Journal of the Taiwan Institute of Chemical Engineers xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

# Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate)

Wen-Chuan Hsieh [a,*], Yuki Wada [b], Tsutomu Mitobe [b], Hiroshi Mitomo [b], Noriaki Seko [c], Masao Tamada [c]

[a] Department of Biological Science and Technology, I-Shou University, Kaohsiung 82445, Taiwan
[b] Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Gunma 376-8515, Japan
[c] Environment and Industrial Materials Research Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency, Gunma 370-1292, Japan

ARTICLE INFO

*Article history:*
Received 16 July 2008
Received in revised form 23 October 2008
Accepted 24 October 2008
Available online xxx

*Keywords:*
Microbial poly(3-hydroxybutyrate)
Radiation grafting
Enzymatic degradation
Hydrophilic property

ABSTRACT

The radiation-induced graft polymerization of hydrophilic [sodium *p*-styrene sulfonate (SSS), acrylic acid (AAc)] and hydrophobic [styrene (St), methyl acrylate (MAAc)] monomers onto microbial poly(3-hydroxybutyrate) (PHB) powder was performed and the characteristics of the both grafting films obtained after remolding were compared. The grafting of various monomers onto the PHB powder increased with reaction time. The degree of grafting ($X_g$) onto the PHB by these various monomers followed the order St, SSS, MAAc and AAc. The PHB films onto which were grafted St, SSS, MAAc and AAc were characterized by measuring the contact angle, the adsorption of enzyme PHB depolymerase, and the biodegradability, as well as by differential scanning calorimetry. Both hydrophobic and hydrophilic monomers affected the contact angle of grafted PHB film with St, SSS, MAAc and AAc monomers. However, the adsorption of enzyme on the grafted PHB film increased with the amount of hydrophilic monomer and degree of grafting. Consequently, the enzyme PHB depolymerase easily approached the PHB film when the hydrophilic groups were grafted onto its surface. The enzymatic degradation of grafted PHB films with hydrophilic monomer proceeded more quickly than that of hydrophobic monomers.



## 1. Introduction

The microbial polyesters poly(3-hydroxybutyrate) P(3HB), is a biocompatible and biodegradable thermoplastic polymer that is produced by various bacteria in nature as an intracellular carbon and energy source (Anderson and Dawes, 1990; Doi, 1990). The degradable P(3HB) had attracted considerable attention for its many agricultural, industrial, and medical applications (Doi, 1990). However, the brittleness and stiffness of PHB limit its range of practical applications, and the enzymatic degradability of PHB must be controlled to enable utilization.

Three techniques have been applied to overcome this shortcoming of PHB. One of the approaches is to biosynthesize copolymers that contain poly(hydroxyalkanoate) (PHA) units. The second is to prepare the blend polymers of PHB and other chemical synthesized polymers. The third is radiation grafting onto PHB. Another application of radiation graft polymerization is developmental research into the separation and refinement of proteins to separate functional materials (Miyoshi *et al.*, 2005). For environmental reasons, materials are being developed as graft adsorbents to remove toxic metals from industrial waste waters and the harmful constituents of the atmosphere (Seko *et al.*, 2003, 2004; Shiraishi *et al.*, 2003). Research into the recovery of useful rare metals from seawater and hot springs is currently being undertaken (Seko *et al.*, 2005). The graft adsorbent has been used to recover rare metals such as scandium (Sc), vanadium (V) and arsenic (As) from the hot springs of Kusatsu in the Gunma Prefecture. These graft adsorbents are improved by changing the functional groups on the graft chains to promote the recovery of required metals.

This authors' research group recently succeeded in controlling the enzymatic degradability of PHB using the radiation graft copolymerization method (Bahari *et al.*, 1997, 1998; Cakmakli *et al.*, 2001; Mitomo *et al.*, 1995, 1996). In an earlier work our group described grafted hydrophilic monomers (2-hydroxyethyl methacrylate (HEMA) (Mitomo *et al.*, 1995), acrylic acid (AAc) (Mitomo *et al.*, 1996) and the hydrophobic monomers, styrene (St) (Bahari *et al.*, 1997, 1998; Cakmakli *et al.*, 2001) and methyl methacrylate (MMA)) onto PHB. These experiments indicated that while grafting

* Corresponding author. Tel.: +886 7 6151100x7319; fax: +886 7 6155150.
*E-mail address:* wenchuan@isu.edu.tw (W.-C. Hsieh).

