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(AIST),
Osaka Research Institute of Industrial Science and Technology(ORIST).

Our group

<u>Theme ④</u>
Degradability & safety evaluation of photoinduced biodegradable plastic （AIST, Kobe Univ., Kagoshima Univ.）

in vitro microbiota model for biosafety （④-2）

<u>Theme ①-2</u>
Development of “ON”-switching photocatalysts (Tokyo Univ. Sci., Kobe Univ.)

Sasaki group

Toxicity of polymer decompositions in marine environments （④-4）
Biodegradability of polymer decompositions in marine environments （③-1）

Okamura group

Ogino group

Safety evaluation of photocatalyst （①-2）

Kawaguchi group

<u>Theme ③</u>
Biodegradability of photoinduced biodegradable plastic in marine environments (AIST, Kobe Univ.)

Itaconic acid production from sorghum biomass （⑥）

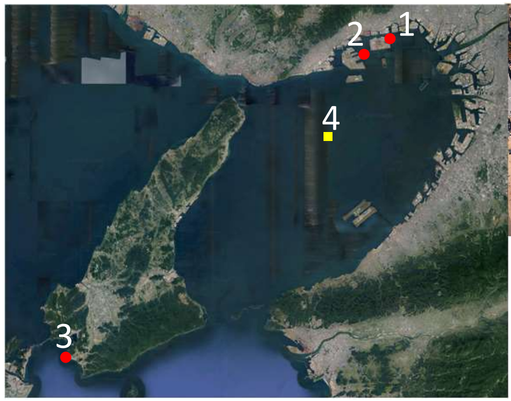
<u>Theme ①-1</u>
Development of photoinduced biodegradable polymers using itaconic acid (JAIST)

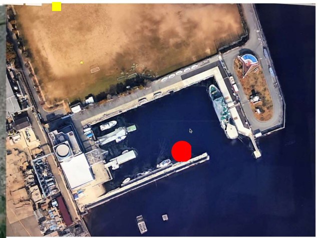
<u>Theme ⑤</u>
Designing and breeding of sorghum suite for producing photoinduced biodegradable polymers (Nagoya Univ.)

Issue ③ Evaluation of marine degradability of photo-switch biodegradable plastic
Issue ④ Evaluation of enzymatic degradability and safety of photo-switch biodegradable plastic

③- 1 Evaluation of degradability in actual sea area (Kobe Univ, AIST)

-R&D content: Evaluate on-site degradability of the polymer developed in Task ①, and feed back the results to Task ①.
-Final goal: Demonstrate that the test sample exhibits ocean degradability according to the depth (sunlight intensity) at several levels of water depth in multiple test sites.
-Main results at present: At two-week immersion of the test plastic in the ship mooring area (water depth 1 m) of Kobe University Fukae Campus, the antibacterial property by the photocatalyst was confirmed from the weight loss measured. In addition, preparations for conducting an immersion test at the 6th breakwater at Kobe Port and the Mega Float in Minamiawaji City have been completed.

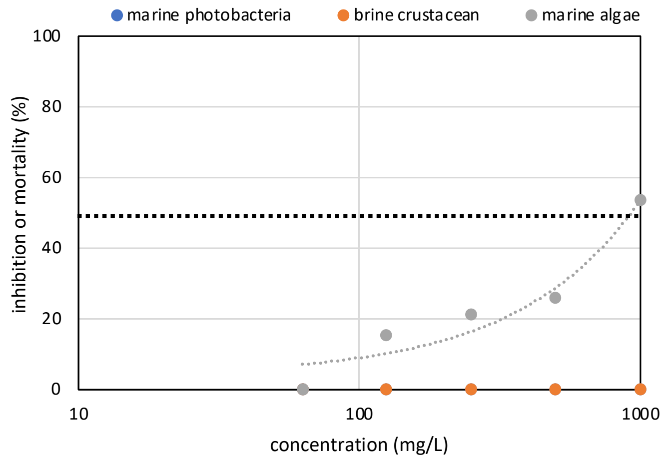




Site 1. University pond, water depth < 4 - 5 m
2. 6th breakwater, < 7 - 8 m
3. Mega Float, < 25 m
4. Candidate site, < 15 m

