SURVIVAL OF ASCARIS SUUM OVA, INDICATOR BACTERIA AND SALMONELLA TYPHIMURIUM PHAGE 28B IN MESOPHILIC COMPOSTING OF HOUSEHOLD WASTE

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Introduction

Organic waste such as household waste and human excreta contains plant nutrients as well as organic material valuable for agriculture. When recycling organic waste products the hygienic quality is of great importance to avoid spreading enteric and other diseases and plant pathogens in the environment. There are different possible routes for transmission of diseases from waste, like when collecting and treating the waste, and from the end product used as fertilizer or soil conditioner, to crops, grazing animals or vector animals (rats, birds etc.)

When treating organic waste the amounts are minimized and the nutrients are mineralised, thus becoming more easily available for plants. There are different ways of treating organic waste, such as anaerobic digestion and composting. The way the treatment is performed highly affects the survival or die off of pathogenic organisms. Temperature is the most important factor, but pH and biological competition also affect the die off rate (Haug 1993). The waste has to be treated in a manner killing or reducing the number of pathogens.

The composting process

Composting is one way of treating organic waste. It has several hygienic advantages such as creating elevated temperature, changes in pH and high biological activity leading to competition.

There are two main types of composting systems, non-reactor systems and reactor systems. The non-reactor systems are open and the material can either be formed in windrows or in piles. The reactor systems are closed. Oxygen can either be supplied by natural ventilation, by gas exchange during turning or by forced aeration from blowers. (Haug 1993)

To get a biologically active compost a bulk material giving a good structure has to be added to secure the oxygen supply, specially in a non-forced aeration system. This is important to get the temperature rise. A more energy rich material, such as kitchen waste, elevates the temperature more than a material poorer of energy, like faecal material. Therefore kitchen waste could be a good input to the composting of toilet waste. To obtain an increase in temperature there also has to be a certain volume of material. Typically for the initial phase of the composting process when composting batch wise, is a slow and oscillating temperature increase (mesophilic phase) (Beck-Friis et al. 2001). After a while the temperature may increase rapidly (thermophilic phase) and the composting process is carried on until the temperature slowly falls (maturing phase).

The level of pH is depending on which kind of material that is composted. Kitchen waste gives a lower initial pH due to production of fatty acids in anaerobic microhabitats (Beck-Friis et al. 2001). Faecal material is more neutral. During the composting process pH is increasing due to release of ammonium, often ending up at a high pH. To avoid big losses of nitrogen from the organic waste it is preferable to use toilets sorting out the nitrogen rich urine and only compost faecal material and kitchen waste.

Both low and high pH may have a positive effect of the die off of pathogenic organisms.

Pathogens in compost

Depending of raw material, a big variety of pathogens may occur in compost. Material of faecal origin may contain bacteria like Salmonella and Shigella, viruses like polioviruses and rotaviruses, protozoan like Giardia and Cryptosporidium and different parasitic helminth eggs (Feachem et al. 1983). With kitchen waste and waste from restaurants etc. different bacteria may be in the material, e.g. Campylobacter , Salmonella, Shigella, Bacillus cereus and pathogenic E. coli. Calicivirus may also be found. Also plant pathogens may be found in compost, like Tobacco mosaic virus.

Hygienisation of compost material

To determine the efficiency in pathogen reduction in a compost process, analyses have to be done of the material. Many pathogens are hard to find due to lack of analyse methods, very costly analyses or densities under detection limit, still making a serious threat. Therefore, the inactivation of indicator organisms that either are naturally present or added to the material, is studied.

Indicator organisms

Faecal bacteria, like coliforms, Escherichia coli and faecal streptococci, occur in high numbers in the intestine. They are often used to determine water quality and waste product quality (Bendixen 1999). Faecal streptococci have shown the highest temperature resistance among these three. But they have been questioned because of bad correlation to other pathogenic bacteria in survival and also to survival of viruses. There may also be a regrowth of the indicator bacteria under favourable conditions.

Viruses are hard and expensive to analyse in organic material. Instead, bacteriophages that are either naturally occuring or added, may be used as indicators (Lasobras 1999). They are similar to other viruses in many ways and offer easier and cheaper analyse methods. Salmonella typhimurium phage 28B has been found to be a resistant and thermotolerant phage (Eller 1995). Other used bacteriophages as indicators for viruses are coliphages and phages of Bacteroides fragilis (Lasobras 1999).

Among helmintic parasites, Ascaris suum is found to be very resistant to environmental factors (Feachem et al. 1983, Lewis-Jones 1991). Ascaris suum infects pigs and is very similar to Ascaris lumbricoides, which infects humans. When adding eggs to a compost as a hygienic indicator, Ascaris suum is used.

Objective

In this investigation the aim was to compare the survival of some commonly or less used indicator organisms in mesophilic composting of municipal household waste.

The chosen temperature range is of interest, as composts do not always reach higher temperatures, especially not small-scale composts, or composts made of energy-poor materials.

