Journal of Industrial Microbiology (1996) 16, 182–188

Putative anaerobic activity in aerated composts

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It has been suggested that anaerobic microenvironments develop in aerobic composts, regardless of the aeration system used, and that anaerobic activity is responsible for odor generation and nitrogen losses. This study was designed to measure levels of microorganisms capable of anaerobic growth in two aerated composts: municipal solid waste, a relatively nutrient-rich compost, and pulp and paper-mill solid waste, which is relatively nutrient-poor. Anaerobic microorganisms were isolated from both composts at mesophilic and thermophilic temperatures. The majority of the anaerobic mesophiles were facultative anaerobes, whereas facultative, anaerobic thermophiles varied from 0 to 100%. Serially-diluted samples were spot-plated onto various media to preserve microbial consortia. Levels of aerobic and anaerobic exoenzyme production on spot-plates were similar on cell-wall, starch, and casein media. Although microbial levels on spread plates indicate that aerobes are present in much higher numbers than anaerobes (in 47 of 56 subsamples, 90% of the population were aerobes), microbial growth levels and exoenzyme production on spot-plates indicate that anaerobes may be responsible for a large portion (greater than or equal to 72%) of the metabolic activity in anaerobic microenvironments of aerobic composts.

Keywords: enzymes; anaerobes; municipal solid waste; pulp and paper

Introduction

Composting is characterized as a microbial process in which solid organic material is degraded and the temperature is maintained in the thermophilic range for a portion of the time [3]. Most modern composting systems are designed to operate aerobically [4]. Hansen *et al* [5] stated that aeration control is critical to successful aerobic, high-rate composting, and that anaerobic activity is responsible for odor generation and ammonia losses during 14 days of composting poultry litter.

Fogarty and Tuovinen [4] stated that because of the nature of the material being composted, the development of anaerobic microsites was inevitable during aerobic composting, regardless of the aeration system used. In bench-scale composting studies we had previously conducted on oxidation ditch sludge and on poultry litter, clumping of the composts was observed even after thorough mixing and addition of sawdust to increase free air space. Clumping could enhance development of anaerobic microenvironments as surfaces of clumps dry out and the interior surfaces remain moist.

Although many researchers have speculated about anaerobic activity [3–5,8], few studies measuring the levels of anaerobic microorganisms in aerobic composts have been published. Diaz-Raviña *et al* [2] found 10^2 to 10^4 anaerobic celluloytic bacteria g^{-1} dry solids compared to 10^4 to 10^6 aerobic cellulolytics in four composted urban refuses. Overall levels of anaerobes were not measured.

The study was conducted as part of two bench-scale aerobic composting experiments in which the biodegradabilities of municipal solid waste (MSW) and of pulp and paper-mill primary solid wastes (PS) were measured. Levels of microorganisms, exoenzyme production, and proportions of microorganisms capable of facultative and strict anaerobic growth were determined.

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Received 7 August 1995; accepted 22 December 1995

Materials and methods

The bench-scale compost reactors were described previously [1]. Materials composted were unsupplemented municipal solid waste (MSW-U), supplemented municipal solid waste (MSW-S), pulp and paper-mill primary solids that were not supplemented until day 22 of the experiment (PSU), and pulp and paper-mill primary solids that were supplemented on days 1 and 22 (PSS). Supplements added were ammonia, phosphate, vitamin supplement, trace minerals, and lactose. All initial moisture contents were adjusted to 60%. When samples were removed periodically from the MSW and PS composts, two 50-g portions were placed into separate Waring blenders containing 500 ml distilled water, then blended to disperse the cells. Ten-milliliter samples were removed from the contents of each blender and serially diluted for spread-plate counts on tryptic soy agar (TSA) and on Brewer's Anaerobic Agar. Initially, samples were also serially diluted in anaerobic broth and spread onto Brewer's Anaerobic Agar for enumeration of anaerobes. Differences in anaerobic growth levels of less than 10-fold were found between blended samples and those diluted directly into anaerobic broth; therefore, blended samples were used subsequently to ensure more uniform, representative sampling. The serially diluted samples were also used for spot-plating onto various media. Briefly, spot-plating involved placing 10 μl from each serial dilution onto agar plates containing 1% (w/v) casein, 1% (w/v) starch, 1% (w/v) α-cellulose, Gram-negative cell wall components, or Gram-positive cell wall components. Cell wall medium preparation was as described previously [1]. Spread plates were inoculated in duplicate. The TSA spread plates were incubated aero-

