RADIATION PROCESSING TECHNOLOGY APPLICATIONS

VOLUME I

A comprehensive text covering various aspects of radiation processing with a focus on its applications in different industry sectors

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Chapter-2

Radiation Technology: Processes and Products – Concepts and Applications

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Abstract

The radiation technology process is based on the physics and chemistry of radiation interactions with matter and the quantities that are used for monitoring radiation energy depositions.

The modifications in a material exposed to ionizing radiation are caused by deposition of energy such as in thermal or chemical processes. However in any thermal or chemical process the energy transfer is relatively small. Taking into account that the binding energies are generally below 12 eV any chemical bond may be broken and/or potential chemical reactions happen during the exposure to ionizing radiation.

The impact of primary radiation energies (≥ 10 keV ≤ 10 MeV) could lead to the degradation and complex interactions with matter which produce a cascade of reactions of secondary lower energies. Ionizing radiation with wavelengths less than 10^{-10} m, such as gamma-rays, X-rays and electron beams have a higher energy, causing electron transitions and atom ionization, but the energy imparted in the system is not enough to change the nucleus into a radioactive isotope.

The primary radiation energies are produced by electron beam accelerators and electromagnetic radiation (X and gamma rays) are produced by machines or by radioisotopes such as cobalt-60 or Caesium-137.

The mean energy, dE imparted by ionizing radiation to an incremental quantity of matter, divided by the mass of that matter, dm, is called the absorbed dose (D). The unit of absorbed dose is joules per kilogram (J/kg) and is expressed in grays (Gy) or multiples of grays (previously rad: 1 Gy = 100 rad).

Ionizing radiation is a clean and environment friendly energy and could be applied to a large range of applications on biology and chemistry fields. Some applications and their ranges of absorbed doses usually applied will be discussed.

Basic Concepts of Ionizing Radiation

The basic concepts presented in this chapter are connected with the application of ionizing radiation on microorganisms and its effects that enables its use for processes and products in Industry.

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The modifications in a material exposed to ionizing radiation are caused by deposition of energy such as in thermal or chemical processes. However, in any thermal or chemical process the energy transfer is relatively small (from a tiny fraction of an eV to less than 10 eV) comparing with ionizing radiation energy that is imparted in quanta of $\geq$ 10 eV. Taking into account that the binding energies are generally below 12 eV any chemical bond may be broken and/or potential chemical reactions happen during the exposure to ionizing radiation. In Figure 1 (In IAEA, 1973 Technical Report series n°149) can be seen a resume of interaction processes of ionizing photons and electron energy less than 10 MeV.

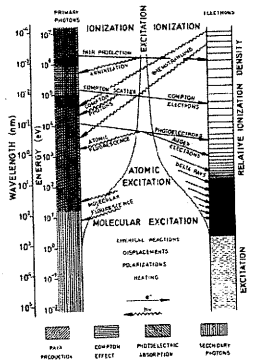

Figure 1 – Interaction processes of ionizing photon and electron energy (< 10 MeV deposited in matter and including important degrade secondary particles and events (in IAEA, Technical Report series n°149, 1973)

The impact of primary radiation energies (≥ 10 keV ≤ 10 MeV) could lead to the degradation and complex interactions with matter which produce a cascade of reactions of secondary lower energies. Ionizing radiation with wavelengths less than 10^{-10} m, such as gamma-rays, X-rays and electron beams have a higher energy, causing electron transitions and atom ionization, but the energy imparted in the system is not enough to change the nucleus into a radioactive isotope.

The primary radiation energies are produced by electron beam accelerators and electromagnetic radiation (X and gamma rays) produced by machines or by radioisotopes such as Cobalt-60 or Caesium-137.

The mean energy, dE imparted by ionizing radiation to an incremental quantity of matter, divided by the mass of that matter, dm, is called the absorbed dose (D). The definition is given strictly for absorbed dose at a point. In radiation processing, it means the average over a finite mass of a given material and is read by a calibrated dosimeter in terms of energy imparted per unit of mass. The unit of absorbed dose is joules per kilogram (J/kg) and is expressed in grays (Gy) or multiples of grays (previously rad: 1 Gy = 100 rad).

