A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

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(Supervised by Professor In-Kyu Yeo)

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## ABSTRACT

Currently, microalgae considered as one of the potent resources for the sustainable biodiesel production. To enhance biodiesel yield by indigenous microalgae via cost-effective process, this study was investigated availability of mammalian cell culture waste (MCCW) as an additive to culture medium. To evaluate growth response of microalgae, aseptically isolated *Chlorella* sp. CKC2 was cultivated in different concentrations (v/v, 0-100%) of MCCW mixed with F/2 medium. As results, maximum 6.2-fold higher dry cell weight (DCW) of *Chlorella* sp. was obtained at 20% compared to control (0%), and concentrations-dependently decreased DCW was observed upper concentrations. In the result of further growth test under 0, 5, 10, and 20%, MCCW concentration-dependently increased daily growth patterns were observed along with gradually increased DCW. Furthermore, more significantly augmented lipid productivities were obtained from MCCW supplemented culture with high accumulation of polyunsaturated fatty acid methyl esters compared to control (0%) which indicated changes of biodiesel properties. From the results of this study, it is suggested that MCCW can be a cost-effective additive for the sustainable production of biodiesel from *Chlorella* sp. CKC2.



**Keywords:** microalgae, waste, cultivation, biodiesel, mammalian cell

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# I. INTRODUCTION

The use of fossil fuel and its derivatives has been negatively affected in environment by producing atmospheric carbon dioxide which considered as causative molecule of greenhouse effect in the earth (Gurney *et al*., 2009). According to previous studies, about 29 Gt of carbon dioxide emission was observed, and only about 12 Gt of carbon dioxide can be removed via natural processes (Bilanovic *et al*., 2009; Brennan and Owende, 2010). Also, due to the exhaustion of fossil fuel, the sharply increased fuel cost play a crucial role in global economy by affecting varying parts of industries (Shafiee and Topal, 2008). To solve these problems, alternative energies such as wind, waves, sunlight and biomass have been attracted many attentions from researchers and investigators, and continuously growing alternative energy production has shown in worldwide, especially in China (Aslf and Muneer, 2007). One of these, the biomass-based fuel production is in the spotlight due to their sustainability and eco-friendly process (Fischer *et al*., 2010).

Biomass are produced from cultivation of energy-producing crops or organisms such as oil seeds, grains, algae and animals, and they can be converted to varying types of biofuels such as biodiesel, bioethanol, biomethane and biohydrogen via different bioprocesses (Amin, 2009; Chandra *et al*., 2012; Kapdan and Kargi, 2006; Kim and Dale, 2004; Ma and Hanna, 1999). The global demands for the biofuel continuously have been augmented along with changes of energy policies in many nations. According to previous report, The US federal government set a target to replace 30% of conventional transportation petrolic fuels such as gasoline and diesel as biofuels until 2030, and it is estimated about 227 billion liters of biofuel should be produced per year in US (Simmons *et al*., 2008). Biodiesel can be produced by trans-esterfication reaction of triglyceride which is considered as a major component of lipid with alcohol and various catalysts, and it can be used as transportation fuel (Fig. 1.) (Chisti, 2007).

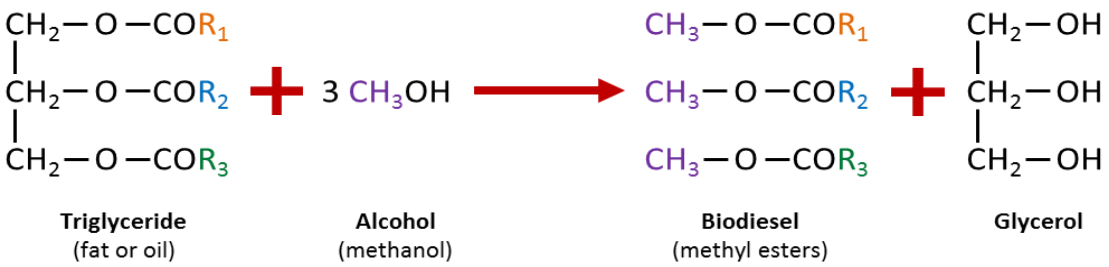


**Fig. 1.** Transesterification reaction.

Microalgae-derived biomass considered as one of the potent biodiesel resources because they can grow faster, accumulate higher lipid in their body, do not compete with food resources, not require wide space or productive soil, and more efficiently sequestrate atmospheric carbon dioxide compared to first generation resources such as food sources, sugarcane and oil seeds, and second generation resources such as lignocellulosic agriculture and forest residues or non-food resources (Chisti, 2007; Mata *et al*., 2010; Brennan and Owende, 2010). For these reasons, microalgae considered as a next generation or third generation biofuel resource (Brennan and Owende, 2010; Wan *et al*., 2012). However, although theoretical calculation has shown that the annual oil production by microalgae is about 30,000 L/year per hectare unit, which is about one hundred-fold of soybeans, lower lipid yields have been made due to lack of information about mass cultivation, and unforeseen environmental factors (Hu *et al*., 2008). Thus, the cost of biodiesel production from microalgae does not meet appropriate level presently. To produce cost-effective biodiesel from microalgae, it is essential to devise a means to increase algal oil productivity and to reduce cost of algal cultivation process and cell harvesting (Chisti, 2007; Mata *et al*., 2010; Brennan and Owende, 2010).

Over the past decades, the studies about microalgal biodiesel production using wastewater has been performed to achieve wastewater disposal and cost-effective energy production simultaneously (Christenson and Sims, 2011; Rawat *et al*., 2011). Because tertiary treatment of wastewater contains excess nutrients such as nitrogen, phosphorus and inorganic matters, it can cause eutrophication in aquatic environment and resulted in harmful algal blooms by cyanobacteria and red tide-forming algae if discharged without chemical, physical or biological treatments (Correll, 1998; Christenson and Sims, 2011). Furthermore, organic carbon sources such as glucose, sugar alcohol, and the variety of wastes such as papaya waste,

molasses waste, waste from yeast production, and sheep's blood were studied for the microalgal biomass production (Heller *et al*., 2015; Ayala and Vargas, 1987; Venkataraman *et al*., 1982; Cho *et al*., 2015; Cheirsilp and Torpee, 2012). According to previous study of Venkataraman *et al*. (1982), blue-green alga *Spirulina platensis* showed sheep's blood concentrations dependently increased growth ranged from 0.1 mL/mL to 1.0 mL/mL, and the result indicated that the nutrient sources of sheep's blood may contain microalgal growth-promoting agents.

