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Evaluation of Mutagenic and Genotoxic Activity in Vinasses Subjected to Different Treatments

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Abstract The mutagenic and genotoxic activity of vinasses collected from a fuel alcohol plant, located in the municipality of Frontino, Northwestern Colombia, were evaluated. Two samples obtained from an 82-L capacity hybrid reactor (UASB-anaerobic filter (AF)-UASB) were studied under laboratory conditions after being treated with biological oxidation, the first, and the second with Fenton reaction consecutively. Mutagenicity was evaluated in vitro by the Ames test using strains TA98 and TA100 with and without S9 metabolic activation. The genotoxic analysis was conducted using the *Allium cepa* roots assay where chromosomal aberrations were used as clastogenic or aneugenic response markers, and micronuclei as mutagenic response. The Ames test results showed a strain-dependent positive linear association with the vinasse sample concentration before treatment (dose–response effect). Unlike TA100, strain TA98 showed a mutagenic effect in both the presence and absence of metabolic enzymes. After the biological oxidation treatment, vinasse mutagenicity significantly decreased. Finally, after Fenton treatment, the sample did not induce any mutagenic event. Genotoxic activity was observed in all three samples, but there was a higher frequency in the vinasse sample before treatment. Concerning the frequency of micronuclei, no clear association was observed with either the concentration or the type of sample.

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1 Introduction

Ethanol production from agricultural feedstocks for alternative fuel usage has been of great interest, mainly due to an increased demand for non-renewable energy sources and high prices of gasoline and natural gas. Large amounts of vinasses are produced in sugar cane ethanol industry. Vinasse is a liquid industrial waste highly pollutant to water and soil as a result of the presence of water-soluble recalcitrant compounds called melanoidins (Parnaudeau et al. 2008). Phenolic compounds, nitrogen, sulfur, phosphorus, potassium, and heavy metals are also present. The amount of the effluent and its properties are highly variable and depend on the feedstocks used and on several aspects in the ethanol production process. Vinasses are characterized by a biochemical oxygen demand (BOD) around 12,500 mg/L and a chemical oxygen demand (COD) around 20,000 mg/L (Mahadevan et al. 2006).

Improper disposal of these wastes into water sources causes oxygen depletion, destroying aquatic wildlife. Therefore, a number of biological alternatives have been proposed to reduce the polluting ability of the effluent, such as UASB bioreactors and chemical treatments like the Fenton reaction, the latter generates hydroxyl radicals (OH·) to oxidize the effluent organic matter (Matilainen and Sillanpää 2010). Although distilling industries use sophisticated techniques and different

ways of treating these wastes, they cannot guarantee a thorough removal of these compounds (Chandra et al. 2008), which are recognized as toxic, genotoxic, and potentially mutacarcinogenic (Christofoletti et al. 2013b). Moreover, genotoxic and mutagenic events caused by these untreated residues have been observed in tests with plants and *Drosophila melanogaster* (Christofoletti et al. 2013a; Yessilada 1999).

The Ames mutagenicity assay, Ames test, employs *Salmonella typhimurium* strains carrying mutations in the histidine operon to determine whether any compound or mixture is mutagenic or not, depending on the number of his^{-} to his^{+} revertant colonies. This assay has been extensively validated, and it is used to detect mutagenic activity in complex mixtures because of its high environmental sensitivity (Maertens et al. 2004). Higher plants are suitable systems for monitoring the presence of mutagens in the environment, as well as for the study of adverse effects of a particular mutagen at known concentrations (Maluszynska and Juchimiuk 2005). The *Allium cepa* test has been widely used for environmental quality assays and for its sensitivity to detect cytotoxic, genotoxic, and mutagenic agents in complex mixtures (Leme and Marin-Morales 2009).

The aim of this research was to study the influence of vinasse treatments, such as aerobic–anaerobic digestion in a UASB-anaerobic filter (FA)-UASB reactor and an advanced oxidation technology as the Fenton reaction, in the transformation or removal of cytotoxic, genotoxic, and mutagenic compounds found in sugar cane vinasses and detected using the Ames test and the *A. cepa* roots assay.

2 Materials and Methods

2.1 Vinasse Sampling

Vinasse samples were collected from a bioethanol production plant located in Frontino, Antioquia. A system of three anaerobic hybrid UASB-FA-UASB reactors was adapted to the laboratory environment for sample treatment; vinasse pH was adjusted in the 6.5–7.5 range. An inoculum was then added (1 % porquinase) and stabilized with vinasse as the only source of organic matter. Once stabilized, it was subjected to digestion, controlling hydrolysis, acidogenesis, and methanogenesis stages at 20, 30, and 52 °C, respectively.

After biological oxidation, the effluent was treated with Fenton reaction, removing the remaining organic matter in it. A combination of hydrogen peroxide and ferrous sulfate (Fenton reagent) was performed at atmospheric pressure and temperatures between 20 and 40 °C. In this case, a mixture of 8 g of $FeSO_4$ and 80 mL of H_2O_2 (50 %) was added per liter of vinasse, radicals generated easily oxidize the organic matter, previous pH 4 adjustment to obtain better oxidation results (Beltrán de Heredia 2005). Finally, pH was raised to 6.51 so that iron bases could precipitate (Novelo et al. 2010).

