MICROBIAL INACTIVATION OF MUNICIPAL SLUDGE USING E-BEAM AND CHEMICAL OXIDANTS

A Thesis

by

CHANDNI VIJAYAKUMARAN NAIR SOBHA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Suresh D. Pillai
Committee Members,	Luc R. Berghman
	Joseph M. Sturino
Head of Department,	John R. Carey

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ABSTRACT

Microbial Inactivation of Municipal Sludge Using E-Beam and Chemical Oxidants.

(December 2009)

Chandni Vijayakumaran Nair Sobha, B.Sc., Kerala Agriculture University, India;

M.Sc., University of Agricultural Sciences, Bangalore

Chair of Advisory Committee: Dr. Suresh D. Pillai

Municipal biosolids generated from waste water treatment plants should be effectively disinfected before being used for beneficial purposes. Novel sewage sludge treatment technologies ensuring efficient microbial inactivation and sludge stabilization can help in reducing adverse environmental and health impacts. The underlying hypothesis of this study was that electron beam irradiation solely, or in combination with chemical oxidants such as ferrate or chlorine dioxide, will effectively inactivate the microbial loads in municipal biosolids. The specific objectives of the research were to determine the inactivation of selected microbial pathogens and indicator organisms(*Salmonella* Typhimurium, *Escherichia coli*, phi X 174, MS2, poliovirus, *Bacillus subtilis* and *Clostridium perfringens* spores) using high energy (10 MeV) e-beam irradiation in the presence and absence of ferrate and or chlorine dioxide. Additionally, a quantitative microbial risk assessment (QMRA) exercise was performed to evaluate the risk reduction using e-beam technology.

Results indicated that susceptibility of different target organisms to the e-beam irradiation varied considerably, with bacteria being most sensitive and bacterial endospores being most resistant in both aerobically and anaerobically treated sludge. Bacteria as well as poliovirus were susceptible to 200 ppm of ferrate. However, coliphages and endospores could be inactivated, only when ferrate was combined with e-beam. Somatic coliphage were susceptible 30 ppm of chlorine dioxide treatment, where as poliovirus and endospores were resistant to 30 ppm chlorine dioxide treatment. Microbial inactivation by e-beam and chemical oxidants depended upon the sludge matrix (aerobic and anaerobic).QMRA results indicate that the application of e-beam technology to sludge applied to lettuce growing field reduced the risk of food borne outbreaks to below detectable level. Overall, the results highlight the efficiency and stability of e-beam and chemical oxidants in ensuring sludge disinfection and safe nutrient recycling.

DEDICATION

This thesis is dedicated to my beloved parents, grandmother, sisters and all my dear ones!!!

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I would like to sincerely thank Dr. Suresh Pillai, chair of my committee, for his strong support and guidance which helped me in completing this project successfully. His creative criticisms and comments helped me to improve the quality of my work. I express my gratitude to my committee members, Dr. Luc Berghman and Dr. Joseph Sturino, were really supportive in all my endeavors.

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Finally, I thank my beloved Amma, achan, unnanammumma (grand mom) and my sisters for their prayers and wishes which had been a great inspiration for me. I’m grateful to Praveen for his encouraging words and for being with me in each and every step of my career and life.

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CHAPTER I

INTRODUCTION

RATIONALE

Municipal sludge harbors different microorganisms of diverse nature. It is estimated that more than 150 known enteric pathogens may be present in the untreated sludge and the number keeps on increasing every year (Gerba and Smith 2005). There is increased concern among all the nations to implement appropriate treatment strategies to prevent any kind of health and environmental issues related to sewage sludge application. This calls for devising an enhanced sludge treatment process which ensures very high rate of microbial disinfection and stabilization of the treated biosolids to facilitate safe recycling.

Irradiation is used as an effective tool for waste water treatment (Jung *et al*. 2002; Breer 1983). Gautam *et al*. (2005) has reported 6-7 log reduction in fecal coliforms in the sludge samples using Cobalt- 60 based gamma irradiation. Studies conducted by Martin *et al*. (2005) suggest the use of electron beam irradiation for sludge treatment as it reduces the bacterial population by 5 log. Since e-beam is generated from non radioactive sources, they are relatively safe and generate less hazardous by-products (Smith and Pillai 2004). This irradiated sludge also has an additional advantage of prev-

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enting pathogen re-growth and degradation of organic pollutants, which makes irradiation an ideal option for sludge treatment (Gautam *et al*. 2005).

Use of chemical oxidizing agents such as ferrate and chlorine dioxide is shown to inactivate the pathogens in the municipal biosolids and also reduces offensive odors by oxidizing various compounds such as sulfides and ammonia into sulfites and nitrates (de Luca *et al*. 1996). As per Riemers *et al*. (2005), the +6 oxidation state of iron, ferrate (VI) reacts with sludge and inactivates *Clostridium* spp., viruses and also helminth ova. Ferrate (VI) is regarded as a multifunctional oxidant owing to its broad spectrum application as a disinfectant, antifoulant, coagulant and oxidant (Waite 1979; Sharma 2002).

Chlorine dioxide (ClO_2) is another powerful oxidizing agent extensively used in treating potable water. ClO_2 has an oxidation state of + 4 and is a neutral compound of chlorine. Pratt *et al*.(2005), reported extensive use of chlorine dioxide in the sludge neutralization process during which notable reduction occurred in case of bacteria, bacterial spores, viruses and helminth ova.

As per US EPA regulations, irradiation, ferrate and chlorine dioxide treatment are regarded as processes which further reduce pathogens (PFRP) or processes which significantly reduce pathogens (PSRP). Based upon the degree of pathogen disinfection and reduction in vector attraction brought about by the treatment processes, the treated sludge is rendered safe for further land or field application. The overall focus of this study was to determine the level of microbial inactivation brought about by different

sludge processing methods such as irradiation and chemical oxidants. The individual efficiency of each of the process was compared to the effect observed when irradiation was combined with the oxidants. This approach was based on the central hypothesis that e-beam irradiation, solely or in combination with ferrate or chlorine dioxide treatment will effectively inactivate the microbial load in the municipal sludge.

OBJECTIVES

1. Determination of log reduction of microorganisms in aerobic and anaerobically treated sludge samples as a function of e-beam irradiation.

2. Determination of microbial inactivation using chemical oxidant ferrate and a combination treatment of ferrate and e-beam.

3. Determination of microbial inactivation using chemical oxidant chlorine dioxide and a combination treatment of chlorine dioxide and e-beam.

4. Quantitative microbial risk assessment of treated sludge application for agricultural purposes.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Globally there exists a great concern about the hazardous pollutants and microbial pathogens reaching the environment through the improper disposal of waste water. Due to the ever increasing world population, enormous quantities of human waste are generated that needs to be treated appropriately to prevent any kind of threat to human and animal health. There are 16,000 waste water treatment plants in US which treat around 150 billion liters of waste water per day, thus producing 5-7 million dry metric tonnes of sludge on an annual basis (US EPA 1997; Pepper *et al*. 1996).

According to US EPA, Sewage sludge refers to 'the solid, semi solid or liquid residue generated during the treatment of municipal sewage in a treatment work' and bio solid refers to 'the sewage sludge which has undergone treatment and which meets the state and federal regulations for land application'. In US, the disposal of treated sewage sludge and other application of biosolids is regulated under 40 CFR Part 503, issued under the authority of Clean Water Act. The treated sewage sludge could be classified as Class A or Class B, depending upon the level of treatment, pathogen load as well the reduction in the vector attraction capability of the sludge. Class A sludge undergoes a more complete disinfection and thus can be safely applied for agricultural purposes where as disinfection is not complete in case of class B sludge, hence has to undergo stringent monitoring while applying the sludge for other purposes.

PATHOGENS IN SLUDGE

Sewage sludge harbors lot of pathogens which are capable of causing disease to humans and animals. Majorly four kinds of pathogens are encountered in the municipal biosolids and effluents which include viruses, bacteria, protozoa and helminths. Table 2.1 provides the concentration of different microorganisms that are typically present in the domestic waste water. The amount of pathogens that are present in the sewage varies with different locations, socio economic status of the communities, incidence of infections and time of the year (Maier *et al*. 2000). Entry of pathogens into waste water system is also possible through effluents from hospitals which usually have a high microbial concentration. Table 2.2 gives an overview of principal pathogens that gain entry into the sewage sludge and the disease as well as symptoms associated with that pathogen (US EPA 1989, Kowal *et al*. 1985 and US EPA 2003). Microbiological analysis for every single pathogen listed in the Table 2.2 is not a practical option. Instead, the microbiological safety level of sewage is determined based on the number of indicator organisms present on the sludge. Indicator microorganisms serve as representative species for the larger set of pathogenic bacteria, viruses and helminthes, which provide an almost accurate level of the survivability of the pathogens in sewage sludge.

Table 2.1 Concentration of microorganisms normally present in untreated domestic waste water (Pepper *et al*.1996; Metcalf and Eddy 1991).

Organisms	Concentration (per ml)
Total coliforms	10^5-10^6
Fecal coliforms	10^4-10^5
Fecal Streptococci	10^3-10^4
Enterococci	10^2-10^3
Shigella	present
Salmonella	10^0-10^2
Clostridium perfringens	10^1-10^3
Giardia cysts	10^{-1}-10^2
Cryptosporidium oocysts	10^{-1}-10^1
Helminth ova	10^{-2}-10^1
Enteric virus	10^1-10^2

Table 2.2 Principal Pathogens of concern in sewage sludge (US EPA 2003).

Organism	Disease/ Symptom
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning),typhoid fever
Shigella sp.	Bacillary dysentery
Yersinia sp .	Acute gastroenteritis (including diarrhea, abdominal pain)
Vibrio cholera	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Enteric Viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk and Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Rotaviruses	Acute gastroenteritis with severe diarrhea
Polioviruses	Poliomyelitis
Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms
Echoviruses	Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.
Reovirus	Respiratory infections, gastroenteritis
Astroviruses	Epidemic gastroenteritis
Caliciviruses	Epidemic gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Balantidium sp.	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Ascaris suum	May produce symptoms such as coughing, chest pain, and fever
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginafa	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Taenia solium	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

Different indicator organisms which are subjected to microbiological analysis include- fecal coliforms, Enterococci, coliphages, *Clostridium sp.*, helminth ova etc. Fecal coliforms are group of enteric bacteria that are relatively non pathogenic to humans, but serve as an indicator for the presence of fecal contamination. The US EPA (2003) regarded fecal coliforms as an overly conservative indicator. Coliforms have the ability to multiply outside host and there are chances of reintroduction of fecal coliforms in sludge from the addition of wood chips, bulking agents etc, thus providing an overestimation of the actual contamination level of the sample being tested (Meckes 1995).

Somatic and male specific coliphages serve as ideal indicator organisms for fecal contamination (Gerba *et al*. 1987; Pillai 2006). Theoretically, coliphages can multiply in environments which support the growth of *E. coli*, which is considered as a limitation of coliphage from being considered as an ideal indicator organism (Muniesa *et al*. 2003). However, no studies have yet been published, which reports the multiplication of coliphages in natural environment. Under natural environment conditions, the appropriate growth conditions such as optimum temperature, host bacterial density and other physiological conditions required for phage replication is rarely found (Muniesa and Joffe 2004; Pillai 2006). Compared to bacterial indicators, bacteriophages serve as a better indicator for the presence of enteric virus owing to the similarity in size, survival and persistence pattern, transport as well as density in environment (Havelaar *et al*. 1986; Gerba 1987; Hsu *et al*. 2002; Endley *et al*. 2003). A subset of the enteric viruses which constitutes Poliovirus, Coxsackie virus, and Echovirus could be assayed to

accurately monitor presence of several enterovirus species. But enteric virus analysis is a time consuming and expensive assay. Coliphage analysis can easily replace the enteric virus analysis, as they give indicative results of enteric virus at a fraction of cost of cell culture infectivity assays (Pillai 2006).

According to US EPA, the results obtained from all the microbiological analyses should be expressed in terms of density of microorganism per unit mass of total solids. The number of organisms present in a unit volume of sewage sludge is expected to change with different processes in the treatment such as dilutions, thickening etc. But the number of microorganisms present in the unit mass remains constant, thus providing a near estimate of pathogen density. Hence, density of different microorganisms present in the sewage sludge is expressed as CFU (Colony forming units), PFU (Plaque forming units) or MPN (most probable numbers) per 4 g of dry solids. This unit came into existence as most of the tests started with an initial volume of 100 ml of sewage sludge which typically contained 4 g of sewage sludge solids on dry weight basis (US EPA, 2003).

HUMAN EXPOSURE PATHWAYS

Poorly treated waste water can contain different viruses, bacteria and protozoans that serve as the source of contamination to humans (Gerba and Smith 2005). Exposure can occur via direct as well as indirect contact. Direct contact majorly occurs by ingestion, inhalation or dermal contact. The bioaerosols generated during land application of sludge also invokes occupational risk (Tanner *et al*. 2008). Indirect exposure to pathogens occurs mainly through consumption of pathogen contaminated crops grown in sewage sludge amended soil, through consumption of meat or animal products obtained from animals grazed in fields amended with sewage sludge, by drinking contaminated water or through recreational water contaminated with pathogens migrating from sludge via run off or ground water aquifers (Haas *et al*. 1996; US EPA 2003; Gale 2005). To minimize the possible risk of pathogen exposure to humans and animals arising from sewage sludge application in agricultural farms, US EPA has established stringent regulations and site restrictions as summarized in Table 2.3.

Table 2.3 Pathways of exposure of pathogens and applicable site restrictions for Class B biosolids (US EPA 2003).

Pathways	Part 503 Required Site Restriction
Handling soil from fields where sewage sludge has been applied	No public access to application sites until at least 1 year after Class B biosolids application.
Handling soil or food from home gardens where sewage sludge has been applied	Class B biosolids may not be applied on home gardens.
Inhaling dust	No public access to application sites until at least 1 year after Class B biosolids application.
Walking through fields where sewage sludge has been Applied	No public access to fields until at least 1 year after Class B biosolids application.
Consumption of crops from fields on which sewage sludge has been applied	Site restrictions which prevent the harvesting of crops until environ mental attenuation has taken place.
Consumption of milk or animal products from animals grazed on fields where sewage sludge has been applied	No animal grazing for 30 days after Class B biosolids have been applied.
Ingestion of water contaminated by runoff from fields where sewage sludge has been applied	Class B biosolids may not be applied within 10 meters of any waters in order to prevent runoff from biosolids amended land from affecting surface water.
Ingestion of inadequately cooked fish from water contaminated by runoff from fields where sewage sludge has been applied	Class B biosolids may not be applied within 10 meters of any waters in order to prevent runoff from biosolids amended land from affecting surface water.
Contact with vectors which have been in contact with sewage sludge	All land applied biosolids must meet one of the Vector Attraction Reduction

WASTE WATER TREATMENT

In centralized waste water treatment systems, wastewater generated from all the households as well as industries, is directed to a single end point where it undergoes different sequential treatments. These treatments result in pathogen reduction as well as the "conditioning" of the biosolids for safe disposal and for agricultural purposes. Fig. 2.1 gives an overview of generation, treatment and further disposal of the sewage sludge.

Waste water should be treated to remove all biodegradable organic matter before the effluent enters water bodies. Presence of such organic matter will result in the rapid growth of microorganisms in the water body, which will consume all available oxygen in the water thus creating an anaerobic atmosphere adversely affecting aquatic ecosystem (Pepper *et al*. 1996). Appropriately treating the waste water reduces the organic content of the effluent. The major steps involved in the sludge treatment process are illustrated in Fig. 2.2. Primary treatment refers to the initial step where in which the larger debris is removed by a physical process. Bar screens are employed for removing the debris and grit chambers helps in settling sand and gravels. The suspended organic matter is further allowed to settle at primary settling tanks. The primary settlement of the sludge helps in removal of helminth eggs as well as bacteria and viruses attached to the soil. This sedimented solid is directed for further treatments and the resultant sludge is referred to as primary sludge, which contains 3-8 % solids (Maier *et al*. 2000).

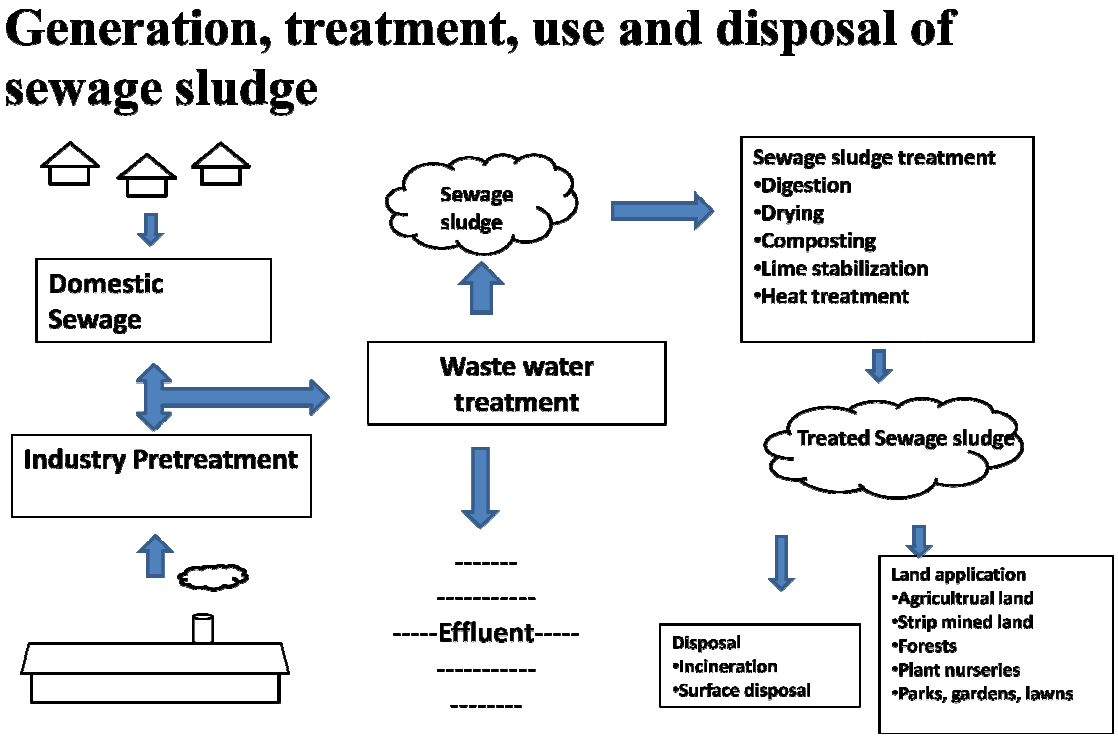


Fig. 2.1 Schematic representation describing generation, treatment, use and disposal of sewage sludge (Adapted from US EPA 2003).

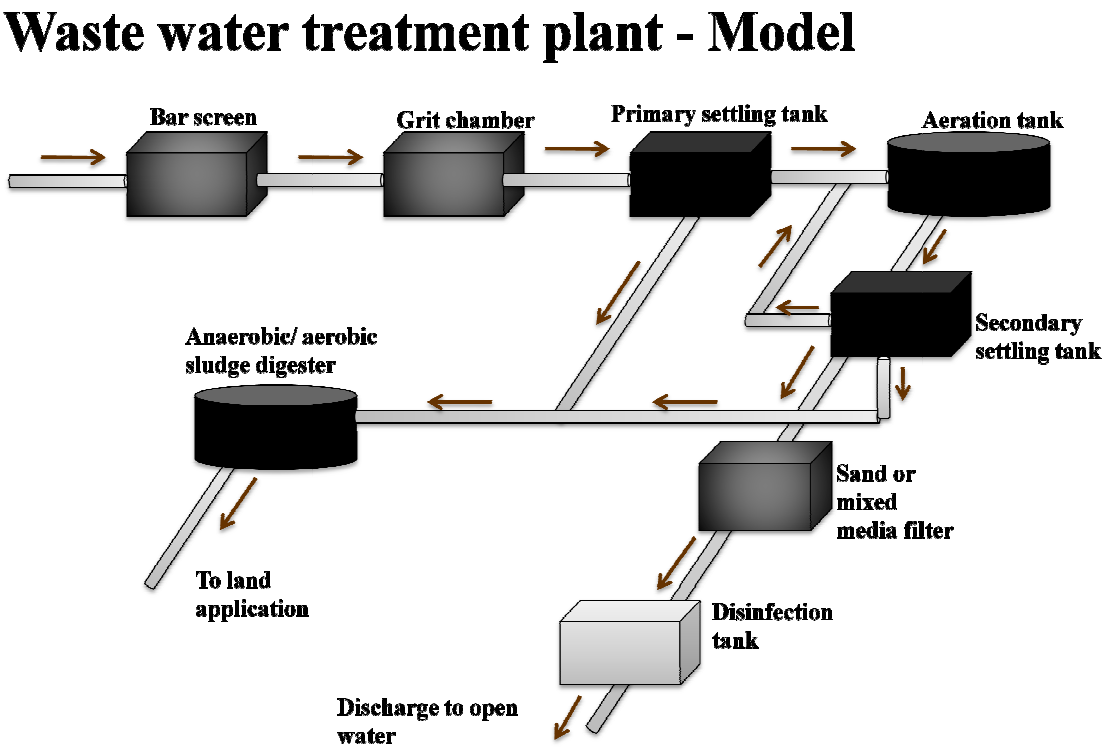


Fig. 2.2 Schematic representation of a waste water treatment plant (Adapted from Pepper *et al*. 1996).

Secondary treatment is carried out using trickling bed filters or conventional activated sludge method. Trickling bed filters are made up of media comprising of stone beds, corrugated plastic, coal or ceramic stacked together to a height of 6-10 ft through which effluent is allowed to trickle down. These trickling bed filters are also referred to as biotowers (Maier *et al*. 2000). The microbes present in the sludge forms a biofilm in the media called as zooleal layer. Another secondary treatment method is the use of activated sludge process, which is considered as a better treatment method as it removes 53-99.92% of enteric viruses (Moore *et al*. 1981, Maier *et al*. 2000). During the activated sludge treatment, the effluent from the primary treatment is aerated with a mixture of bacteria rich slurry. Aeration promotes the microbial growth and favors organic matter decomposition. Bitton (1994) suggested maintaining proper food to the microorganism ratio (F/M ratio) in order to control the organic decomposition level in the activated sludge process. The activated sludge is allowed to settle within secondary settling tanks and the sediments are directed to a sludge digestor. The effluent obtained from the secondary treatment is referred to as secondary sludge and contains 0.5-2 % solids (Pepper *et al*. 1996).

Tertiary treatment refers to the physic-chemical processes of removing the turbidity by employing different filtration techniques and by coagulating the sludge using lime or Iron or Aluminium salts. Coagulation increases the pH of the sludge facilitating a 90-99% reduction of enteric viruses (Rao *et al*. 1986). Reverse osmosis and ultra filtration, UV irradiation are other tertiary treatment methods employed for microbial reduction in the effluent.

The sludge or biosolids generated from various steps of the waste water treatment are subjected to different processing steps including sludge thickening, digestion, conditioning and dewatering, before they are approved for safe disposal or for any other beneficial purposes (Pepper *et al*. 1996) (Fig. 2.3). Thickening involves volume reduction of the sludge basically by removing excess water. Digestion is the key step that helps in pathogen reduction, odor control, and sludge stabilization. Digestion can be carried out either aerobically or anaerobically. Anaerobic digestion of the sewage sludge is the most common sludge processing method, which takes 2-3 weeks and produces methane gas that could be used as an energy source. Aerobic digestion of sewage sludge is carried out by passing air or O_2 in to the sludge in 10-20 ft deep open tanks where sludge is retained for a period of 12- 30 days. Aerobic digestion is relatively cheaper and produces bulk quantities of odorless sludge (Bitton 1994; Maier *et al*. 2000). Table 2.4, briefly compares between two different sludge digestion processes- ATAD (Autothermophilic thermal aerobic digestion) and anaerobic digestion which are employed by College station and Texas A&M University waste water treatment plants respectively. Sludge samples were collected from ATAD and anaerobically digestion plants for the microbiological analyses for this study. Before disposal of the digested sludge, it is conditioned using lime, alum or ferric chloride. Further the stabilized sludge is dewatered by air drying, centrifugation, vacuum filtration etc and is utilized for land application or for other beneficial purposes.

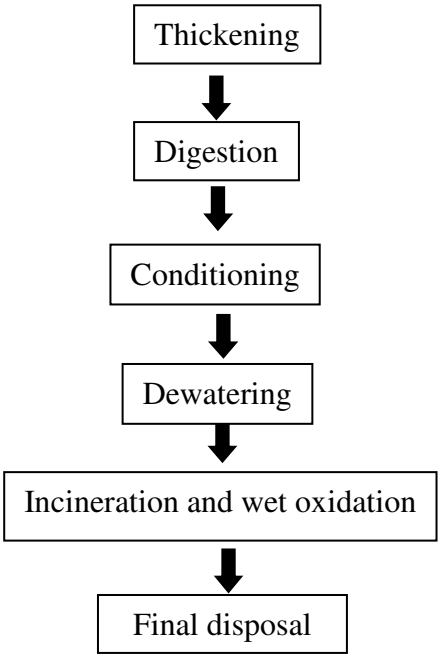


Fig. 2.3 Different sludge treatment processes (Adapted from Pepper *et al*. 1996).

Table 2.4 Comparison between the ATAD and the anaerobic sludge digestion process.

ATAD (Autothermophilic thermal aerobic digestion)	**Anaerobic digestion**
Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean residence time of the sewage sludge is 10 consecutive days at 55°C to 60°C	Sewage sludge is treated in the absence of air for a specific mean residence time at a specified temperature which shall be between 15 days at 35°C to 55°C and 60 days at 20°C
Biological oxidation takes place in an efficiently aerated environment Biodegradable volatile solid content can be reduced by upto 70 % in very short span of time	Biological process involving bacteria that function in an oxygen free environment Volatile solids are reduced by 35% to 60% depending upon the nature of sewage sludge
Pathogenic viruses, bacteria and viable helminth ova are reduced below detectable limits	Reduces bacterial and viral population by 90% or more. No substantial reduction in viable helminth ova is obtained
Recognised as a PFRP (Processes to further reduce pathogens) by US EPA, thus producing class A sludge	Recognised as a PSRP (Processes to significantly reduce pathogens) by US EPA, thus producing class B sludge

CLASS A AND CLASS B SLUDGE REQUIREMENTS

The sewage sludge obtained from the waste water treatment plant should meet standards according to 40 CFR 503, for safe use as well as disposal (US EPA 2003). The subpart D of the regulation, provides stringent guidelines for ensuring pathogen disinfection and reduction in vector attraction. There are two levels of pathogen reduction , namely Class A and Class B. Class A sludge undergoes more complete form of disinfection which ensures the level of pathogens to be below detectable levels (US EPA 2003). Class A sludge is also regarded as stable as it no longer provides food for rapid microbial activity as it meets vector attraction reduction requirements. Class A sludge can thus be put into use without any further site restrictions and monitoring. However, disinfection is incomplete in case of Class B sludge. Hence, Class B sludge may contain certain pathogens and there exists restrictions for crop harvesting, animal grazing and to public access up to a certain time period (Table 2.3). The pathogen reduction and the vector attraction reduction requirements for Class A sludge and Class B sludge are given in Table 2.5 and Table 2.6 (US EPA 2003).

Table 2.5 Pathogen reduction requirements for (a) Class A and (b) Class B sludge.

Pathogen	Pathogen requirement
Salmonella	Less than 3 MPN/4 g total solid biosolid (dry weight basis)
Fecal coliforms	Less than1000 MPN/ g total solids
Enteric viruses	Less than 1 PFU/4 g total solids biosolids
Viable helminth ova	Less than 1 viable helminth ova/ 4 g total solids biosolids

(a)

Pathogen	Pathogen requirement
Fecal coliform	Less than 2 million MPN or CFU/ g total solids

(b)

Table 2.6 Vector attraction requirements determining stability of the treated sewage sludge (US EPA 2003).

