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Effects of Gamma Irradiation on Fungal Growth and Associated Pathogens

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Abstract: Microorganisms from chlorine treated sewage water samples from Al-Ahsa municipal sewage plant were irradiated with gamma rays at doses of 10.0, 15.0, 20.0 and 25.0 kGy. The microorganisms identified were the filamentous fungi, *Aspergillus fumigatus* and *Absedia* spp. and the yeast *Candida silvicola* (Hansenula *holstii*), *Cryptococcus laurentii* and *Candida sake*. Microbial counts were made immediately after irradiation. Although, the damage to microorganisms increased with an increase in irradiation dose but even the highest dose did not completely sterilize the water. The microbiological results revealed that irradiation above 25.0 kGy completely inhibited the growth of all the microorganisms. However, a high dose of irradiation of 25.0 kGy did not show the inhibitory effect on the growth of *Candida sake*. Whereas *Cryptococcus laurentii*, *Aspergillus fumigates* and *Absedia* sp. were killed by10.0 kGy. The results obtained highlighted the potential of this technology for wastewater treatment.

Key words: Gamma rays, fungi, microorganisms, pathogens, wastewater, chlorine

INTRODUCTION

Major cities around the kingdom generate large volumes of wastewater and are generally inefficient users of water (Akers, 1994, 1997; Basel *et al*., 2002; Ben Arie *et al*., 1969). Therefore, not only the consequences of wastewater are critical for the environment, but water is also critically wasted. Furthermore, the potential for environmental pollution is greater due to high quantity of waste materials produced namely the organic matter (Kenaga, 1974). Therefore, due to strict legislation and control to protect health and the environment, new approaches should be taken to overcome this situation, including remediation and the reuse of wastewater. Although, it is under scrutiny, chlorine is still a widely used chemical disinfectant for the treatment of wastewater. In the United States, many municipal discharges are treated in this way, usually after secondary treatment. In the United Kingdom, chlorination has been considered for the disinfection of primary or crude discharges to improve water quality in the vicinity of bathingwater to aid compliance with the European Community's Bathingwater Directive (Ajaz *et al*., 2004). Treated water discharged from sewage treatment plant can be reused for irrigation. Prescribed water quality standards for recreational and other water uses, such as shellfish production, are typically based on contamination levels as indicated by fecal coliform bacteria levels in effluents. Therefore, disinfection is frequently used to improve recreational bathingwater which do not comply with microbiological standards (Maxie *et al*., 1964). With the increase in population in many of the big cities and the growing industrialization in many countries, the pollution load on the environment is increasing. Of particular concern is the waste materials containing pathogenic bacteria, parasites, fungi and viruses. Destruction of microorganism's flora became very important and urgent practical issue. To overcome this problem, fungicides and bacteriocides are applied for the control of these disease generators. However, excessive use of the cidel agents has resulted in serious problems of chemical residues in the environment and of high health risks. Thus, alternative control strategies have encouraged the scientists to do the research. Recently, ionizing irradiation is interested to apply especially gamma ray which is generated from Cobalt-60. An important advantage of gamma radiation over most chemical treatments, which derives from its short wavelength, is its ability to penetrate into the tissues. It was reported that ionizing radiation induces both the degradation of numerous compounds and the inactivation of microorganisms depending on the type of energy, dose rate and absorbed dose (Klieber *et al*., 2002; Drzewicz and Gehringer, 2005; Casimiro *et al*., 2008). Powerful oxidizing and reducing species (e.g., OH, e_{aq}^{A}, H) and molecular products (e.g., H_2, H_2O_2) are produced due to the interaction between gamma radiation and water, so these chain reactions lead to the phenomenon as described earlier by Parker and Darby (1995) and Sommers and Glenn (2006). Therefore, the effects of gamma irradiation on the inactivation of microorganisms, mainly on the fungi and associated pathogens were investigated.

MATERIALS AND METHODS

The experiment was carried out at Department of Microbiology, King Faisal University, Al-Ahs during 2007-2008.

Collection of Water Samples

A total of 28 chlorinated samples were taken from the Wastewater Treatment Plant (WTP), the sub-samples were exposed to γ-irradiation. Wastewater samples were refrigerated after being transported to the laboratory in an ice packed cooler. Biological samples were generally finished within 7 or 8 h of sample collection.

