Comparing the effectiveness of three pretreatment technologies for *Microcystis aeruginosa* inhibition

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ABSTRACT

Several lab experiments were done to compare the effectiveness of three pretreatment technologies for Microcystis aeruginosa inhibition at the drinking water treatment (DWTP): NaClO, K_2FeO_4 and $KMnO_4$. All three technologies showed effectiveness in varying degree to inhibit Microcystis aeruginosa, their performance increased with increasing concentration and time. However, Microcystis aeruginosa cell wall lyses was of concern at higher concentrations.

To provide sufficient information for decision makers at the DWTP, not only should the performance of the technologies be analyzed but also the cost implications.

Index Terms- *Microcystis aeruginosa*, Drinking water treatment, Pretreatment, Growth inhibition

1. INTRODUCTION

Harmful algal blooms (HABs) is a global challenge and it is a real problem faced by many DWTPs (Weirich and Miller 2014, Brooks, Lazorchak et al. 2016). Eutrophication and Climate change are the main causes of HABs which has become the most widespread water quality challenge in many parts of the world (Tian, Lu et al. 2013). The conventional DWTPs shows limited efficiency to solve this water quality problem especially where algae cell wall is broken and harmful algal derivatives released in the water (Ou, Gao et al. 2012, He, Liu et al. 2016). Other challenges with Conventional DWTPs is that some algae species float through the coagulation and sedimentation processes and cause filter clogging (Joh, Choi et al. 2011), also some may escape to the Drinking water distribution system where they will cause biofilms in the pipes (Douterelo, Boxall et al. 2014). There is therefore the need for pretreatment and advanced treatment technologies as strategies to take care of these limitations. During a HABs event, the suspended solids is increased in the raw water, pretreatment can promote the coagulation and sedimentation, improving the filtration process (Wang, Qiao et al. 2013, Naceradska, Pivokonsky et al. 2017).

Globally, Chlorine is comparatively cost effective and it is the most popular disinfectant in DWTPs in developing countries. However, when it comes to treating raw water after a HABs event, chlorine's major problems is that it is prone to cause severe cell lysis releasing intracellular and extracellular algal organic matter (AOM) that provides precursors for DBPs, when the water is subsequently treated with chlorine during pretreatment or during disinfection (Qi, Lan et al. 2016, Goslan, Seigle et al. 2017).

The aim is therefore to explore other oxidants, identify high performance yet cost effective pretreatment technologies that can easily be adopted in developing countries all over the world to solve algal problems.

Just like Chlorine, NaClO is quite cheap disinfectants stable enough to remain in water distribution systems for a long time to prevent water reinfection. (Lebedev, Shaydullina et al. 2004). Unlike Chlorine, a major advantage here of NaClO over chlorine is that the use of chlorine leads to a greater variety and higher concentrations of chlorinated products compared to sodium hypochlorite. (Sinikova, Shaydullina et al. 2014) This property has stimulated research in the possibilities of using NaClO as an alternative for chlorine in algae removal.

K_2FeO_4 is a strong oxide in both acidic and alkaline conditions. K_2FeO_4 has emerged as a novel oxidant and coagulant to remove contaminants in water. The application of ferrate (VI) as a pretreatment oxidant has been evaluated and found to increase the efficiency of flocculation.(He, Liu et al. 2016, Liu, Tang et al. 2017) Ferrate (VI) is also ideal as it is environment friendly and decomposes into non-toxic Fe(III) ions or ferric hydroxide, which simultaneously occurs as a coagulant of water.(Zhou, Shao et al. 2014) The unique properties of Fe(VI) have stimulated a number of researchers to study its performance for water and wastewater treatment.

$KMnO_4$ is has been used as an algaecide and disinfectant, but now more attention is paid to use it as a pre-oxidizer to enhance coagulation. (Wang, Qiao et al. 2013)Pretreatment with $KMnO_4$ provides excellent control of taste and odor compounds, improves flocculation, controls biological regrowth and production of DBPs. (Ou, Gao et al. 2012, He, Liu et al. 2016, Naceradska, Pivokonsky et al. 2017)

In this study, *Microcystis aeruginosa* (*M. aeruginosa*) was chosen as the model algae to be tested. NaClO, K_2FeO_4 and $KMnO_4$ were selected as the pretreatment technologies to inhibit *M. aeruginosa* lab experiments. Their performance was compared in regards of concentration against time. Their pricing was also compared for the purposes of identifying the best performer at the best price so as to help decision makers in technology selection.

2. MATERIALS AND METHODS

2.1 Microorganism and reagents

Cyanobacteria M. *aeruginosa* (FACHB-912) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The algae were cultured with BG11 medium in 2 L conical flasks and incubated in an illumination incubator at 25 ± 0.5 ℃ with the illumination intensity of 2,000 Lux under a 12h of light and 12 h of darkness cycle every day. The stock cultures of M. *aeruginosa* were shaken three times a day. Because the optical density (OD) values were in linear relation to algae concentration, the algae concentrations were determined by a UV-vis spectrophotometer at 680 nm, which is the maximum absorbance band of algal cell suspension (Liang, Qu et al. 2005).

All the reagents and solvents were of analytical grade. NaClO of analytical grade was purchased from Jiangtian industrial chemicals, Tianjin, China. Standard procedures were used to make a stock of NaClO diluted in ultrapure water to 1000 mg/L and stored in an aluminum foil-covered bottle kept at 4 °C until use (Zhai, He et al. 2017). Standard procedures were used to make the stock solution of potassium ferrate (K2FeO4, Sigma–Aldrich, USA) 1000mg/L dispersed in deionized water purified by Millipore (Zhou, Shao et al. 2014). The stock solution of KMnO4 was prepared using standard procedures get dissolved crystal KMnO4 (Sigma–Aldrich, USA) in Milli-Q water at a concentration of 1000mg/L. Fresh solutions were used. (Ou, Gao et al. 2012, Wang, Qiao et al. 2013)

2.2 Experimental Procedures

2.2.1 NaClO oxidation experiments

The M. aeruginosa culture was harvested in the growth exponential phase and diluted by BG11 medium to OD_{680} of 0.12, corresponding to the algae concentration of 2.27×10^6 cells/L, which is extremely equal to the concentration of algae that causes bloom (Wang, Qiao et al. 2013). Prior to the experiments, the solution pH was adjusted to 7 using Sodium Hydroxide solution (NaOH). 90ml of the solution was put in 5 beakers 250ml sizes and put on a magnetic stirrer at slow mix. Predetermined NaClO solutions 0, 2.5, 5,10 and 15 mg/L, prepared in advance from the stock solution, were added to the M. *aeruginosa* suspension. After every 5 minutes of contact time, 5ml samples were harvested for immediate Biomass (cell density), photosynthetic yield and chlorophyll-a analyses. 0.5ml samples were also collected after every 15 minutes for M. *aeruginosa* microscope analysis. For scanning electron microscopy (SEM) cell-wall integrity analyses, at the end of the 60 minutes 5ml samples were collected from each of the concentrations, centrifuged at 6000 rpm for 10 minutes and the precipitates retained for SEM analysis. All experiments were repeated three to four times.

2.2.2 K2FeO4 Oxidation experiments

The M. aeruginosa culture was prepared as mentioned in section 2.2.1 above. Prior to the experiments, the solution pH was adjusted to 7 using 0.1 M HCl (Zhou, Shao et al. 2014). Same procedure as seen in section 2.21 above was used to conduct K_2FeO_4 experiments and collect the samples for biomass, chlorophyll-a, photosynthetic yield, microscope analyses and SEM analyses.

