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Elimination of Potential Pathogenic Microorganisms in Sewage Sludge Using Electron Beam Irradiation

Jean Engohang-Ndong and Roberto M. Uribe

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http://dx.doi.org/10.5772/62705

Abstract

Microbiological analyses on municipal sewage sludge sample treated in a pilot plant process utilizing an electron accelerator with a beam energy of 3 MeV were conducted as a way to show the potential of this technology to decontaminate sludge containing 15% solids. Bacterial counts including total heterotrophic bacterial, total coliform, and fecal coliform counts were performed on sewage sludge samples pre- and postirradiation with the electron beam at doses ranging between 2.7 and 30.7 kGy. At each irradiation dose, bacterial and *Ascaris* ova counts and survival were measured in triplicate as colony forming units (CFUs) per milliliter (ml) of sewage sludge. Experimental results obtained revealed that a dose of 6.7 kGy is enough to reduce bacterial load to consider the treated sewage sludge safe for both the environment and human according to the Environmental Protection Agency standards. However, a dose of 25.7 kGy was needed to reduce the concentration of *Ascaris* ova at levels deemed safe for land applications. This study also showed that electron beam treatment is less energy consuming with shorter processing times than conventional techniques used to decontaminate sludge. Taken altogether, these observations open new avenues for large urban agglomerations to save money on sewage sludge treatment.

Keywords: Electron beam, Irradiation, Microorganisms, Sewage sludge, Decontamination

1. Introduction

Radiation processing has been used in biotechnological applications for more than 50 years. The effect of radiation on pathogenic microorganisms was first initiated in 1956 by Ethicon Inc. (a...
subsidiary of Johnson and Johnson) together with High Voltage Engineering Corp. (a manufacturer of accelerators) in order to sterilize single-use medical devices such as gloves, hypodermic needles, sutures, surgical drapes. Nowadays, it is worldwide used, not only for medical devices, but also for cosmetics. The exact mechanism by which radiation kills microorganisms is not very well understood, but it is certainly related to the damage caused by the radiation to the DNA molecule of the microorganisms. Also it is generally accepted that the smaller the microorganism the larger the dose of radiation needed to kill it. So, the radiation dose needed to kill bacteria will be larger than the dose needed to kill human cells, and it will be smaller than the dose needed to kill a virus.

Sterilization is not the only area in which radiation can be used in biotechnological applications. Radiation is being used to develop new implant materials which are biocompatible. An example of this is the irradiation of water-soluble polymers in aqueous solutions, with or without the addition of another monomer which gives rise to a variety of cross-linked gels which can be used in the biomedical field. Some of these hydrogels can be used to hydrate the skin of patients with severe burns.

Radiation is also used in the area of food preservation. Depending on the dose used on a food commodity, the radiation can either sterilize (e.g., in meat products), kill bacteria including *Salmonella* and *Streptococcus* species, disinfest (e.g., in fruits and grains), kill insects in adult, larvae, or pupae stages, or delay maturation (e.g., in some fruits and vegetables) by decomposing the enzymes responsible for ripening.

2. Application of electron beam technology

The commercial use of irradiation to disinfect sludge started in 1973 when an industrial gamma ray facility from Geiselbullach near Munich (Germany) used Co-60 and Cs-137 sources [1]. The facility used 90,000 Ci of Co-60 and 570,000 Ci of Cs-137 and treated up to 180 m³/day of sludge. More recently, a new technology using electron beam accelerators was developed in Miami, Florida where a 1.5 MeV 50 mA accelerator with a throughput of 645 m³/day, and in Brazil where a 1.5 MeV 37.5 kW accelerator, with a maximum throughput of 45 l/min were described [2]. However, these two studies only addressed the engineering aspects of the facilities and the possibilities to use electron beam irradiation for environmental applications. The radiation effects on the bacterial load and removal of noxious chemical compounds have been performed mostly in small samples of sludge irradiated under laboratory conditions and mainly address either only the microbiological or the chemical effect of radiation in a sample of sludge [3–5]. Processing and disposal of wastewater sludge are a critical problem worldwide [5]; therefore, new technologies to solve this problem are constantly being sought.

