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Effects of sausage industry effluents before and after bacterial biodegradation treatment and cytotoxicity in *Allium*

Efeitos de efluentes da indústria de embutidos antes a após tratamento de biodegradação bacteriana e citotoxicidade em modelo *Allium cepa*

Efectos de los efluentes de la industria de embutidos antes y después del tratamiento de biodegradación bacteriana em el modelo de *Allium cepa*

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Abstract
Water is essential in the food and meat processing industry. The processing industry is one of the major consumers of water, and consequently, generators of a significant amount wastewater effluents which pollute the water bodies heavily. The objective of this study was to evaluate the cytotoxic potential of raw effluent, anaerobic and aerobic ponds, and surface water of the Cleopatra stream pre- and post-discharge of the effluent from a sausage industry by *Allium cepa* L. test. At different sampling timepoints, tests on the roots were conducted, as follows: control samples of the self-bulb (0 h), treatment with effluent or water (24 h), and recovery in filtered water (24 h). The results showed that the raw effluent is cytotoxic, because it inhibited the cell division completely. However, this inhibition was not permanent as, after the recovery time, the cells divided again at a rate greater than that of the control. Wastewater from the anaerobic and aerobic ponds and before and after discharge into the stream was not found to be cytotoxic. At the time of recovery, an increase in cell division was observed in samples treated with wastewater from the anaerobic and aerobic ponds, which is possibly caused due to the presence of organic matter. Despite the efficiency of treatment methods, it is necessary to conduct studies that monitor the effluents from the industry and stream waters to ascertain possible impacts on the environment.
Keywords: *Allium cepa* Test; Environmental monitoring; Food processing; Cytotoxic effect.

Resumo
A água é essencial na indústria de processamento de alimentos e carnes. A indústria de transformação é uma das maiores consumidoras de água e, consequentemente, geradora de uma quantidade significativa de efluentes residuais que poluem fortemente os corpos hídricos. O objetivo deste estudo foi avaliar o potencial citotóxico de efluentes brutos, lagoas anaeróbicas e aeróbicas e águas superficiais do córrego Cleópatra pré e pós-descarga do efluente de uma indústria de embutidos pelo teste Allium cepa L.. Em diferentes momentos de amostragem, foram realizados testes nas raízes, a saber: amostras controle do bulbo próprio (0 h), tratamento com efluente ou água (24 h) e recuperação em água filtrada (24 h). Os resultados mostraram que o efluente bruto é citotóxico, pois inibiu completamente a divisão celular. No entanto, essa inibição não foi permanente, pois, após o tempo de recuperação, as células se dividiram novamente a uma taxa maior que a do controle. As águas residuais das lagoas anaeróbicas e

aeróbicas e antes e depois da descarga no córrego não foram consideradas citotóxicas. No momento da recuperação, observou-se um aumento na divisão celular nas amostras tratadas com águas residuais das lagoas anaeróbias e aeróbicas, o que possivelmente é causado pela presença de matéria orgânica. Apesar da eficiência dos métodos de tratamento, é necessário realizar estudos que monitorem os efluentes da indústria e das águas de córregos para verificar possíveis impactos ao meio ambiente.
Palavras-chave: Teste *Allium cepa*; Monitoramento ambiental; Processamento de alimentos; Efeito citotóxico.

Resumen
El agua es esencial en la industria de procesamiento de alimentos y carne. La industria de procesamiento es uno de los principales consumidores de agua y, en consecuencia, generadora de una cantidad significativa de efluentes de aguas residuales que contaminan fuertemente los cuerpos de agua. El objetivo de este estudio fue evaluar el potencial citotóxico de efluentes crudos, estanques anaeróbicos, aeróbicos y aguas superficiales del arroyo Cleopatra antes y después de la descarga del efluente de una industria de embutidos mediante la prueba de *Allium cepa* L. En diferentes momentos de muestreo, se realizaron pruebas en las raíces de la siguiente manera: muestras de control del autobulbo (0 h), tratamiento con efluente o agua (24 h) y recuperación en agua filtrada (24 h). Los resultados mostraron que el efluente crudo es citotóxico, ya que inhibe completamente la división celular. Sin embargo, esta inhibición no fue permanente ya que, después del tiempo de recuperación, las células se dividieron nuevamente a una velocidad mayor que la del control. No se encontró que las aguas residuales de los estanques anaeróbicos y aeróbicos y antes y después de la descarga en la corriente fueran citotóxicas. En el momento de la recuperación se observó un aumento de la división celular en las muestras tratadas con aguas residuales de las balsas anaerobias y aerobias, posiblemente por la presencia de materia orgánica. A pesar de la eficiencia de los métodos de tratamiento, es necesario realizar estudios que monitoreen los efluentes de la industria y las aguas de los arroyos para conocer los posibles impactos en el medio ambiente.
Palavras clave: Prueba de *Allium cepa*; Monitoreo ambiental; Procesamiento de alimentos; Efecto citotóxico.

