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Evaluation of e-beam irradiation effects on the toxicity of slaughterhouse wastewaters

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ABSTRACT
Slaughterhouse industry produces large volumes of polluted wastewater, which cause negative impacts on the environment. The objective of this study was to assess the effect of electron-beam irradiation on the ecotoxicity of slaughterhouse effluents with absorbed doses up to 35 kGy. Two acute toxicity assays were applied to evaluate the efficiency of irradiation onto toxicity of wastewater. The exposed living-organisms were a luminescent bacteria *Vibrio fischeri*, and a freshwater microcrustacean *Daphnia similis*. Also, the total organic carbon was analysed in order to determine any possible organic carbon removal after irradiation. The ecotoxicological results evidenced that both living-organisms were suitable for the measurements. Therefore, the results demonstrated the toxicity of the effluent and its similarity for both organisms as well as the potential of radiation to reduce these effects. The 35 kGy dose was very effective for reducing toxic effects of slaughterhouse wastewater for daphnids suggesting that ionizing radiation could be used as a tool for removing toxic charge of such effluents. The type of contamination presented by the effluent justify the needs for alternatives of treatment.

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Introduction

Water pollution is a serious global issue that affects the entire world. It has been reported as the major contributor to health problems and disorders in humans and animals. Pollution has been introducing negative impacts on the aquatic environment since a high quantity of pollutants, such as domestic and industrial effluents is discharged directly or indirectly into the water bodies.

Slaughterhouses are important industries worldwide once the main product is essential in the human diet. It is one of the most complicated industries in terms of environmental pollution, since it produces large volumes of wastewaters containing high amounts of total organic carbon (TOC), chemical oxygen demand (COD), total suspended solids (TSS) and biochemical oxygen demand (BOD) [1,2]. Among pollutants there are others, feaces, urine, blood and fat, as the main source of slaughterhouse wastewater.

Although the quality of waste effluents is usually based on the control of the global parameters previous referred, the evaluation of the negative effects on living-organisms is still needed. Based on this, the toxicity tests appear as rapid, simple, cheap and effective strategies to evaluate the biological effects. Therefore, the use of biological assays can provide a direct and appropriate evaluation of toxicity to complement the physico-chemical measures of quality of wastewaters [3,4]. Many types of bioassays are available and the test organism incorporated could include representative microorganisms, plants, invertebrates and fish. The most frequently applied tool for wastewater toxicity screening is Microtox® and it is based on the bioluminescence inhibition of *Vibrio fischeri*. Other alternative assay used to study the acute toxicity of wastewaters is the mobility test with the microcrustacean *Daphnia similis* [5].

Due to the importance of meat production and save water, there are many advances in terms of slaughterhouse wastewater treatment in the world, such as biological treatment, adsorption, electrocoagulation, biological DAF-UASB, and combined AOP processes [6–11]. López-López et al. [12] used a combined anaerobic and aerobic system which was able to remove more than 95% of organic matter as BOD and COD. Orssatto et al. [9] reported the COD removal of 81.01% by the application of the electrocoagulation technique for the treatment of effluents from the pig slaughterhouse and packing plant. Real-Olvera et al. [8] used the powder of *Moringa oleifera* Lam. (Moringaceae) as a natural coagulant for the adsorption of organic pollutants from these wastewaters, with COD removal efficiencies of 64% at

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pH 9. Also, Torres-Pérez et al. [13] showed that natural zeolitic materials were the most efficient material to remove TOC, COD, and colour from slaughterhouse wastewater in central Mexico. Manjunath et al. [10] assessed the performance of upflow anaerobic sludge blanket (UASB) for the treatment of slaughterhouse wastewater, and also proposed their system as a pre-treatment step. Although there is a lack of information about toxicity measurements for such type of effluent, genotoxic effects of two different slaughterhouse effluents were demonstrated in wastewater and in the receiving rivers after the effluents discharges, using A*styanax sp* fish [14].

