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Biomass and lipid accumulation of oceanic microalga *Chlorella* sp. in peritoneal dialysis waste fluid supplemented mixotrophic culture

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ABSTRACT

Interest in the use of microalgal biodiesel production using nutrients from wastewater has been increasing due to the cost-effectiveness of such processes. The aim of this study was to evaluate the feasibility of using peritoneal dialysis waste fluid (PDWF) from renal failure patient as an additive to the algal culture medium used for biodiesel production. The obtained PDWF contained large amounts of glucose (9.78 g L^{-1}), nitrogen, phosphorus, and iron, along with various inorganic elements. Supplementation of the culture medium of *Chlorella* sp. CKC2 with 0–1000 mg L^{-1} of dried-PDWF (DPDWF) resulted in concentration-dependent increase in growth, with the maximum increase in biomass (1.3-fold) at 1000 mg L^{-1}, compared to culture not supplemented with DPDWF (0 mg L^{-1}). The lyophilized biomass exhibited the maximum amount of increased total lipid content (1.31-fold, wt%) at 1000 mg L^{-1}, compared to the control. Differences in the fatty acid composition of supplemented and un-supplemented cultures induced no significant changes in the biodiesel properties. The results suggest that mixotrophic cultivation using readily available DPDWF as a carbon source, could provide a significantly lower-cost method for algal biodiesel production, and at the same time, provide a neat solution for disposal of dialysis-derived wastewater.

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Ⅰ. INTRODUCTION

Microalgae-derived biofuel production has tremendous potential for the replacement of conventional biofuel-producing crops due to their high growth rate, removal of atmospheric carbon dioxide, spatial convenience, high lipid accumulation, and to their not being a food resource [1]. Furthermore, because microalgae can be grown in a variety of wastewaters (e.g., municipal, agricultural and industrial), from which they will remove large amounts of nitrogen, phosphorus, and other metal elements, they can also be used for wastewater treatment [2]. For instance, Chinnasamy *et al* (2010) and Wang *et al* (2010) showed that some microalgae could be grown in carpet-mill-effluent-dominated wastewater, and municipal wastewater, respectively, and could be applied for the production of biofuel [3,4].

Dialysis is a treatment required by renal-failure patients (a kidney disease) and it is reported that approximately 200,000 such patients existed worldwide in 2008 [5]. Furthermore, it is estimated that the number of patients treated using peritoneal dialysis (PD) will increase along with the increasing number of diabetic patients [5,6]. For example, according to a report by the Health Insurance Review and Assessment Service (Republic of Korea), there were about 70,000 dialysis patients in 2013, which was a 22.7% increase since 2009 in Korea. To perform dialysis requires large amounts of water, and it is reported that 1.5–3 L and 8–12 L of fluids are used per person for continuous ambulatory peritoneal dialysis (CAPD), and automated peritoneal dialysis (APD), respectively, per day. This amounts to an estimated total dialysis waste fluid of about 156 billion liters per year [7]. However, the disposal and

harvest of dialysis waste fluid is complicated and requires effort from the government because home-generated PDWF is usually discarded in toilets or sinks without any treatment. This may cause eutrophication because this waste contains large amounts of glucose, urea, phosphorus, trace metals, and other nutrients [8].

Chlorella species comprise a group of widely studied microalga used for commercial applications in the food and nutritional industry. Because such species can grow in organic-carbon-supplemented mixotrophic or heterotrophic modes, and can provide substantial biomass and lipid accumulation, they are considered a potential source of biodiesel [9]. According to previous reports, *C. vulgaris* exhibited the highest biomass and lipid production in mixotrophic culture mode [10]. Also, mixotrophic cultivation of *C. vulgaris* in industrial dairy waste showed greater biomass and lipid accumulation than in photoautotrophic culture [11].

However, the use of organic carbon sources incurs considerable cost, and Li *et al* (2007) estimated that the use of glucose as a heterotrophic growth medium would require about 45.4% of the total biodiesel production cost, when using *Chlorella protothecoides* [12].