Table 1 Mean ($n = 2$) counts of microorganisms g^{-1} dry solids from unsupplemented and supplemented MSW growing on aerobically- and anaerobically-incubated spread plates

MSW-U	*Day*	*Aerobic 23°C*	*s.d.*	*Anaerobic 23°C*	*s.d.*
	1	2.02×10^{10}	2.70×10^{8}	6.87×10^{9}	5.00×10^{6}
	8	4.88×10^{9}	2.10×10^{7}	9.93×10^{7}	5.00×10^{4}
	15	2.31×10^{9}	6.50×10^{6}	1.52×10^{8}	3.30×10^{6}
	22	3.51×10^{9}	1.07×10^{8}	9.46×10^{7}	4.00×10^{5}
	29	7.75×10^{8}	0	6.76×10^{7}	4.50×10^{5}
	36	3.43×10^{8}	1.50×10^{6}	1.77×10^{7}	9.00×10^{4}
	43	8.68×10^{8}	2.00×10^{7}	1.65×10^{8}	3.50×10^{5}
	Day	*Aerobic 55°C*	*s.d.*	*Anaerobic 55°C*	*s.d.*
	1	1.86×10^{10}	0	5.79×10^{8}	4.50×10^{6}
	8	1.06×10^{11}	3.81×10^{9}	2.48×10^{8}	2.65×10^{6}
	15	8.11×10^{9}	1.50×10^{7}	1.15×10^{8}	1.45×10^{6}
	22	7.55×10^{9}	1.45×10^{8}	1.29×10^{8}	1.70×10^{6}
	29	1.51×10^{9}	3.00×10^{6}	1.71×10^{8}	0
	36	2.56×10^{9}	0	1.26×10^{8}	1.40×10^{6}
	43	1.05×10^{9}	5.00×10^{6}	4.04×10^{8}	6.00×10^{6}
MSW-S	*Day*	*Aerobic 23°C*	*s.d.*	*Anaerobic 23°C*	*s.d.*
	1	6.94×10^{10}	6.00×10^{8}	3.65×10^{9}	1.00×10^{7}
	8	9.56×10^{8}	5.00×10^{6}	1.09×10^{8}	2.45×10^{6}
	15	1.89×10^{10}	0	3.51×10^{7}	4.00×10^{5}
	22	1.60×10^{10}	1.45×10^{8}	1.90×10^{8}	1.00×10^{5}
	29	2.51×10^{9}	4.50×10^{6}	1.02×10^{8}	2.50×10^{5}
	36	3.33×10^{9}	3.80×10^{7}	5.92×10^{7}	0
	43	5.73×10^{9}	0	1.10×10^{9}	1.15×10^{7}
	Day	*Aerobic 55°C*	*s.d.*	*Anaerobic 55°C*	*s.d.*
	1	9.38×10^{9}	2.50×10^{7}	6.05×10^{7}	1.65×10^{6}
	8	8.32×10^{9}	4.00×10^{7}	1.27×10^{8}	2.20×10^{6}
	15	1.95×10^{10}	0	1.36×10^{8}	1.70×10^{6}
	22	1.92×10^{10}	2.80×10^{8}	1.22×10^{8}	3.50×10^{5}
	29	5.73×10^{10}	9.50×10^{7}	4.59×10^{7}	0
	36	9.54×10^{9}	4.50×10^{7}	1.07×10^{8}	3.00×10^{5}
	43	7.08×10^{9}	0	1.52×10^{9}	0