2.2.3 $KMnO_4$ Oxidation experiments

The M. aeruginosa culture was prepared as mentioned in section 2.2.1 above. Prior to the experiments, the solution pH was adjusted to 7 using phosphate buffer solution (KH_2PO_4-NaOH) (Ou, Gao et al. 2012). Same procedure as seen in section 2.21 above was used to conduct $KMnO_4$ experiments and collect the samples for biomass, chlorophyll-a, photosynthetic yield, microscope analyses and SEM analyses.

2.3 Sample Analyses

For biomass determination, immediately after harvesting the 5ml suspension samples after every 5minutes they were analyzed at 680 nm by a UV spectrophotometer (UV-1800, Shanghai Science Instrument Company Limited, China).

The chlorophyll-a concentration and the photosynthetic activity of M. aeruginosa were measured by a Phyto-PAM phytoplankton analyzer (Walz, Germany) equipped with an Optical Unit ED-101US/MP. The variable Chlorophyll-a fluorescence (Fv) can be calculated from the maximal fluorescence (Fm) and the minimal fluorescence (Ft) as shown in equation (1). The photosynthetic yield (Y) is an indicator of photosynthesis and assesses the maximal quantum conversion efficiency of Photosystem II. It is calculated as shown in equation (2) (Maxwell and Johnson 2000, Tang, Tian et al. 2015).

$$F_v = F_m - F_t \qquad (1)$$

$$Y = \frac{F_v}{F_m} \qquad (2)$$

Samples for microscope analysis were dropped on glass slides then microscope (LW200-2JT, Shanghai liwei photoelectric technology co. LTD) was used to observe and photograph the specimen treated with NaClO, K2FeO4, and KMnO4.

Analysis of precipitated samples for SEM followed a standard procedure (Ma, Liu et al. 2012). The precipitates were firstly fixed with 2.5% glutaldehyde at 4 degrees Celsius overnight then washed with a phosphate buffer solution thrice. The samples were then treated with 30%, 50%, 70%, 85%, 95% and 100% ethanol for 15 minutes consecutively. A critical point dryer was then used to dry the dehydrated cells before mounting them on copper stubs for sputter-coating with gold-palladium. A scanning electron microscope (SEM, HITACHI S-4800, Japan) was then use to observe and photograph the specimen treated with NaClO, K_2FeO_4, and $KMnO_4$.

3. RESULT AND DISCUSSIONS

3.1 NaClO oxidation experiments

Several experiments were conducted to investigated the potential of NaClO to inhibit algal growth. Figure 1 show the impact of NaClO (at 0.25 to15mg/L) on Biomass (cell density of M. aeruginosa) after 1hour interaction. In the first 5mins there was evidence of biomass decrease across all the concentrations, proving that NaClO is a strong oxidant and works fast. In the graph it can be observed that Biomass decrease improved with increasing time and concentration. The same trend, improving performance with increasing time and concentration, is seen in figure 2 for chlorophyll-a and figure 3 for photosynthetic yields, with best performance achieved at concentrations 10mg/L and 15mg/L.