We hypothesized that algal culture medium supplemented with dried PDWF might increase the biomass and lipid accumulation of microalgae because it contains a large amount of glucose as an organic carbon source, as well as abundant nitrogen, phosphorus, and other required trace metals. Therefore, this study was carried out to determine the effect of dried-PDWF supplemented mixotrophic culture on the growth performance and lipid accumulation of a locally isolated, oceanic microalga (*Chlorella* sp. CKC2) for the sustainable production of biodiesel.

Ⅱ. MATERIALS AND METHODS

1. Algal source and purification

Chlorella sp. CKC2 (KM605130) was isolated from samples taken on the Tongyeong coast (Republic of Korea), and acclimated in silicate removed F/2 (F/2-Si) medium prepared with natural seawater for 2 weeks [13]. To purify the microalgal culture, 1.5% agar containing F/2-Si medium was used for the streaking, and the isolated colony was sub-cultured in 500 mL Erlenmeyer flasks with 200 mL of F/2-Si medium. Cultivation was performed in a VS-3DM plant growth chamber (Vision, Korea) with a regulated 12/12 light/dark cycle under 6000 lux of light intensity, 26 ℃ temperature, and 60% humidity.

2. Preparation of peritoneal dialysis waste fluid (PDWF)

The PDWF used in our experiments was obtained from five patients who regularly performed dialysis using automated peritoneal dialysis equipment (sleep-safe system) with *balance* solution (1.5% glucose, 1.75 mmol L^{-1} calcium; Fresenius Medical Care, Germany). The initial dialysis water is composed of 16.5 g of glucose monohydrate (equivalent to 15 g glucose), 5.64 g of sodium chloride, 3.925 g of sodium lactate, 0.2573 g of calcium chloride dehydrate, and 0.1 g of magnesium chloride hexahydrate. To remove precipitate from raw PDWF, filtration was conducted using a PTFE membrane filter with pore size 0.2 μm (Whatman, USA). The filtrate was evaporated in an OF-22GW drying oven (JEIO TECH, Korea) at 60 ℃ for 48 h. After cooling at room temperature, we obtained sticky brown saccharic residues from the PDWF (Fig. 1).