MSW-U = municipal solid waste, unsupplemented.
MSW-S = municipal solid waste, supplemented.
s.d. = standard deviation.

bically at 23 and 55°C; the anaerobic agar plates were incubated anaerobically using the GasPak System (Becton Dickinson, Cockeysville, MD, USA). Eight plates of each substrate were used for spot-plating: two of each medium were incubated aerobically at 23°C and two at 55°C; two were incubated anaerobically in the GasPak System at each temperature. All plates were examined after 4 days of incubation.

When spot-plates were examined, levels of growth were determined based on the most dilute sample from which microbial growth was observed. Levels of exoenzyme production were determined based on the most dilute sample from which clearing of the medium around microorganisms was observed.

When spread plates were examined, total colonies were enumerated on aerobic and anaerobic plates. To determine oxygen requirements, individual colony types on the anaerobic plates were each described and counted. Representative colonies were subcultured onto TSA and anaerobic agar, and incubated at their original growth temperature. Microorganisms that grew both aerobically and anaerobically when subcultured were termed 'facultative anaerobes.' Microorganisms that grew only anaerobically were termed 'strict anaerobes.' Some of the microorganisms could not be recultured under aerobic or anaerobic conditions. These microorganisms were grouped as 'no regrowth'. Percentages for each group were determined based on the original total colonies on anaerobic spread plates.

Results and discussion

Aerobic and anaerobic spread plate counts

In both unsupplemented and supplemented MSW, levels of mesophiles cultured on anaerobic spread plates represented 34 and 37% of the culturable population in the starting mixes (Table 1). These levels decreased to less than 10% of the population by day 36, then increased to about 19% of the culturable population by day 43. Levels of thermophiles cultured on anaerobic spread plates were initially 3 and $< 1\%$ of the microbial population culturable from the unsupplemented and supplemented MSW compost mixes, respectively. Levels of thermophilic anaerobes remained low ($\leq 11\%$) through day 36. However, by day 43, levels

Table 2 Mean ($n = 2$) counts of microorganisms g^{-1} dry solids from unsupplemented and supplemented PS growing on aerobically- and anaerobically-incubated spread plates

PSU	*Day*	*Aerobic 23°C*	*s.d.*	*Anaerobic 23°C*	*s.d.*
	1	3.57×10^8	3.27×10^7	9.93×10^5	2.89×10^5
	8	8.27×10^8	3.41×10^8	1.89×10^6	5.96×10^5
	15	4.37×10^8	7.73×10^7	1.07×10^6	4.36×10^4
	22	9.41×10^8	1.05×10^8	3.36×10^6	7.09×10^5
	29	8.28×10^9	1.40×10^9	4.20×10^7	3.82×10^6
	36	4.46×10^8	2.73×10^7	9.78×10^5	9.10×10^4
	43	2.82×10^8	1.75×10^7	5.13×10^5	9.46×10^4
	Day	*Aerobic 55°C*	*s.d.*	*Anaerobic 55°C*	*s.d.*
	1	3.27×10^8	1.01×10^5	7.42×10^4	3.77×10^3
	8	5.01×10^7	1.07×10^7	1.10×10^7	1.07×10^7
	15	2.04×10^6	2.29×10^5	9.04×10^4	9.80×10^3
	22	4.30×10^6	2.67×10^6	1.09×10^5	4.65×10^3
	29	9.05×10^6	2.68×10^6	4.78×10^6	3.63×10^6
	36	8.30×10^8	1.71×10^8	1.21×10^6	5.00×10^5
	43	5.49×10^9	1.99×10^9	3.87×10^6	3.86×10^5
PSS	*Day*	*Aerobic 23°C*	*s.d.*	*Anaerobic 23°C*	*s.d.*
	1	3.66×10^8	1.14×10^8	3.05×10^7	9.00×10^5
	8	1.30×10^{10}	1.83×10^9	2.46×10^8	1.03×10^7
	15	2.94×10^8	1.68×10^7	1.96×10^6	8.08×10^5
	22	5.27×10^9	9.31×10^8	2.42×10^7	7.72×10^6
	29	1.79×10^{10}	3.18×10^9	8.65×10^8	5.09×10^7
	36	1.23×10^{10}	4.46×10^8	8.25×10^8	6.69×10^7
	43	9.56×10^9	1.43×10^9	2.00×10^7	7.53×10^6
	Day	*Aerobic 55°C*	*s.d.*	*Anaerobic 55°C*	*s.d.*
	1	1.68×10^7	6.43×10^5	0	0
	8	1.95×10^8	1.03×10^7	2.28×10^3	2.28×10^3
	15	7.14×10^8	8.40×10^7	1.71×10^5	7.35×10^3
	22	5.40×10^9	1.16×10^9	3.91×10^6	3.49×10^6
	29	1.04×10^{10}	2.80×10^9	1.34×10^5	8.90×10^3
	36	1.08×10^{10}	2.79×10^9	3.24×10^4	1.45×10^4
	43	5.23×10^9	8.61×10^8	7.53×10^4	1.31×10^4

