Degradation of Solid and Muddy Organic Matter of Blue Mussels by *Bacillus* sp.

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Abstract- Organic matter contained in 4,000 kg of blue mussels was subjected to microbial degradation. Seawater (1,280 liter) trapped in the shells discharged by smashing shells, and the value of chemical oxygen demand (COD) was 5,800 mg/liter. The remaining solid organic matter (2,720 kg) including broken shells was washed with 6,000 liter of tap water to reduce NaCl concentration. The COD in the wastewater was 550 mg/liter. The washed solid organic matter was subjected to compression treatment by using the Screw-press machine to adjust its water content (around 30%) to that optimal for microbial activity, thereby removing 680 liter (COD = 11,800 mg/liter) of wastewater from solid organic substances. Wastewater obtained at each process was collected together in one pot, and flocculating agents were added there to cause aggregation and precipitation, thereby recovering organic matter in the form of sludge. Water content of sludge after the belt press process was reduced from 56% to 39%, by vacuum drying. The solid organic matter obtained after washing and compressing and the sludge after vacuum drying were subjected to the degradation (45 h) by *Bacillus* sp. As a result, 41% (w/w) of the solid organic matter and 20% (w/w) of sludge disappeared. Total carbon and nitrogen ratios were reduced from 6.0 to 5.1 in the solid organic matter and from 12.8 to 12.5 in the sludge, respectively. The concentrations of contaminated heavy metals such as As, Cd, Hg, Ni, Cr, and Pb in the solid organic matter and the sludge were quite less than those of their tolerance levels.

I. INTRODUCTION

Blue mussels grow in summer on the marine structures where the surface of water bodies is alternatively rising and falling. Actually, blue mussels contribute to the purification of seawater through the filtration and degradation of dissolved organic substances [1]. The organic matter of blue mussels, however, contributes to the eutrophication of the closed areas of seawater such as inland seas and bays after died in late summer to autumn.

Blue mussels also cause an operational trouble at the power plants by stacking in the pipes for pumping in cooling seawater. Therefore, huge amount of blue mussels are removed at certain intervals. After put on the ground, they are spoiling and give off an odor. Consequently, rapid treatment of organic matter in blue mussels is important from the view point of our public health and recycle of organic wastes. Reduction of smell of them also has a high priority [2].

We have succeeded composting of the organic matter contained in blue mussels in a pilot experiment [3]. In that experiment, we used 218 kg of blue mussels, and an 82.5 kg of compost was obtained. We had to add sawdust to the solid organic matter to adjust the water content (around 30%) to that optimal for microbial activity. This process led the volume of compost and the value of carbon/nitrogen (C/N) ratio of the compost increase. The muddy organic matter discharged from blue mussels had not been treated in that experiment.

In this experiment, we tried to keep the value of C/N ration of the organic waste at 7 – 10 to make a high quality of compost so as to use soil fertilizer. Moreover, we tried to increase the net weight of the organic matter in blue mussels to degrade in one batch treatment. Discharged wastewater including suspended organic substances was also tried to degrade after flocculated by the addition of flocculating agents.

II. MATERIALS AND METHODS

A. Strain and culture condition

Bacillus sp., which was isolated from soil, was incubated in nutrient medium. Cell suspension was added to bean curd refuse and cells were cultivated on it. Bean cured refuse (100 kg) was added to the cultivation chamber once in a day and that was continued for 5 days. The product (102 kg) thus obtained was used to degrade organic matter of blue mussels. For the microbial degradation of solid organic matter, 102 kg of the product was used. The product (35 kg) was also applied to the degradation of suspended organic matter, which was collected together in one pot and precipitated by the addition of flocculating agents.

B. Comparison of dominant strain in compost made from bean curd refuse with that of laboratory stock

Cultivation of *Bacillus* sp. was aerobically continued for 5 days. We turned the blower off at the last day when the fermentation temperature was over 70*C*. One hour later, we sampled some part of compost to count a total viable cell number and identify what the dominant strain is.

