



Efficient cell-wall disruption of microalgae *Chlorella Vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide

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ARTICLE INFO

Article history:

Received 26 August 2018

Received in revised form 24 October 2018

Accepted 24 October 2018

Available online 26 November 2018

Keywords:

Cell-wall disruption

Chlorella

Catalytic ozonation

ABSTRACT

Microalgae *Chlorella vulgaris* are industrially important microorganisms that have been studied for producing valuable bioproducts such as feed, food, cosmetics and pharmacy industries. The cell-walls of *Chlorella vulgaris* probably represent the biggest barrier to target bioproduct extraction. Many cell-wall disruption methods have been reported for microalgae in order to maximize the extraction efficiencies. However, but there has been no industrial scale application related to the high costs and electrical energy. This study investigated several parameters for cell-wall disruption from microalgae *Chlorella vulgaris* during catalytic ozonation over microporous carbon-supported titanium oxide, including flow ozone, catalytic time and reactor capacity. At the same time, the cell-wall disruption yield and an active compound yield such as chlorophyll and carotenoid were evaluated for each pretreatment. Pretreatment with 1 minute at 1 liter per minute in 2 liters produced chlorophyll yield by approximately 59.45% and the carotenoid was reduced to 98.18%. Carbon-supported titanium oxide reduces the required O₃ dose and catalytic time for cell-wall disruption, although the chlorophyll yield does not exceed 75.67%. Catalytic ozonation at 1 minute at 4 liters per minute produced 76.47% cell-wall disruption of *Chlorella vulgaris*, chlorophyll 56.75% and carotenoid 89.09%.

1. INTRODUCTION

Microalgae is a unique resource of health and nutrient food that cannot be isolated by common technology, it requires specific technology. Unfortunately, from the high destruction of the bioactive compound of microalgae to its intensive use common technology, efficient cell-wall disruption of microalgae is subject to ever-increasing pressures that affect the bioactive quality and efficiency of the process. For over many years research has been devoted to improve bioactive quality/quantity by developing efficient cell-wall disruption of microalgae for all

applications involving homogenization (Cheng et al., 2013), ultrasonication (Ciudad et al., 2013), microwave (Daly et al., 2011), solvent (Dong et al., 2015), acid/base (Kim et al., 2015), fenton chemical (Siew et al., 2008), hydrolytic enzyme (Taskova et al., 2006) and supercritical CO₂ (ScCO₂) (Wang et al., 2015). Current common technology in cell-wall disruption of microalgae struggles to minimize bioactive destruction and efficiency of the process.

Advanced oxidation processes (AOP), base to their high oxidation potentials, have great potential to be the most efficient solution for the cell-wall disruption of

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microalgae. AOP is aqueous phase oxidation methods consisting of highly reactive species used in the oxidative cell-wall disruption of microalgae. AOP creates a more powerful and less selective secondary oxidant, hydroxyl radicals, in the water (Gottschalk et al., 2000; Li et al., 2010; Zaikov and Rakovsky, 2009). The secondary oxidant can cause the oxidation of most cell-wall organic compounds until they are mineralized as carbon dioxide and water. The hydroxyl radical (2.87 eV) has much higher oxidation potential than ozone (2.07 eV) or hydrogen peroxide (1.78 eV) and usually reacts faster, thus leading to smaller contact time and carbon footprint (Barsanti and Gualtieri, 2018; Gracia et al., 2000; Guo et al., 2012; Posten and Chen, 2016). Common AOP systems use or combine ozone, UV, hydrogen peroxide to create hydroxyl radicals. The high cost and potential for contamination due to the use of UV, hydrogen peroxide in the AOP process to produce hydroxyl radicals must be avoided (Rame et al., 2017).

Catalytic ozonation has been demonstrated to be an effective and efficient advanced oxidation processes used in industry and wastewater treatment for removal of colour (Rame et al., 2017), gaseous pollution (Mastan et al., 2012), pesticides, pharmaceuticals (Rame et al., 2018) and pathogen (Wu et al., 2016). However, its usage for microalgae production is limited by the fast release of the hydroxyl radical ($\bullet\text{OH}$) if natural microalgae concentrations are not significant.

State of the art AOP systems bases catalytic ozonation developed by this paper is on the use of microporous carbon-supported titanium oxide and ozone to create hydroxyl radicals, the ultimate oxidant for cell-wall disruption of microalgae. Ozone is produced when oxygen molecules (O_2) are split into atomic oxygen (O) then recombined into ozone (O_3) (Gottschalk et al., 2000; O'Donnell et al., 2012; Zaikov and Rakovsky, 2009). In this research, ozone is produced when a gas containing oxygen is passed through an electrical field separated by two electrodes. When oxygen molecules in the gas interact with the electrical field, they split and recombine forming ozone. This process is the corona discharge ozone generation method. Catalytic ozonation system uses microporous

carbon-supported titanium oxide to produce hydroxyl radicals from ozone in water, making it the most effective method for hydroxyl radicals production in industrial applications.

