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INACTIVATION AND REGROWTH OF BACTERIA UNDER DIFFERENT HEATING IN SIMULATION OF COMPOSTING

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ABSTRACT

This study investigated the effects of heating patterns on bacterial indicator organisms addressing inactivation and regrowth potential of *Salmonella*, *Escherichia coli*, and Faecal streptococcus. Using a laboratory thermal controller, three patterns of heating were applied: single-impact heating, constant heating, and intermittent heating. The pattern of single-impact heating showed the greatest effect on the inactivation of the organisms and intermittent heating had the least effect. Following the same pattern, single-impact heating had the least microbial regrowth potential while the intermittent heating had the greatest potential. Solids and dissolved organic carbon severely inhibited the inactivation of all the organisms.

KEYWORDS

Inactivation, regrowth, composting, *E. coli*, *Salmonella*, Faecal streptococcus, heating pattern

INTRODUCTION

Although composting is a well developed technology, the potential risk of pathogens, which always exist in raw wastes and the final products of composting, must be further considered as a primary concern for public safety (Abdennaceur *et al*, 2001; Marco *et al*, 1998). The purpose of this research is to use bacterial indicator organisms to investigate the efficiency of the inactivation of the selected indicator bacteria under the different heating patterns followed by evaluation of regrowth rates.

MATERIALS AND METHODS

Bacterial strains *Salmonella* was chosen as a pathogen, and *Escherichia coli* and Faecal streptococcus were employed as indicator microbes. The strains *Salmonella typhimurium* TA 1535 (Fujita *et al*, 1991), *E. coli* K-12, and Faecal streptococcus FS-IFO 33826 were used for inactivation studies.

Sample heating methods A laboratory thermal controller (GTU-1615, TAITEC) was used for cultivating test samples under various thermal conditions. Composting operations were simulated, and by subjecting test samples to designed temperature patterns.

Determination of critical lowest temperature The critical lowest temperatures of the strains *Salmonella typhimurium* TA 1535, *E. coli* K-12, and Faecal streptococcus FS-IFO 33826 were determined for finding the base line of the temperature to be set in the inactivation experiments.

Evaluation of heating patterns Three model patterns of heating were considered to simulate composting as shown in Fig. 1.

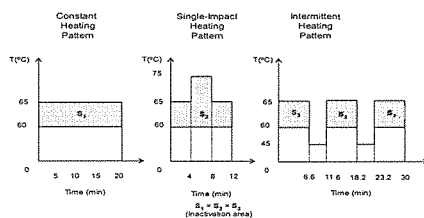


Fig. 1 Heating patterns used in this research.

The first pattern consisted of constant heating at a temperature at 65°C throughout the testing period. The second pattern was single-impact heating, which allowed the temperature of the samples to increase to a maximum of 75°C. The third pattern was intermittent heating which consisted of an oscillating pattern of temperature changes designed to imitate composting conditions. In this case, the temperature was decreased rapidly to 45°C to imitate the turning of a pile and then increased back to 65°C. The three heating patterns were designed so that the areas of time-temperature (S_1 , S_2 , and S_3 in Fig. 1) were the same.

Evaluation of Regrowth Regrowth was assessed by using the optical density (OD660 nm) of the samples. Three extra samples were set with those of the previous inactivation experiment and, after the experiment, the extra samples were taken from the thermal controller.

Effects of solids and dissolved organic carbon (DOC) Effect on the inactivation of the bacterial indicator organisms by solids and DOC was investigated.

RESULTS AND DISCUSSION

Effects of different heating patterns

Constant heating pattern Survival curves of the bacterial indicator organisms with constant heating are shown in Fig. 2. The trends of the survival curves of the three indicator organisms were almost the same although the strain Faecal streptococcus FS-IFO 33826 showed more heat resistance than the other organisms, and the strain *Salmonella typhimurium* TA 1535 had the highest inactivation.