Three individual colonies were selected randomly and subjected to partial 16S rDNA sequencing as well as physiological and biochemical examinations. DNA sequencing was followed by the protocol of Applied Biosystems (USA). Briefly, extracted DNA was amplified with universal primer by PCR, and amplified DNA was sequenced with DNA sequencer (ABI PRISM 377 DNA Sequencer, Applied Biosystems, USA). Microbial Identification Software (Applied Biosystems, USA) was used to carry out the homology analysis based on the sequence database.

C. Smashing blue mussels

Blue mussels (4,000 kg) were subjected to the Smash machine to break shells. This machine has two turning shafts, and shells were broken when passing through the clearance of them.

D. Preparation of solid organic matter for microbial degradation

The solid organic matter with broken shells was washed once with tap water (6,000 liter) to decrease NaCl concentration, and the washed organic matter was subjected to compression treatment by using the Screw-press machine (Fig. 1 and 2) to adjust its water content (around 30%) to that optimal for microbial activity. Punching pore size of holes on the drum in the Screw-press machine was determined after the examination by changing the pore size up to 5.0 mm to give the optimal water content.

E. Flocculation of suspended organic matter in wastewater obtained through the preparation processes of solid organic matter for microbial degradation

Breaking shells (4,000 kg) discharged seawater (1,280 liter). After washing the organic matter with 6,000 liter of tap water, it was subjected to the treatment with the Screw-press machine. In this process, 680 liter of wastewater including muddy organic matter was obtained. Wastewater thus obtained was collected together in one pot to add the flocculating agents. Flocculating agents such as $FeSO_4$ (8.5 g/liter) and acrylamid-acryl acid copolymer (1.12 g/liter) were used to precipitate and to recover the muddy organic matter in the form of sludge. Vacuum drying was carried out to reduce the water content of the sludge until 39% followed by the Belt-press treatment, and the sludge was subjected to the microbial degradation.

F. Analyses of microbial degradation processes

Microbial degradation of the solid and the flocculated organic matter was carried out with the degradation machine (BIOCOSMO BCT-3000, Sanyo-Techno Co., Ltd, Japan). Production of CO_2 and NH_3 was monitored with each inspection tube connected to the gas suction pump. Heat generation was monitored with thermocouple, which had been located in the reaction chamber.

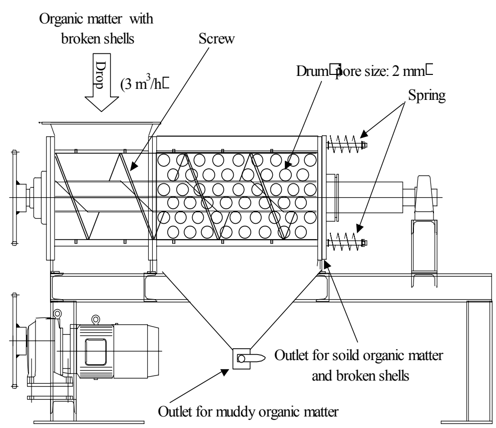

Fig. 1. Scheme of Screw-press machine.

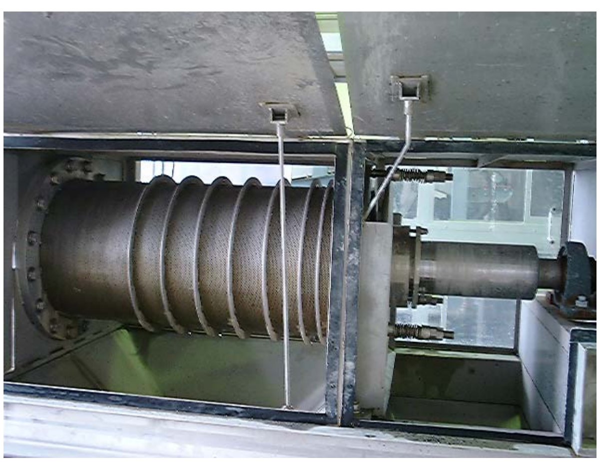

Fig. 2. Drum portion of Screw-press machine.

Screw is located inside the drum to compress the solid organic matter.

G. Measurements of total carbon and nitrogen concentrations in compost made from organic matte of blue mussels

Total carbon and nitrogen concentrations in the sample before and after microbial degradation for 45 h were analyzed. After dried, 2 mg of the samples, which seemed to be excluded broken shells, were subjected to the measurement of the total carbon and nitrogen concentrations with the CN analyzer (MT-5, Yanagimoto Co., Ltd., Japan). We did not treat the samples with hydrochloric acid solution as a pre-treatment process to remove $CaCO_3$, which is the major component of shells.