Sludge is commonly used as a soil amendment and fertilizer but must be treated in order to remove various bacteria, toxic compounds, parasites, and viruses. Many researchers have shown that exposing sludge to high-energy radiation successfully removes all the bacteria and other organisms from the sludge. Thus, the right dose of radiation will ensure proper sludge
disinfection. It has been shown that even a small dose of radiation will remove 99.9% of all bacteria in sludge [6]. In addition to disinfection, irradiation of sludge often accelerates sedimentation and filtration, which helps facilitate removal of water from the sludge. Even while changing the physical makeup of sludge, this does not affect the ability of using sludge as a good fertilizer.

The generation of offensive odors from sewage sludge is also a concern in the subsequent disposal and/or use of sludge. Volatile sulfur-containing compounds (carbon disulfide \( \text{CS}_2 \), dimethylsulfide \( [\text{CH}_3]_2\text{S} \), dimethyldisulfide \( [\text{CH}_3\text{S}]_2 \) and volatile carboxylic acids (acetic acid, propanoic acid, butanoic acid) have been identified as odor causing compounds in sewage sludge [7].

3. Sludge irradiation dosimetry

3.1. Dose mapping: experimental procedure

Prior to the determination of the dose using different accelerator parameters, the scanned beam on top of the sludge delivery system needed to be mapped in order to verify that all the water coming through the weir length of the sludge delivery system would be exposed to the electron beam. The delivery system consists of a stainless steel box 152 cm (59.8 in) long with two compartments, one for the incoming sludge and the other one to drain the irradiated sludge. The sludge is transferred from one compartment to the other through a weir located in the center of the box. Irradiation takes place at the top of the weir (Figure 1).

To map the extent of the irradiation zone along the weir, a CTA film was taped to the top of the delivery system just underneath the scanner system of the accelerator and irradiated for 5 s using the following accelerator conditions: \( E = 3 \text{ MeV}, \ I = 15 \text{ mA}, \) and \( S = 100\% \). After irradiation, the optical absorption at 280 nm was measured along the film using a Genesys 5
spectrophotometer fitted with a driving mechanism to measure film strips. Aerial™ software determined the dose from the absorbance measurements.

3.2. Dose mapping: results and discussion

Figure 2 shows a graph of the dose along the top surface of the weir to treat the sludge. The graph shows two features, the extent of the irradiation zone on top of the weir system and the dose uniformity along the weir. The graph shows an effective irradiation length of 127.5 cm which is shorter than the length of the weir itself (152 cm). In order to ensure that all sludge falling over the weir was irradiated, two pieces of aluminum tabs 10 cm long were fastened to each edge of the weir using C-clamps. These tabs shortened the effective weir by a total of 20 cm and allowed for all the sludge falling over the weir to be irradiated by the electron beam given the fact that the scanning angle of the electron beam is 18.5°. The graph also gives information about the uniformity of the electron beam on top of the weir and shows that the dose at the two ends of the weir is about 25% lower than the dose in the idle section of the weir. However, this measurement was taken under static conditions, and in the case of water or sludge, the liquid will not move on a laminar flow fashion and might have been receiving an average dose with variations of up to ± 12.5% assuming that part of the liquid moved on the middle section and another part on the extreme end of the weir. So, then it is reasonable to assume that with the movement of the sludge as well as with its mixing, this might be the maximum difference in dose achieved by the sludge.

Figure 2. Dose along the sludge delivery system. The measurements were taken using cellulose triacetate film irradiated with 3 MeV electrons, 15 mA of current, 5 s of exposure time and 100% scanning aperture [7].

