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ScienceDirect
Renewable Energy 32 (2007) 904–915
RENEWABLE ENERGY
www.elsevier.com/locate/renene

Microbial conversion of food wastes for biofertilizer production with thermophilic lipolytic microbes

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Received 15 March 2006; accepted 20 April 2006
Available online 5 July 2006

Abstract

Food waste is approximately one quarter of the total garbage in Taiwan. To investigate the feasibility of microbial conversion of food waste to multiple functional biofertilizer, food waste was mixed with bulking materials, inoculated with thermophilic and lipolytic microbes and incubated at 50 °C in a mechanical composter. Microbial inoculation enhanced the degradation of food wastes, increased the total nitrogen and the germination rate of alfalfa seed, shortened the maturity period and improved the quality of biofertilizer. In food waste inoculated with thermophilic and lipolytic *Brevibacillus borstelensis* SH168 for 28 days, total nitrogen increased from 2.01% to 2.10%, ash increased from 24.94% to 29.21%, crude fat decreased from 4.88% to 1.34% and the C/N ratio decreased from 18.02 to 17.65. Each gram of final product had a higher population of thermophilic microbes than mesophilic microbes. Microbial conversion of food waste to biofertilizer is a feasible and potential technology in the future to maintain the natural resources and to reduce the impact on environmental quality.

Keywords: Biofertilizer; Food wastes; Maturity; Thermophilic and lipolytic microbes

1. Introduction

Food waste is approximately 17.94–27.76% of the total garbage in Taiwan [1–3]. It has a high moisture and organic matter content, which is easily decomposed by microbes.

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0960-1481/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.renene.2006.04.019

It produces odor detrimental to environmental quality. Direct landfill of food wastes has created various problems such as putrid smells and leachate polluting ground and surface waters, while incineration treatment has been restricted due to its low calorific value and the cost of fuel supplements for operation [3,4]. Recycling food wastes can benefit the environment by reducing the amount of garbage disposed, promoting the fertility of soil and improving the physical and chemical properties of soil [5]. Moreover, recycling of food wastes reduce the unpleasant odors of garbage, benefits the sanitation of the environment, and decreases garbage collection-related spending.

Food waste is less harmful to the environment than industrial waste. Thus, composting of food waste is attracting considerable attention because it would significantly reduce the amount of waste and the product can be used as compost or biofertilizer which can be handled, stored, transported and applied to the field without adversely affecting the environment [6]. Although various composters are currently commercially available or several types of in-vessel composting systems have been developed for installation in food service establishments to manage food waste as a recyclable resource, it is difficult to maintain steady degradation due to the instability of the microflora within the composter due to the raw material, pH, temperature and other environmental conditions [7–12]. Combining, food waste collection with mechanical composting and inoculation of thermophilic microbes to accelerate the composting has been examined. Food wastes were mixed with sawdust or waste leaves as bulking material, thermophilic microbes were inoculated and biofertilizer was prepared using the mechanical composter. The physical, chemical and microbiological properties of the biofertilizer were analyzed during the incubation. The effects of inoculant and raw material on the quality of biofertilizer were also investigated.

2. Materials and methods

2.1. Raw materials

Food wastes were collected from a National Taiwan University (NTU) restaurant. Despite daily variation of the menu in the restaurant, the compositions of food wastes changed only slightly in different batches as shown in Table 1. Waste leaves were also collected from the NTU Campus, and chopped to about 1 cm pieces using the composter in order to enhance the composting. Sawdust was purchased from the local market. Raw materials of food wastes were mixed with sawdust or waste leaves to adjust the initial moisture content 60–65% and the C/N ratio between 20 and 25.

2.2. Composter operation

The DY-8075E mechanical composter (Biorich Technology Corporation, Taiwan), having capacity of approximately 250 l was used. It has stainless steel blades for intermittent rotary agitation to ensure uniformity of the contents, a heating unit for the inner temperature and a heat sensor for monitoring the inner temperature.

