1952 *Chem. Mater.* **1999,** *11,* 1952−1956

Preparation and Characterization of a Novel Polyethylene−*Chlorella* Composite

Farao Zhang,† Hiroshi Kabeya,†
Ryouichi Kitagawa,† Takahiro Hirotsu,*,†
Masatada Yamashita,‡ and Toshi Otsuki‡

Biological Material Division, Shikoku National Industrial Research Institute (SNIRI), 2217-14 Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan, and Project Center for CO_2 Fixation and Utilization, Research Institute of Innovative Technology for the Earth (RITE), Toyokaiji Bldg. #7, 8F, 2-8-11 Nishishinbashi, Minato-ku, Tokyo 105, Japan

Received January 29, 1999

We have directed much attention to chlorella, a kind of microalga belonging to chlorophyceae, from the viewpoint of fixation of CO_2 by photosynthesis because of its high utilization rate (10−20%) of light energy, compared with common plants (0.1−0.5%).[1,2] Highly efficient photosynthesis with solar light would produce a high-density, large-volume industrial culture of chlorella consuming CO_2 instead of discharging it into the atmosphere, regarded as a main cause of global warming. A fixing rate of CO_2 greater than 1 kg of $CO_2/m^3/d$ has been achieved.[3] However, the problem of how to make effective use of the microalgae following biological fixation of CO_2 still remains. Thus, we have conducted various studies to explore the utilization of the microalga. One of these studies involved using the microalga as a filler in thermoplastics, such as polyethylene (PE), aiming at developing building or other functional materials.

Natural cellulosic fibers such as wood,[4,5] straw,[6] and pineapple leaf[7] have been studied as reinforcers of thermoplastic resins. To overcome their incompatibility with the resin matrix, methods of coating[4,5,8] and grafting[5,8−10] have been studied to improve the surface properties of natural polymers. It has been reported that fiber grafting is better than fiber coating because in the former case chemical bonds are formed between the cellulosic fibers and treating agents such as a silane coupling agent.[11] Compatibility between cellulosic fibers and a resin matrix can also be enhanced by modification of the resin, on the surface of which hydrophilic or active groups are introduced.[12,13] Maleic anhydride has been reported to be an effective modifier for polypropylene[14,15] and polyethylene.[16−18]

However, little study has been reported on composite materials of microalgae−thermoplastic resins. Only *cladophora glomerata*, an alga with a filamentous structure, has been reported as a filler in polystyrene resin, destructurized starch, and polycaprolactones,[19] but no evidence has been presented for the binding between the alga and matrix components. In this study, we proposed a method for improving the compatibility of chlorella and polyethylene by chemical modification of PE with maleic anhydride, by which the resultant PE−chlorella composite exhibits a marked increase in tensile strength, compared with that of the unmodified composite.

Chlorella (Yaeyama Shokusan, Japan) used in the experiment was spray-dried after centrifugal concentration. The dried chlorella had a density of 0.65 g/cm^3, and the grains of the chlorella aggregate were nearly spherical and about 50 μm in average grain size (Figure 1a). The aggregate grains are hollow spheres, with walls about 5−20 μm thick, each sphere consisting of chlorella cells (about 3−5 μm in diameter) bonded together (Figure 1b). It is known that the cell wall is mainly made of α-cellulose (ca. 15.4%), hemicellulose (ca. 31%), protein (ca. 27%), and fatty acid (ca. 9.2%).[2] Polyethylene (high density), of a commercially available grade (grade 7000F, $M_w \doteqdot 40\,000$, Mitsui Sekiyu Kagaku, Japan), was mechanically seived into spherical grains with a size of about 1 mm before modification.

Chemical modification of PE and preparation of the PE−chlorella composite were performed in a roller mixer with a chamber volume of 60 cm^3 (R60, Toyo Seiki Seisakusho, Japan). Maleic anhydride (MA) (Wako Chemicals, Japan) and benzoyl peroxide (BPO) (Nacalai Tesque, Japan) used as an initiator were dissolved in acetone in turn, and the acetone solution was then sprayed onto the PE powder until the weight ratio of MA:BPO:PE became 0.25:0.02:100 (mole ratio: 0.071: 0.0023:100, where the last number is a molar number of ethylene units). After it was well-blended, the mixture was fed into the roller mixer and treated at 160 °C for 30 min to modify the PE. The modified-PE(MPE)−chlorella composite was prepared by feeding chlorella

---

* To whom correspondence should be addressed. Tel.: +81-87-869-3511. Fax: +81-87-869-3553. E-mail: hirotsu@sniri.go.jp.

