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Growth inhibition and oxidative stress caused by four ionic liquids in *Scenedesmus obliquus*: Role of cations and anions

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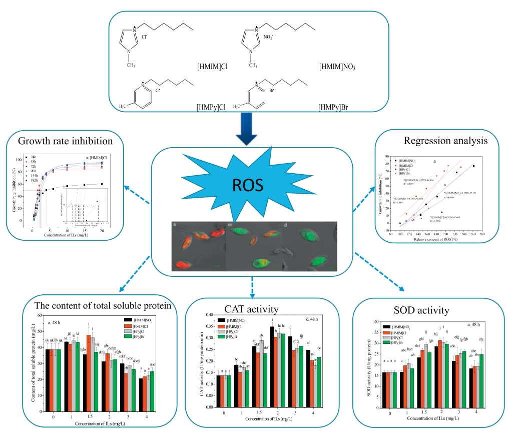
HIGHLIGHTS

- Growth inhibition of *S. obliquus* was noted in treatment with four ionic liquids (ILs).
- The four ILs stimulate ROS, SOD, and CAT in *S. obliquus*.
- Imidazolium-IL had greater effect than pyridinium-IL.
- The nitrate-IL and bromide-IL had a greater effect than chloride-IL.

GRAPHICAL ABSTRACT



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ABSTRACT

Ionic liquids (ILs) are widely used in various industrial applications. However, they are considered potential toxins in aquatic environments because of their physical stability and solubility. The growth inhibition and oxidative stress induced by four ionic liquids with different cations and anions on the green algae *Scenedesmus obliquus* was investigated in this study. The order of growth inhibition was 1 hexyl 3 methylimidazolium nitrate ([HMIM]NO_3) > 1 hexyl 3 methylimidazolium chloride ([HMIM]Cl) > *N* hexyl 3 metylpyridinium bromide ([HMPy]Br) > N hexyl 3 metylpyridinium chloride ([HMPy]Cl). Imidazolium IL had a higher growth inhibition effect than pyridinium IL, nitrate IL and bromide IL had a higher effect than chloride IL. Reactive oxygen species (ROS) level in *S. obliquus* increased with increasing IL concentrations. Green fluorescence in [HMIM]Cl treated algae showed increased brightness compared to the [HMPy]Cl treatment, and [HMIM]NO_3 treatment produced increased brightness compared to the [HMPy]Br treatment, suggesting that higher ROS levels were induced by [HMIM]Cl and [HMIM]NO_3. Soluble protein, catalase (CAT), and superoxide dismutase (SOD) activities were stimulated at lower concentrations but were inhibited at higher concentrations. Regression analysis suggested that ROS level is the main index responsible for oxidative stress induced by the four ILs. The ILs induced oxidative damage on *S. obliquus*, and ROS in high concentration treatments could not be effectively removed by the antioxidant system, leading to oxidative damage and ultimately resulting in growth inhibition and cell death.

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1. Introduction

Ionic liquids (ILs) are the solvents consisting solely of ions, with melting points below 100 °C (Egorova and Ananikov, 2014). They have many intriguing characters such as low vapor pressure, high thermal stability, non-flammability, large electrochemical window, and versatile solvation capacity. These properties make them potential candidates for chemical industries (Kárászová et al., 2014). ILs are suitable compounds for use as media for catalysts, extraction, electrochemistry, pharmaceutical, and biotechnology applications (Cerqueira et al., 2012; Madria et al., 2013; Stradi et al., 2013; Salar-García et al., 2017). ILs are also considered "designer solvents" owing to the option of tailoring its characteristics to many particular process by altering cation and anion combinations. Variation in cation and anion combinations potentially allows the design of considerably high numbers of different ILs, >30,000 imidazolium salts are listed in the CAS database (Amde et al., 2015).

ILs are considered to be relatively environmental friendly solvents as they are unlikely to be an air pollutants or toxic inhalable aerosols. As the production and practical use of ILs increase rapidly, doubts about the so-called "green solvents" and their harmlessness have gradually emerged. However, ILs have been identified as contaminants on the horizon (Richardson and Ternes, 2011), the ecotoxicity of ILs in many organism have been proved (Amde et al., 2015; Egorova and Ananikov, 2014; H.J. Liu et al., 2018; Pham et al., 2010). Mistakes made in the past with agents such as polychlorinated biphenyls (PCB) should be avoided for ILs in the future (Chatel et al., 2017). Owing to their excellent water solubility and poor biodegradability, the ILs release into aquatic environments through discharge of sewage or accidental may result in water pollution and pose a potential hazard to aquatic ecosystems (Bubalo et al., 2014). In fact, the ILs impact to aquatic organisms at different trophic levels, including marine bacteria (Rantamäki et al., 2017), algae (Deng et al., 2017), invertebrates (Zhang et al., 2017), and vertebrates (Ruokonen et al., 2016), was reported recently. The specific toxicity of ILs can equal or even exceed the toxicity of traditional organic solvents (such as benzene, methanol, or chloroform) by two to four orders of magnitude (Bubalo et al., 2014).