2.2 Mutagenicity Using the Ames Test

Mutagenicity of vinasse samples was evaluated using the *Salmonella*/reversion assay (histidine auxotrophic) with strains TA98 and TA100 (100 μL), based on the method described by Maron and Ames (1983). According to established in our laboratory, samples were analyzed with 100 μL of PBS buffer or liver S9 fraction (Moltox, Boone, NC, USA) that incorporate an important aspect of mammalian metabolism within the in vitro test. In addition, samples were directly evaluated (Vargas et al. 1995) in three independent experiments in duplicate petri dishes. Four 100-μL dilutions of vinasses were tested at nontoxic concentrations 20, 30, 40, and 50 %. H_2O was used as a negative control and 100 μL of 0.5 mg/mL of 4-nitroquinoline oxide (Sigma-Aldrich, CAS 56-57-5) for TA98 and 100 μL of 0.05 g/L of sodium azide (Sigma-Aldrich, CAS 26628-22-8) for TA100, both strains without metabolic activation. As positive controls 100 μL of 2-aminofluorene 1 μg/mL (Sigma-Aldrich, CAS 153-78-6) for both strains with metabolic activation were used. Mutagenicity index (MI) was determined to quantify mutagenicity, and this index refers to the number of times mutations produced by each treatment contain mutations of the negative control (spontaneous mutations).

2.3 Genotoxicity in *A. cepa*

Three healthy onion bulbs were employed for concentrations in each treatment; primordia were cut and placed in water for 72 h in the dark. We selected bulbs with root length between 1.5 and 2.0 cm and were subjected to each nontoxic effluent concentrations under the following conditions: 0.25, 0., 0.75, and 1 %, 48 h, approximately two cell cycles. Distilled water was used

as a negative control and 10 mg/mL of methyl methanesulfonate (MMS, Sigma-Aldrich, CAS 66-27-3) as a positive control. Treated roots were cut, measured, and fixed in Carnoy's solution (methanol/acetic acid, 3:1). Three roots of each treatment were hydrolyzed in HCl for 6 min at 55 °C, and 1-mm root apex was cut to assess chromosomal aberrations and mitotic index (MitI) while the second millimeter (F1) was used to observe micronuclei (Ma et al. 1995). Root tip samples were squashed on a glass plate and stained in 2 % acetic orcein, for 8–10 min, pressed gently with a coverslip, and varnished to prevent premature dehydration. The procedure was carried out in triplicate. In order to determine MitI and micronuclei (MN) analysis ((no. of cells in mitosis or with MN/total cells)×100), 2000 cells were counted per plate (6000 in total per treatment), and to observe chromosome alterations, approximately 500 cells per plate were counted.

2.4 Statistical Analysis

Differences between the CODs of each treatment were determined using Dunnett's T3 test. The mutagenic effect in the Ames test was considered positive if MI was greater than 3 and weakly mutagenic or indicative if the MI was greater than 1.5, as long as there was a significant concentration–response curve (Vargas et al. 2001). Significant differences in the frequencies of revertants, chromosomal aberrations, and micronuclei among distinct concentrations were identified employing Duncan's multiple range test (Abdel Migid et al. 2007). Univariate regression analysis was applied to calculate concentration–response effect and correlation analysis between biomarkers (Radic et al. 2010). All these analyses were performed with the statistical package STATGRAPHICS Centurion XV with a significance level of 0.05.

3 Results

3.1 Variation of COD

COD was measured in untreated vinasses, the effluent after biological oxidation, and the effluent after Fenton treatment (Table 1). There was a significant difference ($p<0.05$) between COD before and after treatment with both methods.

3.2 Mutagenicity Using the Ames Test

Four vinasse concentrations were tested using the Ames test (Table 2).

Maximum mutagenic activity was obtained with strain TA98 without S9 in the highest concentration of the untreated sample, and the minimum was obtained with the lowest concentration in Fenton treatment (Fig. 1a). However, it is worth noting that increasing concentration in the Fenton treatment did not generate a mutagenic activity increase, unlike untreated vinasses that indicated a concentration–response effect. Furthermore, there was no mutagenic effect ($p>0.05$) after the biological oxidation treatment at all concentrations tested, except in strain TA98 without S9 (Table 2); however, that mutagenic activity changed slightly between the biological oxidation and the Fenton treatment (Fig. 1a). In TA98 with S9, the greatest mutagenic activity was observed at the highest concentration of the untreated vinasses and the smallest activity was observed at the lowest concentration of the Fenton sample, but unlike the absence of metabolic enzymes, concentration–response effect was not observed in the effluent after the biological oxidation treatment. Nevertheless, the mutagenic activity between the biological oxidation and the Fenton treatment remained homogeneous (Fig. 1b). In strain TA100 without S9, there was mutagenic activity ($p<0.05$) in the highest concentrations of untreated vinasses (Table 2). However, in the presence of metabolic enzymes, the mutagenic behavior was uniform and concentration and type of sample independent, indicating no mutagenicity (Fig. 1c, d).

