Asian Journal of Chemistry; Vol. 27, No. 3 (2015), 870-874

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17157

Variations of Macro-compounds in Cyanobacterium, *Thermosynchococcus elongatus* CL-1 Under Various Nutrients

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Received: 3 February 2014; *Accepted*: 8 May 2014; *Published online*: 19 January 2015; AJC-16687

The photosynthetic algae, *Thermosynchococcus elongatus* CL-1 (TCL-1) purified from an alkaline hot spring (pH 9.3, 62 °C) in eastern Taiwan was applied to enhance the overall CO_2 absorption capacity in aqueous solution. It was observed that the *Thermosynchococcus elongatus* CL-1 can be experimentally cultivated over pH 12 and suggested its high affinity of bicarbonate and adapted ability of alkaline condition. A series of the variations of macro-compounds, including lipids, carbohydrates, proteins, pigments and element compositions (C, H and N) were determined under a batch cultivation at pH 9. Additionally, a combined method with Fourier transform infrared spectrum and curve fitting as well as a wet chemistry method was used to determine the variations of lipids.

Keywords: *Thermosynchococcus elongatus* CL-1, Kinetic, Macro-compounds, FTIR.

INTRODUCTION

The Kyoto protocol was based on the obligation of reducing greenhouse gas emissions, aimed especially at lowering the amount of CO_2[1]. The CO_2 emitted from hot flue gas in thermal sources such as power plants is abundant. However, high temperature is a technical barrier in a biological treatment system for CO_2 mitigation. Cyanobacteria, such as *Thermosynchococcus elongatus* CL-1, which tolerate over 60 ºC and high alkaline, have been obtained from hot springs in eastern Taiwan[2]. In addition to performance of bio-fixation, biomass was an important bio-energy source to balance the energy consumption. However, the composition variations of biomass obtained from different growth time under a batch cultivation is not well understood. On the other hand, the contents of lipids (LI), carbohydrates (CA), proteins (PR), pigments (PG) were not easily analyzed by wet chemistry methods because of long-term analysis period and organic solvent using.

Unlike wet chemistry method, the spectra technology has many advantages. First of all, sufficient sample is needed for analysis. Second, much time can be saved. Finally, no organic solvent is used. Accordingly, FTIR is potentially recognized as one of the important spectra to measure cellular macromolecules[3,4]. Quantitative analysis of those cellular macromolecules was applied *via* standardized methods[5]. The internal standard was usually calcium carbonate or sodium azide whose peaks did not overlap with the peaks of macromolecules such as lipids, proteins and carbohydrates[6]. Which peaks and ranges of spectra taken as the characteristics of lipids, carbohydrates, proteins and pigments were always according to those functional group. The process combined with spectral derivation and curve fitting was common to formulate which peaks belonged to which macromolecules. Higher orders of derivations will produce more detail separated peaks but also more error peaks. Hence we proposed a process to purified the lipids, carbohydrates, proteins and pigments step by step with the chemical method and then identify the separated characteristics peak of spectra. The result of this process was compared with that of curve fitting process.

EXPERIMENTAL

Cultivation: Environmental cyanobacterial material, TCL-1, was obtained from Chin-Lun hot spring (pH 9.3, 62 °C) in eastern Taiwan and purified as well as identified in the laboratory[7,8]. A Modified Fitzgerald medium was adopted as the culture solution. The medium was sterilized by an autoclave at 121 °C and 1.5 atm. The cultivation condition was at 50 ºC, 10,000 Lux and 3 g/L $NaCO_3$ additive (DIC = 28.3 mM). The cultivation with a constant pH value was also carried out by automatically adding 0.1 N of HCl through a feedback system in the constant pH cultivation experiments. Other details of the procedures were previously documented[7].

