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SUPPRESSION OF *APHANIZOMENON FLOS-AQUAE* BY SOLID POLYMER ELECTRODE (SPE) MEMBRANE ELECTROLYSIS WITH AERATED SYSTEMS

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ABSTRAK

Alga biru-hijau terutama sianobakteria tahan-suhu tinggi membawa ancaman kepada kualiti air minum bukan di sahaja di kawasan tropika tetapi juga pada musim panas di kawasan temperat. Sianobakteria berfilamen adalah yang paling sukar dirawat kerana kebanyakan teknologi rawatan mempunyai keberkesanan yang lebih rendah pada suhu panas. Elektrolisis, salah satu *advance oxidative technology* (AOT), menunjukkan potensi yang tinggi dalam merawat spesis yang persisten. Namun, kebanyakan elektrolisis direka untuk airsisa yang sangat tercemar. Dalam kajian ini, teknologi *solid polymer electrolysis (SPE)* telah diuji untuk penindasan sianobakteria air tawar *Aphanizomenon flos-aquae.* Eksperimen kelompok 4-hari menunjukkan bahawa elektrolisis telah menindas *Aphanizomenon* dengan berkesan pada 35°C tanpa sebarang pengurangan dalam keberkesanan berbanding dengan pada 25°C. Bukti penindasan adalah (i) kepadatan sel sianobakteria yang berkurang (ii) pemalar kadar penindasan (*k*) - 0.1269 $\pm$ 0.0805 hari^{-1} pada 35°C dan -0.1486 $\pm$ 0.0592 hari^{-1} pada 25°C, (iii) pelunturan warna media dan (iv) pengurangan panjang filamen ($p<0.05$). Sistem pengudaraan menyebabkan pertumbuhan sianobakteria berfilamen yang signifikan, dibuktikan oleh peningkatan kepadatan sel, kadar pertumbuhan yang lebih tinggi, warna media yang lebih kuat dan panjang filamen yang bertambah. Oleh itu, pengudaraan adalah

teknologi yang kurang digalakkan untuk penindasan sianobakteria persisten yang tahan-suhu tinggi manakala elektrolisis SPE menindas *Aphanizomenon flos-aquae* dengan berkesan.

ABSTRACT

Algal blooms mainly heat-tolerant cyanobacteria pose a threat to drinking water quality not only in tropical regions but also during summer in temperate regions. The filamentous cyanobacteria are the most challenging to remediate as many technologies have reduced efficiencies in warm temperatures. Electrolysis, an advance oxidative technology (AOTs), shows high potentials in remediating persistent species. However, most electrolysis systems have been designed for highly polluted wastewaters. In this study, a solid polymer electrolysis (SPE) technology was tested for the suppression of freshwater cyanobacteria *Aphanizomenon flos-aquae.* The 4-day batch scale experiments revealed that electrolysis significantly suppressed *Aphanizomenon* successfully at 35°C without reduced efficiency compared to at 25°C. The evidence of suppression was indicated by (i) reduced cyanobacterial cell density, (ii) suppression rate constant (*k*) of - 0.1269 $\pm$ 0.0805 day^{-1} at 35°C and -0.1486 $\pm$ 0.0592 day^{-1} at 25°C, (iii) discolouration of media and (iv) reduction in filament length ($p<0.05$). Aerated systems instead significantly enhanced the proliferation of the filamentous cyanobacteria, evidenced by increased cell density, higher growth rate, more intense media colour and elongated filament lengths. Hence aeration is a less recommended technology for suppression of persistent heat-tolerant cyanobacteria while the SPE electrolysis effectively suppresses *Aphanizomenon flos-aquae*.

Keywords*: advance oxidation, aeration, cyanobacteria, degradation, electrolysis*

1.0 INTRODUCTION

Algal blooms, especially from cyanobacteria such as *Aphanizomenon, Cylindrospermopsis, Anabaena, Nodularia* and *Microcystis,* have caused deterioration in water quality and public health risk worldwide. The rising water temperatures enable the proliferation of persistent heat-tolerant species that are challenging to remediate. These hazardous species are reported to cause adverse effects in the development, immunological, neurological, respiratory and reproductive systems of aquatic species, farm animals, birds and shellfish and humans by release of cyanotoxins. Cyanobacteria in reservoirs and lakes led to hypoxic waters resulting in large scale death in aquatic species subsequently causing loss of biodiversity. Economic losses occur from reduced tourism and recreational activities such as fishing, boating and water sports. Infrastructures of water treatment facilities have been damaged from clogging by blooms, consequently reducing the cost-efficiency of water treatment [1-3].

Bloom remediation technologies applied in lakes and reservoirs such as nutrient inactivation, biomanipulation and biological degradation, are not only resource-intensive and time-

consuming, but also inappropriate as water constituents occur as a cocktail in the water as well as due to the complicated food-web interactions in the lakes and reservoir [2, 3]. Many advance technologies at the water treatment plants (WTPs) are expensive, time-consuming and less effective. Coagulation, oxidation, filtration and activated carbon methods may suppress some algae and cyanobacteria but not the filamentous *Aphanizomenon* [1]. Membrane separation/filtration, photocatalytic oxidation and UV irradiation more precisely suppress taste and odour metabolites of algae that have a lower molecular weight [4]. Advance oxidation technologies (AOTs) such as Fenton, ozone and chlorine dioxide are disadvantageous as these introduce chemicals which generate harmful by-products [4, 5].

Electrolysis, had been explored for removal of various pollutants such as bacteria, organics, nutrients, pesticides, and persistent organic pollutants (POPs) from water, wastewater and seawaters. However, electrolysis systems were mainly tested for removal of common unicellular algae such as cyanobacteria *Microcystis aeruginosa*; and in highly polluted wastewaters, less for freshwaters. Membrane-based electrolysis were recently preferred over more common liquid-based electrolysis as they are more rapid, efficient and advantageous. Membrane electrolysis are mechanically stable, enable the use of better current densities, have higher ionic conductivity, better gas impermeability and absent of secondary pollution [6-17].