1. Introduction

Water scarcity has emerged as one of the most pressing problems of the 21st century. Changes in the quantity, distribution, and quality of water resources is a threat for the survival of humans and other species on the planet. Scarcity and poor quality of water impoverishes local populations, destroys healthy alternatives for sustainable development and directly impacts the economy of a country (Tundisi, 2005; Metha, 2014; Hoffmann et al., 2020; Cardoso et al., 2022).

Water pollution is the addition of substances or energy forms that directly or indirectly alter the nature of the water bodies in a way that prejudices their legitimate uses (Von-Sperling, 2016). The sausage industry generates wastewater rich in organic contaminants and is classified as one of the most harmful industries for the environment, because it can cause deoxygenation of the rivers and contamination of the groundwater (USEPA, 2016). Furthermore, the wastewater from sausage industries may contain toxic and non-biodegradable organic substances that are cytotoxic for various organisms (Rahman et al., 2014).

The meat processing industry is responsible for the consumption of 24% of the total freshwater used by the food and beverage industry, further generating large volumes of wastewater rich in organic contaminants (Liu & Haynes, 2011; Thirugnanasambandham et al., 2016; Aziz et al., 2019).

According Bustillo-Lecompte and Mehrvar (2016), the global production of meat is likely to increase considerably by 2050. Consequently, it will lead to an increase in the amount of wastewater, and consequently, an increasing need to treat these extremely polluting effluents (Hilares et al., 2021). The treatments used majorly in the sausage industry include anaerobic or aerobic processing. Particularly, the combined anaerobic-aerobic method is preferred to the conventional methods due to the following advantages: high efficiency of removal of organic matter, lesser generation of aerobic sludge, potential energy recovery (biogas), and a reduction in the operation and maintenance costs (Chan et al., 2009; Mccabe et al., 2014; Aziz et al., 2019; Nowrouzi et al., 2021).

When the wastewater is managed properly, its physical, chemical and biological conditions are improved, and consequently, possible damage to the environment is reduced. Therefore, it is necessary to evaluate the implications of the wastewater on living organisms. *A. cepa* is recognized as an excellent genetic model for monitoring studies that is used to detect environmental mutagens and disturbances in the mitotic cycle (Leme & Marin-Morales, 2009; Iqbal et al., 2019; Martins et al., 2022).

This study aimed to assess the cytotoxic potential in root meristematic cells of *A. cepa*, from the surface of the Cleopatra stream and effluents from a sausage industry located in the urban area of Maringá-PR. Sampling from the sites was done before and after the discharge of effluents from the sausage industry and also tested with raw and treated effluents, and wastewater from the anaerobic and aerobic ponds.

2. Methodology

2.1 Collection points

Raw and treated effluents originating from the sausage industry, which discharged the treated effluents into the Cleopatra stream, both located in the urban area of Maringá-PR, were collected. We collected the five samples as follows: raw effluent (23° 26' 31.88"S, 51° 56' 25.12"W) which is the wastewater generated by the industrial processes, treated effluents, wastewater from anaerobic (23° 26' 33.68"S, 51° 56' 25.12"W) and aerobic ponds (23° 26' 27.01"S, 51° 56' 27.98"W), and water of the Cleopatra stream before (23° 26' 26.93"S, 51° 56' 30.61"W)- and after (23° 26' 28.95"S, 51° 56' 29.45"W)- discharge of the effluent.

