

Biotechnology of intensive aerobic conversion of sewage sludge and food waste into fertilizer

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Abstract Biotechnology for intensive aerobic bioconversion of sewage sludge and food waste into fertilizer was developed. The wastes were treated in a closed reactor under controlled aeration, stirring, pH, and temperature at 60°C, after addition of starter bacterial culture *Bacillus thermoamylovorans*. The biodegradation of sewage sludge was studied by decrease of volatile solids (VS), content of organic carbon and autofluorescence of coenzyme F₄₂₀. The degradation of anaerobic biomass was faster than biodegradation of total organic matter. The best fertilizer was obtained when sewage sludge was thermally pre-treated, mixed with food waste, chalk, and artificial bulking agent. The content of volatile solid and the content of organic carbon decreased at 24.8% and 13.5% of total solids, respectively, during ten days of bioconversion. The fertilizer was a powder with moisture content of 5%. It was stable, and not toxic for the germination of plant seeds. Addition of 1.0 to 1.5% of this fertilizer to the subsoil increased the growth of different plants tested by 113 to 164%. The biotechnology can be applied in larger scale for the recycling of sewage sludge and food wastes in Singapore.

Keywords Aerobic bioconversion; fertilizer; food waste; sewage sludge; thermophilic bacteria

Introduction

Sewage sludge is a general term used to describe the solids produced during the wastewater treatment process at a municipal sewage plant, including sludge removed from an aerobic or anaerobic digester. The main component of sewage sludge is usually dewatered sludge from the anaerobic digester of activated sludge. This sludge is disposed of by landfilling, incineration, in cement production, in agriculture, or by composting (Warman and Termeer, 1996; Krogmann, 2001; Wei *et al.*, 2001). The advantage of using sewage sludge in agriculture is to recycle nitrogen, phosphorus, potassium, calcium, microelements, and organic matter for plant growth by the enhancement of soil. Although applying sewage sludge on land is a concern because of high content of heavy metals, low concentration of potassium, high moisture content, and possible presence of chemical pollutants (Furhacker and Haberl, 1995), there is a trend to increase the use of sludge in agriculture. Utilization of sewage sludge in agriculture in the USA was 20% in 1972 and 55% in 1997; while utilization of sewage sludge in agriculture of European countries in 1990 varied from 10% in Greece to 80% in Portugal and Luxembourg (Vesilind and Spinosa, 2001). In Japan, 2.3 million cubic metres of sewage sludge are produced annually from sewage treatment plants and 24% have been reused on agricultural lands as a fertilizer after composting (Ito *et al.*, 1998). However, sewage sludge in other Asian countries is usually disposed of, not utilized (Vesilind and Spinosa, 2001). For example, more than 50,000 tonnes of sewage sludge and 1,000,000 tonnes of food waste are disposed of in landfill and incineration plants, respectively, in Singapore annually (<http://www.nea.gov.sg>). Due to the proper planning of the industrial and municipal zones, the municipal wastewater and sewage sludge in Singapore have low content of heavy metals and could be converted for agriculture use.

A popular way of sewage sludge utilization is composting. Composting of sewage sludge can enhance the stability of organic matter and provide the inactivation of pathogens

and parasites (Furhacker and Haberl, 1995; Rantala *et al.*, 2000; Krogmann, 2001). In conventional composting the material is aerobically or anaerobically treated for one month, then “cured” and “maturated” in piles or windrows for some months (Furhacker and Haberl, 1995; Matthews, 2001). Starter cultures are rarely used in the composting. The aeration is usually provided only by periodical turning of the composting material. Composting of sewage sludge occupies a large area and is not suitable for application in large scale in a country with a shortage of land such as Singapore.

