

A Novel Polyethylene–Chlorella Composite. I. Characterization of Chlorella Biologically Fixing CO₂

FARAO ZHANG,¹ HIROSHI KABEYA,¹ RYOUICHI KITAGAWA,¹ TAKAHIRO HIROTSU,¹
MASATADA YAMASHITA,² TOSHI OTSUKI²

¹ Biological Material Division, Shikoku National Industrial Research Institute (SNIRI), 2217-14 Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan

² Project Center for CO₂ 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

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ABSTRACT: Chlorella, a microalga massively produced from biological fixation of CO₂, was investigated to evaluate the possibility of processing it with polyethylene. Chlorella grain, a hollow aggregate sphere constituted of chlorella cells binding with each other by hydrogen bonds, exhibits a higher thermal stability than cellulose and strong resistance to cracking under mechanical forces up to 150 MPa, possibly due to the spherical structure and unique manner of assembly of chlorella cells. These characteristics of chlorella strongly indicate that it can resist deformation and thermal decomposition in a compounding process with polyethylene. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 77: 2278–2284, 2000

Key words: microalga; chlorella; composite; polyethylene; characterization

INTRODUCTION

Chlorella is a kind of microalgae consisting of a single cell and belonging to oocystaceae of chlorococcales of green algae. Such a single cell contains all the organs that are necessary for its reproduction, such as genes, chloroplast, mitochondria, and cell membrane. Progenition of chlorella is by asexual reproduction, during which an autospore inside the cell itself forms, grows, and causes fission, using water, carbon dioxide, and various inorganic salts as nutrients. A variety of research has been conducted on the application of chlorella as a source of food because of its high protein content and nutritional value.¹

We have paid much attention to chlorella because of its high ability to fix CO₂. To find a resolu-

tion for the reduction of CO₂ discharged into the atmosphere, regarded as the main reason for global warming, systems for biologically fixing CO₂ have been studied in Japan since 1990.² By collection/transmission of solar light, the most appropriate culture conditions, and highly efficient photosynthesis, a great amount of chlorella can be produced utilizing CO₂ that otherwise would be discharged into the atmosphere. A fixing speed of CO₂ greater than 1 kg CO₂/m³/day or 50 g CO₂/m²/day has already been achieved, and the utilization rate of light energy is over 4%, which is much larger than those of common plants (0.1–0.2%).³

How to make effective use of the resultant chlorella is the key to making the system practicable. Such a large volume of chlorella will gradually putrefy and decompose in the natural environment, resulting in the release of CO₂ again. Thus, we have conducted various studies to explore the utilization of the resultant microalga. One of these studies involves the development of novel chlorella–thermoplastic resin composites:

Correspondence to: T. Hirotsu.

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for example, chlorella-polyethylene composite⁴ and chlorella-polyvinyl chloride composite.⁵

Although there have been many reports on the application of natural fibers as reinforcers of thermoplastic resins,⁶⁻¹¹ little study¹² has been undertaken on the development of composites with microalgae. In a previous communication,⁴ we tentatively reported the preparation and characterization of a novel composite of polyethylene and chlorella. The composite of hydrophobic polyethylene with hydrophilic chlorella has been successfully achieved through solid-phase reactions characterized by premodification of polyethylene with a small amount of maleic anhydride. The formation of ester bonds between maleic-anhydride groups grafted on polyethylene and hydroxyl groups on cell walls of chlorella has realized good interfacial adhesion between chlorella grains and polyethylene matrix. In this study, we describe the characteristics of chlorella and evaluate its application as a filler processed with polyethylene, before discussing in detail the preparation, characterization, and properties of the novel composite elsewhere.

EXPERIMENTAL

Materials

Chlorella that had been spray dried after centrifugal concentration was purchased from Yaeyama Shokusan Co., Ltd. of Japan. Cellulose powder (CF-11) was purchased from Whatman International Co., Ltd. of England. Both chlorella and cellulose were vacuum dried at 50°C for 72 h before use. The other chemicals for this study were purchased from Wako Pure Chemical Industries of Japan, and used without further purification.

