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Effect of nitrogen/phosphorus concentration on algal organic matter generation of the diatom *Nitzschia palea*: Total indicators and spectroscopic characterization

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ABSTRACT

Critical algal blooms in great lakes increase the level of algal organic matters (AOMs), significantly altering the composition of natural organic matters (NOMs) in freshwater of lake. This study examined the AOM's characteristics of *Nitzschia palea* (*N. palea*), one kind of the predominant diatom and an important biomarker of water quality in the great lakes of China, to investigate the effect of AOMs on the variation of NOMs in lakes and the process of algal energy. Excitation–emission matrix fluorescence (EEM) spectroscopy, synchronous fluorescence (SF) spectroscopy and deconvolution UV–vis (D-UV) spectroscopy were utilized to characterize AOMs to study the effects of nutrient loading on the composition change of AOMs. From results, it was revealed that the phosphorus is the limiting factor for *N. palea*'s growth and the generation of both total organic carbon and amino acids but the nitrogen is more important for the generation of carbohydrates and proteins. EEM spectra revealed differences in the composition of extracellular organic matter and intracellular organic matter. Regardless of the nitrogen and phosphorus concentrations, aromatic proteins and soluble microbial products were the main components, but the nitrogen concentration had a significant impact on their composition. The SF spectra were used to study the AOMs for the first time and identified that the protein-like substances were the major component of AOMs, creating as a result of aromatic group condensation. The D-UV spectra showed carboxylic acid and esters were the main functional groups in the EOMs, with $–OCH_3$, $–SO_2NH_2$, –CN, $–NH_2$, –O– and $–COCH_3$ functional groups substituting into benzene rings.

Introduction

In recent years, global climate change led to several hydrological geology disasters in great lakes such as the shrinkage and significant temperature increases that concentrated pollutants and aggravated lake eutrophication, resulted in frequent algal blooms in fresh water lakes and reservoirs (Glibert et al., 2014). Many algal bloom events have been reported in China's great lakes or reservoirs in recent years (Liu et al., 2011b), each of which is followed by a huge release

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of algal organic matter (AOM). As a major contributor to the natural organic matter (NOM) in lakes, these huge inputs of AOMs can have a big impact on a lake's NOM composition because of their different properties (Henderson et al. 2008a). In addition to destroying the geochemical composition of water bodies (Henderson et al., 2010), it also affects their aqueous water chemical behavior (Beaulieu et al., 2005; Vandamme et al., 2012). Generally, AOMs in surface water or lake, would be accounted for color, taste and odor (Dokulil and Teubner, 2000) and can lead to the production of hazardous cyanobacteria toxins. When lakes and reservoirs are used as source water, these variations of the NOM composition will also influence drinking water treatments including coagulation (Alizadeh Tabatabai et al., 2014; Henderson et al., 2010), filtration (Taylor Eighmy et al., 1992) and disinfection (Fang et al., 2010). The released AOM is not totally consumed by the heterotrophic bacteria in lakes, resulting in its concentration buildup. Recently, algal were used as an economic source for biofuel. The composition of algal will influence the harvesting-extraction systems and energy conversion efficiency. It is therefore necessary to assess how AOM in lakes is generated and how its composition changes during or after an algal bloom to better understand the resulting change in a lake's NOM, effects on subsequent drinking water treatment and algae biomass process.

The released AOM is composed of the extracellular organic matter (EOM) releasing from the algal cell and intracellular organic matter (IOM) from cell autolysis, generated during population growth and would be released after dead. Generally, the AOM is mainly composed of polysaccharides, proteins (González López et al., 2010), peptides, amino acids and other organic acids such as fatty acids (Cardozo et al., 2007). Several of these have been used to characterize the composition of the AOM for *Euglena gracilis*, *Microcystis aeruginosa*, *Chlorella vulgaris*, *Asterionella formosa* and *Melosira* sp. by examining their cell concentration, surface area, charge density, dissolved/total organic carbon (DOC/TOC), hydrophilic properties, carbohydrate, protein, molecular weight fractionation, specific UV absorbance (SUVA) (Labanowski and Feuillade, 2011), and UV absorbance at 254 nm (Leloup et al., 2013). However, most researchers focused on the EOM generated from the exponential or stationary phases of cyanobacteria and *chlorella* (Li et al., 2012). However, little has yet been done to characterize the properties of IOM and there have been no reports about the total amino acid content or the use of spectroscopic techniques such as synchronous fluorescence and UV–vis spectra to identify the composition of IOM under different growth phases.