Nevertheless, the need for improving wastewaters treatment promotes the development of new technologies for pollution decomposition. Ionizing radiation has been proved to be effective for destroying the organic pollutants of effluents [15] although few works have included the evaluation of ecotoxicity. Melo et al. [2] reported the efficiency of gamma radiation on wastewater treatment from a slaughterhouse industry. Electron-beam irradiation technology, EBI, has been already used to enhance the biodegradability of wastewaters [16,17] and also to remove the acute toxicity of industrial and municipal effluents [18]. Controlling toxicity may be an important strategy for the evaluation of treatment processes, also for achieving legal and safety discharges after a suitable treatment as well as for industrial reuse [14,19]. In terms of organic load in wastewater, some authors have based their studies in total organic carbon removals once it is an instrumental analysis that is faster than other methods and less variable, specialty if compared to biological oxygen demand [20].

The aim of this work was to study the effect of electron-beam irradiation (EBI) on the ecotoxicity of slaughterhouse effluents. Two acute toxicity tests were applied to evaluate the efficiency of irradiation to diminish wastewater toxicity. Two different organisms (a luminescent bacteria *Vibrio fischeri*, and a freshwater microcrustacean *Daphnia similis*) were used.

Methods

Sampling and irradiation

The sample used for the study was collected from CASO-Centro de Abate de Suínos do Oeste Lda., a company located in the West of Portugal. The sample was untreated slaughterhouse wastewater, which was submitted to mechanical pre-treatment screening only. The sampling was punctual, and after the slaughter period to use the most polluted water (with high concentration of blood). The samples were stored at 4°C until experiments.

The wastewater samples were irradiated at the e-beam facility (Dynamitron model) using an electron-beam accelerator (1.50 MeV, 37.50 kW) located at the Instituto de Pesquisas Energéticas e Nucleares—IPEN-CNEN/SP. In order to achieve the desired absorbed doses, the wastewater sample has to be submitted to several ways on the irradiator (7 kGy each way). The absorbed doses were: 7, 14, 21, 28 and 35 kGy.

Total organic carbon analysis

The total organic carbon was measured by a Shimadzu TOC- 5000A analyzer. The TOC was determined indirectly by the difference between total carbon content of the sample (TC) and inorganic carbon (IC) content. The analysis were made in triplicate.

Ecotoxicological assays

The Ecotoxicology assays were carried out in order to assess the level of toxicity for the raw effluent and if ionizing radiation may reduce the toxic charge of such effluent. The acute toxicity evaluation was performed on irradiated and non-irradiated samples, and expressed as EC-50 value, which means the sample concentration that reduces the measured effect by 50%. The *Vibrio fischeri* luminescent bacteria and *Daphnia similis* water flea were the exposed living-organisms. The validation of these assays were performed by a negative control for each sample and one complete assay using a reference substance at the same time. Phenol was the reference substance for the *V. fischeri* assays, while sodium potassium was used for the daphnids.

Vibrio fischeri assays

The *Vibrio fischeri* assays are based on the luminescence of bacteria *V. fischeri,* which expontaneously produce light as suitable metabolism. Any inhibition of the normal metabolism, such as that caused by toxins or the presence of toxic compounds, results in a decreased rate of luminescence. *V. fischeri* bacteria were exposed for 15 min to wastewater samples. The higher the level of toxicity, the greater the inhibition of light production. These assays were performed with a Microtox System (model M-500, Microbics).

For EC-50 calculation the statistical method used was a linear regression, using the sample concentrations versus the gamma effect (the ratio of light lost to light remaining after exposure of the reagent to a sample). In this case, the EC-50 is the concentration at which gamma equals 1, i.e. the light lost equals the light remaining (Microbics Corporation 1994).

Daphnia similis assays

Daphnia similis, commonly reported as water fleas, is an aquatic daphnid useful for toxicity measurements. Several other species may be applied. For these assays, immobility effects induced by the effluents were measured. Young *D. similis* (6–24 h) were exposed to different concentrations of wastewater during 48 h.

The EC-50 values were calculated from the number of immobile organisms per sample concentration and the statistical analysis were determined by Trimmed Spearman Karber method, which is based on the integrated normal (probit) or logistic (logit) models [21]. These models describe the relationship between mean mortality and concentration of toxic compound [22].

The evaluation of irradiation process on the acute toxicity reduction of the samples was performed by transforming the obtained values of EC-50 into Toxicity Units (TU - that are directly proportional to the toxicity - TU = 100/EC-50). The percentage of the acute toxicity reduction between the raw and the irradiated sample were calculated from the TU values for each applied radiation doses.