Methods

Distribution of Particle Size

The distribution of particle size of original chlorella was measured with a LMS-24 Laser Micron Sizer (Seishin Co.) after it was treated by an ultrasonicator using ethanol as a medium. Also investigated was the change of distribution of particle size of chlorella with time after ultrasonic-dispersion in water or ethanol.

Thermal Properties

The thermal stability of chlorella was determined using a MAC Science 2000 Thermal Analyzer

(MAC Science Co.). The measurements were undertaken both in a nitrogen and in an air atmosphere at a heating rate of 5°C/min⁻¹ from 50 to 550°C. Samples of pure cellulose and leucine were also analyzed for comparison. Some measurements on the chlorella were also carried out under isothermal conditions at 140, 160, and 180°C in an air environment.

Microstructure

Scanning electron microscopy (SEM) of chlorella was conducted with a S-2460N SEM microscope (Hitachi Co.), after a chlorella sample was sprinkled on to a double-sided adhesive tape attached to an aluminum stub and then coated with gold.

Functional Groups

Functional groups of chlorella were determined by FTIR spectroscopy with KBr pellets using a Spectrum 2000 spectrometer (Perkin-Elmer Ltd.) in the spectral range of 4000-370 cm⁻¹, with a resolution of 2 cm⁻¹.

Cracking Resistance

The resistance of chlorella to cracking was examined with a SSP-10A Handy Press (Shimadzu Co.). Chlorella (100 mg) was put into a molding set of a cylindrical shape, and pressed at a constant pressure to form a disc. The resultant disc was abraded, and the separated chlorella grains were observed by SEM.

RESULTS AND DISCUSSION

The principal aim of this work is to consider the structure and properties of chlorella with a view to its application as a filler for polyethylene. Hence, great emphasis has been laid on whether the characteristics of the microalga permit it to successfully undergo the anticipated melt-mixing and heat-presurizing processes necessary for the preparation of a novel chlorella-polyethylene composite.

General Profile and Aggregate Characterization

Chlorella used in this study contains mainly protein (53-60 wt %), lipid (6-15 wt %), and carbohydrates (10-20 wt %).¹³ The protein consists of leucine, glutaminic acid, and aspartic acid in the main. The lipids are mainly made of palmitic acid (41.5 wt %), oleic acid (19.2 wt %), stearic acid (14.5 wt %), and palmitoleic acid (9.5 wt %). The

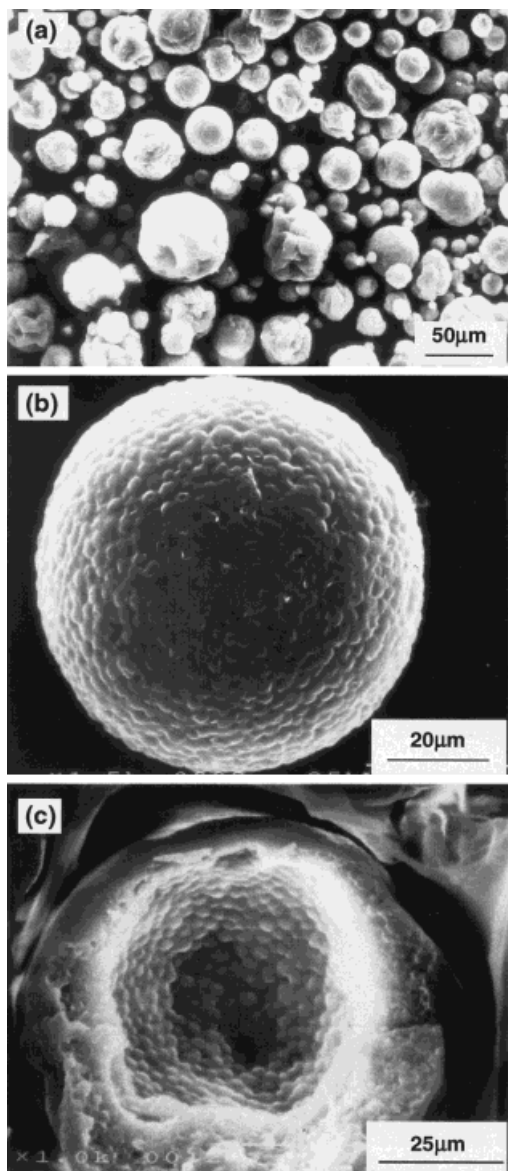


Figure 1 SEMs of chlorella: overall appearance (a), a typical aggregate grain (b); a profile of a chlorella grain displaying its hollow spherical shape and wall, and consisting of chlorella cells of about 3–5 μm (c).