In the current study, a novel solid polymer electrolyte (SPE) electrolysis named Oxygen Productive Electrode (OPE) was tested for suppression of *Aphanizomenon flos-aquae*, a freshwater filamentous cyanobacteria. This species was found dominant with a cell density of 6.6 x 10^5 cells mL^{-1} in a tropical reservoir and at the pre-aeration tank of a water treatment plant in Malaysia [18]. As water temperatures reach a maximum of 35°C in Malaysia [19] and growth rate of *Aphanizomenon flos-aquae, A. ovalisporum* and *A. gracile* is maximum at 32 to 35°C, the suppression rate of *Aphanizomenon flos-aquae* was evaluated at 35°C in comparison with room temperature of 25°C [2, 20, 21]. The efficiency of OPE in suppressing *Aphanizomenon flos-aquae* was compared to aerated systems, widely used for algal remediation and lake and reservoir restoration [3, 22, 23].

2.0 METHODOLOGY

2.1 Electrolysis device (OPE)

The OPE consists of a Nafion® 117 solid polymer electrode (SPE) membrane (E.I. DuPont de Nemours & Co, U.S.) sandwiched in between a Ti/Pt anode and a stainles steel cathode (Figure 1). It was developed by the University of Tsukuba and Ishizaki Corporation, Japan; modified by Seto Engineering Co. Ltd. (Chiba, Japan); and currently available from Imai Seisakusho Co. Ltd. (Ibaraki, Japan). In the previous studies, OPE used Ti/Pt for both anode and cathode [5]. The titanium anode was coated with platinum (Ti/Pt), while stainless steel

was used as cathode in order to lower the cost of OPE device. Platinum and stainless were chosen for their ability to produce highest oxygen mass transfer coefficient (K_La) similar to titanium [24]. Mesh-shape electrodes, able produce a higher K_La was used instead of the previous 15-hole 16 mm round [5, 24]. Nafion® SPE membrane was selected for its higher mechanical stability, ionic conductivity and gas impermeability compared to many other membranes [5, 13, 25].

2.2 Experimental set-up

Laboratory-scale batch experiments were conducted in a large environmental incubator (Koitotron, Koito, Japan) with temperature maintained at a good precision of $\pm$ 2°C. Light intensity of 25 to 35 µmol photon $m^{-2}s^{-1}$ at a 12-hour light / 12-hour dark (12:12 LD) cycle, was monitored using Li-189 photometer (Licor, Japan) throughout all experiments. The OPE was operated using a PMC18-5A DC power supply unit (0 to 18V/0 to 5A) (Kikusui Electronics, Japan) to provide optimum current value of 0.06 A and a resulting current density of 40 mA cm^{-2} [5]. Bebicon Oil-Free air compressor (Hitachi, Japan) was used to generate compressed air for the air aeration system, while oxygen gas supply tank (99.99%) was used for the oxygen aeration. Each of the aeration systems had flow regulators to control the air/oxygen supply diffused through fine bubble diffuser sized 1.5 cm diameter.

Value of K_La of OPE was determined prior to suppression experiments. Nitrogen gas was supplied at a flow rate of 100 mL min^{-1} to the 1.5 L Milli-Q water in a 2 L beaker, to displace its DO concentration to near zero. OPE was placed into the beaker and DO determined at an interval of 5 minutes using a ProODO™ optical DO meter (YSI Nanotech, Japan). Continuous stirring was done at optimum 500 rpm to prevent fast dissolution of DO into water-air interface by excessive turbulence, while preventing settling of cyanobacteria which could reduce contact between cyanobacteria and the active species from the OPE [6]. DO values were measured at the first 30-minute using the formula shown in Equation 1.

$$K_La = \frac{2.303}{t_2 - t_1} \times log\ log\ \frac{(C_s - C_1)}{(C_s - C_2)} \qquad \text{Equation 1}$$

where, K_La is oxygen mass transfer coefficient ($hour^{-1}$)

t_1 is start time 0 hour

t_2 is end time 0.5 hour

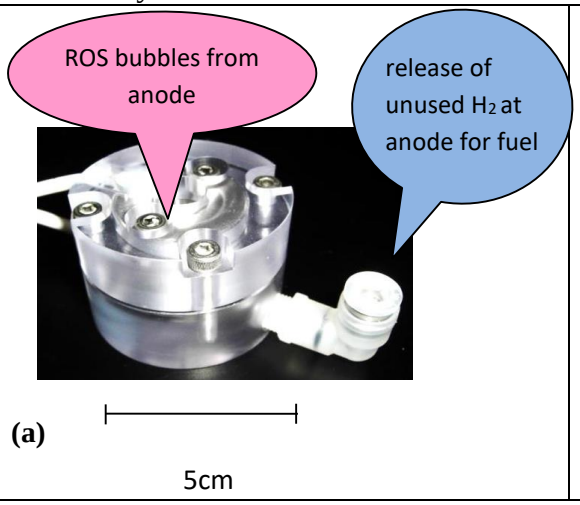
C_s is saturated DO concentration (mg L^{-1})

C_1 is initial DO concentration at start time 0 hour (mg L^{-1})

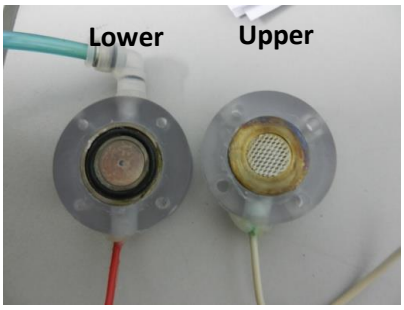
C_2 is DO concentration at end time 0.5 hour (mg L^{-1})

Rate of gas production from OPE was 0.6 mL min^{-1} and the K_La was 1.00 $hour^{-1}$. This K_La value was applied to aeration systems to ensure the transfer of oxygen was of equal strength with OPE. Each of the aeration systems were tested at various air flow rates. As a result, the flow rates of air aeration and oxygen aeration systems that achieved the K_La value of 1.00

hour^{-1} were 4.8 mL min^{-1} and 0.5 mL min^{-1}, respectively. K_La experiments were conducted four times for each unit of OPE, air aeration and oxygen aeration to ensure reproducibility and validity of the results.