3.3. Dose measurements: experimental procedure

The dose delivered to the sludge was determined from temperature measurements made on the sludge before and after irradiation, after calibration in terms of dose with alanine pellets
and films. The irradiation of alanine dosimeters produces free radicals that become trapped inside the solid matrix of the dosimeter and can be measured by electron spin resonance (ESR) spectrometry. The trapped radicals are stable over long periods of time, and their concentration can be directly related to the absorbed dose as determined from a calibration curve.

For the experiments described in this chapter, a Bruker eScan ESR spectrometer using an insert FL0041 to measure the alanine films and an insert PH0027 to measure the alanine pellets were used to measure the free radicals. In a first experiment, 40 alanine pellets and 40 alanine film strips were randomly selected and irradiated in order to make a calibration curve of the pellets. The pellets would be used to measure the dose in sludge once they were calibrated with the alanine films.

The 40 alanine pellets were divided into ten groups of four and were placed in small plastic bags 1.5 cm long and 0.5 cm wide, and sealed with heat. The bags with the pellets were placed on a piece of cardboard, one at a time, on top of one of the carts that would be conveyed through the electron beam. On the side of the individual bags, four alanine film strips were placed, to measure the dose. The cardboard was irradiated in the cart conveyor system of the NEO Beam facility Dynamitron electron accelerator and irradiated using the following beam parameters: 3 MeV electron energy and 100% scanning angle; the dosimeters were moving under the beam at a constant speed of 20.32 cm/s, and the current changed to give different dose values ranging from 2 to 40 kGy, according to Table 1. Once all the dosimeters were irradiated, the alanine films were measured to determine the dose in each run, and with this information and the measurement of the intensity of the ESR signal of the irradiated pellets, a calibration curve was constructed.

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Beam current (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>8.4</td>
</tr>
<tr>
<td>8</td>
<td>13.4</td>
</tr>
<tr>
<td>10</td>
<td>16.8</td>
</tr>
<tr>
<td>13</td>
<td>21.8</td>
</tr>
<tr>
<td>16</td>
<td>26.9</td>
</tr>
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<td>27</td>
<td>45.3</td>
</tr>
<tr>
<td>30</td>
<td>50.4</td>
</tr>
</tbody>
</table>

Table 1. Electron beam current values needed to produce the selected doses for alanine pellets running under the beam at 20.32 cm/s.
A second experiment consisted in irradiating a set of pellets in a pyrex baking dish containing cold tap water at a depth of 1.1 cm (7/16 in). The purpose of this experiment was to simulate the accelerator conditions needed to irradiate the sludge. Four vials per run were used, each containing three alanine pellets. These vials were placed into the baking dish and floated on top of the water. The electron beam parameters were set up such that the electron energy was 3 MeV and five runs were conducted underneath the electron beam. Each run had a constant speed of the samples equal to 23.3 cm/s and the following beam currents: 3.8, 9.6, 19.1, 38.3, and 45.9 mA. After irradiations, the dose from the ESR intensity of each alanine pellet using the Bruker eScan instrument was determined.

The next experiment measured the dose for a sample of water running through the delivery system to irradiate sludge and to relate those measurements to the temperature of the water coming in and going out of the system as measured by a set of thermocouples installed near the sludge delivery system in the influent and effluent pipes. Small sealed plastic bags containing two alanine pellets were introduced into the system through a “Tee” connection into the pipe where the water flowed and sent them through the irradiation zone. At this point, 300 gallons of water was being recirculated through the system at 50 GPM. Two sets of five runs were conducted. Beam conditions for each set were as follows for each run: \( E = 3 \text{ MeV}, \ S = 100\%, \ I = 3.8, 9.6, 19.1, 38.3, \) and \( 46.2 \text{ mA}, \) respectively. After irradiation, the plastic bags with the alanine pellets were collected in a catch basket that would separate the sealed bags from the water. Some of the bags leaked water when they passed through the water pump that removed the irradiated water from the system. The bags that did not show water leaks were used to measure the dose. Dose measurements were then related to the temperature measurements from the thermocouples.