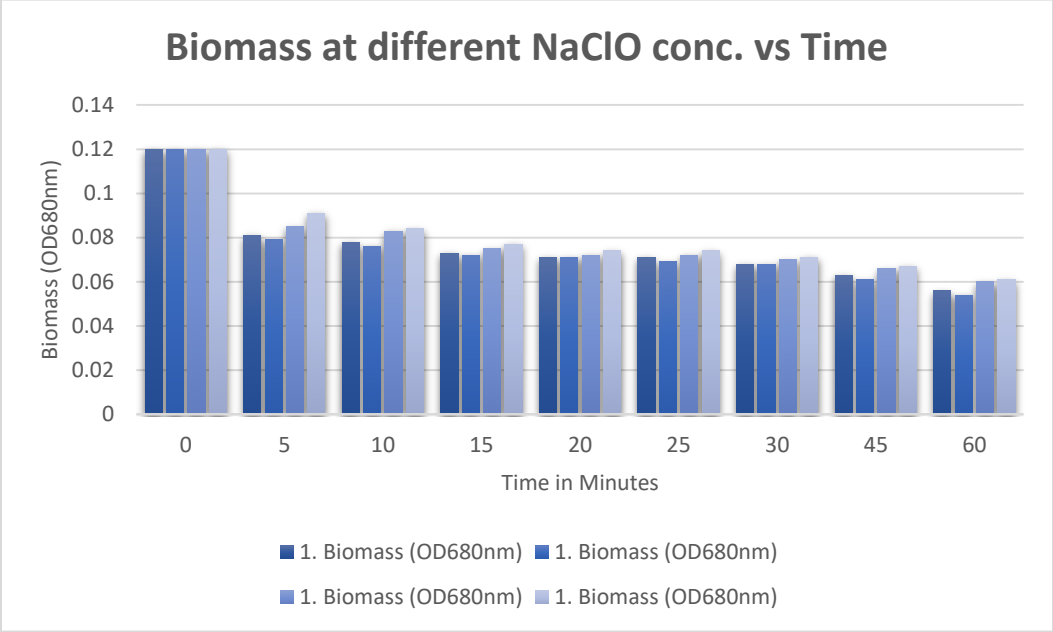

Figure 1:Biomass at different NaClO conc. Vs time

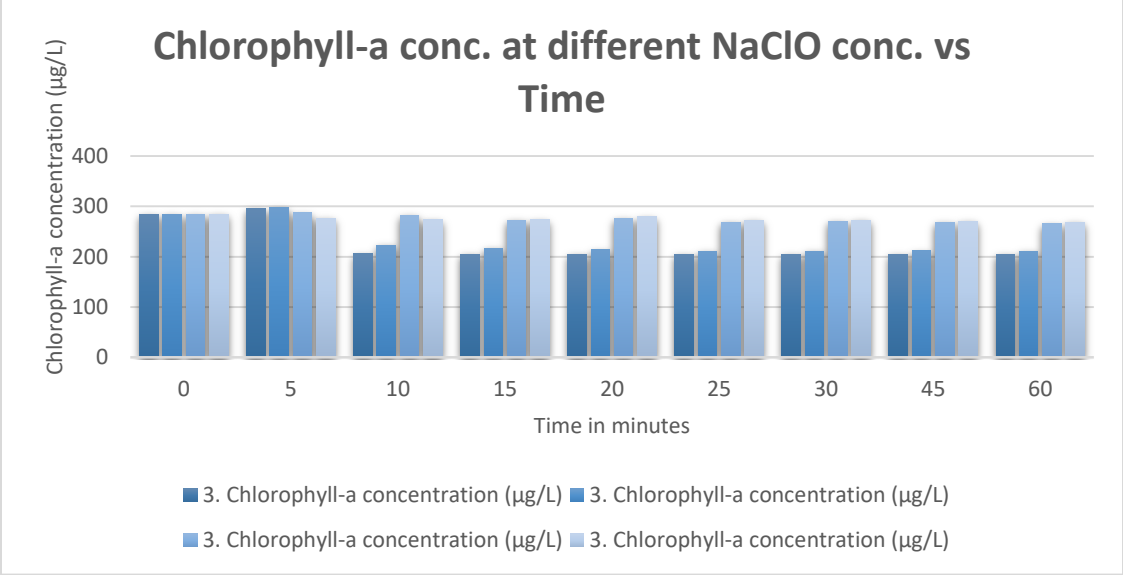

Figure 2: Chlorophyll-a at different NaClO conc. Vs time

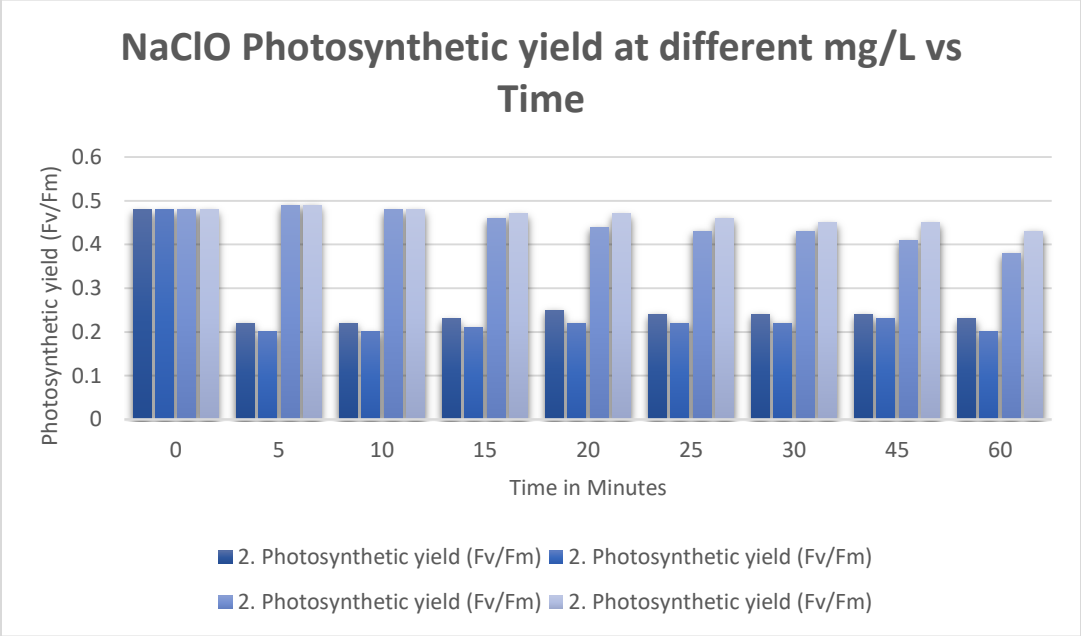

Figure 3:**Photosynthetic yield at different NaClO conc. Vs time**

SEM images of algae treated with NaClO (at 0.25 to 15mg/L) were analyzed. The algae kept their structure and morphology at 0.25, 5 and 15mg/L. But, at 10mg/L the cells were severely ruptured, figure 4. Microscope pictures show a reduction in the number of mobile algae cells on a field as the concentration increased, Figure 5.

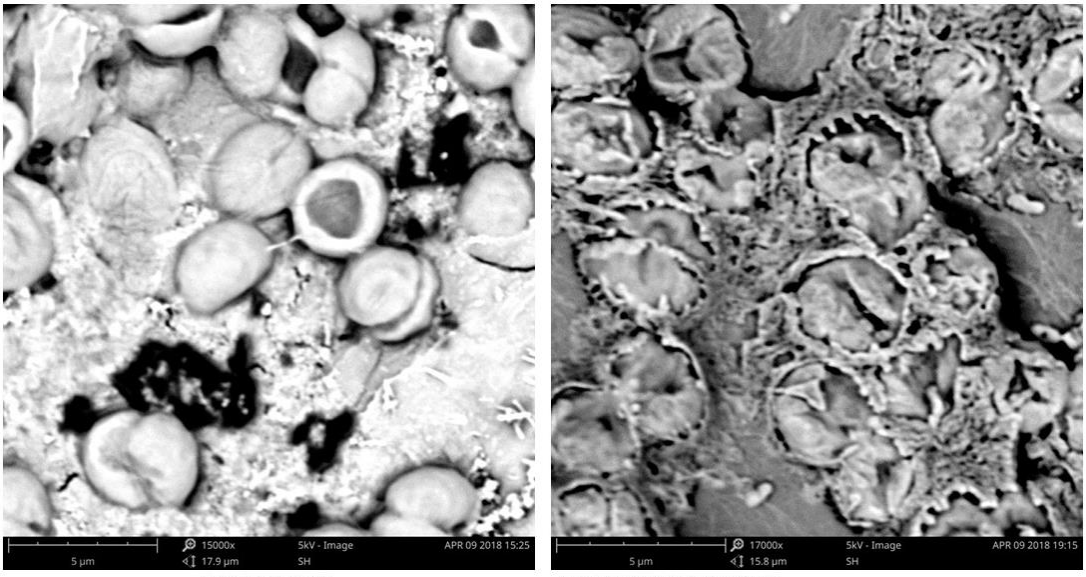

Figure 4:SEM pictures: A, blank sample; B, sample treated with NaclO 10mg/L