*Abbreviations:* P(3HB), poly(3-hydroxybutyrate); SSS, sodium *p*-styrene sulfonate; AAc, acrylic acid; St, styrene; MAAc, methyl acrylate; $X_g$, degree of grafting; Sc, scandium; V, vanadium; As, arsenic; HEMA, 2-hydroxyethyl methacrylate; MMA, methyl methacrylate; BOD, biochemical oxygen demand; KGy, irradiation dose; Torr, unit of pressure; $\Delta H_m$, enthalpy of melting; $(\Delta H_m)_{corr}$, corrected enthalpy of melting; $T_m$, melting point.

1876-1070/$ – see front matter © 2008 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jtice.2008.10.005

PHB into the hydrophobic MMA sharply reduced its enzymatic degradability, introducing hydrophilic HEMA increased enzymatic degradability (Mitomo *et al.*, 1995). Accordingly, the effect of the hydrophilicity of the grafting chains on the enzymatic degradation of the PHB was approximately determined.

In this work, onto microbial PHB was grafted various hydrophilic monomers, including sodium *p*-styrene sulfonate (SSS) and acrylic acid (AAc), and hydrophobic monomers, such as styrene (St) and methyl acrylate (MAAc), using γ-rays. The thermal properties of grafted PHB were determined. Furthermore, the enzymatic degradability, contact angle and enzymatic adsorption of the grafted samples were discussed.

## 2. Materials and methods

### 2.1. Preparation of materials

PHB was purchased from Aldrich Chemical Co., Ltd. (USA). PHB was purified as follows: PHB (20 g) was dissolved in chloroform (1000 mL) and then poured into a solvent that consisted of *n*-hexane and methanol (2000 mL, 1:1 vol%). The precipitated PHB powder was then isolated by filtration and dried under vacuum. The mean particle size of the obtained PHB powder was about 20 μm. The St monomer, purchased from Kanto Chemical Co., Inc., was used after the inhibitor was removed at graft proceeding. The monomers of SSS, AAc (purchased from Tokyo Kasei Kogyo Co., Ltd.) and MAAc (purchased from Kanto Chemical Co., Inc.) were used without further purification.

### 2.2. Radiation-induced graft polymerization on PHB powder

The PHB-g-SSS, PHB-g-St, PHB-g-AAc, and PHB-g-MAAc powders were prepared by the pre-irradiation before radiation-induced graft polymerization. The PHB powder (2.0 g) was placed on one side of an H-shaped glass ampoule that was sealed at reduced pressure ($10^{-3}$ Torr). The PHB was then pre-irradiated with $^{60}$Co γ-rays (dose rate = 10 kGy/h) at 10 kGy in the glass ampoule at −70 °C. Following irradiation, the monomer (10 mL) was poured into the opposite side of the ampoule, and it was resealed and evacuated ($10^{-3}$ Torr). The degree of grafting ($X_g$) given by the following equation (Basuki *et al.*, 2003):

$$X_g(\%) = (W_g - W_0) \times \frac{100}{W_0} \quad (1)$$

where $W_0$ and $W_g$ are the weights of the PHB powder before and after the graft polymerization, respectively.

The grafting reactions of the St, SSS, AAc and MAAc monomers proceeded in a temperature-controlled water bath at 60 °C for 10 min, 30 min and 3 h before the grafted powders were immersed in a solvent to terminate the grafting reaction and to remove the homopolymers. The concentration of monomer in the St was denoted 100 wt.% solution in this study. The monomer concentration of the SSS solution was adjusted to 0.5 M using water as the solvent and the monomer concentrations of the AAc and MAAc solutions were adjusted to 30 and 10 wt.%, respectively, using methanol as the solvent.