The experiments were run under three different control regimes and the conditions are shown in Table 2. The first batch was run using the method provided by the Biorich Technology Corporation without inoculation, the second and third batches each with two bioreactors, were operated with inoculation, one with the thermophilic microbial

Table 1
The properties of raw materials for the preparation of biofertilizer

Material	pH	Moisture content (%)	Ash (%)	Total organic carbon (%)	Total nitrogen (%)	Crude fat (%)	Germination rate (%)	C/N ratio
(a) The first batch								
Food	4.67	76.53	12.59	41.86	3.01	15.31	0	13.91
waste	±0.64	±0.34	±0.89	±0.65	±0.08	±0.32		±0.79
Sawdust	7.11	61.15	33.45	29.79	0.33	0.46	80.0	92.81
	±0.02	±0.07	±0.42	±0.33	±0.06	±0.08	±3.0	±0.53
(b) The second batch								
Food	4.08	73.55	11.66	43.58	3.93	17.08	0	12.01
waste	±0.03	±0.26	±0.41	±0.77	±0.02	±1.07		±0.34
Waste	6.15	11.64	25.19	45.43	0.71	4.01	67.0	64.00
Leaves	±0.01	±0.27	±1.55	±0.18	±0.02	±0.82	±1.0	±1.02
(c) The third batch								
Food	4.70	81.12	12.95	39.27	2.36	12.62	0	15.30
waste	±1.65	±0.73	±0.25	± 0.16	±0.13	±0.25		±1.12
Waste	6.13	5.78	22.78	46.22	0.92	4.26	80	50.23
Leaves	±0.01	± 0.07	±2.72	±1.64	±0.01	±0.20	±3	±2.83

Mean ± SD ($n = 3$).

inoculation (isolate SC6 in batch 2, and isolate SH169 in batch 3) (bioreactor A) and the other without inoculation as the control (bioreactor B). Five liters of inocula of test microbes ($>10^7$ CFU ml^{-1}) were applied at the beginning. The detailed operation conditions are listed in Table 2.

2.3. Culture media and conditions

Bacteria were counted at 30 or 50 °C after 2 days incubation on nutrient agar containing ($g l^{-1}$) beef extract 3.0, peptone 5.0, and agar 15.0 at pH 6.8 ± 0.1. Cellulolytic microbes were plated at 30 or 50 °C after 5 days incubation on Mandels-Reese medium with carboxymethylcellulose (CMC, Sigma) as the sole carbon source and sprayed with Congo red to show clear zones around the colonies [13]. Lipolytic microbes were assayed at 30 or 50 °C after 3 days incubation on Tributyrin agar with tributyrin (Merck) as a reactant. Degradation of this compound gave rise to clear zones surrounding the lipolytic colonies in the otherwise turbid culture medium. All the experiments were carried out in triplicate.

2.4. Growth rate and enzyme activity

The thermotolerant *Streptomyces thermonitrificans* NTU-88, isolate CH18 and *Brevibacillus borstelensis* SH168 were isolated from vegetable and fruit waste compost, animal manure compost and food waste compost, respectively. The *Bacillus stearothermophilus* ATCC 10149 was the indicator strain. Growth rate and enzyme activity were

Table 2
The operation conditions of each batch to prepare biofertilizer

Item	The first batch	The second batch	The third batch
Pre-treatment (days)		7	7
The frequency of agitation rotate		Continuous agitation (agitate 10 min, then stop for 20 min)	Continuous agitation (agitate 10 min, then stop for 20 min)
Temperature (°C)		50	50
Feeding day (Amount of feeding in kg)	35	21	4
	3(5) 6(10) 9(15) 12(25) 15(35) 18(50) 21(55) 24(55) 27(55) 30(55) 33(55)	7(40) 14(40) 21(50)	1(70) 2(70) 3(70) 4(70)
The frequency of agitation rotate	Continuous agitation (agitate 10 min, then stop for 20 min)	Daily agitation once for 10 min	Continuous agitation (agitate 10 min, then stop for 20 min)
Temperature (°C)	50	40	50
Composting periods (days)		49	28
The frequency of agitation rotate		Once daily for 10 min	Once every 2 to 3 days for 10 min
Temperature (°C)		40	40
Bulking materials	Sawdust 25 kg	Waste leaves 15 kg	Waste leaves 35 kg
Food wastes (kg)	415	130	280
Gross weight of raw materials (kg)	440	145	315
Gross time of experiment (days)	35	77	39

measured as follows: nutrient agar for growth, Mandels-Reese medium for cellulase, Tributyrin agar for lipase, soluble starch-yeast extract medium for amylase and skim milk agar for protease activity.

