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Short Communication

Harvesting of microalgae cell using oxidized dye wastewater

Yeong Hwan Seo [a], Doyoung Park [a], You-Kwan Oh [b], Sukhwan Yoon [a], Jong-In Han [a,*]

[a] Department of Civil and Environmental Engineering, KAIST, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Clean Fuel Department, Korea Institute of Energy Research, 152 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea

HIGHLIGHTS

- Amine group in oxidized dye wastewater could be utilized as microalgae coagulant.
- Dye having azo group is a better option for amine formation during oxidation.
- Oxidized wastewater and ferric ion had a synergistic effect in harvesting process.

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ABSTRACT

In this study, oxidized dye wastewaters were tested for their potential to be used as a cheap coagulant for microalgae harvesting. Two dyes (methylene blue (MB) and methyl orange (MO)) were selected as model dyes, and the Fenton-like reaction under high temperature (90 °C, 1 min) employed as an oxidative treatment option. A maximum harvesting efficiency over 90% was obtained with both MB and MO at a dilution ratio of 5:1 (dye wastewater: cell culture), when the optimal oxidation condition was 20 mg/L of dye, 1 mM of $FeCl_3$, and 0.5% of H_2O_2 concentration. This phenomenon could be explained by the possibility that amine groups are formed and exposed in oxidized dyes, which act as a kind of amine-based coagulant just like chitosan. This study clearly showed that dye wastewater, when properly oxidized, could serve as a potent coagulant for microalgae harvesting, potentially rendering the harvesting cost reduced to a substantial degree.

1. Introduction

Microalgae is thought of as feedstock of choice for biodiesel production, because this type of microbe grows rapidly and can accumulate high content of lipid. However, the typical concentration of biomass after cultivation is too low for downstream processing to be effective. It is this reason that an increase in biomass concentration, namely harvesting, is prerequisite.

There are a good many techniques that have been developed thus far, such as centrifugation, filtration, air flotation, and flocculation. Among these, flocculation is seen as one of the most implementable options, which is exceedingly simple and relatively cheap (Vandamme et al., 2012). The flocculation of microalgae is preceded differently according to types of flocculants, that is, metal salts like $FeCl_3$ and amine-containing polymers like chitosan. A metal salt can promote the coagulation through the neutralization between positive metal ion and negative cell surface, whereas chitosan does it using amine groups (Ahmad et al., 2011; Seo et al., 2015). Despite their advantage of high efficiency, however, a large amount of chemical consumption and as a result high cost are not easily solvable issues. For example, a typical dosage of ferric salt required for acceptable coagulation is known to be larger than 500 mg per liter of biomass solution (Kim et al., 2015); and chitosan is $10 to $1000 per kilogram depending on the product quality (Mahe et al., 2015). It is therefore of absolute necessity that an alternative flocculant, which is equally effective yet vastly cheap, is sought for such high potential of flocculation to be realized and to become a commercially adopted means of harvesting.

One promising source is wastewater, especially one with oxidized dyes. Dyes are used in various industrial areas such as textile, food, cosmetics, and paper printing, and making up 15% of the total world production of wastewater (Hsueh et al., 2005). From an environmental perspective, the dye wastewater must be treated before disposal, as it contains various toxic structures such as aromatic or azo types. This wastewater is commonly treated via oxidation either biologically or chemically (e.g., via the Fenton-like reaction) (Liu et al., 2013). During the oxidation process, the nitrogen linkage is broken, becoming amine groups with the unique color of the dye disappearing (Houas et al., 2001; Devi et al., 2009; Benetoli et al.,

* Corresponding author. Tel.: +82 42 350 3629; fax: +82 42 350 3610.
E-mail address: hanj2@kaist.ac.kr (J.-I. Han).

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2011). The newly formed amine groups are expected to have flocculating activity, just as a potent amine-based coagulant of chitosan. To the best of our knowledge, the potential of oxidatively treated dye wastewater has never been beneficially utilized thus far.

In this study, a dye wastewater, after being oxidized, was explored for its possibility as a flocculant of microalgae. To this end, artificial waste waters were made using two representative dyes, specifically methylene blue (MB) and methyl orange (MO), and oxidized using the Fenton-like reaction, which has a distinctive advantage of simplicity (Duarte et al., 2009). After oxidization, the efficacy for algae harvesting was tested and conditions were optimized.