Material and methods

Municipal household waste was mixed with straw and seeded with E. coli (ATCC 25922), Enterococcus faecalis (ATCC 29212) and Salmonella typhimurium phage 28B. At the beginning of the investigation the concentration of E. coli was 2*107 cfu/g dw (colony forming units per gram dry weight), Enterococcus faecalis 3*106 cfu/g dw and Salmonella typhimurium phage 28B 2*108 pfu/g dw (plaque forming units). Ascaris suum eggs were enclosed in nylon bags, permeable for bacteria and fungi, and added to the compost. The compost material was divided into four parallel composts, one litre each, and one as control. Ten nylon bags with Ascaris suum eggs were added to each parallel and to the control compost, and two bags were taken out at each sampling. The four parallels were kept in an isolated box with aeration and stored at room temperature. Two types of controls were used. The control compost was kept at 4°C. The indicator organisms were also kept in suitable media stored at 4°C.

The composts were mixed nearly every day. Temperature and pH was measured during the experiment period. Samples were analysed regularly during a 31-day period. All analyses followed standard methods. After sampling, Ascaris suum eggs were kept in 25°C in 0,1 N H2SO4 to develope to larvae. The eggs were aerated weekly. After three weeks 300 eggs from each bag were counted and the percentage that had developed to larvae was calculated.

Results

Temperature

The temperature increased from 25°C to 29°C during the first 5-day period and during the rest of the investigation it varied between 29°C and 30°C.

pH

The pH level was low at start, around 4.5 (Fig. 1). From day 5 to day 8 it increased rapidly to a neutral value. During day 10 to day 16 the pH was stable at 7.3 and then slowly increased ending at a level of 8.6. In the control, compost pH stayed acidic for a longer period. At day 10 it slowly started to increase, ending at 8.4.

Survival of bacterial indicators

The faecal indicator bacteria survived for a very short period in the compost. E. coli had a 6-log10 reduction and Enterococcus faecalis had a 5-log10 reduction during the first 3 days. Both E. coli and Enterococcus faecalis was under detection limit after 5 days. In the control compost both E. coli and Enterococcus faecalis decreased with less than 1 log10 during the first 5 days. There was no reduction in the control medium.

Survival of Salmonella typhimurium phage 28B

The bacteriophages were reduced with 2 log10 during the first 8 days (Fig. 2). This reduction coincides with the acidic period of the compost. The remaining 23 days, the reduction was 1 log10. In the control compost and in the control medium there was no reduction of Salmonella phages.

Survival of Ascaris suum ova

The viability for Ascaris suum ova in the beginning of the experiment was 91% and after 31 days the viability was still as high as 70%. In the control compost the viability was lower, 60% after 31 days. Ascaris suum ova kept in solution in 4°C showed no reduction in viability.