2.5. Chemical analysis

Moisture content was determined by drying the sample at 105 °C overnight to a constant weight. pH was measured in five times volume of distilled water equilibrated with sample for 1 h with pH meter (Good digital pH meter model 2002, Taiwan). Temperatures were determined under 50 cm depth of compost with a thermometer. Ash content in the dried sample was measured at 550 °C. Total nitrogen was determined with a modified Kjeldahl method [14]. Total organic carbon determination was followed by the Walkley–Black method [15]. Crude fat was determined by extracting an oven-dried sample with hexane using Soxhlet extraction for 8 h [16]. Germination rate of alfalfa seed was assayed using 1:10 (W/V) aqueous suspensions of sample [17]. Experiments were carried out to obtain three measurements and statistical analysis of the results was performed using analysis of variance and Duncan's multiple range tests ($p = 0.05$) with the help of the Statistical Analysis System as previously described [18].

3. Results and discussion

3.1. Isolation of thermophilic microbes

There were 228 thermophilic isolates that had been isolated from animal waste compost, vegetable and fruit waste compost and food waste compost. It included 100 bacterial isolates, 52 actinomycete isolates and 76 fungal isolates. After series testing for cellulolytic, lipolytic, proteolytic and amylolytic activities, *Brevibacillus borstelensis* SH168 isolated from food waste compost, *Streptomyces thermonitrificans* NTU-88 and actinomycete isolate CH 18 isolated from animal waste compost and *Bacillus stearothermophilus* ATCC 10149 purchased from Culture Collection and Research Center of Food Research and Development Institute, Hsin-Chu, Taiwan were used for biofertilizer preparation.

3.2. Growth rate and enzyme activity of tested microbes

The growth rates and enzymatic activities on the nutrient agar, Mendels-Reese medium, tributyrin agar, skim milk medium and soluble starch-yeast extract medium at 50 °C were measured for thermotolerant *Bv. borstelensis* SH168, *B. stearothermophilus* ATCC 10149, *S. thermonitrificans* NTU-88, and the actinomycete isolate CH18. *Bv. borstelensis* SH168 showed significantly high growth rates and enzyme activities on the nutrient agar, tributyrin agar, skim milk medium and soluble starch-yeast extract medium (Table 3). Therefore, *Bv. borstelensis* SH168 was used as inoculum to accelerate biofertilizer preparation of food wastes.

3.3. Food waste biofertilizer prepared with eliminated type bioreactor

Rapid and entire humification of a substrate essentially depends on its initial C/N ratio which should be between 25 and 35 and a pH of 6.0–7.5 [19]. The food wastes used here had a C/N ratio of 12.01–15.30, while the pH values of the food wastes were between 4.08 and 4.70. Therefore, biofertilizer preparation with food wastes needs supplementation of bulk materials to adjust the initial C/N ratio and the initial pH. The food waste of university dormitory restaurant was mixed with sawdust and treated in the eliminated type bioreactor for 35 days using the method provided by Biorich Technology Corporation at 45–55 °C. It was found that the pH dropped from 7.11 to 4.90–5.00, moisture content decreased from 61.15% to 4.79%, ash decreased from 33.45% to 14.44% and then increased to 18.72%, total organic carbon increased from 29.79% to 50.90%, total nitrogen content increased from 0.33% to 2.23%, the C/N ratio decreased from 92.81 to 20.68–24.10, and the germination rate of alfalfa seed also dropped from 80% to 27% (Table 4). Microbial populations had a tendency to decrease suddenly from day 0 to day 7 due to the acidic environment, and then remain steady during the biofertilizer preparation. Total mesophilic microbes and mesophilic cellulolytic microbes decreased from 6.11×10^7 and $1.96 \times 10^7\,\mathrm{CFU\,g^{-1}}$ at day 0, to 7.00×10^3 and $6.00 \times 10^3\,\mathrm{CFU\,g^{-1}}$ at day 7, respectively. Total thermophilic microbes and thermophilic cellulolytic microbes also decreased from 7.30×10^6 and $8.25 \times 10^5\,\mathrm{CFU\,g^{-1}}$ at day 0, to 2.75×10^5 and $7.70 \times 10^4\,\mathrm{CFU\,g^{-1}}$ at day 7, respectively. From the physical and chemical properties, food waste biofertilizer produced with the eliminated bioreactor was not a good quality product.

