Impact of Ionizing Radiation on Slaughterhouse Wastewater
- a study case -

M. Luisa Botelho¹, Rita Melo², Joaquim Branco³, Vanda Farinha², Iris Sousa²

¹ Physics Dept., Nuclear and Technological Institute, (ITN) E.N. 10, apartado 21, 2686-953 Sacavém, Portugal
² Post and Pre-Graduated student on leave in Physics Dept., Nuclear and Technological Institute, (ITN) E.N. 10, apartado 21, 2686-953 Sacavém, Portugal
³ Chemical Dept., Nuclear and Technological Institute, (ITN) E.N. 10, apartado 21, 2686-953 Sacavém, Portugal

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Abstract
A preliminary study using gamma radiation for the treatment of CASO slaughterhouse wastewater was undertaken. The samples were subjected to γ-radiation from a ⁶⁰Co source facility, activity 276 kCi (Dec. 2003), in ITN. The results were obtained at dose rate 0.9 kGy per hour and absorbed dose range was 7 up to 25 kGy. After treatment, the wastewater analysis has shown a COD that is inferior (mean 35±4 %) to that of the unirradiated samples, whereas the BOD decreases until 50±5 %. The intensity of the wastewater red colour also decreases after irradiation at 30 kGy and is comparable to that of the CASO anaerobic lagoon. Results on the microorganisms response to γ-radiation, at two doses rate (0.9 kGy h⁻¹ and 3 kGy h⁻¹) were analyzed. An inactivation of 5 log of c.f.u., corresponding to efficiency of 99,999 %, was found for 7 kGy absorbed dose at higher dose rate (3 kGy h⁻¹). At lower dose rate (0.9 kGy h⁻¹), for the same dose, an inactivation of 4 log c.f.u was observed, corresponding to less efficiency (99,986 %).

Therefore, the irradiation of slaughterhouse wastewater with γ-radiation decreases the organic matter and microbiota on the effluent, an important goal of the slaughterhouse industry. Moreover, ionizing radiation could be a useful tool for bioremediation.

1. Introduction
Contamination of surface water and groundwater from industrial waste and anthropogenic activities is a serious problem nowadays and could have drastic consequences for society in the near future. Since the Industrial Revolution, pollution of the environment was considered as being a “price” to pay for progress.
Among the possible wastewater treatments alternatives, remediation is complementary to conventional systems as a means to improve the yields of degradation/destruction of contaminants in both the ground and the water. Portuguese legislation (Decree-Law n.º 47/94, 22th February) related to effluent discharge is becoming increasingly demanding. Therefore the development of new technologies able to minimize discharges and water consumption seem to be the only option. As an example, effluent discharge from slaughterhouses has caused deoxygenation of rivers (Quinn and Farlane, 1989) and contamination of groundwater (Massé, 2000). Not only this has critical consequences for the environment, but also water is critically overspent in the process. The potential pollution is due to the high quantity of wastes produced in “line production”, namely organic matter such as blood, which degrades difficultly. The water wasted in the process is due to the demands of attaining GMP (Good Manufacturing Practice). Therefore, new challenges are needed to rebound this, leading to remediation and reuse of water.

Ionizing radiation technologies are currently used in a number of industrial processes including sterilization (Becker, 2002; Nogueira, et al., 1998; Botelho, et al., 1987) food radiation (Cabo Verde, 2004a, b; Trigo and Fraqueza, 1998), cross-linking of polymers (Casimiro, 2004) and contaminated wastes treatment (Ahlstrom, 1985; Borrely et al., 1998a, b; Farooq et al., 1992, 1993; Levaillant and Gallien, 1979; Mann, 1971; Nikonorova et al., 1976; Duarte et al., 2002) that insure disinfection and increase water quality. Furthermore, research by Kurucz (Kurucz et al., 2002) shows that gamma radiation degradation yields could be better than electron beam irradiation.

Besides the inactivation effect of ionizing radiation, this technology could be a rather useful instrument for bioremediation if a low dose rate is used. This technology is useful for transforming a complex substratum to soluble small molecules that can be metabolized by microbiota (Hurst et al., 1997).