This study investigated several parameters for cell-wall disruption from microalgae *Chlorella vulgaris* during catalytic ozonation over microporous carbon-supported titanium oxide, including flow ozone, catalytic time and reactor capacity. To assess whether the parameter changes the effectiveness of the cell-wall disruption, at the same time, the cell-wall disruption yield and an active compound yield such as chlorophyll and carotenoid were evaluated for each pretreatment. The microalgae *Chlorella vulgaris* was chosen because it is the second largest microalgae used after *Spirulina*.

2. METHODS

2.1. Materials

Chlorella vulgaris was obtained from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. Briefly, *Chlorella vulgaris* were cultivated with sea water on the open pond (Jepara). *Chlorella vulgaris* were fed daily with nutrient and aeration periodically. *Chlorella vulgaris* used in the catalytic ozonation experiment was harvested after 6 days of cultivation.

2.2. Experiments

The experimental set-up for the ozonation was based on a 10 g/h ozone generator from BUMA, Semarang, Indonesia, which was supplied with dry oxygen gas. A diffuser was used to disperse the generated O_3 into a collection bottle.

The experimental design used a central composite design (four levels) with three variables: flow ozone, catalytic time and reactor capacity. The flow ozone set at three different levels: 1, 2, 3, 4 liters per minute, catalytic time of 1,2,3,4 minutes and reactor capacity 500 mL, 2000 mL, 10000 mL.

In this study catalytic ozonation of microporous carbon-supported titanium oxide were used to determine the effect of approach on bioactive destruction and cell-wall disruption of microalgae *Chlorella vulgaris*.

2.3. Analysis

2.3.1. Quantification of ozone concentration

The output O₃ concentration of the ozone generator was measured with a UV-Vis benchtop at λ 260 nm in a quartz cuvette. Specific volumes of the O₃ stock solution were added in each batch bottle to give the desired O₃ concentration. Ozone gas flow is regulated by flow meter. Ozone gas flows for 30 seconds with an ozone flow of 1 liter per minute in each batch bottle. The actual concentration O₃ gas dissolved in water was quantified with the titrimetric method. The O₃ concentration was analyzed by two different methods. Spectroscopy analysis was performed by benchtop O₃ meter for gas phase, while the titrimetric method was used for quantitative analysis for aqueous phase (Gottschalk et al., 2000; Zaikov and Rakovsky, 2009).

2.3.2. Cell-wall disruption

We performed spectroscopy analysis using scanning electron microscope (SEM) and transmission electron microscopy (TEM) to obtain data of cell-wall disruption microalgae *Chlorella vulgaris*.

2.3.3. Bioactive analysis

Determination of bioactive concentration was performed via spectroscopy UV/Vis, which was measured in a UV/Vis detector (based on the method of Tsaloglou, 2016). The carotenoid was detected by the detector at λ 452 nm, chlorophyll was detected by the detector at λ 663 nm and a specific pattern was detected by the detector at λ 490 nm (Cuellar-bermudez et al., 2014; Posten and Chen, 2016; Tsaloglou, 2016).

3. RESULT AND DISCUSSION

Spectroscopy UV/Vis analysis was performed to find out the bioactive concentration from *Chlorella vulgaris*. Analysis visible spectroscopy was done for calculation of chlorophyll concentration, specific pattern and carotenoids, also cell-wall disruption level. The results of the cell-wall disruption of microalgae *Chlorella vulgaris* using spectroscopy UV/Vis is shown in **Figure 1** and **Figure 2**.

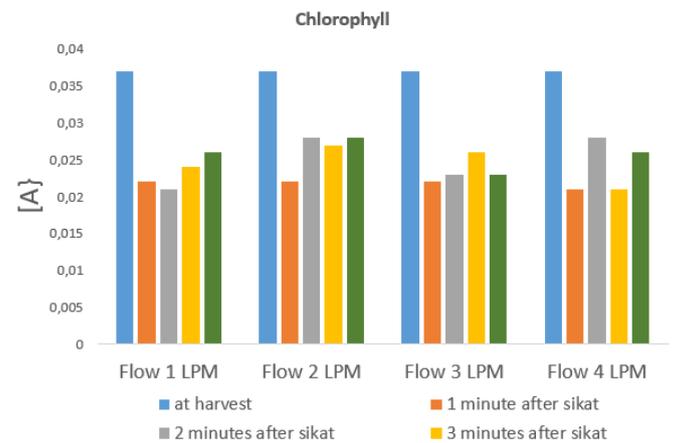


Figure 1. Data characterization of chlorophyll of the *Chlorella* from the ozonation catalytic from minute 0 to minutes 4 was analyzed on the day of harvesting, after 1 minute of ozonation catalytic and after 2, 3, 4 minutes of further ozonation catalytic

