OZONATION AND CULTIVATION OF GREEN ALGAE *CHLORELLA ZOFINGIENSIS* IN DOMESTIC WASTEWATER

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Abstract: *Ozone has been used in treatment of municipal wastewater effluents primary for disinfection. However, its powerful capacities to react with organic matter in wastewater led to another application of ozone in water treatment. Heterotrophic cultivation of green algae Chlorella zofingiensis in ozonated waste water was used to remove nutrients and also to collect Astaxanthin. Results of this study shows ozonation did not remove but produce transformation of organic matters; increase capacity of dissolved organic carbon and dissolved nitrogen removal of Chlorella zofingiensis as well. The ozonation - heterotrophic cultivation of Chlorella zofingiensis process could be used in domestic wastewater treatment. However, as the dual-goal of algae cultivation, astaxanthin production in this process was not effective.*

Keywords: *Ozonation, algae cultivation, domestic wastewater, astaxanthin.*

1. Introduction

Domestic (also called sanitary) wastewater is wastewater discharged from residences and from commercial, institutional, and similar facilities. It is handed by wastewater treatment plans and discharged into received water bodies (rivers, sea, etc). General terms used to describe different degrees of treatment are preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment. In some countries, disinfection to remove pathogens sometimes follows the last treatment step (FAO). Disinfection is used in water treatment process to reduce pathogens to acceptable level. There are three normal categories of human enteric pathogens: bacteria, viruses, and amebic cysts. Powerful disinfectant must destroy all three. The common disinfection process using in wastewater treatment are chlorination, ozonation, and ultraviolet radiation.

Ozone has been used in treatment of municipal wastewater effluents primary for disinfection. However, its powerful capacities to react with organic matter in wastewater led to another application of ozone in water treatment. Beside disinfection, ozonation can improve the general physical and chemical quality of effluents, such as reducing chemical oxygen demand (COD), biological oxygen demand (BOD_5), color, and UV absorbance, and increasing dissolved oxygen (DO) [6]. Because of its high oxidation potential, ozone reacts with a wide range of organic and inorganic compounds in water. Chemical oxidation by ozone occurs by two distinct reaction mechanisms, namely a molecular ozone reaction pathway and a hydroxyl radical ($^{\bullet}OH$)

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reaction mechanism. While ozone is a very selective oxidant which reacts quickly with double bonds, activated aromatic compounds and deprotonated amines, OH^- radicals react with most water constituents with nearly diffusion controlled rates. Many previous studies of ozonation indicated that ozone attacks aromatic and unsaturated compounds, thereby affecting the chemical composition and the overall quality of the water [7].

However, at the low level of ozone dose used in wastewater treatment plant, the total organic carbon is not significantly affected [5]. The limited dissolved organic carbon removal that was observed in the ozonation stage shows that oxidation leads to form the transformation products rather than mineralization [8]. The ozonated organic compound is readily biodegradable the original compounds. So ozone oxidation is a promising process as a supplementary method for biological treatment [9].

Algae *Chlorella*sp. was widely applied for wastewater treatment and had proven abilities of removing nitrogen, phosphorus, and chemical oxygen demand (COD) with different retention times ranging from 10 h to 42 days, mixing with bacteria or not, which shows the potential of replacing activated sludge process in a secondary or tertiary step in view of nutrient reduction and biomass production [11].

Moreover, astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione), a product of *Chlorella zofingiensis's* metabolism with a high-value ketocarotenoid with a broad range of applications in food, feed, nutraceutical, and pharmaceutical industries, has been gaining great attention from science and community in recent years. The green microalgae *Chlorella zofingiensis* represents the most promising producers of natural astaxanthin. *C. zofingiensis* grows fast phototrophically, heterotrophically and mixtrophically, is easy to be cultured and scaled up both indoors and outdoors, and can achieve ultrahigh cell densities. These robust biotechnological traits provide *C. zofingiensis* with high potential for mass astaxanthin production [4].

