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Succession of the Community of Dominant Growth Bacteria in an Aerobic Biodegradation Machine for Garbage Which Uses a Wood Matrix

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Abstract

In order to evaluate the microbial community in a garbage degradation machine which uses wood matrix, culturable dominant microorganisms were isolated from the matrices. Sample matrices were taken during the course of the degradation experiment upon various moisture content conditions of the matrices (20%, 30%, 40%, 50%, 60%, 70%, and 80%) and with various environmental temperatures (10°C, 20°C, 30°C, 40°C, and 50°C), at 3, 5, 10, 20, and 30 days. Isolates were identified by cell morphology, Gram staining, and utilization pattern of 95 different carbon sources. The microbial community for each temperature and moisture condition and time was analyzed. It was found that *Bacillus subtilis* acts as a pioneer and that *Cellulomonas turbata* and *Xanthomonas campestris* appeared predominantly in the community at the optimum conditions of moisture content and environmental temperature to confer the highest degradation rate.

Key words: *Bacillus subtilis*, *Cellulomonas turbata*, garbage biodegradation machine, microbial community, *Xanthomonas campestris*, wood matrix

Introduction

The problem of waste in modern society has become acceleratingly serious. The total amount of waste in Japan has rapidly increased nationwide due to the economic growth since the 1960's. The total amount per day had reached about 134,500 tons in 1992. About 75% of processing of wastes is carried out by incineration, and landfill is used approcimately 15% of the time (Ministry of Health & Welfare 1995), so the rate of incineration in Japan is high compared to the rest of the world (Takeda 1996). Recently, serious problems have arisen due to damage of the incineration facilities caused by high incineration temperatures or corrosion from the acid gas generated by high calorization from the increase of paper and plastic wastes. Further cause for concern are air, water, and soil pollution by chemical substances such as sulfur oxides, hydrogen chlorides, nitrogen oxides, heavy metals, and fine dust, which cause grave problems both for humans and for the environment (Tchobanoglous *et al.* 1998). Furthermore, dioxin, a carcinogen, is formed through the incineration process, and is discharged as exhaust gas and incinerated ash. Excess wastes are carried every day into the landfill, which is the final disposal of the waste treatment system. Municipalities must therefore always be in the process of preparing new places to use as landfill areas. To solve these problems, resource recovery and weight reduction of the waste are crucial.

Composting, a method of waste processing that has been used for centruies, has been employed with various organic substances as raw materials, such as agricultural waste, forestry waste, lumbering and papermaking waste, sludge, and municipal waste. The traditional composting method, which tales several months to complete, is to pile up and occasionally turn over the raw material. In modern times, high-speed composting technologies on an industrial scale have been developed and the ways to effectively utilize organic wastes have been greatly expanded (Fujita 1993).

Additionally, the oligotrophication of farmland has become a problem in an age of increased food production and high economic growth. This is a result of heightened dependence on agricultural chemicals and chemical fertilizers. A recycling system to counteract this situation is needed. Organic materials taken from the farm as agricultural products should be returned to nourish farms and forests as compost.

The composting of municipal wastes in modern Japanese cities is not well-developed. One possicle explanation is the difficulty of gathering and storing the garbage because it easily rots. To solve thin problem, a small scale processing machine for organic wastes (especially food wastes) was developed in order to process wastes where they are

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generated (Terazawa *et al.* 1999, Fujino et al. 1994, Inoue *et al.* 1996). This machine degrades garbage in a container in which microorganisms proliferate in a granular carrier such as wood chips or sawdust. The decomposition rate is faster than with previous composting methods because the degradation condition becomes extremely aerobic when the granular matrix is used. Most of organic substances degrade into carbon dioxide and water, while degradation residues such as humus and minerals accumulate in the matrix. Residual matrix is considered useful as organic fertilizer and soil conditioner. Therefore, both weight reduction and resource recovery of the wastes become possible through the use of this equipment. The requirements for the ideal matrix of the machine are the following: it must be cheap and easily obtainable abundance, an organic material so that it can be recycled, it must be difficult to degrade by bacteria, it must be able to absorb and hold water, and it must be low bulk density for easier agitation. Wood meets all of these requirements (Horisawa *et al.* 1999).