PSU = pulp and paper-mill primary solids, unsupplemented.
PSS = pulp and paper-mill primary solids, supplemented.
s.d. = standard deviation.

of thermophilic anaerobes increased to 38% in unsupplemented MSW and 21% in supplemented MSW. This reflects a relative increase in the proportions of mesophilic and thermophilic anaerobes in the population, as well as an increase in the total number of anaerobes in the population. The increases in levels of thermophilic anaerobes observed on day 43 may indicate that the thermophiles were better adapted to anaerobic conditions.

In unsupplemented and in supplemented PS, the proportions of mesophilic and thermophilic anaerobes cultured on anaerobic spread plates were less than 1% on most sampling days (Table 2). In contrast to the levels of anaerobes observed in MSW, there was no comparable increase in the proportion of anaerobes in the culturable population at the end of the composting period. This may be the result of lower nutrient value of the PS.

Proportions of anaerobes cultured from MSW were generally higher than were those cultured from PS (Tables 1 and 2). Particles in MSW were much larger than particles in PS and tended to form larger clumps, which could account for the presence of anaerobic microsites as surfaces of the clumps dried and inner surfaces retained moisture. Decreasing the size of MSW particles used in composting would provide more surface area for microbial activity and could lessen formation of anaerobic pockets.

In both unsupplemented and supplemented MSW, levels of aerobically-incubated microorganisms growing on spread plates were as much as 1000-fold higher than microorganisms growing on anaerobically-incubated spread plates (Table 1). Aerobic counts were up to 10^3-fold higher than anaerobic counts in unsupplemented PS and up to 10^7-fold higher than anaerobic counts in supplemented PS (Table 2). The portion of the microbial population that can be cultured on anaerobic spread plates is, therefore, small compared to the portion that can be cultured aerobically. If microbial levels on spread plates correspond to metabolic activity in the composts, these data suggest that aerobes are primarily responsible for composting under aerobic conditions.

Facultative and strict anaerobes (phenotypes)

Generally, in both unsupplemented and supplemented MSW or in PS composts, greater than 90% of mesophilic microorganisms subcultured from anaerobic spread plates

Table 3 Mean spread plate counts of microorganisms g^{-1} incubated anaerobically (n = 2) and proportions of microorganisms growing as facultative anaerobes and strict anaerobes