From this point of view, we study the combination of ozonation and heterotrophic cultivation of *Cholorella zofingiensis* in domestic wastewater treatment and astaxanthin production.

2. Materials and methods

2.1. Materials

Raw wastewater samples were collected from the Koto Domestic Wastewater Treatment Plant (Okayama city, Japan). In every sampling, wastewater was taken at the influent to the primary sedimentation tank which was taken from 2014, July 4thuntil 2015, January 29th. The experiment was conducted at natural pH conditions of wastewater; pH is close to it.

Ozone was applied to the coagulation supernatant; the supernatant was derived after coagulation and sedimentation of the wastewater with coagulation conditions of 4 mg/L chitosan dosage without pH adjustment. Ozone was generated by Mitsubishi OS-1N ozonizer.

2.2. Methods

Ozonation experiment was conducted at 3 different electricity occurrence of Ozonize, which would produce different ozone doses. The *Chlorella zofingiensis* was cultivated in

250mL of ozonated wastewater. The cultivation is continued in three weeks.The culture conditions are shown in table 1.

***Table 1**. Culture conditions of C. zofingiensis in ozonatedwastwater*

Valuables	Conditions
Ozone consumption	0; 2.6; 4.33; 5.72mg O_3 L^{-1}
Temperature	27^oC

(1) Daily sampling.

A 3mL of sample was taken from every flask every day for measurement of turbidity

(2) Sampling at every 3 days.

A 10mL of sample was taken from every flask every 3 days. The samples were to be filtered with glass microfiber filter GF/B. The filters were to be used for the measurement of suspended solid, while the filtrates are subjected for the measurement of dissolved nitrogen (DN), dissolved phosphorus (DP), dissolved organic carbon (DOC).

(3) Sampling on the final day.

A 5 mL of samples is subjected for the measurement of astaxanthin. The remaining in the flasks is used for the measurement for the items mentioned in (2).

Analytical methods

In order to determine the physical-chemical characteristics of the effluents and treated effluents, a large number of analyses based on Standard Methods for the Examination of Water and Wastewater (APHA, 2005) were conducted on each sample and the following parameters were measured: pH, Zeta Potential, Turbidity, Total Organic Carbon (TOC), Total Phosphorus (TP), Total Nitrogen (TN).

3. Results and discussions

3.1. Ozonation

Raw wastewater was taken from Koto Domestic Wastewater Treatment Plant, Okayama city, Japan. The characteristics of sample are shown in table 2.

***Table 2**. Characteristics of raw wastewater*

Parameters	**Average**	**Range**
pH	6.93	6.46 - 7.2
Turbidity (Absorbance at 660nm)	0.10	0.031 - 0.18
Zeta potential	-17.95	-20.5 - -14.1
UV254	0.60	0.131- 1.087
Total Nitrogen (mgL^{-1})	26.10	11.83 - 37.60
Total Phosphorus (mgL^{-1})	5.22	1.19 - 11.19
Total Organic Carbon (mgL^{-1})	27.38	4.724 - 47.57

Before conducting ozonation to waste water, seawage was removed solid, turbidity by chitosan coagulation without pH adjustment. The properties of coagulated wastewater are shown in table 3.

***Table 3**. Characteristics of coagulated wastewater*

Parameters	Average	Range	Removal rate (%)
pH	6.91	6.64 - 7.18	-
Turbidity (Absorbance at 660nm)	0.02	0.009 ≈ 0.045	74.30
Zeta potential	-15.55	-19.4 ≈ -1.61	-
UV254	0.31	0.083 ≈ 0.717	45.66
Total Nitrogen (mgL^{-1})	23.30	11.18 ≈ 36.03	11.57
Total Phosphorus (mgL^{-1})	3.76	0.56 ≈ 5.94	21.68
Total Organic Carbon (mgL^{-1})	19.12	1.83 ≈ 30.7	39.16

3.2. Effects of ozonation on total organic carbon of coagulated wastewater

In this study, ozonation was applied to coagulated wastewater at 4mg/L of chitosan of domestic wastewater taken weekly from December 12th, 2014 to January 29th, 2015.