In composting, straw, rice hulls, paper, and/or sawdust are added to the compost pile to improve the properties of raw material with a high water content such as sludge (Fujita 1993). However, the fermentation time must then be extended, because the composting speed decreases with the addition. Wood sawdust is the most difficult to decompose. Since the biodegradation rate of lignin is remarkably slow, there is also the concern of growth injury to crops if unstabilized compost is used. In the case of the garbage degradation machine, the fact that lignin in wood is difficult to degrade by microorganism is an advantage. In view of the above, wood seems to be the best material for the degradation machine.

The use of the garbage degradation machine has gradually spread since the 1990's, and many manufacturers have developed various types of machines. However, optimum conditions for degradation according to the various requirements of the individual user were not determined, so there have been many instances in which the degradation rate decrease or odor was generated. To effectively use the garbage degradation machine, it is necessary to determine the decomposition mechanism in the machine and to establish optimum conditions. Factors such as temperature, moisture content, oxygen, pH, and nutrients had to be examined. We investigated the effects of environmental temperature and moisture content of the matrix on the degradation rate using laboratory scale equipment, and we determined that the optimum conditions were a temperature of 30-50°C and moisture content of 30-60% (Horisawa *et al.* 2000 and 2001). However, the bacterial communities formed under those conditions have not yet been studied. It is necessary to analyze the bacterial community to degrade garbage efficiently. In this study, bacteria were isolated sequentially from the matrix of the degrading machine. The isolates were identified by cell morphology and utilization pattern of 95 carbon sources. The changes in viable count and population of bacterial flora with time were also analyzed.

Materials and Methods

Sample collection

Biodegradation experiments using small-scale degradation reactors and simulated waste were carried out according to the protocol described in a previous report (Horisawa *et al.* 2000 and 2001). A 1000-ml Kjeldahl flask was used as a degradation reactor, and cedar (*Cryptomeria japonica* (L. fil.) D. Don) particles sieved in a grain size of 0.5-1.0 mm were used as a matrix in the degrading machine. Every 24 hours, 3.5 g of formula rabbit feed (Hi-Pet Co., Osaka, Japan) was added to each reactor as the simulated waste. An incubator was set to 30°C and the moisture content (MC) of matrix was adjusted 20%, 30%, 40%, 50%, 60%, 70% and 80%, when the effect of the MC of the matrix on the degradation rate was under examination. On the other hand, when the effect of environmental temperature (ET) on the degradation rate was examined, the MC of the matrix was set at 60% and the incubator was adjusted to 10°C, 20°C, 30°C, 40°C, and 50°C. Three duplicated experiments lasted for 30 days on each condition except the experiments at the MC of 80%. The duration of the latter was shortened because the strength of the equipment could not bear the reactor weight for more than about 20 days. One gram of wood matrix was taken from the reactor at 3, 5, 10, 20, and 30 days.

Isolation and identification of bacteria

One gram of matrix was suspended in 100 ml of sterilized water. The suspension, diluted serially, was applied to a standard method agar (SMA) plate (Nissui, Tokyo, Japan) containing yeast extract (0.25%), peptone (0.5%), glucose (0.1%), and agar (1.5%). The viable cells were counted after several days of incubation at 30°C. Each agar plate on which 20-200 colonies appeared independently was selected for further experiments since those were considered to be the predominant species of bacterial community in each matrix. Each isolate was confirmed to be pure culture and then was subcultured.

Isolates were examined according to cell morphology and Gram staining. In order to identify the isolates, utilization patterns of 95 carbon sources were used to determined the nearest species from a database (Bochner 1989, Miller and Rhoden 1991). The composition of carbon sources is shown in Table 1. A relative importance (%) of each species was defined as the ratio of the species in the microbial community. The frequency of appearance of each species was determined by its average of relative importance across three duplicate experiments.

