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Ozonation as cyanophyta control method for water treatment

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Abstract

A problem with the use of chemical oxidants for algae control in water treatment processes is an incomplete destruction of algal cells, leading to the clogging of the filter media. Ozone, with its high oxidizing power, can be used not only for treating many types of organic contaminants but also for destroying microorganisms. This study investigated the effect of ozone in controlling an algal population. The sample for the study, obtained from a raw water reservoir of the MWA Bangkhen Water Treatment plant (Thailand), was cultured in Bold's Basal medium and used as a study population. Morphological identification of algae in raw water sample and cultured sample (study population) revealed that the predominant organisms were Chlorophyta, Cyanophyta and Bacillariophyta. Identification by PCR-DGGE and DNA sequencing of Cyanophyta in raw water and in the study population showed that the predominant algae were *Oscillatoria* sp. (99% similarity), *Limnothrix* sp. (99% similarity) and *Merismopedia* sp. (93% similarity). Ozonation was conducted using ozone gas from a corona discharge type generator, generated at the rate of 3.7 mg/min. The results showed that ozone successfully destroyed algae. With 15.4 mg O_3/L, the initial population of 1,021 µg chlorophyll-a/L was reduced by 72.4%. This was equivalent to the removal rate of 47µg chlorophyll-a/mg O_3. The re-culturing of the ozonated algae population, both in supernatant and sludge, yielded no change in chlorophyll-a content. Also no presence of a cyanophyta DNA fingerprint was observed on agarose gel. This indicated that regrowth did not occur and ozonation completely destroyed all the algae.

Keywords: Ozonation, Cyanobacteria, Algae control, Water treatment

1. Introduction

Algae blooms are a common phenomenon in fresh water reservoirs. Many studies on the population of the blooms in various regions indicated that blue-green algae (cyanophyta) was the main group forming the blooms. The predominant species was found to be *Microcystis aeruginosa* [1-3]. Algae blooms are common in many areas of Thailand [4-5]. From the studies on algal population in freshwater reservoirs in Chonburi Province, *M. aeruginosa*, *M. wesenbergii* and *Anabaena affinis* were reported as the predominant cyanobacteria. Large populations of *Aulacoseira granulate*, a diatom species, were also found [6-7]. A recent study, in 2013, on algae proliferation in the same reservoir reported the presence of *Synechococcus* sp. (Cyanophyta) and Bacillariophyta as predominant [8]. An investigation (2003-2004) on seasonal variation of phytoplankton in Doi Tao Lake, Chiangmai Province, revealed that Cyanophytes dominated in winter and summer [9]. Cyanophyta is of the most concern since various species of Cyanophyta can produce toxins harmful to humans and domestic animals [10-12].

In addition to producing harmful toxins, the clogging effect of algae can also cause problem to coagulation and filtration units of water treatment plants. Algae can be controlled or destroyed by chemical oxidants or by advanced oxidation processes [13]. The normal practice is to remove algae together with suspended particles in a coagulation process. When algae is not totally destroyed during coagulation and subsequently enters the filtration system, it can later grow and clog the filtration media. Algae control is thus an important process in a water supply system. The use of copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$) and calcium hypochlorite ($Ca(OCl)_2$) for algal control in the alum coagulation process were compared in a previous study [14]. The results showed that these oxidants did not completely destroy algae since algae regrowth was observed when treated supernatant and sludge were re-cultured.

Ozone is a good alternative to the commonly used oxidants in controlling algae populations. With high oxidizing power it can be used to oxidize organic substances and destroy microorganisms. Destruction of microbial cells has been reported to occur at cell membranes and cell walls [15-16].

The current study aimed at application of ozone as an algae control method for water treatment system. Algae samples were collected from a raw water reservoir of the MWA Bangkhen water treatment plant. Algae identification was done by a direct microscopic examination technique and confirmed by a molecular technique (PCR-DGGE). The samples were cultured in Bold's Basal medium and used as

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test (ozonated) population. An ozonated algae population was investigated to determine its degree of destruction and capability of regrowth. Molecular identification of algae in the initial and ozonated populations was done to determine which algae species that are resistant to ozonation.