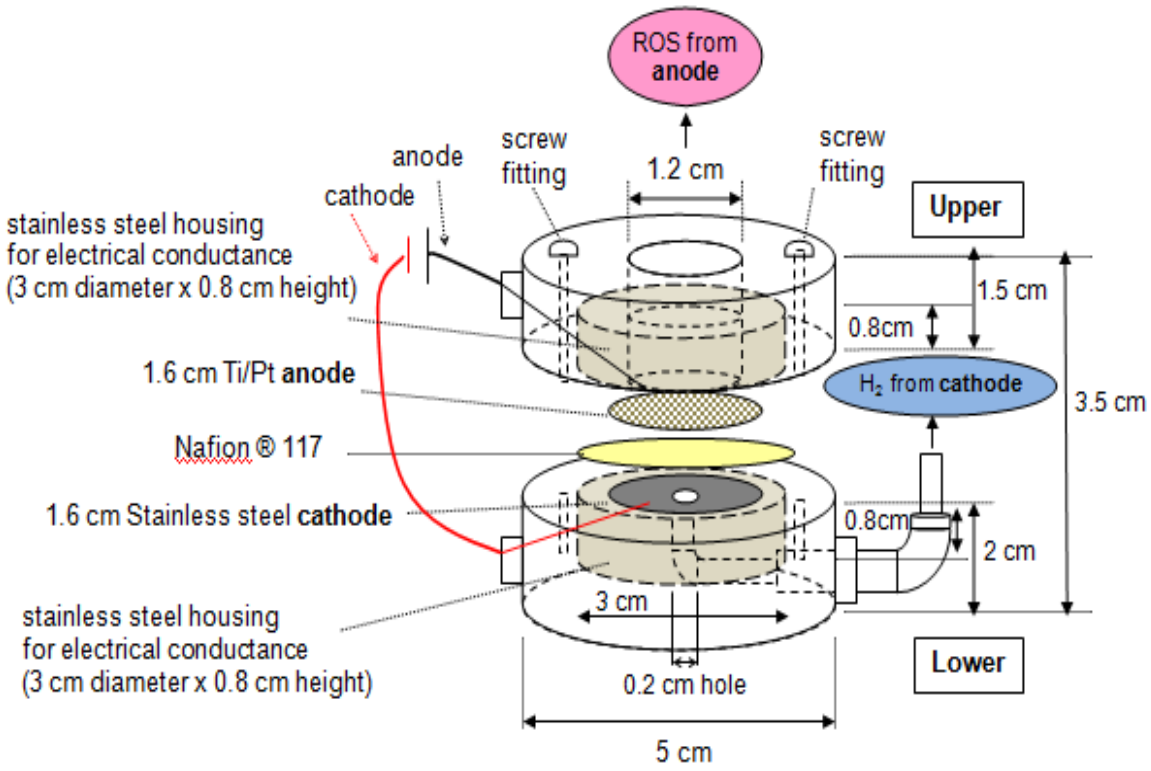


(a)



Inner view
(Right: Upper part housing the anode;
Left: Lower part housing the cathode)

(b)



(c)

Figure 1: OPE device (a) outer view (b) inner view (c) schematic diagram

2.3 *Aphanizomenon* Suppression Experiment at 25°C and 35°C

Aphanizomenon flos-aquae (unialgal, clonal and non-axenic NIES-1258; isolated from Lake Suigetsu, Fukui, Japan) (Figure 2), was obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. The NIES-1258 strain was cultivated in CT medium at 25 $\pm$ 2°C, 12:12 LD cycle and at a light intensity of 25 to 35 µmol photon $m^{-2}s^{-1}$ during the light phase, to achieve logarithmic phase of growth.

Figure 2: *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault microscope image (400 times magnification) using Olympus BX-250 inverted microscope with a coupled digital camera (CCD) and image analysis software (Olympus, Japan)

The 1.5 L CT medium was inoculated with 45 mL NIES-1258 culture and stirred at 500 rpm in 2L beakers to achieve initial cyanobacterial density of 8,000 to 9,000 filaments mL^{-1}. The 4-day batch experiment was conducted twice, each time with two units of each OPE, air aeration and oxygen aeration running concurrently at 25 $\pm$ 2°C temperature, 12:12 LD cycle and with a light intensity of 25 to 35 µmol photon $m^{-2}s^{-1}$ during light phase. A control, which did not have any remediation technology, was operated at the same conditions and initial cyanobacterial density. This 4-day experiment was also conducted at a temperature of 35 $\pm$ 2°C. Each set of experiment was conducted twice at each temperature to ensure reproducibility and validity of the data.

DO concentrations and pH were closely monitored within the first 24 hours and at regular intervals till the 4th day. All measurements were duplicated and averaged. For cyanobacteria cell density determination, 4.5 mL was sampled regularly and preserved with 0.5 mL of 25% glutaraldehyde to obtain a sample to preservative ratio of 1:9 v/v [26]. Samples were pipetted (300 µL) into the MPC-200 plastic plankton counter (Matsunami Glass, Japan) and allowed to settle for an hour prior to enumeration using BX-250 inverted microscope (Olympus, Japan). The Whipple grid was used to select a total of 40 fields to ensure a statistically significant number of fields were counted. The stage of the microscope was moved vertically to ensure all settled or buoyant cyanobacteria filaments were counted to minimize counting errors. Samples were taken three times and each sample was counted twice, obtaining six counts which were then averaged and accounted for dilution factor to produce cyanobacterial density values. Measurement of filament length was taken for 20 filaments of *Aphanizomenon flos-aquae* before and after each remediation. In addition, changes in colour were observed and compared upon remediation at both temperatures 25°C and 35°C.

The growth/suppression rate constant (k), also known as specific growth rate (SGR) was determined using a first-order rate expression based on trend-line of natural log calculations of cyanobacterial density, $ln\ ln\ C(t)$ plotted against time (t) for each 25ºC and 35°C. First order rate expression (Equation 2) was analytically integrated to produce Equations 3-5 [26, 27].

$$\frac{dC(t)}{dt} = k \cdot C(t) \qquad \text{Equation 2}$$

Equation 2 analytically integrated between t_0 and t; yielding;

$$ln\ ln\ C(t) - ln\ ln\ C(t_0) = k \cdot (t - t_0)\ \text{, or}$$

$$C(t) = ln\ C(t_0) + k \cdot (t - t_0) \qquad \text{Equation 3}$$

$$C(t) = C(t_0) \cdot e^{k \cdot (t - t_0)} \qquad \text{Equation 4}$$

$$k = \frac{ln\ C(t) - ln\ C(t_0)}{t - t_0} \qquad \text{Equation 5}$$

where

k is the growth / suppression rate constant (day $^{-1}$)
t_0 and t are the initial time and a time, respectively (day)
$C(t_0)$ and $C(t)$ are the densities of cyanobacteria observed at the initial time t_0 and at a time, t, respectively (filaments mL^{-1})

All data analysis was performed using Minitab version 16 (Kozo Keikaku Engineering, Japan).

