MOLECULAR ASSESSMENT OF INOCULUM EFFICACY AND PROCESS REPRODUCIBILITY IN COMPOSTING USING ARISA

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ABSTRACT. *Traditional analysis of composting process dynamics has focused on changes in physical variables such as temperature, pH, and effluent gas composition. However, to better understand the effect and value of microbial inoculation for reducing process variability, it is necessary to employ techniques that allow for the measurement of changes in community composition. In this study, automated ribosomal intergenic spacer amplification (ARISA) analysis was performed to characterize the ability of inoculated microorganisms from primary effluent wastewater to persist during the initial stages of composting in micro–scale reactors. We found that while the initial microbial communities differed based on inoculation level, after 24 h the microbial community no longer resembled the initial community. In addition, the effect of inoculation was no longer apparent at this point. There was a clear relationship between the variability observed between physical process variables and the composition of the microbial communities. The largest source of variation was due to seasonal changes, suggesting that despite the addition of an inoculum, a source of inoculation that varies between reactor groups is not being controlled. It is hypothesized that this source of inoculation is either from the air being forced through the reactors or from sparsely populated microbial populations in the initial substrate that become active at different rates in different reactor groups. Furthermore, this analysis demonstrates that it is possible to employ a simple molecular technique to understand the effects of inoculation and process reproducibility in a traditional agricultural waste treatment bioprocess.*

Keywords. *Automated ribosomal intergenic spacer analysis (ARISA), Composting, Inoculum, Reproducibility.*

Detailed analysis of microbial communities in engineered ecosystems, such as composts, has tremendous potential to improve process design and yield industrially important biochemicals. However, much of what is currently known about composting microbiology is based on studies that have employed culturing techniques. These methods are problematic since they are limited in their ability to adequately sample the microbial environment. Early studies predicted a community of several bacteria closely related to the *Bacillus* (Strom, 1985a, 1985b), but it has recently been found that only about 1% of bacteria in an environmental sample are culturable (Amann et al., 1995). In light of the inability to culture a sample's entire microbial community, microbiologists have developed a series of molecular identification tools that are based on the sequence variability found within the rRNA genes (rDNA). These three genes code for the nucleic acid component in ribosomes. They are located together in the bacterial genome and are separated by intergenic regions that are highly variable in sequence and in length. Since all organisms contain ribosomes and some version of the rRNA genes, it is possible to compare the 16S rRNA gene sequences of unknown organisms to previously identified organisms. Several of the 16S rRNA gene analysis techniques that exploit this sequence variability have been reviewed elsewhere (e.g., Head et al., 1998).

The application of molecular tools to study agriculturally relevant bioprocesses has been minimal compared to the quantity of soil and marine microbiology studies. One of the few bioprocesses to be studied using these tools is composting. Several investigations have used molecular tools to assess the dynamics of microbial populations during composting (Ishii et al., 2000; Peters et al., 2000), while others have been merely descriptive (Dees and Ghiorse, 2001; Kowalchuk et al., 1999; Malik et al., 1994). A number of factors have contributed to the limited number of publications that have applied these techniques to bioprocesses, including the difficulty in extracting nucleic acids from samples contaminated with humic substances (Miller et al., 1999), poor experimental reproducibility (Michel Jr. et al., 1996; Schloss and Walker, 2001), and the sheer complexity of interpreting the results of a highly coupled, nonlinear bioprocess. Despite these limitations, composting provides an excellent experimental system for applying molecular techniques to improve process design.

One of the more straightforward molecular tools that can be applied to understand the relationship between physical process variables and changes in the microbial community is automated rDNA intergenic spacer analysis (ARISA; Fisher and Triplett, 1999). In ARISA, fluorescently labeled polymerase chain reaction (PCR) primers are designed to anneal to conserved regions within the 16S and 23S rRNA genes of

bacteria. Since the intergenic region is highly variable in length, when it is amplified using PCR and observed on a polyacrylamide gel, a series of fragments varying in length is observed (Fisher and Triplett, 1999).

We previously hypothesized that part of the observed process variability common to composting was due to variability in the initial inoculum (Schloss et al., 2000; Schloss and Walker, 2000). In these earlier studies, the inoculum was introduced primarily through the added moisture, the substrate, and the air used to aerate the substrate. Since replicate reactors initiated at different times showed high levels of variability in physical measurements, it was hypothesized that there was a salient variable that was not being controlled. An initial study showed that a wastewater inoculum resulted in slightly improved process reproducibility compared to non–inoculated reactors (Schloss and Walker, 2000). While the finding of reduced variability was an important conclusion, the inability to significantly affect the temporal changes in important process variables has been repeatedly observed in other studies (Golueke et al., 1954; Schloss and Walker, 2000). Process reproducibility was so poor that it was not possible to detect differences of 15˚C reliably using statistical tests.

This study built on our previous studies by using ARISA to assess qualitative differences between the microbial community compositions of reactors operated at different times. In addition, the effect of a wastewater inoculum on community composition variability was investigated. The primary objective of this study was to test the hypothesis that the initial microbial community structures in reactors operated at different times was different. ARISA was used to determine possible explanations for the inability of inoculums to affect the trajectory and variability of process variables in composting studies. We also demonstrated that a simple molecular tool can be used to better understand the microbial ecology of an important bioprocess in an unbiased manner.

