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Application of Gamma irradiation in treatment of Waste Activated Sludge to Obtain Class a Biosolids

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Abstract: Problem statement: The main objective of the current study was investigation of the possible application of Gamma irradiation for treatment of the activated sludge generated wastewater treatment stations, to achieve the standard requirements in term of pathogens content. **Approach:** Activated sludge samples were collected from Riyadh wastewater plant and analyzed quantitatively for the presence of important bacterial parameters including fecal coliforms and *Salmonella* spp. The collected samples were treated with various doses of Gamma irradiation and bacterial count was determined. **Results:** The results indicated that all tested sludge samples were positive for the presence of fecal coliforms and *Salmonella* spp, with different counts in different stages of wastewater treatment. The raw sludge showed to have the highest coliforms and *Salmonella* spp counts of 1.1×10^8 and 2×10^3 MPN g^{-1} dry sludge, respectively. Furthermore, coliforms and *Salmonella* spp were detected in final resulted sludge with count of 2.5×10^7 and 6×10^2 MPN g^{-1} dry sludge, respectively. It was found that treatment of samples with gamma irradiation was able to reduce the fecal coliforms and *Salmonella* spp effectively and the reduction efficiency was increased by increasing the irradiation dose. Fecal coliforms and *Salmonella* counts were reduced to less than 100 MPN g^{-1} dry sludge by exposing to 1.5 and 0.25 kGy respectively. Furthermore, Gamma radiation dose of 2.0 kGy was able to remove both fecal coliforms and *Salomnell*a spp completely. In addition, D_{10} values were determined and was found to be 0.25 and 0.24 kGy for fecal coliforms and *Salmonella* spp., respectively. **Conclusion/Recommendations:** The results indicating that the resulted activated sludge generated from Riyadh wastewater plant are rich with important pathogens and therefore further treatment procedures are necessary to achieve the required standards, before any land application. Application of Gamma irradiation in treatment of the activated sludge showed to be a promising safe technology for this purpose.

Key words: Colony Forming Units (CFU), Most Probable Numbers (MPN), Dry Solids content (DS), Gamma radiation, gamma irradiation, bacterial parameters, fecal coliforms, generated wastewater, pathogens entering, dry sludge, *Salmonella* spp

INTRODUCTION

Wastewater treatment plants generate huge amounts of residual sludge whose treatment and disposal is receiving increasing attention. Treatment and disposal of waste activated sludge is a major problem for municipal wastewater treatment facilities due to its health and environmental effects (De la Rubia *et al*., 2005). Sludge can be used as fertilizers for agricultural land applications owing to its high contents of organic materials, different nutrients and metals (Eddy, 2003; Yu *et al*., 2010; Forster-Carneiro *et al*., 2010). However, land application of sludge may have a serious health threat due to the possible increase in soil transmitted diseases. Furthermore, pathogens entering the soil may also lead to both surface and ground water contamination as any member of the soil microflora will be ultimately deposited either in aquatic environment or to be dispersed in the atmosphere (Santamaria and Toranzos, 2003; Yu *et al*., 2010). Consequently, sludge now must meet stringent pathogen reduction regulations (which is specified in the Standards for the Use or Disposal of

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Sewage Sludge), before it can be used for any land applications (USEPA, 1999; Lang and Smith, 2008). The pathogen reduction requirements are divided into two levels, Class A and Class B, depending on the extent of pathogen reduction (Carneiro and Perez, 2010). In class A, disinfection is almost complete, where fecal coliform levels are less than 1000 Most Probable Numbers (MPN) per gram of total solids dry weight and there is a complete removal of pathogens from three major classes: viable helminth eggs, enteric viruses and *Salmonella* spp. (Wang *et al*., 2008). While in class B, disinfection is incomplete, where fecal coliform levels are reduced to below two million Colony Forming Units (CFU) per gram of total solids dry weight and there only reduction of the major pathogens. Therefore analysis of the level of pathogens like faecal coliforms and *Salmonella* spp., helminth eggs, viruses and other potential pathogens indicators are necessary to characterize the risks associated with the treated sludge befor use (Perez-Elvira *et al*., 2006; Carneiro and Perez, 2010). Thus, several countries have developed legislations about the use of residual sludge. Therefore, the main objective of the current study was investigation of the possible application of Gamma radiation for treatment of the residual activated sludge of Riyadh wastewater treatment (Riyadh, Saudi Arabia) to achieve the standard requirements in term pathogens content, before any land application of the resulted sludge.

MATERIALS AND METHODS

Samples collection: Different samples were collected from six sites in Riyadh wastewater treatment plant (Riyadh, Saudi Arabia), from the raw and anaerobically digested sludge from two stations (north and south station). In addition, samples were collected from two sites in the final common treatment step of the two stations, including the mixing and collecting digested sludge tank and the final resulted sludge.