3.0 RESULTS AND DISCUSSION

3.1 Suppression of *Aphanizomenon*

The initial drastic reduction for all OPE, air aeration, oxygen aeration and control within first 12 hours, was due to the adaptation phase of the species in the new medium. Hence the density for the first 24 hours was not used in the calculation of *k* value. Cyanobacteria was suppressed by OPE as indicated by the reduced density (Figure 3) and the negative *k* value (Table 1). In four days, *Aphanizomenon* sp. level dropped from 8,000 and 9,000 filaments mL^{-1} to 3,000 and 4,000 filaments mL^{-1} at 25°C, and from 7,000 and 7,500 filaments mL^{-1} to 3,500 and 4,500 filaments mL^{-1} at 35°C. The average of net *k* of cyanobacteria NIES-1258 were -0.1486 day^{-1} at 25ºC (n=4) and -0.1269 day^{-1} at 35°C (n=4). Statistical analysis using General Linear Model (GLM) for *k* values, showed that OPE significantly suppressed cyanobacteria, at both 25°C and 35°C (Table 2). An accuracy of *k* values was ensured from the regression equation which had strong regression values (R^2 = 0.9 to 1.0).

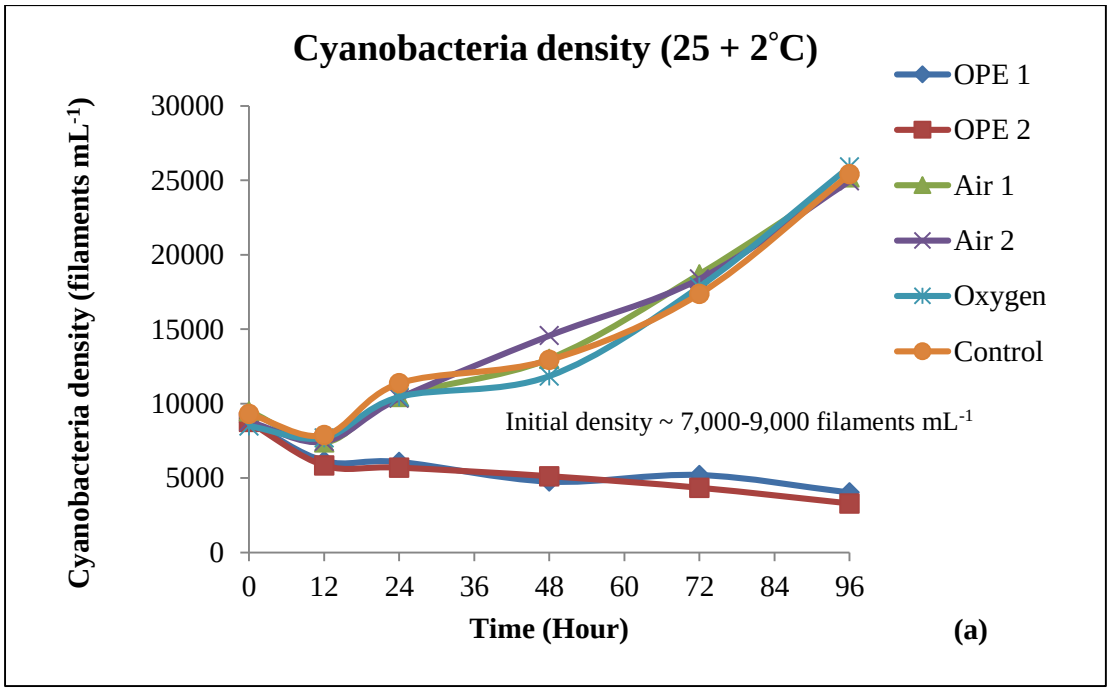
On the contrary, for both air and oxygen aeration, cyanobacterial density increased, proving the inability of these technologies to suppress cyanobacteria. The *k* values were slightly lower at 35°C than at 25°C for each OPE, air aeration, oxygen aeration and control. However, significance test demonstrated that the differences in *k* values at the two different temperatures were insignificant except for oxygen aeration (Table 1 and 2). Significant difference for oxygen aeration was a result of a more rapid air-supersaturation with oxygen at higher temperature (35°C) enabling early inhibition of photosynthesis and sinking of cyanobacteria through collapse of gas vesicles (when it is heavy with carbohydrates). This phenomenon led to a reduced growth rate of cyanobacteria [3].

Based on selection of two best OPE experiments with highest *k* values, the average *k* (n=2) was -0.21 day^{-1}. Based on previous studies, *k* value was 0.40 day^{-1} for *Phormidium tenue* [28]; 0.19 day^{-1} for *Dictyosphaerium subspicatus*, 0.99 day^{-1} for *Aphanizomenon smithii* and 0.95 day^{-1} for *Phormidium tenue* [29]; 0.76 for *Microcystis aeruginosa* [5] compared to 0.21 day^{-1} for *Aphanizomenon flos-aquae* from the current study. This shows that *Aphanizomenon flos-aquae* is indeed a species which is difficult to remediate, as seen in the lower suppression rate constant obtained compared to other species, except *Dictyosphaerium subspicatus* to which *k* value was similar.

3.2 DO Productions

There was an initial rise in DO for all technologies and control during the first 12 hours to air-supersaturation level, 8.2 mg L^{-1} at 25°C (1 atm) and 6.9 mg L^{-1} at 35°C (1 atm) due to dissolution of DO from atmosphere into the water at the water-air interface [26]. However, DO levels for only OPE and oxygen aeration continued to rise above the air-supersaturation

level, leading to a significantly higher net DO compared to air aeration and control at both temperatures (Figure 4, Table 3 and 4). Net DO was lower at 35°C, compared to 25°C, for all systems as a result of the lower air-saturation level at this temperature (6.9 mg L^{-1}), in other words, a more rapid air-supersaturation as well as a higher oxygen dissolution kinetics at higher temperature as explained by van't Hoff's rule. This led to faster inhibition of photosynthesis resulting in lower net DO at 35°C [30].



