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Analysis of toxicity from the effluent generated in a furniture industry spray booth using the species *Lactuca sativa* and *Allium cepa*

Análise da toxicidade do efluente de cabine de pintura de indústria moveleira por meio das espécies *Lactuca sativa* e *Allium cepa*

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Abstract: This study evaluated the toxicity of effluent generated in a furniture industry spray booth, before and after treatment in a system composed of an anaerobic sequencing batch (ASBR) followed by an aerobic sequencing batch reactor (SBR). The toxicity tests were carried out with raw (with and without dilutions) and treated effluent to evaluate the toxic potential using *Allium cepa* and *Lactuca sativa* as bioindicators. The toxicity tests, using *Allium cepa* and *Lactuca sativa*, indicated that the anaerobic-aerobic treatment performed was efficient to reduce the toxicity of the paint booth effluent. The raw effluent, undiluted and diluted (at 1:10; 1:8 and 1:6 dilutions), showed toxic effect on the root growth of *Allium cepa*, as it inhibited root growth by 100%. In the tests with *Lactuca sativa* seeds, there was partial inhibition, between 44% and 63%, for 1:10 and 1:8 dilutions, but for the 1: 6 dilution and without dilution of effluent the inhibition was 100%. The treated effluent, in an ASBR followed by an SBR, presented a small percentage of inhibition for tests with *Allium cepa* (13%) and *Lactuca sativa* seeds (4%). The effluent treated by the anaerobic system followed by aerobic presented low toxicity without generating lethal or sub-lethal effects to the test organisms, which indicates the efficiency of the treatment process.

Keywords: Bioassay, formaldehyde, wastewater..

Resumo: Este estudo avaliou a toxicidade do efluente gerado na cabine de pintura da indústria moveleira, antes e após o tratamento, em um sistema composto por um reator anaeróbio em bateladas (ASBR) seguido de um reator aeróbico em batelada sequencial (SBR). Os testes de toxicidade foram realizados com efluente bruto (sem e com diluição) e tratado para avaliação do potencial tóxico utilizando como bioindicador *Allium cepa* e *Lactuca sativa*. Os testes de toxicidade, utilizando *Allium cepa* e *Lactuca sativa*, indicaram que o tratamento aeróbio-anaeróbico realizado foi eficiente para reduzir a toxicidade do efluente da cabine de pintura. O efluente bruto, não diluído e diluído (diluições de 1:10; 1: 8 e diluição 1: 6), apresentou efeito tóxico no crescimento radicular de *Allium cepa*, pois inibiu o crescimento radicular em 100%. Nos testes com sementes de *Lactuca sativa*, houve inibição parcial, entre 44% e 63%, para diluições de 1:10 e 1: 8, mas para a diluição de 1: 6 e sem diluição do efluente a inibição foi de 100%. O efluente tratado, em ASBR seguido de SBR, apresentou pequena porcentagem de inibição para testes com sementes de *Allium cepa* (13%) e *Lactuca sativa* (4%). O efluente tratado pelo sistema anaeróbio seguido de aeróbio apresentou baixa toxicidade sem gerar efeitos letais

ou subletais aos organismos testados, o que indica a eficiência do processo de tratamento realizado.

Palavras-chave: águas residuárias, bioensaio, formaldeído..

1. INTRODUCTION

The wastewater produced in spray booths of the furniture industry change according to the type of the production. The most-used products in the final finishing step, besides dyes, were varnishes and thinner (solvent). Paints and varnishes contain urea-formaldehyde resins, so formaldehyde is one of the organic compounds in higher concentrations (up to 400 mg.L⁻¹) in this type of wastewater, and this compound can be toxic to humans and animals (Lu e Hegemann, 1998).

However, formaldehyde can be biodegradable in concentrations lower than 100 mg HCHO. L⁻¹ (Pereira and Zaiat, 2009). In higher concentrations, it causes the inhibition of the microorganisms, and consequently reduces the capacity of organic matter removal in the treatment process. In this case, it might be necessary to dilute the effluent to avoid inhibition of the process (Lu and Hegemann, 1998).