carbohydrates consist mainly of glucose, galactose, and arabinose. The cell wall of chlorella amounts to about 13.6 wt % of the whole cell, and is principally made of α -cellulose (ca. 15.4 wt %), hemicellulose (ca. 31 wt %), protein (ca. 27 wt %), and fatty acid (ca. 9.2 wt %).¹³

The shape and structure of spray dried chlorella are shown in Figure 1. The chlorella grains are nearly spherical, and different in particle size from each other [Fig. 1(a)]. A grain is an aggregate of a large number of chlorella cells, which are tightly bound to each other [Fig. 1(b)]. A cross-

section of a grain reveals that the grain of chlorella is a hollow sphere [Fig. 1(c)], and that its wall thickness is several ten times as large as one cell size of 3–5 μm , depending on the whole grain size.

To investigate the aggregation properties of chlorella grains, we examined the change in particle size distribution of chlorella grains on standing under ultrasonic dispersion in water and ethanol. The particle size distributions of chlorella grains dispersed in water and ethanol are shown in Figure 2. Table I summarizes the average particle sizes of chlorella grain with ultrasonic dispersion time in water and ethanol.

The particle-size distribution of original chlorella exhibits two peaks around particle sizes of 4 and 100 μm . The former is due to individual chlorella cells, and the latter is due to aggregates of chlorella. From Figure 2(a), the peak due to chlorella aggregates decreases more in intensity and shifts to a smaller particle size with ultrasonic dispersion over time in water; on the other

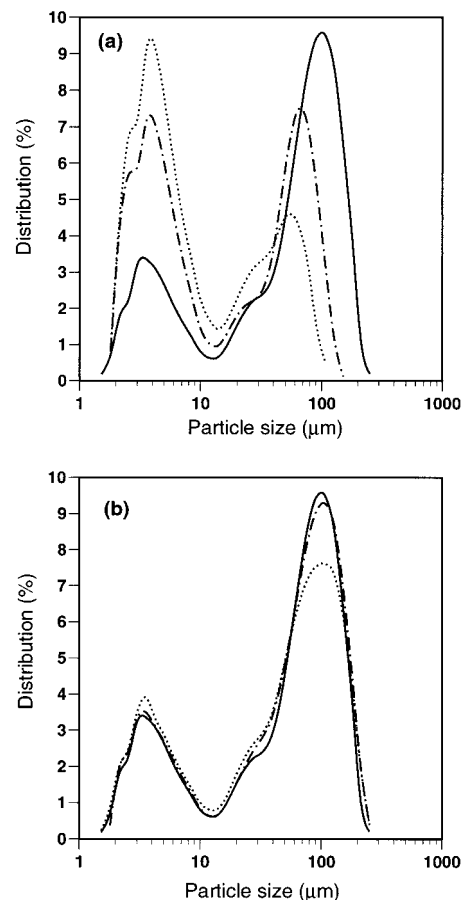


Figure 2 Changes over particle-size distribution of chlorella with ultrasonic-dispersion time in water (a) and ethanol (b). The dispersion times are 0 min (—), 30 min (---), and 120 min (----).

Table I Changes in Average Particle Size of Chlorella Grains with Ultrasonic-Dispersion Over Time in Water and Ethanol

Time (min)	Average Particle Size (μm)	
	in Water	in Ethanol
0	63.8	63.8
30	12.3	63.6
120	5.7	53

hand, the peak due to individual chlorella cells increases in intensity without any shift. This indicates that the chlorella grains are easily broken into chlorella cells in water.

In contrast, the particle-size distribution of chlorella in ethanol displays hardly any change after ultrasonic treatment for 30 min, and only a small change even after the 120-min treatment [Fig. 2(b)]. Thus, the average particle size of chlorella drops only slightly from 63.8 to 53 μm in ethanol, in strong contrast to a marked decrease from 63.8 to 5.7 μm in the case of water (Table I). This suggests that the binding between chlorella cells is mainly due to hydrogen bonds or ionic interactions.