A case study will be presented, which is an extension to the results obtained in the scope of IAEA-Research Project 302-F2-POR-12017 "Impact of e-beam and gamma Radiation on the treatment of wastewater and drinking water by radiation"). These results indicate an ionising radiation influence on wastewater quality (Botelho et al., 2002). In this work, we carried out such a study on wastewater from a slaughterhouse industry. This work presents the results of the impact of gamma radiation, at dose rate 0,9 kGy h\(^{-1}\), on some relevant parameters as Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand after 5 days (BOD\(_5\)), and BOD\(_5\) versus microbiological survivors. It is also presented the total coliforms and the microbiota radiation inactivation at higher dose rate (3 kGy h\(^{-1}\)).
2. Experimental

2.1. Irradiation

Samples were subjected to γ-radiation from a $^{60}$Co source facility in ITN with an activity about 276 kCi (December, 2003). The local dose rate was previously determined by Fricke solution (International Atomic Energy Agency, 1977). Samples in a 0.5 L beaker were irradiated at γ-ray dose rate between 0.9 kGy and 3 kGyh$^{-1}$. Doses were established from 5 kGy up to 30 kGy. Two routine dosimeters (Harwell Red Perspex, Batch HA Type 4034; and Gammachrome YR), with nominal uncertainty limits of about 5% (McLaughlin et al., 1989) were placed in each beaker before irradiation. For inactivation studies two dose rates were applied ($3 \pm 0.9 \text{kGy h}^{-1}$).

2.2. Sampling

2.2.1. Case Study

The effluent used for the study was CASO untreated wastewater, which was submitted to a mechanic screening pre-treatment. CASO is a slaughterhouse located in Mafra, near Lisbon. The plant produces approximately 143 ton of hog meat daily, this is an activity that generates solid residues and wastewaters. Part of the solid residues are collected and reuse; the bones, the guts and the excess fat removed from the screening unit of the wastewater treatment plant to use in a unit to produce pet food, hog’s fat and dehydration blood used to fertilizers. Water consumption is higher comparing with others (Thompson, 1999). Wastewater treatment takes 7 days since pre-treatment until discharge on the river and it is a continuous process.

2.2.2. Analytical Methods

**BOD, COD and TSS**

BOD (Method 5210 B), COD (Tritimetric Method 5220 C) and TSS (Method 2540 B) were measured accordingly with standard methods (Standard Methods for the Examination of Water and Wastewater, 20$^{th}$ Edition). BOD and COD were measured before and after filtration with a 1.2 µm filter.

The gamma radiation efficacy was calculated in terms of %COD, BOD$_5$ and TSS reduction. As an example, Eq. 1 shows the case of %COD reduction where COD$_x$ is the value measured after a given dose and COD$_{initial}$ the COD value for the non irradiated sample.
\[
\%\text{COD}_{\text{reduction}} = \left(\frac{\text{COD}_{\text{initial}} - \text{COD}_x}{\text{COD}_{\text{initial}}}\right) \times 100 \quad (\text{Eq. 1})
\]

Moreover, the chemical and biological reduction of organic matter is ascribed to \(\%\text{COD}_{\text{reduction}}\) and \(\%\text{BOD}_{5\text{reduction}}\) (Gaudy and Gaudy, 1980), respectively, whereas the ratio between COD and BOD_5 defines the amount of biodegradable matter (Wang et al., 1993), or in other words, the bio effect added values over the chemical one.

**Inactivation of Microorganisms**

To determine “total coliforms”, before and after irradiation, the Most Probable Number (MPN) technique was applied. The growth conditions were incubation at 37 °C for 7 days in Lauryl Sulphate Broth (LSB). Results were observed at 24 h and 48 h. Confirmative methods was carried using Brilliant Green Lactose Bile

To determine the mesophilic microbiota before and after irradiation, the spread plates after dilution technique was used. The growth conditions were aerobic incubation at 30 °C for 14 days on Tryptic Soy Agar (TSA) plates. Colony forming units (c.f.u.) were counted after 24 h, 48 h, 72 h, 7 and 14 days.

These protocols were based on (Standard Methods for the Examination of Water and Wastewater, 20th Edition). Identification was worked out by API system and based in macroscopic and microscopic analysis (Holt et al., 1994)

Data Analysis was done using Excel 97 (e.g.: regression analysis and descriptive statistics).

