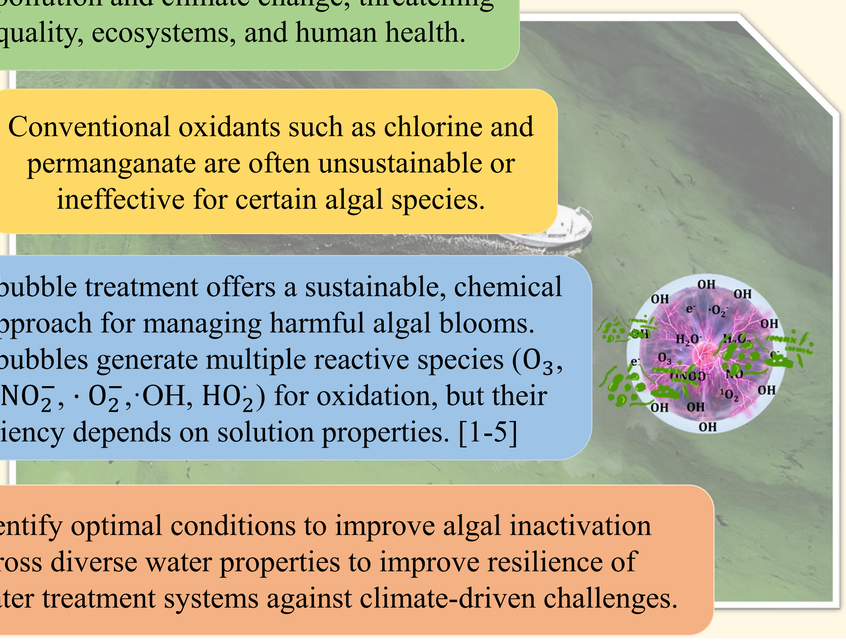
Investigating plasma bubble treatment of algae under diverse water conditions

Angelina[1], Rao N.R.H.[1], Leslie G.L.[1], Cullen P.J.[2], Henderson R.K.[1]
1. University of New South Wales, Sydney, NSW, Australia
2. University of Sydney, Sydney, NSW, Australia

INTRODUCTION

Harmful algal blooms are increasing due to nutrient pollution and climate change, threatening water quality, ecosystems, and human health.

Conventional oxidants such as chlorine and permanganate are often unsustainable or ineffective for certain algal species.



Plasma bubble treatment offers a sustainable, chemical free approach for managing harmful algal blooms. Plasma bubbles generate multiple reactive species (O_3, H_2O_2, NO_2^-, $\cdot O_2^-$,$\cdot OH$, $HO_2^{\cdot}$) for oxidation, but their efficiency depends on solution properties. [1-5]

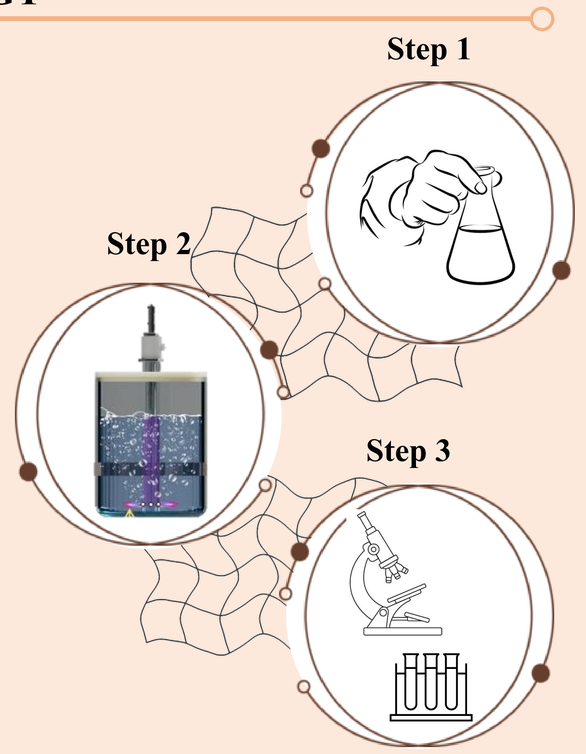
AIM

➢ Identify optimal conditions to improve algal inactivation across diverse water properties to improve resilience of water treatment systems against climate-driven challenges.

METHODOLOGY

1. **Preparation**: *Chlorella vulgaris* CS 42/7 (CSIRO ANACC) was cultivated in Jaworski medium; harvested at late exponential phase (19–21 days) and re-suspended in phosphate buffer saline (PBS). Algal suspensions adjusted to:
   - Cell number: 1×10^4, 1×10^5, 2.5×10^5, 5×10^5 cells·mL^{-1}
   - pH: 5, 6, 7.5, 9
   - Salinity: 0, 1, 2.5, 7.5 g·L^{-1}
2. **Treatment**: Each 250 mL sample treated with plasma bubbles for 10 min. Untreated samples served as controls.
   - ➢ Plasma setup: Leap100 generator (PlasmaLeap Technologies, Australia), air feed gas, 150 V input, 8.91 max output, 60 kHz resonance, 100 Hz discharge.
3. **Analysis**: Flow cytometry (BD Accuri™ C6) and PI-FDA staining for cell number, integrity and viability, O_3, H_2O_2, NO_2^-, and NO_3^- measured to assess mechanism. Measurements were collected over contact times up to 168 h.

