Using ozonation to eliminate the inhibition of soluble algal products (SAP) of *Scenedesmus* sp. LX1 on its growth in microalgal cultivation for biomass/bioenergy production

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**ABSTRACT**

Water recycling is an effective way to reduce water consumption in the industrialization of microalgal-based biomass/bioenergy production. The soluble algal products (SAP) which inhibit the microalgae growth will accumulate in the recycled water. Therefore, the ozone oxidation treatment of SAP produced by *Scenedesmus* sp. LX1 was studied to reduce the inhibition of SAP. The experimental results showed that there was almost no change in the content of SAP (counted by dissolved organic carbon) after ozonation, but the inhibition of SAP on microalgae growth disappeared. The intrinsic growth rate (*r*) of *Scenedesmus* sp. LX1 in the cultivation solution containing untreated SAP was 0.52 d⁻¹, and it rose to 0.95 d⁻¹ after SAP was ozonized. The maximum population growth rate (*R*ₘₐₓ) followed a similar trend, increasing from 9.19 × 10⁵ to 13.0 × 10⁵ cells mL⁻¹ d⁻¹. It was suggested that the changes of fluorescence and hydrophilic–hydrophobic/acid–base property of SAP after ozonation leads to the disappearance of SAP inhibition on microalgae growth.

**Key words**| algal biomass production, microalgae *Scenedesmus* sp. LX1, ozone oxidation treatment, soluble algal products (SAP), water reuse

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**INTRODUCTION**

Petroleum and coal have been the dominating energy sources in the past centuries, but as non-renewable natural resources, their reserve is sharply declining. Alternative energy sources have been studied and used in response to the global energy crisis. Microalgal-based bioenergy has become increasingly anticipated due to the microalgal rapid growth and efficient photosynthesis (Chisti 2007; Weyer et al. 2009; Kirrolia et al. 2013). Some microalgae have a high lipid content which could be extracted and used as biodiesel for automotive power, and so on.

In the meantime, there are some difficulties hindering the development of this bioenergy on a large scale. One of the difficulties is the vast amount of water consumed during microalgal cultivation (McGraw 2009; Kirrolia et al. 2013; Tian-Yuan et al. 2013; Zhang et al. 2013a, b). To produce 1 kg biodiesel, 3,726 kg of fresh water is required (Yang et al. 2011). Moreover, the soluble algal products (SAP), released from microalgae into the cultivation medium, may inhibit the growth of microalgae. The inhibiting effect will be accumulated with water recycling (Zhang et al. 2013a). After microalgal recovery, the water containing SAP would cause water pollution if it is discharged, such as odors and taste problems, increasing biochemical oxygen demand (BOD) content, fouling of membrane filtration and rising demand of disinfectant in the downstream water treatment process (Paralkar & Edzwald 1996; Her et al. 2004; Li et al. 2012).

Water reuse can decrease the water consumption to a great extent. However, when the cultural solution is being reused, the SAP will accumulate and aggravate the inhibition of SAP on microalgal growth in water (Zhang et al. 2013a).
Before the water reuse, some water treatment processes should be done to reduce SAP content or modify SAP properties and ultimately decrease SAP inhibition on microalgal growth. Coagulation, activated carbon adsorption processes are commonly used in water supply treatment (Pivokonsky et al. 2006). Ozone oxidation is an alternative method to eliminate dissolved organic matters and microbes (Paralkar & Edzwald 1996). Hence, whether these treatment methods are applicable to SAP removal needs to be investigated.

As physico-chemical treatments, coagulation and activated carbon adsorption treatments can remove some macromolecules, but cannot change the properties of the molecules left in the water, such as functional groups and the molecular weight (MW). In our previous study, many experiments were conducted, and it has been proved that coagulation and activated carbon adsorption treatments had no effect on SAP of Scenedesmus sp. LX1. In the coagulation experiments, two kinds of coagulants, poly aluminium chloride and poly ferric chloride, were used with the dosage ranging from 0 to 20 mg/L. However, dissolved organic carbon (DOC) of SAP had a negligible change after coagulation. Similar results were found in the active carbon absorption experiments.

Ozone oxidation is used in water treatment for disinfection or advanced oxidation. The influence of ozonation on organic matter has been widely studied and reported. However, whether ozone has effects on SAP and whether ozone-treated SAP has influences on microalgae require investigation. Furthermore, the research on using ozone treatment to eliminate the inhibition of SAP on Scenedesmus sp. LX1 for water reuse in microalgae cultivation for biomass/bioenergy production is a brand new research topic which our team first studied.

