Note

Chromatographic Analysis of Bean Curd Refuse Decomposed by *Bacillus* sp. HR6

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Chromatographic analyses were carried out on bean curd refuse decomposed by *Bacillus* sp. HR6 to evaluate the decomposition process. The water-soluble fraction possessed strong absorption bands (251 - 259 nm). Gel filtration chromatography showed that one of the major peaks, at a molecular weight of 1170, disappeared and that two peaks with molecular weights of 1040 and 1310 were newly detected. In the ether- and chloroform-soluble fractions, a novel peak at the molecular weight of 8890 was observed after 24 h of decomposition, although we did not detect peaks from such higher molecular weights in the original sample. These results indicate that not only the decomposition of many kinds of water-soluble compounds but also the polymerization of hydrophobic ones were carried out in this process.

Key words: *Bacillus* sp. HR6 / Microbial decomposition / Bean curd refuse / Chromatographic analysis.

A large amount of food waste is produced daily by the food industry and in our daily lives. Since this waste generally contains a high amount of water, it takes much time and fuel to burn it up completely. The combustion of fossil fuel is directly related to the CO₂ emission which causes the greenhouse effect (Hammitt et al., 1996). A high amount of water in the food waste causes the combustion temperature in the chamber to decrease, which leads to the initiation of the synthesis of highly toxic and carcinogenic compounds such as dioxin (Hansen, 1991) when food waste is burned with the compounds containing chloride. Therefore, the study of biological decomposition of food waste has become of increasing importance in order to preserve the natural environment and conserve resources.

When *Bacillus* sp. HR6 was mixed with bean curd refuse (e. g., 30 kg) and incubated for 24 h in the organic waste decomposition machine (BIO COSMO 100A, Sanyo Techno Co., Ltd.), the net weight was reduced by about 70% (Mimura et al., 1995). To apply the final product to the agricultural fertilization, it seems necessary to estimate the decomposition process through quantitative analyses. For example, gel chromatography is one of the best methods to measure compost maturity quantitatively (Hirai et al., 1983). Hinata and Takeuchi (1995) reported that the water-soluble compounds with a molecular weight (MW) < 1500, which had the absorption at 280 nm, were decreased with the improvement of compost maturity. Therefore, in this study, each water-, diethyl ether- (ether-), or chloroform-soluble fraction was analyzed by the column chromatography to evaluate the changes of the compounds present in the bean curd refuse before and after decomposition by *Bacillus* sp. HR6.

Cells were grown in a medium containing 5 g of Bacto peptone (Difco, USA) and 1 g of yeast extract (Difco, USA) per 1 liter for 24 h at 30°C. The pH of the medium was adjusted by NaOH.

In the reaction chamber, 500 kg of the fresh bean curd refuse was mixed with both 500 ml of cell suspension, containing 9.5 x 10⁸ cells, and 100 kg of...
sawdust which was used to adjust the water content to about 50% in the mixture. After 24 h, 500 kg of the fresh bean curd refuse was added to the vessel, and the reaction was continued for another 24 h. This process was repeated five times. Twenty grams of the final product obtained were treated for 3 d in the drying oven at 104°C to evaporate the water before use.

Distilled water at 200 and 300 ml was added to the fresh and decomposed materials to extract the water-soluble compounds, respectively. After 1 h at 50°C, the mixture was filtrated by 0.45 μm pore size of membrane filter (Sartorius, Germany), and the water-insoluble fraction was further mixed with 200 ml of ether. Thus, we obtained both ether-insoluble and soluble fractions, and the former was mixed with 100 ml of chloroform. Then, the chloroform-soluble fraction was separated by filtration, and elementary analysis was applied to the remaining chloroform-insoluble fraction.

The water-soluble fraction was analyzed by high performance liquid chromatography (HPLC, HP-1100 HPLC system, Hewlett-Packard, USA). The column used was Waters μ BONDASPHERE 5 μ C18 (Waters, USA). The column oven was kept at 40°C. The eluant used was 0.01 N H2SO4 and its flow rate was 1.0 ml/min. As a detector, we used the photodiode array (HP PhotoDiode Array Detector, Hewlett-Packard, USA).

