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Chlorella vulgaris cultivation with an additive of magnesium-aminoclay

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ABSTRACT

In integral microalgal biofuel production consisting of microalgal culturing, harvesting, lipid (oil) extraction, oil-to-biodiesel conversion and biodiesel quality improvement, cultivation to improve lipid (oil) content in microalgal cells without inducement of transformants is a booming field of experimentation and application. In the present study, water-solubilized, transparent and cationic-charged and clustered magnesium-aminoclay (MgAC) was utilized as a co-additive in *Chlorella vulgaris* cultivation. The MgAC effected both an increase of cell size (from ~3.524 μm to ~4.175 μm size) with low cell density (from 1260×10^6 cells/mL to 511×10^6 cells/mL) and an increase of total lipid content without microalgal cell transformation according to various MgAC loadings (0, 0.25, 0.50, and 1.0 g/L) at the 2.0% CO_2 supply. The cationic-charged MgAC clusters wrapped the microalgal cells to create a stressful microenvironment, increasing the intracellular reactive oxygen species (ROS) content. Therefore, additive-MgAC-based microalgal cultivation is herein suggested in a novel microalgal biofuel production.

1. Introduction

As a replacement for depleted petroleum oils, environmentally and economically sustainable biofuel from microalgal biodiesel production is the subject of intensive interest in the academic and industrial sectors [1]. Microalgae-based biofuel production is confronted by important issues involving cultivation, harvesting, dewatering, lipid (oil)-to-biodiesel conversion by microalgal cell disruption in harvested wet biomass, biodiesel-quality upgrading, and others [2,3]. Moreover, for each down-stream step including microalgal cultivation, harvesting and oil-to biodiesel conversion, cost reduction is necessary to achieve market survival [4].

With application of nanotechnology to this bioenergy area, recently Lee et al. reviewed nanoparticles (NPs)-engineering-based microalgal biofuel production, particularly microalgal cultivation and harvesting in down-stream processes [5]. For instance, *Chlorella vulgaris* cultivated with $MgSO_4$ NPs as a micronutrient supplement, compared with the case of $MgSO_4$ salt supplement, showed an increased oil yield per glycerol consumed [6]. Kadar et al. reported the direct use of *Pavlova lutheri* and *Tetraselmis suecica* as inducers of lipid accumulation in the presence of nanoscale zero-valent iron (nZVI), which enhanced lipid contents with normal growth [7]. Very recently also, a commercial TiO_2 photocatalyst as a co-additive in *C. vulgaris* UTEX 265 cultivation increased lipid productivity (~15%) under a stressful condition versus no TiO_2 reference [8]. However, effect on microalgal lipid content enhancement was related on those NPs' toxicity. This creates a bottleneck due to the consequent difficulty of NP recovery in consideration of succeeding downstream processes such as microalgal harvesting, lipid extraction from harvested wet-biomass, and oil-to biodiesel conversion, among others [9–11].

Importantly, 3-aminopropyl-functionalized magnesium phyllosilicate (i.e., Mg-aminoclay [MgAC]) was formulated by Mann et al. in 1997 [12]. Aminoclays (ACs), made of cationic metals (Mg^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, and Ce^{3+}), can be functionalized with $–NH_2$ organic moiety [13] by simple one-pot sol-gel reaction under mild conditions [14]. In aqueous solution, MgAC is water-solubilized and transparent. It functions as cationic-charged organo-building blocks (average hydrodynamic diameter: 30 nm) [15] due to the protonated amine groups, manifesting in sheet form in aqueous solution and interacting with negatively charged biomolecules such as DNA, enzyme, lipid (etc.) and resulting thereby in mechanical/thermal-properties-enhanced, self-assembled bio(nano)composites [16]. Utilizing cationic-charged MgAC over a wide pH range (2.0–12.0) [17], Lee et al. trialed efficient and effective microalgae harvesting in freshwater and seawater by means of a sweep flocculation mechanism [4]. Additionally, lipid extraction efficiencies from wet microalgae biomass were achieved by ACs due to the cell-destabilization effect of MgAC coating and the high density of Brønsted basicity in MgAC [10,13]. MgAC's destabilizing effect on cell membranes/walls prompted us to investigate microalgae [18] transformation by stick spreading with

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mixture of MgAC and plasmid DNA on an agar plate which allows DNA penetrate into the internal cells with the protection of enzymatic nucleic acid cleavage, revealing an efficient nucleic acids delivery carrier.

However, in the viewpoint of microalgae-based biofuel production, the effects of MgAC addition on microalgal cultivation in integral biorefinement have as yet gone unreported. Specifically, the purpose of this study is focused on enhancement of lipid content in microalgae biomass by cultivation with co-additive of MgAC NPs without inducement of microalgal transformants.

2. Materials and methods

2.1. Materials

3-aminopropyltriethoxysilane (APTES; ≥98%, 221.37 g/mol), DCFDA (2′,7′-dichlorodihydrofluorescein diacetate, 487.29 g/mol), dipyridamole, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, USA). Magnesium chloride hexahydrate (98.0%, 203.30 g/mol) was acquired from Junsei Chemical Co. Ltd. (Tokyo, Japan). Ethanol (18L, 95%) was obtained from Samchun Pure Chemicals (Pyungtack, Korea). All of the chemicals were used as received. Distilled deionized water (DI water; resistance >18 mΩ) was employed throughout the experiments.