The MI at the 50 % vinasse concentration sample before treatment was 5.1 and 3.7 in strain TA98 without and with S9, respectively (Table 2). It is clear that the MI decreases as depuration treatments are applied (Fig. 2).

3.3 Cytotoxicity, Genotoxicity, and Mutagenicity in *A. cepa* Roots

The presence of cytotoxic, genotoxic, and mutagenic substances in all three vinasse samples (one before treatment and the other two after treatment consecutively) was determined using chromosome aberration frequency analysis in metaphase and anaphase, and also by determining the presence of micronuclei during interphase of *A. cepa* root cells (Table 3). Nonsample and concentration-independent cytotoxic effect was found since MitI did not vary significantly in any condition.

Table 1 COD values in milligrams per liter of vinasse effluents before and after treatments

Treatment	COD (mg/L)	Removal percentage	pH
Untreated	15121.15±1321.2	–	6.5
Biological oxidation	816.66±106.72[a]	94.5 %	9.2
Fenton	63±31.42[a]	99.6 %	6.9

[a] Significant difference compared with the untreated effluent ($p<0.05$) using Dunnett's T3 test

It was globally observed that purification treatments significantly reduced the frequency of chromosomal aberrations (Table 3). A concentration–response effect ($p<0.05$) was also noticed in the frequency of the following: C-metaphases before and after treatment (Fig. 3a); lagging chromosomes after biological oxidation treatment (Fig. 3b); micronuclei in the Fenton sample (Fig. 3c); and cells with sticky chromosomes before treatment (Fig. 3d), indicating that chromosomal aberrations increased with sample concentration increasing.

Multivariate analysis showed significant correlations ($p<0.05$) among frequencies of chromosomal aberrations induced by different vinasse samples before and after biological oxidation treatment (Table 4). A negative correlation was also observed between MitI and C-metaphases in the vinasse sample treated by biological oxidation, indicating that when MitI increases, C-metaphases frequency decreases. The other correlations were positive: MitI–chromosome breaks, lagging chromosomes–anaphase bridges and breaks-anaphase bridges in vinasse sample before treatment, and C-metaphases–sticky chromosomes in samples before and after treatment by biological oxidation.

4 Discussion

Results showed that concentrations of organic matter, expressed in terms of COD, decreased significantly after treating vinasses with biological oxidation and Fenton.

Table 2 Mutagenicity of different concentrations of vinasse before and after treatment on *Salmonella typhimurium* strains (TA98 and TA100) in the absence and presence of S9

Sample	Concentration (%)	TA98						TA100					
		− S9			+ S9			− S9			+ S9		
		Mean	±SD	MI	Mean	±SD	MI	Mean	±SD	MI	Mean	±SD	MI
Negative control	0	10.5	1.7	1	11	2.3	1	71	11.1	1	79.8	4.9	1
Untreated vinasses	20	26.3[a,c]	5.9	2.5	34.3[a,c]	13.1	3.1	72.3	9.6	1	78.3	14.2	0.9
	30	28.8[a,c]	12	2.7	48.3[a,c]	13.7	4.4	71.5	10.1	0.9	75	8.6	0.9
	40	38.8[a,c]	3.5	3.7	45.3[a,c]	7.3	4.1	95[c]	8.4	1.3	74.3	13.6	0.9
	50	53.8[a,c]	15.4	5.1	40.8[a,c]	10.9	3.7	87.8[c]	7.1	1.2	63	10	0.8
Biooxidation-treated effluent	20	12.8[c]	1.7	1.3	10.3	1	0.9	73.8	1.7	1	75	1.7	0.9
	30	16.3[b,c]	0.95	1.8	10	2.4	0.9	66.8	11.5	0.9	78	9	1
	40	13.8[b,c]	0.5	1.5	10.3	0.95	0.9	69.2	7.6	1	83	2.8	1
	50	13.3[c]	1.7	1.4	12.5	0.57	1.1	66.3	5.2	0.9	75	9.8	0.9
Fenton-treated effluent	20	9.5	1.4	0.9	9.7	1.6	0.9	71	5.5	1	79.3	8.3	0.9
	30	11	2	1	9.3	1.2	0.8	70	5.2	0.9	76	4.7	0.9
	40	10	1.4	0.95	9.2	1.3	0.8	71.5	6.3	0.9	76.3	5.3	0.9
	50	10.5	2.3	1	9.7	2	0.9	76.3	9.4	1.1	75.5	4.8	0.9

[a] MI greater than 3.5

[b] MI greater than 1.5, complemented with a significant concentration–response curve

[c] Significant difference between the number of revertants of treatment relative to the negative control ($p<0.05$)

