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Research article

Assessment of water samples with complex compositions using microalgal bioassay based on the community level physiological profiling (CLPP)

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ABSTRACT

The ability to effectively characterize the response of microalgal communities to changes in water quality is limited. Earlier, a microalgal bioassay was developed based on community level physiological profiling (CLPP). The efficacy of this assay was evaluated using three wetland water samples, a surface water sample, and two wastewater samples (i.e. primary and secondary), all collected from southwestern Ontario, Canada. In addition, the assay was applied to untreated and activated carbon treated oil sand process water (OSPW). YT (Yeast Identification Test Panel) and Biolog plates were successfully utilized for defined microalgal community under both heterotrophic and mixotrophic growth conditions to characterize the changes in the defined microalgal community due to the changes in water type. It was found that, although the degrees of changes in the algal community varied, all tested water samples were distinguished under both growth regimes using principal component analysis (PCA). The variations in the algal community were caused by the differences of the water samples. The response of the assay due to changes in the algal community caused by different waters was found to be very sensitive and could be used to differentiate different water bodies. It further can be used to monitor temporal changes of water quality of the same water body.

1. Introduction

Functional or metabolic characterization of microbial community has been well developed after a method was first published by Garland and Mills (1991). Community level physiological profiling (CLPP) uses a commercially available Biolog 96-well plates containing up to 95 different carbon sources. Different microbial communities are compared and classified based on carbon source utilization patterns (CSUPs) (Garland and Mills, 1991). The relatively simple protocol and ease of use make it very practical for various applications (Weber and Legge, 2010).

CLPP has been applied on a variety of areas and expanded from prokaryotic bacteria to eukaryotic fungi and microalgae (Dobranic and Zak, 1999; Kim et al., 2017). The perturbation or change in the microbial community has been observed in terrestrial and aquatic environments caused by a plant interaction (Zhang et al., 2010), root secretion (Grayston et al., 1998), spill of hydrocarbon (Maila et al., 2006), metal contamination (Yang et al., 2006), water pollution with acid mine drainage (Weber et al., 2008), and differently fed bluegills in guts (Uchii et al., 2006). This technology has been further modified by the simultaneous use of antibiotics or cycloheximide to isolate the response of a particular group of interest in environmental samples by selectively repress signal interferences from either bacteria or fungi (Buyer et al., 2001; Pérez-Piqueres et al., 2006). It was then used as an assay to study the community tolerance to antibiotic sulfachloropyridazine (Schmitt et al., 2004) and toxicity to gold nanoparticles and ciprofloxacin (Weber et al., 2014).

Recently, a bioassay was developed based on CLPP using defined algal communities for the detection of micropollutants (Kim et al., 2017). The objective of the current study is to determine the performance of the microalgal bioassay based on CLPP by characterizing the changes in the defined microalgal community exposed to various water sources with complex compositions. Often, it is difficult to differentiate the effects of water quality from different water bodies using a standard single algae growth inhibition test (OECD 201, 2011). Moreover, there are unfavorable conditions in the environment where algal community exist in dark (e.g. arctic (Zhang et al., 1998) and deep ocean (Kamp et al., 2011)), therefore, it is intriguing to investigate the variable effects of mixed water under different growth conditions. In this study, wetland water samples, primary and secondary effluents from municipal wastewater treatment plant, river water, and oil sand process water (OSPW) were assessed using YT Biolog plates in a defined

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microalgal community under both heterotrophic and mixotrophic growth regimes. Samples with presumably different water quality were chosen to evaluate the efficacy of a microalgae CLPP based assay and not to remove certain nutrients (Shi et al., 2007; Shen et al., 2017).

2. Materials and methods

2.1. Water samples

Wetland surface water samples were collected from three sites in London, Ontario; Walkers pond (42°57′00.7″N 81°13′22.1″W), Pond mills (42°56′50.6″N 81°11′36.9″W), and Redmond's pond (42°58′18.0″N + 81°19′31.4″W) in July 2016. Primary and secondary wastewater samples were obtained from the Adelaide wastewater treatment plant (43°00′56.6″N 81°14′47.7″W) located in London, Ontario in April 2017. River water was collected from the North Thames River at a discharge point from the Adelaide wastewater treatment plant (43°00′48.4″N 81°15′11.5″W). Untreated oil sand process water (OSPW) was supplied by Suncor Energy (Calgary, AL, Canada). The OSPW was treated with granular activated carbon where most of the dissolved organic carbon (DOC) was removed. All the samples were sterilized by passing through 0.22 μm filters (Acrodisc® Pall, NY, USA), prior to addition to YT Biolog plates. The prepared samples were checked frequently for bacterial contamination by streaking onto Lysogeny broth (LB) agar plates and no contaminations were found (n = 5). Preliminary water assessment was conducted for different water quality. Table 1 summarizes fundamental characteristics of the water samples. A detailed analysis of the dissolved organic carbon was not conducted, however the ultra-violet (UV) spectra reveal substantial differences (Fig. 1). The total organic carbon (TOC) of the water samples was determined using a TOC analyzer (TOC-V analyzer connected with an ANSI-V auto sampler, Shimadzu, Japan). Nitrate (method 10242) and phosphate (method 10209) concentrations in water were analyzed using Hach kits (Hach, CO, USA).