Fungal Isolation and Identification

Aspergillus fumigatus, *Absedia* sp., *Hansenula holstii*, *Cryptococcus laurentii* and *Candida sake* were isolated from sewage water. The fungal mycelium was transferred to fresh medium. The fungi genus were identified under light microscope. Pure culture of each fungus was grown in PDA and used throughout in this experiment to evaluate the microbial population. Water samples were vortex mixed and then serial tenfold dilution were prepared with sterile buffered water (Daubner, 1967) to reach final concentrations. Each sample was spread, in triplicate, onto Petri dishes containing Sabouraud Agar (SA) added of chloramphenicol (500 mg L^{-1}). Each water sample (0.5 mL, undiluted) was also spread onto Petri dishes as above. The plates were incubated at 28°C. As soon as the first colonies were developed, they were transferred to test tubes containing SA. After the purity of the colonies was confirmed, they were sub-cultured onto potato dextrose agar, malt agar or czapeck, in glass tubes. Fungal identification was carried out by macroscopic and microscopic observation of colonies and also by micro-culture on a microscope glass slide for species identification according to the procedure of Rapper and Fennell (1977).

The yeasts were routinely isolated and cultured on PDA medium (potato, 20%; glucose 2%; agar, 2%), YM medium (peptone, 0.5%; yeast extract, 0.3%; malt extract, 0.3%; glucose, 1%; agar, 2%) or Czapek-Dox-glucose medium (NaNO, 0.3%; K_2HPO_4, 0.1%; $MgSO_,$. 7H, 0.5%; KCl, 0.05%; $FeSO_4$-$7H_2O$, 0.001%; glucose, 3%; agar, 2%) at 7°C, 22°C or 30°C aerobically for 3 to 14 days. Fermentation and utilization of carbohydrates by yeasts were carried out by following the methods of Goto *et al*., 1969. The characterization of yeasts was mainly performed according to Kreger-Van Rij (1984).

Gamma Irradiation

Prior to chlorine inactivation, samples of primary effluent were taken from sewage treatment works at Al-Ahsa, kingdom of Saudi Arabia. The samples were exposed to gamma radiation with four doses such as 0 (control), 10.0, 15.0, 20.0 and 25.0 kGy from a Cobalt-60 gamma source at the Office of Atoms for Peace, King Abdulaziz City for Science and Technology (KACST), Riyadh, Kingdom of Saudi Arabia. The treated samples wer kept at room temperature (25°C). The absorbed radiation doses were confirmed by reading the dosimeter with spectrophotometer (Bausch and Lomb, 1001 Plus, Spectronic) at the wavelength of 530 nm. The plates were incubated at room temperature (25°C). The daily radial measurements of growth were recorded until the fungus reached the edge of the plate. Each treatment was replicated 8-times. Percentage of mycelial growth was calculated in comparison with growth in the control plate and exposed to gamma irradiation from a cobalt 60 source at four different doses i.e., 10, 15, 20 and 25 kGy.