Extraction of lipids, carbohydrates, proteins: The extraction procedure of lipids was to add 5 mL of chloroform-

methanol solution (2:1 v/v) to the 10 mg of dry biomass. Stand 0.5 h at room temperature and put ultrasonic probe into it 5 min. Then, left for 1 h at room temperature, centrifuge 6,000 ×g 5 min and collect the suspended solution. The procedure above was repeated two times. Dry the pellets from the extraction above. The DNA isolation was carried out with phenol extraction method. The proteins was taken from the proteins and carbohydrates was obtained from the residues.

FTIR analysis: 10 mg of the algal sample (or extractors or residues from its 10 mg biomass) was mixed with 1,000 mg of potassium bromide by grounding in a vessel. 200 mg of the mixture was taken and placed on a pan with a 13 mm diameter and was compressed to a disc shape by an oil pressure machine. 0.5 mg of $CaCO_3$ (an internal standard) was mixed with 199.5 mg of KBr and compressed to a disc shape, following the same procedure. For the quantitative analysis, spectra were obtained with an FTIR spectrometer (Perkin Elmer Spectrum One): from 4,000 to 450 cm^{-1}, transmittance type of spectra, a resolution of 2 cm^{-1}, a scan speed of 0.2 cm s^{-1} and scans of 100 duplications. The procedure was repeated for three times.

Peak fitting: The fourth derivative, curve fitting and peak area integration were performed using the software Origin 6.1. First, the fourth derivative of the spectra was obtained using the Savitsky-Golay function and a second degree polynomial with 50 convolution points. Then, the identified peak positions and widths from the fourth derivative were used for the purpose of curve fitting and peak area integration.

The analysis of lipids, carbohydrates and proteins *via* other methods. The total lipids of the algal cells was estimated from the extract by conventional gravimetric analysis[9]. Analytical method of carbohydrates was followed by the colourimetric method[10]. The content of proteins was determined *via* N contents × 6.25.

Elemental analysis of biomass and determination of ion concentrations of carbonate, nitrate and sulfate in the medium. The concentrations of four elements (C, N, H) of biomass were determined using elemental analyzer. The concentrations of CO_3^{2-}, NO_3^- and SO_4^{2-} within the media under different conditions were determined with ion chromatography (IC).

RESULTS AND DISCUSSION

TCL-1 adapted high alkaline condition: The pH values increased as the increase of the cell densities due to the photosynethetic alkaline mechanism. Before the initial 25 h, the pH value approached to 12 and this value seems to inhibit the growth of TCL-1 algae. Note that the activation of TCL-1 was observed when the titration of HCl was conducted to pH 7. The cell density and pH value increased simultaneously to 300 mg L^{-1} and 12.2, respectively. After titration of HCl, the TCL-1 algae remained the high activation even over pH 11. Regarding $CO_2/HCO_3^-/CO_3^{2-}$ equilibrium system in water, carbonate is the main role over pH 11.3 and not the useful carbon source in any research[11] (Fig.1). Low concentration of bicarbonate under the equilibrium of HCO_3^-/CO_3^{2-} may not limit the growth seriously. This species may have high affinity of bicarbonate and high ability of mitigation of CO_2 at high levels of alkaline.

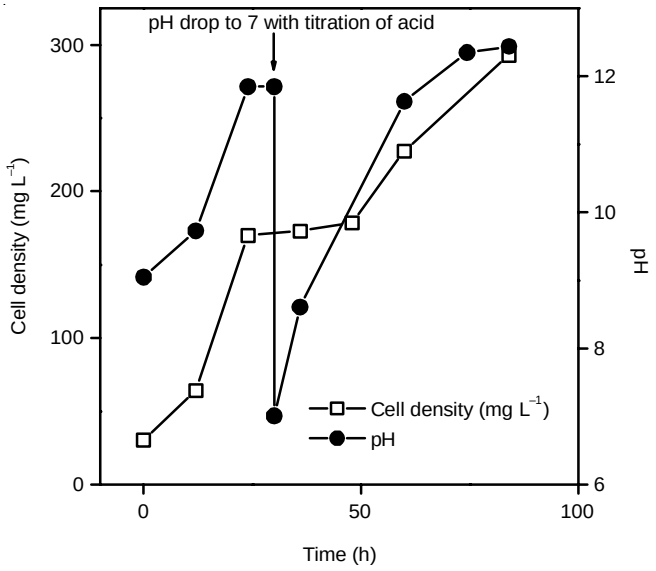

Fig. 1. Growth curve of upper pHs physiological limitations *via* batch cultivation and titrating HCl one time

