Radiation Technologies: Processes and Products

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Radiation Technologies: Processes and Products is an interdisciplinary group that uses the holistic approach as the key to conceptualize a research or a service. This interdisciplinarity, using Biology, Chemistry and Physics science, allows the study of a subject from various angles and methods unified by a common goal: the validation of methodologies to understand the subject of study.

The group *modus operandi* permits a constant connection with Industries, Universities and other Research groups applying its "way of knowing" in response to requested services, as a collaborator in a research project or in the transmission of knowledge.

Following this concept in 2009 a collaboration was started with a multidisciplinary group named "Investigação em Aplicações Avançadas de Potência Pulsada" (GIAAPP- http://sites.isel.ipl.pt/giaapp/) that intends to apply high-intensity pulsed electric fields (PEF) as a decontamination/sterilization process for liquid products, in food and pharmaceutical line production. The group activities focus on the delineation, development, validation and application of technologies and processes in various fields, such as Environment, Food and Pharmaceutics. As a fundamental part of the validation studies, Risk Analysis is being applied as a process management tool either in production lines of studied products (*e.g.*: food, devices and pharmaceuticals) or in environmental control (*e.g.* hospitals rooms, pharmaceutical industries and buildings energetic certification).

In the scope of ITN mission the group is solicited by the authorities or private industries to undertake a consultant role on sterilization and decontamination procedures mainly applying ionising radiation. The group also develops work with the National and International normalization, standardization and certification bodies (IPQ, CEN and ISO).

Being aware of society's current needs and the demand of Quality, Innovation and Development, the upgrading and renewal of facilities are being carried out in the scope of project REEQ/996/BIO/2005. In the course of this project, modelling tools (Monte Carlo simulations) have been applied to the pre-upgrading phase of ionizing radiation equipments (*e.g.* gamma experimental facility). Other domain of this project has been the design of a renewed layout of an existing building transforming it in an interdisciplinary laboratory with controlled environment in order to assist new applications for radiation technology, among others. These facilities together with the inclusion of automation/robotic systems, in a further stage, have as main purpose to allow researchers of National and International Institutions and Industries to develop radiation technologies and/or to suppress the need of environmental control areas (clean areas) for their work.

The Group's main R&D activities are focused at employing ionising radiation technologies to new processes and applications in Agriculture, Food, Pharmaceutical, Wastewater Treatment and other areas. In order to improve our understanding of the Radiation effects in products integrated methodologies composed by Analytical Methods of Biology, Microbiology, Chemistry and Physics are being used. Molecular Biology new trends based on PCR technique are being developed as a diagnostic tool (*e. g.* potential pathogenic micro-organisms) and as well as fingerprinting methods to assess the bio-diversity profile of environmental samples.

Training and "know-how" diffusion are one of the main issues of this Group reflecting in the attainment of academic degrees (Graduation, M.Sc. and Ph.D.) and in the dissemination of obtained results in the scientific community (publications, workshops and conferences).

The financial support of the group is based on projects, sponsored by National (e.g. FCT, AdI) and International (e.g IAEA) science foundations and expertise services to Industrial Companies.

Research Team

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LETAL up to date

S. Cabo Verde, R. Melo, T. Silva, H. Marcos, S. Oliveira, I. Nunes, V. Dores and M. L. Botelho

Objectives

The Laboratory of Technological Assays in Clean rooms (LETAL) is a multidisciplinary laboratory renewed under the scope of project REEQ/996/BIO/2005. Benefiting of the new infrastructures capabilities microbiologic studies are being developed, either as research experiments, or as a requested service. The outputs and milestones of these studies will be further detailed.

