Radiation Technologies: Processes and Products

Maria Luisa Botelho

The **Radiation Technologies: Processes and Products** activities focus on the development and demonstration of radiation processing applications. Nowadays these activities are closely related with the gamma radiation facility (UTR) whose main applications are the sterilisation of medical devices and pharmaceuticals and the decontamination of other products.

The group, therefore, supports the sterilisation and the decontamination procedures being carried out at the UTR. This work is carried out in collaboration with the Food, Medical Devices and Pharmaceutical industries. The group develops also work with the National and International bodies of normalisation, standardisation and certification (IPQ, CEN and ISO). UTR upgrade and maintenance and performed in compliance with ISO 9002 in the perspective of obtaining accreditation.

In order to develop the procedures at UTR, based on the Quality System, studies of dose distribution and determination of D_{min} and D_{max} are performed taking into account the Safety Assurance Level and the safety of the product.

The Group develops work studying the impact of radiation on viable (e.g.: micro-organisms) and nonviable (e.g.: synthetic and natural polymers) materials.

The main purpose of the **microbiological** work (**viable materials**) is to develop and implement the validation technologies for inactivation procedures of micro-organisms mainly by radiation (e.g.: γ and ε beam). These technologies are based on microbiological studies on bioburden in/on the products in order to improve the quality in this field. Thus, the hazard analysis and the control of critical points in the production lines of the products take part of the validation studies. Environmental control in surgical operation theatres at hospitals is also carried out.

The action mechanisms of the lethal agents in micro-organisms are studied in order to improve our understanding of the Procedures.

The **nonviable materials** studies aim to use γ radiation for developing new materials or improving their quality and to understand the interaction of radiation with matter in order to avoid damage to the products. Copolymerisation, reticulation and others effects induced by γ radiation are carried out (performed and characterised) in order to improve the properties and biocompatibility of new materials to be used for biomedical and environmental applications.

The Group collaborates with the University of Lisbon (Sciences Faculty/ Plant Biology Department/ Molecular Biology and Genetics Group) and the University of Coimbra (Chemical Engineering Department) .

Research Team

Researchers

M. Eduarda Andrade (Principal Researcher) (Group Leader until her retirement on June 2000)
M. Luisa Botelho (Aux. Researcher) (**Group Leader**)

Luis M. Ferreira* (Research Assistant**) (Responsible for the UTR**)

Students

Sandra Cabo Verde (PRAXIS **PhD** Student)
M.Helena Casimira* (**PhD** Student)

* Doing PhD in the field of Polymers under the supervision of Prof. M.Helena Gil of the University of Coimbra.

Technical Personnel

Helena M. Marcos
Pedro Pereira
Vasco Damas
José Venâncio

Rita Ribeiro (ITN grant)
Ana Paula Marques (ITN grant)

Publications

Journals:	2
Proceedings:	1 in press
Conf. Communications:	2

Funding

	$\times 10^3$ PTE
Research Projects[(a)]:	800
Services:	8344
TOTAL:	9144

(a)

	$\times 10^3$ PTE
- Project BAT under Contract SINDEPEDIP meas. 3.2 (Proc. no. 25/00243) (BAT/sub-Project – Products Sterilisation) – (800$\times 10^3$ PTE) ITN/Co-ordinator**: Luisa Botelho**, Partners: OFTALDER (Ana Baptista), INETI/LMI and DTIA	800

Influence of Matrix on the Response of Microorganisms

M.L. Botelho, H.M. Marcos, S. Cabo Verde, R. Ribeiro, A.P. Marques

Objectives

The objectives are to study the matrix influence on the response of two strains isolated from garments irradiated by γ and ε beams using traditional methodologies [e.g.: direct spread on TSA plates and count of colony forming units (c.f.u.)]. This work follows the BIOSTER Project L 1347/Medical device Industry.

