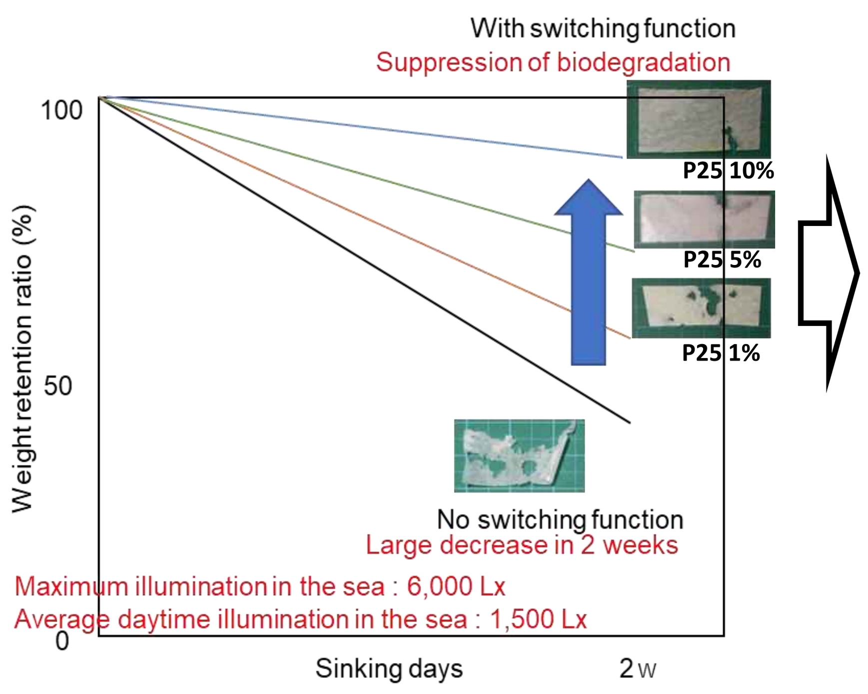


Marine immersion test

KOBE UNIVERSITY ORIST 産総研

Immersion test in Winter

Kobe (2021.11.26-2021.12.9)				
PCL	Weight retention ratio (%)	12.9	84.6	42.8
		46.8		
PCL＋ Anatase 5%	Weight retention ratio (%)	89.6	79.4	89.8
		86.3		
PCL＋ g-CN 5%	Weight retention ratio (%)	48.1	69.1	51.1
		56.1		
PCL＋ P25 5%	Weight retention ratio (%)	69.3	70.9	74.1
		71.4		



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Immersion test in Spring

Dirt on film : Intense

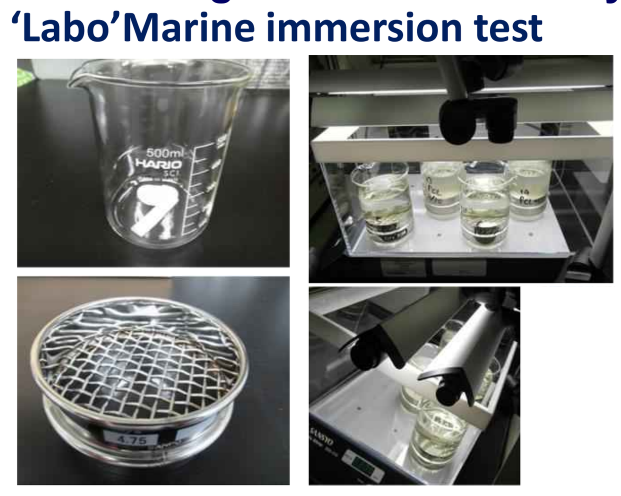
4/7-4/28	room1	room2	room3	Av
PCL	72.7	85.1	83.2	80.3
PCL+Anatase 5%	77.2	86.2	84.6	82.7
PCL + P25 5%	83.0	86.6	90.6	86.7
PCL+g-C_3N_4 5%	85.7	90.1	94.7	90.2

From March to May, marine organisms are active, so a lot of dirt adheres to the film surface, making it difficult for the switch to work.

☛Resin-adhered sludge hinders evaluation

Considering new evaluation system

'Labo' Marine immersion test



☛Evaluation of switch performance under conditions without interference by marine organisms

Evaluation in the field (multi)

☛Less dirt on the sample surface

No. A-13-8E
PJ : Development of Photo-switching Ocean-degradable Plastics with Edibility
Theme: Sorghum: development of a dedicated biomass crop and its utilization for itaconic acid production for the synthesis of biodegradable plastic
Organization: Nagoya U, Kobe U
Contact: kawaguchi_h@port.kobe-u.ac.jp

#5 Development of sorghum varieties optimized for biorefinery

Biorefinery crop : Sorghum

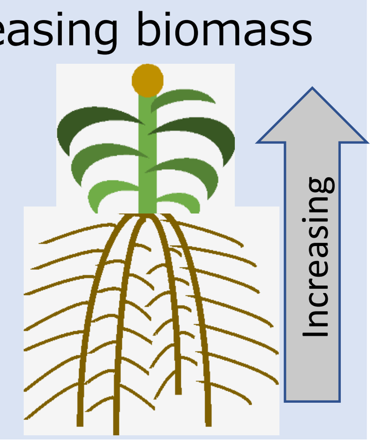
- **High biomass** → **>85t/ha**
- **Sweet** → **Pressed sugar solution is also available.**
- **It can be grown in converted fields.→ fallow land measures**
- **Wide growing region→ equatorial ～ temperate**
  → It can be grown in semi-arid areas.
- **C4 plant** → **Large CO2 fixation capacity**
- **There is a mechanized seeding and mechanized harvesting system.**

Development of sorghum varieties with root systems that contribute to high biomass.