The aim of this research was to develop intensive in-vessel bioconversion of sewage sludge under controlled aeration, stirring, pH, and temperature at 60°C. Such an intensive system requires small space and minimizes odour problems. Sewage sludge is not easily utilized by microorganisms (Warman and Termeer, 1996; Fang *et al.*, 2001). It is accepted that the best ratio of carbon to nitrogen (C/N) in composting material is in the range from 20:1 to 30:1, whereas the C/N ratio in sewage sludge is approximately 7:1. In addition, the texture of the composting material must allow effective oxygen transfer into the bulk of the compost, but it is difficult due to the viscous texture of sewage sludge. An approach, which was used in this research, was to mix sewage sludge with solid food waste to allow simultaneous bioconversion in the compost. Through the mixing, both the C/N ratio and potassium content in the raw material could be increased and the texture of the mixture be made less viscous than if sewage sludge alone was used. To enhance the intensive bioconversion, the starter culture was selected, identified and used; plastic rings were also added to the treated matter to improve its aeration; and pH of the treated material was buffered by CaCO₃.

Materials and methods

Dewatered anaerobic sludge was acquired from a local municipal water reclamation plant. Vegetable food waste was collected from a university canteen. Bioconversion of the mixture of sewage sludge and solid food waste was carried out in a polyacrylic cylinder reactor having a volume of 3.6 l. The contents were stirred at 10 rpm and air was supplied at 0.3 l/min. The temperature of the mixture was maintained at 60°C. Sewage sludge was first pre-treated at 100°C for 15 minutes to inactivate the pathogens and parasites for hygienic considerations. The heating pre-treatment was also designed to enhance the sludge cell disruption and the hydrolysis of insoluble macromolecules so as to increase the efficiency of the subsequent process. Solid food waste was mixed with sewage sludge in the ratio of total solids 1:1 to improve both the chemical composition and the texture of the raw material. The C/N ratio of the dry solid food waste was approximately 21. Due to this mixing, the C/N ratios in the raw and finished materials were increased. This mixing also had the advantage that the texture of the mixture was less viscous than that of sewage sludge. To improve aeration of the mixture, plastic rings with diameter of 10 mm and width of 8 mm were added as artificial bulking agent to give a weight of the rings/total solids (TS) of 25%. Water was added to the reactor daily to maintain the moisture content approximately at the 75–80% level except during the last two days of the process. This was to dry the final product, which was a grey powder with a moisture content of approximately 5%.

Biomass of the thermophilic bacterial strains *Bacillus thermoamylovorans* SW25 and SW09, which were isolated previously from sludge compost, was used as the starter culture for the bioconversion (Wang *et al.*, 2003). The bacterial strains were grown on tryptic soy broth (Difco, USA) in a shaker at 130 rpm for 24 hours at 60°C. Biomass was recovered by centrifugation at 4,000 rpm for 20 min in Eppendorf centrifuge 5810R and added into the reactor. The bioconversion was carried out for 12 days. The enumeration of thermophilic bacteria on nutrient and tryptic soy agar (Difco Laboratories, USA) was carried out by a spread-plate method from a serial ten-fold dilution of the suspension produced by vortexing 1 g of mixture in 9 ml of phosphate-buffered saline (PBS). The Petri dishes were

incubated at 60°C for one day under aerobic conditions.

Enumeration of enterobacteria in sewage sludge was done by whole-cell hybridization with the Ent1432 oligonucleotide probe (Sghir *et al.*, 2000), which is specific for enterobacteria, including genera *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*. Counts were performed with a FACSCalibur flow cytometer (Becton Dickinson, USA). The oligonucleotide probe was labelled by CY5. Whole cell *in situ* hybridization with this probe was performed at 46°C for 2 h, and then washing was done by double-filter-sterilized hybridization buffer at 35°C for 0.5 h.

The pH of the samples was measured in a suspension of 1 g of the matter in 10 ml of distilled water. The content of dry matter was determined by a standard method (Standard Methods, 1998). The content of organic matter was determined as volatile solids by the weight loss after ignition of the samples (approximately 1 g of dried sample) at 500°C for 20 minutes. The contents of carbon and nitrogen in the samples were determined using an Elemental Analyzer CHNS/O 2400 (Perkin Elmer, USA). The content of K and P was determined using an Inductively Coupled Plasma Atomic Emission Spectrometer (Perkin-Elmer ICP-AES).