Test site in Osaka Bay

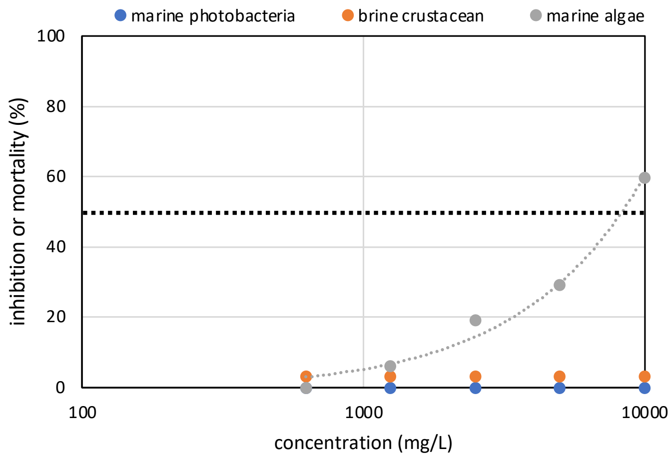
Immersion experiment at sea



Ecotoxicity of dicarboxylic acid type product

④–4 Ecotoxicity evaluation of degradation products to aquatic species (Kobe Univ, AIST, Kagoshima Univ)

- R&D content: Evaluate the ecological risk of degradation products derived from the polymer developed in Task ①, and feed back the results to Task ①.
-Final goal: Evaluate the effects of degradation products on aquatic species such as algae, crustaceans, and fish, and calculate the predicted no-effect concentration (PNEC). The predicted environmental concentration (PEC) is calculated in consideration of persistency, and ecological risk is estimated by the PEC/PNEC ratio according to production amount.
-Main results at present: From the ecotoxicity of two types of degradation products to aquatic species (right graphs), PNEC was calculated as 0.9 mg/L and 8.0 mg/L with an assessment factor 1000. If it remains in the aquatic environment above the concentrations, it is judged that there is an ecological risk.



Ecotoxicity of amino acid type product

④-2 Degradability and safety assessments by *in vitro* microbiota model

Construction of a degradability and safety assessment system using *in vitro* intestinal microbiota model of marine mammals

Research objective and Results（2020-2022）

・Construction of a method for degradability and safety assessment using an *in vitro* microbiota culture system of human feces

Kobe University Human Intestinal Microbiota Model

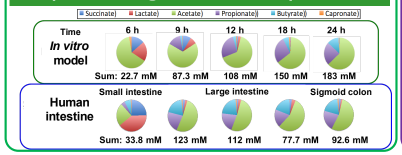
~Assessment of effect on human intestinal microbiota ~

In vitro human colonic microbiota model

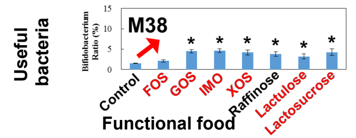
・About 1000 species
・Maintaining microbial diversity

	ID	Species number	Shannon index
Healthy subject Model:	HS	1043	5.23
Feces:		943	4.94

Reproducing metabolic profile



Response of commensal organisms

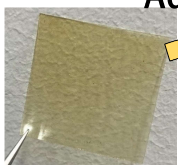


Conventional study method
・Animal trial
・Human intervention study
costly, ethical constraint

To construct the simple pre-evaluation system.

Degradation of plastic materials by human gut microbiota

Addition



・Nylon 6i
・Nylon 6i-Low

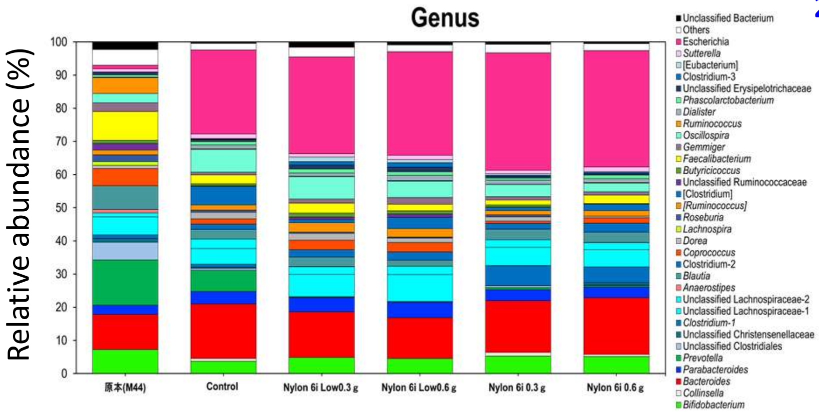
In vitro intestinal microbiota model

1. Molecular phylogenetics
→ 16S rRNA gene sequencing by NGS

2. Metabolomics
→ Detection of short-chain fatty acids (SCFAs) in culture supernatants

3. physiochemical analysis
→ Detection of total organic carbon (TOC) in cultures

1. No effect on structure and diversity of gut microbiota



2. No effect on production of SCFAs

	Control	Nylon 6i L 0.3%	Nylon 6i L 0.6%	Nylon 6i 0.3%	Nylon 6i 0.6%
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