Requirement	What is Required?	Most Appropriate For:
Option 1 503.33(b)(1)	At least 38% reduction in volatile solids during sewage sludge treatment	Sewage sludge processed by: Anaerobic biological treatment, Aerobic biological treatment
Option 2 503.33(b)(2)	Less than 17% additional volatile solids loss during bench-scale anaerobic batch digestion of the sewage sludge for 40 additional days at 30°C to 37°C (86°F to 99°F)	Only for anaerobically digested sewage sludge that cannot meet the requirements of Option 1
Option 3 503.33(b)(3)	Less than 15% additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20°C (68°F)	Only for aerobically digested liquid sewage sludge with 2% or less solids that cannot meet the requirements of Option 1 - e.g., sewage sludges treated in extended aeration plants. Sludges with 2% solids must be diluted
Option 4 503.33(b)(4)	SOUR at 20°C (68°F) is ≤1.5 mg oxygen/hr/g total sewage sludge solids	Liquid sewage sludges from aerobic processes run at temperatures between 10 to 30°C. (should not be used for composted sewage sludges)
Option 5 503.33(b)(5)	Aerobic treatment of the sewage sludge for at least 14 days at over 40°C (104°F) with an average temperature of over 45°C (113°F)	Composted sewage sludge (Options 3 and 4 are likely to be easier to meet for sewage sludges from other aerobic processes)
Option 6 503.33(b)(6)	Addition of sufficient alkali to raise the pH to at least 12 at 25°C (77°F) and maintain a pH ≥12 for 2 hours and a pH≥11.5 for 22 more hours	Alkali-treated sewage sludge (alkaline materials include lime, fly ash, kiln dust, and wood ash)
Option 7 503.33(b)(7)	Percent solids ≥ 75% prior to mixing with other materials	Sewage sludges treated by an aerobic or anaerobic process (i.e., sewage sludges that do not contain unstabilized solids generated in primary wastewater treatment)
Option 8 503.33(b)(8)	Percent solids ≥90% prior to mixing with other materials	Sewage sludges that contain unstabilized solids generated in primary wastewater treatment (e.g., heatdried sewage sludges)

Table 2.6 Continued

Requirement	What is required?	Most Appropriate For:
Option 9 503.33(b)(9)	Sewage sludge is injected into soil so that no significant amount of sewage sludge is present on the land surface 1 hour after injection, except Class A sewage sludge which must be injected within 8 hours after the pathogen reduction process	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, a forest, or a reclamation site, or placed on a surface disposal site
Option 10 503.33(b)(10)	Sewage sludge is incorporated into the soil within 6 hours after application to land or placement on a surface disposal site, except Class A sewage sludge which must be applied to or placed on the land surface within 8 hours after the pathogen reduction process	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, forest, or a reclamation site, or laced on a surface disposal site
Option 11 503.33(b)(11)	Sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day	Sewage sludge or domestic septage placed on a surface disposal site
Option 12 503.33(b)(12)	p H of domestic septage must be raised to ≥12 at 25°C (77°F) by alkali addition and maintained ≥ 12 for 30 minutes without adding more alkali	Domestic septage applied to agricultural land, a forest, or a reclamation site or placed on a surface disposal site

Processes which produce the Class A sludge are termed as PFRP (Processes to further reduce pathogens) and the processes resulting in Class B sludge production are termed as PSRP (Processes to significantly reduce pathogens). These processes not only reduce pathogens but also aid for reduction in vector attraction properties of the sludge. Vectors are attracted to the sludge due to the presence of putrescible objects that serve as food source for vectors (Acquisto *et al*. 2006). Chemical or biological oxidation of the sludge will considerably reduce the available food source and render the sludge stable and free from offensive odors. Table 2.7 lists various physical, biological and chemical processes involved in the disinfection and stabilization of the sewage sludge. In this review, focus will be given to discuss about physical stressor- electron beam and chemical stressors- ferrate and chlorine dioxide and their use in waste water treatment and sludge stabilization.

Table 2.7 Key processes in sludge disinfection and stabilization (Adapted from Acquisto *et al*. 2006).

Biological Methods	**Physical methods**	**Chemical methods**
Aerobic digestion	Thermal treeatment	Alkaline treatment
Anaerobic digestion	Pasteurization	Lime stabilization
Composting	Heat drying	Ferrate (VI) oxidation
	Air drying	Ozone
	Gamma rays	Chlorine dioxide
	Electron beam	Hydrogen peroxide
	Microwaves	Acid liming
	Homogenization	
	Ultrasonics	

ELECTRON BEAM IRRADIATION

There exists majorly two types of irradiator systems for radiation processing – e-beam and gamma irradiators. Gamma rays are emitted by radioactive source such as ^{137}Cs and ^{60}Co, where as e-beam does not involve radioactive sources. In e-beam, electrons are accelerated under controlled electric and magnetic fields in a single direction to penetrate the target. Thus e-beam does not have the public acceptance issue as compared to the gamma rays obtained from radioactive source (Gehringer *et al*. 2003). The effect caused by e-beam depends upon certain key factors such as dose, dose distribution, stopping power, range and penetration of the e-beam.

When electrons travel through a target material, they tend to lose energy due to excitation and ionization of atoms present within the material. This average linear rate of energy loss of these electrons in a particular medium is referred to as "stopping power". Stopping power is generally expressed in terms of MeV per cm. It is also referred to as the linear energy transfer (LET) of the particle with units generally expressed as keV per μm (Turner 1995). Stopping power and LET are closely related to the dose imparted to the material by the electrons traveling through that material. Dose refers to the energy deposition per electron colliding with an atom in the target surface thereby generating secondary, tertiary electrons to dissipate off the energy. Absorbed dose is defined as the quantity of the ionizing radiation energy which is imparted per unit mass of the specified material being irradiated (Sommers and Fan 2006). The SI unit of absorbed dose is the gray (Gy). One Gy represents one joule of energy deposited per kilogram of material.

Depth dose distribution profile depicts the energy absorbed by a unit mass at particular depth. This dose- depth distribution curve consists of energy absorbed from the incident electron or the absorbed dose as the ordinate and the depth of penetration as the abscissa. The dose distribution profile over different depths varies based upon the source of electron. Since the electron distribution is not constant, certain positions in the target material receives more energy compared to other positions. The uniformity of electron deposition is thus represented by a D_{max}/D_{min} ratio, where D_{max} and D_{min} correspond to depths receiving maximum and minimum dose respectively.

The electron distribution pattern is markedly different in case of single and double sided e-beam. In case of a double sided dose distribution curve energy deposition occurs from both the sides of the target material. Hence the range of the particle can be increased by using a dual e-beam which allows for the electrons to travel more distance inside the target material before it comes to rest (Turner 1995). The dual beams overlap and would result in a synergistic effect at the point of convergence which maintains the dose distribution uniform throughout the thickness of the material.

When microbial cells are exposed to ionizing radiations as in case of electron beam, it brings about several direct as well as indirect effects which make it possible for e-beam to be used for the microbial reduction. The primary effects of the e-beam is due to the collision of electrons into the microbial cell which results in breakdown of the vital cellular components such as nucleic acid where as the secondary effects are due to the production of radiolytic species causing indirect damage to the cell.

Primary effects of E-beam irradiation. The high energy radiation upon entering into the cell, targets the DNA molecules which constitute the major molecular structure of the cell. Ionization by the electron beam causes stripping off of electrons from the DNA molecules thereby causing breakages in the DNA double strand (Smith and Pillai 2004). Single strand DNA as well as most of the double stranded (not directly opposed) DNA repair is carried out by the cell with the help of the DNA polymerase enzyme owing to its proof reading activity, where as directly opposed double stranded DNA repair is hard to undertake as there doesn't exist a corresponding complementary base to provide the correct nucleotide information to repair the strand breakage. This kind of DNA strand damage could possibly accumulate together to bring about lethal mutations in the nucleic acid of the microbial cell (Bartek and Lukas 2003).

Secondary effect of Ionizing radiation. Apart from the DNA damage there also occurs production of a range of radiolytic species as a result of the series of oxidation reduction reactions occurring in the course of electron transfer from one atom to another. Water constitutes a major portion of the microbial cell and hence would suffer from the radiolytic breakdown by the incoming e-beams producing several reactive oxygen species (ROS). The ROS thus produced are toxic to the cell and also causes damage to the other cell components (Grecz *et al*. 1983). Major ROS produced includes hydroxyl radical, superoxide radical, hydrogen peroxide molecules etc. Cells do have innate systems such as catalase, peroxide reductase, superoxide dismutase etc which scavenges these ROS being formed (Arena 1971).

Another important effect is the production of mutagenic lesions in the DNA such as the GO lesion (Michaels and Miller 1992). The 8 hydroxyguanine (GO) or the 7, 8 dihydro-8-oxoguanine (8-oxoG) refers to the damaged form of the Guanine residue in the nucleic acid. The GO lesions are very stable product of oxidative damage of DNA which can be due to ionic radiation, electron transport or lipid peroxidation. Cells have evolved different strategies such as GO system comprising of mutM, mutt and mutY genes to enhance the proof reading of the DNA polymerase III to encounter such lesions in the nucleic acid (Michaels and Miller 1992).

IRRADIATION IN MUNICIPAL BIOSOLIDS TREATMENT

Irradiation is regarded as a process which further reduces pathogens in sludge as per the part 503 D regulation. Initial studies employing irradiation in waste water treatment was carried out by Lowes *et al*. in 1956, where ^{60}Co gamma source was used to disinfect effluent and to remove organic compounds from waste water. Gamma irradiators were installed at Geiselbullach treatment plant, Germany for disinfection of sewage sludge (Lessel *et al*. 1975). The United States initiated establishment of irradiator using Cs-137 source for sewage treatment at Sandia Laboratories, New Mexico in 1979. Co-60 was also used as the source for a municipal wastewater treatment plant built by the Isotope Division of the Bhabha Atomic Research Center in collaboration with M.S. University of Baroda, Gujarat Water Supply and Sewerage Board, and Municipal Corporation of Baroda, India (Shah *et al*. 2001). First electron accelerator was built to treat biosolids as a demonstration project at the Deer Island

Wastewater Treatment Plant in Boston in 1976 under supervision of experts from Massachusetts Institute of Technology (Wang 2007). Another e-beam plant was established in Virginia Key, Miami, Florida during 1981-1983 (Wang 2007). Pikaev (1995), estimated that globally, there exists more than 180 gamma irradiators and nearly 1000 electron beam accelerators serving different industrial, medical and agricultural purposes.

When sewage sludge undergoes irradiation lot of changes occur in terms of sludge disintegration, average floc size and floc distribution (Yuan *et al*. 2008). There was a shift in the floc distribution from 80-100 µm to 0-40 µm with a gamma irradiation dose of 0-30 kGy. This clearly indicated that the sludge disintegration occur with increased dose of irradiation (Yuan *et al*. 2008). It was also observed that the microbial cells get disrupted due to the increased irradiation dose, resulting in the release of protoplasm into the sewage thereby increasing the soluble chemical oxygen demand (SCOD). The microbial cell rupture as well as sludge disintegration occurring due to pretreatment with irradiation effectively enhance the digestibility of the sewage sludge (Yuan *et al*. 2008). In 1983, Keller reported the potential of bacterial re-growth after being subjected to irradiation. Aggregation or clumping of the bacteria and viruses in the sludge make them inaccessible to gamma and e-beam penetration, thus causing re-growth after treatment. Studies conducted by Gautam *et al*. (2005), provide an insight about the use of irradiation to prevent bacterial regrowth. By slightly modifying the sludge treatment system design from an entirely closed loop to an open loop system, 6-7 log reduction in the fecal coliform population was achieved and bacterial regrowth was

effectively prevented. This effective reduction was obtained as a result of utilizing oxygen's radio- sensitizing properties (Gautam *et al*., 2005). Possible reactivation or regrowth of e-beam inactivated organisms were studied in our lab. The results show that there is no regrowth or reactivation of either *Salmonella* or *E. coli* even when incubated at room temperature for up to 6 weeks (unpublished data), proving the efficacy of the e-beam irradiation in preventing bacterial regrowth.

The disinfection capacity of gamma and e-beam irradiators in waste water treatment has been widely studied (Jung *et al*. 2002; Pikanev 2001; Sakamoto *et al*. 2001). Gamma rays have a high penetration depth compared to e-beam irradiation of low energy levels. Hence, gamma rays are effective for treating sludge introduced into a vessel that surrounds the radiation source. E-beam has a relatively lower penetration capability thereby requiring the sewage sludge to be introduced as thin layers as shown for irradiation. Studies conducted by our laboratory suggest that the range of electron penetration could be vastly improved by the use of double beam (unpublished data). E-beam irradiators are more suitable to the waste water treatment plants due to their low cost and versatility. The economic feasibility analysis showed that gamma irradiation costs 4 times more than e-beam irradiation due to high cost and the low productivity of the radioactive source. Moreover e-beam has a better public acceptance as it does not involve any radioactive source and can be turned on and off instantaneously facilitating better process control compared to gamma irradiators.

CHEMICAL OXIDANTS – FERRATE (VI)

Ferrate (VI) serves as a powerful oxidizing agent in waste water treatment providing excellent disinfection and sludge stabilization (Jiang and Llyod 2002; Sharma 2002; Sharma *et al*. 2005). Common disinfectant used for treatment includes chlorine, chloramines, ozone etc that transforms organic compounds to produce disinfection byproducts such as trihalomethanes that are toxic or carcinogenic in nature (Mitch and Sedlak 2002). However, the final reaction product of ferrate (VI) treatment is a non toxic compound that is found to inactivate even chlorine resistant organisms like aerobic spore formers and sulfite reducing *Clostridia* (Sharma 2007).

The +6 oxidation state of ferrate can be synthesized by three different synthetic processes (Perfliev and Sharma 2004). A dry technique suggested by Scholder (1962) of ferrate synthesis involves heating of Fe_2O_3- NaOH- Na_2O_2-O_2 system at different temperatures. This would result in less than 50% yield of ferrate. Potassium ferrate could also be synthesized from dehydrated ferrous sulfate in a dry process (Neveux *et al*. 1999). Lapique and Valentine (2002) suggested ferrate synthesis by electrolyzing an alkaline solution with an anode. The anodic iron in NaOH solution is oxidized to ferrate (VI) using an appropriate anodic potential. This approach generates pure dissolved ferrate with a yield of less than 50%. Wet synthesis involves oxidation of a basic solution using Fe (III) salt by hypochlorite (Thompson *et al*. 1951, Sharma 2007). Potassium hydroxide is added to a solution of sodium ferrate to precipitate potassium ferrate (VI). The ferrate thus obtained is more than 90% pure but the process

results in very low yield of only 10-15% of ferrate (VI). An innovative technique of synthesizing ferrate on-site using equipments referred as ferrators which improved the ease and cost of ferrate application. Ferrator**TM** are patent protected on-site ferrate synthesis technique which generates high quality ferrate widely being used for waste water treatment (Matheickal *et al*. 2005; Jessen et al. 2008).

Ferrate (VI) is regarded as a multifunctional oxidant owing to broad spectrum application as a disinfectant, antifoulant, coagulant and oxidant (Waite 1979; Sharma 2002). The Fe (VI) ions have a characteristic violet color similar to the permanganate in aqueous solution. The diluted Fe (VI) solutions are stable in nature and have high redox potential under acidic condition (Sharma 2002). In water, Fe (VI) undergoes spontaneous decomposition releasing molecular oxygen and nontoxic Fe (III) (Goff and Murmann 1971) according to the reaction equation given as follows

$$2FeO_4^{2-} + 5H_2O \rightarrow 2Fe^{3+} + \frac{3}{2}O_2 + 10\ OH^-$$

Ferrate (VI) inactivates coliforms at a relatively lower dose, contact time and over wide pH range (Sharma 2007). Up to 3 log reduction in aerobic spore formers and sulfite producing clostridia population was achieved by treating river water with 2 ppm ferrate which was found to be chlorination resistant (Franklin 1998). Several other bacterial species were also reported to be susceptible to ferrate which includes *Streptococcus bovis, Staphylococcus aureus, Shigella flexneri, Streptococci faecalis, and Salmonella typhimurium* (Murmann and Robinson 1974; Gilbert *et al*. 1976; Sharma *et al*. 2005).

Ferrate (VI) treatment results in irreversible inactivation of many crucial enzymes (Basu *et al*. 1987). The enzymes include DNA polymerase and murine leukaemia virus reverse transcriptase (MuLV RT), reverse transcriptase enzyme expressed in *E. coli* (Basu *et al*. 1987; Reddy *et al*., 1991; Kotewicz *et al*. 1985). The polymerization and 3'-5' exonuclease activity of DNA polymerase is lost as a result of ferrate oxidation, where as MuLV RT enzyme loses the DNA polymerase and RNAase H activity required for reverse transcription of mRNA to DNA (Kotewicz *et al*. 1985). Oxidation by ferrate also resulted in degradation of deoxyribonucleosides dG and dT in the DNA molecule causing nicks and lesions making the DNA unstable at alkaline pH (Stevenson and Davies 1995). Apart from the direct impact, ferrate (VI) oxidation also results in production of +5 oxidation state of ferrate which is a highly reactive species capable of oxidizing pollutants and pathogen inactivation (Sharma 2002).

The multifunctional properties of ferrate enable it to inactivate wide range of microorganisms and toxins (Sharma *et al*. 2006). Being an efficient coagulant and oxidant, ferrate can be effectively utilized for recycling and reuse of waste water (Eng *et al*. 2006; Sharma *et al*. 2006).

CHEMICAL OXIDANTS – CHLORINE DIOXIDE

Chlorine dioxide is increasingly preferred as a disinfectant over chlorine as they produce much less harmful organic byproducts compared to the toxic trihalomethanes and chloramines produced by chlorine (Weinberg and Narkis 1992). According to the reports by Hoehn (1992), approximately 700-800 public water systems use chlorine

dioxide to treat potable water. Chlorine dioxide serves as a disinfectant and a pre-oxidant to control odor and taste during the water treatment process (US EPA 1999).

Chlorine dioxide (ClO_2) has an oxidation state of + 4 and is a neutral compound of chlorine. It remains stable as free radicals in aqueous solution. Aieta and Berg (1986) showed that even though ClO_2 is highly soluble in water it does not undergo hydrolysis, instead remains as a dissolved gas in water. In contrast to the chlorine gas which has a high hydrolysis rate, ClO_2 reacts 7-10 million times slower in water (Gates 1989). For most of the commercial purposes, ClO_2 is generated by reacting sodium chlorite with an acid such as hypochlorous acid, hydrochloric acid or sulfuric acid. Reaction between Sodium chlorite and chlorine also yields ClO_2, as given by following equations (Gates 1998).

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$$

$$2NaClO_2 + HOCl \rightarrow 2ClO_2 + 2NaCl + NaOH$$

$$5NaClO_2 + 4HCl \rightarrow 4ClO_2 + 5NaCl + 2H_2O$$

Chlorine dioxide is being used as a stressor in Neutralizer™ process, which is referred to as an acid-oxidative process (Pratt *et al*. 2005). During the process, ClO2 is added into a closed system and allowed to mix continuously for certain period of time. Further acid such as sulfuric acid is added on to the system to reduce the pH around 2.5. Later, addition of nitrite induces formation of nitrous acid and the pH of the system drops due to the formation of more acidic substances. Lime is added to restore the pH of

the treated sludge and it is subjected to dewatering. During the Neutralizer™ process, the sludge undergoes through three different stressors such as ClO2, acidic pH conditions, and nitrous acid which together ensures complete disinfection of pathogenic organisms in the sludge.

Unlike, irradiation and ferrate, which predominantly affects the nucleic acids, ClO_2 disrupts the protein synthesis mechanism (Roller *et al*. 1980). It interacts with proteins and lipids in the cell membrane and thus alters the membrane permeability (Aieta and Berg 1986). Hence, ClO_2 causes viral inactivation not by degrading the viral RNA or DNA but by altering viral capsid proteins (Noss *et al*. 1983). But there has been some reports of ClO_2 affecting the RNA synthesis of Poliovirus (Alavrez and O'Brien 1982).

The efficiency of ClO_2 as a waste water disinfectant was studied by Roberts *et al*. (1980). It was found that ClO_2 brings about rapid coliform inactivation with lesser contact time compared to that of Chlorine. Upto 4 log inactivation in f2 coliphage virus was achieved with less than 1 ppm of ClO_2 in water distribution systems (Oliveri *et al*. 1984). ClO_2 was found equally effective against various bacterial pathogens and indicator species such as *E. coli*, *Salmonella typhosa, Bacillus subtilis* etc (Trakhtman, 1949; Ridenour *et al*. 1949; Bedulivich *et al*. 1954). Viricidal properties of ClO_2 was also studied in case of poliomyeletis I (Cronier *et al*. 1978), echovirus 7 and coxsackievirus B3. Narkis *et al*. (1995) suggested that the inactivation of viruses or bacteria will be complete only if they are released out of the suspended particles as the

microorganisms get entrapped inside the flocs and are inaccessible to the action of ClO_2. By effective pretreatment, sewage sludge becomes vulnerable to the ClO_2 treatment. Thus ClO_2 serves as a better alternative for chlorine as a disinfectant. For obtaining class B level of sewage sludge, ClO_2 treatment alone or in combination with other stressors such as irradiation could be used as PSRP.

Sewage sludge should be treated according to the federal and state regulations before using it as landfill or for agricultural purposes. According to the National Research Council report (2002), land application is the most preferred method of biosolids application in US, which accounts to 60% of the treated sludge. Application of biosolids for agricultural purposes is also a preferred way of nutrient and organic matter recycling. All the treated sludge applied for landfills as well as for agricultural use, must comply at least with the class B sludge standards. Currently only 0.1% of the treated biosolids is being used for agricultural purposes (NRC 2002). There exists an enormous opportunity to utilize the treated sludge for land application. By employing appropriate sludge processing techniques such as irradiation, chemical oxidants etc, stable sludge could be generated which ensures pathogen reduction, elimination of putrification and offensive odor thus meeting the criteria of safe sludge.

CHAPTER III

DETERMINATION OF MICROBIAL INACTIVATION IN AEROBICALLY AND ANAEROBICALLY TREATED SLUDGE SAMPLES USING ELECTRON BEAM IRRADIATION

INTRODUCTION

Use of radiation as a tool for waste water treatment has been effectively utilized by various countries all around the world. The United States, Austria, and Japan use electron beam irradiation, where as Germany, India, China predominantly use gamma sources such as Co-60 or Cs- 137 for irradiation (Wang 2007).Radiation processing of sewage sludge is considered as one of the best alternative owing to various reasons such as effective reduction of pathogenic microorganisms, oxidation of organic pollutants odor elimination properties, biodegradability enhancement properties etc (Wang 2007). Apart from these, irradiation also alters the surface properties of the sludge particles rendering them amenable to other disinfection as well as sludge conditioning processes (Borrely *et al*. 1995). This particular study focuses on the use of electron beam radiation as a tool for bacterial, viral as well as spore inactivation in sewage sludge processed via aerobic and anaerobic treatment plants.

MATERIALS AND METHODS

Sludge collection. Sludge samples were collected from two different waste water treatment plants. Aerobically digested sludge was obtained from Carter Creek waste

water treatment plant, College Station. Anaerobically treated sludge was collected from Texas A&M University waste water treatment plant. Samples were collected in sterile bottles (Nalgene, Rochester, NY) and transported to laboratory in a cooler and were maintained at 4℃ until analysis. Dry weight data of the samples were recorded to determine the percentage of total solids and dry weight equivalent of the sludge.

Spiking with microorganisms and sample preparation for electron beam irradiation. The samples were spiked with high titer of laboratory grown strains of *Salmonella* Typhimurium (accession # 87-26254, obtained from National Veterinary Service Laboratory, Ames, Iowa), *E. coli* phages – phi X 174 (ATCC # 13706-B1) and MS-2 (ATCC # 15597- B1) and Enteric virus – Poliovirus-1 (VR- 1562). Apart from these spiked organisms, microbiological analyses were conducted to enumerate other common indigenous organisms such as *Escherichia coli*, aerobic spore formers and anaerobic spore formers. The samples were mixed evenly and 20 ml of samples were triple packaged in whirl pak bags (Nasco, NY) in triplicates, to make it leak proof and to provide adequate protection while irradiating using e-beam. The inoculated samples were further subjected to different target doses of e-beam irradiation based on the type of microorganisms present in the sample. Lower dose ranging from 0.2- 1 kGy were provided for samples spiked with bacteria, where as higher doses ranging from 1-10 kGy was given for samples with viruses, spores and phages. E-beam irradiation was carried out at National Center for Electron Beam Research, Texas A&M University using 10 MeV LINAC source. The absorbed dose was measured using L-α-alanine dosimeter tablets and Electron spin paramagnetic resonance spectroscope (Bruker Biospin Corp.,

Billerica). Irradiated samples were stored at 4℃ until they were subjected to microbiological analysis.

Microbiological analyses. The irradiated bags were opened under sterile conditions and the samples were analyzed for the presence of the spiked microorganisms

(1) *Salmonella* Typhimurium – The sludge samples were serially diluted in 1X PBS and 0.1 ml of dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates containing Nalidixic acid (25 µg/ml) (Sigma, St. Louis, MO) and Novobiocin (25µg/ml) (Sigma, St. Louis, MO). The plates were incubated overnight at 37 ℃ and the characteristic *Salmonella* colonies were enumerated.

(2) *Escherichia coli* - Irradiated samples were serially diluted and 0.1ml of the dilutions was plated in EC-MUG media (Difco Laboratories, MI) and plates were incubated overnight at 37℃. The plates were read under long wave (366 nm) ultra violet light and the fluorescent colonies were enumerated.

(3) Aerobic spores (*Bacillus subtilis*) – Sludge samples were thermally inactivated at 64℃ for 15 minutes using a hot water bath. The inactivated samples were serially diluted and 0.1 ml of the dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates and incubated overnight at 37℃ .

(4) Anaerobic spore formers (*Clostridium perfringens*) – Thermal inactivation of the sludge samples were carried out for 15 minutes at 64℃ and the inactivated samples were serially diluted in 1X PBS. Perfringens agar base, including TSC and SFP (Oxoid, Hampshire) media was prepared and m-CP selective

supplement I (Fluka, Buchs, Switzerland) was added (1 vial/ 500 ml). The media was dispensed into petri plates along with 1 ml of the samples and swirled. The plates were then incubated overnight in anaerobic jars at 37℃. Black colored colonies were enumerated which indicated the presence of *Clostridium perfringens*.

(5) Phi X 174/ somatic coliphage – Virus extraction from the sludge samples were carried out using 3% beef extract and the extracts thus obtained were filtered using 0.22 µm filters (Millipore, Billerica, MA). The viral extracts thus obtained were serially diluted for analysis of phages as well as enteric virus, Enumeration of somatic coliphages were carried out using Single Agar Layer method (Method 1602,US EPA, 2001) with the host bacteria *E. coli* CN- 13. The plates were incubated overnight at 37℃ and plaques were counted after 24 hours.

(6) MS-2/ male specific coliphage – Serially diluted viral extracts were analyzed using Single Agar Layer method (Method 1602, US EPA, 2001) with host bacteria *E. coli* F_{amp}^{+} specific for male specific coliphages. After overnight incubation at 37℃, plaques were enumerated.

(7) Enteric virus – The viral extract obtained from the sludge samples were also used for Poliovirus estimation using tissue culture methods. Infectivity assay was carried out in 6 well plates using BGMK (Buffalo Green Monkey Kidney) cell lines). 0.2 ml of the samples as well as dilutions was used for infection of the BGMK cells and the plates were incubated at 37℃ at 5% CO_2 atmosphere for 24 hours. Plaques were enumerated after staining the plates with 0.1% crystal violet.

D-10 value calculation. Based on the counts obtained for each of the microorganism from the samples irradiated with different doses, a survivor curve was plotted using linear regression function. The slope of the survivor or the pathogen inactivation curve was determined and D-10 value was calculated by taking the negative reciprocal of the slope (Ic *et al*. 2007).