### 2.3. Analysis

The thermal properties of the PHB powder and film were investigated using a Shimadzu DSC-50 (Japan). About 2 mg of the sample was packed in an aluminum pan and heated from 25 °C to 190 °C at a rate of 10 °C/min in a 30 mL/min nitrogen flow. The enthalpy of melting ($\Delta H_m$) was determined by DSC from the area under the endothermic peak. The temperature was calibrated using highly pure benzoic acid standards.

The $\Delta H_m$ value was corrected using the weight fraction of PHB in the grafted samples and the following equation (Mitomo *et al.*, 1995, 1996) (corrected enthalpy of melting $(\Delta H_m)_{corr.}$).

$$\frac{\Delta H_m}{(\Delta H_m)_{corr.}} = \frac{100 - X_g}{100} \quad (2)$$

The contact angles of various monomers on the grafted PHB films were measured using a contact-angle meter (Kyowa Scientific Co., Ltd.). The contact angles were determined using a drop of 0.05 M NaOH solution at room temperature.

The grafted PHB powder was formed into a film by hot-pressing at 190 °C. The obtained films were 150 μm thick and were isothermally crystallized at 90 °C for 1 week before use. The enzymatic degradation of the irradiated PHB films was evaluated at 37 °C in 0.1 M phosphate buffer (pH 7.4). PHB depolymerase purified from *Ralstonia pickettii* T1 (Yamada *et al.*, 1993) was used. The irradiated PHB films (150-μm thick) were cut into squares of 10 mm × 10 mm and then placed in small test tubes that contained 1.0 mL of buffer solution. The reactions were initiated by adding 4 μg of PHB depolymerase. The weight loss of the film was periodically measured after it was removed and washed using methanol and distilled water. In this work, the weight loss (mg/cm$^2$ h) was calculated from the weight after 48 h of enzymatic degradation.

The enzymatic adsorption test involved a U-3310UV spectrophotometer from HITACHI. The grafted PHB film adsorbed enzyme at 10 °C in 0.1 M phosphate buffer (pH 7.4). The grafted PHB films (initial weight, about 10 mg; film dimensions, 10 mm × 10 mm; film thickness, 0.09 mm) were placed in small test tubes that contained 1.0 mL of the buffer. The reactions were initiated by adding aqueous PHB depolymerase (1 μg). The reaction solutions were incubated with shaking. The film of enzyme-adsorbing PHB was then periodically removed, and the reduced solution (1 μL) was moved to another buffer solution that contained the PHB matrix at 37 °C. The reaction solution was assayed by measuring the drop in turbidity at 650 nm and 37 °C.

## 3. Results and discussion

### 3.1. Grafting PHB powder with acrylic acid

Acrylic acid (AAc) was grafted onto the PHB powder with 20 wt.% (▲), 30 wt.% (○), and 50 wt.% (●) monomer solutions at 10 kGy. Fig. 1 plots the relationship between the reaction time and

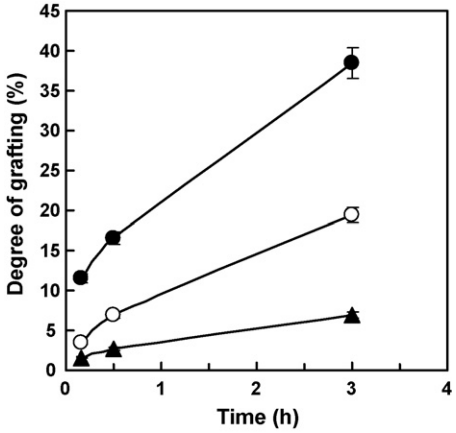


**Fig. 1.** Relationship between reaction time and degree of grafting ($X_g$). Monomer concentration: (▲) 20 wt.% AAc solution, (○) 30 wt.% AAc solution, and (●): 50 wt.% AAc solution.

