UNSW SYDNEY

Impact of coagulants and flocculants on cyanobacterial cell viability and integrity

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Acknowledgements

MICROSCOPIC PLANTS

ALSO KNOWN AS BLUE-GREEN ALGAE

TOXIC AND CAN CAUSE MASS FISH KILLS

HUMAN LIFE IN DANGER


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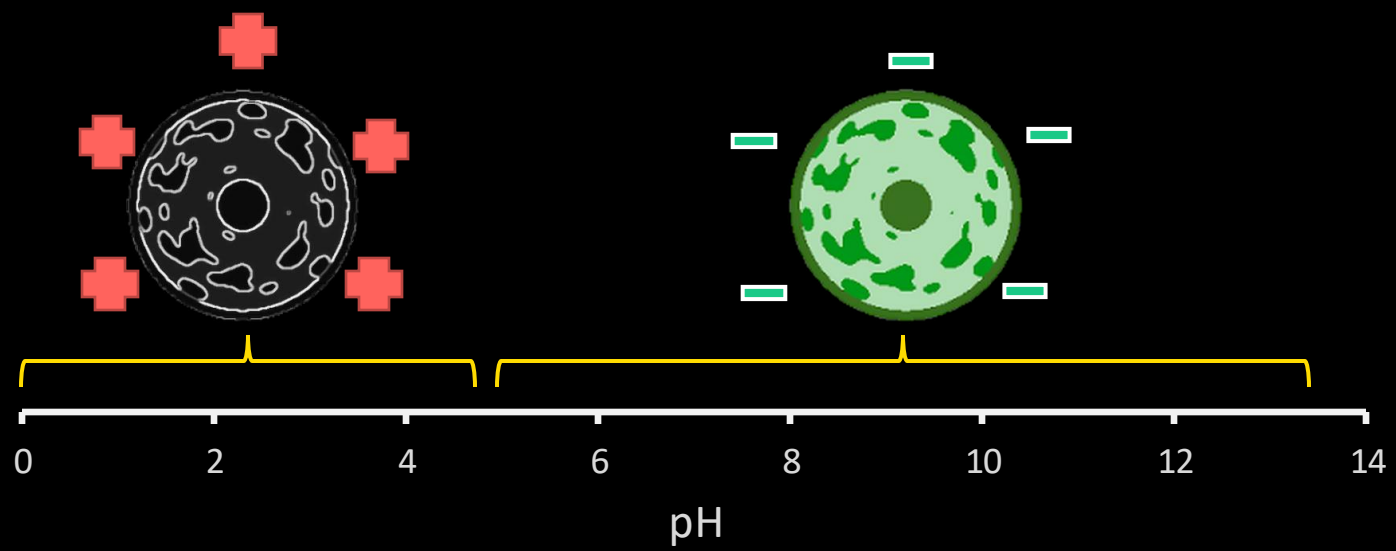
Another approach is to aggregate cells by dosing cationic chemicals

WHY USE CATIONIC CHEMICALS SPECIFICALLY?

- In the working pH range, algal cells are negatively charged



WHY AGGREGATE CELLS?

- Individual cells are small (3-15 µm)
- Aggregates are > 300 µm

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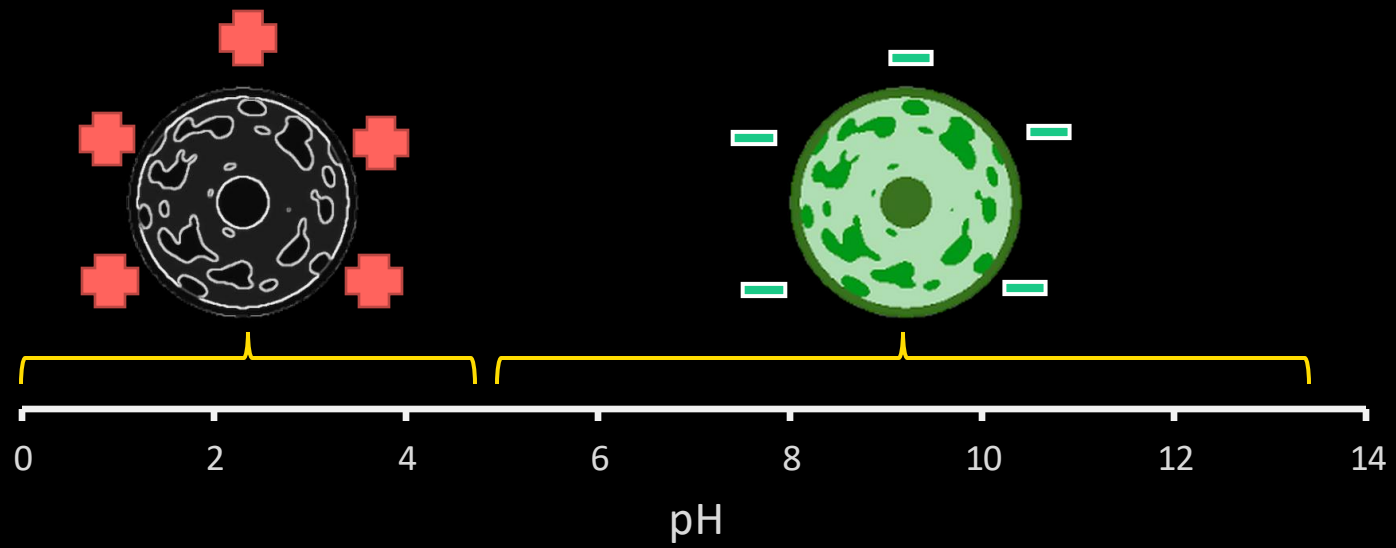