The significance of this study lies in its finding of the effectiveness of ozonation as a Cyanophyta control alternative in water treatment plant, thus avoiding clogging of filtering media and contamination of water with toxic substance from the algae.

2. Materials and methods

2.1 Preparation of study population

Algae samples were collected from the raw water reservoir of MWA (Metropolitan Water Work Authority) Bangkhen water treatment plant. The samples were examined under a microscope (direct microscopic examination) for morphological identification of microorganisms. The samples were then cultured in Bold's Basal medium at a ratio of 1:4 (sample: medium) under fluorescent light for 12 hours/day at ambient temperature. After 5 weeks, an algae population was cultivated and used in ozonation experiments. The population density was determined by its chlorophyll-a content (APHA&AWWA standard method). Samples for chlorophyll-a determination were centrifuged. The solid portion was ground into solvent (1:9 of 0.1N NH_4OH:80%acetone) then centrifuged. The supernatant was measured for absorbance at 663 and 645 nm using HACH DR/4000 UV-VIS spectrophotometer and chlorophyll-a content determined. The predominant group of Cyanophyta was identified by PCR-DGGE and DNA sequencing methods.

2.2 Ozonation of algae

Ozonation of algae was conducted in a batch reactor consisting of a sample jar and three flasks serially connected by silicone tubes (Figure 1). Ozone from a generator (with generation rate of 3.7 mg O_3/min, as determined by wet chemistry iodide method [17]) was fed into the jar containing the algae culture. Excess ozone was passed through the tube into three consecutive flasks (trap A, B and C) containing 2% potassium iodide (KI) solution for ozone trapping. The amount of ozone used in the reaction was determined by subtracting the amount of excess ozone from the amount fed into the sample jar. Ozonated algae samples were collected after 5, 10, 20, 30 and 40 min of ozonation, equivalent to treatments of 15.4, 29.4, 60.9, 85.7 and 113.2 mg O_3/L, respectively. The samples were then left to stand, allowing the algae to settle. The remaining population in the supernatant was subjected to chlorophyll-a determination. Algae removal efficiency was evaluated. To investigate the influence of pH, experiments were done at initial pH values of 3, 7 and 9.

To determine if ozone completely destroy the algae, the ozonated samples, both supernatant and sludge portions, were re-cultured in Bold's Basal medium under the conditions previously described. Regrowth of algae indicated resistance of the algae to ozone. The regrown population was then identified using PCR-DGGE and DNA sequencing methods.

2.3 Identification of Cyanophyta *by molecular technique*

Cyanophyta was the major algae group of concern in this study since it can be harmful to human and aquatic life. DNA extraction of Cyanophyta was done using a Genomic DNA Extraction Mini Kit (RBC Bioscience), following the Rinta-Kanto protocol [18]. Extracted DNA was amplified by a polymerase chain reaction using FastStart SYBR Green Master (Roche). The primers for cyanobacteria 16S rRNA genes used were CYA359F and CYA781R (a), (b) [19]. Amplification was performed in Light Cycle Nano (Roche) with 5 min hold at 95°C followed by 35 cycles of 60s denaturing at 94°C, 60s at 60°C annealing, 60s at 72°C extension. Final chain elongation was at 72°C for 7 min. Amplification products were then analyzed using Agarose Gel Electrophoresis and Denaturing Gradient Gel Electrophoresis. DNA fragment bands from DGGE were analyzed for nucleotide sequence by a commercial sequencing facility (AIT Biotech, Singapore). The sequences obtained were compared to genetic sequence databases (GenBank at NCBI - the National Centre for Biotechnology Information) using the BLASTn search option.

3. Results and discussion

3.1 Predominant algae in MWA Bangkhen raw water reservoir

Algae predominating in MWA Bangkhen raw water reservoir during the study period (October 2015 to January 2016) were found to be Cyanophyta, Chlorophyta and Bacillariophyta. Some examples of predominant Cyanophyta are shown in Figure 2. The species found are common in freshwater reservoirs in Bangkok [20-21] and in freshwater estuaries in southern Thailand [22].