The samples were collected in the morning in the month of March, under conditions of good weather, sunshine, average temperature of 24° C, 71 % relative humidity and approximately 90 mm of average rainfall recorded for the month of March according to the Institute National Meteorology (INMET, 2010).

2.2 Physico-chemical analysis

After sampling, the pH, turbidity, sedimentable solids, chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), and levels of oils and greases present in each effluent as well as in the Cleopatra stream water were determined using standard methods (Rice et al., 2012) in the Laboratory of Environment and Sanitation.

2.3 *Allium cepa*-test

The cytological preparation that were applied in this study was described by Kihlman (1971), with modifications (Silva et al., 2018). Small onion bulbs of *A. cepa* (2n = 16), were purchased from a commercial source and placed in bottles with filtered water at room temperature (± 25 °C) for growth. The bottles were aerated and roots of 1 to 1.5 cm length were obtained.

Before each treatment, some roots (3-5) were collected and fixed in a solution of methanol and acetic acid in a proportion of 3:1 to serve as controls of the self-bulb. This is considered as the initial timepoint 0 h (Co-0 h). The roots were then placed in the respective treatment solutions for 24 hours (Tr-24 h). After the treatment period, some roots were removed (3-5) and fixed. The remaining roots were washed again, and the bulbs were placed in filtered water for more than 24 hours to allow recovery of any damage. After this, the roots were removed and fixed in the methanol: acetic acid solution (Re-24 h). In the negative control group, the bulbs were placed in filtered water throughout time of sampling and were collected every 24 hours. After fixation, the roots were washed with distilled water. The material was then hydrolyzed in 1N HCl at 60 °C for 10 min followed by two washes with distilled water and transferred to a dark area in containers with Schiff's reagent for 45 min. At the end, the slides were prepared by crushing and permanent mounting.

The slides were analyzed by a "blind test" under a light microscope with a 40X objective. To assess the cells on the basis of their morphological and structural characteristics, and determine the mitotic index (MI), five bulbs were used for the

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control and effluent treatments, each. Per bulb, 1,000 cells were analyzed, totaling 5,000 cells per group. MI was calculated as: MI= a/b x 100%, where a: total dividing cells and b: total counted cells per group. Statistical analysis was performed by the Chi-square test ($\alpha = 0.05$).

3. Results and Discussion

The results showed that the cytotoxic potential of the raw effluent was high because it drastically inhibited the cell division by 99.9 % in the meristematic cells of *A. cepa*. Statistically different mitotic indices of the control (Co-0 h), recovery (Re-24 h) and negative control (24 h), and the outcome of treatment (Tr-24 h) with the effluent from the aerobic pond, respectively, were observed. Unlike other groups no significant differences were observed in the results when compared with each other and the negative control for the rate of cell division (Table 1). Cellular and chromosomal alterations were not verified in this study; therefore, results on mutagenic effects of the treatments are not presented.

Table 1. Mitotic index obtained in the three sample times.

Collection points	Mitotic Index ± SD		
	Co-0 h	Tr-24 h	Re-24 h
Negative Control	7.78±1.01	5.02±1.15	7.4±1.24
Raw Efluent	5.16±0.72	0.06±0.008[abcd]	8.58±1.3
Anaerobic pond	2.58±0.39	3.02±0.78	8.06±0.77[b]
Aerobic pond	3.12±1.54	4.04±0.99	6.6±1.19[b]
Pre-discharge	3.06±0.86	2.1±0.56	4.6±0.76
Post- Discharge	5.04±0.88	2.4±0.41	5.08±1.28

a Result statistically significant compared to negative control. b Result statistically significant compared to the control of the self-bulb (Co-0 h). c Result statistically significant compared to recovery (Re-24 h). d Result statistically significant compared to treatment (Tr-24 h) with the effluent of aerobic pond. Source: Research data.

Complex mixtures consisting of various types of effluents have a wide variety of components that can interact with each other and with the water bodies causing cytotoxic and genotoxic effects (Amaral et al., 2007). This occurs mainly with the wastewater discharges from industrial or domestic sources which contain many substances that cause acute effects on the organisms, reducing their long-term survival (Silva et al., 2003; Aziz et al., 2019).