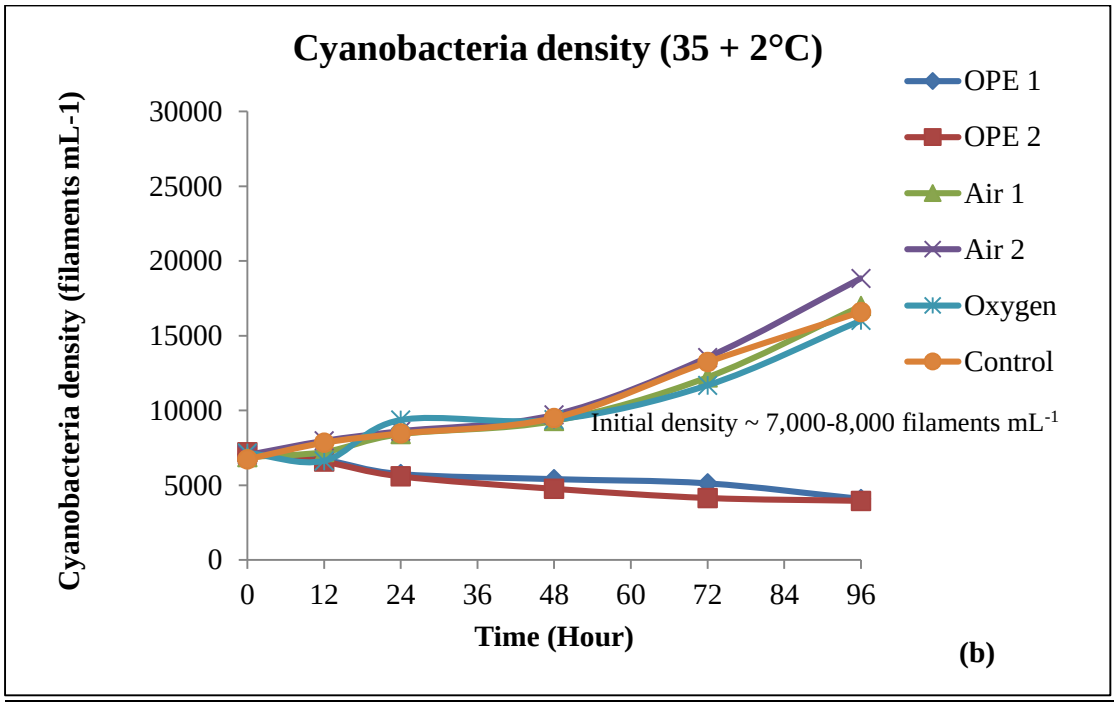


Figure 3: Cyanobacteria density (n=2) (a) 25 ^{O}C (b) 35 ^{O}C

Table 1: Comparison of *k* values of *Aphanizomenon* for the technologies at 25°C and 35°C

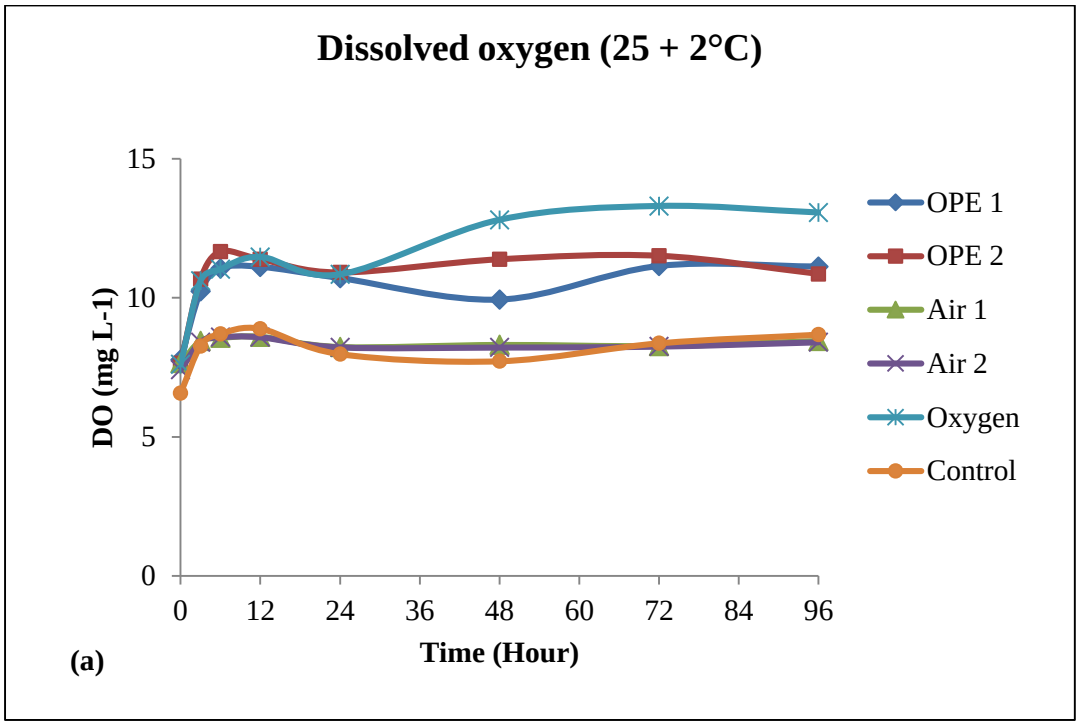
Technology	***k* ± SD* (day^{-1})**		**F-statistics ** (p value)**
	25 ± 2°C	**35 ± 2°C**	
OPE	-0.1486 ± 0.0592 (n=4)	-0.1269 ± 0.0805 (n=4)	0.01 (0.942)
Air aeration	0.2947 ± 0.0550 (n=4)	0.2616 ± 0.0883 (n=4)	1.63 (0.249)
Oxygen aeration	0.3060 ± 0.0972 (n=2)	0.1841 ± 0.0342 (n=2)	9.72 (0.021) ***
Control	0.2646 ± 0.0296 (n=2)	0.2423 ± 0.0935 (n=2)	1.12 (0.330)

* SD: standard deviation
** F-statistics from GLM to compare significance mean difference of *k* between 25°C and 35°C
*** indicating significance, $p < 0.05$

Table 2: Mean difference of *k* values between technologies at 25°C and 35°C

Between Technologies	**Mean difference (p value)***	
	25 ± 2°C	**35 ± 2°C**
OPE • **OPE** • **Air aeration** • **Oxygen aeration** • **Control**	1 -0.4544 (p=0.0002) ** 0.4148 (p=0.0004) ** -0.3922 (p=0.0006) **	1 -0.3884 (p=0.0000) ** 0.3110 (p=0.0003) ** -0.3692 (p=0.0001) **
Air aeration • **Air aeration** • **Oxygen aeration** • **Control**	1 -0.0621 (p=1.0000) -0.0395 (p=1.0000)	1 -0.0775 (p=0.9323) -0.0192 (p=1.0000)
Oxygen aeration • **Oxygen aeration** • **Control**	1 0.0226 (p=1.0000)	1 -0.0582 (p=1.0000)

