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Pyrolysis of *Arundo donax L.* to produce pyrolytic vinegar and its effect on the growth of dinoflagellate *Karenia brevis*

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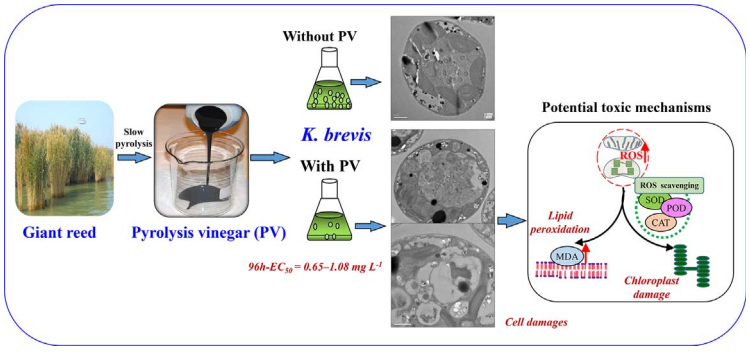
GRAPHICAL ABSTRACT



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ABSTRACT

Harmful algal blooms (HABs) have become global environmental issues, and the demand for alternative algaecides is urgent. Pyrolytic vinegars (PVs) were pyrolyzed from giant reed at 300–600 °C to investigate the underlying mechanisms of their inhibitory effect on the red tide dinoflagellate *Karenia brevis* by sub-chronic toxicity experiments. The major components of PVs were acetic acid, phenols, aldehyde, ketone, and esters. The 96 h median effective concentration (96 h-EC_{50}) values of PVs were 0.65–1.08 mL L^{-1}, and PV300 showed the strongest inhibitory effect. The increased contents of reactive oxygen species (ROS) and malondialdehyde, antioxidant enzymes activities indicated that *K. brevis* cells were suffering from oxidative stress, leading to lipid oxidation and cell structure damage. The sites of ROS accumulation in the treated cells were chloroplasts and mitochondria. These results suggest the suitability of PVs as potential algaecides for HAB control, and also provide a new direction for biomass valorization.

1. Introduction

Harmful algal blooms (HABs), involving explosive proliferation and accumulation of algae at extremely high cell densities (> 1000 cells mL^{-1}), have been increasing worldwide (Kudela and Gobler, 2012). These have resulted from global climate change and

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exacerbating eutrophication as a result of expanding human activities such as wastewater discharge, aquaculture, and fertilizer leaching (Chen et al., 2012; Mcfarland et al., 2016). The regions in which HABs occur are increasing annually. Approximately 107 places suffered from HABs worldwide in 2016, although they were only observed in 15 regions in 1970 (WHOI, 2016). China is one of the countries that have suffered from severe HABs. According to China's Marine Environment Quality Bulletin in 2016, the occurrence of red tide increased from 46 times (within 4070 km^2) in 2013–68 times (within 7484 km^2) in 2016. These blooms led to severe water quality deterioration and high fish mortality by a combination of reduced dissolved oxygen in the water and production of algal toxins. They were responsible for annual losses of about 726 million USD. *Karenia brevis* (*K. brevis)* is a single-celled planktonic organism called a dinoflagellate, and one of the harmful red tide species that occur worldwide in oceans (Fleming et al., 2011). During blooms, *K. brevis* may cause neurotoxic poisoning of marine organisms like fish and mollusks (Mcfarland et al., 2016). In consequence, the effective control of *K. brevis* HABs has become an urgent task for protection and exploitation of marine ecosystems.

Many strategies, including the use of chemical algaecides, physical adsorption, biological control, and mechanical collection of bloom biomass, have been applied to avoid and reduce the potential risks associated with HABs (Chen et al., 2012; Jančula and Maršálek, 2011). As an emergency measure, mechanical collection was effective and widely used in freshwater lakes such as Lake Taihu during bloom seasons in China (Chen et al., 2012). However, ecological risks to terrestrial plants (e.g., crops and wild grass) due to absorption and accumulation of microcystins (MCs), and risks to human health associated with consumption of MC-contaminated groundwater and crops occurred once the toxic bloom organisms and toxin-contaminated water were transferred to soil (Chen et al., 2006, 2012). One of the most promising physical strategies has been the use of suspended clay particles over the blooms to flocculate the planktonic cells, but the high clay load results in high mortalities among suspension-feeding bivalves like *Argopecten irradians* (bay scallop) and *Mytilus edulis* (blue mussel) (Archambault et al., 2004). The use of algaecides like ferric chloride, copper sulfate, and hydrogen peroxide is a quick and efficient approach, but these poisons may indiscriminately kill other organisms in the aquatic ecosystem and cause secondary pollution (Jančula and Maršálek, 2011). For instance, copper compounds (copper oxychloride, cuprous oxide, and copper sulfate), which have been intentionally introduced into water bodies as aquatic plant herbicides or algaecides, have been shown to result in water pollution owing to their elevated concentrations in the water (de Oliveira-Filho et al., 2004). Thus, eco-friendly algaecides that would act on only certain species instead of causing widespread mortality among organisms are urgently needed.