RESULTS

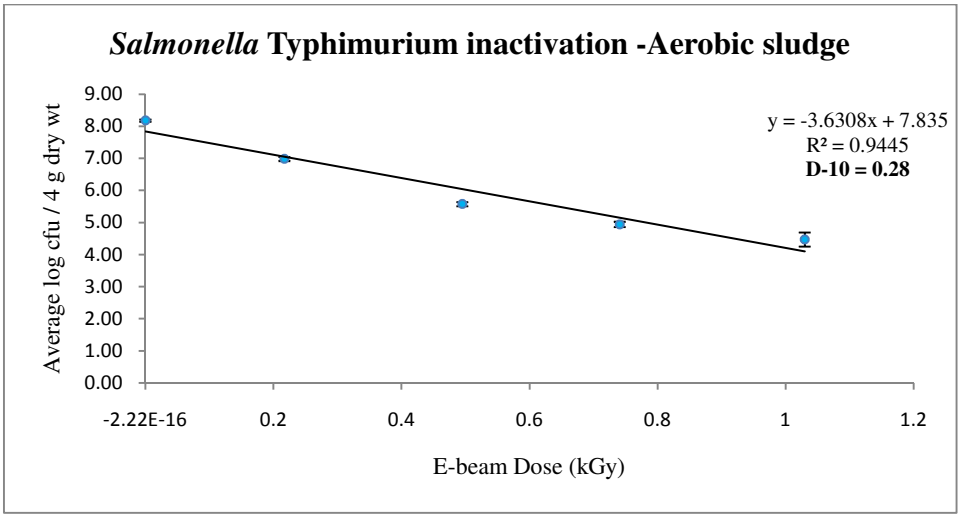
The radiation sensitivity of specific microorganisms is expressed in terms of its decimal reduction dose or D-10 value, which indicates the amount of absorbed dose required to kill 90% of the microbial population (Borrely *et al*. 1998). The D-10 value (in aerobic and anaerobic sludge samples) was calculated for all the organisms that were included in this study. The inactivation curve for different target organisms present in aerobically and anaerobically treated sludge samples were plotted. Table 3.1 summarizes the estimated D-10 values of the target organisms in aerobically and anaerobiocally treated sludge samples.

Table 3.1 Estimated D-10 values of microorganisms present in aerobically and anaerobically treated sludge samples.

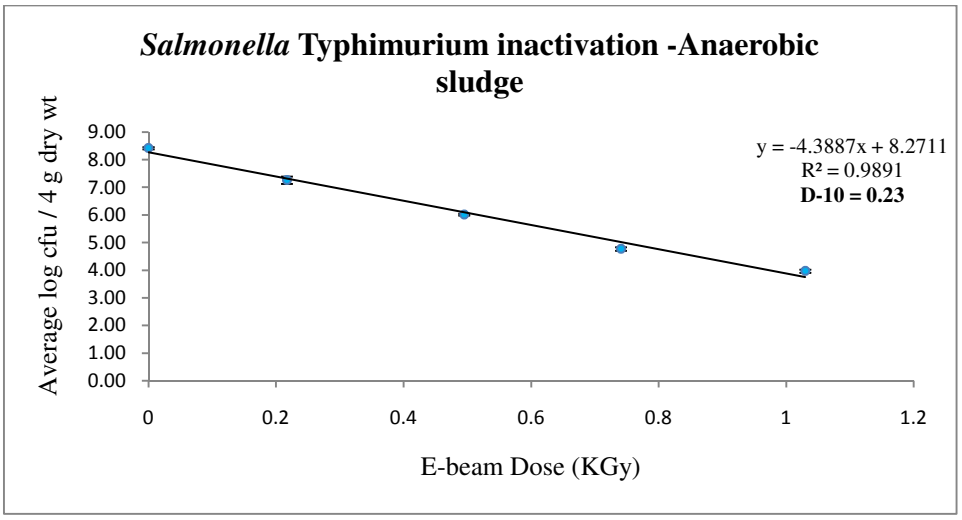
Target Organisms	Aerobically treated sludge (kGy)	Anaerobically treated sludge (kGy)
Salmonella typhimurium	0.28	0.23
Escherichia coli	0.31	0.25
Aerobic spore formers	3.74	4.02
Anaerobic spore formers	5.13	3.12
Somatic coliphages	4.12	4.17
Male speific coliphages	2.31	2.51
Poliovirus		2.69

DISCUSSION

The results obtained from this study indicate that the radiation sensitivity differs between different groups of organisms and is also a function of the matrix in which the organisms are present. The D-10 value of 0.28 and 0.23 kGy estimated for *Salmonella* Typhimurium (Fig. 3.1) lies within the range of 0.14-2.5 kGy reported for the sludge pathogens by Bitton *et al*. (1994). Borrely et al. (1995) studied the decimal reduction dose of bacterial pathogens such as *Salmonella* Typhimurium and *E. coli* in buffer solutions using e-beam irradiation and reported a D-10 value of 0.30 and 0.34 kGy respectively. Results obtained from this study are in agreement with these values (Fig. 3.1 and Fig. 3.2). D-10 value does not remain constant for a particular target organism, but is a function of initial microbial population, innate characteristics of the target organism and the properties of the matrix on which the organisms are present and are irradiated.

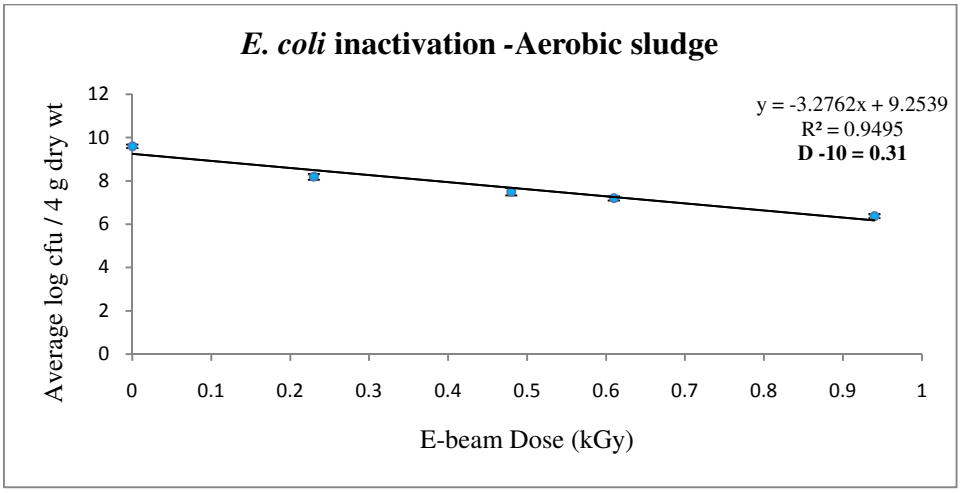


(a)

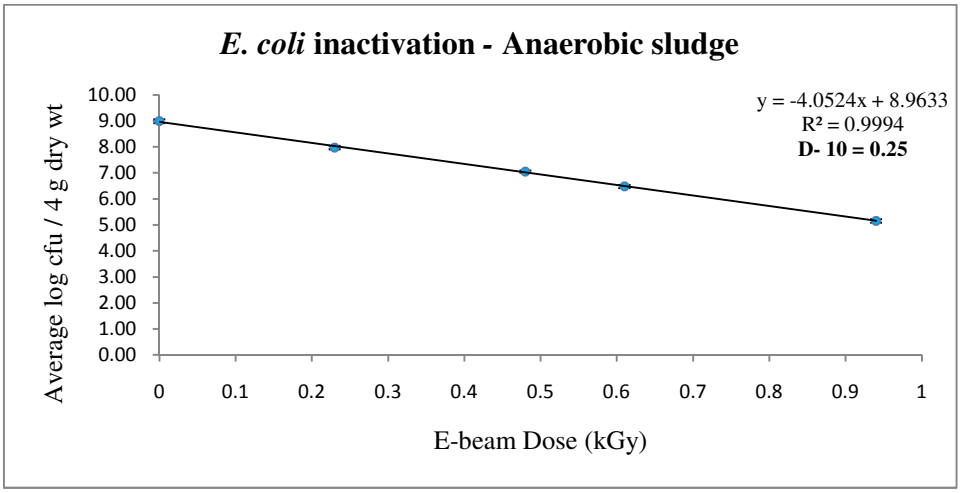


(b)

Fig. 3.1 Response of *Salmonella* Typhimurium to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.

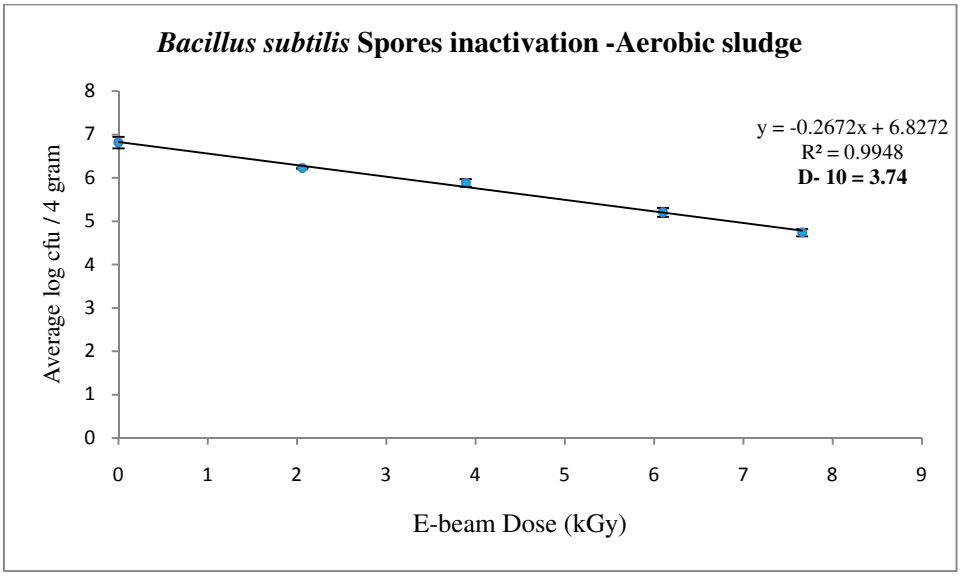


(a)

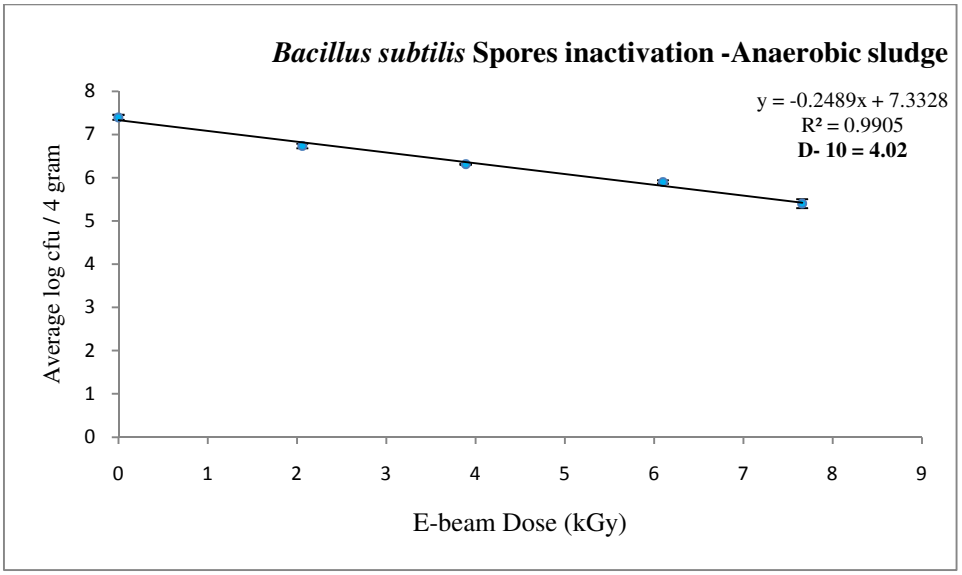


(b)

Fig. 3.2 Response of *E. coli* to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.

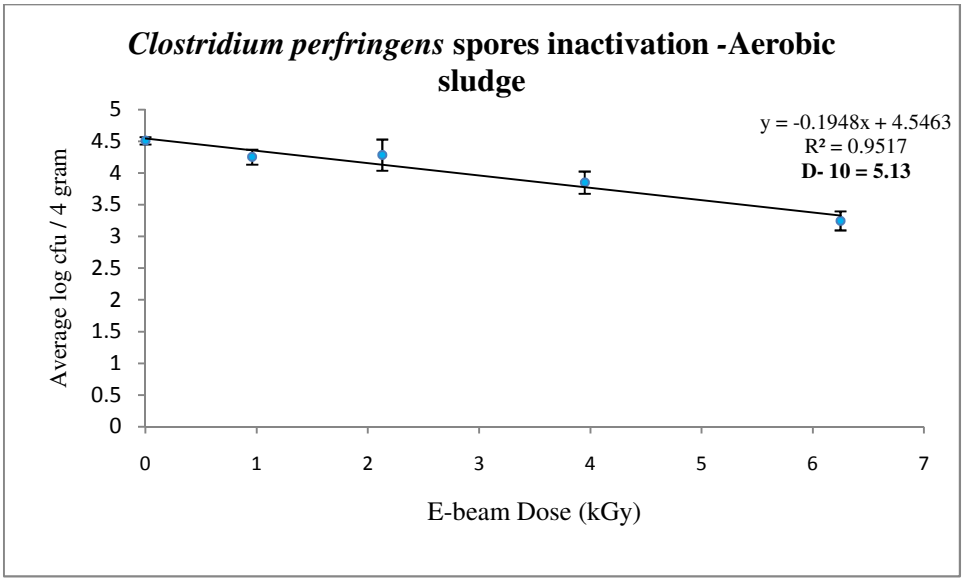


(a)

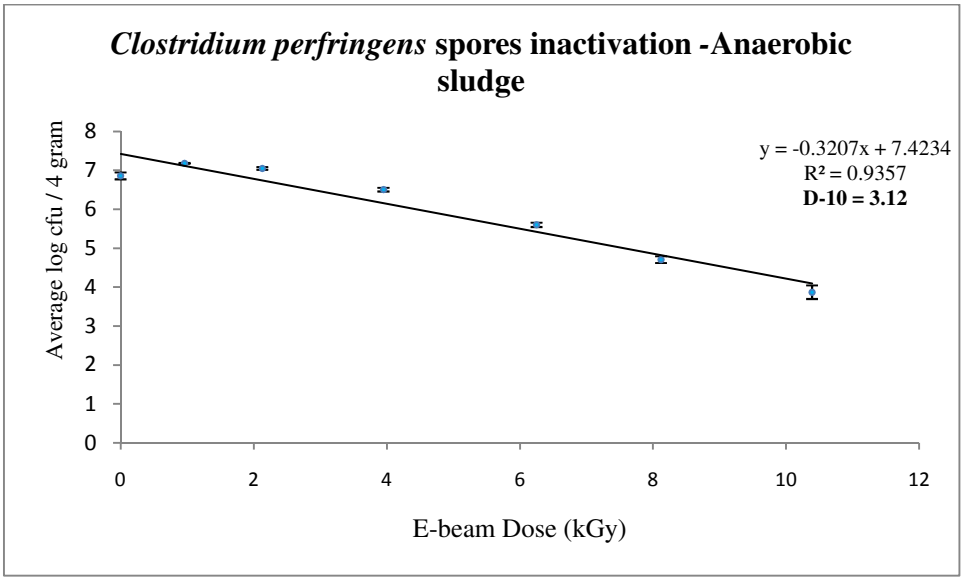


(b)

Fig. 3.3 Response of *Bacillus subtilis* to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.



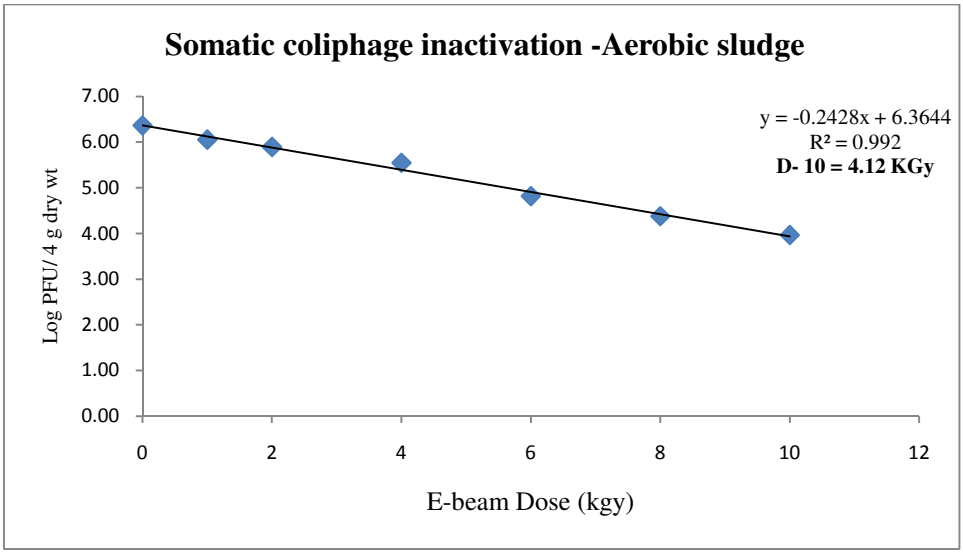
(a)



(b)

Fig. 3.4 Response of *Clostridium perfringens* to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.

Table 3.1 clearly indicates that D-10 values of bacteria (*E. coli and Salmonella* Typhimurium) were strikingly lower compared to viruses as well as spore formers (Fig. 3.3 and Fig. 3.4). Gehringer *et al*. 2003, carried out an experiment to study the D-10 values of different bacteriophages in tap water using both gamma and e-beam irradiation. The results showed that phi X 174 was very resistant to radiation treatment compared to malespecific coliphages. Somatic coliphage required a dose of 340 Gy of gamma radiation and 700 Gy of e-beam to bring about 1 log reduction in the phage population where as male specific coliphage, MS-2 was found to be more susceptible to radiation requiring only 45 Gy and 20 Gy of gamma and e-beam irradiation respectively. As per our findings, estimated D-10 values of somatic coliphage in aerobically and anaerobically treated sludge samples were relatively high i.e., 4.12 kGy and 4.17 kGy (Fig. 3.5), where as that of male specific coliphage is 2.31 and 2.51 kGy (Fig. 3.6). This data supports the argument by Gehringer *et al*. of choosing somatic coliphages as an ideal indicator organism for assessing the virological quality of water and sewage sludge treated by different ionizing radiation. Aerobic as well as anaerobic spore formers were also found to be resistant to radiation processing indicated by their higher D-10 value compared to that of the bacterial cells. D-10 values of spore formers were on par with that of somatic coliphages implying their potential as indicator organisms for radiation treatment of sludge.



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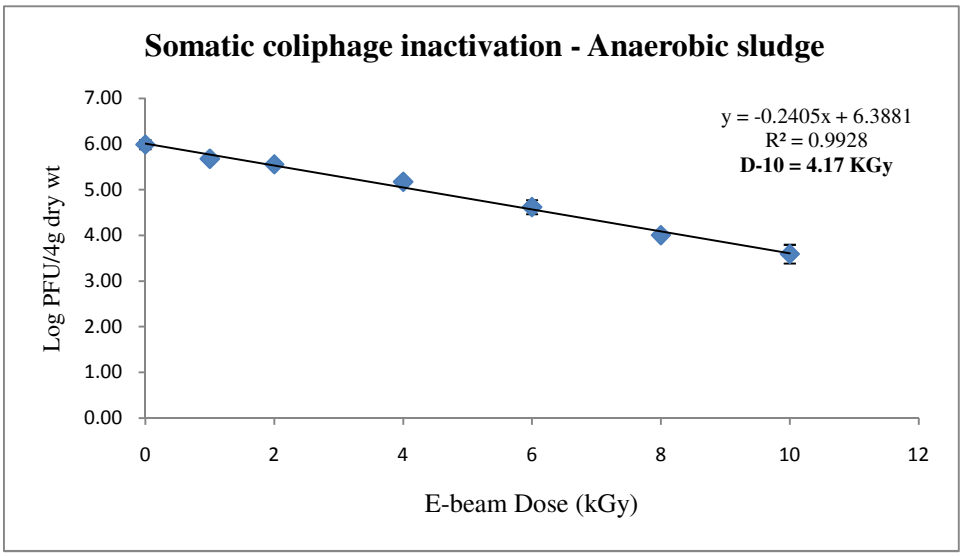

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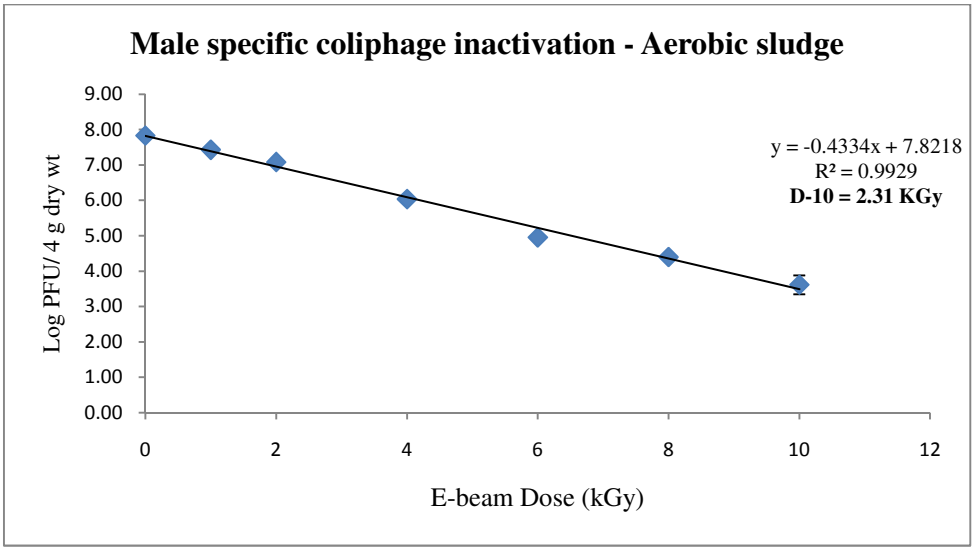
Fig. 3.5 Response of somatic coliphage to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.



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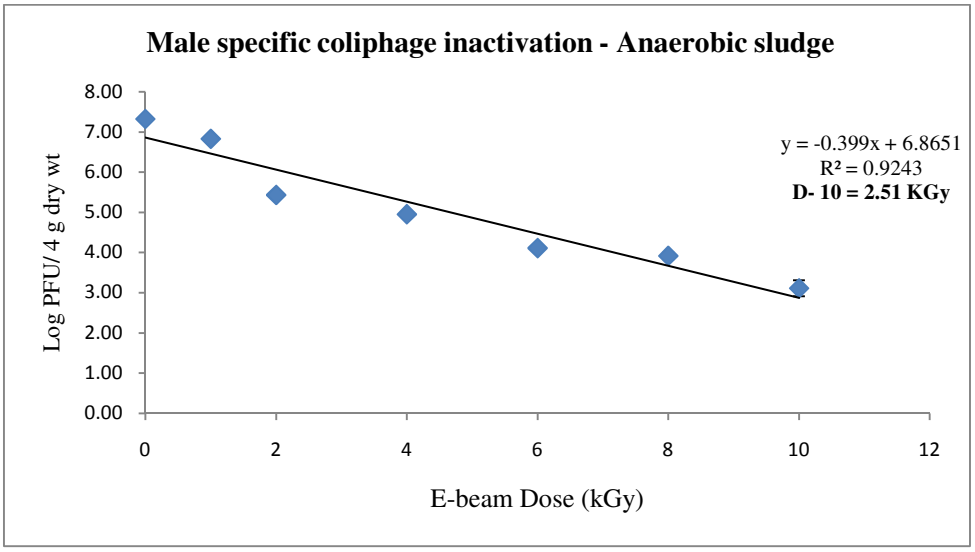

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Fig. 3.6 Response of male specific coliphages to varying doses of e-beam in (a) aerobically treated sludge and (b) anaerobically treated sludge.

There are published reports demonstrating that enteric viruses require a higher ionizing irradiation dose compared to bacteria. When subjected to e-beam irradiation, Poliovirus suspended in buffer solution showed a D-10 value of 1.85 kGy. Table 3.1 indicates the D-10 value of poliovirus in sludge to be 2.6 kGy (Fig. 3.7), which falls in the range of enteric virus D-10 value of 1.65- 3.5 kGy in sludge as suggested by Bitton *et al*. (1994). In general the higher D-10 values can be attributed to the difference in size as well as the innate susceptibility of the microorganism to the ionizing radiation (Gehringer *et al*. 2003). When microbial cells are exposed to ionizing radiations as in case of electron beam, it brings about several direct as well as indirect effects which make it possible for e-beam to be used for the microbial reduction. The primary effects of the e-beam is due to the collision of electrons into the microbial cell which results in breakdown of the vital cellular components such as nucleic acid where as the secondary effects are due to the production of radiolytic species causing indirect damage to the cell (Sommers and Fan 2006).

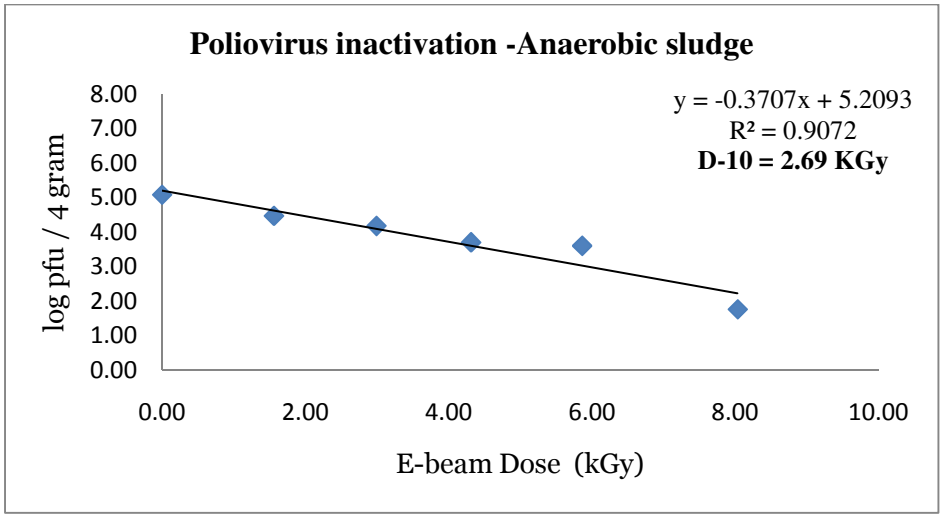


Fig. 3.7 Response of Poliovirus to varying doses of e-beam in anaerobically treated sludge.

Irradiation not only reduces the microbial population but also brings about degradation of many organic pollutants. It provides better aeration to the samples being irradiated thereby enhancing the pathogen destruction and prevents subsequent re-growth (Gautam *et al*. 2005). Irradiation augments sludge disintegration and microbial cell rupture making the sludge amenable for further disinfection treatments (Yuan *et al*. 2008). Hence e-beam irradiation singly or in combination with other disinfection methods can serve as an ideal PFRP for producing class A sludge.

CHAPTER IV

DETERMINATION OF MICROBIAL INACTIVATION USING CHEMICAL OXIDANT FERRATE AND COMBINATION OF FERRATE AND E-BEAM IRRADIATION

INTRODUCTION

Use of multifunctional oxidants such as ferrate (VI) is being increasingly used as a viable option for drinking water and waste water treatment reference. Ferrate has an added advantage of being environmental friendly compared to other common disinfectants like chlorine, in use for water treatment (Sharma *et al*. 2005). Unlike halogenated disinfectants like bromine, iodine, chloramines, and chlorine, Ferrate does not produce carcinogenic or mutagenic byproducts (Sharma *et al*. 2005) which make it a better alternative for conventional water treatment. High reactivity and selectivity enables ferrate to achieve desired level of disinfection with low dose and less contact time over a wide range of pH (Sharma *et al*. 2007). The antifoulant and coagulant properties of ferrate allows it to be used as a good sewage sludge conditioning agent and thus helps in the stabilization of sludge. In this study, we have focused on studying the disinfectant properties of ferrate alone and in combination with e-beam. A combination treatment is intended to bring forth a higher degree of microbial inactivation of even resistant microbes, thereby projecting the synergistic effect of the treatments.

MATERIALS AND METHODS

Sludge collection. Sludge samples were collected from two different waste water treatment plants. Aerobically digested sludge was obtained from Carter Creek waste water treatment plant, College Station. Anaerobically treated sludge was collected from Texas A&M University waste water treatment plant. Samples were collected in sterile bottles (Nalgene, Rochester, NY) and transported to laboratory in a cooler and were maintained at 4℃ until analysis. Dry weight data of the samples were recorded to determine the percentage of total solids and dry weight equivalent of the sludge.