2. Methods

2.1. Microalgae and culture conditions

A locally isolated freshwater microalgae species *Chlorella* sp. KR-1 was obtained from the Korea Institute of Energy Research (KIER), and the cultivation conditions were the same as in other previous studies (Lee et al., 2013; Kim et al., 2015). This microalgae species was cultivated at 30 °C in a Pyrex bubble-column reactor (length: 1,180 mm; diameter: 85 mm; working volume: 6 L). The reactor was supplied with 5% (v/v) CO_2 in air at a rate of 0.75 L/min, and filled with a medium having the following composition: KNO_3 3 mM, KH_2PO_4 5.44 mM, Na_2HPO_4 1.83 mM, $MgSO_4{\cdot}7H_2O$ 0.20 mM, $CaCl_2$ 0.12 mM, FeNaEDTA 0.03 mM, $ZnSO_4{\cdot}7H_2O$ 0.01 mM, $MnCl_2{\cdot}4H_2O$ 0.07 mM, $CuSO_4$ 0.07 mM, and $Al_2(SO_4)_3{\cdot}18H_2O$ 0.01 mM. The pH was maintained at around 6.5 during cultivation. A 7 day-old algae culture (1.7 g/L of biomass) was used.

2.2. Preparation of artificial dye wastewater and oxidation

The artificial dye wastewaters were made according to Mo et al. (2007) with a slight modification, and were diluted 10–200 times for a final dye concentration ranging from 5 mg/L to 100 mg/L. The initial dye wastewater contained 1000 mg/L of dye (MB or MO), 2 mM of NaCl, and 2 mM of Na_2SO_4. All chemicals were purchased from Sigma–Aldrich (USA).

To investigate what effect the Fenton-like reaction had on the dye wastewater, experiments were done with various concentrations of $FeCl_3$ (0, 0.5, 0.7, 1, and 2 mM) and H_2O_2 (0.05, 0.1, 0.2, 0.5, and 1%) at room temperature and 90 °C. All samples were adjusted to pH 3.0, which is the optimal pH for a Fenton-like-reaction (Seo and Han, 2014). The degradation of dye wastewater was measured by color removal, and simply calculated with the following equation:

$$\text{Decolorization efficiency } (\%) = (1 - A_t/A_i) \times 100 \quad (1)$$

where A_i is the initial absorbance of the wastewater, and A_t is the absorbance at time t. The absorbances of MB and MO were measured at 664 nm, and 463 nm, respectively (Chen et al., 2014). The amine group formation in oxidized wastewater was qualitatively analyzed according to Song et al. (2015), which was based on 2,4,6-trinitrobenzenesulfonic acid.

2.3. Harvesting of microalgae using degraded dye wastewater

After the Fenton-like reaction, the dye wastewater was sampled and diluted at a ratio between 2:1 and 10:1 (dye wastewater:cell culture, v/v). The cell suspension mixture was shaken for 30 s and left to settle for 30 min. The harvesting efficiency (HE) was calculated with the following equation (Lee et al., 2013):

$$\text{HE } (\%) = \left(1 - \frac{OD_f}{OD_i}\right) \times 100 \quad (2)$$

The OD_i and OD_f were optical densities (OD) of each sample, which was taken at the middle of the tube, before and after harvesting of the microalgae cells. The optical density was measured at 680 nm, and all experiments were done in triplicate. All data had low p-values (<0.05), which were calculated using ANOVA program (state-ease, Inc. USA) (Seo et al., 2015).

3. Results and discussion

3.1. Degradation of dye wastewater

As expected, the Fenton-like reaction was found to effectively degrade the dye wastewater; as long as two Fenton chemicals ($FeCl_3$ and H_2O_2) were simultaneously supplied, the dyes were bleached even with minimal doses in a short time (data was not shown). No significant difference in degradation degree was observed with amounts of Fenton reagents, and only an result at one point (20 mg/L of dye concentration, 1 mM of $FeCl_3$ concentration, and 0.5% of H_2O_2 concentration) is shown in Fig. 1. Most of the reactions were completed within 5 min and the formation of amine group was detected; and the fast reaction rate is the most important advantage, making chemical treatment competitive to the

