Ankara Üniv Vet Fak Derg, **58**, 55-59, 2011

Inactivation of indicator bacteria in treated municipal wastewater and biosolids by gamma irradiation

Zişan EMRE[1], Gülay ALTAY ÇAPIN[1], Seyit CANPOLAT[1], Hülya MERT[1]

[1] Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Department of Nuclear Applications on Animal Science, Ankara, Turkey.

Summary: Increasing growth of the world's population, waste minimization policies and agricultural needs make the recycling of domestic wastewater quite a desirable practice. Factors like environmental and public health risks must be taken into account when considering treated wastewater for field irrigation and biosolids for land application. Wastewater disinfection is achieved via chlorination or UV-irradiation. In this study, an alternative disinfectant, gamma radiation is investigated. It is found that ^{60}Co-gamma irradiation could be effectively used for the disinfection of effluents from the municipal wastewater treatment plants, and gamma radiation dose of at least 5000 Gy was required for the inactivation *Enterococcus* sp., the most resistant bacteria among the indicator bacteria tested.

Key words: Disinfection, gamma irradiation, indicator bacteria, municipal wastewater.

Belediye arıtılmış atıksu ve biyokatılarında bulunan indikatör bakterilerin gama ışınlama ile inaktivasyonu

Özet: Dünya nüfusundaki artış, atıkların azaltılması politikası ve tarımsal gereksinimler evsel nitelikli atıksuların arıtılarak tekrar kullanımını istenen bir uygulama haline getirmiştir. Arıtılmış atıksuların tarım arazilerinin sulanmasında ve biyokatıların gübre olarak kullanımında ortaya çıkabilecek çevre ve halk sağlığına ilişkin risk faktörleri dikkate alınmalıdır. Atıksuların dezenfeksiyonu klorlama veya ultraviyole ışınlama ile sağlanmaktadır. Bu çalışmada gama ışınlama alternatif bir dezenfektan olarak araştırılmıştır. Sonuç olarak ^{60}Co-gama ışınlamanın belediye arıtılmış atıksularının dezenfeksiyonunda etkili olduğu ve incelenen indikatör bakteriler içinde en dirençli olan *Enterokok* türleri dikkate alındığında en az 5000 Gy radyasyon dozunun uygulanması gerektiği belirlenmiştir.

Anahtar sözcükler: Belediye atıksuyu, dezenfeksiyon, gama ışınlama, indikatör bakteri.

Introduction

Effects of global warming and population growth cause the countries concern about the use and preservation of water and to look for alternative water resources. Use of wastewater can increase the available water supply, however, factors like environmental and public health risks must be taken into account. Pathogens present in wastewater and biosolids may remain active after treatment and can easily cross-contaminate the surface and ground water (31,35). Besides, there is a great risk of transmission of bacterial and parasitic infections via consuming crop and vegetables grown in this wastewater-irrigated and/or biosolid-applied agricultural fields (8,37). Bacterial pathogens in domestic wastewater include many species of fecal coliforms, *Salmonella* spp. and fecal streptococci (5,27). Cases related to waterborne infections have not really been estimated in Turkey (2,19), and very limited information is available on occurrence and survival of microorganisms in treated wastewater (4,11).

Within all types of treatment, disinfection plays an important role because it guarantees the elimination of indicator organisms, especially pathogens, to safe levels (24,25). Wastewater disinfection is achieved via chlorination, ozonation or ultraviolet (UV)-radiation (14,18). Another technique, potentially useful for wastewater treatment, is ionizing radiation including high-energy electrons and gamma radiation (13,17). Studies on the use of electron beams in treating wastewater and municipal biosolids have been conducted at several locations around the world (16,36), and gamma irradiation was identified as an alternative method for disinfection (12,21,30).

The work reported herein represents, (i) the population densities of indicator bacteria in treated municipal wastewater and biosolid samples, and (ii) the impact of gamma irradiation on these bacterial population.

Materials and Methods

Sampling: Treated municipal wastewater and biosolid samples were collected on monthly basis at the Ankara Central Municipal Wastewater Treatment Plant from May 2005 to April 2006 (except for January 2006). The collected 5 lt wastewater sample from the final settling tanks and 1 kg biosolid sample from the mechanical sludge dewatering station were processed and analysed within the same day.

The plant is located about 40 km. from the city center and about 90% of the city population is connected to the network. The treatment capacity of the plant is 765000-1530000 m^3/day of wastewater. The sewage sludge is generated from the wastewater during primary and secondary treatment and it undergoes mesophilic anaerobic digestion (35±1°C for about 20 days). The digested sludge is dewatered to produce a semi-solid cake and named as biosolid (~650m^3/day) (4).