* Mean difference of *k* from GLM
** indicating significance, $p < 0.05$


Dissolved oxygen (25 + 2°C)
DO (mg L-1)
15
10
5
0
0
12
24
36
48
60
72
84
96
Time (Hour)
OPE 1
OPE 2
Air 1
Air 2
Oxygen
Control
(a)

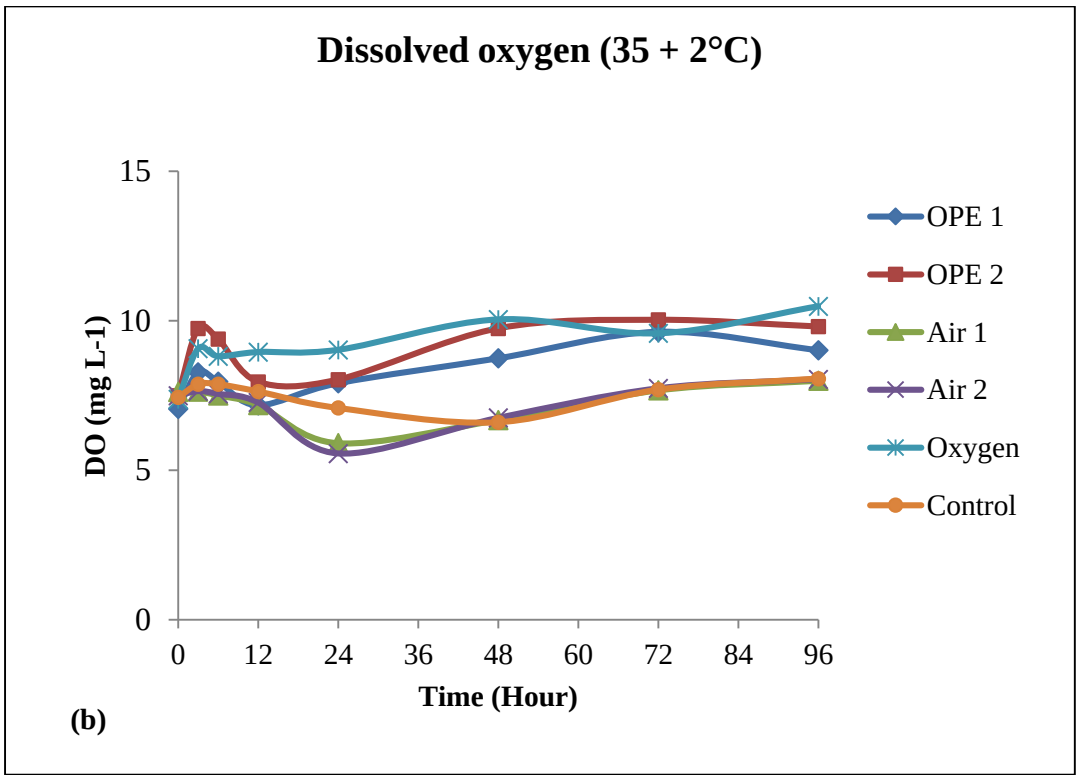

Dissolved oxygen (35 + 2°C)
DO (mg L-1)
15
10
5
0
0
12
24
36
48
60
72
84
96
Time (Hour)
OPE 1
OPE 2
Air 1
Air 2
Oxygen
Control
(b)

Figure 4: DO concentration (n=2) (a) 25˚C (b) 35˚C

Table 3: DO concentrations for the technologies at 25°C and 35°C

Technology	DO (mg L^{-1}) (0 - 96 hours)					
	25 ± 2°C			35 ± 2°C		
	Initial DO	Final DO	Net Difference	Initial DO	Final DO	Net Difference
OPE	7.71	10.99	3.28	7.29	9..42	2.13
Air aeration	7.54	8.43	0.89	7.57	8.03	0.46
Oxygen aeration	7.61	13.07	5.46	7.38	10.49	3.11
Control	6.57	8.68	2.11	7.44	8.07	0.63

Table 4: Mean difference of DO between technologies at 25°C and 35°C

Between Technologies	Mean difference (p value)*	
	25 ± 2°C	35 ± 2°C
OPE • **OPE** • **Air Aeration** • **Oxygen Aeration** • **Control**	1 -2.2404 (p=0.0000) ** 1.057 (p=0.0796) -2.426 (p=0.0000) **	1 -1.3896 (p=0.0000) ** 0.6954 (p=0.5553) -1.415 (p=0.0000) **
Air Aeration • **Air Aeration** • **Oxygen Aeration** • **Control**	1 3.2971 (p=0.0000) ** -0.1854 (p=1.0000)	1 2.0850 (p=0.0000) ** -0.02542 (p=1.0000)
Oxygen Aeration • **Oxygen Aeration** • **Control**	1 -3.483 (p=0.0000) **	1 -2.110 (p=0.0000) *

* Mean difference of DO from GLM

** indicating significance, $p < 0.05$

3.3 Discolouration

The light green media was entirely discoloured upon remediation by OPE (at both temperatures) as seen in other algae and cyanobacteria removal studies. This is caused by lyses or decomposition of cyanobacterial cells, as well as membrane and organic compounds

such as chlorophyll and other photosynthetic pigments [16, 28, 29, 31]. Discolouration also occurred in a study on removal of microcystin-LR cyanotoxin and humic acid [32]. Cyanobacteria pigments (phycocyanins and chlorophyll a) were destroyed in some electrolysis technologies which explains the reason for discolouration [6, 7]. Air aeration, oxygen aeration and control instead turned greener indicating that proliferation of cyanobacteria had occurred.

3.4 Changes in Filament Length

Another interesting finding was that filament lengths of *Aphanizomenon flos-aquae* were reduced (at both 25°C and 35°C) as a result from growth inhibition or suppression by OPE. On the contrary, filament lengths for air aeration, oxygen aeration and control increased at both temperatures, indicating proliferation (Figure 5). Significant test performed using General Linear Model (GLM) shows that filament length reduction by OPE was significant ($p < 0.05$), without any significant difference between filament lengths at 25°C and 35°C. Filament lengths were significantly longer at 35°C compared to 25°C for both air aeration and oxygen aeration as well as control. At 35°C, filament length significantly increased for air aeration compared to oxygen aeration and control, while no significant difference between oxygen aeration and control. This could be due to the presence of 79% nitrogen in air aeration which could serve as nutrient source that enhance the growth of the nitrogen-fixing cyanobacteria [3].

