

Cell-wall disruption and characterization of phycocyanin from microalgae: *Spirulina platensis* using Catalytic ozonation

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Abstract. *Spirulina* is one key material in pharmacy and nutraceutical industries. The cell-wall disruption of *Spirulina* have been studied for many years, resulting in diverse methodologies with a range of yields and grades of quality. In this paper we report the cell-wall disruption and characterization of phycocyanin from *Spirulina platensis* using catalytic ozonation. Various parameters of cell-wall disruption such as flow ozone and catalytic time of the reaction were varied to identify the optimum ones. We obtained the phycocyanin with homogeneous size distribution and high ratio could be obtained at flow ozone of 4 LPM, catalytic time of 1 minute and 89.31% cell-wall disruption yield with 90% phycocyanin yield. The method reported here is very attractive and potential for production of large scale phycocyanin from microalgae for industrial applications.

Keywords: Cell-wall disruption; phycocyanin; spirulina; catalytic ozonation.

1 Introduction

Spirulina have a large potential for producing valuable substances for the feed, food, cosmetics and pharmacy industries[1]. Microalgae spirulina are industrially important microorganisms that have been studied largely for bioproducts. Microalgae have been mainly considered for large-scale commercial production, especially for *Spirulina*. *Spirulina* is rich in linolenic acid and a good source for polyunsaturated fatty acid (PUFA). *Spirulina* contains many pigments, including chlorophyll a, xanthophyll, beta-carotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, osceol-xanthin, c-phycocyanin, and allophycocyanin. In addition, *Spirulina* contains about 13.5% carbohydrates, which is mainly composed of glucose, along with rhamnose, mannose, xylose, galactose, and two unusual sugars, including 2-O-methyl-L-rhamnose and 3-O-methyl-L-rhamnose.

Spirulina also contains over 10-fold more beta-carotene than any other food, including carrots, and more vitamin B12 compared to any fresh plant or animal food source. Compared to green algae, spinach, and liver, this genus represents the richest source of vitamin E, thiamine, cobalamin, biotin, and inositol [2]. Among these compounds, there is C-phycocyanin (C-PC), a photosynthetic pigment found in *Spirulina*. Phycocyanin is composed of a linear prosthetic groups (bilins) that are linked to specific cysteine residues. Phycocyanin is

contain of an apoprotein and a non-protein component (corresponding to the chromophore moiety) known as phycocyanobilin (PCB) [3].

Phycocyanin has been widely studied as nutraceutical and health properties that can be used in many industrial applications. In this respect, the use of spirulina and phycocyanin as food supplements for showing antitumor, anti-inflammatory, hepatoprotective, and other health-promoting properties. Other potential industrial applications of spirulina and phycocyanin are used as a natural dye and a good alternative to highly toxic and carcinogenic artificial colorings. Because of its potentially high commercial value, many studies have been directed to improve the efficiency and economics of extraction, isolation and purification processes to produce phycocyanin[2].

Spirulina is known to accumulate high concentrations of phycocyanin. The value of 1 kilogram of natural phycocyanin has been estimated as high as \$500[4]–[6]. In microalgae-based biorefinement, high-energy and cost-intensive downstream processes such as cell disruption and intracellular products extraction are considered to be major techno-economic bottlenecks.

Recently, the extraction of intracellular phycocyanin from *Spirulina*, one of most desirable alternative organic nutraceutical, has been a primary focus due to several advantages including high protein productivity, proper nutrition composition for supplement, robustness under outdoor culturing conditions, as well as applications to industrial flue gas utilization and wastewater treatment.

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On the other hand, tons of *Spirulina platensis* biomass has been produced annually as a natural source of the high-value strong antioxidant, phycocyanin.

The cell-walls of *Spirulina platensis* probably represent the biggest barrier to target-phycocyanin extraction. Multi-layered cell-walls in *Spirulina platensis* inhibit conventional organic solvents such as hexane's entry to the cell, thereby preventing the proper contact between the organic solvent and intracellular /phycocyanin component. Many cell-wall disruption methods have been reported for microalgae in order to maximize the extraction efficiencies of target bioproducts such as phycocyanin. However, the selection of appropriate cell disruption and extraction methods is largely dependent on the cell-wall characteristics. The efficiencies of those methods, moreover, are affected significantly by operational conditions such as technology, temperature, pressure, biomass condition and scale.