Table 3
Colony size and clear zone of thermotolerant isolates on Mandels-Reese, tributyrin, skim milk and soluble starch-yeast extract media at 50 °C for 4 days

Test strain	Colony size (mm)	Clear zone (mm)	CZ/CS
(a) Mandels–Reese medium			
Brevibacillus borstelensis SH168	10.0±1.0	10.7±0.6	1.08±0.16
B. stearothermophilus ATCC 10149	5.7±0.6	26.7±1.2	4.73±0.44
S. thermonitrificans NTU-88	21.3±0.6	26.0±1.0	1.22±0.06
Isolate CH18	22.7±0.6	34.7±1.2	1.53±0.05
(b) Tributyrin medium			
Brevibacillus borstelensis SH168	38.7±0.6	40.1±0.6	1.04±0.06
B. stearothermophilus ATCC 10149	6.0±0.6	7.0±1.2	1.17±0.14
S. thermonitrificans NTU-88	8.7±0.6	10.0±1.0	1.15±0.06
Isolate CH18	8.7±0.6	11.0±1.2	1.27±0.05
(c) Skim milk medium			
Brevibacillus borstelensis SH168	18.7±0.6	31.3±0.6	1.68±0.05
B. stearothermophilus ATCC 10149	8.0±0.0	16.7±0.6	2.08±0.07
S. thermonitrificans NTU-88	10.3±0.6	20.0±1.0	1.94±0.19
Isolate CH18	17.3±0.6	27.3±0.6	1.58±0.07
(d) Soluble starch-yeast extract medium			
Brevibacillus borstelensis SH168	32.3±0.6	33.7±0.6	1.04±0.02
B. stearothermophilus ATCC 10149	13.7±0.6	21.3±0.6	1.56±0.06
S. thermonitrificans NTU-88	12.7±0.6	21.0±1.0	1.66±0.11
Isolate CH18	14.3±0.6	28.7±0.6	2.00±0.12

Mean±SD ($n = 5$).

Table 4
The properties of food waste biofertilizer in mechanical composter during the first batch preparation

Incubation period (day)	pH	Moisture content (%)	Ash (%)	TOC (%)	TN (%)	Germination rate (%)	C/N ratio
0	7.11±0.02	61.15±0.07	33.45±0.42	29.79±0.33	0.33±0.06	80±3	92.81±4.24
7	5.78±0.01	13.63±0.18	17.44±0.75	49.53±0.39	2.72±0.04	77±1	20.83±1.32
14	5.33±0.01	6.41±0.58	18.47±0.52	54.92±0.59	2.62±0.10	76±3	20.95±0.45
21	5.20±0.02	4.55±0.59	14.37±0.48	53.58±0.76	3.06±0.29	55±4	17.58±1.12
28	4.90±0.00	9.98±0.00	18.16±0.41	50.57±1.10	2.45±0.04	45±4	20.68±1.53
35	5.00±0.03	4.79±0.06	18.27±0.47	50.90±0.16	2.23±0.72	27±4	24.10±0.89

Mean±SD ($n = 3$).