Improving root systems→Increasing biomass

- Using 250 accessions
- Identification of QTLs（GWAS）
- Pyramiding of the QTLs using MAS

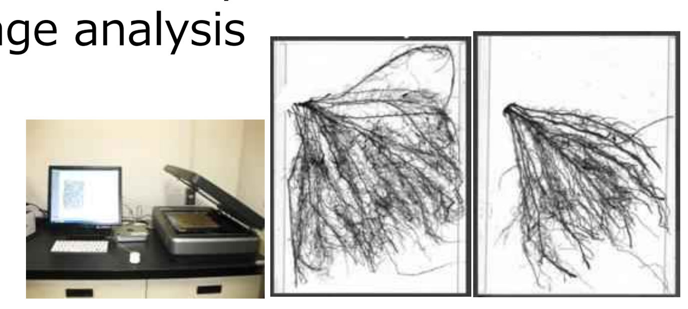
Optimization for producing bioplastic.

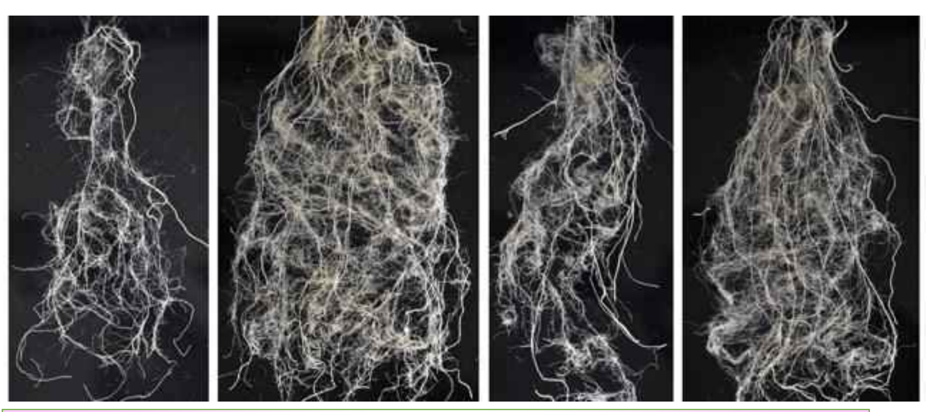


（1）Evaluation of root traits using Nagoya university sorghum panel

Using 250 accessions collected around the world

Evaluation of root system using image analysis

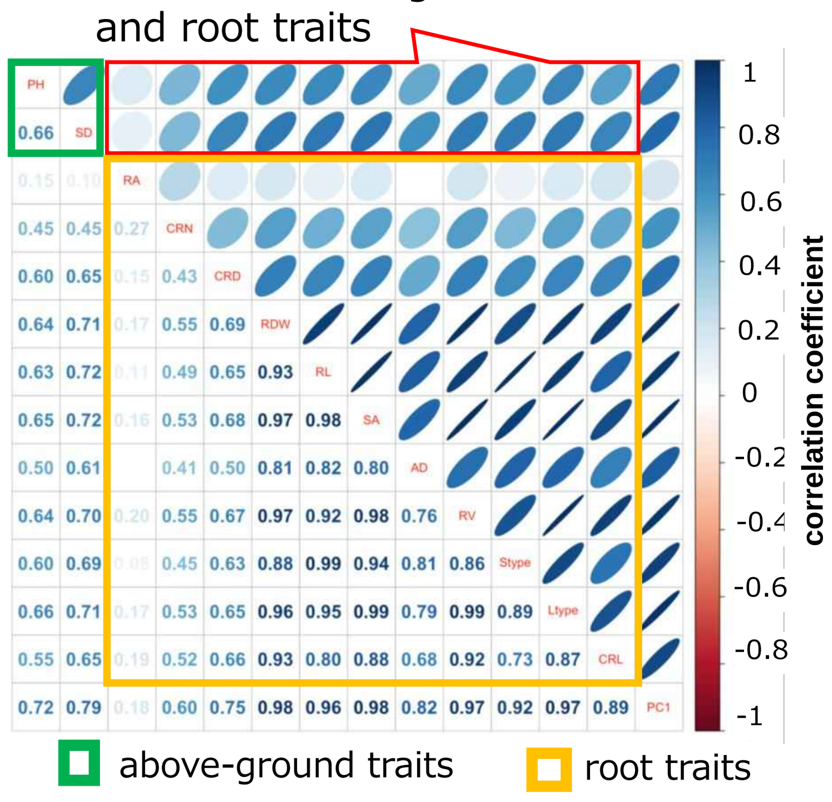




This population showed high diversity of root system.

・Correlation analysis

between above-ground traits and root traits



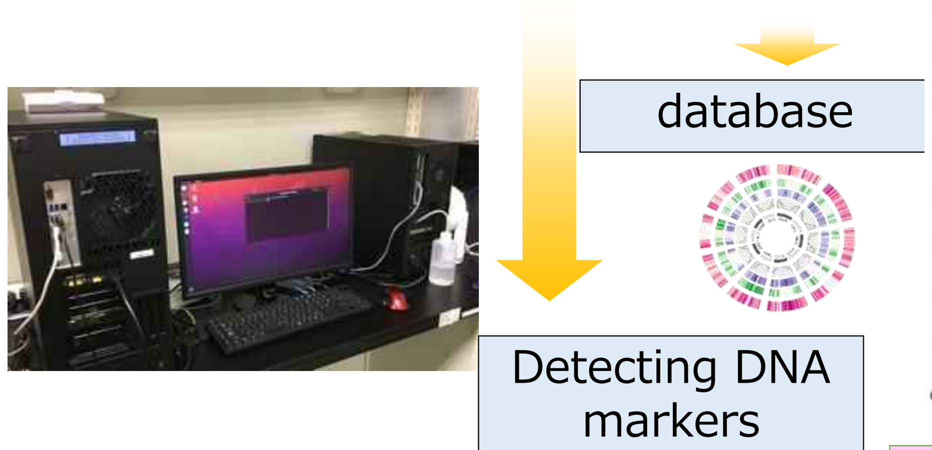
※ The fineness and color of the ellipses represent the magnitude of the correlation coefficient.