Experiments in triplicate using different culture batches. Results reported as a mean with one standard deviation.



RESULTS

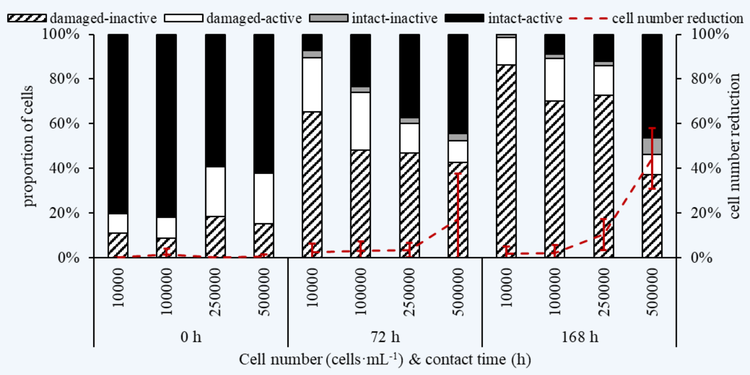


Figure 1. Effect of cell number on the change in viability, integrity, number reduction of *C. vulgaris* after 10 min of air-plasma bubble treatment, followed by a contact time of up to 168 h.

Effect of initial cell number on plasma oxidation of cells

- Lower initial cell number (1×10^4 – 1×10^5 cells·mL^{-1}) → slower initial effect but sustained higher inactivation (>85% at 168 h).
  - ➢ Fewer initial interactions occurred, but higher reactive species availability per cell enabled sustained oxidation over time
- Higher initial cell number (2.5×10^5 – 5×10^5 cells·mL^{-1}) → greater immediate inactivation (~18%) and damage (~40%), but long-term oxidation was limited due to rapid reactive species depletion.
  - ➢ Greater initial reactive species interactions within the same volume cause rapid reactive species depletion.
  - ➢ Previous study showed longer discharge times (≥15 min) are required for high cell loads, while shorter treatments are effective only at low densities [6].

✓ **Maintaining sufficient reactive species per cell is necessary for optimal results.**

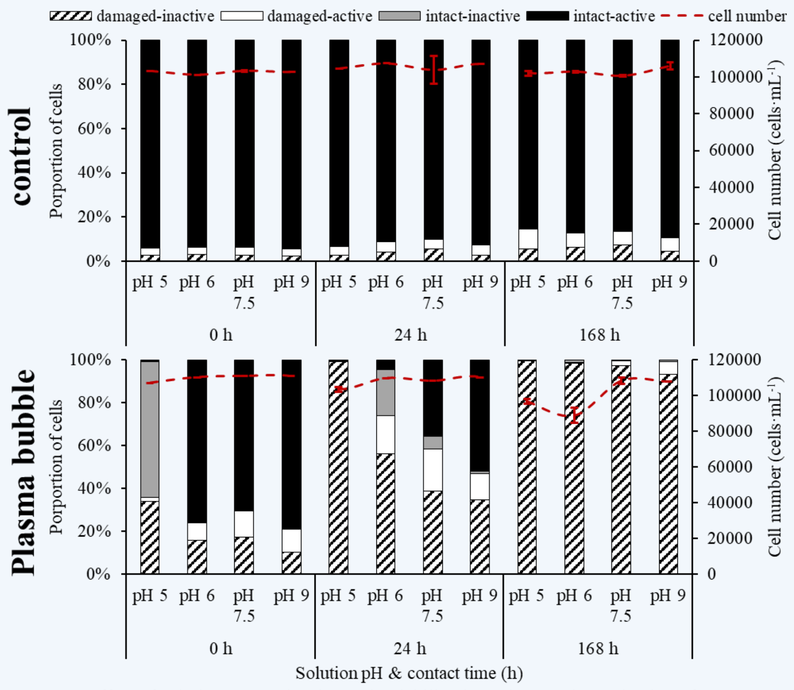


Figure 2. Effect of solution pH on the change in viability, integrity, number reduction of *C. vulgaris* after 10 min of air-plasma bubble treatment, followed by contact time of up to 168 h.

Effect of solution pH on plasma oxidation of cells

- Control experiments at varying pH showed negligible impact on cells.
- Acidic conditions enhanced oxidation efficiency by ~65% compared to neutral-alkaline (pH 7.5–9), particularly during early contact time (0–48 h).
  - ➢ Acidic pH led to protonation of algal cell surfaces, reducing repulsion against reactive species [7], while promoted the formation of stronger oxidations [8].
- At prolonged contact time (>72 h), pH has a negligible impact on cell oxidation.
  - ➢ At neutral-alkaline pH, sustained ·OH generation from long-lived reactive species decomposition (O_3 & H_2O_2) supported gradual inactivation over time.
  - ➢ At lower pH, highly reactive species were rapidly consumed, limiting secondary oxidative effects.