From the results shown in Figure 3.1, the total organic carbon was hardly removed, but increased. This result is similar to that Asano's work [1]. The TOC removals were varied, and no apparent trend was observed. In the ozonation process, to observe the TOC level reduction, a significant part of the organic carbon must be completely oxidized to CO_2. However, the ozone dose examined in this study was low, and consequently it is considered unlikely that any significant degree of the complete oxidation occurred.

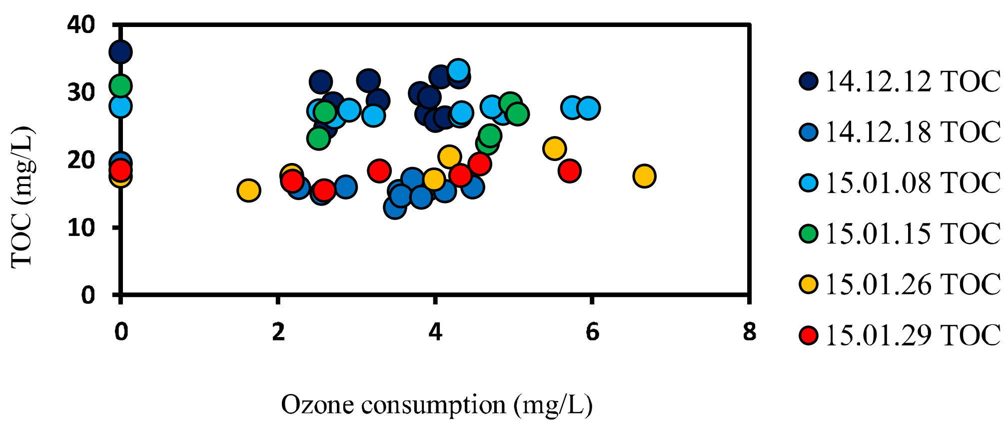


***Figure 1.** Total organic carbon profiles of different wastewater samples at diffirent ozonation conditions*

The value of the UV absorbance at 254 nm is indicative of organic species having double bonds and an aromatic structure. The reduction of this parameter is consistent with established reaction mechanisms whereby molecular ozone readily reacts with both unsaturated and aromatic compounds [6]. SUVA- specific ultraviolet light absorbance, an indicator of the aromaticity of organic matter in water, was defined by UV254 divided by TOC.

Figure 2 shows the change of SUVA during the treatment of domestic wastewaters with different ozone doses. This value could provide insights into the characteristics of water such as aromatic contents per unit concentration of organic carbon, hydrophobicity, and molecular weight distribution of DOC. Unlike TOC, the SUVA value of wastewater were decreased with the increasing of ozone consumptions , indicating that may be occur destruction of unsaturated bonds in organic matter. According to previous studies, limited reduction of DOC but dramatic decrease in SUVA was used as an evidence for the destruction of the unsaturated bonds by ozonation [10].

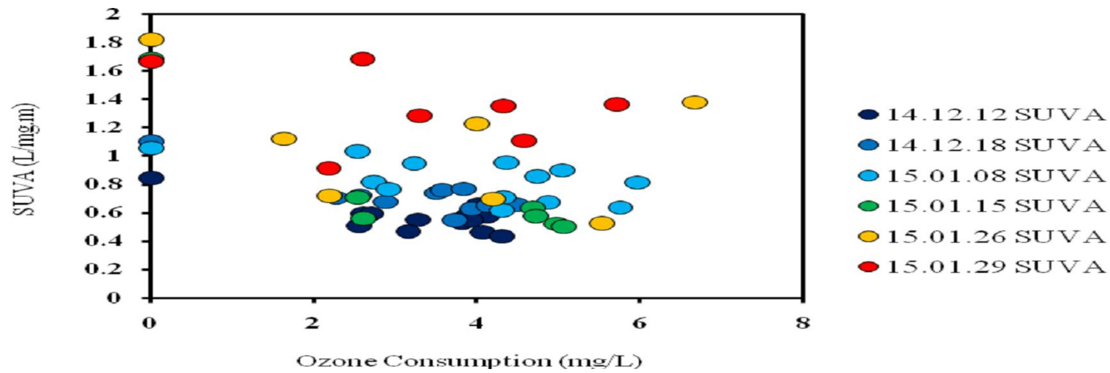


Figure 2. *SUVA of different wastewater samples at different ozonation treatment condition*