Dry weight determination: The dry mass of the activated sludge was determined as previously reported (Eaton *et al*., 2005). The activated sludge samples were mixed and 20 mL was filtered through Whatman filter paper (Whatman GF/C, Pore Size 1.2 µm, UK) .The filter papers with solid materials were then kept for dryness in oven at 105°C till the weight become constant and the dry weight was calculated. The Dry Solids Content (DS) sludge content of the sludge was expressed as mass percentage.

Bacterial indicators analysis: The collected sewage samples were analyzed in term of fecal coliforms and *Salmonella* spp as the following.

Determination of fecal coliform concentrations: Fecal coliform concentrations were determined using the multiple-tube fermentation direct test method as described previously for the EPA Standard Method 9221 E (Redlinger *et al*., 2001; Eaton *et al*., 2005). In which, eleven grams of the sludge sample was mixed with 99 mL of sterile saline solution (0.9% wt/v) and blended for one minute in a sterile blender at low speed for one minute. Then, serial dilutions of the slurry were prepared and 10 ml of the10^{-2}, 10^{-3} 10^{-4}, 10^{-5} and 10^{-6} dilutions was added to 1 ml of A-1 medium (Oxoid). The concentration of the A-1 medium was adjusted according to the standard medium described for the EPA Standard Method 9221 E (Eaton *et al*., 2005). The Tube contents were incubated at 37°C for 3 h, any bubbles were removed and the tubes were again incubated at 44.5°C for 21 h before results were recorded. Any gas production in the inverted Durham tube and turbidity of the medium indicated positive results and the Most Probable Number (MPN) was estimated using standard curve of MPN table (Redlinger *et al*., 2001).

Detection of *Salmonella* spp: *Salmonella* spp was detected according to the Standard Methods (9260 B), (Carneiro and Perez, 2010) in three steps, including pre-enrichment, enrichment and isolation and confirmation. (A) Pre-enrichment: Sludge samples were serially diluted in sterile saline solution. Aliquots, from different dilutions, were transferred to peptone solution (3%, w/v) and incubated at 37°C for 3 h. (B) Enrichment and isolation: Serial dilutions, from the above culture, were made up to 10^{-6} and one mL of each dilution were incoculated into 9 ml of Rappaport-Vassiliadis culture medium (Difico) and incubated at 40°C for 24-28h. Appearance of a characteristic green colour colonies, is an indication of the presence of *Salmonella* spp. (C) For further confirmation of the presence of *Salmonella* spp, samples from the positive tubes were inoculated into XLD-agar (Xylose Lysine Deoxycolate agar) plates and incubate at 37°C for 24 h. The appearance of red colonies with black centers is a positive reaction that indicates the presence of *Salmonella* spp (Carneiro and Perez, 2010).

Irradiation treatment: Samples irradiation were carried out using modification of a previously reported method (Clavero *et al*., 1994; Chiang *et al*., 2010), in the Nuclear Research Institute, King Abdul- Aziz City for Science and Technology (Riyadh, Saudi Arabia) using ^{60}Co Gamma Cell 220, from MDS-Nordian International. Three boxes fitted with lids were prepared. Each box constituted one replicate. Gammachrome YR dosimeters were placed in a central position on the top external side of the lids of boxes

(three replicate). The three boxes were centered on top of a 10-cm-thick styrofoam block placed on a turntable (2.3 rpm) approximately 220 cm from the ^{60}Co irradiation source. The samples were exposed to gamma irradiation at the desired doses of 0.25, 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5 and 3.0 kGy. Un-irradiated sludge samples were subjected to the same storage, transport and handling conditions as the irradiated samples to served as the control (0 irradiation dose). D_{10} were calculated representing the dose of gamma radiation required to kill 90% of the initial bacterial count (Chiang *et al*., 2010).

RESULTS AND DISCUSSION

Treatment and disposal of excess sludge generated by wastewater treatment plants is a bottleneck in plant operation especially in both developing and industrial countries due to more stringent quality requirements regarding landfilling, ocean disposal, agricultural use and incineration (Eskicioglu *et al*., 2009). The analysis of the level of pathogens like faecal coliforms and Salmonella spp., helminth eggs, viruses and other potential pathogens indicators are necessary to characterize the risks associated with the treated sludge us. In this study, sludge samples were collected from different sites in Riyadh wastewater treatment station (Riyadh, Saudi Arabia) and were analyzed quantitatively for the presence of important bacterial parameters, including fecal coliforms and *Salmonella* spp. The results indicated that all samples were positive for the presence of fecal coliforms and *Salmonella* spp, with different counts in different stages of wastewater treatment (Table 1). The raw sludge from the south station showed the highest coliforms count of 1.1×10^8 MPN/g dry sludge (Table 1).