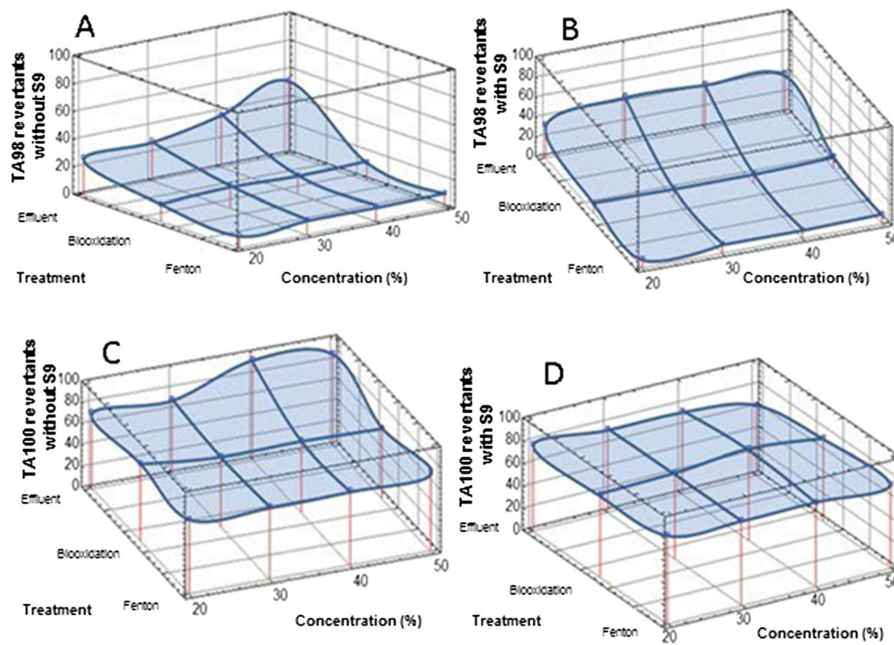

Fig. 1 Response surface according to the concentration effect of untreated vinasses over the number of strain revertants. **a** TA98 in the absence of S9; **b** TA98 in the presence of metabolic enzymes; **c** TA100 in the absence of S9; **d** TA100 in the presence of metabolic enzymes

In both treatments, degradation mechanisms of organic matter were observed (Satyawali and Balakrishnan 2008). It has been proposed that the products in the process of biological oxidation include low-molecular-weight and high-molecular-weight compounds (Ohmomo et al. 1988). The Fenton oxidation process is based on the production of hydroxyl radicals which oxidize organic matter, degrading it to CO_2 and thus decolorizing the effluent (Pala and Erden 2005).

Anaerobic treatments have been operated successfully in the presence of high organic loads (Nandy et al. 2002) and the usage of UASB reactors has been reported for treatment of distillery waste in various studies, achieving a COD removal of around 90 % (Wolmarans and De Villiers 2004), similar to the one reported in this study. Possibly, there was a higher percentage of organic matter removal in this study because the growth rate of methanogenic bacteria increases in thermophilic conditions (>42 °C) compared to mesophilic conditions (<42 °C). The pH increases from 6.5 to 7.5 (adjusted due to reactor setup before starting treatment) to 9.2 (after biooxidation treatment) as a result of organic acid degradation and oxidation to form CH_4 and CO_2 and the reaction between CO_2 and

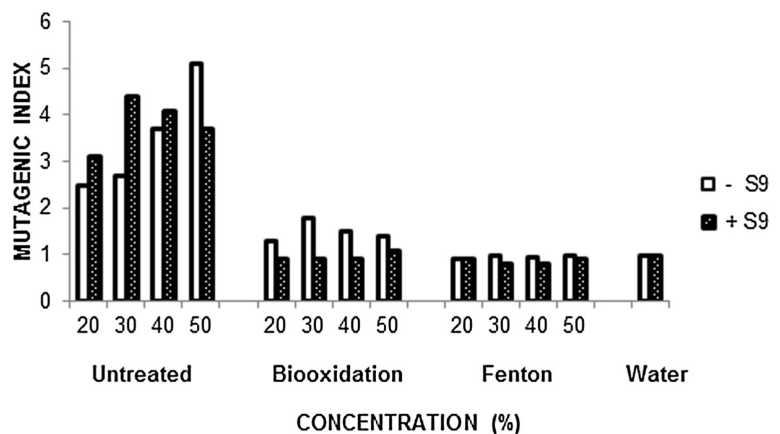

Fig. 2 Effect of different concentrations of three samples on the mutagenic index in strain TA98 with and without metabolic activation

Table 3 Chromosomal aberration averages in 6000 *A. cepa* root cells induced by different concentrations of the three vinasse samples