In this research, the water tested had a relatively low SUVA value (1.87±1.09 L/mg-m), indicating that the water contained hydrophilic and low-molecular-weight materials. SUVA decreased with increasing ozone consumption. This means that ozone processes could alter hydrophobic to hydrophilic and high-molecular-weight to low-molecular-weight organic matter.

From this result, it was confirmed that low concentrated ozone had a limitation of organic oxidation due to the selective reaction and the partial oxidation with organics by ozone. The ozone process alone could be proposed as a pre-treatment for the biological treatment because ozonation was able to enhance the biodegradability in the water.

3.3. Cultivation of Chlorella zofingiensis

To evaluate effects of ozonation on the growth of *Chlorella zofingiensis*, the algae were cultivated in ozonated wastewaters with different ozone dosages. The ozonated wastewater was prepared by ozonation of coagulated wastewater taken on January 29th, 2015.

In heterotrophic cultivation, *C. zofingiensis* used dissolved organic matters as carbon source. The concentration and component of organic carbon would affect *C. zofingiensis'* growth.

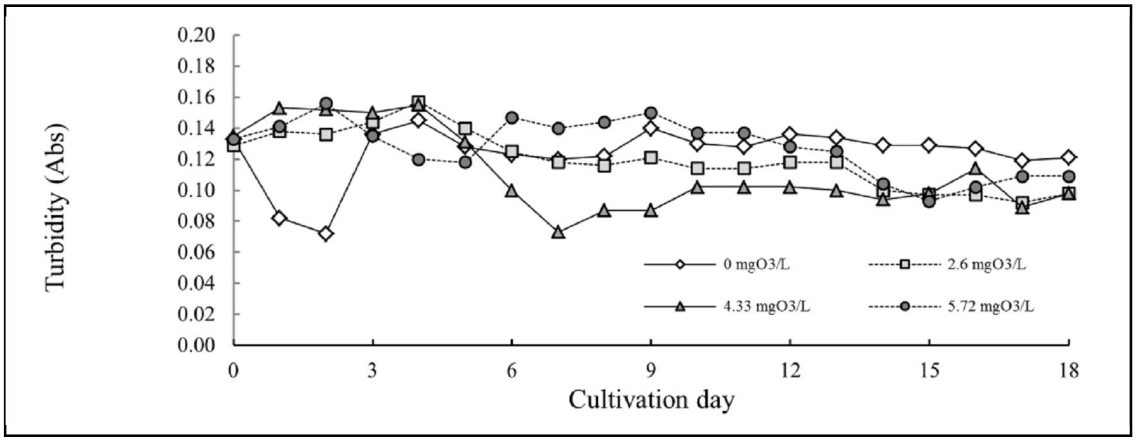
In this experiment, the carbon source for *C. zofingiensis*is dissolved organic carbon in wastewater. Especially the organic carbons have transform from high-molecular-weight to low-molecular-weight compounds. The dissolved organic carbon of ozonated wastewater was presented in table 4.

Table 4. *Dissolved organic matter of ozonated wastewaters*

Ozone dosage (mgO_3/L)	Dissolved Organic Carbon (mg/L)
0	20.94
2.60	19.97
4.33	20.03
5.72	21.56

The figure 3 shows the growth of *Chlorella zofingiensis* in different ozonated wastewaters. The algae adapted well and grown fast in 3 first days of cultivation. The algae exhibited the highest specific growth rate at day 3 of cultivation with 0.084; 0.076; 0.151; 0.061 (day^{-1}) in different ozonated wastewaters; and the highest biomass are 0.006; 0.005;

0.012; and 0.003 mg/day. Microalgae also uptakes dissolved organic carbon, nitrogen and phosphorus compounds for growing. Therefore DOC, DN, and DP are removed in wastewater. The nutrient removal of *C. zofingiensis* is shown in figure 4. The DOC removed with the highest rate at day 6 of cultivation. The DOC removal rates in ozonated wastewaters were higher than non-ozonated wastewater, 47.26% - 53.47% to 35.55%; and the highest removal rates of DOC were 53.47% for 4.33 mgO_3/L ozonated wastewater.