MATERIALS AND METHODS

SUBSTRATE PREPARATION

A synthetic food waste substrate was created by combining Big Red Puppy Food (Pro–Pet, Syracuse, N.Y.) with maple wood chips (Costal Lumber, Cayuta, N.Y.) to obtain a carbon–nitrogen ratio of 18:1 (VanderGheynst et al., 1997). Before loading the substrate into the reactors, the moisture content was raised to 55% (wet basis, w.b.) with the appropriate inoculum mixture. Primary effluent wastewater was obtained from the Cayuga Heights, New York, wastewater treatment plant during the first week of September 2000. Aliquots of wastewater were stored at –20˚C between experimental trials. The viability of the inoculum was confirmed by plating wastewater samples on R2A agar plates. Each reactor received 240 g of wet substrate, and the remaining substrate was analyzed for its moisture content and pH and stored at –20˚C.

REACTOR OPERATION

The reactor system utilized in this study has been described elsewhere (Beltz, 2000; Richard and Walker, 1998). Each 500 mL reactor was placed within a section of pipe insulation with a 9 × 9 cm piece of foam insulation (12.7 mm thick with an R–value of 10.5 K/W, Armaflex AP, Lancaster, Pa.) as a lid. The reactors were autoclaved prior to each trial. Nine reactors were housed in an upright incubator (model 1560, Sheldon Manufacturing, Cornelius, Oregon) with the temperature controller disabled so the reactors would auto–heat. Humidified air was passed through each reactor at a rate of 150 mL/min.

Following each trial, the contents of each reactor were unloaded and mixed by hand until the material was homogenized. Three 2 g samples of the initial substrate and of the processed compost were mixed with 3 mL of deionized/distilled H_2O (ddH_2O) and the pH was immediately determined (Corning Science Products, Corning, N.Y.). The average of the three values was recorded as the pH for the entire reactor. The remaining substrate and compost was frozen.

EXPERIMENTAL DESIGN

Since it was not possible to remove samples from the reactors without altering the structure of the organic matrix, the entire contents of each reactor were used for pH and moisture content determinations and for DNA extraction. In order to create a time course for pH, moisture, and biological data, reactor runs were ended after 12, 24, 36, 48, 60, 72, 84, or 96 h (fig. 1). For each time span, three trials were carried out, with the second trial initiated 24 h after the conclusion of the initial trial, and the third trial initiated 50 days after the conclusion of the initial trial. The date of the initial trial for each time span was randomly assigned, and all trials were performed between 14 September and 19 December 2000. Within each trial, three replicates for each of the three treatments were used to address variability found among sets of reactors initiated at the same time. A total 216 separate reactors were operated during this study. Three levels of wastewater inoculation (0%, 50%, and 100%) were obtained by mixing the wastewater and tap water and using this mixture to raise the initial moisture content of the substrate (7% w.b.) to 55% (w.b.).

DNA EXTRACTION

A 5 g homogenized compost sample was combined with 5 g of 170 to 180 μm diameter glass beads, 10 mL of TE buffer [10 mM Tris–Cl (pH 8.0), 1 mM EDTA (pH 8.0)], and 500 μL of 10% SDS in 50 mL conical centrifuge tubes. The samples were thoroughly mixed by hand and incubated for 60 min in a 70˚C rotary water bath, with mixing every 10 min. The samples were vortexed twice for 2 min, stored on ice, and centrifuged at 4500× g and 10˚C for 10 min in a Marathon 21K/BR benchtop centrifuge (Fisher Scientific, Inc., Hampton, N.H.). Supernatants were recovered and transferred to fresh 50 mL conical centrifuge tubes, which were stored on ice. An additional 5 mL of TE was used to resuspend compost debris, and samples were centrifuged as before for 5 min. The second set of supernatants was combined with the first. This step was repeated a third time, and the pooled samples were centrifuged at 4500× g and 10˚C for 10 min. DNA was purified and precipitated by following the CTAB (hexadecyltrimethyl ammonium bromide) extraction with polyethylene glycol precipitation procedure (Ausubel et al., 1987). The resulting DNA pellet was resuspended in 1 mL of TE. The remaining humic acids were removed by transferring 250 μL of the DNA solution to a 1.5 mL micro–centrifuge tube with
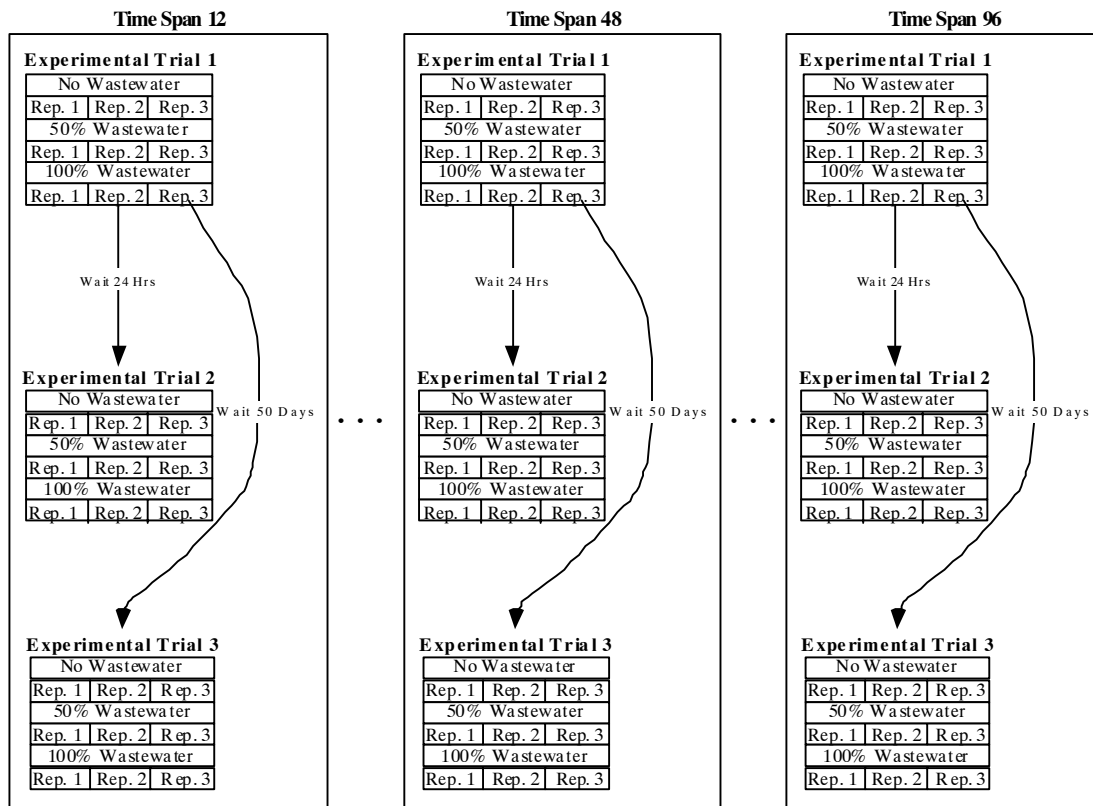
Figure 1. Graphic description of experimental design used to construct a 96 h time course of the composting process. Each block represents the experimental trials and reactors run for each of the eight time spans.