RESULTS AND DISCUSSION

The treatment of gamma irradiation on growth of microorganisms showed that The radiation doses above 10.0 kGy completely killed the *Cryptococcus laurentii* (Fig. 1 A, B), whereas the highest dose of 25.0 kGy could not kill *Candida sake* (Fig. 1 C-E). Irradiation above 10.0 kGy completely inhibited the growth of *Cryptococcus laurentii, Hansenula holstii, Absedia* spp. and *Aspergillus. Fumegatous* (Fig. 1 A-B). The minimum lethal effective dose of 6 kGy (600 krad) was established for the *Aspergillus spp. Mold.* However, cultured candida was not affected by a radiation dose of 6 kGy. The same results were obtained in Roumania by Beljajevova (1960) and Paun *et al*. (1978) on testing sterilization of 30 types of molds who found a minimum effective dose of 7 kGy. The most resistant were cultures of *Stemphylium* and *Stachybotritis atra*. Urban (1983) tested four types of molds- *Aspergillus flavus, Penicillium spinulosum, Chartonium globosum* and *Aspergillus niger* and found that the dose of 6 kGy eliminated all cultures tested. It was found that 8 kGy is the minimum effective radiation dose (Urban, 1983). In our study, it was found that the radiation dose of 10.0 kGy completely inhibited the growth of *Aspergillus. Fumegatous*. The complete inhibition of fungal growth was reported by Smith and Pillai (2004) who found that the gamma ray destroys DNA structure of cells and cells cannot continue their function, while incomplete inhibition may result from a little injury of cells. Tauxe (2001) reported that the high energy rays of irradiation directly damage the DNA of living organisms, inducing cross-linkages and other changes that make an organism unable to grow or reproduce. When these rays interact with water molecules in an organism, they generate transient free radicals that can cause additional indirect damage to DNA. Moreover, other factors may be involved to the sensitivity or tolerance of fungi on gamma ray. It was also reported that multi-cellular spore or bi-cellular spores are more tolerant to gamma radiation than the unicellular spore (Sommer *et al*., 1964; Satish *et al*., 2005). Thus, this may be one of the reasons that unicellular spore of *Cryptococcus laurentii* showed more the sensitive to gamma radiation than the multi-cellular spore of *Candida sake*. Moreover, the number or density of mycelium in the inoculum exposed to radiation may affect the radiation dose required for the inactivation of microorganism. Generally, the increased mycelium density in the inoculums need to elevate radiation dose (Barkai-Golan, 1992). Since, characteristic of *Candida sake* colonies on PDA medium is similarly to the slime and high density. This result may explain that the survival of *Candida sake* after exposing to high dose (25 kGy) irradiation may cause by the characteristic of this. Sometime, irradiation cannot kill pathogens completely but it may results in cell damage (Smith and Pillai, 2004) and directly harm the chromosomal DNA of living cell (Barkai-Golan, 2001). The damaging of nuclear DNA may cause the mutagenesis and some genetic materials of pathogens probably mutate to be more high or low virulence. Many pathogens produce extracellular products that may influence their growth, be determinant factors in their pathogenic capability, or contribute to their pathogenicity or virulence (Kolattukudy, 1985; Salmond, 1994). The mechanisms associated with resistance have received uneven attention (Bank and Corrigan, 1995). For most disinfectants, the studies are largely on phenomenological descriptions of the occurrence. Much less is known about the frequency with which resistance develops and the impact of environmental factors