Diatom, an important algal bloom species all over the world, is a useful ecological indicator of aqueous ecosystem health for the rapid growth rate and the role in aqueous food web (Chen et al., 2016), however, their AOM has rarely been characterized in either lakes or reservoirs, which will affect the aqueous quality and sediment aggregation. To date, only four marine diatom species, *Skeletonema costatum*, *Achnanthes brevipes*, *Chaetoceros affinis*, and *Cylindrotheca fusiformis*, have been characterized (Granum et al., 2002; Guerrini et al., 1998, 2000; Myklestad and Haug, 1972). The AOM composition is linked to the algae, growth phase, the age of the culture, the environmental conditions including nutrient loading (Pivokonsky et al., 2006). Although EOMs of *S. costatum* as a species of the marine diatom (Granum et al., 2002) and AOMs of *A. brevipes* blooming in Italian lakes were studied, only the carbohydrate, chlorophyll and enzyme composition were concerned, with scant attention being paid to spectroscopic analyses.

The objective of this work was, therefore, (1) to study the characteristics of the EOM and IOM produced by *Nitzschia palea*, the predominant diatom species in lakes and reservoirs in China (Ishikawa and Furuya, 2004; Yang et al., 2012); and (2) to investigate the influence of nutrients (nitrogen or phosphorus concentration) on the AOM profile of *N. palea* for all four growth phases.

1. Materials and methods

1.1. Algae cultivation

N. palea purchased from the Chinese Research Academy of Environmental Sciences was cultured in the D1 medium (the detailed composition is shown in Appendix A Table S1) of pH = 8.00–8.15 in an incubator (25°C, 12 hr/12 hr dark/light cycle; luminance = 2200 lx), and used for subsequent experiments once the cell concentration reached 1.0×10^6 cells/mL. To study the influence of nutrient salts (type and/or concentration) on the growth of the algae and the generation of AOM, the algae cells were pretreated in a starvation culture for 3 days and then spiked into the appropriate phosphate or nitrogen medium (details of the nutrient concentration are shown in Appendix A Table S2) at an initial concentration of 1.0×10^5 cells/mL. Each treatment set consisted of three parallel samples.

1.2. AOM extraction

Centrifugal-filtration was used to extract the EOM and IOM of *N. palea*. The AOM extraction method was as follows: (a) 10 mL of cell suspension was centrifuged at 5000 r/min, 2400 ×*g* for 15 min, and the supernatant was filtered through a 0.45 μm polyethersulfone membrane filter to obtain the EOM sample (Leloup et al., 2013); (b) 10 mL ultrapure water was then added to the same volume of the residue remaining in the centrifuge tube after the EOM extraction to wash the surface of cell twice and then the residual cell was resuspended in the ultrapure water. The cells were subjected to freeze–thawing (−80°C in ultra-low freezer, 35°C in water bath) for three cycles before being centrifuged at 10,000 r/min, 9600 ×*g* for 15 min, after that the supernatant was filtered again through a 0.45 μm polyethersulfone membrane filter to obtain the IOM sample (Zhu et al., 2015). The samples were prepared up to 3 days in advance and stored in a refrigerator at 4°C until use.

1.3. AOM characterization

1.3.1. Cell concentration and specific growth rate

The cell density of the algae was determined under an optical microscope using hemocytometers every 2 days until the beginning of the decline phase. The specific growth rate (μ) is calculated as Eq. (1):

$$\mu = \frac{\ln C_t - \ln C_0}{t} \quad (1)$$