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Measurement of process performance and variability in inoculated composting reactors using ANOVA and power analysis

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Abstract

The variability associated with composting can limit the ability to detect statistically significant differences between treatments. This study investigated the effect of a wastewater inoculum on the process dynamics and variability associated with temporal changes in temperature, moisture content, and effluent oxygen concentration, as well as spatial changes in temperature and moisture content. Statistical tests suggested that the inoculum had little effect on the above variables although the statistical power or the ability to detect statistical differences for small, medium, and large differences between treatments for temperature and moisture content was typically below 80% for the differences tested. Comparison of the temperature data showed that the inoculum was able to decrease the experimental variation, but had no positive effect on moisture content variability. Calculation of the statistical power associated with temperature data from a previous study showed that the statistical power found for this study was much higher than typical composting studies because of the decreased variability caused by the inoculum. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Composting; Bench-scale; Power analysis; ANOVA; Inoculum

1. Introduction

Composting is characterized by the degradation of heterogeneous organic wastes by a mixed culture of aerobic microorganisms. Statistical studies of experimental results have reported large variability in replicated state variables, such as temperature, oxygen concentration, and moisture content [1-3]. This variation makes it difficult to infer true effects between experimental treatments and limits the ability to infer how changes in operational and design variables cause changes in process behavior. The ability to detect these differences is called statistical power [4,5].

As the result of an earlier study [3], it was hypothesized that the use of an inoculum from a single source might increase the statistical power to detect true differences between treatments and improve process dynamics. The objective of this investigation is to assess the

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influence of a wastewater inoculum on composting process dynamics and the ability to detect real differences between treatments. Past studies involving the effects of an inoculum on process variables have used previously generated compost [6-13], soil [1,6,14,15], cultured microbial populations [15-19], manure [6,20], commercial 'starters' [6,11,13,21] and chemical additives [22] to increase the populations of effective microbial groups. A common conclusion from most of these studies is that an inoculum increases the rate of metabolic activity during the climb to higher temperatures. However, they also found that as the process continues, the differences in degradation and time spent at elevated temperatures were negligible when the extra operational costs incurred from creating and introducing the inoculum were considered [6,11,13,21]. A survey of the current literature concerning the effects of an inoculum on the composting process revealed no studies that used wastewater as an inoculum. Wastewater was expected to help accelerate the composting process since wastewater treatment processes exploit microorganisms to aid in the degradation of its contaminants.

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Fig. 1. Diagram of bench-scale aerated static bed reactor.



Fig. 2. Temperature profiles representing average values for each reactor and experiment for inoculated and non-inoculated reactors at 30 cm above the reactor floor. No significant differences were detected between treatments at any time or height within the reactors.



Fig. 3. Average temperature spatial gradients at 108, 192, and 264 h for inoculated and non-inoculated reactors. All inoculated gradients presented are significant while only the gradient at 192 h in the non-inoculated is significant.



Fig. 4. Within experiment mean squared error values (MS_{within}) from each treatment for statistical tests to detect temperature spatial gradients.

2. Methods and materials

2.1. Substrate preparation

Big Red Puppy Food (Pro-Pet, Syracuse, NY, USA) was mixed with air dried maple wood chips (Coastal Lumber, Cayuta, NY, USA) to obtain a carbon to nitrogen (C:N) ratio of 18 and a dry bulk density of 280 kg/m³. The moisture content of this mixture was 7% wet basis (w.b.). Detailed descriptions of preparing the reactor feed are provided elsewhere [23]. The initial substrate for non-inoculated reactors was brought to its initial moisture content (51-55% w.b.) with 100% tap water and the inoculated reactors used a primary

wastewater-tap water mix (1:3) to bring the substrate to the same moisture content as those using tap water exclusively. Primary wastewater was obtained from the Cayuga Heights (Cayuga Heights, NY, USA) wastewater plant during the first week of December 1998. Between the beginning of the first and second experiments, the wastewater was stored at -20° C.

2.2. Reactor configuration

Fig. 1 is a drawing of the 30 l bench-scale reactors used in this investigation. The body of the reactors was made of schedule 40 PVC pipe. Thermocouples were inserted at heights of 10, 20, 30 and 40 cm above the



Fig. 5. Between experiment mean squared error values (MS_{exper}) from each treatment for statistical tests to detect temperature spatial gradients.



Fig. 6. Average moisture content profiles at 10 and 30 cm above the reactor floor for inoculated and non-inoculated reactors. Vertical bars represent significant differences that were found between the two treatments.



Fig. 7. Average moisture content spatial gradients at 120, 168, and 408 h into the process. All gradients are significant and the magnitudes of the gradients at 168 are the highest for the entire process for both treatments.



Fig. 8. Within experiment mean squared error values (MS_{within}) from each treatment for statistical tests to detect moisture spatial gradients.

perforated plate. Solids sampling ports were also located at heights of 10, 20, 30, and 40 cm above the perforated plate. Two O_2 sensors were located in the effluent air stream. Compressed air was used to aerate the reactor at a flow rate of 5.25 lpm and a temperature of 23.9°C (S.D. = 2.5). Details of the reactor design, control, and data acquisition can be found elsewhere [3].

