DIGESTION OF MUNICIPAL SEWAGE SLUDGE
BY A MIXTURE OF THERMOPHILIC BACILLI
AND THEIR CULTURE EXTRACT

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Organic fractions in municipal sewage sludge were markedly digested by a mixture of 11 strains of thermophilic bacilli (9 strains of *Bacillus stearothermophilus* and 2 strains of *Thermus* sp.) isolated from sewage sludge compost. The organic fractions were also digested by the culture extract of the mixture of the bacilli grown on a medium containing the sewage sludge. The culture extract contained some lytic enzymes, with digestive activities on freeze-dried sewage sludge and on scleroproteins, so it was concluded that the proteolytic activity may be useful as a specific measure for composting activity and that these thermophilic bacilli can partially digest or mineralize municipal sewage sludge.

The amount of sewage sludge from the treatment units is rapidly increasing with the increase of sewage in towns and cities. The sewage sludge is usually about 70% organic matter and this organic fraction can be stabilized by composting, which makes it possible to recover and reuse a major portion of the nutrient and organic fraction. During the stabilization stage in aerobic composting, the temperature usually rises to a thermophilic level. The organic fraction can be digested and partially mineralized by a mixture of thermophilic bacteria capable of metabolizing the organic fraction. In sewage sludge composting, the function of the composting microorganisms are still not clear. Many subjects remain to be explored.

To elucidate the composting mechanism of sewage sludge, 11 thermophilic bacilli have been isolated from sewage sludge compost. Here we report on the function of those bacilli in sewage sludge composting.

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MATERIALS AND METHODS

Microorganisms. Eleven thermophilic bacilli were used in this experiment. These bacilli were isolated from a municipal sewage sludge compost, which was produced at a sewage treatment center in Fukuoka city, Japan, using dewatered cake with lime and FeCl₃. Using Bergey's Manual of Systematic Bacteriology, vol. 1 (Brock, 1984) and vol. 2 (Claus and Berkeley, 1986), the 11 isolates were identified as 9 B. stearothermophilus and 2 Thermus sp. Their optimal growth temperature was 65°C.

Municipal sewage sludge. The sewage sludge was obtained from the activated sludge process in a sewage treatment unit (centrifugate of the sludge from an aeration tank) in Fukuoka, Japan. The sludge was 70% water and 30% solids. The dried sludge was 65.7% organic (measured by ignition loss) and 34.3% inorganic.

Cultivation of thermophilic bacteria and preparation of culture extract. A mixture of 11 thermophilic bacteria was cultured at 60°C for 10 days on an autoclaved medium (at 121°C for 20 min) containing 10% sewage sludge (freeze-dried matter), 1% filter paper (Toyo No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan), 0.5% yeast extract, 0.1% polypeptone in tap water. The initial pH was adjusted to 7.5–8.0. The culture was used as the inoculum for the sewage sludge digestion test and to prepare culture extract.

The culture was centrifuged and 2 volumes of cold acetone was added to the centrifugate. The mixture was kept at 4°C for 24 hr with gentle stirring. The precipitate formed was centrifuged and dissolved in an adequate amount of deionized water. The resulting solution was dialyzed against deionized water at 4°C for 24 hr. The dialyzate was used as the culture extract for sewage sludge or protein digestion tests.

Sewage sludge digestion by thermophilic bacilli. Ten percent of the mixed culture of the 11 strains was inoculated into a test medium containing 20% sewage sludge (dry basis), 0.5% yeast extract, and 0.1% K₂HPO₄, at an initial pH of 7.5. The test medium was incubated aerobically at 60°C and a 20 ml sample was taken for analysis every 24 hr. The solid fraction in the sample was completely separated by centrifugation. The dry weight of the centrifuged solid was determined after drying at 105°C for 24 hr. The control medium without inoculum was prepared and incubated in the same procedures as that for the test medium. The solid weight decrease with incubation time was defined as a measure of sewage sludge digestion.

Analysis of protein and amino acids. A portion of the above centrifugate was filtered using a 0.45 μm filter and the dissolved protein in the filtrate was assayed by Bio-Rad protein assay (γ-globulin as a standard, Bio-Rad Laboratories Japan, Tokyo, Japan). Two ml of 0.44 M TCA (trichloroacetic acid) was added to 2 ml of the filtrate and kept it for 24 hr at 4°C. The formed precipitate was filtered with filter paper (Toyo No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The free amino acids fraction (as a soluble fraction in 0.22 M TCA) was determined by the ninhydrin method of Muro et al. (6) using glycine as a standard.
Digestion of sewage sludge by culture extract. The culture extract was mixed with 20% (dry basis) of sewage sludge suspended in phosphate buffer (pH 6, or 7.5, 0.1 M) and boric–NaOH buffer (pH 10.5, 0.1 M). The sewage sludge suspension and control suspension (without culture extract) were incubated at 60°C for 2 days. The analytical methods were like those for bacterial digestion.

Mycelium/cell mass and scleroprotein digestion. Since the culture extract contained considerable proteolytic activity (as an indicator of lytic activity), digestion tests were carried out on the various substrates using acetone treated *Rhizopus* mycelium, alkali treated baker’s yeast and freeze-dried sewage sludge to test for microorganism mycelium/cell (mixture of mycelia and cells), and casein, albumin, fibrinogen, collagen, keratin, hemoglobin, elastin, and fibrin as test proteins. The lysis of the mycelium/cell mix was assayed at pH 6.0, 7.5, or 10.5, at 60°C for 30 min by the method of Iwamoto et al. (5). One unit of digestion was defined as 1% reduction of transmittance at using turbidimetry 660 nm. Proteolysis of various proteins was assayed at pH 6.0, 7.5, or 10.5, at 75°C for 20 min by the ninhydrin method of Muro et al. (6). The reaction mixture was 0.1% (w/v) of each protein and 0.1 ml of culture extract. One activity unit was defined as 1 μg of glycine per min per ml of reaction mixture.

