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Short communication

Photostimulation of sequential degradation and assimilation of recalcitrant carbonaceous organics in *Scenedesmus quadricauda*

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ABSTRACT

This study investigated the photostimulation of organic degradation with *Scenedesmus quadricauda* microalga, which is capable of assimilating organic carbon as a carbon source under mixo– and hetero–trophic growth conditions. The assimilability of carbonaceous organics in the microalga was significantly inhibited for polymeric organic compounds (a mixture of humic substances and polysaccharides) due to their recalcitrant characteristics when compared to using glucose. However, continuous illumination (150 μmol photons $m^{-2} s^{-1}$) under non-aerated conditions stimulated the microalga to increase the degradability of the organic mixture. The subsequent algal cultivation in the light using aeration with 10% (v/v) CO_2 achieved a significant assimilation of the carbonaceous organics along with nitrate, which was comparable to that observed when using glucose as a carbon source. This is the first time that the photostimulation of *S. quadricauda* has been shown to induce the degradation of humic-like substances that are typically resistant to microbial decomposition.

1. Introduction

The concentration and recalcitrance of wastewater effluent organic matter (EfOM) can be affected by both the characteristics of the raw sewage and the performance of the reclamation processes typically involved in biological treatment methods. The EfOM consists of a heterogeneous mixture of organic compounds that are polyfunctional polymers formed through the synthesis of products by chemical and biological processes [1], known also as the browning reaction transformations in wastewater treatment plants. The major fraction of the EfOM is constituted of humic substances that are typically resistant to microbial decomposition and strongly associated with the fate and transport of persistent organic pollutants with potential harmful impacts on human health and the environment. In practice, a wide range of recalcitrant organic compounds are found in the concentrate resulting from reverse osmosis (RO) filtration used in the tertiary treatment and/or indirect reuse of the secondary effluent from urban wastewater [2]. Due to their recalcitrance to microbial degradation, common biological treatments may be inefficient for robust treatment of RO concentrate; thus, it is necessary to utilize complementary technologies to degrade and mineralize them accumulated in the RO concentrate.

Some studies have reported that electrochemical oxidation can improve the degradation of dissolved organic matter in RO concentrate produced from municipal wastewater [3,4], and the combination of UV-based photochemical and electrochemical processes has also been investigated [5]. More recently, Umar et al. [6] suggested a sequence consisting of coagulation, UV/H_2O_2, and biological treatment to remove organic matter from a highly saline RO concentrate. Despite recent advances in remediation technologies, effective and economic strategies still need to be developed. Alternatively, algae-mediated wastewater treatment is effective in removing nutrients and heavy metals, reducing chemical oxygen demand, and removing and/or degrading xenobiotic compounds and other contaminants [7]. Despite previous demonstrations of algae as useful mediators for wastewater remediation, limited research has been carried out in removing non– or slowly–biodegradable organic matter in wastewater to date. To the best of our knowledge, it has not yet been reported that continuous illumination can stimulate *Scenedesmus quadricauda* microalga to sequentially degrade and assimilate the recalcitrant carbonaceous organic matter, along with also removing nutrients from wastewater in a single bioreactor. This observation has important implications for assessing algae-mediated photolysis in water environment, and further study may open an opportunity to develop an artificial biomimetic biophotolysis system that is believed to be cheap and safe to remediate contaminated environments. In this study, different phycoremediation modes were evaluated, and we also used advanced techniques to characterize their impacts on the degradation of carbonaceous organics.

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2. Experimental methods

2.1. Synthetic RO concentrate

Stock solutions of inorganic constituents were individually prepared by dissolving $NaHCO_3$, Na_2SO_4, $CaCl_2{\cdot}2H_2O$, KCl, $NaNO_3$, $MgCl_2{\cdot}6H_2O$, and NaH_2PO_4 in deionized water. Synthetic RO concentrate was prepared by adding stock solutions in commercially available mineral water (Samdasu, Korea) to achieve the following characteristics: HCO_3^- 488 mg L^{-1}, SO_4^{2-} 250 mg L^{-1}, NO_3^- 133 mg L^{-1}, Ca^{2+} 100 mg L^{-1}, K^+ 70 mg L^{-1}, PO_4^{3-} 31 mg L^{-1}, Mg^{2+} 20 mg L^{-1}. The above chemicals and NaCl were used to adjust the chloride concentration in synthetic RO concentrate to 1000 mg L^{-1}. Synthetic RO concentrate was autoclaved for 30 min and was cooled to room temperature prior to adding organic compounds. D–(+)–Glucose (i.e., dextrose), dextran (15–25 kDa) from *Leuconostoc* spp., and humic acid sodium salt were used to identify the extent of degradation and assimilation during algae treatment. Dextran has frequently been used as a good surrogate for polysaccharide-like substances present in secondary effluent [8]. Aldrich humic acid was purified by repeated pH adjustment, precipitation, and centrifugation to remove inorganic impurities. Experiments have used synthetic wastewater prepared with dextrose alone or by blending 60% dextran and 40% purified humic acid. The organic concentration was shown as dissolved organic carbon (DOC). All chemicals used to prepare synthetic RO concentrate were of analytical grade and were supplied by Sigma-Aldrich (St. Louis, MO). The pH of the mixture ranged between 7.5 and 7.8 (Orion Star A215, Thermo Scientific). The final concentrations of organic and inorganic components in the synthetic RO concentrate were selected based on previously reported observations with real RO concentrate [5,9,10].