Results

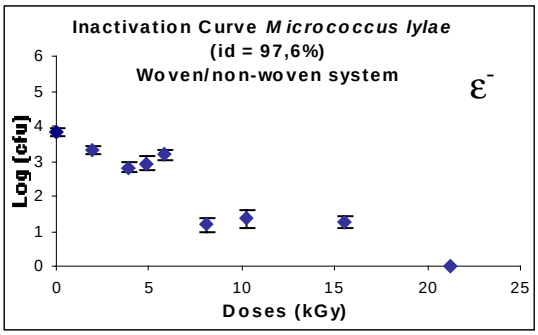
The identification of the two strains using the API system shows that they are of the same genus and species *Micrococcus lylae* (i.d. 97,6 % and 99,9 % respectively).

Droplets (0,02 ml) of suspensions of each strain were dried in polyethylene and woven-non woven test pieces respectively and inactivation curves were worked out (Dose rate = 0,8 kGy/h in September 2000). The results obtained based on the responses to γ radiation are shown in Fig. 1. They indicate that: a) the microorganisms identified as *Micrococcus lylae* showed different D_{10} b) the variations of data and the presence of a "shoulder" point for the presence of agglomerates mainly in woven-non-woven matrices c) the matrix influenced the efficiency of the microorganisms removal and/or the response to the lethal agent → D_{10}.

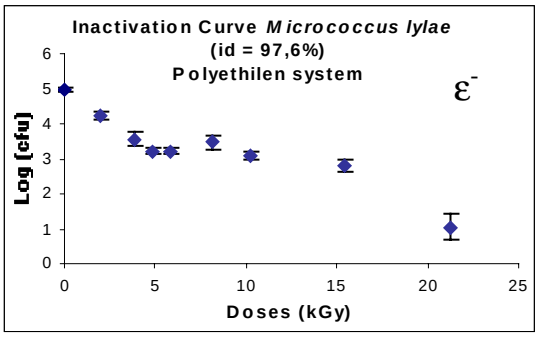
In conclusion indirect methods as the "traditional methodologies" could lead us to an incorrect number of survivors, mainly due to the agglomerated cells.

Further Work

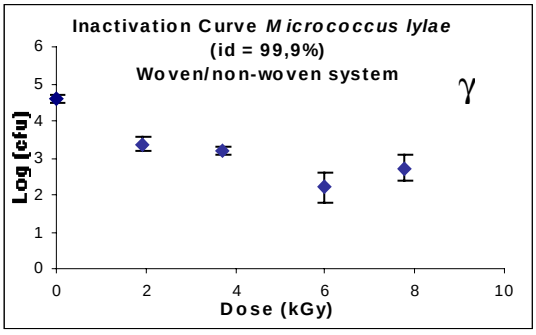
Alternative methodologies to study microoganisms kinetics will be developed (e.g. microcalorimetry) to improve knowledge about the growth and inactivation systems.



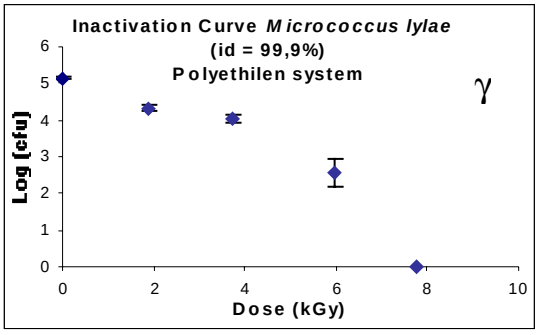
y =-0,1883x + 3,6593;R^2 = 0,7738;n = 76;**D_{10} = 5,3 ± 1,2**



y = -0,1457x + 4,4459; R^2 = 0,8561; n = 71; **D_{10} = 6,9 ± 1,1**



y = -0,2536x + 4,2004 ; R^2 = 0,7601; n = 45; **D_{10} = 3,9 ± 1,4**



y = -0,4120x + 5,2179; R^2 = 0,949; n = 43; **D_{10} = 2,43 ± 0,41**

Fig. 1 - The influence of a matrix in the response of two strains isolated from garments.