The predominant algae were identified by direct microscopic examination. The Cyanophyta found included *Mastigocladus* sp., *Merismopedia* sp., *Oscillatoria* sp. and *Pseudanabaena* sp. Those of Chlorophyta included *Chlamydomonas sp*., *Chlorella* sp., *Dictyosphaerium* sp., *Monoraphidium sp*., and *Scenedesmus* sp. Bacillariophyta included *Aulacoseira* sp.

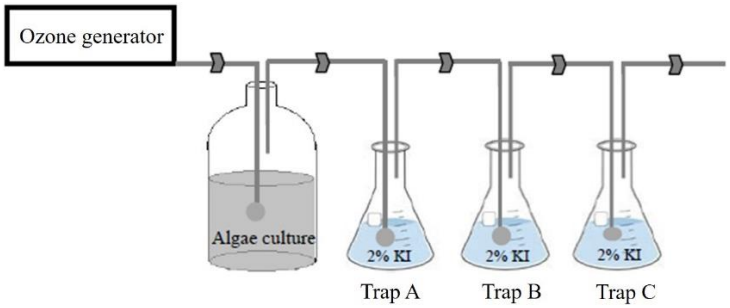


Figure 1 Batch reactor used for ozonation of algae cultures with traps for excess ozone (A, B, C)

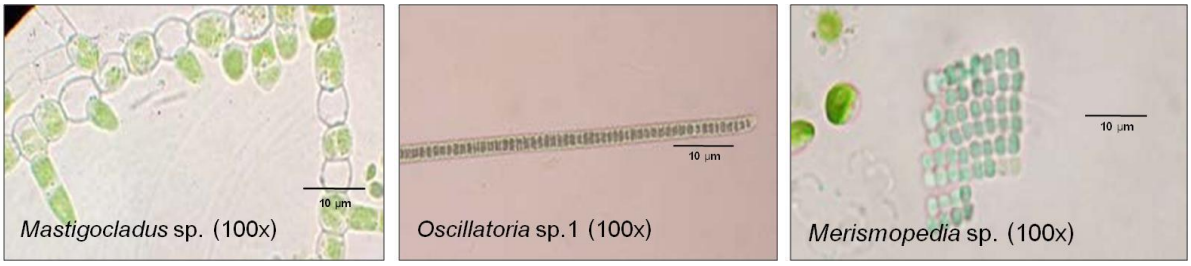


Figure 2 Cyanophyta were predominant in the Bangkhen raw water reservoir (optical microscope images)

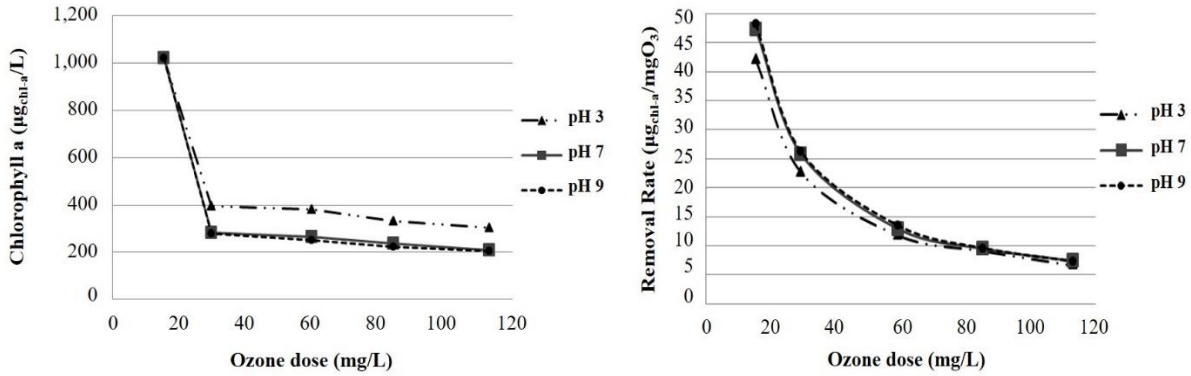


Figure 3 The results of ozonation of the cultured algae population at pH 3, 7 and 9. (a) The changes in chlorophyll-a content, (b) Ozone consumption (µg chlorophyll-a removed: mg O_3)

