Extraction of allelochemicals from poplar alkaline peroxide mechanical pulping effluents and their allelopathic effects on *Microcystis aeruginosa*

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**Keywords:** Allelopathic inhibition, Allelochemicals, Organic solvent extraction, *Microcystis aeruginosa*, Alkaline peroxide mechanical pulping (APMP) effluents

**Abstract**

In this study, allelochemicals were extracted from pulping effluents rather than from the raw material of plants. Herein, five organic solvents (ethyl acetate (EAC), methyl tert-butyl ether (MTBE), dichloromethane (DCM), carbon tetrachloride (CTC), and petroleum (PE)) were applied to separately extracting the allelochemicals from alkaline peroxide mechanical pulp (APMP) effluents. The results from the algal density, inhibition ratio, and optical density of 446 nm (OD446nm) concluded that the extracts from the APMP effluents can act as effective allelochemicals and showed noticeable allelopathic inhibition effects on *Microcystis aeruginosa* growth. The results indicated that organic solvent extraction could be a practical approach to isolate the allelochemicals from the APMP effluents, which would broaden the potential application of the APMP effluents in the production of antimicrobial agents and other value-added materials.

1. Introduction

The *Microcystis aeruginosa* Kützing (MA), as one of the most common and harmful freshwater algae (Zou et al., 2018), can cause serious cyanobacterial blooms in lentic aquatic ecosystems, such as lakes or rivers, that are polluted by an excess of nutrient elements (e.g., N, P, K) all over the world (Ma et al., 2015; Sun et al., 2018). Such harmful algae blooms (HABs) would pose expanding threats to the environment by blocking sunlight and depleting oxygen in the water, killing other aquatic plants and animals; thus, decreasing the biodiversity and sustainability of freshwater ecosystems (Wu et al., 2010; Paerl et al., 2011; Liu et al., 2018a).

Allelochemicals, which belongs to a class of allelochemic that can induce chemical interactions between organisms or species to affect their growth, health, behavior, or even population biology (Whittaker and Feeny, 1971). Allelochemicals so far have at least several hundred different organic compounds, such as 2-propyl phenol (Jiang et al., 2014), ethyl 2-methyl acetooacetate (EMA) (Li and Hou, 2007), and other lignin and polyphenols related organics (Popa et al., 2008). It has been reported that allelochemicals released from plants and microbes can generate an effective allelopathic inhibition on the growth of cyanobacteria strains and control the HABs (Li and Hu, 2005; Zhang et al., 2013). For example, some anti-algal allelochemicals (mainly including indoles, ketones, esters, alcohols, etc.) had been extracted from *Arundo donax* L. through organic solvent extraction, the methanol extract was fractioned into neutral and acidic fractions, and both these fractions were found to have an allelopathic inhibition on the growth of the MA (Hong et al., 2010). Linoleic acid (LA) sustained-release microspheres were used for anti-algal growth and the optimal dose of the LA microspheres was 0.3 g/L with an inhibitory ratio over 90% (Ni et al., 2012).

It is well known that the process to extract allelochemicals from bioresources is as follow according to the literatures (Coll et al., 1982; Barnes et al., 1987; Turlings et al., 1991; Li and Hu, 2005): 1) pre-extraction of raw materials with hot-water or steam
to obtain a coarse aqueous solution of allelochemicals; 2) further purification of allelochemicals from the above solution by organic solvents extraction for several times to obtain an aqueous solution of allelochemicals with a highly purified concentration; and 3) rotary evaporation and subsequent vacuum drying in an N2 atmosphere to obtain dried allelochemicals for further application. However, there are two issues for the extraction of allelochemicals from biomass materials directly, such as rye herbage (Barnes et al., 1987) and Eucalyptus urophylla (Liu et al., 2018b): 1) large amounts of solid waste residues would be generated, thus increasing the environmental load; and 2) some treasured biomass species for isolation of allelochemicals would increase the production cost (Gahukar, 2012). Alkaline peroxide mechanical pulp (APMP) effluents are obtained from a newer pulping process for the pulp and papermaking industries (Liu et al., 2011c). The APMP effluents contain dissolved organics with high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Freitas et al., 2009), in which the content of the extractives is 50–800 mg/L and approximately 30%–70% of the extractives are organic acids (Li, 2016). These acids (e.g., abietic acid, gallic acid, catechinic acid, shikimic acid) mainly come from lignin or lignin derivatives and have been proven to have allelopathic inhibition on microorganism growth (Nakai et al., 2001; Ni et al., 2012; Liu et al., 2018b). Interestingly, a typical pretreatment process for the production of the APMP is that the wood chips are treated with hot water at 70°C–95°C for 30–90 min from which the APMP effluents are mainly released (Hideno, 2017), which is similar to the main extraction process of allelochemicals from the bioresources stated above. That is why the extractives (i.e., organic acids) from the APMP effluents can be regarded as allelochemicals. In addition, no solid wastes are generated from the APMP effluents during the extraction process. Furthermore, the extraction of organic acids from the APMP effluents will decrease the BOD and biological toxicity of effluents simultaneously (Liu et al., 2011b). Therefore, it is feasible to extract allelochemicals (organic acids) from the APMP effluents for the allelopathic inhibition study of allelochemicals on the MA.