3.4. Food waste biofertilizer prepared with the inoculation of thermophilic cellulolytic microbes

The food waste of the university dormitory restaurant was mixed with waste leaves and treated with the composting bioreactors for 70 days, one was inoculated with thermophilic cellulolytic isolate SC6 (bioreactor A) and the other was without inoculation as the control (bioreactor B). Waste leaves (15 kg) were put in the bioreactor and blended for 5 days, then one of the bioreactors was inoculated with isolate SC 6 (2.0×10^7 CFU g^{-1}). After 2 days,

both of them were fed with food waste 6.2 kg daily for 21 days and then composted for another 49 days. It was found that the pH dropped from 6.13–6.17 to 4.00–4.06, moisture content decreased from 67.08–71.46% to 18.41–25.15%, ash content decreased from 32.02–36.25% to 16.78–18.49% and then increased to 21.39–21.95%, total organic carbon increased from 41.45–44.70% to 51.09–55.42% and then decreased to 43.23–46.64%, total nitrogen content increased from 0.80–0.97% to 3.34–3.66%, crude fat content increased from 2.79–4.51% to 23.65–24.62%, the C/N ratio decreased from 46.22–52.13 to 12.76–12.94, and the germination rate of alfalfa seed dropped from 85–91% to 0% after day 14. Products from both bioreactors had similar physical and chemical properties except total organic carbon (Table 5). Total organic carbon of bioreactor A with the inoculation of thermophilic cellulolytic microbes decreased more rapidly than that of bioreactor B without inoculation due to the degradation of organic matter by the inoculated microbes. The germination rate of alfalfa seed decreased to 0% after day 14 due to the low pH value of the biofertilizer (pH 3.85–4.20) and the high crude fat content (16.49–17.38%). The same phenomenon was also reported by Aoshima et al. [8]. The pH drop-off at the early stage of composting was associated with the degradation of organic matter and the formation of acidic metabolites. Low pH value inhibited severely the microbial and plant growth [20]. Beck-Friis et al. [9] indicated that short chain fatty acids, particularly lactic acid, were found at the beginning stage of composting.

Microbial populations had a tendency to decrease suddenly from day 0 to day 7 in the food waste mechanical composters during the second batch biofertilizer preparation. Total mesophilic microbes, mesophilic cellulolytic microbes and mesophilic lipolytic microbes decreased from $8.34–9.04 \times 10^8$, $5.67–7.02 \times 10^8$ and $4.12–5.97 \times 10^8$ CFU g^{-1} at day 0; $1.95–2.71 \times 10^8$, $8.90 \times 10^7–2.02 \times 10^8$ and $1.89–2.27 \times 10^8$ CFU g^{-1} at day 21, to $6.00 \times 10^6–1.10 \times 10^7$, $1.00 \times 10^4–5.00 \times 10^5$ and $5.00–7.00\,10^6$ CFU g^{-1} at day 56, respectively. While total thermophilic microbes, thermophilic cellulolytic microbes and thermophilic lipolytic microbes decreased from $7.97–8.99 \times 10^8$, $7.26–8.37 \times 10^8$ and $1.94–4.19 \times 10^8$ CFU g^{-1} at day 0, $3.33–4.46 \times 10^8$, $1.49–1.75 \times 10^8$ and $9.40 \times 10^7–2.34 \times 10^8$ CFU g^{-1} at day 21, to $4.30–6.80 \times 10^7$, $2.00–4.00 \times 10^5$ and $1.10–4.30 \times 10^7$ CFU g^{-1} at day 56, respectively. From the physical and chemical properties, food waste biofertilizer preparation with the inoculation of thermophilic cellulolytic microbes in high fat content raw material did not yield a good quality product.

3.5. *Food waste biofertilizer prepared with the inoculation of thermophilic lipolytic microbes*

To investigate the production of biofertilizer with food waste containing high crude fat raw material, food waste of the university dormitory restaurant was treated with the composting bioreactors for 28 days, one was inoculated with thermophilic lipolytic *Bv. borstelensis* SH 168 (bioreactor A) and the other was without inoculation as the control (bioreactor B). Waste leaves (35 kg) were put in the bioreactor and blended for 5 days, then one of the bioreactors was inoculated with *Bv. borstelensis* SH 168 (2.0×10^7 CFU g^{-1}). After 2 days, both of them were fed with food waste 70 kg daily for 4 days and then composted for another 28 days. The biofertilizer temperature in bioreactor A increased from 43 to 70 °C at day 3 due to the heavy growth of thermophilic lipolytic microbes, and then decreased gradually to 52 °C at day 28 because of the maturity of biofertilizer; while it was 56 °C at day 3 in bioreactor B and 45 °C at day 28. Temperature has been widely