Measurement of co-enzyme F₄₂₀, which is an essential component of methanogens and can be used as a relative parameter of their concentration, was made using a Luminescence Spectrometer LS-50B (Perkin Elmer, UK) in 3 ml quartz cuvette. The measurement was performed in the regime of synchronous scan. The difference between excitation and emission wavelengths was 20 nm. The excitation wavelength was changed from 360 to 480 nm and the emission wavelength was synchronously changed from 380 to 500 nm.

Stability of the fertilizer was measured by the carbon dioxide evolution rate (*Q*) over a period of 4 days (Test Methods for the examination of composting and compost materials, 2001). The fertilizer stability index was evaluated according to the following range of CO₂ evolution rate, mg CO₂/g of organic matter/d: very stable (*Q* < 2); stable (*Q* = 2–8); and unstable (*Q* = 8–15). An influence of the fertilizer on the plants was determined by the modified germination and root Elongation Test (Test Methods for the examination of composting and compost materials, 2001).

The plant growth experiments were performed by the common scheme of the research on agricultural use of sewage sludge and compost (Wong and Su, 1997). Three plant species, cucumber, tomato and kangkong, were used to study the influence of the fertilizer on plant growth in the pots. Two kilograms of fresh subsoil with moisture of 14% were placed in every pot. No fertilizer was added to the control. The fertilizer was added in the experimental pots at an application rate of 0.2, 0.5, 1.0, 1.5, and 1.75% (weight of dry matter/ weight of soil). Each mixture was incubated one week prior to plant growth experiments for the release of nutrients. The growth of the plants was observed and measured over a period of 5 weeks.

Results and discussion

The addition of vegetable waste to sewage sludge, with the ratio of 1:1 by total solids, was made to improve C/N ratio in the initial material for bioconversion, to increase the potassium content, to dilute the content of heavy meals and to reduce the viscous texture of sludge. The characteristics of the raw materials are shown in Table 1.

The data show that the mixing of sewage sludge and food waste could improve the quality of the raw material and the end product. The nitrogen content is high in sewage sludge (5.5%) and C/N ratio is low (6.8). An addition of food waste with high content of carbon (44%), low content of nitrogen (2.2%), and C/N ratio of 18.3 could improve the initial C/N of the mixture. The content of phosphorus is low (0.1%) in the food waste, but high in the sludge (1.8%). The content of potassium is low in the sludge (0.2%), but high in the food waste (4.5%). Therefore, addition of the food waste to sewage sludge will improve the

Table 1 Characteristics of the raw materials, initial matter and final product of bioconversion

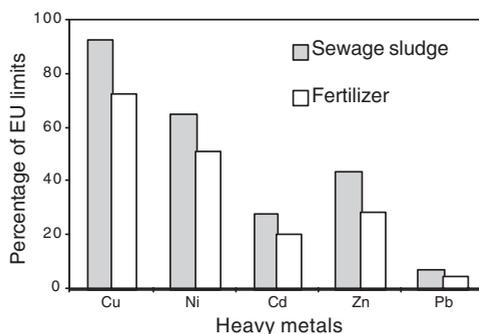
Characteristics	Raw materials		Bioconversion	
	sewage sludge	food waste	initial matter	final product
pH	7.9	7.1–7.3	7.3	7.0
TS, %	21.7 ± 0.4	7.7 ± 1.5	16.6 ± 0.3	95.9 ± 0.3
VS, % of TS	73.6 ± 0.6	91.7 ± 1.5	79.4 ± 0.7	63.3 ± 0.2
Content of carbon, %	37.2 ± 1.0	40.4 ± 3.4	35.9 ± 0.4	33.7 ± 0.4
Content of nitrogen, %	5.5 ± 0.1	2.2 ± 0.4	4.4 ± 0.2	3.9 ± 0.3
C/N ratio	6.8	18.3	11.2	7.7
Content of phosphorus, %	1.8 ± 0.3	0.1 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
Content of potassium, %	0.2 ± 0.0	4.5 ± 3.5	2.1 ± 0.3	2.1 ± 0.1

Note: mean ± standard deviation for three independent measurements are shown

chemical characteristics of the initial matter for the bioconversion and the end product of bioconversion.