Sample	Concentration (%)	MitI	C-metaphase	Chromosome losses	Chromosome breaks	Anaphase bridges	Sticky chromosomes	Total chromosome alterations	Micronuclei
Negative control[b]	0	5.22	0.66	0.16	0.41	0.16	0.08	1.47	0.416
Untreated vinasses	0.25	4.78	0.66	1[a]	1	1.66	1	5.32	0.66
	0.5	6.09	2.66[a]	1.33[a]	1.33	2.33[a]	1	8.65	0.66
	0.75	4.65	3.33[a]	0.33	0.33	0.33	2[a]	6.32	0.33
	1	4.77	6.0[a]	0	0	0.33	2[a]	8.33	0.66
Biooxidation-treated effluent	0.25	4.06	0.33	0	1.33	0.66	0	2.32	1.33
	0.5	4.84	1	1	1.33	1.66	0.33	5.32	0.66
	0.75	4.5	2	0.66	0.33	0.33	0.33	3.6	0.33
	1	3.63	2.33[a]	1.66[a]	0.33	0	1.33	5.65	0.66
Fenton-treated effluent	0.25	4.46	1	0.33	0.33	0.66	0	2.32	0.66
	0.5	4.4	1.33	0	1.33	0.33	0.33	3.99	0.66
	0.75	4.4	1.33	0.33	1.66	0.33	0.33	3.65	1.66[a]
	1	5.43	2.33[a]	1[a]	1	0.66	1	5.99	1.33

[a] Statistically significant difference with respect to the negative control ($p<0.05$), according to Duncan's multiple range test

[b] Distilled water

basic compounds to form carbonates and bicarbonates (Beltrán et al. 1999).

De Heredia et al. (2005), unlike our work, used Fenton treatment directly in biologically untreated vinasses, obtaining a COD removal of 74 %. Some authors (Mohana et al. 2009) argue that these types of advanced oxidation processes should be conducted after the primary anaerobic treatment for better removal of color and COD, which is in agreement with the results of this work. Vinasse effluents before and after treatment showed predominance of TA98 mutagenic activity, as observed in most of the complex environmental mixtures which have greater activity in this strain compared to the TA100, except for chlorinated water. Several

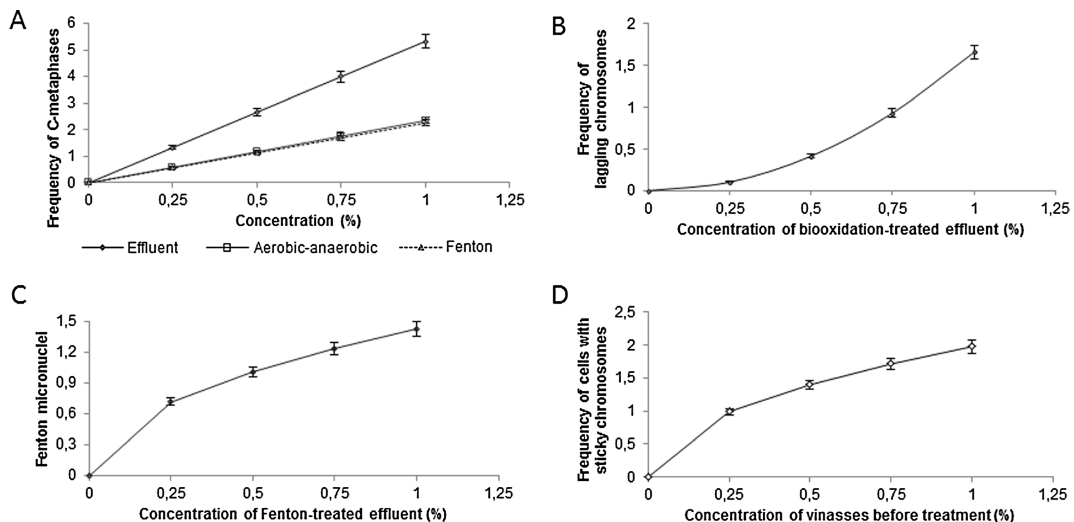

Fig. 3 Significant correlation between concentration and the frequency of chromosome aberrations. **a** C-metaphases in all three samples. **b** Lagging chromosomes following biological oxidation. **c** Micronuclei after Fenton treatment. **d** Cells with sticky chromosomes before treatment

Table 4 Significant correlations ($p<0.05$) among chromosomal alterations induced by different samples of vinasses

	MitI	Sticky chromosome	Anaphase bridges	C-metaphase	Chromosome breaks	Lagging chromosome
C-Metaphase	X[b]	O[a,b]		–		
Chromosom breaks	O[a]		O[a]		–	
Lagging chromosomes			O[a]			–

X negative correlation (−), *O* positive correlation (+)

[a] Untreated vinasse sample

[b] Biooxidation-treated vinasse sample

studies have used these two strains to assess the mutagenicity of different complex environmental samples (Oñate and Paruma 2007; Zuleta et al. 2004).

Distillery industry waste is a major source of water and soil pollution due to the presence of water-soluble recalcitrant polymeric pigments (melanoidins) (Parnaudeau et al. 2008) which are formed by the Maillard reaction from a variety of sugars (carbohydrates) and proteins (amino group). Kitts et al. (1993), observed that some Maillard reaction products exhibited mutagenic activity in strains TA98 and TA100, but only in the absence of S9, since in the presence of enzyme, the mutagenic activity was reduced in TA100 and disappeared in TA98. Melanoidins could be responsible of the mutagenic activity generated by untreated vinasses in strain TA98 without S9. Strain TA98 mutagenicity with metabolic activation can be attributed to the presence of polycyclic aromatic hydrocarbons (PAHs), such as benzo-a-pyrene (Ames et al. 1975), which may be present in the waste (Raghukumar et al. 2004) because of burning of cane crops for planting.