The common technique to isolate organics from organic-contained mixtures is the organic solvent extraction, which is an energy-efficient process (Wang et al., 2007). Ethanol, acetone, chloroform, carbon tetrachloride, ethyl acetate, methyl tert-butyl ether, and petroleum are the main organic solvents applied in the chemical engineering process (Smallwood, 1996; Covington et al., 2012). It is well known that polar organic solvents have a better extraction ratio for polar organic materials, and vice versa (Dachuri et al., 2016). In the literature, Chen et al. (2017) successfully screened the most promising extractant with high extraction efficiency and good physical properties by extracting phenol from its aqueous waste solutions via the model of conductor-like screening model segment activity coefficient (COSMO-SAC) from 40 organic solvents, during which, ketones were found to be promising solvents for extracting phenol from wastewater. Yang et al. (2014) obtained the allelochemicals (phthalate n-octyl ester and phthalate 2-ethylhexyl ester) from the root of Flaveria bidentis culturing solution by dichloromethane (DCM) extraction and found that the obtained allelochemicals from the culturing solution of root exudates had an effective allelopathic inhibition on seed germination.

In this study, five organic solvents, EAC, methyl tert-butyl ether (MTBE), DCM, carbon tetrachloride (CTC), and petroleum (PE) were used separately to extract allelochemicals from APMP effluents. Hence, the allelopathic effects of the extracted allelochemicals on the MA were further studied. In addition, the extract yields of the allelochemicals by the five organic solvents at different solvent concentrations were discussed via gas chromatography-mass spectrometry (GC-MS) analysis. The growth and inhibition ratio of the MA influenced by the five solvent-extracted allelochemicals were also studied.

2. Materials and methods

2.1. Materials

The poplar APMP effluent sample was obtained from Sun Paper in Shandong Province, China. The effluent was a combination of several wastewater streams from the APMP process, including chip washing, hot water impregnation, chemical impregnation, pressing, and mechanical refining, to name a few. The effluent sample was passed through a 200-mesh screen to remove fibers, fines, and debris, and then stored in a refrigerator until its use.

Organic solvents, including EAC, MTBE, DCM, CTC, PE, and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (Shanghai, China). Deionized water was utilized throughout the experiments. All of the reagents in the experiments were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Organic solvent extraction of allelochemicals from APMP effluents

The EAC, MTBE, DCM, CTC, and PE were utilized as organic solvents to isolate the allelochemicals from the APMP effluents, successively. The detailed extraction processes were shown as follows according to the previous reports (Li, 2016): first, the pH of poplar APMP effluents were adjusted to 2.0 using 1% NaOH solution or 1% H2SO4 solution followed by an extraction process with organic solvent. The extraction was completed with sharp shaking for 5 min followed by a centrifugation operation for 10 min. Therefore, the organic phase was collected from the multiple liquid/liquid systems according to a method described in Wang et al. (2007). The obtained residual aqueous phase was adjusted to a pH of 9.0 and subjected to an extraction process for the second time with a half amount of the original organic solvent according to the same procedures mentioned above. And then, five fractions were obtained: EAC (including allelochemicals), MTBE (including allelochemicals), DCM (including allelochemicals), CTC (including allelochemicals), and PE (including allelochemicals). Next, the obtained allelochemical-contained organic solvent solution was concentrated by rotary evaporation, in which the evaporation temperature was set to be 1°C–3°C higher than the boiling point of each organic solvent followed by a drying process in a vacuum dryer in N2 atmosphere for more than 48 h. The prepared dried allelochemicals were
saved in a refrigerator at −20 °C for further experiments. The GC-MS analysis was performed using a VARIAN4000us (Varian Medical Systems, Lincolnshire, IL, USA) as described in the literature (Li, 2016).