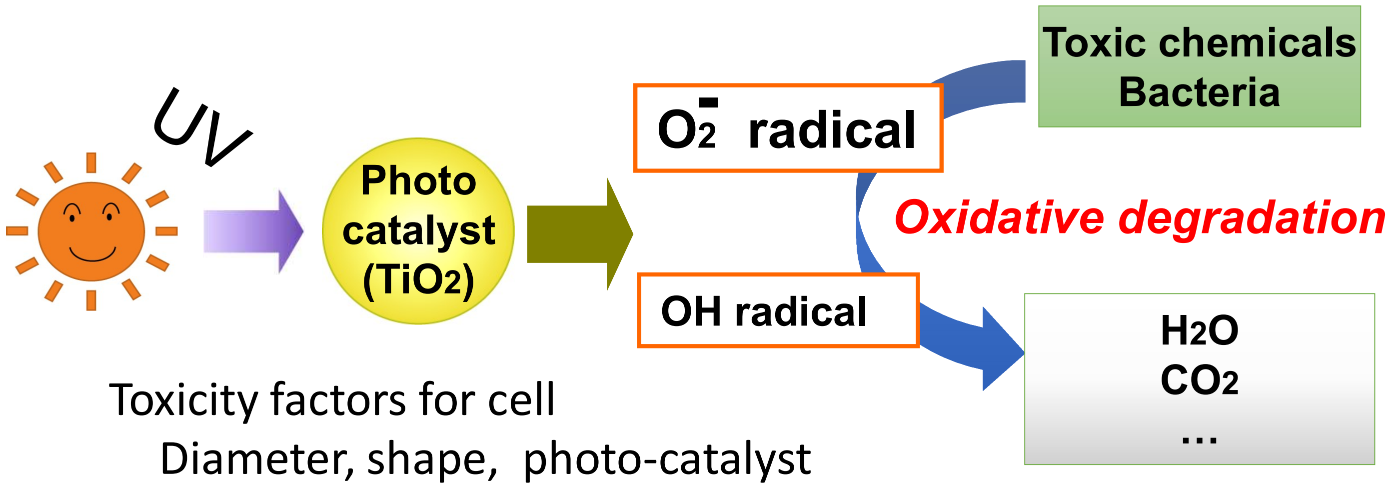
3. No effect on removal of TOC

	Pre-culture（mg/L）	Post-culture（mg/L）	Removal（mg/L）
Control (no-addition)	16,000	13,500	2,500
Nylon-6i-L(0.3%addition)	16,500	15,000	1,500
Nylon-6i-L(0.6%addition)	19,500	17,000	2,500
Nylon-6i(0.3%addition)	15,500	12,500	3,000
Nylon-6i(0.6%addition)	16,500	14,179	2,321

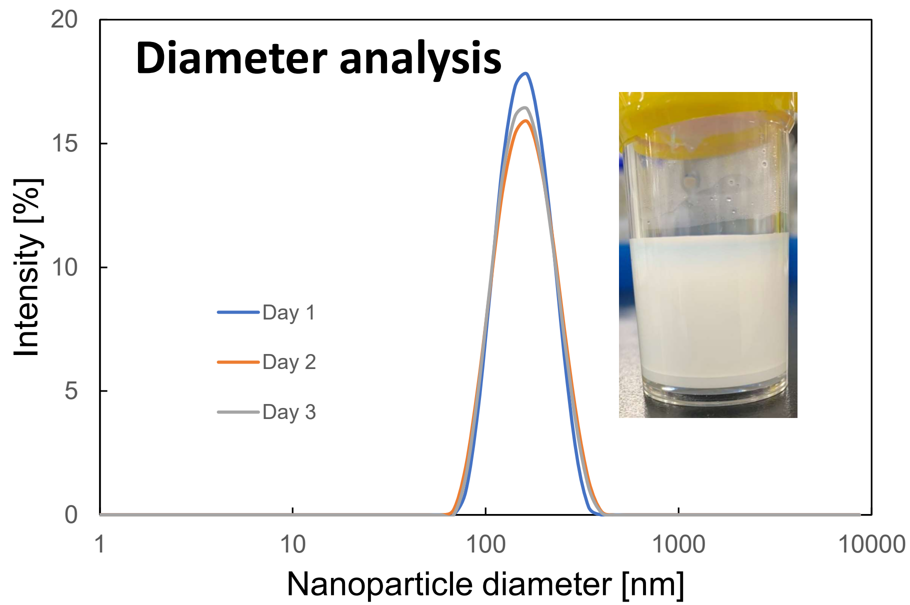
→ Human administration of plastic material was predicted to have less effect on gut microbiota.