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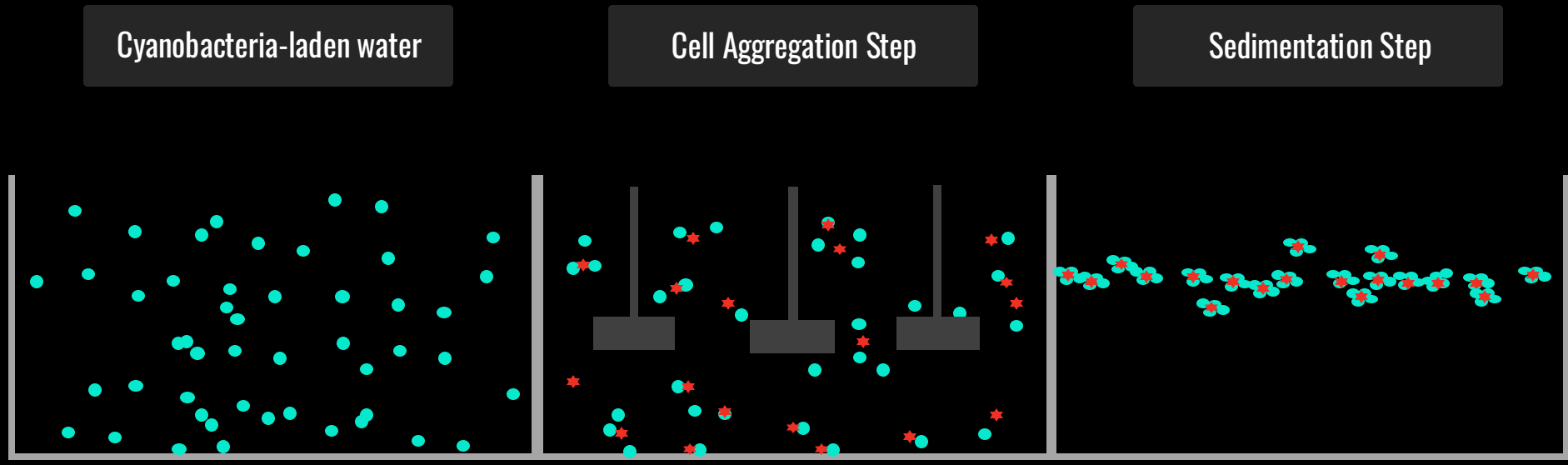
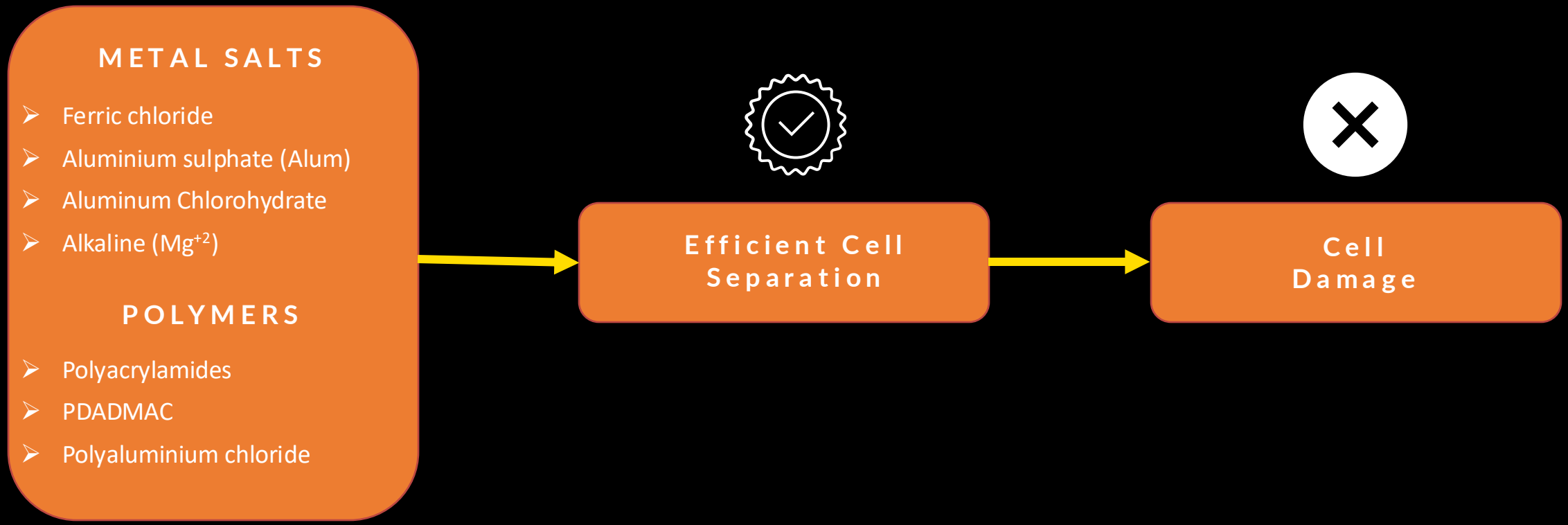


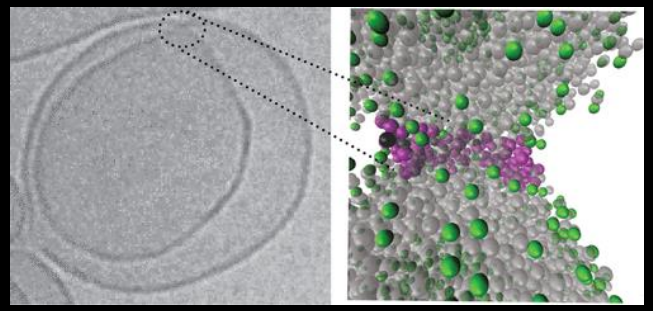
Different types of chemicals have been used for cyanobacterial cell aggregation

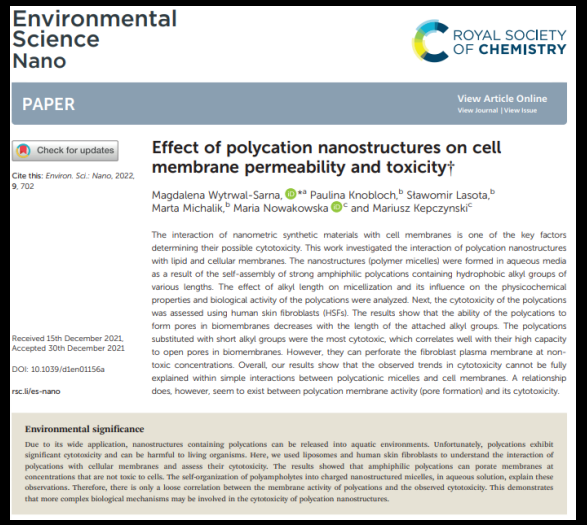


Why is this evaluating and understanding this important?