Spiking of sludge with microorganisms. The samples were spiked with high titer of laboratory grown strains of 7 different organisms, which include bacteria - *Salmonella* Typhimurium (accession # 87-26254, obtained from National Veterinary Service Laboratory, Ames, Iowa), *Escherichia coli* (ATCC # 25922), coliphages phi X 174 (ATCC # 13706-B1) (somatic) and MS-2 (ATCC # 15597- B1) (Male-specific), enteric virus – Poliovirus-1 (VR- 1562), aerobic spore former – *Bacillus subtilis* (ATCC #6633) and anaerobic spore former – *Clostridium perfringens* (ATCC # 13124).

Treatment of sludge with ferrate. The spiked samples were mixed evenly and subjected to different concentrations of ferrate. Ferrator^{TM} equipment was obtained from Ferrate Treatment Technologies, LLC with the assistance of Dr. Bob Reimers, Tulane University. Both College Station and TAMU sludge samples were treated with 50 ppm, 100 ppm and 200 ppm of ferrate. After addition of ferrate, the samples were mixed gently to allow for sufficient contact with the sludge matrix. Spiked controls were

maintained without ferrate treatment to enumerate the amount of spiked microorganisms present in each of the sample.

E-beam irradiation of ferrate treated sludge. The ferrate treated samples were subjected to e-beam dose of 8 kGy at National Center for Electron Beam Research, Texas A&M University using 10 MeV LINAC source. The absorbed dose was measured using L-α-alanine dosimeter tablets and the Electron spin paramagnetic resonance spectroscopy (Bruker biospin Corp., Billerica). Irradaited samples were stored at 4 ˚C until they were subjected to microbiological analysis. Another set of ferrate treated samples were maintained without e-beam irradiation to study the effect of the oxidant alone in microbial inactivation. Those samples were packaged similarly as for e-beam irradiation but were not subjected to irradiation and were labeled as 0 kGy. Fig. 4.1 provides a schematic representation of the ferrate and e-beam + ferrate treatment given to aerobically and anaerobically treated sludge samples.

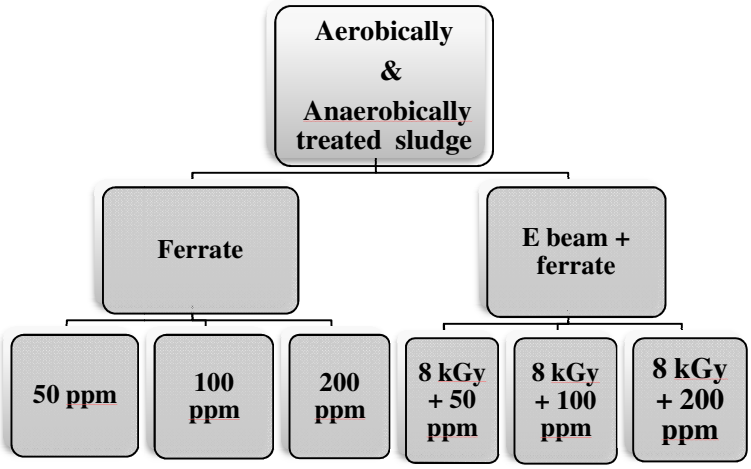


Fig. 4.1 Schematic representation of ferrate and e- beam + ferrate treatment provided to aerobically and anaerobically treated sludge samples.

Microbiological analyses. The irradiated and non-irradiated bags were opened under sterile conditions and the samples were analyzed for the presence of the spiked microorganisms.

(1) *Salmonella* Typhimurium – The sludge samples were serially diluted in 1X PBS and 0.1 ml of dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates containing Nalidixic acid (25 µg/ml) (Sigma, St. Louis, MO) and Novobiocin (25µg/ml) (Sigma, St. Louis, MO). The plates were incubated overnight at 37 ℃ and the characteristic *Salmonella* colonies were enumerated.

(2) *Escherichia coli* - Irradiated samples were serially diluted and 0.1ml of the dilutions was plated in EC-MUG media (Difco Laboratories, MI) and plates were

incubated overnight at 37℃. The plates were read under long wave (366 nm) ultra violet light and the fluorescent colonies were enumerated.

(3) Aerobic spores (*Bacillus subtilis*) – Sludge samples were thermally inactivated at 64℃ for 15 minutes using a hot water bath. The inactivated samples were serially diluted and 0.1 ml of the dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates and incubated overnight at 37℃ .

(4) Anaerobic spore formers (*Clostridium perfringens*) – Thermal inactivation of the sludge samples were carried out for 15 minutes at 64℃ and the inactivated samples were serially diluted in 1X PBS. Perfringens agar base, including TSC and SFP (Oxoid, Hampshire) media was prepared and m-CP selective supplement I (Fluka, Buchs, Switzerland) was added (1 vial/ 500 ml). The media was dispensed into petri plates along with 1 ml of the samples and swirled. The plates were then incubated overnight in anaerobic jars at 37℃. Black colored colonies were enumerated which indicated the presence of *Clostridium perfringens*.

(5) Phi X 174/ somatic coliphage – Virus extraction from the sludge samples were carried out using 3% beef extract and the extracts thus obtained were filtered using 0.22 µm filters (Millipore, Billerica, MA). The viral extracts thus obtained were serially diluted for analysis of phages as well as enteric virus, Enumeration of somatic coliphages were carried out using Single Agar Layer method (Method 1602,US EPA, 2001) with the host bacteria *E. coli* CN- 13. The plates were incubated overnight at 37℃ and plaques were counted after 24 hours.

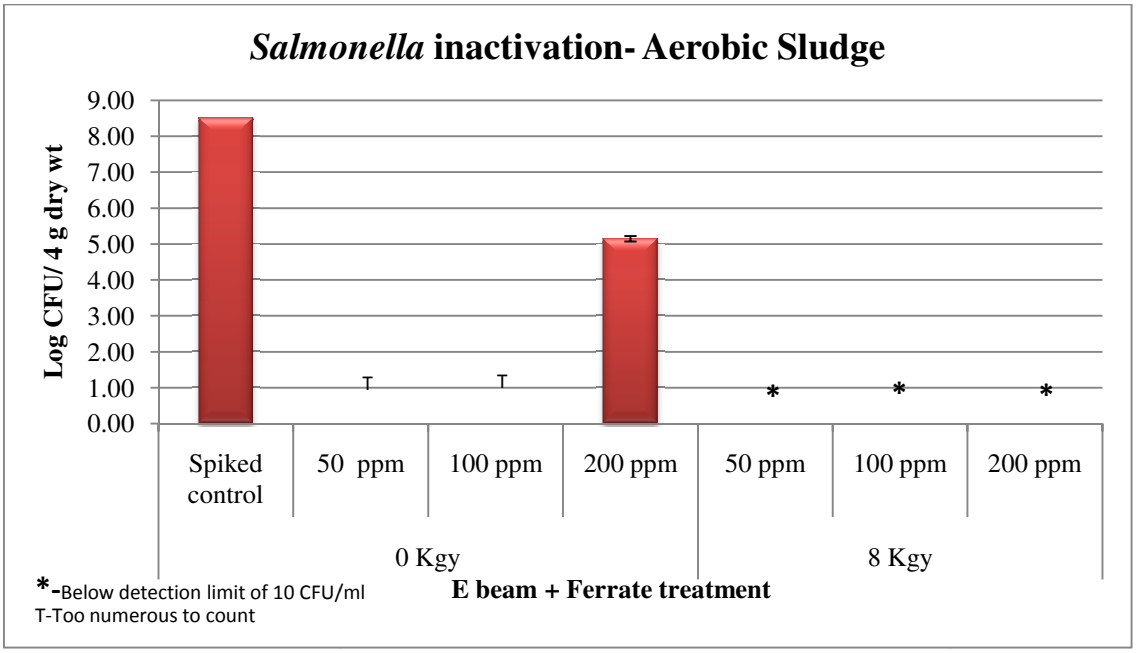
(6) MS-2/ male specific coliphage – Serially diluted viral extracts were analyzed using Single Agar Layer method (Method 1602, US EPA, 2001) with host bacteria *E. coli* F_{amp}^{+} specific for male specific coliphages. After overnight incubation at 37℃, plaques were enumerated.

(7) Enteric virus – The viral extract obtained from the sludge samples were also used for Poliovirus estimation using tissue culture methods. Infectivity assay was carried out in 6 well plates using BGMK (Buffalo Green Monkey Kidney) cell lines) (Appendix A). 0.2 ml of the samples as well as dilutions was used for infection of the BGMK cells and the plates were incubated at 37℃ at 5% CO_2 atmosphere for 24 hours. Plaques were enumerated after staining the plates with 0.1% crystal violet.

Statistical analysis. The values obtained from the inactivation studies were converted to $\log_{10}x$ values and plotted against respective doses of ferrate and e-beam + ferrate. The disinfection efficiency of ferrate and the combination treatment of ferrate and e-beam were determined by analyzing the $\log_{10}$ reduction in the microbial population subjected to different treatments compared to that of the spiked control, which did not receive any disinfection treatments. In order to compare the pathogen reduction between different treatments and within treatments Paired–t–tests were carried out using statistic software package SPSS.

RESULTS

The effect of ferrate on *Salmonella* and *E. coli* in aerobically and anaerobically treated sludge is represented in Fig. 4.2 and Fig. 4.3 respectively. The inactivation of bacterial populations showed almost similar trend following ferrate and e-beam + ferrate treatment. There is a gradual reduction in bacterial population with respect to increase in ferrate concentration, but a significant difference was observed with the introduction of e-beam component that resulted in complete reduction of bacterial population. At 50 ppm and 100 ppm, the colonies obtained were too numerous to count which makes it difficult to arrive at an actual number for the surviving microbial population. Based on the dilutions we made the reduction could be approximately 3 log in the case of *S.* Typhimurium in both aerobically and anaerobically treated sludge, whereas *E. coli* was found to be more susceptible to ferrate with 4 log reduction in both the sludge samples. Bacterial colonies were totally absent or were below detection limit of 10 CFU/ml in case of the combined e-beam and ferrate treatment. This clearly indicates that the synergistic effect of the combination treatment brought about 8 log reduction of *Salmonella* and *E. coli* population in sludge.



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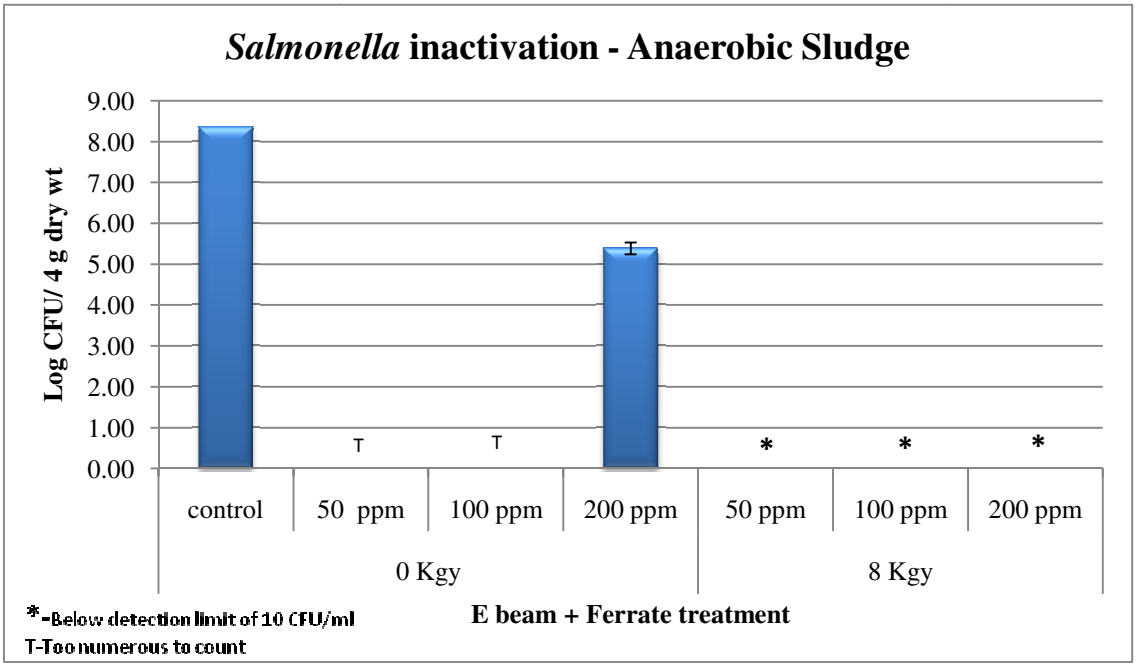

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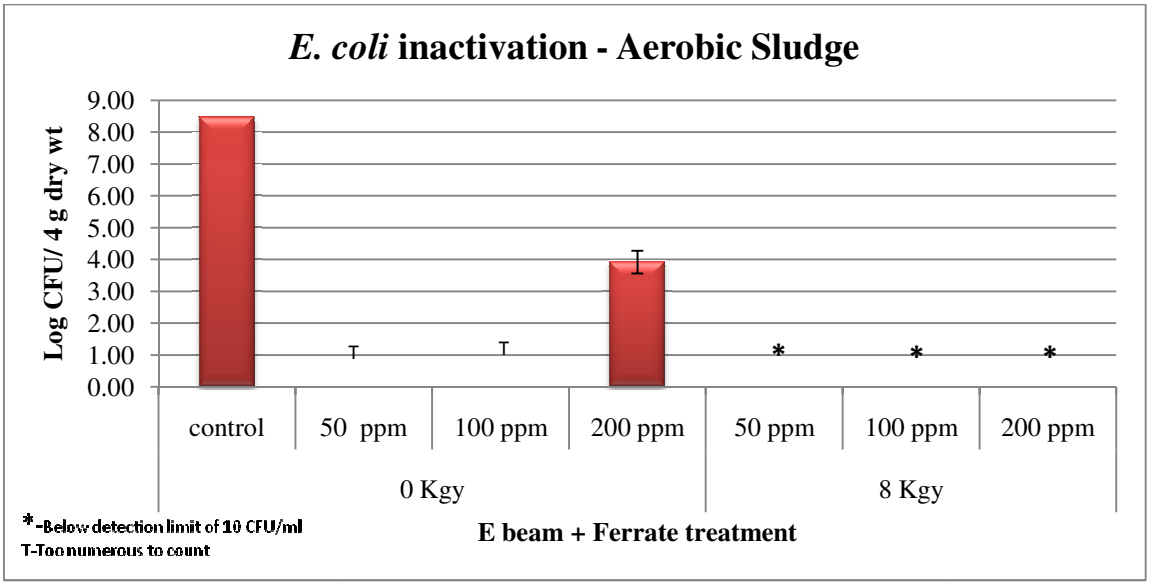
Fig. 4.2 Inactivation of *Salmonella* Typhimurium when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically treated and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).



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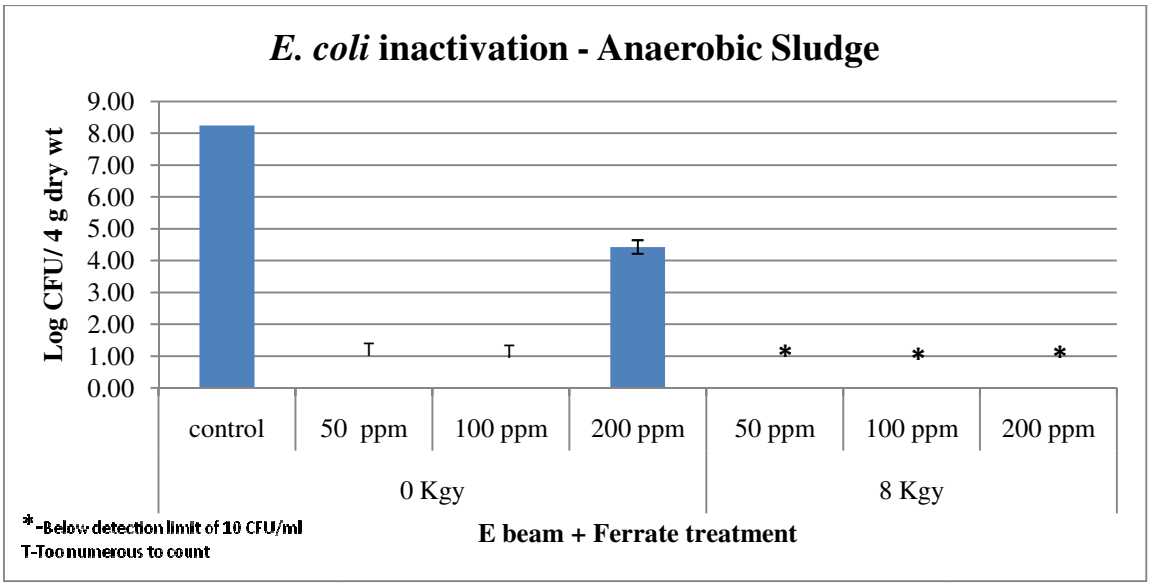

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Fig. 4.3 Inactivation of *Escherichia coli* when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).

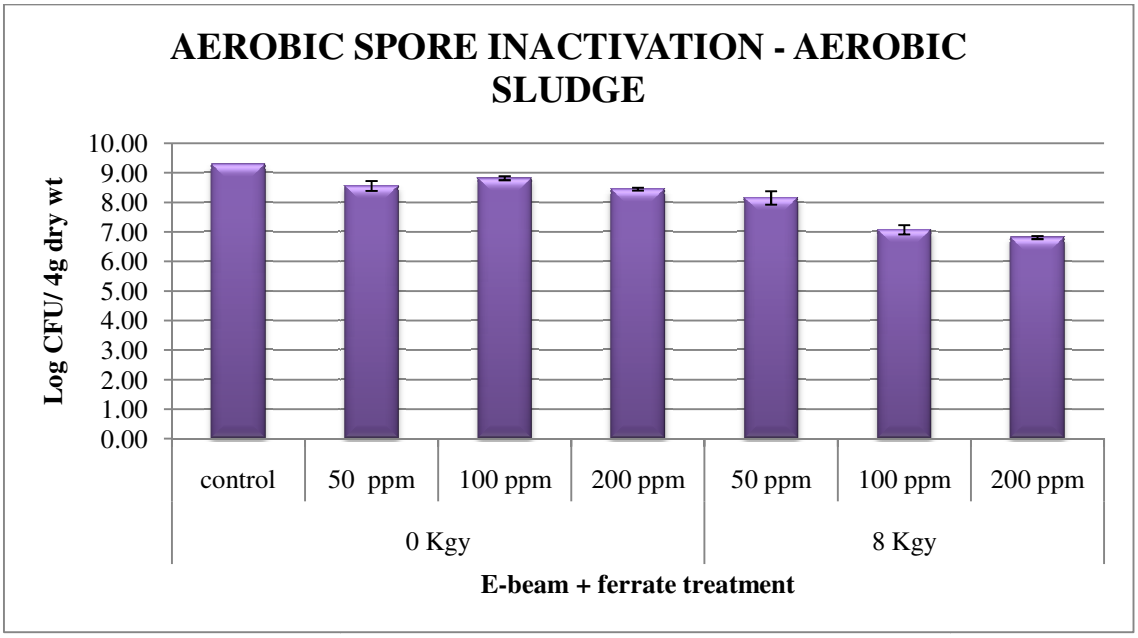
Aerobic and anaerobic spores were comparatively more resistant to ferrate and e-beam treatment compared to the bacterial population. When the concentration of ferrate was increased from 100 ppm to 200 ppm, significant reduction was observed in case of aerobic spores in aerobically and anaerobically digested sludge with a p value of 0.018 and 0.034 respectively. As shown in Fig. 4.4 the spore population did show up 1-2 log reduction upon increase in ferrate concentrations from 0 to 200 ppm. 1-2 log reduction was observed when treated with 8 kGy e-beam in case of College Station sludge. TAMU sludge showed almost similar pattern, but 3 log reduction was observed in case of the combination treatment of ferrate with e-beam. Combination of e-beam and ferrate was compared statistically with the ferrate treatment using paired t-tests and the results suggests statistically significant reduction as illustrated in Table 4.1.

The anaerobic spore inactivation profile had a similar inactivation pattern as that of aerobic spores (Fig. 4.5). Table 4.1 describes the statistical significance of the e-beam+ ferrate treatment compared to that of ferrate treatment alone. In both aerobically and anaerobically digested sludge samples, significant difference were observed upon combining the ferrate with e-beam ($p<0.05$ and $p<0.01$).

Table 4.1 Statistical comparison between ferrate and the e-beam + ferrate combination treatment of aerobic spores and anaerobic spores in aerobically and anaerobically treated sludge.

Treatment comparison	t	Df	p-value
Aerobic spores- aerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	1.107	2	0.384
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	7.885	2	0.016*
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	17.846	2	0.003**
Aerobic spores- anaerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	2.273	2	0.151
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	4.637	2	0.043*
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	6.569	2	0.022*
Anaerobic spores- aerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	22.215	2	0.002**
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	3.639	2	0.068
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	11.261	2	0.008**
Anaerobic spores- anaerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	4.516	2	0.046*
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	14.401	2	0.005**
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	6.862	2	0.021*

* - Significance level 0.05, ** - Significance level 0.01



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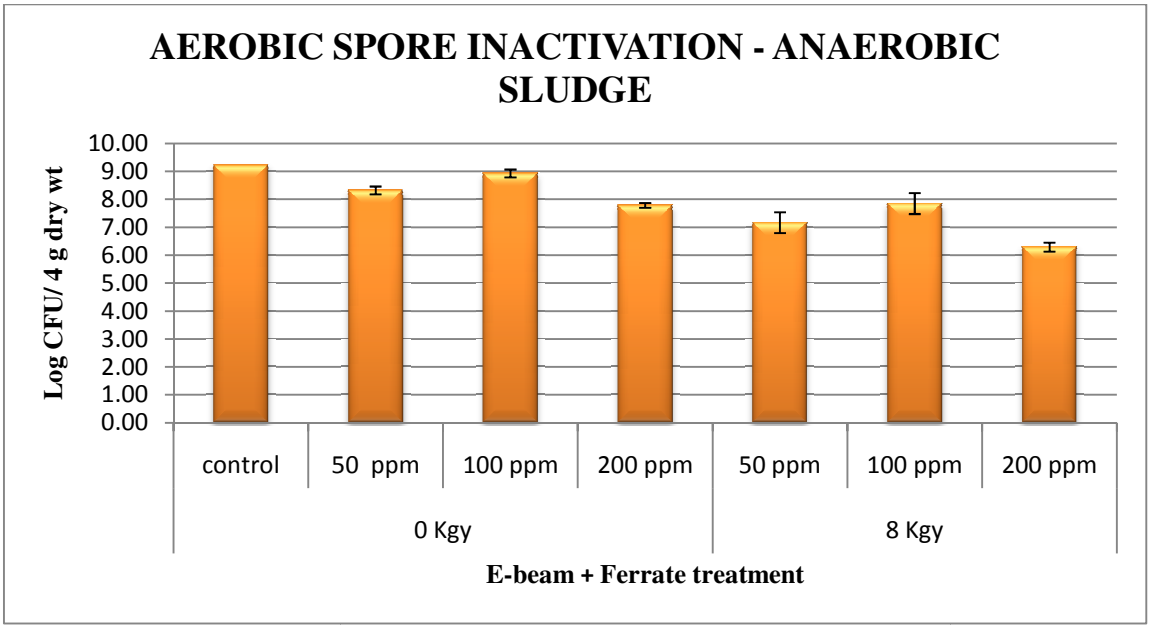

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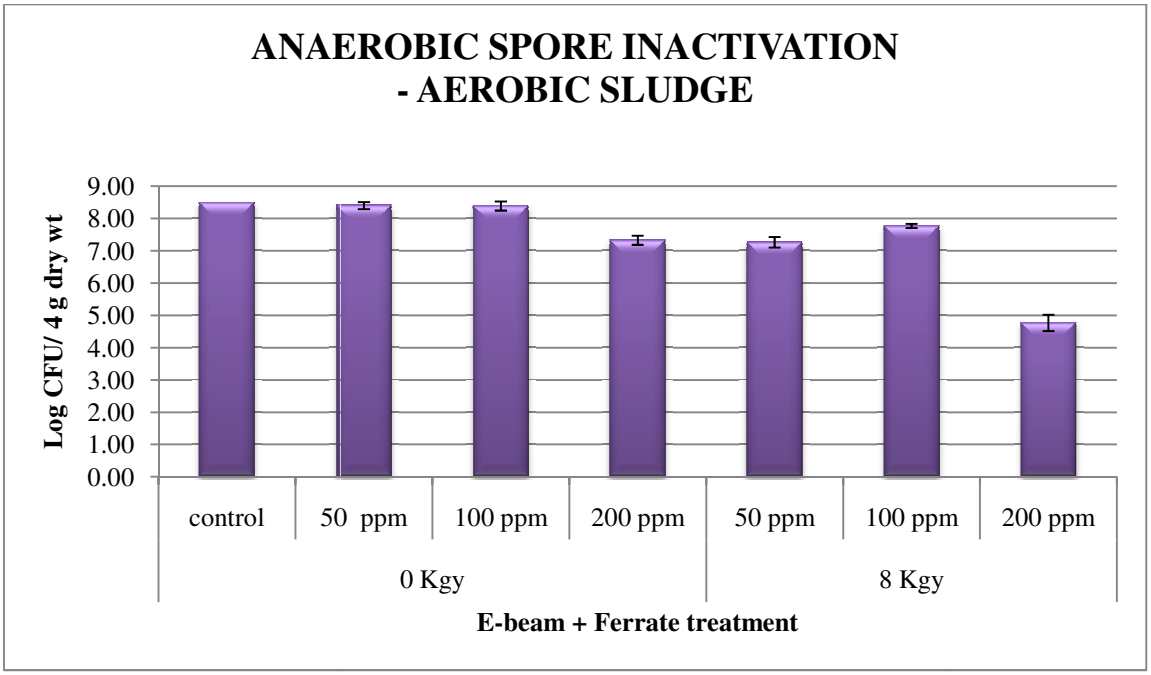
Fig. 4.4 Inactivation of aerobic spore - *Bacillus subtilis* when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).