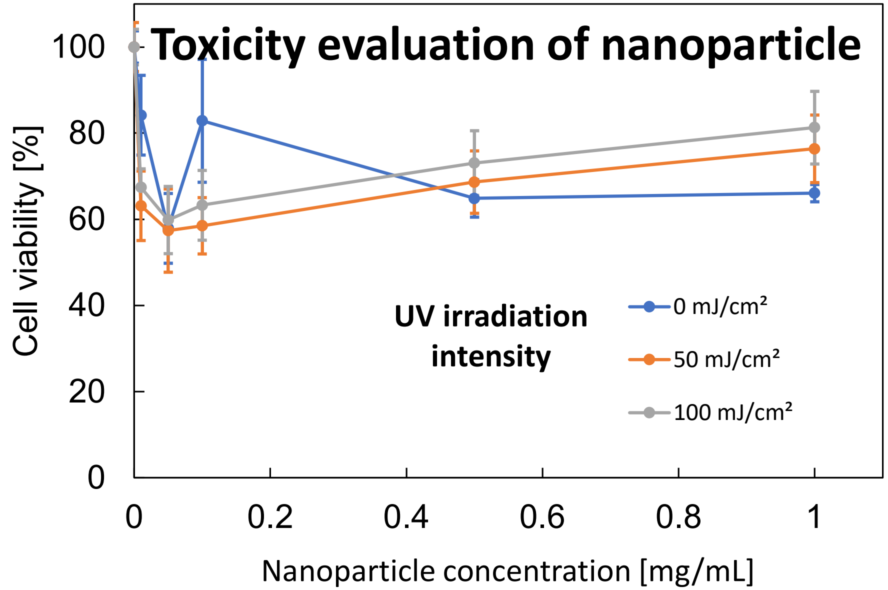
Theme ①-2 Toxicity evaluation of photocatalyst

Catalysis mechanism of general photocatalyst

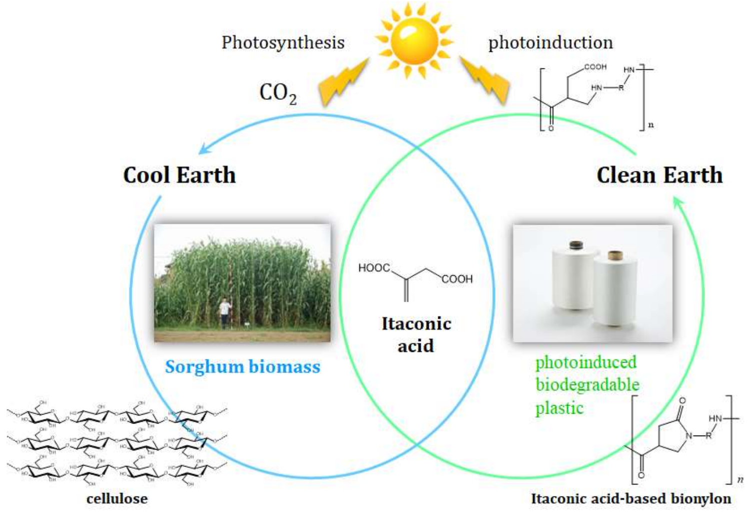


Characterization of ON-type photo-catalyst (Sodium niobate)