(1) Project of biological CO_2 fixation and utilization. Pamphlet of the Research Institute of Innovative Technology for the Earth (RITE), 1997, available from RITE, Toyokaiji Bldg. #7, 8F, 2-8-11 Nishishinbashi, Minato-ku, Tokyo 105, Japan.

(2) Takechi, Y. *Fundamentals and Applications of Chlorella*, 1st ed.; Gakushu Kenkyusha: Tokyo, 1973; p 28, 85.

(3) Yamada, B. *Research Report about UK001 Chlorella*, 114 Division of RITE, available from RITE, Toyokaiji Bldg. #7, 8F, 2-8-11 Nishishinbashi, Minato-ku, Tokyo 105, Japan, 1995.

(4) Coutinho, F. M. B.; Costa, T. H. S.; Carvalho, D. A. *J. Appl. Polym. Sci.* **1997**, *65* (6), 1227.

(5) Maldas, D.; Kokta, B. V.; Raj, R. G.; Daneault, C. *Polymer* **1988**, *29*, 1255.

(6) Hornsby, P. R.; Hinrichsen, E.; Tarverdi, K. *J. Mater. Sci.* **1997**, *32*, 1009.

(7) UMA Devi, L.; Bhagawan, S. S.; Thomas, Sabu. *J. Appl. Polym. Sci.* **1997**, *64* (9), 1739.

(8) Felix, J. M.; Gatenholm, P.; Schreiber, H. P. *Polym. Compos.* **1993**, *14* (6), 449.

(9) Gaylord, N. G. *J. Macromol. Sci.-Chem.* **1989**, *A26* (8), 1211.

(10) Mansour, O. Y.; Nagaty, A.; Beshay, A. D.; Nossier, M. H. *J. Polym. Sci.* **1983**, *21* (3), 715.

(11) Beshay, A.; Hoa, S. V. *Sci. Eng. Compos. Mater.* **1992**, *2* (2), 85.

(12) Price, G. J.; Clifton, A. A. *Macromolecules* **1996**, *29* (17), 5664.

(13) Gao, S.; Zeng, Y. *J. Appl. Polym. Sci.* **1993**, *47* (11), 2065.

(14) Karnani, R.; Krishnan, M.; Narayan, R. *Polym. Eng. Sci.* **1997**, *37* (2), 476.

(15) Gaylord, N. G.; Mishra, M. K. *J. Polym. Sci.: Polym. Lett. Ed.* **1983**, *21*, 23.

(16) Vijayalakshmi, N. S.; Murthy, R. A. N. *J. Appl. Polym. Sci.* **1992**, *44* (8), 1377.

(17) Gaylord, N. G.; Mehta, R.; Kumar, V.; Tazi, M. *J. Appl. Polym. Sci.* **1989**, *38*, 359.

(18) Liu, N. C.; Baker, W. E.; Russell, K. E. *J. Appl. Polym. Sci.* **1990**, *41*, 2285.

(19) Khanolkar, A.; Shivkumar, S. *Proc. Soc. Plast. Eng. Ann. Technol. Conf.* **1995**, *53* (2), 2120.