Efficiency was calculated according to Equation 2:

\[
\text{Efficiency (\%) = } \left(\frac{N_0 - N_d}{N_0}\right) \times 100 \quad (\text{Eq. 2})
\]

Where \(N_d\), is the number of survivors after irradiation at several doses and \(N_0\) the initial count (non-irradiated sample). D value was estimated based on the linear regression of the inactivation curve and corresponds to the inverse of its slope (Block, 1983)

**Biodegradation studies**

A preliminary study of biodegradation was carried out with *Xantomonas maltophilia*, one of the most frequent microorganisms isolated after irradiation at high doses. The isolated bacterium was identified by API system (i.d. 98 %) and the growing rate was studied into the same substrata after two different treatments: a) irradiation of slaughterhouse wastewater sample 30 kGy, dose rate 3 kGy h\(^{-1}\); b) treatment of an aliquot of same sample by steam at 121 °C 30’. Inoculation of bacteria was carried out with a concentration of cells approx. \(10^2\) /ml. The growth conditions were water bath with mechanical agitation temperature at 30 °C. The rate of growing was measured by turbidity [Spectronic 21- Bausch & Lomb] at
wavelength 490 nm. The different phases of growing (e.g.: lag, log increasing, stationary, decelerating autodigestion, log decreasing) are being confirmed by spread plates technique.

3. Results

3.1. Wastewater parameters for measurement of quality

COD, BOD5, TSS and colour were the parameters selected to measure efficiency of slaughterhouse wastewater treatment by gamma radiation. They were obtained at a dose rate of 0.9 kGy, whereas the inactivation rate of total coliforms and total microorganisms, was studied at two dose rate: 0.9 kGy h\(^{-1}\) and 3 kGy h\(^{-1}\). Preliminary studies on biodegradation of irradiated wastewater and steamed wastewater by *Xantomonas maltophilia*\(^1\) will be also presented.

3.1.1. Chemical Oxygen Demand

The COD was measured in the irradiated and non irradiated wastewater before and after filtration (Fig. 1 and Fig. 2, respectively). The COD values are the average of 3 replicates (\(\alpha = 0.05\)). As shown in Fig 1, results at 22 kGy and 35 kGy as high dispersion of values that point to inconclusive result. Therefore, a filtration step, before the chemical analysis, was introduced.

![COD before filtration](image)

Fig. 1. COD before filtration.

\(^1\)isolated during the experiments, after irradiation of slaughterhouse wastewater samples (i.d. 98 %)
Fig. 2 shows that at 7 kGy the %COD reduction decreases (mean -60 ± 6 %), whereas at higher dose the reduction increase (mean 35±4 %) when compared with the unirradiated sample value. With the exception of 7 kGy absorbed dose, the gamma radiation effect is associate with the decrease of COD and therefore point to a better water quality (the higher the dose absorbed, the lower the COD value). Moreover, since the %COD reduction indicates also the chemical degradation of organic mater (Kurucz et al., 2002), the decrease at 7 kGy can be explained by the gamma radiation scissor effects that increase the number molecular low weight substrates. Therefore, results showed that high gamma radiation doses are adequate to lower the organic matter weight on the effluent, which is an important goal for the slaughterhouse industry.

At our knowledge, this is the first time that the gamma radiation effect on wastewater is reported for a case study where the effluent was only submitted to a mechanical screening. The closest approach to our work is that of Caixeta (Caixeta et al., 2002) who reported ≥80% COD removal efficacy using an up flow anaerobic sludge blanket (UASB) reactor. In our case study, CASO has implemented a 4 step wastewater treatment: a) mechanical screening pre-treatment, b) anaerobic lagoon, c) aerobic lagoon and d) sedimentation. Interesting, the COD measured at high gamma radiation dose (≥25 kGy), mean 2000±500 mgO₂/l, is comparable to
that reported by CASO after the anaerobic lagoon treatment. Therefore, it is easy to anticipate that combining ionising radiation with bioremediation process could lead to better results.

### 3.1.2. Biochemical Oxygen Demand

For irradiated and non-irradiated wastewater samples the 5-day BOD was measured and the results summarized in Fig. 3. All samples were filtrate before analysis. The BOD5 values are the average of 2 replicates ($\alpha = 0.05$).