The study of the content of the main heavy metals in sewage sludge from Singapore municipal water reclamation plant showed that their content was generally lower than the US and EU limits for the biosolids. Food waste addition to sewage sludge provides higher quality of final product (Figure 1). The average content of heavy metals in fertilizer produced from the mixture of sewage sludge and food waste in ratio 1:1 by TS, mg/kg was as follows: 913 ± 132 for Cu; 156 ± 25 for Ni; 1,060 ± 104 for Zn; 5.3 ± 1.1 for Cd; 150 ± 27 for Cr; 44 ± 6 for Pb. The content of heavy metals in fertilizer produced from the mixture of sewage sludge and food waste in ratio 1:1 by TS was lower than or within the US and EU limits for the biosolids: 21% and 52–91% of the limit for Cu; 37% and 39–52% of the limit for Ni; 14% and 27–42% of the limit for Zn; 6% of the limit for Cd; 5% of the limit for Cr and 5% and 4–6% of the limit for Pb. Therefore, there is no limitation by the content of heavy metals to use the fertilizer from sewage sludge and food waste in agriculture.

Removal of pathogens and parasites is an important task in the thermophilic treatment of sewage sludge. The content of viable and dead cells detected by Ent1432 oligonucleotide probe, specific for enterobacteria, was measured by flow cytometry. It was shown that the treatment under 60°C did not completely remove enterobacteria from the treated material. The content of enterobacterial cells increased from 78 × 10⁶ cells/g of dry matter to 740 × 10⁶ cells/g of dry matter after 4 days of the biodegradation and then dropped to 36 × 10⁶ cells/g of dry matter at the end of the process. The share of viable enterobacterial cells (EBC) was changed from 3% to 12% of total viable bacterial cells (Table 2). Sewage sludge contains a significant number of viral, bacterial, protozoan, fungal, and helminth pathogens (Straub *et al.*, 1993). It is well known that the content of pathogens and enterobacteria could be reduced during the conventional composting of sewage sludge. Hussong and co-authors

**Figure 1** The content of heavy metals in sewage sludge and fertilizer, % of EU limits

(1985) analysed sewage sludge composts from 30 municipalities and found four samples (12%) contained salmonellae (two samples contained fewer than 0.3/g, and the other two had 21/g and 1.7×10^4 /g). In other studies, different systems of sewage sludge composting have been evaluated bacteriologically. Faecal coliforms and salmonellae were removed completely during the first two weeks in the case of forced aeration, but these bacteria were still present till near the end of the experiment with natural aeration. Salmonellae disappeared after a few days from initiating treatment (Shaban, 1999). There are no known thermophilic enterobacteria. Therefore, their cells must be destroyed at 60°C, which was maintained in the studied biodegradation of sewage sludge and food waste. The presence of viable enterobacteria in the described process may be due to their survival and even growth in mesophilic microniches in the treated matter and in the reactor. Similar data on the survival of mesophilic nitrifying bacteria during the thermophilic composting of animal wastes were also explained hypothetically by the presence of mesophilic microniches (Kowalchuk *et al.*, 1999). It was observed that *Salmonella typhimurium* and *Escherichia coli* survived for 59 days at about 60°C in industrial compost, and at least 5–9 days at 60–70°C in a food waste compost or sewage sludge compost (Droffner and Brinton, 1995). These results and our data suggest that the mechanism for removal of enterobacteria during aerobic composting is not only the result of the temperature in the reactor. The elimination of pathogenic microorganisms from the sewage sludge biodegraded at 60°C must probably be pasteurised or thermally sterilised and then biodegraded by starter culture(s).