Cationic charges can destabilise anionic cell membranes and rupture them

Knowledge is not new – approaches routinely used in drug delivery, anti-microbial studies





Environmental Science Nano

ROYAL SOCIETY OF CHEMISTRY

PAPER

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Effect of polycation nanostructures on cell membrane permeability and toxicity†

Magdalena Wytrwal-Sarna, *a Paulina Knobloch,[b] Sławomir Lasota,[b] Marta Michalik,[b] Maria Nowakowska [c] and Mariusz Kepczynski[c]

The interaction of nanometric synthetic materials with cell membranes is one of the key factors determining their possible cytotoxicity. This work investigated the interaction of polycation nanostructures with lipid and cellular membranes. The nanostructures (polymer micelles) were formed in aqueous media as a result of the self-assembly of strong amphiphilic polycations containing hydrophobic alkyl groups of various lengths. The effect of alkyl length on micellization and its influence on the physicochemical properties and biological activity of the polycations were analyzed. Next, the cytotoxicity of the polycations was assessed using human skin fibroblasts (HSFs). The results show that the ability of the polycations to form pores in biomembranes decreases with the length of the attached alkyl groups. The polycations substituted with short alkyl groups were the most cytotoxic, which correlates well with their high capacity to open pores in biomembranes. However, they can perforate the fibroblast plasma membrane at non-toxic concentrations. Overall, our results show that the observed trends in cytotoxicity cannot be fully explained within simple interactions between polycationic micelles and cell membranes. A relationship does, however, seem to exist between polycation membrane activity (pore formation) and its cytotoxicity.

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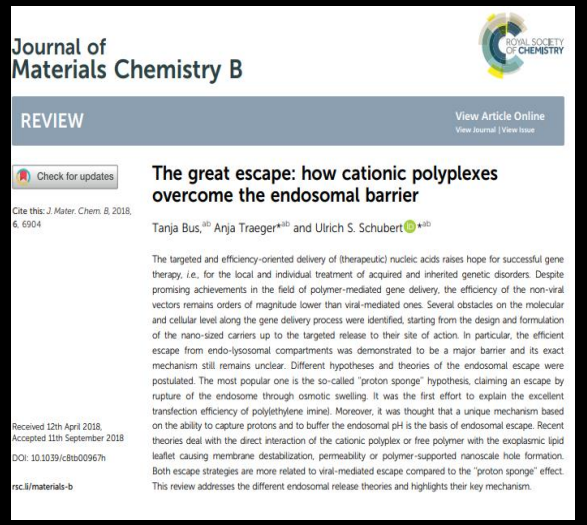
Environmental significance

Due to its wide application, nanostructures containing polycations can be released into aquatic environments. Unfortunately, polycations exhibit significant cytotoxicity and can be harmful to living organisms. Here, we used liposomes and human skin fibroblasts to understand the interaction of polycations with cellular membranes and assess their cytotoxicity. The results showed that amphiphilic polycations can porate membranes at concentrations that are not toxic to cells. The self-organization of polyampholytes into charged nanostructured micelles, in aqueous solution, explain these observations. Therefore, there is only a loose correlation between the membrane activity of polycations and the observed cytotoxicity. This demonstrates that more complex biological mechanisms may be involved in the cytotoxicity of polycation nanostructures.



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REVIEW

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The great escape: how cationic polyplexes overcome the endosomal barrier

Tanja Bus,[ab] Anja Traeger*[ab] and Ulrich S. Schubert*[ab]

The targeted and efficiency-oriented delivery of (therapeutic) nucleic acids raises hope for successful gene therapy, *i.e.*, for the local and individual treatment of acquired and inherited genetic disorders. Despite promising achievements in the field of polymer-mediated gene delivery, the efficiency of the non-viral vectors remains orders of magnitude lower than viral-mediated ones. Several obstacles on the molecular and cellular level along the gene delivery process were identified, starting from the design and formulation of the nano-sized carriers up to the targeted release to their site of action. In particular, the efficient escape from endo-lysosomal compartments was demonstrated to be a major barrier and its exact mechanism still remains unclear. Different hypotheses and theories of the endosomal escape were postulated. The most popular one is the so-called "proton sponge" hypothesis, claiming an escape by rupture of the endosome through osmotic swelling. It was the first effort to explain the excellent transfection efficiency of poly(ethylene imine). Moreover, it was thought that a unique mechanism based on the ability to capture protons and to buffer the endosomal pH is the basis of endosomal escape. Recent theories deal with the direct interaction of the cationic polyplex or free polymer with the exoplasmic lipid leaflet causing membrane destabilization, permeability or polymer-supported nanoscale hole formation. Both escape strategies are more related to viral-mediated escape compared to the "proton sponge" effect. This review addresses the different endosomal release theories and highlights their key mechanism.

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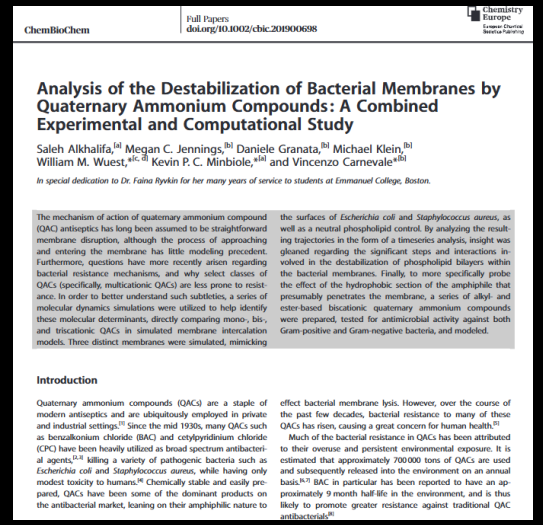

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Full Papers
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Chemistry Europe

Analysis of the Destabilization of Bacterial Membranes by Quaternary Ammonium Compounds: A Combined Experimental and Computational Study

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In special dedication to Dr. Faina Ryvkin for her many years of service to students at Emmanuel College, Boston.

The mechanism of action of quaternary ammonium compound (QAC) antiseptics has long been assumed to be straightforward membrane disruption, although the process of approaching and entering the membrane has little modeling precedent. Furthermore, questions have more recently arisen regarding bacterial resistance mechanisms, and why select classes of QACs (specifically, multicationic QACs) are less prone to resistance. In order to better understand such subtleties, a series of molecular dynamics simulations were utilized to help identify these molecular determinants, directly comparing mono-, bis-, and triscationic QACs in simulated membrane intercalation models. Three distinct membranes were simulated, mimicking the surfaces of *Escherichia coli* and *Staphylococcus aureus*, as well as a neutral phospholipid control. By analyzing the resulting trajectories in the form of a timeseries analysis, insight was gleaned regarding the significant steps and interactions involved in the destabilization of phospholipid bilayers within the bacterial membranes. Finally, to more specifically probe the effect of the hydrophobic section of the amphiphile that presumably penetrates the membrane, a series of alkyl- and ester-based biscationic quaternary ammonium compounds were prepared, tested for antimicrobial activity against both Gram-positive and Gram-negative bacteria, and modeled.