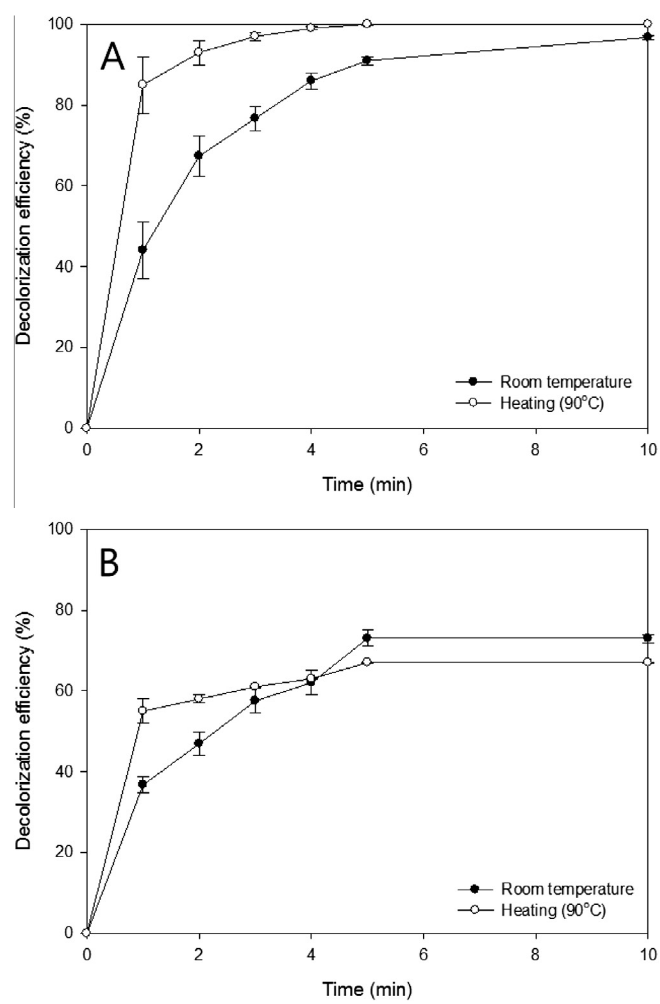

Fig. 1. Decolorization of dye wastewater (20 mg/L) using Fenton-like reaction ($FeCl_3$: 1 mM and H_2O_2: 0.5%) at room temperature and 90 °C. (A) Methylene blue, (B) Methyl orange.

biological method (Szpyrkowicz et al., 2005). The already fast reaction was further accelerated by heating; only 1 min was required for complete degradation. This result is in good agreement with previous studies using heating or microwave to enhance the Fenton reaction (Liu et al., 2013; Seo and Han, 2014). At an elevated temperature, H_2O_2 was rapidly decomposed into OH radicals and increasing the possibility of the reaction between OH radicals and dye molecules to occur. Therefore, the dye wastewater oxidized at 90 °C for 1 min was used for the subsequent microalgae harvesting.

3.2. Harvesting of microalgae cells using degraded dye wastewater

To achieve effective microalgae harvesting, various factors were controlled, and the optimal conditions were found (Table 1). First, the dye concentration and dilution ratio were optimized. Second, the Fenton-like reaction conditions such as $FeCl_3$ and H_2O_2 concentration were controlled. To optimize one factor, other experimental factors were fixed at the optimal conditions.

3.2.1. The effect of the dye concentration and dilution ratio

Wastewaters containing various dye concentrations were oxidized at fixed Fenton-like reaction conditions and used for microalgae harvesting (Fig. 2A). There was an optimal dye concentration of about 20 mg/L at which a higher than 90% harvesting efficiency was achieved. As the dye concentration increased over this dosage, however, the harvesting efficiency greatly decreased; a negligible harvesting effect was obtained with 50 mg/L of MO. An optimal flocculant concentration of about 20 mg/L and the decreasing tendency were almost the same as that with the results from the microalgae harvesting using chitosan (Ahmad et al., 2011). This phenomenon could be explained by the amine group, which has a positive charge at low pH. For a less than optimal dye concentration, the amine group neutralizes the negative charge of the microalgae cell and coagulated the cells effectively. However, the excessive cations by over dosage caused repulsion and hindered the formation of flock (Pan et al., 1999).

The relation between the dilution ratio and harvesting efficiency also showed a similar trend, which had an optimal ratio (5:1, dye wastewater:cell culture, v/v) (Fig. 2B). This was somewhat an expected result because an increase or decrease in the dilution ratio had the same effect as when controlling the dye concentration. At the optimal dye concentration (20 mg/L) and dilution ratio (5:1), which had a harvesting efficiency of over 90%, 1 mg of dye could coagulate 17 mg of microalgae cells. This efficiency was higher than previous results using metal ion coagulants for the same microalgae species (Table 2). When considering the input amount of $FeCl_3$ (1 mM, 162 mg/L) to oxidize the dye wastewater, the dosage of metal ion was greatly reduced, and it can be concluded that dye wastewater could indeed enhance the coagulant efficiency of metal ion coagulants to a substantial degree.

Table 1
Experimental design for microalgae harvesting using oxidized dye wastewater.