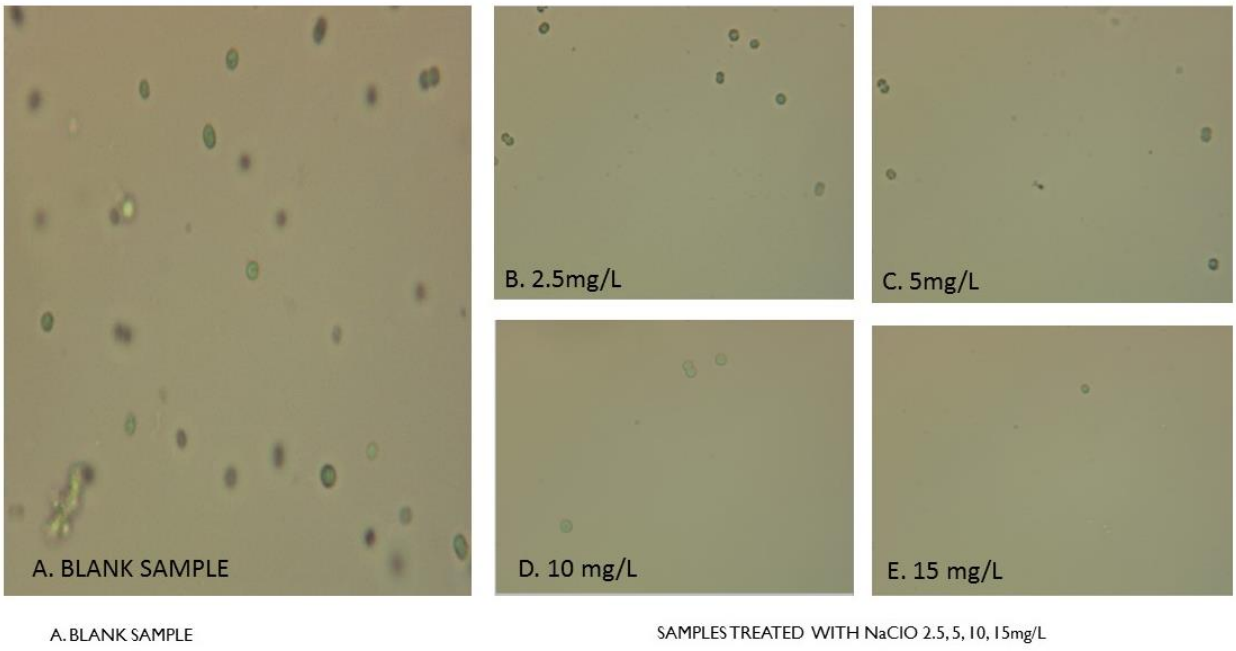

Figure 5: Microscope photos, 0-15mg/L from A-E

In this work the low concentrations of NaClO decreased the value of Biomass, Chlorophyll-a, and Photosynthetic yield, indicating that the preoxidation by NaClO can effectively inhibit growth of *Microcystis aeruginos*a. With increasing concentration and time, algae growth was inhibited as evident on the decreasing biomass and a corresponding decrease in chlorophyll-a content and photosynthetic yield. A possible phenomenon is that NaClO inhibited the algae by inducing oxidative stress and inhibiting cell recovery at higher concentrations (Ebenezer and Ki 2014, Ebenezer, Lim et al. 2014).