10.1021/cm990041y CCC: $18.00

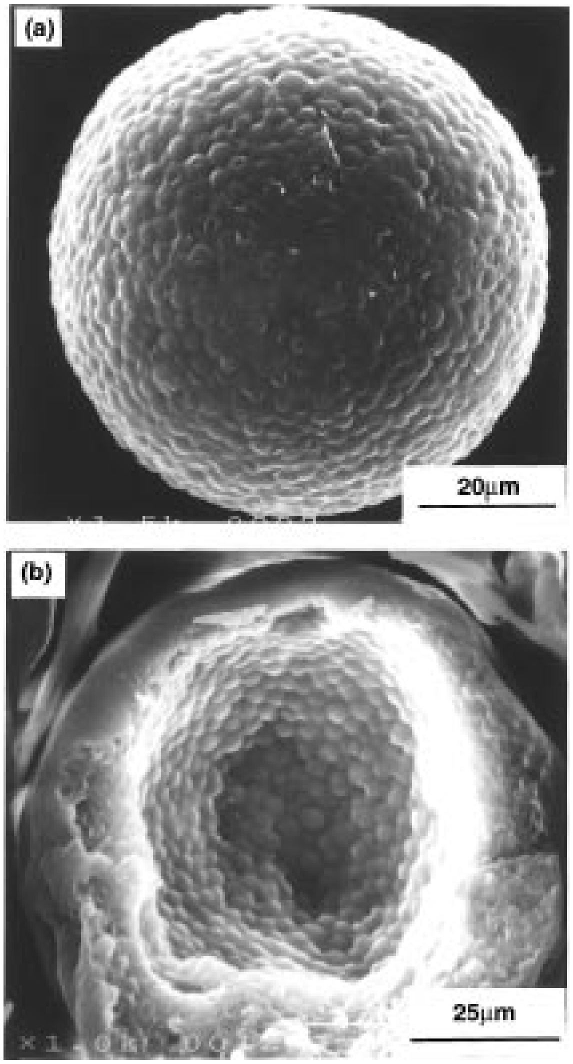


Figure 1. SEM micrographs of a chlorella aggregate grain (a) and the profile of a chlorella grain displaying its hollow spherical shape and wall (b). Chlorella grains consist of chlorella cells of about 3−5 μm.

into the modified PE, mixing at 160 °C for 10 min, and finally shaping it into pellets with a size of less than 3.36 mm. In the case of the unmodified-PE(UPE)−chlorella composite, PE and chlorella were directly fed into the mixer and mixed at 160 °C for 10 min.

Test sample sheets of the PE−chlorella composites were prepared by heat pressurizing (160 °C, 2.2 MPa, 2 min) the pellets. The samples were conditioned at 20 °C and 65% relative humidity in an environmental chamber for 24 h before measuring the tensile strength and elongation with a tensile tester (AG-100A, Shimadzu, Japan) in accordance with the testing method for tensile properties of plastics (at a strain speed of 50 mm/min).[20] The microstructure of composite fractures was observed with a S-2460N scanning electron microscope (SEM) (Hitachi, Japan). IR spectra of MPE, chlorella, and UPE−chlorella and MPE−chlorella composites were obtained by a KBr-pellet method at a resolution of 2 cm^{-1}, using a Perkin-Elmer model Spectrum 2000 spectrometer.