Introduction

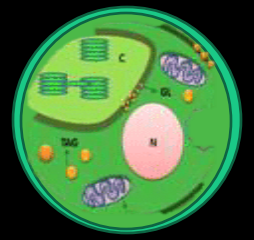
Quaternary ammonium compounds (QACs) are a staple of modern antiseptics and are ubiquitously employed in private and industrial settings.[1] Since the mid 1930s, many QACs such as benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC) have been heavily utilized as broad spectrum antibacterial agents,[2,3] killing a variety of pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*, while having only modest toxicity to humans.[4] Chemically stable and easily prepared, QACs have been some of the dominant products on the antibacterial market, leaning on their amphiphilic nature to effect bacterial membrane lysis. However, over the course of the past few decades, bacterial resistance to many of these QACs has risen, causing a great concern for human health.[5]

Much of the bacterial resistance in QACs has been attributed to their overuse and persistent environmental exposure. It is estimated that approximately 700 000 tons of QACs are used and subsequently released into the environment on an annual basis.[6,7] BAC in particular has been reported to have an approximately 9 month half-life in the environment, and is thus likely to promote greater resistance against traditional QAC antibacterials[8]

Coagulants and flocculants are cationic and used for cyanobacterial bloom management heavily

Used not just in WTPs, but also in remote/recreational lakes and catchments

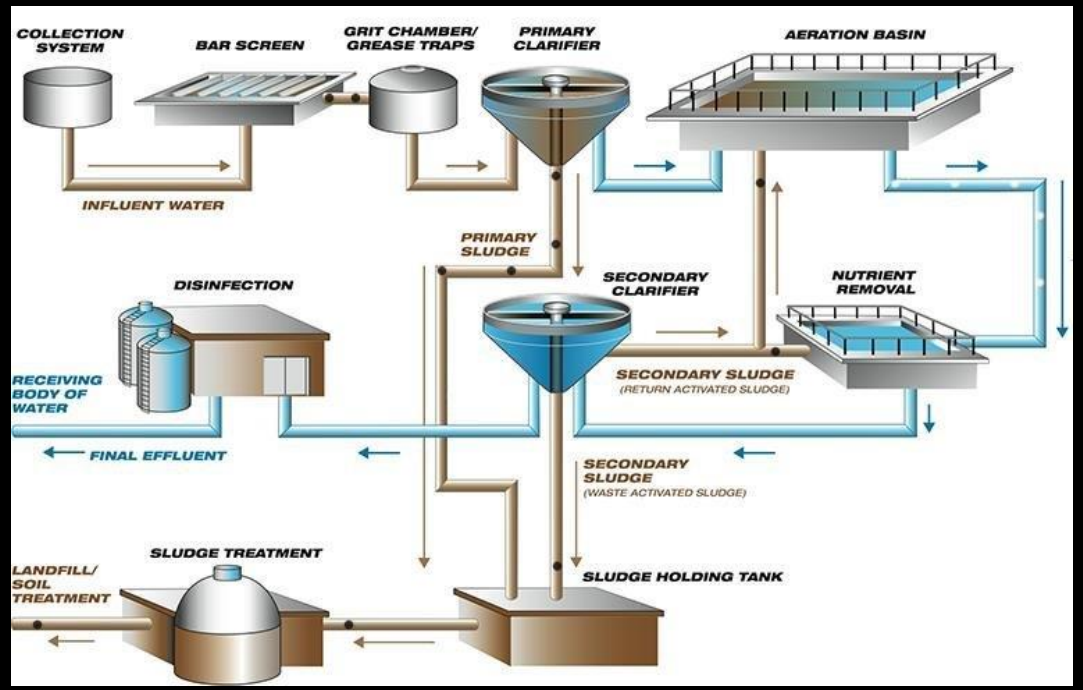
What happens when cells get damaged?



Cell Lysis

Release of

- Toxins
- Nutrients (N & P)
- Organic C-compounds
- T&O compounds



Cell lysis can cause release of intra-cellular matter within process train and sludge holding tanks and lagoons

"Perpetual" cyanobacteria blooms in Dutch lakes. Lurling (2020), Hydrobiologia, 847 (21), p:4447-4467

What are we doing in this study?

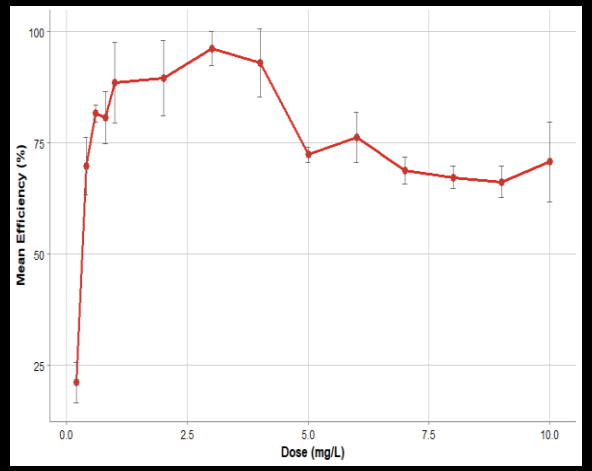
- Evaluate intracellular material release when using 8 commonly employed coagulants and flocculants
  - Ferric chloride
  - Aluminium sulphate (Alum)
  - Aluminum Chlorohydrate
  - Alkaline (Mg^{+2})
  - Polyacrylamides (LT22S and Poly 4190)
  - PDADMAC
  - Polyaluminium chloride

- Over a time period of 0–168h, assess cell damage and release of:
  - Toxins
  - Nutrients
  - Intracellular organic carbon

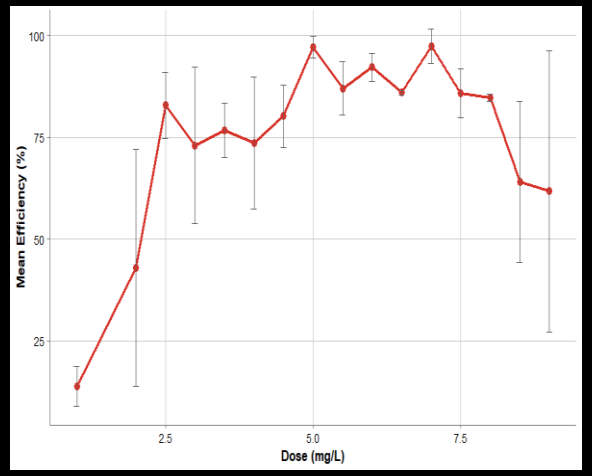
- Toxic strain of cyanobacteria examined: *Microcystis aeruginosa* (CS-555/1)
  - Cell concentration: $6\text{-}8\times10^{5}$ cells/mL