Please cite this article in press as: Hsieh, W.-C., et al., Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate). J. Chin. Inst. Chem. Eng. (2009), doi:10.1016/j.jtice.2008.10.005

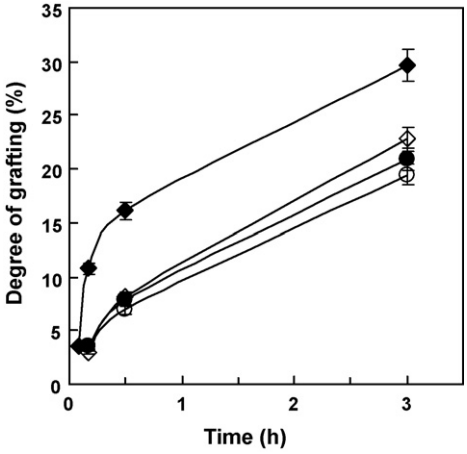


**Fig. 2.** Relationship between reaction time and degree of grafting ($X_g$). Monomer concentration: (◆) 100 wt.% St, (◇) 0.5 M SSS, (●) 10 wt.% MAAc, and (○) 30 wt.% AAc.

degree of grafting. The $X_g$ 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. The $X_g$ of PHB increased with the monomer concentration (●: 50 wt.% AAc > ○: 30 wt.% AAc > ▲: 20 wt.% AAc) over a reaction time of 3 h.

### 3.2. Hydrophobic/hydrophilic monomers grafted on PHB powder

To evaluate the effect of the grafting of hydrophobic/hydrophilic monomers onto the PHB powder, St (100 wt.%; ◆), SSS (0.5 M; ◇), AAc (30 wt.%; ○), and MAAc (10 wt.%; ●) were grafted onto the PHB powder using various concentrations of the monomer solutions. Fig. 2 plots the relationship between the reaction time and the degree of grafting. The grafting ratios of grafted PHB powder to various monomers increased with the reaction time to 3 h. The degree of grafting onto the PHB by these various monomers decreased in the order St, SSS, MAAc, and AAc. Grafted PHB powders, such powders were made by hot pressing to evaluate their contact angle, adsorption of enzyme and biodegradability.

Table 1 presents the grafting rates and thermal properties of the various PHB and PHB-grafted monomers (St, SSS, AAc, and MAAc) films. The melting point ($T_m$) and corrected enthalpies of melting ($(\Delta H_m)_{corr.}$) of all grafted PHB samples were almost independent of the grafting ratio, suggesting that the graft chains barely affected the crystalline structure.

**Table 1**
Grafting ratio and thermal properties of various PHB grafted monomer (St, SSS, MAAc, and AAc) films.

| Sample | $X_g$ (%) | $T_m$ (°C) | $\Delta H_m$ (J/g) | $(\Delta H_m)_{corr.}$ (J/g) |
|---|---|---|---|---|
| PHB | 0 ± 0 | 172.6 ± 0.1 | 77.6 ± 0.3 | 77.6 ± 0.3 |
| PHB-g-St | 3.6 ± 0.2 | 171.8 ± 0.0 | 73.4 ± 0.1 | 76.1 ± 0.0 |
| | 10.8 ± 0.3 | 171.7 ± 0.2 | 69.9 ± 0.3 | 78.4 ± 0.4 |
| | 29.7 ± 0.5 | 170.6 ± 0.1 | 54.9 ± 0.4 | 78.1 ± 0.3 |
| PHB-g-SSS | 3.0 ± 0.1 | 172.6 ± 0.3 | 70.9 ± 0.3 | 73.1 ± 0.3 |
| | 8.2 ± 0.3 | 172.3 ± 0.2 | 66.9 ± 0.2 | 72.9 ± 0.2 |
| | 22.8 ± 0.4 | 172.2 ± 0.0 | 57.2 ± 0.1 | 74.1 ± 0.5 |
| PHB-g-MAAc | 3.5 ± 0.6 | 172.2 ± 0.1 | 73.2 ± 0.2 | 75.9 ± 0.3 |
| | 7.8 ± 0.4 | 171.8 ± 0.0 | 71.2 ± 0.2 | 77.2 ± 0.4 |
| | 20.9 ± 0.3 | 171.1 ± 0.2 | 55.6 ± 0.1 | 79.9 ± 0.2 |
| PHB-g-AAc | 3.5 ± 0.2 | 172.8 ± 0.0 | 74.0 ± 0.2 | 76.7 ± 0.1 |
| | 6.9 ± 0.5 | 173.1 ± 0.2 | 73.1 ± 0.3 | 78.5 ± 0.1 |
| | 19.5 ± 0.3 | 173.2 ± 0.3 | 64.3 ± 0.4 | 79.9 ± 0.2 |

The results are the mean ± standard error of mean of three separate experiments.

