



Acidic Anaerobic Fermenting Eliminates Pathogens:

One of the concerns with an enriched food waste feedstock is the potential for pathogens like *E. coli* and *Salmonella* to proliferate and contaminate compost.

It is well known and established in composting operations that piles do not everywhere achieve sufficient temperatures to destroy these pathogens. Many pathogens survive composting temperatures. This is a major concern as the chances of pathogens ending up in the final product (compost) are improved as more and more food waste enters into the composting process.

Bokashi (Acidic anaerobic) fermenting results in the accumulation of numerous carboxylic acids including butyric, valproic and caproic acids, and these metabolites are highly lethal to pathogens. Fermenting is done by excluding oxygen. The pH shifts into the range 3.5 to 5.5 and the end points in fermenting are typically observed near pH 4.0.

Many tests with 3M petri film and laboratory cultures reveal coliforms including *Salmonella* do not survive in this fermenting environment and pathogens are rapidly destroyed. The process is so efficient that it is even used to destroy pathogens in petwaste.¹

Many reports in the literature confirm that pathogens are rapidly destroyed in a low pH environment in the presence of butyric and caproic acids.

Fukushi et. al showed that *Salmonella* spp are completely eliminated in less than 2 days in the presence of these acids when the pH is less than or equal to 5.5.²

E. coli O157 does not do well with organic acids at low pH and with the addition of metabolites forming in the fermenting do not survive.³ Acids produced in bokashi fermenting all had profound killing impact on *E. coli* O157.

Even acid resistant rare forms fall off rapidly by the hour in acid media. Note that adding additional factors like anaerobic conditions, competing organisms (non-pathogens) multiplying rapidly in the acidic anaerobic environment, natural

¹ <http://www.bokashicycle.com/blog/bokashi-food-waste-and-pet-poop-disposal-%E2%80%93-chemical-analysis-and-biology/>

² K Fukushi, S Babel, S Burakrai Survival of *Salmonella* spp. in a simulated acid-phase anaerobic digester treating sewage sludge; <http://www.sciencedirect.com/science/article/pii/S0960852402001463>

³ G J Leyer, L L Wang, E A Johnson, Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC167674/>



antibiotics secreted and expressed by fungi further have lethal impact on pathogens.

Zhao revealed even with highly selected adapted organisms, the killing is abundant and evident. ⁴

E. coli are killed very effectively by butyric acid (one of the metabolites observed as fermentation progresses in both pet waste and food waste). This is also very effective at killing *Salmonella* and other pathogens and is used in preventing pathogen growth on meat products. ⁵

Fecal contaminated drinking water containing *E. coli* O157 and other *E. coli* spp. was also shown to be efficiently treated with carboxylic acid metabolites found in the ferment. ⁶

It is the combination of acids (butyric, caproic, etc.) at low pH, lack of oxygen, natural antibiotics formed by fungi growing in the fermentation process, and likely nitrite derivatives formed in the reducing environment all contributing to effective elimination of pathogens that accounts for this efficient pathogen eliminating capacity. The real evidence is of course in the actual testing where none of the coliforms survive 24 hours in pet waste processing with fermenting.

These efficient pathogen killing mechanisms appeared active in an early pilot study in Armstrong BC in a study monitored by the BC Ministry of the Environment. In this study no pathogens were detected in the fermented end products and when that material was mixed with soil, the native coliforms in the soil were further reduced by 50%.⁷

3M petri film coliform analysis for potential pathogens:

Fecal coliform counts were obtained at the CARO Analytical Services (Kelowna) and those results for the IPE protocol will be discussed in more detail under the laboratory results section in this report.

⁴ <http://www.ugacfs.org/research/pdfs/Water2003.pdf>

⁵ Falcini et. al, A systems biology approach sheds new light on *Escherichia coli* acid resistance
<http://nar.oxfordjournals.org/content/early/2011/06/19/nar.gkr338.full>

⁶ Tong Zhao, Ping Zhao, Joe W. West, John K. Bernard, Heath G. Cross, and Michael P. Doyle Inactivation of Enterohemorrhagic *Escherichia coli* in Rumen Content- or Feces-Contaminated Drinking Water for Cattle. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1472328/>

⁷ K. Trusdale, L. Green, An Efficient Cost Effective Alternative Organic Waste Treatment Pilot Study Supporting Sustainable Farming Practices and Soil Restoration. Sep 2010.
http://www.bokashicycle.com/canada/Armstrong_Pilot_Sep012010_No_AS09012010_Summary%20Report_web.htm

bokashi cycle

Butyric acid, propionic acid, and other small chain fatty acids are toxic to coliforms including *E. Coli* and *Salmonella* (known pathogens that do contaminate ground water) under the conditions of this fermentation. The pathogens die off in a fermenting system while other microbes are breaking down the poop and waste material.

