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Modification of Biodegradable Polyester using Irradiation

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Background

Global environmental issues

- Global warming
- Acid deposition
- Ozone layer depletion
- Tropical deforestation
- Desertification
- Endangered species
- Marine pollution
- Pollution problems in developing countries

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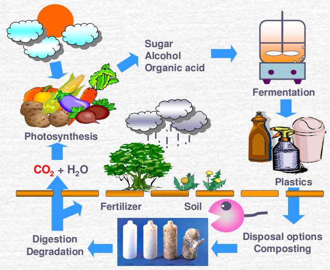

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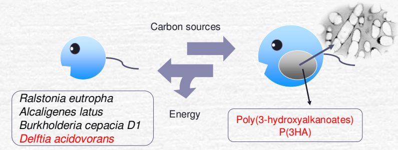
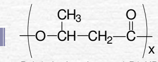


Typical biodegradable plastic

- Biodegradability
- Biocompatibility
- Thermoplasticity



Poly(3-hydroxybutyrate) P(3HB)

Fig. Sketch of microbial synthesis and typical biodegradable polyester produced by microorganism.

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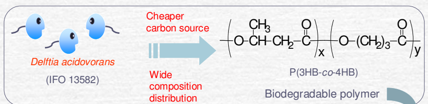
Propose



- Biosynthesis & Fractionation
- Physical properties & Enzymatic degradation
- Mechanism of Enzymatic degradation & Cocrystallization

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Experimental

First-step multiplication → Second-step biosynthesis → Extraction & Fractionation → P(3HB) P(3HB-*co*-4HB) → Analysis

Delftia acidovorans (IFO13582)
Carbon sources: *n*-butyric acid, 1,4-butanediol
Fermentation: 26° C, pH 7.0, 72 h

Fig. The fermentation apparatus used in this study.

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Table .Composition of mineral medium in two-step fermentation.

1st step		2nd step		Microelement solution	
$(NH_4)_2SO_4$	5 g	$Na_2HPO_4 \cdot 12H_2O$	7.16 g	$CoCl_2 \cdot 6H_2O$	217 mg
Meast extract	5 g	KH_2PO_4	2.65 g	$FeCl_3 \cdot 6H_2O$	16.2 mg
Yeast extract	10 g	$MgSO_4 \cdot 7H_2O$	0.2 g	$CaCl_2 \cdot 2H_2O$	7.8 mg
Polypeptone	10 g	$(NH_4)_2SO_4$	0.1 g	$NiCl_2 \cdot 6H_2O$	118 mg
H_2O	1 L	$CaCl_2 \cdot 2H_2O$	1.0 g	$CrCl_3 \cdot 6H_2O$	135 mg
		Citrate	5.0 g	$CuSO_4 \cdot 5H_2O$	156 mg
		Microelement solution	1 ml	0.1 N-HCl	1 L
		H_2O	1 L		

Y. Saito *et al.* *Macromolecules*, 1994;16:99.

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4HB fraction (mol%)

$$\frac{\text{4HB peak (8)}}{[2 \times \text{3HB peak(3)} + \text{4HB peak (8)}]} \times 100$$

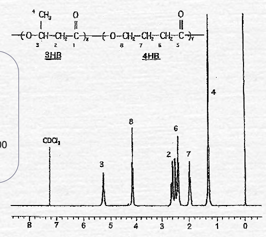

Fig. 1H-NMR spectrum of P(3HB-*co*-65 mol% 4HB).

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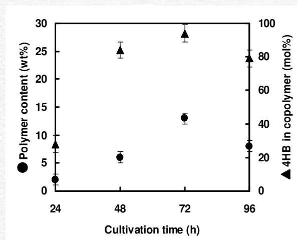

Fig. 3 Dependence of polyester content and 4HB fraction in copolymer on cultivation time by *D. acidovorans*.

