COMMUNICATION

Cold plasma oxidation of harmful algae and associated metabolite BMAA toxin in aqueous suspension

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Abstract:

We report experiments in which reactive oxygen species (ROS) from a 20 kHz HV discharge in Ar/O₂ (90/10) gas mixture at atmospheric pressure were directly bubbled into highly concentrated aqueous suspensions of cyanobacteria *Dolichospermum*, green algae *Scenedesmus* and BMAA toxin, simulating extreme algal blooms. It has been found that even quite short treatment durations, up to 6 minutes, could greatly reduce the numbers of viable cells and completely destroy the BMAA toxin. Perhaps even more important, “plasma activated water” (PAW) was found to continue its effectiveness after 24 hours, even 4 days after terminating the discharge.

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1. Introduction

Since 1906 the water of Nice, France, has been sterilized by ozone, O_3 , a powerful oxidant that is generated in large quantities by dielectric barrier discharge (DBD “cold” plasma) systems operating at atmospheric pressure (AP).^[1] Large ozone installations normally function with dried clean air or dry oxygen, can reach input powers of several MW, and can generate more than 100 kg/hr. Ozone concentrations up to 18% can be reached using O_2 feed, at least three times more than with dry air.^[2] In spite of these impressive performance criteria, however, there is reason to believe that these can be improved even further: O_3 , a relatively long-lived meta-stable compound, is only one among other plasma-chemical reaction products that are generated in AP O_2 plasma, especially if the discharge is in direct contact with water or water vapor, but those have far shorter lifetimes. These so-called “reactive oxygen species” (ROS) include, for example, $OH\bullet$ and $O\bullet$ radicals, along with ions and molecular excited species, all of which can possess equal or greater oxidizing potency as O_3 itself.

In a recent special issue of this journal, *Plasma Processes and Polymers*, which was devoted to the theme “plasma and liquids”, there were several articles that dealt with AP plasmas immersed in, or in direct contact with a liquid, water being the liquid of choice in most cases.^[3] We shall later be referring in more detail to one of those articles, namely that

by F. Tampieri *et al.*^[4] They reported a clever bench-top AP plasma reactor system based on a hollow needle electrode in contact with (deliberately contaminated) water, through which the oxidant gas was fed. They then mapped the morphology of the plasma/gas/liquid interface, identified short-lived excited species, determined oxidants in solution and monitored the degradation of their organic pollutants.

Climate change, human activities and eutrophication of water bodies have degraded the quality of freshwater sources worldwide.^[5, 6] Increased simultaneous presence of nuisance algal and cyanobacterial blooms, pesticides, and fecal contaminants in water supply systems constitute a substantial part of our new environmental challenges.^[7-11] Current operational oxidation processes apply only to a limited number of reactive species simultaneously, thereby restricting the ability to degrade combinations of harmful cells and toxins. For example, water that is contaminated by cyanobacterial cells, saxitoxins, microcystins and pesticides would require both chlorination and ozonation.^[6, 12, 13] Ozonation would oxidize microcystins and pesticides, but with little or no effects on saxitoxins.^[7, 14, 15] β -N-methylamino-L-alanine (BMAA) oxidation has been studied only in the past year under limited experimental conditions.^[16] For all these reasons it is becoming increasingly urgent and important to develop new AP plasma-based technologies that can help resolve these problems effectively and economically. The objective of this present investigation has therefore been to assess the capability of “cold” AP plasma oxidation (i) for inactivation / lysis of cyanobacteria and algae cells in water suspension, and (ii) for oxidation and removal of BMAA cyanotoxin from water.