The raw effluent is characterized by a high organic load, high fat content, pH fluctuations due to the use of cleaning agents, acids and bases, significant healing levels of various salts (relating to the conservation of a product by adding salt), compounds used as color fixatives (nitrates and/or nitrites), sugars and spices (Tritt & Schuchardt 1992; Liu & Haynes, 2011; Léon-Becerril et al., 2016; Thirugnanasambandham et al., 2016).

In the present study, measurement of the physico-chemical parameters of the raw effluent (Table 2) revealed that, except the pH, 6.35 (6-9), none of the parameters complied with the CONAMA 357/2005 (CONAMA, 2005) environmental regulatory tolerance limits for discharge into streams with water class 3. The values of our results and those of the stipulated tolerance limits for the different parameters are as follows: turbidity, 463 µT (should be not exceed 100 µT), solid sedimentary cone IMHOOF, 7 mL L h (should be virtually absent), COD (chemical oxygen demand), 1580 mg L (should be not exceed 90 mg L), BOD_5 (biochemical oxygen demand), 1317 mg L (should not exceed 10 mg L), and oils and greases, 160.8 mg L (should be virtually absent).

Table 2. Physico-chemical analysis of effluents collected from the sausage industry and water of the Cleopatra stream.

Collection points	pH	Turbidity (uT)	Sedimentable solids (mL L h)	COD (mg L)	BOD_5 (mg L)	Oils and greases (mg L)
Raw effluent	6.35	463	7	1580	1317	160.8
Anaerobic pond	6.53	89	0.09	386	311	6.9
Aerobic pond	7.30	53.8	0.01	208	67	1.7
Pre-discharge	7.08	8.73	0	4.8	3	1.8
Post-discharge	7.01	8.52	0	20.6	8.2	0.7

COD=Chemical oxygen demand, BOD_5=Biochemical oxygen demand, Sedimentable solids IMHOFF cone. Source: Research data.

Physico-chemical analysis of the effluents from slaughterhouses have high values of BOD, suspended solids and grease floatable material due to the presence of blood, pieces of meat, fat, entrails and guts (Scarrasati et al., 2003; Léon-Becerril et al., 2016). Oils and greases are commonly present in the effluents of many diverse origins. In this case, they are a part of the raw materials used in the industry (Giordano, 2004; Nowrouzi et al., 2021).

Possibly because of these characteristics, the raw effluent analyzed in this study exhibited high cytotoxic potential due to the presence of oil and grease involved in the process of decomposition which reduced the concentration of dissolved oxygen and consequently increased the COD and BOD_5, causing changes in the aquatic ecosystem. Additionally, reduction in the availability of dissolved oxygen to the cells of *A. cepa*, can lead to hypoxia followed by cell death. Possibly, this effect was observed on the onion cells when in direct contact with the raw effluent. However, when the cells were put back into the water during recovery time, they divided again, and at a higher rate than those of the controls.

According to Smack-Kincl and collaborators (1996), lethal and sub-lethal effects in test organisms are caused when the mitotic index reduces to less than 50 % and 22 % of the negative control, respectively. This level, in which 50 % reduction of mitotic index is observed, is called the threshold of cytotoxicity. In the present study, less than 50 % rate of cell division was observed in the raw effluent group while the effluent was cytotoxic when in contact with the meristematic cells of *A. cepa*. However, according Scarrasati and collaborators (2003), the effluent from slaughterhouses, for the most part, do not contain hazardous waste but consist primarily of organic matter. Indeed, when the bulbs of *A. cepa* were removed and placed in the filtered water during recovery time, the meristematic cells showed the same pattern observed in the negative control and the bulbs. That is, during the recovery time, transfer of the bulbs from the raw effluent into filtered water restored the osmotic conditions in the meristematic cells and provided them with adequate oxygen for division. However, it is important to highlight that, for this to occur, contact with the effluent must be ceased.