From those results, it is suggested that the mammalian cell culture waste (MCCW) which discharged from laboratory may also have algal growth-promoting agents. Although its composition is not consistent due to different cultivation time of different mammalian cells and mixing of variety of treated agents, all the MCCW basically contains algal growth-promoting ingredients including organic carbon source and fetal bovine serum (FBS) along with bactericidal antibiotics. Also, because cell culture become an essential technique in biological laboratory, and mass cultivation of animal cells plays a significant role in vaccines or bioactive compounds production more and more, it is estimated that the amount of MCCW will increase in the future (Kretzmer, 2002). Therefore, this study was investigated to verify whether growth of microalgae increase in MCCW supplemented culture medium compared to un-supplemented medium, and further studies about biodiesel application of MCCW supplemented culture was evaluated via analysis of lipid accumulation and fatty acid methyl esters of locally isolated microalgae.

# II. MATERIALS AND METHODS

## 1. Isolation and identification of strain

To perform experiment using indigenous microalgae, seawater was obtained from Tongyeong, Korea (Fig. 2.), and isolation was performed by agar plate streaking method using sterilized and silica removed F/2 medium containing 2% of agarose (w/w) (Guillard, 1975). After plating 10 µm of eutrophicated (with F/2 medium) sample, it was incubated for two weeks and generated green-colored colony was transferred to sterilized 50 mL of liquid F/2 medium which was filled in 100 mL of Erlenmeyer flask. The cultivation was performed in plant growth chamber (JSR, KOR) regulated with 26℃, 12/12 of light/dark cycle, and 150 µmole $m^{-2}$ $s^{-1}$ of light intensity (cool-white fluorescence light). To prevent precipitation of algal cells, hand shaking was performed every twice a day during the experiment. To identify isolated strain, DNA was extracted using DNeasy® Blood & Tissue Kit (Qiagen, The Netherlands) and 18S rDNA was amplified by polymerase chain reaction (PCR) using C1000 Thermal Cycler (Bio-Rad, USA) with two eukaryotic primers; 18S1F (forward), 5'-GGTTGATCCTGCCAGTAGTC-3' and 18S1R (reverse), 5'-GATCCTTCTGCAGGTTCACC-3'. The sequence of amplified rDNA PCR product was analyzed by SolGent Co., Korea, and the identity of obtained sequence data was verified by BLAST (Altschul *et al*., 1997). The morphological identification of isolated strain was performed by optical microscopy (Olympus, JPN) after fixation with 2% of Lugol's solution, and for the specific morphological observation, images were obtained using field emission-scanning electron microscopy (FE-SEM, Carl Zeiss, GER).

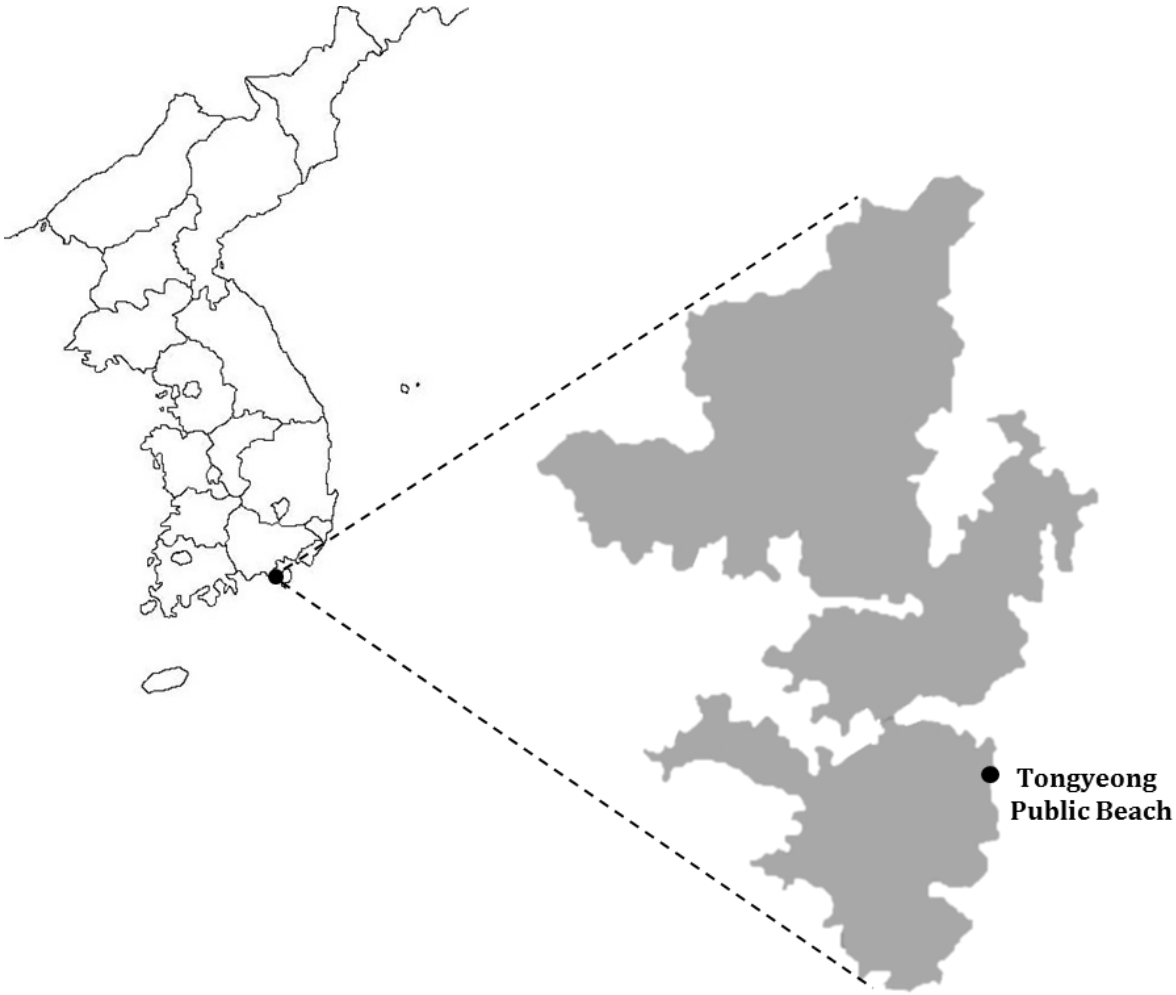


**Fig. 2.** A map of sampling site.

2. Preparation of mammalian cell culture waste (MCCW)

MCCW was obtained from biological lab of Korea Basic Science Institute (KBSI), Jeju center. To prevent bacterial contamination, MCCW was sterilized using autoclave (JSR, KOR) with 121℃ for 15 min. After cooling in a room temperature, MCCW was filtered through 0.2 μm pore-size of of PTFE syringe filter (LaboGene, Denmark). The prepared MCCW was stored at 5 ℃ before starting experiment.