an equal volume of glass milk (Express Matrix, Bio101, Inc., Vista, Cal.). Purification using the glass milk followed the manufacturer's protocol, and samples were resuspended in ddH$_2$O. This extraction and purification procedure was performed in triplicate for each reactor's finished compost and initial substrate material. The DNA from the three extractions was pooled for subsequent analysis to reduce intra–sample variability.

ARISA

The ARISA–PCR protocol of Fisher and Triplett (1999) was employed in this study with several modifications. The 2× *Taq* PCR Master Mix (Qiagen, Inc., Valencia, Cal.) was used in a final reaction volume of 20 μL. The final concentrations of the master mix constituents were 1.0 U of *Taq* DNA polymerase, 1× PCR Buffer, 1.5 mM MgCl$_2$, and 200 μM each dNTP. Approximately 30 ng of DNA, 0.5 μM of each primer, and ddH$_2$O were added to each reaction. The bacterial–specific primers and thermalcycling program were the same as those used previously (1406f, 5'–TG(C/T)ACA-CACCGCCCGT–3' and 23Sr, 5'–GGGTT(C/G/T)CCCCA TTC(A/G)G–3', PTC–100 Thermalcycler, MJ Research, Waltham, Mass.; Fisher and Triplett, 1999). Primer 1406f was end labeled with the fluorescent dye TET.

Samples were analyzed on an ABI 377 DNA sequencer with Long Ranger polyacrylamide gels (Perkin–Elmer, Inc., Boston, Mass.) at the Cornell University Bio–Resource Center. Fragment size determination was performed by comparison to an internal rhodamine–labeled size standard (MapMarker 1000, Bioventures, Murfreesboro, Tenn.). Elec-

tropherograms were generated using GeneScan and analyzed using GenoTyper (Perkin–Elmer, Inc., Boston, Mass.).

STATISTICAL ANALYSIS

Nested analysis of variance (ANOVA) was used to test the differences in the final temperature, moisture content, concentration of O$_2$ and CO$_2$, and pH recorded for each reactor run between treatments using the SPSS statistical package (SSPS, Inc., Chicago, Ill.; Schloss et al., 2000; Sokal and Rohlf, 1995). A power analysis was performed as described previously to determine the ability to detect a physically meaningful difference as being statistically significant (Lindman, 1991; Schloss and Walker, 2000).

COMMUNITY STRUCTURE COMPOSITION ANALYSIS

ARISA electropherogram peaks between 390 and 900 base pairs (bp) with intensities greater than 5% of the greatest peak intensity were scored as 1, while those below 5% were scored as 0. Justification for only including peaks between 390 and 900 bp is discussed elsewhere (Fisher and Triplett, 1999). Fragments scored as 1 are described as OTUs (operational taxonomic units), and the assemblage of OTUs for a sample describes the microbial community composition. The Jaccard distance coefficient (D–value) was calculated as a value between 0 and 1 describing the difference found between two community compositions (Legendre and Legendre, 1998):