![Fig. 3. BOD vs. absorbed radiation dose.](image)

Fig. 3 shows that at 7 kGy the $\%$BOD5$_{\text{reduction}}$ decreases (mean -20±2 %), whereas at higher dose it increases (mean +50±5 %) when compared with the non irradiated sample value. Therefore, with the exception of the lowest dose, the gamma radiation effect can be associated to the decrease of BOD5 and therefore to a better water quality. Moreover, since the $\%$BOD5$_{\text{reduction}}$ is a parameter that measures also the bio-degradation of organic mater (Wang et al., 1994.), the decrease at 7 kGy can be explained by the gamma radiation scissor effect reported before for COD where, as a consequence of the interaction with gamma radiation, the transformation of substrata into soluble small molecules better metabolized by microorganisms, occurs. Therefore, the nutrients assimilation is easily and better done which correlates the increase of BOD5 at 7 kGy.

As described by other authors (Getoff, 1996) powerful oxidizing and reducing species (e.g., OH, $e^{-}_{\text{aq}}, H$) and molecular products ($H_2, H_2O_2$) are produced as a sequence of the interaction between $\gamma$-radiation and water, so these chain reactions induce to degradation of pollutants.
3.1.3. Reduction of organic matter: chemical and biodegradation

Taking in account our case study where the organic matter degradation is an important parameter, the chemical and biological reduction of organic matter was ascribed to $\%\text{COD}_{\text{reduction}}$ and $\%\text{BOD}_5_{\text{reduction}}$, respectively, whereas the ratio between COD and BOD$_5$ defines the of biodegradable matter (Wang et al., 1994), or in other words, the bio effect added value over the chemical one.

Fig. 4 shows the relationship between $\%\text{COD}_{\text{reduction}}$ and $\%\text{BOD}_5_{\text{reduction}}$.

![Graph showing the relationship between COD reduction and BOD5 reduction vs. absorbed radiation dose.](image)

In both cases, a decrease of organic matter with the same curve shape, ascribed to chemical and bio factors, was observed. Therefore, the changes in COD and BOD$_5$ values caused that the ratio COD/BOD$_5$ change only slightly with the gamma radiation dose (Fig. 5).

![Graph showing the ratio of COD/BOD vs absorbed radiation dose.](image)

As could be seen in Fig. 5 the ratio increases from 2 to 4.5 into the range of 7 kGy up to 17 kGy applied doses. At higher doses the ratio shows approximately the same value of the unirradiated samples.

3.1.4. Total Suspended Solids
Total Suspended Solids (TSS) was determined at irradiated and non irradiated wastewater samples. Fig. 6 shows that the \%TSS\text{reduction} increases up to 25% at 7 kGy. As described before at this dose the COD increases also up to 25%. So it means that in this case, solid particles and COD are related. Some authors suggested (Nieuwenhuijzen, 2001) a direct influence of the filtration as a compact treatment system to produce a high quality primary effluent with a constant composition where the pollutants integrated into or adsorbed onto particulate matter are non negligible and are the major contribution to the wastewater COD. At higher dose, the \%TSS\text{reduction} attains a plateau (+10±5%). Therefore, in all cases the TSS values are inferior to that of the non irradiated sample. Such tendency is not compensated by the BOD parameter and future analysis of the solids is required to full understand this unusual behaviour.

![Fig. 6. TSS vs. absorbed radiation dose.](image)

3.1.5. Inactivation of microorganisms

Total coliforms

Total coliforms are an indicator of pollution by fecal origin and, in our point of view, are also the representatives of the lesser resistsants bacteria to ionizing radiation.
As we can observe in Fig. 7, and taking into account the Dvalue\(^2\) definition, 0, 2 kGy is the absorbed dose needed to inactivate 90 % of the population, and is calculated based on the linear regression of the inactivation curve and corresponds to the inverse of its slope. In this case, the Dvalue does not depend on the dose rate applied.

Fig. 7. Inactivation curve of total coliforms at two dose rate.

**Microbiota**

Fig. 8 shows the results of the total wastewater microorganisms inactivation at lower dose rates that could be seen to be somewhat less efficient than at higher dose rates. After irradiation, at higher dose rate (3 kGy. h\(^{-1}\)), determination of Dvalue points to 90 % of the population is inactivated at 2.2 kGy, whereas at lower dose rate (0.9 kGy.h\(^{-1}\)) 90 % of inactivation was found at 4 kGy. These results are in agreement with those previously reported by the authors (Botelho *et al.* 2002) for Municipal waste water samples.

