

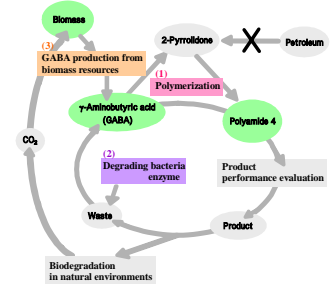
# Novel Biodegradable Polyamide 4

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Polyamide (nylon) has excellent thermal and mechanical properties but it is known to be nondegradable in the natural environment. One of polyamides, polyamide 4 is a linear polymer of  $\gamma$ -aminobutyric acid (GABA). It has also excellent properties based on its high melting point (260 ° C). Unlike other polyamides, it can be biodegraded in an activated sludge as we reported. From these properties, polyamide 4 becomes a new attractive biodegradable material. Furthermore polyamide 4 has the possibility as a novel bio-based polymer because it is synthesized from 2-pyrrolidone, a lactam of GABA, and GABA can be made from glutamate. Glutamate fermentation system using biomass as a raw material is already developed. Our objective is establishment of new recycling system of novel bio-based and biodegradable polymer, polyamide 4.



### (1) The synthesis of the branched polyamide 4 and their properties.

In order to apply in wide practical use, we attempted to improve the properties of polyamide 4. We clarified that introducing branched structure into the polyamide 4 chains it makes possible to improve the tensile strength.

#### Properties and features

##### Properties

- high melting point
- high tensile strength

##### Environment

- biodegradability
- monomer through fermentation process

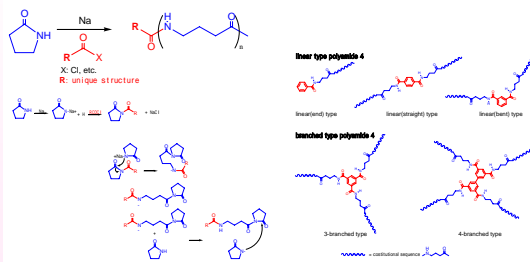
##### Synthesis

- ability of introducing several structures
- polymerizing at room temperature

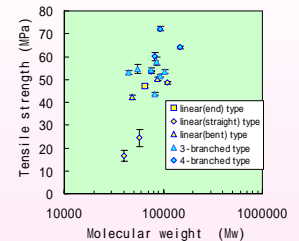
	Melting point ( ° )	Tensile strength (MPa)
Poly(caprolactone)	64 <sup>a)</sup>	68 <sup>b)</sup>
Poly(butylene succinate)	114 <sup>a)</sup>	32 <sup>b)</sup>
Poly(lactic acid)	184 <sup>a)</sup>	53 <sup>b)</sup>
Polyethylene	137 <sup>a)</sup>	30 <sup>b)</sup>
Polyamide 6	223 <sup>a)</sup>	80 <sup>b)</sup>
<b>Polyamide 4</b>	<b>265</b>	<b>~100</b>

reference. a) Polymer Handbook 4th ed. b) maker's catalog

#### Reaction scheme



#### Effect of branching on tensile strength



### (2) Polyamide 4 degrading bacteria isolated from activated sludge.

Isolated bacteria were identified as *Pseudomonas* sp. strain ND-10 and 11. From the detection of GABA as an intermediate product it was suggested that it degraded polyamide 4 by hydrolysis of amide bonds.

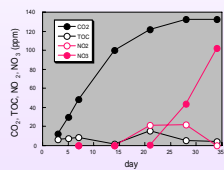
#### Polyamide 4 degradation by activated sludge

Medium: 500 mL  
Polyamide 4: 200 mg  
Activated sludge: 30 mL

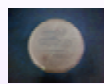


Theoretical CO<sub>2</sub>: 226 ppm

evolved CO<sub>2</sub>  
total organic carbon (TOC)  
(water-soluble products concentration)  
NO<sub>2</sub>, NO<sub>3</sub> concentration



#### Isolation of polyamide 4 degrading bacteria

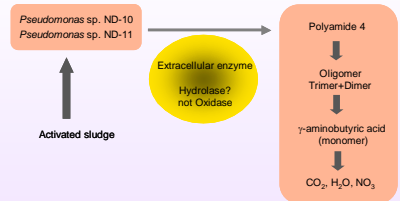


Polyamide 4 degrading bacterium strain ND-11

#### Characteristics of polyamide 4 degrading bacteria

	ND-10	ND-11
Colony color	cream	yellow
Colony shape	circular, low convex, shiny, smooth	circular, convex, shiny, undulated margin
Cell shape	rod	rod
Gram stain	-	-
Catalase	-	-
Motility	-	-
Reduction of nitrate	-	-
Stable from hyposulfite	-	-
Acid from glucose	-	-
O-F test	-	-
Arginine dihydrolase	-	-
Urease	-	-
Oxidase	+	+
Catalase	+	+
$\beta$ -Galactosidase	+	+
Esculin hydrolysis	+	+
Gelatin hydrolysis	+	+
Utilization of		
Glucose, D-mannose, D-fructose, D-xylose, D-cellobiose, sodium citrate	+	+
L-arabinose, D-sorbitol, potassium gluconate	-	-
Inorganic acid, adipic acid, stearic acid	-	-

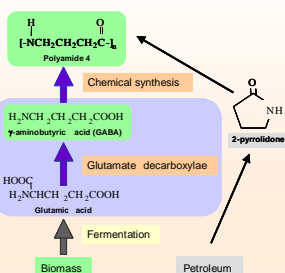
#### Degradation of polyamide 4 by isolated bacteria



### (3) Glutamate decarboxylase (GAD) derived from archaea.

It catalyzes the reaction from glutamate to GABA. We cloned the GAD gene from archaea, *Pyrococcus horikoshii* and prepared recombinant GAD. Prepared GAD was thermostable and could react at high temperature (>95 ° C).

#### Polyamide 4 as bio-based polymer



#### Glutamate decarboxylase (GAD)

##### Strategy

Archaeal genomic database  
↓  
Glutamate decarboxylase (GAD) gene  
(*Pyrococcus horikoshii* PH0937)  
↓  
Expression and detection of enzyme activity

##### Enzyme assay

**Reaction mixture**  
20 mM phosphate buffer pH8.0  
50 mM glutamic acid  
0.25 mM PLP  
Enzyme solution  
↓ incubate

**GABA detection by HPLC**  
column: Wakosil-PTC  
buffer: acetonitrile / 60 mM Sodium acetate buffer pH6.0  
A: 6/94(v/v), B: 60/40(v/v)  
wave length: 254 nm  
sample: PTC derivatization

##### Characterization

Optimum temperature: > 95  
Thermostability: > 90% 85 , 6 hr