Influence of Different Dose Rates on the Radioresistance of Microorganisms

M. L. Botelho, H. M. Marcos, S. Cabo Verde, R. Ribeiro, A.P. Marques,

Objectives

The objectives are a) to study the influence of different dose rates 0,8 kGy/h and 0,02 kGy/ h (September 2000) on the radioresistance of microorganism isolated from a pharmaceutical product composed of sea water (similar to saline water). b) the influence of two substrates (dried and saline water) on spores of *B. Pumilus* E601 after irradiation at the same Dose rate (0,8 kGy/ h Sept. 2000).

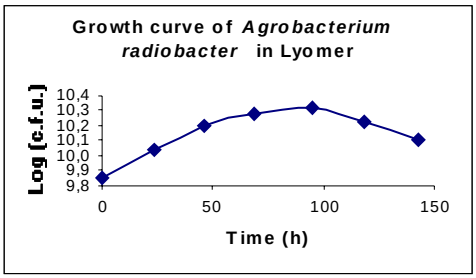
Results

Kinetic studies of the natural contaminant identified as *Agrobacterium radiobacter* (i.d. 85,0 %) were carried out on the pharmaceutical product in order to build up the growth curve (Fig. 1a) and the inactivation curves (Figs 1 b, 1c) [1,2] at the two dose rates.
The inactivation curves of *B. pumilus* suspended in physiological serum (Saline nonviable menstruum) and in a dried state were worked out at the UTR dose rate equal to 0,8 kGy/ h (Sept. 2000) (Fig. 2).

The results indicate that: a) contaminants of the product showed growth using the substrates (residues of components of sea e.g. algae, however, after 4 days the number of survivors decreased); b) after irradiation at two dose rates (Figs. 1b, 1c) it was observed a decrease in the inactivation rate at a lower dose rate (0,02 kGy/h) as shown by the D_{10} values. Higher Dose rate → D_{10}= 0,063 ± 0,004; Lower dose rate → D_{10} = 0,09 ± 0,01. [2]. The D_{10} of *B. pumilus* are similar in both substrates for the same dose rate. Dried system → D_{10}= 3,0 ± 0,2; Saline → D_{10} = 2,8 ± 0,1.
Low Dose rate and/or low activity of ^{60}Co could lead to a low inactivation rate of micro-organisms namely with substrates that allow their growth.

Internal Reports

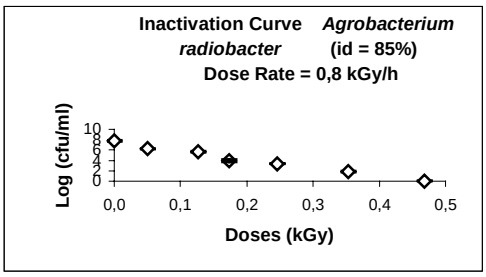
[1] Cabo Verde, S., Growth Kinetic of Natural Contaminants Strains Lyomer Product, Internal Report, October 2000.
[2] Ribeiro, R., Inactivation Curves of Lyfilt 3ª Strain. Internal Report, December 2000.



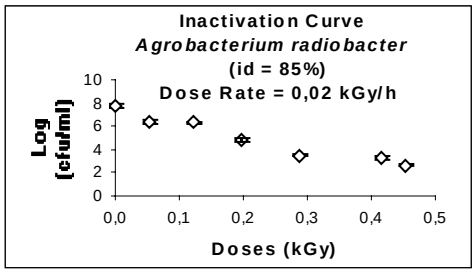
a)

Growth rate = 0,0074 h^{-1} ; Generation time = 41 h

b)



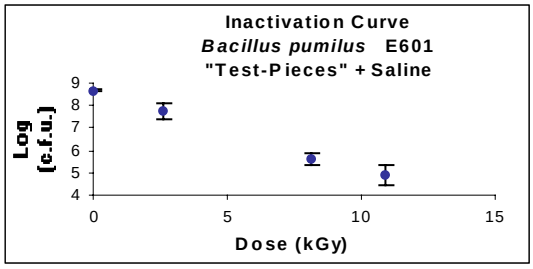
y = -15,936x + 7,3553 ; R^2 = 0,9798 ; n = 72 ; **D_{10} = 0,063 ± 0,004**