Finally, the dose in the sludge was determined from the temperature measurements with the thermocouples, after correcting for the dose measured by the alanine pellets.

3.4. Dose measurements: results and discussion

As mentioned earlier, the dose absorbed by the sludge was determined from temperature measurements in the sludge after a calibration with alanine pellets was performed. Figure 3 shows the result of the dose calibration of the pellets when irradiated with alanine films in the cart conveyor system of the NEO Beam facility.

As stated in the experimental section, the pellets were calibrated using alanine films calibrated at Risø National Laboratory and then used to calibrate the in-house Bruker eScan spectrometer that measured the doses. After this, the calibrated pellets were used to determine the dose in the experimental setup to irradiate the sludge with the electron accelerator using different beam currents. Thus, the graph in Figure 4 shows the dose recorded by the pellets run through the irradiation dispositive using water at different beam currents of the electron accelerator.
Figure 3. Calibration curve for the alanine pellets used to measure dose in this experiment. The dose was measured by alanine films and the response of the pellets as the ratio of the ESR intensity of the alanine to the internal marker of the pellet holder. The eScan instrument performed a trendline analysis on the experimental data obtaining a 4th polynomial as the best fit to the experimental data with a standard error of 0.0076 and an $R^2 = 0.9989$.

Figure 4. Doses of electron beam irradiation in water. Doses were measured by alanine pellets as a function of the electron beam current of the accelerator. Water was running in the system at a rate of 50 gpm, and the electron energy was 3.0 MeV [7].

At the same time, the increase in temperature of the water running through the sludge delivery system at constant flow rate of 50 gpm and different beam currents was recorded and compared with the dose given by the alanine pellets. This relationship was later used to determine the dose absorbed by the sludge when irradiated with the electron beam.

The flow rate during the irradiation of the sludge sample was 30 gpm instead of the 50 gpm originally selected for this experiment. In order to keep the doses within the interval selected for this experiment, it was decided to run the experiment at a reduced level of electron beam.
currents to compensate for this effect. The dose was determined then from temperature increase of the sludge by measuring the temperatures at the input and exit ports of the irradiation setup. Table 2 presents results of the temperature increments and dose measurements as a function of the beam currents for the sludge sample running through the delivery system.

<table>
<thead>
<tr>
<th>Beam current (mA)</th>
<th>Temperature increase (°C)</th>
<th>Dose (kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3</td>
<td>0.6</td>
<td>2.7</td>
</tr>
<tr>
<td>5.8</td>
<td>1.6</td>
<td>6.7</td>
</tr>
<tr>
<td>11.5</td>
<td>3.1</td>
<td>13.2</td>
</tr>
<tr>
<td>23.0</td>
<td>6.1</td>
<td>25.7</td>
</tr>
<tr>
<td>27.6</td>
<td>7.3</td>
<td>30.7</td>
</tr>
</tbody>
</table>

Table 2. Irradiation conditions used to achieve targeted doses. Sludge samples were flowing at a rate of 30 gpm [7].

4. Elimination of potential pathogenic bacteria in municipal sludge

4.1. Sampling: experimental procedure

Sample collection, transport, and storage are crucial when studying the effect of electron beam irradiation on microbial population found in municipal sewage sludge. In these experiments, sewage sludge samples were collected in two separate batches. First batch contains pretreated municipal sewage sludge or influent samples, and a second batch is made of municipal sewage sludge treated with electron beam irradiation or effluent samples. In the case reported here, since the sludge was treated with different doses of electron beam irradiation, samples were collected prior to (influent) and after (effluent) irradiation of sludge at each dose. Several 100 ml influent and effluent samples of sewage sludge were harvested in sterile-capped plastic vials for bacterial count and survival. Each sample was then placed on ice immediately after collection and transported in an isotherm ice container (a cooler) from the electron beam irradiation facility to the microbiology laboratory for microbial analysis. For accurate observation of the direct effect of electron beam irradiation on bacterial population, samples should be analyzed as soon as possible after treatment.