3.2 Ozonation of algae

The population obtained after five weeks of cultivation in Bold's Basal medium was used in the experiment. After ozonation, a portion of algae population settled to the bottom of the culture vessel, resulting in decreased turbidity. The turbidity of the ozonated samples was significantly lower than that of the sample that was not ozonated. The chlorophyll-a content decreased with ozonation time. This decrease in chlorophyll-a content and turbidity of the supernatant together with the increase in the amount of sludge indicated that algae was removed. As can be seen in Figure 3(a), variation in pH slightly affected removal. Within the first 5 min of ozonation (14.9-15.4 mg O_3/L) at pH 3, 7 and 9, the initial population of 1021 µg chlorophyll-a/L substantially decreased to 394.1, 281.7 and 278.8 µg chlorophyll-a/L, respectively. Extension of ozonation time further decreased the algae population, although the rates were slower. At 40 min. (111.9-113.2 mg O_3/L), the algae populations remaining in the supernatant had 287.6, 198.1 and 193.9 µg of chlorophyll-a/L at pH 3, 7 and 9, respectively.

A number of previous studies on ozonation to remove algae suggested destruction mechanisms of ozone on algae cell. Ozonation of green algae and diatoms at 3 mg/L O_3-dose resulted in cell wall alterations and damage and a release of dissolved organic carbon [23]. This was confirmed by the change in algae cell morphology and the increase in extracellular organic matter (EOM) found in a study of phytoplankton [24], green algae, diatoms and cyanobacteria [25].

Considering ozone consumption or algae removal rate, as the ratio of algae removed: ozone administered in mg chlorophyll a: mg O_3, it was noted (Figure 3(b)) that the ratio was highest in the early period with the least amount of ozone but highest population. During the first 5min. (14.9-15.4 mg-O_3/L) the removal rate was 42-48 µg chlorophyll-a/mg O_3. Later, at a lower population, the rate decreased to 6.6-7.4 µg chlorophyll-a/mg O_3. This showed that the population density had significant influence on removal efficiency. However the surviving population is an important factor that must be taken into account when evaluating the performance of a treatment method.

3.3 The test of regrowth of the ozonated algae population

Both portions of ozonated samples (supernatant and sludge) were re-cultured in Bold's Basal medium for 10 days and the population determined by measuring its chlorophyll-a content at 2 day intervals. Figure 4 shows the effect of ozonation (under various conditions – pH 3, 7, 9 for 5-25 min.) on the regrowth of algae in the supernatant (sp) and sludge (sl). The chlorophyll-a contents in all ozonated samples either remained unchanged or slightly decreased after re-culturing. This indicated that there was no regrowth of algae after the ozone treatment. At the same time, the chlorophyll-a contents in un-ozonated samples increased over the incubation period (data not presented). Successful control of algae by ozone treatment in this study was due to the high ozone dosages (15-115 mg/L). The dosage of ozone was found to have an important influence on algae removal. The studies by Miao and Tao [25] and Huang et al. [15] showed complete algae removal with an O_3 dose of 5 mg/L while regrowth was observed with doses of 1-3 mg/L.

3.4 Identification of cyanophyta by molecular technique

This study focused on Cyanophyta, which could also pose problems to human health. To confirm its presence, a molecular technique (PCR-DGGE) was employed for identification. The result obtained is as shown in Figure 5.

