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Microbial-growth inhibition during composting of food waste: Effects of organic acids

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ABSTRACT

Factorial designs were employed to analyze the inhibitory effects of acetic, butyric, lactic, and propionic acids on composting microorganisms. Compost samples were withdrawn on different days of composting and treated with acids alone and in combination (at 0 and 0.5 mmol/g). Microorganisms were enumerated to determine degree of growth inhibition. Generally, inhibition was more severe on the day when pH decreased rather than on the day when pH started to increase. Butyric or lactic acid alone, and the combination of butyric, lactic, and propionic acids, significantly inhibited thermophilic bacteria. Only 51.0–65.0% of the thermophilic bacteria exist if 0.5 mmol/g of these acids were present in compost. Temperature, microbial populations, and microbial growth phase might cause variation in the inhibitory effects of acids. These findings are useful not only in the study of microorganisms in acidic microenvironments, but also in preventing microbial-growth inhibition by predicting population via a regression model.

1. Introduction

Many solid wastes, such as food, paper, wastewater sludge, and garden waste, are amenable to biodegradation by composting as they have major concentrations of heterogeneous organic substrates, including sugars, fats, proteins, hemicelluloses, celluloses, and lignin (Gray et al., 1971a; Rhyner et al., 1995). When these organic wastes are composted in reactors, or in piles, the acidity of the waste material increases and the pH significantly decreases to a range of 4–5 during the initial phase (or the mesophilic stage) of aerobic composting (Beck-Friis et al., 2003; Brinton, 1998; Eklind et al., 1997; Gray et al., 1971b; Krichmann and Widen, 1994; Nakasaki et al., 1993; Sundberg et al., 2004; Sundberg and Jönsson, 2005). This reduced pH is caused by the presence of organic acids (Golueke, 1977; Haug, 1980), which are intermediate by-products of microbial breakdown of easily degraded substrates such as sugars, fats, starch, and greases (Brinton, 1998; de Bertoldi et al., 1983; Smars et al., 2002; Sundberg, 2003). Acetic, butyric, lactic, and propionic acids have been detected in compost by several studies (Eklind et al., 1997; Robertsson, 2002; Sundberg and Jönsson, 2005).

Organic acids are main products contributing odor in anaerobic composting, but they can be produced in aerated composting. It has been suggested that some microbial species, such as *Lactobacillus* and *Escherichia coli*, can oxidize organic substrates to produce organic acids in the presence of oxygen (Brinton, 1998; Sundberg, 2003; Sundberg and Jönsson, 2005). Another suggestion is that anaerobic microenvironment can be developed in aerobic compost (Brinton, 1998; Reinhardt, 2002). Because of the restriction of oxygen flow between pore particles and water film in compost matrices, it is attributed to the co-existence of aerobic and anaerobic microenvironments. Obligate or facultative anaerobes produce organic acids when oxygen is depleted during aerated composting (Reinhardt, 2002; Sundberg, 2003).

The presence of organic acids and low pH values (less than 5) usually coincide with reduced microbial activities (Beck-Friis et al., 2001; Day et al., 1998; Smars et al., 2002), which adversely affect the degradation rate of compost and thereby the composting efficiency (Beck-Friis et al., 2001, 2003; Kubota and Nakasaki, 1991; Nakasaki et al., 1993; Smars et al., 2002; Sundberg and Jönsson, 2005). Therefore, organic acids are considered antimicrobial agents and detrimental to microorganisms (Reinhardt, 2002; Sundberg and Jönsson, 2005). Once optimum conditions for microbial activity are reached in the later stages of composting, the organic acids become easily biodegradable and can be readily consumed by microorganisms (Brinton, 1998; Sundberg and Jönsson, 2005). During this phase, the pH increases to 8 to 9, while the concentrations of organic acids decline and even disappear (Beck-Friis et al., 2001, 2003; Gray et al., 1971b; Smars et al., 2002).

Low microbial activities are probably caused by inhibition of active microorganisms under the combined conditions of acidic pH

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and thermophilic temperatures above 40 °C (Smars et al., 2002). Generally, two main classes of microorganism are considered with respect to temperatures: mesophiles and thermophiles. Mesophiles grow optimally at temperatures of 20–35 °C, and the minimum and maximum tolerable temperatures for mesophile survival are 10 and 45 °C, respectively. Thermophiles are best adapted to temperatures between 50 and 60 °C, although they can tolerate temperatures as low as 30–40 °C and as high as 80–90 °C (Henis, 1987; Lester and Birkett, 1999; Lyles, 1969; Swatek, 1967). During the initial and thermophilic phases of composting, bacteria, rather than fungi and actinomycetes, are responsible for most of the decomposition and heat generation (Nakasaki et al., 1985; Ryckeboer et al., 2003).

The microbial inhibition mechanism induced by organic acids is primarily based on undissociated molecules [HA] of organic acids. At a pH of less than 4.50, large amounts of [HA] can enter bacterial cells and release their dissociated protons [H^+] and anions [A^-] inside the cells. To maintain a pH gradient between extracellular and intracellular environments, [A^-] are pumped across the cell membrane while [H^+] accumulate inside the cells. Because the energy for pumping [A^-] is exhausted and the cells are acidified by excess [H^+], the intracellular pH of the cells decreases and further activities are hampered (Cherrington et al., 1991; Lueck, 1980; Ray, 2003). Sundberg and Jönsson (2005) suggested that the inhibitory effect of organic acids depends on pH, acid type, acid concentration, and microbial species. However, few experimental studies have examined microbial activity or growth responses to specific organic acid types and concentrations in composting, and the relationship between compost microbiology and organic acids is still poorly understood.