Results

Succession of the microbial community with MC

The succession of the microbial community when the MC of the matrix was adjusted to 20%, 30%, 40%, 50%, 60%, 70%, and 80% with an ET of 30°C are shown in Tables 2-8. In the initial stage, *Bacillus subtilis* was the dominant species at MCs of 20-60%. *Pseudomonas* at an MC of 50% and *Enterobacter* at 60% also appeared as important species in the initial stage. Frequency of *Enterobacter* increased at an MC of 70% and 80% in the initial stage, while *B. subtilis* remained stable. In the later stage, *Enterococcus* and yeast were abundant at an MC of 20%. However, *Xanthomonas campestris* and *Cellulomonas turbata* were frequent at an MC of 30-60% in the later stage. Also in the later stage, *X. campestris* and *X. maltophilia* were dominant and *Enterobacter* increased while *C. turbata* and *Bacillus* decreased at an MC of 70% and 80%.

Succession of the microbial community with ET

The succession of the microbial community when the ET of the matrix was adjusted to 10°C, 20°C, 30°C, 40°C, and 50°C with an MC of 60% are shown in Tables 9-12 (see Table 6 about 30°C). At 10°C, *Bacillus* was dominant in the initial stage; however, Gram-negative bacteria such as *Pseudomonas*, *Enterobacter agglomerans*, *Pantoea agglomerans*, and *X. maltophilia* became important species in the later stage. At 20°C and 30°C, *Bacillus*, *Pseudomonas*, and *Enterobacter* were pioneers, and finally, the community in which *C. turbata* and *X. campestris* were dominant species was formed. The communities at 40°C and 50°C were formed primarily by *Bacillus* throughout the experiment. *B. brevis* in particular appeared at a high frequency from the initial stage to the final stage. *C. turbata* was frequent at 40°C but not at 50°C.

Discussion

Previous reports have shown that the degradation rate declines at an MC of 20%. According to the report by Kaneko and Fujita (1986), an MC in of 20% in wood particles is converted to a water activity (a_w) of 0.909, which is the minimum a_w for bacteria in general (Yoshii 1975). Therefore, yeast, which can grow at a low a_w (minimum 0.88), should be dominant at an MC of 20%. It was found that *Bacillus subtilis* propagates initially, then the community is formed mainly from *C. turbata* and *X. campestris* at the MCs of 30-60% that are the optimum MCs for biodegradation of waste. The reason for the high frequency of *Enterobacter* at MCs of 70-80% is that the container is becoming anaerobic. The water holding capacity of wood particle is 66.1% on a wet weight basis (Horisawa 1999), so water is assumed to fill the spaces between the grains of wood particles at an MC of more than 70%, thus cutting off air.

The community at 10°C changed drastically, but the reason is not clear. At ETs of 20-40°C, which is the optimum temperature (Horisawa 2001), *Bacillus* is the pioneer and *C. turbata* and *X. campestris* were dominant species in the later stage and this situation is similar in the optimum MC. The frequency appearance of *Bacillus* increased at 40°C and 50°C because it can grow at a higher temperature. The strains of *Bacillus* isolated in this study are able to grow at temperatures higher than 40°C (Claus and Berkeley 1986). Specifically, *B. brevis* and *B. coagulans* have been reported to grow at 55°C. On the other hand, the optimum temperature for growth of *X. campestris* is 25-30°C (Bradbury 1984) and *C. turbata* are not able to grow at above 42°C (Lechevalier and Lechevalier 1986).

It was found that the community formed from *B. subtilis* in the initial stage and *C. turbata* and *X. campestris* in the later stage at the both optimum MC of the matrix and the optimum ET for degradation rate. This fact indicates the possibility of applying *B. subtilis* as a starter for a biodegradation machine. *C. turbata* is a Gram-positive bacterium that is generally isolated from soil and decayed plants (Lechevalier and Lechevalier 1986). *X. campestris* is a known plant pathogen and exists in composting of organic agricultural substrates (Peters et al. 2000). The community found in this study is considered to be characteristic of this simulated waste made from vegetables. It is assumed that this type of community is formed during the degradation of vegetal-rich waste.