Results

Monitoring of Bonelike® product bioburden – The sterilization process of Bonelike product was previously established by the Group in 2008. As recommended by ISO 11137-2:2006, once the sterilization dose has been established, periodic audits shall be carried out to confirm appropriateness of the sterilization dose. This work is being carried out (requested service for Lusomedicamenta and Medmatinnovation) based on the validated methodology to assess Bonelike bioburden. These assays permit a review of environmental and manufacturing controls and if this follow up indicates lack of control, appropriate action should be taken. Until now the product bioburden has ranged between < 1 and 3 cfu/sample and the determined dose of 26 kGy remain in conformity to sterilize the Bonelike product.

D_{min} establishment of biomaterial based products – In the scope of ionizing radiation processes application it was requested by Ceramed® to establish the sterilization dose (D_{min}) of six pharmaceutical biomaterial products. The followed methodology was based on the validation of specific procedures to characterize products microbiota. The bioburden knowledge was used to correct the production lines and further on to estimate gamma radiation doses that guarantee products safety and quality. As outputs it was identified the critical control points for each product production line and corrective actions were implemented (*e.g.* use of nonpyrogenic water; raw material autoclavation) in order to lower and homogenize the products bioburden. For all the products it was able to validate a Sterilization Dose that guarantees the probability of a non sterile product item in one million processed items (SAL = 10^{-6}). The estimated D_{min} ranged between 17 and 25 kGy. The applied dose establishment methodology allowed a product specific sterilization dose, reducing potential gamma radiation effects on functional and mechanical proprieties of the analyzed products. A strict collaboration with Ceramed continues to improve the stability to ionizing radiation of one of the studied products. This work is being developed by a common fellow.

MycoArchive project – A collaboration project with Coimbra University is being developed since 2007. This project aims to establish a disinfection process for a book archive using gamma radiation. Presently, book contamination sources are being followed up. Archive air and insects were collected and analysed to establish a contamination link with books microbiota. The most frequent microorganism type found were the fungi (63%) and the isolates are being morphologically and genotypically characterized to analyse their relatedness. The archive air point out to be a significant cross source in book contamination.

LPM/MDN PIDDAC project – This collaboration project intended to study the microbiological environment of operating rooms of the Portuguese Military Hospital (HMP) for the prevention of nosocomial infections. During past years a microbial collection of surgical room environment has been collected and typified by bacteriological and molecular methods. The hospital microbial isolates showed to be very genetically diverse (Fig. 1) although it was noticed the persistence of some airborne microorganisms. As project milestone a database of nosocomial microorganisms was construct to be used in the HMP as infection control tool.

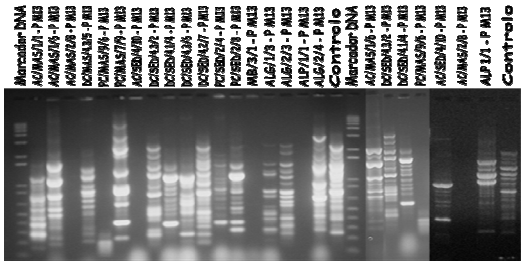

Fig.1 M13 fingerprinting of some airborne nosocomial microorganisms (n = 25) of HMP surgical rooms.

TradeLabor partnership – The air bioburden in national health care services is being evaluated for Air Quality maintenance. All surgical rooms tested presented airborne contamination bellow 10^2 ufc/m^3. Comparing with corresponding previous results the surgical rooms air bioburden is decreasing, suggesting the effectiveness of the proposed corrective actions.

HomeEnergy service - The European Directive no. 2002/91/CE relative to energetic certification of buildings was transposed to the Portuguese law with the inclusion of Indoor Air Quality requirements, such as the evaluation of microbial parameters. As a requested service by HomeEnergy it is being evaluated the bacterial, fungal and *Legionella* spp. bioburden in several building air samples.

Published work

Internal Reports to LusoMedicamenta; February to December 2009: Sterilization dose certificates of Bonelike product batches (n=4).
Internal Report to Ceramed; August 2009: "Validation of sterilization process of biomaterial based products", pp. 1 - 32.
Internal Report to "Clínica do Poetas"; October 2009: "Monitorization of Microbial Indoor Air Quality", pp. 1 - 10.
Internal Reports to HomeEnergy (n=80); since October 2009. Microbial parameters of Indoor Air Quality.