**MATERIALS AND METHODS**

**Algal cultivation and acquirement of SAP**

The Scenedesmus sp. LX1 (collection no. CGMCC 3036 in China General Microbiological Culture Collection Center) was cultivated in mBG11 medium, at 25 °C in the presence of light (dark/light = 14:10) (Li et al. 2010). After cultivation of 15 days, the microalgae cultural solution was filtered using a 0.45 μm filtration membrane. The cells were held on the membrane in the form of solid phase and the SAP was obtained in the form of liquid phase through the membrane. The SAP solution was kept in the refrigerator at 4 °C for further use.

**Experimental set-up**

In the ozone oxidation experiments, the ozone was produced by an ozone generator and aerated to the SAP solution (1 L) at a dosage of 30 ± 1 mg/L for 10 minutes (flow rate of 0.01 L/min) at a constant pressure of 0.3 Mpa in order to obtain the ozone dosage of 3 mg/L, which was in accordance with Paralkar’s research (Paralkar & Edzwald 1996). Pure oxygen was then aerated into the SAP solution to expel the rest of the ozone.

The SAP solution and ozonized SAP solution, each with a volume of 500 mL, were freeze-dried by a freeze dryer (FDU-1100) to be transformed into their respective powder forms. Then, the SAP powder and ozonized SAP powder were put into the 500 mL mBG11 solution for Scenedesmus sp. LX1’s cultivation, respectively. The dosage of SAP powder was 10 mg/L. The mBG11 solution without any form of SAP powder was set as a blank control. During the Scenedesmus sp. LX1’s cultivation, the algal density was calculated using a blood counting chamber. All tests were carried out in triplicate (n = 3) and t-test was conducted by statistical package SPSS to examine the consistency between different groups. A schematic diagram of the experimental procedure is shown in Figure 1.

**Analytical methods**

A logistics model was used to describe the growth kinetics of algae with a limited resource. The expression is as follows:

\[
N = \frac{K}{1 + \exp \left( a - rt \right)}
\]

\[
R_{\text{max}} = \frac{rK}{4}
\]

where \( N \) (cells mL\(^{-1}\)) is the algal density at time \( t \) (h), \( K \) (cells mL\(^{-1}\)) is the maximum algal density reached in the culture), \( r \) (d\(^{-1}\)) is the intrinsic growth rate, \( a \) is a constant, and \( R_{\text{max}} \) (cells (mL d\(^{-1}\)) is the maximum population growth rate.

The DOC was tested by TOC-VCPH (Shimadzu). Before the test, samples were filtered using a 0.45 μm filtrations
membrane to segregate the particular matter and settle the pH at about 5 by hydrochloric acid to exhaust the inorganic carbon.

The distribution of MW of the SAP was tested by ultrafiltration system (Millipore, XFUF07601). In this experiment, ultrafiltration membranes with nominal molecular weight limits (NMWL) of 3 kDa and 30 kDa were used to divide SAP into three fractions: <3 kDa MW, between 3–30 kDa MW, and >30 kDa MW, respectively. The ultrafiltration membranes were immersed in the NaOH/NaClO solution for 30 minutes and filtrated the high purity water until the DOC of effluent was under 0.5 mg/L.

Resin chromatography technology was used to determine the hydrophilic–hydrophobic/acid–base property as referred to in Wu et al. (2010). XAD-8, MSC-H, and Duolite A-7 resins (Sigma, USA) were used to divide SAP into six fractions: hydrophilic acid (HIA), hydrophilic base, hydrophilic neutral, hydrophobic acid (HOA), hydrophobic base (HOB), and hydrophobic neutral (HON), respectively.

Fluorescence excitation–emission matrix measurements (EEMs) were conducted using a F-7000 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence spectrogram was divided into five regions by excitation–emission wavelengths, by which means all the fluorescence organic matter was classified into five fractions, such as aromatic protein, soluble microbial by-products, and so on (Chen et al. 2005).

DOC, distribution of MW, the hydrophilic–hydrophobic/acid–base property and the fluorescence determination were all tested to describe the changes of SAP properties.

RESULTS AND DISCUSSION

Inhibitory effect of SAP on algae after ozonation

The microalgae *Scenedesmus* sp. LX1 were cultivated in 500 mL mBG11 solution with 5 mg dissolved ozonized
SAP powder, 5 mg untreated SAP powder, and no SAP, respectively. It was obvious, through the observation under microscope during the cultivation, that the Scenedesmus sp. LX1 had more quadruplets in the ozonized SAP group than in the other two groups. The algal density was measured every 2 days, and the growth curves were fitted by the logistics model mentioned previously. Then, the parameters $K_{\text{max}}$ and $r$ were calculated and are listed in Table 1.