References

Bochner, B.R. (1989) Sleuths out bacterial identities. Nature 339: 157-158.

Bradbury, J.F. (1984) Genus Xanthomonas Dowson 1939, 187^{AL}. In: Krieg NR (ed.) Bergey's Manual of Systematic Bacteriology vol. 1, Williams & Wilkins, Baltimore, 199-210.

Claus, D. and Berkeley, R.C.W. (1986) Genus Bacillus Cohn 1872, 174^{AL}. In: Sneath, P.H.A. (ed.) Bergey's Manual of Systematic Bacteriology vol. 2, Williams & Wilkins, Baltimore, 1104-1139.

Fujino, E., Shinbo. H., Minoura, H. and Kumada, K. (1994) Garbage Decomposer. Matsushita Electric Works technical report, 48: 81-85 (in Japanese).

Fujita, K. (1993) Composting technology. Gihodo, Tokyo, Japan, pp. 196 (in Japanese).

Horisawa, S., Tamai, Y., Sakuma, Y., Doi, S. and Terazawa, M. (2000) Effect of Moisture Content of Matrix on a Small Scale Bio-degradation System for Organic Solid Waste. J. Wood Sci., 46: 317-321.

Horisawa, S., Sakuma, Y., Tamai, Y., Doi, S. and Terazawa, M. (2001) Effect of Environmental Temperature of Matrix on a Small Scale Bio-degradation System for Organic Solid Waste. J. Wood Sci., 47: 154-158.

Horisawa, S., Sunagawa, M., Tamai, Y., Matsuoka,

Y., Miura, T. and Terazawa, M. (1999) Biodegradation of Non-lignocellulosic Substances II: The physical Properties and Deterioration of Sawdust as an Artificial Soil J. Wood Sci., 45(6), 492-497.

Inoue, T., Kimura, T., Morishita, T., Kumada, K. and Sekiguchi, T. (1996) Changes of the State in a Small Scale Bio-degradation System. In Fujita, K. (ed.) Proceedings of 7th ISWA International Congress and Exhibition, No.2, Japan Society of Waste Management Experts, Tokyo, Japan, 149-150 (in Japanese).

Kaneko, H. and Fujita, K. (1986) The Moisture Limit for Optimum Composting. Proceedings of the Japan Society of Civil Engineers 369: 303-309. (in Japanese with English summary).

Lechevalier, H.A. and Lechevalier, M.P. (1986) Genus Oerskovia Prauser, Lechevalier and Lechevalier 1970, 534; emended Lechevalier 1972, 263^{AL}. In: Sneath, P.H.A. (ed.) Bergey's Manual of systematic Bacteriology vol. 2, Williams & Wilkins, Baltimore, 1489-1491.

Miller, J.M. and Rhoden, D.L. (1991) Preliminary Evaluation of Biolig, a Carbon Source Utilization Method for Bacterial Identification. J. Clin. Microbiol., 29: 1143-7.

Minist. Health & Welfare, Environ. Health Bureau., Water Supply & Environ. Sanitation Dept., Planning Div. (1995) Abstract of Waste Management in Japan. J. Japan Waste Management Association, 48: 417-442 (in Japanese).

Peters, S., Koschinsky, S., Schwieger, F. and Tebbe, C.C. (2000) Succession of Microbial Communities during Hot Composting as Detected by PCR-Single-Strand-Conformation Polymorphism-Based Genetic Profiles of Small-Subunit rRNA Genes. Appl Environ Microbiol, 66: 930-936.

Takeda, N. (1996) Present condition and management of waste. In Suzuki Y. (ed) Recycle engineering. Japan Society of Energy and Resources, Osaka, Japan, 30 (in Japanese).

Tchobanoglous, G., Theisen, H. and Vigil, S. (1998) Integrated solid waste management. NTS, Tokyo, Japan, pp.862 (in Japanese).