Figure 3 shows an SEM micrograph of chlorella ultrasonic-treated in water for 24 h. The chlorella grains are completely deaggregated to single cells, which are not broken further into pieces. It was observed that single cells following ultrasonic-dispersion in water will aggregate together again once they are dried. This also supports the binding with hydrogen bonds of chlorella cells.

Thermal Stability

In the present investigation, the thermal properties of chlorella were regarded as of great impor-

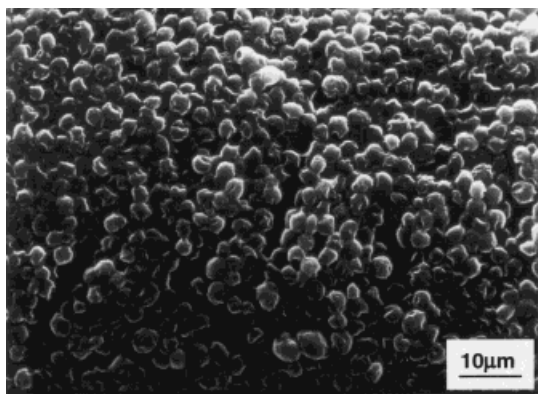


Figure 3 An SEM of chlorella after ultrasonic treatment for 24 h in water, showing that chlorella grains have been deaggregated into separate cells.

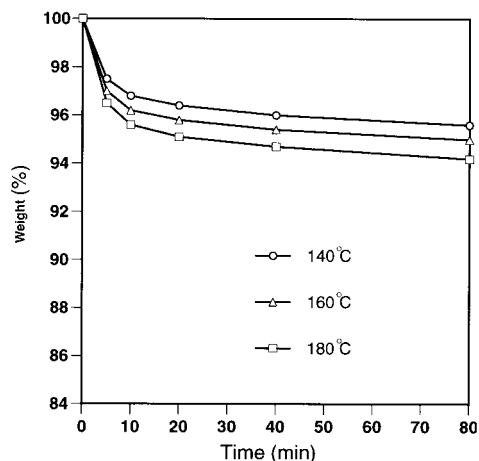


Figure 4 Isothermal gravimetric analysis of chlorella in air at 140°C (○), 160°C (△), and 180°C (□).

tance, because the processing of chlorella in combination with polyethylene would require the exposure of chlorella to temperatures up to ca. 180°C during both compounding and heat-presurizing operations.

Figure 4 shows the isothermal weight-loss curves of chlorella obtained in air at three temperatures. The sharp weight loss in the initial 10 min is mainly attributed to loss of combined water. Thus, the small weight loss in the heating time from 10 to 80 min, regardless of the temperatures, clearly indicates that chlorella has good thermal stability at temperatures from 140 to 180°C even in air.

TG curves of chlorella up to 550°C in air and nitrogen environments are shown in Figure 5(a) and 5(b), respectively, including corresponding results with leucine and cellulose for comparison. From the results in Figure 5, the initial weight losses (due to loss of combined water), the amounts of residues at 550°C, and the onset temperatures for the main weight loss were determined as in Table II.

The results regarding initial weight loss show that, even after being vacuum dried, chlorella still contains a considerable amount of water, reflecting the hygroscopic nature of chlorella. The lower onset temperature for decomposition of chlorella than for cellulose and leucine implies that chlorella contains some volatile and/or decomposable substances. The slow weight loss of chlorella reflects the diversity of substances in chlorella, as described earlier. The larger amount of residues of chlorella rather than cellulose and leucine above 350°C indicates that chlorella is more stable than leucine and cellulose, which decompose significantly in narrow tempera-