Analyses: In treated wastewater samples, total coliforms, fecal streptococci and *Enterococcus* sp. were all enumerated by using the Most Probable Number (MPN) Method as described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association,1998) (1,3). In biosolid samples, fecal coliform tests and *Salmonella* spp. analysis were performed by using American Environment Protection Agency, Method No.6260/2003 (1,34).

Gamma irradiation: Gamma irradiation was performed at Sarayköy Nuclear Research and Training Center by placing the samples inside the irradiation chamber of a Gamma Cell (Tenex-Issledovatel) at 18°C. A ^{60}Co source with an average dose rate of 1.34 kGy/h was used for the irradiation. Samples of 100 ml treated wastewater and 100 g of biosolids in glass vials were irradiated for the appropriate time to achieve the desired gamma irradiation doses. After irradiation survival microbial counts were determined and the D_{10} values were calculated.

D_{10} value calculations: The D_{10} value is the dose of radiation needed to achieve one log cycle or 90% reduction of the initial bacterial population. The D_{10} values from MPN data were determined using the equation

$$D_{10} = D / \log No - \log N$$

in which, D is the dose of radiation used, log No is the initial bacterial population and log N is the amount of survivors at dose D. The relationship between dose and survival rate is logarithmic (6,26).

Results

In all tested treated wastewater samples, total coliforms, *Enterococcus* sp. and fecal streptococci were detected. *Salmonella* spp. were detected in 7 samples. The bacterial population densities of the treated wastewater samples were tabulated in Table 1. The Table 1 includes the annual average densities (geometric means and standart deviations) of the bacteria as well.

In biosolids, fecal coliforms were determined throughout the year. *Salmonella* species in biosolids were detected in 8 samples. The population densities and annual average of fecal coliforms and *Salmonella* spp. were presented in Table 2.

The results indicated that gamma irradiation was very effective in eliminating pathogenic bacteria in treated wastewater and biosolids. The D_{10} values for the pathogens in treated wastewater and biosolid samples ranged from 900 Gy for *Salmonella* spp. to 5000 Gy for *Enterococcus* sp. The irradiation doses required for the inactivation of indicator bacteria tested in treated wastewater and biosolids were tabulated in Tables 3 and 4.

Table 1. Population densities of indicator bacteria in treated wastewater samples
Tablo 1. Arıtılmış atıksu örneklerinde indikatör bakterilerin yoğunlukları

Months	Total Coliforms MPN / 100 ml	Salmonella sp. MPN / 100 ml	Enterococcus sp. MPN / 100 ml	Fecal Streptococci MPN / 100 ml
May 2005	$3.61x10^2$	(-)	$<1.60x10^4$	$3.30x10^2$
June 2005	$3.59x10^2$	$1.3x10^2$	$<1.60x10^4$	$1.63x10^2$
July 2005	$3.47x10^2$	$2.0x10^1$	$1.70x10^3$	$2.13x10^3$
August 2005	$1.66x10^2$	$4.0x10^1$	$5.50x10^2$	$1.24x10^3$
September 05	$1.88x10^2$	(-)	$1.25x10^2$	$<1.60x10^4$
October 2005	$2.93x10^2$	$4.0x10^1$	$3.19x10^2$	$1.70x10^3$
November 05	$2.94x10^2$	(-)	$4.84x10^2$	$2.56x10^2$
December 05	$1.43x10^2$	$1.3x10^2$	$<1.60x10^4$	$2.20x10^3$
January 2006	*NA	*NA	*NA	*NA
February 2006	$4.09x10^2$	$8.0x10^1$	$\geq 1.60x10^4$	$\geq 1.60x10^4$
March 2006	$\geq 1.6x10^4$	(-)	$\geq 1.60x10^4$	$\geq 1.60x10^4$
April 2006	$2.34x10^2$	$4.0x10^1$	$\geq 1.60x10^4$	$5.98x10^1$
Annual average (GM±SD)	$3.84x10^2$±0.56	$1.30x10^1$±0.91	$2.21x10^3$±0.86	$2.14x10^3$±0.80

* NA : Not analysed

Table 2. Population densities of indicator bacteria in biosolid samples
Tablo 2. Biyokatı örneklerinde indikatör bakterilerin yoğunlukları