2.2. Strain and batch experiments

The *S. quadricauda* strain (AG 10003) was obtained from the Korea Collection for Type Culture of the Korea Research Institute of Bioscience and Biotechnology (Jeongeup, Korea). The stock culture of *S. quadricauda* was grown in 2 L flasks containing 1.5 L of sterilized BG-11 medium [11] in air enriched with 5% CO_2 under continuous white fluorescent light illumination (75 μmol photons $m^{-2}\,s^{-1}$) at 25 °C. *Scenedesmus* is a dominant genus of algae commonly found in wastewater ponds [12], and can engage in mixotrophic growth, in addition to the common photoautotrophic growth.

A wide range of batch experiments were conducted to determine the removal of organic matter with *S. quadricauda* in the RO concentrate, and Table 1 shows the culture conditions in more detail. The algal cells in the exponential growth phase were collected via centrifugation (3000 rpm, 10 min), washed by mineral bottled water, and inoculated at 200 mg L^{-1} (as dry weight) into flasks containing 800 mL of synthetic RO concentrate. The flasks were incubated at 25 °C while shaking at 130 rpm for 48 h. In some experiments, the incubation was conducted under continuous illumination with a light intensity of 150 μmol photons $m^{-2}\,s^{-1}$, and aeration was optionally applied with and without supplemented CO_2 (10%, v/v) at a flow rate of 40–50 mL min^{-1}. During incubation, 50 mL of mixed liquor was collected from each flask at 24 and 48 h, and manually filtered with 0.45 μm polyethersulfone membranes, prior to measuring the water quality. The persulfate digestion method was used to measure the total nitrogen (TN) in the water samples using a DR/5000 spectrophotometer. Identical spectrophotometer was also used to measure the UV absorbance at 254 nm (UV254). The DOC was determined (TOC-V CPN, Shimadzu, Japan), and the specific UV absorbance (SUVA) value was calculated from the UV254 divided by the DOC of the water sample. Each measurement was carried out in triplicate, and the average values were reported. The cell growth was also monitored by determining the algal cell concentration using fluorescence-based flow cytometry. The analysis was immediately carried out using a Partec CyFlow® Cube6 flow cytometer (Partec GmbH, Görlitz, Germany) equipped with a 20 mW blue diode pumped solid-state laser emitting at 488 nm. The autoclaved cellular suspension was also analyzed for negative control to differentiate intact cells from non-viable cells and non-algal particles. The flow cytometer operated at a constant flow rate of 300 μL min^{-1}, and at least 10^5 events (number of fluorescent particles) were tested for each sample. All the collected fluorescence data were processed using FCS Express 4 Cytometry software (De Novo Software, Glendale, CA). Control batches without algal biomass were also incubated under the identical conditions applied to the algal cultures. The control batches were prepared using the RO concentrate with added glucose which is more biodegradable than a mixture of humic acid and dextran. The DOC concentration was monitored at intervals during the control experiments.

A separate batch test was performed to identify and characterize the algae-induced degradation of dissolved organic matter in the RO concentrate. The test was conducted using an identical apparatus to that described above, but the flask was incubated under continuous illumination (150 μmol photons $m^{-2}\,s^{-1}$) with sequential non-aeration (12 h) and aeration (48 h) while shaking at 130 rpm. The mixed liquor was collected after the first 12 h incubation from the flask to perform an advanced characterization of the dissolved organic constituents. Fluorescence spectra were collected using a Shimadzu RF-5301PC fluorescence spectrometer with a 150W xenon lamp source. Three-dimensional spectra were obtained by repeatedly measuring the emission (Em) spectra within a range of 280–600 nm, with excitation (Ex) wavelengths from 200 to 400 nm spaced at 10 nm intervals in the excitation domain. The spectra were then concatenated into an excitation-emission matrix (EEM). Typical surface water and wastewater effluent organic matter may contain two major fluorescence peaks, described as tryptophan-like (T1 and/or T2) and humic-like (A and/or C) fluorescence maxima [13,14]. The EEM components determined in this study were T1 (Ex/Em 220–240/330–360 nm), T2 (Ex/Em 270–280/330–360 nm), A (Ex/Em 230–260/400–450 nm), and C (Ex/Em 300–340/400–450 nm) [15]. Dissolved organic matter was also characterized by liquid chromatography with online organic carbon

Table 1
Culture conditions applied in this study for the treatment of RO concentrate with *S. quadricauda*.

Culture ID	Light[a]	Aeration[b]	CO_2 supplement[c]	Organic matter applied	Description
A	On	No	No	Humic acid (40%) + dextran (60%)	Culture in the light under non-aerated conditions without supplemented CO_2
A+	"	"	"	Glucose (100%)	"
B	Off	Yes	No	Humic acid (40%) + dextran (60%)	Culture in the dark under aerated conditions without supplemented CO_2
B+	"	"	"	Glucose (100%)	"
C	On	Yes	Yes	Humic acid (40%) + dextran (60%)	Culture in the light under aerated conditions with supplemented CO_2
C+	"	"	"	Glucose (100%)	"

All the culture media were freshly prepared prior to cultivation of *S. quadricauda* (200 mg L^{-1}) for 48 h under the given conditions.

[a] Light intensity: 150 μmol photons $m^{-2}\,s^{-1}$.

[b] Flow rate: 40–50 mL min^{-1}.

[c] 10% (v/v).