The water-soluble fraction was also analyzed by gel filtration chromatography (GFC, Waters 600 HPLC / GPC system, Waters, USA). The columns used were connected in tandem with Waters Ultrahydrogel 250 Å and 120 Å. The column oven was kept at 40°C. The eluant used was 0.1 M NaNO3 and its flow rate was 1.0 ml/min. The differential refractometer (RI Waters 410, Waters, USA) was used as a detector. The molecular weights of the water-soluble compounds were calculated as a logarithmic value from the equation $y = -0.0102x^3 + 0.4944x^2 - 8.1648x + 49.0986$. The correlation coefficient was > 0.99. The minimal MW of polyethylene glycol used was 400 to draw the standard curve. Consequently, the compounds of MW < 400 were not detected by this method.

Both ether- and chloroform-soluble fractions were analyzed by gel permeation chromatography (GPC, Waters 600 HPLC / GPC system, Waters, USA). The columns used were Waters Ultrastyragel Linear (one piece) and Waters Ultrastyragel 100 Å (two pieces), three of which were connected in tandem. The column oven was kept at 35°C. The eluant used was chloroform with the flow rate of 0.8 ml/min. The photodiode array (HP PhotoDiode Array Detector, Hewlett-Packard, USA) was used as a detector. These fractions were dried by heating and dissolved by chloroform to obtain concentrations twice as high as the original. To estimate the molecular weights of

![FIG. 1](image-url). High performance liquid chromatography analysis of the water-soluble fraction obtained from fresh bean curd refuse (A) and from bean curd refuse decomposed by Bacillus sp. HR6 (B). The absorption intensity was classified as follows: — , <4 mV; --- , <8 mV; ---- , <12 mV; --- --- , <16 mV; and ---- ---- , <20 mV.
the ether- and chloroform-soluble compounds, the equation $y = 0.0041x^3 - 0.3257x^2 + 8.0963x - 57.7860$ was used, in which the correlation coefficient was > 0.99. The minimal detection limit was the MW of 580.

The chloroform-insoluble fractions were dried in a vacuum at 45°C for 72 h, and then the elements of carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) were analyzed by CHN-O-Rapid elemental analyzer (Elemental, Germany).

The results of HPLC analysis of the water-soluble fractions obtained from bean curd refuse before and after the bacterial decomposition are shown in Figs. 1A and 1B, respectively. In the original sample, absorption bands were mainly observed from 220 to 290 nm at less than 6 min of the retention time, but these bands were not detected at > 6 min. The bacterial decomposition of the original sample brought about the appearance of a new spot between 251 to 259 nm with the intensity of 8 - 11 mV at 25.5 min. In addition, absorption between 240 - 277 nm was observed at around 12 min. A peak due to the compounds which had the wide range of UV absorption up to 286 nm was detected at 4.8 min with a high intensity of 16 - 19 mV.

According to Hinata and Takeuchi (1995), the amount of compounds with the absorption at 280 nm (MW < 1500) decreased with the progress of the compost maturation. In the present experiment, however, the amounts of the water-soluble compounds which had the UV absorption at 220 - 290 nm increased at longer retention times (> 6 min). Although 24 h was long enough to decompose the bean curd refuse by Bacillus sp. HR6 (Mimura, et al., 1995), it might take a longer time to obtain a perfect mature compost without lower-molecular weight compounds having an absorption at 280 nm.

In this analytical system, a longer retention time was required for the elution of the relatively more hydrophobic compounds than hydrophilic ones, because there exist the hydrophilic interactions between the former compounds and octadodecyl silica used as a packed material in the column. Therefore, we concluded that the compounds eluted at around 12 and 25.5 min, which indicated that they were weakly hydrophobic, were synthesized by this strain in the decomposition process.