Ratios of C/H and C/N varied under batch cultivation: The batch cultivation under pH 9 and 28.3 mM of DIC concentration was carried out. Only pH 9 and 10,000 Lux of light intensity was controlled. S1 happened before 50 h (Fig. 2a) and S3 was after 125 h (Fig. 2a and 2b). The acidification at S3 was observed due to the increment of acetic acid (data not shown) and pH decrease from 9 to < 5 (Fig. 2b). The cell density was also down below 200 mg L^{-1} due to the degradation of biomass (Fig. 2a). As shown in Fig. 2c, the molar ratios of C/H increased at S1. The source of inoculation was cultivated under air bubbling. Air bubbling provided not enough carbon and may provide the activation of carbon concentration mechanism (CCM). As transfer to 28.3 mM of DIC concentration, molar ratio of C/H may increase due to the increase of carbon supply. After this stage, this ratio remained a constant even at S3. At S2, the condition of cells was stable and C and H contents were also stable. At S3, the degradation of cell will decrease the C and H contents but the ratios were very similar. Regarding molar rations of C/N, the increases were observed at initial 50 h (S1) but decreased after this time (S2 and S3). Although several proteins must be produced at S1 to adapt different growth condition (air bubbling to 28.3 mM of DIC concentration) but the increment of N was smaller than one of C. At S2 and S3, the increment was approach to nothing or even negative, the ratios of C/N decreased. In summarize those results, related to the activation cultivation under air bubbling, 3 g L^{-1} of the additive Na_2CO_3 was the more abundant carbon source. The variations of molar ratios of C/H and C/N seem the stimulus of higher bicarbonate concentrations. The concentration of carbonate, nitrate approached to 0 and 120 mg L^{-1} after 50 h (the end of S1) and carbonate was limited nutrient. Regarding the uptake of sulfate, two stages of uptake were observed through S1 to S2 but the mechanism was not easy understood.

Micro- and macro-compound ratios were identified with FTIR: As shown in Fig. 3a, the separated spectra of micro-compounds and four macro-compounds were identified. One of the extracted lipids included 3000-2800, 1700-1500

