

# 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).

## ④ – 5 Development of nylon-degrading enzymes and evaluation of their in vitro degradation

Issue 1 : Enhanced functionality of nylon (oligomer) hydrolase (NylA~C) toward itaconate derived nylon

Issue 2 : Screening of new microorganisms degrading itaconate derived nylon from Kagoshima ocean resources

Final target for FY2029 :

We will attempt to create nylon-degrading enzymes with high degradation activities that can be used for objective ④-6, by various genetic recombination technologies based on the three-dimensional structural information toward nylon-degrading enzymes (NylA-C) in our laboratory and newly obtained from screening.

## ④ – 6 Construction of composting system using nylon-degrading enzymes

Issue 1 : Optimization of pretreatment methods for solubilized itaconate derived nylon polymers and oligomers with enhanced solubility

Issue 2 : Construction of composting systems using itaconate derived nylon-degrading microorganisms and enzymes

Final target for FY2029 :

We will construct an itaconate derived nylon-degrading microbial composting system that can reduce the molecular weight of 100 g of highly durable composite in about 20 days by combination of soil and marine microorganisms and/or enzymes.

### Members

KATO Dai-ichiro : Director

FUJIEDA Shigeru : Marine

YOKOGAWA Yukiko : Synthesis

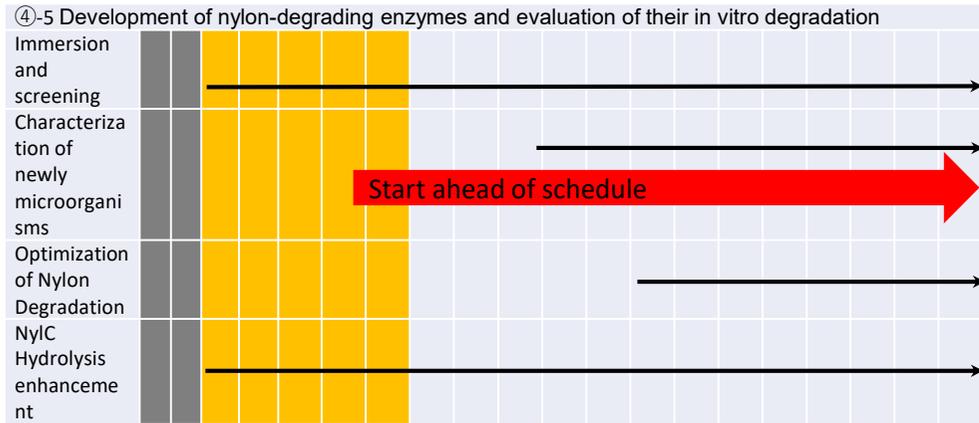