Terazawa, M., Horisawa, S., Tamai, Y. and Yamashita, K. (1999) Biodegradation of Non-lignocellulosic Substances I: A System for the Complete Decomposition of Garbage using Sawdust and Aerobic Soil Bacteria, J. Wood Sci., 45: 354-358.

Yoshii, H. (1975) The Water Activity of Food. Miso Sci. Tech. 260: 5-13 (in Japanese)

Table 1. The 95 carbon sources used in the Biolog microplates (8x12 wells) for Gram-negative and Gram-positive bacteria.

	GN	GP		GN	GP
1	Water	Water	35	methyl pyruvate	β -methyl glucose
2	α-cyclodextrin	α-cyclodextrin	36	Mono-methyl succinct	α-methyl D-glucoside
3	Dextrin	β-cyclodextrin	37	Acetic acid	β-methyl D-glucoside
4	Glycogen	Dextrin	38	Cis-aconitic acid	α-methyl D-mannoside
5	Tween 40	Glycogen	39	Citric acid	Palatinose
6	Tween 80	Inulin	40	Formic acid	D-psicose
7	N-acetyl-D-galactosamine	Mannan	41	D-galactonic acid lactone	D-raffinose
8	N-acetyl-D-glucosamine	Tween 40	42	D-galacturonic acid	L-rhamnose
9	Adonitol	Tween 80	43	D-gluconic acid	D-ribose
10	L-arabinose	N-acetyl-D-glucosamine	44	D-glucosaminic acid	Salicin
11	D-arabitol	N-acetyl-D-mannosamine	45	D-glucuronic acid	Sedoheptulosan
12	Cellobiose	Amygdalin	46	α-hydroxybutyric acid	D-sorbitol
13	i-erythrutol	L-arabinose	47	β-hydroxybutyric acid	Stacyose
14	D-fructose	D-arabitol	48	γ-hydroxybutyric acid	Sucrose
15	L-fucose	Arbutin	49	p-hydroxyphenylacetic acid	D-tagalose
16	D-galactose	Cellobiose	50	Itaconic acid	D-treharose
17	Gentiobiose	D-fructose	51	α-keto butyric acid	Turanose
18	α-d-glucose	L-fucose	52	α-keto glutaric acid	Xylitol
19	m-inositol	D-galactose	53	α-keto valeric acid	D-xylose
20	α-D-lactose	D-galacturonic acid	54	D,L-lactic acid	Acetic acid
21	Lactulose	Gentiobiose	55	Malonic acid	α-hydroxybutyric acid
22	Maltose	D-gluconic acid	56	Propionic acid	β-hydroxybutyric acid
23	D-mannitol	α-D-glucose	57	quinic acid	γ-hydroxybutyric acid
24	d-mannose	m-inositol	58	D-saccharic acid	p-hydroxyphenyl acetic acid
25	D-melibiose	α-D-lactose	59	Sebacic acid	α-keto glutamic acid
26	β-methyl-D-glucoside	Lactulose	60	Succinic acid	α-keto valeric acid
27	D-psicose	Maltose	61	Bromo succinic acid	Lactamide
28	D-raffinose	Maltrotiose	62	Succinamic acid	D-lactic acid methyl ester
29	L-rhamnose	D-mannitol	63	Glucuronamide	L-lactic acid
30	D-sorbitol	D-mannose	64	Alaninamide	D-malic acid
31	Sucrose	D-melezitose	65	D-alanine	L-malic acid
32	D-trehalose	D-melibiose	66	L-alanine	Methyl pyruvate
33	turanose	α -methyl D-galactoside	67	L-alanyl-glycine	Mono-metyl succinate
34	xylitol	β -methyl D-galactoside	68	L-asparagine	Pripionic acid

Table 1. Continued.