2.2. Microalgal strains and mixed algal community

Five axenic cultures of freshwater green algae (*Chlorella vulgaris* (UTEX 2714), *Chlamydomonas reinhardtii* (CC 125), *Desmodesmus subspicatus* (CCAP 276/20), *Selenastrum capricornutum* (CCAP 278/4), and *Scenedesmus obliquus* (CPCC 5)), were obtained from collections at University of Texas at Austin, Chlamydomonas Centre, Culture Collection of Algae and Protozoa, and Canadian Phycological Culture Centre. Cultures were maintained and formulated into a synthetic mixed algal community of equal proportions as proposed by Kim et al. (2017). The reagent reservoir (Axygen® Biosciences, CA, USA) was used for the mixture of algal strains and the mixed cultures were brought up to a final volume of 15 mL to obtain the density of 1.5×10^6 cells/mL by a modified HS media dilution. An inoculum cell density of 1×10^6 cells/mL was used for the control and subsequent CLPP water sample assessment experiments as it was the cell density that showed a short lag in the growth for heterotrophic condition but still allowed data to be collected for the wells that reached fast growth under mixotrophic condition. To maintain an axenic condition, the individual and mix cultures were checked regularly for the bacterial contamination by streaking onto Lysogeny broth (LB) agar plates.

Table 1
Total organic carbon (TOC), nitrate, and phosphate concentrations were measured for various water samples.

Samples	Water	TOC (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Control	Modified HS media	97.3	0.4	123.6
Sample 1	Walkers pond	29.9	0.4	0.004
Sample 2	Pond mills	49.6	0.4	0.04
Sample 3	Redmond's pond	46.7	0.3	0.1
Sample 4	Primary effluent	120.0	0.3	15.3
Sample 5	Secondary effluent	–	–	–
Sample 6	North Thames River	58.2	1.2	0.3
Sample 7	Untreated OSPW	81.4	0.5	0.2
Sample 8	Treated OSPW	–	–	–

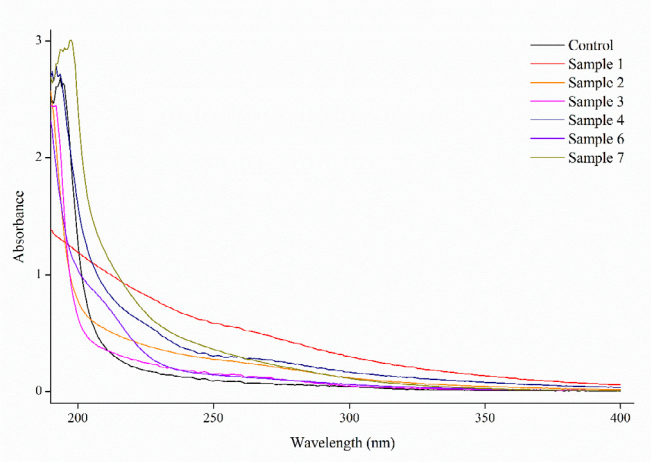

Fig. 1. Ultra-violet (UV) spectrum of various waters. Descriptions of the samples are presented in Table 1.

2.3. Sample assessment using community level physiological profiling (CLPP)

Prefilled 96-well microtiter plates (YT microplate; Biolog, CA, USA) were used to characterize different microalgal growth profiles of water samples under heterotrophic and mixotrophic growth conditions. The YT Biolog plates were selected as they have both sections of wells with or without included tetrazolium dye (Biolog YT microplate; available from Biolog, CA, USA). Only sixty bottom wells without tetrazolium dye were used for the analysis as tetrazolium dye can be light sensitive (Biolog redox dye mixes; available from Biolog, CA, USA). The sample assessment using CLPP was performed by exposing a synthetic mixed algal community of equal proportions to different water samples. The Biolog plates contained 100 μL of mixed algal community in 50 μL of water samples. The control represents a modified HS minimal medium. The Biolog plates were kept in dark at 25 °C for heterotrophic condition and incubated at 25 °C (Bechet et al., 2013; Cassidy, 2011) on shaking (150 rpm) with a light intensity of 140 $\mu E\, m^{-2}\, s^{-1}$ using an incubator (Infors HT Multitron, Basel, Switzerland) for mixotrophic condition. The growth of the algae was determined using a M1000 pro infinite series microplate reader (Tecan, Männedorf, Switzerland) measuring a fluorescence (excitation at 470 nm and emission at 650 nm) after orbital shaking for 15 s at 6 mm amplitude.

2.4. Analysis

The well fluorescence development measured through five days of treatment was corrected by subtracting the initial fluorescence readings (a fixed gain between 10 and 15 in t = 0 day was selected). Five days were chosen as it was desirable to assess the growth while algal cells were still actively growing as they reached a stationary phase at 6th day with the wells E1: D-Cellobiose and F3: α-D-Glucose for mixotrophic condition. One-dimensional metric of Euclidean distance (ED) analysis was performed following a method suggested by Weber and Legge (2009). The Euclidean distances of carbon source utilization data for algal communities by fluorescence developments of 59 wells or dimensions for each water sample between each time point and time zero were computed. Natural-log transformed fluorescence data in profiles