The groups treated with the effluent of anaerobic and aerobic ponds presented statistically significant differences between the results obtained after 24 h of recovery (8.06 and 6.6, respectively) and those of the controls themselves (2.58 and 3.12, respectively), with increased rates of cell division. The reason for this increase may be related to the environment of the effluent ponds which is rich in organic matter, serving as a source of nutrients for the induction of cell division. Moreover, according to the physico-chemical analysis, a reduction in the values was observed for the parameters evaluated for the two ponds in relation to those of the raw effluent. The reduction of COD, BOD, and oils and greases was 386 mg/L to 208 mg L, 311 mg L to 67 mg L, and 6.9 mg L to 1.7 mg L, respectively. This reduction in the values of the evaluated parameters of the water from anaerobic to aerobic ponds represents the effectiveness of their treatments; however, they still showed values above those permitted by the Resolution 357/2005 of CONAMA (2005). For the anaerobic and aerobic ponds, the respective values of pH (6.53 and 7.3), turbidity (89 µT and 53.8 µT) and settleable solids in the Imhoff cone (0.09 mL L h and 0.01 mL L h) were within the permissible limits.

The anaerobic ponds are usually deep, with a depth ranging from 4 to 5 meters. The depth is intended to prevent the oxygen produced on the surface layer from penetration into the lower layers. To ensure anaerobic conditions, a large amount of effluent per unit volume of the pond is discharged, and with it, oxygen consumption takes place in the upper layers. As the length of the surface of the pond is smaller than its depth, the oxygen produced by the algae and atmospheric re-aeration are considered negligible. The process of anaerobic decomposition of organic matter generates sub-products of high energy (biogas) and, thus, the availability of energy required for the reproduction and metabolism of bacteria is lower than that of the aerobic process (Von-Sperling, 2002; Sivamani et al., 2021).

However, when the BOD of the effluent from an anaerobic pond is high, it implies that there is a need for a subsequent treatment unit (Von-Sperling, 2016), which is usually an aerobic pond. This pond serves not only to ensure oxygenation of the medium, but also to keep the suspended solids (biomass) dispersed in the liquid medium (Scarrasati et al., 2003; Aziz et al., 2019).

In aerobic ponds, oxygen is supplied by mechanical aerators rather than employing the conventional method in which it is produced as a result of photosynthesis in algae. Mechanical aerators are equipment with rotating vertical axis turbines that cause great turbulence in the water by rotating it at a high speed. Movement of water facilitates penetration and dissolution of the oxygen (Chernicharo, 2006; Pardi et al., 2006).

As mentioned by Scarrasati and collaborators (2003) and Aziz et al. (2019), aerobic and anaerobic ponds are commonly employed for the treatment processes while debugging the dumps of slaughterhouses, the first being more appropriate, given the nature of the discharges. High loads of BOD and suspended solids, which are characteristics of these discharges, are the basic requirements for successful anaerobic treatment. The industry evaluated in the present study made use of these two types of ponds and correctly followed the recommendations for the treatment of effluents produced in their processes.

The COD is very useful when used in conjunction with BOD_5 to observe the biodegradability of evictions. The BOD_5 measures only the biodegradable fraction, and the closer this value is to that of the COD, it is indicative of a correspondingly higher effluent biodegradability. However, it should be noted that the values of COD/DBO_5 are different for different effluents, but for the same effluent, the relationship changes depending on the treatment, particularly biological. Thus, a raw effluent with COD/DBO_5 equal to 3/1, may, for example, change to 10/1 after biological treatment which operates to a greater extent on the BOD_5 (CETESB, 2009).

According to physico-chemical analyses, the parameters of pH, turbidity and oil and greases for the water before discharge into the Cleopatra stream were 7.08, 8.73 µT and 1.8 mg L, and those post-discharge were 7.01, 8.52 µT and 0.7 mg L, respectively, which were within the levels permitted by CONAMA 357/2005 for class 3 waters (pH 6-9, turbidity not exceed the up 100 µT, and oils and greases virtually absent).

The values of COD (20.6 mg L) and BOD (8.2 mg L) post-discharge were higher than those found pre-discharge (4.8 mg L and 3.0 mg L, respectively). This was possibly because the aerobic pond effluent, which has higher levels of these factors, was released into the stream.

Turbidity measures the amount of light reflected from a water sample. In the present work, turbidity in the waters of the Cleopatra stream as well as that of the effluents from two treatment ponds is within acceptable levels, which suggests the effectiveness of the treatment carried out by industry. This parameter is adopted for monitoring the control of water pollution and verification of the physical parameters of waters considered potable.