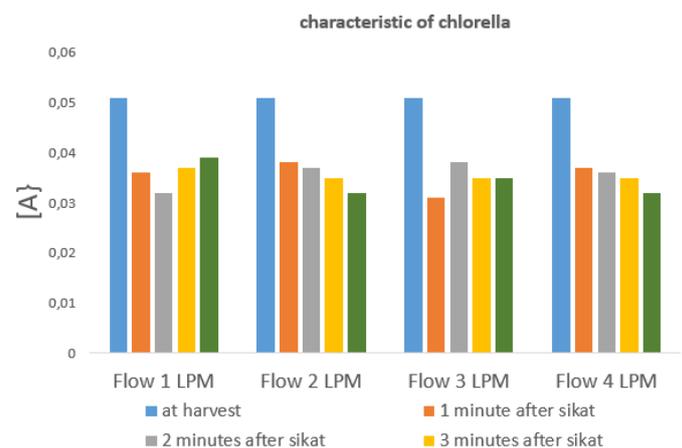


Figure 2. Characterization of the specific pattern of the *Chlorella* from the catalytic ozonation from 0 to 4 minutes on the day of harvesting

The results of microalgae testing using a scanning electron microscope (SEM) is shown in **Figure 3**.

SEM images of samples microalgae are shown in **Figure 3**: from *Chlorella vulgaris* cells before treatment (pictured left) and prepared from *Chlorella vulgaris* cells after treatment (pictured right). After treatment with catalytic ozonation, *Chlorella vulgaris* split into several pieces. This indicated that cell-wall of microalgae was catalytically disrupted by ozonation. In the sample prepared before treatment, the cell shape is still intact and the cell wall

surrounds the cells evenly with size distribution centered around 3.35 μm was observed.

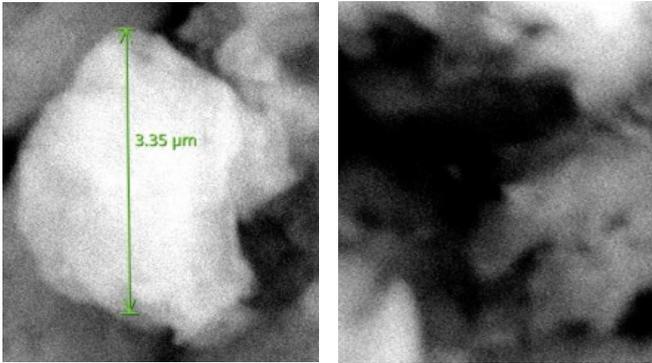


Figure 3. SEM image of *Chlorella vulgaris* cells after augmented aquades (left) and after catalytic ozonation with MCTO catalyst (right)

Microalgae test results using transmission electron microscopy (TEM) is shown in Figure 4.

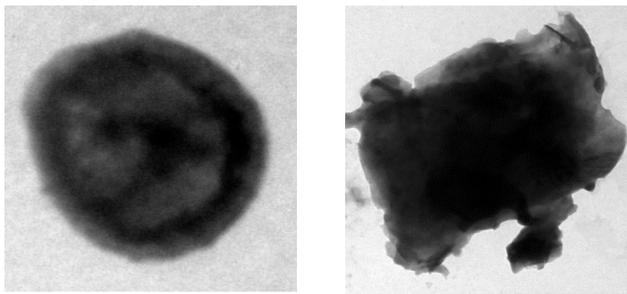


Figure 4. TEM image of *Chlorella vulgaris* cells without (left) and with catalytic ozonation treatment (right)

The TEM image of the microalgae samples is shown in Fig. 4. In the sample prepared from *Chlorella vulgaris* cells before treatment (pictured left), the cell is still intact, where the cell shape is still intact and the cell wall surrounds the cells evenly were observed. In the sample prepared from *Chlorella vulgaris* cells after treatment (pictured right), only left part of the cell which has changed, where the cell shape is not complete and the cell wall surrounds the cell unevenly were also observed. A similar result has been obtained by (Zheng et al., 2011) that disrupted by microwaves and grinding in liquid nitrogen. This indicated that there has been cell-wall disruption of microalgae *Chlorella vulgaris* by catalytic ozonation.