Some similar studies show that NaClO is indeed a fast, efficient and inexpensive approach to inhibit algae problems. Cyanobateria Akinetes 'dormant cells' (Kaplan-Levy, Hadas et al. 2010) were effectively treated using NaClO (Vaz, Bastos et al. 2014) , in another study beta-N-methyl amino-L-alanine (BMAA) a Cyanobacteria neurotoxin was degraded using NaClO (Cao and Xian 2016).

3.2 K_2FeO_4 Oxidation experiments

Several experiments were conducted to investigated the potential of K_2FeO_4 to inhibit algal growth. Figure 6 show the impact of K_2FeO_4 (at 0.25 to15mg/L) on Biomass (cell density of M. aeruginosa) after 1hour interaction. In the first 5mins there was evidence of biomass decrease across all the concentrations, proving that K_2FeO_4 is a strong oxidant and works fast. In the graph it can be observed that Biomass decrease improved with increasing time and concentration. The same trend, improving performance with increasing time and concentration, is seen in figure 7 for chlorophyll-a and figure 8 for photosynthetic yields, with best performance achieved at concentration 15mg/L.

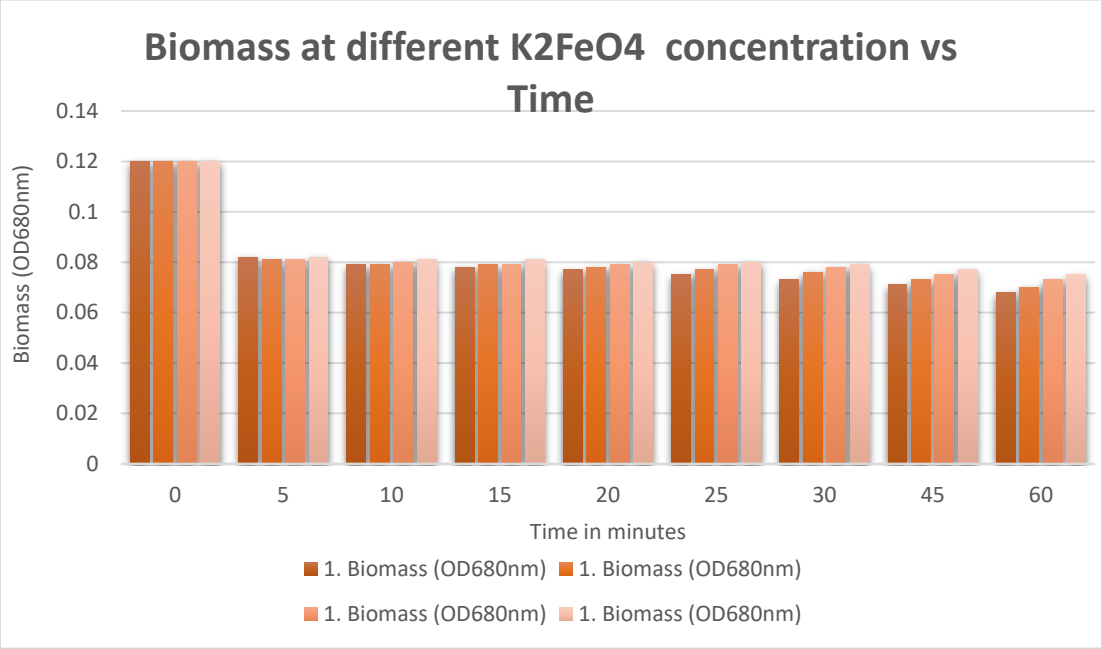

Figure 6: Biomass at different K_2FeO_4 conc. Vs time

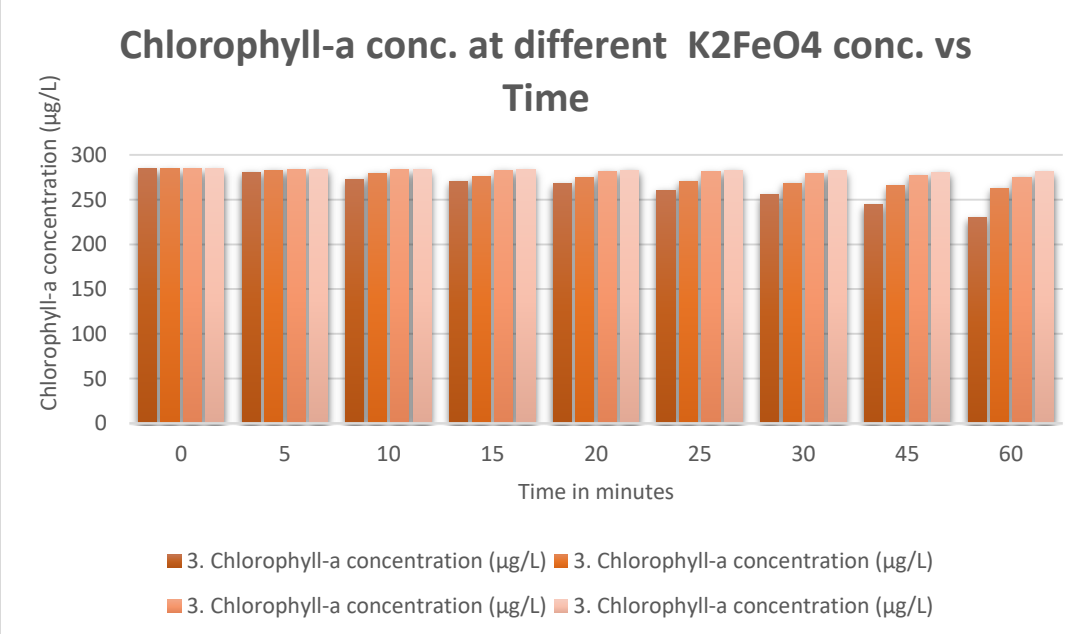

Figure 7: Chlorophyll-a at different K_2FeO_4 conc. Vs time

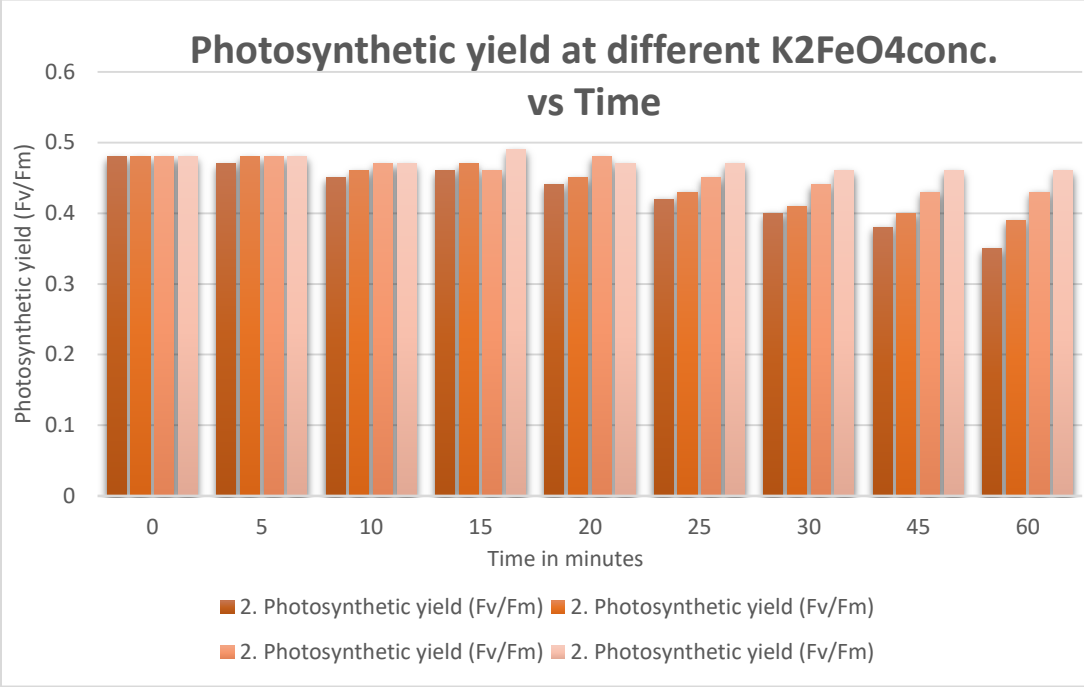

Figure 8: Photosynthetic yield at different K_2FeO_4 conc. Vs time

SEM images of algae treated with K_2FeO_4 (at 0.25 to 15mg/L) were also analyzed. The algae kept their structure and morphology at all these concentrations. Microscope pictures show a reduction in the number of mobile algae cells on a field as the concentration increased, similar to figure 5.

In this work the low concentrations of K2FeO4 decreased the value of Biomass, Chlorophyll-a, and Photosynthetic yield, indicating that the oxidation of K_2FeO_4 can effectively inhibit growth of *Microcystis aeruginos*a. With increasing K_2FeO_4 concentration and time, algae growth was inhibited as evident on the decreasing biomass and a corresponding decrease in chlorophyll-a content and photosynthetic yield. A possible phenomenon for this is that, treating with K_2FeO_4 caused oxidative stress on the algal cells which lead to an increase in membrane permeability and eventually death of *Microcystis aeruginos*a (Liu, Li et al. 2011)

Similar studies agree that a quick inactivation of Microcystis aeruginosa was achieved after K_2FeO_4 oxidation with no obvious cell lysis at concentrations up to K_2FeO_4 7mg/L (Zhou, Shao et al. 2014), chlorophyll-a and photosynthetic yield decreased with increase of K_2FeO_4 **(Liu, Li et al. 2011).**

3.3 $KMnO_4$ Oxidation Experiments

Several experiments were conducted to investigated the potential of $KMnO_4$ to inhibit algal growth. Figure 9 show the impact of $KMnO_4$ (at 0.25 to15mg/L) on Biomass (cell density of M. aeruginosa) after 1hour interaction. In the first 5mins there was evidence of biomass decrease across all the concentrations, proving that $KMnO_4$ is a strong oxidant and works fast. In the graph it can be observed that Biomass decrease improved with

increasing time and concentration. The same trend, improving performance with increasing time and concentration, is seen in figure 12 for chlorophyll-a and figure 13 for photosynthetic yields, with best performance achieved at concentration 15mg/L.