The inoculation of composting organic waste by starter cultures is not practically used at present. Usually, finished compost, or the compost from the thermophilic phase, is used as an inoculum to speed up the process (Furhacker and Habel, 1995; Beffa *et al.*, 1996). The use of a pure starter culture gives control over desirable processes and lower risk of accumulation of harmful microorganisms in the final product of the bioconversion. Thermophilic bacterial strains SW25 and SW09, identified from partial sequences of 16S rRNA gene as *Bacillus thermoamylovorans*, with high digestion activity of sewage sludge, were selected and used as starter culture to enhance bioconversion of sewage sludge and food waste (Wang *et al.*, 2003). GenBank accession numbers for identified partial 16S rRNA gene sequences are AY197332 and AY197333 for the strains SW09 and SW25, respectively. The biomass of the starter culture was added to the reactor for initial content of the cells 4.2×10^7 to 6.0×10^8 c.f.u./g dry matter. The starter culture was only applied at the beginning of the bioconversion process. Similar maxima, in the range 10^8 – 10^9 cells/g of the composted matter, have been found during composting of anaerobic sewage sludge mixed with wood chips in a closed 300 m³ bioreactor, using aeration, and temperature control from 65°C to 82°C (Beffa *et al.*, 1996). It was the same as the highest content of bacterial cells in hot synthetic compost (Dees and Ghiorse, 2001).

Drop of pH from 7.3–7.5 to 5.3–6.1 was observed during the bioconversion process. To

Table 2 Changes in the number of dead and live enterobacterial cells during the bioconversion

Duration of the process, days	Total number of enterobacterial cells, 10 ⁶ /g	Share of dead enterobacterial cells in the population of enterobacteria, %	Share of alive enterobacterial cells in the population of alive bacteria, %
0	78 ± 5	25 ± 2	12 ± 1
1	72 ± 8	73 ± 4	8 ± 1
2	300 ± 22	73 ± 4	3 ± 1
4	740 ± 95	85 ± 7	5 ± 2
6	670 ± 68	95 ± 4	1 ± 1
8	510 ± 67	36 ± 4	14 ± 4
13	36 ± 8	4 ± 4	7 ± 2

maintain a neutral pH, the buffering substance, such as chalk, was added to adjust pH. Chalk was added at the beginning of bioconversion in a quantity of 5% by total solids to ensure pH stability.

The best fertilizer was obtained when the ratio of the sewage sludge and food waste was 1:1 by total solids with sewage sludge thermally pre-treated. The content of volatile solids and organic carbon satisfactorily decreased by 24.8% and 13.5% of total solids, respectively, after 10 days of bioconversion (Table 1, Figure 2).

It is commonly agreed that the fluorescence of coenzyme F₄₂₀ can be used for the conventional measurement of methanogenic biomass, which is the main component of sewage sludge from the anaerobic digester. The fluorescence of F₄₂₀ decreased during the bioconversion of sewage sludge and food wastes, which demonstrated the degradation of methanogens in anaerobic sludge. It was correlated also with the decrease of volatile solids content in the treated matter (Figure 2).

The final product of bioconversion was a grey powder with moisture content of 5%. The product was confirmed as stable and non-toxic matter for the plants tested based on the results of the phytotoxicity test. The stability index was 1.3 mg CO₂/g organic matter/d (considered as “very stable”). The quality of fertilizer, determined in germination and the root elongation test, was almost the same and better than the quality of commercial organic fertilizer.

The plant tests indicated that the produced fertilizer can be considered as good as a commercial-grade fertilizer. Addition of 1.0% to 1.5% of fertilizer to the subsoil tested showed that the yield and growth of different plants increased by 113 to 164%, respectively, depending on the types of plants tested. The results of tomato growth, for example, are shown in Figure 3.

The fertilizer produced by the bioconversion of sewage sludge and food waste was com-

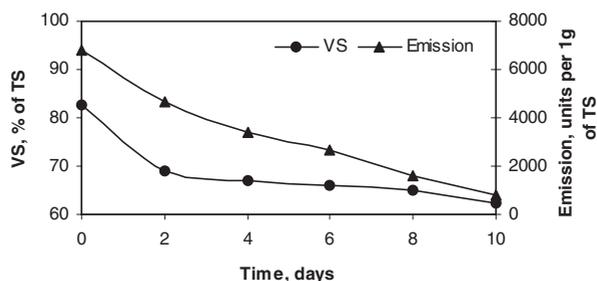


Figure 2 The changes in the content of volatile solids and autofluorescence F₄₂₀ in relative units, during the bioconversions of sewage sludge and food wastes