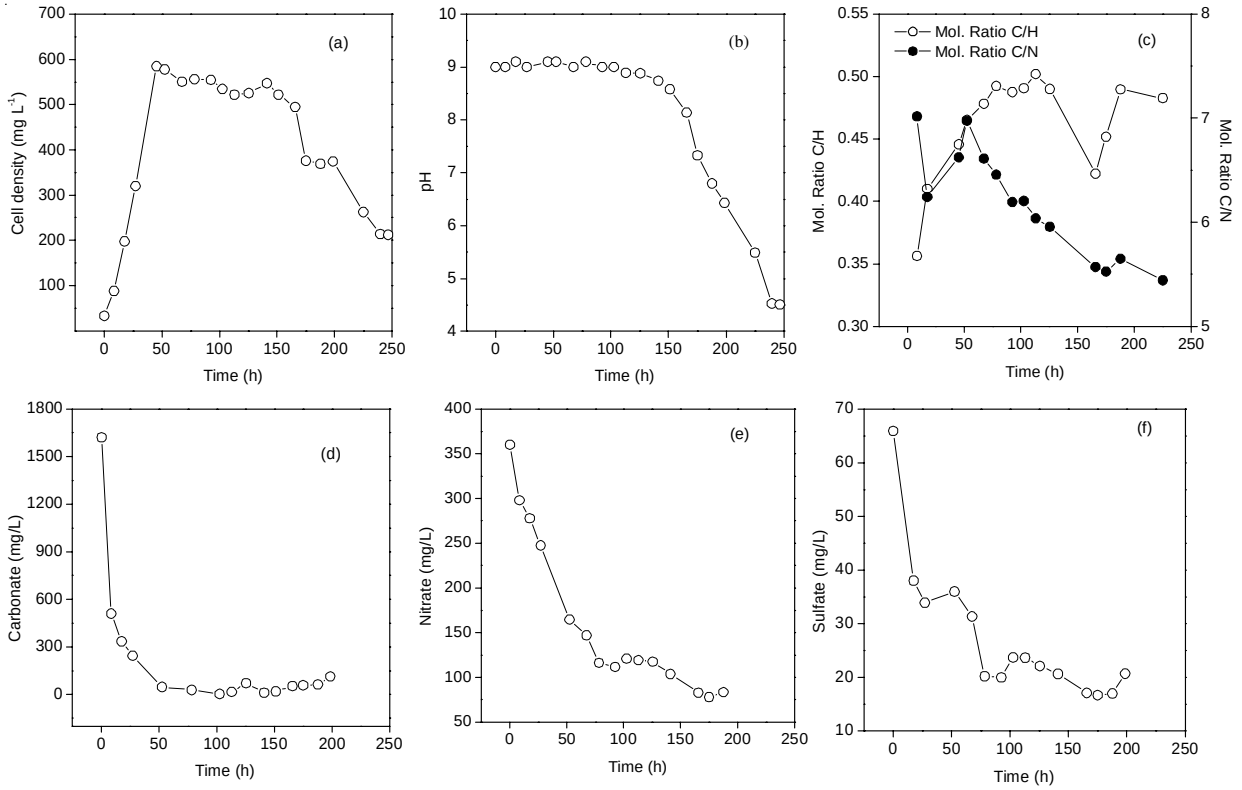

Fig. 2. (a) Growth curve under pH = 9 and DIC = 28.3 mM. (b) The variations of pHs. (c) The variations of ratios of C/H and C/N of biomass. (d) The variations of carbonate concentration in the medium. (e) The variations of nitrate concentration in the medium. (f) The variations of sulfate concentration in the medium