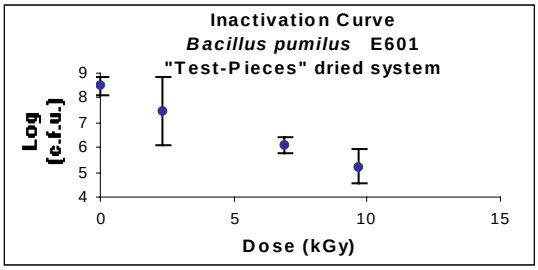
c)

y = -10,794x + 7,3164; R^2 = 0,9392 ; n = 72 ; **D_{10} = 0,09 ± 0,01**

Fig. 1 – The influence of the dose rate on a natural contaminant of a pharmaceutical product composed of sea water and its growth kinetic with the same menstruum.



y = -0,3560x + 8,6500; R^2 = 0,996; n = 96; D_{10} = 2,8 ± 0,1



y = -0,3298x + 8,3800; R^2 = 0,994 ; n = 96 ; D_{10} = 3,0 ± 0,2

Fig. 2–The influence of two substrates on the response of *B. pumilus* at the same dose rate.

Study of the Viability of Radiosterilization for Terricil

M. L. Botelho; H. M. Marcos; S. Cabo Verde; R. Ribeiro; A.P. Marques;

Objectives

Determination of the sterilization dose (D_{min}) versus D_{max}, taking into account the impact on the antibiotic activity of Terricil. This work was part of the Project SINDEPEDIP (proc. Nº 25/002243-Pharmaceutical Industry).

Results

The D_{min} of 13 kGy was determined for the Radiosterilization of Terricil based on the study of the bioburden in three simulated batches of the product. During the first stage, the dose sterilisation of 17 kGy was suggested considering the results obtained by means of a Hazard Analysis of Critical Control Points on the production line. After correction of the main critical points and once the reproducibility of the results has been assured the Sterility Assurance Level of 10^{-6} will be attain with 13 kGy.

Biocontamination Evaluation at Hospital D. Estefânia

M.L. Botelho, H.M. Marcos, S. Cabo Verde, R. Ribeiro, A.P. Marques

Objectives

Biocontamination evaluation of the environment in the surgical operating theatre in the gynaecology ward of Hospital D. Estefânia.

Results

The results obtained in the biocontamination analysis of the operating theatre environment showed us: a) a critical point for future controls in three rooms; b) alert values obtained by two method : settle plates and bio-collectores and c) the contamination of of three stages: "as-built", "at-rest ", and "operational" (see Fig. 1) indicating that the main sources of contamination are the personel and tissues.