3. Analytical methods

3. 1. Daily growth and dried cell weight determination

To verify growth-responses of isolated strain to MCCW supplemented medium under batch culture condition, the different concentrations (0-100%) of MCCW were mixed with F/2 medium with $2 \times 10^5$ cells/mL of initial cell number. To analyze the dried cell weight of isolated strain, each 6 mL of culture aliquot was harvested by centrifugation at 12,000 × *g* for 5 min after 7 days, and then washed with deionized water. The washed residue was freeze-dried by a CoolSafe freeze drier (LaboGene, Denmark), and precisely weighed by an XP-205 analytical balance (METTLER TOLEDO, Switzerland). The daily growth test of isolated strain was performed in 6-well plates with total 8 mL of different concentrations MCCW (0-20%) supplemented medium, and absorbance (OD) at 600 nm wavelength was determined using a synergy microplate reader (BioTek, USA).

3. 2. Monosaccharide composition analysis

The monosaccharide composition of MCCW was analyzed using a HPAEC-PAD (Dionex, USA) with a CarboPack$^{TM}$ PA1 column. After a 1 mL of MCCW was mixed with a 1 mL of deionized water, it was filtered through a PTFE syringe filter (0.2 μm pore-size, LaboGene, Denmark), and repeatedly diluted with deionized water (DW). A 15 μL of sample was injected by auto-sampler, and flow of mobile phase (18 mM NaOH) was regulated at 1.0 mL/min with 25℃ temperature. The monosaccharides including glucose, galactose, fructose, xylose, mannose, and fucose were used for generating standard curve, and obtained result was compared with retention times of standard peaks.

3. 3. Total lipid and fatty acid methyl esters (FAME) analysis

To obtain sufficient amount of dried algal cells for lipid analysis, scale-up culture was performed in 5 L Erlenmeyer flasks with 2.5 L of MCCW supplemented or un-supplemented F/2 culture medium under same culture conditions described above. After 15 days of incubation time, the algal cells were harvested by centrifugation at 10,000 × *g* for 10 min and washed twice with DW. The freeze-dried residue was used for lipid and FAMEs analysis. The total lipid content was analyzed by modified gravimetric methods of Blight & Dyer (1959) and Chiu *et al*. (2009). In brief, after a 20 mL of methanol and a 10 mL of chloroform were added to glass vials which containing dried algal cells, extraction was performed by sonication (60 min) at 60℃ using a Power sonic 520 sonicator (HWASHIN, KOR). After extraction, DW was added to mixture and chloroform phase was collected from layer.

Subsequently, obtained chloroform phase was evaporated by nitrogen gas and precisely weighed using an XP-205 electronic balance (METTLER TOLEDO, Switzerland).

FAME compositions were determined using a proposed method by Breuer *et al*. (2013). In brief, a 3 mL of methanol solution which containing 0.5 N of NaOH was mixed with extracted lipids and incubated at 90℃ for 5 min. After 95% hexane (1 mL) was added to mixture, it was incubated at room temperature for 30 min. The aliquots of hexane phase (upper layer) were transferred to vial with dilution and analyzed by gas chromatograph (GC-2010 plus) with a flame ionization detector (FID) (Shimadzu, Japan). The SP$^{TM}$-2560 Fused silica capillary column (100 m × 0.25 mm × 0.2 μm film thickness) was used for analysis, and the flow of helium (carrier gas) was 1.03 mL/min. The both temperatures of injector and detector were 260℃, and 1.0 μL was injected with a split ratio of 100:1. The oven temperature was regulated initially 100℃ for 5 min, and gradient was made from 100℃ to 240℃ (4℃/min), and maintained at 240℃ for 20 min. Each FAME was determined according to retention time comparison of FAMEs standard curves, and glyceryl triundecanoate was used for internal standard.

### 3. 4. Determination of biodiesel properties

The biodiesel properties were determined by calculating values from FAMEs composition using previously proposed equations. Both saponification value (*SV*) and Iodine value (*IV*) were calculated according to equations of Kalayasiri *et al*. (1996), and cetane number (*CN*) was calculated by an equation of Krisnangkura (1986), and degree of unsaturation (*DU*) was calculated by an equation of Francisco *et al*. (2010).

$$SV = \Sigma(560 \times F)/M_w \quad (1)$$

$$IV = \Sigma(254 \times F \times D)/M_w \quad (2)$$

$$CN = (46.3 + 5458/SV) - (0.225 \times IV) \quad (3)$$

$$DU = MUFA + (2 \times PUFA) \quad (4)$$

Where, *F* is percentage of each FAME, *Mw* is molecular weight, *D* is number of double bonds in FAME, *MUFA* is monounsaturated fatty acids (wt%), and *PUFA* is polyunsaturated fatty acids (wt%) respectively.

4. Statistical analysis

One-way ANOVA and subsequent t-test was performed by MS Excel 2007 (Microsoft, USA) software. All the experiments were performed in triplicate and $P<0.05$ was considered as significant differences.

# Ⅲ. RESULTS AND DISCUSSION

## 1. Strain identification

As shown Fig. 3A and 3B, images of microscopic observation of isolated strain was shown green-colored and spherical-shaped morphology with 6-9 μm of diameter. The results of BLAST search indicated that the similarity of isolated strain is highly related to *Chlorella* sp. WT1 (KX109776), *Chlorella* sp. TNBR1 (KR869729), and *Chlorella vulgaris* (KJ561358) with 97% of identity along with 1,234, 1,218, and 1,218 of max scores respectively. From the results of morphological and genetic similarities, we named isolated strain as *Chlorella* sp. CKC2, and sequence data was registered to NCBI Genbank and obtained accession number (KM605130) (Table 1).