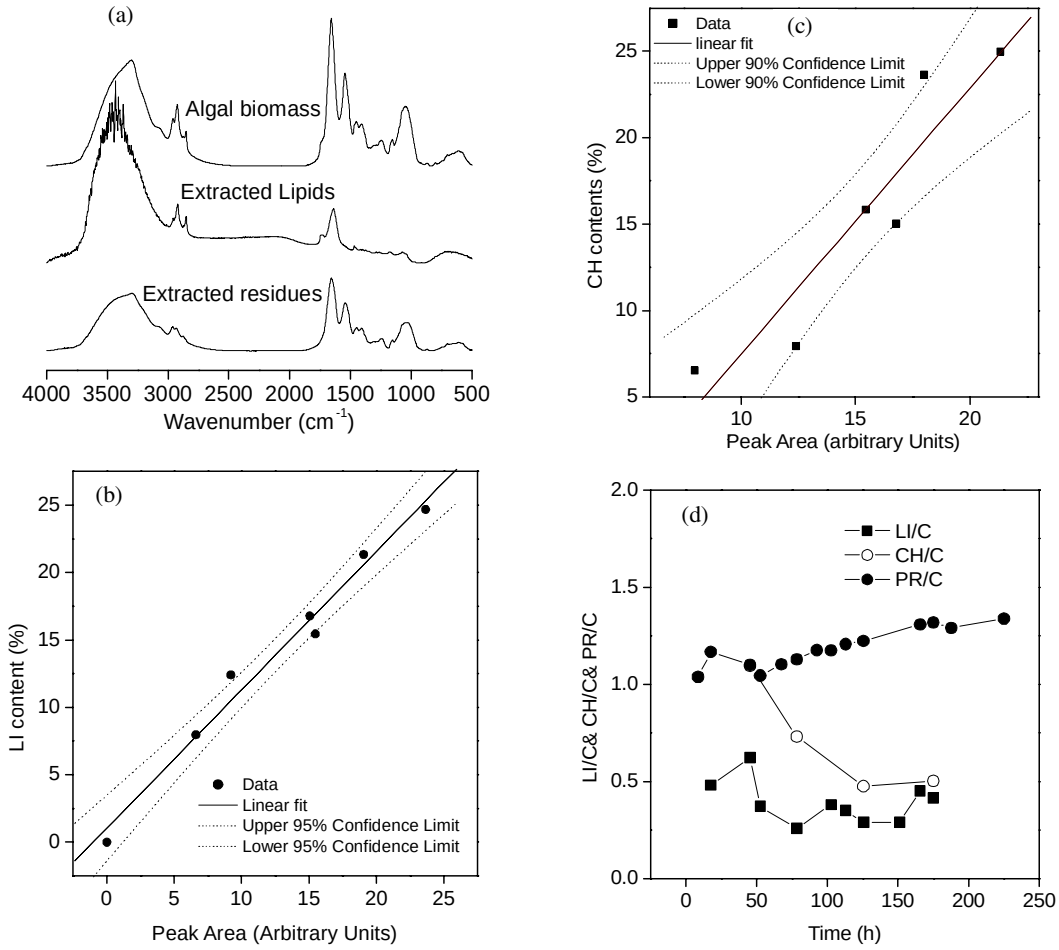

Fig. 3. (a) FTIR spectra of algal biomass, extracted LI and extracted residues from algal biomass. (b) The calibration curve between peak area and LI contents. (c) The calibration curve between peak area and CA contents. (d) The variations of LI, PR and CH under the batch cultivation

cm^{-1}. 3000-2800 cm^{-1} of range was unique and determined to fit and obtain the contents of lipids. The range of 1700-1500 cm^{-1} contained also one of amide I and not good to take as the characteristic peak of lipids. The ranges of 1200-950 cm^{-1} was determined to fit and obtain the contents of carbohydrates. The $CaCO_3$ was taken as internal standard. The lipids peak was normalized based on $CaCO_3$ peak. The calibration curve between normalized peak area and lipids contents from wet chemistry analysis was obtained. For a case of carbohydrates, the regression ratio of calibration line was not good enough to take as the tool to get carbohydrates contents. As shown in Fig. 3c, the ratio of LI/C remained a constant but the CH/C decreased mostly (81 %) and PR/C increased slightly (30 %). Regarding lipids, CH, proteins based on H, similar trend was observed (Fig. 3d).

Pigment ratios were identified with UV-visible spectrum. The spectra of lipids extraction compared clearly with one of algal biomass (Fig. 4a). For 300-500 nm of spectra, several intensity peaks were observed. However, 7 clear absorption peaks (338, 380, 437, 492, 579, 627 and 678 nm) of algal biomass can be revealed after fourth derivation (Fig. 4b). Also, 7 clear absorption peaks (336, 378, 433, 489, 572, 618 and 667 nm) of lipids extraction also observed under the same fourth derivation process (Fig. 4c). Obviously, red movement happened due to some interference such as cell membrane. As shown in Fig. 4d, relative amounts of pigments based on 678 nm of spectrum peak decreased as the increase of cell intensity. It is due to the effect of shelter from the increment of cell density. As the stable stage reached, the relative amounts also remained a constant. After stable stage, cell begun to decompose and pigments also disappeared. The green of cultivation solution changed into white and relative amounts were not easy to be estimated.