The development of cell-wall disruption responsible for phycocyanin extraction is an important step in advancing process technologies towards future commercialization of phycocyanin. Many cell-wall disruption methods such as homogenization, ultrasonication, microwave, solvent, acid/base, fenton chemical, hydrolytic enzyme and supercritical CO₂ (ScCO₂) is widely used in studies aimed to develop efficient cell-wall disruption process for phycocyanin extraction [7]–[12]. Although technical scale cell-wall disruption for phycocyanin extraction had been evaluated, a systematic study to examine efficiency parameters important for phycocyanin extraction is still lacking and much needed. This is especially imperative in view of the low yields phycocyanin obtained in a recent study. Catalytic ozonation is a process of AOP (Advanced Oxidation Processes) has been widely utilized in the industry [13]–[17].

We report in this paper our in-depth research on flow ozone preference and catalytic time to improve the efficiency of cell-wall disruption from microalgae spirulina. The information resulted from this research is very attractive and potential for production of large scale phycocyanin from microalgae for industrial applications. This approach is very critical for future development of sustainable cell-wall disruption processes that use low-cost to produce phycocyanin, which is needed to ensure commercial viability of production.

2 Materials and Method

Microalgae (*Spirulina platensis*) biomass contained 15% moisture content was obtained from CV. Neoalga (Sukoharjo, Indonesia). The biomass was stored under dry and dark conditions.

The experimental design used a central composite design (four levels) with two variables: flow ozone and time of catalytic. The total run was 16 trials. The flow ozone set at three different levels: 1, 2, 3, 4 LPM and catalytic time of 1,2,3,4 minutes.

In this study catalytic ozonation of 10 g/hour kHz ozone and platinum catalyst with a power of 200 W were used to determine the effect of the frequencies on phycocyanin yield and cell-wall disruption of microalgae

Spirulina platensis. The conventional ozone method was also conducted to compare yield and cell-wall disruption data. We performed digital microscope analysis and spectroscopy analysis using visible spectrophotometer, scanning electron microscope (SEM), transmission electron microscopy (TEM) to obtain data of phycocyanin yield and cell-wall disruption yield of microalgae *Spirulina platensis*.

3 Results

The results of microalgae observation using digital microscope:

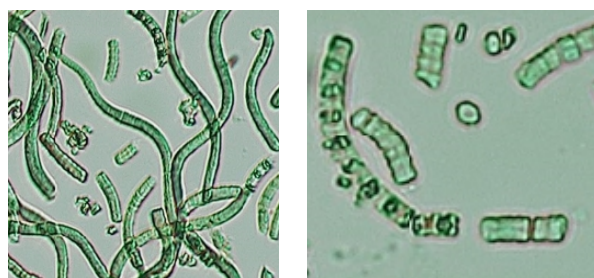


Fig. 1. Digital microscope analysis of *Spirulina platensis* liquid phase (left) and after catalytic ozonation with Pt catalyst (right).

The results of microalgae testing using scanning electron microscope (SEM):

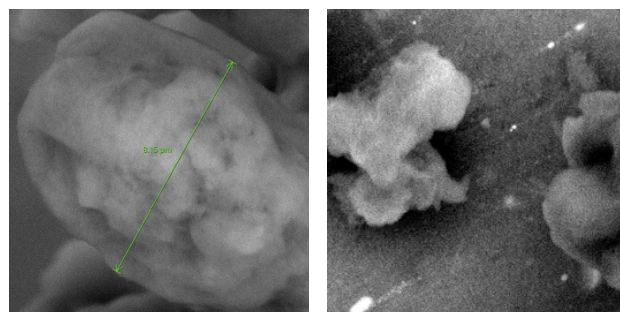


Fig. 2. SEM image of *Spirulina platensis* cells after augmented aquades (left) and after catalytic ozonation with Pt catalyst (right).

Microalgae test results using Transmission electron microscopy (TEM):

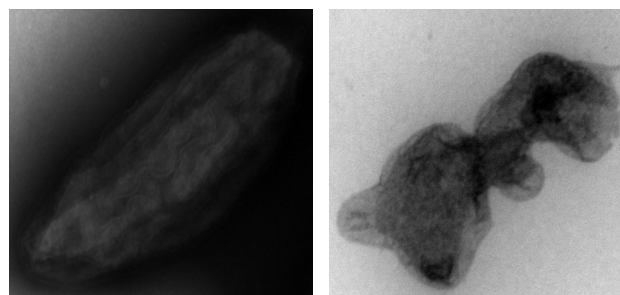


Fig. 3. TEM image of *Spirulina platensis* cells before treatment (left) and after catalytic ozonation (right).