*Chlorella* is one of the important micro organisms because it has been studied widely over the years, and exceeds 2,000 tones of biomass currently produced by more than 70 companies (Spolaore *et al*., 2006). *Chlorella* mostly used for food, neutraceutical and aquaculture industry due to their high amounts of protein or bioactive compounds. Also, it can be used for biofuel production due to its high amounts of lipid accumulation under various stress conditions and applicability to wastewater (Guccione *et al*., 2014). Thus, to verify availabilities of isolated strain in biodiesel production under MCCW supplemented culture, we aseptically cultivated isolated strain for the further investigations.

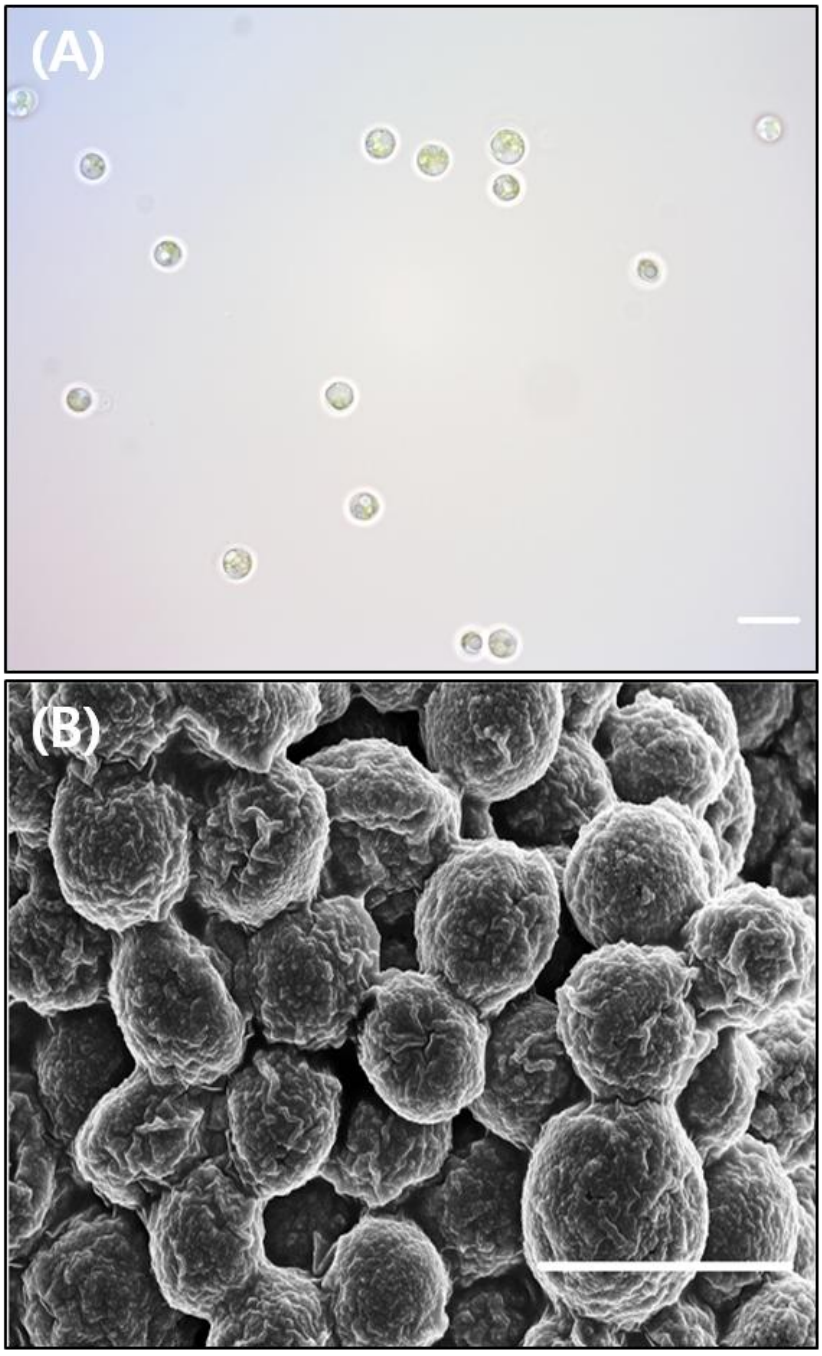


**Fig. 3.** Optical microscopic (A) and field emission-scanning electron microscopic (FE-SEM) (B) images of *Chlorella* sp. CKC2. Scale bar represents 10 μm of length.

**Table 1.** BLAST identity of rDNA sequence alignments of *Chlorella* sp. CKC2 (KM605130).

| Species | Identity (%) | GenBank Accession No. |
|---|---|---|
| *Chlorella* sp. CKC2 | - | KM605130 |
| *Chlorella* sp. KAS603 | 97 | KT886087 |
| *Chlorella vulgaris* BDUG 92001 | 97 | KT893862 |
| *Chlorella* sp. WT1 | 97 | KX109776 |
| *Chlorella* sp. TNBR1 | 97 | KR869729 |
| *Chlorella vulgaris* UMT-M1 | 96 | KJ561358 |
| *Chlorella* sp. ACL1 | 96 | KF746947 |

