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Inactivation of Bacteria in Sewage Sludge by Gamma Radiation

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ABSTRACT

The survival of certain bacterial cultures suspended in sewage sludge and exposed to gamma-radiation was studied. The inactivation patterns of most of the organisms were significantly different when irradiation was performed using sewage samples collected in the summer and monsoon seasons. The summer sample collected from the anaerobic digestor afforded significant protection to both Gram negative and Gram positive organisms. This was evident by the increase in dose required to bring about a 6 log cycle reduction in viable count of the bacterial cultures, when suspended in sewage samples instead of phosphate buffer. The observations made using monsoon digestor samples were quite different. This sewage sludge greatly enhanced inactivation by gamma-radiation in most cases. The effects of certain chemicals on the inactivation patterns of two organisms—Salmonella typhi and Shigella flexneri—were examined. Arsenate, mercury and lead salts sensitised S. typhi, while barium acetate and sodium sulphide protected this culture against gamma-radiation. In the case of Sh. flexneri, barium acetate and iodacetamide proved to be radioprotectors. The effects of some chemicals on the inactivation pattern of Sh. flexneri cells irradiated in sludge are also discussed.

INTRODUCTION

The use of waste water sludge as a land fertilizer or soil conditioner is a common method of resource conservation practised worldwide. In addition to beneficial ingredients, sludge may also contain an indigenous population of human enteric pathogens (Yeager & Ward, 1981). A typical analysis of

Environmental Pollution 43 (1987) 281-290

raw sewage effluent of an Indian city shows that it often contains a high count of pathogenic bacteria and viruses (Ramkrishnan, 1971). A large number of such pathogens can become associated with the sewage solids, and hence may not be completely removed during conventional treatment processes (Dudley et al., 1980; Farrh & Bitton, 1983). Irving and Smith have reported the presence of a large number of viruses in sewage samples collected from all stages of treatment in an activated sludge purification plant (Irving & Smith, 1981). It is, therefore, imperative that sewage sludge be suitably disinfected before land application.

Various methods of sludge disinfection are known to be used. Heat treatment, although effective, has a number of disadvantages. It is an energy intensive and expensive process (Krishnamurthy, 1980). Other treatments, such as chlorination, have been reported to be inefficient, as viruses associated with solids have been found to be protected against inactivation (Hejkal et al., 1979, 1981). Composting of sewage solids is routinely used as a means of reducing organisms to very low counts. However, repopulation of certain bacteria is a serious drawback of this treatment process (Russ & Yanko, 1981).

A very effective method for rendering sewage and sludge free of pathogens is irradiation with ionising radiations. Studies have clearly demonstrated the removal of pathogens in raw and digested sludges, waste activated primary, secondary and composted sludges given ionising radiation treatment (Etzel et al., 1969; Wizigmann & Wiirsching, 1975). Various factors affect the removal of pathogenic microorganisms in sewage by irradiation, the major one being moisture content (Lowe et al., 1956; Ward et al., 1981).

We have reported earlier that the total bacterial and mold count of sewage samples collected from the Atladara plant during various seasons of the year varied (Kapila et al., 1981). Disinfection by gamma radiation was found to be more effective in final effluent than in digestor sludges. Dose requirements for inactivation of some bacterial cultures in sewage sludge were found to be very high (Puranam et al., 1981). In this study, we have examined the effects of some chemicals on the gamma-radiation inactivation of some bacteria, in an attempt to find potential radiosensitisers, especially for human bacterial pathogens.

MATERIALS AND METHODS

Collection of sewage sludge samples

Sewage sludge samples were collected at regular times intervals from the Sewage Treatment Plant, Wadi, Baroda. Samples at various stages of treatment, i.e. grid channel and anaerobic digestor, were taken.
Bacterial cultures

Bacterial strains, i.e. *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus* were obtained from the Medical College, Baroda. The radioresistant *Bacillus* sp. was our isolate, obtained as mentioned in a previous paper (Kapila *et al.*, 1981). All cultures were maintained on nutrient agar slants.

Irradiation and enumeration of the organisms

Nutrient-broth-grown cultures (18-h old) were centrifuged aseptically and resuspended, either in sterile sewage sludge or in sterile, 0.05M phosphate buffer, pH 7.0, to get a final suspension of about 10^7 organisms per millilitre. The culture suspensions were subsequently distributed aseptically, and irradiated in 5 ml aliquots in 15 ml capacity test tubes at ambient temperature. Gamma radiation was provided by a 60Co containing Gamma Chamber 900 (Unit No. 43) provided by BARC, Bombay. The samples received doses at the rate of 6.6 krad min^-1 at the beginning of the study. After the culture samples had been irradiated at various doses, viable counts were made to estimate the number of survivors.