When the ozonized SAP powder was added into the culture medium, the properties of algae were better than those cultivated in the untreated SAP solution. Comparing the $K_{\text{max}}$ and $r$ in different groups, the $K_{\text{max}}$ and $r$ of the microalgae Scenedesmus sp. LX1 with ozonized SAP solution were both higher than those in other groups. The results of $t$-test by SPSS show that the ozonized SAP could not only eliminate the inhibiting effect on the microalgae, but could also improve the growth of microalgae.

The results mentioned above could be caused by the change of SAP properties which involved the DOC, molecular distribution, EEMs fluorescence property, and hydrophilic–hydrophobic/acid–base properties of SAP released by Scenedesmus sp. LX1. These properties of SAP before and after ozonation were further investigated.

**DOC removal of SAP during ozonation**

The SAP production of different microalgal species ranged from 3 mg/L to 70 mg DOC/L (Pivokonsky et al. 2006; Henderson et al. 2008). The SAP of Scenedesmus sp. LX1 was about 10 mg DOC/L after a cultivation of 15 days under the conditions of this study which was harvested in the initial stationary phases of microalgae. After the ozonation of SAP, the DOC content remained at about 10 mg/L. Similar results were shown in Bijan’s and Tang Xin’s research into ozonation of reclaimed water (Bijan & Mohseni 2005; Tang et al. 2014), which proved that low dosage of ozone could not oxidize the organic matters into carbon dioxide or carbonate form.

**Change of EEMs fluorescence of SAP during ozonation**

Three dimension fluorescence determination was applied to qualitatively test some active components of SAP, such as benzene series. This method was more sensitive to the changes of water property than the conventional methods such as DOC, BOD, and so on (Chen et al. 2005). The result of fluorescence is shown in Figure 2. The peaks of untreated SAP were 225 nm/340 nm (EX/EM) and 275 nm/340 nm (EX/EM), which corresponded to aromatic protein, and tryptophan-like/protein-like, respectively (Coble 1996). However, the substance mentioned above disappeared after the ozonation. The result showed that ozonation had changed the properties of SAP significantly and might reduce SAP inhibition effect on microalgae.

**Composition change of SAP during ozonation**

The MW distribution of SAP was dependent on algal species. Some SAP was bimodally distributed, such as the cyanobacteria, but some algal species did not match this distribution. In this research, SAP was divided into three fractions by MW: <5 kDa, 3–50 kDa, and >30 kDa, respectively. The proportions (counted by DOC) of different fractions are shown in Figure 3(a). The fraction with MW <5 kDa occupied 76% of the untreated SAP. The results of MW were in agreement with research by Henderson and Lüsse (Lüsse 1985; Henderson et al. 2008). The MW distribution did not change significantly after ozonation.

The hydrophilic–hydrophobic/acid–base property of SAP is shown in Figure 3(b). The percentage of the neutral fraction was the largest among the six fractions, the HIA fraction was the second largest proportion (22%), and the HOB fraction occupied the lowest percentage of 4%. The percentage of the hydrophilic fraction was slightly higher than the hydrophobic fraction which matches the results of Henderson et al. (2008). After ozonation, the fraction of HON increased while the fraction of HOA decreased and the other four fractions showed almost no changes.

<table>
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<th>Conditions</th>
<th>$R_{\text{max}}$</th>
<th>$R_{\text{max}}$</th>
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<tr>
<td>mBG11 blank without SAP</td>
<td>0.69 ± 0.02</td>
<td>9.19 ± 0.40</td>
</tr>
<tr>
<td>mBG11 with SAP</td>
<td>0.52 ± 0.01</td>
<td>11.2 ± 0.66</td>
</tr>
<tr>
<td>mBG11 with O$_3$-SAP</td>
<td>0.95 ± 0.07</td>
<td>13.0 ± 1.25</td>
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The dosage of treated and untreated SAP was 10 mg/L, respectively.
Although DOC and MW distribution of SAP showed almost no change after ozone oxidation, the other properties of SAP changed significantly. Figure 3 demonstrates that the percentage of the HON fraction increased sharply to 34% of the whole SAP. Furthermore, the fluorescence absorption of SAP almost disappeared after ozone oxidation. Thus, it could be concluded that low dosage of ozone could damage the functional group of organic compounds (Paralkar & Edzwald 1996) which had the fluorescence response, change the hydrophilic–hydrophobic/acid–base property of SAP, and...
then eliminate the inhibition of SAP on *Scenedesmus* sp. LX1.

**CONCLUSIONS**

The properties of SAP of *Scenedesmus* sp. LX1 changed significantly after the treatment of ozone oxidation. The growth inhibition of *Scenedesmus* caused by SAP disappeared after the ozone treatment.

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**REFERENCES**


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