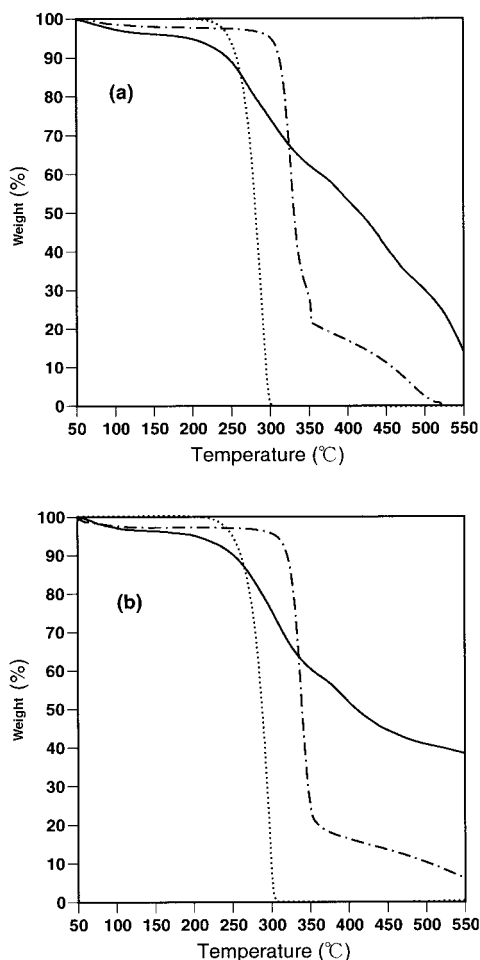


Figure 5 Thermogravimetric analysis of chlorella (—), cellulose (---), and leucine (····) in air (a) and nitrogen (b).

ture ranges, and completely up to 310°C (leucine) and 530°C (cellulose) in air [Fig. 5(a)].

SEM micrographs of chlorella heated to 250 and 550°C are shown in Figure 6. Chlorella grains remain almost unchanged at 250°C both in air and in nitrogen [Fig. 6(a-1),(b-1)]. This shows that chlorella grains are stable enough to be resistant

to thermal destruction below 250°C. On the other hand, at 550°C, chlorella grains completely decompose into fragments in an air environment [Fig. 6(a-2)], although in a nitrogen environment some whole spheres of chlorella grains still remain even if the wall thickness becomes thinner [Fig. 6(b-2)].

It is evident that chlorella has greater thermal stability than leucine and cellulose. The characteristic composition of chlorella and the unique assembly of spherical chlorella cells might give rise to such thermal resistance.

Cracking Resistance

Taking into account the molding process of potential chlorella–polyethylene composites, the resistance to cracking of chlorella grains was examined under different pressures. Figure 7 shows the shapes of chlorella grains after being subjected to different pressures. The shape of chlorella grains does not exhibit any change at 75 MPa, but undergoes some deformation at 150 MPa. The deformation of chlorella grains is more intensive, yielding cracks in the grains observed at 300 and 600 MPa. Considering that the highest processing pressure of plastics is generally not greater than 150 MPa, it can be concluded from these results that the structure of chlorella grains is sufficiently stable to resist cracking during the compounding process with thermoplastic resins.

Functional Groups

Interfacial features often determine the mechanical properties of composites. Thus, from FTIR spectroscopy, we characterized functional groups of chlorella, which would play an important role in interfacial interactions with thermoplastic resins. In Figure 8, the strong absorbance peaks at 3312, 1654, 1543, and 1242 cm^{-1} are due to aliphatic secondary amide or benzoguanamine

Table II Results of Thermal Analysis of Chlorella, Cellulose, and Leucine

Material	Initial Weight Loss (wt %)	Residue at 550°C (wt %)	Onset Temperature for Main Decomposition (°C)
Chlorella	3.5 (3.8)	13.9 (38.42)	225 (228)
Cellulose	1.9 (2.4)	0 (6.09)	315 (325)
Leucine	0 (0)	0 (0)	265 (267)

^a The numbers with and without parentheses refer to the results in nitrogen and air, respectively.