R&D in Chemical Field

R. Melo, J.P. Leal[1], S. Cabo Verde and M. L. Botelho

The applicability of ionizing radiation in the environmental remediation of wastewater is being researched. The presence of biorecalcitrant compounds in wastewater blocks the biological processes. Therefore, radiation degradation of complex compounds is a promising technology to achieve the aforementioned goal. Gallic acid (GA) is a biorecalcitrant phenolic compound and could be considered as a model pollutant of the wastewater generated in the cork boiling process. The chemical oxidation of GA is being studied but there is a lack of information on its radical reactions pathways and degradation products. The evaluation of rate constants as well as the establishment of the partial contributions of the direct and radical reactions pathways to the global process and the identification of potential stable by-products was studied using pulse radiolysis technique and Electrospray ionization (ESI), respectively. The $OH^{\bullet}$ radical and $H^{\bullet}$ atom intermediates of water radiolysis react with the GA molecules yielding cyclohexadienyl type radicals which could react by two ways: (1) loss water molecule forming phenoxyl type radical or (2) reacts with superoxide leading to benzenic ring opening and formation of stable structures. These structures could rearrange and reacts with phenoxyl radical generating stable by-products. The main stable by-products were identified by ESI. Biodegradation studies are being developed using four different carbon sources in a minimal growth medium (Colby&Zatman): (a) without carbon source; (b) with 0,1% methanol; (c) with 0,1 mM gallic acid solution and (d) with irradiated 0,1 mM gallic acid solution. The different substrata were inoculated with *Methylobacterium extorquens* and kinetic growth curves are under study. The present results suggest that there is no effect of carbon source on *M. extorquens* growth. Other Chemical field of study is the validation of dose rate by reference Fricke dosimeter in the upgraded experimental Co-60 source. In the highest dose rate local, the value is approximately 1.1 kGy/h. Several studies are being made to establish isodose curves to a better definition of the irradiator and chamber geometry.

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Advanced applications of high-intensity pulsed electric fields – A2P2

V. Dores, B. Batista, H. Canacsinh[1], L. Redondo[1] and M. L. Botelho

Recent advances in the field of high-intensity pulse power technology allowed the development of new nonthermal sterilization methods. This technology has as main advantages an energy saving, environmental friendly, increase of added-value products, process optimization and cost reduction. The aim of present research in this field is the application of high-field electric pulse technology to inactivate microorganisms in order to develop an industrial scale non thermal sterilization method. Presently the performed studies focus the perception of critical parameters and potential microorganism's inactivation mechanisms for an effective method optimization. This R&D work is under a Protocol in development between several entities and could be seen the participants at http://sites.isel.ipl.pt/giaapp/.

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Implementation of techniques to assess virus infectivity

S. Cabo Verde, S. J. Nascimento[1] and M. L. Botelho

Environmental virology is an emergent field due to the importance of food- and water-transmitted viruses. This area of research is being implemented at LETAL with the purpose of study the inactivation of enteric viruses (e.g. norovirus and adenovirus) by ionizing radiation for disinfection purposes. Murine norovirus (MNV) and human adenovirus 2 (HAd2) stocks were propagated using RAW 264.7 and A549 cell lines, respectively. The viral stocks yields were estimated by plaque assay that is the gold-standard method to measure virus infectivity. This method relies upon (1) the use of confluent monolayers of cells which are susceptible to the virus, (2) the induction of a visible cytopathic effect by the virus and (3) the use of a semisolid overlay which prevents virus diffusion from one infected cell to other nearby cells. As a result, small round plaques (clear areas) form in the cell monolayer as the virus replicates. The estimated MNV titre was 10^6 PFU/ml and sub-lethal gamma irradiations will be carried out further on. For HAd2 the plaque assay technique is being optimized, namely in the type of cell vital staining, for an enhanced visualization of plaques forming units.

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