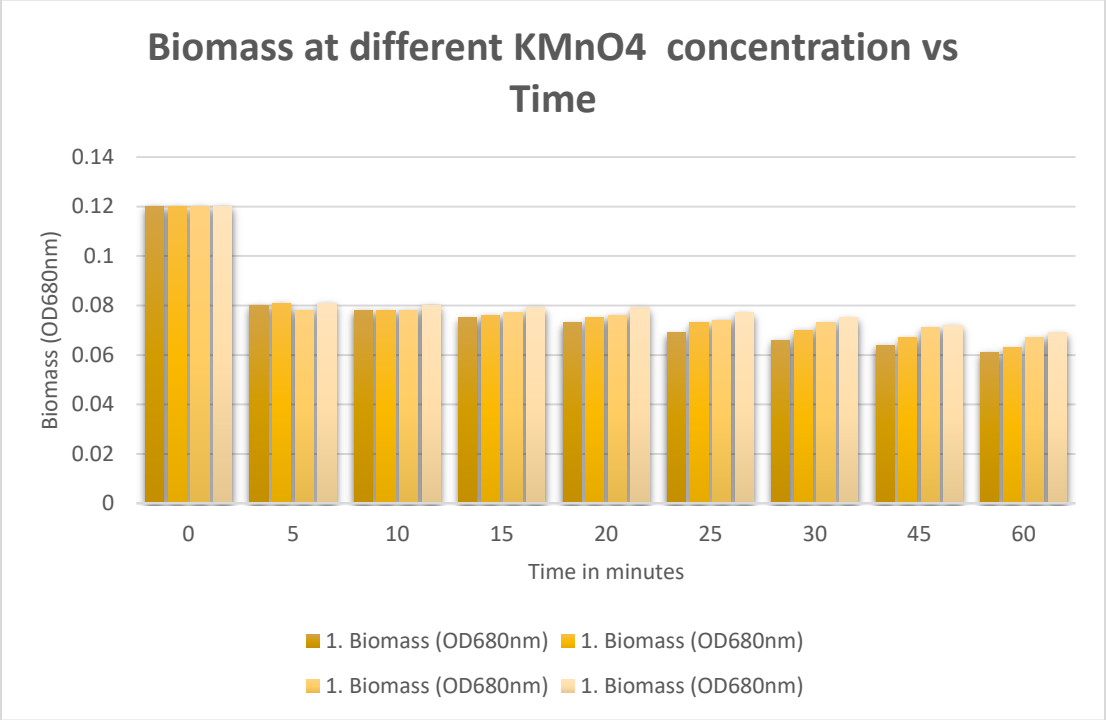

Figure 9: **Biomass at different $KMnO_4$ concentration vs Time**

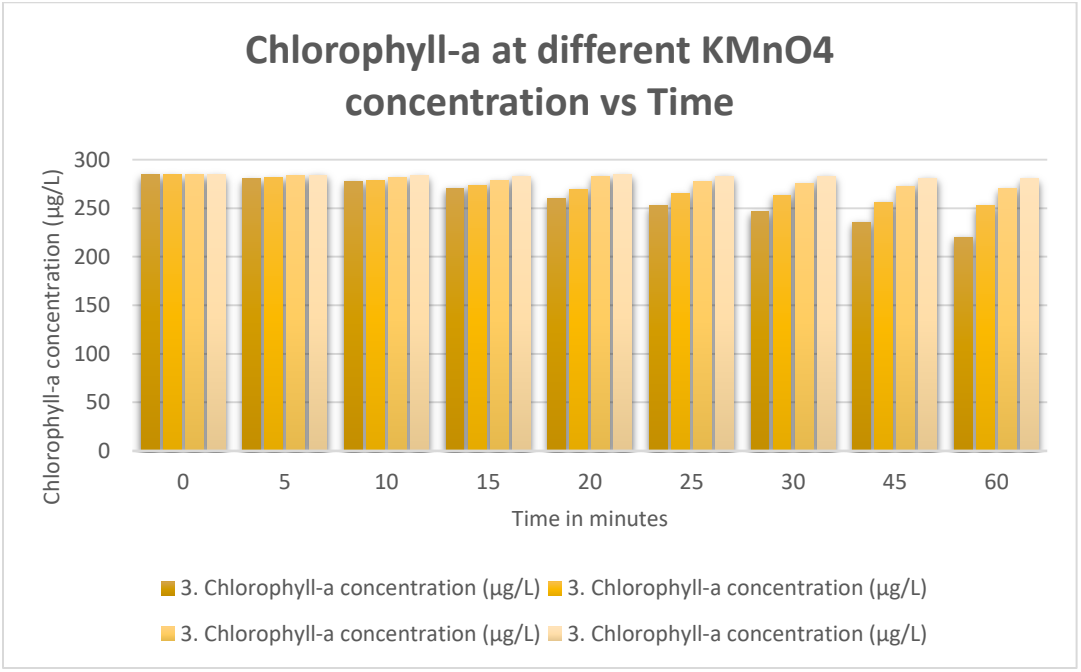

Figure 10: ***Chlorophyll-a at different $KMnO_4$ concentration vs Time***

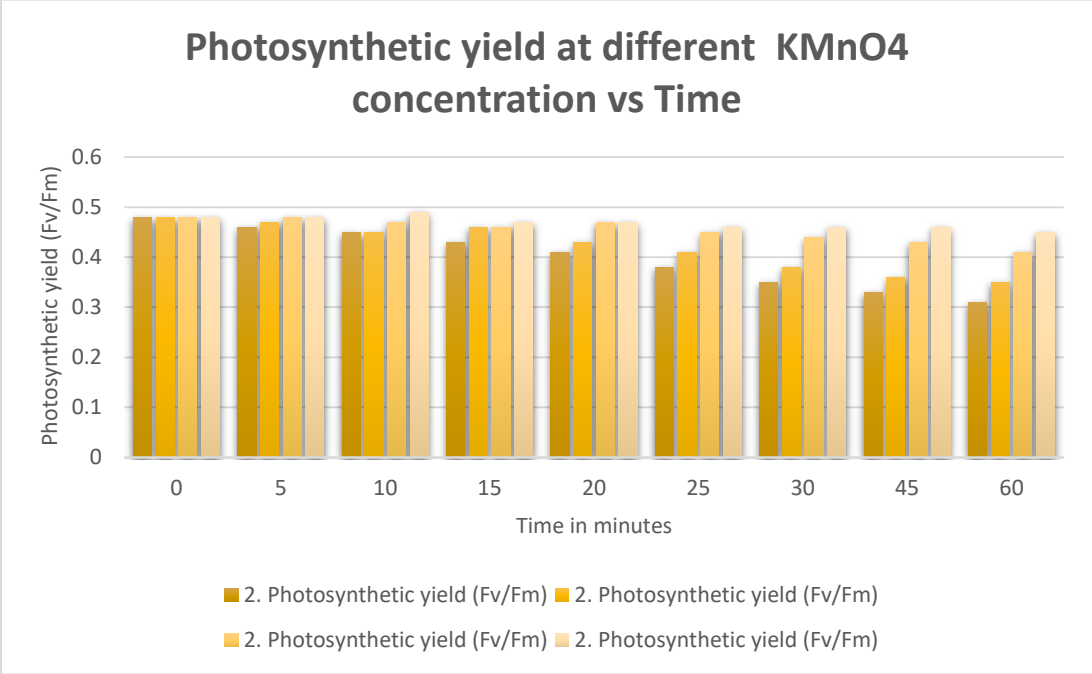

Figure 11: Photosynthetic yield at different $KMnO_4$ concentration vs Time

SEM images of algae treated with $KMnO_4$ (at 0.25 to 15mg/L) were also analyzed. The algae kept their structure and morphology at all these concentrations. Microscope pictures show a reduction in the number of mobile algae cells on a field as the concentration increased, similar to figure 5.

In this work the low concentrations of $KMnO_4$ decreased the value of Biomass, Chlorophyll-a, and Photosynthetic yield, indicating that the oxidation of $KMnO_4$ can effectively inhibit growth of *Microcystis aeruginos*a. With increasing $KMnO_4$ concentration and time, algae growth was inhibited as evident on the decreasing biomass and a corresponding decrease in chlorophyll-a content and photosynthetic yield. Reduction of these parameters reflected a compromise of the photosynthetic capacity, a phenomenon likely due to the suppression of photosynthesis primary reactions in *M. aeruginosa* cells. (Ou, Gao et al. 2012)

Similar studies agree that biomass and photosynthetic parameters were reduced to different degrees after various dosages $KMnO_4$ oxidation (2–20 mg/L) (Ou, Gao et al. 2012). However, the integrity of *M. aeruginosa* cells is compromised with increasing time and concentrations of $KMnO_4$ oxidation, this would eventually lead to cell lyses and release of toxins in the water (Wang, Qiao et al. 2013). More studies need to be done to determine the optimum concentration of $KMnO_4$ that will inhibit algae growth yet keep the relative integrity of algal cell-wall to maintain the integrity of the cell-walls to avoid release of algal derivatives; toxins, AOM and taste and odor compounds.