2.3. Data analysis: hypothesis testing

The method of testing for differences for this study was a nested mixed design analysis of variance (ANOVA) [24]. The sums squared of the errors (SS) and mean squared errors (MS) for a nested analysis of variance (ANOVA) test were calculated every 12 h as described previously [3]. Briefly, the data recorded in the 2 h prior to the point of interest were pooled in order to increase the total degrees of freedom. For example, if an ANOVA were to be performed at 24 h, the analysis at 24 h would include data from 22.00, 22.50, 23.00, 23.50 and 24.00 h, since the data was recorded every half-hour. The pooled data was analyzed by a runs-test to insure that the data was independent [3]. It was not possible to pool the moisture content data since it was only collected every 24 h. There were a total of 35 groups of temperature and oxygen concentration data and 18 groups of moisture content data.

The effect of the inoculum on temporal variations for temperature, moisture content, and effluent oxygen



Fig. 9. Between experiment mean squared error values (MS_{exper}) from each treatment for statistical tests to detect moisture spatial gradients.



Fig. 10. Average percent of oxygen in the effluent air stream for inoculated and non-inoculated reactors. Significant differences detected between 24 and 166 h into the process.

concentration was determined using two treatments (i.e. inoculated/non-inoculated), two experimental groupings (i.e. December and January), two reactors per experimental grouping, and five data points per reactor which resulted in 39 total degrees of freedom. Separate ANOVAs were performed at each height for temperature and moisture content data. To detect the formation of temperature and spatial gradients in the reactor for each treatment there were three treatments (i.e. 10, 20 and 30 cm), two experimental groupings (i.e. December and January), two reactors per experimental grouping, and five data points per reactor resulting in 59 total degrees of freedom. Because of a limited number of O_2 sensors, it was not possible to perform a balanced nested ANOVA with oxygen data. Instead, a one-way ANOVA was performed to detect differences in O_2 concentration between the two treatments [24]. Under this design, there were two treatments (i.e. inoculated/ non-inoculated), three reactors and the five points pooled at each 12-h increment for each of the reactors so that there were a total of 29 degrees of freedom. All significance tests were conducted using an α , the probability of falsely rejecting the null hypothesis, of 0.05.

In order to determine the variability between treatments, between each experiment, and within each experiment, as well as to perform the ANOVA, the total SS were partitioned into possible sources of variation. These sources of variation included variation due to the treatment, variation due to separate experiments, and the variability within each experiment. The MS for each source of variation at each 12-h increment was calculated using Eq. (1) through Eq. (3).

$$MS_{treat} = \frac{nb}{a-1} \sum_{i=1}^{a} (\overline{Y}_{A} - \overline{\overline{Y}})^{2}$$
(1)

$$MS_{exper} = \frac{n}{a(b-1)} \sum_{i=1}^{a} \sum_{j=1}^{b} (\overline{Y}_{B} - \overline{Y}_{A})^{2}$$
(2)

$$MS_{\text{within}} = \frac{1}{ab(n-1)} \sum_{i=1}^{ab} \sum_{j=1}^{b} (Y - \overline{Y}_{B})^{2}$$
(3)

where,

Y = value of observation,

 \overline{Y}_{A} = mean value for observations within treatment, $\underline{\overline{Y}}_{B}$ = mean value for observations within experiment,

- \overline{Y} = mean value for entire data set,
- a = number of treatments,
- b = number of experiments,
- n = number of observations per experiment.

2.4. Data analysis: power analysis

Failure to detect a difference between treatments when a true difference exists is a Type II error. The

| Table 1 | | | | | | | | |
|-------------------|--------------|--------------|-----------|------|------|-----|----------|--------------------|
| Mean temperatures | and standard | d deviations | estimated | from | Fig. | 2 o | f Michel | study ^a |

| Time (days) | Piles 1A-1E | | Piles 2A-2E | | Piles 3A-3E | | Overall mean | Overall S.D. | |
|-------------|-------------|------|-------------|------|-------------|------|--------------|--------------|--|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. | | | |
| 3 | 45 | 1.9 | 49 | 2.2 | 59 | 2.2 | 51.0 | 10.8 | |
| 5 | 55 | 2.6 | 60 | 2.6 | 71 | 1.3 | 62.0 | 11.1 | |
| 10 | 57 | 1.7 | 55 | 2.1 | 70 | 1.0 | 60.7 | 8.2 | |
| 13 | 57 | 2.1 | 57 | 1.9 | 69 | 1.3 | 61.0 | 9.1 | |
| 20 | 58 | 2.0 | 56 | 1.8 | 68 | 2.3 | 60.7 | 10.4 | |
| 31 | 60 | 1.8 | 60 | 2.3 | 69 | 1.5 | 63.0 | 9.6 | |
| 33 | 56 | 1.6 | 62 | 2.0 | 71 | 0.8 | 63.0 | 7.5 | |
| 38 | 56 | 1.3 | 61 | 2.0 | 69 | 1.5 | 62.0 | 8.2 | |
| 41 | 55 | 1.2 | 59 | 1.8 | 65 | 2.8 | 59.7 | 9.9 | |
| 48 | 56 | 1.6 | 58 | 1.8 | 67 | 1.7 | 60.3 | 8.7 | |
| 57 | 54 | 1.3 | 55 | 1.6 | 66 | 1.7 | 58.3 | 7.9 | |
| 59 | 53 | 1.2 | 59 | 2.0 | 68 | 1.3 | 60.0 | 7.7 | |
| 63 | 52 | 1.2 | 57 | 1.4 | 69 | 1.2 | 59.3 | 6.5 | |
| 66 | 53 | 1.0 | 59 | 1.4 | 70 | 1.3 | 60.7 | 6.3 | |
| 69 | 52 | 1.5 | 58 | 1.3 | 69 | 1.4 | 59.7 | 7.2 | |
| 74 | 51 | 1.2 | 58 | 1.2 | 64 | 2.1 | 57.7 | 7.7 | |
| 85 | 52 | 1.8 | 53 | 1.0 | 59 | 3.8 | 54.7 | 11.3 | |
| 92 | 43 | 1.3 | 52 | 1.6 | 69 | 2.0 | 54.7 | 8.4 | |
| 99 | 45 | 1.2 | 56 | 1.9 | 65 | 2.0 | 55.3 | 8.7 | |
| 106 | 45 | 1.2 | 55 | 2.0 | 59 | 2.6 | 53.0 | 9.9 | |
| 113 | 44 | 1.5 | 50 | 1.3 | 62 | 2.3 | 52.0 | 8.7 | |
| 136 | 41 | 2.0 | 41 | 2.8 | 50 | 2.3 | 44.0 | 12.1 | |