4.2. Sample analysis using membrane filtration method: experimental procedure

Each sample was thoroughly mixed, and serial dilutions were performed in 1× phosphate-buffered saline. Influent samples were diluted up to $10^8$, while effluent samples were diluted up to $10^6$. Diluted samples were filtered using disposable filter funnels. For filtration, 10 ml of the diluted sample was transferred with a sterile pipette into the middle of a sterile 45 mm (diameter) and 0.45 μm (pore size) gridded membrane filter. After filtration, the filter was washed with three volumes of 1× phosphate-buffered saline. The filter was then removed and transferred on a 50-mm (diameter)-padded Petri dish plate containing 2 ml of culture medium
for total heterotrophic bacterial (THB), total coliform (TC), and fecal coliform (FC) counts. In order to perform THB counts, mHPC Heterotrophic medium was used and for TC counts, mEndo medium was used, while m-FC medium supplemented with Rosolic acid was used for FC counts. Plates were placed in plastic bags containing moistened paper towels and transferred in an incubator. Heterotrophic plates were placed in an incubator for $35 \pm 0.5^\circ C$ and TC plates were incubated for $22 - 14$ h at $35 \pm 0.5^\circ C$, while FC were incubated at $44.5 \pm 0.2^\circ C$. Known positive and negative controls were used in order to verify accuracy of analytical procedures for identification and counts of heterotrophic, TCs, and FCs. Thus, for TC media, Escherichia coli and Enterobacter aerogenes were used as positive controls, while Staphylococcus aureus and Pseudomonas aeruginosa were used as negative controls. The positive control for the FC media was E. coli, and the negative control was E. aerogenes. Prior to testing, test organisms were grown in tryptone soy broth and incubated overnight at $37^\circ C$. After growth, these cultures were treated according to the procedure used for sewage sludge samples. Only dilution plates with a density of 20-100 colonies were counted. FC colonies appeared as different shades of blue, while other non-FC colonies appeared as gray or cream-colored. Bacterial counts were performed in triplicate to verify the reproducibility of results for total heterotrophic counts, TC counts, and FC counts.

4.3. Results and discussion

Bacterial counts before and after irradiation were performed with the electron beam at doses 2.7, 6.7, 13.2, 25.7, and 30.7 kGy. The counts were done specifically for THB, TC, and FC. Figure 5 shows the effect of electron beam irradiation on bacterial survival in municipal sewage sludge after treatment. It appears that THB, TC, and FC counts decreased in a dose-dependent manner. This decrease in bacterial population is directly associated with the ionizing effect of electron beam irradiation that damages bacterial DNA and biomembranes, and the production of reactive oxygen species which also damage cell components. A similar observation was recently made by Cao and Wang [8] when they treated municipal sludge with electron beam irradiation. However, these authors did not count specific types of bacteria.

Figure 5. Effect of electron beam irradiation on bacterial survival in municipal sewage sludge samples. (a) Survival of total heterotrophic bacteria, (b) survival of total coliforms, and (c) survival of fecal coliforms [7].