- Lab-scale study using a bench-top flocculator

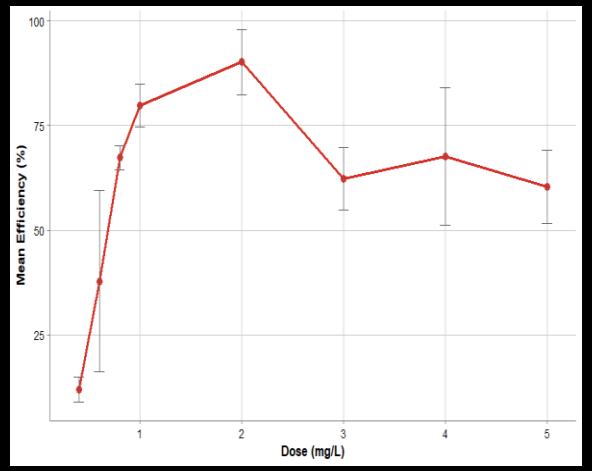
All the test coagulants/flocculants achieved >90% cell removal



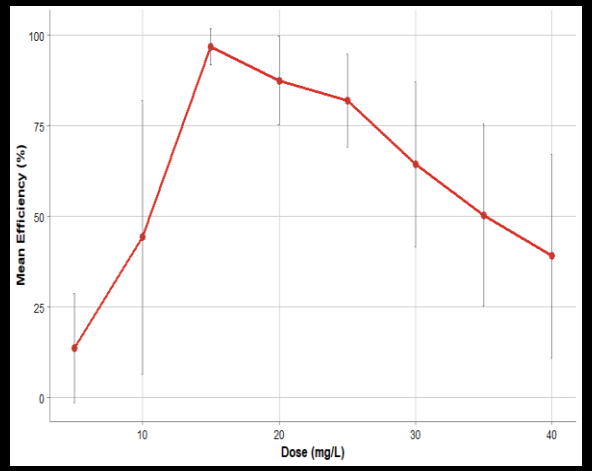
PDADMAC – 96.1±3.7%



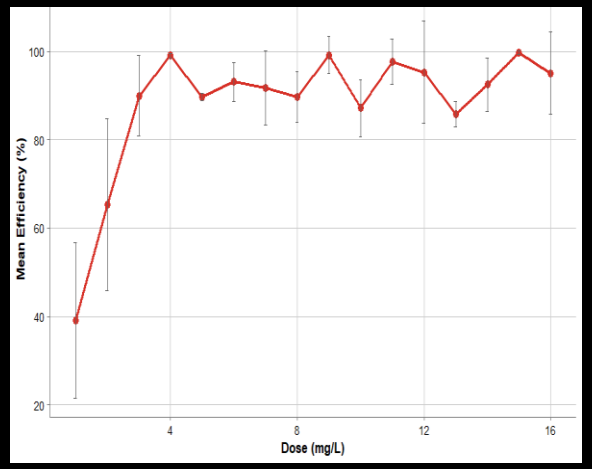
ACH – 97.2±2.6%



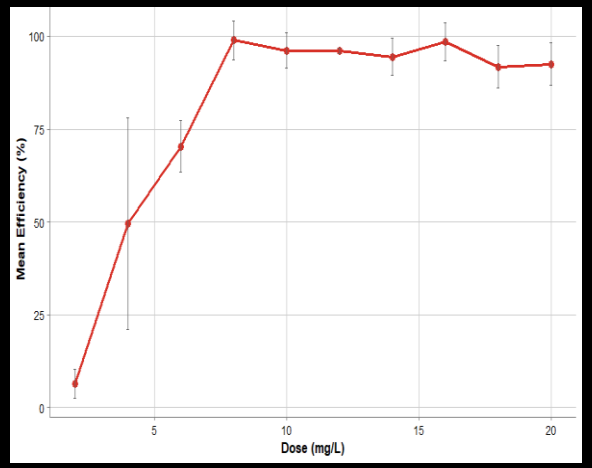
Poly4190 – 90.1±7.8%