The toxicity of a compound, such as formaldehyde, can be analyzed by means of a toxicity test. According to Kapanen and Itävaara (2001), toxicity tests are classified by time of exposure (acute or chronic), the effect mode (death, growth or reproduction) or the effect response (lethal and sub-lethal). The acute and subacute exposure tests differ from the chronic because they evaluate the effects upon an organism during a short time, unlike the chronic test, which is based on a longer exposure. Subacute toxicity tests, known as low-duration tests, are developed with a focus on quantitative assessments, such as the effects on the growth of organisms tested. (Kapanen and Itävaara, 2001).

Plants have been shown to be useful when used as bioindicators to monitor the presence of toxic compounds in rivers and lakes. *Allium cepa* (onion) has been used in ecotoxicological tests to evaluate several compounds (Düsman et al., 2014).

Düsman et al. (2014) analyzed the cytotoxic potential of the surface water of the Quatorze River in Francisco Beltrão, Paraná, Brazil by means of tests with the roots of *Allium cepa*. Besides root growth, the authors verified possible alterations in meristematic cells of the plant. The authors affirmed that among the advantages of using the test with *Allium cepa*, it is worth noticing that it is a low-cost, easy-to-handle test with no prior preparation required.

According to Fiskesjö (1985), the *National Water Research Institute* recommends the use of lettuce seeds (*Lactuca sativa*) in toxicity tests in effluents, soils or sediments, due to its rapid grow and the fact that little energy is required to germinate it. The advantages of the usage of theses vegetables are their low-cost, easy-cultivation, availability during the whole year and the possibility of using them both in acute and chronic toxicity tests, in laboratory and field conditions (Fiskesjö, 1985).

This study evaluated the toxicity of the effluent generated in a spray booth of the furniture industry, before and after treatment in a system composed of an anaerobic sequencing batch (ASBR) followed by an aerobic reactor operated under an SBR mode. The toxicity tests were carried out with raw (without and with dilutions) and treated effluent for evaluating the toxic potential using as bioindicators *Allium cepa* and *Lactuca sativa*.

2. MATERIALS AND METHODS

2.1. Experimental apparatus and procedures

For the biological treatment of the furniture industry spray booth effluent, a bench-scale system was used (Figure 1), composed of an anaerobic sequencing batch reactor (ASBR) followed by an aerobic reactor operated under an SBR mode. The ASBR was constructed of Polyvinyl chloride, with an internal diameter of 10 cm and height of 33.5 cm with total volume of 2.5 L and working volume of 2.0 L. The aerobic SBR was a glass vessel with a total volume of 2 L and useful volume of 1.1 L. Continuous aeration was performed by means of an aquarium aerator with porous stone at the end of the hose connected to the system.

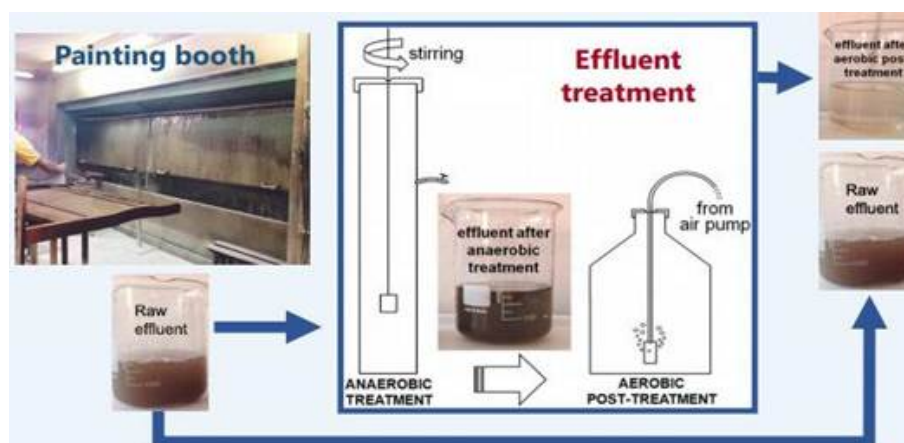


Figure 1.

Schematic diagram of the experimental system.