Looking more into details, it was shown that when irradiating sludge with electron beam, a dose of 2.7 kGy, 93.3 $\pm$ 8.5% THB survived the treatment, while only 21.1 $\pm$ 11.4% of TC and 67.2 $\pm$ 1.8% of FC survived at the same irradiation dose. At a dose of 6.7 kGy, while 31 $\pm$ 15%
of THB survived the treatment, only 0.85 ± 0.23% and 1.85 ± 0.65% of the initial populations of TC and FC survived, respectively. At doses of 13.2 kGy and above, neither TC bacteria nor FC were detected. Nevertheless, at a 13.2 kGy irradiation dose, 8.9 ± 1.3% of THB from the initial population survived the treatment. At a dose of 25.7 kGy and above, no significant THB from the initial population were left in treated sewage sludge samples [7]. Table 3 summarizes bacterial counts per gram of sludge dry weight at different electron beam doses. From these results, D_{90}-values were determined as 8.94, 3.16, and 3.17 kGy for THB, TC, and FC respectively. D_{90}-values are defined as doses necessary to kill 90% of the bacterial populations in the sample for irradiation conditions applied, or the dose needed to reduce the bacterial population by a factor of 10. A close look at Table 3 shows that dose 6.7 kGy reduces the FC counts to 180 colony forming unit (CFU) per gram of sludge dry weight, a count that is within the Environmental Protection Agency (EPA) norm to classify such treated municipal sewage sludge as class A sludge utilizable for land application in agriculture [9]. However, from the D_{90}-value determined for FC, based on initial population of FC in influent samples, the dose required to convert this sludge to class A was estimated to be 4.5 kGy. Although no previous work similar to this one is known to perform a comparison with our estimated D_{90}-value, nevertheless, water-based and surface membrane Bacillus spore killing D_{90}-values were reported to be 6 and 6.9 kGy, respectively [8, 9]. These values are about twice lower than the 3.17 kGy determined in our case. This difference could be attributed to the presence of a large amount of organic and inorganic materials that make our sample relatively thick and slightly viscous compared to water and a surface membrane.

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Sludge dry weight (gram) percent</th>
<th>Total heterotrophic bacteria Counts (CFU) per gram of dry weight</th>
<th>Total coliforms</th>
<th>Fecal coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>15.00%</td>
<td>1.4 x 10^6</td>
<td>1.7 x 10^6</td>
<td>2.0 x 10^6</td>
</tr>
<tr>
<td>2.7</td>
<td>25.75%</td>
<td>8.6 x 10^5</td>
<td>8.2 x 10^4</td>
<td>1.5 x 10^4</td>
</tr>
<tr>
<td>6.7</td>
<td>20.46%</td>
<td>3.2 x 10^5</td>
<td>9.3 x 10^4</td>
<td>1.8 x 10^4</td>
</tr>
<tr>
<td>13.2</td>
<td>12.29%</td>
<td>4.5 x 10^4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>25.7</td>
<td>3.67%</td>
<td>1.3 x 10^4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30.7</td>
<td>3.25%</td>
<td>6.1 x 10^3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 3. Bacterial counts in sludge samples at different irradiation doses [7].

5. Elimination of *Ascaris* ova in municipal sludge

5.1. Sampling: experimental procedure

Compared to bacterial analyses of influent and effluent sewage sludge samples, *Ascaris* ova analyses required much larger volumes of biosolids (sewage sludge). Thus, several 1 l samples
of sludge were collected in sterile glass bottles. Some samples were obtained as influent samples, while others were obtained as effluent samples, transferred on ice immediately after collection, and transported to the microbiology laboratory for prompt *Ascaris* ova counts.