Two Support Staffs

Laboratory students

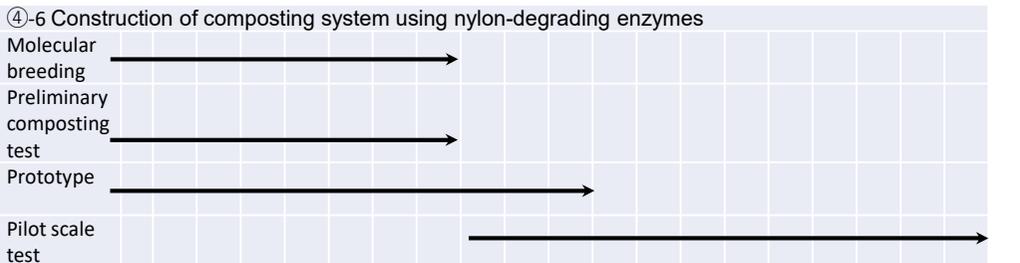
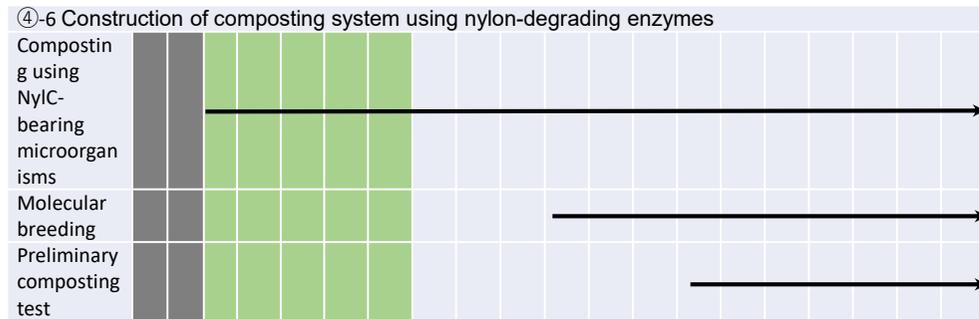
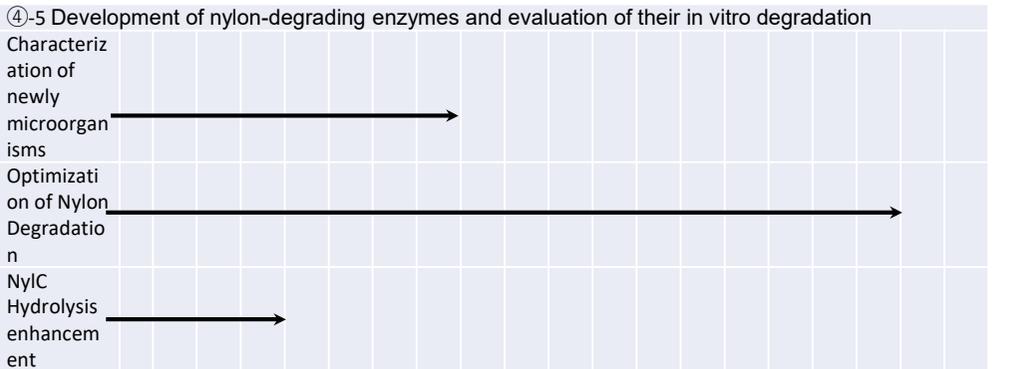


# Details of R&D issues and schedule

| Issue | FY2020 |    |    |    | FY2021 |    |    |    | FY2022 |    |    |    | FY2023 |    |    |    | FY2024 |    |    |    |
|-------|--------|----|----|----|--------|----|----|----|--------|----|----|----|--------|----|----|----|--------|----|----|----|
|       | Q1     | Q2 | Q3 | Q4 |



| 項目 | FY2025 |    |    |    | FY2026 |    |    |    | FY2027 |    |    |    | FY2028 |    |    |    | FY2029 |    |    |    |
|----|--------|----|----|----|--------|----|----|----|--------|----|----|----|--------|----|----|----|--------|----|----|----|
|    | Q1     | Q2 | Q3 | Q4 |



## Research Progress :

The study of each issue is smoothly progressed.  
Some items are being considered ahead of schedule.

## Focus Points :

- ① We are developing a biodegradation system for itaconate-derived nylon using a nylon hydrolase, which is the only enzyme possessed only by our laboratory in the world.
- ② We are attempting to isolate new marine bacteria in order to construct a complete nylon degradation system.

# Our Nylon Oligomer Degrading Bacteria

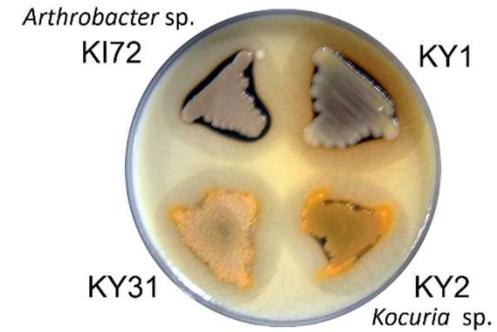
About 10 species of oligomer-degrading microorganisms were obtained from activated sludge of nylon manufacturing plant (since 1970s).