Root traits and above-ground traits were positively correlated

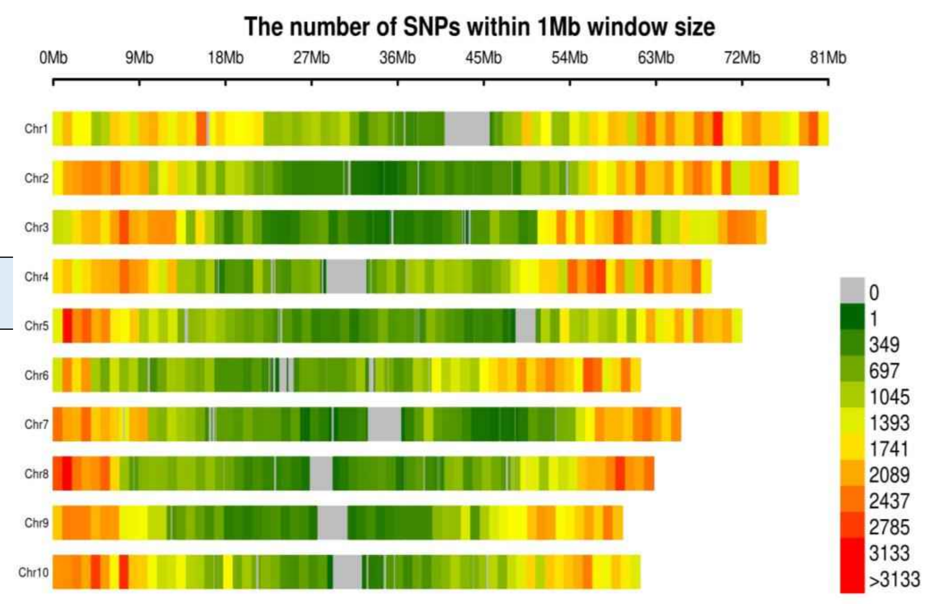
It is possible that improving root system lead to increasing biomass.

（2）GWAS

Whole genome re-sequencing, 7.1Gb/1line (genome size : about732Mb)

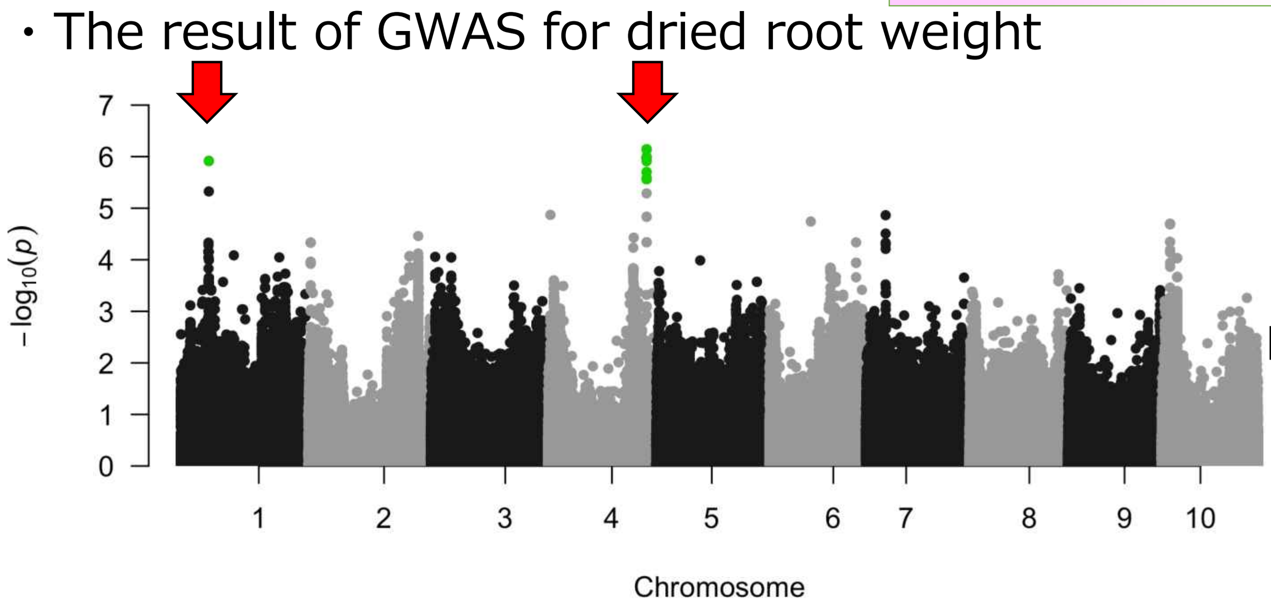


・The number of DNA markers in the 1Mb



・DNA markers were present in high density.