Sludge of an upflow anaerobic sludge blanket reactor from the Sewage Treatment Plant - ETE Norte / Sanepar -Londrina-PR was used as inoculum for the start-up of an ASBR. The sludge inoculum (30.48 g TVS.L⁻¹) was adapted for 28 days with the diluted (1:10) wastewater. For the start-up of the SBR the seed sludge was obtained from the aeration 300 mL of treated sewage in a structured-bed reactor, in bench-scale with intermittent aeration. The 300mL were mixed with 800 mL of effluent from the ASBR and kept in aeration for adaptation. The biomass adaptation occurred within 1 week and was monitored by COD (mg O₂. L⁻¹).

The hydraulic detention time (HDT) of operation was 4 days for the ASBR and 2 days for the SBR. The ASBR was fed with raw effluent and the SBR was fed with the effluent coming out of the ASBR. The main characteristics of the raw effluent were COD of $10550 \text{ mg O}_2\text{.L}^{-1}$ and formaldehyde of $400 \text{ mg HCHO.L}^{-1}$.

The ASBR-SBR system was operated in three distinct phases according to the concentrations of influent COD, so it was necessary to dilute the wastewater to feed the ASBR. Dilutions to obtain the desired COD concentrations were performed with tap water. The operating conditions of the ASBR-SBR system were, according to COD concentration: Phase 1 - 1:10 dilution ratio ($700 \pm 70 \text{ mgO}_2\text{. L}^{-1}$ and $34 \pm 5 \text{ mg HCHO. L}^{-1}$); Phase 2 - 1: 8 dilution ratio ($1856 \pm 200 \text{ mg O}_2\text{. L}^{-1}$ and $157 \pm 7 \text{ mg HCHO. L}^{-1}$); Phase 3 - 1: 6 dilution ratio ($3960 \pm 100 \text{ mg O}_2\text{. L}^{-1}$ and $232 \pm 10 \text{ mg HCHO.L}^{-1}$). There was no adaptation period between the different phases.

The SBR was fed with 600 mL of the ASBR effluent every 48 hours. To coincide with the 4-day HDT of the ASBR, the remaining 600 mL of the ASBR effluent - once 1.2 L of the ASBR was withdrawn - were kept in a refrigerator programmed to reach room temperature for each new feed after 48h

The concentrations of COD were determined (potassium dichromate oxidation method- COD 5220 -D) according to APHA (2005). Formaldehyde was determined by the colorimetric method according to Bailey and Rankin (1971).

2.2. Toxicity tests using *Allium cepa*

The toxicity tests were performed with samples of the undiluted and diluted raw effluent (dilutions with distilled water - 1:10; 1:8 and 1:6) and with samples of the treated effluent of Phase 2. The treated effluent from Phase 2 was used because it was the phase that presented better stability of operation of the ASBR-SBR system.

The tests were conducted as described by Fiskesjö (1985) with modifications. The onion bulbs were bought commercially in the supermarket and kept in a place free of moisture and protected from light. The surface peels were removed and the onions were placed in flasks (small vessels) with the root portion immersed in 50 mL of mineral water for hydration for 48 hours. After this period, those that showed root growth were selected, excluding those that did not show any growth. The roots were then carefully cut so that they were not more than 5 mm.

The bulbs were placed into vials, with their root portions exposed to 50 mL of the raw effluent with and without dilution and the treated effluent from the ASBR system followed by the SBR. The characteristics of the samples tested are shown in Table 1.

Table 1.
Concentrations of organic matter and formaldehyde in the samples tested.

| Samples tested | Dilution | Characteristics of the samples tested | |
|-------------------------------|------------------|--|-----------------------------|
| | | COD (mg O ₂ . L ⁻¹) | HCHO (mg. L ⁻¹) |
| Raw Effluent | 1:10 | 700 | 36 |
| | 1:8 | 1960 | 157 |
| | 1:6 | 4060 | 235 |
| | Without dilution | 10550 | 400 |
| Treated Effluent from Phase 2 | Without dilution | < 25 | 0.8 |

The test had as negative control onions exposed only to mineral water. After seven days of exposure at a temperature of 25°C and in the absence of light, root growth was observed. The tests were performed with six replicates for each sample and each set of tests was repeated three times.