MSW-U		Total	%	%	%		Total	%	%	%
23°C	Day	cells	F An	S An	N R	*55°C*	cells	F An	S An	N R
	1	6.87×10^9	94.1	5.9	0		5.79×10^8	63.6	33.1	3.3
	8	9.93×10^7	98.1	1.9	0		2.48×10^8	24.5	42.0	33.5
	15	1.52×10^8	88.8	11.3	0		1.15×10^8	65.1	34.9	0
	22	9.46×10^7	100.0	0	0		1.29×10^8	35.2	52.8	12.0
	29	6.76×10^7	98.4	1.6	0		1.71×10^8	91.6	1.9	6.5
	36	1.77×10^7	99.1	0.9	0		1.26×10^8	100.0	0	0
	43	1.65×10^8	94.6	4.5	0.9		4.04×10^8	79.6	0	20.4
MSW-S										
23°C						*55°C*				
	1	3.65×10^9	99.1	0.9	0		6.05×10^7	69.4	11.7	18.9
	8	1.09×10^8	100.0	0	0		1.27×10^8	74.2	0	25.8
	15	3.51×10^7	83.3	16.7	0		1.36×10^8	17.7	69.2	13.1
	22	1.90×10^8	100.0	0	0		1.22×10^8	12.1	24.8	63.1
	29	1.02×10^8	100.0	0	0		4.59×10^7	62.5	37.5	0
	36	5.92×10^7	100.0	0	0		1.07×10^8	99.0	1.0	0
	43	1.10×10^9	91.6	6.1	2.3		1.52×10^9	44.4	33.3	22.3
PSU										
23°C						*55°C*				
	1	9.93×10^5	100.0	0	0		7.42×10^4	100.0	0	0
	8	1.89×10^6	100.0	0	0		1.10×10^7	100.0	0	0
	15	1.07×10^6	100.0	0	0		9.04×10^4	31.3	12.0	56.7
	22	3.36×10^6	100.0	0	0		1.09×10^5	74.1	0	25.9
	29	4.20×10^7	100.0	0	0		4.78×10^6	10.1	0	89.9
	36	9.78×10^5	100.0	0	0		1.21×10^6	100.0	0	0
	43	5.13×10^5	70.5	29.5	0		3.87×10^6	46.8	0	53.2
PSS										
23°C						*55°C*				
	1	3.05×10^7	44.4	50.7	4.9		0	0	0	0
	8	2.46×10^8	100.0	0	0		2.28×10^3	100.0	0	0
	15	1.96×10^6	100.0	0	0		1.71×10^5	100.0	0	0
	22	2.42×10^7	100.0	0	0		3.91×10^6	91.3	0	8.7
	29	8.65×10^8	100.0	0	0		1.34×10^5	55.4	0	44.6
	36	8.25×10^8	33.8	0	66.2		3.24×10^4	17.3	44.8	37.9
	43	2.00×10^7	100.0	0	0		7.53×10^4	1.6	7.9	90.5

MSW-U & MSW-S = municipal solid waste, unsupplemented and supplemented, respectively. PSU & PSS = pulp and paper-mill primary solids, unsupplemented and supplemented, respectively. Total cells = cells g^{-1} dry solids growing on anaerobic spread plates. % F An = % of total cells that regrew anaerobically and aerobically. % S An = % of total cells that regrew anaerobically but not aerobically. % NR = % of total cells that did not regrow.

were facultative anaerobes (Table 3). In contrast to the mesophiles, proportions of thermophiles from anaerobic spread plates that were facultative anaerobes varied from 0 to 100%. There were also many more instances of thermophilic microorganisms that could not be recultured from the anaerobic spread plates. Non-reculturable organisms may have been present on the initial spread plate as a result of nutrient carry-over and, thus, represent a group of uncertain physiology. It is also possible that some of these microorganisms were strict anaerobes that could not survive exposure to oxygen during counting and reculturing. However, based on preliminary studies comparing growth levels of anaerobes from blended compost samples with those from samples diluted in anaerobic broth, it is unlikely that oxygen sensitivity could account for a large portion of the no regrowths.