・The result of GWAS for dried root weight

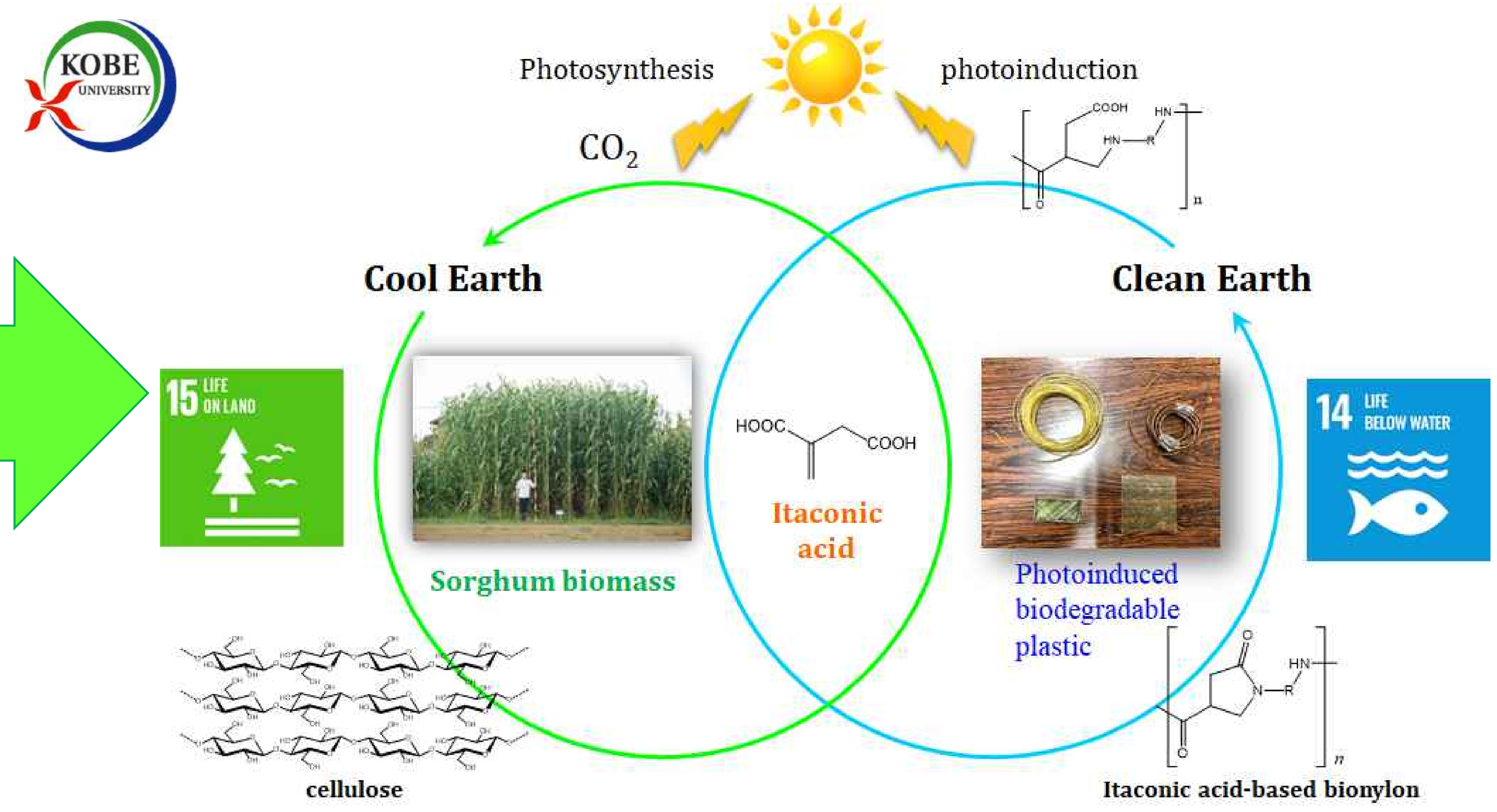


・Two QTLs on chromosome 1 and 4 were detected.

Since dried root weight is also positively correlated with aboveground traits, these QTLs may also increase aboveground biomass.

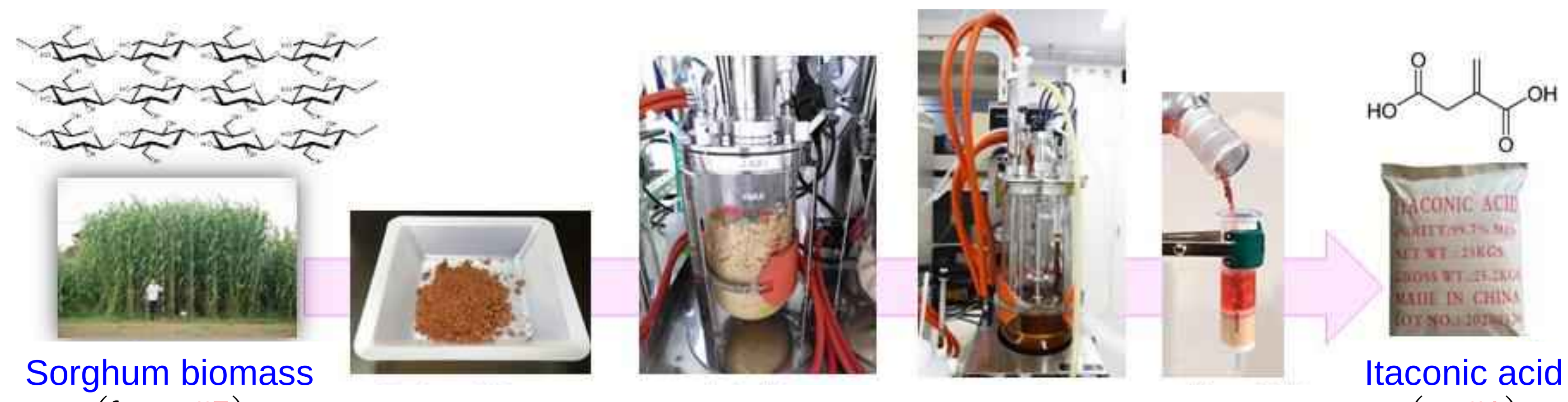
Summary： GWAS successfully detected root system-related QTLs that can contribute to increasing above-ground biomass.