Months	Fecal coliforms MPN / g	Salmonella sp. MPN / 4 g
May 2005	$1.1x10^4$	$1.22x10^0$
June 2005	$2.0x10^5$	$0.32x10^0$
July 2005	$3.4x10^5$	(-)
August 2005	$3.1x10^5$	$0.67x10^0$
September 2005	$2.2x10^4$	$2.26x10^0$
October 2005	$1.3x10^5$	(-)
November 2005	$3.4x10^5$	$0.68x10^0$
December 2005	$9.5x10^3$	$0.68x10^0$
January 2006	*NA	*NA
February 2006	$1.4x10^6$	$1.51x10^0$
March 2006	$6.4x10^5$	$1.60\ x10^0$
April 2006	$5.6x10^4$	(-)
Annual average (Geometric mean ± SD)	$1.54x10^5 \pm 0.84$	$0.88x10^0 \pm 0.23$

* NA : Not analysed

Table 3. Effect of gamma irradiation on bacteria in treated wastewater
Tablo 3. Gama ışınlamanın arıtılmış atıksuda bulunan bakteriler üzerine etkisi

Bacteria	Irradiation Dose (Gy) / Percent Inactivatıın								
	500	700	900	1000	1500	2000	2500	3000	5000
Total coliforms	-	-	45	99.30	99.99				
Fecal streptococci	-	-	-	-	-	99.50	99.50	99.90	99.99
Salmonella sp.	30	70	99.99						
Enterococcus sp.	-	-	-	-	-	82	99.50	99.90	99.99

Table 4. Effect of gamma irradiation on bacteria in biosolids
Tablo 4. Gama ışınlamanın biyokatıda bulunan bakteriler üzerine etkisi

Bacteria	Irradiation Dose (Gy) / Percent Inactivatiın								
	500	700	900	1000	1500	2000	2500	3000	5000
Fecal coliforms	62.50	99.90	99.97	99.99					
Salmonella sp.	45	82	99.99						

Discussion and Conclusion

The annual average population densities of total coliforms, *Salmonella* spp. fecal streptococci and *Enterococcus* sp. in treated wastewater were $3.84x10^2 \pm 0.56$, $1.30x10^1 \pm 0.91$, $2.14x10^3 \pm 0.80$ and $2.21x10^3 \pm 0.86$ MPN/100ml respectively. Relatively similar results were reported as $3.20x10^2$ MPN/100ml total coliforms in Canada and Spain (9,15), 7-15 MPN/100ml Salmonella spp. in Wisconsin (10), $2.81x10^4$ MPN/100ml fecal streptococci in Spain and Greece (15,23), and $7.30x10^6$ MPN/100ml *Enterococcus* sp. in Antarctica (22).

In biosolids, the mean concentration of fecal coliforms was found to be $1.54x10^5 \pm 0.84$ MPN/g. This estimated value is in the range of values reported by Lisle and coworkers (22) and Mavridou and coworkers (23). The mean concentration of Salmonella spp. in biosolids was $0.88x10^0 \pm 0.23$ MPN/4g. The result is in accordance with the results reported by other researchers (10,20,28).

Disinfection process using gamma irradiation was very effective on bacteria from treated wastewater and biosolids. The effect of gamma irradiation on bacteria interacts directly with a sensitive site in the organism, usually the deoxyribonucleic acid (DNA) that directs cellular reproduction and synthesis of cell components rather than the relatively radiation resistant constituents (33). Zagory (38) reports that bacteria have smaller DNA and so are more resistant to irradiation, and would require 1500-3500 Gy for inactivation. In the present study, the lowest lethal dose of *Salmonella* spp. was 900 Gy while the dose of 5000 Gy was effective on *Enterococcus* sp. This study confirmed the findings of

the previous investigations and the irradiation doses given by other researchers were in accordance with our findings (7,29,32).

In conclusion, this study shows that gamma irradiation could be effectively used for the disinfection of effluents from the municipal wastewater treatment plants, especially if reuse in agricultural practice is to be considered, and considering *Enterococci*, the dose of at least 5000 Gy is necessary to inactivate the indicator pathogens from treated wastewater and biosolids. Electron beam radiation which is characterized by its low penetration and high dosage rates would improve the economics of the process and reduce any public objection related to the use of radioisotopes.

Acknowledgements

The authors are grateful to the Ankara Central Wastewater Treatment Plant, for facilities and support provided for the completion of this research. The authors also acknowledge the financial support provided by Turkish Atomic Energy Authority.