Table 5
The properties of the food waste biofertilizer in mechanical composter during the second batch preparation

Incubation period (day)	Temp. (°C)	pH	Moisture content (%)	Ash (%)	TOC (%)	TN (%)	Crude fat (%)	Germination rate (%)	C/N ratio
(a) Bioreactor A with inoculation									
0	42	6.13	7.96	26.39	39.39	0.85	3.53	85	46.85
	±1	±0.07	±0.72	±2.54	±4.93	±0.01	±0.27	±0	±3.83
2	43	5.80	71.46	32.02	41.45	0.80	4.51	88	52.13
	±2	±0.16	±0.30	±2.56	±3.03	±0.02	±0.19	±2	±2.59
7	48	5.22	72.83	18.80	46.01	2.17	9.85	91	21.18
	±1	±0.04	±2.40	±2.16	±3.35	±0.05	±0.67	±1	±1.24
14	46	3.85	67.38	16.82	53.04	2.78	17.38	0	19.10
	±2	±0.00	±1.04	±1.36	±4.36	±0.00	±0.12		±1.42
21	48	3.91	66.94	16.78	48.18	2.54	19.36	0	18.94
	±2	±0.11	±0.40	±0.82	±2.01	±0.01	±0.18		±1.35
28	49	3.89	61.75	17.45	45.79	2.89	21.02	0	15.86
	±2	±0.24	±0.29	±0.74	±2.05	±0.08	±0.21		±1.62
35	51	3.98	57.21	17.85	51.09	3.37	19.35	0	15.17
	±3	±0.12	±1.07	±0.91	±0.19	±0.16	±0.16		±0.84
42	52	3.96	47.24	17.93	51.04	3.17	20.32	0	16.12
	±2	±0.21	±1.80	±0.67	±0.83	±0.16	±0.19		±0.56
49	56	3.96	38.28	18.63	50.02	3.38	20.55	0	14.78
	±2	±0.11	±0.45	±0.41	±3.72	±0.15	±0.30		±0.23
56	48	4.05	25.97	19.65	49.41	3.25	23.83	0	15.22
	±1	±0.14	±0.04	±0.54	±0.36	±0.01	±0.38		±0.28
63	43	3.90	26.61	21.26	42.71	3.23	22.19	0	13.23
	±1	±0.11	±0.74	±0.86	±0.64	±0.04	±0.30		±0.22
70	41	4.00	18.41	21.39	43.23	3.34	23.65	0	12.94
	±2	±0.14	±1.04	±0.92	±0.79	±0.17	±0.47		±0.75
(b) Bioreactor B without inoculation									
0	44	6.17	8.73	30.36	42.27	0.92	3.68	89	46.16
	±3	±0.23	±0.34	±1.80	±3.20	±0.08	±0.06	±2	±2.13
2	45	5.68	67.08	36.25	44.70	0.97	2.79	88	46.22
	±3	±0.15	±1.64	±3.63	±0.48	±0.50	±0.23	±7	±2.42
7	48	5.34	63.81	21.92	42.86	2.31	9.12	91	18.57
	±3	±0.10	±0.10	±1.81	±0.94	±0.02	±0.32	±1	±0.48
14	50	4.20	61.73	17.63	52.81	3.00	16.49	0	17.59
	±3	±0.04	±0.15	±0.44	±4.12	±0.08	±0.18		±0.84
21	51	3.79	61.08	18.49	47.42	2.91	22.80	0	16.33
	±2	±0.04	±1.71	±0.69	±1.40	±0.13	±0.02		±0.23
28	48	3.94	46.09	19.13	45.58	2.96	22.32	0	15.42
	±2	±0.06	±3.21	±0.46	±2.42	±0.03	±0.29		±0.37
35	49	3.98	33.84	19.48	55.42	3.74	22.56	0	14.83
	±2	±0.10	±1.78	±0.42	±0.48	±0.03	±0.31		±0.65
42	46	4.01	24.40	19.75	50.55	3.55	25.50	0	14.25
	±2	±0.13	±0.29	±0.66	±1.90	±0.15	±0.53		±0.73
49	44	4.02	23.27	19.83	48.10	3.59	24.49	0	13.44
	±2	±0.11	±1.72	±1.21	±3.24	±0.16	±0.54		±0.48
56	43	4.13	16.02	21.63	46.42	3.47	26.29	0	13.36
	±1	±0.12	±0.23	±1.38	±0.70	±0.03	±0.37		±0.21
63	42	4.03	27.94	22.97	37.26	3.67	24.84	0	10.15
	±1	±0.08	±0.21	±1.20	±0.25	±0.01	±0.70		±0.18
70	42	4.06	25.15	21.95	46.64	3.66	24.62	0	12.76
	±1	±0.09	±0.49	±1.45	±1.01	±0.59	±1.02		±0.11