4 Discussion

We observed a significant increase in the phycocyanin yield and cell-wall disruption yield of microalgae *Spirulina platensis* when using platinum catalyst for catalytic ozonation. This observation contradicted with the results reported previous research [7]–[12]. This difference was likely caused by difference in previous research was many stages, more chemical, and more energy consumed. Previous research used homogenization, ultrasonication, microwave, solvent, acid/base, fenton chemical, hydrolytic enzyme and supercritical CO₂ (ScCO₂). Although no industrial analysis was reported by previous research.

However we worried lots of energy, chemicals, and time wasted during process. Microwave and supercritical CO₂ requires very high investment costs, a lot of energy and resource competencies with less satisfactory results [6], [18]–[20]. On the other hand, solvent, acid/base and hydrolytic enzyme requires chemicals with high purity and setting conditions that are quite difficult to achieve [6], [11], [21], [22]. Low cell-wall disruption yield was confirmed using homogenization, ultrasonication, and fenton chemical [6]. Our results was consistent with green industry, where using materials that are environmentally friendly, low energy, and low cost.

The successful isolation of the desired phycocyanin is determined by the disruption of the microalgae cell wall at the catalytic reactor used. Characterization of microalgae cell walls is a very important information of this research. To obtain microalgae cell wall-disruption data, visible spectrofotometer, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were done. The evaluation of the research was determined based on visible spectrofotometer test results of phycocyanin content on various operating conditions. The result of catalytic ozonation is optimum if the amount of phycocyanin obtained to the raw material and other operational elements is obtained the highest value based on the data analysis from the visible spectrofotometer test results.

Digital microscope observation was performed to find out the breakdown of cell wall as shown in Fig. 1. *Spirulina platensis* cells before treatment (picture left) appears intact as filamentous nondifferentiated and spiral-shaped. In the sample prepared from *Spirulina platensis* cells after treatment (picture right), only left part of the cell and the cell wall surrounds the cell unevenly were also observed. Analysis visible spectroscopy was done for calculation of ficocyanin concentration and cell-wall disruption level. The optical properties of fikosianin were measured using a spectrophotometer at 615 nm and 652 nm wavelengths, the optical density (OD) values obtained at each wavelength were used to determine the concentration of fikosianin [12].

SEM images of samples microalgae are shown in Fig. 2: from *Spirulina platensis* cells before treatment (picture left) and prepared from *Spirulina platensis* cells after treatment (picture right). After treatment with catalytic ozonation, *Spirulina* formed flakes smaller than 2 μm in size. This indicated that there has been activity cell-wall disruption of microalgae *Spirulina platensis* by catalytic ozonation. In the sample prepared before treatment, the cell is still intact, where the cell shape is still intact, and the cell wall surrounds the cells evenly with size distribution centered around 6 μm was observed. The TEM image of the microalgae samples are shown in Fig. 3. In the sample prepared from *Spirulina platensis* cells before treatment (picture 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 *Spirulina platensis* cells after treatment (picture 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. This indicated that there has been activity cell-wall disruption of microalgae *Spirulina platensis* by catalytic ozonation.

Based on the result herein gathered, a protocol was proposed with a one-step cell-wall disruption process in to obtain both a good phycocyanin yield and a high cell-wall disruption. This was accomplished by using an aqueous catalytic ozonation with a Pt catalyst, giving phycocyaninin a yield of 90 % and 89.31 % cell-wall disruption yield, thus better the results of previous research. It can be clearly seen that the result value derived from phycocyaninin in the form of crude, so further research is needed for phycocyanin purification.

Summary

To maximize the phycocyanin benefits that may be obtained from microalgae *Spirulina platensis*, that essential to seek innovative methods for their cell-wall disruption, and thus preserve the valuable phycocyanin. The current research achieve good phycocyanin yield and cell-wall disruption yield of microalgae *Spirulina platensis*. The results showed the breakdown of microalgae cell walls can be done through the process of catalytic ozonation. The best catalytic ozonation operating conditions on *Spirulina platensis* with Pt catalyst at ozone 4 LPM flow for 1 minute with 89.31% cell-wall disruption yield and 90% phycocyanin yield. Electricity consumption for catalytic ozonation operations is relatively low compared to other technology so that it can be an alternative in the effort to realize the development of low carbon communities in the microalgae industry.

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