#6 Itaconic acid production from sorghum biomass



Development of bioprocessing for the utilization of inedible lignocellulosic feedstock of sorghum bagasse for microbial production of itaconic acid (IA) serving as building block of photo-induced biodegradable plastic(s)

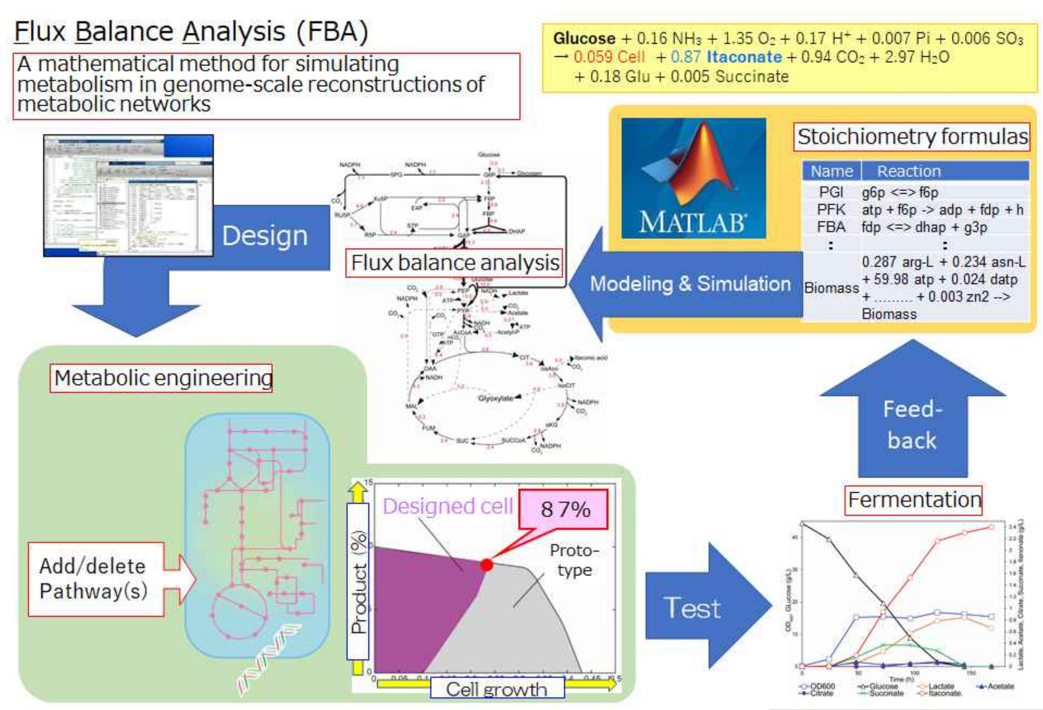
(1) Preparation of enzymatic hydrolysate of sorghum bagasse



Sorghum biomass (from #5) Pretreatment / Enzymatic hydrolysis / Fermentation / Purification Itaconic acid (to #1)

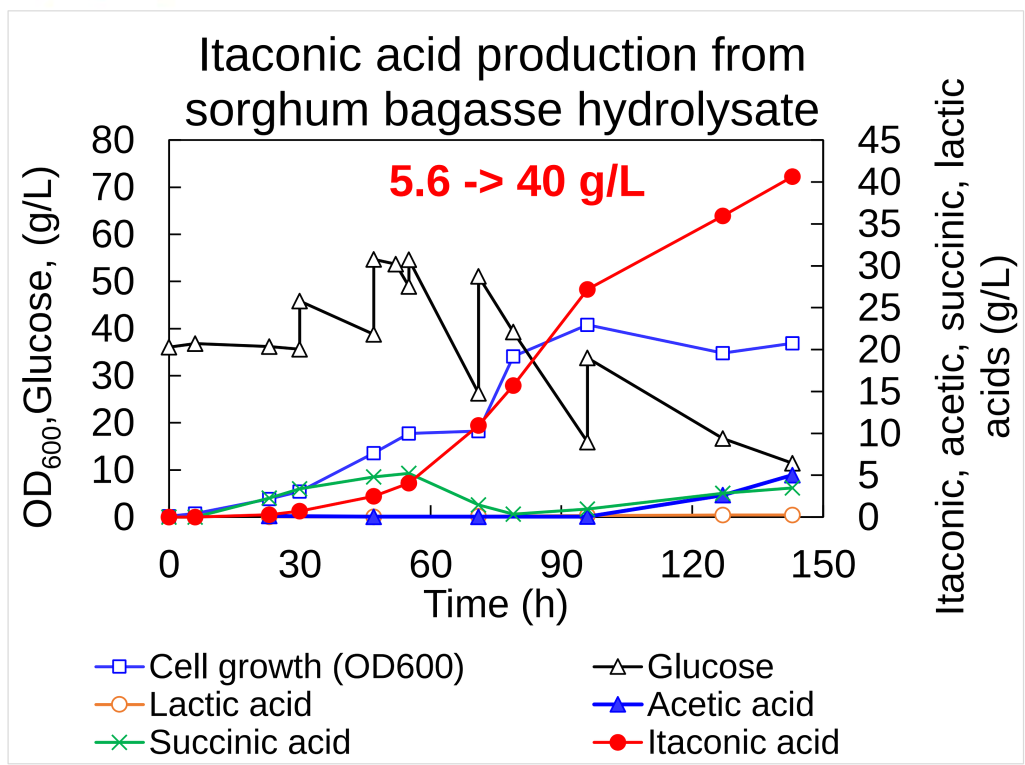
Optimized enzymatic hydrolysis process for sorghum bagasse with >90% sugar yield