^a Temperatures expressed as °C.

Table 2

Results of power analysis using temperature profile data showing the number of tests that had an observed statistical power for a given range to detect three effect sizes for this study and the Michel Study^a

| Power | Small effect size 5°C difference | | | | Medium effect size 10°C difference | | | | Large effect size 15°C difference | | | |
|-----------|----------------------------------|-------|-------|--------|------------------------------------|-------|-------|--------|-----------------------------------|-------|-------|--------|
| | 10 cm | 20 cm | 30 cm | Michel | 10 cm | 20 cm | 30 cm | Michel | 10 cm | 20 cm | 30 cm | Michel |
| 0.99– | 3 | 2 | | | 8 | 3 | 1 | | 15 | 9 | 3 | |
| 0.95-0.98 | | 1 | | | 4 | 2 | 1 | | 8 | 1 | 5 | |
| 0.90-0.94 | 2 | | | | | 3 | | | 1 | 1 | 1 | |
| 0.80-0.89 | 2 | | 1 | | 7 | 2 | 4 | | 3 | 4 | 2 | 3 |
| 0.70-0.79 | 1 | | | | 4 | 1 | 2 | | 3 | 5 | 3 | 1 |
| 0.60-0.69 | 2 | | 1 | | 2 | 2 | 1 | | 1 | 1 | 2 | 6 |
| 0.50-0.59 | 2 | 2 | | | 2 | 4 | 3 | | 1 | 5 | 2 | 5 |
| 0.40-0.49 | 2 | 3 | | | 3 | 3 | 3 | 3 | 1 | 1 | 3 | 4 |
| 0.30-0.39 | 6 | 2 | 5 | | 1 | 4 | 1 | 7 | | 5 | 3 | 3 |
| 0.20-0.29 | 6 | 3 | 3 | | 2 | 4 | 5 | 9 | 1 | 1 | 7 | |
| 0.10-0.19 | 5 | 8 | 6 | 3 | 1 | 5 | 10 | 3 | | 1 | 2 | |
| 0.05-0.09 | 4 | 14 | 19 | 19 | 1 | 2 | 4 | | 1 | 1 | 2 | |
| N | 35 | 35 | 35 | 22 | 35 | 35 | 35 | 22 | 35 | 35 | 35 | 22 |
| Mean | 0.425 | 0.264 | 0.177 | 0.0805 | 0.729 | 0.516 | 0.395 | 0.307 | 0.864 | 0.695 | 0.568 | 0.600 |
| Median | 0.34 | 0.14 | 0.08 | 0.08 | 0.81 | 0.47 | 0.27 | 0.30 | 0.98 | 0.76 | 0.51 | 0.60 |
| S.D. | 0.31 | 0.28 | 0.18 | 0.01 | 0.28 | 0.32 | 0.30 | 0.10 | 0.23 | 0.28 | 0.317 | 0.16 |

^a That is, 2 of 35 ANOVAs had a statistical power between 0.90 and 0.94 when attempting to detect a 5°C difference at 10 cm.

probability of detecting a Type II error is called β , and is typically expressed as statistical power or $1 - \beta$. While not universally used, a statistical power of 0.80 is typically considered sufficient. A statistical power of 0.80 means that if an experiment with a real difference between two of its treatments were repeated ten times, the difference would be detected eight times. A detailed discussion of the theory of statistical power calculations for ANOVA models is presented elsewhere [25]. We will describe the mechanics of the power analyses used on the data collected from this study and for a one-way ANOVA design.