Single-impact heating pattern The survival trends were different for the strain Faecal streptococcus FS-IFO 33826 and the other two indicator organisms with single-impact heating (Fig. 2). Among the three patterns of heating, the single-impact heating showed the highest efficiency on inactivation of the three bacterial indicator organisms, and all indicator organisms reached a population level of below 10^5 cfu/ml in twelve minutes.

Intermittent heating pattern The trends of the survival curves with intermittent heating followed the typical sigmoid decay pattern (Haug, 1993)(Fig. 2). This heating pattern showed the lowest efficiency on inactivation among the three indicator organisms.

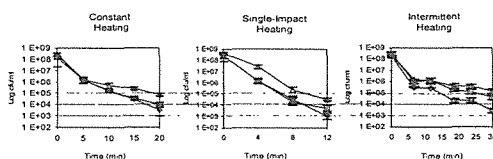


Fig. 2 Effect of the different heating patterns on the inactivation of the bacterial indicator organisms.
□ Strain *E. coli* K-12, ◇ Strain *Salmonella typhimurium* TA1535,
△ Strain Faecal streptococcus FS-IFO 33826

Regrowth of the bacterial indicator organisms The regrowth curves for strain *E. coli* K-12 are shown in Fig. 3. The regrowth curves of the intermittent heating pattern were recovered first among the three heating patterns, and those of the single impact heating showed the slowest recovery, which corresponded with the efficiency of inactivation of the bacterial indicator organisms. High efficiency of inactivation of the indicator organisms led to slow regrowth of the indicator organisms.

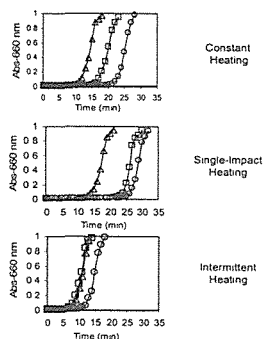


Fig. 3 Regrowth curves of strain *E. coli* K-12 at 37°C after the three thermal inactivation.
○:right after inactivation, □:after 1 hour-incubation at 37°C, △:after 2 hour-incubation at 37°C

Effects of solids and DOC

Effect of solids The results of experiments evaluating the effect of solids on thermal inactivation are shown in Fig. 4. The trends of inactivation were similar in spite of the heating patterns and the bacterial indicator organisms used. The highest

inactivation was shown for 0 % of kaolin and the lowest inactivation was shown for both 10 and 20 % of kaolin, being virtually the same.

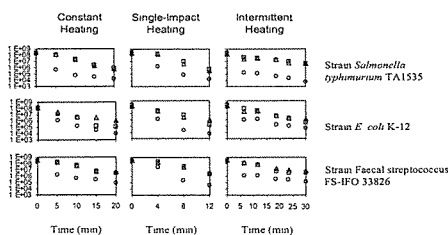


Fig. 4 Effect of solids on thermal inactivation.
Kaolin contents: ○ 0 %, □ 10%, △ 20%

Effect of DOC Experiments to evaluate the effect of DOC concentration were done only with a constant heating pattern (data not shown). 20% kaolin concentration was used for all experiments. According to the results, it was found that there were two trends. One trend was for the strain *E. coli* K-12 where inactivation strongly depended on the concentration of DOC. The inactivation of the strain *E. coli* K-12 was the largest when the DOC concentration was the smallest. The other trend was the same for the strains of *Salmonella typhimurium* TA 1535 and Faecal streptococcus FS-IFO 33826 where inactivation was not influenced by the concentration of DOC.

CONCLUSIONS

From a hygienic point of view, it was found that turnings of compost piles during composting should be minimized and that achieving higher temperatures in the piles results in more effective inactivation of the bacterial indicator organisms. As Tateda *et.al.* (2002) reported, greatly uneven temperature distributions would be expected if turning is not conducted during composting. Therefore, effective and optimal turning schemes should be investigated by minimizing the number of turnings during operation.

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