*Arthrobacter* sp. KI72

*Agromyces* sp. KY5R

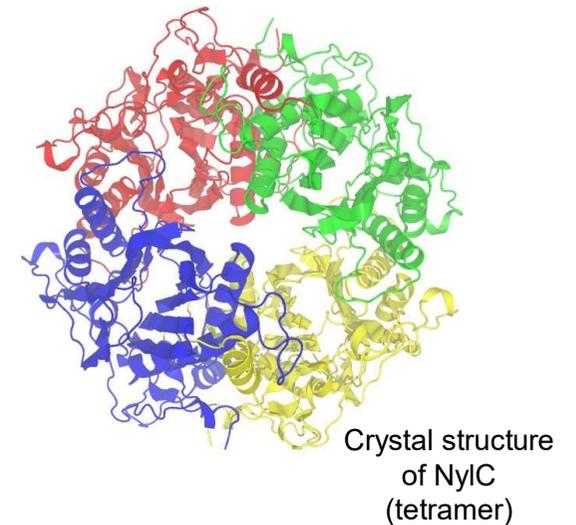
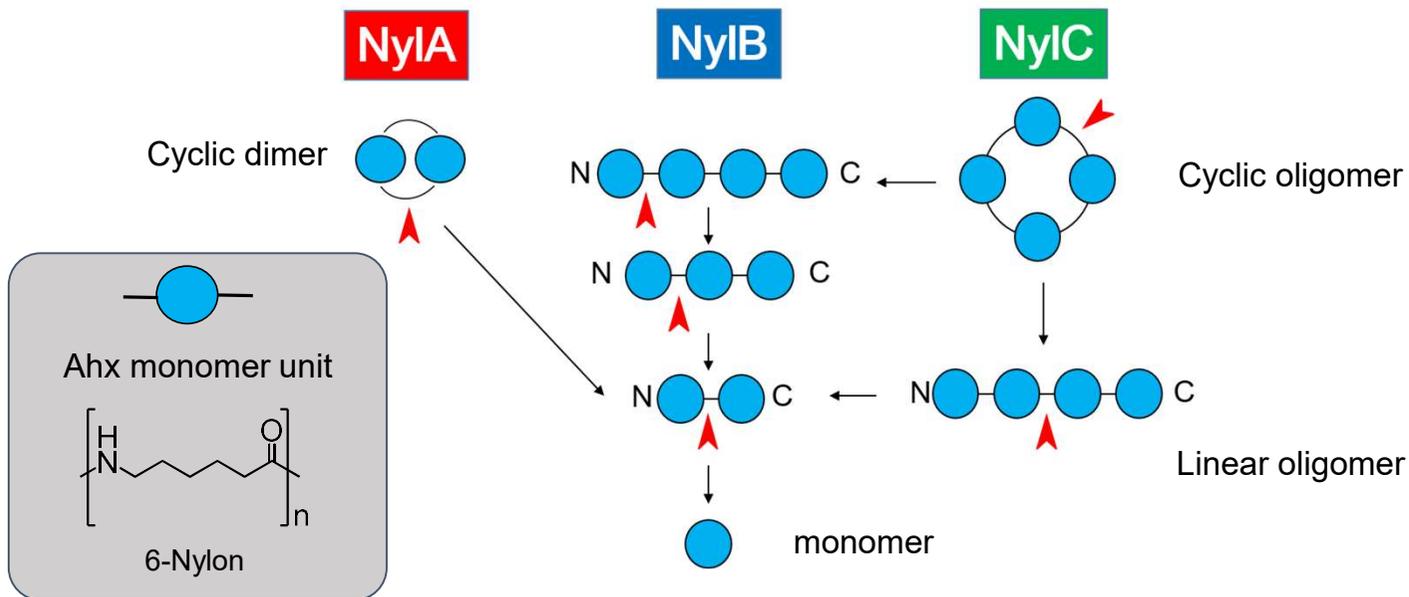
*Kocuria* sp. KY2

→ Related enzymes are widely distributed in different bacteria.



Bacterial growth on nylon oligomer containing agar

## Nylon Oligomer Degrading Enzymes - Three Modes



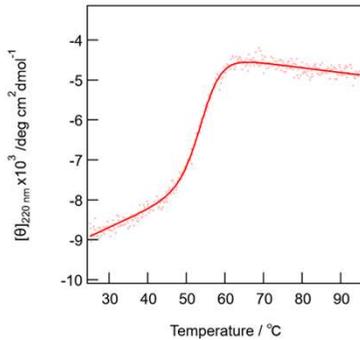
➔ **NylC mutant can hydrolyze polyamide nylon!**

*J. Biol. Chem.*, 2012, 287, 5079.