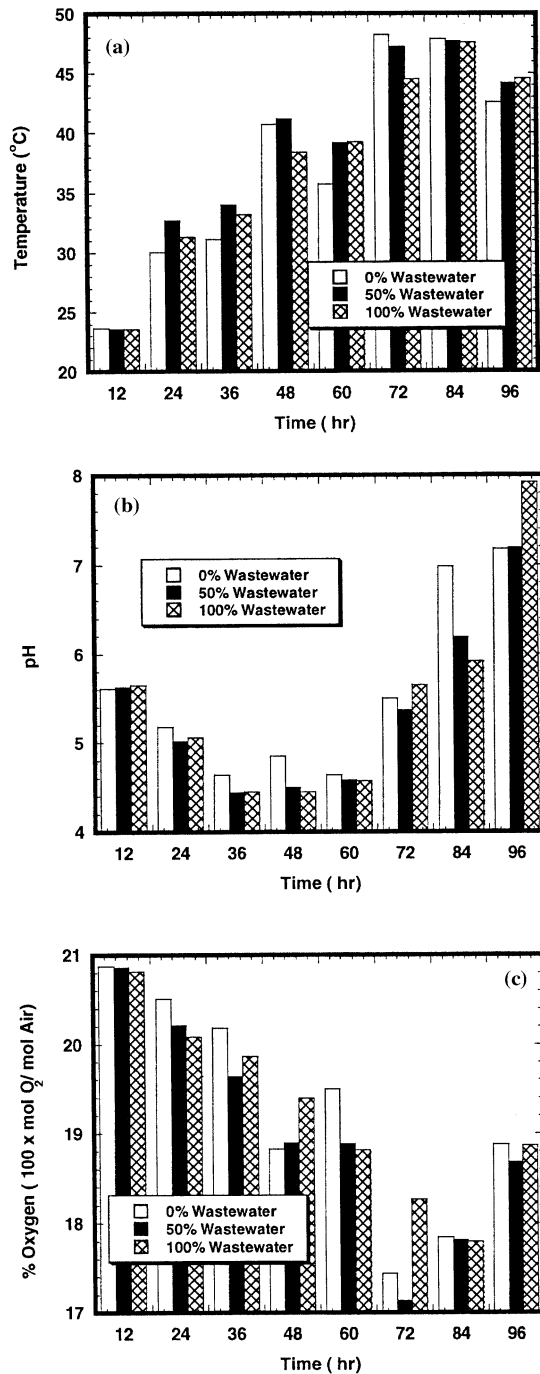$$D(i,j) = 1 - \frac{a}{a+b+c} \qquad (1)$$

Figure 2. Changes in average temperature, pH, and effluent O_2 concentration. No significant differences were found between treatments. Each bar represents the average of nine reactors.

where

a = number of fragments contained in both samples

b = number of fragments contained in sample i, but not in sample j

c = number of fragments contained in sample j, but not in sample i

The resulting matrix was symmetric about a diagonal of zeros and was interpreted with dendrograms, which were generated by TreeView (version 1.6.5) using the unweighted pair–group method using arithmetic averages (UPGMA) algorithm contained within PHYLIP (version 3.5). The
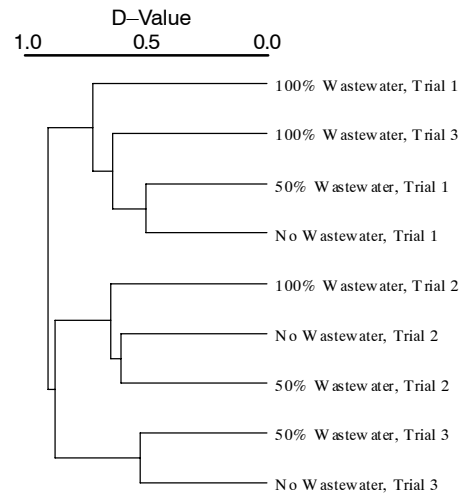

Figure 3. Differences in initial community structure compositions found in the substrate used for reactors run 96 h.

scales above each dendrogram describe how different two samples or clusters are from one another. A low value (i.e., below 0.5) indicates that two samples are more similar to each other, while a high value indicates that they are different.

RESULTS AND DISCUSSION

PROCESS DYNAMICS

Despite the higher level of inoculation used in this study, inoculation had no statistically significant effect on temperature, O_2 and CO_2 concentration, or pH at any time during the process (all $p > 0.18$, fig. 2; CO_2 data not shown). However, there was insufficient power to detect meaningful physical differences between treatments (all power < 0.30). Therefore, it was not possible to conclude with confidence what effect inoculation had on changes in the measured physical parameters. These results are similar to those found in our previous study (Schloss and Walker, 2000).

INITIAL MICROBIAL COMMUNITY STRUCTURE

Dendrograms were used to graphically cluster groups that have similar characteristics based on information contained within a distance matrix. While the interpretation of dendrograms can appear overwhelming, it is easiest to think of their structure as being similar to a family tree. For example, the initial samples collected from reactors run for 96 h are shown in figure 3. The samples that are clustered together are most similar to each other, e.g., "No Wastewater, Trial 1" and "50% Wastewater, Trial 1" have a D–value of 0.44. But samples that do not form a cluster are more distantly related, e.g., "No Wastewater, Trial 1" and "100% Wastewater, Trial 2" have a D–value of 0.86. Even though "No Wastewater, Trial 1" and "100% Wastewater, Trial 2" are ordered consecutively in this figure, in order to compare the two samples, it is necessary to trace the dendrogram branches to the common node and then to the second sample being compared.