✓ **Plasma bubble is effective under varying pH, but acidic pH is favourable for faster oxidation of cells.**

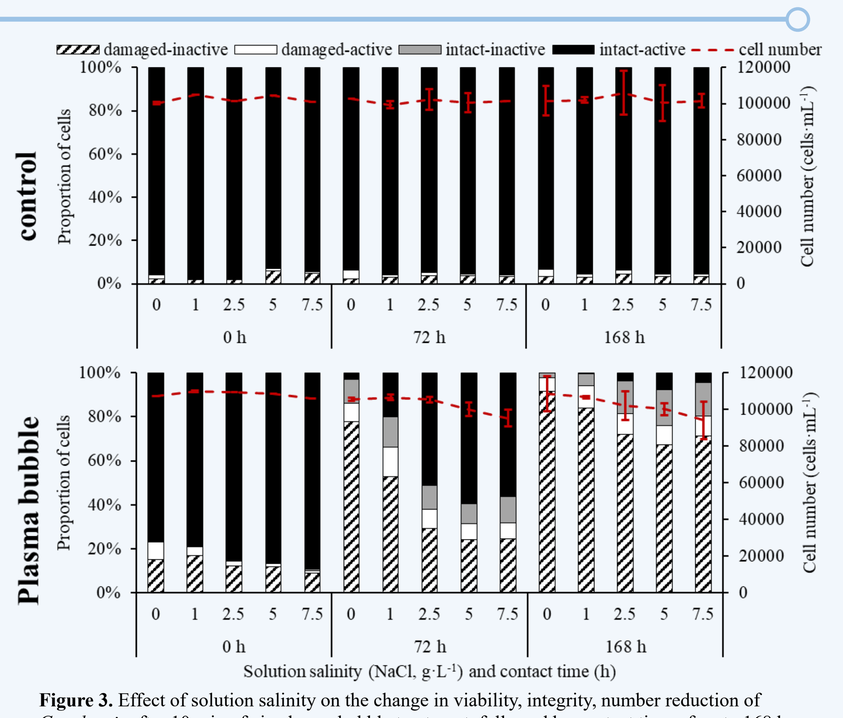


Figure 3. Effect of solution salinity on the change in viability, integrity, number reduction of *C. vulgaris* after 10 min of air-plasma bubble treatment, followed by contact time of up to 168 h.

Effect of solution salinity on plasma oxidation of cells

- Control experiments at varying salinity showed negligible impact on cells.
- Short-term effect of salinity on algal oxidation was negligible.
- As the contact time progressed to 168 h, the proportion of damaged-inactive cells decreased with increasing salinity.
  - ➢ Cl^- ions scavenged reactive species (OH, O_3 & H_2O_2), forming less reactive Cl^- compounds [4].

✓ **Treatment in brackish or saline waters may require optimisation strategies.**

CONCLUSION

- Plasma bubbles are a promising alternative to conventional oxidants for algal inactivation.
- Cell number, pH, and salinity affect treatment efficiency through changes in reactive species availability and stability.
- Lower cell number and acidic pH favour prolonged oxidation, while high salinity suppresses performance through species quenching.
- Effective application requires optimising discharge time and operating conditions to match diverse water qualities.
- These findings provide guidance for scaling plasma bubble technology toward sustainable algal bloom management in diverse waters.

Acknowledgement

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Reference

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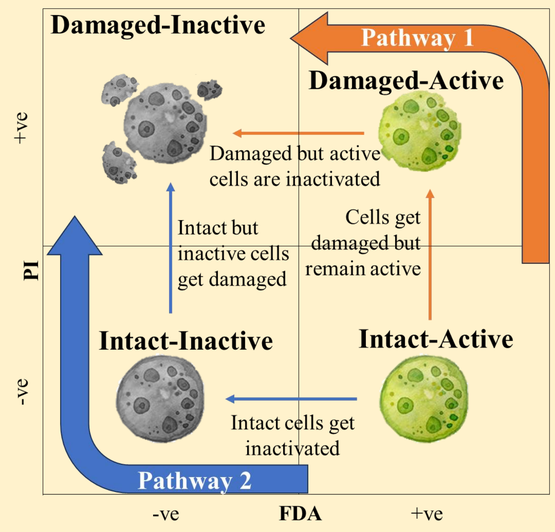
Supporting Information



Differentiation of cell integrity and viability by PI-FDA staining illustrates the pathways of cell inactivation and damage

Contacts

Angelina: angelinatan98@gmail.com, 0410203435.
Naras Rao: n.hanumanthrao@unsw.edu.au, 0449615913.
Greg Leslie: g.leslie@unsw.edu.au, 0293856092.
Patrick Cullen: Patrick.Cullen@sydney.edu.au, 0291141183.
Rita Henderson: r.Henderson@unsw.edu.au, 0419474468.