Fragmentation followed by cell division and elongation of the filaments to form longer filaments, is a growth strategy of cyanobacteria seen occurring from 27.5°C to 35°C [33, 34]. This explains why longer filaments were obtained for air aeration, oxygen aeration and control after the 4-day experiment especially at 35°C. In addition, specific growth rate (SGR) for *Aphanizomenon* was highest at 35°C [21, 33, 35].

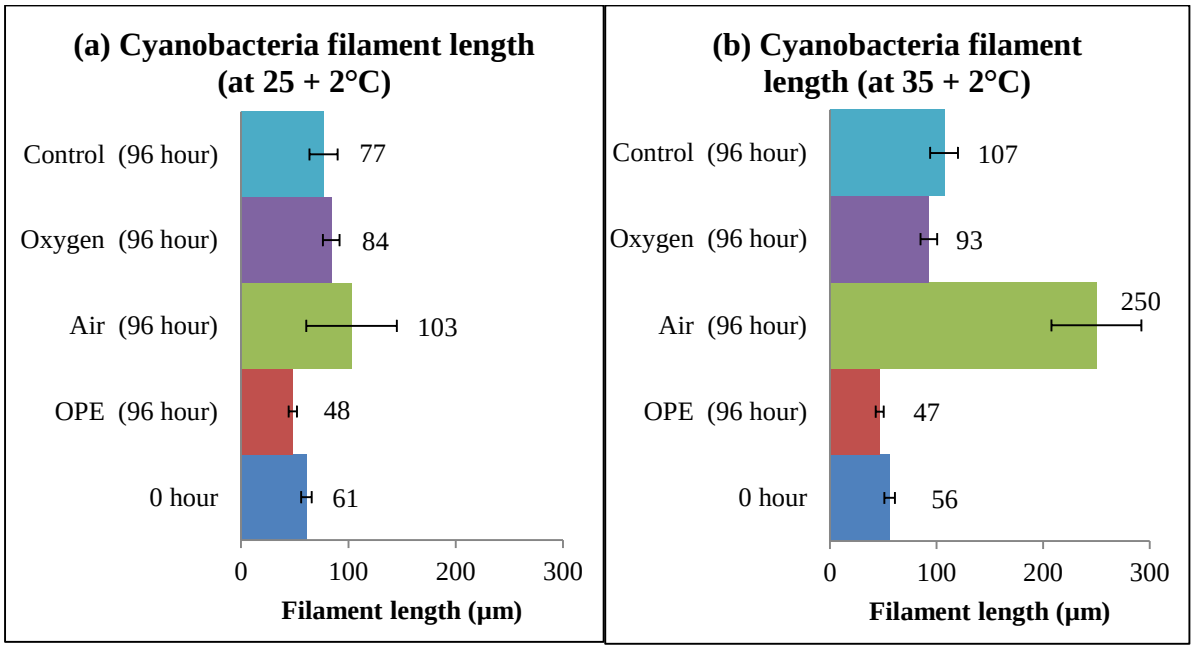


Figure 5: Filament length (n=20 cyanobacteria) upon 96 hours of remediation.

3.5 Mechanism and advantages of OPE system

As cyanobacteria was significantly suppressed by OPE only, despite the rise of oxygen for both OPE and oxygen aeration, it was concluded that oxygen is not the mechanism for the suppression of algae or cyanobacteria. Oxygen aeration which has 100% oxygen at a partial pressure of 1 atm was used to compare with air aeration which has 21% oxygen. Previous studies demonstrated that ozone and ·OH radicals are the active species responsible for suppression as indicated by the positive association between cyanobacterial suppression and the rise in ozone and ·OH radicals; while in oxygen aeration, air aeration and control, there was no ·OH radical or ozone produced [5, 24].

The OPE is sustainable as it continuously produces strong oxidants known as Reactive Oxygen Species (ROS) through water-splitting reactions (Equation 6-8). When in water, the sulfonic group ($-SO_3^-$) of the Nafion® membrane transports hydrogen ions (H^+) from anode to cathode, while releasing oxygen at the anode [25].Ozone (O_3) is formed through a direct reaction followed by ·OH as an indirect reaction [5, 27, 36].

$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$ Equation 6

$4H^+ + 4e^- \rightarrow 2H_2$ Equation 7

$O_2 \rightarrow$ ROS Equation 8

The mechanism of the oxygen evolution is clarified based on various literature. Oxygen receives an electron to form superoxide anion radical ($O_2^{\bullet-}$) (Equation 9) subsequently

reacting with H^+ to form perhydroxyl radical ($HO_2^{\bullet}$) (Equation 10). Perhydroxyl radical is reduced electrochemically to hydroperoxide anion (HO_2^-) which accepts a proton (H^+) to chemically oxide to hydrogen peroxide (H_2O_2) as shown in Equation 11 [15]. Hydrogen peroxide in aqueous form also dissociates partially to hydroperoxide anion (HO_2^-) (Equation 11). Hydroxyl radicals are produced directly from water (Equation 12) and dissociate electrochemically generating oxygen radical ($O^{\bullet}$) (Equation 13). Two oxygen radical ($O^{\bullet}$ **)** react with each other to form oxygen as in Equation 14. One oxygen radical reacts with oxygen to produce ozone (Equation 15) [15]. Oxygen also produces hydroxyl radicals ($OH^{\bullet}$**)** and hydroxide ions (OH^-) (Equation 16). Two hydroxyl radicals ($OH^{\bullet}$**)** then react to produce H_2O_2 (Equation 17) [15, 37]. H_2O_2 reacts with ozone resulting in a series of chain reactions which include formation of hydroxyl radical ($OH^{\bullet}$**)** and oxygen (Equation 18) as well as perhydroxyl radical ($HO_2^{\bullet}$) and ozonide anion radical ($O_3^{\bullet-}$) (Equation 19) [27, 36, 37].