References

1. **American Public Health Association** (1998): *Standard Methods for the Examination of Water and Wastewater*. 20th ed., Am. Publ. Health Assc., Inc., New York.
2. **Aslan G** (2005): *Sularla Bulaşan İnfeksiyonlar*. IV. Ulusal Sindirim Yoluyla Bulaşan İnfeksiyonlar Simpozyumu, 16-20 Mayıs 2005, Mersin, pp.152-163.
3. **Ayres RM, Mara DD** (1996): *Analysis of Wastewater for use in Agriculture*. WHO, Geneva.
4. **Bilgin N, Eyüpoğlu H, Üstün H** (2002): *Biyokatıların (Arıtma Çamurlarının) Arazide Kullanımı*. Ankara Büyükşehir Belediyesi, Su ve Kanalizasyon İdaresi Genel Müdürlüğü Arıtma Tesisi Dairesi Başkanlığı ve Köy Hizmetleri Genel Müdürlüğü Ankara Araştırma Enstitüsü Ortak Yayını, Ankara.
5. **Bitton G** (2005): *Wastewater Microbiology*. 3rd Edition, Wiley-Liss, Hoboken, New Jersey, USA.
6. **Black JL, Jaczynski, J** (2008): *Effect of water activity on the inactivation kinetics of Escherichia coli O157:H7 by electron beam in ground beef, chicken breast meat, and trout fillets*. Int J Food Sci Tech, **43,** 579–586.
7. **Brandon JR, Burge WD, Enkiri NK** (1977): *Inactivation by ionizing radiation of Salmonella enteritidis serotype montevideo grown in composed sewage sludge.* Appl Environ Microbiol, **33**, 1011-1012.
8. **Cecile Willert** (2005): *Biosolids Pellet Review Study, Human Health and Ecological Risk Assessment*. Prepared for Toronto Public Health, Project No. ONT36194, Jacques Whitford Ltd., Toronto, Ontario, Canada. Reaching: http://www.toronto.ca/health/hphe/pdf/abtp_presentation2.pdf Access date: 04 July 2006
9. **Chauret C, Springthorpe S, Sattar S** (1999): *Fate of Cryptosporidium oocysts, Giardia cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion.* Can J Microbiol, **45**, 257-262.
10. **Cheng CM, Boyle WC, Goepfert JM** (1971): *Rapid quantitative method for Salmonella detection in polluted waters.* Appl Microbiol, **21,** 662-667.
11. **Çeber K, Aslan G, Otağ F, Delialioğlu N ve ark.** (2005): *Mersin ilinde içme suyu, kullanma suyu, atık su ve deniz sularında Cryptosporidium spp. oocystlerinin araştırılması.* Türkiye Parazitol Derg, **29**, 224-228.
12. **Etzel JE, Born GS, Stein J, Helbing TJ, Baney G** (1969): *Sewage sludge conditioning and disinfection by gamma irradiation.* Am Public Health Assoc, **59**, 2067-2076.
13. **Farooq S, Kurucz CN, Waite TD, Cooper WJ** (1993): *Disinfection of wastewaters: high energy electron vs gamma irradiation*. Water Res, **27**, 1177-1184.
14. **Gehr R, Wagner M, Veerasubramanian P, Payment P** (2003): *Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater*. Water Res, **37**, 4573-4586.
15. **Howard I, Espigare E, Lardelli P, Martin JL, Espigares M** (2004): *Evaluation of microbiological and physicochemical indicators for wastewater treatment.* Environ Toxicol, **19,** 241-249.
16. **International Atomic Energy Agency** (1990): *Technical Document, Prooceedings of the Joint ASCE-IAEA Meeting on Radiation Treatment*, Washington DC.
17. **International Atomic Energy Agency** (2001): *Use of Irradiation for Chemical and Microbial Decontamination of Water, Wastewater and Sludge.* Technical Document-1225, Vienna, Austria.
18. **Keller R, Passamani F, Vaz L, Cassini ST, Goncalves RF** (2003): *Inactivation of Salmonella spp. from secondary and tertiary effluents by UV irradiation.* Water Sci Technol, **47,** 147-150.
19. **Köksal F** (2002): *Kaynak sularının Giardia ve Cryptosporidium yönünden incelenmesi.* Türk Mikrobiyol Cem Derg, **32,** 275-277.
20. **Lemarchand K, Lebaron P** (2003): *Occurrence of Salmonella spp. and Cryptosporidium spp. in a french coastal watershed: relationship with fecal indicators.* FEMS Microbiol Lett, **218,** 203-209.
21. **Lessel T, Suess A** (1984): *Ten-year experience in operation of a sludge treatment plant using gamma irradiation.* Radn Phys Chem J, **24,** 3-16.
22. **Lisle JT, Smith JJ, Edwards DD, McFeter GA** (2004): *Occurrence of microbial indicators and Clostridium perfringens in wastewater, water column samples, sediments, drinking water, and weddell seal feces collected at McMurdo Station, Antarctica.* Appl Environ Microbiol, **70,** 7269-7276.
23. **Mavridou A, Kouloubis P, Vassalou E, Rigas F, Vakalis N** (2001): *Microbiological quality of sewages sludge in Greece disposed for agricultural use.* Int J Environ Health Res, **11**, 275-279.
24. **Oppenheimer JA, Jacangelo JG, Laine JM, Hoagland JE** (1997): *Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards.* Water Environ Res, **69**, 14-24.
25. **Rawat KP, Sharma A, Rao SM** (1998): *Microbiological and physical analysis of radiation disinfected municipal sewage.* Water Res, **32**, 737-740.
26. **Richter SG, Barnard J** (2002): *The radiation resistance of ascospores and sclerotia of Pyronema domesticum*. J Industrial Microbiol Biotech, **29,** 51 –54.