After performing an ANOVA, the non-central parameter (ϕ^2) is calculated. This parameter describes the difference between the distribution of a randomly occurring population and the distribution representing the data used in the ANOVA. It is a function of the effect size or the true difference between two treatments

(δ), the sample variance (σ^2), the number of observations (*n*), the number of treatments (*a*), and the ANOVA design. The ϕ^2 for a one-way ANOVA is calculated using Eq. (4) while the ϕ^2 for the current study was calculated using Eq. (5) [25].

$$\phi^2 = v_1 \left(\frac{\mathrm{MS}_{\mathrm{treat}}^1}{\sigma^2}\right) \tag{4}$$

$$\phi^{2} = v_{1} \left(\frac{\mathrm{MS}_{\mathrm{treat}}}{\mathrm{MS}_{\mathrm{exper}}} - 1 \right)$$
(5)

Table 3

Results of power analysis using moisture content profile data showing the number of tests that had an observed statistical power for a given range to detect three effect sizes^a

| Power | Small effe | ect size 5% w.t | b. difference | Medium e | effect size 10% | w.b. difference | Large effe | .b. difference | |
|-----------|------------|-----------------|---------------|----------|-----------------|-----------------|------------|----------------|-------|
| | 10 cm | 20 cm | 30 cm | 10 cm | 20 cm | 30 cm | 10 cm | 20 cm | 30 cm |
| 0.99– | 1 | | 2 | 3 | 3 | 3 | 8 | 9 | 8 |
| 0.95-0.98 | | | | 1 | | 3 | 2 | 3 | 1 |
| 0.90-0.94 | | | | 1 | 4 | | 3 | | 1 |
| 0.80-0.89 | | 1 | | 4 | 2 | 2 | 1 | 1 | 1 |
| 0.70-0.79 | 2 | 1 | | 2 | 3 | 2 | 1 | | 1 |
| 0.60-0.69 | | 1 | 2 | 3 | | | 1 | 1 | |
| 0.50-0.59 | 2 | 3 | 2 | | 1 | 1 | | | 1 |
| 0.40-0.49 | 1 | 2 | 1 | 1 | | 1 | 1 | 2 | |
| 0.30-0.39 | 3 | 3 | 2 | 1 | 1 | | | | 1 |
| 0.20-0.29 | 5 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 2 |
| 0.10-0.19 | 2 | 1 | 2 | 1 | 2 | 3 | | | 1 |
| 0.05-0.09 | 2 | 4 | 6 | | | 2 | | | 1 |
| N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| Mean | 0.372 | 0.367 | 0.365 | 0.713 | 0.691 | 0.609 | 0.870 | 0.823 | 0.725 |
| Median | 0.31 | 0.35 | 0.29 | 0.78 | 0.82 | 0.74 | 0.96 | 0.98 | 0.95 |
| S.D. | 0.26 | 0.25 | 0.31 | 0.26 | 0.32 | 0.38 | 0.20 | 0.27 | 0.35 |

^a That is, 2 of 18 ANOVAs had a statistical power between 0.70 and 0.79 when attempting to detect a 5% (w.b.) difference at 10 cm.

Table 4

Results of power analysis using temperature gradient data showing the number of tests that had an observed statistical power for a given range to detect three effect sizes^a

| Power | Small effect size | 5°C difference | Medium effect si | ze 10°C difference | Large effect size 15°C difference | | |
|-----------|-------------------|----------------|------------------|--------------------|-----------------------------------|-------------|--|
| | Inoculum | No inoculum | Inoculum | No inoculum | Inoculum | No inoculum | |
| 0.99– | 1 | 1 | 2 | 3 | 7 | 9 | |
| 0.95098 | | | 1 | 4 | 1 | | |
| 0.90-0.94 | 1 | | 2 | 2 | 9 | 1 | |
| 0.80-0.89 | | | 2 | | | 1 | |
| 0.70-0.79 | | 1 | 1 | | 2 | 1 | |
| 0.60-0.69 | | 1 | 7 | 1 | 4 | 2 | |
| 0.50-0.59 | 1 | 1 | 2 | 1 | 3 | 5 | |
| 0.40-0.49 | 1 | 4 | 2 | | 5 | 2 | |
| 0.30-0.39 | 1 | 1 | 4 | 3 | 2 | 7 | |
| 0.20-0.29 | 3 | | 6 | 7 | | 3 | |
| 0.10-0.19 | 10 | 2 | 4 | 10 | 1 | 3 | |
| 0.05-0.09 | 17 | 24 | 2 | 4 | 1 | 1 | |
| N | 35 | 35 | 35 | 35 | 35 | 35 | |
| Mean | 0.194 | 0.202 | 0.490 | 0.407 | 0.721 | 0.580 | |
| Median | 0.10 | 0.08 | 0.43 | 0.24 | 0.78 | 0.52 | |
| S.D. | 0.22 | 0.24 | 0.29 | 0.36 | 0.27 | 0.31 | |

^a That is, 1 of 35 ANOVAs had a statistical power between 0.70 and 0.79 when attempting to detect a 5°C difference in an inoculated reactor.

