Agrotechnology
ISSN: 2168-9881

Hirayama, Agrotechnology 2017, 6:3
DOI: 10.4172/2168-9881.1000171

Research Article Open Access

Decolorization of Organic Compounds by Controlled Microalgal Culture

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Abstract

This study demonstrates that hydroxyl radicals (·OH) are generated by *Chlorella vulgaris* under culture conditions where only air is used for aeration, and that these free radicals can be used to degrade organic compounds. Methylene blue was used as a model organic compound and added to a *Chlorella* culture illuminated with visible light. As a result, methylene blue was essentially bleached entirely. Decolorization was likely due·OH because it was ameliorated by the addition of a radical scavenger (ethanol). Because this system does not require high-energy ultraviolet irradiation or hydrogen peroxide, it is more environmentally friendly than current wastewater treatment methods. Based on these results, we propose a system for decomposing organic matter in a micro algae culture tank, which has a lessened environmental impact than current methods.

Keywords: Microalgal cultivation; Hydroxyl radical; Visible light; Decolorization; Decomposition; Lessened environmental impact

Introduction

Decomposition or decolorization of organic compounds, especially dyes, by ozone and various other active oxygen species has been used for wastewater treatment. Among these species, hydroxyl radicals (·OH) have been reported to be a key to the decomposition of organic compounds due to their high reactivity [1-4].

The reactions depicted in equations 1 and 2 are well known producers of ·OH, and effective decomposition of organic compounds has been reported due to their use [4].

$$Fe^{2+} + H_2O_2 \rightarrow + Fe^{3+} + \cdot OH + OH^- \quad (1)$$

$$H_2O_2 + h\nu \rightarrow \cdot OH + \cdot OH \quad (2)$$

When Fenton's reaction (Equation 1) is used for decomposition or decolorization of organic compounds, Fe^{2+} ions must be added to H_2O_2. Also, the addition of these compounds becomes a factor of increasing COD from the environmental viewpoint. Additionally, irradiation of hydrogen peroxide with ultraviolet radiation to generate ·OH (Equation 2) requires large amounts of energy.

Therefore, the author has been seeking a method of ·OH generation that does not require H_2O_2 or ultraviolet irradiation, and describes here the generation of ·OH by photosynthesis occurring in microalgae.

If this phenomenon can be applied to decomposition or decolorization of organic compounds, especially dyes, it may not be necessary to use high-energy ultraviolet rays or H_2O_2 for wastewater treatment. The author has been detecting ·OH in the microalgae at visible light condition. Since this phenomenon utilizes visible light, it can be considered that it can be ·OH generation system with less energy consumption compared to ultraviolet irradiation. Therefore, this microalgae method can be used for decomposing or decoloring organic compounds, especially dyes, and is considered to be a system having characteristics of both lower energy consumption and less environmental impact.

Materials and Methods

Cultivation of microalgae

The microalgae *Chlorella vulgaris* var. *vulgaris* was used in this study. Culturing was performed in a vessel with a height of 370 mm, width of 120 mm, and a depth of 28 mm. The culture was illuminated with visible light using a fluorescent lamp at 290 μmol $m^{-2}s^{-1}$. The growth medium contained 5 g KNO_3, 1.25 g KH_2PO_4, 0.1 g K_2HPO_4, 2.5 g $MgSO_4 \cdot 7H_2O_2$ 1.8 g NaCl, 2.8 mg $FeSO_4 \cdot 7\ H_2O$, and 1 ml of trace-metal mixture A_5 solution in 1L of deionized water. Trace-metal mixture A_5 solution contained 2.86 g H_3BO_3, 1.81 g $MnCl_2$, 0.22 g $ZnSO_4 \cdot 7H_2O$, 80 mg $CuSO_4 \cdot 7H_2O$, 21 mg Na_2MoO_4, and one drop of concentrated H_2SO_4 in 1L of deionized water. Cultures were grown at 25% and continuously aerated with air or air containing 0.5% CO_2.

Reagents and estimation of decolorization

The spin trapping reagent 5, 5-dimetyl-1-pyrroline N-oxide (DMPO) was purchased from Labotec., Ltd.(Japan). All other reagents were of the highest grade commercially available. Methylene blue was used as a model organic compound and separated from the *Chlorella* culture broth by centrifugation at 2,000 x g for 10 min. The optical density of the supernatant was determined at methylene blue absorbance maximum of 661 nm using a spectrophotometer. Decolorization was determined by a reduction in this absorbance and the percentage of decolorization was calculated as follows:

Percentage decolorization = (Initial Absorbance – Final Absorbance) x 100/Initial Absorbance

Equipment

A model RE-3X ESR spectrometer (JEOL) was used for ·OH detection, using an aqueous quartz flat cell (inner dimensions, 60 mm x 10 mm x 0.31 mm) and the spin trapping reagent 5, 5-dimetyl-1-pyrroline N-oxide (DMPO). Mn^{2+} cations fixed in the ESR cavity were used as an internal standard to calculate relative amounts from the ESR signal intensity. The *g* values of the peaks were 2.0334 and 1.9810 at the

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Received November 13, 2017; **Accepted** December 12, 2017; **Published** December 19, 2017

Citation: Hirayama S (2017) Decolorization of Organic Compounds by Controlled Microalgal Culture. Agrotechnology 6: 171. doi: 10.4172/2168-9881.1000171

resonance frequency of 9450.0MHz. ESR conditions were as follows; microwave power, 6 mW; modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; response time, 0.03 sec; gain, x 200–790; and sweep times, 2.5 mT/min.