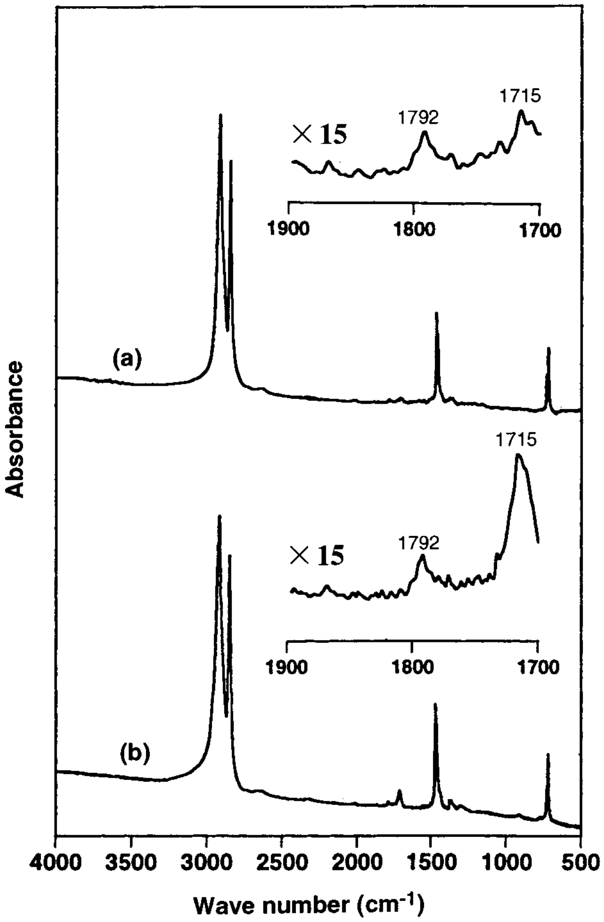


Figure 2. FT-IR spectra of MPE prepared in this study (a) and rMPE from Aldrich (0.85 wt % MA, catalog number 43720-4) (b).

The aim of modification of PE with MA is to graft MA onto PE. To avoid cross-linking reactions between PE macroradicals as well as homopolymerizations of MA during the grafting of PE, extremely low molar quantities of benzoyl peroxide and maleic anhydride were adopted compared to that of monomer units of PE. Figure 2 shows IR spectra of MPE (a) and a reference PE-*graft*-MA (rMPE) from Aldrich (0.85 wt % MA, catalog number 43720-4)[21] for comparison (b). The absorption bands at 2918, 2850, 1473, and 719 cm^{-1} in both of the spectra are due to the same backbone of PE. The magnified spectrum of MPE between 1900 and 1700 cm^{-1} clearly exhibits absorption bands due to saturated succinic anhydride and its hydrolysis derivative at 1792 and 1715 cm^{-1}, respectively,[22] with no bands due to poly(maleic anhydride) and free maleic anhydride which may be expected to appear at 1784 and 1780 cm^{-1}, respectively.[22] Little reduction in weight after recrystallization of MPE, in which MPE was dissolved in boiling 1,2-dichlorobenzene followed by precipitation in acetone, also supports no existence of poly(maleic anhydride) and free maleic anhydride in MPE, which are soluble in acetone. These characteristics are in good agreement with those of rMPE from Aldrich in Figure 2b, which exhibits only saturated succinic anhydride

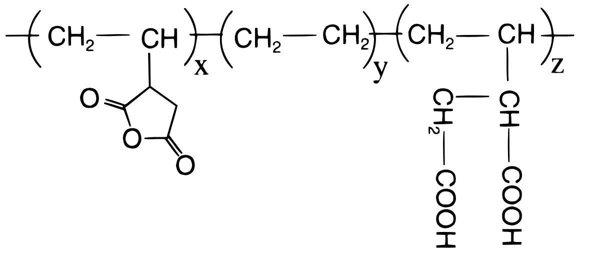
---

(20) Japan Standard Society, *JIS K7113-1995 Testing method for tensile properties of plastics*; Japan Standard Society: Tokyo, 1995.

(21) *Products Catalog of Aldrich* (Japanese edition); Aldrich Chemical Co.: Tokyo, 1998−1999; p 1368.

(22) Roover, B. D.; Sclavons, M.; Carlier, V.; Devaux, J.; Legras, R.; Momtaz, A. *J. Polym. Sci.* **1995**, *33*, 829.

groups.[21] The bands at 1715 cm^{-1} result from the hygroscopic nature of maleic anhydride, and the difference in absorption intensity is attributed to the difference in the degree of hydrolysis of the anhydride groups. Accordingly, the characteristics of MPE prepared in this experiment can be expressed by the following molecular structure:



The lack of occurrence of homopolymerization of maleic anhydride during the modification of PE is probably due to the extremely low molar quantities of maleic anhydride and peroxide compared with ethylene units of PE. Because the experimental conditions are the same, a majority of grafted MA monomers and their hydrolysis derivatives is to be expected in MPE, on the basis of the results obtained by Heinen et al.[23] On the basis of the IR spectroscopy described in Figure 2, MPE is estimated to contain about 0.23 wt % MA, in agreement with 92% of added MA being grafted onto PE. Another 8% is expected to be volatilized during the modification.