Dendrograms comparing the community composition of the initial composting material showed segregation between the three different experimental trials. Typical segregation among the initial samples collected for each time point is
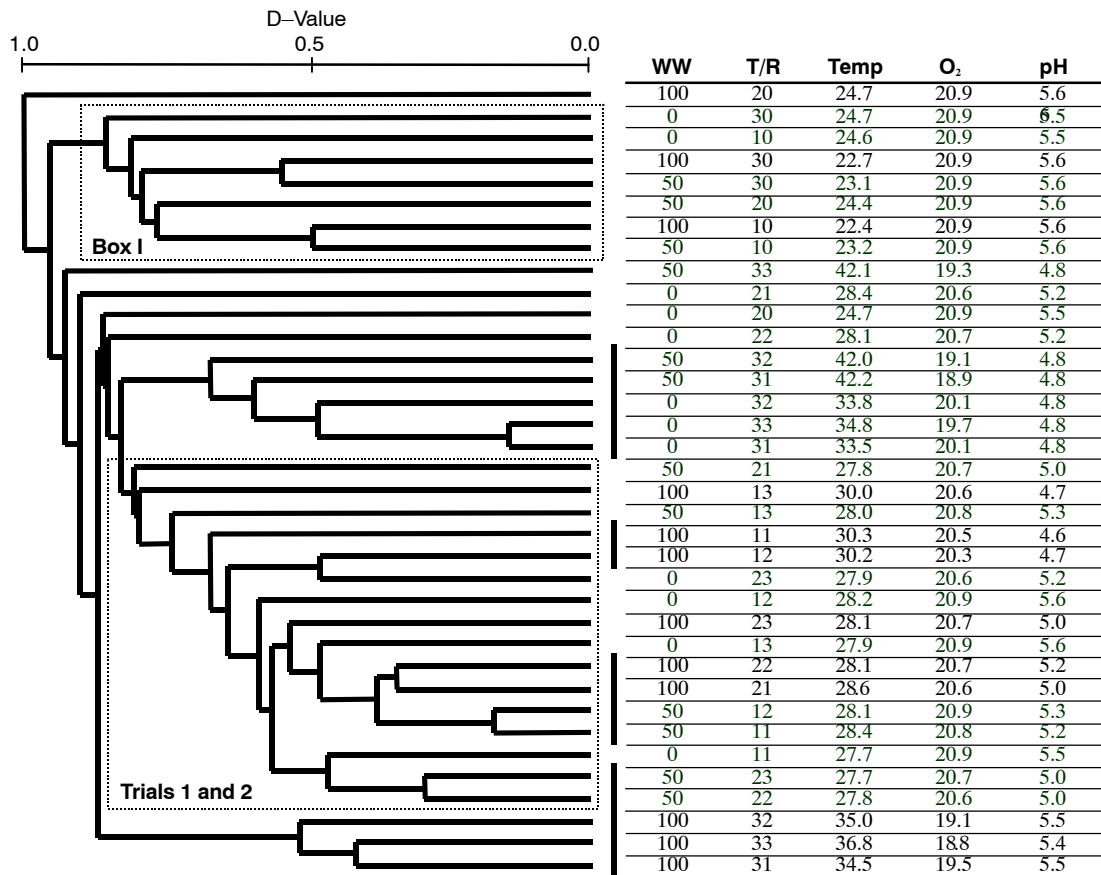
Figure 4. Differences between community structure compositions at the beginning and end of 24 h reactor runs with a table describing each sample's environment. "Box I" outlines the cluster of initial composting material and their segregation from the other samples. "Trials 1 and 2" outlines the segregation found among the different experimental trials performed. The vertical bars between the dendrogram and the table group replicates of the same treatment and trial that segregate together. "WW" denotes the percentage of wastewater used (0%, 50%, or 100%), and "T/R" denotes the experimental trial and replicate number (i.e., T/R 32 means replicate 2 of trial 3).

WW	T/R	Temp	O$_2$	pH
100	20	24.7	20.9	5.6
0	30	24.7	20.9	6.5
0	10	24.6	20.9	5.5
100	30	22.7	20.9	5.6
50	30	23.1	20.9	5.6
50	20	24.4	20.9	5.6
100	10	22.4	20.9	5.6
50	10	23.2	20.9	5.6
50	33	42.1	19.3	4.8
0	21	28.4	20.6	5.2
0	20	24.7	20.9	5.5
0	22	28.1	20.7	5.2
50	32	42.0	19.1	4.8
50	31	42.2	18.9	4.8
0	32	33.8	20.1	4.8
0	33	34.8	19.7	4.8
0	31	33.5	20.1	4.8
50	21	27.8	20.7	5.0
100	13	30.0	20.6	4.7
50	13	28.0	20.8	5.3
100	11	30.3	20.5	4.6
100	12	30.2	20.3	4.7
0	23	27.9	20.6	5.2
0	12	28.2	20.9	5.6
100	23	28.1	20.7	5.0
0	13	27.9	20.9	5.6
100	22	28.1	20.7	5.2
100	21	28.6	20.6	5.0
50	12	28.1	20.9	5.3
50	11	28.4	20.8	5.2
0	11	27.7	20.9	5.5
50	23	27.7	20.7	5.0
50	22	27.8	20.6	5.0
100	32	35.0	19.1	5.5
100	33	36.8	18.8	5.4
100	31	34.5	19.5	5.5

shown in figure 3 for reactors run 96 h. Inoculation level did not substantially affect the segregation pattern, indicating that the inoculum was not sufficiently dense to impact the initial microbial community composition.

However, when the community composition of the initial samples is compared to the community composition observed at any time after 24 h, there was a clear distinction between the community structure composition found in the initial and composted samples (e.g., fig. 4). In addition, there was no discernable effect of inoculation on community composition segregation at any time point.

PROCESS REPRODUCIBILITY

Because replicates within a trial showed high levels of reproducibility in previous studies, it was hypothesized that trials performed within a short time span would be more reproducible than trials with a long interval between them. This hypothesis was addressed by performing a pair–wise power analysis of each trial at each time point. The median power to detect a 5°C temperature difference using the two trials performed 24 h apart was 0.38, while it was below 0.17 for both of the comparisons made with the trial performed 50 days after the first. While not as pronounced, the same effect was found for power analyses performed using pH and O$_2$ and CO$_2$ concentration data. While the decreased time between trials did not produce acceptable power levels, the power did increase measurably. The high level of variability

between trials was largely dependent on the timing of the trials. This led to the hypothesis that the initial variability in microbial community structure was a salient source of process variability.