H. Measurement of heavy metals as environmental pollutants in compost

The concentrations of heavy metals such as arsenic, cadmium, mercury, nickel, chromium, and lead were analyzed after the samples were dried. Measurement was carried out commercially by a company, Kanken (Japan).

III. RESULTS AND DISCUSSION

A. Comparison of the dominant strain isolated from the microbial product made from bean curd refuse with that of laboratory stock

Bean curd refuse was easily handled, so that it has been used as bed for the strain, *Bacillus* sp. The viable cell number on it was calculated as 1.7 x 10^7 (cfu/ml). Three individual colonies on the plate were subjected to the identification and comparison with that of the laboratory stock. As a result, the dominant strain was identified as genus *Bacillus*, and partial rDNA sequences fitted 100% with those of the laboratory stock of *Bacillus* sp. These results indicate that the microbial degradation product made from bean curd refuse contains *Bacillus* sp. as the major strain.

In general, *Bacillus* sp. shows high tolerance to dry and heat stress, which depends on spores in the cells. High secretion activities of extracellular proteases and amylases are also the typical properties of genus *Bacillus*. Therefore, genus *Bacillus* is useful for the degradation of organic wastes [4]. When the strain, *Bacillus* sp., was cultivated in bean curd refuse, fermentation temperature increased more than 70*C* [5]. Such a high temperature seems to contribute the thermal inactivation of contaminated environmental microorganisms and to support the strain dominant through cultivation.

B. Compression of solid organic matter

Relationship between punching pore size of the Screw-press machine and water content of the solid organic matter is shown in Fig. 3. Reduction of water content was observed with an increase in punching pore size from 0 to 2 mm, although enough reduction of the water content was not observed at the punching pore size of both 0.3 – 1 mm and more than 2mm. Water content showed the minimum value of 26.5 ± 1.1% when the punching pore size was adjusted to 2 mm.

Adjustment of water content to a certain value is one of the most important factors to degrade organic wastes efficiently. As for the degradation of the organic matter by *Bacillus* sp., around 30% of the water content seems to be better than the other values because the

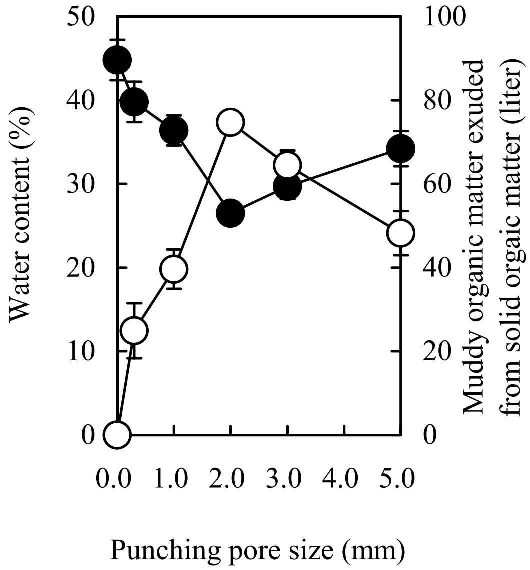

Fig. 3. Effect of punching pore size on the drum in Screw-press machine on the reduction of water content of solid organic matter as well as the increased volume of exuded wastewater discharged from solid organic matter.

Punching pore size was changed up to 5.0 mm. Changes in water content of solid organic matter (●) as well as muddy organic matter exuded from solid organic matter (○) were measured after treated with Screw-press machine. The experiments were carried out three times independently, and the data shown here is the average ± SD (n = 3).