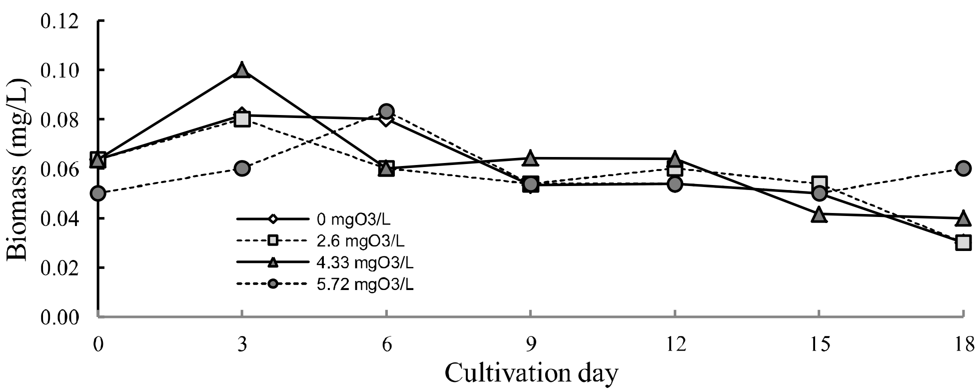
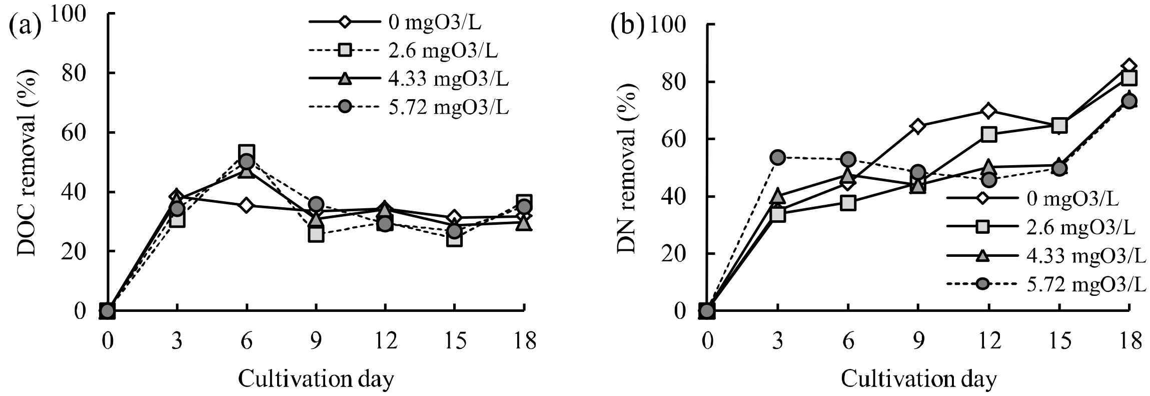


Figure 3. *The growth (a) and biomass (b) of Chlorella zofingiensis when cultivated in different ozonated wastewaters*

On day 6 of cultivation, the DP of wastewater were removed with 34.51%; 32.12%; 33.02%, and 9.98% removal ratios for 0 ; 2.6 ; 4.33; and 5.72 mgO_3/L, respectively. The removal ratios of DP in ozonated wastewater with 4.33 and 5.72 mgO_3/L were higher in the day 9 of cultivation but not significant (39.7 and 12.0% respectively). Unlike the DOC and DP, DN of wastewater continued to be removed even the algal growth and biomass decreased. On day 6 of cultivation, the DN removal ratios were 37-52%. And at the final day of cultivation, DN removals for different ozonated wastewaters increased to 85.34; 81.33; 74.13; and 73.46%, respectively.