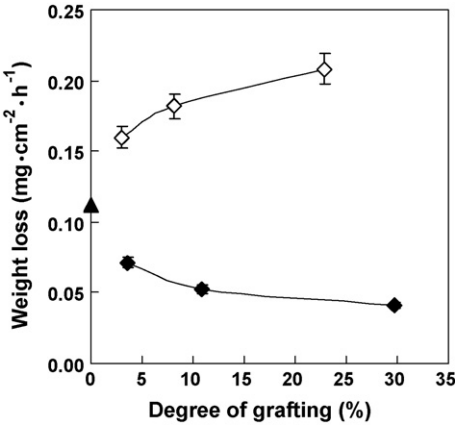


**Fig. 3.** Enzymatic degradation of PHB and grafted PHB films against degree of grafting (%). (◇) PHB-g-SSS film, (▲) PHB film, and (◆) PHB-g-St film.

### 3.3. Enzymatic degradation of PHB film with various grafted monomers

The hot-pressed PHB and various grafted monomer (St, SSS, MAAc, and AAc) films were degraded using the enzyme PHB depolymerase. Fig. 3 plots the relationship between $X_g$ [PHB-g-St (◆) and PHB-g-SSS (◇)] and weight loss after 48 h of enzymatic degradation. The weight loss of PHB-g-St significantly declined and that of PHB-g-SSS substantially increased as $X_g$ increased. This difference between the weight loss characteristics of the two grafted films was related to the presence or absence of the hydrophilic side chains on the graft chains, indicating that the grafting of St (PHB-g-St) and SSS (PHB-g-SSS) onto PHB powders affected their enzymatic degradability, although St and SSS have similar chemical structures, they have different hydrophilic properties.

Fig. 4 plots the relationship between $X_g$ [PHB-g-AAc (○) and PHB-g-MAAc (●)] and weight loss after 48 h of enzymatic degradation. The difference between the enzymatic degradability of the PHB powder to which was grafted AAc (PHB-g-AAc) and that to which was grafted MAAc (PHB-g-MAAc) was examined. These results demonstrate that the weight loss of PHB-g-MAAc was lower

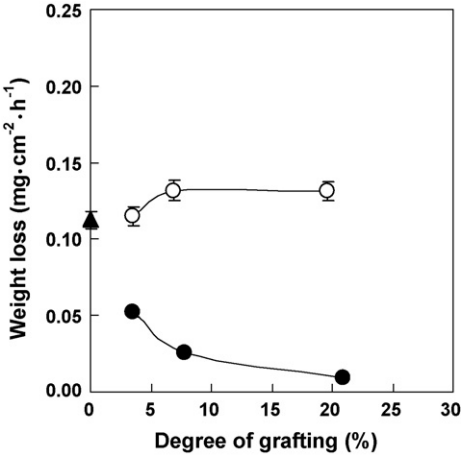


**Fig. 4.** Enzymatic degradation of PHB and grafted PHB films against degree of grafting (%). (○) PHB-g-AAc film, (▲) PHB film, and (●) PHB-g-MAAc film.

Please cite this article in press as: Hsieh, W.-C., et al., Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate). J. Chin. Inst. Chem. Eng. (2009), doi:10.1016/j.jtice.2008.10.005