Results and discussion

The slaughterhouse wastewater samples are characterized by high organic matter content, which may contribute to high levels of toxicity [13]. Previous studies performed by the authors [2] showed the following values for wastewaters organic matter: 2368 ± 221 mgO_2/l for COD, 1031 ± 52 mgO_2/l for BOD and 805 ± 40 mg/l for TSS. These values are 20- to 40-fold above the EU standard limits for slaughterhouse effluent discharge [1]. Other authors already reported the high levels of pollution due to organic and inorganic matter [13,23]. TOC is one of the most important parameter providing an assessment of global organic pollution in wastewaters [24]. Thus, in this study, TOC and IC were the selected parameters to evaluate the organic matter concentration in the wastewater. The irradiation effects on slaughterhouse wastewaters were also studied by TOC and IC.

Figure 1 represents the results concerning the effects of ionizing radiation on organic and inorganic carbon. At 14 kGy TOC increases considerably (550 ppm) in relation to non-irradiated sample (368 ppm). Inorganic matter (IC), however, decreases approximately 60% at 14 kGy in comparison with non-irradiated sample. The obtained results support the idea of an ionizing radiation scissor effect [2] that originates more and easily degradable matter in this type of sample.

Concerning the pH of slaughterhouse effluents, the common range is between 4.90 and 8.10, with 6.95 as average value [1]. During this work, the pH of non-irradiated samples were 7.20 ± 0.30, decreasing 0.60 after 7 kGy.

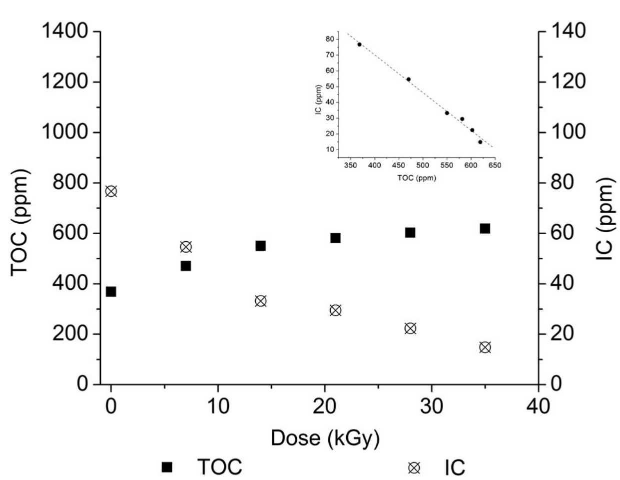

Figure 1. Electron-beam radiation effects on organic (TOC) and inorganic (IC) matter. Inset: Linear correlation between TOC and IC ($R^2 = -0.996$).

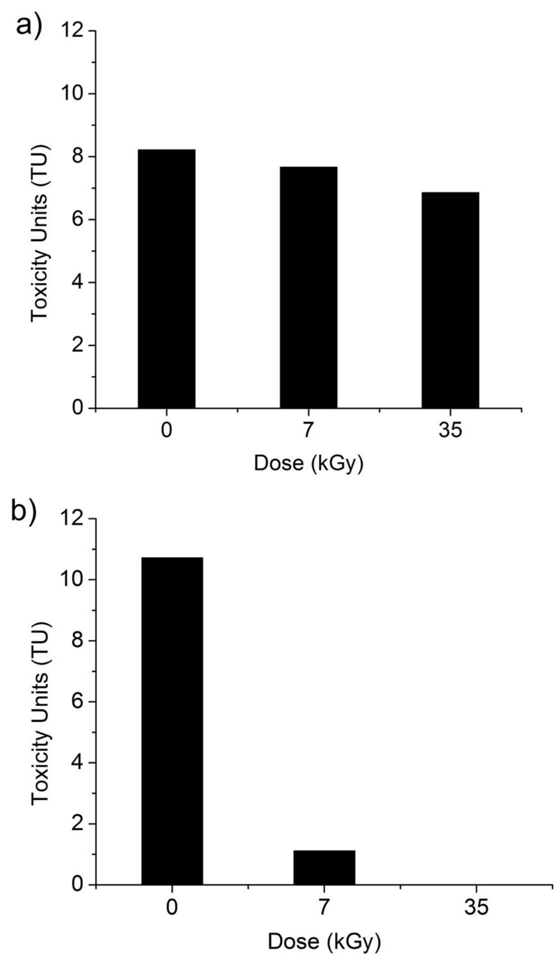

Figure 2. Acute toxicity (in toxicity units, TU) of slaughterhouse samples for electron-beam irradiation at different doses for a) *Vibrio fischeri* and b) *Daphnia similis*.