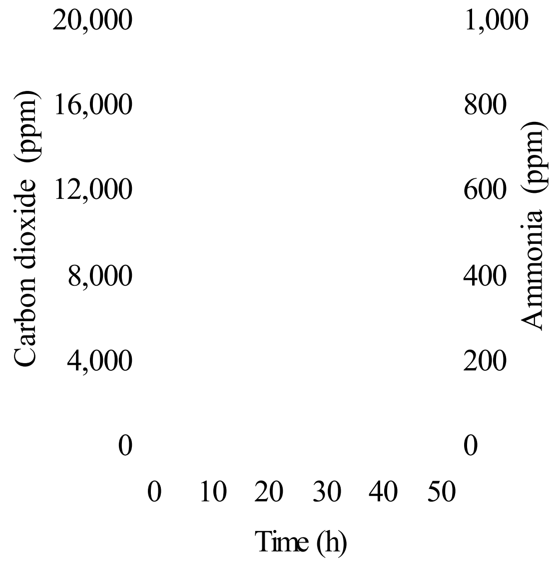

Fig. 4. Changes in the concentrations of carbon dioxide as well as ammonia production during composting of organic matter by *Bacillus* sp.

Production of carbon dioxide (○, ●) and ammonia (Δ, ▲) were measured when the solid (closed symbol) and the muddy organic matter (open symbol), which was flocculated by flocculating agents, were degraded by *Bacillus* sp. The experiments were carried out three times independently, and the data shown here is the average ± SD (n = 3).

peak value of fermentation temperature (over 65C) was observed at about 30% of water content [3]. Therefore, we decided the punching pore size to adjust 2 mm for the following experiments.

C. Production of carbon dioxide and ammonia as well as generation of fermentation temperature through the microbial degradation of solid and flocculated organic matter

Through the degradation of the solid organic matter and the sludge by *Bacillus* sp., carbon dioxide and ammonia were produced (Fig. 4). When the solid organic matter was degraded, the peak value of carbon dioxide (16,000 ppm) and ammonia (810 ppm) were detected after 12 h and 21 h, respectively. When the sludge was subjected to the microbial degradation, the peak values of carbon dioxide and ammonia were 2,800 ppm for 18 h and 130 ppm for 27 h, respectively. Figure 5 shows the changes in fermentation temperature through the microbial degradation of the solid organic matter and the sludge. Through the microbial degradation of the solid organic matter, fermentation temperature showed the maximum, 68*C*, after 12 h. As for the sludge degradation, fermentation temperature reached 62*C* after 18 h. It took further several hours to reach the peak value for ammonia production than that for the production of carbon dioxide, indicating that carbohydrate included in the organic wastes of blue mussels was primarily degraded to make carbon dioxide and water, and as a second step, proteins were degraded into small molecules and ammonia. The time taken to the peak value of carbon dioxide production was the same as that of fermentation temperature. This result indicates that heat generation was closely related to the microbial consumption of carbohydrate.

The values of carbon dioxide and ammonia production as well

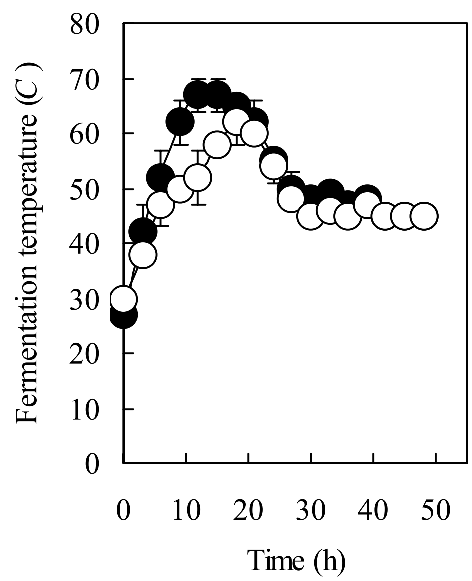

Fig. 5. Heat generation during the degradation of solid and muddy organic matter of blue mussels by *Bacillus* sp.

Fermentation temperature was measured when the solid (●) and the flocculated muddy organic matter (○) were degraded by *Bacillus* sp. The experiments were carried out three times independently, and the data shown here is the average ± SD (n = 3).

as fermentation temperature for the microbial degradation of the sludge were lower than those for that of the solid organic matter. The flocculating agents used for the precipitation of the suspended organic matter might give some damage on the microbial activity.