This was done by serially diluting the culture suspensions in sterile 0.85% saline and plating on nutrient agar plates, which were subsequently incubated at 37°C for 24h.

Addition of chemicals to check radiomodification effects

All chemicals were added aseptically into sewage or buffer suspended cultures prior to irradiation, as required. Additions were made in order to obtain final concentrations as mentioned in the 'Results' and 'Discussion' sections.

RESULTS

Gamma radiation effects on bacterial cultures suspended in sewage samples

In our previous study with sewage sludge samples from the Atladara Treatment Plant, we had observed that sludge afforded protection against ionising radiation to a number of bacteria (Puranam *et al.*, 1981). In the case of *V. cholerae*, we had found that dilution reduced the extent of protection. Here we present results obtained with sewage samples from the Wadi Treatment Plant.
The Dose Requirements for Inactivation (by 6 Log Cycles) of Some Bacterial Cultures
Gamma Irradiated in Sewage Sludge Samples Collected during May 1983

<table>
<thead>
<tr>
<th>Bacterial culture</th>
<th>Dose required for 6 log cycles reduction in viable count (krad). Cultures were suspended in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>65</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>360</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>290</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>360</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>440</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>400</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>1000</td>
</tr>
</tbody>
</table>

Table 1 shows the doses required to bring about a 6 log cycle reduction in viable counts of some bacterial cultures. It can be seen that, with the exception of *S. typhi*, sewage afforded protection to the cultures against gamma radiation. Furthermore, inactivation was more rapid when cultures were irradiated in grid channel samples than when irradiated in digestor sewage. Protection was most pronounced in the case of *Staphylococcus aureus*, a Gram positive organism.

Table 2 represents the D_{10} values (dose required for 90% inactivation) for the various bacterial cultures. Here again, with the exception of *S. typhi*...
Inactivation of bacteria in sewage sludge by gamma radiation

TABLE 3
Dose Requirements for 6 Log Cycle Reduction in Viable Count and D_{10} Values for Bacterial Cultures Gamma-Irradiated in Digestor Sewage Collected During July 1983

<table>
<thead>
<tr>
<th>Bacterial culture</th>
<th>Dose required for 6 log cycle reduction in viable count (krad)</th>
<th>D_{10} values (krad) for cultures irradiated in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate buffer</td>
<td>Digestor sewage</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>440 240</td>
<td>65 40</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>300 100</td>
<td>55 20</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>350 85</td>
<td>65 15</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>&gt;1000 1000</td>
<td>250 180</td>
</tr>
</tbody>
</table>

and K. pneumoniae, the organisms were more resistant to gamma radiation when exposed using sewage as suspension medium.

The results obtained with sewage samples collected during the monsoon months of July to September were very different. In the case of digestor sewage, marked sensitisation towards gamma radiation was afforded to both Gram positive and Gram negative organisms, as shown in Table 3. The dose required for a 6 log cycle reduction in viable count dropped by about 200–270 krad in all cases; D_{10} values were also greatly reduced, even in the case of the radio-resistant Bacillus species. The D_{10} value for S. typhi was as low as 15 krad.

Effects of chemicals on radiolethality of bacterial species

Since a 3-year survey of sewage from different treatment plants revealed that, generally, dose requirements for bacterial inactivation are rather high, we have searched for radiomodification by some chemicals often found in industrial effluents. For this purpose, we selected two bacterial pathogens, S. typhi and Sh. flexneri, for a detailed study.

Table 4 shows the effects of some chemicals on the survival pattern of S. typhi gamma-irradiated in phosphate buffer. Salts of heavy metals, such as lead and mercury, were found to sensitise this culture quite markedly. Sodium arsenate and copper sulphate also sensitised S. typhi towards gamma radiation. Another well known sensitiser, iodoacetamide (Nair et al., 1976) was found to enhance the radiation lethality of S. typhi. Sodium sulphide and barium acetate, on the other hand, increased the D_{10} value for this culture.
TABLE 4
Di0 Value for *Salmonella typhi* Gamma Irradiated in Phosphate Buffer Containing Various Chemicals

<table>
<thead>
<tr>
<th>Chemical added</th>
<th>Concentration* (mg litre^{-1})</th>
<th>D_{10} (krad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>65</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Lead nitrate</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Mercury iodide</td>
<td>0.01</td>
<td>30</td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>0.05</td>
<td>35</td>
</tr>
<tr>
<td>Sodium sulphide</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>Potassium chromate</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>3</td>
<td>65</td>
</tr>
<tr>
<td>Barium acetate</td>
<td>3</td>
<td>70</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>10</td>
<td>65</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>18.5</td>
<td>50</td>
</tr>
</tbody>
</table>

* The concentrations used were those known to be present in industrial sewage and were essentially non-toxic.

