Measurement of lipid content of compost in the fermentation process using near infrared spectroscopy

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Introduction

Compost fermentation is one of the key technologies for waste treatment and recycling of the waste matter and residual products from food processing. It is important to measure the compost composition and condition of the fermentation process. In particular, the amount of organic compounds, such as lipids, cellulose and protein, is an important factor in the management of the compost fermentation.

Tofu (soybean-curd) made from soybeans is a traditional food, which is normally eaten in East Asia. tofu refuse, a residual product from tofu processing, has a high moisture and nutrient content and putrefies very quickly with an unpleasant odour. In the past, tofu refuse has been utilised as a feedstuff. However, disposal of this refuse has recently become a serious problem because the amount utilised as feedstuff is decreasing. Because it does not contain toxic or harmful substances, it is very suitable as a compost material and this may be the most appropriate treatment for tofu refuse.

Near infrared (NIR) spectroscopy has already been used for the measurement of cell mass for mushroom cultivation in solid media,[1] measurement and control of moisture content[2] and measurement of carbon and nitrogen content of compost during fermentation.[3] In the present study, the suitability of lipid content as an indicator of compost fermentation of tofu refuse was studied and NIR was used to determine the lipid content of a compost sample during the fermentation process.

Materials and methods

Compost material, fermentation and composter

Fresh tofu refuse, used as a raw material for compost in this study, was supplied by a tofu factory. Compost fermentation was performed in a domestic composter (Model EH4381B-H, Matsushita Electronic Co., Tokyo). The temperature of the compost in the composter was kept between 65–70°C by on-off control of the heater and by mixing with a triple impeller fitted in the composter. To accelerate the fermentation process, compost made from tofu refuse in our laboratory was added as a seed.[2]

The starting mixture placed in the composter consisted of tofu refuse (71% moisture content, 9.65 kg of wet weight), sawdust (7% moisture content, 3.00 kg of wet weight) and the seed (39% moisture content, 200 g of wet weight). During fermentation, the moisture content of the compost was controlled by supplying additional water.[2]

Measurement of lipid, carbon and nitrogen contents

Lipid content of the compost samples was measured by the Soxhlet extraction method. The dried sample (about 10 g, W_1) was extracted with chloroform/methanol (2 : 1 v / v) solvent for 21 h at 75°C

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using a Soxhlet extractor. The extract in the flask was dried for 24 h at 80°C and the weight of the flask was measured (W_2). The lipid content of the compost sample was calculated using the weight of the flask and the weights W_1 and W_2. Each sample was measured by taking the average value of six measurements.

Carbon and nitrogen content were measured by the Pregie–Dumas combustion method using CN analyzer (MT-700, Yanaco Co., Kyoto, Japan).[3]

Near infrared spectroscopy

Six runs of compost fermentation were performed. During the fermentation process, compost was regularly sampled to produce 95 samples for measuring NIR spectra. These samples were divided at random to make a calibration sample set (n = 60) and a validation sample set (n = 35). The measurement of the NIR spectrum of the compost was carried out by the same procedure described in the previous study.[2] A polyethylene bag, filled with the compost ,was placed in a sample holder after being held at 25°C for 30 min. Reflectance values at wavelengths ranging from 400 to 2500 nm were measured at 2 nm intervals using an NIR spectrometer (NIRS6500SPL, Nireco Co., Hachioji, Tokyo, Japan). All samples were measured in triplicate. To correct for baseline shift in the spectra, second derivatives were calculated from the raw spectra.[2]

Least squares multiple linear regression (MLR) was carried out between the NIR spectral data of the calibration set (n = 60) and the lipid content of the compost obtained by Soxhlet extraction. Calculation of the second derivatives and the regression analysis were carried out using NSAS software supplied by the Nireco Co.

To evaluate the performance of the calibration equation, validation was carried out using a separate sample set (n = 35), which had not been used in the calibration. In addition, time courses of the values of the lipid content during the compost fermentation were predicted using the calibration equation.