These data suggest that thermophilic anaerobes are more likely to be strict anaerobes than are mesophilic anaerobes. In composts that are not properly aerated, temperatures well into the thermophilic range would be expected, and oxygen would be less available to the aerobic microorganisms because of the reduced solubility of oxygen. This implies that conditions would favor activity by thermophilic, strict anaerobic microorganisms.

Microbial growth and exoenzyme production on spot plates

In general, levels of growth and exoenzyme production by MSW microorganisms were high (counts were between 10^8 and 10^{10}) on cell-wall media, on casein, and on starch plates incubated aerobically and anaerobically throughout the composting process (Table 4). The level of aerobes in

Table 4 Microbial growth/exoenzyme production on spot-plates incubated aerobically and anaerobically

Day	Substrate	1	8	15	22	29	36	43
Aerobic					**MSW-U**			
23°C	Gram– wall	11/10	9/8	9/9	9/9	10/10	8/8	9/9
	Gram+ wall	10/10	9/9	9/9	9/9	9/9	9/9	9/9
	Casein	9/9	9/7	10/8	10/9	9/9	8/8	9/9
	Starch	10/10	8/8	9/9	8/8	9/9	8/8	9/9
	Cellulose	7/–	6/6	–/–	8/8	10/6	8/7	9/9
55°C	Gram– wall	10/10	10/10	11/7	9/8	9/9	10/9	9/8
	Gram+ wall	9/9	10/10	9/8	8/8	9/9	9/9	8/8
	Casein	9/9	11/11	9/7	9/9	10/9	10/8	9/7
	Starch	10/9	9/9	8/8	8/8	9/9	9/9	9/9
	Cellulose	8/8	9/8	–/–	9/5	8/7	9/8	7/7
Anaerobic								
23°C	Gram– wall	9/8	10/7	9/7	8/8	8/8	8/8	8/8
	Gram+ wall	10/8	9/7	9/8	9/7	9/8	8/8	9/7
	Casein	10/8	9/7	8/6	7/7	7/7	7/7	10/7
	Starch	8/8	8/8	7/7	8/8	8/8	8/8	8/8
	Cellulose	6/–	8/–	–/–	6/–	7/–	7/–	8/–
55°C	Gram– wall	10/10	10/8	10/8	10/8	8/8	10/9	10/8
	Gram+ wall	9/9	11/9	9/8	10/8	9/9	9/9	9/9
	Casein	9/6	11/9	8/7	9/8	10/7	8/8	8/7
	Starch	9/9	9/9	9/8	9/8	9/9	9/9	9/9
	Cellulose	8/–	7/–	9/–	6/–	8/–	8/–	9/8
Aerobic					**MSW-S**			
23°C	Gram– wall	10/10	9/9	10/10	10/9	10/10	9/9	10/10
	Gram+ wall	10/10	9/9	9/9	9/9	9/9	9/9	10/10
	Casein	9/9	8/8	9/9	9/9	8/8	10/9	9/9
	Starch	11/10	9/9	9/9	10/10	8/8	9/9	9/9
	Cellulose	8/–	6/–	6/–	7/–	8/8	8/8	7/6
55°C	Gram– wall	9/7	10/9	9/9	10/10	10/9	10/10	10/9
	Gram+ wall	8/8	9/9	10/10	10/9	10/9	10/10	8/8
	Casein	9/9	11/11	10/10	10/10	9/9	10/9	10/9
	Starch	8/8	9/9	9/9	9/7	10/9	9/9	10/10
	Cellulose	–/–	7/–	6/–	6/–	7/–	10/9	9/8
Anaerobic								
23°C	Gram– wall	10/8	8/8	11/9	9/7	9/8	8/7	10/9
	Gram+ wall	11/9	8/7	9/9	10/9	8/7	8/8	10/9
	Casein	10/8	8/7	9/8	10/8	8/7	7/7	8/8
	Starch	9/9	7/7	8/8	9/9	8/8	8/8	9/9
	Cellulose	7/–	5/–	–/–	9/–	8/–	–/–	9/–
55°C	Gram– wall	9/9	10/9	7/7	8/8	9/8	7/7	10/9
	Gram+ wall	7/7	8/7	7/7	7/7	8/8	9/8	7/7
	Casein	10/9	8/5	9/9	9/8	9/9	6/–	9/9
	Starch	9/9	9/8	7/7	7/7	7/7	9/9	9/9
	Cellulose	7/–	6/–	9/–	7/–	8/–	6/6	6/6