A similar study was performed with *Shigella flexneri* and the results are presented in Table 5. Unlike *S. typhi*, no chemical could bring about marked sensitisation of *Sh. flexneri* towards gamma radiation. Copper sulphate had no significant radiomodifying effect at a concentration of 3 mg litre^{-1}. All the other compounds used showed protection of *Sh. flexneri* towards gamma radiation inactivation. Among these, barium acetate and iodoacetamide showed marked radioprotection. We also tried

TABLE 5
The Effect of Various Chemicals on D_{10} Values for *Shigella flexneri* Gamma Irradiated in Phosphate Buffer

<table>
<thead>
<tr>
<th>Chemical added</th>
<th>Concentration* (mg litre^{-1})</th>
<th>D_{10} (krad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>30</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Mercury chloride</td>
<td>0.01</td>
<td>40</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.1M*</td>
<td>55</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>0.1M*</td>
<td>45</td>
</tr>
<tr>
<td>Barium acetate</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>18.5</td>
<td>70</td>
</tr>
</tbody>
</table>

* The concentrations used were those known to be present in industrial sewage and were essentially non-toxic.

* Concentration used is in terms of Molar.
Inactivation of bacteria in sewage sludge by gamma radiation

The Effect of Various Chemicals on the D_{10} Values of Sh. flexneri Gamma Irradiated in Digestor Sewage (May 1983 Sample)

<table>
<thead>
<tr>
<th>Chemical added</th>
<th>Concentration* (mg litre^{-1})</th>
<th>D_{10} (krad) when cells were irradiated in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sewage alone</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td>Mercury chloride</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Lead nitrate</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Barium acetate</td>
<td>5</td>
<td>85</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>0.02 M*</td>
<td>90</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>5</td>
<td>85</td>
</tr>
</tbody>
</table>

* The concentrations used were those known to be present in industrial sewage and were essentially non-toxic.

b Concentration used is in terms of Molar.

to see whether the addition of chemicals into digestor sludge (May sample) could modify the inactivation dose for Sh. flexneri. All the chemicals, except lead nitrate, protected the culture (Table 6).

DISCUSSION

The results obtained indicate that the presence of chemicals in sewage could be a major factor contributing to the unusual effects of ionising radiations on bacterial pathogens. The total solids content also can play a significant role in radiolethality of organisms. The rapid inactivation of bacterial cultures, when irradiated in grid channel samples as compared to digestor samples, is probably due to the higher solids content of the digestor sewage sample. Protection was more pronounced in Gram positive organisms. Streptococcus faecalis, a gram positive organism, has been consistently found to be more resistant to ionising radiation than other sewage associated bacteria (Ward et al., 1981). The D_{10} values for most of the organisms indicated that protection was offered by sewage constituents towards radiation inactivation. Ward and co-workers have reported their strain of S. typhimurium to be significantly protected in dried sludge (Ward et al., 1981).
The radiation inactivation studies carried out for several organisms using July sewage samples projected a different picture. Even in the case of a radioresistant *Bacillus* sp., the $D_{10}$ value was greatly reduced. The $D_{10}$ value for *S. typhi* was found to be as low as 15 krad, being as significant as that found when ascorbic acid was used as a sensitisier for *S. typhi* (Kapila *et al.*, 1983). It has been reported that a great deal of variation was found in the $D_{10}$ value for total coliforms in dried raw sludge (Brandon & Neuhauser, 1978). The observations made with monsoon digester sewage led us to believe that some radiosensitising chemical(s) was present in it. This idea seemed to be plausible, as the sewage contains some industrial effluent.