2. Effect of mammalian cell culture waste (MCCW) on the growth of *Chlorella* sp. CKC2

To test algal growth responses of MCCW supplemented culture medium, 0%, 20%, 40%, 60%, 80%, and 100% (v/v) of MCCW were prepared with F/2 culture medium. After 7 days cultivation time (exponential phase), algal cells were harvested and dry cell weight (DCW) was precisely determined. As shown Fig. 4, a minimum DCW was obtained from MCCW un-supplemented control medium (0%, 0.18 g/L). The maximum DCW was obtained from 20% (1.13 g/L) and it showed about 6.2-fold increased DCW compared to control, and gradually decreased DCWs were shown with MCCW concentrations increased at upper 20% of concentrations. Therefore, we set up a maximum concentration as 20% and performed further growth test at the MCCW concentration ranged from 0% to 20% for 15 days of incubation time. As shown Fig. 5A, changes of daily growth at each concentration based on optical density (OD) were represented concentrations-dependently increased patterns. During the experimental period, the growth curves of isolated strain in MCCW supplemented medium were shown increased patterns after 1 day compared to control. This result indicated that the MCCW immediately can promote algal growth without long term of incubation time. Also, after 15 days, concentrations-dependently increased DCWs were obtained from tested culture (Fig. 5B), and maximum 3.48-fold increased algal biomass was shown at 20%. Because of varying availabilities of microalgae, many studies about algal growth-promoting effects have been performed by researchers over the years. According to Cho *et al*. (2015), *myo*-inositol was promoted *Dunaliella salina* biomass yield up to 1.48-times along with changes of lipid and fatty acid composition. Also, alginate oligosaccharide mixture which is a digestion material of alginate polymer by bacterial alginate lyase significantly increases growth of *Nannochloropsis oculata* up to about 5-times (Yokose *et al*., 2009). In the other studies,

growth and lipid-promoting effects of microalgae under heterotrophic or mixotrophic culture conditions by organic carbon sources such as glucose, corn powder hydrolysate have been reported (Cheirsilp and Torpee, 2012; Xu *et al*., 2006). Those growth-promoting agents are highly expensive and require another bioprocess such as hydrolysis or enzymatic engineering. According to Li *et al*. (2007), it is estimated that about 80% of total media cost will require if glucose used as organic substrate for the microalgae biodiesel production. Also, because microalgae effectively absorb nutrients such as nitrogen, phosphorus, carbon, and varying inorganic elements, it can easily be applicable for purification of wastewater. According to Biohazardous Waste Management Plan, cell culture media waste should be chemically treated or autoclaved, and it can be discharged to a sanitary sewer. This disposal method not only requires expensive cost of disinfectants, but also can affect water quality if discharged to aquatic environment without adequate processing. Therefore, if we use MCCW as a supplement agent for microalgae culture media, cost-effective biomass production and wastewater treatment will be simultaneously achieved because mammalian cell culture medium generally contains high amounts of nutrients. In this study, MCCW showed significantly increased growth of *Chlorella* sp., and it is not requires further bioprocess. Currently, mammalian cells are considered as important platform to produce therapeutic protein and varying molecules such as vaccines (Farzan *et al*., 2017). Thus, large scale bioreactors have been developed and capacities of manufacturing sites also have increased up to 200,000 L (Farzan *et al*., 2017). Although specific amounts of discharge of MCCW are not calculated yet, it is estimated that the MCCW will increase along with demand for biochemicals from mammalian cells.

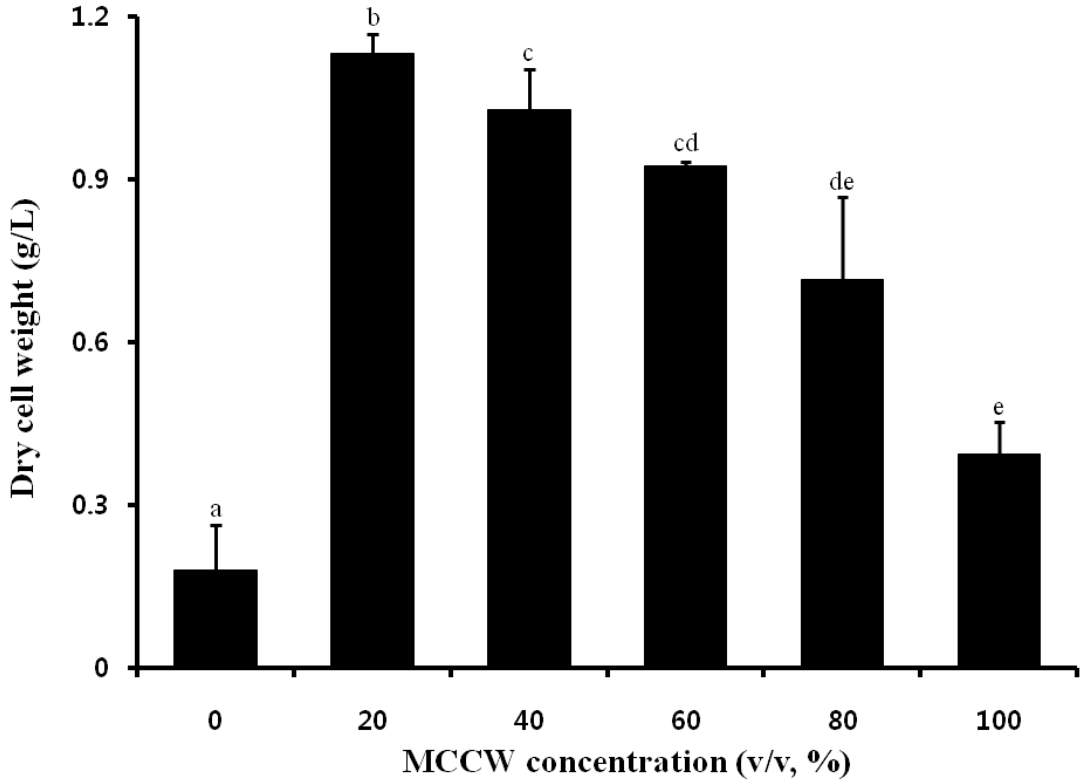


**Fig. 4.** Effect of different concentrations (0-100%) of mammalian cell culture waste (MCCW) on the dry cell weights of *Chlorella* sp. CKC2 after 7 days of incubation time. Error bars represent mean ± standard deviation (SD) and different letters exhibit significant difference ($P$<0.05)