Astaxanthin production was also objective of heterotrophic *Chlorella zofingiensis* cultivation. The result is shown in figure 5. The astaxanthin production increased with the increasing of ozone consumption. The highest production was 0.009 $mg.L^{-1}$ when algae cultivated in 5.72 $mgO_3.L^{-1}$ wastewater. However, the astaxanthin productions were low, may be due to the low concentration of DOC in wastewaters.



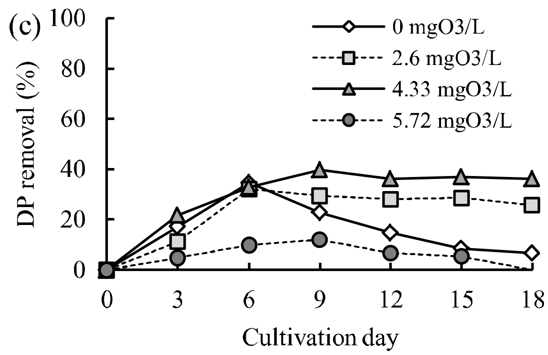


Figure 4. *Nutrient removal capacity of Chlorella zofingiensis when cultivated in diferrent ozonated wastewaters. a, Dissolved organic carbon removal rate; b, Dissolved nitrogen removal rate; c, Dissolved phosphorous removal rate.*

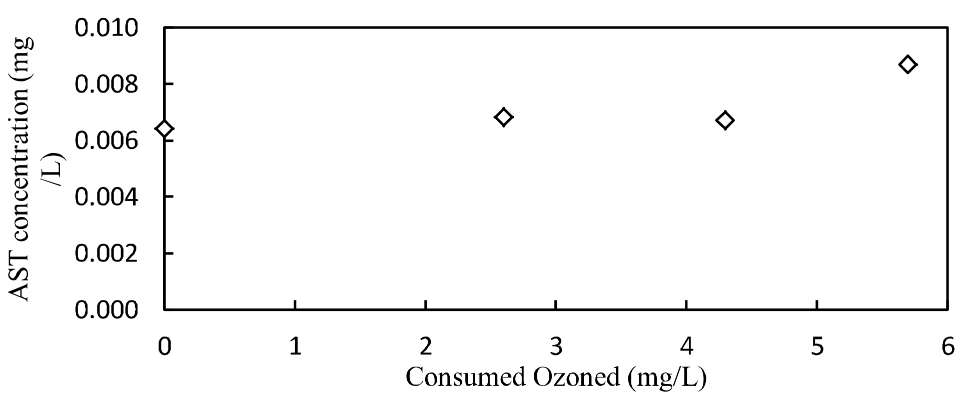


Figure 5. *The astaxanthinproduction of Chlorella zofingiensis when cultivated in different ozonated wastewater after 18 days of cultivation. (This result was obtained with the help of Hirotaka Komatsu)*

4. Conclusion

Ozonation not only removed but produced transformation of organic matters; also increased capacity of dissolved organic carbon and dissolved nitrogen removal of *Chlorella zofingiensis* as well. On day 6 of *Chlorella zofingiensis* cultivation, dissolved organic carbon removal ratios in ozonated wastewaters were higher than non-ozonated wastewater, with 47.26% - 53.47% to 35.55%. And the dissolved nitrogen removal ratios were 47.38 and 52.90% if 4.33 and 5.72 mgO_3/L applied compared to 44.59% of non-ozonated wastewater. The astaxanthin production of *Chlorella zofingiensis* in heterotrophic cultivation was the highest (0.009 mg/L) when cultivated in ozonated wastewater with the highest ozone dose applied (5.72 mgO_3/L). Therefore, the ozonation - heterotrophic cultivation of *Chlorella zofingiensis* process could be used in domestic wastewater treatment. However, as dual-goal of algae cultivation, astaxanthin production in this study is not effective.

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