2.2.2. Algal culturing of MA
The MA was provided by the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China) (FACHB). The algae were cultured in a BG11 (Blue-Green) medium at (25 ± 2) °C under (2800 ± 100) lx. Light was cycled to provide 12 h of light and 12 h of dark conditions in the phytotron. Cultures were grown in a triplicate for a minimum of seven days. The tested organisms were cultivated to exponential growth phase (approximately 5.0 × 10^5 cells/mL) for further use.

2.2.3. Allelopathic effects test
The prepared allelochemicals derived from above five organic solvent extraction processes were dissolved and diluted to given concentrations with the DMSO, respectively. Next, 0.2 mL of the diluted allelochemicals solutions was added into the flask, which has been inoculated with 150 mL of the MA suspension with an initial algal density of 5.0 × 10^5 cells/mL. For comparison, 150 mL MA solution was added into 0.2 mL DMSO without allelochemicals addition. To analyze the growth and inhibition ratio of the MA influenced by the five solvent-extracted allelochemicals with different concentrations, allelochemicals with different concentrations (50 mg/L vs. 100 mg/L) were added into the MA solution for culturing study. All the flasks were cultivated at the same conditions as described earlier. The number of the MA cells of each sample was counted every day using microscopy with a hemocytometer (1492; Hauser Scientific, Horsham, PA, USA) (Men et al., 2007).

During the culturing process, a 500 µL sample of each culture was removed every 24 h under sterile conditions for the measurement of optical density at 446 nm (OD_{446nm}) of the algal suspension using a Bio-Tek Synergy H1 Hybrid Reader (GoIndustry DoveBid, Bethesda, MA, USA) (Geller et al., 2018). Growth curves (concentration vs. time) were plotted using the daily biomass data and biomass productivity rates (mg/(L·day)), which were determined as the slope of a linear regression of the linear phase of these growth curves using Origin software (OriginLab, v.8.5, Northampton, MA, USA).

3. Results and discussion
Component analyses of the extracts from the APMP effluent isolated by five organic solvents were performed according to the results from the GC-MS technique shown in Table 1. It was clearly found that the number of extractive ingredients from the APMP effluents extracted by five organic solvents were similar and ranged from 18 to 23, although the yields of the five solvents extracts varied from 75 mg/L to 510 mg/L as shown in Table 1. Due to the different polarities of the solvents, the yield of allelochemicals is also different. According to the order of polarity, EAC > MTBE > DCM > CTC > PE. It was evidenced that the extractives isolated from the APMP effluent by the EAC organic solvent had the highest yield of extractives when compared with those of the other four organic solvents. It can be found from Table 1 that the yield of the EAC extract is 510 mg/L, and the yield of the MTBE is 375 mg/L. Though the MTBE has a lower relative polarity, which might be due to the fact that the molecule structures of the extractive isolated by the MTBE are similar to those of the MTBE (Li et al., 2018). As the polarity decreases, the extraction yield also decreases. The extraction yield of the DCM is 330 mg/L, the extraction yield of the CTC is 136 mg/L, and the smallest extraction yield is the PE with relatively low polarity of 75 mg/L.

The data of the top five major components and percentage contents of organics in the extractives shown in Table 1 indicate that the organic acids are the main components of the extractives from the APMP effluents, and the contents varied from 55% to 79% for the five different solvents. In addition, amines and ketones are also the major components of the extractives. The total percentage content of organic acids, amines, and ketones of the five groups of extractives was almost 95% or more, and it was reported that the above three organics could play an effective role in the allelopathic inhibition of algae growth (Ni et al., 2012). As shown in Table 1, the organic acid content of the APMP effluent extractives was approximately 55%–79%. Additionally, many publications have also proven that the organic acids coming from wood plants have effective allelopathic inhibition on algae growth (Wu et al., 2006; DellaGrecia et al., 2010).