Pyrolysis is the thermal degradation of biomass under anoxic conditions, which may produce syngas, biochar or charcoal, and bio-oil (also called pyrolytic vinegar: PV) (Collard and Blin, 2014; Saikia et al., 2015). Biochar, as the main product of biomass pyrolysis, has been studied extensively in relation to carbon (C) sequestration (Zheng et al., 2018), soil improvement (Zheng et al., 2017), and alternative energy supply (Saikia et al., 2015). However, PVs are usually ignored during biochar production using slow pyrolysis technology (Rahmat et al., 2014). Even if PVs are recycled, researchers are mainly concerned with PV energy characteristics and values (Saikia et al., 2015). In addition to energy values, PVs are considered potentially valuable chemicals that could be utilized as bactericides (Yang et al., 2016), soil amendment (Lashari et al., 2015), and botanical protection (Rahmat et al., 2014) because of their high antimicrobial and antioxidant activity (Yang et al., 2016). In addition, the macrophyte allelochemicals such as polyphenols, esters, and alkaloids extracted from wetland plants (e.g., *Phragmites communis*, *Eichhornia crassipes*, *Stratiotes aloides*), of which the chemical compositions were similar to PVs (Temiz et al., 2013), were successfully used to control algal growth (Meng et al., 2015; Gao et al., 2017). Therefore, it is reasonable to hypothesize that the PVs may have similar anti-algal effect and may be potential algaecides useful for HAB control.

Therefore, the specific objectives of this study were to: 1) characterize the physico-chemical properties of the PVs formed by pyrolysis of giant reed (*Arundo donax L.*) at 300–600 °C, 2) investigate the effects of these PVs on *K. brevis* growth, and 3) illustrate the potential underlying mechanisms responsible for the inhibited growth of *K. brevis*. These findings will provide information useful for producing PV-derived alternative algaecide for HAB control, and will also provide a new way of biomass valorization.

2. Materials and methods

2.1. Preparation and characterization of PVs

Giant reed is a common wetland plant that is widely applied in water purification and ecological restoration in China owing to its rapid rate of growth and good resistance to drought and floods (Zheng et al., 2013). The reeds used to produce the PV in this study were collected from Nansi Lake wetland (37° 24′ 43″ N, 118° 39′ 49″ E) in August. The reed shoots were cut into small pieces (1 × 3 cm), and dried at 80 °C for 24 h. The PV was produced using slow pyrolysis as described by Zheng et al. (2013). In brief, 50 g of giant reed stems were pyrolyzed at 300, 400, 500, or 600 °C for 4 h using a vacuum-quartz-tube furnace (GSL-1300, MTI, China) under a N_2 flow of 200 mL min^{-1}. The PV samples were collected via a condenser filled with an ice-water mixture, and then filtered through a 0.45 μm cellulose acetate membrane. The corresponding treatments are hereafter referred to as PV300, PV400, PV500, and PV600, respectively. The yields of PV and biochar were recorded and all the PV samples were kept at 4 °C in the dark for further analysis.

The density of the PV was measured using an optical density meter (LS117, Linshang, China). Moisture content was measured using Karl Fischer volumetric titration with a moisture analyzer (870 KF Titrino plus, Metrohm, Switzerland). The chemical composition of the PV was determined using an Agilent Gas Chromatography-Mass Spectrometer (GC–MS, 7890A-5957C, Agilent, USA). The GC–MS analysis was carried out with the following oven temperature programming (Yang et al., 2016): initial temperature 50 °C for 2 min, ramped at 15 °C min^{-1} to 250 °C; then held at 250 °C for 5 min. One microliter of PV sample was injected into the column with a split ratio of 20:1, and the electron impact mass spectrum was obtained at 70 eV. The relative contents of the compounds were determined by the corresponding peak areas (Wu et al., 2015).

2.2. Exposure of K. brevis to PVs

The *K. brevis* tested were obtained from the Institute of Oceanology, Chinese Academy of Science. The algae were cultivated in 200 mL f/2 medium. The culture vessels were 500 mL Erlenmeyer flasks, which were autoclaved (121 °C) for 20 min before inoculation. The algae were cultivated under a light/dark cycle of 14:10 h at 23 ± 1 °C with 4000 lux illumination in an intelligent artificial climate box (GXZ-380Z, Jiangnan, China). Every flask was gently shaken by hand three times every day.

For all the experiments, 200 mL of *K. brevis* was taken from the stock culture at log phase of growth (1.2×10^5 cells mL^{-1}) and added to 500 mL Erlenmeyer flasks under sterile conditions. PV portions of (0, 40, 80, 120, 16, and 200) μL were diluted in 200 mL portions of *K. brevis* solution to reach the desired concentrations of (0, 0.2, 0.4, 0.6, 0.8, and 1.0) mL L^{-1}, respectively. The treatments are hereafter referred to as CK, PV-0.2, PV-0.4, PV-0.6, PV-0.8, and PV-1.0, respectively. Each treatment was replicated three times. The algae were sampled and the densities were counted using a hemocytometer with a Nikon microscope (E100-LEO, Nikon, Japan) every 24 h for 7 d. The inhibitory ratio (*IR*, %) of *K. brevis* upon PV exposure was calculated