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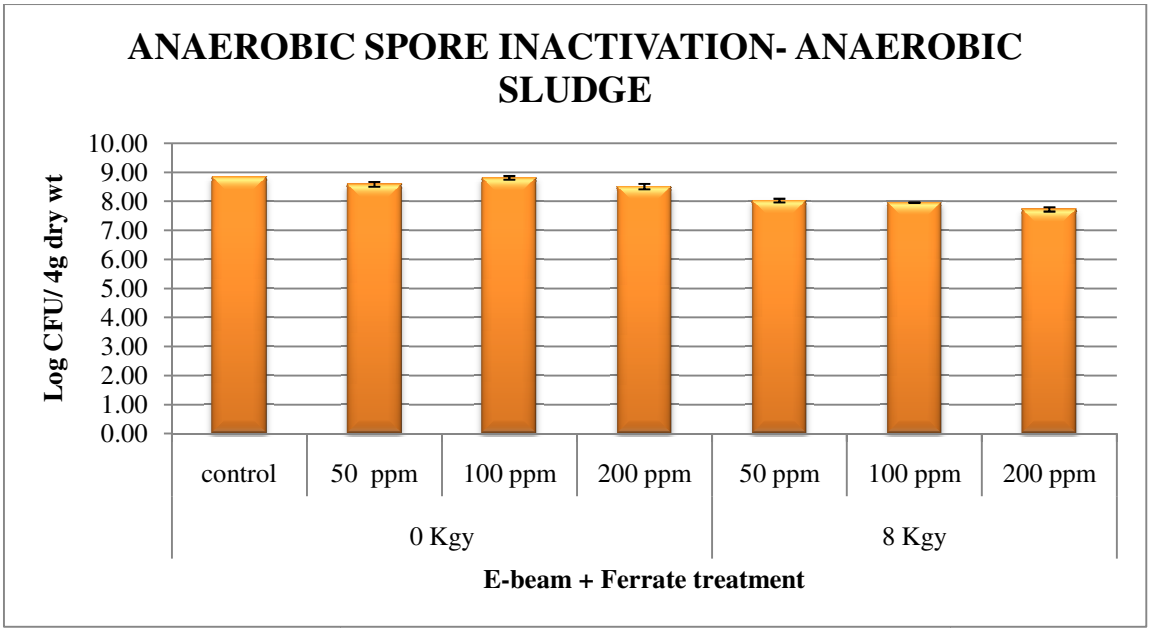

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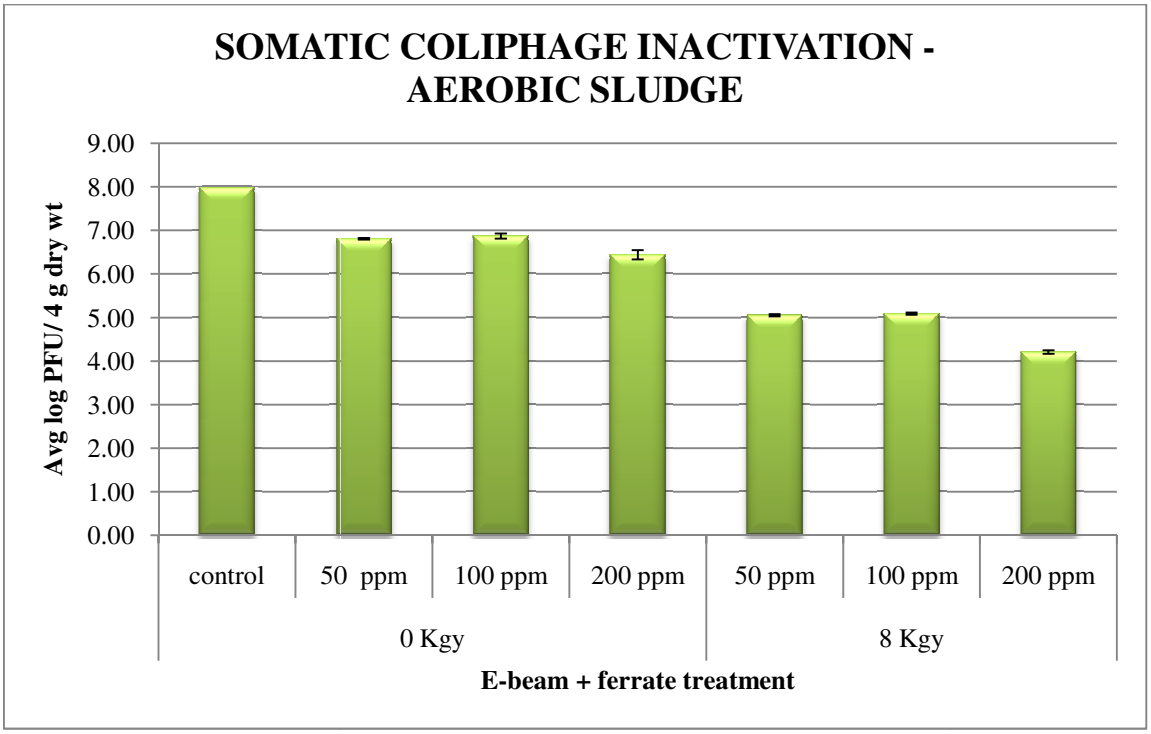
Fig. 4.5 Inactivation of anaerobic spore – *Clostridium perfringens*, when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).

Inactivation of somatic phages in both aerobically and anaerobically treated sludge is represented in Fig. 4.6. Aerobically treated sludge samples were more susceptible to phi X 174 inactivation using ferrate and e-beam. About a 2 log reduction was observed with 200 ppm of ferrate (p= 0.029) and a progressive reduction of 4 log with 200 ppm ferrate and a dose of 8 kGy e-beam irradiation. A 3 log reduction of somatic phage population was observed with the combination treatment in TAMU samples. Table 4.2 statistically compares the significant difference attained by the ferrate and e-beam + ferrate combination treatment on somatic coliphage inactivation. Results of male specific coliphage exposed to ferrate and e-beam are illustrated in Fig. 4.7.Complete inactivation of male specific coliphage was observed with 200 ppm ferrate in case of aerobically treated sludge samples (p=0.00). The combination of e-beam with 50 ppm ferrate resulted in 3 log reduction whereas 100 ppm and 200 ppm resulted in approximately 4 and 7 log reduction of male-specific coliphage population indicating that male-specific coliphage are susceptible to ferrate (Table 4.2).

Table 4.2 Statistical comparison between ferrate and the e-beam + ferrate combination treatment of somatic and male specific coliphages in aerobically and anaerobically treated sludge.

Treatment comparison	t	Df	p-value
Somatic coliphage- aerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	54.751	2	.000**
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	42.777	2	.001**
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	32.424	2	.001**
Somatic coliphage- anaerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	30.137	2	.001**
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	18.113	2	.003**
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	13.379	2	.006**
Male specific coliphage- aerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	9.197	2	.012*
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	10.698	2	.009**
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	-	2	.000**
Male specific coliphage- anaerobically digested sludge			
50 ppm ferrate Vs 50 ppm ferrate + 8kGy e-beam	3.569	2	.070
100 ppm ferrate Vs 100 ppm ferrate + 8kGy e-beam	3.871	2	.061
200 ppm ferrate Vs 200 ppm ferrate + 8kGy e-beam	3.019	2	.094

* - Significance level 0.05, ** - Significance level 0.01



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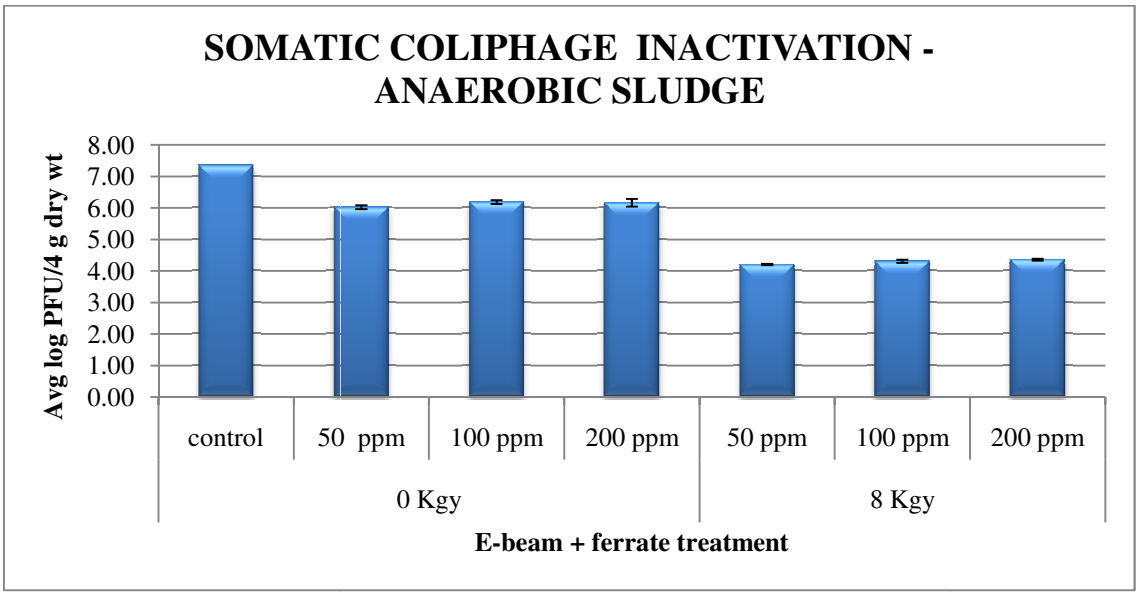

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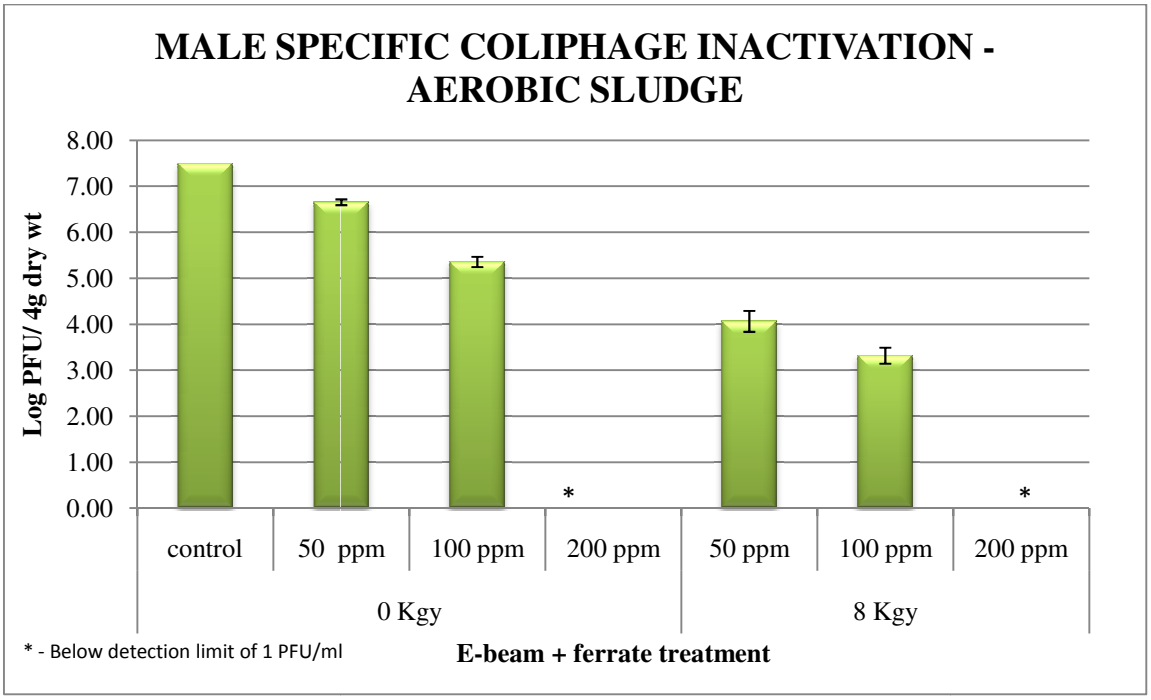
Fig. 4.6 Inactivation of Somatic coliphage – phi X 174, when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).



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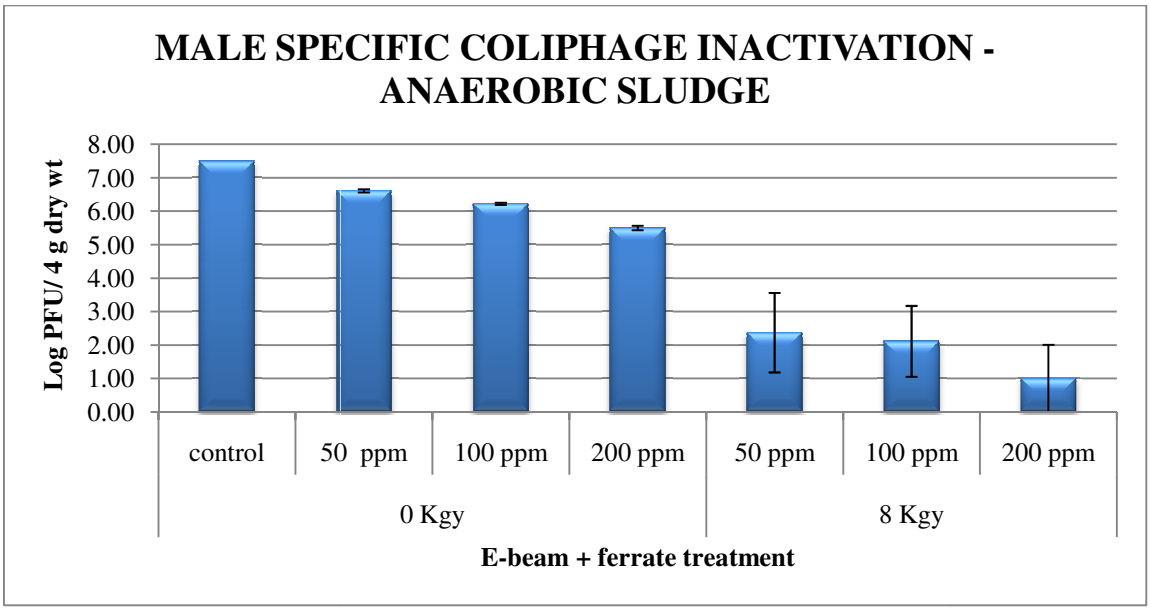

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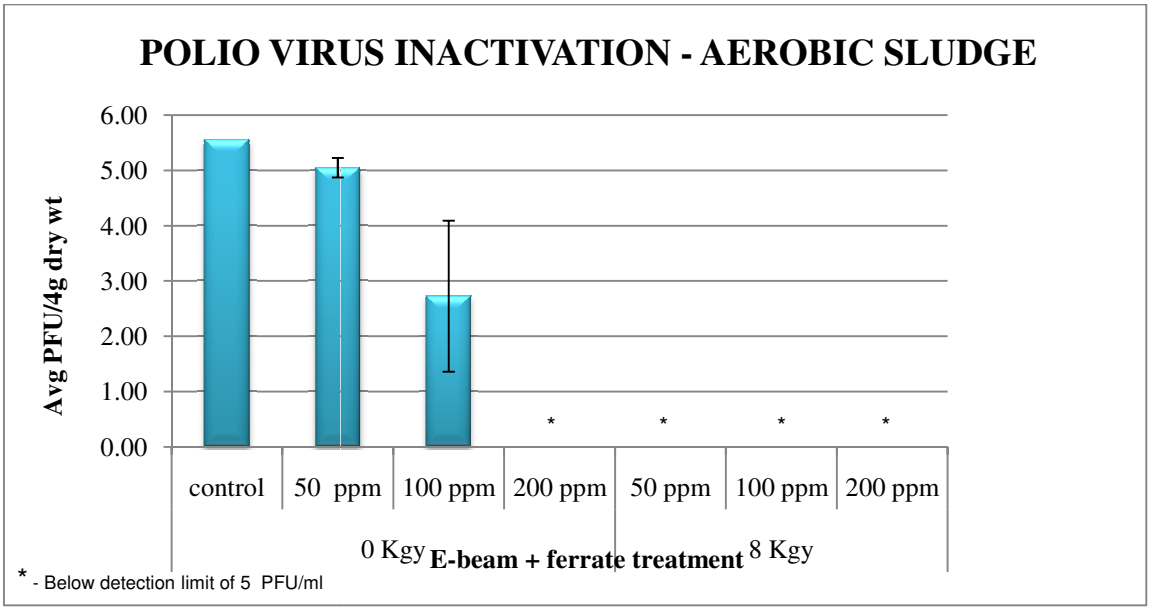
Fig. 4.7 Inactivation of Male specific coliphage, MS2 when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).

Anaerobically treated sludge samples also showed about 6 log reduction in with ferrate and e-beam treatment. When paired t test was carried out to assess the significant difference between the ferrate and combination treatment, anaerobically treated sludge did not show statistically significant difference for male specific coliphage inactivation. On the other hand aerobically treated sludge showed significant difference between the ferrate and combination treatment (Table 4.2).

Ferrate was found to be highly effective against enteric viruses (Fig. 4.8). The poliovirus spiked in aerobically treated sludge samples showed >2 log reduction when treated with 100 ppm of ferrate where as anaerobically treated samples were highly susceptible as there was approximately 6 log reduction with 100 pm ferrate. Both the samples showed complete reduction of enteric virus population with the combination of ferrate and e-beam.



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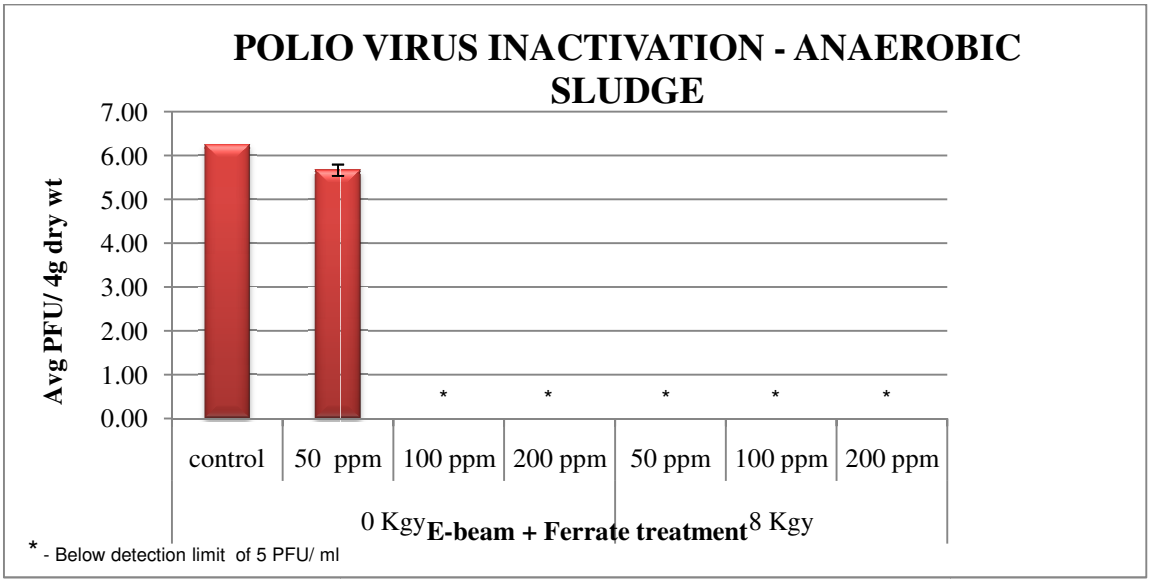


(b)

Fig. 4.8 Inactivation of Poliovirus, when exposed to 50, 100, 200 ppm of ferrate and combination of ferrate and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to ferrate treatment alone and control refers to 0ppm ferrate and 0 kGy e-beam (untreated spiked sludge sample).

DISCUSSION

Ferrate was reported as an effective disinfectant against *E. coli* (Waite *et al*. 1979; Jing and Wang 2003). The results from the current study also supports that there was a significant reduction both *E. coli* and *S*. Typhimurium by 3-4 log with ferrate (Fig. 4.2 and 4.3). Franklin *et al*. (1998) reported that a 3 log reduction of aerobic spore formers in river water upon treating with 2 ppm ferrate. Current study showed that the aerobic spore formers were resistant in sludge compared to the river water as only a 200 ppm concentration of ferrate could bring about a log reduction in the spore population. But with the combination of ferrate and e-beam the reduction was approximately 2-3 log. The spore population could be reduced only by combination of these two stressors. In general, aerobic and anaerobic spore formers are more resistant to inactivation by ferrate compared to bacteria and enteric viruses. Under such circumstances, combination of ferrate and e-beam would definitely be a beneficial option for sludge disinfection. Significant reduction of phage population was observed with the synergistic effect of ferrate and e-beam (Fig. 4.6 and Fig. 4.7). There is a difference in the response of male-specific coliphage depending upon the sludge matrix (Fig. 4.7) suggesting that matrix play a role in sludge inactivation using ferrate. Compared to the somatic coliphages, male-specific coliphage had a low D-10 value (Fig. 3.5), which suggests the susceptibility of male-specific coliphage to irradiation. Studies conducted by Gehringer *et al*. 2003 also state that the innate susceptibility of male-specific coliphage towards radical attack is responsible for the low decimal dose reduction. D-10 value of Poliovirus

was also on par with that of the male-specific coliphage (Fig. 3.7), which explains for a similar response of both the organisms to the ferrate and e-beam combination treatment.

In general, we observed that all the organisms targeted in this study showed a significant reduction with a combination of ferrate and e-beam compared to that of ferrate alone. Irradiation causes sludge disintegration and cell rupture which hastens the disinfection process by ferrate (Yuan *et al*. 2008). Ferrate exactly complements the e-beam treatment by the production of highly reactive species of Iron such as +6 and +5. The +5 oxidation state of Iron enables better inactivation of biological species as well as toxins and other pollutants which cannot be achieved by +6 oxidation state alone (Sharma 2007). Pretreatment with ferrate also has an advantage of removing humic acid and helps in coagulation of the sludge which helps in better conditioning of biosolids (Liu *et al*. 2002). When combined with e-beam, ferrate provides better sludge disintegration, reduced floc size, and increased microbial cell break down, oxidation of organic matter that enhances microbial inactivation and stability of the treated sludge enabling appropriate use of treated byproducts for agricultural purpose and for nutrient recycling.

CHAPTER V

DETERMINATION OF MICROBIAL INACTIVATION USING CHEMICAL OXIDANT- CHLORINE DIOXIDE AND COMBINATION OF CHLORINE DIOXIDE AND E-BEAM IRRADIATION

INTRODUCTION

Chlorine is one of the most common disinfectants used in drinking water as well as waste water treatment (Li *et al*. 2002). One of the major problems accompanying with chlorination is the production of harmful products such as trihalomethanes, as a result of high reactivity between the chlorine and organic constituents present in the sewage sludge (Narkis *et al*. 1995). Chlorine dioxide is a better replacement for chlorine since they induce production of very less toxic or carcinogenic byproducts (Weinberg and Narkis 1992). The mode of action of chlorine dioxide is unique because of the one electron exchange mechanism. This enables chlorine dioxide to attack the electron rich centers in the organic molecule and transfer 1 electron to reduce chlorine dioxide to chlorine (Baribeau *et al*. 2002). This provides an advantage for chlorine dioxide over the other halogenated disinfectant, as a selective oxidizer and efficient disinfectant. In this chapter focus is given to study the microbial inactivation capacity of chlorine dioxide in different sewage sludge matrix and to evaluate the added advantage of combining e-beam treatment with chlorine dioxide in reducing microbial population and maintaining the sludge stability.

MATERIALS AND METHODS

Sludge collection. Sludge samples were collected from two different waste water treatment plants. Aerobically digested sludge was obtained from Carter Creek waste water treatment plant, College Station. Anaerobically treated sludge was collected from Texas A&M University waste water treatment plant. Samples were collected in sterile bottles (Nalgene, Rochester, NY) and transported to laboratory in a cooler and were maintained at 4°C until analysis. Dry weight data of the samples were recorded to determine the percentage of total solids and dry weight equivalent of the sludge.

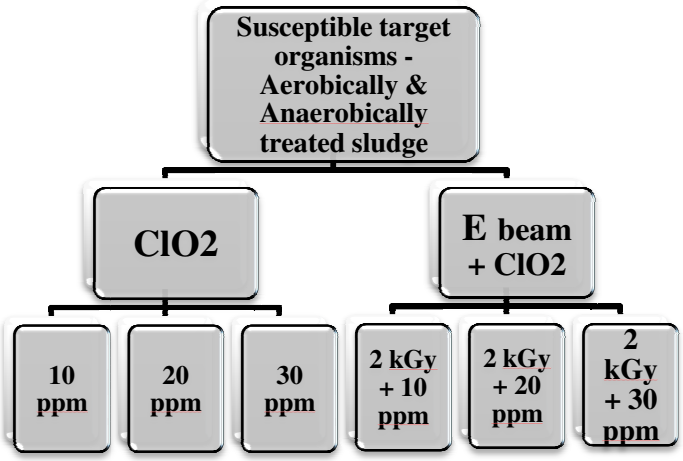
Spiking of sludge with microorganisms. The samples were spiked with high titer of laboratory grown strains of different organisms, which include bacteria - *Salmonella* Typhimurium (accession # 87-26254, obtained from National Veterinary Service Laboratory, Ames, Iowa), *Escherichia coli* (ATCC # 25922), coliphages phi X 174 (ATCC # 13706-B1) (somatic) and MS-2 (ATCC # 15597- B1) (Male-specific), enteric virus – Poliovirus-1 (VR- 1562), aerobic spore former – *Bacillus subtilis* (ATCC #6633) and anaerobic spore former – *Clostridium perfringens* (ATCC # 13124). For the ClO_2 and e-beam treatment, microorganisms were spiked into 2 different groups – Susceptible and resistant groups. Susceptible group for both aerobically and anaerobically treated sludge samples were spiked with *Salmonella* Typhimurium, *E. coli* and poliovirus. Resistant group for both the sludge samples were spiked with somatic coliphage, male specific coliphage, aerobic spores and anaerobic spores. ClO_2 and e-beam treatments were provided differently for susceptible and resistant groups.

Chlorine dioxide treatment. The spiked samples were mixed evenly and further subjected to different doses of chlorine dioxide treatment. Chlorine dioxide was prepared *in situ* through a direct reaction between 1.2 ml of 15% Sodium chlorite solution and 1.2 ml of 50 % H_2SO_4. The resultant solution is dissolved in 500 ml of water to obtain approximately 300 ppm of chlorine dioxide solution .The protocol for preparation of chlorine dioxide was provided by BCR Environmental, St. Augustine, Fl. In this process the chlorine dioxide gas that is produced as a result of reaction between sodium chlorite and sulfuric acid is dissolved in water to prevent the escape of gas. Dissolved chlorine dioxide is more stable compared to the gaseous compound. The concentration of chlorine dioxide produced was measured using spectrophotometer (HACH DR/2010, Loveland, CO.) with a program specific for measuring chlorine dioxide concentration. The accuracy of spectrophotometric readings were ensured by calibrating the readings with digital titration which is a colorimetric iodine titration for determining the oxidant titration of chlorine dioxide as per the standard methods for the examination of water and waste water, 1995 guidelines.

The susceptible group was subjected to 10, 20, and 30 ppm of chlorine dioxide where as resistant group received higher doses – 25, 50, 75 ppm. After the addition of different concentrations of chlorine dioxide, the samples were mixed gently to allow for sufficient contact with the sludge matrix for 2 hours at room temperature. Spiked controls were maintained without chlorine dioxide treatment to enumerate the amount of spiked microorganisms present in each of the sample. Matrix control of the sludge samples was also stored without chlorine dioxide or e-beam treatment to quantify the

indigenous population of different target microorganism. After 2 hours of chlorine dioxide treatment, the samples were neutralized using 2% sodium thiosulphate which inactivated chlorine dioxide present in the treated samples. The samples were mixed evenly and 20 ml of samples were triple packaged in whirl pak bags (Nasco, NY) in triplicates, to make it leak proof and to provide adequate protection while irradiating using e-beam.

E-beam treatment. The chlorine dioxide treated samples were subjected to e-beam irradiation at National Center for Electron Beam Research, Texas A&M University using 10 MeV LINAC source. Susceptible group of both aerobically and anaerobically treated sludges were irradiated at a dose of 2 kGy where as resistant group received a dose of 8 kGy. The absorbed dose was measured using L-α-alanine dosimeter tablets and the electron spin paramagnetic resonance spectroscopy (Bruker biospin Corp., Billerica). Irradiated samples were stored at 4 ˚C until they were subjected to microbiological analysis. Another set of chlorine dioxide treated samples were also maintained without e-beam irradiation to study the effect of the oxidant alone in microbial inactivation. Those samples were packaged similarly as for e-beam irradiation but were not subjected to irradiation and were labeled as 0 KGy. Fig. 5.1 provides a schematic representation of the ferrate and e-beam + ferrate treatment given to (a) susceptible group and (b) resistant group in aerobically and anaerobically treated sludge samples.



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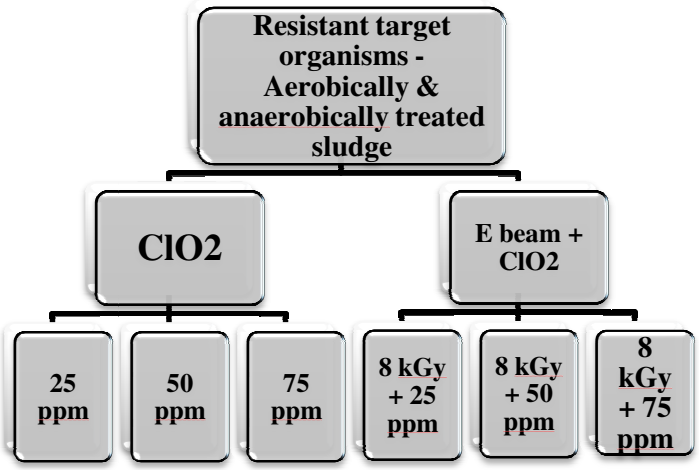


(b)

Fig. 5.1 Schematic representation of ferrate and e-beam + ferrate treatment provided to (a) Susceptible and (b) resistant groups in aerobically and anaerobically treated sludge samples.