2.2. Preparation of magnesium-aminoclay (MgAC)

MgAC was prepared according to the procedure outlined in the literature [12]. Briefly, ~8.4 g of $MgCl_2 \cdot 6H_2O$ was dissolved in 200 mL of ethanol solution. After 10 min magnetic stirring, ~13 mL of APTES was drop-wise added to the mixture for a ~1.34 M ratio of Si in APTES to Mg in $MgCl_2 \cdot 6H_2O$ [19], and the resultant solution was stirred overnight. The obtained white-slurry MgAC was centrifuged at 3000 rpm for 15 min, afterwards the separated MgAC was washed twice with 100 mL ethanol solution. It was then oven-dried at 60 °C overnight, and finally, the gravel-shaped MgAC was powdered by pestle and mortar preparatory to use. For confirmation of its delamination of MgAC in aqueous solution, 10 mg/mL MgAC in a 100-mL plastic bottle was bath-sonicated for 5 min, showing its water-solubilized and transparent appearance.

2.3. MgAC-based C. vulgaris cultivation

Microalgae *C. vulgaris* (UTEX-265) was obtained from the UTEX Collection Center of the University of Texas at Austin (USA). A *C. vulgaris* seed culture was grown in a 500 mL glass flask using 300 mL of a modified BG-11 medium (with half the sodium nitrate concentration [0.75 g/L] of the original recipe [1.5 g/L]) with 2% CO_2 (v/v) bubbling at a flow rate of 150 mL/min, under a light intensity of 100 μmol/$m^2 \cdot s$ and 125 rpm orbital-shaker mixing. In order examine lipid induction by only MgAC, the recipe was modified to avoid nutrient starvation. The pH of the culture medium was adjusted to 7.5 using 1.0 N HCl [20]. For the MgAC-assisted cultivation, *C. vulagris* was grown in a BG-11 medium modified and subjected to the same conditions as noted above, except for the addition of MgAC in various concentrations (0, 0.25, 0.5 and 1.0 g/L) with 2.0% CO_2 gas purging and atmospheric air purging. All culturing are performed in triplicates and plotted with averaged values and standard deviation (Sigma-Plot version 9.0).

2.4. Water reuse for C. vulgaris cultivation

Water recycling in the course of microalgal cultivation is one of the principal elements of economically viable commercialization of microalgae-based biofuel production [21]. In the present study, after MgAC-based harvesting, water was recycled and reused three times in the harvest of microalgal cells, addition or adjustment of 1.0 g/L MgAC, monitoring the microalgal growth at five times. In addition, water-reuse experimentations were repeated until distinct inhibition of microalgae growth due to accumulated MgAC concentration in the culturing supernatant solution. All culturing are performed in triplicates and plotted with averaged values and standard deviation (Sigma-Plot version 9.0).

2.5. Cell counting, measurements of cell size and imaging

Microalgal cell growth was monitored by cell count using a hemocytometer under optical microscopy, and the optical density (OD) of the microalgal cells was measured at 680 nm by UV-VIS spectrophotometry. The cell size was measured using the Coulter counter (Beckman Coulter, Multisizer 4, USA). Microalgae cell imaging was performed both before and after cell staining with Nile red dye [22] under light microscopy (Leica DM 25000, Leica Microsystems, Switzerland).

2.6. Measurements of cell weight and lipid contents

Microalgal-cell biomass was calculated by cell weight using Whatman® filter paper. Subsequently, biomass samples were vacuum-freeze-dried for 24 h at −70 °C and 1.0 mm torr vacuum pressure. Using dried 10 mg samples, a lipid analysis was carried out by a gas chromatography (GC) technique, applying the 2:1 chloroform:methanol (v/v) ratio for the extraction along with sulphuric acid (H_2SO_4) and methanol (MeOH) for the transesterification reaction (Folch method; [23]). All lipid contents are performed in triplicates and plotted with averaged values and standard deviation (Sigma-Plot version 9.0).

2.7. Measurement of reactive oxygen species (ROS) in the presence and absence of MgAC

According to the MgAC concentrations, reactive oxygen species (ROS) assay was conducted as specified in literature [24]. Briefly, in the presence of MgAC concentrations (0, 0.25, 0.5, and 1.0 g/L) or 0 mg/L MgAC with dipyridamole (10 μM) as a negative control, ROS generation was evaluated in triplicate runs. Samples were withdrawn at 4, 8, and 12 days, immediately stained with 10 μM DCFDA in DMSO for 60 min at room temperature, and then washed with phosphate-buffered saline (PBS) (50 mM, pH 7.0). The photoluminescence spectra were recorded by an RF 5301 PC spectrofluorophotometer (485 nm excitation/530 nm emission, 150 W Xenon lamp, Shimadzu). All tests are performed in triplicates and plotted with averaged values and standard deviation (Sigma-Plot version 9.0).

2.8. Other characterizations

MgAC dispersed in DI water (0.25 mg/mL) was morphologically examined by field-emission transmission electron microscopy (FE-TEM, 200 kV, Tecnai F20, Philips). MgAC concentrations were calculated by calibration of Si concentration by measurement of Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, OPTIMA 7300 DV, Perkin-Elmer, USA). A pH meter (ORION STAR A211, Thermo Scientific™, USA) was used for monitoring in pH values.

3. Results and discussion

3.1. Rudimental information on MgAC structure

In order to check rudimental MgAC structure, Fig. 1a depicts the ideal approximate unit structure of MgAC (chemical composition: $[H_2N(CH_2)_3]_8Si_8Mg_6O_{12}(OH)_4$) [18]. An octahedral brucite ($Mg(OH_2)$) sheet exists in the middle sandwiched between tetrahedral silica sheets, thus forming 2:1 smectite phyllosilicate. At the tetrahedral silica sheets, organic pedants of $-(CH_2)_3NH_2$ are densely functionalized. TEM image