(2) Itaconic acid (IA) production from sogrhm bagasse

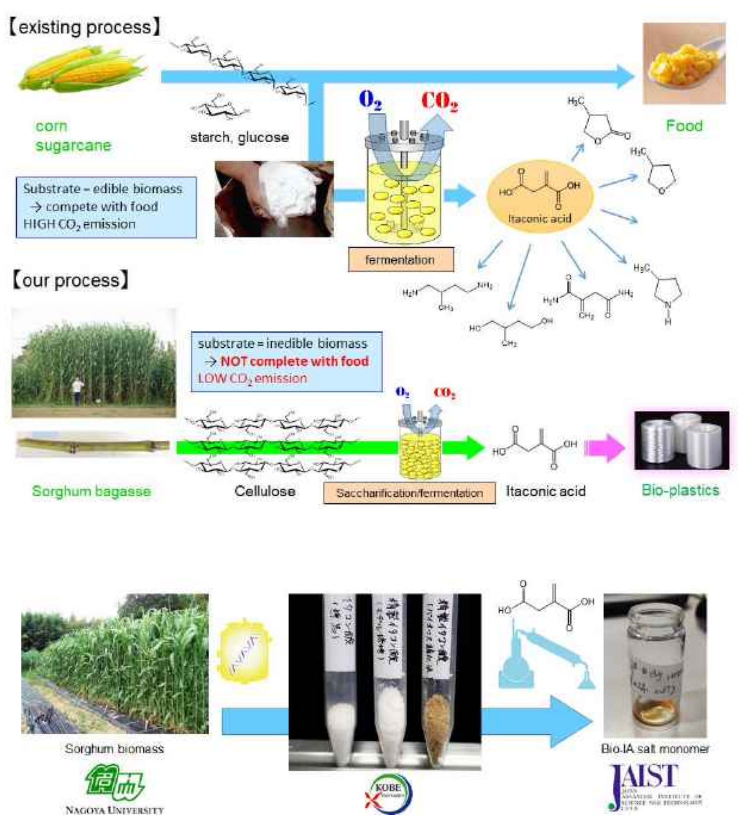


✓ Developed a computational model for metabolic network
✓ Developed ***designed cells*** with high IA yield
✓ IA production from enzymatic hydrolysate of sorghum bagasse

✓ Designed a metabolic pathway with 87% yield
✓ 7-fold increased production



(3) Low-carbon bioprocess for enhanced IA production



Substrate	Microorganism	Titer (g/L)	Rate (g/h/L)	Yield (g/g)	References
glucose	*Aspergillus terreus*	86.2	1.2	0.62	Kuenz, et al. (2012) Appl Microbiol Biotechnol., 96(5), 1209-1216.
glucose	*Escherichia coli*	0.086		0.01	Vuoristo, et al. (2015 AMB Express, 5:61.
glucose	*E. coli*	32		0.49	Harder, et al. (2016). Metabolic Engineering, 38, 29-37.
glucose	*Corynebacterium glutamicum*	7.8	0.273	0.29	Otten, et al. (2015). Metabolic Engineering, 30, 156-165.
starch	*E. coli*	0.017	0.0007		Yamamoto, K. et al. (2015) Bioengineered 6(5): 303-306
Potato starch waste	*A. terreus* strain C1	29.7	0.138		Bafana, et al. (2019). Biotechnology Progress, 35(3).
Organosolv beech wood	*A. terreus*	*7.2*	0.1	0.3	Tippkötter, et al. (2014). Bioresource Technology, 167, 447-455.
Corn stover	*A. terreus*	19.3		0.36	Li X, et al. (2016) Bioresources 11(4):9047-9058.
Sorghum (Glucose)	*C. glutamicum*	**40.6** **(24.8)**	0.57 (0.49)	0.25 (0.37)	***This work***

✓ Developed low-carbon bioprocess
✓ Purified bio-based IA for polymer synthesis

No. A-13-9E

PJ: Development of Photo-switching Ocean-degradable Plastics with Edibility

Theme: Commercialization Studies of Photo-switching Biodegradable Plastics

Organization: TUS, JAIST (subcontractor: JMAC)

Contact: osato@rs.tus.ac.jp, shuta_ishizuka@jmac.co.jp

Backgrounds

Promote commercialization with the aim of both solving social issues and creating disruptive innovations.

Project: Development of edible photoswitching ocean-degradable plastics
PM: Dr. Tatsuo Kaneko
Participating Organization
- 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

Period (Budget): 2020-2029 (2.5 billion yen)
Final goal:
Development of photoswitchable marine degradable edible plastic using itaconic acid produced from a new sorghum variety and a newly developed high-performance photocatalyst

⑦-1 LCA : JMAC
⑦-2 Strengthen business foundation and foster environment: TUS

Activities for commercialization

Long-term R&D and social implementation activities are essential to create disruptive innovations from basic research

(1) R&D in the natural sciences

- Establishment of technological basis
- Creation of technological seeds
- Elucidation of operating principles

(2) Application exploration and business model creation

- Search for needs that match the seeds
- Establishment of a business model

(3) Creative justification of resource mobilization

- Obtaining approval to continue innovative projects in the organization

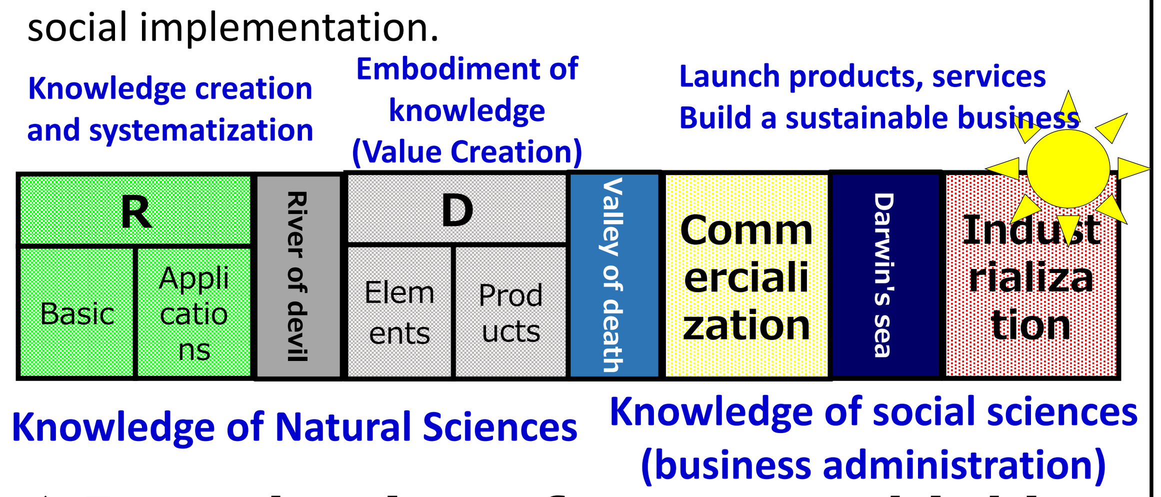
(4) Social acceptability

- Gaining public understanding for innovative technologies and concepts

Promotion Policy (1)

◆ Integration of natural and social sciences

Integration of natural and social sciences is indispensable for the integrated strategic planning from basic research to social implementation.