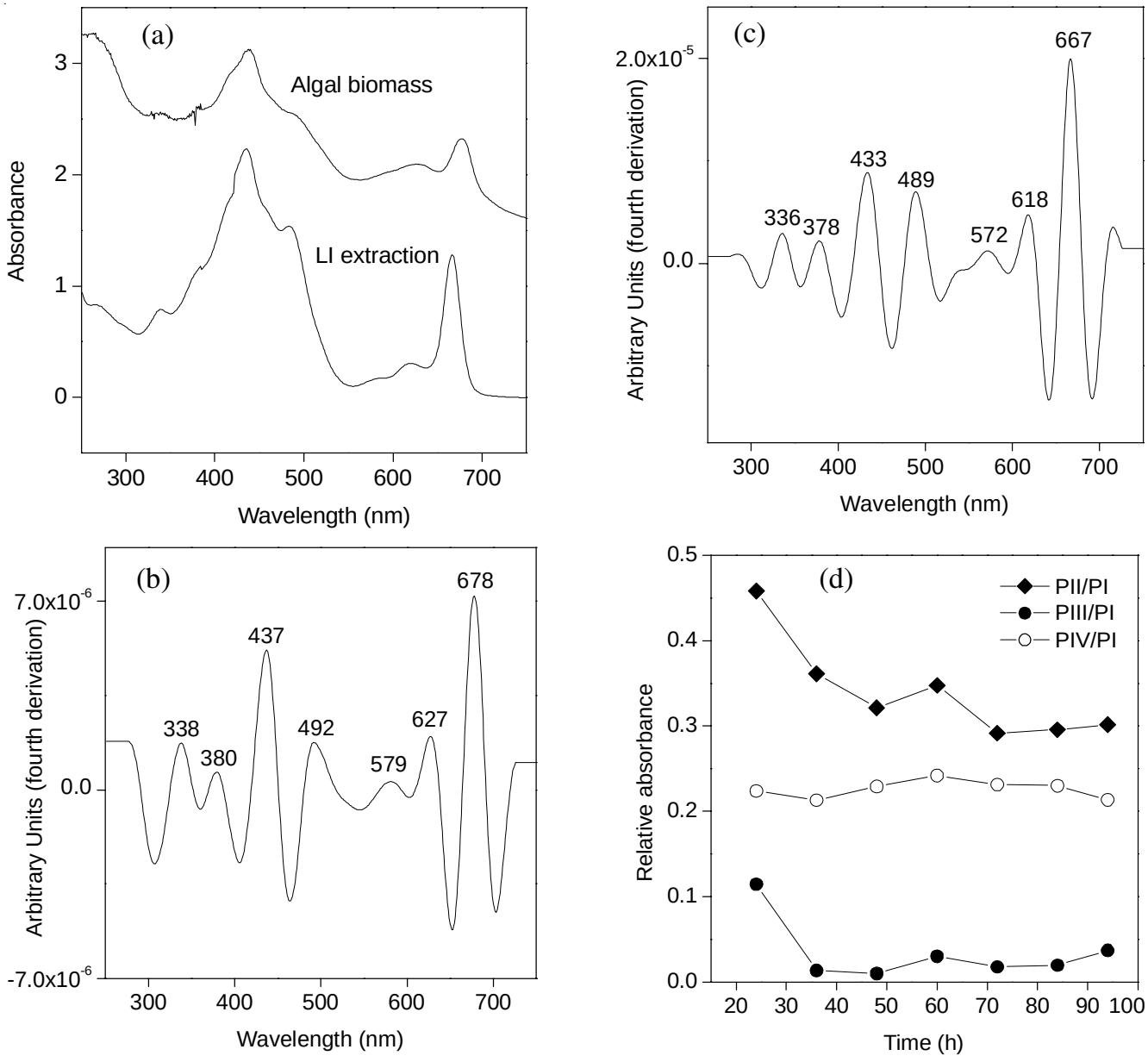

Fig. 4. (a) UV-visible spectra of algal biomass and its extracted LI. (b) The fourth derivative of UV-visible spectra of algal biomass. (c) The fourth derivative of UV-visible spectra of its extracted LI. (d) The variations of pigments ratios based on OD 680 nm

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