Fig. 1 shows the results of algal density of the MA in the culturing system for seven days at the existence of allelochemicals extracted by organic solvents from the APMP effluent. It was clearly noted that the addition of allelochemicals effectively impeded the algal growth of the MA, which showed that allelochemicals extracted from the APMP effluents had a good allelopathic inhibition on the MA growth. The allelopathic inhibition from 100 mg/L of the allelochemical concentration was stronger than that from 50 mg/L, compared with the results of Fig. 1a and b.

Similar conclusions can be obtained from the literature. Xiao et al. (2014) isolated a pair of chiral flavonolignans as allelochemicals against Microcystis sp. from barley straw (Hordeum vulgare) extract using a bioassay-guided isolation procedure and found that the novel anti-cyanobacterial allelochemicals exhibited significant allelopathic inhibition on Microcystis sp. Additionally, the inhibition effects increased along with the concentration of the chiral flavonolignans.

The results from Fig. 1 also showed that allelochemicals extracted by the MTBE had the strongest allelopathic inhibition in the first two days of the culturing of the MA. Meanwhile, the allelochemicals extracted by the PE had the weakest effect on the MA growth, which might have been due to the distinct difference of extractive yields from the MTBE and PE, as shown in Table 1.

Fig. 2 shows that the inhibition ratio (%) of the MA was affected by the allelochemicals from the APMP effluent with different extractive dosages (50 mg/L vs. 100 mg/L). It was noted that the growth inhibition ratios of allelochemicals extracted by the five organic solvents ranged from 7.0% to 38.2% when the extract dosage was 50 mg/L, while the growth inhibition ratios increased from
Table 1
The GC-MS analysis of extractives from alkaline peroxide mechanical pulp (APMP) effluents isolated by different organic solvents.