### 5.2. Sample analysis: experimental procedure

From each 1 l sample, 500 ml of well-mixed sludge was transferred in a blender, then 200 ml of sterile water was added, and the mixture was blended for 1 min at high speed. The blended mixture was transferred to a 1-l tall beaker to which 1% 7x detergent was added in order to reach 900 ml final volume. The same procedure was repeated for the second half the sludge sample, and the homogenized mixtures were combined and allowed to settle overnight in a cold (4°C) room or in a refrigerator. At this stage, some floating materials may be observed; therefore, stirring occasionally the mixture with a wooden applicator has shown to help settle the material. The supernatant was discarded by vacuum aspirating it to right above the layer of biosolids. The settled sediments were then transferred into a blender to which 500 ml of sterile water, blended again for 1 min at high speed, and transferred to a beaker. The blender was rinsed, and 1% 7x detergent was added to reach 900 ml final volume. Samples were allowed to settle for 2 h at 4°C after which the supernant was discarded by vacuum aspirating it to right above the layer of biosolids. The biosolids were resuspended into 300 ml of 1% 7x detergent and stirred for 5 min using a magnetic stirrer. Homogenized sample was then strained through a 50 mesh (300 μm) sieve placed in a funnel over a beaker. Samples were washed through the sieve with a spray of 1% 7x detergent from a spray bottle. The sample volume in the beaker was adjusted to 900 ml by adding the necessary amount of 1% 7x detergent and allowed to settle for 2 h at 4°C. The supernatant was discarded using a vacuum, while the sediments were mixed and equally distributed in 50-ml centrifuge sterile tubes. In each tube, the sample volumes were adjusted to 50 ml with sterile water and centrifuged for 10 min at 1000×g. The supernatant was then discarded, and the pellet (biosolids) that should not exceed 5 ml was resuspend in 10 ml of MgSO₄ (specific gravity 1.2). Each tube was vortexed for 2 min, and more MgSO₄ was added to each tube to reach a volume of 50 ml. The tubes were then centrifuged for 10 min at 1000×g. The top 25–35 ml of supernatant of each tube was poured through a 400 mesh (38 μm) sieve supported in a funnel over a beaker. Biosolids retained on the sieve were washed, rinsed, and collected into a 100 ml beaker. The suspension of biosolids was then transferred into 15 ml centrifuge tubes. Tubes were centrifuged for 3 min at 800×g, and supernatants were discarded. If the previous step generated more than one tube for one initial sample, the sediments should be transferred into one single 15 ml tube and the centrifugation step repeated. Finally, after discarding the supernatant, the biosolids were resuspended in 4 ml 0.1 N H₂SO₄. The vials were incubated at 26°C for 3 weeks. After 24 days of incubation, when the majority of the controls were fully embryonated, samples were ready to be examined microscopically (10×) using a Sedgwick Rafter cell to enumerate the detected ova. Ova were classified as either nonviable (unembryonated) or viable (embryonated to the first, second, or third larval stage, those with the potential to become adult *Ascaris*). The percent moisture of the sample was determined by analyzing a separate portion of the sample, so that the final calculation of ova per gram dry weight could be determined. This was done by measuring the weight of the sludge samples before and after incubating at 45°C for 4 days.
until dry by observation. Categories of ova per 4 grams per weight were calculated in the following manner:

\[
\text{Ova/g dry wt} = \frac{(NO) \times (CV) \times (FV)}{(SP) \times (TS)}
\]

where \(NO\) = no ova, \(CV\) = chamber volume (=1 ml), \(FV\) = final volume in ml, \(SP\) = sample processed in ml or g, \(TS\) = % total solids.

5.3. Results and discussion

In order to determine *Ascaris* ova viability per four grams of sludge dry weight, the average dry weight of untreated sewage sludge samples was first determined. Hence, the dry weight of untreated sewage sludge samples was \(\bar{w} \pm R\%\) total solids. On average, untreated sewage sludge contained \(312 \pm 24\) *Ascaris* ova per four grams of dry weight. Figure 6 shows percentages of *Ascaris* ova viability after treatment of sewage sludge samples with electron-beam doses of 2.7, 6.7, 13.2, and 25.7 kGy.

![Figure 6. Effect of electron beam irradiation on Ascaris ova survival in municipal sewage sludge. Percentages of Ascaris ova survival after irradiation of samples [7].](image)

Similar to bacterial counts, the results indicate that the viability of *Ascaris* ova decreases in a dose-dependent manner [7] with a \(D_{50}\)-value of 7.93 kGy. At dose 2.7 kGy, 23 ± 8% (72 ± 24 *Ascaris* ova per four grams of dry weight) viable ova survived the treatment, while only 11 ± 1.6% (34 ± 4 *Ascaris* ova per four grams of dry weight) ova survived at dose 6.7 kGy. At dose 13.2 kGy, the survival rate dropped to 2 ± 0.03% (6 ± 1 *Ascaris* ova per four grams of dry weight). No *Ascaris* ova were detected in sewage sludge samples irradiated at 25.7 kGy. However from
our counts, we estimated the electron-beam dose 14.5 kGy to be necessary to obtain a sewage sludge containing less than one *Ascaris* ova per four grams of sludge dry weight, meaning that the dose of 25.7 kGy applied during our experiments was high enough to achieve a class A sludge. Indeed, according to EPA standards, to be considered class A, sewage sludge must contain less than one *Ascaris* ovum per four grams of sludge dry weight [9].