An IR spectrum of chlorella is shown in Figure 3a. The absorption band at 3440 cm^{-1} is assigned to OH groups, which the absorption bands at 3312, 1654, and 1543 cm^{-1} are due to second amide groups. The existence of hydroxyl groups suggests that chlorella contains cellulose and hemicellulose. It is obvious from Figure 3 that the MPE−chlorella composite exhibits a lower intensity of absorption at 3440 cm^{-1} than does chlorella. To evaluate the reactivity of hydroxyl groups toward MPE, we examined the change of the intensity ratio I_{3440}/I_{1654}, where I_{3440} and I_{1654} denote the absorbances at 3440 and 1654 cm^{-1}, respectively, and show the results in Table 1. The value of I_{3440}/I_{1654} for the UPE−chlorella composite remains unchanged, but that of the MPE−chlorella composite exhibits a significant decrease, compared with that of chlorella. This suggests that a part of the hydroxyl groups of chlorella in the MPE−chlorella composite take part in a reaction with MA groups grafted on PE.

The difference spectrum between the spectra of the MPE−chlorella composite and chlorella with respect to the band at 1543 cm^{-1} is shown in the top-right part of Figure 3. The disappearance of the band at 1792 cm^{-1} and the appearance of the new band at 1735 cm^{-1} indicate that, after MPE is compounded with chlorella, a reaction is connected to the OH groups of chlorella by esterification. Considering that the MA groups of maleated polyolefins are readily bonded to cellulose by ester linkages,[14,24] we can deduce that MPE takes part in ester linkages to cellulose or hemicellulose components on the surface of chlorella grains in the MPE−chlorella composite.

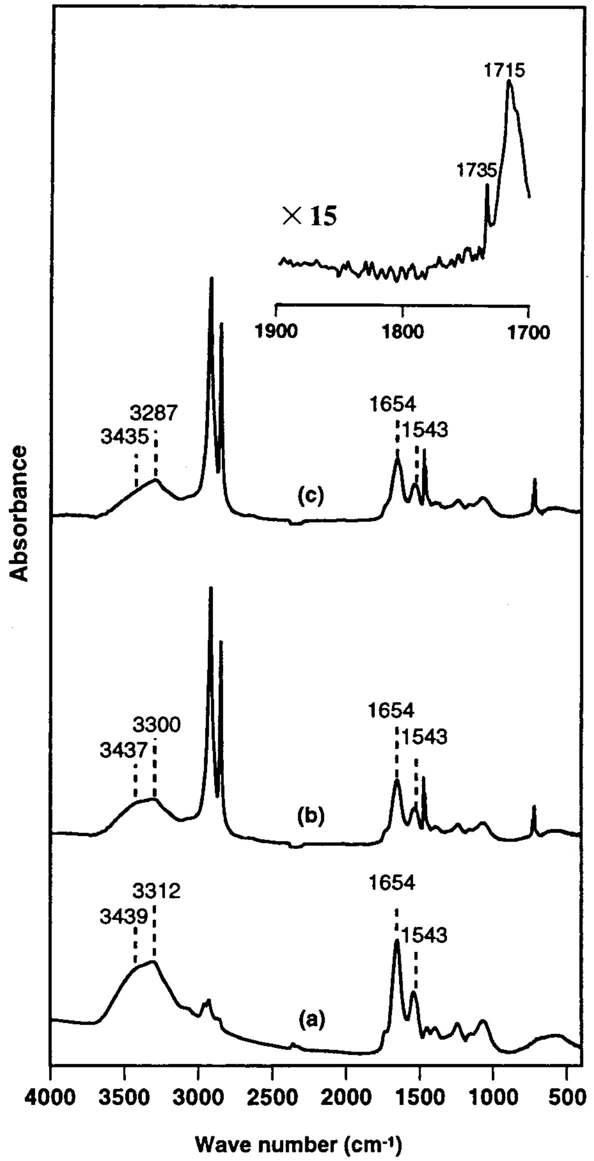


Figure 3. FT-IR spectra of chlorella (a), UPE−chlorella (30%) composite (b), and MPE−chlorella (30%) composite (c). The top-right spectrum represents the subtraction spectrum between the MPE−chlorella composite (Figure 3c) and chlorella (Figure 3a).