69	L-aspartic acid	Pyrvic acid	83	D,L-carnitine	2,3-butanediol
70	L-glutamic acid	Succinamic acid	84	γ-amino butyric acid	Glycerol
71	Glycyl-L-asparic acid	Succinic acid	85	Urocanic acid	Adenosine
72	Glycyl-L-glutamic acid	N-acetyl L-glutamic acid	86	Inosine	2'-deoxyadenosine
73	L-histidine	Alaninamide	87	Uridine	Inosine
74	Hydroxy L-proline	D-alanine	88	Thymidine	Thymidine
75	L-leucine	L-alanine	89	Phenyl ethylamine	Uridine
76	L-ornithine	L-alanyl-glycine	90	Putrescine	Adenosine-5-manophosphate
77	L-phenylalanine	L-asparagine	91	2-aminoethanol	Tymidine-5'-manophosphate
78	L-proline	L-glutamic acid	92	2,3-butanediol	Uridine-5'-manophospate
79	L-pyroglutamic acid	Glycyl-L-glutamic acid	93	Glycerol	Frctose-6'-phospate
80	D-serine	L-pyroglutamic acid	94	D,L-α-glycerol phosphate	Glucosamine-1-phospate
81	L-serine	L-serine	95	Glucose-1-phosphate	Glucose-6-phosphate
82	L-threonine	Putrescine	96	Glucose-6-phosphate	D-L-α-glycerol phosphate

Table 2. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 20% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus subtilis	72.2	49.5	5.1	7.4	4.7
Enterococcus sp.	27.8	0.5	5.1	32.1	32.5
Candida entomophia		50.0	33.3	26.9	22.2
Yeast1*			43.6	28.0	30.8
B. megaterium			7.7		
B. licheniformis			5.1	0.9	0.9
Flobacterium esteroaromaticum				2.8	1.7
Alcaligenes latus				0.9	
Xanthomonas campestris				0.9	
Enterobacter asburiae					7.2

Notes: *: unidentified yeast.

Table 3. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 30% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus subtilis	63.6	77.8	39.8	0.3	
?1**	29.3	13.9			
Rothia dentrocariosa	7.1	5.6	1.1		
Yeast2*		2.8	11.3		
Corynebacterium aquaticum			17.3	28.8	1.8
Xanthomonas campestris			17.1	7.9	20.2
Cellulomonas turbata			6.8	29.4	42.3
Staphylicoccus spp.			3.3	5.6	6.3
Bacillus marcocanus			3.3		
Enterobacter asbriae				12.0	
B. gordinae				12.0	
B. brevis				4.0	24.2
B. thermoglicosidasius					5.3

Notes: see Table 2; **: unidentified bacteria.

Table 4. Frequency of appearance (%) of bacteria isolated from the matrix at an moisture content of 40% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus subtilis	85.3	50.7	2.2	0.8	2.4
Bacillus coagulans	13.3				
B. licheniformis	1.4	4.4		0.0	
Enterobacter cloacae		26.7		4.0	
B. megaterium		10.5	1.3		
Rothia dentrocariosa		4.2		1.3	
Xanthomonas campestris		3.5	82.8	74.9	55.7
E. agglomerans			7.6		
E. cloacae			3.0		
E. tayloae			2.7		
Cellulomonas turbata			0.4	14.0	12.7
?2**				2.5	
?3**				1.2	
Enterobacter asbriae				1.2	
?4**					9.5
X. maltophilia					7.9
?5**					4.8
A. rhizogenes					3.2
Micrococcus diversus					1.3
Agrobacterium tumefaciens					1.3
Corynebacterium aquaticum					1.3

Notes: see Table 3.

Table 5. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 50% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus subtilis	56.8	21.7	7.0		1.6
Pseudomonas sp.	30.6	24.2			
Enterobacter taylorae	9.5				
Rothia dentrocariosa	2.0	3.6			
B. licheniformis	1.1	0.4			
Xanthomonas campestirs		16.0	6.7	41.1	24.2
E. cloacae		15.6			
Cellulomonas turbata		12.4	25.0	12.5	36.1
Pichia sp. (yeast)		6.1			
X. maltophilia			27.3	25.2	5.1
Alcaligenes latus			15.9		
Corynebacterium aquaticum			14.9	14.0	19.7
?6**			2.4		
Ochirobacterum anthropi			0.8		1.9
Comamonas testosteroni				2.3	
B. alcalophilus				1.9	
B. azotoformans				1.9	
B. azotoformans				1.2	
?7**					4.0
B. badius					3.3
B. sphaericus					2.8
Cryptococcus luteolus					0.9
?8**					0.5

Notes: see Table 3.