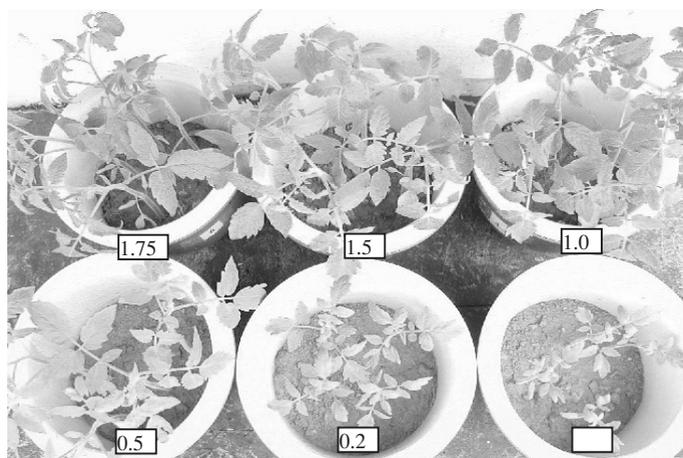


Figure 3 Growth of tomato in soil with different contents of fertilizer

Table 3 The influence of raw sewage sludge, dry sewage sludge, fertilizer, and commercial organic fertilizer on the plant growth

Parameters	Growth on subsoil with			
	raw sewage sludge	dry sewage sludge	fertilizer	commercial organic fertilizer
Length of stem, cm	26.2 ± 0.6	27.0 ± 2.6	38.3 ± 6.1	34.0 ± 5.9
Length of root, cm	8.1 ± 2.6	9.1 ± 1.3	11.0 ± 1.2	5.6 ± 1.4
Dry weight of stem, g	0.56 ± 0.07	0.57 ± 0.08	0.91 ± 0.34	0.65 ± 0.35
Dry weight of root, g	0.12 ± 0.08	0.09 ± 0.04	0.20 ± 0.10	0.11 ± 0.10

Table 4 The content of heavy metals in the tomato plant, mg/kg of dry matter

Content	Growth on subsoil with			
	raw sewage sludge	dry sewage sludge	fertilizer	commercial organic fertilizer
Cu	59	41	32	26
Ni	30	14	3	4
Zn	220	84	70	63
Cd	2.4	1.8	0.8	0.2
Cr	4.6	2.0	2.0	2.0
Pb	1.8	1.7	1.6	0.8

pared with that of raw sewage sludge, dried sewage sludge, and commercial organic fertilizer in the germination and root elongation test and in the plant experiments. The fertilizer, raw sludge and dried sludge were added to the subsoil at a dosage of 1% (weight of dry matter/weight of soil). Commercial organic fertilizer was added to the subsoil in quantity 0.7% (weight of dry matter/weight of soil) to equalize the content of nitrogen in the fertilizer and in commercial organic fertilizer. Best growth of the plants was obtained when fertilizer was applied. The parameters of the tomato growth in the subsoil with addition of the raw and dry sludge fertilizer produced from sewage sludge and food waste and commercial organic fertilizer are shown in Table 3.

The lengths of stems and roots of tomato grown in soil with fertilizer were bigger than those of tomato grown in soil with raw sludge, dry sludge and commercial organic fertilizer by 146 and 136, 141 and 121, 113 and 196%, respectively.

The content of heavy metals in the tomato plant grown in soil with fertilizer was lower than that with addition of raw sludge or dry sludge, but this content was higher when commercial organic fertilizer was applied (Table 4).

Conclusions

A new biotechnology for intensive aerobic bioconversion of the mixture of sewage sludge and vegetable food waste into fertilizer was developed. The fertilizer obtained from the mixture of pre-treated sewage sludge and vegetable food waste with a ratio of 1:1 by TS was non-toxic, stable, and its application to the soil resulted in faster growth and development of agricultural plants.

The proposed bioconversion technology appears to be promising to resolve the sludge disposal problem in the populated metropolitans or those countries that are short of land resources, such as Singapore, while the produced fertilizer can be used in urban landscaping and agriculture.

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