Therefore ionizing radiation is a clean and environment friendly energy and could be applied to a large range of applications on biology and chemistry fields. Figure 2 shows some applications and their ranges of absorbed doses usually applied.)

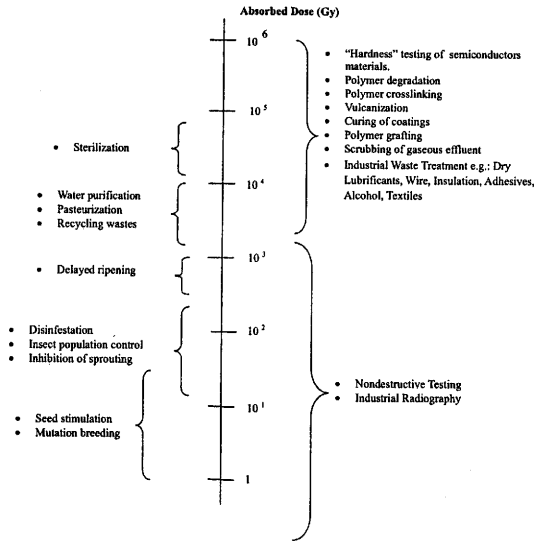

Figure 2 – Various industrial applications of ionizing radiation and doses applied (adapted from Mclaughlin et. al., 1989)

In spite of various industrial applications, this chapter will focus mainly on the microbiological inactivation and some factors that could interfere on its response.

Microbiological Inactivation by Ionizing Radiation

When ionizing radiation is absorbed by biological material, there is a probability that it will act on the critical targets in the cell and induce inactivation. Now, it is generally accepted that the deoxyribonucleic acid (DNA) represents the most critical target of ionizing radiation.

The biomolecules may be ionized or excited by energy deposition, inducing a chain of events that leads to biological change and to cell death. This phenomenon is called the direct effect of radiation, which is the dominant process occurring when dry spores of spore-forming microorganisms are irradiated. Radiation can also interact with other atoms or molecules in the cell, particularly water, to produce free radicals, which include hydrogen radicals ($H^{\cdot}$) hydroxyl radicals ($OH^{\cdot}$) and solvated electrons (e_s^-), which can diffuse through the cell. This effect is designated the indirect effect of radiation and has major importance on vegetative cells, since 80% of the cell is made up of water. These reactive intermediates can then interact with the cell biomolecules. When such systems are irradiated in the presence of oxygen the radicals formed in the biomolecules are converted into the corresponding peroxyl radicals [1].

The cumulative amount of absorbed radiation energy required to inactivate microorganisms depends on several factors. Thus, the dose required for each individual application should be established by risk analysis, taking into consideration the contamination level, the hazard involved, irradiation temperatures, oxygen presence, and the efficiency of the radiation treatment and the fate of critical organisms during formation and storage [2].

Radiation resistances, even under comparable conditions, vary widely among different microorganisms. The resistance can differ from species to species and between strains of the same species [3]. These radiation sensitivity differences among similar groups of microorganisms are correlated to their inherent diversity with respect to the chemical and physical structure as well as their capacity to recover from radiation injuries.

Inactivation Kinetics

Radiation survival follows in most cases exponential kinetics. In order to characterize organisms by their radiation sensitivity, the D_{10} value is used, which is defined as the dose required to inactivate 90% of a population or the dose of irradiation needed to produce a 10-fold reduction in the population. Considering N_o as the initial number of the organisms

present, N the numbers of organisms surviving the radiation dose D and the D_{10} the decimal reduction dose, the exponential survival plot can be represented mathematically by the equation (1): [4]

$$\log N = -\frac{1}{D_{10}} D + \log N_0 \quad (1)$$

The value of D_{10} can be achieved by calculating the inverse of the slope of the regression line obtained.

