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CYCLOPIAZONIC ACID DEGRADATION BY AQUEOUS OZONE

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ABSTRACT

Ozone is a chemical agent with great potential to reduce mycotoxins, it was effective against to reduce some mycotoxins. In view of this it was aimed of this work study the Cyclopiazonic acid (CPA) degradation by aqueous ozone. The degradation of exogenously CPA introduced in mobile phase was confirmed by High performance liquid Chromatography (HPLC). In parallel it was tested the effect of sodium formate (SF), to evaluate the influence of this chemical to neutralize ozone after adding CPA to ozone reaction, since it is a chemical used to stop ozone reaction. Results shown that SF did not influence aqueous ozone to degrade the CPA, since the quickness of ozone reaction.

INTRODUCTION

Cyclopiazonic acid (CPA), an indole tetramic acid mycotoxin (Figure 1), which is toxic to a wide variety of animals and also has been implicated in human poisoning (Chang et al., 2009). *Aspergillus* section *Flavi* are among the main CPA producers, however, strains from this section vary considerable in their ability to produce CPA (Rodrigues et al., 2009). Although CPA is not as acutely toxic as aflatoxin B_1 (Chang et al., 2009); the combination of both can produce degenerative changes, inactivity, tremors, necrosis of liver, pancreas, spleen and kidney. Until now, no specific regulations or recommendations exist for CPA. The prevention of mycotoxin contamination in the field is the ideal solution to the health hazards that mycotoxins pose. However, despite of all efforts devoted in that direction, a high incidence of mycotoxins in many commodities is still observed. So, decontamination/detoxification procedures are still a challenge for re-use mycotoxin-contaminated commodities and feeds (Shapira & Paster, 2004).
The application of ozone (O_3) as a strong anti-microbial agent is well known and its use for mycotoxin destruction has been studied earlier in various commodities (Freitas-Silva & Venâncio, 2010). This present work is focused on the use of aqueous ozone to evaluate the control level and degradation of CPA.

MATERIAL & METHODS

Aqueous ozone solutions

Water saturated with ozone was prepared by bubbling gas, generated by passing *extra-dry oxygen through* an air-cooled corona discharge generator (Model CD-COM-HF-4) for 10 minutes (with the power generator at 100%; gas flow of 25 L / hours) in a bottle with 1000 mL of Milli-Q water, at 3 ºC. The final ozone concentration in water was determined by a colorimetric method in a spectrophotometer (λ_{max} = 258 nm and ε = 2900 M^{-1} cm^{-1}). A concentrated ozone solution with around 40 mg/L was obtained and this was diluted with ozone demand free water as necessary.

CPA reagents and solutions for HPLC analysis

The CPA standard was provided by Sigma (St. Louis, MO), and a 1 mg/mL stock solution was prepared in methanol. Standard and working solutions were prepared by taking an appropriate volume of the stock solution to clean vial, drying under a nitrogen stream and dissolving in a known volume of mobile phase. All solvents (acetonitrile, methanol) used were HPLC grade. CPA solution was stored at ±4 °C.

Samples Preparation

The degradation of CPA by aqueous ozone was tested at 5 defined aqueous ozone levels: 0 (control), 1, 10, 20 and 40 mg/L. Two hundred microliters of these ozone solutions were applied in 2 mL vials containing 20 µL CPA solution, to obtain the final CPA concentrations A (61 ng/mL) and B (264 ng/mL). Ozone

was allowed to react, and samples were analyzed every 5 seconds. To stop ozone reaction, the solution of sodium formate (SF). Briefly, at the end of the prescribed contact time, 100 µL of 1 M reagent-grade SF solution was added to the flask to neutralize the remaining dissolved ozone. Before analysis, 980 µL of mobile phase was added. Each experiment was repeated six times.

Cyclopiazonic acid detection by HPLC

Samples were analyzed using a HPLC equipped with a Jasco UV detector (284 nm). Chromatographic separations were performed on a EuroSpher 100 NH_2 column (Knauer, 4.6 mm x 250 mm, 5 µm), fitted with a precolumn with the same stationary phase. The mobile phase used was pumped at 1.0 mL/min and consisted of an isocratic program as follows: acetonitrile: 50 mM ammonium acetate (3:1, v/v), pH 5. The injection volume was 100 µL. Samples were taken as positive if they yielded a peak at a retention time similar to the CPA standard. The detection and quantification limits by this approach were 4 and 10 ng/mL.

RESULTS

The CPA was degraded by aqueous ozone (Figure 1A). Samples were well classified by their area from HPLC chromatograms signals (Figure 2). The retention time and peak area detected by HPLC served as useful parameters for classification of potential degradation in contaminated water samples. SF has no effects on stopping reaction to CPA degradation (Figure 1B).

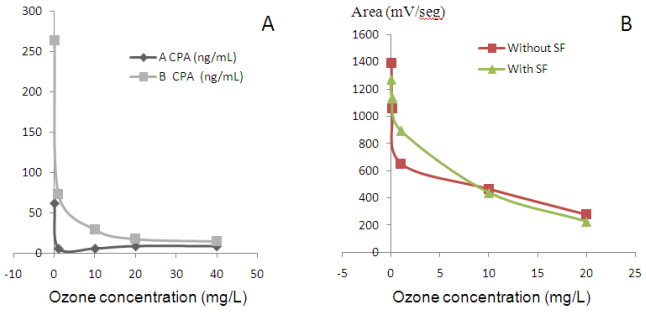

Figure 1 - CPA degradation curves by ozone under two CPA concentrations (A) and with and without SF (B).

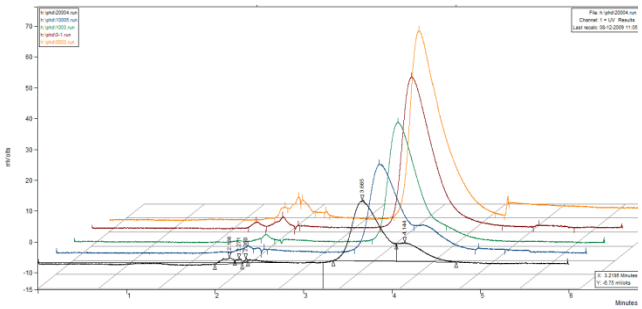

Figure 2 – Chromatogram showing CPA degradation at 0, 1, 0.1, 10 and 20 mg/L of ozone.

CONCLUSIONS

Aqueous дzone can be used as a treatment on the CPA decontamination. Apart from its foaming ability which permits ozone to be trapped in a better way in water, since water is a vehicle for washing and decontamination process.

However, only a limited volume samples was used in this study and this limitation may influenced the result classification, further experiments with biological samples are recommended in order to determine and confirm the aqueous ozone potential ability to destroy mycotoxins. The additional gain of this work is to show that the aqueous ozone use is possible to decontaminate not only CPA standards but also contaminated raw material as well laboratory equipment/reagent for disposal.

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