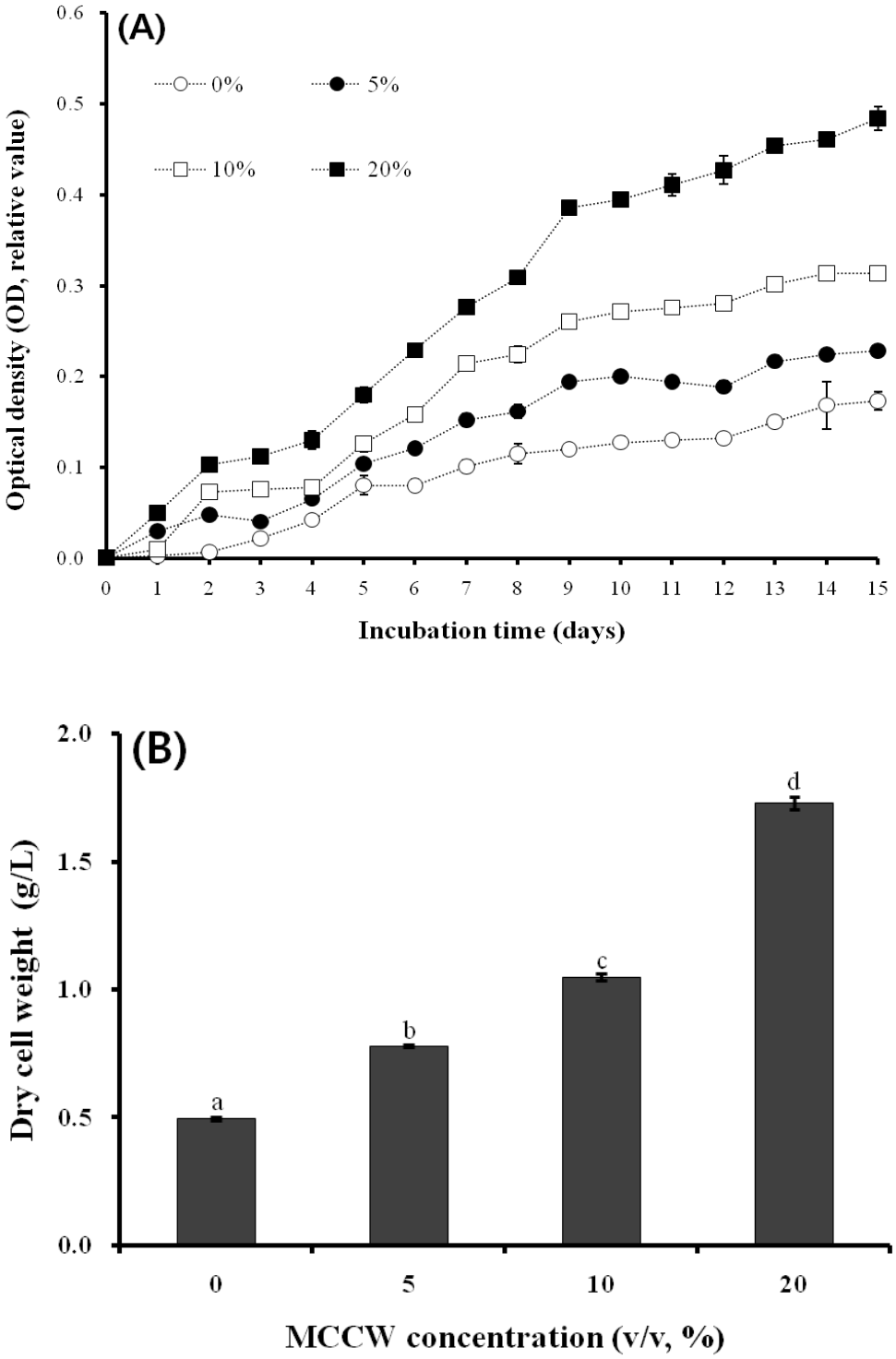


**Fig. 5.** Effect of different concentrations (0-20%) of mammalian cell culture waste (MCCW) on the daily growth (optical density value, 600 nm) (A) for 15 days of incubation time, and dry cell weight after 15 days of incubation time (B). Error bars represent mean ± standard deviation (SD) and different letters exhibit significant difference ($P$<0.05)

3. Monosaccharide composition of MCCW

To verify possible algal growth-promoting factor, monosaccharide composition of MCCW was analyzed by HPAEC-PAD (Dionex, USA) system with a CarboPack$^{TM}$ PA1 column. As shown Fig. 6A, standard curve showed peaks of diverse monosaccharides including fucose, rhamnose, galactose, glucose, mannose and fructose, and as shown Fig. 6B, MCCW included total about 5.5 g/L of monosaccharides which composed of 3.5 g/L of glucose, 0.3 g/L of mannose, and 1.7 g/L of fucose. Glucose was shown the highest amount of monosaccharide in MCCW, and it is considered as major organic carbon source to achieve heterotrophic or mixotrophic growth of microalgae to achieve high biomass and lipid production simultaneously (Perez-Garcia *et al*., 2011). According to previous report, effect of initial glucose concentrations (0-20 g/L) on the growth and lipid accumulation by marine *Chlorella* sp. was tested, and strain exhibited high cell dry weight (about 3.7 g/L) at 10 g/L of glucose concentration (Cheirsilp and Torpee, 2012). From our results, although sufficient amounts of carbon sources were not included in MCCW, it is considered that the detected monosaccharides may affect growth-promoting effect to strain. However, further studies are required to investigate specific growth-promoting factors of MCCW.