Table 1: Most probable number (MPN) of fecal coliforms and *Salmonella* spp in different sludge samples, generated from Riyadh wastewater treatment plant. All counts are means of three experiments.

	Bacterial indicator		
Sample source	Faecal coliforms (MPN g^{-1} dry sludge)	*Salmonella* spp (MPN g^{-1} dry sludge)	Water content (%)
Raw sludge (South station)	1.5×10^8	7×10^2	94
Raw sludge (North station)	1.1×10^8	2×10^3	92
Anaerobically digested sludge (south station)	3.5×10^7	1×10^2	89
Anaerobically digested sludge (North station)	7×10^7	7×10^2	91
Digested sludge from the buffered tank	2.2×10^8	7.1×10^2	95
Final sludge	2.5×10^7	6×10^2	83

This result is relatively similar to that reported by Lasobras *et al*. (1999), that the concentrations of faecal coliform were 5.3×10^8 MNP/g dry sludge samples obtained from the sedimentation process. However, less faecal coliform concentration in activated sludge was also reported by others. According to Carneiro *et al* (2010), the highest concentrations of faecal coliform were detected in raw sludge samples with an average of 5×10^4 MPN g^{-1} dry sludge. In addition, Mandilara *et al*. (2006) as well as Lucero-Ramirez and Molina (1998) reported detection of highest concentrations of faecal coliform in raw sludge samples with an average of 1.2×10^4 to 3.6×10^5 MNP/g dry sludge.

Similarly, *Salmonella* spp was detected in all sludge samples with highest concentration in raw sludge of the north station with count of 2×10^3 10^3 CFU/g dry sludge (Table 1). This result is much higher than previously reported by Carneiro *et al*. (2010), who indicated detection of highest *Salmonella* spp concentration in raw sludge and digested sludge with values in the order of 23.7 MPN/4 g TS and 10.3 MPN/4 g TS, respectively. Furthermore, *Salmonella* densities in raw sludge samples (feedstock) were between 2 and 12 MPN/4 g TS (Cheunbarn and Pagilla 2000).

Irradiation treatment: Quantitative bacteria analysis of the sludge samples indicated detection of fecal coliforms and *Salmonella* spp, in final resulted sludge with high count of 2.5×10^7 and 6×10^2 MNP g^{-1} dry sludge, respectively (Table 1). These results indicating that further treatment procedures are necessary to achieve the required standards, before any land application of the activated sludge generated from Riyadh wastewater treatment plants. In this study, the efficacy of gamma irradiation of the activated sludge was investigated for reduction and/or removal of important pathogens like faecal coliforms and *Salmonella* spp to achieve either class A or class B standards. In class A, disinfection is almost complete, where fecal coliform levels are less than 1000 Most Probable Numbers (MPN) per gram of total solids dry weight and there is a complete removal of pathogens from three major classes: viable helminth eggs, enteric viruses and *Salmonella* spp. (Wang *et al*., 2008). While in class B, disinfection is incomplete, where fecal coliform levels are reduced to below two million Colony Forming Units (CFU) per gram of total solids dry weight and there only reduction of the major pathogens. Gamma radiation is a physical process commonly used for the eradication of microorganisms distributed in different ingredients. Gamma Irradiation is known to initiate a chain of events leading to the impairment of structural or metabolic functions, such as fragmentation of DNA and the eventual death of microbial cells (Chiang *et al*., 2010).

Table 2: Effect of Gamma radiation on the fecal coliforms and *Salmonella* spp content in the activated sludge generated from Riyadh wastewater treatment plant

	Bacterial indicator (MPN g^{-1} dry sludge)	
Dose of Gamma irradiation (kGy)	Fecal coliforms	*Salmonella* spp
0	2.2×10^{8}	7.1×10^{2}
0.25	1.7×10^{7}	66
0.5	4.8×10^{5}	13
1.25	3.6×10^{2}	3
1.5	75	1
1.75	7	0
2	0	0

Table 3: D_{10} for Fecal coliforms and *Salmonella* spp for sludge samples generated from Riyadh wastewater treatment plant. D_{10} is defined as dose of gamma radiation required to kill 90% of the initial bacterial count

	D_{10}	
Sample	Fecal coliforms	*Salmonella* spp
Sample 1	0.27	0.26
Sample 2	0.22	0.24
Sample 3	0.26	0.22
Average	0.25	0.24

The effect of sludge treatment with different dose of gamma radiation on the bacterial content was investigated (Table 2). It was found that treatment of sludge samples with gamma irradiation was able to reduce the fecal coliforms and *Salmonella* spp effectively and the reduction efficiency was increased by increasing the radiation dose. Fecal coliforms and *Salmonella* counts were reduced to less than 100 MPN g^{-1} dry sludge by exposing to 1.5 and 0.25 kGy respectively. Furthermore, Gamma radiation dose of 2.0 kGy was able to remove both fecal coliforms and *Salomnell*a spp completely (Table 2). In addition D_{10} values (defined as the dose of Gamma radiation able to kill 90% of the indicator bacteria) were determined and was found to be 0.25 and 0.24 kGy for fecal coliforms and *Salmonella* spp., respectively (Table 3)