Point mutations decreased after biological oxidation treatment and disappeared after Fenton, showing that these treatments are entirely effective in removing contaminants that originate this type of mutations. It has been proposed (Chandra et al. 2008) that the possible mechanism for anaerobic degradation of melanoidins and PAHs can be carried out by some anaerobic bacteria common in bio-digesters, including esulfococcus *oleovorans*, *Dechloromonas* sp., *Geobacter metallireducens*, *Acidovorax*, *Bordetella*, and *Methanosarcina* sp.; these are responsible for degradation processes where enzymes are involved, such as sugar oxidase, sorbose oxidase, laccase, lignin peroxidase, and peroxidase-dependent manganese. Furthermore, previous reports showed that mutagens were completely degraded after 1 h of treatment with Fenton reagent and there was no residual mutagenicity, examined by the Ames test with or without metabolic enzymes (Castegnaro et al. 1997; Hansel et al. 1997).

No significant differences were observed in the MitI of different treatment concentrations, indicating a scarce presence of toxic substances. The results concerning chromosome aberrations showed high reliability and sensitivity of the bioassay, as the sum of these aberrations in treatments differed significantly compared with the negative control (Table 3). The most frequent aberrations were caused by chromatin dysfunction (sticky chromosomes and anaphase bridges) and mitotic spindle malfunction (C-metaphases). Anaphase bridges, chromosome breaks, and sticky chromosomes indicated clastogenic effects, while lagging chromosomes and C-metaphases indicated aneugenic effects (Leme and Marin-Morales 2009).

Christofoletti et al. (2013a) observed several chromosomal alterations in roots of *A. cepa* that were induced by different concentrations of vinasses. Another study also assessed chromosomal alterations in sugarcane cuttings induced by different residues from the distillery industry before and after being treated. As in this work, the authors found that after treating vinasses some genotoxic effects decreased, but cytotoxic characteristics were seen in the samples before and after treatment (Srivastava and Jain 2010).

The increasing frequency of C-metaphases and lagging chromosomes, induced by the augmented concentration of the vinasse samples before and after treatment, indicates the existence of chemical agents interfering with the behavior of microtubules (Fernandes et al. 2007) or kinetochores (Seth et al. 2008), which cannot be eliminated by the treatments. The mitotic spindle malfunction is mainly attributed to the reactivity of metal ions with the sulfhydryl groups of tubulins (Dash et al. 1988). On the other hand, there are compounds capable of inducing sticky chromosomes with

increasing concentrations of the untreated sample, indicating that these contaminants act upon degradation or depolymerization of chromosomal DNA, DNA condensation, and the entanglement of interchromosomal chromatin fibers that generate subchromatid connections among chromosomes (Yildiz et al. 2009).

We observed that a high frequency of C-metaphase cells was associated with a mitotic index decrease. A reduction in the mitotic activity suggests mitodepressive effects caused by chemical agents produced or transformed in the biological oxidation treatment. These agents can also generate C-metaphases, preventing that a number of cells enters prophase (Rustenbil and Poortvliet 1992) or promoting rapid mitosis exit (Murray and Kirschner 1989), thereby increasing the number of cells in interface. Bridges are generated from chromosome breaks; this is shown in a positive correlation between these two variables in the sample of untreated vinasses (Table 4). Although the frequency of breaks is not significant in any sample, this would imply that most breaks become anaphase bridges as a result of repair by homologous recombination (Acilan et al. 2007).

MN have been considered as true chromosome mutation effects (Corvi et al. 2008). Induction of MNs in *A. cepa* root meristems or in any other organism cell is the manifestation of chromosome breaks and anomalies in the mechanism of the spindle (El-Shahaby et al. 2003). However, the effluent only had one significant incidence with respect to the frequency of MN, at the 0.75 % concentration of the Fenton sample. In addition, mutagenicity detection did not match between the two assays used in this study because *Allium* test only showed mutagenic activity in the sample treated with Fenton, while the Ames test showed activity in all samples, except Fenton. This may be due to the ability of the *Allium* test to detect a low concentration of contaminants dissolved in aqueous media that can only be identified by chromosomal aberrations and not through MN (Grant et al. 1992), possibly because the DNA damage can be repaired. Another hypothesis is that depending on whether the MN are composed of lagging chromosomes or nonviable chromosome fragments, they can lead to cell death, suggesting that MN elimination may be an indicator of cellular physiology maintenance, since removing any disabled or erroneous structure produces a physiological response. Also, as the overall chromosome alteration frequency was high, the death of these aberrant cells can reduce the total number of cells, preventing the formation of cells with MN in F1 (Leme and Marin-Morales 2008).

Finally, the two bioassays used in this research provide a sensible approach for the proposed analysis, representing an efficient model for detecting a wide presence of compounds in these three vinasse samples. Undoubtedly, these treatments are very effective in the removal of substances that can be dangerous to any living organism and thus have an impact on the improvement of methods for a comprehensive wastewater management.

