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Electron beam inactivation of selected microbial pathogens and indicator organisms in aerobically and anaerobically digested sewage sludge

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HIGHLIGHTS

- Municipal sewage sludges contain a variety of microbial pathogens.
- Sewage sludges need to be adequately disinfected before land disposal.
- Electron beam (e-beam) technology is an environmentally sustainable technology.
- E-beam irradiation causes significant reduction of bacterial and viral pathogens.

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ABSTRACT

Microbial pathogens in municipal sewage sludges need to be inactivated prior to environmental disposal. The efficacy of high energy (10 MeV) e-beam irradiation to inactivate a variety of selected microbial pathogens and indicator organisms in aerobically and anaerobically digested sewage sludge was evaluated. Both bacterial and viral pathogens and indicator organisms are susceptible to e-beam irradiation. However, as expected there was a significant difference in their respective e-beam irradiation sensitivity. Somatic coliphages, bacterial endospores and enteric viruses were more resistant compared to bacterial pathogens. The current US EPA mandated 10 kGy minimum dose was capable of achieving significant reduction of both bacterial and viral pathogens. Somatic coliphages can be used as a microbial indicator for monitoring e-beam processes in terms of pathogen inactivation in sewage sludges.

1. Introduction

The spread of human pathogens through improper disposal of wastewater effluent and sludge is a global concern. With increasing urbanization and resulting population increases, cities around the world have to manage enormous quantities of human wastes. These wastes have to be adequately treated to prevent the transmission of diseases and prevent environmental impacts. In the United States, more than 16,000 waste water treatment plants are in operation today treating around 150 billion liters of waste water per day. In total, these treatment plants produce approximately 5.6 million dry metric tons of treated sewage sludge (termed "biosolids") annually, of which, approximately 60% is land-applied (NRC, 2002). Land application of biosolids is a disposal option that is used around the world and is often recommended as an economical and sustainable method of disposal (Nappier et al., 2006; Pepper et al., 2006; US EPA, 2003).

In the United States, the land disposal of biosolids is federally regulated by the US EPA (US EPA, 1994). This regulation classifies biosolids as either Class A or Class B biosolids based on the level of treatment, pathogen loads, and their potential to attract disease vectors. Class A biosolids undergo a more complete disinfection process with the aid of specific treatment processes referred to as Processes to Further Reduce Pathogens (PFRP). In the United States, the EPA approved PFRP processes are heat treatment, drying, composting, thermophilic aerobic digestion, pasteurization, and ionizing irradiation. Ionizing radiation using either cobalt-60 or electron beam (e-beam) at a minimum dose of 10 kGy is considered as an effective PFRP to produce Class A biosolids. Class

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A biosolids should contain no detectable pathogens (in specified quantities of biosolids), and is considered safe for environmental disposal without restrictions. However, in Class B biosolids, disinfection is incomplete, and so Class B biosolids will contain detectable levels of pathogens. Consequently, there are strict regulations as to how the Class B biosolids are managed (NRC, 2002; US EPA, 1993).

A cobalt-60 based sludge treatment plant is currently in operation in India (Gautam et al., 2005). In the United States, however, there are no ionizing radiation based municipal treatment plants. A pilot-scale low energy e-beam wastewater treatment plant was operational in Florida in the early 90's (Wang and Wang, 2007). The advantage of using e-beam irradiation is that this process is based on commercial electricity, and does not involve the use of radioactive isotopes. Hence issues surrounding radioactive isotopes such as transport, storage, disposal and security are non-existent. There are no published reports describing the inactivation of specific microbial pathogens in municipal sludge under high energy e-beam irradiation conditions. In addition to the difference in the source of ionizing radiation between cobalt-60 and e-beam, these two types of ionizing radiation differ in terms of energy (measured in MeV) and dose-rate (measured in grays/min). The energy profile of the gamma rays from a cobalt-60 source ranges between 1.17 and 1.33 MeV while high energy e-beam is normally around 10 MeV. The dose rate of gamma rays from cobalt-60 is often in the range of hundreds of grays per minute, while in the case of e-beam the dose rate is in the range of tens of millions of grays per minute (Miller, 2005). Given the significant differences in dose rate and energy levels between current e-beam and cobalt-60 processes there is a need to build an information base regarding the inactivation profiles of specific microbial pathogens under e-beam irradiation conditions.