D. Measurements of total carbon and nitrogen concentrations in compost

Total carbon (C) and nitrogen (N) concentrations were measured before and after microbial degradation. The C/N ratios obtained from the solid organic matter and the sludge are shown in Table 1 and Table 2. After 45 h of degradation of the solid organic matter by *Bacillus* sp., the value of C/N ratio, 6.0, was reduced 5.1. As for the sludge degradation, the C/N ratio reduced from 12.8 to 12.5. It is necessary to keep the C/N ratio in compost around 7 - 10 to obtain a high quality compost, which is available to use as a soil fertilizer. So far, we could keep the C/N ratio near 7 for compost made from the solid organic matter of blue mussels. As for the reduction of the water content and the volume of compost, we could get a big progress by compressing the solid organic matter with the Screw-press machine rather than the addition of huge amount of sow dust. This compression process also contributed to prevent the contamination of extra carbon, which was contained in sow dust. The C/N ratio obtained from the degraded sludge, however, was higher than 10. The organic flocculating agents added to precipitate muddy organic matter caused to an increase in carbon concentration in compost. Biodegradable organic flocculating agents are required to be developed.

E. Analyses of heavy metals in compost

Heavy metals such as arsenic, cadmium, mercury, nickel, chromium, and lead were detected from compost (Table 3). The concentrations of them, however, were quite lower than those of tolerance level, indicating that compost obtained is available to use as a soil fertilizer, although the technical development to remove such heavy metals is necessitated.

Table 1. Total carbon and nitrogen ratio of compost obtained from microbial degradation of the solid organic matter.

	Before degradation[a, b] (g/kg (dry weight))	After degradation[a, b] (g/kg (dry weight))
Total carbon (C)	43.3 ± 2.2	34.0 ± 1.8
Total nitrogen (N)	7.2 ± 0.4	6.7 ± 0.3
C/N ratio	6.0 ± 0.1	5.1 ± 0.1

[a]Experiments were carried out three times independently. Each sample obtained was measured three times and averaged. The data shown here is the average ± SD (n = 3).
[b]The sample included microbial product of bean curd refuse by *Bacillus* sp.

Table 2. Total carbon and nitrogen ratio of compost obtained from microbial degradation of the flocculated muddy organic matter.

	Before degradation[a, b] (g/kg (dry weight))	After degradation[a, b] (g/kg (dry weight))
Total carbon (C)	75.7 ± 6.8	68.6 ± 4.9
Total nitrogen (N)	5.9 ± 0.4	5.5 ± 0.3
C/N ratio	12.8 ± 0.3	12.5 ± 0.2

[a]Experiments were carried out three times independently. Each sample obtained was measured three times and averaged. The data shown here is the average ± SD (n = 3).
[b]The sample included microbial product of bean curd refuse by *Bacillus* sp.

Table 3. Analyses of heavy metals in compost obtained from the solid and the flocculated muddy organic matter of blue mussels.

	Solid organic matter[a]	Muddy organic matter[a]	Tolerance level[a]
Arsenic	0.6	2	50
Cadmium	< 0.5	< 0.5	5
Mercury	0.1	0.06	2
Nickel	21	8	300
Chromium	35	7	500
Lead	35	11	100

[a]mg/kg(dry weight)

F. An overview of preparation processes of the organic matter of blue mussels for microbial degradation

Blue mussels (4,000 kg) was used to the microbial degradation. Seawater (1,280 liter) trapped in the shells discharged by smashing shells, where the value of chemical oxygen demand (COD) was 5,800 mg/liter. The remaining solid organic matter including broken shells (2,720 kg) was washed by 6,000 liter of tap water. The COD in that wastewater was 550 mg/liter. After that, compression of the solid organic matter was carried out with the Screw-press machine to reduce the water content around 30%. Through this process, 680 liter (COD = 11,800 mg/liter) was removed from the solid organic matter. Wastewater obtained at each process was gathered and flocculating agents were added in there in order to precipitate muddy organic matter in the form of sludge. After the Belt-press treatment and vacuum drying, water content in the sludge was reduced 39%. The solid organic matter and the sludge were subjected to the degradation by *Bacillus* sp. After 45 h, 41% (w/w) in the solid organic matter and 20% (w/w) in the sludge were disappeared.

ACKNOWLEDGMENT

Partial sequencing of 16S rDNA and its homology analysis for *Bacillus* sp. were carried out commercially by NCIMB Japan.

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