For heterogeneous microbial population another mathematical model [5] (equation 2) can be applied to describe the microbiota inactivation response to ionizing radiation that can be used to express the Inactivation Assurance Level (IAL):

$$IAL = 1 - \sum_{i=1}^{n} f_i \times \left[1 - \sum P_j (10)^{-\frac{D}{D_j}} \right]^{N_i} \quad (2)$$

in which,

IAL – is the probability of non non-activation (n° of survivors) after exposure to an inactivation process (Inactivation Assurance Level).

Dj – is the resistance response of microbiota present (mixed population) to lethal agent.

Pj – is the probability of occurred of Dj.

Ni – is the centre of class contamination.

fi – is the frequency of Ni.

D – is the absorbed dose (or time of treatment).

However, deviations to the exponential inactivation kinetics are observed experimentally. The most frequent types of inactivation curves that are obtained are schematically presented in Figure 1. Inactivation curves could present curvilinear survival plots which can present an initial shoulder (convex curves), an ending tail (concave curves) or both (sigmoid curves). [6]

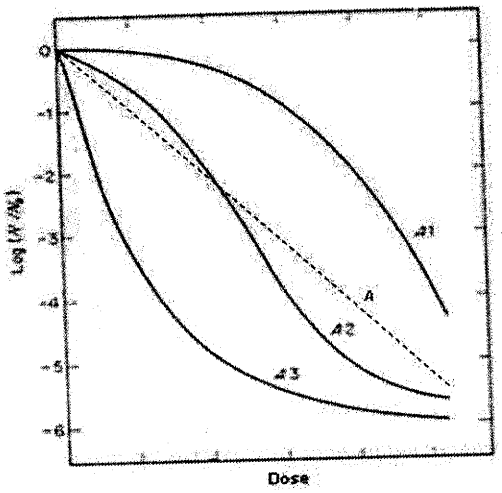

Figure 3 – Representative inactivation curves of most frequent types of response of microorganisms to ionising radiation [6] A – Exponential inactivation curve; A1 – Convex inactivation curve; A2 – Sigmoid inactivation curve; A3 – Concave inactivation curve.

In convex curves a shoulder is observed at low doses and an exponential phase at higher doses. The shoulder may be explained by multiple targets and/or certain repair processes being effective at low doses and becoming inoperative at higher doses.[7] The concave curves can be interpreted as caused by microbial population which are non-homogeneous with regard to resistivity. A higher portion of the less resistant cells are inactivated first, leaving the more resistant cells to tail out.[8] The sigmoid curves can be regarded as a combination of both convex and concave inactivation curves.

Effect of Environmental Parameters

The effectiveness of a given dose depends on intrinsic factors, as reported previously, but also on extracellular environment parameters, such as temperature, gaseous environment, water activity, pH and chemical components of the substrate, as well as dose rate and post-irradiation conditions.

Generally, irradiation at low temperatures increases, while at higher temperatures decreases the resistance of bacteria and viruses [9]. Elevated temperature treatments synergistically enhance the bactericidal effects of ionizing radiation on vegetative cells, possibly due to the repair systems that normally operate at or slightly above ambient temperature that become damaged at higher temperatures.[10] Vegetative microorganisms are considerably more resistant to irradiation at subfreezing temperatures than at ambient temperatures. [11] The decrease in water activity and the restriction of the diffusion of radicals in the frozen state are

possible explanations. Otherwise, bacterial spores are less affected by subfreezing temperatures; [12] since their core has low moisture content and appreciable effect on the already restricted diffusion of radicals would not be probable.

The presence of oxygen increases the lethal effects of ionizing radiation on microbial cells.[13] In anaerobic and wet conditions, the resistance levels of vegetative bacteria may be expected to increase by factors ranging from 2 to about 5 as compared to those in aerated systems. [14] However, this oxygen effect is not always so evidently observed because irradiation itself causes more or less anoxic conditions in a sample, especially when electron radiation is used.

Since part of the effect of ionizing radiation on a microorganism is due to indirect action mediated through radicals, the nature of the medium in which the microorganisms are suspended can play an important role in determining the dose required for a given microbiocidal effect. The more complex the medium, the greater is the competition from its components for the radicals formed by irradiation within the cell, thus "sparing" or "protecting" the microorganisms [15].