CONCLUSION

Quantitative bacteria analysis of the sludge samples indicated that all sludge samples generated from Riyadh wastewater plant were positive for the presence of fecal coliforms and *Salmonella* spp, with different counts in different stages of wastewater treatment. Furthermore, the final resulted activated sludge was rich with both coliforms and *Salmonella* spp. The efficacy of gamma irradiation of the activated sludge was investigated for reduction and/or removal of such important pathogens. Gamma radiation dose of 2.0 kGy was able to remove both fecal coliforms and *Salomnell*a spp completely. Therefore, application of gamma irradiation in treatment of the activated sludge showed to be a promising safe technology to to achieve either class A or class B standards of biosolids before any land application.

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REFERENCES

Cheunbarn, T. and K.R. Pagilla, 2000. Anaerobic thermophilic/mesophilic dual-stage sludge treatment. J. Environ. Eng., 126: 796-801.

Chiang, Y., G. Huang, Y. Ho, P. Hsieh and H. Chung *et al*., 2010. Influence of gamma irradiation on microbial load and antioxidative characteristics of *Polygoni Multiflori* Radix. Process Biochem (In Press).

Clavero, M.R., J.D. Monk, L.R. Beuchat, M.P. Doyle and R.E. Brackett, 1994. Inactivation of Escherichia coli 0157:H7, Salmonellae and *Campylobacter jejuni* in Raw Ground Beef by Gamma Irradiation. Applied Environ. Microbiol., 60: 2069-2075.

De la Rubia, M.A., L.I. Romero, D. Sales and M. Perez, 2005. Temperature conversion (mesophilic to thermophilic) of municipal sludge digestion. Environm. Energ. Eng. AIChE J., 51: 2581-6.

Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E. Greenberg and M.H. Franson, 2005. Standard Methods for the Examination of Water and Wastewater. 21th Edn. American Public Health Association (APHA), Washington, D.C.

Eddy, M., 2003. Wastewater Engineering Treatment Disposal and Reuse, 4th Edn., McGraw Hill, New York,

Eskicioglu, C., K.J. Kennedy and R.L. Droste, 2009. Enhanced disinfection and methane production from sewage sludge by microwave irradiation. Desalination, 248: 279-285.

Forster-Carneiro, T., V. Riau and M. Perez, 2010. Mesophilic anaerobic digestion of sewage sludge to obtain class B biosolids: Microbiological methods development. Biomass Bioenergy, pp: 1-8

Lang, N.L. and S.R. Smith, 2008. Time and temperature inactivation kinetics of enteric bacteria relevant to sewage sludge treatment processes for agricultural use. Water Res., 42: 2229-41.

Lasobras, J., J. Dellunde, J. Jofre and F. Lucena 1999. Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. J. Applied Microbiol., 86: 723-9.

Lucero-Ramirez, B. and J.F. Malina, 1998. The effect of standard municipal sludge treatment processes on the reduction of indicator and pathogenic organisms. Proceeding of WEFTEC'98. Orlando, pp: 95-103.

Mandilara, G.D., E.M. Smeti, A.T. Mavridou, M.P. Lambiri and A.C. Vatopoulos *et al*., 2006. Correlation between bacterial indicators and bacteriophages in sewage and sludge. FEMS Microbiol. Lett., 263: 119-26.

Perez-Elvira, S.I., P. Nieto and F. Fernandez-Polanco, 2006. Sludge minimization technologies. Rev. Environ. Sci. Biotechnol., 5: 375-98.

Redlinger, T., J. Graham, V. Corella-Barud and R. Avitia, 2001. Survival of fecal coliforms in dry-composting toilets. Applied Environ. Microbiol., 67: 4036-4040.

Santamaria, J. and G.A. Toranzos, 2003. Enteric pathogens and soil: a short review. Int. Microbiol., 6: 5-9.

USEPA, 1999. Standards for the Use or Disposal of Sewage Sludge, Code of Federal Regulations, Part 503, Title 40.

Wang, H., S.L. Brown, G.N. Magesan, A.H. Slade and M. Quintern *et al*., 2008. Technological options for the management of biosolids. Environ. Sci. Pollut. Res., 15: 308-317.

Yu, Y., W.I. Chan, P.H. Liao and K.V. Lo, 2010. Disinfection and solubilization of sewage sludge using the microwave enhanced advanced oxidation process. J. Hazard. Mater., 181: 1143-1147.