At all time points, the primary difference in community composition was typically due to the timing of trials. Variability between trials was high at all time points, as indicated by the consistent clustering of intra–trial replicates (see box "Trials 1 and 2" in fig. 4). The vertical bars between the dendrogram and the environmental conditions in figure 4 group replicates of a single treatment within a trial. Similar to the physical data results, replicates within a trial were more likely to cluster with one another than with similar replicates from other trials.

As shown in figures 3 and 4, however, the D–values between replicates were generally high. Upon inspection of figure 5, it appears that the analysis inflates the difference between samples. For example, the D–values between initial samples collected from trial 2 were between 0.52 and 0.57 (table 1), while the electropherograms in figure 5 for those samples appear identical. While some variability between replicates performed simultaneously was expected, the ability of PCR to amplify fragments at low concentrations probably resulted in the inclusion of sparsely populated OTUs in the analysis. Amplification of sparsely populated OTUs could be variable from sample to sample, resulting in the larger than expected D–values. Our analysis included
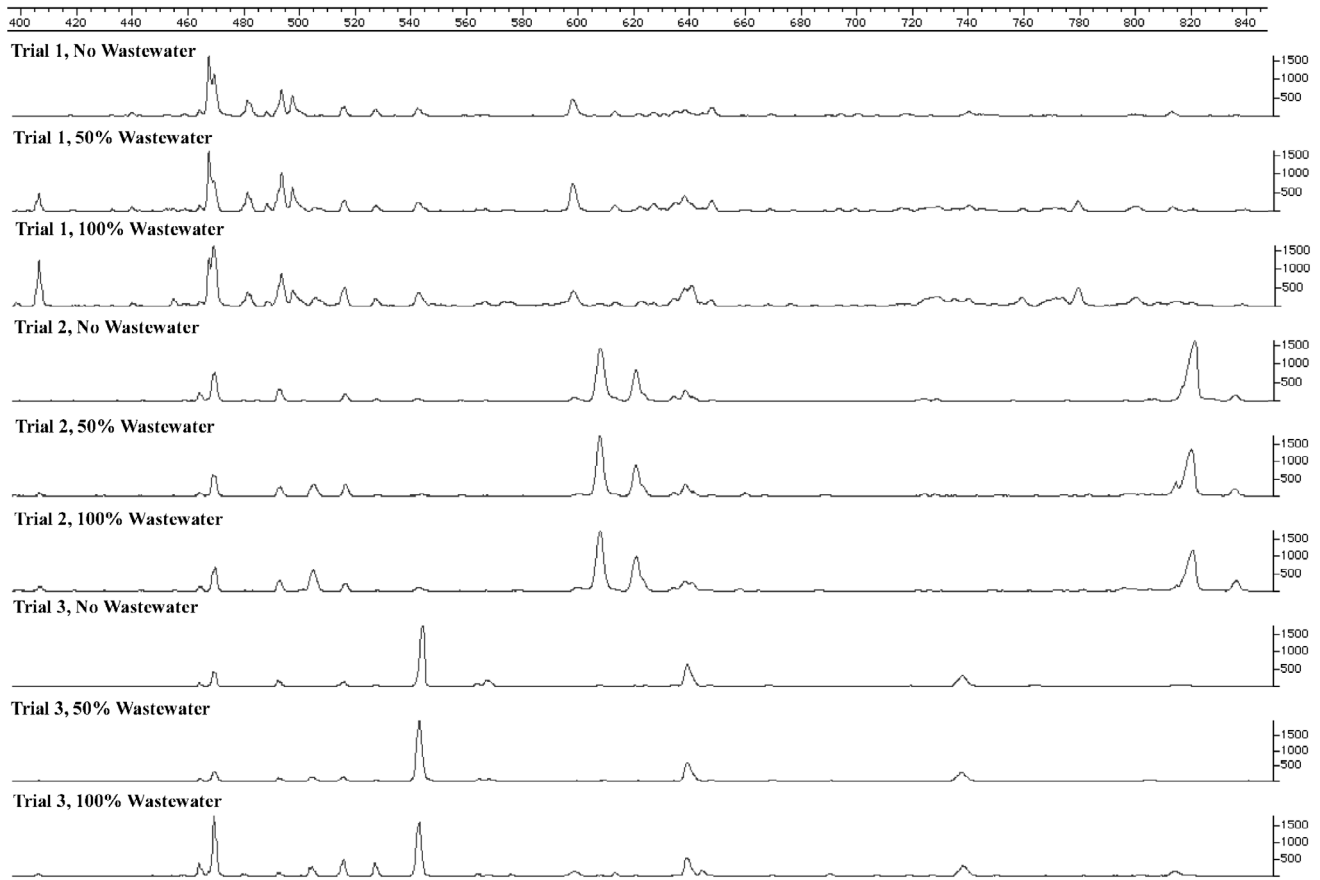
Figure 5. Electropherogram of initial composting substrate OTU composition in inoculated and non–inoculated reactors. Horizontal axis is expressed in number of DNA base pairs, and vertical axes are the relative abundance of each fragment in the final PCR mixture.

Table 1. D–values between the microbial community compositions observed in the initial samples used to load the reactors run for 96 h and are expressed graphically as a dendrogram in figure 3.