Table 1. I_{3440}/I_{1654} **Values of chlorella, UPE−chlorella, and MPE−chlorella Composites**

sample	chlorella	UPE−chlorella composite	MPE−chlorella composite
I_{3440}/I_{1654}	0.810	0.812	0.787

Figure 4 shows a proposed mechanism for the formation of chemical bonds between chlorella grains and MPE from the above FT-IR spectroscopy. Benzoyl peroxide (BPO) initiates radical formation on the PE backbone by H abstraction. The radicals then react with maleic anhydride (MA) to form modified PE (MPE). In the last step, MPE reacts with hydroxyl groups of the cellulosic component in the cell wall of chlorella to form chemical bonds by esterification.

Tensile properties of PE−chlorella composites are summarized in Table 2. The results show that the tensile strength and elongation of the composite decrease with an increase in chlorella content in the composite. Compared with the UPE−chlorella compos-

---

(23) Heinen, W.; Rosenmoller, C. H.; Wenzel, C. B.; de Groot, H. J. M.; Lugtenburg, J.; van Duin, M. *Macromolecules* **1996**, *29*, 1151.

(24) Felix, J. M.; Gatenholm, P. *J. Appl. Polym. Sci.* **1991**, *42*, 609.

Table 2. Effects of PE Modification and Chlorella Content on Mechanical Properties of PE−Chlorella Composites[a]

chlorella content (wt %)	PE modification	tensile strength (MPa)	Young's modulus (GPa)	elongation (%)	density (g/cm^3)	specific tensile strength[b] (MPa)/(g/cm^3)
0	no	24.40 ± 0.08			0.92	26.52
0	yes	24.92 ± 0.11				
10	no	19.96 ± 0.27	0.36 ± 0.02	26.3 ± 0.2	0.89	22.43(−15.4%)[c]
10	yes	22.53 ± 0.32	0.47 ± 0.05	14.1 ± 0.2		25.31(−4.6%)
20	no	16.12 ± 0.21	0.33 ± 0.03	21.8 ± 0.3	0.87	18.53(−30.1%)
20	yes	21.03 ± 0.16	0.53 ± 0.03	8.7 ± 0.4		24.17(−8.9%)
30	no	12.72 ± 0.14	0.29 ± 0.02	17.2 ± 0.2	0.85	15.14(−42.9%)
30	yes	20.72 ± 0.07	0.60 ± 0.01	5.8 ± 0.3		24.67(−7.0%)
40	no	9.7 ± 0.36	0.24 ± 0.06	10.5 ± 0.1	0.82	11.97(−54.9%)
40	yes	19.83 ± 0.48	0.67 ± 0.10	3.3 ± 0.1		24.48(−7.7%)

[a] Six samples were tested. [b] The ratio of tensile strength over density of the composite. [c] Percentage reduction of tensile strength compared with that of PE.

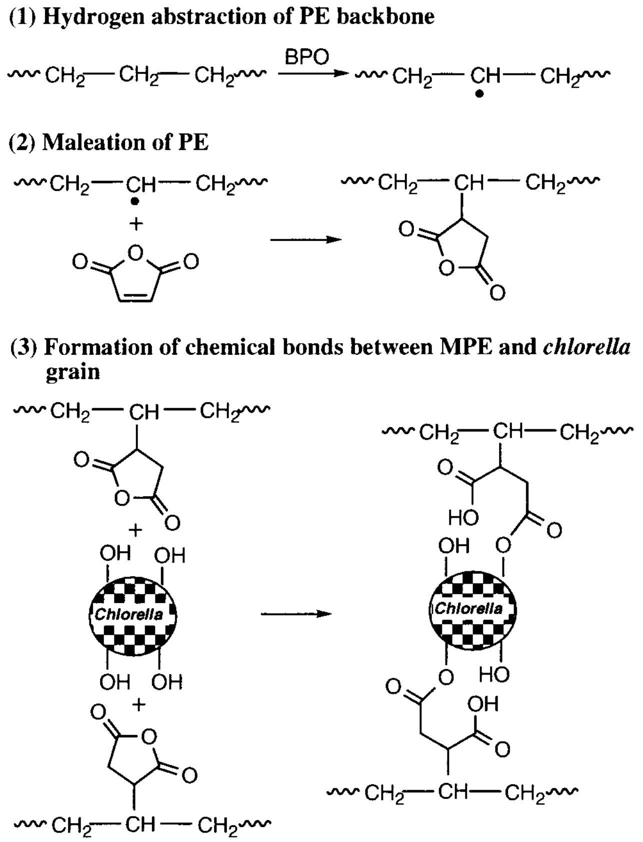


Figure 4. A proposed reaction mechanism for the formation of chemical bonds between chlorella grains and a MPE matrix.