◆ Introduction of systems thinking

- Viewing the complex connections of various elements as a "system" and capturing the overall picture of the structure
- Understand its complex behavior to improve the system

Pursue leverage points for issues

Promotion Policy (2)

◆ Activities along global megatrends

- Reduction of fossil fuels
- Expansion of the Circular Economy
- Growing interest in environmental issues

Promotion Policy (3)

◆ Search for applications and business models suitable for the basic research stage: Developmental stage

- Conceptualization of a broad framework for solving the marine plastic problem
  - Establishment of measures to prevent outflow into the ocean
  - Realization of plastics that decompose safely and completely even if they outflow into the ocean

Explore applications and business models based on operating principles

Examples of Promotion Policies (3)

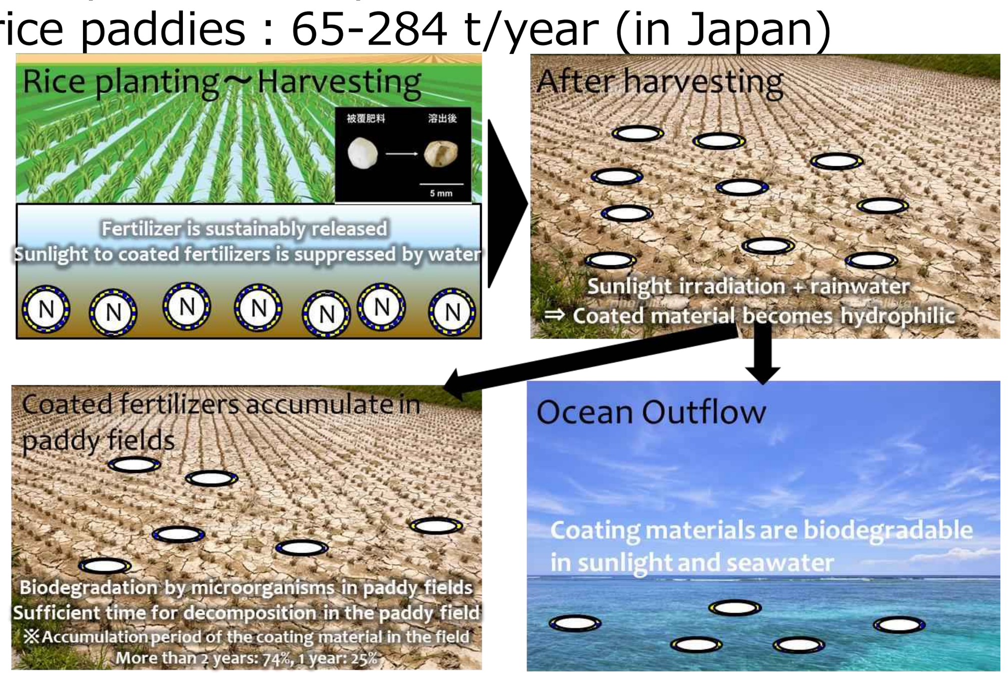
◆ Operating principle of photoswitchable resin

- Light irradiation ⇒ Ring opening reaction ⇒ Hydrophilization ⇒ Biodegradation

◆ Proposed Applications of Photoswitchable Resins

- Coating material for covered fertilizers

Complete decomposition of coated fertilizer in rice paddies : 65-284 t/year (in Japan)

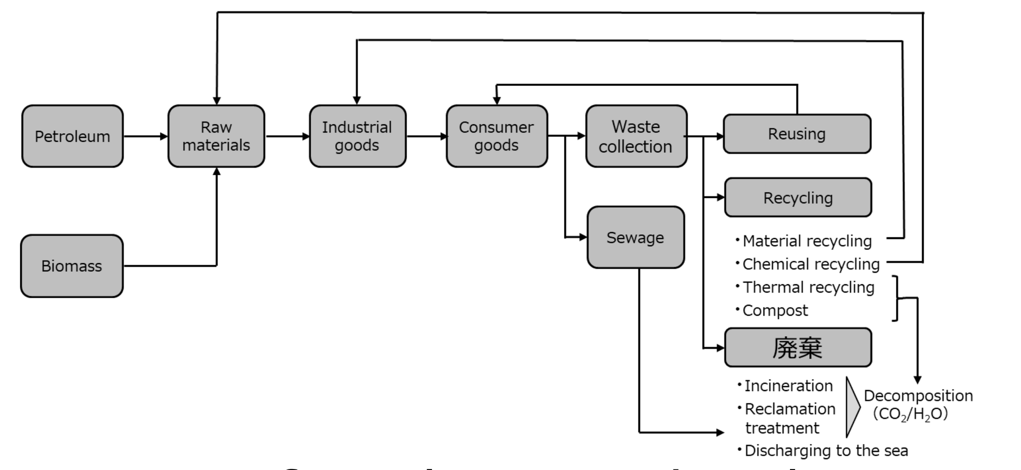


※ Prepared based on data from Dr. Naoya Katsumi

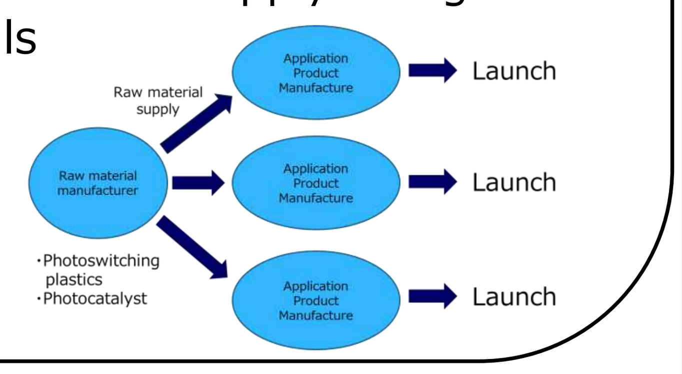
Promotion Policy (4)

