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Synergistic interaction between metal ions in the sea salts and the extracellular polymeric substances for efficient microalgal harvesting

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ABSTRACT

Artificial sea salts were found to cause microalgal flocculation and was so rather effectively in the presence of extracellular polymeric substances (EPS). EPS-producing species *Ettlia* sp. YC001 formed flocs at relatively low salt concentration: at 3.5 g/L, it reached up to 90% of the flocculation within 2 h. EPS non-producer *Chlorella vulgaris* UTEX 265, on the other hand, was less responsive to the salts even at 7 g/L and it had only 42% of floc formation. This phenomenon of salt-mediated flocculation appeared to be brought about mainly by calcium ion, as it has had greater affinity to the EPS than any other ions including the divalent magnesium ion. This sea salt-based coagulation may serve as one environmental-friendly alternative to commonly used chemical flocculants, which also potentially possess an economic merit.

1. Introduction

As a third generation biofuel source, microalgae have entered the mainstream of energy research in recent years, aiming to possibly replace at least some of petroleum. However, a current cost of fuel production from microalgae is far from being able to be commercialized: every step of the whole process is either energy-intensive or inefficient. Harvesting, which is a critical step for the subsequent oil extraction to be energetically viable, is nevertheless expensive in itself due partly to large investment costs [12] and energy consumption, accounting for 20–30% of total biomass production cost [7].

Among a good many means of concentrating microalgal biomass developed thus far [4], flocculation is advantageous particularly for bulk biomass production, because it consumes less energy and also requires less capital costs and energy than competing harvesting techniques [15]. The flocculation is driven by 3 distinctly different mechanisms, namely, charge neutralization, sweeping flocculation, and bridge formation. Charge neutralization occurs through the electrostatic interaction between positively charged ions or polymers and negatively charged cell surface. Sweeping flocculation is an entrapment of cells by metal precipitates in a culture [15]. It can happen because metal precipitates are large enough to be able to trap microalgal cells regardless of their charges. The last mechanism, bridge formation, is especially interesting in that it appears to be mediated by the natural polymers referred to as extracellular polymeric substances (EPSs); so this phenomenon is termed autoflocculation. This EPS-mediated autoflocculation was rather well known to take place in self-flocculating microalgal species such as *Ankistrodesmus falcatus*, *Scenedesmus obliquus*, *Tetraselmis suecica*, and *Ettlia texensis* [10,11], by way of EPS binding to cell surfaces and inducing large aggregates.

The purpose of this study was to investigate the sea salt-induced flocculation of *Ettlia* sp. YC001, particularly focusing on interactions between EPS and metal ions. Underlined mechanisms were speculated by comparison of EPS-producing species *Ettlia* sp. YC001 and EPS non-producer *Chlorella vulgaris* UTEX 265.

2. Materials and methods

2.1. Microalgal strain and cultivation condition

A microalgal strain *Ettlia* sp. YC001 was obtained from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) [17]. *Chlorella vulgaris* UTEX 265 was obtained from University of Texas at Austin Culture Collection of Algae. Both strains were cultivated in two different 2 L Duran laboratory glass bottles containing 2 L of tris-acetate-phosphate (TAP) medium which was comprised of 2.42 g L^{-1} $H_2NC(CH_2OH)_3$, 375 mg L^{-1} NH_4Cl, 100 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 50 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 288 mg L^{-1} K_2HPO_4, 144 mg L^{-1} KH_2PO_4, 50 mg L^{-1} $Na_2EDTA \cdot 2H_2O$, 22 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 11.4 mg L^{-1} H_3BO_3, 5 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 5 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 1.6 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 1.6 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 1.1 mg L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$

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and 1 ml L^{-1} acetic acid under continuous illumination at a light intensity of 100 μmol photons m^{-2} s^{-1}. Cell growth was monitored every 24 h by a UV/Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 680 nm and also by way of measuring dry cell weight. For the dry weight measurement, a culture sample was filtered through a GF/C glass-fiber filter (Whatman) paper and dried at 60 °C for 24 h, after which the final weight of the filter paper was compared with the initial value. The plotted values to determine cell growth are the average of three independent measurements of two different culture bottles.

2.2. Microalgal flocculation by artificial sea salt and metal salts

The effects of sea salt concentration and extracellular polymeric substances (EPS) on microalgal harvesting were examined by adding different concentrations of artificial sea salts (Sigma Aldrich, Korea) to algae cultures 9 days after inoculation (Fig. 1.). The concentrations of major metal cations from the sea salts are provided by the Sigma Aldrich Korea (g L^{-1}), which are converted into mmol L^{-1} (Table 1). For a systematic study of the possible role of calcium and magnesium, 20, 50 and 80 mg L^{-1} of calcium chloride and 65, 160 and 260 mg L^{-1} of magnesium sulfate (Sigma Aldrich, Korea) were used, which are equivalent to 1.75, 4.25, and 7 g L^{-1} of the artificial sea salts. Harvesting efficiency was measured every 30 min upon the salt added, by using an UV/Vis spectrophotometer at 680 nm (UV-1800, Shimadzu, Japan). The efficiency was estimated as follows:

$$\text{Harvesting efficiency at time t}\ (\%) = (OD_{680}\ (t_0) - OD_{680}\ (t))/(OD_{680}\ (t_0)) \times 100\%,$$

where OD_{680} (t_0) represents the initial cell concentration of a sample at time zero, and OD_{680} (t) is the cell concentration of the sample at time t.