A survey of various chemicals to select an effective radiosensitiser for bacterial pathogens in sewage was aimed at making the radiation treatment process more effective. Use of a radiosensitiser will also lower the dose requirements for sewage disinfection, making the process more rapid. We find that mercury and lead salts sensitisise *S. typhi*. *E. coli* cells have been reported to have a drastically lowered $D_{10}$ value when gamma irradiated in the presence of *cis*-platinum complexes (Zimbrick *et al.*, 1979). However, our results indicate that not all organisms respond to chemicals in the same manner. The exact mechanism of radiomodification brought about by chemicals on *S. typhi* or *Sh. flexneri*, in our case, is not clearly understood. Thus, this study clearly indicates that, although treatment with gamma radiation could be a feasible method of ridding sewage sludge of indigenous pathogenic organisms, the constitution of individual sewage systems should be given careful consideration before applying the method on a large scale.

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**REFERENCES**


Inactivation of bacteria in sewage sludge by gamma radiation


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HYGIENIZATION OF SEWAGE-SLUDGE BY GAMMA-RADIATION

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Faculty of Science
M.S. University of Baroda
BARODA

Ionizing radiations have been successfully employed for hygienization of sewage-sludge in many countries. It has been clearly demonstrated that pathogenic viruses, bacteria and parasite ova can be easily eliminated from various types of sewage sludges by the use of such radiations. In an attempt to investigate the possibility of employing such a method for treating sewage of an Indian city, we have found that gamma-rays can be effectively used to inactivate pathogenic bacteria in sewage-sludge obtained from two local treatment plants. In this connection studies have been performed to examine the removal of both, indigenous sewage flora as well as known bacterial pathogens. The dose requirements varied, depending upon the season, stage of treatment and load and types of pathogens present in the sewage. Some chemicals were found to have radiomodification effects on bacterial inactivation. The implications of the presence of chemical effluents on hygienization efficacy will be discussed.
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SOUVENIR
&
ABSTRACTS

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Hygienisation of sewage-sludge by gamma-radiation is a comparatively unknown technology in India. The possible use of this process in the large-scale management of municipal sewage-sludge requires a careful investigation of the effects of these ionizing radiations on the various microbial pathogens likely to be present in the sludge. We have earlier, performed a systematic study of the seasonal variation in the total bacterial and mold count of sewage sludge from a local treatment plant. The dose requirements for removal of bacterial pathogens have also been established. In continuation with this study, we have examined sewage sludge samples from the Wadi Municipal Sewage Treatment Plant. A number of gram-positive and gram-negative bacteria were selected for this purpose. They were added into sterile sewage samples and the effect of various doses of gamma-radiation on their inactivation was checked. The inactivation patterns of most of the organisms were significantly different when irradiation was performed using summer samples as compared to monsoon samples. The summer (May 1983) sample collected from the anaerobic digester afforded significant protection to most of the bacterial species checked. The dose required to bring about a 6 log cycle reduction in viable count increased by about 150 Krad when Shigella flexneri cells were exposed in digester sludge instead of in phosphate buffer. Similarly in the case of Proteus vulgaris, E. coli and S. typhi cultures, this dose requirement increased by about 80, 60 and 30 krad respectively. The survival of Klebsiella pneumoniae was not significantly affected. However, both the gram-positive species checked viz. Staph. aureus and a Bacillus isolate were afforded, marked protection by digester sludge. The sewage sample collected from the grid channel afforded slight sensitization to Sh. flexneri and the Bacillus isolate, while protecting E. coli, Proteus vulgaris, S. typhi and Staph. aureus to some extent.

The observations made, using monsoon July 1983 digester samples were quite different. This sludge greatly enhanced the killing of both gram-negative and gram-positive organisms. The dose needed for a 6 log cycle reduction in viable count...
was about 300 krad less for the Bacillus spp., 180 krad less for Proteus vulgaris and Staph. aureus and 225 krad less in the case of S. typhi. This indicated the possible presence of some sensitizer(s) in the sludge. Further investigation revealed that sensitizing activity was present in the soluble fraction (sludge supernatant). Also, the sludge did not itself inactivate the bacteria.

Two gram-negative organisms were studied in further detail for the effect of various chemicals on their inactivation pattern. Arsenate and Mercury salts proved to be good sensitzers for S. typhi when exposed in phosphate buffer. Phenol afforded slight sensitization while barium acetate protected this culture against gamma-radiation. Mercury had no significant effect on the inactivation pattern of Sh. flexneri cells. In this case also, barium acetate protected the culture against gamma-radiation. Copper sulphate behaved as a protectant, for this organism.

We have also examined the effects of some of these chemicals when sludge was used as suspension medium.