The dose rate of the irradiation processes is another parameter that can influence the radiation response of microorganisms. The effect on resistivity usually decreases at high rates [15, 16], probably due to inability of the repair system to respond quickly to the constant induced damages.

Sublethal damage to microorganisms taking place during irradiation can increase their sensitivity to environmental stress factors and other injurious agents (temperature, pH, nutrients, inhibitors, etc.) and synergistic effects of irradiation and other processes could be encountered. [17]

Influence of Type of Radiation on Microorganisms Response to Ionizing Radiation

The type of ionizing radiation used to inactivate microorganisms could lead to different responses and consequently to different treatment efficiencies. The arguments for the relative biological effectiveness (RBE) of the different types of radiation based on radiation biology studies can come to very different and opposite conclusions as shown in the following examples.

The inactivation kinetics of the microbial population of drinking water samples was studied in a Cobalt-60 facility (276 kCi) and in a LINAC accelerator (9 MeV). Dosimetric studies using the Fricke dosimeter were carried out in order to ensure similar irradiation conditions. The drinking water samples were irradiated at sub lethal doses (0.5 and 3 kGy) and at dose

rates of 1.3 kGy/h and of 1.0 kGy/h for gamma and electron beam radiation, respectively. The survival curves obtained are presented in Figure 4.

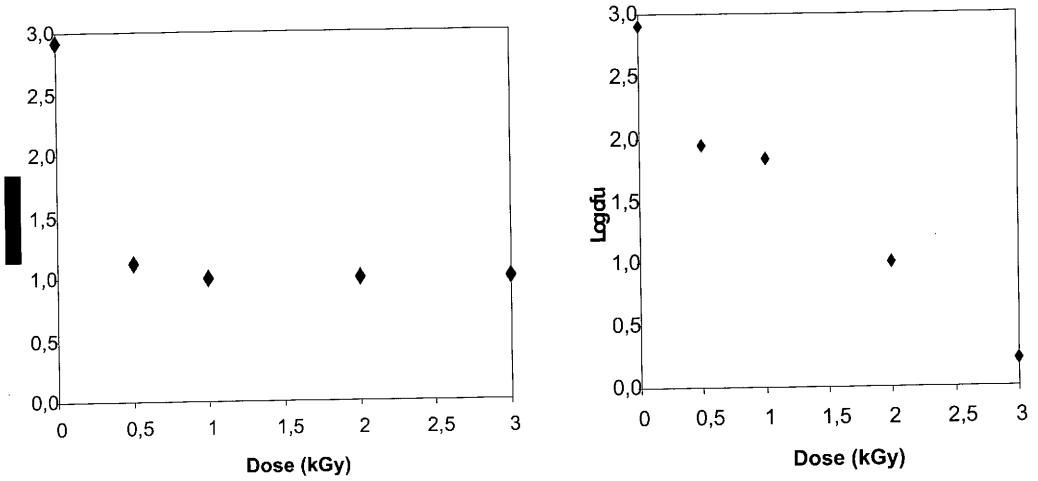

Figure 4 – Inactivation curve of total microrganisms of a drinking water sample by: A) gamma radiation and a dose rate of 1.3 kGy/h and B) electron beam and a dose rate of 1.0 kGy/h. For each point n = 6.

The inactivation response of drinking water natural microbiota was found to be different for both type of radiation. Whereas for gamma radiation the survival curve obtained presented a tail, for electron beam the total microbiota seem to follow linear inactivation kinetics. These dissimilarities can also be numerically observed in the estimated inactivation efficiencies and D_{10} values presented in the Table 1.

Table 1 – Inactivation Efficiencies and D_{10} Values Estimated for Total Microbiota Population of Drinking Water Samples Submitted to Sub Lethal Doses Of Gamma Radiation and Electron Beam.