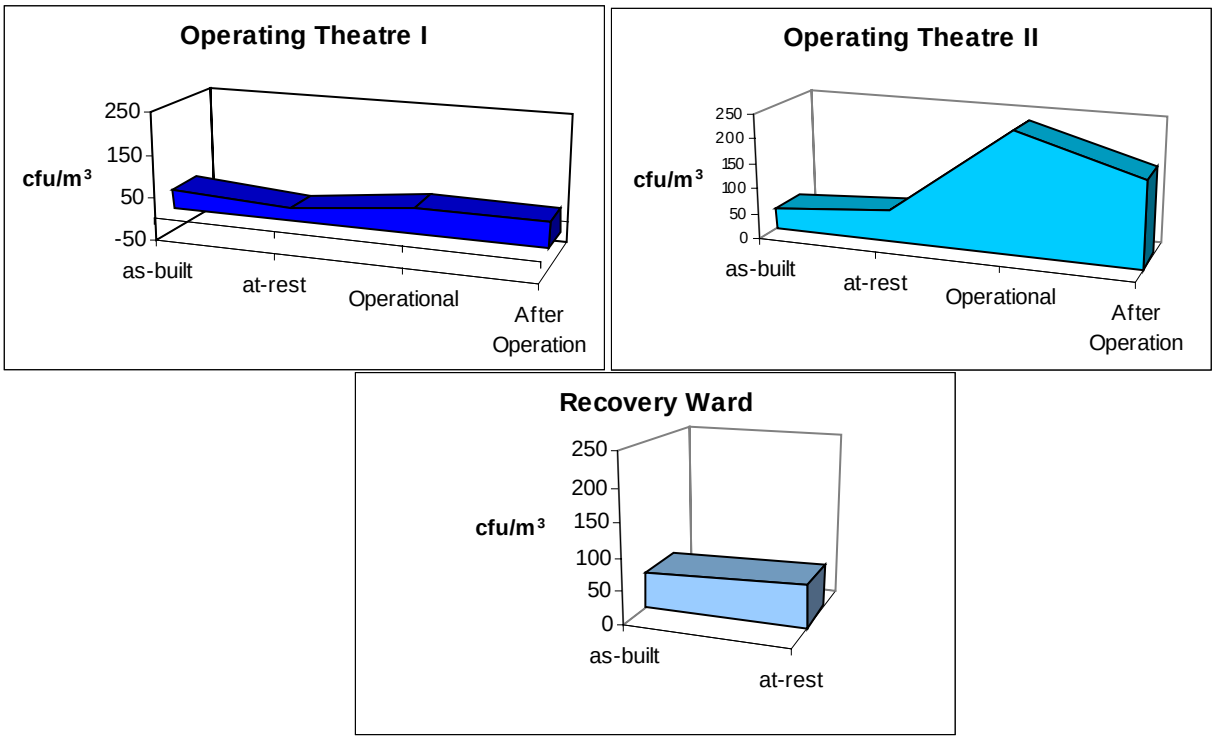


Fig. 1 - Results of the Biocontamination analysis of the operating theatre environment.

Elimination of 2,4,6-Trichloroanisole in Cork Stoppers with Gamma Radiation

M. H. Casimiro, L. M. Ferreira, M. L. Botelho, F. Coutinho[1]

Objectives

Preliminary studies to remove 2,4,6-trichloroanisole (TCA) from cork stoppers by fragmentation induced by gamma radiation.

Results

TCA immobilised in KBr was irradiated at 13, 26, 52, 65, 91, 143 and 195 kGy. Molecular characterisation before and after irradiation was carried out by FTIR. Results point to there is no fragmentation in TCA molecule even after irradiation at 195 kGy (see Figs.1 and 2). Finger tips of TCA at FTIR are characterised beneath 2000 cm^{-1}.

A second approach was developed to extract TCA by vacuum. Sequential extractions in a vacuum line and in a vacuum stove of 20 cork stoppers from each two contaminated lots were made. Results obtained point out to a decrease of TCA.

Based on these results and in order to future application in a semi-industrial scale, a further experimental development was worked out. Three hundred (300) units from a contaminated lot were irradiated with 15 kGy (microbiological decontamination dose). After irradiation, corks were submitted to ethanol washing followed by evacuation and stabilisation at $1.5x10^{-2}$ mbar. The procedure was repeated for three times. Before sealing cork stoppers were submitted to sterile air. The samples were sent to Cork Supply Co. for sensorial analysis (blind essay).
The results obtained showed no TCA decrease.

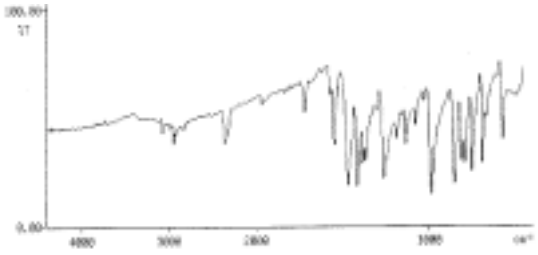


Fig.1: FTIR spectrum of TCA before irradiation

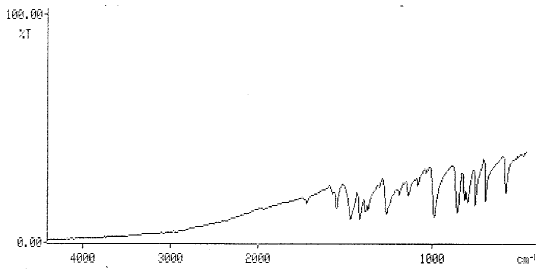


Fig. 2. FTIR spectrum of TCA after irradiation at 195 kGy.