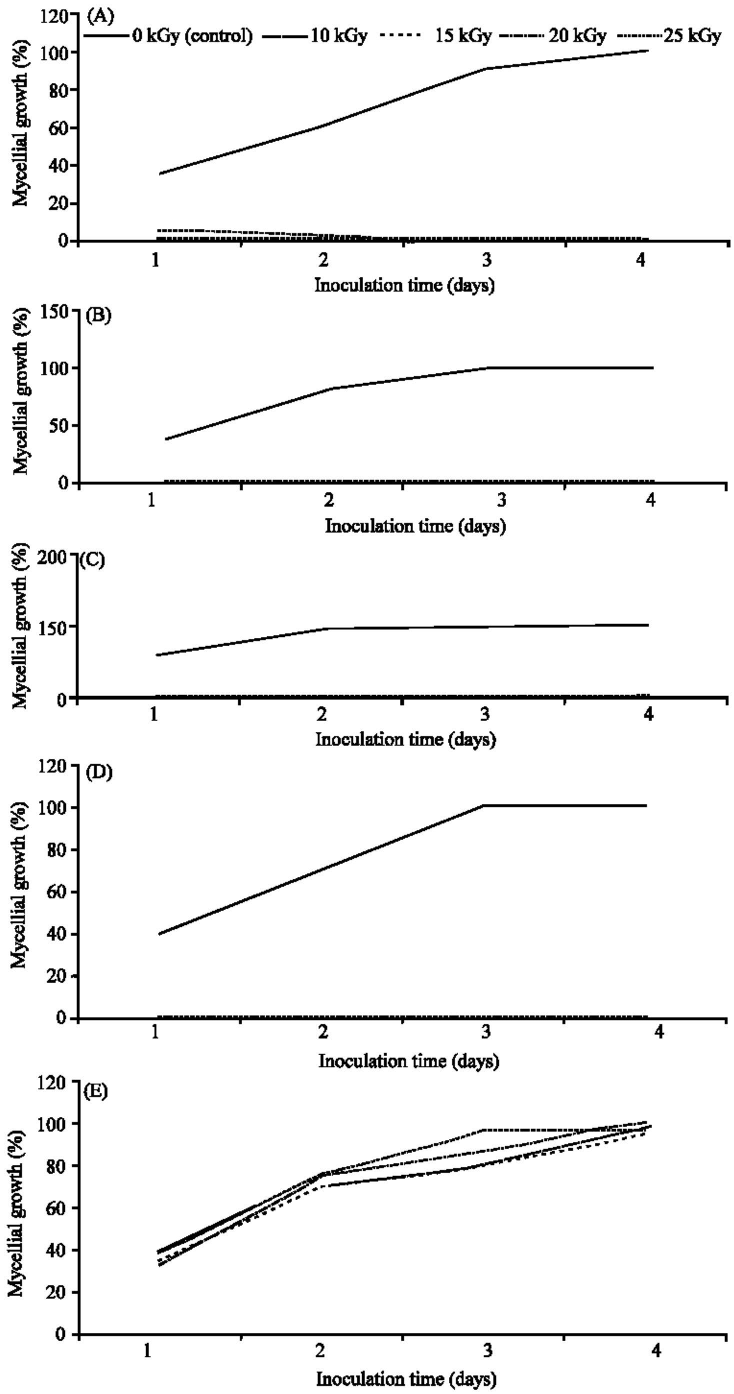

Fig. 1: Effect of gamma irradiation at 0.0, 10.0, 15.0, 20.0 and 25.0 kGy on mycelial growth of (A) Absedia spp., (B) *Aspergillus fumegatous*, (C-E) *Cryptococcus laurentii*, *Hansenula holstii* and *C. sake*, on potato dextrose agar at days 1-4, respectively

Table 1: Contribution of various organisms to the total biota found in water samples (Percent contribution)

Dose (kGy)	*Cryptococcus laurentii*	*Hansenula holstii*	*Candida sake*	*Aspergillus fumegatous*	Absedia sp.
0	10	5	5	10	5
10.0	0	0	5	0	0
15.0	0	0	5	0	0
20.0	0	0	5	0	0
25.0	0	0	5	0	0

on resistance development (Chapman, 2003). The thick wall of a cell or spore is a reasonable explanation for resistance to irradiation because this kind of non-ionizing radiation penetrates weakly (Gherbawy, 1998). It seems that physiological or genetic changes, such as phenotypic adaptation, genetic alteration, or genetic acquisition, must have been developed (Cloete, 2003). It has been reported that some genes special resistant phenotypes are located together on mobile genetic elements such as a plasmid, transposon, or integron (Chapman, 2003; Barkai-Golan *et al*., 1971). Therefore, the development of resistance to one antibacterial agent is always accompanied by the appearance of resistance to another agent (Dukan and Touati, 1996; John *et al*., 1994; Pavon, 1975-76). Contribution of various organism is shown in Table 1.

CONCLUSION

The development and implementation of alternative technologies for the cleanup of industrial wastewater, municipal water, groundwater and drinking water is critical to the sustainability of many countries. Among the possible water treatment alternatives radiation processing, a very effective form of energy use, can degrade toxic organic compounds and biological contaminants. Conventional wastewater disinfection is typically accomplished via chlorination, ozonation or ultraviolet (UV) irradiation. For each of these disinfectants, changes in the composition of the disinfected water are known to occur and have been linked to changes in the toxicity responses of the disinfected effluent. The toxicity responses of wastewater effluent samples exposed to an alternative disinfectant, gamma (γ) radiation were studied (Prakash *et al*., 2000; Barkai *et al*., 1969). The radiation treatment of sewage effluent offers an efficient, simple and reliable method to produce pathogen-free sludge, which can be further upgraded to produce bio-fertilizers. The important pathogens of *Cryptococcus* sp. (Paull *et al*., 1998) cause human meningitis. To overcome this problem, fungicides are applied for controlling this disease. However, excessive use of these fungicides will contaminate the ecosystem. while a radiation dose of 10.0 kGy from a high power accelerators are used as well-controlled sources for wastewater treatment to save parameter. Currently, radiation processing is used in a number of industrial processes including sterilization, cross linking of polymers, food irradiation, rubber vulcanization in the manufacturing of tires, contaminated medical waste etc. These are examples of well-established economical applications of radiation processes employing gamma and electron beam sources

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