		Gamma Radiation			Electron beam		
	Dose (kGy)	Max. Effic. (%)	Min. Effic. (%)	Dvalu (kGy)	Max. Effic. (%)	Min Effic. (%)	D10 value (kGy)
Total Microorganisms	0.5	99.7636	99.1864	1.20	99.9727	99.1864	2.25
	1.0	100.0000	99.9661		100.0000	96.2373	
	2.0	100.0000	99.9972		100.0000	100.0000	
	3 0	100.0000	99.9989		100.0000	99.4407	

The same approach (irradiation equipments and dosimetry) was applied to study the influence of the type of radiation (gamma vs. electron beam) in natural microflora of shell eggs. The survival curves obtained for both types of radiation (Figure 3).fit exponential inactivation kinetics ($r2 \geq 0.96$) with estimated Dvalues of 1.15 ± 0.06 kGy and 1.36 ± 0.19 kGy for gamma and electron beam radiation, respectively. The statistical analysis of the results indicated that the inactivation kinetics of natural contaminants of shell eggs by gamma radiation and electron beam was not significantly different ($P>0.05$). In opposition to the drinking water measurements, the results of this study suggested that the type of radiation did not influence the microorganism's inactivation response.

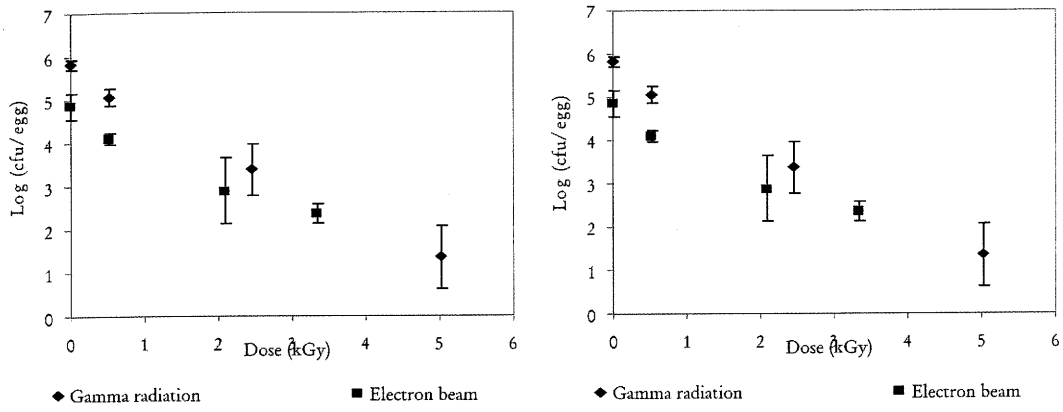

Figure 5 Inactivation curves of total microorganisms of shell eggs by gamma radiation (dose rate = 1.2 kGy/h) & electron beam (dose rate = 1.1 kGy/h). ($12 \leq n \leq 24$; $\alpha = 0.05$).

In an investigation performed by Lowy et al. [18] the effects of neutrons and gamma photons were compared during influenza A virus inactivation. In accordance to the results obtained by these authors the D_{10} values for neutron exposure were found to be consistently greater than those for gamma (Table 2). It was concluded that the neutrons were much less effective in inactivating influenza A virus in comparison to gamma rays.

Table 2 – D_{10} values for radiation inactivation by gamma rays and neutrons (dose rate = 3.9 kGy/h) of influenza A virus strains. [18]

	D10 (kGy) ± SE	
Virus strain	Gamma rays	Neutrons
X31	2.46 ± 0.152	5.49 ± 0.267
PR8	2.82 ± 0.094	4.89 ± 0.535

The results presented previously suggested that there is no pattern related to the presence/absence of the effect of type of radiation in the inactivation of microorganims. Possibly this effect correlates with other effects, for instance the substrate effect.

Influence of Irradiation Substrate on Microorganisms Response to Ionising Radiation

As already noted, the nature of the medium in which the microorganisms are suspended could have an important role in determining the dose required for a given microbiocidal effect. Some chemical components of the substrate medium could have a protective effect (increase the radioresistance) and others a sensitizing effect (lower the radioresistance). As examples of protective components are alcohols, carbohydrates, proteins and sulphydryl containing compounds; on the opposite side there are the nitrites, nitrates and quinones. [15] Table 3A compares food irradiation applications and shows the results [19] obtained by various authors [20, 21]. From Tables 3A and 3B it is noticeable that the same microorganism could present different D_{10} values depending the product in which is present.