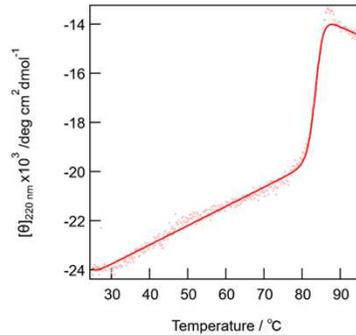
# Development of a practical enzymatic nylon-degradation method

## Success of improving the thermal stability

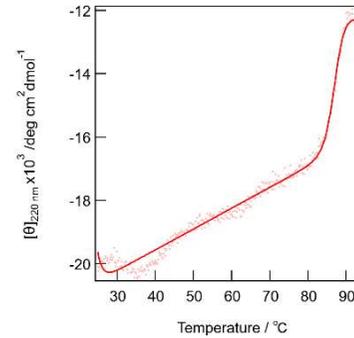
CD spectra : NylC



GYAQ



GYAQT



NylC WT :  $T_m = 52^\circ\text{C}$

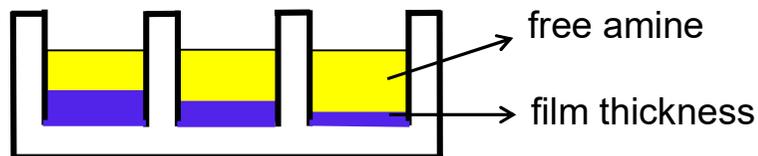
GYAQ mutant :  $T_m = 83^\circ\text{C}$

GYAQT mutant :  $T_m = 88^\circ\text{C}$

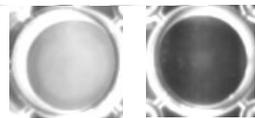
Retain the enzymatic activity even at  $T_g$  of polyamide

## Establishment of quantitative tracking of polyamide hydrolysis

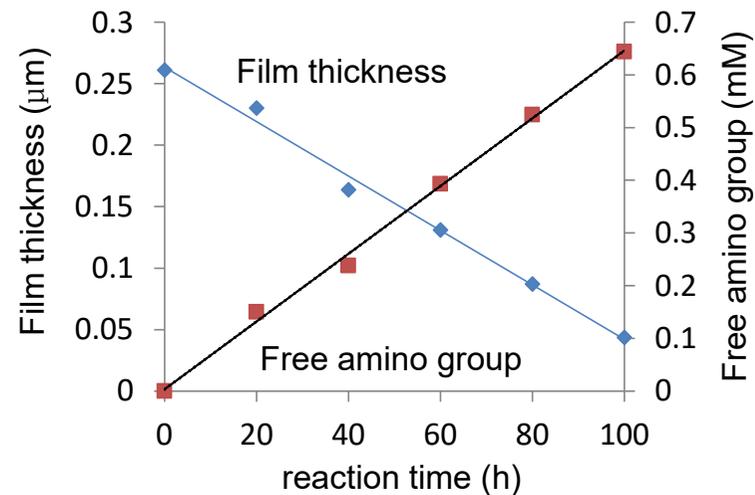
Use of thin-film polymers



Nylon thin film on microplate



before and after reaction



The thermo-stabilized NylC mutant exhibits degradation activities, including not only nylon 6, but also nylon 66 and copolymer bodies.

# Degradation Behavior of Itaconate-derived nylon in the Marine Environment

2021.01.27~ Collecting seawater

Azuma-cho station



Kamoike Fishing Park



2021.02.12~ Collecting seawater, sludge  
2021.08.06~ Starting degradation test

水深5 m



海底から2 m (水深~20 m)



木製漁礁マリンクルードル

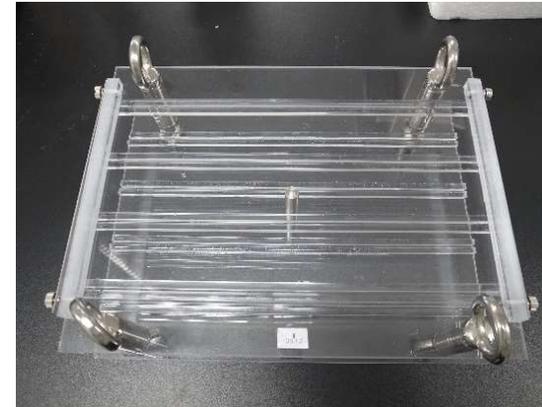


Bacterial flora analysis toward ocean immersed itaconate derived nylon film

Adopted the method used by NITE in NEDO Technology Development Project for Social Implementation of Marine Biodegradable Plastics / Establishment of evaluation method for marine biodegradability (NEDO standardization project)

Reference: "Latest Research Trends in Marine Biodegradable Plastics".  
Technosystems Co. Ltd.

Immersion jig



Film mount



40days



Differences in bacterial flora at depth  
Comparison with Seawater and Sediment Samples