The results obtained by treating *A. cepa* with water of the Cleopatra stream pre- and post-discharge of the effluent did not differ from each other, indicating that the rate of cell division had not been affected by the effluent from the industry. It is important to highlight that Cleopatra stream have been affected by the industrial sectors of the region, whereby they receive several effluents of different origins from different sources (Silva et al., 2018), as well as other streams. In the study by Düsman and collaborators (2011), effects of the waters of the Corregozinho, Isalto, Morangueira and Ozório streams located in the urban area of Maringá, Paraná were evaluated on the same test system. In this work as well, their cytotoxic potential has not been verified.

Düsman and collaborators (2012), evaluated samples of water from the streams Mandacaru, Maringá, Miosotis and Nazareth located in the urban region of Maringá, Paraná and found that these waters did not show cytotoxic activity in the meristematic root cells of *A. cepa*. According to the study, these results were considered to be dependent on climatic variations, the concentration of harmful substances, and the time and place of sampling. Peron and collaborators (2009), found that the waters of the Rio Pirapó-Paraná collected during rains showed no cytotoxic potential; however, in periods of water scarcity, significantly reduced rates of cell division were observed in the root meristematic cells of *A. cepa*.

Thus, one factor that needs to be considered and may also explain the outcomes of this study are the rains that took place in the region before sample collection which may have diluted the effluent treatment ponds and stream water.

4. Conclusion

The results obtained in this study, including those of the cytotoxicity and physico-chemical parameters indicate that the treatment process employed for the anaerobic and aerobic ponds by the industry significantly reduced the potential damage to the environment that could be caused by these effluents when discharged into the stream. The validity of these data are strengthened when compared to the results of the water from the Cleopatra stream collected pre- and post-discharge, as there was no difference between the outcomes of the evaluation conducted at the two collection points and those of the effluent ponds.

The results show that there are many variables that can influence the components in water and their reactions with xenobiotics. Environmental variables such as pH, BOD_5, COD, water temperature and air, transparency, turbidity, salinity and presence of organic matter can interfere with the reactions of toxic agents and the biotic and abiotic components influencing the toxicity of the chemical compounds. These reactions are also influenced by the occurrence of rainfall and its effect on the total depth of the river, as well as the possibility of trawl in soils changing the physical and chemical composition of water. Due to the above reasons, environmental genotoxicity studies should also consider these variables, as they are permanent and systematic.

Thus, despite absence of cytotoxic potential, in *A. cepa* meristematic cells, of the effluents, it is known that the Cleopatra stream suffers constant degradation through anthropogenic processes, mainly from industrial activities. Therefore, it is extremely important that environmental monitoring studies in the region must be developed with the aim of preserving and maintaining water quality, and consequently, the ecosystems. However, the present generations need a new culture regarding the use of water because it is the required for their well-being and survival. The concern of environmental contamination is very important, and research aimed at identifying the presence of toxic agents, assessing their bioavailability through the analysis of their components, and their activities in different bioassays needs to be carried out.

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References

Amaral, A. M., Voltolini, J. C., Barros, L., & Barbério, A. (2007). Avaliação preliminar da citotoxicidade e genotoxicidade, da água da bacia do rio Tapanhon (SP- Brasil) através do teste *Allium* (*Allium cepa*). *Rev Bras Toxicol*, 20, 65-72.

Aziz, A., Basheer, F., Sengar, A., Irfanullah, Khan, S. U., Farooqi, I. H. (2019). Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. *Sci. Total Environ*. 686, 681-708.

Bustillo-Lecompte, C. F., & Mehrvar, M. (2015). Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *J Environ Manage,* 161, 287-302.

Bustillo-Lecompte, C. F., & Mehrvar, M. (2016). Treatment of an actual slaughterhouse wastewater by integration of biological and advanced oxidation processes: Modeling, optimization, and cost-effectiveness analysis. *J Environ Manage,* 182, 651-666.

Cardoso, N. L. L., Silva, F. F., Silva, A. K. M., Ribeiro, J. A. T., Valinhas, R. V., Penido, W. D., Souza, I. B. S. de, Silva, J. A. da, Granjeiro, P. A., Magalhães, J. T. de, & Gonçalves, D. B. (2022). Utilização de bactérias para biorremediação de resíduos lácteos: uma revisão panorâmica. *Res., Soc. Dev*., 11(7), e30311729830.