6. Economic benefits of using electron beam irradiation for the treatment of municipal sewage sludge

An important aspect in the implementation of a new technology such as an electron accelerator in a wastewater treatment plant is to anticipate its impact on the operation costs of the facility and on the environment. In a recent investigation, the number of kWh used during the irradiation process of the sewage sludge was considered. Data were obtained from the electrical supply company delivering power to the NEO Beam accelerator facility (Toledo Edison, Toledo, Ohio) on the day of the experiment. Energy consumed by the electron accelerator recorded in Figure 7 shows a relatively stable plateau in the power consumed at the facility prior to sample irradiation. The dosimetry calibration of the irradiation setup started at 9:30 am with an increase of the beam current from 0 to 46.2 mA in equal time intervals. From 9:30 am until the end of the irradiation procedure, we observed a constant increase in power consumption. However, the graph only shows electricity consumption from 9:30 am until 10:00 am.

Figure 7. Average power consumed at the NEO Beam electron facility on the day of the experiment (information provided by Toledo Edison, Ohio, USA) [7].
The power consumed at a specific irradiation dose was obtained in terms of the beam current used, and the cost was determined to be $0.115/kWh. Therefore, at irradiation doses 6.7 and 25.7 kGy, the costs were $1.10 and $1.26 per m³ of sludge, respectively. This represents only 15% of increase of the cost when quadrupling the dose of irradiation to achieve the required *Ascaris* ova reduction to a class A biosolids, suggesting that when selecting a higher dose of irradiation of sludge, the increase in cost due to the use of higher beam currents should not be a concern [7]. Similar results were obtained by other authors who estimated the cost of using electron beam and gamma radiation for the disinfection of sludge. However, experiments developed were performed under different considerations. Indeed, a team in Florida reported a cost of $2.50 per 1000 gallons of sludge for a 1.5 MeV electron irradiation facility running at 160 gallons per minute, while another group compared gamma and electron beam irradiations for a sample of activated sludge and obtained treatment costs of $4.20/m³ for gamma irradiation and $2.10/m³ for electron beam irradiation, which are lower compared with $4.85–$5.19 when using conventional technology at the Central District Wastewater Treatment Facility in Miami Dade County [12]. Furthermore, a comparison was made between irradiation at dose 6 kGy and incineration of sludge samples and showed a cost of $60.87/m³ for this latter compared with $3.12/m³ when using gamma radiation. In both instances, gamma and electron beam irradiations prove to be more economic than incineration [13]. Taken altogether, these observations show that electron beam irradiation of sludge is less energy consuming, with shorter processing times, and a more environmental friendly technology compared to methods such as incineration.

7. Conclusion

Electron beam irradiation technology is able to decrease microbial populations in a dose-dependent manner. In the experiments described in this chapter, it has been estimated that 4.5 kGy of irradiation is sufficient to reduce bacterial populations to safe levels for agricultural use. However, a dose of 14.5 kGy is required to eliminate risks of infection by helminths. Altogether, these observations suggest that irradiation of municipal sludge with electron beam requires at least a dose of 14.5 kGy to eliminate risks of microbial infection. Furthermore, electron beam technology is more cost-effective and less time-consuming than incineration in order to achieve a class A sludge according to EPA standards.

Author details

Jean Engohang-Ndong and Roberto M. Uribe

*Address all correspondence to: jengohan@kent.edu*

1 Kent State University at Tuscarawas, New Philadelphia, Ohio, USA

2 Kent State University, Kent, Ohio, USA
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