Ozone reacts with hydroxide anions (OH^-) and hydrogen ion producing superoxide anion radical ($O_2^{\bullet-}$) and perhydroxyl radical ($HO_2^{\bullet}$). Ozone subsequently reacts with $O_2^{\bullet-}$ to form ozonide anion radical ($O_3^{\bullet-}$) which immediately decomposes to hydroxyl radical ($OH^{\bullet}$**)**. This reaction is displayed in Equation 20. Ozone also decomposes to form hydroxyl radical, superoxide anion and oxygen as in Equation 21. Ozone is commonly known to react with hydroxide ions (OH^-) leading to generation of hydroperoxide anion (HO_2^-) and oxygen (Equation 22). Ozone decomposition reactions are highly favoured under alkaline pH conditions, leading to production of hydroxyl radical (Equation 18, 20 and 21) [27, 36, 37].

$O_2 + e^-$	$\rightarrow O_2^{\bullet-}$	Equation 9
$O_2^{\bullet-} + H^+$	$\rightarrow HO_2^{\bullet}$	Equation 10
$HO_2^{\bullet} + e^-$	$\leftrightarrow HO_2^- + H^+ \leftrightarrow H_2O_2$	Equation 11
H_2O	$\rightarrow OH^{\bullet} + H^+ + e^-$	Equation 12
$OH^{\bullet}$	$\rightarrow O^{\bullet} + H^+ + e^-$	Equation 13
$2O^{\bullet}$	$\rightarrow O_2$	Equation 14
$O_2 + O^{\bullet}$	$\rightarrow O_3$	Equation 15
$O_2 + 2H^+ + 3e^-$	$\rightarrow OH^{\bullet} + OH^-$	Equation 16
$2OH^{\bullet}$	$\rightarrow H_2O_2$	Equation 17
$2O_3 + H_2O_2$	$\rightarrow 2OH^{\bullet} + 3O_2$	Equation 18
$O_3 + HO_2^-$	$\rightarrow HO_2^{\bullet} + O_3^{\bullet-}$	Equation 19
$3O_3 + OH^- + H^+$	$\rightarrow 2OH^{\bullet} + 4O_2$	Equation 20
$O_3 + HO_2^-$	$\rightarrow OH^{\bullet} + O_2^{\bullet-} + O_2$	Equation 21
$O_3 + OH^-$	$\rightarrow HO_2^- + O_2$	Equation 22

As the previous *Microcystis* suppression experiment using OPE was operated in the same conditions as this study, the production of ·OH radical and ozone of this study is expected to be similar to previous OPE study [31]. Production rate of ·OH at a current density of 40 mA cm^{-2} was approximately 0.1 nM s^{-1} while production rate of O_2 was approximately 0.15 mL cm^{-2} min^{-1}.

Ozone had the highest rate constants in oxidization compared to free chlorine, chlorine dioxide and chloramines, as well as UV/TiO_2 system [38]. In addition, ozone forms less harmful byproducts or secondary pollutants compared to chlorine [39] and permanganate [27]. Whilst ozone which has a relative oxidation power of 1.52, the $^{\cdot}OH$ radical is stronger oxidant with relative oxidation power of 2.05 able degrading all forms of organics including cyanobacteria organic matter and toxins [37].

Electrolysis has been proven more efficient in producing both $^{\cdot}OH$ and ozone compared to the conventional corona discharge ozonation [27]. It betters the conventional technologies that are not only incomplete in suppressing cyanobacterial growth and destroying the toxins but enable undesired lyses of cells releasing hazardous toxins into the treated water [5]. In addition, electrolysis, unlike chlorination and ozonation, is successful in degrading odour metabolites such as geosmin at high and low concentrations [5, 6]. Minimal sludge is produced by OPE as it breaks double bonds of organics including cyanobacterial cells and toxins which can be easily removed by sedimentation and filtration [9].

It was also observed that in order to achieve the same K_La as OPE (1.00 ± 0.16 $hour^{-1}$), a higher gas flow rate of 4.8 mL min^{-1} was required by air aeration compared to the 0.6 mL min^{-1} and 0.5 mL min^{-1} required by oxygen aeration and OPE. Although a higher air flow would mean higher oxygen dissolution, many studies have proven against a higher air flow rate as it increases bubble size leading to reduced oxygen transfer efficiency (OTE), higher cost of operation, reduced mechanical efficiency and reduced mixing effectiveness. This is because when higher flow rate is introduced, input energy gets wasted for creating turbulence at the surface as air bubbles move faster, while reducing contact time between bubbles and ambient water [22, 40]. Previous studies showed increase in flow rate in aerators did not produce cause reduction of chlorophyll-a [41, 42]. Therefore, aeration shown to be ineffective for suppression of algae, consistent with current and other previous studies [5, 22, 23]. Proliferation of *Anabaena* and *Aphanizomenon* upon aeration in shallow reservoirs could be due to circulation of nutrients, which serve as food source of algae/cyanobacteria, from the sediments or hypolimnion to the upper layers of the reservoir [41]. Studies in Malaysia, also showed that algal blooms persist upon hypolimnetic diffuser aeration in Sg. Terip reservoir in Negeri Sembilan and Upper Layang reservoir in Johor, Malaysia [45, 46]. Therefore, the existing practice of aeration should be seriously re-considered.

The operation of OPE does not cause increase in voltage as seen this and previous studies, it gradually increased from 2.8 to 3.3 V forming a stable plateau and reaching a maximum voltage of 4.0V at both temperatures, 25°C and 35°C [5, 8]. Meanwhile presence of salts in seawater, can cause resistance, leading to an increased voltage [43]. Therefore, OPE is only suitable for freshwater or treated water at the water treatment plant and is highly advantageous and sustainable for remediation of cyanobacteria in freshwaters. In addition, it was found that regular cleaning of the membrane is sufficient and change of membrane only

required if suppression efficiency reduced as seen through reduction in K_La or active species. As OPE is able to degrade organics and prevent generation of disinfection by-products (DBPs) such as THM, it is most appropriate to be installed at the pre-treatment stage of the WTP, thus reducing the cost of water treatment [3, 23, 44].

4.0 CONCLUSION

This study demonstrates that OPE is equally efficient in suppressing the persistent filamentous cyanobacteria, *Aphanizomenon*, at both 25°C and 35°C, hence widening its applicability to tropical and temperate regions. Continuous generation of the active species by OPE and its ability to degrade all forms of organics makes it a more sustainable, practical and cost-effective technology. However, as *Aphanizomenon flos-aquae* is one of the most difficult species to remediate, further studies to enhance the suppression efficiency of OPE to be carried out. This may include simulation of this batch-scale finding to a continuous-scale pilot plant with modification of operational factors such as current densities, retention time and newer electrode or membrane materials to ensure a high-throughput OPE technology.

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