◆ Co-creation from the research stage

- Industry-government-academia co-creation to link supply chains
- Establishment and Operation of Study Group
  - ✓ Integration of natural/social sciences and
  - ✓ Search for applications and business models
  - ✓ Creative justification and Social Acceptance



- Promotion of application development
  - Establish systems for mass supply of high quality raw materials



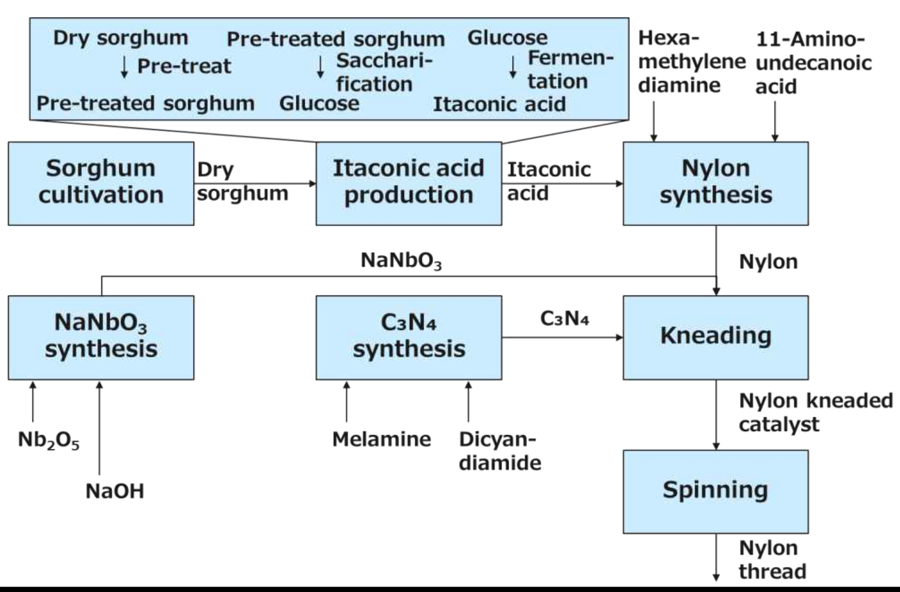
Directions of LCA

Product LCA : GHG emission in the raw material production stage (sorghum cultivation–spinning) was calculated collecting laboratory data.

GHG emission reduction contribution : GHG emission reduction contribution by spread of our new material was quantified as far as possible.

Product LCA improvement

Subject flow of product LCA



Process	GHG emission per 1 kg of final product[kg]		
	1st	2nd	3rd
Spinning	138.58	138.58	138.58
Kneading	519.39	519.39	519.39
NaNbO3 synthesis	16.23	15.66	15.66
C_3N_4 synthesis	53.71	53.71	53.71
Nylon synthesis	2,033.97	75.30	49.90
Itaconic acid production (Fermentation)	1,131,860.62	12,682.53	1,283.09
(Saccharification)	151,797.45	1,663.39	133.92
(Pre-treatment)	1,032,747.89	6,767.94	1,099.02
Sorghum cultivation	0.70	0.70	0.70
Total	2,319,168.53	21,917.19	3,293.97

1/700

LCA calculation was conducted three times. GHG emission was significantly reduced by improvement of itaconic acid (IA) yield and scale-up of nylon synthesis.

Superiority of sorghum

	GHG emission[t/year]
Sorghum	33,002
Thinned wood (Chip)	77,703
Rice straw	13,889,385

GHG emission of sorghum is less than other non-edible biomass as material of IA.

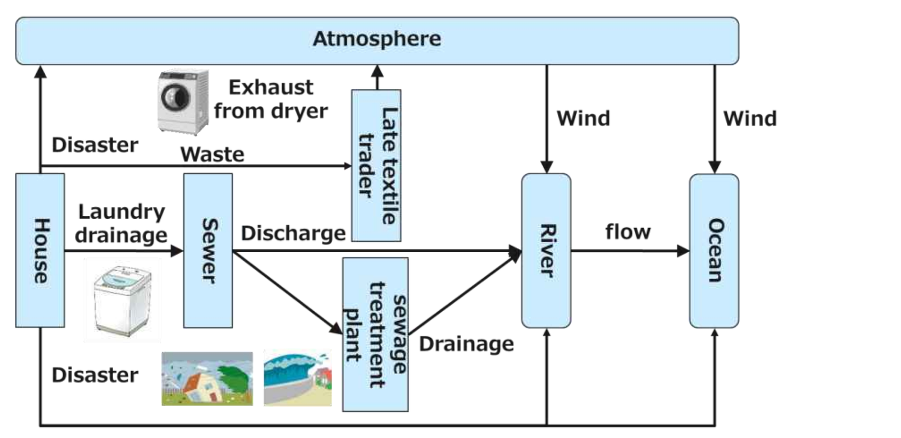
	Value	Unit
Required dry sorghum amount	410,913	t/year
Amount of water (Sorghum)	70.00%	
Required sorghum amount	1,369,709	t/year
Sorghum yield	85	t/ha
Required arable land area	16,114	ha/year
Deserted arable land area In Japan	423,000	ha

Sorghum can be cultivated in deserted arable land area in Japan

GHG emission reduction contribution

Subject	Effort	GHG emission	Unit
Clothing	Laundry net	13,761,066	kg-CO2/year
coated fertilizers	Drain net	3,816,093	kg-CO2/year
Total		17,577,158	kg-CO2/year

Example of the marine pollution mechanisms (Clothing)



While referring to the marine pollution mechanisms, "actual" pollution prevention efforts which become unnecessary by spread of our new material were selected and combined them to formulate scenarios to calculate the reduction contribution. GHG emissions were quantified from these scenarios and totalized to determine the GHG emission reduction contribution by spread of our new material. As a result, the reduction contribution was calculated to be approximately 17.58 million kg-CO2/kg/year.