RESULTS

Digestion test of sewage sludge by a mixture of 11 thermophilic bacteria

Figure 1 shows the weight decrease during incubation at 60°C obtained by a

![Graph](image)

Fig. 1. Digestion of municipal sewage sludge by a mixture of 11 thermophilic bacilli. The test medium, inoculated with a mixture of 11 bacilli, composed of 10% sewage sludge (freeze-dried matter), was incubated at 60°C for 10 days. ●: Test medium, ▲: Control test.
mixture of 11 thermophilic bacilli compared with control tests without the bacilli. The weight decrease increased linearly up to day 6 of incubation and then the curve gradually leveled off to the 10th day. The solid fraction of sewage sludge was decreased about 25% after 10 days of incubation. Since the solid fraction was 34.3% inorganic matter, which was not digested by the microorganisms, the decrease of the organic fraction was 38.9% of the initial fraction. In the control test only about 7% of the solid fraction was decreased after day 10 and 11.7% of the organic fraction was solubilized. The weight decrease by a mixture of the 11 thermophilic bacilli was higher than those of by the control test.

**Digestion test of sewage sludge by culture extract**

Table 1 shows the digestion of sewage sludge by culture extract (bacteria free) at pH 6.0, 7.5, or 10.5, for 2 days of incubation at 60°C. Compared with the initial solid content of the sewage sludge, the solid fraction after digestion by culture extract was markedly decreased at pH 6.0 and 7.5 but at pH 10.5 the decrease was small. The weight decrease in the control test was almost same at pH 6.0, and 7.5, but was considerably decreased at pH 10.5. The solubilized protein (%, w/w) based on the initial solid fraction, including the amino acid fraction, was also determined as a measure of sewage sludge digestion. In the incubation, the solubilized protein was markedly increased at pH 6.0 and 7.5, but not at pH 10.5. In the incubation at pH 10.5 (in Table 1), the amino acid fraction was almost the same as the solubilized protein, but the amino acid contents of culture extract at pH 10.5 was the highest contents. This indicates a fragmentation of sewage sludge by the culture extract below pH 7.5 and further hydrolysis into smaller molecules at pH 10.5.

**Digestion of mycelium/cell mass by culture extract**

Finely powdered acetone treated *Rhizopus* mycelium, alkali treated baker’s yeast, and freeze-dried sewage sludge were digested by the culture extract at pH 6.0, 7.5, and 10.5. The results, Table 2, are given as digestion activity based on the reduction of the transmittance at 660 nm of suspended reaction mixture. The digestion was indicated by the amount of solubilized substrate. The digestion at

<table>
<thead>
<tr>
<th>pH 6.0</th>
<th>pH 7.5</th>
<th>pH 10.5</th>
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<tbody>
<tr>
<td>Solid</td>
<td>Protein</td>
<td>Amino acid</td>
</tr>
<tr>
<td>Initial</td>
<td>98.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Culture extract</td>
<td>79.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Control</td>
<td>95.4</td>
<td>1.5</td>
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Weight decreases in the digestion by the culture extract and control were calculated based on initial total sum (100%) of solid (insoluble), protein (soluble), and amino acid (soluble) matter. The tests were carried out with incubation at 60°C for 2 days.
pH 6.0 and 7.5 were almost the same for all substrates. However, at pH 10.5 it was about half as much as it was at pH 6.0 or 7.5. This indicates that the lysis of mycelium/cell mass takes place mostly below pH 7.5.

Hydrolysis of various proteins by culture extract

As shown in Table 3, proteolysis of the soluble or insoluble proteins by the culture extract occurred at pH 6.0, 7.5, and 10.5. At pH 6.0, all protein were digested reasonably well, including scleroproteins. Keratin and elastin were not digested at pH 7.5 and collagen, keratin and elastin were not digested at pH 10.5. However, fibrin and hemoglobin as insoluble proteins were all digested by the culture extract at all pH tested.

DISCUSSION

The municipal sewage sludge used was 65.7% organic matter which originated from various microorganisms present in the sludge. Despite many recent studies on
composting (7, 8), little is known about microbial mineralization in sewage sludge composting. To elucidate the mineralization, the digestion of sewage sludge samples was studied using a mixture of 11 thermophilic bacilli isolated from sewage sludge compost. The first digestion step was the breaking of large tissue or microbial cell aggregates into small fragments, then the fragments were digested and solubilized into giant polymers or macromolecules. Finally these latter entities were solubilized and hydrolyzed into oligomers or monomers. The oligomers/monomers were then consumed as substrates by the composting microorganisms. For this, the composting microorganisms must secrete extra-cellularly lytic enzymes. To further test this concept, the culture extract was tested to determine its effectiveness in digesting sewage sludge and microbial mycelium/cell mixes (Tables 1 and 2). The culture extract had considerable digestive ability in a broad pH range for the insoluble mycelium/cells.

In composting, the effects on nitrogen compounds (1, 4) are important. So along with the present digestion tests, we studied the proteolytic activity as an important indicator of the lytic activities of the culture extract. The proteolysis was examined at a wide pH range with various proteins. It was found that the protease in the culture extract hydrolyzes some scleroproteins, such as fibrin/fibrinogen (Table 3).

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