Table 5

Power Small effect size 5% w.b. difference Medium effect size 10% w.b. difference Large effect size 15% w.b. difference Inoculum No inoculum Inoculum No inoculum Inoculum No inoculum 0.99-1 1 2 4 6 0.95-0.98 2 2 1 5 2 0.90-0.94 1 1 0.80-0.89 2 1 1 2 0.70-0.79 3 3 3 0.60-0.69 1 2 1 0.50-0.59 1 2 2 1 0.40 - 0.491 2 1 2 2 0.30-0.39 4 1 0.20-0.29 2 3 1 3 1 0.10-0.19 2 5 4 1 1 2 2 0.05-0.09 11 4 18 18 18 18 N 18 18 Mean 0.202 0.27 0.456 0.650 0.657 0.833 Median 0.09 0.20 0.39 0.70 0.73 0.96 0.25 0.21 0.33 0.30 0.32 0.27 S.D.

Results of power analysis using moisture content gradient data showing the number of tests that had an observed statistical power for a given range to detect three effect sizes^a

^a That is, 1 of 18 ANOVAs had a statistical power between 0.50 and 0.59 when attempting to detect a 5% (w.b.) difference in an inoculated reactor.

where,

 ϕ^2 = non-central parameter, v_1 = degrees of freedom used for MS_{treat}, MS_{treat}^I = Eq. (1) with b = 1, σ^2 = population variance, MS_{treat} = Eq. (1), MS_{exper} = Eq. (2). To perform the power analysis it was pecess

To perform the power analysis it was necessary to vary δ to determine the ability of the ANOVA to detect different effect sizes between any two treatments. Eq. (1) was rewritten so that δ equaled twice the difference between the mean of one treatment and the mean for all the data combined. Using this method, the $\overline{Y}_A - \overline{\overline{Y}}$ for one treatment equaled $-\frac{\delta}{2}$, while for another treatment the value was $\delta/2$. $\overline{Y}_A - \overline{\overline{Y}}$ was set equal to zero for all remaining treatments [25]. Once this was done, Eq. (1) could be rewritten for the power analysis:

$$MS_{treat} = \frac{nb}{2v_1}\delta^2$$
(6)

Once ϕ^2 was calculated, the non-central *F* value, *F'*, and the non-central degrees of freedom were calculated using the approximations given by Lindman [25]. Using these values from Eq. (4) or Eq. (5) the probability of committing a Type II error test was calculated using Eq. (8).

$$\beta = \Pr[F^{(\nu,\nu_2)} < F'] \tag{8}$$

where,

 β = probability of committing a Type II error or 1-power,

F' = approximation of the non-central *F* distribution, *F* = ratio of the MS_{treat} term to the MS_{exper} or MS_{error} terms,

v' = first degree of freedom for use with non-central *F* distribution,

 v_2 = degrees of freedom for the MS_{exper} or MS_{error} terms.

For the power analysis used in this report, three effect sizes were used to analyze each ANOVA. When analyzing temperature data, effect sizes of 5, 10, and 15°C were used. The largest effect size was chosen since a 15°C difference in temperature accounts for differences large enough to span between the thermophilic and mesophilic temperature ranges. Analysis of moisture content used differences of 5, 10, and 15 percent points (w.b.). A difference of 15 percentage points was selected as the largest effect size since this difference could account for the differences between a biologically uninhibited reactor (i.e. above 45%) and biologically inhibited reactor (i.e. below 35%) [2]. The small and medium effect sizes were selected since they were evenly spaced points between the largest effect size and zero. The MS_{exper} terms were calculated from the data in this study using SPSS (Chicago, IL, USA). The power analyses were performed using a spreadsheet (Excel '97; Microsoft; Redmond, WA, USA).

2.5. Experimental design

Two experiments were conducted between December 1998 and January 1999. Each experiment consisted of four reactors, two of which were inoculated with the wastewater mix and two of which exclusively used tap water. Each experiment was run for 408 h.

3. Results and discussion

3.1. Effect of inoculum on temperature profile

Consistent with the investigations cited earlier, no statistically significant differences were detected (all P > 0.05) between the temperature profiles of inoculated and non-inoculated reactors at any time or height inside the reactors. Fig. 2 demonstrates the similarity of the average temperature data collected at 30 cm above the reactor floor for both treatments. These data are presented since the highest temperatures were found at 30 cm.

The dip found between 30 and 50 h is of particular interest for understanding one potential effect of an inoculum on composting microbiology. Between these times, the temperature fluctuated between 45 and 50°C, the temperature range that differentiates between mesophilic and thermophilic bacteria. The inoculum did not appear to introduce a sufficient quantity of thermophilic bacteria to shorten the transition from mesophilic to thermophilic temperatures. Contrary to our hypothesis, the introduction of a wastewater inoculum appeared to have no effect on the initial lag period or the time required to reach maximum temperatures. However, it is not known what the effect would be if more of the moisture added to the original sample were from the wastewater.

3.2. Effect of inoculum on temperature spatial gradients

Spatial temperature gradients are common to composting processes and were detected during the hottest parts of the temperature profile. The largest gradients were detected at 192 h into the process for both the inoculated (P < 0.001) and non-inoculated (P < 0.025) reactors. These profiles are shown in Fig. 3. Five statistically significant gradients were detected in the non-inoculated reactors at 144, 156, 180, 192, and 360 h. The reactors found to have the longest lasting gradients were those in which an inoculum was introduced. A continuous string of gradients were detected in the inoculated reactors between 108 and 264 h into the process.