Mean ± SD ($n = 3$).

recognized as one of the most important parameters in the composting processes. The temperature patterns have been reported to correlate with the microbial activities [21,22].

Initial moisture content was 64.81% in bioreactor A, this increased slightly to 67.72% due to metabolic water formation by the tested microbes [23–25] and then decreased gradually to 41.96% due to vaporization during preparation, while the initial moisture content was 65.23% in bioreactor B, and then increased slightly during the incubation for the low temperature and vaporization. The pH decreased slightly due to the production of acidic metabolites, and then increased to 6.83–6.98 because of the assimilation of these acidic compounds. The pH changes were closely related to the decomposition of the food wastes [4]. The pH increased with degradation of soluble carbon began. Similar results were also found in animal waste compost and vegetable waste compost [26–28].

Ash content increased from 17.29–18.39% to 24.94–29.21%, total organic carbon decreased slightly from 38.39–42.25% to 36.21–37.09%, total nitrogen content increased from 1.40–1.59% to 2.01–2.10%, crude fat content decreased from 7.99–8.02% to 1.34–4.88%, the C/N ratio decreased from 26.55–27.51 to 17.65–18.02, and the germination rate of alfalfa seed increased from 10.50–34.00% to 97.50–98.00% (Table 6). Organic carbon decomposed to gaseous inorganic carbon which represented complete degradation [4]. The crude fat content decreased 83.23% during the incubation in bioreactor A with the inoculation of thermophilic and lipolytic *Bv. borstelensis* SH 168, while crude fat content

Table 6
The properties of the food waste biofertilizer in mechanical composter during the third batch preparation

Incubation period (day)	Temp. (°C)	pH	Moisture content (%)	Ash (%)	TOC (%)	TN (%)	Crude fat (%)	Germination rate (%)	C/N ratio
(a) Bioreactor A with inoculation									
0	43	4.55	64.81	17.29	42.25	1.59	7.99	0	26.55
	±3	±0.01	±1.96	±0.29	±0.99	±0.03	±0.07		±0.77
7	70	4.22	67.72	21.14	41.10	1.84	5.72	10.50	22.28
	±5	±0.01	±0.23	±0.56	±2.86	±0.47	±0.17	±1.41	±1.25
14	61	4.21	65.53	23.22	40.63	1.97	5.56	48.00	20.63
	±4	±0.06	±1.06	±0.16	±0.25	±0.02	±0.10	±2.83	±0.29
21	55	4.84	57.77	24.84	39.75	2.06	4.84	95.00	19.33
	±3	±0.02	±0.86	±0.23	±0.75	±0.06	±0.21	±1.41	±0.83
28	52	6.83	41.96	29.21	37.09	2.10	1.34	97.50	17.65
	±3	±0.06	±0.64	±0.89	±0.35	±0.08	±0.09	±0.71	±0.61
(b) Bioreactor B without inoculation									
0	41	3.91	65.23	18.39	38.39	1.40	8.02	0	27.51
	±3	±0.04	±1.74	±1.71	±1.11	±0.01	±0.13		±1.02
7	56	3.99	67.49	21.90	37.39	1.74	6.61	0	21.55
	±3	±0.01	±1.00	±1.09	±0.98	±0.08	±0.10		±0.97
14	50	5.33	68.03	22.17	37.08	1.80	6.22	34.00	20.65
	±3	±0.02	±0.86	±0.45	±0.31	±0.02	±0.09	±2.83	±0.53
21	47	6.23	69.01	24.96	36.39	1.92	5.35	87.00	18.95
	±2	±0.11	±0.81	±1.71	±0.66	±0.03	±0.10	±1.41	±0.37
28	45	6.98	69.47	24.94	36.21	2.01	4.88	98.00	18.02
	±2	±0.01	±0.35	±1.56	±0.22	±0.04	±0.06	±2.83	±0.21