<table>
<thead>
<tr>
<th>Component</th>
<th>EAC</th>
<th>MTBE</th>
<th>DCM</th>
<th>CTC</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of extractives (mg/L)</td>
<td>510</td>
<td>375</td>
<td>330</td>
<td>136</td>
<td>75</td>
</tr>
<tr>
<td>Number of extractive Ingredients</td>
<td>21</td>
<td>18</td>
<td>23</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>2-amino-isobutyric acid</td>
<td>2-undecenoic acid</td>
<td>2-amino-isobutyric acid</td>
<td>2-amino-isobutyric acid</td>
<td>L leucine</td>
<td></td>
</tr>
<tr>
<td>2-undecenoic acid</td>
<td>Ethylamine</td>
<td>2-undecenoic acid</td>
<td>Ethylamine</td>
<td>2-amino-isobutyric acid</td>
<td></td>
</tr>
<tr>
<td>Ethylamine</td>
<td>Ethanolamine</td>
<td>Ethylamine</td>
<td>Ethanolamine</td>
<td>2-undecenoic acid</td>
<td></td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>2-hydroxypropionic acid</td>
<td>Benzoic acid</td>
<td>Polydiimine carbide</td>
<td>ethylamine</td>
<td></td>
</tr>
<tr>
<td>2-hydroxypropionic acid (lactic acid)</td>
<td>Glycolic acid</td>
<td>1, 5 - butyl glycol</td>
<td>Ethanolamine</td>
<td>Polydiimine carbide</td>
<td></td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>Benzoic acid</td>
<td>4-carbonyl pentanoic acid</td>
<td>Benzoic acid</td>
<td>ethanolamine</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>2, 3, 4-trihydroxybutyric acid</td>
<td>Butylene acid</td>
<td>Palmitic acid</td>
<td>Glycolic acid</td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>2, 3, 4-trihydroxybutyric acid</td>
<td>Butylene acid</td>
<td>Polydiimine</td>
<td>3-hydroxydecanoic acid</td>
<td></td>
</tr>
<tr>
<td>Butylene acid</td>
<td>2-hydroxysebacic acid</td>
<td>p-hydroxyphenylacetone</td>
<td>Stearic acid</td>
<td>Palmitic acid</td>
<td></td>
</tr>
<tr>
<td>Pimelic acid</td>
<td>2-hydroxysebacic acid</td>
<td>Caprylic acid</td>
<td>11-icosenoic acid</td>
<td>9, 12-octadecarboxylic acid</td>
<td></td>
</tr>
<tr>
<td>p-hydroxyphenylacetone</td>
<td>2-hydroxysebacic acid</td>
<td>4-hydroxy-3-methoxybenzonic acid</td>
<td>Twenty acid</td>
<td>9, 12-octadecarboxylic acid</td>
<td></td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>3, 5-dimethoxy4-hydroxybenzoic acid</td>
<td>Azelaic acid</td>
<td>12,13-dihydroxy-11-methoxy 18-carbon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-hydroxy-3-Methoxybenzoic acid</td>
<td>2, 3-dihydroxy-5-allyl methyl ether</td>
<td>Sebacic acid</td>
<td>-9- methyl enate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 4-dihydroxybenzoic acid</td>
<td>Palmitic acid</td>
<td>9, 12-octadecarboxylic acid</td>
<td>Denoprost</td>
<td>3-hydroxy-2-decenedioic acid</td>
<td></td>
</tr>
<tr>
<td>Sebacic acid</td>
<td>2-hydroxysebacic acid</td>
<td>9-octadecenoic acid</td>
<td>2-hydroxyoctanoid acid</td>
<td>2-hydroxyoctanoid acid</td>
<td></td>
</tr>
<tr>
<td>3, 5-dimethoxy4-hydroxybenzoic acid</td>
<td>Stearic acid</td>
<td>Stearic acid</td>
<td>Twenty-two acid</td>
<td>Twenty-two acid</td>
<td></td>
</tr>
<tr>
<td>2, 3-dihydroxy-5-allyl methyl ether</td>
<td>2-hydroxysebacic acid</td>
<td>11, 12-dihydroxy-11-methoxy 9-eichenoic acid</td>
<td>Cerotic acid</td>
<td>Cerotic acid</td>
<td></td>
</tr>
<tr>
<td>2-hydroxysebacic acid</td>
<td>2-hydroxysebacic acid</td>
<td>2-hydroxysebacic acid</td>
<td>Twenty-two acid</td>
<td>Denoprost</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>2-hydroxysebacic acid</td>
<td>2-hydroxysebacic acid</td>
<td>Twenty-two acid</td>
<td>Twenty-two acid</td>
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</tr>
<tr>
<td>Stearic acid</td>
<td>2-hydroxysebacic acid</td>
<td>2-hydroxysebacic acid</td>
<td>Twenty-two acid</td>
<td>Cerotic acid</td>
<td></td>
</tr>
</tbody>
</table>

Percentage content of organics in extractives

- Organic acids: 58% 55% 79% 74% 75%
- Amines: 8% 7% 13% 19% 21%
- Ketones: 32% 38% 3% 0% 0%
- Esters: 0% 0% 0% 7% 4%
- Others: 2% 0% 5% 0% 0%

Notes: EAC, ethyl acetate; MTBE, methyl tertiary butyl ether; DCM, dichloromethane; CTC, carbon tetrachloride; PE, petroleum ether.

Fig. 1. Algal density (10^6 cells/mL) of MA as a function of culture time (Day) at allelochemical concentration of (a) 50 mg/L and (b) 100 mg/L extracted by five organic solvents. Allelopathic inhibition effect of five organic solvents extracting allelochemicals from alkaline peroxide mechanical pulp (APMP) effluents on growth of Microcystis aeruginosa Kützing. EAC: ethyl acetate; MTBE: methyl tertiary butyl ether; DCM: dichloromethane; CTC: carbon tetrachloride; PE: petroleum ether.
23.0% to 39.6% when the dosage was 100 mg/L. It was concluded that the allelochemicals extracted by the organic solvents had effective allelopathic inhibition on the MA growth, which agreed well with the results from Fig. 1.