Microbiological analyses. The irradiated and non-irradiated bags were opened under sterile conditions and the samples were analyzed for the presence of the spiked microorganisms.

(1) *Salmonella* Typhimurium – The sludge samples were serially diluted in 1X PBS and 0.1 ml of dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates containing Nalidixic acid (25 µg/ml) (Sigma, St. Louis, MO) and Novobiocin (25µg/ml) (Sigma, St. Louis, MO). The plates were incubated overnight at 37 ℃ and the characteristic *Salmonella* colonies were enumerated.

(2) *Escherichia coli* - Irradiated samples were serially diluted and 0.1ml of the dilutions was plated in EC-MUG media (Difco Laboratories, MI) and plates were incubated overnight at 37℃. The plates were read under long wave (366 nm) ultra violet light and the fluorescent colonies were enumerated.

(3) Aerobic spores (*Bacillus subtilis*) – Sludge samples were thermally inactivated at 64℃ for 15 minutes using a hot water bath. The inactivated samples were serially diluted and 0.1 ml of the dilutions were plated in Tryptic Soy Agar (Difco Laboratories, MI) plates and incubated overnight at 37℃ .

(4) Anaerobic spore formers (*Clostridium perfringens*) – Thermal inactivation of the sludge samples were carried out for 15 minutes at 64℃ and the inactivated samples were serially diluted in 1X PBS. Perfringens agar base, including TSC and SFP (Oxoid, Hampshire) media was prepared and m-CP selective supplement I (Fluka, Buchs, Switzerland) was added (1 vial/ 500 ml). The media was dispensed into petri plates along with 1 ml of the samples and swirled. The

plates were then incubated overnight in anaerobic jars at 37℃. Black colored colonies were enumerated which indicated the presence of *Clostridium perfringens*.

(5) Phi X 174/ somatic coliphage – Virus extraction from the sludge samples were carried out using 3% beef extract and the extracts thus obtained were filtered using 0.22 µm filters (Millipore, Billerica, MA). The viral extracts thus obtained were serially diluted for analysis of phages as well as enteric virus, Enumeration of somatic coliphages were carried out using Single Agar Layer method (Method 1602,US EPA, 2001) with the host bacteria *E. coli* CN- 13. The plates were incubated overnight at 37℃ and plaques were counted after 24 hours.

(6) MS-2/ male specific coliphage – Serially diluted viral extracts were analyzed using Single Agar Layer method (Method 1602, US EPA, 2001) with host bacteria *E. coli* F_{amp}^{+} specific for male specific coliphages. After overnight incubation at 37℃, plaques were enumerated.

(7) Enteric virus – The viral extract obtained from the sludge samples were also used for Poliovirus estimation using tissue culture methods. Infectivity assay was carried out in 6 well plates using BGMK (Buffalo Green Monkey Kidney) cell lines). 0.2 ml of the samples as well as dilutions was used for infection of the BGMK cells and the plates were incubated at 37℃ at 5% CO_2 atmosphere for 24 hours. Plaques were enumerated after staining the plates with 0.1% crystal violet.

Statistical analysis. The values obtained from the inactivation studies were converted to $\log_{10}x$ values and plotted against respective doses of chlorine dioxide and e-beam + chlorine dioxide. The disinfection efficiency of ClO_2 and the combination treatment of ClO_2 and e-beam were determined by analyzing the $\log_{10}$ reduction in the microbial population subjected to different treatments compared to that of the spiked control, which did not receive any disinfection treatments. In order to compare the pathogen reduction between different treatments and within treatments, paired–t–tests were carried out using statistic software package SPSS.

RESULTS

The disinfection efficiency of chlorine dioxide was studied by dividing the spiked sludge samples into groups. Susceptible group comprised of *Salmonella* Typhimurium, *E. coli* and poliovirus where as resistant group included somatic coliphage, male specific coliphage, aerobic and anaerobic spores. Fig. 5.2 and Fig. 5.3 illustrate the bacterial inactivation as a result of chlorine dioxide treatment. *Salmonella* showed any significant reduction with chlorine dioxide treatment of up to 30 ppm upon pair wise comparison with 10 ppm of ClO_2 ($p= 0.036$) .

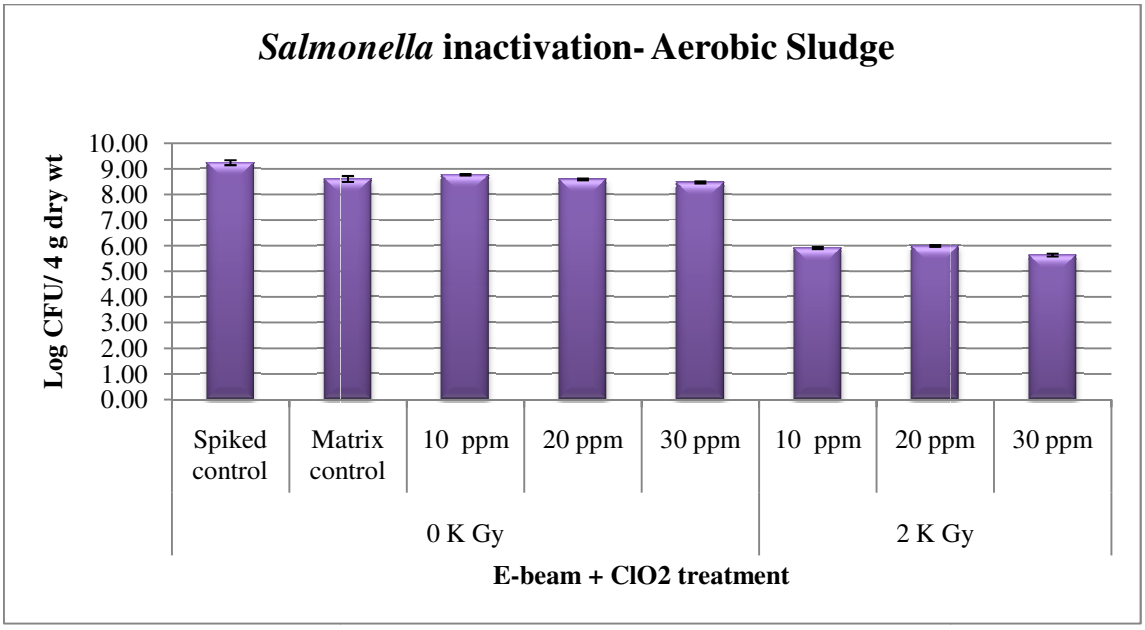
Interestingly with a combination of 10 ppm chlorine dioxide and 2 KGy of e-beam irradiation, 3 log reduction was observed for both aerobically and anaerobically treated sludge with p value 0.00 and 0.010 respectively (Table 5.1).

E. coli was found to be more susceptible to chlorine dioxide as well as combination treatment with e-beam. 1-2 log reduction was observed with 30 ppm of chlorine dioxide in case of anaerobically treated sludge. Aerobically treated sludge samples showed complete reduction of *E. coli* (approximately 8 log) with the synergistic effect of chlorine dioxide and e-beam where as a 4-5 log reduction was observed in case of anaerobically treated samples (Fig. 5.3). Table 5.1 illustrates pair wise comparison of ClO2 and combination treatment of Salmonella and *E. coli* inactivation in aerobically and anaerobically treated sludge.

Table 5.1 Statistical comparison between ClO_2 and the e-beam + ClO_2 combination treatment of *Salmonella* Typhimurium and *E. coli* in aerobically and anaerobically treated sludge.

Treatment comparison	t	df	p-value
***Salmonella* Typhimurium - aerobically digested sludge**			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	54.466	2	.000**
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	37.017	2	.001**
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	37.705	2	.001**
***Salmonella* Typhimurium - anaerobically digested sludge**			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	9.794	2	.010*
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	45.654	2	.000**
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	3.462	2	.074
***E. coli*- aerobically digested sludge**			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	3.347	2	.079
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	148.063	2	.000**
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	203.027	2	.000**
***E. coli*- anaerobically digested sludge**			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	20.579	2	.002**
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	33.206	2	.001**
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	3.600	2	.069

* - Significance level 0.05; ** - Significance level 0.01



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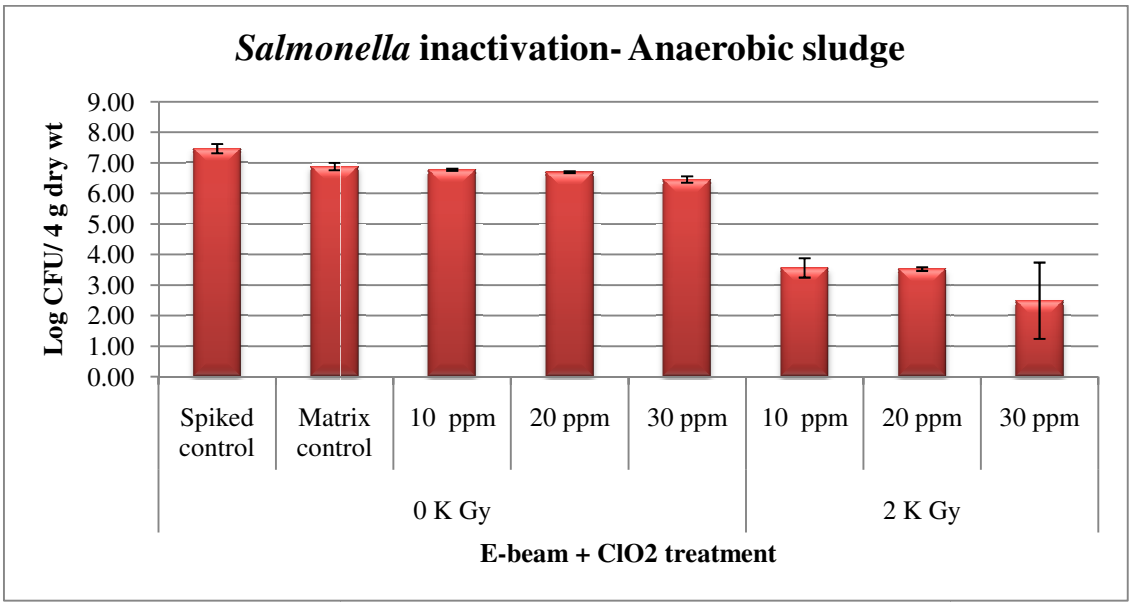

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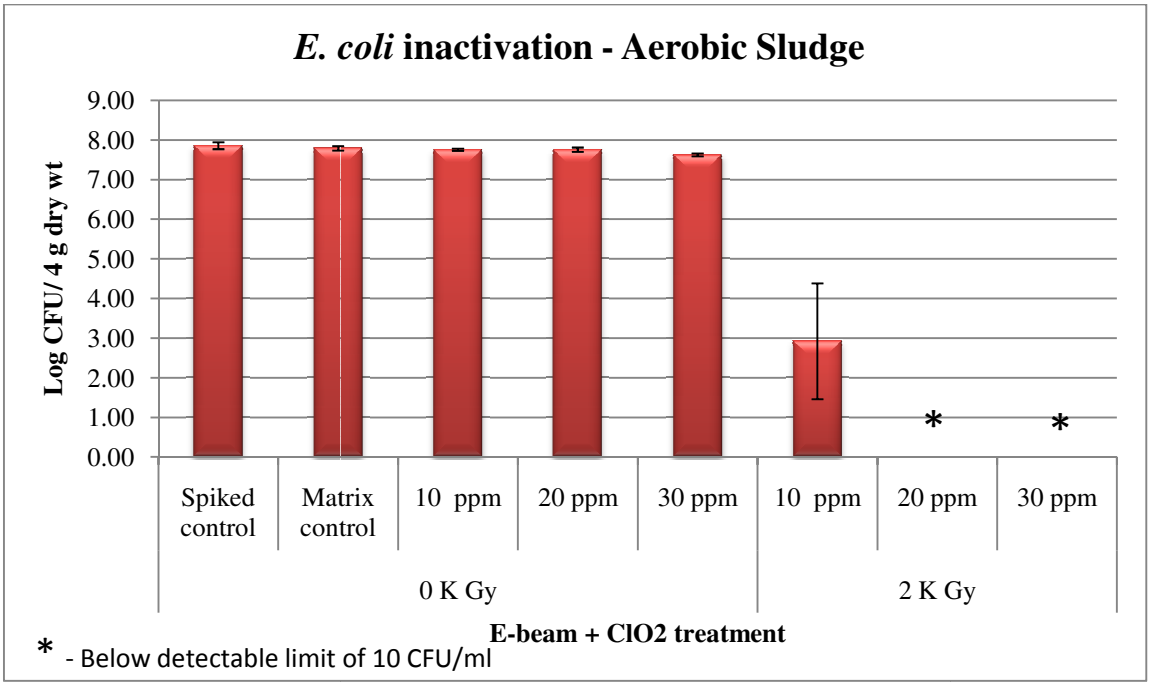
Fig. 5.2 Inactivation of *Salmonella* Typhimurium when exposed to 10, 20, 30 ppm of chlorine dioxide and combination of chlorine dioxide and 2 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.



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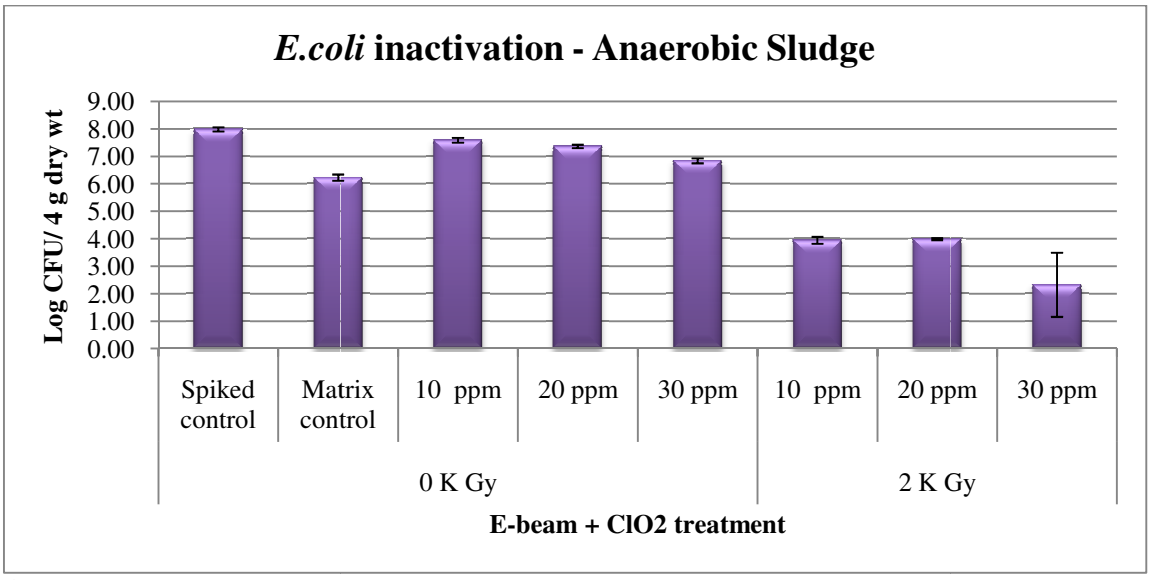

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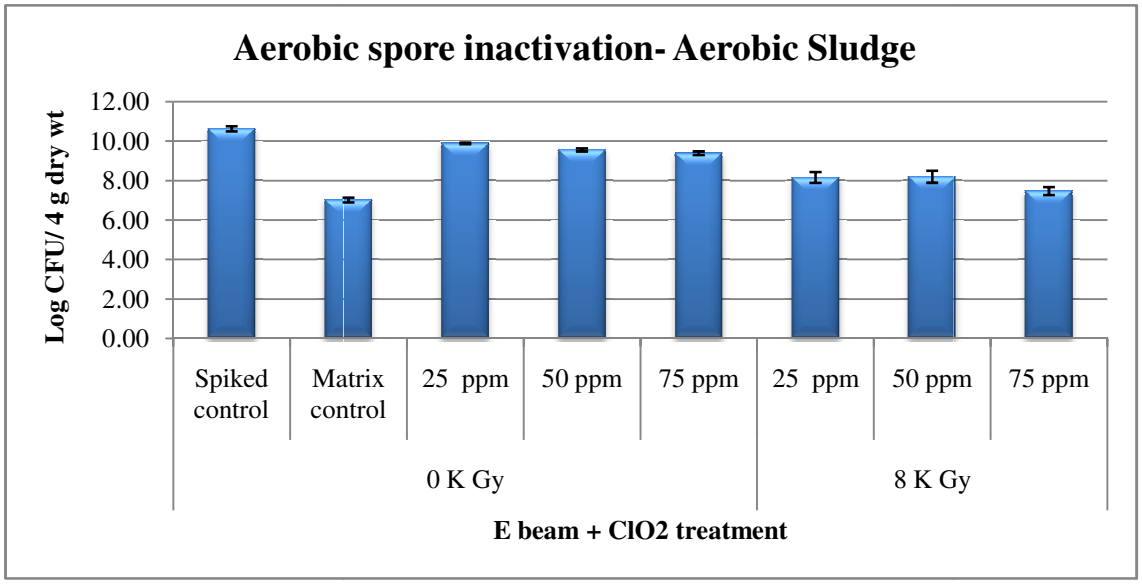
Fig. 5.3 Inactivation of *Escherichia coli* when exposed to 10, 20, 30 ppm of chlorine dioxide and combination of chlorine dioxide and 2 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.

Aerobic and anaerobic spores were subjected to higher doses compared to bacteria – 25, 50, 75 ppm of ClO_2 and 8 kGy of e-beam irradiation. Both aerobic and anaerobic spore were resistant to the ClO_2 with not more than 1 log reduction even at 75 ppm and with e-beam dose of 8 kGy. Approximately 2 log reduction was observed in the case of aerobic spores (Fig. 5.4). Reduction in the aerobic spore population could be observed with an increase in ClO_2 concentration to 75 ppm in aerobically digested sludge ($p=0.013$). But the difference is not significant in case of anaerobically treated sludge ($p>0.05$). Table 5.2 depicts the pair wise comparison of ClO_2 and the corresponding combination treatment. Significant difference could be observed in both aerobically and anaerobically treated sludge samples with $p<0.05$. Increase in the concentration of ClO_2 alone did not bring any significant difference in anaerobic spore population, given by $p>0.05$ in both aerobically and anaerobically treated sludge samples.

Table 5.2 Statistical comparison between ClO_2 and the e-beam + ClO_2combination treatment of aerobic spores and anaerobic spores in aerobically and anaerobically treated sludge.

Treatment comparison	t	df	p-value
Aerobic spores- aerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	5.583	2	.031*
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	5.979	2	.027*
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	10.842	2	.008**
Aerobic spores- anaerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	11.040	2	.008**
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	8.533	2	.013*
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	3.084	2	.091
Anaerobic spores- aerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	3.869	2	.061
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	6.773	2	.021*
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	4.198	2	.052
Anaerobic spores- anaerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	2.550	2	.125
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	4.303	2	.050
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	5.596	2	.030*

* - Significance level 0.05, ** - Significance level 0.01



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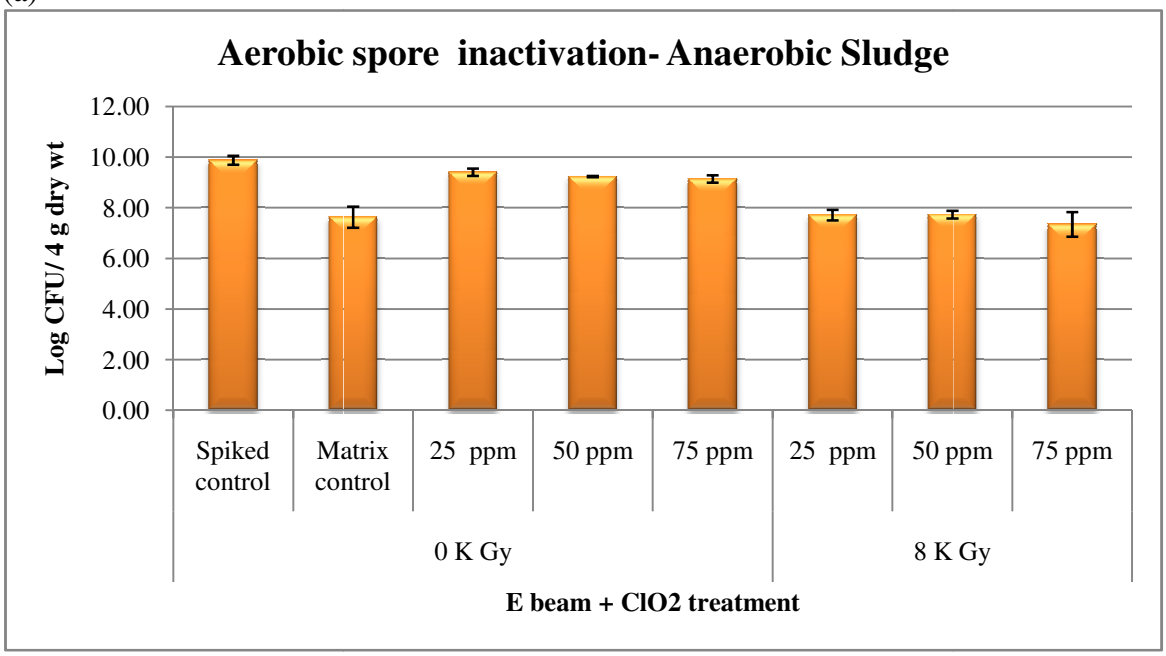

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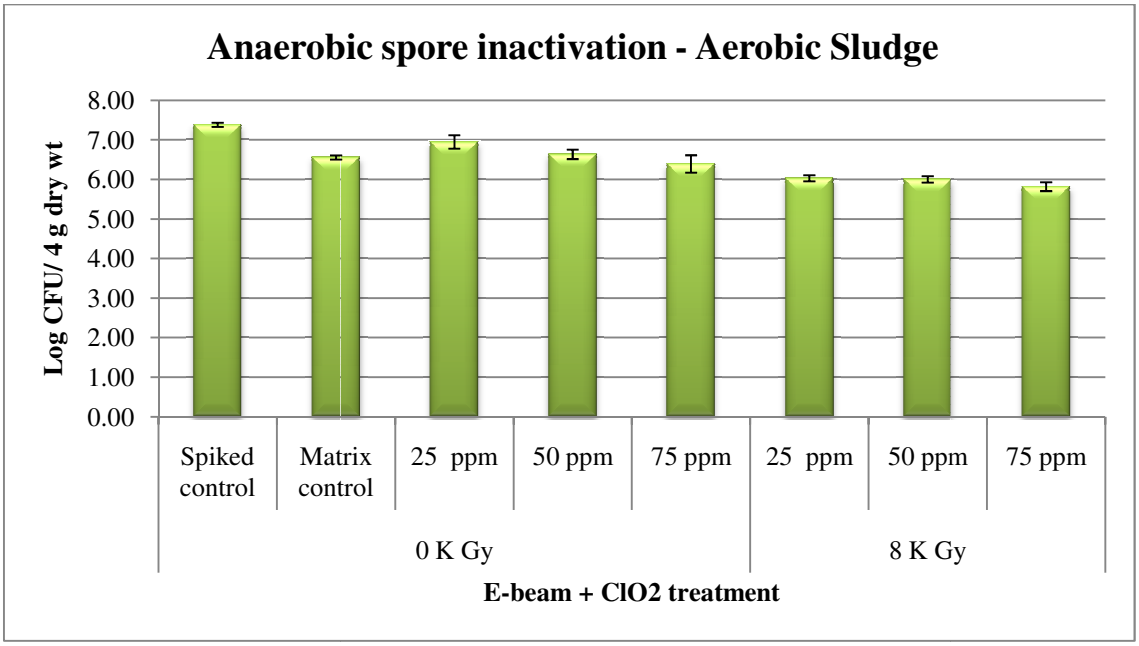
Fig. 5.4 Inactivation of aerobic spore – *Bacillus subtilis*, when exposed to 25, 50, 75 ppm of chlorine dioxide and combination of chlorine dioxide and 8 kGy of e beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.



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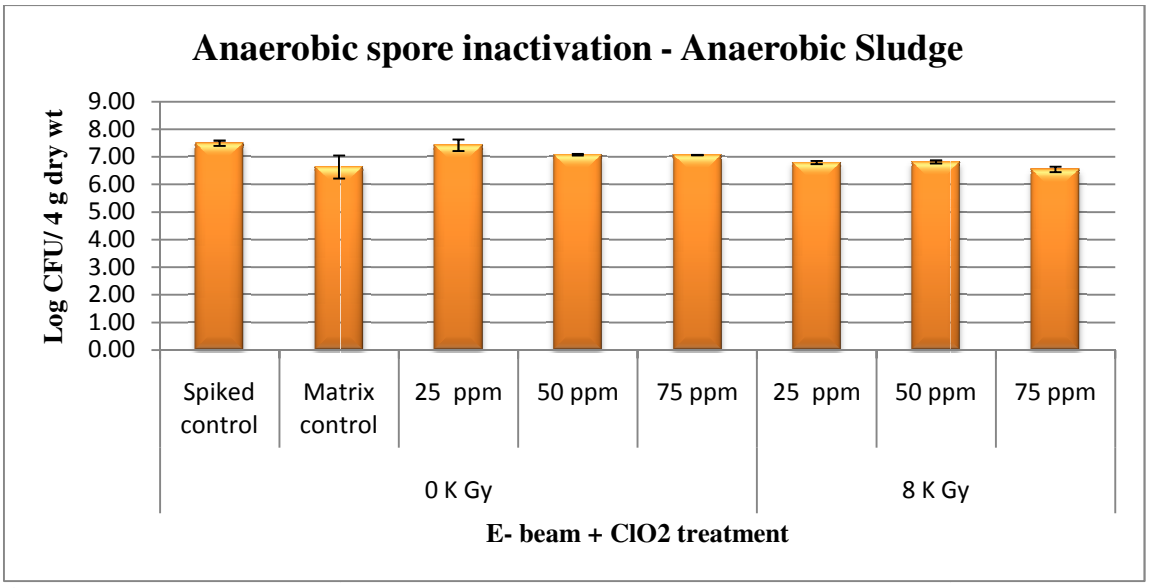


(b)

Fig. 5.5 Inactivation of anaerobic spore – *Clostridium perfringens*, when exposed to 25, 50, 75 ppm of chlorine dioxide and combination of chlorine dioxide and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.

Even the combination treatment could not bring significant reductions in anaerobic spore in both the aerobic and anaerobic sludge (Table 5.2). This clearly shows the resistance of anaerobic spores towards ClO_2 as well as the e-beam treatment. No significant reduction was observed for anaerobic spore with ClO_2 and the combination treatment (Fig. 5.5).

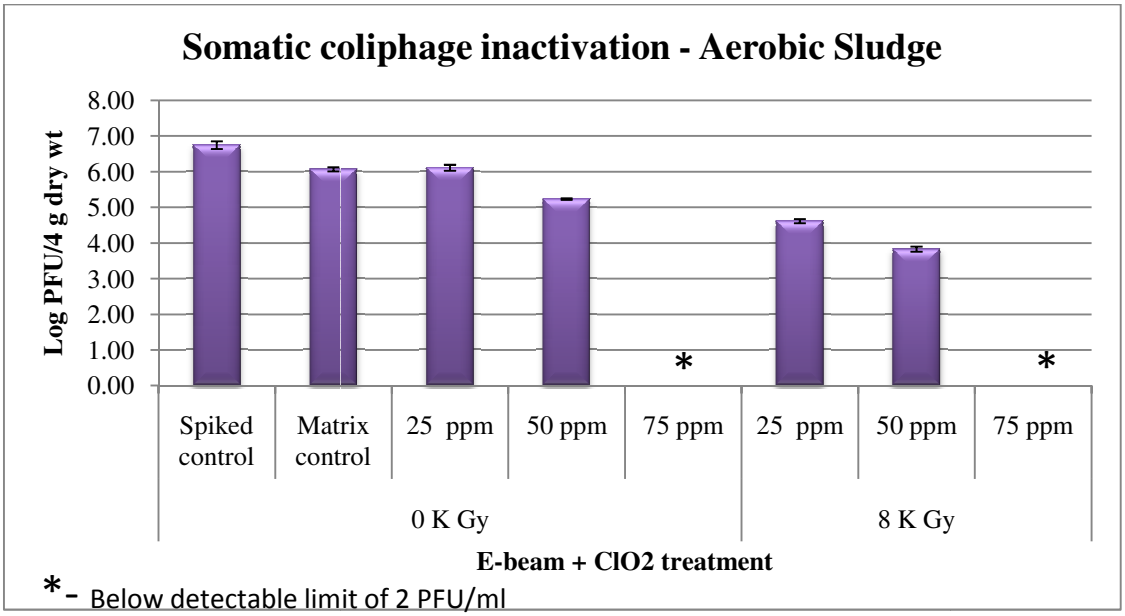
Somatic and male-specific coliphages were effectively reduced by the ClO_2+ e-beam treatment (Fig. 5.6 and Fig. 5.7). Aerobically treated College Station sludge supported better phage elimination with 75 ppm ClO_2 alone. With the combination of 8 kGy of e-beam, approximately 7 log reduction of male-specific population was observed in aerobically treated sludge samples (Table 5.3). In contrast to this, male-specific in TAMU sludge showed only 2 log reduction with the combination treatment (Fig. 5.7).