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Table . Optimum condition of fermentation by *D. acidovorans*.

pH	7.0
Incubation temperature (° C)	26
Incubation time (h)	72
Concentration of carbon source (g l^{-1})	10

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Radiation-induced graft polymerization for pre-experimental

- Using sodium *p*-styrene sulfonate (SSS) and acrylic acid (AAc), and hydrophobic monomers, such as styrene (St) and methyl acrylate (MAAc) with 60Co γ-rays (dose rate = 10 kGy/h) at 10 kGy in the glass.
- Acrylic acid (AAc) was grafted onto the PHB powder with 20 wt% (▲), 30 wt% (○), and 50 wt% (●) monomer solutions at 10 KGy.
- Figure 4 plots the relationship between the reaction time and degree of grafting (Xg). The Xg of PHB increased with the reaction time on the grafted PHB powder for each concentrations of monomer solution. Moreover, the monomer concentration affected the degree of grafting.

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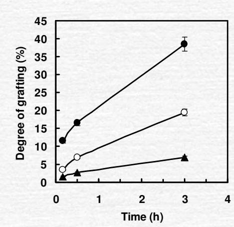

Figure 4: Relationship between reaction time and degree of grafting (Xg). monomer concentration: (▲) : 20 wt% AAc solution, (○) : 30 wt% AAc solution, (●) : 50 wt% AAc solution.

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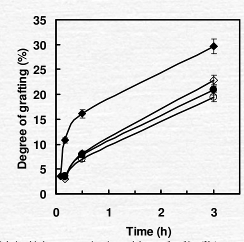

Figure 5: Relationship between reaction time and degree of grafting (Xg). monomer concentration: (◆): 100wt% St; (◇): 0.5 M SSS; (●): 10 wt% MAAc; and (○): 30 wt% AAc.

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Sample	Xg(%)	Tm(℃)
PHB	0	172.6
PHB-g-SSS	3.0	172.6
	8.2	172.3
	22.8	172.2
PHB-g-AAc	3.5	172.8
	6.9	173.1
	19.5	173.2

Grafting ratio and thermal properties of various PHB grafted monomer. (SSS and AAc)

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Sample	Xg(%)	Tm(℃)
PHB	0	172.6
PHB-g-St	3.6	171.8
	10.8	171.7
	29.7	170.6
PHB-g-MAAc	3.5	172.2
	7.8	171.8
	30.4	171.1

Grafting ratio and thermal properties of various PHB grafted monomer. (St and MAAc)

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Conclusion

- The fermentation conditions to obtain the highest polymer content at the two-step fermentation by *D. acidovorans* were examined by changing the concentration of carbon source, pH and incubation temperature or time. The result of the most optimum condition decided experimentally was as follows:

pH	7.0
Incubation temperature (° C)	26
Incubation time (h)	72
Concentration of carbon source (g l^{-1})	10

Where the highest polymer content was around 13% per dry cell weight.

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Conclusion

- The grafting of various monomers to the PHB powder increased with the monomer concentration and reaction time. The degree of grafting (Xg) onto the PHB by these various monomers decreased in the order St, SSS, MAAc, and AAc.
- The thermal properties of the PHB-g-St, PHB-g-SSS, PHB-g-MAAc and PHB-g-AAc films were very similar. A comparison of the enzymatic degradability of the pair of PHB-g-St and PHB-g-SSS films with the pair of PHB-g-MAAc and PHB-g-AAc films demonstrated that the presence of hydrophilic groups in the grafting chains affected the enzymatic degradability of the grafted PHB films.
- The grafting of various monomers onto the PHB powder increased with reaction time. The degree of grafting (Xg) onto the PHB by these various monomers followed the order St, SSS, MAAc and AAc.

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Future

- It's possible to use this method of radiation induced graft polymerization onto PHB powder.
- Further ,we can rise the biocompatibility of PHB powder by this way. we will take citoson ,carrangene , alginate and gelatin to radiation induced graft polymerization.
- In order to make it possible that biomaterial use in human , we are researching now and taking cell experiment.

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Reference

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