Several of the temperature differences between the top and bottom of the non-inoculated reactors were not statistically significant while many of the gradients at the same times were statistically significant in the inoculated reactors. However, the gradients in both reactors were of similar magnitude. For example, at 108 h the average difference between 30 and 10 cm in the inoculated reactors was 8.8° C (P = 0.032) while in the non-inoculated reactors it was 7.1° C (P = 0.142). Another example was the gradients at 264 h. The average difference difference between 30 and 10 cm in the non-inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated reactors was 18.0° C (P = 0.064) while in the inoculated was the gradients was the gradients while was the gradients while was the gradients while was the gradients was the gradien

reactors it was 16.7°C (P = 0.047). Interestingly, the gradient in the second example was larger for the non-inoculated than the inoculated reactors yet it was not statistically significant. These results suggest the use of an inoculum decreases the variability between experiments and within experiments.

3.3. Effect of inoculum on intra- and inter-experimental temperature variability

Results reported in the previous section demonstrate the effect of the inoculum on the variability within and between experiments. While the magnitudes of the average gradients were similar, the variability within non-inoculated reactor observations may limit the ability to detect significant temperature differences within the non-inoculated reactors.

The values of the mean squared error (MS) within each experimental unit for each treatment (MS_{within}) are shown in Fig. 4. Except for three points, the MS_{within} for the inoculated reactors is either lower than or comparable to the MS_{within} of the non-inoculated reactors demonstrating the ability of an inoculum to decrease the inter-experimental variability. Fig. 5 compares the values of the MS between experiments (MS_{exper}) for each treatment. While this figure does not show the data at 24 h (inoculum = $2464^{\circ}C^{2}$, no inocu $lum = 2998^{\circ}C^{2}$), it is again clear that for most of the composting process the inter-experimental variability of the inoculated reactors is either lower than or comparable to the non-inoculated reactors. Although the inoculum appeared to have no effect on the temporal temperature profiles, it did reduce the variability between and within each experiment. Reduction of interand intra-experimental variability means that the reproducibility within and between experiments was improved using wastewater as an inoculum.

3.4. Effect of inoculum on moisture content profile

Fig. 6 shows the average drying profiles for samples collected at 10 and 30 cm above the floor of the reactor in inoculated and non-inoculated reactors. The vertical bars represent the points at which significant differences in moisture content were detected between the two treatments. No differences were detected between treatments at 20 cm (data not shown, all P > 0.10).

At 10 cm above the floor of the reactor, the only significant difference between treatments occurred at 168 h. The average moisture content values were 17.5% (w.b.) and 24.7% for inoculated and non-inoculated reactors, respectively. This was a difference of 7.2 percentage points (P < 0.05). In contrast, the moisture content at 30 cm in the inoculated reactors was higher than the non-inoculated reactors. After 240 h, the average moisture content values at 30 cm were 39.4 and

32.6% in the inoculated and non-inoculated reactors, respectively. This was a difference of 6.8 percentage points (P < 0.001). The final significant difference occurred at 336 h, when the average moisture content values were 37.1 and 29.6% in the inoculated and non-inoculated reactors, respectively. This was a difference of 7.5 percentage points (P < 0.026).

Because no significant temperature differences were detected between the two treatments, it is difficult to ascribe meaning to these three moisture content differences. Changes in moisture levels are associated with changes in temperature since as air moves through the composting matrix, it is heated and can absorb more moisture. The amount of moisture absorbed by the air is a strong function of the air temperature. The most logical explanation for higher moisture levels may lie outside of the effects of the inoculum. It is possible that moist air condensed at the top of the reactor and trickled down to the region of the 30-cm sampling port. This explanation is supported by fluctuations in the moisture content profile associated with higher levels of the reactor toward the end of the process (Fig. 6). Unfortunately, this explanation does not account for the differences found between treatments at 30 cm and those detected at 10 cm. The condensation of moisture on the ceiling of the reactor would account for increased variation between reactors and increased moisture content levels.

3.5. Effect of inoculum on moisture content spatial gradients

Similar to the formation of temperature gradients, spatial moisture content gradients are common to composting and are known to have a profound effect on the composting process [2]. Both treatments developed statistically significant moisture content gradients across the reactor bed between 120 and 408 h into the process (all P < 0.05). However, the gradients at 216 and 312 h in the inoculated reactors and the gradients at 216 and 360 h in the non-inoculated reactors were not significant. Also, a gradient at 72 h was detected in the inoculated reactor although the magnitude of this gradient of 0.70% (w.b.) was not of any physical significance. Gradients at 120, 168, and 408 h are shown in Fig. 7. The gradient at 168 h was selected since it was the largest detected gradient under both treatments. The other two time points represent the initial and final detectable gradients.

3.6. Effect of inoculum on intra- and inter-experimental moisture content variability

While the inoculum appears to hold constant or decrease inter- and intra-experimental temperature variability, the inoculum did not have a positive effect on moisture content variability. Figs. 8 and 9 show the mean squared errors within each experiment (MS_{within}) and between the experiments (MS_{exper}) for each treatment, respectively. The variation shown in Fig. 8 suggests that while there are time periods where intra-experimental variability is decreased in the inoculated reactors, there are also periods where the variability increased. The inter-experimental variation shown in Fig. 9 suggests that the wastewater inoculum treated reactors experienced increased inter-experimental variability.