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References

Abdel Migid, H. M., Azab, Y. A., & Ibrahim, W. M. (2007). Use of plant genotoxicity bioassay for the evaluation of efficiency of algal biofilters in bioremediation of toxic industrial effluent. *Ecotoxicology and Environmental Safety, 66*, 57–64.

Acilan, C., Potter, D. M., & Saunders, W. S. (2007). DNA repair pathways involved in anaphase bridge formation. *Genes, Chromosomes and Cancer, 46*, 522–531.

Ames, B. N., McCann, J., & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research, 31*, 347–363.

Beltrán de Heredia, J. (2005). Tratamiento de las aguas residuales de destilerías de vino mediante un proceso combinado delodos activos y oxidación química. *Alimentación Equipos Y Tecnología, 198*, 55–59.

Beltrán, F. J., García-Araya, J. F., & Álvarez, P. M. (1999). Wine distillery wastewater degradation. 2. Improvement of aerobic biodegradation by means of an integrated chemical (ozone)-biological treatment. *Journal of Agricultural and Food Chemistry, 47*, 3919–3924.

Castegnaro, M., De Méo, M., Laget, M., Michelon, J., Garren, L., Sportouch, M. H., & Hansel, S. (1997). Chemical degradation of wastes of antineoplastic agents. 2: Six anthracyclines: idarubicin, doxorubicin, epirubicin, pirarubicin, aclarubicin, and daunorubicin. *International Archives of Occupational and Environmental Health, 70*, 378–384.

Chandra, R., Bharagava, R. N., & Rai, V. (2008). Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. *Bioresource Technology, 99*, 4648–4660.

Christofoletti, A., Pedro-Escher, J., & Fontanetti, C. (2013a). Assessment of the Genotoxicity of Two Agricultural