		No WW[a]			50% WW			100% WW		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
No WW	Trial 1	0.00	0.83	0.83	0.44	0.89	0.90	0.63	0.86	0.53
	Trial 2	0.83	0.00	0.70	0.76	0.52	0.79	0.81	0.57	0.70
	Trial 3	0.83	0.70	0.00	0.79	0.75	0.45	0.88	0.79	0.68
50% WW	Trial 1	0.44	0.76	0.79	0.00	0.75	0.81	0.55	0.74	0.58
	Trial 2	0.89	0.52	0.75	0.75	0.00	0.78	0.85	0.56	0.74
	Trial 3	0.90	0.79	0.45	0.81	0.78	0.00	0.92	0.81	0.70
100% WW	Trial 1	0.63	0.81	0.88	0.55	0.85	0.92	0.00	0.81	0.75
	Trial 2	0.86	0.57	0.79	0.74	0.56	0.81	0.81	0.00	0.77
	Trial 3	0.53	0.70	0.68	0.58	0.74	0.70	0.75	0.77	0.00

[a] WW = wastewater.

OTUs if their peak represented at least 5% of the most intense peak in the sample, while others included peaks that represented at least 5% of the total signal for a sample (Fisher and Triplett, 1999). For samples with a large OTU richness, the former method is best since it is less likely to exclude physiologically important OTUs.

SOURCES OF EXPERIMENTAL TRIAL VARIABILITY

While undocumented, it has been previously hypothe–sized that the influent air stream and the water used to moisten the substrate were the primary sources of inoculation in composting. In addition, it has been assumed that the maple wood chips and dog food would be relatively sterile or at least contain stable microbial diversity over time because of the low moisture content (7% w.b.). However, in light of the results shown in figure 4, it appears that the primary source of microbial variability is the substrate, indicating that our previous assumption was incorrect.

Considering the substrate was stored in an area that has poor environmental control and that is exposed to an external wall, it was hypothesized that changes in temperature could account for changes in microbial diversity. In order to test this hypothesis, we incubated separate dog food and wood chip samples at 4°C and 30°C. These samples were then homogenized in phosphate–buffered saline, and the number of colony forming units (cfu) was determined by plating

dilutions on agar plates containing heterotrophic growth agar. Fungal colonies created a lawn on the wood chip plates but were not observed on the dog food plates. Additional plates containing 100 µg/mL of cyclohexamide, a eucaryotic growth inhibitor, were then used for plate counts so that fungal populations could not grow. Plate counts of bacteria isolated from dog food samples stored at 4°C and 30°C were 6.7×10^2 and 5.7×10^3 cfu/g of dog food, respectively, while there were 3.2×10^3 and 1.3×10^4 cfu/g of wood chips at 4°C and 30°C, respectively. These data indicate why the wastewater inoculum failed to have a significant effect on the initial microbial communities. Plate counts showed there were approximately 10^3 cfu/mL in the wastewater. Considering the mix of dog food, wood chips, and wastewater was roughly 1:1:2, the organisms contained within the wastewater only accounted for between 10% and 35% of the initial microbial population. The relatively large number of fungi and bacteria that were already colonized in the initial substrate would have had a competitive advantage in the composting ecosystem over those introduced through the wastewater.

In addition, DNA was directly extracted from the dog food and wood chip samples and amplified using the 1406f–23Sr primer pair in order to observe if there was a difference in the community composition growing on the substrates incubated at 4°C and 30°C. Figure 6 shows the banding pattern of these samples on an agarose gel. While there did not appear to be an effect of incubation temperature on the community composition within dog food, there was a clear difference for the wood chips. There are two 1406f–23Sr PCR fragments in the wood chip sample incubated at 30°C approximately 600 and 750 bp long that were not found for the sample incubated at 4°C. It is possible that visualization of the PCR products with an ABI 377 sequencing gel would have shown even more differences.

Based on plate counts and fingerprinting analysis, it is clear that the difference in storage temperature had a profound effect on the microbial diversity of the initial dry substrate. While the differences between 4°C and 30°C are extreme, it is expected that much of the process variability would be reduced if the substrate were stored at a constant temperature in future experiments.

ASSESSMENT OF ARISA

Of the 288 ARISA electropherograms collected, all but one contained discernable peaks. This indicated that the DNA purification technique reliably yielded high–quality DNA for PCR reactions. Among the 288 samples analyzed by ARISA, 373 unique OTUs were found. On average, there were 16.6 OTUs per sample (s.d. = 10.0). The average OTU was represented in 12.9 samples (s.d. = 21.6) and did not change with treatment or time. This level of OTU richness agrees with previous studies in which compost samples were analyzed using denaturing gradient gel electrophoresis (Ishii et al., 2000) and single–strand–conformation polymorphism (Peters et al., 2000).