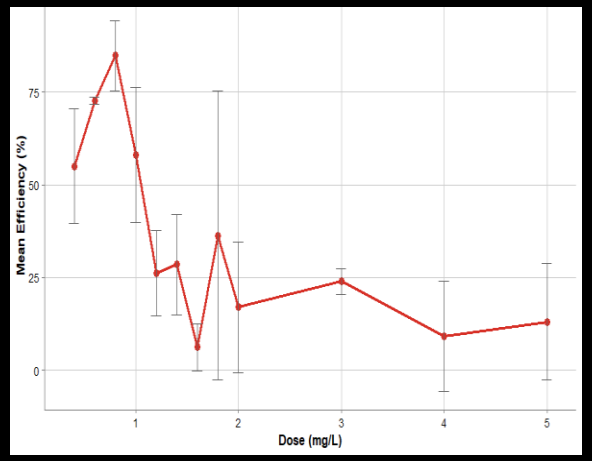
$FeCl_3$ – 96.9±2.7%



PACl – 99.1±0.3%



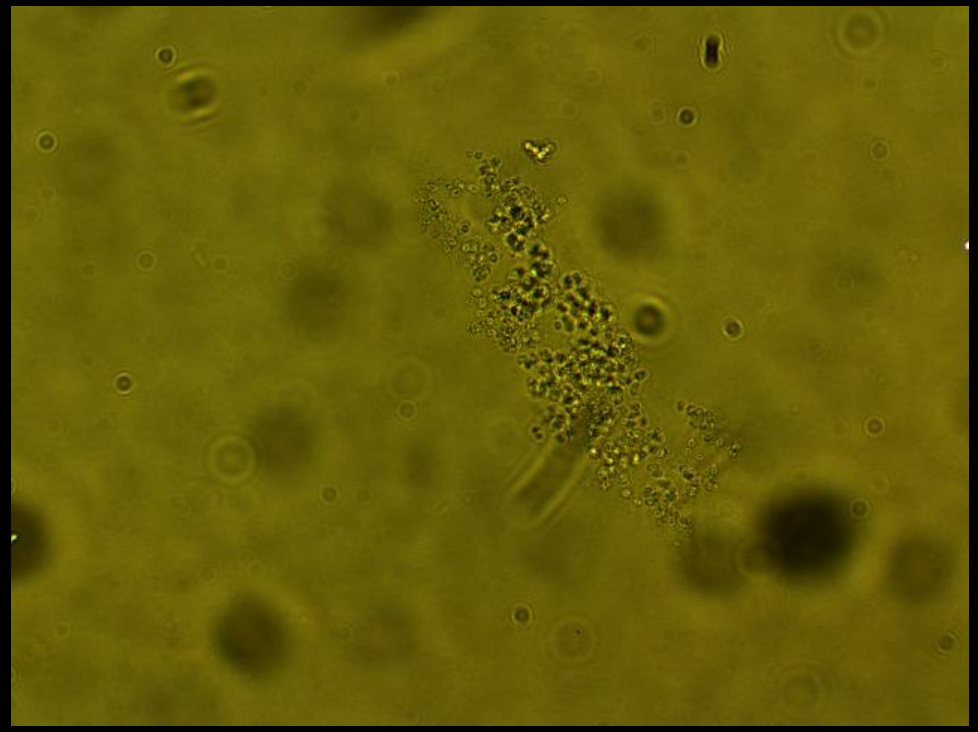
Alum – 98.5±0.7%



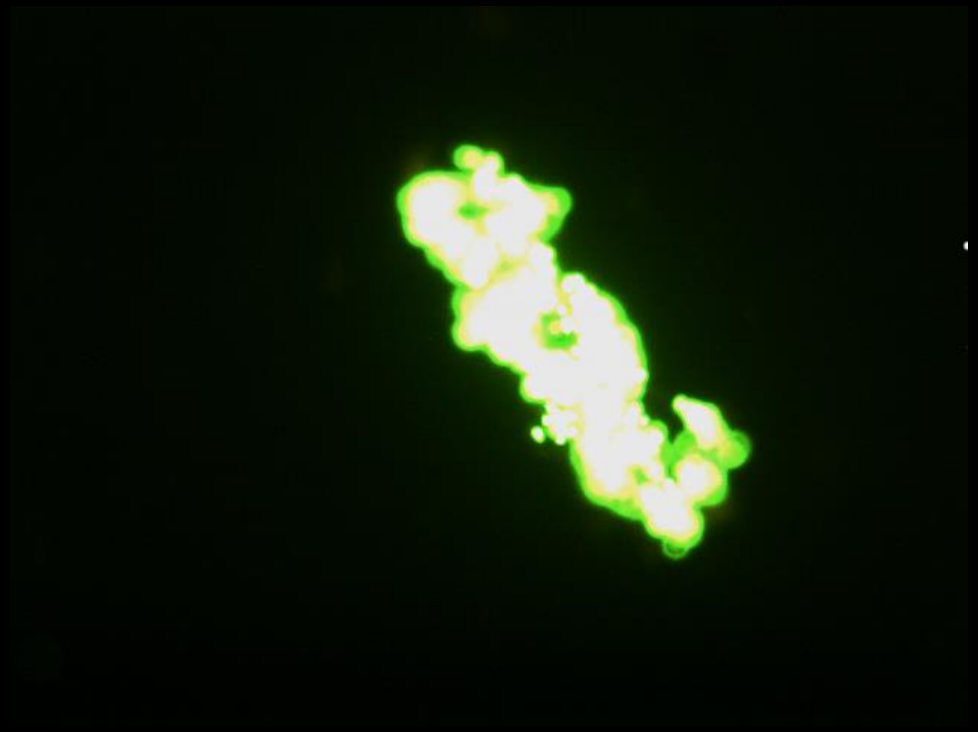
LT22S – 84.7±9.5%

NaOH – 95.8±4.1%

All coagulants/ flocculants caused immediate cell damage



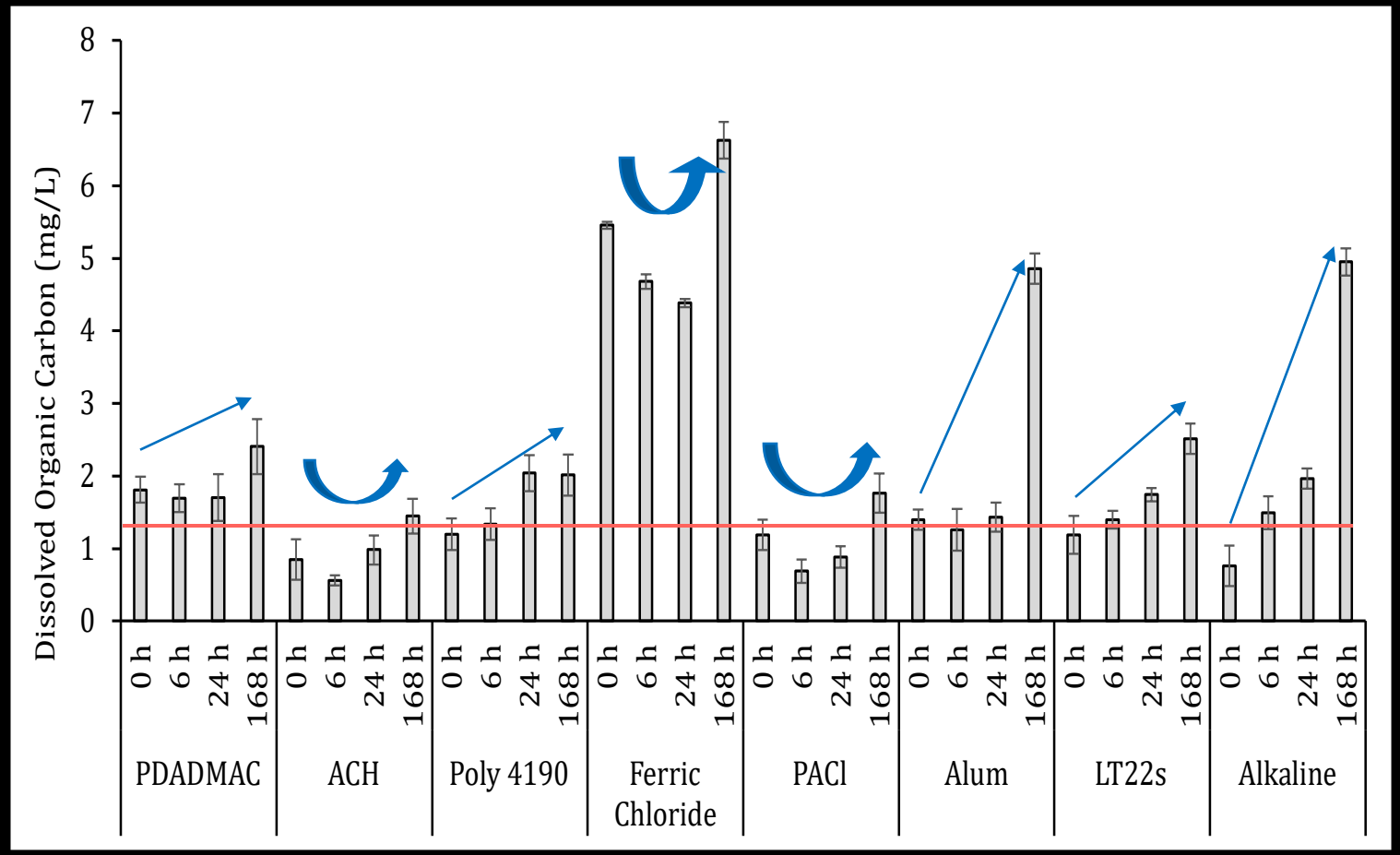
Unstained floc (PDADMAC)