The tests were performed to analyze the subacute toxicity, checking at the end of the procedure the length of the highest root of each onion, performing the measurement with ruler. The root growth of the *Allium cepa* in the control condition was used as a parameter, and from that parameter root growth inhibition ratios (ICR) exposed to treated effluent and untreated effluent were developed, using Equation 1, according to the methodology proposed by Palácio et al. (2012):

$$\% ICR = \frac{NRCA}{NRCC} * 100 \quad (1)$$

Being:

- : Root growth inhibition;
- : Number of grown roots in the samples;
- : Number of grown roots in the control.

2.3. Toxicity test used in *Lactuca sativa* seeds

The test was performed following the methodology described by Sobrero and Ronco (2004) and Palácio et al. (2013) with some adaptations. *Lactuca sativa* (mimosa lettuce) seeds with the germination percentage of 98% were used.

The used samples followed the conditions presented in Table 1, as used in the tests with *Allium cepa*. Mineral water was used as a negative control. Petri dishes 9 cm in diameter were prepared with filter paper, where twenty seeds of lettuce moistened with the samples were prepared previously and deposited. The tests were carried out in triplicate.

The plates were packed in plastic bags to avoid the loss of humidity and taken to the bacteriological oven with a temperature of 20°C ± 2, in the dark, for a period of 120 hours. After the incubation period, the number of germinated seeds was counted and the roots and the radicle lengths

were measured. The percentage of relative germination for each dilution was calculated using Equation 2.

$$\%GR = \frac{NSGA}{NSGC} * 100 \quad (2)$$

Being:

- : The relative germination percentage;
- : Number of germination seeds in samples;
- : Number of germinations seeds in control.

Percentages of relative roots growth inhibition were calculated by the medium values for each sample, using Equation 3.

$$\% ICRRZ = \frac{MCRzC - MCRzA}{MCRzC} * 100 \quad (3)$$

Being:

- : Root growth average in control;
- : Root growth average in samples;

3. RESULTS AND DISCUSSION

The anaerobic treatment followed by aerobic (ASBR-SBR) provided a 98% efficiency in the removal of COD for Phases 1 and 2 (dilutions: 1:10 and 1:8). As shown in Table 1, the treated effluent from Phase 2 had organic matter content lower than 25 mg O₂.L⁻¹ (COD) and formaldehyde less than 0.8 mg HCHO.L⁻¹.

It should be noted that for Phase 3 (dilution of 1:6), the ASBR presented instability with less organic matter and formaldehyde removal (efficiency of 75% and 64%, respectively) compared to Phases 1 and 2. This must have been due to the inhibition of the action of microorganisms caused by the high concentration of formaldehyde (Pereira and Zaiat, 2009). However, the polishing in the aerobic reactor (SBR) was efficient in the removal of the organic matter (COD) and formaldehyde with a final concentration lower than 50 mg O₂. L⁻¹ and 50.9 mg HCHO. L⁻¹.

3.1. Tests with *Allium cepa*

The tests found that the toxicity of the raw effluent and its dilutions had sub-lethal effects, and caused root-growth inhibition, especially for the raw effluent without dilution. It was observed that in the raw effluent, with and without dilution, there was no root growth, and the roots had sizes smaller than 5 mm, as shown in Figure 2. The results showed the toxicity of raw effluent (without dilution) in all tested dilutions. Further, the results found that the raw effluent without dilution had lethal effects

(inhibiting germination) that deteriorated the roots and left them with sizes smaller than 5 mm (Figure 2 (d)).

The treated effluent tests showed growth of the *Allium cepa* root similar to the root growth of the control samples kept in contact with only mineral water (Figure 2 (e) (f) (g)).