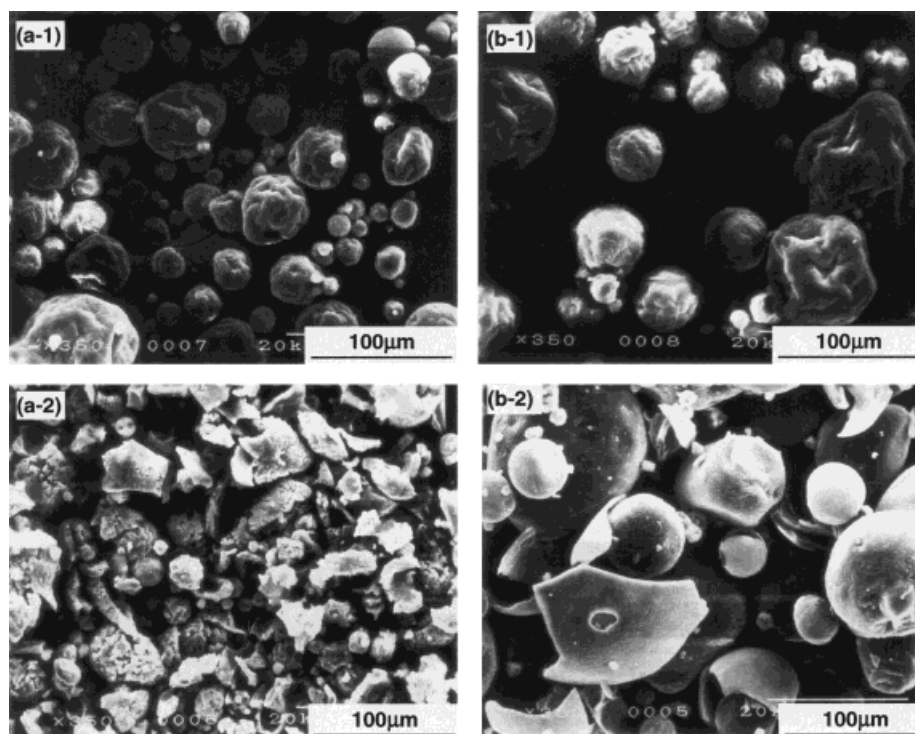


Figure 6 SEMs of chlorella residues at different temperatures and environments: 250°C-air (a-1), 550°C-air (a-2), 250°C-nitrogen (b-1), and 550°C-nitrogen.

groups, consistent with the notion that proteins form the main composition as described earlier. The existence of carbohydrates also is confirmed

from absorption bands at 3440 and 1072 cm^{-1} ascribed to OH groups. These main functional groups (amide and hydroxyl) explain well the

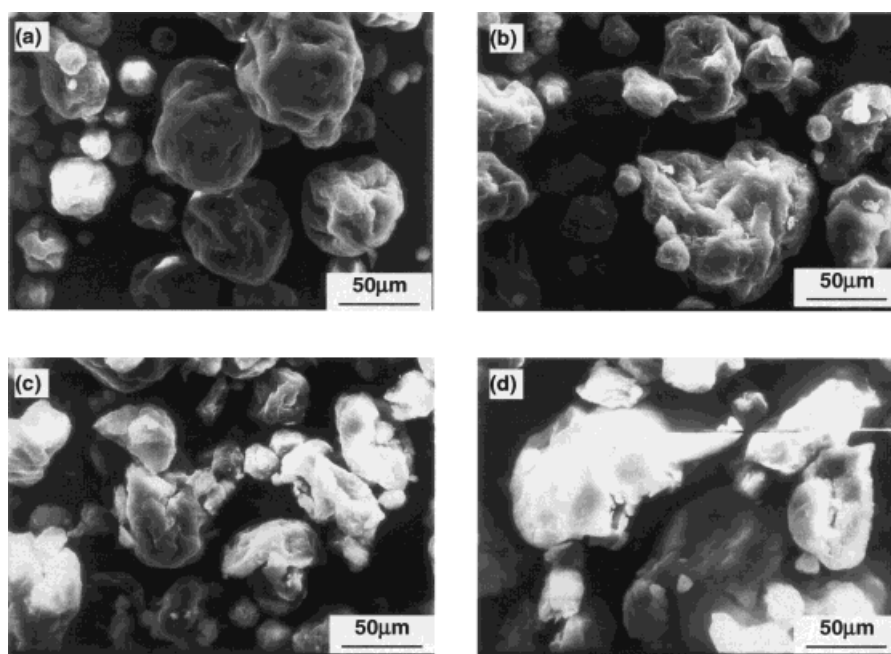


Figure 7 SEMs of chlorella grains under different pressures: 75 MPa (a), 150 MPa (b), 300 MPa (c), and 600 MPa (d).