Therefore, the main objectives of this study are (1) to investigate the inhibitory effects of acetic, butyric, lactic, and propionic acids on total mesophilic and thermophilic microorganisms during the initial low-pH phase of food-waste composting; and (2) to compare any variations in the inhibitory effects of acids between the growth of total mesophilic and thermophilic microorganisms in acidic microenvironments.

2. Methods

2.1. Compost material preparation

Two duplicated batches of compost materials (10.5 kg wet weight) were prepared to carry out the preliminary and factorial experiments in this study. The compost materials were customarily made up of food components, which have been widely tested in composting (Sundberg and Jönsson, 2005). A mixture of 1.96 kg peeled and chopped carrots, 1.96 kg steamed soybeans, 0.35 kg ground pork, 1.27 kg peeled and chopped potatoes, 2.01 kg steamed rice, and additional materials such as 0.46 kg leaves, 2.00 kg soil, and 700 g water were composted in identical laboratory-scale 30-L in-vessel reactors. The leaves were added as a bulking agent and garden soil was used as an inoculant to provide additional microorganisms. The carrots, potatoes, soybeans, and leaves were ground into smaller pieces, and then mixed with the rest of the materials before being loaded into the reactors. The two batches of compost materials initially had a pH of 5.99–6.06, a C/N ratio of 18.1–20.6, a moisture content of 64.0–67.0%, an ash content of 3.00–8.00%, and an organic content of 92.0–97.0%, in which these initial properties were in respect to the acceptable ranges for proper and practical composting performances (Gray et al., 1971a; Haug, 1980; Ryckeboer et al., 2003).

2.2. In-vessel composting reactor system

The materials were composted in reactors under microbial self-heating conditions. The reactors (R1 and R2) were heat-insulated by wrapping with several layers of aluminum foil and Styrofoam. The layout of a rector system is shown in Fig. 1. Each reactor was equipped with a vacuum air pump and an airflow meter continuous air supply at a flow rate of 3 l/min, and the flow rates were regulated throughout the composting process. Two thermocouples were installed at two different heights inside each reactor to daily determine the temperatures of the compost materials in the middle and lower sections. A desiccator was used to collect evaporated moisture, which discharged from the exhaust gas outlet of the reactor along a plastic tube. The remaining escaped moisture was trapped as condensate in a collector. The discharging gases were determined for oxygen concentration using a M40 multi-gas monitor (Industrial Scientific Corp., Oakdale, PA) and then emitted outside through a plastic tube. Before taking samples, the compost material was homogenized daily by tuning several times with a shovel to promote uniformity of the material inside the reactors. Compost material was withdrawn as a pooled sample and manually stirred to create a well-distributed, consistent, and highly representative sample for analysis.

2.3. Full and fractional factorial designs

Factorial design was employed to screen factors that may have significant effects on response(s). It allowed examining how a response changes as a factor at predefined value, expressed in terms of *level*, change via regression model (Box et al., 1978; Montgomery, 2001). Two-level factorial design (2^k) is the most common design to analyze the number of "k" factors at two levels: low '−' and high '+'. When four factors ($k = 4$) are considered in the two-level full (2^k) and fractional (2^{k-1}) factorial designs, there have a total of 16 ($2^4 = 2 \times 2 \times 2 \times 2 = 16$) and 8 ($2^{4-1} = 2^3 = 8$) treatment combinations, respectively. Each treatment combination represents the response is examined under the condition of specific factors at specific levels. A series of treatment combinations can be notated by Yates order and represented by designated run numbers as shown in Tables 1 and 2.

Two-level factorial design is an efficient way to analyze the "main effect" of single factors and "interaction effect" of factors in combinations simultaneously on response(s). The "main effect" is defined as the average differences in response between the effects of a factor at high and low levels, while the "interaction effect" is defined as the average difference in response between the effects of one factor at high and low levels of other factors (Box et al., 1978; Montgomery, 2001). For example, the main effect of factor A and interaction effect of factors AB in 2^4 and 2^{4-1} factorial designs can be computed by Eqs. (1)–(4), respectively.

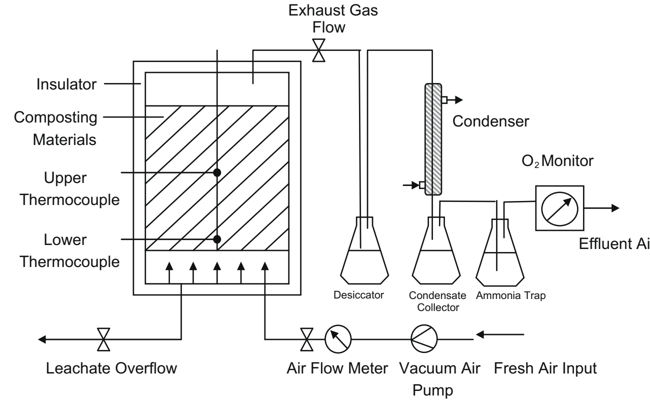

Fig. 1. Layout of the in-vessel composting reactor system.