In general, the toxicity of ILs is correlated with the respective alkyl chain length, cation family, and anion moiety (Amde et al., 2015). The alkyl chain length, which determines the hydrophobicity of ILs, correlates positively with IL toxicity to organisms such as *Vibrio fischeri* (Viboud et al., 2012), *Daphnia magna* (Roy et al., 2014), and *Chlorella vulgaris* (Zhang et al., 2017). Furthermore, head-groups have been investigated as a potential main factor contributing to the toxicity of ILs on a diverse array of organisms: compared to ILs containing quaternary ammonium salts and morpholinium cations, those containing aromatic cations proved to be more toxic to marine bacteria (*Vibrio fischeri*), algae (*Scenedesmus vacuolatus*), and duckweed (*Lemna minor*) (Stolte et al., 2007; Biczak et al., 2014). However, the general understanding of the role of cation and anion regarding IL toxicity is still limited, and few studies have done about the impact of IL structure and compound toxicity on oxidative stress of algae (Dołżonek et al., 2017; Yun et al., 2015).

Algae are widely distributed in various ecosystems and are typically characterized by a short life cycle and a quick response to environmental changes, which make them intriguing candidates for studies on ecotoxicology (Liu et al., 2017; Deng et al., 2017). Our previous study published the effect of four ILs on chlorophyll contents, the chlorophyll fluorescence parameters of photosynthetic system II (PSII), cell membrane permeability and cellular ultrastructure of *S. obliquus* (Xia et al., 2018). In this study, growth inhibition and oxidative stress of these four commonly used ILs, which are imidazolium- and pyridinium-based IL derivatives of Br^-, Cl^-, and NO_3^-, on *S. obliquus* was examined to complement the toxicological mechanism. Growth inhibition was calculated as half inhibition concentration (IC50) by using logistic models. Reactive oxygen species (ROS) level and ROS distribution were measured in *S. obliquus* cells to assess the oxidative damage caused by the four different types of ILs. Total soluble protein concentrations and the antioxidant enzymes activity (catalase (CAT) and superoxide dismutase (SOD)) were detected to evaluate oxidative stress caused by the four ILs. The correlation of ROS level and growth inhibition, cellular membrane permeability, chlorophyll content, SOD and CAT activity were analyzed. The results obtained from this study will help to better understand the role IL structure plays in toxic effects, and provide information on their environmental effects to better design ILs for use.

2. Materials and methods

2.1. Chemicals

Four ILs, 1 hexyl 3 methylimidazolium chloride ([HMIM]Cl), 1-hexyl-3-methylimidazolium nitrate ([HMIM]NO_3), *N*-hexyl-3-metylpyridinium chloride ([HMPy]Cl), *N*-hexyl-3-metylpyridinium bromide ([HMPy]Br) were purchased from Chengjie Chemical Co. Ltd. (Shanghai, China), with respective purity of 99%. The ILs structures are shown in Xia et al. (2018). Fluorescein acetate (FDA) was bought from Sigma-Aldrich. All the other reagents are analysis purity.

2.2. The cultivation of algae

An initial inventory of the microalga *S. obliquus* was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). An HB-4 culture medium was prepared for *S. obliquus* cultivation according to the 201 Guidelines of China National Environmental Protection Agency (CNEPA, 1990). The HB-4 medium was sterilized by an autoclave (Hirayama HVE-50). Algae were cultured in illuminated incubators with a constant temperature of 25 °C, 3000–4000 lx illumination, and a 16:8 h light: dark photoperiod (Liu et al., 2017).

2.3. Growth inhibition experiment

All the toxicity tests were based on the OECD (Organisation for Economic Cooperation and Development) guidelines (OECD, 2006). *S. obliquus* cells were pre-cultured in the sterilized HB-4 medium to induce the exponential growth phase. The final batch cultures (100 mL) used in the experiments were inoculated into sterilized glass Erlenmeyer flasks (250 mL) to achieve a final density of 8.0×10^5 cells/mL. Concentrations of ILs ranging from 0.50 to 20.00 mg/L were added to each flask, respectively, to obtain complete concentration-response curves, including control treatments with no ILs added. The IL concentrations used in this study were based on preliminary experiments (data not shown). The density of *S. obliquus* cells was detected after 24, 48, 72, 96, 144, and 192 h treatment, and algal growth was assessed on a daily basis using absorbance values related to a standard curve measured with a UV–visible spectrophotometer (Shimadzu UV-2401PC) at 680 nm. The number of algal cell was counted with a hemocytometer, and the linear relationship between the algal biomass and absorbance at 680 nm was recorded. The algal cell biomass was calculated according to the linear equation: $Y = 310.8X - 2.173$, where Y is the algal cell biomass (10^5 cells/mL), X is the absorbance at 680 nm. The relative inhibition (RI) of algal growth was calculated, and the IC_{50} value was obtained through logistic model.

2.4. Intracellular reactive oxygen species (ROS) measurement

2.4.1. Measurement of ROS level

Algae cells exposed to ILs for 48, 96, and 144 h were collected for ROS level measurement. Intracellular ROS levels were detected with a fluorescent probe 2,7 dichlorofluorescein diacetate (H_2DCF-DA)