Table 3A D_{10} for Selected Pathogens

MICROORGANISM D_{10} VALUES

Organism	Group	D10 Value (kGy)	Substrate	Temp. (°C)	Ref.
Campylobacter jejuni		0.16 to 0.20	Poultry	5	[1]
Escherichia coli O157:H7		0.30 ± 0.02	Beef Poultry	or 5	[2,3]
Listeria monocytogenes		0.45 ± 0.03	Beef	5	[3]
Salmonella spp.		0.70 ± 0.04	Beef	5	[3]
Staphylococcus aureus		0.46 ± 0.02	Beef	5	[3]
Bacillus cereus spore		2.46 ± 0.31	Beef	5	[4]
Clostridium botulinum spore		3.56	Poultry	-30	[5]
Moraxella nonliquifaciens		5.3 - 5.8	Beef	-30	[6]
Bacillus anthracis		5.5	(war strains)	Ambient	[7]
		3.8	(war strains)	Ambient	[8]
Foot & mouth disease	Picorna	5.3		-68	[9]
Swine vesicular disease	Picorna	5		-68	[9]
	Picorna	1.1-5.2		Ambient	[10]
	Entero				
	Picorna	2.8		Ambient	[11]
Vesicular stomatitis	Rhabdo	2.9		-68	[9]

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Newcastle	Paramyxo	5.2		Ambient	[10]
Rinderpest	Paramyxo	?			
Peste des petits ruminants	Paramyxo	?			
Rift Valley fever	Bunya	<2.0		Ambient	[11]
Bluetongue	Reo	8.3		-68	[9]
African horse sickness	Reo	?			
Sheep/goat pox	Orthopox	2.2		Ambient	[11]
Hog Cholera	Toga	5.5		Ambient	[9]
Fowl Plague	Orthomyxo	?			
Influenza A	Orthomyxo	2 5-7 1		-40	[10 12]

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Table 3B – D_{10} values for potential patoghenic microorganisms in food substrates.

Microorganisms	Substrate	D10 values (kGy)
Salmonella Enteritidis	Shell eggs	0.2 0.3 19
	Prawn (surface)	0.5 20
Salmonella Typhimurium	Shell eggs	0.3 0.4 19
	Meat	0.4 0.8 21
Campylobacter jejuni	Shell eggs	0.07 0.2 19
	Meat	0.08 0.3 21

These results point out the vulnerability of the D10 value as the only parameters to rely in the establishment of an irradiation treatment process.

Influence of dose rate on microorganims response to ionising radiation

As noted previously, the substrate effect could be linked to other irradiation parameters such as dose rate. There is still a debate relating to the presence/absence of a dose rate effect. [15, 16].

In order to find out the influence of dose rate and substrate in the waste water treatment process, an experimental design was used. Briefly, a suspension of pink-pigmented bacteria (isolated from a wastewater sample irradiated at 30 kGy) was inoculated in two different substrates. Tryptic Soy Broth (TSB) is usually used as a nutrient complete culture medium, and waste water (WW) sample sterilized, and irradiated at three different dose rates (0.08, 0.8 and 9 kGy/h). The response of the radioresistant microorganism to the six different irradiation conditions and the determination of the D_{10} values, demonstrate a higher resistance with the wastewater substrate than at TSB, is probably due to protecting

components of wastewater, and an increasing D_{10} value with the decrease of dose rate (Table 4). The results also demonstrate an increasing difference between the D_{10} values of the two substrates with the diminishing of the dose rate.

Table 4 Radiation response (D_{10} value) of microorganism suspended in two substrates and three dose rates.

Dose rate (kGy/h)	Substrate	D10 value (kGy)
9	TSB	2.9
	WW	2.0
0.8	TSB	14.1
	WW	19.4
0.08	TSB	21.0
	WW	43.9

TSB – Tryptic Soy Broth a nutrient liquid medium. WW – Wastewater.