Results

Analysis of hydroxyl radical behavior by the spin trapping method

To determine factors that could increase ·OH formation, two *Chlorella* cultures, whose turbidities were both adjusted to 6.1 at OD_{750}, were illuminated with visible light, while aerating with air or air containing 0.5% CO_2.

Using ESR to monitor the cultures, only DMPO–OH adducts, which were assigned as the hydroxyl radical (DMPO–OH; aN = 1.49mT, aH = 1.49 mT), were observed in the absence 0.5% CO_2 (Figure 1) [5,6] and the intensity of this signal increased slowly with time [7,8].

Conversely, the presence of 0.5% CO_2 resulted in a decrease in DMPO–OH adducts, as shown in Figure 2.

Therefore ·OH production is substantially more efficient in the presence of air than that in not absence of 0.5% CO_2 (Figure 3). In addition, 5% CO_2 also led to a decrease in DMPO–OH adducts [9]. These data suggest that the ·OH generated in *Chlorella* culture aerated with air might be able to decompose or decolorize organic compounds within the culture medium.

Decolorization of methylene blue by microalgae *Chlorella vulgaris* cultivation

To obtain higher growth rates of *Chlorella* cells, algal cultures were continuously aerated with air mixed with 0.5% CO_2. Methylene blue was then added to act as a model organic compound and a target for oxidation by the generated radicals. Methylene blue color intensity

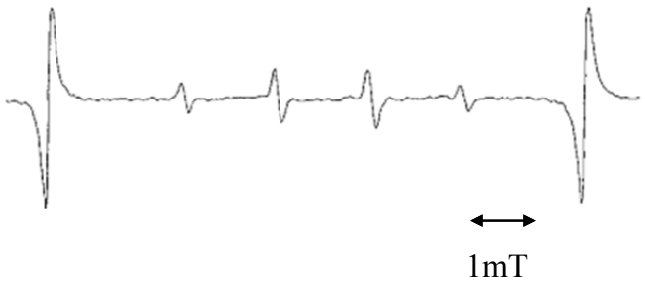

Figure 1: ESR spectra of spin adducts of ·OH produced using visible light irradiation of *Chlorella* cultures without 0.5% CO_2 aeration.

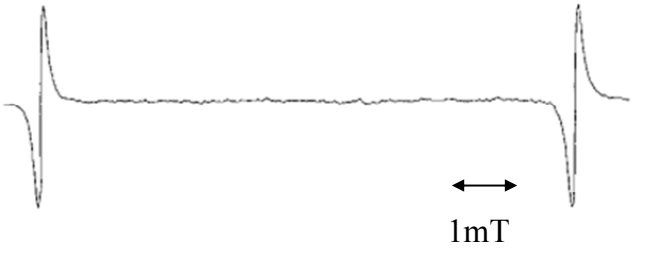

Figure 2: ESR spectra of spin adducts of ·OH produced using visible light irradiation of *Chlorella* cultures with 0.5% CO_2 aeration.

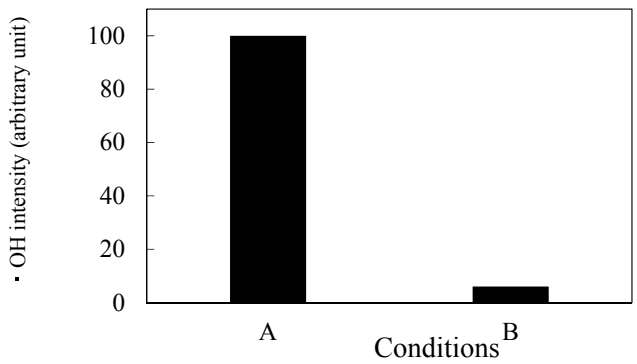

Figure 3: Comparison of ·OH intensities under two conditions of *Chlorella* culture. Cultures were aerated with either air (A), or air + 0.5% CO_2 (B).