An inactivation of 5 log of c.f.u., corresponding to efficiency of 99,999 %, was found at 7 kGy absorbed dose, whereas higher dose rate was used (3 kGy h\(^{-1}\)). For the lower dose rate, at same dose (7 kGy), an inactivation of 4 log c.f.u, was observed, corresponding to less efficiency (99,986 %)

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\(^2\) Dvalue is the time (dose required to inactivate 90 % of the cells present or to reduce microbial population to one tenth its number (in Block, 1983).
3.1.6. Biodegradation studies

After 17 kGy, the microorganisms of irradiated samples reveal a homogeneous population. These microbiota were detected in lower numbers during the determination of $N_0$ and at lower doses (e.g.: 7 kGy). This fact leads to the hypothesis that γ radiation selects the more resistant species if the geometry and doses applied are suitable. Therefore, ionizing radiation could be also used selectively to improve bacteria population and this technology could be a rather useful instrument for bioremediation.

3.1.6. Biodegradation studies

Fig. 9 presents the experimental results obtained by the measurement of turbidity due to the growth of *Xantomonas maltophilia*\(^3\) in a wastewater samples from the slaughterhouse industry, one sample was previously sterilized by gamma radiation while the other one was treated with steam.

\(^3\)isolated during the experiments, after irradiation of slaughterhouse wastewater samples (i.d. 98 %)
As we can observe, there are different rates in the different phases. The lag phase is shorter in irradiated wastewater (IR) than in wastewater sterilized by steam (AC). Furthermore, the biomass growth is higher in irradiated substrata than in the steam sterilized substrata. They confirm our hypothesis that ionizing radiation makes the nutrients more available for bacteria growth. Therefore, this technology could be a useful instrument for bioremediation.

Moreover microorganisms and chemical species are dynamically inter-dependent, thus studies on effluents need multidisciplinary groups in order to fully understand them. As showed in Fig.: 10, the tendency of BOD5 parameter could be explained by the behavior of microbiota after the several doses applied depending on the number of survivors, as is demonstrated in similar platform at 17 kGy and 25 kGy.
3.1.7. Colour

Blood, oil and greases gives to slaughterhouse wastewater a red colour. This parameter is one of the major problems in this kind of effluents. As shown in Fig. 12 at 30 kGy intensity of colour change turning off on similar colour to the sample after anaerobic process (Fig.12).
3. Conclusion

Preliminary experiments were conducted to evaluate the effects of gamma radiation on slaughterhouse industry wastewater. Results obtained at a low dose rate (0.9 kGyh\(^{-1}\)) show a substantial improvement in waste water quality with an efficient decrease in COD, BOD5 and TSS. For doses superior to 25-35 kGy, the COD is about 35% inferior to that of the non irradiated samples, whereas the BOD5 decreases to at least 50%.

Interestingly, the COD measured at these doses, mean 2000±500 mgO\(_2\)/l, is comparable to that reported by the CASO analytic laboratory after anaerobic lagoon treatment. Therefore, it is easy to conceive that combining ionising radiation with the bioremediation process could lead to better results.

At 7 kGy absorbed dose, the higher dose rate showed an inactivation of 5 log of c.f.u. corresponding to an efficiency of 99,999 %, whereas for the lower dose rate a 4 log c.f.u was observed, corresponding to an efficiency of 99,986%.

Results showed that lower dose rates lead to a better bioremediation process. This is more effective for the wastewater substrata due to the irradiation effect on its degradation, changing it into molecular forms that are more easily metabolized by microbiota. Furthermore, a large irradiation period allows the repair mechanisms of microbiota to respond more easily and efficiently to biodegradation.

The high dose rate was shown to be more efficient for the cleanliness process of the tertiary treatment.

Therefore, this study stresses the advantages of the use of gamma radiation for slaughterhouse wastewater remediation. Moreover, the authors defend the use of ionizing radiation in two ways for the treatment of wastewater: 1) lower dose rates for helping bioremediation and 2) higher dose rates for disinfection during the tertiary phase.
4. References