Table 6. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 60% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus subtilis	37.6	5.2	9.0	5.2	
Enterobacter sp.	24.8	38.2	18.7		1.4
Pseudomonas spp.	17.5	1.0	2.8		
Cellulomonas turbata	9.5	9.5	11.1	36.1	36.4
B. brevis	4.8		4.8		1.3
Xanthomonas campestris	4.2	27.6	38.9	41.2	37.7
Acinetobacter calcpaceticus	1.6	3.9		2.3	
Agrobqacterium sp.		6.6			
B. megaterium		4.2		2.3	7.5
B. pasteurii		1.0			
?10**		1.0			
X. maltophilia		0.9	10.1	6.1	4.6
Sphingobacterium sp.		0.9			
B. thermoglucosidasius			3.1		
*Yeast3**			1.4		
Gilardi pink gram negarive rod				1.5	1.4
B. licheniformis				2.1	
B. sphaericus				1.8	3.8
Enterococcus sp.				1.5	3.5
Corynebacterium sp.					0.9
Rhodococcus sp					0.9
?9**					0.7

Notes: see Table 3.

Table 7. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 70% at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Enterobacter agglomerans	24.1	1.9			
Burkholederia cepacia	15.7	9.0			
Pseudomonas sp.	15.5	9.9	5.3		
Bacillus licheniformis	13.6		1.4		
Enterobacter asburiae	7.4				3.0
Xanthomonas maltophilia	6.2	18.9	20.6	23.4	4.5
E. cloacae	6.2	5.9			
Salmonella subspecies	4.9				
X. campestris	2.5	7.7	33.0	29.5	37.5
Rothia dentrocariosa	2.0		1.1	2.6	6.9
B. cepacia	2.0				
E. tayloae		25.9			
B. subtilis		7.0	1.4	7.0	5.6
Sphingobacterium sp.		3.6	7.0		
Flabobacterium esteroaromaticum		2.2	24.6		4.5
Cellulomonas turbata		2.0		15.5	9.8
?11**		2.0			
?12**		1.9			
B. coagulans		1.3			
?13**		1.0			
Comamonas acidovorans			1.8		
Corynebacterium aquaticum			1.4	2.9	12.1
Agrobacterium tumefaciens			1.1		
?14**			1.1		
Alcaligenes latus				14.5	
Acinetobacter sp.				2.9	
B. megaterium				1.8	
B. azotoformans					5.6
Ochrobactrum anthropi					4.2
Yeast4*					3.0
B. brevis					1.9
B. megaterium					1.4

Notes: see Table 3.

Table 8. Frequency of appearance (%) of bacteria isolated from the matrix at a moisture content of 80% at 3, 5, 10, and 20 days, respectively.

	3	5	10	20
Enterobacter agglomerans	42.4	26.4	14.1	6.1
?15**	11.8			
Salmonella sp.	8.3		9.1	
Xanthomonas campestris	7.4	15.1	18.6	27.6
?16**	7.4			
Corynebacterium mitrilophilus	5.6			
?17**	3.9	5.3		
E. taylorae	3.9		12.3	15.3
Acinetobacter johnsonii	3.7		4.6	
E. cloacae	2.8	30.9		
X. maltophilia	2.8	1.0	21.9	33.0
Comamonas testosteroni		7.8		
Yeast5*		3.5		
Bacillus coagulans		3.0		
Yeast6*		2.9		
B. licheniformins		1.5		
Sphingobacterium thalpophilum		1.5		
B. subtilis		1.0	6.1	
E. asburiae			5.7	
Cellulomonas turbata			2.4	5.2
Corynebacterium aquaticum			2.4	
Yeast7*			1.5	
Cryptcoccus albudus			1.2	
?18**				7.7
Pseudomonas putida				5.1

Notes: see Table 3.