2. Experimental Methodology

2.1. Plasma activation of water: setup and operating conditions

The reactor used in the present work was in several important aspects similar to the one recently described by Tampieri et al.^[4]: It consisted of a 50 mL Pyrex beaker with a top cover made of (electrically insulating) polytetrafluoroethylene, PTFE, traversed by a ca. 20 cm long stainless steel (*s.s.*) tube (1/4" Swagelok™; ID = 4.0 mm; OD = 6.3 mm) that served both as high-voltage (HV) and gas feed conduit. Unlike Tampieri et al., we elected to immerse our beaker in a larger (ca. 500 mL) Pyrex vessel that contained cooling water at ambient temperature, after observing a rapid rise in temperature, T , during HV discharge experiments with 15 mL water samples. Another important modification of Tampieri's published design was our HV electrode. Having noted visual and electrically-measurable changes in the plasma during HV discharge experiments, which we attributed to erosive / corrosive degradation of the electrode-tip, we chose to resolve this problem as follows: The bottom ID of the *s.s.* tube was suitably threaded so as to enable one to easily screw into place (and then subsequently replace) a pointed commercial 3D-printer tip (MK8-type nozzle; ID = 0.5 mm, *s.s.*, <http://www.zyltech.com>). Its great advantages of (a) near-perfect reproducibility and (b) low cost allowed us to replace this key component of the experimental setup at appropriate intervals, well before discharge characteristics might be about to change. However, like in Tampieri's design, the HV electrode tip was mounted axially within a suitable length of Pyrex glass tube that fit tightly onto the outer diameter of the *s.s.* tube, and that could be slid upwards or downwards on it. This tight fit minimized possible air contamination of the Ar+O₂ feed gas stream, but may not have completely eliminated its effects [see **Figure 2(a)** below].

Figure 1. Scale drawing of the replaceable HV electrode tip and surrounding components

Figure 1 is a scale drawing of the electrode assembly and its associated components. The Pyrex tube was then partially submerged in the water sample in such a way that a 6.0 mm wide spacing was created between the electrode tip and the lower edge of the Pyrex tube. When a gas flow was initiated in the *s.s.* conduit / electrode assembly and the latter was positioned so that the tip was exactly level with the water sample, gas bubbles formed in the 6.0 mm space, then escaped downwards into the water sample. When HV was applied, streamer discharges formed at the electrode tip and propagated within the gas bubbles, carrying the (ROS) active species directly into the water. Like in Tampieri's case, the

grounded electrode was a thin copper disk (0.15 mm thickness; $\varnothing = 65$ mm) in intimate contact with the bottom of the Pyrex beaker.

High voltage (14.0 kV, 20 kHz) AC power was supplied from a source comprising an AC generator (Hewlett Packard 3310 A), a power amplifier (QSC Ltd. Model RMX2450), an HV transformer (Enercon, Model LM2727-03), and an impedance matching unit. The voltage was monitored with an HV probe (Tektronix P6015A) and oscilloscope (GW Instek, Model GDS-2204A). Unlike Tampieri's choice of air, we elected to use 100 mL min⁻¹ of Ar/O₂ (90:10 v/v) reagent gas mixture, for reasons explained below. It should, however, be underlined that solution volume (15 mL), gas flow rate (100 sccm) and geometry of the reaction zone (see **Figure 1**) were all nominally the same as Tampieri's, and that these conditions were then used in all experiments. To help further minimize heating of the water sample during plasma treatment, beside heat transfer to the bath surrounding the Pyrex beaker, HV discharges were applied in the form of one-minute bursts, followed by typically three-minute cooldown periods, judged to be an optimal compromise. In order to quantitatively evaluate energy consumed during the treatment process as accurately as possible, the following procedure was adopted: Tampieri et al. reported power measurements carried out in their HV AC air discharges under conditions otherwise quite comparable to ours, as discussed above. They also carried out degradation experiments of an organic dye, Rhodamine B, by reactive species generated in their HV air plasmas. On the basis of those combined sets of data, power and degradation kinetic measurements, we have used discoloration of Rhodamine B aqueous solutions by ROS generated in our Ar/O₂ mixture as a means to estimate power consumption here. For this,

spectral transmittance of untreated and plasma-treated Rhodamine B (Sigma-Aldrich® ≥95%, HPLC grade, CAS 81-88-9) solutions were measured using a Perkin Elmer Lambda 1050 spectrophotometer operating in the 250-2500 nm range, as described in the Results and Discussion section.