							Optimal condition
Dye concentration (mg/L)	0	5	10	20	50	100	20
Dye:Algae solution (v/v)	2:1	3:1	4:1	5:1	10:1		5:1
$FeCl_3$ concentration (mM)	0	0.5	0.7	1	2		1
H_2O_2 concentration (%)	0	0.05	0.1	0.2	0.5	1	0.5

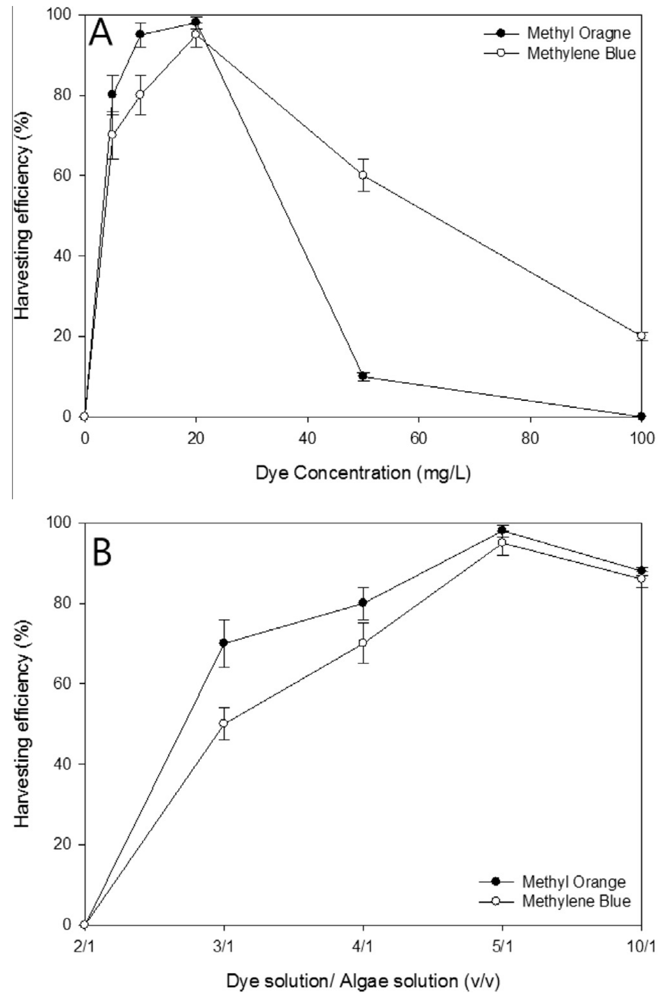

Fig. 2. Microalgae harvesting using oxidized waste water, and different harvesting efficiencies were obtained depending on (A) dye concentration and (B) dilution ratio.

Table 2
Harvesting efficiency using various coagulants for same species *Chlorella* sp. KR-1.

Coagulants	Concentration (mg/L)	Microalgae species	Harvesting efficiency (%)	References
$Al_2(SO_4)_3$	900	*Chlorella* sp. KR-1	60	Lee et al. (2013)
Fe_3O_4	4000	*Chlorella* sp. KR-1	>95	Lee et al. (2014)
$FeCl_3$	500	*Chlorella* sp. KR-1	90	0
$Fe_2(SO_4)_3$	1000	*Chlorella* sp. KR-1	~100	Kim et al. (2015)
Oxidized dye waste water	20	*Chlorella* sp. KR-1	>90	This study

3.2.2. The effect of the Fenton-like reaction condition

To minimize the input amount of $FeCl_3$ to oxidize the dye wastewater, bleached dye made by less than 1 mM of $FeCl_3$ was used for microalgae harvesting (Fig. 3). Unfortunately, microalgae cells were not coagulated effectively even though the color of the dye was completely removed with a low concentration of $FeCl_3$. There might be two possible causes for this: (1) less than 1 mM of $FeCl_3$ was enough to be able to discolor the dye, but not high enough to be able to transform the structure of dye into a form