ite, the MPE−chlorella composite exhibits a marked increase in tensile strength and Young's modulus. This supports our conjecture that chemical bonds are formed between chlorella grains and the MPE matrix, since the MPE matrix itself exhibits little increase in tensile strength even after modification. The SEM micrographs of composite fractures reveal that chlorella grains in a MPE−chlorella composite are well inlaid in the MPE matrix, with no intervening air gaps between the chlorella grains and the MPE matrix (Figure 5a-1), in strong contrast to those in a UPE−chlorella composite which show no adhesion with the UPE matrix, leaving air gaps in the interface with the UPE matrix (Figure 5b). Figure 5a-2 shows the binding between a chlorella grain and its surrounding MPE matrix in the MPE−chlorella composite. A feature here is that when a MPE−chlorella composite was strained to failure, the chlorella grains on the fracture of the composite were broken (Figure 5a) which indicates that the binding strength between the MPE matrix and chlorella grains

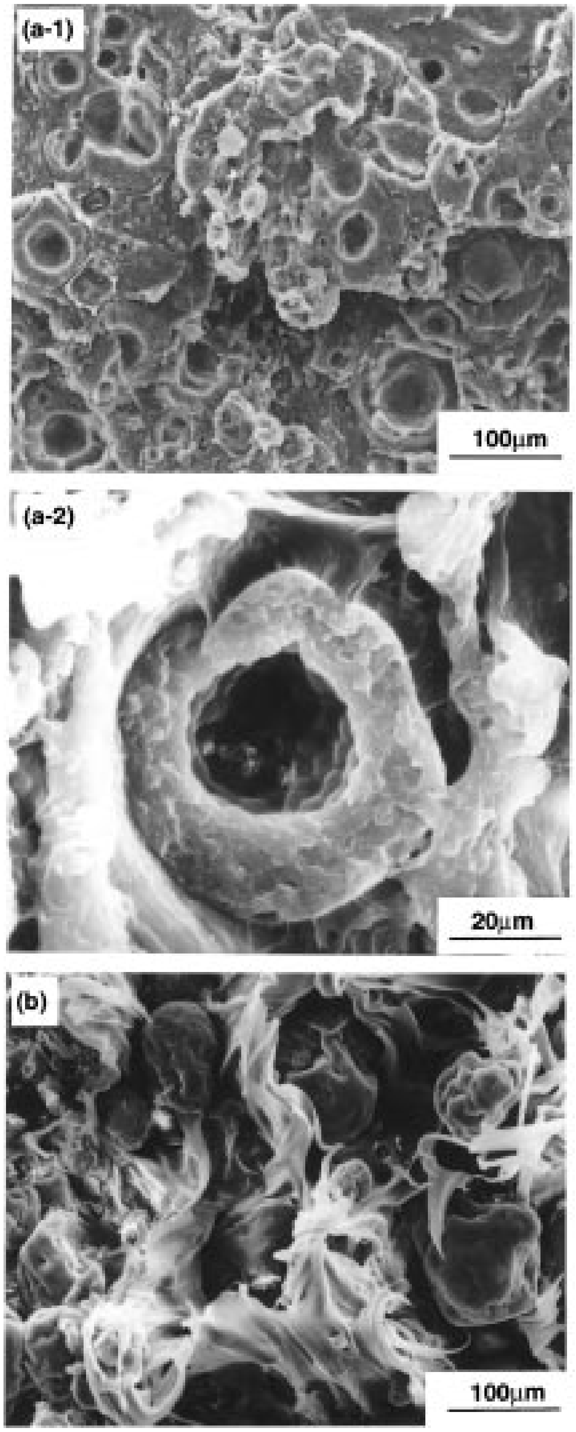


Figure 5. SEM micrographs of fractures of PE−chlorella composites containing 30% chlorella: MPE (a-1), a chlorella grain displaying binding with MPE matrix (a-2), and UPE (b).