NIR spectra of polyethylene bag, dried tofu refuse, residual and extract of Soxhlet extraction

To select a wavelength used for a calibration equation, NIR spectra of polyethylene bags, dried tofu refuse, as well as residual and extract of the Soxhlet extraction of tofu refuse were all measured. Fifteen polyethylene bags were placed in a sample holder and the reflectance measured. The tofu refuse and residual, after Soxhlet extraction, were dried for 12 h at 80°C before the dried samples were packed into a standard sample cup (Nireco Co.) and NIR reflectance spectra were collected. The extracts (in the form of a syrup after removal of the organic solvent) were injected into a cuvette with a 2 mm light path length and measurements taken as NIR transmittance spectra.

Results and discussion

Changes in the carbon, nitrogen and lipid content of compost during fermentation

Figure 1 shows time courses for (a) moisture content, (b) dry weight of the compost in the composter, (c) carbon and nitrogen contents and (d) lipid content of the compost during fermentation. In Figure 1(b), total dry weight of the compost in the composter decreased because compost fermentation is a biodegradation process in which the organic compounds in the compost material were changed to carbon dioxide and ammonia by microorganisms. The nitrogen (protein) in the compost material was decomposed at an early stage of compost fermentation [Figure 1(c)]. On the other hand, lipid content of the compost only begins to decrease in the middle stage of fermentation [Figure 1(d)]. The time course of the lipid content of the compost during fermentation was related to the decrease in total dry weight of the compost in the composter. These phenomena suggested that degradation of the

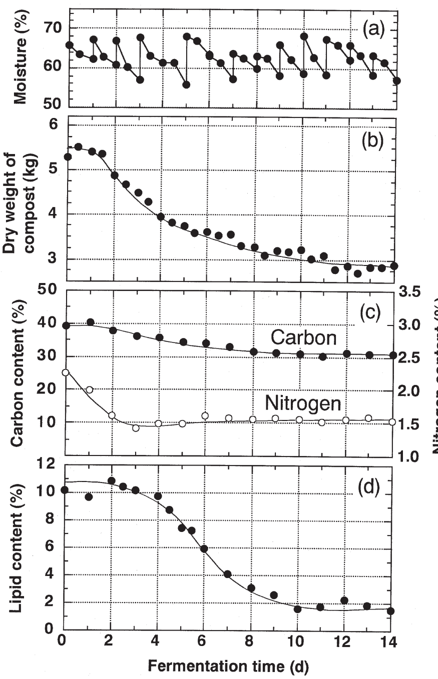

Figure 1. Time courses of (a) moisture content (b) dry weight of compost in the composter, (c) carbon and nitrogen contents and (d) lipid content of the compost during fermentation.

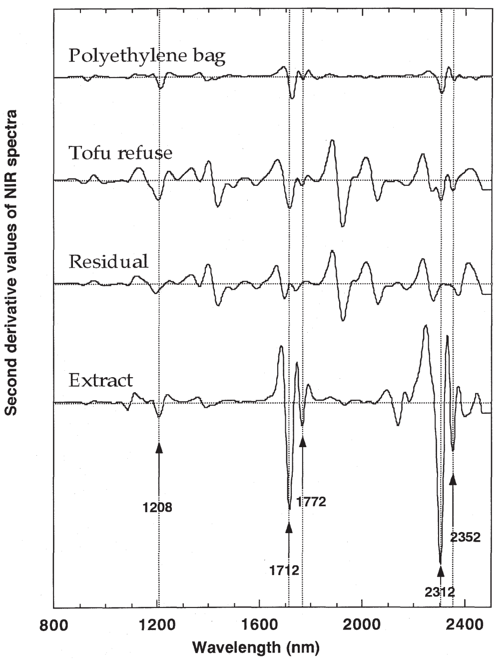

Figure 2. Second derivative NIR spectra of (a) polyethylene bags, (b) tofu refuse, (c) residual after Soxhlet extraction of tofu refuse, and (d) Soxhlet extract of tofu refuse.

lipids in the compost material was slower than that of other biodegradable organic compounds, and the change in lipid content in the compost could be one of the indicators to determine the end point of the thermophilic compost fermentation.