CESTEB, Companhia Ambiental do Estado de São Paulo. (2009). *Qualidade das águas interiores no estado de São Paulo*. http://cetesb.sp.gov.br/aguas-interiores/wp-content/uploads/sites/32/2013/11/variaveis.pdf

Chan, Y. J., Chong, M. F., Law, C.L., & Hassell, D. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chem Eng J,* 155,1-18.

Chernicharo, C. (2006). Post-treatment options for the anaerobic treatment of domestic wastewater. *Rev Environ Sc. Biotechnol*, 5, 73-92.

CONAMA – Conselho Nacional do Meio Ambiente. (2005). *Resolução n° 357/2005*. http://www.mma.gov.br/port/conama/res/res05/res35705.pdf

Düsman, E., Faria, J. S., Toledo, F., Mazeti, C. M., Gonçalves, M. E. K., & Vicentini, V. E. P. (2011). Vegetal test-system investigation on cytotoxicity of water from urban streams located in the northeastern region of Maringá, Paraná State, Brazil. *Acta Sci Biol Sci*, 33, 71-77.

Düsman, E., Gonçalves, L. A., Reusing, A. F., Martin, P. G., Mariucci, R. G., & Vicentini, V. E. P. (2012). Cytotoxic potential of waters of the streams Mandacaru, Maringá, Miosótis and Nazareth in the urban area of Maringá PR Brazil. *Acta Sci Biol Sci*, 34, 311-318.

Giordano, G. (2004). Tratamento e Controle de Efluentes Industriais. *Rev Abes*, 1-81.

Hoffmann, S., Feldmann, U., Bach, P. M., Binz, C., Farrelly, M., Frantzeskaki, N., Hiessl, H., Inauen, J., Larsen, T. A., Lienert, J., Londong, J., Lüthi, C., Maurer, M., Mitchell, C., Morgenroth, E., Nelson, K. L., Scholten, L., Truffer, B., & Udert, K. M. (2020). A Research Agenda for the Future of Urban Water Management: Exploring the Potential of Nongrid, Small-Grid, and Hybrid Solutions. *Environ Sci Technol*, 54 (9), 5312-5322.

Hilares, R. T., Atoche-Garay, D. F., Pinto Pagaza, Muhammad, A. A., Colina Andrade, G. J., & Santos, J. C. (2021). Promising physicochemical technologies for poultry slaughterhouse wastewater treatment: A critical review. *J. Environ. Chem. Eng*., 9 (2), 105174.

INMET – Instituto Nacional de Meteorologia. (2010). *Monitoramento das Estações Automáticas*. http://www.inmet.gov.br/sonabra/maps/automaticas.php.

Iqbal, M., Abbas, M., Nisar, J., Nazir, A., & Qamar, A. (2019). Bioassays Based on Higher Plants As Excellent Dosimeters for Ecotoxicity Monitoring: A Review. *Chem Int,* 5 (1), 1-80.

Kihlman, B. A. (1971). Root tips for stuying the effects of chemicals on chromosomes. *Chemical Mutagens: Principles and Methods for theis Detection*. Hollaender, A. New York-London: Plenum Press.

Leme, D. M., & Marin-Morales, M. A. (2099). *Allium cepa* test in environmental monitoring: A review on its application. *Mut Res*, 682, **7**1-81.

Liu, Y. Y., & Haynes, R. J. (2011). Origin, nature, and treatment of effluents from dairy and meat processing factories and the effects of their irrigation on the quality of agricultural soils. *Crit Rev Environ Sci Technol*, 41,1531-1599.

Martins, G. A., Corrêa, L. B., Guidoni, L. L. C., Lucia Jr, T., Gerber, M. D., Silva Jr, F. M. R., & Corrêa, E. K. (2022). Toxicity and physicochemical parameters of composts including distinct residues from agribusiness and slaughterhouse sludge. *J. Waste Manag,* 138, 75-82.

McCabe, B.K., Harris, P., Baillie, C., Pittaway, P., & Yusaf, Y. (2013). Assessing a new approach to covered anaerobic pond design in the treatment of abattoir wastewater. *Aust J Multi-Disciplinary Eng*, 10, 81-93.