Mean ± SD ($n = 3$).

decreased only 39.15% in bioreactor B without inoculation. In addition, inoculation of thermophilic and lipolytic *Bv. borstelensis* SH 168 also reduced the organic acid concentration and stimulated the germination rate of alfalfa seed. Excess oil in the food waste had an inhibitory effect on the biodegradation and caused oil accumulation within the composter [9]. From these observations, the time course of degradation of food wastes could be deduced. Inoculation of fat tolerant and decomposing microbes, or adjusting the substrate pH, may increase the decomposition rate of these fat-containing materials. The results of the composting experiments revealed that as compared to control, a significant reduction in percentages of crude fat, total organic carbon and the C/N ratio took place. Therefore, inoculation of thermophilic and lipolytic microbes enhanced the organic matter degradation, decreased total carbon content and the C/N ratio, increased ash, total nitrogen content and the germination rate. Inoculation of appropriate microbes to food waste could thus shorten the period for maturity and improve the quality of biofertilizer [21,26,29,30]. Nakasaki et al. [31,32] also reported that liming to control the pH or inoculation of *Bacillus licheniformis* clearly enhanced the carbon turnover by shortening the low pH phase when composting in a laboratory-scale composting reactor at 60 °C. Total organic carbon content of bioreactor A with the inoculation of thermophilic and lipolytic microbes decreased more rapidly than that of bioreactor B without inoculation due to the degradation of organic matter by the inoculated microbes. Proper composting effectively destroys pathogens and weed seeds through the metabolic heat generated by microorganisms during the process [21,33]. Such biofertilizers are not only suitable for use as a soil conditioner and fertilizer, but can also suppress soil-borne and foliar plant pathogens [21,34,35].

Total mesophilic microbes, mesophilic cellulolytic microbes and mesophilic lipolytic microbes were 1.40×10^7–7.05×10^8, 7.00×10^6–4.03×10^8 and 7.00×10^6–6.13×10^8 CFU g^{-1} at day 0; and 1.90×10^7–1.02×10^9, 2.00×10^6–1.20×10^7 and 2.00×10^6–1.20×10^7 CFU g^{-1} at day 28, respectively. While total thermophilic microbes, thermophilic cellulolytic microbes and thermophilic lipolytic microbes were 1.36–8.18×10^8, 8.70×10^7–4.54×10^8 and 5.10×10^7–4.18×10^8 CFU g^{-1} at day 0, and 7.40×10^7–1.90×10^9, 4.00×10^6–1.82×10^9 and 4.00×10^6–1.82×10^9 CFU g^{-1} at day 28, respectively. The thermophilic microbe count was higher than that of the mesophilic microbes in bioreactor A due to the inoculation of the thermophilic lipolytic microbe *Bv. borstelensis* SH 168. Low fat content product favored microbial and plant growth. From the physical and chemical properties, biofertilizer prepared with food waste using thermophilic lipolytic microbes is a feasibile and potential method for the future to maintain nature resources and to reduce the impact of waste on environmental quality.

Acknowledgments

The authors thank Professor C. M. Lai for his technical assistances, The Biorich Technology Corporation for offering the mechanical composter, and the National Science Council of the Republic of China (NSC 91-2317-B005-006) and the Bureau of Environmental Protection of Taipei City Government for financial support.

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