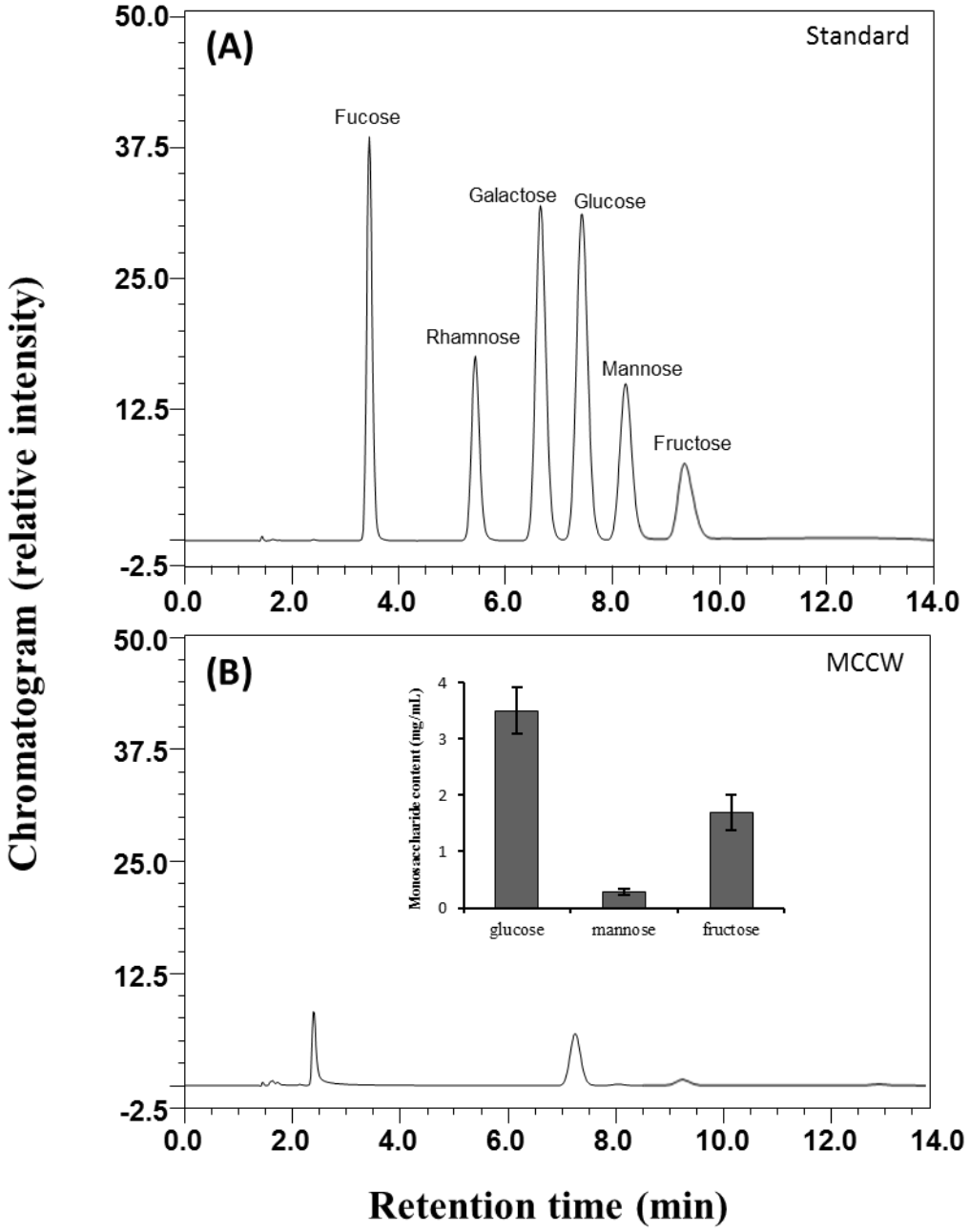


**Fig. 6.** The standard peaks of monosaccharide including fucose, rhamnose, galactose, glucose, mannose, and fructose (A) and monosaccharide composition of mammalian cell culture waste (MCCW) (B). Error bars represent mean ± standard deviation (SD).

4. Lipid accumulation and fatty acid methyl esters (FAMEs)

To evaluate availability of MCCW in microalgal biodiesel production, changes of total lipid and FAME composition under different MCCW concentrations were analyzed to verify changes of biodiesel productivity and properties. As shown Fig. 7, although total lipid accumulations by isolated strain were decreased under MCCW supplemented culture medium, lipid productivity increased in a concentrations-dependent manner. The reason of lipid productivity increment in MCCW supplemented culture is due to the increased biomass, and the result indicated that MCCW increase biodiesel productivity per unit culture media by *Chlorella* sp. CKC2. The result of FAME composition changes under different MCCW supplemented cultures were represented in Table 2. The major FAMEs of *Chlorella* sp. CKC2 were methyl palmitate (C16:0), methyl linoleate (C18:2(n-6),cis), and methyl linolenate (C18:3(n-3)). Whereas the saturated FAMEs were concentration-dependently decreased, polyunsaturated FAMEs were concentration-dependently increased in MCCW supplemented culture. The increased accumulation of polyunsaturated fatty acids in enhanced algal growth conditions have been reported by previous studies (Cho *et al*., 2015; El Arroussi *et al*., 2015; Cho *et al*., 2016). Microalgal lipid categorized by two groups; storage lipids (non-polar lipids) which are composed of predominant saturated fatty acid, and structural lipids (polar lipids) which are mostly composed of polyunsaturated fatty acids (Sharma *et al*., 2012). The structural lipids play an important role to form cell membranes, maintain specific functions of membrane, intermediate cell signaling pathway and cell fusion (Sharma *et al*., 2012). Thus, it is suggested that the high accumulation of polyunsaturated fatty acids in MCCW supplemented culture is may due to the metabolic function of microalgae to prepare fast cell fusion and membrane formation under optimal growth condition.