SYTOX Green stained floc

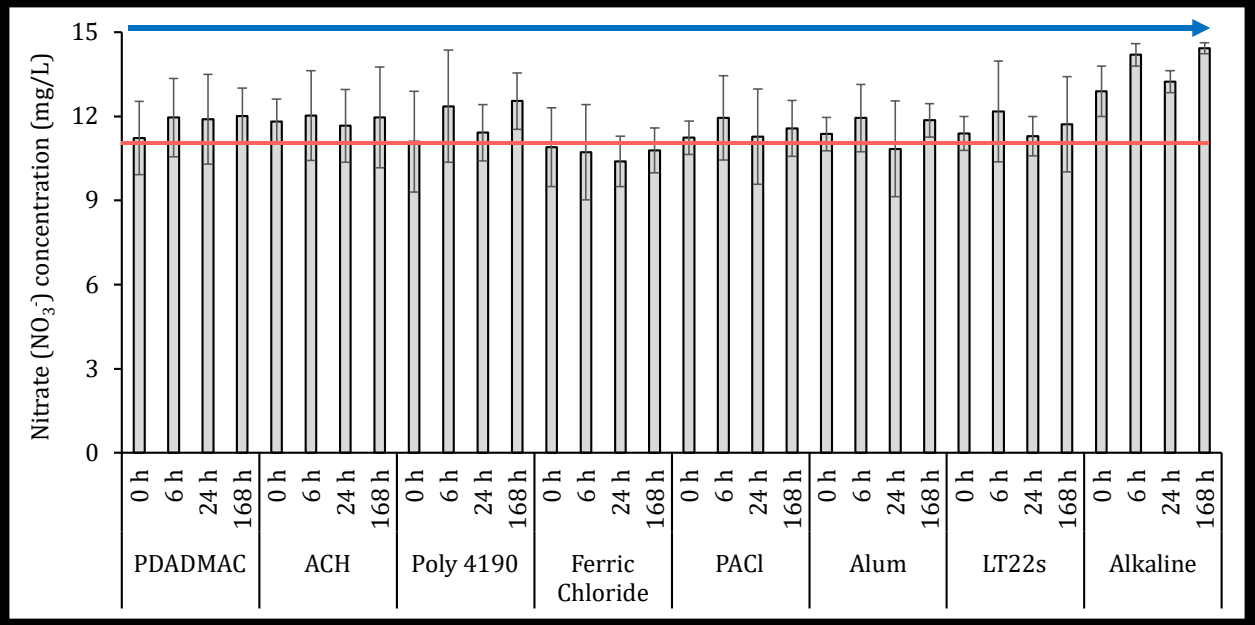
- Flocs with cells that are damaged appear green due to stain penetration
- Undamaged cells appear red (none were detected in any samples)
- All fluorescence microscopy assays conducted at 0h post-sedimentation

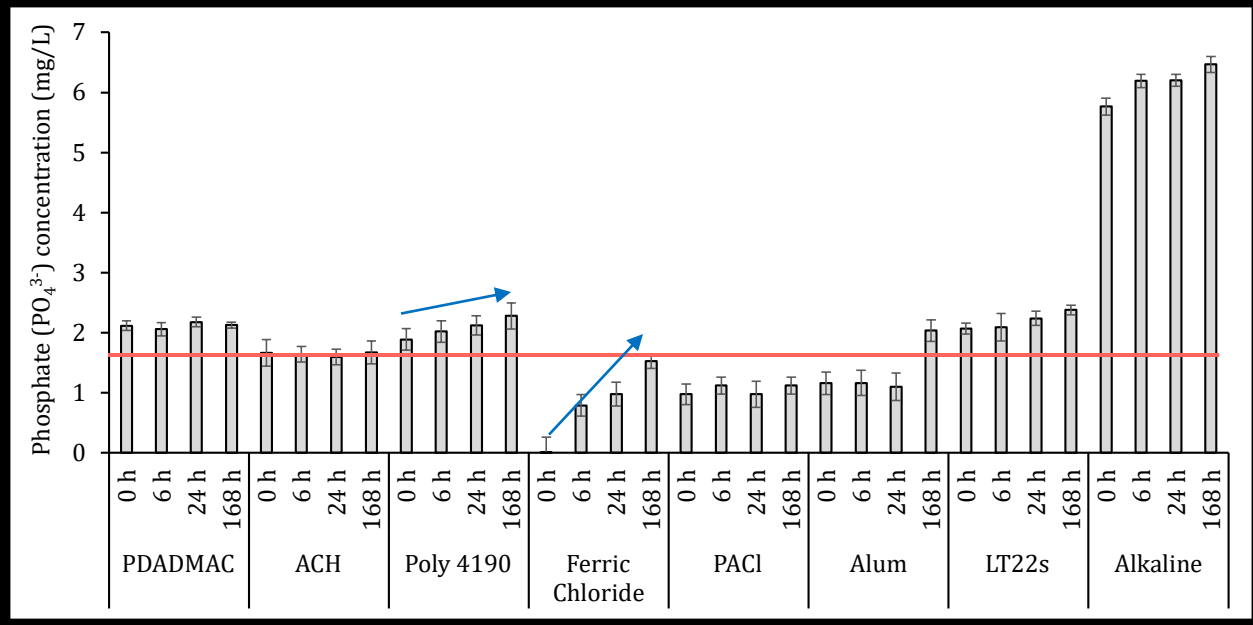
DOC increased with storage – indicative of IOM release