'–' indicates below detection limit of method (10^4). Means (n = 3) are expressed as log 10 on a g^{-1} dry solids basis. MSW-U, MSW-S = municipal solid waste, unsupplemented and supplemented, respectively.

MSW was greater than that of anaerobes in only about 20% of the plated samples. On only 22 of 112 occasions were levels of aerobes at least 10-fold higher than anaerobes.

The level of aerobes in PS was greater than that of anaerobes in about 38% of the plated samples (Table 5). On only 42 of 112 occasions were levels of PS aerobes at least 10-fold higher than anaerobes. These data suggest that anaerobic microorganisms could participate in degrading macromolecules like cell-wall components, protein, or starch in aerobic composts.

On cellulose plates, levels of growth and exoenzyme production were lower and more variable than on the other macromolecules (Tables 4 and 5). In only about 40% of the samples from MSW and PS was the level of aerobes greater than that of anaerobes. The level of growth by aerobes from MSW was greater than 10-fold higher than anaerobic growth on 8 of 28 occasions. Exoenzyme production by aerobic microorganisms was greater than 10-fold higher on 13 of 28 occasions. In PS, levels of growth by aerobes were at least 10-fold higher than for anaerobes on 11 of 28 occasions. Exoenzyme production by aerobes was greater than 10-fold higher on 13 of 28 occasions. In contrast, Palmisano *et al* [7] found lower levels of cellulase activity, and Diaz-Ravinã [2] found lower levels of cellul-

Table 5 Microbial growth/exoenzyme production on spot-plates incubated aerobically and anaerobically

Day	Substrate	1	8	15	22	29	36	43
Aerobic					**PSU**			
23°C	Gram– wall	9/9	9/9	9/9	8/8	10/10	7/7	9/8
	Gram+ wall	10/10	10/10	9/9	8/8	9/9	9/8	9/9
	Casein	9/9	10/10	8/8	8/8	10/10	8/8	9/9
	Starch	9/9	10/9	8/8	9/9	10/10	9/9	9/9
	Cellulose	8/–	8/–	–/–	–/–	9/8	8/8	9/9
55°C	Gram– wall	5/5	6/–	6/6	6/5	6/–	8/7	9/9
	Gram+ wall	7/7	6/6	7/7	5/5	6/5	8/6	8/8
	Casein	5/5	6/6	7/6	6/5	6/6	9/9	9/9
	Starch	6/5	5/5	7/7	6/5	6/5	7/7	9/9
	Cellulose	5/4	5/5	7/6	6/6	6/6	7/7	8/8
Anaerobic								
23°C	Gram– wall	6/6	7/6	6/–	5/5	7/7	8/7	8/8
	Gram+ wall	5/5	7/7	8/6	6/5	8/7	7/6	7/7
	Casein	6/–	7/–	7/5	5/–	7/–	6/6	6/6
	Starch	6/6	6/6	8/7	6/6	7/7	8/8	7/7
	Cellulose	5/–	8/–	–/–	–/–	7/–	7/–	5/5
55°C	Gram– wall	5/–	8/7	6/–	6/5	9/8	8/7	9/8
	Gram+ wall	8/5	7/7	8/6	7/6	7/7	8/8	9/8
	Casein	5/–	6/6	7/–	7/6	6/6	9/7	8/7
	Starch	5/5	6/6	7/5	5/5	7/7	7/7	8/8
	Cellulose	6/–	7/5	7/5	8/5	6/6	7/6	6/5
Aerobic					**PSS**			
23°C	Gram– wall	9/9	10/10	8/8	8/8	9/9	10/9	10/10
	Gram+ wall	9/9	10/10	9/9	8/8	10/10	10/9	9/9
	Casein	10/10	9/9	9/8	8/8	8/8	11/10	9/9
	Starch	9/9	10/10	8/8	9/8	9/9	10/9	9/9
	Cellulose	7/7	11/6	6/6	8/7	9/9	8/6	8/8
55°C	Gram– wall	6/6	6/6	8/7	11/10	10/10	10/9	9/9
	Gram+ wall	7/7	8/8	8/8	10/10	9/9	9/9	9/9
	Casein	5/–	8/7	8/8	10/10	9/9	10/9	9/9
	Starch	5/5	6/6	8/8	9/9	8/8	10/9	9/9
	Cellulose	5/5	7/6	8/8	9/9	10/8	10/10	8/8
Anaerobic								
23°C	Gram– wall	9/9	8/8	6/5	6/6	8/8	9/8	9/9
	Gram+ wall	8/8	9/8	7/6	7/6	8/8	10/10	9/9
	Casein	9/9	10/–	7/–	5/5	10/–	9/8	9/7
	Starch	8/8	8/8	7/7	8/7	9/9	9/9	9/8
	Cellulose	7/–	9/5	6/5	5/5	10/–	6/–	7/7
55°C	Gram– wall	5/5	5/–	9/–	10/8	8/7	6/6	7/7
	Gram+ wall	7/6	–/–	9/–	9/9	8/8	6/5	9/8
	Casein	5/–	5/–	9/7	7/7	10/9	–/–	7/7
	Starch	5/5	5/5	7/7	7/7	7/7	6/6	7/7
	Cellulose	5/–	–/–	7/–	6/6	8/–	6/6	7/7