These observations could lead to the conclusion that there is a dose rate effect. The results obtained in a study with another pharmaceutical product corroborates this hypothesis. The survival curves of Agrobacterium radiobacter (bacteria isolated from a pharmaceutical product) in the pharmaceutical product obtained for two dose rates, 0.8 and 0.02 kGy, pointed out to higher radioresistances at lower dose rate (Figure 6).

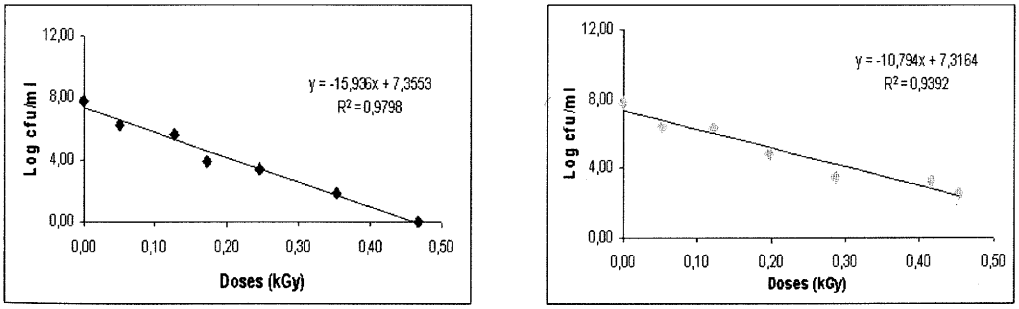

Figure 6 – Inactivation curve of a strain of Agrobacterium radiobacter in a pharmaceutical product at two dose rates: A) 0.8 kGy/h and B) 0.02 kGy/h.

This result could indicate that growth had occurred during the irradiation at the lower dose rate. To examine this possibility, measurements were undertaken of the absorbance at 550 nm of the non-irradiated and irradiated products at sub-lethal doses. The results for a dose rate of 0.02 kGy showed an increase in absorbance of the product suspension during irradiation, which is opposite to the non variation observed in the product absorbance for the dose rate of 0.8 kGy/h (Figure 7). These results suggest growth during irradiation at lower dose rates.

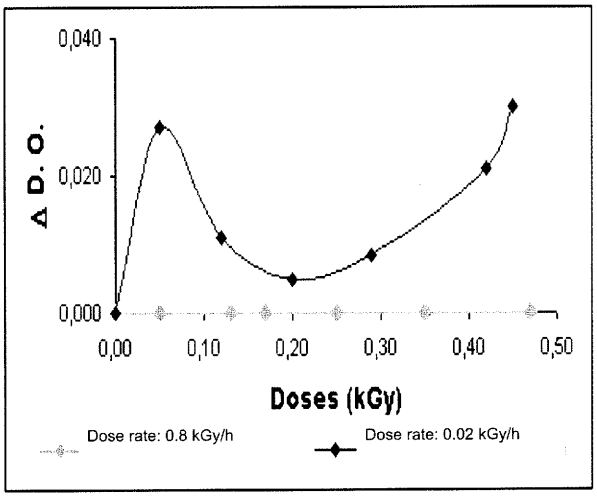

Figure 7 – Variation of the differences of absorbance at 550 nm for the pharmaceutical product irradiated at sub-lethal doses at two dose rates.

Conclusion

The results presented substantiate the influence of dose rate and substrate on the radiation inactivation response of microorganisms. The use of gamma radiation could lead to the selection of radioresistant microorganisms if the irradiation geometry and doses applied are not suitable or adequate. Otherwise, the ionizing radiation technology could be a useful instrument for bioremediation, since microorganisms and chemical species are dynamically inter-dependent [23] [24]. Nowadays, the application of radiation sterilization to medical devices by the Industry is aware of these factors due to the standards developed by International Organization Standardization that guide the validation of the processes [25] [26] [27] in order to guarantee the safety of products for health.

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