2.2. *Cyanobacteria culture, chemical and analytical methods:*

Cyanobacteria belonging to genus *Dolichospermum* (formally known as *Anabaena*) and green algae belonging to genus *Scenedesmus* were isolated from Canadian algal blooms by the Canadian Phycological Culture Centre (CPCC), and cultured in Z8 Medium. Cultured cells at stationary growth phase were spiked in dechlorinated tap water to reach a total cell concentration of 200,000 cells/mL, approximately 150,000 and 50,000 cells/mL of cyanobacteria and of the green algae, respectively. All taxonomy samples were enumerated on a compound microscope in a Sedgewick-Rafter counting chamber after preservation in Lugol's iodine.^[17, 18] These cell concentrations were selected because they represent a total that doubles the highest guideline threshold and mixed suspension ratio observed in North American water bodies.^[19] It is precisely on these suspensions that the plasma-based oxidation experiments were conducted.

Standard solution of BMAA was purchased from Sigma-Aldrich (Missouri, USA). This solution was diluted to 1 mg/L with HPLC-grade MeOH and kept in amber vials at -20°C. Working solutions of the toxin for sample use were prepared via appropriate dilutions with HPLC grade H₂O and were kept at 4°C prior to and during analysis. All chemicals and reagents were analytical grade, from various suppliers. For the BMAA oxidation

experiments, samples were analyzed using an Abraxis BMAA ELISA Plate (an enzyme-linked immunosorbent assay – Abraxis LLC, Pennsylvania, USA). The concentrations were measured as $\mu\text{g/L}$ of BMAA. Cyanobacteria, algae and BMAA were treated together. The initial concentration of the “working solutions” of BMAA used during the treatment were 200 $\mu\text{g/L}$. After predetermined times, samples were taken for analyses. For cell oxidation assays two sub-samples were used to determine cell viability and -lysis in triplicate. The first sub-sample was employed for cell integrity tests using the cell pigment’s auto-fluorescence.^[20] These analyses were conducted a maximum of 30 minutes after ending the experiment on non-preserved 2 mL samples. The second sub-sample (2 mL each) was taken for total cell counts after preservation in Lugol’s iodine. For toxin oxidation assays, 10 mL sub-samples were taken and stored at -80°C for BMAA analysis using the ELISA kit.

3. Results and Discussion

3.1. Characterization of Plasma Treatment Effects

In **Figure 2(a)** we have plotted the measured pH values of 15 mL distilled water samples after they had been exposed for indicated durations to HV AC discharges in (i) air; and (ii) Ar/O_2 (90:10) gas mixture, under conditions described in section 2, Experimental Methodology. Note that “plasma duration” does not include cooldown, only “plasma on”.

Figure 2. (a) pH values of 15 mL distilled water samples after indicated exposure durations to air and Ar/O₂ HV AC discharge effluents; (b) Rhodamine B degradation versus Ar/O₂ plasma treatment time. The diamond symbols correspond to data reported by Tampieri *et al.* using air discharges.^[4]

We note that systematic decreases in pH occurred in both cases during approximately 5 minutes of cumulative discharge exposure duration, asymptotic minimum pH values of ca. 3.5 and 2.5 then being attained, respectively, for the cases of Ar/O₂ and air plasmas. This can be explained very simply as follows: In the case of Ar+O₂, the (non-degassed) water was in contact with the atmosphere, which permitted interaction with air and carbon dioxide, both of which might account for the observed slight acidification. On the other hand, it is well known and documented^[4, 21] that air plasma generates NO_x as major reaction products, in other words “RONS” (reactive oxygen and nitrogen species); in the presence of water these form nitrous and nitric acids, the reason for observed low pH values. Because those acids and other RONS can also partake in reactions with organic constituents in the water, we opted to use Ar/O₂ as feed gas for this study, one that yields only reactive oxygen species (ROS) for degrading the organic contaminants, hence leading to presumably less ambiguous interpretation of resulting data.

Figure 2(b) portrays Rhodamine B oxidative degradation kinetics with the present Ar/O₂ discharges, and with those in air reported by Tampieri et al.^[4] With appropriate caution regarding simplifying assumptions, we interpret the near-identical slopes (up to ca. 200 s of plasma exposure duration) as follows: Assuming that ROS in both cases were the principal reactive species responsible for observed oxidative discoloration, and that energy consumptions in both Tampieri's (air) and the present (Ar/O₂) HV AC discharge plasmas were comparable, we estimate an average power of about 6 W, close to the value determined by Tampieri. In future work, already started, this will be determined more precisely.