4. Comparison of NaClO, K_2FeO_4 and $KMnO_4$ algae pretreatment technologies

4.1 Performance on Algae Growth Inhibition

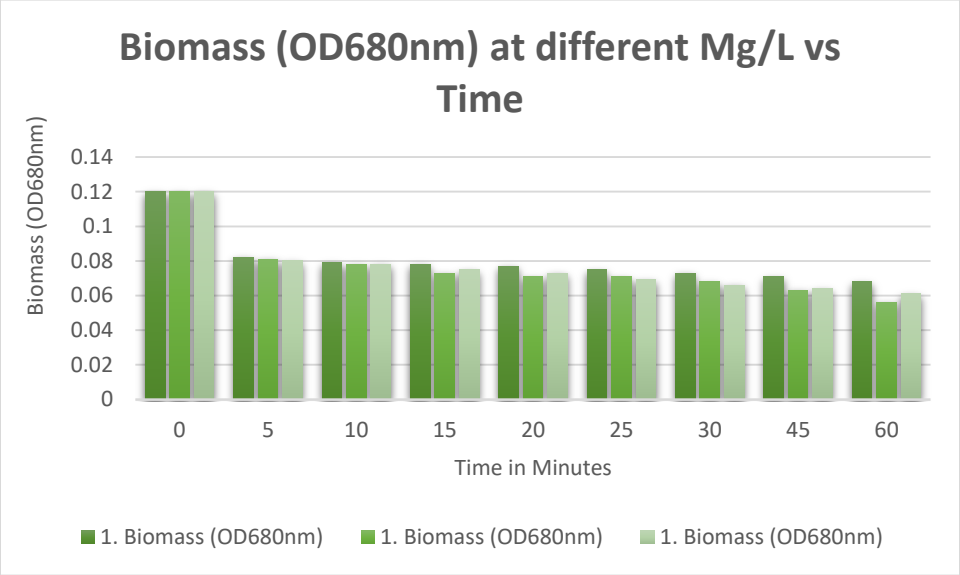

Figure 12: **The three oxidants compared; Biomass**

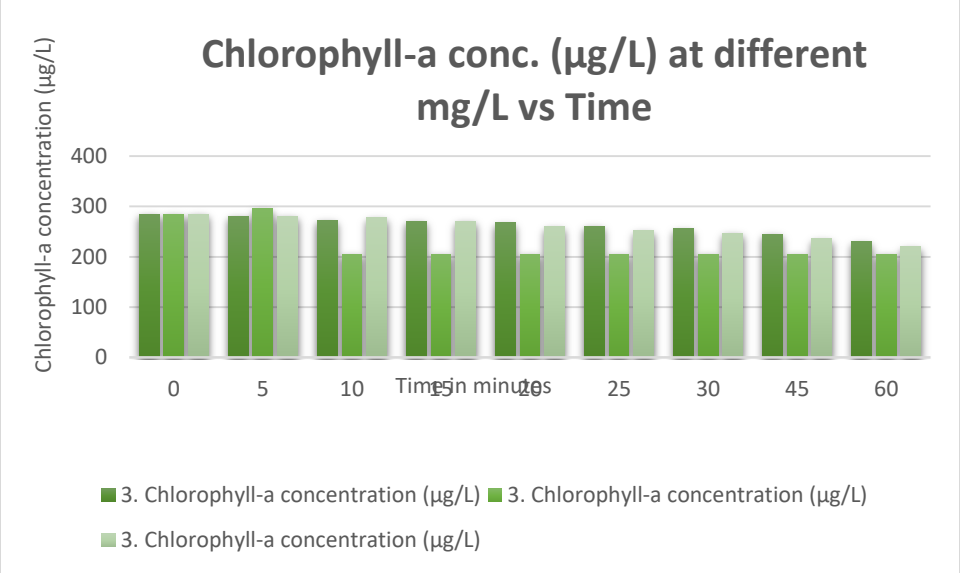

Figure 13: The Three Chemical Compared; Chlorophyll-a

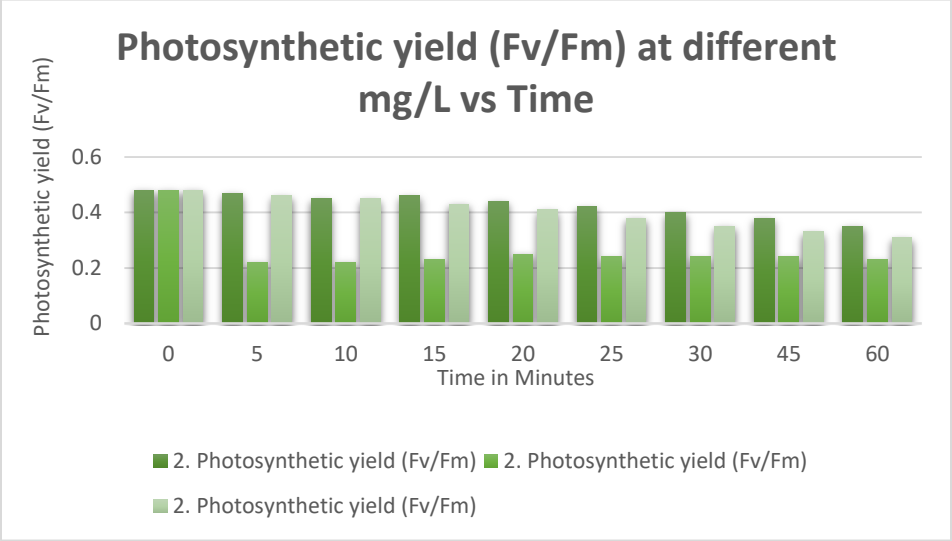

Figure 14: The Three Chemicals Compared; Photosynthetic yield

The figures above show that NaClO concentrations performed the best in reducing the biomass, photosynthetic yield and chlorophyll-a concentrations. These results show that second best in performance and third best for reducing Biomass, photosynthetic yield and chlorophyll-a are KMnO4 and K2FeO4 respectively.

SEM pictures results show that K2FeO4 performed the best in maintaining the cell-wall integrity, second best is KMnO4 and lastly NaClO. This is summarized in figure below.

The discussions of the phenomenon about these technologies have been discussed in section 3 above.

The Performance of three pretreatment technologies compared

Best Performance	NaClO	K_2FeO_4	$KMnO_4$
mg/L vs time	✓✓✓	✓	✓✓
Biomass (OD680nm)	✓✓✓	✓	✓✓
Photosynthetic yield (Fv/Fm)	✓✓✓	✓	✓✓
Chlorophyll-a conc. (µg/L)	✓✓✓	✓	✓✓
SEM (cell integrity)	✓	✓✓✓	✓✓

Performance : NaClO > $KMnO_4$ > K_2FeO_4

Figure 15: The Three Chemicals Compared; Performance

4.2 Capital costs

To do a complete Cost Analysis on a water treatment technology, study is needed to find out:

- the capital cost,
- operation costs: the energy demand, sludge production, and chemical consumption
- Effluent quality evaluation, kg pollution units/d
- Net present value calculations. (capital + operational costs) (Verrecht, Maere et al. 2010)

In this paper, the scope of our lab experiment allows us to do only Capital costs of the three pretreatment technologies.

To evaluate the capital investment costs, pricing information was obtained from suppliers, as shown in table below.

Chemical	Price/Ton (USD)	Reference
NaClO	200-250	Supplier
K2FeO4	10-50	Supplier
KMnO4	985 - 1300	Supplier

Table 1: Capital cost of the three chemical

Figure 16: The Three Chemicals Compared; Capital Cost

Chapter 5: Conclusion and Recommendation

Several experiments were done to compare and identify the best performance among three pretreatment technologies for algae inhibition at the DWTP. In the previous chapter, all the three (NaClO, KMnO4, K2FeO4) have proved to be effective to inhibit algae growth, however there is need to identify the best performer and also the most cost effective for the purposes of making it easier for decision makers when they consider which algae pre-treatment technologies to use at the DWTP.

The results show that NaClO performed the best to inactivate Microcystis aeuriginosa and it also has the best price among the three. However, the results also show that cell wall integrity was most compromised with increasing concentrations of NaClO as evident by SEM pictures showing severe cell lyses at NaClO 10mg/L. cell lyses will release harmful algal derivatives in the water that will compromise the quality of the treated drinking water. More studies is require

The results of this study show that KMnO4 can inactivate M. aeruginosa and its price is comparatively better than that of K2FeO4. KMnO4 showed better performance than NaClO to maintain cell wall integrity as seen in SEM pictures. However, with increasing KMnO4 concentration the color gets more purple and this may affect the aesthetic acceptability of the treated drinking water. Mn is a heavy metal and increased concentrations of KMnO4 may cause high residual manganese in the treated drinking water and sludges (Naceradska, Pivokonsky et al. 2017).

The results show that K2FeO4 can inactivate M. aeruginosa, even though its performance was not better than the other two pretreatment technologies. The price of K2FeO4 is also the most effective among the two. SEM results however show that it was the best in maintain algae cell wall integrity. K2FeO4 has also been generally termed by other studies as the “environmental friendly

oxidant" because it decomposes into non-toxic Fe (111) ions or ferric hydroxide, which at the same time is a coagulant of water treatment (Zhou, Shao et al. 2014, Liu, Tang et al. 2017).

This experiment had some limitations and as such can be improved upon in the future to properly identify the dynamics and kinetics of M. aeruginosa inhibition. Environmental factors like water temperature and pH ought to have been considered. In the results, other parameters

The findings of this study point out important factors that decision makers need to consider in choosing a pretreatment technology for algae treatment. Performance, Cost and Environmental safeness. When considering the performance, the emphasis should not only be on the best concentration/best time, but also close attention to prevent cell lyses that would release harmful algal derivatives in the water. Other than the performance, a thorough cost analysis covering the capital cost, operation cost, and effluent quality evaluation.