Calibration equation for measuring lipid content

To produce a suitable calibration equation, it is very important to select a wavelength having the absorption assigned to the target compound. Figure 2 shows the second derivative NIR spectra of (a) polyethylene bags, (b) tofu refuse, (c) the residual after Soxhlet extraction of tofu refuse and (d) the Soxhlet extract from tofu refuse. The second derivative NIR spectrum of the Soxhlet extract from tofu refuse had five negative peaks at 1208, 1712, 1772, 2312 and 2352 nm representing absorption of lipids. However, the second derivative NIR spectrum of the polyethylene bag also had the negative peaks at 1214, 1726, 1772, 2312 and 2352 nm.

The wavelength used to formulate a calibration equation of lipid content should, therefore, be selected from 1208, 1712, 1772, 2312 and 2352 nm. MLR was carried out between the second derivative NIR spectral data and the lipid contents of the compost (C_{act}) obtained by the reference method. A calibration was made to predict the carbon content (C_{pre}). The results are shown in Table 1. Where a calibration equation was formulated using only one wavelength, an equation using 1712 nm was better than that any other wavelength. The reason for this was that, while the three absorptions at 1772, 2312

Table 1. Calibration and validation results for prediction of lipid content of compost.

Wavelength (nm)			Calibration (n = 60)		Validation (n = 35)	
λ_1	l_2	λ_3	R (–)	SEC (%)	r (–)	SEP (%)
1208	—	—	0.726	2.17	—	—
1712	—	—	0.971	0.754	0.955	0.902
1772	—	—	0.176	3.10	—	—
2312	—	—	0.543	2.650	—	—
2352	—	—	0.542	2.650	—	—
1208	1712	—	0.975	0.698	0.964	0.815
2312	1712	—	0.974	0.720	0.955	0.902
2352	1712	—	0.975	0.710	0.956	0.897
1208	2312	—	0.888	1.46	—	—
1208	2352	—	0.889	1.45	—	—
1208	2312	2352	0.889	1.45	—	—

and 2352 nm depend mainly on the lipids, the C–H bonds from the polyethylene bags also has absorption bands at these wavelengths.[4,5] The absorption at 1208 nm may relate to the vibration of the C–H stretching second overtone of the lipid.[4] As a result, the wavelengths at 1208 and 1712 nm were selected as the first and second terms in a calibration equation for lipid content. The values obtained for *R* and *SEC* were 0.975 and 0.698%, respectively.

$$C_{\text{pre}} = -1.11 - 129\text{A}_{1208} - 497\text{A}_{1712} \quad (1)$$

Validation

The lipid content in the independent validation sample set (n = 35) was predicted using the calibration equation (Equation 1) and compared with the values of C_{act}. A good agreement between the values obtained by the conventional method and those obtained by NIR was observed and the values of r and standard error of prediction (*SEP*) were 0.964 and 0.815%, respectively (Table 1).

Monitoring of the lipid content during compost fermentation was performed using this equation (Equation 1). In Figure 3, a good agreement between the values obtained by the conventional method and those obtained by NIR was observed during compost fermentation. These results suggest that the calibration equation would be highly suitable for this purpose.

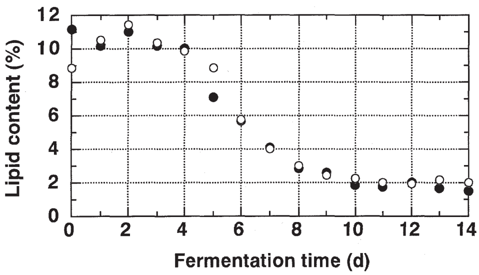

Figure 3. Monitoring of the lipid content during compost fermentation using NIR. Symbols: ●, measurement of the lipid content by the Soxhlet extraction method; ○, predicted value of the lipid content by NIR.

Conclusion

The lipid content of the compost was an important indicator of compost fermentation and could help to detect the end-point of the fermentation process. Furthermore, the validity of the NIR method was shown by applying it to predict the time course of lipid content during compost fermentation. The operational procedure involved in NIR is very simple and nondestructive and the time required for the measurement is but a few minutes. To develop high performance in the composting process, simultaneous measurement of the moisture,[2] carbon,[3] nitrogen[3] and lipid contents of the compost would be a powerful tool.

Acknowledgments

The authors are very grateful to the Eishoku Foods Co. for generously providing fresh tofu refuse. This work was supported by a Hiroshima City University Grant for Special Academic Research (Encouragement for Researchers in 1999 and 2000).

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