Table 5.3 Statistical comparison between ClO_2 and the e-beam + ClO_2combination treatment of somatic coliphage and male specific coliphage in aerobically and anaerobically treated sludge.

Treatment comparison	t	df	p-value
Somatic coliphage- aerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	8.210	2	.015*
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	64.074	2	.000**
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	22.716	2	.002**
Somatic coliphage- anaerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	8.210	2	.015*
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	64.074	2	.000**
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	22.716	2	.002**
Male specific coliphage- aerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	-	2	0.00**
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	-	2	0.00**
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	-	2	0.00**
Male specific coliphage- anaerobically digested sludge			
25 ppm ClO_2 Vs 25 ppm ClO_2 + 8 kGy e-beam	18.448	2	.003**
50 ppm ClO_2 Vs 50 ppm ClO_2 + 8 kGy e-beam	19.000	2	.003**
75 ppm ClO_2 Vs 75 ppm ClO_2 + 8 kGy e-beam	27.373	2	.001**

* - Significance level 0.05, ** - Significance level 0.01

- Male specific coliphage in aerobically treated sludge were below detectable limits to carry out a paired-t- test



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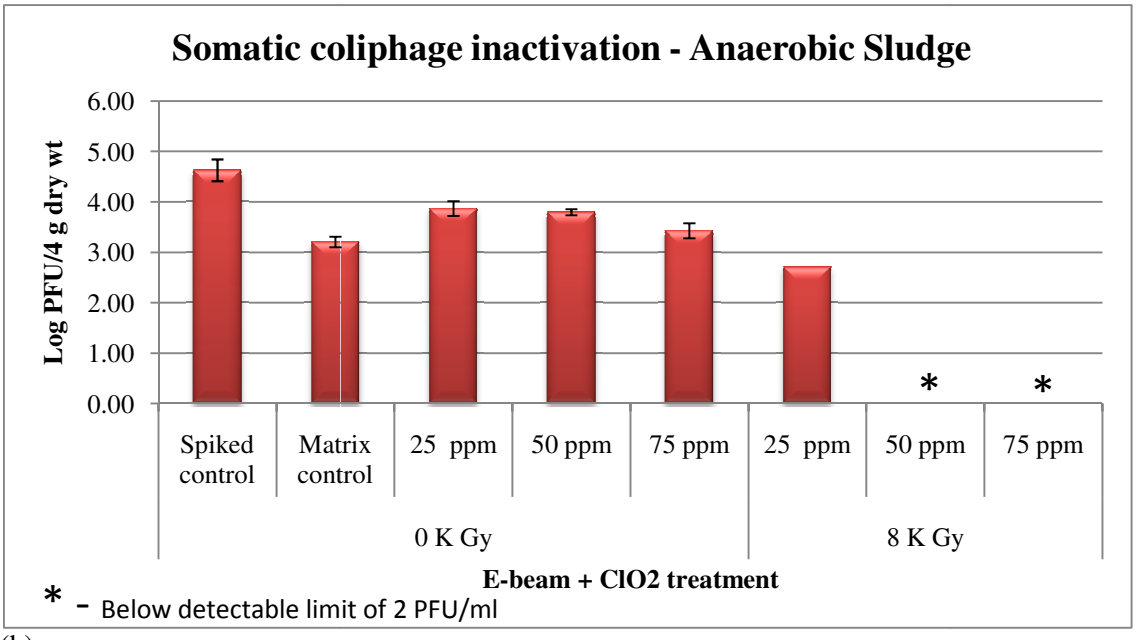

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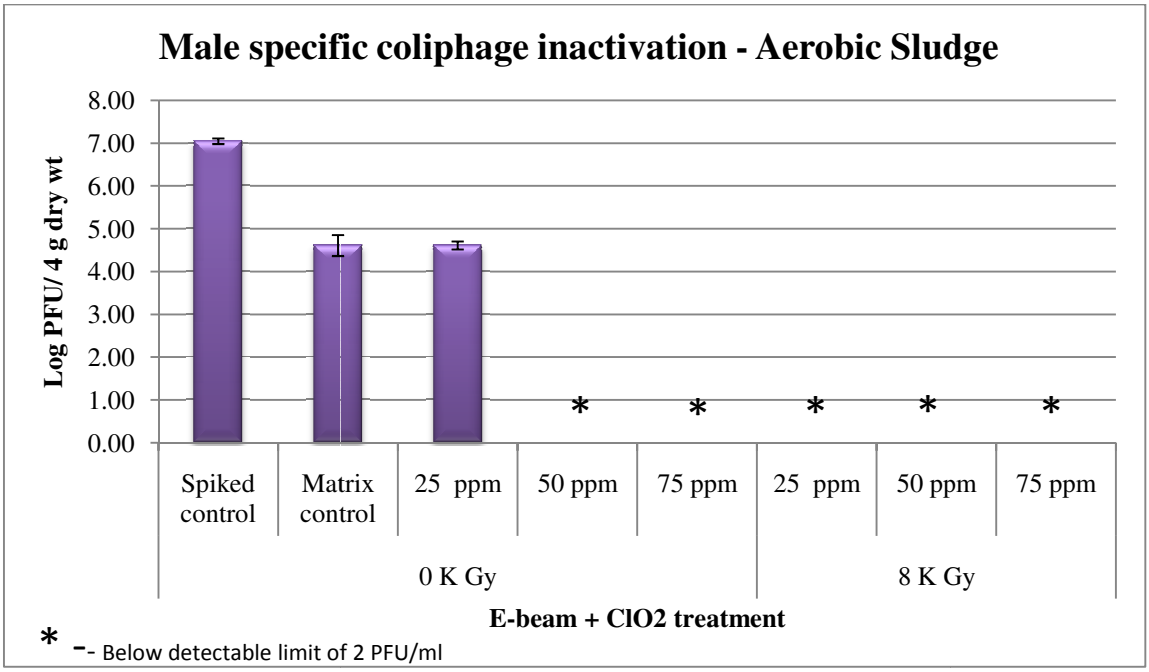
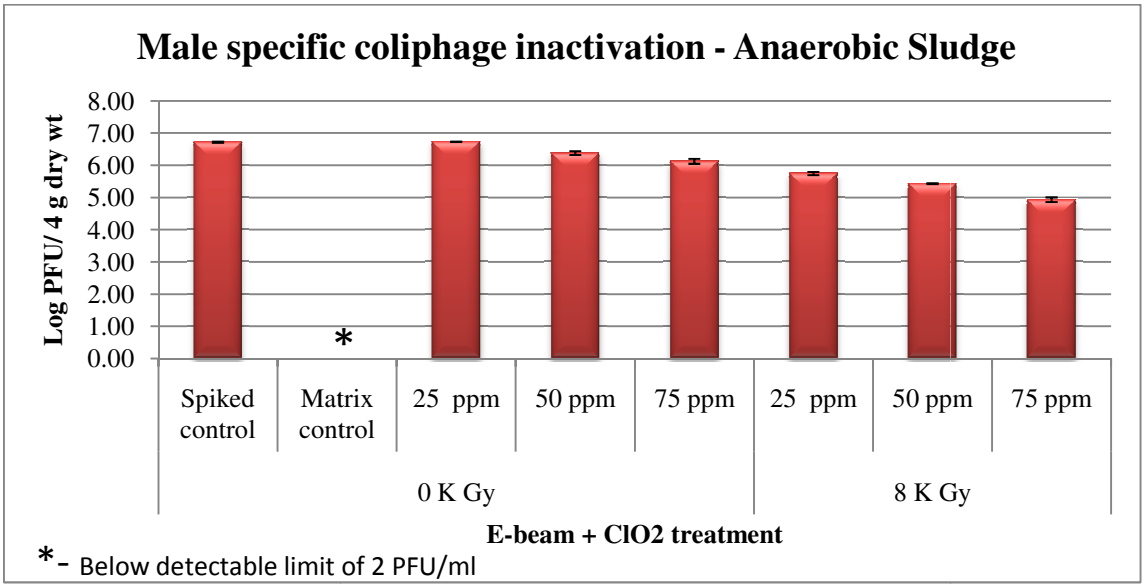
Fig. 5.6 Inactivation of somatic coliphage – phi X 174, when exposed to 25, 50, 75 ppm of chlorine dioxide and combination of chlorine dioxide and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.



(a)



(b)

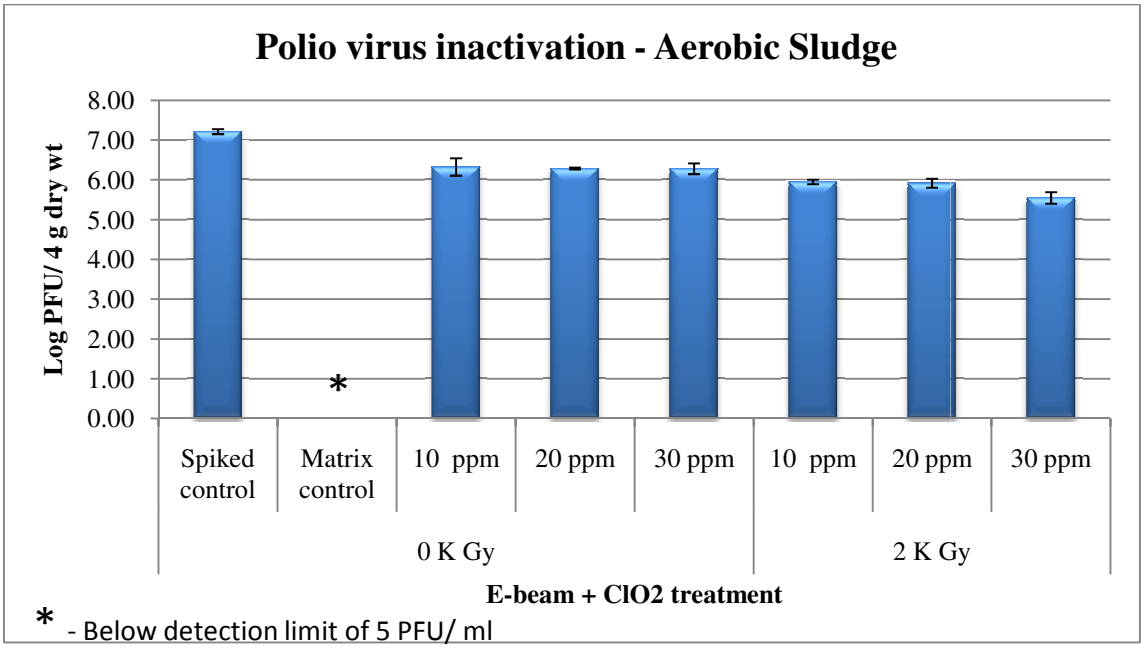
Fig. 5.7 Inactivation of male specific coliphage – MS2, when exposed to 25, 50, 75 ppm of chlorine dioxide and combination of chlorine dioxide and 8 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.

Similar trend was observed in case of both aerobically and anaerobically treated sludge samples for the somatic coliphage inactivation. Complete phage elimination of up to 5-6 log was obtained in case of somatic coliphage with ClO_2 + e-beam treatment ($p<0.01$) (Fig. 5.6). Fig. 5.8 illustrates that increase in ClO2 concentration alone did not bring any difference in the poliovirus population, given by $p>0.05$ in both aerobically and anaerobically treated sludge samples. Addition of e-beam has brought slightly significant difference in poliovirus as indicated in Table 5.4. But this cannot be compared directly with that of the phage inactivation because poliovirus was grouped under the susceptible group and hence received only low doses- 10, 20, 30 ppm of ClO_2 and 2 kGy of e-beam irradiation. But an interesting finding was both aerobically and anaerobically digested sludge samples were devoid of indigenous enteric virus, where as considerable amount of indigenous bacteria, spore as well as phage population was noted in this study.

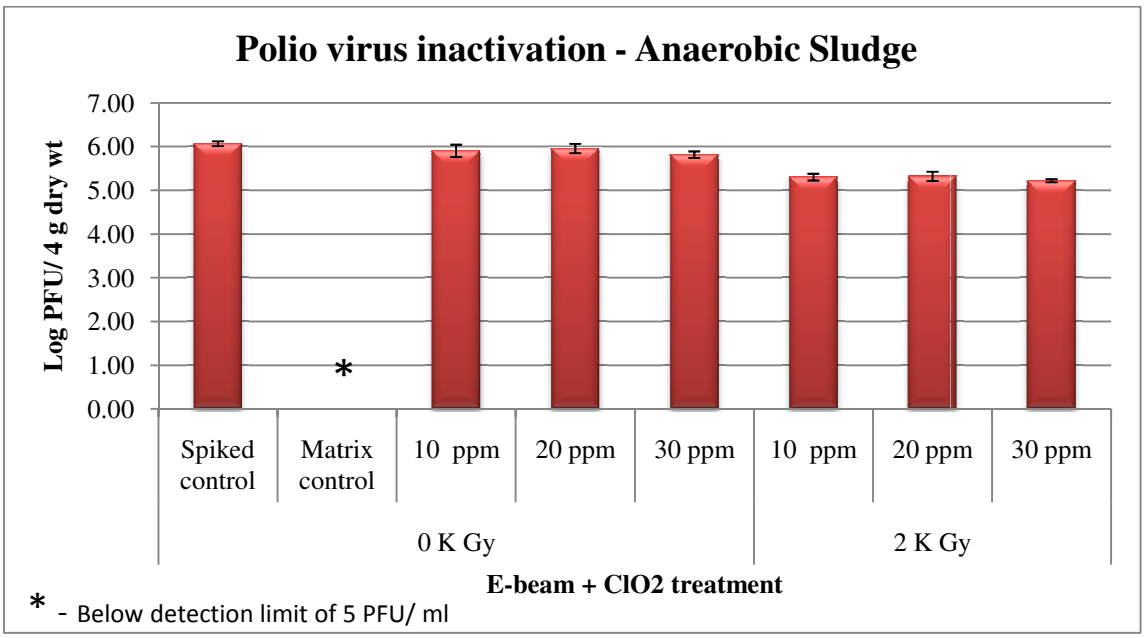
Table 5.4 Statistical comparison between ClO_2 and the e-beam + ClO_2 combination treatment of Poliovirus in aerobically and anaerobically treated sludge.

Treatment comparison	t	df	p-value
Poliovirus- aerobically digested sludge			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	1.462	2	.281
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	4.078	2	.055
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	4.933	2	.039*
Poliovirus- anaerobically digested sludge			
10 ppm ClO_2 Vs 10 ppm ClO_2 + 2kGy e-beam	5.437	2	.032*
20 ppm ClO_2 Vs 20 ppm ClO_2 + 2kGy e-beam	3.017	2	.095
30 ppm ClO_2 Vs 30 ppm ClO_2 + 2kGy e-beam	6.480	2	.023*

* - Significance level 0.05; ** - Significance level 0.01



(a)



(b)

Fig. 5.8 Inactivation of Poliovirus, when exposed to 10, 20, 30 ppm of chlorine dioxide and combination of chlorine dioxide and 2 kGy of e-beam in (a) aerobically and (b) anaerobically treated sludge. 0 kGy refers to chlorine dioxide treatment alone and spiked control refers to 0ppm chlorine dioxide and 0 kGy e-beam (untreated spiked sludge sample), Matrix control refers to untreated sludge without spiking.

DISCUSSION

The bactericidal properties of chlorine dioxide as compared to that of chlorine was studied extensively even from 1949. Trakhtman *et al*. reported the effective inactivation of *E. coli* as well as *Bacillus anthracoides* in secondary effluents using 1-2 ppm of chlorine dioxide. ClO_2 was proved as a better disinfectant compared to chlorine with respect to the reduction of *Salmonella* Typhimurium and *Bacillus* sp. (Bedulivich *et al*. 1954, Ridenour *et al*. 1949). Up to 5 log reduction in fecal coliform population was obtained in secondary effluents treated with 10 ppm of chlorine dioxide for a contact time of 30 minutes (Roberts *et al*. 1980). The reduction in the target microorganism population in response to the chlorine dioxide treatment could be affected by the presence of suspended matter in the matrix being treated. In the current study, we did not see a significant reduction in bacteria, aerobic and anaerobic spores due to effect of ClO_2 alone (Fig. 5.2 and Fig. 5.3). Narkis *et al*. (1995) stated that the presence of suspended particles in the sludge protects the bacteria as well as viral particles from being destroyed by the chlorine dioxide treatment. Under such a condition, e-beam irradiation could be supplemented with the chlorine dioxide treatment. E-beam irradiation disintegrates the sludge particles and cause reduction in the floc size, thus exposing the microbes to better disinfection effect. 3-6 log reduction achieved from the ClO_2 + e-beam treatment (Fig. 5.2 and Fig. 5.3) could be due to the above effect. The amount of suspended particles varies with different type of sludge samples being analyzed. In this study, 2 different sludge samples one from College Station and the other from TAMU were examined. Aerobically treated College Station sludge had comparatively lower

total solid content of 1.3% compared to that of the 3.12 % for anaerobically treated TAMU sludge. The inactivation profile of bacteria, phages, spores as well as enteric viruses shows that e-beam + ClO_2 treatment was more effective for aerobically treated sludge compared to anaerobically treated sludge samples. As reported earlier by Narkis *et al*. (1992), bacteriophages were considerably affected by chlorine dioxide. Based on our study, the incorporation of 8 kGy of e-beam dose accelerated the inactivation of phages in sludge. Poliovirus was subjected to only low dose of ClO_2 and e-beam, but it was found that there was considerable degree of protection to the viral capsid as well as genome by the suspended particles. Brigano *et al*. (1978), showed that chlorine dioxide took 2.7 times longer to inactivate clumped poliovirus I aggregates compared to that of single state virus, which clearly supports the results obtained from the current study (Fig. 5.8).

The disinfection properties of chlorine dioxide depend upon the effective penetration of compound into the floc particles and also upon the innate resistance of various microorganisms to the treatment (Narkis *et al*. 1995). Hence supplementing the ClO_2 treatment with e-beam is a better option to augment the microbial inactivation in sewage sludge. Incorporation of e-beam also prevents the requirement of addition of excess chlorine dioxide that may induce production of toxic byproducts, thus providing a safe and eco-friendly alternative for municipal biosolids disinfection and conditioning.

CHAPTER VI

QUANTITATIVE MICROBIAL RISK ASSESSMENT OF APPLICATION OF TREATED SLUDGE FOR AGRICULTURAL PURPOSES

INTRODUCTION

Treated waste water and biosolids can be applied to fields for agricultural purposes. The humus and the nutrient content present in the sewage sludge helps in crop growth. Addition of sewage sludge also has an added advantage as it can impart moisture to the field, thus reducing the need of irrigation (Tierney *et al*. 1977). In United States, the treated biosolids must meet the US EPA regulations for pathogen reduction and vector control, before being used for agricultural purposes (US EPA 2003). It is estimated that worldwide around 20 million ha of agricultural fields use raw, treated or diluted waste water (Future harvest 2001). But the validity of this statistics still remains a debatable issue (Hamilton *et al*. 2006). Chances of food borne out breaks are extremely high in regions where sewage is applied to fields without appropriate treatment. Irrigation water, soil and manure comprise major preharvest source of microbial contamination in crops which aggravates the chances of food borne outbreaks (Beuchat 1995). Concerns about such food borne outbreaks are too high among public especially in developed nations such as United States where land application of sludge is banned in states like California (Pepper *et al*. 2008). This calls for verifying the sustainability of land application as well as field application of treated sludge in a scientific manner and assessing the risk involved in order to take a pragmatic decision.

QUANTITATIVE MICROBIAL RISK ASSESSMENT

Quantitative microbial risk assessment (QMRA) is a powerful tool for assessing the magnitude of microbial risk involved using probabilistic models. The principle of risk assessment is employed to estimate the consequence from an actual or a planned microbial exposure scenario (Haas *et al*. 1999). QMRA follows the frame work suggested by National Academy of Sciences (NRC 2008). The frame work includes key steps such as hazard identification, dose-response, pathogen exposure and risk characterization. The first step of QMRA is to identify a problem associated with human health or animal health and to trace back the factors leading to the particular scenario. After identifying various factors, the response of those factors upon the health effect is analyzed. Exposure assessment involves the study of susceptible population and the possible route of exposure of pathogen to the population. Finally all the information about factors, dose, exposure are gathered and the risk involved is assessed using transmission models and probabilistic analyses such as Monte Carlo simulation. The three major areas of risk analysis are risk characterization, risk management, and risk communication. The risk characterized using dose response models should be effectively managed and also communicated to the population to prevent the exposure to hazardous factors (Haas *et al*. 1999).

In order to assess the safety of reuse of sewage sludge, the risks associated with worst case scenarios needs to be evaluated. In this chapter, a worst case scenario was been formulated where in which raw/untreated sludge was applied on to the field where

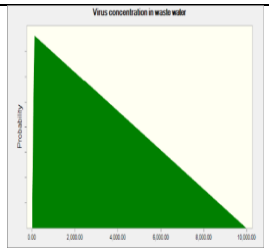
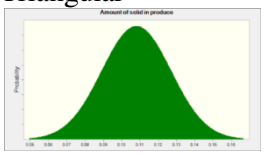
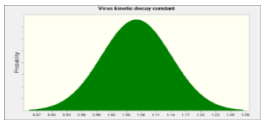
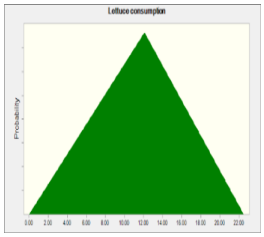
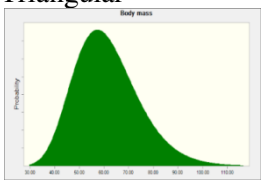
minimally processed leafy vegetable such as lettuce was cultivated. The same scenario was re-evaluated considering the application of treated sludge in order to show the reduction in the associated risk with implementation of appropriate sludge treatment processes.

PATHOGEN EXPOSURE SCENARIO- BACKGROUND

In case of developing nations, only less than 10% of the sewage sludge generated undergoes proper sludge treatment. This fact led to development of the scenario mentioned in this chapter of applying raw or primary sludge to the agricultural field. Even though the raw sludge contains numerous pathogens, enteric virus was selected for the QMRA analysis as their infectivity dose can be as low as 1infective unit (Gale 1995). Moreover viral illnesses associated with food borne disease outbreaks outnumber that caused by bacteria and protozoa (Mead *et al*. 1999). The green leafy vegetable, lettuce was chosen as the crop fertilized by sludge due to various factors. Lettuce is consumed raw without much processing and it is also capable of enhancing the persistence of viruses by protecting the viral particles in the lettuce head against desiccation and inactivation by light (Petterson *et al*. 2001). The event tree leading to the consumption of enteric virus contaminated lettuce and the assumptions made are described below.

Scenario – 1 – Raw sludge application. Raw sludge or primary sludge without any treatment was used for field application. The enteric virus concentration in primary sludge was 10^2-10^4 PFU/ g dry weight of sludge (Maier *et al*. 2000) and the sludge application was carried out in a lettuce field from which the leaves could be harvested after 2 weeks. The rate at which enteric virus undergoes decay due to environment factors was considered as a point estimate 0.69 (Hamilton *et al*. 2006). Virus kinetic decay constant (k) follows normal probability distribution with mean 1.07 and standard deviation of 0.07 (Hamilton *et al*. 2006). During the growth period, virus in the sludge gets transferred into lettuce through irrigation or through soil particles. The probability distribution and the fit parameters associated with each of the pathogen transfer event are mentioned in Table 6.1. The lettuce crop was harvested after 14 days of sludge application. The post harvest viral inactivation was not considered in this scenario as it was assumed negligible (Badaway *et al*. 1985). The daily lettuce consumption data was obtained from the continuing survey of food intakes by individuals of US Department of Agriculture (Hamilton *et al*. 2006) and the body mass data was taken from the Exposure factors handbook (US EPA 1997).

Table 6.1 Distribution and the fit parameters used in the model for QMRA.

Model parameter	Distribution	Fit parameters	Unit	Reference
Virus concentration in waste water (C)	 Triangular	Minimum =0 Likely = 100 Maximum = 10000	PFU/ g dry weight	Maier *et al*. 2000
Amount of solid in produce (V_{prod})	 Normal	Mean = 0.108 SD = 0.019	g solids/ g of lettuce	Hamilton *et al*. 2006
Virus kinetic decay constant (k)	 Normal	Mean = 1.07 SD = 0.07	per day	Hamilton *et al*. 2006
Lettuce consumption (M_i)	 Triangular	Minimum = 0 Likely = 12.1 Maximum = 22.5	g/kg body weight/ day	USDA 2006
Body mass (M_{body})	 Log normal	Mean = 61.429 SD = 13.362	kg	US EPA 1997; Hamilton *et al*. 2006

The daily dose ingested was calculated (Hamilton *et al*. 2006) as a function of the model parameters given in Table 6.1,

$$\textbf{Dose} = \boldsymbol{M_i \times M_{body} \times C \times V_{prod} \times e^{-kt}} \quad \rightarrow \text{ Equation 1}$$

where, t = time between sludge application and harvest = 14 days (assumed)

C = Virus concentration in waste water

V_{prod} = Amount of solid in produce

k = Virus kinetic decay constant

M_i = Lettuce consumption

M_{body} = Body mass

The beta-Poisson model dose-response model was considered to characterize the enteric virus risk involved (Haas *et al*. 1999)

$$\textbf{Dose response, } \boldsymbol{p_i = 1 - [1 + N/N_{50}(2^{1/\alpha} - 1)]^{-\alpha}} \quad \rightarrow \text{ Equation 2}$$

where, p_i = Probability of infection

N= Dose or exposure calculated in equation 1

Median infectious dose (N_{50}) = 5.597

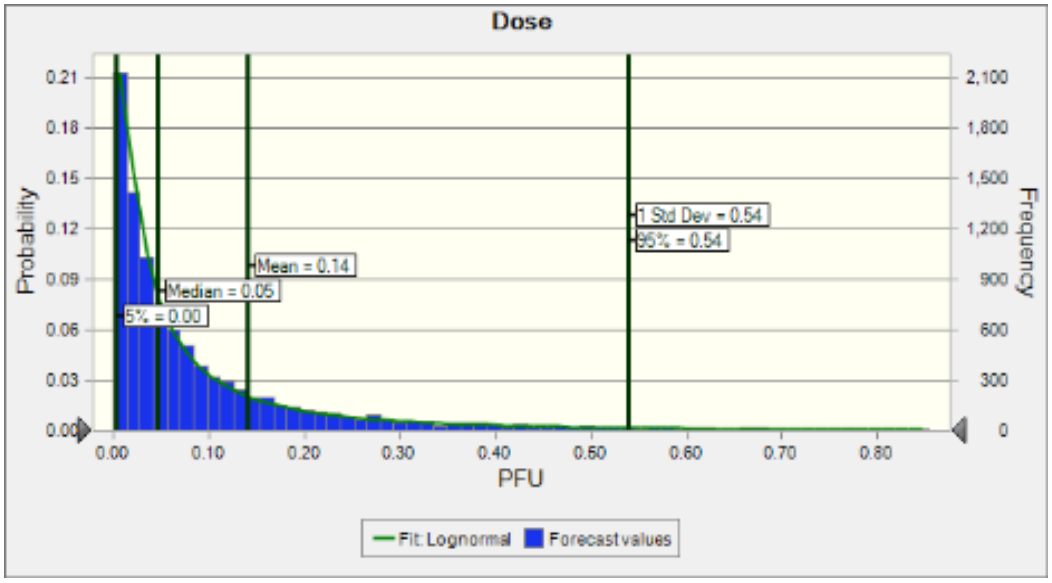
Parameter defining dose- response curve (α) = 0.265

Scenario – 2 – Treated sludge application. A second scenario was considered for risk assessment where all the other model parameters were the same as the first, except for the fact that instead of raw sludge being used, treated sludge was land applied. It is assumed that Electron beam treated sludge irradiated at a dose of 8 kGy was applied to the lettuce field. As per the studies conducted by e-beam described in chapter III, estimated D-10 value for enteric virus in sludge was 2.69 kGy. Hence by the application of 8 kGy of e-beam dose, 3 log reduction in the viral population is expected. Considering this 3 log reduction in the viral population in sludge, virus concentration in the treated sludge is assumed to be 10^{-1}-10 PFU/ g dry weight.