(σ_{p-c}) is stronger than that between chlorella cells (σ_{c-c}) which originates primarily from hydrogen bonds. This feature strongly indicates the formation of chemical bonds between chlorella grains and the MPE matrix. The formation of chemical bonds is also indicated by the phenomenon where the chlorella grains on the surface or fracture of a MPE–chlorella composite do not break away from the MPE matrix even after immersion in water for a week or repeated flexing, compared with the ease with which chlorella grains break off in the case of a UPE–chlorella composite under the same conditions. It is worth noting that the difference in tensile strength between a MPE–chlorella composite and a UPE–chlorella composite increases with an increase in chlorella content. The strength of the MPE–chlorella composite is more than 2 times greater than that of the UPE–chlorella composite at a chlorella content of 40%.

As mentioned earlier, in a UPE–chlorella composite chlorella grains are, on one hand, completely separate from the UPE matrix with air gaps at the interface; on the other hand, in a MPE–chlorella composite, chlorella grains are in tight contact with the MPE matrix because of chemical bonds with no air gaps at the interface. According to this model, the tensile strength (σ_m) of a UPE–chlorella composite model depends entirely on the strength of the UPE matrix (σ_{upe}) because of the lack of adhesion between chlorella and UPE. Thus, σ_m decreases with an increase in chlorella content because it is determined by $\sigma_{upe}A_p/A$, where A_p is the section area of the UPE matrix and A is the total cross-section area. This is consistent with the sharp drop in tensile strength of the UPE–chlorella composite shown in Table 2. On the other hand, the tensile strength of a MPE–chlorella composite depends not only on the strength of the MPE matrix (σ_{mpe}) but also on that of the chlorella grains (σ_{c-c}), because of the formation of strong chemical bonds between MPE and chlorella (σ_{p-c}), where $\sigma_{p-c} > \sigma_{c-c}$. From Table 2, there is no difference in tensile strength between UPE and MPE: $\sigma_{upe} = \sigma_{mpe}$. Accordingly, the σ_m value of a MPE–chlorella composite is determined by $\sigma_{upe}A_p/A + \sigma_{c-c}A_c/A$, where A_c is the section area of chlorella grains. This is consistent with the marked increase in the tensile strength of the MPE–chlorella composite compared to that of the UPE–chlorella composite. Also, this model can interpret the marked decrease in elongation of the composite by modification of the PE matrix, as shown in Table 2. Furthermore, if the specific tensile strength (the ratio of tensile strength over density of the composite) is referred to as an index of property evaluation, it drops only 7.7% when the chlorella content increases from 0% to 40%, and moreover shows no drop from 20% to 40% because of the decrease in density of the foam structure of the PE–chlorella composite and the increasing role of $\sigma_{c-c}A_c/A$ in σ_m.

In conclusion, we have demonstrated the preparation of a novel composite consisting of highly hydrophilic chlorella grains and highly hydrophobic PE. The adhesion between chlorella grains and the PE matrix is significantly improved by chemical modification of PE with maleic anhydride because of the formation of chemical bonds between chlorella grains and the PE matrix. Thus, the modified-PE–chlorella composite exhibits a much greater tensile strength than the unmodified-PE–chlorella composite. Another characteristic of this composite is its interesting structure characterized by cellular foamy grains of chlorella which are well-incorporated in the PE matrix. The overall properties and application of the composite are now being explored further. How to best utilize the cellular foamy structure of chlorella grains to optimize the properties of this composite is of great interest. Such novel biological materials containing microalga are worth further exploration from the standpoint of material science as well as for their applications.

Acknowledgment. This project was funded by the New Energy and Industrial Technology Development Organization (NEDO) in Japan. The author (F.Z.) thanks Dr. Endo (SNIRI) for giving assistance in the experiments and helpful discussions.

CM990041Y