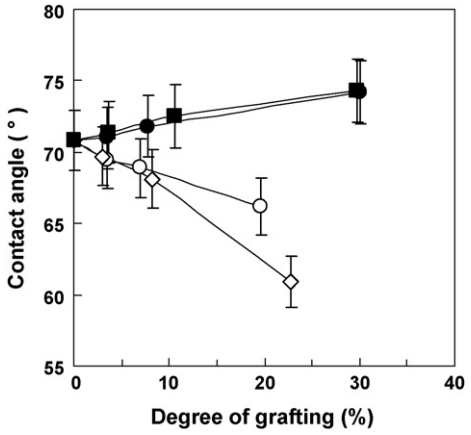


**Fig. 5.** Contact angle of the PHB grafted with various monomer films. (○) AAc, (●) MAAc, (◇) SSS, and (■) St.

than that of the PHB film, while that of PHB-g-AAc was higher, in each case to an extent that increased with the $X_g$ of the samples: the degradation of grafted PHB-g-AAc polymer increased with the amount of hydrophilic monomer (AAc) after grafting, while that of grafted PHB-g-MAAc polymer declined as the amount of hydrophobic monomer (MAAc) increased after grafting. Similar results were obtained, as indicated above, for the grafting of St and SSS monomers onto PHB powder.

### 3.4. Contact angle of PHB film grafted various monomers

Fig. 5 plots the contact angles between the PHB films to which had been grafted various monomers and the monomers themselves. The contact angles of PHB-g-AAc (○) and PHB-g-SSS (◇) films were markedly reduced by increasing $X_g$, perhaps because the surface of the PHB film was completely covered with the grafted acrylic acid or styrenesulfonate. However, the contact angles of the PHB-g-MAAc (●) and PHB-g-St (■) films significantly increased with $X_g$. As described above, the grafting of hydrophilic monomer increased the enzymatic degradability of grafted PHB. Therefore, the grafting chains were homogenously distributed in the remolded film, suggesting that the hydrophilic property on the surface of the grafted PHB film influenced the contact angle of the grafted PHB film. Our research group has been detecting the crosses-section of remolded PHB-g-AAc film using the SEM-EDX technique to observe the distribution of adsorbed elements (Wada *et al.*, 2006). The surface of the remolded grafted PHB sample was almost covered with the grafted monomer. The region close to the surface of the PHB film was overcrowded with graft chains as $X_g$ was increased (data not shown).

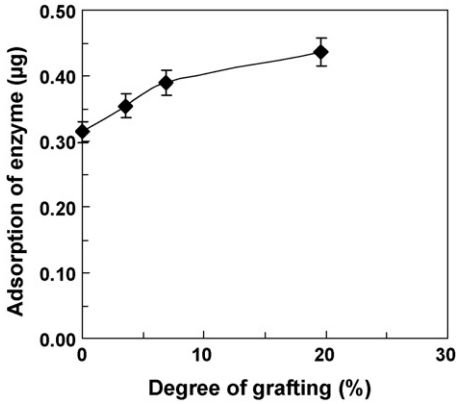


**Fig. 6.** Adsorption of enzyme on the PHB-g-AAc film with various degrees of grafting.

### 3.5. Adsorption of enzyme on PHB-g-AAc film

Fig. 6 plots the adsorption of the enzyme on the PHB-g-AAc film with various degrees of grafting. The adsorption increased with grafting, suggesting that an enzyme easily approaches the film when a hydrophilic group is added to a graft chain, indicating that the grafting of a hydrophilic chain to the PHB film increased its degradability.

## 4. Conclusions

The grafting of various monomers to the PHB powder increased with the monomer concentration and reaction time. The degree of grafting 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 grafted PHB film was hydrophilic because of the grafting of hydrophilic monomers. Therefore, the enzyme PHB depolymerase easily approached the surface of the PHB film. Accordingly, the enzymatic degradability of these grafted PHB films exceeded that of films to which were grafted with hydrophobic monomers.

The contact angles of PHB grafted with various monomer films were influenced by the hydrophilic or hydrophobic character of the monomers. The adsorption of the enzyme on the PHB-g-AAc film increased with the degree of grafting, indicating that the enzyme PHB depolymerase easily approached the surface of the PHB film and that the hydrophilic chain grafted onto the PHB film improved its degradability.

## Acknowledgements

The authors would like to thank the National Science Council of the Republic of China, Taiwan (NSC 97-2221-E-214-014) and I-Shou University, Taiwan (contract no. 97-04-03) for partially supporting this research.

## References

Anderson, A. J. and E. A. Dawes, "Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates," *Microbiol. Mol. Biol. Rev.*, **54**, 450 (1990).