3.7. Effect of inoculum on effluent oxygen concentration

Fig. 10 shows the average effluent O_2 concentration profiles for both treatments. Significant differences were found between treatments from 24 to 164 h into the process. During this period, non-significant differences were detected at 36, 60, 96, and 156 h. At all of the points where significant differences existed, the O2 concentration in the effluent air stream from the inoculated reactors was less than that from the non-inoculated reactors suggesting an increased rate of O₂ consumption with inoculation. During this period, the average significant difference between treatments was 0.33 percentage points (S.D. = 0.09, n = 9). It is difficult to interpret why differences were detected for O2 data but not for temperature data. Since not all of the reactors were used to obtain O₂ concentrations, it is possible that the variability within treatments was smaller than for temperature data allowing for the detection of more differences.

In order to assess the physical importance of these differences, the cumulative O_2 consumed per unit of initial dry mass was calculated as follows:

$$COU = \int_{0}^{384} \frac{F\rho M_{air}}{m_{solids} M_{O_2}} (X_{O_2, influent} - X_{O_2, effluent}) dt$$
(9)

where,

COU = cumulative oxygen uptake per unit initial dry mass,

F = volumetric flow rate,

 $\rho = dry$ air density,

 $M_{\rm air} =$ molecular weight of dry air,

 $m_{\rm solids} = dry$ mass of solids,

 $M_{\rm O_2}$ = molecular weight of oxygen,

 X_{O_2} = mole fraction of influent and effluent air stream.

The average COU values were 286.04 and 294.00 g O_2/kg dry solids for the non-inoculated and inoculated reactors, respectively. The difference of 7.96 is not of practical relevance. While the inoculum increased the rate of oxygen consumption between 24 and 164 h into the process, calculation of the total O_2 consumed shows the overall difference is physically insignificant.

3.8. Power analysis

The effect of an inoculum on temperature and moisture content at a given time and position in a reactor has been shown to be statistically insignificant at all but a few points. Before an assertion can be made that an inoculum has no effect on composting, it is necessary to perform the power analysis described earlier to insure the study had adequate statistical power to detect true differences.

Tables 2 and 3 present the number of ANOVAs that had a statistical power within different ranges for each height in the reactor for temperature and moisture content data, respectively. Several observations can be made from these two tables. First, as the effect size is increased, the average statistical power at each height increases for both dependent variables. This result was expected since it is easier to detect a larger difference than a smaller difference. Second, the statistical power decreases as the height in the reactor increases. This result may be due to increasing temperatures with height in the reactor causing greater variability reducing the statistical power. Finally, there was no relationship between statistical power and time (data not shown). This demonstrates there are no predictable times or temperature ranges when it is easier to detect differences between treatments.

The data in Table 2 suggests that many of the tests performed in this study lacked the statistical power (power ≥ 0.80) to detect significant effects of the treatment on temperature. At 10 cm 20% of the tests had statistical power to detect differences of 5°C while 77% of the tests could detect differences of 15°C at 10 cm. Of the tests performed at 30 cm, only 3% had ample statistical power to detect differences of 5°C and 31% of the tests had enough statistical power to detect differences of 15°C. In other words, between 31 and 77% of the tests could detect a difference large enough to differentiate between mesophilic and thermophilic temperatures at heights between 10 and 30 cm in the reactor. If temperature is used as an indicator of biological activity, it is essential that these statistical power values be higher since it is not possible to reliably detect differences between the two temperature ranges important in composting under these conditions.

Since a power analysis has never been performed for a composting study, we performed an exhaustive search of recent composting literature for a study that included a hypothesis test for temperature data and provided sufficient data to replicate the hypothesis test and perform a power analysis. The only paper we were able to find was by Michel et al. [26], this study will be called the 'Michel study' for the remainder of this report. The study attempted to determine the effects of the composting pile size, mixing frequency, and feed composition on several variables including temperature.

The Michel study presented average temperature values and standard deviations for all experiments using three different pile size and mixing frequency combinations in Fig. 2 of their paper. They report recording temperatures within the section just above the middle of the pile. There are several limitations of using this data set. First, the actual numerical temperature and standard deviations were not presented at each time point, so estimates of the mean and S.D. at each time point were taken and are reported in Table 1. Table 1 represents the 22 time points that were clear and those time points that provided mean values and S.D. for all three treatments. Second, the investigators report having performed an ANOVA, although the specifics of the analysis are not stated. Their hypothesis tests were unable to detect any significant differences for any set of treatments. It will be assumed that their method of analysis was a one-way ANOVA with three treatments and five observations per treatment at each time point. Despite these limitations, comparison of the statistical results of this study to the Michel study will give insight into the effects of an inoculum on the composting process. It is assumed that the Michel study was analyzed using a one-way ANOVA at each time point since it is not explicitly stated in the article.

The results of the power analyses performed for the data in Table 1 are shown in Table 2. It is clear that while the overall statistical power found for the current study is low, the statistical power of the Michel study is even lower. None of the tests performed using the Michel study had a statistical power greater than 0.80 to detect differences of 5 or 10°C and only 14% of the tests could detect differences of 15°C with a statistical power of 0.80. It appears that the statistical power to other studies, based on standard deviations provided between replicates provided elsewhere [27].