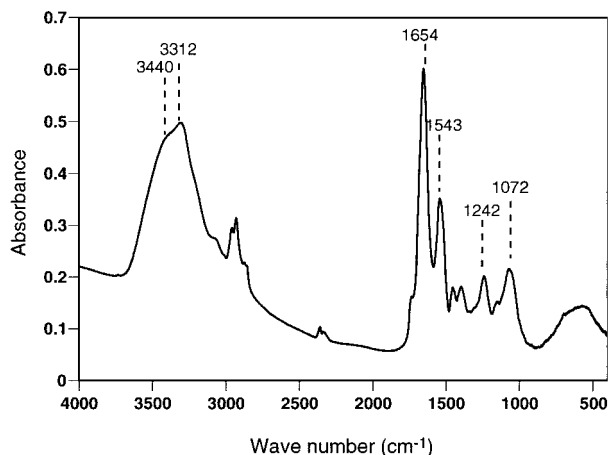


Figure 8 An FTIR spectrum of chlorella.

characteristics of chlorella grains, the strong binding between chlorella cells, the ready deaggregation in water, and the strong hygroscopic nature, all due to the hydrogen bonds originating from these functional groups.

CONCLUSIONS

Chlorella grains are aggregates of chlorella cells binding with each other through hydrogen bonds. The aggregates appear as hollow spherical grains. As a result, chlorella grains exhibit strong resistance to cracking under mechanical forces and stability in ethanol, but can easily be deaggregated in water with ultrasonic treatment.

TG analyses demonstrate that chlorella has a higher thermal stability than cellulose. It exhibits only a little weight loss below 180°C, which is attributed to the loss of combined water and volatile substances. The assembly structure of the grains remains intact below 250°C; moreover, the intact structure can be observed for some chlorella grains even at 550°C in nitrogen.

The high resistance to cracking and thermal stability of chlorella grains strongly indicate that chlor-

ella can resist deformation and thermal decomposition in a process compounding chlorella with thermoplastic resins. These characteristics are most likely attributable to the spherical structure and unique manner of assembly of chlorella cells.

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REFERENCES

1. Takechi, Y. *Fundamentals and Applications of Chlorella*; Gakushu Kenkyusha: Tokyo, 1973, 1st ed.
2. Pamphlet of Research Institute of Innovative Technology for the Earth (RITE), available from RITE, Toyokaiji Bldg. #7, 8F, 2-8-11 Nishishinbashi, Minato-ku, Tokyo 105, Japan, 1999.
3. Yamada, B. Research Report about UK001 Chlorella, available from RITE, Toyokaiji Bldg. #7, 8F, 2-8-11 Nishishinbashi, Minato-ku, Tokyo 105, Japan, 1995.
4. Zhang, F.; Kabeya, H.; Kitagawa, R.; Hirotsu, T.; Yamashita, M.; Otsuki, T. *Chem Mater* 1999, 11, 1952.
5. Zhang, F.; Kabeya, H.; Kitagawa, R.; Hirotsu, T.; Yamashita, M.; Otsuki, T. *J Mater Sci*, to appear.
6. Garcia-Ramirez, M.; Cavaille, J. Y.; Dupeyer, D.; Peguy, A. *J Polym Sci Part B Polym Phys* 1994, 32, 1437.
7. Coutinho, F. M. B.; Costa, T. H. S.; Carvalho, D. A. *J Appl Polym Sci* 1997, 65, 1227.
8. Hornsby, P. R.; Hinrichsen, E.; Tarverdi, K. *J Mater Sci* 1997, 32, 1009.
9. Uma Devi, L.; Bhagawan, S. S.; Sabu, T. *J Appl Polym Sci* 1997, 64, 1739.
10. Karnari, R.; Krishman, M.; Narayan, R. *Polym Eng Sci* 1997, 37, 476.
11. Felix, J. M.; Gatenholm, P. *J Appl Polym Sci* 1993, 50, 699.
12. Khanolkar, A.; Shivkumar, S. *Proceed Soc Plast Eng Ann Technol Conf* 1995, 53, 2120.
13. Pamphlet of Introduction to Chlorella Product; Yaeyama Shokusan Co., Ltd.: Japan.