Bahari, K., H. Mitomo, T. Enjoji, S. Hasegawa, and F. Yoshii, "Radiation-induced Graft Polymerization of Styrene onto Poly(3-hydroxybutyrate) and Its Copolymer with 3-Hydroxyvalerate," *Die Angew. Makromol. Chem.*, **250**, 31 (1997).

Bahari, K., H. Mitomo, T. Enjoji, and F. Yoshii, "Degradability of Poly(3-hydroxybutyrate) and Its Copolymer Grafted with Styrene by Radiation," *Polym. Degrad. Stab.*, **61**, 245 (1998).

Basuki, F., M. Tamada, N. Seko, T. Sugo, and T. Kume, "Direct Synthesis of Adsorbent Having Phosphoric Acid and Radiation Induced Graft Polymerization," *J. Ion Exchange*, **14**, 209 (2003).

Cakmakli, B., B. Hazer, and M. Borcakli, "Poly(styrene peroxide) and Poly(methyl methacrylate peroxide) for Grafting on Unsaturated Bacterial Polyesters," *Macromol. Biosci.*, **1**, 348 (2001).

Doi, Y., *Microbial Polyesters*, , VCH Publishers, New York, NY (1990).

Mitomo, H., T. Enjoji, Y. Watanabe, F. Yoshii, and T. Saito, "Radiation-induced Graft Polymerization of Poly(3-hydroxybutyrate) and Its Popolymer," *J. Macromol. Sci. A*, **32** (3), 429 (1995).

Mitomo, H., T. Sasaoka, F. Yoshii, and T. Saito, "Radiation-induced Graft Polymerization of Acrylic Acid onto Poly(3-hydroxybutyrate) and Its Copolymer," *Sen'i Gakkaishi (Japan)*, **52**, 623 (1996).

Miyoshi, K., K. Saito, T. Shiraishi, and T. Sugo, "Introduction of Taurine into Polymer Brush Grafted onto Porous Hollow-fiber Membrane," *J. Membr. Sci.*, **264**, 97 (2005).

Please cite this article in press as: Hsieh, W.-C., et al., Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate). J. Chin. Inst. Chem. Eng. (2009), doi:10.1016/j.jtice.2008.10.005

Seko, N., A. Katakai, S. Hasegawa, M. Tamada, N. Kasai, H. Takeda, T. Sugo, and K. Saito, "Aquaculture of Uranium in Seawater by a Fabric-Adsorbent Submerged System," *Nucl. Technol.*, **144** (2), 274 (2003).

Seko, N., A. Katakai, M. Tamada, T. Sugo, and F. Yoshii, "Fine Fibrous Amidoxime Adsorbent Synthesized by Grafting and Uranium Adsorption-Elution Cyclic Test with Seawater," *Sep. Sci. Technol.*, **39** (16), 3753 (2004).

Seko, N., M. Tamada, and F. Yoshii, "Current Status of Adsorbent for Metal Ions with Radiation Grafting and Crosslinking Techniques," *Nucl. Instr. Meth. Phys. Res. B*, **236**, 21 (2005).

Shiraishi, T., M. Tamada, K. Saito, and T. Sugo, "Recovery of Cadmium from Waste of Scallop Processing with Amidoxime Adsorbent Synthesized by Graft-Polymerization," *Radiat. Phys. Chem.*, **66**, 43 (2003).

Wada, Y., H. Mitomo, K. Kasuya, N. Nagasawa, N. Seko, A. Katakai, and M. Tamada, "Control of Biodegradability of Poly(3-hydroxybutyric acid) Film with Grafting Acrylic Acid and Thermal Remolding," *J. Appl. Polym. Sci.*, **1021**, 3856 (2006).

Yamada, K., Y. Mukai, and Y. Doi, "Enzymatic Degradation of Poly(hydroxyalkanoates) by *Pseudomonas pickettii*," *Int. J. Biol. Macromol.*, **15**, 215 (1993).

Please cite this article in press as: Hsieh, W.-C., et al., Effect of hydrophilic and hydrophobic monomers grafting on microbial poly(3-hydroxybutyrate). J. Chin. Inst. Chem. Eng. (2009), doi:10.1016/j.jtice.2008.10.005