2.3. Separation and quantification of extracellular polymeric substances (EPS)

EPSs were first separated by centrifugation at 3500 rpm for 10 min. The subsequent separation method was similar to the reported methods [3,11]. The supernatant was then filtered through a GF/C glass fiber filter with a pore size of 0.45 μm to remove any cell debris. The cell pellet collected by the centrifugation was resuspended in deionized water; the solution was then agitated with a magnetic stirrer at 1000 rpm for 2 h and centrifuged at 5500 rpm for 5 min. This supernatant, together with the one from the initial separation, was used to quantify total carbohydrates in the EPS, which was done by the phenol-sulfuric acid assay of [1] using glucose as a standard.

Table 1
Concentrations (mM) of the metal cations in the sea salts used as flocculants.

	Na^+	Mg^{2+}	K^+	Ca^{2+}
Artificial sea salts	468.90	54.30	10.74	9.98

2.4. Scanning electron microscope energy dispersive spectroscopy (SEM-EDS)

For SEM-EDS analysis, the centrifuged pellets were lyophilized but without being immobilized by glutaraldehyde solution, as the primary aim of this analysis was to detect ions and measure their concentrations on the cell surface, rather than to take cell images. The lyophilized cells were spotted on a silicon-wafer by carbon adhesive tabs. After CO_2 drying for 10 s, the sample was sputtered with osmium under 4 Pa for 15 s. The cells were then analyzed at 10 kV in a high-resolution scanning electron microscope with the energy dispersive spectroscopy detector to quantify relative concentration of ions on the cell surface.

3. Results and discussion

3.1. Sea salt induced microalgal flocculation

The addition of artificial sea salts caused flocculation in a species-dependent manner (Fig. 2). *Ettlia* sp. YC001 responded quite sensitively to the salt: it formed flocs as little as 3.5 g/L. *Chlorella vulgaris* UTEX 265, on the other hand, was less responsive: flocculation was seen at 7 g/L and was so to a much reduced degree. *Ettlia* sp. reached up to 90% of flocculation within 2 h, whereas *Chlorella vulgaris* showed only 42%, suggesting that the sea salts could indeed be used as a natural flocculant at least for *Ettlia sp.*

The sea salts used in this study contained relatively high concentrations of magnesium and calcium (Table 1); these divalent metal cations were almost only ligands that possibly brought about such floc formation. Microalgal flocculation is typically proceeded by using trivalent metal ions such as aluminum and ferrous ions in the forms of $Al_2(SO_4)_3$ and $FeCl_3$, respectively [9,16]. These trivalent cations are known to give rise to flocculation by way of charge neutralization. The positively charged metal ions are attached to the negatively charged carboxyl groups on the cell surface [8] and in so doing the aggregated cells are left with nearly no surface charge. Divalent metal cations such as magnesium and calcium ions can also act as flocculants, but they are effective only at pH higher than 11 [14]. At this strong alkaline

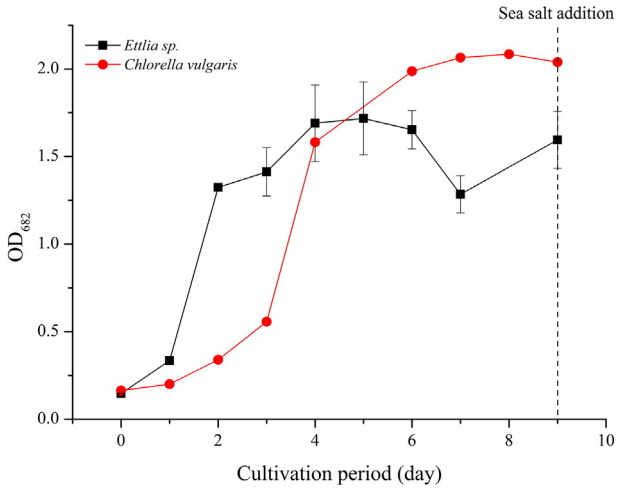

Fig. 1. Growth curves of *Ettlia* sp. YC001 and *Chlorella vulgaris* UTEX 265. Some of the standard errors of the two replicates are smaller than their symbol size.

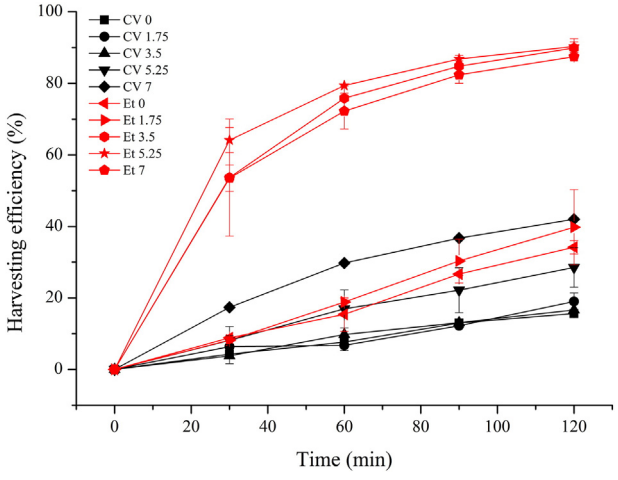

Fig. 2. Harvesting efficiencies of *Ettlia* sp. YC001 (Et) and *Chlorella vulgaris* UTEX 265 (CV) in terms of sea salt concentrations (g/L). Some of the standard errors of the three replicates are smaller than their symbol size.