Residues After Processing by Diplopods Using the *Allium cepa* Assay. *Water, Air, & Soil Pollution, 224*, 1523–1537.
Christofoletti, C. A., Escher, J. P., Correia, J. E., Marinho, J. F., & Fontanetti, C. S. (2013b). Sugarcane vinasse: environmental implications of its use. *Waste Management, 33*, 2752–2761.
Corvi, R., Albertini, S., Hartung, T., Hoffmann, S., Maurici, D., Pfuhler, S., van Benthem, J., & Vanparys, P. (2008). ECVAM retrospective validation of in vitro micronucleus test (MNT). *Mutagenesis, 23*, 271–283.
Dash, S., Panda, K. K., & Panda, B. B. (1988). Biomonitoring of low levels of mercurial derivatives in water and soil by Allium micronucleus assay. *Mutation Research/Environmental Mutagenesis and Related Subjects, 203*, 11–21.
de Heredia, J. B., Dominguez, J., & Partido, E. (2005). Physico-chemical treatment for the depuration of wine distillery wastewaters(vinasses). *Sustainable Viticulture and Winery Wastes Management, 51*, 159–166.
El-Shahaby, O., Migid, H. M. A., Soliman, M., & Mashaly, I. (2003). Genotoxicity screening of industrial wastewater using the Allium chromosome aberration assay. *Pakistan Journal of Biological Sciences, 6*, 23–28.
Fernandes, T., Mazzeo, D., & Marin-Morales, M. (2007). Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to triXuralin herbicide. *Pesticide Biochemistry and Physiology, 88*, 252–259.
Grant, W., Lee, H., Logan, D., & Salamone, M. (1992). The use of Tradescantia and Vicia faba bioassays for the in situ detection of mutagens in an aquatic environment. *Mutation Research, 270*, 53–64.
Hansel, S., Castegnaro, M., Sportouch, M. H., De Méo, M., Milhavet, J. C., Laget, M., & Duménil, G. (1997). Chemical degradation of wastes of antineoplastic agents: cyclophosphamide, ifosfamide and melphalan. *International Archives of Occupational and Environmental Health, 69*, 109–114.
Kitts, D. D., Wu, C. H., Stich, H. F., & Powrie, W. D. (1993). Effect of glucose-lysine Maillard reaction products on bacterial and mammalian cell mutagenesis. *Journal of Agricultural and Food Chemistry, 41*, 2353–2358.
Leme, D. M., & Marin-Morales, M. A. (2008). Chromosome aberration and micronucleus frequencies in Allium cepa cells exposed to petroleum polluted water–A case study. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 650*, 80–86.
Leme, D. M., & Marin-Morales, M. A. (2009). Allium cepa test in environmental monitoring: a review on its application. *Mutation Research/Reviews in Mutation Research, 682*, 71–81.
Ma, T. H., Xu, Z., Xu, C., McConnell, H., Rabago, E. V., Arreola, G. A., & Zhang, H. (1995). The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants. *Mutation Research, 334*, 185–195.
Maertens, R., Bailey, J., & White, P. (2004). The mutagenic hazards of settled house dust: a review. *Mutation Research-Reviews in Mutation Research, 567*, 401–425.
Mahadevan, B., Lucha, A., Atkin, J., Nguyena, T., Sharma, A., Aminc, S., & Baird, W. (2006). Investigation of the genotoxicity of dibenzo[c, p]chrysene in human carcinoma MCF-7 cells in culture. *Chemico-Biological Interactions, 164*, 181–191.
Maluszynska, J., & Juchimiuk, J. (2005). Plant genotoxicity: a molecular cytogenetic approach in plant bioassays. *Arhiv za Higijenu Rada i Toksikologiju, 56*, 177–184.
Maron, D., & Ames, B. (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Research, 113*, 173–215.
Matilainen, A., & Sillanpää, M. (2010). Removal of natural organic matter from drinking water by advanced oxidation processes. *Chemosphere, 80*, 351–365.
Mohana, S., Acharya, B. K., & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials, 163*, 12–25.
Murray, A. W., & Kirschner, M. W. (1989). Dominoes and clocks: the union of two views of the cell cycle. *Science, 246*, 614–621.
Nandy, T., Shastry, S., & Kaul, S. (2002). Wastewater management in a cane molasses distillery involving bioresource recovery. *Journal of Environmental Management, 65*, 25–38.
Novelo, R. I. M., Reyes, R. B. G., Borges, E. R. C., & Riancho, M. R. S. (2010). Tratamiento de lixiviados por oxidación Fenton. *Ingeniería e Investigación, 30*, 80–85.
Ohmomo, S., Daengsubha, W., Yoshikawa, H., Yui, M., Nozaki, K., Nakajima, T., & Nakamura, I. (1988). Screening of anaerobic bacteria with the ability to decolorize molasses melanoidin (microbiology & fermentation industry). *Agricultural and Biological Chemistry, 52*, 2429–2435.
Oñate, J. F., & Paruma, A. F. (2007). *Potencialidad Mutagénica y Genotóxica de Afluentes de una Planta de Potabilización del Suroccidente de Medellín (planta "A")*. Popayán: Universidad del Cauca.
Pala, A., & Erden, G. (2005). Decolorization of a baker's yeast industry effluent by Fenton oxidation. *Journal of Hazardous Materials, 127*, 141–148.
Parnaudeau, V., Condom, N., Oliver, R., Cazevieille, P., & Recous, S. (2008). Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. *Bioresource Technology, 99*, 1553–1562.
Radic, S., Stipanicev, D., Vujcic, V., Rajcic, M. M., Sirac, S., & Pevalek-Kozlina, B. (2010). The evaluation of surface and wastewater genotoxicity using the Allium cepa test. *Science of the Total Environment, 408*, 1228–1233.
Raghukumar, C., Mohandass, C., Kamat, S., & Shailaja, M. (2004). Simultaneous detoxification and decolorization of molasses spent wash by the immobilized white-rot fungus Flavodon flavus isolated from a marine habitat. *Enzyme and Microbial Technology, 35*, 197–202.
Rustenbil, J., & Poortvliet, T. (1992). Copper and zinc in estuarine water: chemical speciation in relation to bioavailability to the marine planktonic diatom Ditylum brightwellii. *Environmental Toxicology and Chemistry, 11*, 1615–1625.
Satyawali, Y., & Balakrishnan, M. (2008). Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. *Journal of Environmental Management, 86*, 481–497.
Seth, C., Misra, V., Chauhan, L., & Singh, R. (2008). Genotoxicity of cadmium on root meristem cells of Allium cepa: cytogenetic and Comet assay approach. *Ecotoxicology and Environmental Safety, 71*, 711–716.
Srivastava, S., & Jain, R. (2010). Effect of distillery spent wash on cytomorphological behaviour of sugarcane settlings. *Journal of Environmental Biology, 31*(5), 809–812.

Vargas, V. M. F., Guidobono, R. R., Jordão, C., & Henriques, J. A. P. (1995). Use of two short-term tests to evaluate the genotoxicity of river water treated with different concentration/extraction procedures. *Mutation Research/ Genetic Toxicology, 343*, 31–52.

Vargas, V. M. F., Miglivaca, S. B., Melo, A. C., Horn, R. C., Guidobono, R. R., Sa Ferreira, I. C. F., & Pestana, M. H. D. (2001). Genotoxicity assessment in aquatic environments under the influence of heavy metal and organic contaminants. *Mutation Research, 490*, 141–158.

Wolmarans, B., & De Villiers, G. H. (2004). Start-up of a UASB effluent treatment plant on distillery wastewater. *Water SA, 28*, 63–68.

Yessilada, E. (1999). Genotoxic activity of vinasse and its effect on fecundity and longevity of Drosophila melanogaster. *Bulletin of Environmental Contamination and Toxicology, 63*, 560–566.

Yildiz, M., Ciğerci, I. H., Konuk, M., Fidan, A. F., & Terzi, H. (2009). Determination of genotoxic effects of copper sulphate and cobalt chloride in Allium cepa root cells by chromosome aberration and comet assays. *Chemosphere, 75*, 934–938.

Zuleta, M., Uribe, Y., Valencia, C., Vargas, H., Orozco, L. Y., & Lopez, C. (2004). Contribución de la contaminación y cloración en la Mutagénicidad, genotoxicidad y presencia de mutágenos en agua potable. *Actualités Biologiques, 26*, 125–136.