MONTE CARLO SIMULATION

Monte Carlo simulation was conducted using software Crystal ball® (Haas *et al*. 1999). The Monte Carlo method (MCM) is used to evaluate the effect of variability and uncertainty of the parameters used to define the dose response model. In MCM, the model inputs are used to generate a simulation output and the simulation is repeated a number of times to get a distribution pattern which depicts the fidelity of the overall uncertainty (Haas *et al*. 1999). The model inputs are entered as a probability distribution into the MCM rather than individual values in order to reduce bias in the simulation, thereby giving a robust estimate. The outputs from the Monte Carlo simulation for scenario 1 are given in Fig. 6.1 and that of scenario 2 are given in Fig. 6.2.

(a)



(b)

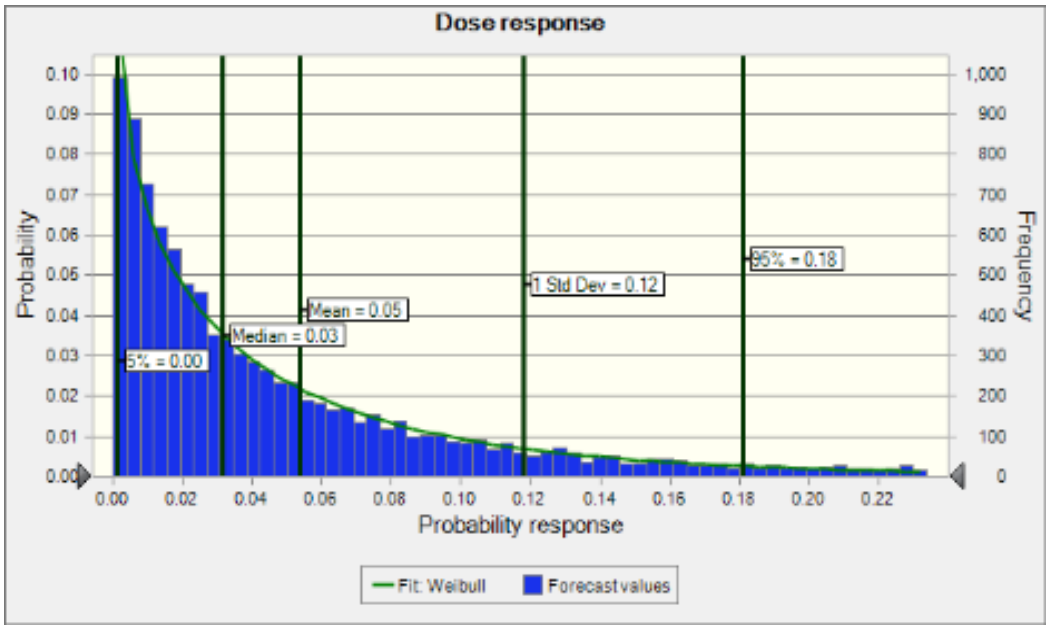
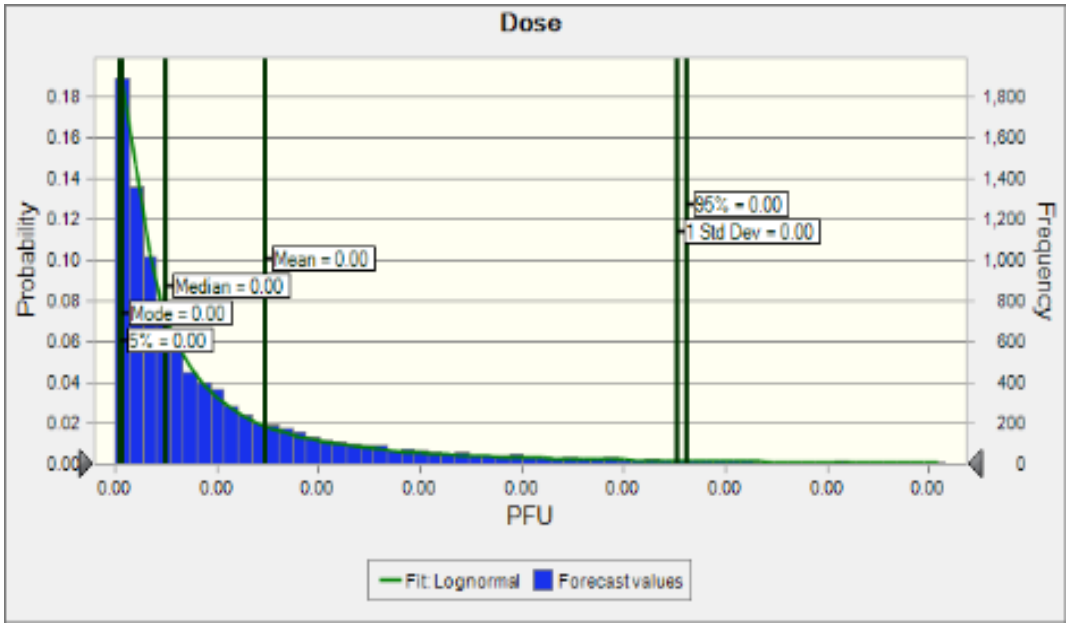


Fig. 6.1 Simulation output forecasting (a) dose and (b) dose response in case of scenario 1 where raw sludge was applied to the lettuce field.

(a)



(b)

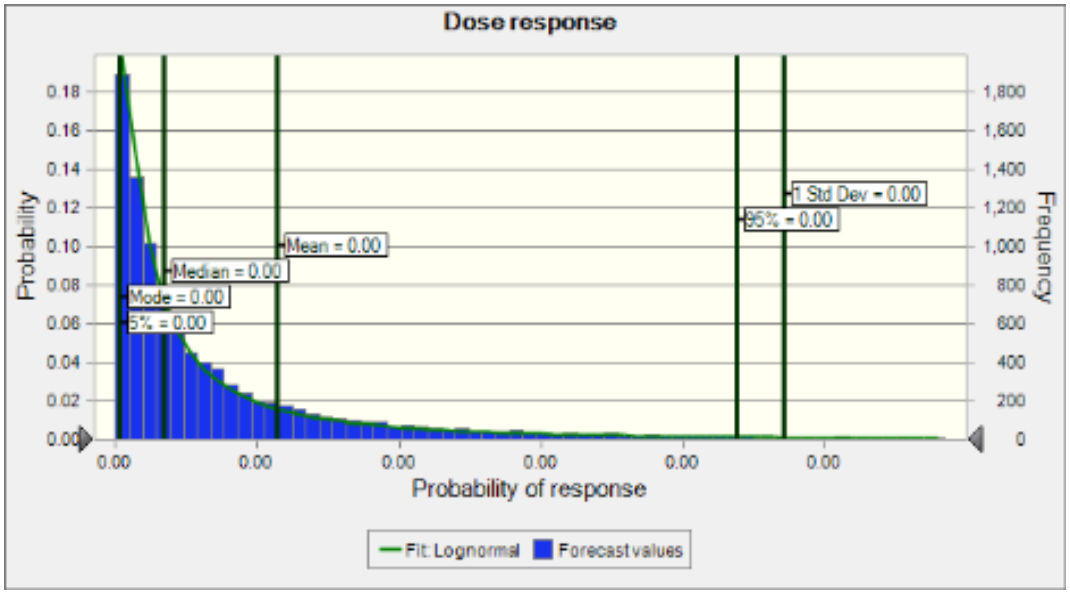


Fig. 6.2 Simulation output forecasting (a) dose and (b) dose response in case of scenario 2, where e-beam treated sludge was applied to the lettuce field.

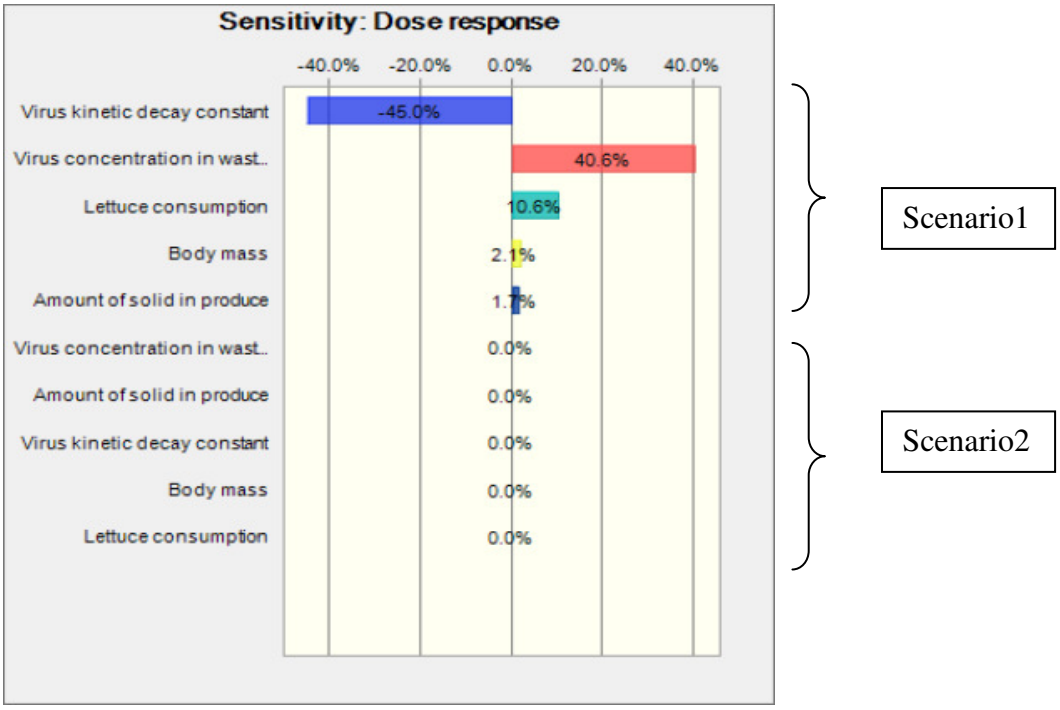


Fig. 6.3 Sensitivity analysis chart specific for the forecast of dose response in scenario1 and scenario 2.

DISCUSSION

On comparing the statistics obtained for the simulations for scenario1 and scenario2, it is evident that there is a marked reduction in the daily dose of enteric virus being ingested and the response to the ingested dose in scenario 2. When raw sludge was applied to the lettuce field, it was found that on an average an individual would ingest 0.14 PFU of enteric virus upon lettuce consumption. This ingestion dose becomes negligible (near zero), when e-beam treated sludge was applied to the lettuce field The probability of developing a response upon encounter with a mean enteric viral ingestion dose of 0.14 PFU was found to be 0.05. Upon treatment the probability of dose response also lowered as the ingested dose was negligible.

Dose –response – Scenario 1- Raw sludge application

Mean = 0.05

Probability of infection = 0.05 per day

Annual probability of infection= 0.05 x 365 = 18.25

Annual probability of illnesses allowed as per US EPA = 1 in 10000 = 0.0001

The annual probability of infection from raw sludge application is 18.25 infections per year which is far greater than the limit of 0.0001 prescribed by US EPA (Haas *et al*. 1999). This projects high risk of enteric virus infection due to consumption of lettuce obtained from field where raw sludge was applied.

Dose –response – Scenario 2- E-beam treated sludge application

Mean = 0.00

Probability of infection = 0.00 per day

Annual probability of infection= 0.00 x 365 = 0.00

Annual probability of illnesses allowed as per US EPA = 1 in 10000 = 0.0001

When e-beam treated sludge was applied to the lettuce field, annual probability of infection reduced to 0.00, which is acceptable as per US EPA regulations. Thus by employing efficient sewage sludge treatment measures such as e-beam irradiation, risk of enteric virus infection could be reduced to a great extent.

The sensitivity analysis chart (Fig. 6.3) gives an account of the influence of the individual model parameters that influence the dose response results. It could be inferred from the first scenario that virus concentration in the waste water and the virus decay constant in the soil play a key role in deciding the dose response distribution pattern. Virus decay shows a negative sensitivity or correlation, which shows that as the environmental decay of the virus increases the probability of viral ingestion as well as development of response or outbreak decreases. The virus concentration in the soil due to application of sewage sludge follows an entirely opposite trend, showing a positive correlation with the ingested enteric virus and dose response. When treated sludge is applied to the field, there is only very low number of viruses present in the soil; this concentration is negligible as inferred from the sensitivity chart showing almost 0%

sensitive to virus concentration in soil and virus decay. Hence, from the Monte Carlo simulations, it can be concluded that by employing appropriate intervention strategies significant reduction in the pathogen concentration and the resultant response is possible. In this chapter, a worst case scenario was considered in terms of application of raw sludge, lettuce harvest after 14 days of sludge application, and minimal processing of lettuce before consumption. These factors augment the risk of enteric virus infection considerably. From the sensitivity analysis the major contributing factor was found to be viral decay in soil, which in turn was dependent upon virus concentration in the sludge. By providing appropriate treatment, the main cause of the risk (virus concentration in soil due to application of raw sludge) was removed. Thus, it is possible to assess the suitability of the treated biosolids by carefully analyzing the quantitative microbial risk involved using appropriate dose response models.

CHAPTER VII

DISCUSSION

Effective disinfection and stabilization of sewage sludge before disposal or for land application is highly necessary. It is estimated that out of $6.9x10^9$ kg of the dry waste water solids generated annually in the United States, $1.7x10^8$-$2.76x10^9$ kg (dry weight) is used for land filling (Haas *et al*. 1996; Sobsey *et al*. 1989; US EPA 1988). Another practice of disposing treated biosolids is to utilize it for land farming where in which the soil will be benefitted due to the nutrient content and the moisture percentage in the sewage solids (Maier *et al*. 2000). There is increased concern among the nations to implement appropriate treatment strategies to prevent any kind of health and environmental issues related to sewage sludge application. US EPA has established stringent regulations which allow land application of biosolids that satisfy the criteria for or class A or class B sludge only (US EPA 2003). The applicability of several stressors such as irradiation, chemical oxidant treatment, thermal treatment, composting, digestion, lime stabilization etc for significantly reducing the pathogen concentration and vector attraction was reviewed by EPA (US EPA 2003). With this context our studies were directed towards evaluating the extent of microbial inactivation brought about by key stressors such as electron beam irradiation, ferrate and chlorine dioxide. Individual effects of each of these methods as well as their combination effects were determined for inactivating bacteria, viruses, spores and other indicator organisms in both aerobically and anaerobically treated sewage sludge.

E-beam irradiation of sewage sludge showed that the radiation sensitivity is different for different microorganisms. Bacteria such as *E. coli* and *Salmonella* Typhimurium were more susceptible than enteric virus, bacteriophages and spores. Among all the organisms analyzed, aerobic and anaerobic spore were found to be most resistant. Both aerobic and anaerobic spores exhibited a relatively high D-10 value ranging between 3-5 kGy, where as D-10 value of bacteria was in between 0.2-0.3 kGy. Such a huge difference in the D-10 value indicates the resistance offered by spores towards irradiation treatment. Bacterial spores are very small in size, 0.8-1.2µm in length. These spores are coated with laminated proteinaceous coating called spore coat which is resistant to organic solvents and lysozymes. The single bacterial chromosome present inside the spore is condensed within the center called spore core (Ricca and Cutting 2003).This spore core is further surrounded by lipid membranes and peptidoglycan layers that ensures high protection to the bacterial nucleic acids present inside the spore. When such condensed bacterial spores are subjected to e-beam irradiation, the chances of escape of nucleic acids from being hit by the colliding electrons are high. Protection by the spore coat could be another reason for the requirement of high dose of e-beam to ensure penetration into the nucleic acids located inside spore core.

D-10 value of the male specific coliphage (2.3-2.5 kGy) was significantly less than that of the somatic coliphage (4.12-4.17 kGy) implying the use of somatic coliphage as an ideal indicator organism. The estimated D-10 value of enteric was 2.69 kGy, which was quite close to that of male specific coliphage. Radiation sensitivity of

organisms also differs with the matrix of the sludge which is subjected to irradiation. The D-10 values of the viruses and spores present in sludge samples were markedly different from the D-10 value obtained from previous studies where the organisms were suspended in river water or effluent (Gehringer *et al*. 2003). This difference is due to the presence of suspended particle, which to a greater level protects the microorganisms from inactivation. When sludge samples are subjected to irradiation, the suspended particles in the sludge undergoes disintegration. This predisposes the suspended microbial cells to cell rupture and release of cytoplasm (Yuan *et al*. 2008). This served as justification for combining e-beam with chemical oxidants.

The effect of combining e-beam with ferrate and chlorine dioxide resulted in very significant reduction in the microbial population. Different doses of chlorine dioxide and ferrate were used for the microbial inactivation. Fifty, 75, 100 ppm of ferrate was complemented with 8 kGy of e-beam irradiation. Ferrate + e-beam treatment resulted in complete reduction (below detectable limits) of *E. coli*, *Salmonella* and poliovirus population. Hence, when chlorine dioxide + ferrate were tested, *E. coli*, *Salmonella* and poliovirus was grouped as susceptible and were provided low doses- 10, 20 and 30 ppm of ClO_2 and 2 kGy of e-beam dose. Aerobic spores, anaerobic spores, somatic and male specific coliphage comprised the resistant group, which was given relatively high dose – 25, 50 and 75 ppm of ClO_2 and 8 kGy of e-beam irradiation.

While comparing the results obtained from both ferrate and ClO_2 treatments, it can be inferred that, ClO_2 + e-beam treatment is more effective in case of bacterial

inactivation. *E. coli* was found more susceptible to ClO_2 + e-beam treatment as there was approximately 7 log reduction with low dose of 20 ppm and 2 kGy of e-beam in aerobically treated sludge. Anaerobically treated sludge also supported this observation, but was found to be slightly resistant than aerobic sludge as it required 30 ppm ClO_2 and 2 kGy electron beam to reduce the *E. coli* population by 6 log.

Addition of e-beam augmented the inactivation caused by chemical oxidants for all the target organisms studied. This was well evident in case of bacteria, where 8 log reduction was obtained by combining two treatments. Somatic coliphage was resistant compared to male specific coliphage and poliovirus when treated with ferrate and e-beam, but the trend seemed to reverse with addition of chlorine dioxide. A 3-7 log reduction in the phi X 174 population was observed with chlorine dioxide treatment indicating the susceptibility of somatic phages to chlorine dioxide. Poliovirus gave almost 6 log reduction with the ferrate by itself, and was considered susceptible to the oxidant treatment. But upon treatment with 30 ppm ClO_2 and 2 kGy e-beam dose, poliovirus showed only 1 log reduction.

The difference in the microbial inactivation is due to the difference in the response of microorganisms to the stressors. Susceptibility of a particular target organism appears to differ depending upon the matrix. Table 7.1 summarizes the susceptibility of different target organisms present in aerobically and anaerobically digested sludge to e-beam + chemical oxidant combination treatment.

Table 7.1 Susceptibility of different target organisms in aerobically and anaerobically treated sludge samples to e-beam + ferrate and e-beam + ClO_2 combination treatments.

Target organism	**Ferrate + e-beam susceptible**		**ClO_2 + e-beam susceptible**	
	Aerobically treated sludge	**Anaerobically treated sludge**	**Aerobically treated sludge**	**Anaerobically treated sludge**
Salmonella Typhimurium	Yes	Yes	Yes	Yes
E. coli	Yes	Yes	Yes	Yes
Bacillus subtilis spores	No	No	No	No
Clostridium perfringens spores	No	No	No	No
Somatic coliphage	Yes	No	Yes	Yes
Male specific coliphage	Yes	Yes	Yes	No
Poliovirus	Yes	Yes	No	No

"Yes"- Susceptible to the treatment; "No"- Resistant to the treatment

It is clear from Table 7.1 that response of somatic and male specific coliphages vary to a great extend depending upon the matrix of the sludge. Somatic coliphage present in aerobically treated sludge sample was susceptible to both ferrate+ e-beam and ClO_2 + e-beam treatments, whereas the same organism was resistant to ferrate + e-beam treatment in anaerobically treated sludge. Male specific coliphage present in the anaerobically digested sludge sample was found resistant to ClO_2 + e-beam treatment. Poliovirus showed marked difference with respect to its susceptibility to different stressor. Irrespective of the matrix, poliovirus was susceptible to ferrate + e-beam treatment, where as it was found resistant to the ClO_2 + e-beam treatment.

The information from Table 7.1 gives comparative susceptibility of different target microorganisms to various oxidant + e-beam treatments. Based on this indicator microorganisms could be chosen for a specific treatment on a specific sludge matrix. Most resistant as well as susceptible organisms provide an approximate range of the effect of treatment.

Based on the results obtained from the study, a quantitative microbial risk assessment was carried out to analyze the effect of sewage sludge treatment in reducing the probability of causing food borne outbreaks. Risk of enteric virus infection arising from the consumption of lettuce grown in fields which were amended with raw Vs treated sewage sludge was determined. Upon application of treated sewage sludge, the annual risk of infection reduced from 18.25 to 0.00. This translates to the efficiency of the sewage sludge treatment method employed. Thus, by employing appropriate sewage sludge treatment methods suitable for different target microorganisms and type of matrix, effective microbial inactivation could be achieved. Municipal biosolids treated using appropriate methods could then be used for land application or for agricultural use with much reduced risk of microbial infection.

CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Electron beam irradiation inactivates microbial populations in aerobically and anaerobically digested sewage sludge. Bacteria are the most susceptible while bacterial endospores are most resistant. The susceptibility of viruses (coliphages and poliovirus) lies in between that of bacteria and endospores. The D10 value ranges for the target microorganisms (Table 8.1) are as follows:

Table 8.1 Estimated D-10 values of microorganisms present in aerobically and anaerobically treated sludge samples.

Target Organisms	Aerobically treated sludge (kGy)	Anaerobically treated sludge (kGy)
Salmonella Typhimurium	0.28	0.23
Escherichia coli	0.31	0.25
Aerobic spore formers	3.74	4.02
Anaerobic spore formers	5.13	3.12
Somatic coliphages	4.12	4.17
Male speific coliphages	2.31	2.51
Poliovirus	-	2.69

2. When e-beam was combined with the chemical oxidant ferrate (VI), there was increased inactivation of the target microorganisms. *E. coli*, *S. typhimurium* and poliovirus showed reduction with an increased concentration of ferrate itself. When combined with e-beam, both bacteria and enteric viruses showed a 6 to 8 log reduction as compared to that of the microbial reductions obtained from the treatment of oxidants alone. Aerobic and anaerobic spores were still resistant to the combination of e-beam and ferrate with the combination treatment resulting in only 1 to 2 log reduction. The synergism in microbial inactivation resulting from combining e-beam with ferrrate (VI) (Table 8.2) is shown below.

Table 8.2 Log reductions in pathogen concentration by ferrate & e-beam treatment in (a) aerobically treated and (b) anaerobic treated sludge.

Target Organism	Aerobic sludge – ferrate (50-200 ppm)	Aerobic sludge – Ferrate + e-beam(50 ppm+2 kGy – 200 ppm+2 kGy)
Salmonella	**0-3 log**	**8 log**
E. coli	**0-4 log**	**8 log**
Aerobic spores	0-1 log	1-2 log
Anaerobic spores	0-1 log	1-3 log
Somatic coliphage	1-2 log	3-4 log
Male specific coliphage	**1-7 log**	**3-7 log**
Poliovirus	**1-6 log**	**6 log**

(a)

Target Organism	Aerobic sludge – ferrate (50-200 ppm)	Aerobic sludge – Ferrate + e-beam(50 ppm+2 kGy – 200 ppm+2 kGy)
Salmonella	**0-3 log**	**8 log**
E. coli	**0-4 log**	**8 log**
Aerobic spores	0-1 log	1-3 log
Anaerobic spores	0-1 log	1 log
Somatic coliphage	1 log	2-3 log
Male specific coliphage	**1-2 log**	**5-6 log**
Poliovirus	**1-6 log**	**6 log**

(b)

3. Combination of e-beam and chlorine dioxide also resulted in significant reduction in the populations of the target bacteria, viruses and spores. The effectiveness of the combination treatment of e-beam and chlorine dioxide is shown in Table 8.3 and Table 8.4. However, significant reduction in somatic coliphage population was obtained with ClO2 + e-beam treatment compared to that of ferrate + e-beam. Response of poliovirus to ClO2 + e-beam treatment was different from that of ferrate + e-beam treatment.

Table 8.3 Log reductions in pathogen concentration by ClO_2 & e-beam treatment for susceptible group in (a) aerobically and (b) anaerobically treated sludge.

Target Organism	Aerobic sludge – ClO2 (10-30ppm)	Aerobic sludge – ClO2+ e-beam(10 ppm+2 kGy – 30 ppm+2 kGy)
Salmonella	**0-1 log**	**3 log**
E. coli	**0 log**	**5-8 log**
Poliovirus	**1 log**	**1-1.5 log**

(a)

Target Organism	Anaerobic sludge – ClO2 (10-30ppm)	Anaerobic sludge – ClO2+ e-beam(10 ppm+2 kGy – 30 ppm+2 kGy)
Salmonella	1-2 log	3-5 log
E. coli	0-1 log	4-6 log
Poliovirus	0 log	1 log

(b)

Table 8.4 Log reductions in pathogen concentration by ClO_2 & e-beam treatment for resistant group in (a) aerobically and (b) anaerobically treated sludge.

Target Organism	Aerobic sludge – ClO2 (25-75 ppm)	Aerobic sludge – ClO2+ e-beam (25 ppm+8 kGy – 75 ppm+8 kGy)
Aerobic spores	0-1 log	2 log
Anaerobic spores	0-1 log	1 log
Somatic coliphage	**1-7 log**	**3-7 log**
Male specific coliphage	**3-7 log**	**7 log**

(a)

Target Organism	Anaerobic sludge - ClO2 (25-75ppm)	Anaerobic sludge – ClO2 + e-beam (25 ppm+8 kGy – 75 ppm+8 kGy)
Aerobic spores	0-1 log	2 log
Anaerobic spores	0-0.5 log	0-1 log
Somatic coliphage	**1-2 log**	**2-5 log**
Male specific coliphage	**0-0.5 log**	**1 log**

(b)

4. Quantitative Microbial Risk Assessment (QMRA) exercise was performed to determine the reduction in health risk that could be achieved with the use of e-beam treatment of sewage sludge. The application of untreated and e-beam treated sewage sludge on lettuce fields and the potential ingestion of enteric viruses were used in the hypothetical scenario. Annual risk of infection reduced from 18.25 to 0.00, when treated sludge was applied to fields in place of raw sludge.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. Changes in the basic biology of target microorganisms upon treatment with e-beam and chemical oxidants could be an interesting field of study.
2. Effectiveness of mixed oxidant treatment could be studied for the target microorganisms.
3. The economic viability of employing various sewage treatment techniques on a commercial basis could be evaluated.
4. Combination of e-beam with other chemical and physical stressors for achieving microbial inactivation could be studied.

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VITA

Name:	Chandni Vijayakumaran Nair Sobha
Address:	Room 411 Kleberg Center 2472 TAMU College Station, TX-77843-2472
Email Address:	chandni.vs@gmail.com
Education:	B.Sc (Ag), Kerala Agriculture University, Thrissur, December 2005
	M.Sc(Crop Physiology), University of Agricultural Sciences, Bangalore, June 2008
	M.S., Poultry Science, Texas A&M University, 2009