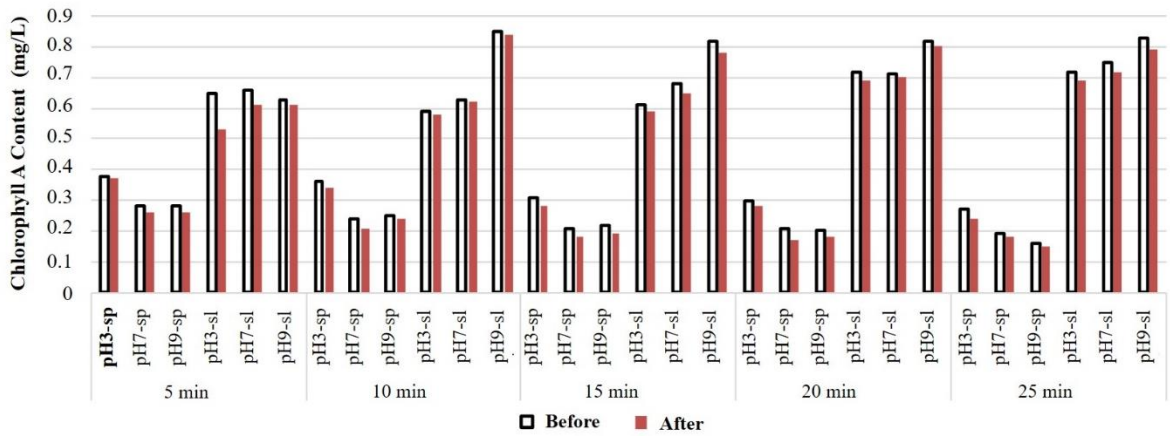
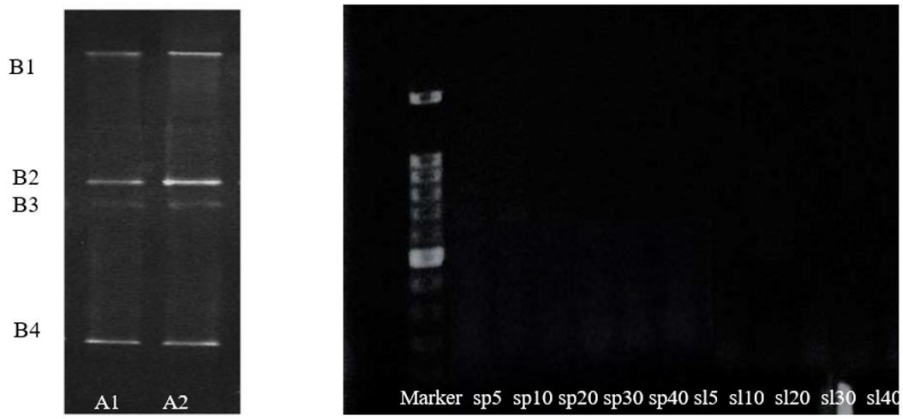


Figure 4 Algae populations (ozonated under various conditions - pH3, 7, 9 for 5-25 min.) before and after reculture in Bold's Basal Medium



(a) (b)

Figure 5 DNA fingerprints, (a) DNA fingerprints from DGGE *(lane A1 = raw water, A2 = culture in Bold's Basal medium)*; (b) DNA band from agarose gel electrophoresis (sp=supernatant at 5-40 min., sl=sludge at 5-40 min.)

The DNA fingerprints (obtained from DGGE) of Cyanophyta predominant in the Bangkhen raw water reservoir are shown in Figure 5(a). The distinguish bands of DNA appeared at the same position in both raw water (lane A1) and the culture in Bold's Basal medium (lane A2) indicating the presence of the same species in both raw water and the cultured sample. The results of nucleotide sequencing identified band B1 as *Oscillatoria sp.* (at 99% similarity), band B2 as *Limnothrix* sp. (at 99% similarity) and band B4 as *Merismopedia* sp. (at 93% similarity). The B3 band could not be identified due to the low similarity of nucleotide sequence matching.

For the samples (both supernatant and sludge) tested for algae survival, no DNA band appeared on agarose gel after electrophoresis (Figure 5(b)). This agreed with the results measuring chlorophyll-a content and confirmed that ozone successfully controlled the algae population.

4. Conclusions

The predominant algae in the raw water reservoir of MWA Bangkhen water treatment plant were Chlorophyta, Cyanophyta and Bacillariophyta. Identification by PCR-DGGE and DNA sequencing of Cyanophyta in raw water and the study population showed that the predominant species were *Oscillatoria sp*. (99% similarity), *Limnothrix sp.* (99% similarity) and *Merismopedia sp.* (93% similarity). It was also found that ozone successfully destroyed algae. An initial population with 1,021 µg chlorophyll-a/L was reduced by 72.4% after ozonation at 15.4 mg O_3/L. This was equivalent to the removal rate of 47 µg chlorophyll-a/mg O_3. Culturing algae from ozonated water resulted in no regrowth. This showed that ozone completely destroyed that algae population. This was confirmed by PCR-DGGE results, in which no Cyanophyta DNA fingerprint was observed after ozonation. Thus ozonation is a good alternative in controlling algae.

5. Acknowledgements

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