'–' indicates below detection limit of method (10^4). Means (n = 3) are expressed as log 10 on a g^{-1} dry solids basis. PSU & PSS = pulp and paper-mill primary solids, unsupplemented and supplemented, respectively.

ase-producing microorganisms under anaerobic than aerobic conditions. Increased levels of exoenzyme production as composting of MSW and PS progressed suggests that cellulose is degraded after the rapidly-degradable compounds have been metabolized during the early stages of composting. Kannan *et al* [6] found that maximum cellulose degradation in liquid cultures of paper-mill sludge occurred after day 16.

Cell numbers on aerobic and anaerobic spread plates indicated that the proportion of anaerobes in the culturable microbial population was less than 1% in about half of the samples. However, when levels of anaerobic growth and exoenzyme production on spot plates were compared with aerobic levels, anaerobic growth and exoenzyme production were generally greater than or equal to aerobic levels. This indicates that anaerobic microorganisms could play an important role in the degradation of macromolecules. Therefore, the formation of anaerobic microsites may not be completely undesirable in composting as long as there is aerobic activity to metabolize endproducts of anaerobic metabolism.

Conclusion

Microorganisms capable of anaerobic growth can be cultured from aerobic composts. In both MSW and PS composts, mesophiles were primarily facultative anaerobes, whereas thermophiles were variable and ranged from 0 to 100%. On spread plates, the portion of the microbial population culturable under anaerobic conditions is small compared to the aerobic population. However, growth levels on anaerobic cell-wall, starch, casein and cellulose spot-plates were greater than or equal to levels on aerobic spot-plates 72% of the time. Therefore, it is possible that anaerobic microorganisms in microenvironments within particles may be responsible for a significant portion of the metabolic activity in aerobic composts.

Acknowledgements

The authors express their sincere appreciation to John Bentley, Dennis Gentle, Stepanie McElwain, and Gloria Robinson for their dedication to this project and for the technical assistance each provided. This project was supported in part by PWT Waste Solutions, Inc, Birmingham, AL, and the Department of Biology, University of Alabama at Birmingham, Birmingham, AL, USA.

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