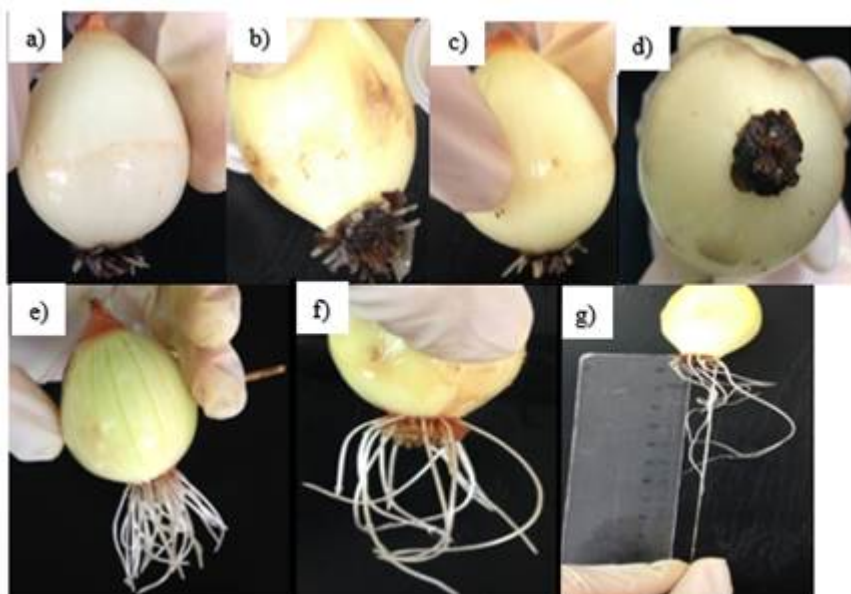


Figure 2.

Image of the *Allium cepa* root grown after the incubation to the toxicity test with the raw effluent for dilutions of a) 1:10; b) 1:8; c) 1:6; and d) without dilution; e) control; f) treated effluent; g) measurement of the bulb root growth immersed in treated effluent.

In Table 2, the medium values of the length of the roots are presented for the samples tested and the mentioned inhibition, in relation to the observed growth in the control. An inhibition of 100% was verified for the raw effluent and for all its tested dilutions. For the treated effluent, there was root growth under conditions similar to the control bulbs, with maximum inhibition of 13%.

Table 2.

Root length (cm) and growth inhibition (%) after 7 days.

| Samples tested | Dilution | Root length (cm) | Growth inhibition - ICR (%) |
|----------------|------------------|------------------|-----------------------------|
| Control | | 9.1 ± 2.3 | - |
| Raw Effluent | 1:10 | 0 | 100 |
| | 1:8 | 0 | 100 |
| | 1:6 | 0 | 100 |
| | without dilution | 0 | 100 |
| Treat effluent | without dilution | 8.3 ± 2.5 | 13.4 |

Fiskesjö (1985) observed that root growth was inversely proportional to concentrations of determined toxic compounds and heavy metals,

such as mercury, copper, zinc and cadmium present in industry effluent. Santos et al. (2010) evaluated formaldehyde toxicity by means of biotests with *Allium cepa* and, besides growth inhibition, the tests indicated the appearance of micronuclei in bulbs exposed to toxic components caused by mutagenicity.

Butler et al (2011) observed toxicity reduction in tests with *Allium cepa* of metallurgical industry effluent after biological treatment. According to the authors, the toxicity reduction occurred due to the removal of metals and of other toxic substances in the effluent treatment process. In this study, despite of the different dilutions tested for the raw effluent, all toxicity tests with *Allium cepa* indicate 100% growth inhibition while the treated effluent inhibition was only 13.4%.

3.2. Tests with *Lactuca sativa*

The tests with *Lactuca sativa* were performed with the same samples of the effluent used for the *Allium cepa* test. The soaked seeds in the treated effluent had germination potential in all the prepared samples and had root growth similar to the that obtained in the controls. The germination potential and root growth obtained after 4 days in the tested samples can be observed in Figure 3. The relative germination index and the root growth inhibition index are presented in Table 3.