This paper is covering the cell-wall disruption and characterization of bioactive from *Chlorella vulgaris* using

catalytic ozonation. In order to study the method of extraction of bioactive, the biomass from own culture with well-known characteristic is essential. It due to the content of the pigment in the biomass itself determine the efficiency of the method. In this study, we used specific pattern concentration extracted from *Chlorella vulgaris* to assess the cell disruption efficiency. The results showed that specific pattern concentration represented the disruption efficiency. Previous research found the shortest disruption time was 2 minute by grinding in liquid nitrogen and the disruption efficiency of *Chlorella vulgaris* were 100% (71.76% unsaturated and 28.24% saturated fatty acid) respectively (Zheng et al., 2011). But chemical using dimethyl carbonate found disruption efficiency of *Chlorella vulgaris* were 82,90% (Young et al., 2018) and acid treatments were approximately 86% (Chao et al., 2018), also ScCO_2 extraction using ethanol (10% v/v) as co-solvent led to the efficiency of 97% (Sara et al., 2018).

Our study found out that catalytic ozonation with an appropriate dosage disrupted the cell wall of *Chlorella vulgaris* which disruption efficiency of *Chlorella vulgaris* was 76.47% in 1 minute. The processing time was a bit fast, and not require a relatively stable reaction temperature or other condition. This cell disruption method is also easy to scale-up. The cell wall of *Chlorella vulgaris* only contains three component, algenan base layer, fibrillar layer and cell membrane (D'Hondt et al., 2018). The lack of components and the thinness of the cell wall of *Chlorella vulgaris* cause the cell wall to be easily destroyed compared to other microalgae. Excess hydroxyl radicals in the catalytic ozonation process can destroy bioactive microalgae compounds. This causes a decrease in cell-wall disruption efficiency. The similar result has been obtained by present study provides the impact of pulsed electric fields and high-pressure homogenization treatments on the disintegration efficiency of *Chlorella vulgaris* were relatively low and did not exceed 5.2% (Daniele et al., 2018).

Evaluation of cell-wall disruption of microalgae *Chlorella vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide (Figure. 1 and 2). (a) A specific pattern of *Chlorella* in the output

decreased until 76.47%, to 0.037 from highest input 0.051 in catalytic ozonation with flow of 4 liters per minute for 1 minutes. (b) chlorophyll decreased from 0.037 to 0.028 and 75.67% in catalytic ozonation with flow of 2 liters per minute for 2 minutes. (c) carotenoids from input decreased from 0.055 to 0.054 on output. Whilst the efficiency 98.18 % in catalytic ozonation with flow 1 liters per minute for 1 minute. Carotenoids were trapped around cell-wall and very difficult to destroy (Posten and Chen, 2016)(d) Also, the chlorophyll from input decreased from 0.037 to 0.021. Whilst the efficiency 56.75 % in catalytic ozonation with flow of 4 liters per minute for 1 minute. (e) The number of chlorophyll from input decreased from 0.037 to 0.022 on output. Whilst the efficiency 59.49% at catalytic ozonation with flow 1 liters per minute for 1 minute. Catalytic ozonation is very reactive to organic such as chlorophyll (Zaikov and Rakovsky, 2009) (f) carotenoids decreased from 0.055 to 0.049 and 89.09% in efficiency at catalytic ozonation with flow of 4 liters per minute for 1 minute. (g) The percentage difference of bioactive concentration from reactor capacity 500 mL, 2000 mL, 10000 mL was no significant difference. These data further indicate that cell-wall disruption of microalgae *Chlorella vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide until 10000 mL are functional.

Altogether, we have presented a promising approach to cell-wall disruption of *Chlorella vulgaris*, with reduced process-time, handling steps and chemical free. This system is easily scalable and can be adapted to automatization and online control. This study has the potential to bring the environmentally friendly, green technology and efficient of manufactured bioactive of *Chlorella* one step closer to reality. The experiment took 1 minute to get 76.47% cell-wall disruption of *Chlorella vulgaris*. No special handling steps are needed for catalytic ozonation processes. Because the harvest of *Chlorella vulgaris* is already in the solution phase. The catalytic ozonation unit only requires oxygen gas as an input ozone generator and microporous carbon-supported titanium oxide as a catalyst classified as environmentally friendly.

4. CONCLUSION

The required delivered flow ozone to achieve 76.47% cell-wall disruption of *Chlorella vulgaris* was 1 minute at 4 liters per minute, which produced chlorophyll 56.75% and carotenoid 89.09%. Microporous carbon-supported titanium oxide reduces the required O₃ dose and catalytic time for cell-wall disruption. However, it limited chlorophyll yield did not exceed 75.67%. Pretreatment with 1 minute at 1 liter per minute in 2 liters produced carotenoid yield by approximately 98.18%, though it reduced chlorophyll to 59.45%. Catalytic ozonation over microporous carbon-supported titanium oxide was found to be very efficient of cell-wall disruption *Chlorella vulgaris* and minimize bioactive destruction.

ACKNOWLEDGEMENTS

This work was supported by the Agency for Research and Industry (BPPI), a grant funded by the Indonesia government Ministry of Industry (2017). The authors are grateful to Hadi Pranoto, for his assistance and insightful input on the catalytic ozonation and the technicians laboratory for their assistance with the analysis.

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