For the water-soluble fractions, GFC analysis was carried out to evaluate the molecular weights. For the fresh bean curd refuse, we observed three peaks derived from the compounds with MW of 660, 1170 and 1840 (Fig. 2A). From the comparison between original and decomposed samples, we noticed the appearance of two novel peaks at MW of 1040 and 1310 and the disappearance of the peak at a MW of 1170 in the decomposed material. Therefore, the spot detected at the retention time of 25.5 min by HPLC (Fig. 1B) is ascribed to MW of 1040 or 1310 (Fig. 2A), because no peaks were newly detected except for them by GFC analysis.

The chromatographic patterns for ether- and chloroform-soluble fractions resembled each other (Figs. 2B and 2C). In the GPC analysis for the ether-soluble fraction of the fresh bean curd refuse, we detected the peak at a MW of 4090 and the shoulder
between MW of 1170 and 1800. During 24 h of decomposition, those two peaks broadened and a new intense peak at a MW of 8890 appeared. As for the chloroform-soluble fraction, a large peak at a MW of 3710 and a small peak at a MW of 1290 were obtained in the original sample. Like in the case of the ether-soluble fraction, both peaks broadened with the progress of the bacterial decomposition. At the same time, we also detected a novel peak at a MW of 8890.

The presence of the compounds with a MW of 8890 was commonly confirmed in both ether- and chloroform-soluble fractions for the decomposed bean curd refuse. It seems plausible that organic compounds after decomposition are partially dissolved in ether, probably due to their solubility, and the remaining ones were finally dissolved in chloroform. Thus, both fractions showed a similar pattern in their spectra.

The total peak area was calculated based on the data before normalization of the differential molecular weight distribution curve. These analyses showed that the ratio of the peak area before and after bacterial decomposition of the bean curd refuse was 1 : 0.55 in the water-soluble fractions (data not shown), suggesting that the compounds of MW > 400 were decomposed to about half. On the contrary, the ratios obtained for the ether- and chloroform-soluble fractions were 1 : 1.44, and 1 : 1.34, respectively (data not shown). These results suggest that hydrophobic compounds are synthesized via bacterial activity during the decomposition processes. Then, we tried to identify the compounds in ether-soluble fractions by the use of gas-chromatograph-massspectrometer (GC-MS, GCQ, Thermoquest Co., Ltd., USA). Some compounds of MW at around 300 were detected, but compounds of MW > 4000 were not detected. Although the mass-spectra of these compounds obtained did not fit completely with the typical compounds listed in the data base (data not shown), some compounds were identified to be methyl esters of fatty acid with C$_{16}$ - C$_{19}$. The reason why the compounds of MW greater than 4000 were not detected by GC-MS is ascribed to the fact that such compounds might exist as oligomers or polymers, or have higher boiling points.

The weight of an element as a percentage of the total weight of the sample was calculated on the basis of the dry weight for the analyses (Table 1). No specific reduction of the element was observed, although the sum of the weight percentages of C, H, N, and O was reduced by 1.9% after 24 h of decomposition.

**TABLE 1.** Elementary analysis for chloroform-insoluble fraction of bean curd refuse before and after decomposition by Bacillus sp. HR6.

<table>
<thead>
<tr>
<th>Element</th>
<th>Before (weight %)</th>
<th>After 24 h (weight %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>48.8±0.3</td>
<td>47.9±0.2</td>
</tr>
<tr>
<td>H</td>
<td>6.6±0.04</td>
<td>6.4±0.06</td>
</tr>
<tr>
<td>N</td>
<td>5.1±0.07</td>
<td>4.9±0.04</td>
</tr>
<tr>
<td>O</td>
<td>32.7±0.3</td>
<td>32.1±0.6</td>
</tr>
<tr>
<td>Total</td>
<td>93.2</td>
<td>91.3</td>
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</tbody>
</table>

*The data shown are the average ± S. D. (n=5).*

The total dry weights of the final materials of both fresh and decomposed bean curd refuse were 16.04 and 15.26 g, respectively. This result indicates that 0.78 g in 20 g (3.9%) of the solvent-insoluble materials, e.g., insoluble fibers, was decomposed. Some part of the weight loss described above (Table 1) are attributed to the diffusion into the atmosphere as gaseous compounds.

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**REFERENCES**