The efficiency of ionizing radiation to diminish slaughterhouses wastewater toxicity was also demonstrated. Figure 2 shows the results obtained for the toxicity of slaughterhouse wastewater of samples irradiated at 7 and 35 kGy in comparison with non-irradiated samples. For a better understanding the EC-50 values were transformed into toxicity units (TU). In the case of non-irradiated slaughterhouse wastewater, the sensitivity of both organisms was not that different, showing its high toxicity. Regarding radiation effect on toxicity parameter, the efficacy of the treatment processes was very effective for *D. similis* assays (both doses). The toxicity removal at 7 kGy was higher for *D. similis* than *V. fischeri*. The toxic effect of slaughterhouse wastewater decreased 89.53% and 6.64% for *D. similis* and *V. fischeri*, respectively. A favourable aspect is that *V. fischeri* appeared to be as sensible to the irradiated samples as to the non-irradiated ones, indicating that the irradiation of slaughterhouse wastewater did not increase the toxicity of treated samples. Moreover, the toxicity of studied wastewaters for *D. similis* seems to decrease in accordance to increasing dose, indicating that the by-products formed by irradiation are less toxic than the parent ones. At 35 kGy there was non-toxic effect, suggesting that radiation could be used as a tool for removing toxic charge of such a type of effluent.

In Table 1, the average EC-50 values were compared for four types of industrial effluent, including those slaughterhouse effluents.

The toxicity values for the non-irradiated slaughterhouse wastewater samples demonstrated relatively high toxicity for both organisms (EC-50 = 9.70% for *D. similis* and EC-50 = 12.50% for *V. fischeri*). Comparing with the other industries, the data evidenced that chemical effluent was the more toxic, followed by pharmaceutical and slaughterhouse. This type of data may be important for the establishment of influent equalization at biological treatment system and also for the control and discharges of effluents into aquatic environment [19].

Water is an important input and also output for food production and environmental pollution issues, which induced the development of many advanced technologies for cleaning wastewaters, such as electron-beam technology. It should be noted that in certain cases there must be combination of technologies for a safe discharge of wastewater [10,23]. EBI could be applied as a previous treatment and before a biological system. Regarding the application of EBI for effluents' cleaning, radiation processing was quite effective for removing colour of textile effluents [26]. For slaughterhouse effluent, another benefit could be the wastewater disinfection once radiation processing could drastically reduce microorganisms. Concerning combined processes for treating wastewater, a cost-effectiveness analysis of TOC removal from slaughterhouse was carried out using combined anaerobic–aerobic and UV/H_2O_2 processes [23].

Table 1. Toxicity of real effluents for daphnids and bacteria expressed by EC-50 number (%, v/v), according to the literature.

Industrial effluent	Daphnids (48 h)	*V. fischeri* (15 min)	Reference
Pharmaceutical	6.70–7.20	3.00	[25]
Chemical (n = 11)	1.36 ± 8.59	2.12 ± 5.87	[26]
Textile (n = 6)	22.46 ± 7.19	41.71 ± 27.10	[26]
Slaughterhouse*	9.70	12.50	Present study

Conclusions

Slaughterhouse wastewater may be an important source of contamination to the environment and this fate accounted for the development and search for a distinct combination of treatment for this liquid residue. In terms of acute toxicity, both exposed organisms were quite sensitive to the contaminants in the effluent, resulting in important level of acute toxicity. After irradiation the toxicity strongly decreased after 7 kGy (*D. similis*) and no effective removal of toxicity was noted for *V. fischeri* bacteria, showing how important it is to run more than one toxicity assay. Both acute assays were suitable for the purpose of this paper and EB irradiation was effective for TOC reduction at the studied conditions.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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