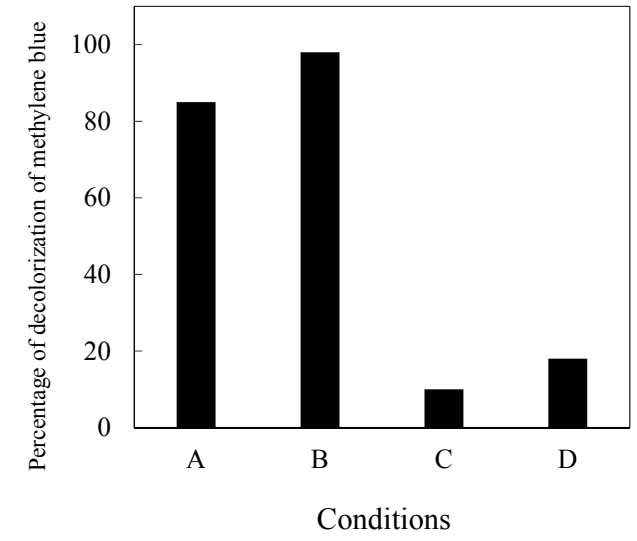

Figure 4: Decolorization of methylene blue by *Chlorella* microalgal culture. Data show the percentage of methylene blue decolorization under different conditions: (A): air for 60 min; (B): air for 120 min; (C): air + 0.5% CO_2 for 60 min; (D): and air + 860 mM ethanol for 60 min

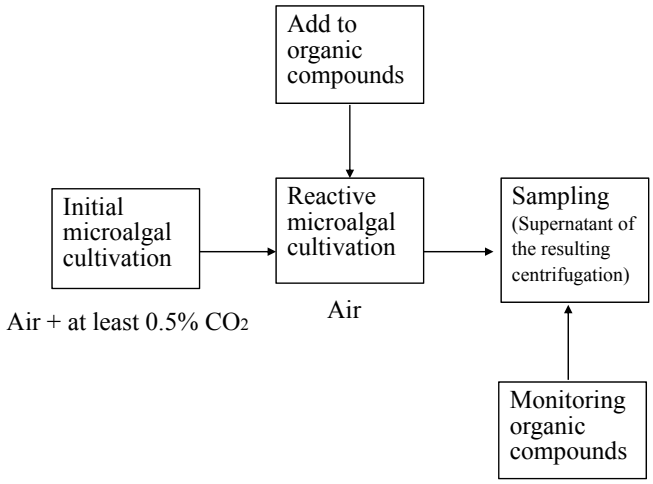

Figure 5: System for the decolorization of organic compounds system using microalgal cultivation.

was measured at 60 min and 120 min following addition. The intensity decreased at 60 min, and the percentage of decolorization at 120 min was approximately 97%. Supplementation of air with 0.5% CO_2, however, inhibited color bleaching (Figure 4). When 860 mM ethanol, a ·OH scavenger, was added [10] the color of methylene blue in the culture barely changed. In addition, this system was also able to reduce the color of humic acid, which does not decompose readily (data not shown).

These data suggest that ·OH produced by the *Chlorella* was responsible for the color loss and therefore, decomposition of methylene blue in this system. Studies on the mechanism of methylene blue decomposition are now in progress.

Based on the above results, the authors designed an organic compound degradation system using microalgae shown in Figure 5. The proposed degradation technology is depicted in Figure 5 and consists of the following: initial microalgal cultivation, reactive microalgal cultivation, sampling of the supernatant following centrifugation, and monitoring organic compounds.

Discussion

Based on the observation of methylene blue decomposition by microalgae, we propose a new system for the degradation of organic compounds, and particularly the decolorization of chromophores, using microalgal cultivation as previously reported [11-14].

The proposed degradation technology is depicted in Figure 5 and consists of the following: initial microalgal cultivation, reactive microalgal cultivation, sampling of the supernatant following centrifugation, and monitoring organic compounds.

The *Chlorella vulgaris* can be considered as the first candidate for the microalga to be used, but if microalgae which can confirm the occurrence of ·OH, it is not limited to the *Chlorella vulgaris*.

Initial microalgal cultivation utilizes aeration with air containing at least 0.5% CO_2 for the purpose of maximizing growth rate under illumination until microalgal concentration reaches approximately 500 mg/L. Next, the supply of CO_2 is stopped by closing the CO_2 supply valve, and the generation of ·OH is promoted by supplying air. Finally, organic compounds are added to the culture and incubated for least 60 min. The decomposition or decolorization of organic compounds are then monitored at their absorption maxima in a supernatant resulting from centrifugation of the culture medium. When microalgae are cultured in the daytime with this system, ·OH can be generated by irradiating with sunlight. Also, when culturing microalgae at night, ·OH can be generated by irradiating visible light. By using this system at night, it is expected to improve the rate of degradation per day. Moreover, in order to reduce the area of the microalgal cultivator to reduce its size, it can be expected that the deep part of the microalgal cultivator is irradiated with light by an optical fiber to lead the light to the inside of the microalgal cultivator to generate ·OH. Because it is unnecessary to irradiate the sample with high-energy ultraviolet rays or use H_2O_2, this system provides an option for the decomposition of organic compounds, and especially the decolorization of chromophores, which has a substantially lower environmental impact (15, 16).

Conflicts of Interest

No conflict of interests declared.

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