Compared with the results from Table 1, Figs. 1 and 2, it was found that there was no linear relationship between the allelopathic inhibition and the yield of extractives isolated by the organic solvents. Additionally, the inhibition ratios on the MA were close to each other although the yields of the allelochemicals extracted by the five organic solvents varied widely from 75 mg/L to 510 mg/L as shown in Table 1. It may be ascribed to the different species and contents of the extractive ingredients isolated by different solvents that played similar allelopathic inhibition roles in the MA culturing process, which could be discussed in a future study.

In the literature, Meng et al. (2015) reported a comparable result from a 5-day culturing experiment of MA inhibited by allelochemicals from Ailanthus altissima. They found that the inhibition ratios on the MA growth induced by 50 mg/L A. altissima ranged from 16.8% to 30.9%, and the range of inhibition ratios increased from 28.7% to 66.3% when the allelochemical concentration increased to 100 mg/L. In another study, the allelopathic inhibition of ferulic acid and p-hydroxybenzoic acid on Chlorella pyrenoidosa were studied separately and found that the inhibition rate of 100 mg/L ferulic acid and 194 mg/L p-hydroxybenzoic acid on C. pyrenoidosa were 57% and 84%, respectively (Zhang et al., 2007).

The optical density at 446 nm of algae suspension can be affected by two main factors (Liu et al., 2011a; Geller et al., 2018): 1) a high algae cell density in suspension responds to high optical density when the algal is in the logarithmic growth phase; and 2) more dissolved color compounds released from algae cells that are degraded and lysed in suspension would induce higher optical. Fig. 3 shows the results of OD_{446nm} of the MA culturing system along with the culture time at an allelochemical concentration of (a) 50 mg/L and (b) 100 mg/L extracted by five organic solvents. It is known that the effect of dissolved color compounds released from disintegrated algal cells plays a more important role in increasing the OD_{446nm} of algal suspension than that of the cell density caused by algal growth. It was clearly noted that the OD_{446nm} of the control MA sample was lower than that of the MA samples with the addition of allelochemicals extracted by the five organic solvents, which indicated that allelochemicals from the APMP effluents had a noticeable allelopathic inhibition effect on the MA growth. The OD_{446nm} of the MA sample with the addition of allelochemicals extracted by the CTC solvent was the highest. This is different from the inhibition ratio of algal cells in Figs. 1 and 2, mainly because...
the optical density of the algal suspension is affected by many factors, and the reasons need to be further analyzed. We will explore it further in the future experiments.

It was concluded from Fig. 3a and b that allelochemicals with a 100 mg/L concentration had a higher OD_{446nm} than that of the allelochemicals with a 50 mg/L concentration, which indicated that the allelochemicals with higher concentration would have a stronger allelopathic inhibition on algae growth. This agreed well with the conclusions from Figs. 1 and 2. In the literature, Pereira et al. (2018) studied the effects of two toxic cyanobacterial crude extracts as allelochemicals containing microcystin-LR and cylindrospermopsin on the allelopathic inhibition of the microalga *Parachlorella kessleri*, and found that the allelopathic inhibition of allelochemicals with a concentration of 150 µg/L was higher than that of the allelochemicals with a concentration of 55 µg/L.

4. Conclusions

In this study allelochemicals were successfully isolated from the APMP effluents by five common organic solvents, i.e., EAC, MTBE, DCM, CTC, and PE. The conclusions are as follows:

1. The GC-MS analyses showed that the EAC had the highest yield, in which the organic acids were the major ingredients in the extractives. The extracted allelochemicals mainly contained organic acids, amines, ketones, and esters, which occupied more than 90% of the extraction.

2. The extractives from the APMP effluents can act as effective allelochemicals and showed noticeable allelopathic inhibition effects on the MA growth. The allelopathic effects of the extract on the growth of the MA were investigated and the growth inhibition ratios of the extracted allelochemicals from the five above solvents ranged from 7.0% to 38.2% when the extract dosage was 50 mg/L, while the growth inhibition ratios increased from 23.0% to 39.6% when the dosage was 100 mg/L.

3. The organic solvent extraction method was used to extract the allelochemicals from the APMP effluents, and the results showed that the allelochemicals extracted by the MTBE exhibited the highest algal inhibitory ratio compared with those extracted by the other extractants.

Declaration of Competing Interest

There are no conflicts to declare.

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