This analysis was made possible by the use of a digital data acquisition system that could detect the presence of fluorescent DNA fragments. Use of this system allowed for the differentiation between two OTUs that were similar in length. The same accuracy would not have been possible using traditional agarose or manually inspected acrylamide gels. PCR–based methods such as ARISA have several shortcomings. When PCR is used to amplify a heterogeneous set of templates within a single reaction, the final product mixture proportions can be different from the initial com–
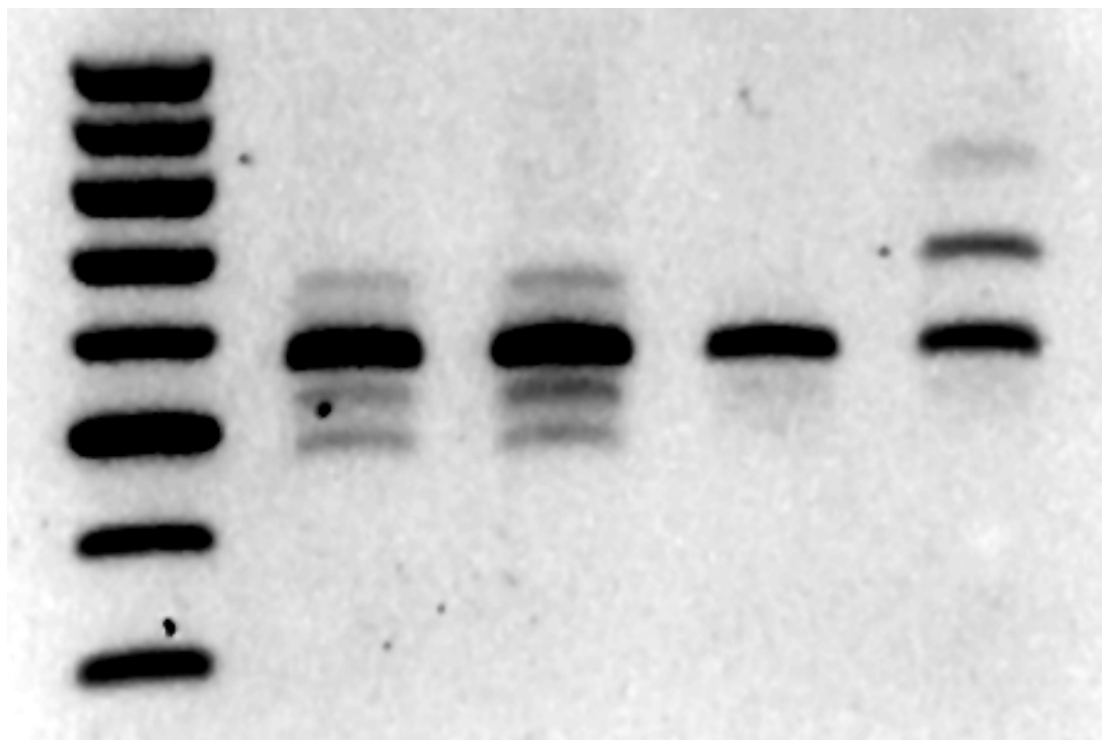

Figure 6. rDNA intergenic spacer amplification products using 1406f–23Sr primer pair to amplify DNA extracted from dog food incubated at 4°C (lane 2) and 30°C (lane 3) and from wood chips incubated at 4°C (lane 4) and 30°C (lane 5). Size marker in lane one steps from 300 to 1000 bp in 100 bp increments.

position (Chandler et al., 1997; Suzuki and Giovannoni, 1996). PCR amplification does not perfectly amplify the template DNA by doubling the product concentration after each cycle (Chandler et al., 1997; Suzuki and Giovannoni, 1996). Both limitations make it impossible to determine the proportional abundance of individual populations in a community using PCR. Occasionally, ARISA of a single microbial strain can produce multiple fragments because bacteria typically have multiple copies of the rRNA genes within their genome. In addition, analysis of two different organisms can occasionally yield fragments that are the same length (Garcia–Martinez et al., 1999). However, fragment size redundancy is also a limitation with other PCR–based community analysis techniques (Moyer et al., 1996).

In spite of these limitations, ARISA has several advantages. First, other fingerprinting methods require a restriction digest step, making the ARISA analysis faster and more efficient. Second, ARISA makes use of a sequencing gel interfaced with a digital data acquisition system, allowing resolution of single base pair differences between fragments. This circumvents the fragment resolution problem typically encountered in gradient–based electrophoresis techniques such as temperature and denaturing gradient gel electrophoresis (T/DGGE) (Muyzer et al., 1993). Finally, the banding profiles (i.e., electropherograms or fingerprints) generated by this data acquisition system can be easily converted into a numerical description of the microbial community composition, allowing for a simple yet highly resolved analysis of the community composition among many samples.

CONCLUSION

In this study, we have shown that the OTUs initially populating the reactors do not persist into the process. Independent of inoculum type, previous studies have been unable to improve process dynamics through inoculation. However, researchers have been unable to determine whether this is due to the lack of environmentally fit microorganisms or because the inoculum was not sufficiently dense. Here we have shown that the most easily amplified populations in the initial substrates, which are perhaps the most abundant populations, do not persist during the initial stages of the process since the community composition found after 24 h shows little resemblance to the initial community composition (fig. 4). In addition, the inability of the inoculated organisms to substantially effect the initial community structure composition indicates that the inoculum, which represents 10% to 35% of the initial microbial population, was not sufficient to dominant the evolution of the microbial community structure (fig. 5). This inconclusive outcome might have been different if an approach were used to identify which population are the most dominant during the initial stages and to ensure that they are present in sufficient numbers. Under this scenario, researchers should be able to develop a better inoculum. In addition, by controlling all sources of variability in the initial substrate, including the temperature and humidity of the environment surrounding the dog food and wood chips, the process should be more reproducible.

The presence of a diverse microbial community in the initial substrate may also account for some variability. The composting substrate is an ideal medium for a broad spectrum of ubiquitous microorganisms that can grow in a moist, nutrient–rich, aerobic environment. Therefore, the substrate would not exert a strong selection pressure on the microbial populations. Considering the well–documented non–linear nature of the process, it is possible that slight differences in initial conditions or reactor operation would permit different populations to dominate the substrate to the exclusion of others in different reactor settings. This study has shown, however, that the composting process is robust. Despite significant differences in the microbial community composition, all reactors reached the elevated temperatures and experienced the swing in pH that characterizes the early stages of composting (fig. 2).

The data presented in this study were consistent with our previous investigations on the effects of inoculation on process dynamics. The slight increase in reproducibility as measured by statistical power indicated that if a denser inoculum is used in the future, substantial increases in process reproducibility should be observed. The molecular analysis performed in this study will be useful for future studies investigating the relationship between microbial ecology of composts and process reproducibility.

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