Theme ⑥: Itaconic acid production from sorghum biomass



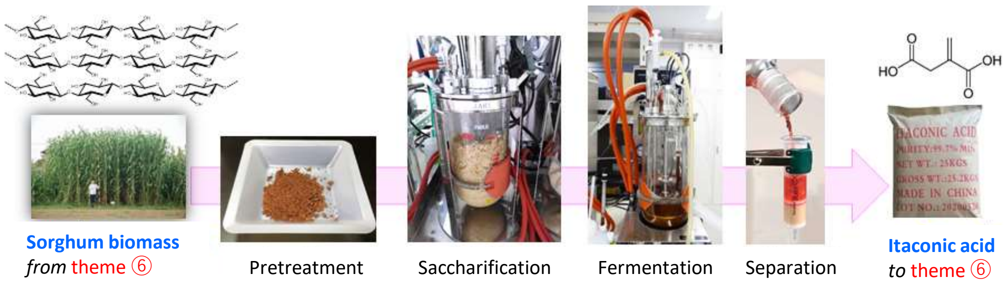
Development of bioprocess to produce itaconic acid, a key intermediate to synthesize photoinduced and biodegradable bioplastics from renewable and inedible sorghum biomass

⑥-1 Saccharification of sorghum biomass

・ Activities：Development of saccharification process suite for cellulosic biomass of sorghum designed in theme ⑤.

・ Goals：
(1) Preparation of enzymatic hydrolysate with attenuated fermentation inhibition for itaconic acid production.
(2) Clarification of mechanism of the attenuated fermentation inhibition of designed sorghum biomass.

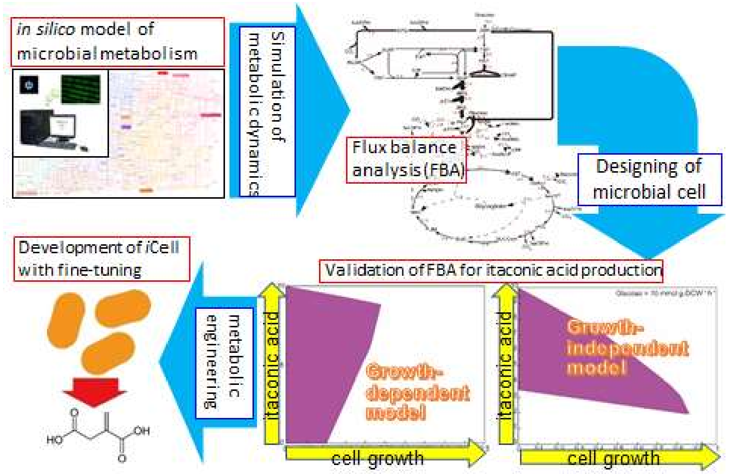
・ Achievements：(1) Over 90% of saccharification efficiency was achieved for all sorghum biomass. (2) One research article has been published （Kawaguchi, *et al* (2022) Bioresour Technol., **344**(Pt B):126165.）



⑥-2 Metabolic engineering to produce itaconic acid

・ Activities：
(1) Development of flux balance analysis (FBA) model.
(2) Metabolic engineering of microibial cell for enhanced itaconic acid production.

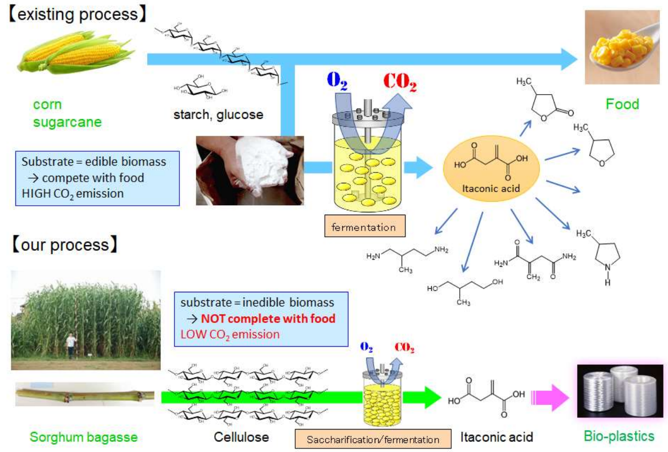
・ Goals：Development of a smart *i*Cell with a fine-tuning metabolism designed by FBA.



・ Achievements：Two FBA model with both cell growth-dependent and growth-independent was developed to maximize itaconic acid production.

⑥-3 Low carbon bioprocess to produce itaconic acid from sorghum biomass

・ Activities：
(1) Development of bioprocess under oxygen limitation to mitigate CO_2 emission for low carbon society.
(2) Development of microbial cell adapted for the oxygen limitation.



・ Goals：The yield of 0.3 g of product/ g of substrate to supply sufficient amounts of biomonomers for bioplastic synthesis.

・ Achievements：Improved itaconic acid production was achieved by development of a microbial cell adapted for oxygen limitation.

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