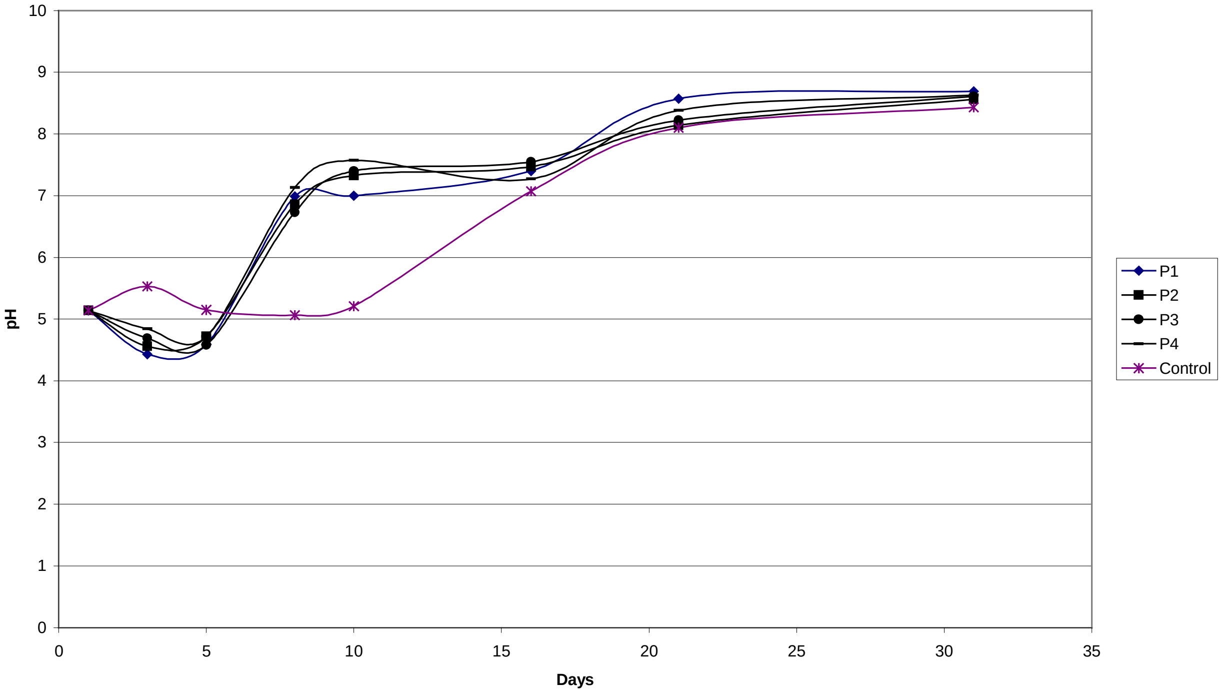

Figure 1. *Development of pH in a mesophilic household waste compost.*

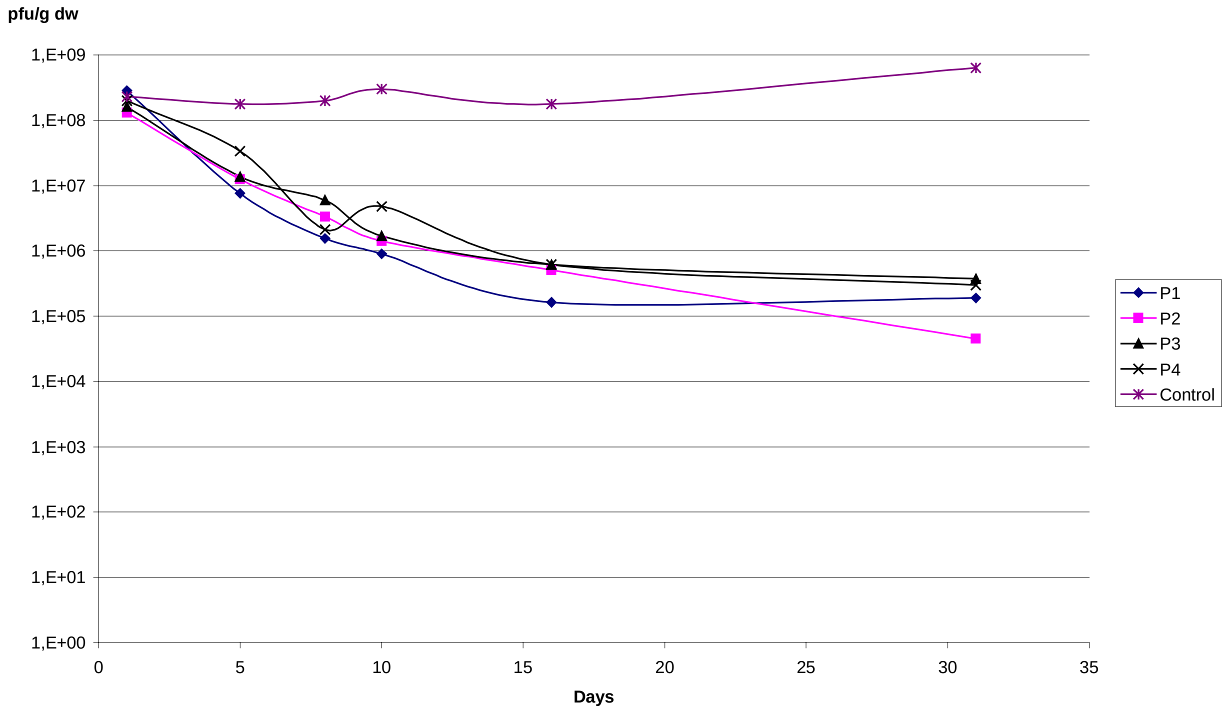

Figure 2. *Reduction of Salmonella typhimurium phage 28B in a mesophilic household waste compost.*

Discussion

Anaerobic micro environments may occur in compost resulting in a production of fatty acids. These may be toxic to different micro organisms. The Salmonella phages were reduced mostly during the acidic period of the compost. The viability was lower for the Ascaris suum ova in the control compost, which had been exposed to a lower pH for a longer period. The bacterial indicators died very rapidly in the start up phase. There seems to be a correlation between a low pH in the starting up phase and the reduction of added indicator organisms.

It is remarkable that the bacterial indicators, very commonly used, died after only a few days under mesophilic conditions. These results question the reliability of these indicator organisms being used to determine the hygienic quality of compost. Though the indicator bacteria have been effectively reduced, other more resistant pathogens, such as enteric viruses and parasitic helminth eggs may have survived. If organic waste is to be recirculated to farmland there has to be a safe way to control the hygienic quality of the compost.

Therefore, other more resistant indicator organisms have to be more thoroughly evaluated. Under mesophilic conditions both bacteriophages and Ascaris eggs may be used. During thermophilic composting bacteriophages may be preferred, since Ascaris eggs have a rapid die-off at temperatures above 45°C (Feachem et al. 1983). There are also other advantages with bacteriophages. They are simple to analyse and non-pathogenic for humans.

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