The underlying hypothesis was that high energy (10 MeV) e-beam irradiation is capable of inactivating a variety of microbial pathogens at significant levels. The experimental objective was to obtain empirical microbial inactivation data of selected pathogens and other target organisms in aerobically and anaerobically digested sewage sludge samples (biosolids) under 10 MeV e-beam irradiation conditions.

2. Methods

2.1. Biosolid samples

Aerobically and anaerobically digested biosolid samples were collected over multiple days from two different waste water treatment plants in Texas. The samples were collected directly from the digester outlets in sterile bottles (Nalgene, Rochester, NY) and transported on blue-ice to laboratory in a cooler. The samples were maintained at 4 °C until analysis. Separate collections were made for different irradiation trials. Moisture content and solids concentration (%) of the biosolid samples were determined (in triplicate) by drying 20 ml of wet samples at 102 °C for 24 h. Basic chemical parameters of the sludge samples was determined (Table 1).

2.2. Sample preparation for e-beam irradiation trials

The starting levels of *Escherichia coli*, aerobic and anaerobic spore formers were analyzed immediately or within 24 h of sample collection. The samples had significant levels of *E. coli*, aerobic, and anaerobic spore formers. However, the density of other indicator organisms and specific pathogens were low. Hence, *Salmonella typhimurium*, coliphages, and enteric viruses had to be spiked into the samples in the laboratory at sufficient titers for the inactivation studies. One set of samples were spiked with high titer of laboratory grown strains of nalidixic acid and novobiocin resistant *Salmonella enterica* serovar *typhimurium* (*S. typhimurium*-accession # 87-26254, obtained from National Veterinary Service Laboratory, Ames, Iowa). Similarly, the samples were spiked *with E. coli* phages, ϕX174 (ATCC # 13706-B1) and MS-2(ATCC # 15597-B1) and enteric viruses, poliovirus-1 (VR-1562) and rotavirus (SA-11). The bacterial pathogen was grown to high titers using an overnight culture which was then centrifuged and washed multiple times to remove media components. The washed cells were then re-suspended in sterile phosphate-buffered saline (PBS) prior to sample seeding. The titer of the inoculum was quantified. For the phages and enteric virus samples, the high titers were prepared and quantified using standard protocols. Each of these microorganisms was spiked into separate samples to facilitate accurate quantification.

2.3. E-beam irradiation trials

Twenty milliliter of evenly mixed biosolids samples were placed (in triplicate) in Whirl-Pak® bags (Nasco, New York, NY) and heat sealed. Each Whirl-Pak® bag in turn was triple bagged. The triple bagging was required to meet the University regulations regarding handling potentially bio hazardous samples at the e-beam irradiation facility. The e-beam irradiation was performed at the National Center for Electron Beam Research's e-beam facility on the Texas A&M University campus.

Irradiation dose measurements were performed using alanine dosimetry that was validated to international standards. The dosimeters (Harwell Dosimeters, Oxfordshire, UK) were placed at different locations within the sample (using heat-sealed pouches) to verify the delivered e-beam dose. Preliminary studies were performed to ensure that the samples could be irradiated effectively with dose-uniformity ratio (DUR) $\sim$1.0. The DUR is an important criterion when performing irradiation experiments. A DUR of $\sim$1.0 signifies that the ratio between maximum and the minimum doses anywhere within the sample bag is uniform. The dosimeters were measured using the Bruker E-scan spectrometer (Bruker, Billerica, MA). The measured dose was used for data plotting and calculation of inactivation kinetics. Each inactivation experiment was carried out with triplicates on multiple days.

The inoculated samples were subjected to different target doses of e-beam irradiation. Experiments involving *E. coli* and *S. typhimurium* were irradiated at lower doses of e-beam ranging from 0.2 to 1.0 kGy. Samples that were spiked with bacteriophages, enteric viruses and bacterial spore formers were irradiated at higher doses ranging from 1 to 10 kGy (due to their known intrin-

Table 1
Basic chemical characteristics of the aerobic and anaerobic sludge samples used in the study.

Sample	Solids (%)	[a]BOD_5	[b]TSS	[c]TVSS	[d]SOUR test
Aerobic digester sample	2	4510	15,700	12,000	5.86
Anaerobic digester sample	2	996	29,000	16,900	2.64

[a] 5-Day biological oxygen demand test (mg/l).
[b] Total suspended solids (mg/l).
[c] Total volatile suspended solids (mg/l).
[d] Specific oxygen uptake rate (mg O_2/h/g total solids).