3.2. Degradation of cyanobacteria, algae cells and BMAA toxin by HV plasma ROS

Having determined the HV reactor's characteristics and estimated energy consumption characteristics in the preceding sub-section, we now present its performance in regard to degradation of harmful living cells and dissolved BMAA toxin in aqueous suspensions.

Six minutes of cumulative plasma exposure time led to 87% reduction in the total number of cells (**Figure 3a**). *Dolichospermum* was selected for these experiments on account of its filamentous shape; however, *Scenedesmus* was observed to be the microorganism more resistant to plasma oxidation, in spite of its lower number density (**Figure 3a**). Keeping the untreated cellular suspension for 24 hours had no measurable impact on cell numbers (see "0 minutes", **Figure 3a** and **3b**), but after 4 days of stagnation a 10% reduction was observed (see "0 minutes", **Figure 3c**). It is noteworthy that 13% of cells could still be counted under the microscope after 6 minutes of plasma treatment (**Figure 3a**), but 80% of

these were non-viable (**Figure 4**); more important, for both 24 h and 4-day stagnation time experiments, no viable cells could be observed corresponding to 2 minutes of initial plasma treatment. **Figure 4** presents these assembled cell-viability measurements in more readily understandable form. In summary, despite the large initial cell numbers, after 6 minutes of plasma-contact 87% of cells were completely lysed (**Figure 3**), and 80% of the remaining cells were not viable (**Figure 4**).

Regarding BMAA, completely destructive oxidation was observed within the first minute of plasma treatment, from 200 $\mu\text{g/L}$ to below the detection limit; this indicated a very rapid BMAA oxidation rate compared to other cyanotoxins and oxidizing agents [6, 12]. However, the stability of BMAA has several unknowns, including precision of the analytical methods and the associated oxidation products. This research presents only the first set of results for BMAA, but it is planned to fill those knowledge gaps in upcoming future work.

Figure 3. Total cell numbers (a) after 1 hour; (b) 24 hours; and (c) 4 days after HV plasma-induced oxidative degradation.

Figure 4. Cell integrity (viability) after HV plasma-induced oxidative degradation. The data clearly demonstrate that PAW is effective for a long time (days) after the plasma was turned off.

On the basis of what has just been described above, data presented in **Figure 3** and **4** were found to possess the following remarkable common characteristics: Although destruction of harmful cells and BMAA toxin took place during their multi-minute exposures to HV discharge plasma-originated ROS, as expected, they were also observed to continue this

trend for extended periods of time (up to 4 days, or more) in solution after the discharge had been extinguished. This unexpected phenomenon may be attributed to the properties of so-called “plasma-activated water” (PAW), a relatively new discovery that it now attracting much research attention.^[22, 23] Because in the present case the plasma gas was Ar/O₂, we can clearly eliminate long-lived RONS like nitric acid from having contributed to these observations. Error bars on the data in **Figure 3** depict the degree of reproducibility encountered in performing these experiments.

In conclusion, this work has shown that direct contact between an AP “cold” oxidizing plasma source and water that contains very high concentrations of cyanobacteria and green algae, along with associated BMAA toxin, could be quite rapidly (in minutes) decontaminated with modest energy input. The laboratory-scale experimental apparatus used here is believed suitable for eventual scale-up, for example using large arrays of hollow electrodes shown in **Figure 1**. We are now refining the procedures and data in such a way as to better assess the process economics for large-scale implementation. Longer-duration contact of residual contaminants with PAW will certainly contribute to favour economic viability.

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We report the outstanding effects of “*plasma activated water*” (PAW), obtained with Ar/O₂ (90/10) atmospheric pressure discharges generated at water/bubbles interfaces, on suspensions of cyanobacteria *Dolichospermum*, green algae *Scenedesmus* and BMAA toxin, simulating extreme algal blooms. Quite short treatment durations (up to 6 minutes) could greatly reduce the numbers of viable cells and completely destroy the BMAA toxin. Perhaps even more important, PAW was found to continue its effectiveness even 4 days after terminating the discharge.

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