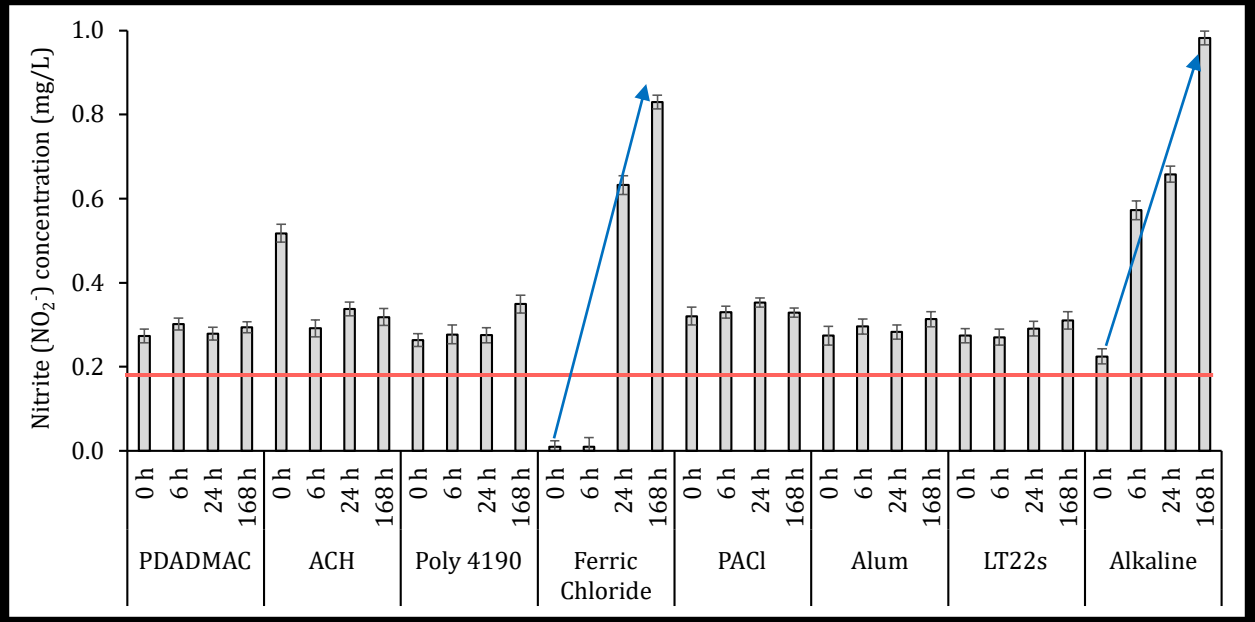


- Red line is baseline DOC just before starting jar tests
- Storage of separated sludge upto 168 h causes definitive increases in DOC
- Sharp steady increase for most coagulants/flocculants
- U-shaped increase for ACH, $FeCl_3$ and PACl (likely due to complexation)
- More problematic for lakes/ catchments

No big changes in nutrients (Nitrite, Nitrate, Phosphate)

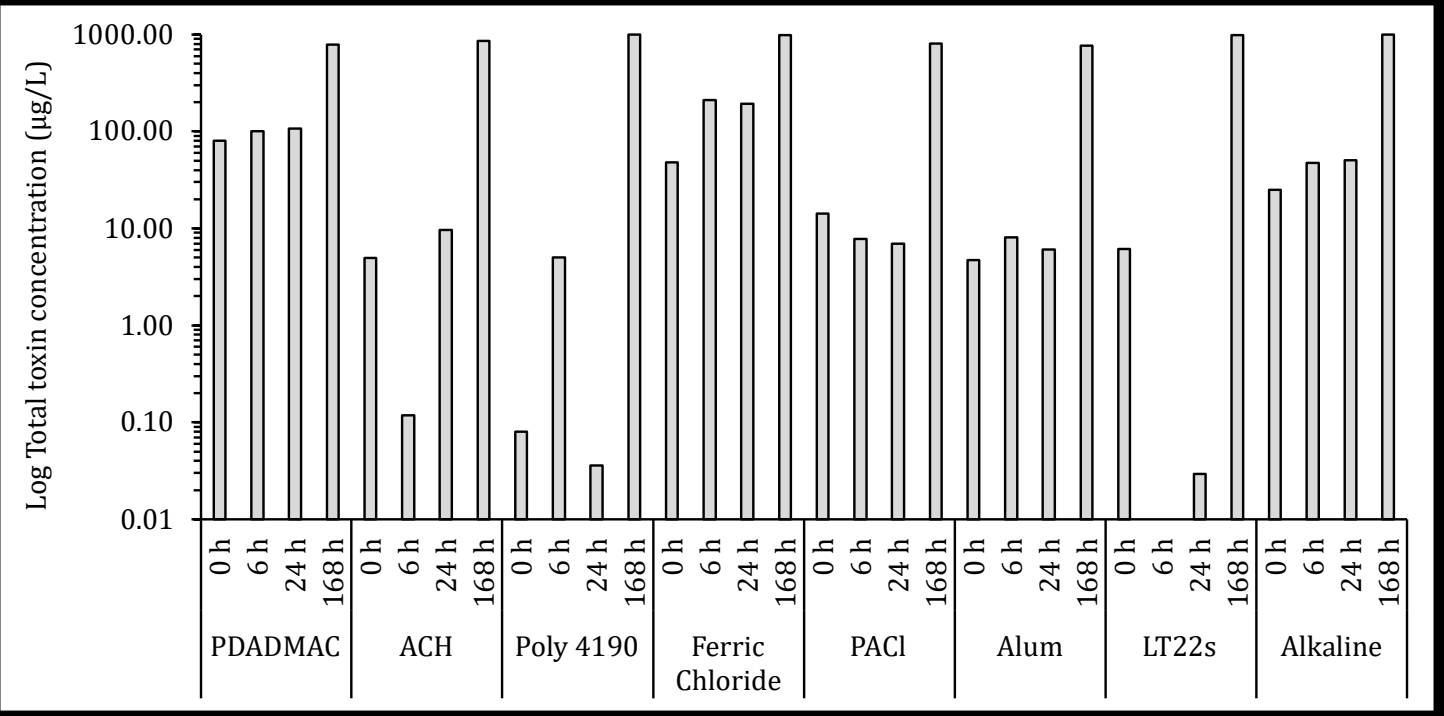






- Red line is baseline just before starting jar tests
- Nitrites increased (sometimes with storage)
- Phosphates generally reduced following jar tests (increased sometimes with storage)
- Ferric chloride good for short-term

Total Microcystin concentrations increased significantly with storage



- 9 types of toxins identified including MC-LR, MC-LR demeth, MC-RR, MC-RR demeth, MC-YR,MA-LA, MC-LW, MC-LF, Nodularin
- All coagulants/ flocculants contribute to toxin release
- Toxin concentrations fluctuate in the first 24h; likely due to complexation with the coagulants/ flocculants (or column interactions)
- Storage of separated sludge degrades cells, causing significant toxin release
- MC-RR demethylated contributed to >90% of total toxins

Key takeaways from this study

Questions and unknowns

- WTPs use pre-oxidation and coagulant + flocculant
- Assess proportions of cell damage within flocs
- Correlation between charge density and proportions of cell damage – is there a sweet spot?
- Interference from background OM
- U-shaped vs linear increase in DOC not observed for all coagulants and flocculants
- Preferential removal/ complexation of certain toxins?
- Does cell damage mean complete disintegration or just a crack on the cells?

Thank you