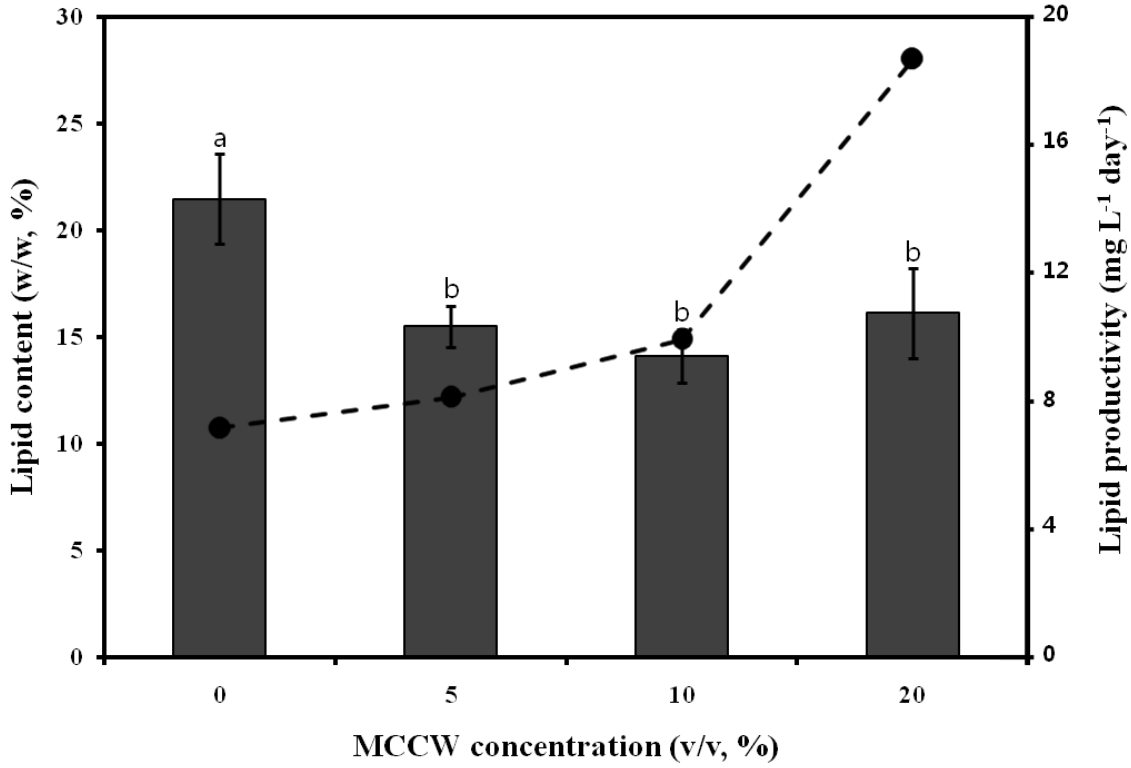


**Fig. 7.** Effect of different concentrations (0-20%) of mammalian cell culture waste (MCCW) on the total lipid accumulation (bars) and lipid productivity (●) of *Chlorella* sp. CKC2. Error bars represent mean ± standard deviation (SD) and different letters exhibit significant difference ($P$<0.05)

**Table 2.** Changes of fatty acid methyl ester (FAME) composition of *Chlorella* sp. CKC2 in different concentrations of mammalian cell culture waste (MCCW) supplemented culture medium.

| FAME (wt.%) | MCCW concentrations (v/v, %) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 20 |
| C13:0 | 0.14 | 0.16 | 0.17 | 0.21 |
| C14:0 | 0.34 | 0.25 | 0.22 | 0.20 |
| C15:0 | 0.17 | 0.25 | 0.15 | 0.16 |
| C16:0 | 22.77 | 18.26 | 17.45 | 16.88 |
| C16:1 | 2.45 | 2.82 | 2.51 | 2.23 |
| C17:0 | 0.28 | 0.29 | 0.22 | 0.22 |
| C18:0 | 3.46 | 1.60 | 1.46 | 0.93 |
| C18:1(n-9),cis | 0.63 | 0.41 | 0.51 | 0.91 |
| C18:2(n-6),cis | 8.50 | 17.89 | 25.14 | 34.46 |
| C20:0 | 7.92 | 6.02 | 5.48 | 5.27 |
| C18:3(n-6) | 0.32 | 0.25 | 0.22 | 0.21 |
| C18:3(n-3) | 52.11 | 50.58 | 45.52 | 37.47 |
| C22:0 | 0.44 | 0.92 | 0.68 | 0.56 |
| C20:4(n-6) | 0.10 | 0.18 | 0.16 | 0.16 |
| C24:0 | 0.38 | 0.11 | 0.12 | 0.14 |
| Saturated | 35.89 | 27.86 | 25.94 | 24.55 |
| Monounsaturated | 3.08 | 3.23 | 3.02 | 3.14 |
| Polyunsaturated | 61.03 | 68.91 | 71.04 | 72.30 |

5. Changes of biodiesel properties

As shown Table 3, biodiesel properties including saponification value (SV), iodine value (IV), cetane number (CN) and degree of unsaturation (DU) were calculated from FAME compositions by equations described above, and the lower SV and CN, and higher IV and DU were shown in MCCW supplemented culture medium. The value of DU is closely related to the oxidative stability and cold flow of biodiesel, and directly affected by unsaturated FAME composition and it show significantly high values in MCCW supplemented culture medium because of high amounts of polyunsaturated fatty acids (Table 2). Also, SV which is defined as the amount of KOH (mg) required to saponify a 1 g of produced diesel, is required to estimate CN (Predojević *et al*. 2012). The IV is considered as an amount of iodine (g) in biodiesel, and it can significantly affect engine deposition if show high value than standard (Knothe 2012; Mandotra *et al*., 2016). The CN is important value to evaluate engine performance, generation of nitrous oxide, and combustion of diesel (Arias-Peñaranda *et al*. 2013). The biodiesel standard EN 14214, published by the European Committee for Standardization, is prescribed standard values of IV (<120) and CN (>51). From the results of this study, isolated strain shown higher IV (154.42) and lower CN (39.76) compared to biodiesel standard. Furthermore, the produced algal biodiesels from MCCW supplemented culture medium exhibited more increased IV and decreased CN. Although the results indicated that MCCW supplementation to culture medium decrease biodiesel quality, it will not cause significant problems because most of biodiesel can be used by mix with petroleum diesel.

**Table 3.** Changes of properties including saponification value (SV), iodine value (IV), cetane number (CN) and degree of unsaturation (DU) of *Chlorella* sp. CKC2 biodiesel in different concentrations of mammalian cell culture waste (MCCW) supplemented culture medium.

| MCCW con. (v/v, %) | SV (mg $KOHg^{-1}$) | IV ($gI_2$ $100g^{-1}$ fat) | CN | DU |
|---|---|---|---|---|
| 0 | 193.53 | 154.42 | 39.76 | 125.14 |
| 5 | 193.13 | 166.86 | 37.02 | 141.03 |
| 10 | 193.05 | 165.84 | 37.26 | 145.10 |
| 20 | 192.89 | 161.00 | 38.37 | 147.74 |

# IV. CONCLUSION

In the present study, responses of growth and lipid accumulation by mammalian cell culture waste (MCCW) of locally isolated microalgae were investigated under batch culture condition. Both growth and lipid productivity of isolated *Chlorella* sp. CKC2 showed significantly increased values in MCCW concentrations dependent manner, and changes of FAME composition was observed along with changes of biodiesel properties. From the results, it is suggested that MCCW can be used for potential growth promoting agent to produce cost-effective diesel production from microalgae. However, application of MCCW to mass cultivation system should be performed along with further studies about quality improvement of biodiesel. Also, to collect MCCW efficiently from laboratory or mass cultivation facilities, it is required to develop efficient collection system of discharged MCCW from industrial facilities or laboratories.

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