27. **Rimhanen-Finne R, Vuorinen A, Marmo S, Malmberg S, Hanninen ML** (2004): *Comparative analysis of Cryptosporidium, Giardia and indicator bacteria during sewage sludge hygienization in various composting processes.* Lett Applied Microbiol, **38**, 301-305.
28. **Saleem M, Al-Malack H, Bukhari AA** (2001): *Seasonal variations in the microbial population density present in biological sludge*. Environ Technol, **22**, 255-259.
29. **Snyder OP, Poland DM** (1995): *Food irradiation today.* Reaching: http://www.hi-tm.com/Documents/Irrad.html#killingdoses Access date: 17 May 2006
30. **Taghipour F** (2004): *Ultraviolet and ionizing radiation for microorganism inactivation.* Water Res, **38**, 3940-3948.
31. **Teltsch B, Kemdi S, Bonnet L, Borenzstajn-Rotem Y, Katzenelson E** (1980): *Isolation and identification of pathogenic microorganisms at wastewater-irrigated fields: ratios in air and wastewater.* Appl Environ Microbiol, **39**, 1183-1190.
32. **Thayer DW, Rajkowski KT, Boyd G, Cooke PH, Soroka DS** (2003): *Inactivation of Escherichia coli O157:H7 and Salmonella by gamma irradiation of alfalfa seed intended for production of food sprouts*. J Food Protect, **66**,175-181.
33. **Thompson JE, Blatchley ER** (2000): *Gamma irradiation for inactivation of C.parvum, E.coli and Coliphage MS-2.* J Envir Engrg, **126**,761-768.
34. **US-EPA** (2003): *Environmental Regulations and Technology*, *EPA/625/R-92/0., Appendix F- Sample preparation for fecal coliform tests and Salmonella sp. analysis*. Environmental Protection Agency, Washington, DC. Reaching: http://www.epa.gov/nrmrl/pubs/625r92013/625R92013.pdf Access date : 28 January 2005
35. **US-EPA** (2004): *Use of Reclaimed Water and Sludge in Food Crop Production, Chapter 5- Public health concerns about infectious disease agents*, pp 89-99. Reaching : http://www.epa.gov/owmitnet/mtb/biosolids/useofmid/mstr-ch5.pdf Access date : 03 June 2005
36. **Wizigmann, I., and E. Wursching.** (1975): *Experience with a pilot plant for the irradiation of sewage sludge: bacterial and parasitological studies after irradiation*. p. 477-484. In proceedings of the IAEA symposium on radiation for a clean environment. International Atomic Energy Agency, Vienna, Austria.
37. **World Health Organization** (1989): *Health guidelines for the safe use of wastewater in agriculture and aquaculture.* Tech Rep Series 778. WHO, Geneva.
38. **Zagory, D.** (2000): *Produce irradiation – The not-so-silver-bullet.* Reaching: http://www.davisfreshtech.com/articles_pro_irradiation.pdf Access date : 08 May 2006

Geliş tarihi: 10.06.2009 / Kabul tarihi: 01.02.2010

Address for correspondence
Zişan Emre, DVM, Ph.D.
Sarayköy Nükleer Araştırma ve Eğitim Merkezi
Hayvancılık Birimi
Saray Mah. Atom Cad. No.27
06983 Kazan, Ankara, Turkey
e-mail : zisan.emre@taek.gov.tr