Table 9. Frequency of appearance (%) of bacteria isolated from the matrix at an environmental temperature of 10°C at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus brevis	55.0	38.4			
B. subtilis	31.7	31.4			
B. licheniformis	6.7				
Cellulomonas turbata	3.3			1.1	9.1
Acinetobacter jonsonii	3.3				
B. megaterium		26.9	2.9		
B. azotoformans		3.3			
Rhodotorula sp.(yeast)			4.9		
Klebsiera pneumoniae			17.8		
Pseudomonas sp.			15.6	0.4	
Enterobacter agglomerans			25.5	5.3	3.0
Pantoea aggromerans			33.3	32.2	30.8
Xanthomonas maltophilia				33.0	32.0
?19**				25.0	21.2
Micrococcus sp.				3.0	
X. campestris					3.2
B. coagulans					0.7

Notes: see Table 3.

Table 10. Frequency of appearance (%) of bacteria isolated from the matrix at an environmental temperature of 20°C at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus megaterium	46.4				
Pseudomonas sp.	34.0	37.4		12.4	
Enterobacter sp.	18.6	14.4	8.2	10.3	5.6
Cellulomonas turbata	1.0	23.1	13.4	11.9	28.9
Xanthomonas campestris		15.2	28.7	20.0	36.6
B. brevis		9.9			
Escherichia		8.7	5.9		
Xanthomonas maltophilia			38.6	12.9	
Corynebacterium			5.2	18.4	5.8
Klebsiella				4.3	
B. pasteurii				4.3	
B. sphaericus				2.2	6.0
Rhodococcus equi				2.2	
Giraldi pink gram negative rod				1.6	7.1
?20**				1.6	
Alcaligenes latus					8.0

Notes: see Table 3.

Table 11. Frequency of appearance (%) of bacteria isolated from the matrix at an environmental temperature of 40°C at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus licheniformis	50.3	1.8			1.9
B. subtilis	18.5	2.6	4.4	2.4	7.4
Cellulomonas turbata	16.7	29.8	41.7	62.4	43.5
B. brevis	6.1	10.5	19.5	22.0	22.2
B. amyloliquifaciens	3.7				
Yeast8*	2.4				
B. coagulans	1.1	28.8		1.8	
? 21**	1.1				
Acinetobacter calcoaceticus		17.5	1.2		
Pseudomonas sp		5.1	3.7		
Enterobacter cloacae		2.1			
Xanthomonas campestris		1.8	1.2		
B. pasteurii			6.5		14.8
? 22**			6.2	1.5	
Enterococcus sp.			6.1		
B. licheniformis			4.9		
Comamonas teststeroni			3.7	0.9	2.8
B. megaterium			0.7		1.9
?23**				3.6	
Acidovorax delafieldii				3.2	
Rothia dentrocariosa				1.1	
?24**				1.1	
Corynebacterium sp.					2.8
Acinetobacter jonsonii					2.8

Notes: see Table 3.

Table 12. Frequency of appearance (%) of bacteria isolated from the matrix at an environmental temperature of 50°C at 3, 5, 10, 20, and 30 days, respectively.

	3	5	10	20	30
Bacillus brevis	63.3	72.6	77.4	63.3	31.3
B. coaglans	10.7				12.5
Promicromonospora enterophila	7.2	9.0			7.8
B. pasteruii	6.4				
?25**	4.4				
B. subtilis	3.6			11.7	4.7
B. licheniformis	2.9		4.2		
B. thermoflucosidasius	1.5				
B.megaterium		14.3			39.9
Xanthomonas campestris		2.5			1.6
?26**		1.6			
?27**			9.5		
Rothia dentrocariosa			6.5	8.3	
Yeast9*			2.4		
?28**				6.7	
?29**				5.0	
B. macerans				2.5	
?30**				2.5	
B. laevolacticus					0.7

Notes: see Table 3.