The statistical power (power ≥ 0.80) to detect significant effects of the treatment on moisture content is higher than that observed for temperature data, as shown in Table 3. At 10 cm, 6% of the tests had statistical power to detect a difference of 5 percentage points while 77% of the tests could detect differences at 10 cm of 15 percentage points. Of the tests performed at 30 cm, 11% had ample statistical power to detect differences of 5 percentage points and 61% of the tests had enough statistical power to detect differences of 15 percentage points. These results can be summarized by stating that between 61 and 77% of the statistical tests were capable of detecting the difference between an active and inactive reactor between 10 and 30 cm in the reactor. Because of a lack of available data from other studies, it was not possible to draw comparisons in order to assess the meaning of these values.

A power analysis of the ANOVAs performed to measure the effect of height in the reactor on tempera-

ture and moisture content demonstrated the effect of the inoculum on statistical power. Tables 4 and 5 show how often the ANOVAs attained a specified level of statistical power for the temperature and moisture content data, respectively, when attempting to detect gradients in inoculated and non-inoculated reactors. The number of tests with sufficient statistical power (power ≥ 0.80) to detect differences between 5 and 10°C does not differ greatly between inoculated and non-inoculated reactors. However, the median statistical power to detect differences of 10°C in inoculated reactors is nearly twice the median statistical power found in non-inoculated reactors. Finally, the number of tests that were able to detect differences of 15°C with ample statistical power in inoculated reactors is 49% while it is 31% in the non-inoculated reactors. While the effect of the inoculum does not appear to have a profound effect on the ability to obtain statistical power greater than 0.80, the use of an inoculum does increase the overall levels of statistical power for detection of temperature differences. These results suggest that the inability to reliably detect differences between treatments in this study is largely due to the variability in the non-inoculated reactors. It is suspected that if this study were repeated to compare the differences between two inoculation levels, the statistical power to detect differences between treatments would be higher.

Table 5 shows the inoculum had a negative effect on the statistical power to detect differences in moisture content. For every effect size analyzed, the median statistical power obtained in the non-inoculated reactors was higher than the inoculated reactors. The number of tests performed with a statistical power greater than 0.80 was greater in the non-inoculated reactors when attempting to detect differences greater than 10 percentage points. The inability of the inoculum to improve the statistical power to detect differences between treatments suggests that variability in moisture is primarily due to the heterogeneity in non-biological factors such as substrate preparation, aeration conditions, and reactor differences although the level of heterogeneity was small.

4. Conclusions

This study was conducted to determine the effect of wastewater inoculum on process variables important to composting and to determine the effect of the inoculum on experimental variability. Application of the inoculum does not appear to produce a substantial increase in rate of temperature increase, rate of drying, magnitude of spatial gradients, or cumulative O_2 consumption over an non-inoculated process, although the statistical power to detect differences between treatments was routinely below 0.80.

Previous investigators have revealed extensive experimental variation between and within replications in composting studies [1-3]. However, no studies have suggested a method of reducing this variation. A review of accepted practices for the statistical treatment of sample data emphasizes the importance of reducing variation within and between experiments. As emphasized by Sokal and Rohlf, within sample, variability obscures differences between treatments [24]. This reduces statistical power, or the ability to detect differences that actually exist. The less variable the results are in a single treatment causes the effect size required to detect differences between treatments to decrease. Consequently, the failure of researchers to detect differences may be as much a function of limited statistical power derived from variability within samples, as from the lack of a 'true' difference [4,5].

The use of an inoculum decreased the overall variability in temperature data as demonstrated by the MS values shown in Figs. 4 and 5. This reduction in variability for temperature data translates into a more reproducible system, as shown by the increase in overall power. While a positive effect was found for variability in temperature data, the inoculum had little positive effect on the reproducibility of moisture content data. In fact, the inoculum slightly increased the inter-experimental moisture content variability, maintained the intra-experimental moisture content variability, and decreased the ability to detect differences in moisture content. Despite this contradiction, the inoculum is still of great value since most investigators focus on temperature as an indicator of biological activity and environmental conditions, while moisture content is predominantly used as an environmental indicator.

A power analysis using the Michel study [26] showed that while the statistical power of the current study was low, it was greater than typical composting experiments. Although, the inoculum was only 25% of the supplemental moisture, it is not known what effect a larger percentage would have. Based on the results of the studies cited earlier, it is doubtful that a larger percentage would change the process dynamics of the system. However, it is possible that a higher level of inoculation could further reduce the variability found between reactors since the population of microorganisms in the inoculated reactor would become more representative of the microorganisms in the inoculum. Further, if an inoculum were used in future studies attempting to describe the effects of a variable on the composting process, the reduced variability due to the inoculum would increase the statistical power to detect differences between different levels of the tested variable.

Although the statistical power values of this study are lower than the desired 80% level, future studies using an inoculum to reduce variability between reactors would have a greater level of statistical power since the power calculations of this study included the variation due to the non-inoculated reactors. This study has demonstrated the need for reducing variability in compost experimental systems and the ability of a wastewater inoculum to reduce this variability and improve the ability to detect statistically significant differences between reactors. Furthermore, we have shown that the use of a power analysis is a useful method for quantifying this variability and for adding another dimension to the statistical analysis of composting data.

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