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REFERENCES

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REFERENCES

1. Brooks, B. W., et al. (2016). "Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems?" Environmental toxicology and chemistry **35**(1): 6-13.

2. Cao, Y. and Q. Xian (2016). "Chlorination degradation of neurotoxin beta-N-methylamino-L-alanine." Environmental Chemistry **35**(2): 267-272.

3. Douterelo, I., et al. (2014). "Methodological approaches for studying the microbial ecology of drinking water distribution systems." Water Research **65**: 134-156.

4. Ebenezer, V. and J. S. Ki (2014). "Biocide sodium hypochlorite decreases pigment production and induces oxidative damage in the harmful dinoflagellate Cochlodinium polykrikoides." Algae **29**(4): 311-319.

5. Ebenezer, V., et al. (2014). "Effects of the algicides CuSO4 and NaOCl on various physiological parameters in the harmful dinoflagellate Cochlodinium polykrikoides." Journal of Applied Phycology **26**(6): 2357-2365.

6. Goslan, E. H., et al. (2017). "Carbonaceous and nitrogenous disinfection by-product formation from algal organic matter." Chemosphere **170**: 1-9.

7. He, X., et al. (2016). "Toxic cyanobacteria and drinking water: Impacts, detection, and treatment." Harmful Algae **54**: 174-193.

8. Joh, G., et al. (2011). "Problematic algae in the sedimentation and filtration process of water treatment plants." Journal of Water Supply Research and Technology-Aqua **60**(4): 219-230.

9. Problematic algae, species that do not settle or are not easily removed by water treatment processes, are common in many water treatment plants (WTPs). We recorded algae in the water that overflowed from sedimentation basins (SBs) to filtration basins of WTPs in South Korea. Diatoms were common and other algae were not discernible in the flocs. Our field observation indicated that long and needle-shaped algae were less likely to settle and more likely to be present in overflow water. Many diatom cells or colonies that were extremely deformed from spherical had high overflow rates. Another long alga, Phormidium sp. (Cyanobacteria), originated from periphytic biofilms attached to SB walls. Algae that form long cells or colonies are less compact and less likely to settle as poor flocs. Species that overflowed the basin also clogged the sand filters, leading to a need for repeated backwashing, thus limiting the production of clean water. Species that clogged the sand filters included the needle-shaped diatom Synedra acus and the discoid diatom Stephanodiscus hantzschii f. tenuis. We also observed two cases where S. acus clogged WTP filters, requiring frequent backwashing that resulted in reduced production of drinking water and economic loss.

10. Kaplan-Levy, R. N., et al. (2010). Akinetes: Dormant Cells of Cyanobacteria. Dormancy and Resistance in Harsh Environments. E. Lubzens, J. Cerda and M. Clark. Berlin, Heidelberg, Springer Berlin Heidelberg**:** 5-27.

11. Cyanobacteria are an ancient and morphologically diverse group of photosynthetic prokaryotes, which were the first to evolve oxygenic photosynthesis. Cyanobacteria are widely distributed in diversed environments. In the case of members of the orders Nostocales and Stigonematales, their persistence and success were attributed to their ability to form specialized cells: heterocysts, capable of fixing atmospheric nitrogen and spore-like cells, the akinetes. This review focuses on akinetes of Nostocales, emphasizing environmental triggers and cellular responses involved in differentiation, maturation, dormancy, and germination of these resting cells. Morphological and structural changes, variation in akinete composition, and metabolism are summarized. Special attention is given to the genetic regulation of the differentiation process in an attempt to

close gaps in our understanding of the dormancy phenomenon in cyanobacteria and to identify open questions for future research.

12. Lebedev, A. T., et al. (2004). "GC–MS comparison of the behavior of chlorine and sodium hypochlorite towards organic compounds dissolved in water." Water Research **38**(17): 3713-3718.

13. Liang, W. Y., et al. (2005). "Inactivation of Microcystis aeruginosa by continuous electrochemical cycling process in tube using Ti/RuO2 electrodes." Environmental Science & Technology **39**(12): 4633-4639.

14. Liu, L., et al. (2011). Physiological responses to ferrate (VI) stress in Microcystis aeruginosa.

15. Liu, S. Y., et al. (2017). "Parameters influencing elimination of geosmin and 2-methylisoborneol by K2FeO4." Separation and Purification Technology **182**: 128-133.

16. Ma, M., et al. (2012). "Chlorination of Microcystis aeruginosa suspension: Cell lysis, toxin release and degradation." Journal of Hazardous Materials **217**: 279-285.

17. Maxwell, K. and G. N. Johnson (2000). "Chlorophyll fluorescence - a practical guide." Journal of Experimental Botany **51**(345): 659-668.

18. Naceradska, J., et al. (2017). "The impact of pre-oxidation with potassium permanganate on cyanobacterial organic matter removal by coagulation." Water Research **114**: 42-49.

19. Ou, H., et al. (2012). "Immediate and long-term impacts of potassium permanganate on photosynthetic activity, survival and microcystin-LR release risk of Microcystis aeruginosa." Journal of Hazardous Materials **219-220**: 267-275.

20. Ou, H., et al. (2012). "Immediate and long-term impacts of potassium permanganate on photosynthetic activity, survival and microcystin-LR release risk of Microcystis aeruginosa." Journal of Hazardous Materials **219**: 267-275.

21. Qi, J., et al. (2016). "Prechlorination of algae-laden water: The effects of transportation time on cell integrity, algal organic matter release, and chlorinated disinfection byproduct formation." Water Research **102**: 221-228.

22. Sinikova, N. A., et al. (2014). "Comparison of chlorine and sodium hypochlorite activity in the chlorination of structural fragments of humic substances in water using GC-MS." Journal of Analytical Chemistry **69**(14): 1300-1306.

23. Tang, Y. L., et al. (2015). "Combined effects of graphene oxide and Cd on the photosynthetic capacity and survival of Microcystis aeruginosa." Science of The Total Environment **532**: 154-161.

24. Tian, C., et al. (2013). "Seasonal dynamics of phytoplankton and its relationship with the environmental factors in Dongping Lake, China." Environmental Monitoring and Assessment **185**(3): 2627-2645.

25. Vaz, M., et al. (2014). "Use of sodium hypochlorite solutions to obtain axenic cultures of Nostoc strains (Cyanobacteria)." Brazilian Journal of Botany **37**(2): 115-120.

26. Verrecht, B., et al. (2010). "The cost of a large-scale hollow fibre MBR." Water Research **44**(18): 5274-5283.

27. Wang, L., et al. (2013). "Pre-oxidation with KMnO4 changes extra-cellular organic matter's secretion characteristics to improve algal removal by coagulation with a low dosage of polyaluminium chloride." Journal of Environmental Sciences **25**(3): 452-459.

28. Weirich, C. A. and T. R. Miller (2014). "Freshwater Harmful Algal Blooms: Toxins and Children's Health." Current Problems in Pediatric and Adolescent Health Care **44**(1): 2-24.

29. Zhai, H. Y., et al. (2017). "Disinfection byproduct formation in drinking water sources: A case study of Yuqiao reservoir." Chemosphere **181**: 224-231.

30. Zhou, S. Q., et al. (2014). "Removal of Microcystis aeruginosa by potassium ferrate (VI): Impacts on cells integrity, intracellular organic matter release and disinfection by-products formation." Chemical Engineering Journal **251**: 304-309.