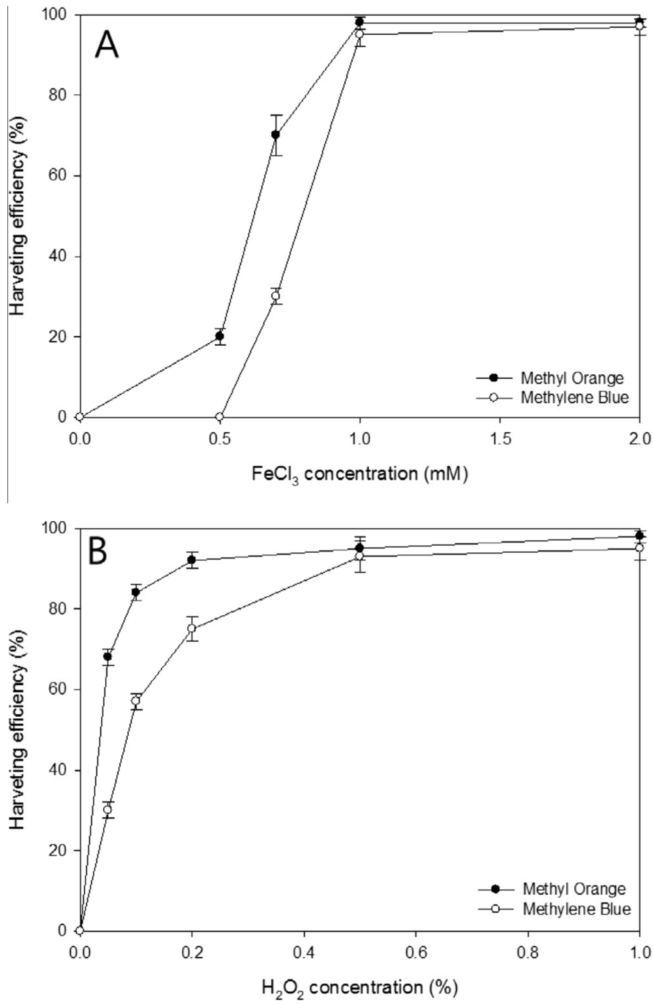

Fig. 3. Oxidized dye wastewaters, which were made by different concentrations of Fenton chemicals, were used for microalgae harvesting. (A) $FeCl_3$ concentration and (B) H_2O_2 concentration.

suitable for microalgae harvesting; (2) the oxidized dye only had the weak coagulating activity ability but the presence of $FeCl_3$ enhanced algae coagulation to a substantial extent.

An evidence of the first hypothetical reason could be found with a difference in harvesting efficiency between MB and MO: at 0.7 mM $FeCl_3$, higher than 70% harvesting efficiency was obtained with MO, whereas only 23% with MB (Fig. 3A). MO has simpler structure than MB, and an N-linkage in the azo group (N=N) is supposedly converted to an amine group more easily, leading to the better coagulation (Devi et al., 2009). For MB to be converted to have an amine group, on the other hand, a ring structure containing an N-linkage has to be first opened; for that, hydroxylation must be preceded (Fig. S1, Houas et al., 2001; Oliveira et al., 2007; Benetoli et al., 2011). The low amount of $FeCl_3$ (less than 1 mM) appeared to be able to open the ring structure of MB partially and yet this did not lead to effective harvesting of microalgae. The low harvesting efficiency, however, was also observed when the bleached MO with 0.5 mM of $FeCl_3$ was used; and in this case harvesting efficiency was only 20%. Despite the complete formation of the amine group in MO, harvesting efficiency was found to be weak, and this could take place possibly by the second hypothesis. The oxidized dye solution seemed to have weak coagulating power, but the harvesting efficiency could be greatly increased by $FeCl_3$ in a synergistic fashion.

The effect of another important factor in the Fenton-like reaction, H_2O_2 concentration, was examined and a similar trend with the result for $FeCl_3$ obtained (Fig. 3). MO showed a higher harvesting efficiency with smaller H_2O_2 concentrations: MO and MB required 0.2% and 0.5% H_2O_2 concentration to achieve an over 90% harvesting efficiency, respectively. This phenomenon might occur possibly by the different structure between MB and MO. To degrade the ring structure of MB, more H_2O_2 was required compared to the azo group in MO. All this result led to conclude that the dye having an azo group is indeed a better option to make amine-containing dye intermediates via oxidation. Azo compounds constitute the largest class (70%) of synthetic dyes (Robinson et al., 2001), and there are various types such as congo red, direct blue 15, direct blue 71, methyl red, disperse yellow, and others. Considering huge amounts of dye wastewaters are oxidized for treatment and then just discharged, this finding that oxidized dye wastewaters can beneficially be utilized in microalgae harvesting can be used in a variety of fields.

4. Conclusions

This study proposed the use of the oxidatively treated dye wastewaters, particularly ones with amino groups exposed, as a coagulant for microalgae harvesting. Both methyl orange (MO) and methylene blue (MB) showed over 90% harvesting efficiency at an optimal condition especially with $FeCl_3$. Commonly used flocculants for microalgae harvesting have a high cost, and thus the proposed approach of using the dye wastewater could be a cheap alternative. What is better, this technology enables to reuse the otherwise problematic pollutant in a beneficial way, which can serve as a paradigm of the future direction of wastewater treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.05.074.

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