If the system is sealed and given time to complete its digestion which takes about 7 days, the chances of getting ground water contamination are very much reduced. We provide in this report results of other studies that demonstrate volatile fatty acids can beneficially inhibit and destroy certain pathogens and fecal coliforms in particular.^{8[1]}

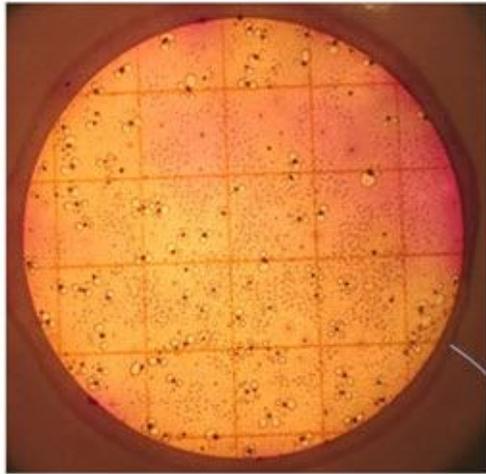
A properly functioning fermenting system is very efficient because it converts the poop or food waste to something of value and gets rid of the heavy load of pathogens that would have otherwise been sent to the landfill or left in the soil to contaminate ground water. Fermenting returns nutrients to the soil. It keeps water from getting contaminated.

3M makes a simple Petri film kit that can be used to rapidly test for coliforms including *E. coli*. In the last two pictures you can see what happens to the *E. coli* /coliforms in the fermenting system compared to a non-fermenting system. They don't make it through the system. In this experiment 10 pounds of dog poop was mixed with 2.5 gallons of water.

A sample of this slurry was set aside and tested on Day 1 and Day 7. A second sample identical to the first except the culture mix powder was added was also tested on Day 1 and Day 7. On Day 1 as would be expected the colonies of microbes were very high and easily detected in both samples.

bokashi cycle

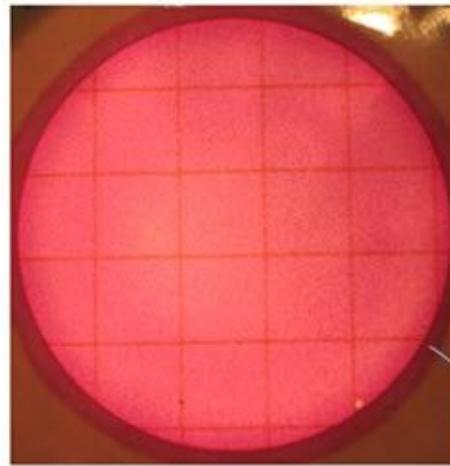
Time = 7 days, sample collection and culture 100 μ L to 500 mL, then 1 mL on film at 35 C Dog waste slurry 10#/2.5 gallons water 3M Petri film Enterobacteracea @ 24 hours



Enterobacteracea (includes *E. coli*) 94

Non - Fermented
Poop Lots of
Pathogens

Time = 7 days, sample collection and culture 100 μ L to 500 mL, then 1 mL on film at 35 C Dog waste slurry 10#/2.5 gallons water + 350 mL Accelerant + Culture mix 3M Petri film Enterobacteracea @ 24 hours



Enterobacteracea (includes *E. coli*) 0

Fermented Poop
Pathogens gone

On Day 7, the sample that had been fermented (right hand image) showed no evidence of surviving pathogens but the sample that was not fermented (left hand image) had abundant colonies of microbes showing.

We have tested fermented food waste systems on numerous occasions and find no evidence of pathogenic microbes in our cultures on Petri film surviving the fermenting cycle.

Fecal Coliform Analysis of Bokashi Tea:

Bokashi tea samples were completely devoid of fecal coliforms as noted in test results below (Table 1). We've already made note of the fact that fermented product put into the soil also results in a reduction in soil fecal coliforms.

bokashi cycle

Table 1: Bokashi Tea Microbiological Analysis for Fecal Coliforms

Bokashi Tea _ Analysis		
Microbiological Parameters		
Compost Tea (K0J1067-01) Matrix: Water Sampled: Oct-12-10 09:00		
Coliforms, Total (MPN)	<3.0	3.0 MPN/100mL
Coliforms, Fecal (MPN)	<3.0	3.0 MPN/100mL

Summary:

Bokashi (acidic anaerobic) fermenting eliminates pathogens efficiently.