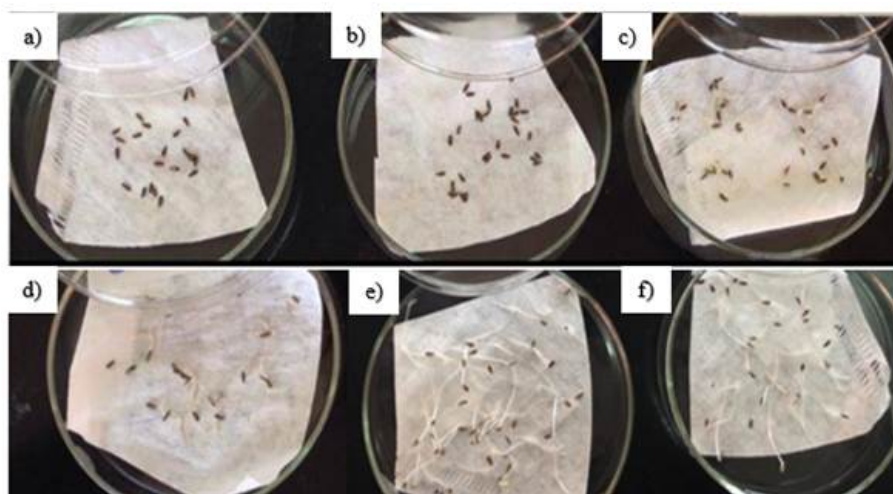


Figure 3.

Petri Dishes contain *Lactuca sativa* seeds soaked in the raw effluent a) without dilution and with dilutions of b) 1:6; c) 1:8; d) 1:10; e) treated effluent; f) control, after 120 hours.

Table 3.

Relative Germination (RG) and Root Growth Inhibition (ICRRz) for the raw effluent without dilution and for tested dilutions (1:10; 1:8; 1:6) and for the treated effluent.

| Samples tested | Dilution | RG (%) | ICRRz (%) |
|----------------|------------------|--------|-----------|
| Raw effluent | 1:10 | 79 | 44 |
| | 1:8 | 22 | 63 |
| | 1:6 | 0 | 100 |
| | without dilution | 0 | 100 |
| Treat effluent | without dilution | 100 | 4 |

Considering that values of ICCR below 80% indicate a root-growth inhibition effect (Young et al., 2012), it was verified that the raw effluent without dilution and with a dilution of 1:6 showed lethal toxicity to the lettuce seeds, with 100% inhibition of relative germination. However, for dilutions of 1:8 and 1:10 the toxic effect was less aggressive, classified as sub-lethal, making the seeds' germination and root growth possible. For a dilution of 1:8, only one of the triplicates showed germination and root growth for 12 of 60 seeds, while for a dilution of 1:10 potential germination and root growth was found in the all of the triplicates.

There was not germination in tests of raw effluent without dilution and for the dilution of 1:6, probably due to the presence of toxic components, such as formaldehyde. Palácio et al. (2012) obtained 100% inhibition of the potential germination of *Lactuca sativa* seeds for the textile industry, with a decrease of the inhibition percentage for the same diluted effluent.

Borba et al. (2008), in a test with *Lactuca sativa*, identified a reduction of 50% in the toxicity of furniture industry effluent after the treatment of the effluent by the photo-fenton method. According to the authors, the reduction was due to the removal of organic toxic compounds, such as formaldehyde.

The test with *Lactuca sativa* showed less resistance of the lettuce seeds to the toxic effect of the effluent when compared to the test performed with *Allium cepa*. However, both tests showed a low toxicity potential of the treated effluent.

Duffeck et al. (2017) analyzed toxicity of the same effluent used in the present research: raw without dilution and diluted (1:6, 1:8, 1:10), and treated, using the Fish Embryo Toxicity test (FET) in *Danio rerio* (zebrafish). The authors verified that the raw effluent, even diluted, was toxic to *Danio rerio* embryos, causing high lethality as early as the first 24 hours of exposure. However, the treated effluent did not cause lethality, nor any sublethal alteration, indicating a decrease of the toxic load after the biological treatment, as was verified in the tests with *Allium cepa* e *Lactuca sativa* in this study.

4. CONCLUSIONS

Toxicity tests using *Allium cepa* and *Lactuca sativa* indicated that the anaerobic followed by aerobic treatment performed was efficient to reduce the toxicity of furniture industry wastewater. The raw effluent without dilution and diluted (1:10, 1:8, 1:6) showed a toxic effect on the root growth of *Allium cepa*, since it inhibited root growth by 100%. The raw effluent without dilution and for the dilution of 1:6 inhibited the germination of *Lactuca sativa* seeds (100% inhibition); however, there was a partial inhibition, between 44% and 63%, for dilutions of 1:10 and 1:8 of the raw effluent. The effluent treated by the anaerobic-aerobic process (ASBR-SBR) presented a small percentage of inhibition for tests with *Allium cepa* and *Lactuca sativa* seeds (13% and 4%, respectively).

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Author notes

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