Further Work

Procedures and performance should have further development.

[1] Cork Supply Portugal S.A.

Technical Upgrade of the Radiation Technology Unit (UTR) - 2nd Year

L.M. Ferreira, C. Cruz, P. Pereira, V. Damas, J. Venâncio

Objectives

To endow the UTR with updated technical means to provide a safer operational control and a wider and profitable commercial activity.

Results

The intense technical revision of UTR initiated in 1999 have continued in 2000. The most important developments achieved were:

- Electrification of the workshop area
- Replacement of the entire illumination system inside the irradiation camera
- Identification and reparation of the most critical problems detected in the Cobalt-60 source water-cooling system
- Building of a balneary and a coffee-break area (almost finished)
- Building of a new road to access UTR product admittance gate
- Development and fit up of an operation stop alarm device which allow to contact the operator in prevention
- Introduction of new specifications in the OMRON PLC control program (to overcome some operational needs)
- Repair of the irradiator lift engine
- Repair of the product boxes transport engine (conveyer motor)
- Repair and fit of entire product boxes transport system (transport chain, position sensors and chain tension sensors)
- Assembly of a compressed air pipe line for robotic pneumatic equipment
- Installation of a rehabilitated experimental Cobalt-60 source, for R&D work (still in progress)

Furthermore, the educational program for Cobalt-60 facility operators have continued.

Further work

- Modification /reparation of the facility fight-fire system
- Substitute the old pipes of the Cobalt-60 water-cooling system
- Finish the educational program for new cobalt-60 facility operators
- Complete the installation of the experimental Cobalt-60 source
- Adjust and improvement of the load/unload/re-arrangement product boxes system

Fig.1 - The new road access to UTR product Admittance gate.

Fig.2 - A view of the experimental Co-60 source.

Services

- Technical Audit to IPQ (M. Luísa Botelho)
- Biocontamination evaluation of the environmental of the operation theatre of Hospital D. Estefânia (M. Luísa Botelho)

***Radiation Technology Unit* - Irradiation Services**

Industry

	Type of service
Jaba Farmacêutica, S.A.	Sterilisation of pharmaceutical devices
Vidrolab, Lda	Sterilisation of PET flasks
Oftalder - Prod. Farmacêuticos, S.A.	Sterilisation of ophtalmical dressings
Hovione - Sociedade Química, S.A.	Sterilisation of laboratory packing devices
Lecifarma, Lda	Decontamination of aromatic herbs
Hikma Farmacêutica, Lda	Decontamination of pharmaceutical raw materials
Falcão Teles,Lda	Decontamination of raw materials
Others	Sterilisation of medical devices and pharmaceuticals

Universities and Research Institutes

	Type of service
CERN	Irradiation tests of polymeric samples for high doses dosimetry
INIA	Irradiation tests of ready-to-eat meals
University of Évora	Irradiation tests of acrylic fibers
Others	Irradiation tests of raw materials

ITN

	Type of service
Radiation Technologies Group	Microbiological validation of radiosterilisation and decontamination Kinetic studies of microorganisms
Radiation Technologies Group	Irradiation tests of natural and synthetic polymeric materials
Nuclear Instruments and Methods Group	Irradiation tests for Monte Carlo simulations
Environmental Analytical Chemistry Group	Irradiation of liofilised vegetable tissues
Project STERITEX (Nuclear Instruments and Methods Group)	Dosimetric assays and irradiation tests of disposable medical devices
Project Microstructure Investigation of Organic-Inorganic Hybrid Materials (Condensed Matter Physics Group)	Irradiation tests for the preparation of hybrid solid materials

Irradiation services distribution at UTR during 2000 :