Metha, L. (2014). Water and Human Development. *World Dev*, 59, 59-69.

Nowrouzi, M., Abyar, H., & Rostami, A. (2021). Cost coupled removal efficiency analyses of activated sludge technologies to achieve the cost-effective wastewater treatment system in the meat processing units. *J. Environ. Manage*, 283, 111991.

Pardi, M. C., Santos, I. F., Souza, E. R, & Pardi, H. S. (2006). *Ciência, higiene e tecnologia da carne*. 2th ed. Goiânia: UFG and Eduff.

Peron, A. P, Canesin, E. A., & Cardoso, C. M. V. (2009). Mutagenic potential of the Pirapo River (Apucarana, Paraná, Brazil) water in meristematic root cells of *Allium cepa* L. *Rev Bras Biocienc*, 7(2), 155-159.

Rahman, U., Sahar, A., & Khan, M. A. (2014). Recovery and utilization of effluents from meat processing industries. *Food Res Int*, 65, 322-328.

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *APHA, Standard methods for the examination of water and wastewater*, 22 ed.

Scarrasati, D., Carvalho, R. F., Delgado, V. L., Coneglian, C. M. R., Brito, N. N., Tonso, S., Dragoni Sobrinho, G., & Pelegrini, R. (2003). *Tratamento de Efluentes de Matadouros e Frigoríficos*. In III Fórum de Estudos Contábeis. http://www.ctec.ufal.br/professor/elca/TRATAMENTO%20DE%20EFLUENTES%20DE%20MATADOUROS%20E%20FRIGOR%C3%8DFICOS.pdf

Silva, J. S., Heck, M. C., Buzo, M. G., Almeida, I. V., & Vicentini, V. E. P. (2018). Evaluation of textile laundry effluents and their cytotoxic effects on *Allium cepa*. *Environ Sci Pollut Res*, 25, 27890-27898.

Silva, J., Erdtmann, B., & Henriques, J. A. P. (2003). Estudos Toxicológicos no Ambiente e na Saúde Humana In: Silva, J., Erdtmann, B., Henriques, J. A. P. editors. *Genética Toxicológica*. Porto Alegre: Alcance, p. 70-84.

Sivamani, S., Saikat, B., Naveen Prasad, B. S., Baalawy, A. A. S., & Al-Mashali, S. M. A. (2021). A comprehensive review on microbial technology for biogás production. In: Srivastava, M., Srivastava, N., & Sing, R. (eds). *Bioenergy Research: revisitng latest developmente. Clean Energy Production Tecnologies*. Springer, Singapore.

Smaka-Kincl, V., Stegnar, P., Lovka, M., & Toman, M. J. (1996). The evaluation of waste, surface and ground water quality using *Allium cepa* L. test procedure. *Muta Res*, 368, 171-179.

Thirugnanasambandham, K., Sivakumar, V., Sruthi, B. (2016). Recovery of biogás from meat industry wastewater using continuously stirred tank reactor (CSTR): Modeling and optimization. *In J Chem React Eng*, 14 (1), 125-132.

Tritt, W. P., Schuchardt, F. (1992). Materials flow and possibilities of treating liquid and solid wastes from slaughterhouses in Germany. A review. *Bioresour Technol*, 41, 235-245.

Tundisi, J. G. (2005). *Água no Século XXI: enfrentando a escassez*. 2nd ed. São Carlos: Rima.

USEPA (2016). *Effluent limitations guidelines and new source performance standards for the meat and poultry products point source category*. U. S. Environ.Prot. Agency (US EPA) Fed. Regist. 69 (173). http://nepis.epa.gov/Exe/ZyPDF.cgi/20002F0Q.PDF?Dockey¼20002F0Q.PDF.

Von Sperling, M. (2002). *Princípios do tratamento biológico de águas residuárias: lagoas de estabilização*. 3th ed. Belo Horizonte: Departamento de Engenharia Sanitária e Ambiental -UFMG.

Von-Sperling, M. (2016). *Princípios do tratamento biológico de águas residuárias: princípios básicos do tratamento de esgotos*. 2nd ed. Belo Horizonte: Departamento de Engenharia Sanitária e Ambiental -UFMG.