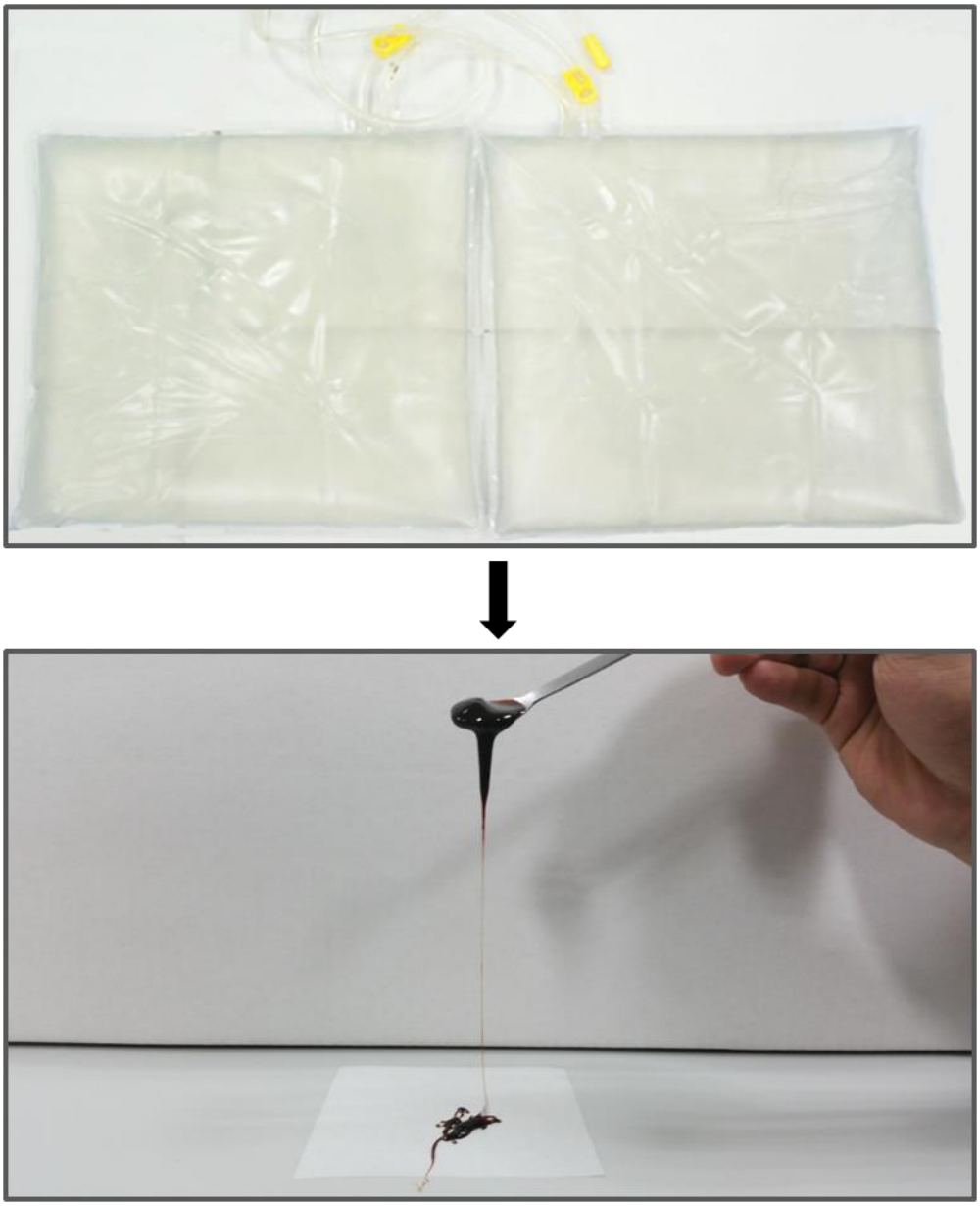

Fig. 1. Pictures of raw peritoneal dialysis waste fluid (up) and dried-PDWF (down).

3. Lab-scale growth test and biomass determination

To evaluate algal growth-responses to dried peritoneal dialysis waste fluid (DPDWF) under batch culture conditions, 5 g of DPDWF was dissolved in 10 mL of F/2 medium prepared with artificial seawater [14]. The concentrations (0, 250, 500, and 1000 mg L^{-1}) were adjusted using F/2 medium, and cultivation was performed in transparent 6-well plates with 8 mL total volume. The initial cell concentration was 2 × 10^5 cells mL^{-1}, and the daily growth was determined by measurement of optical density (OD) at 600 nm of wavelength using a Synergy microplate reader (BioTek, USA). To determine the algal biomass, a 4 mL culture aliquot was centrifuged at 12,000 × *g* for 2 min using a 1.5 mL vial, and then washed twice with deionized water. The residues were lyophilized using a CoolSafe freeze drier (LaboGene, Australia), and the biomass was weighed precisely using an XP-205 analytical balance (METTLER TOLEDO, Switzerland).

4. Analytical methods

4. 1. Inductively coupled plasma mass spectrometer (ICP-MS) analysis of PDWF

The inorganic components in PDWF were determined using a 7700S ICP-MS system (Agilent, USA). To remove precipitate from PDWF, 10 mL of PDWF was filtered through 0.2 μm pores of a PTFE syringe filter (LABOGENE, Denmark) and diluted 1–2 × 10^3-fold using deionized water. The samples were loaded using an auto-sampler by peristaltic pump pressure and a standard curve was generated using a Multi-Element Calibration Standard (Agilent Technologies, USA). The radio frequency was set to 1550 W using the ICP-MS Top MassHunter Workstation

Software (ver. A.01.02, Agilent, USA), and the flow rate of argon gas was 0.40 L min^{-1}.

4. 2. Monosaccharide composition, total nitrogen (TN) and total phosphorus (TP) determination

To determine the monosaccharide composition of PDWF, a HPAEC-PAD (Dionex, USA) with a CarboPack^{TM} PA1 column was used with preparation as follows. In brief, 1 mL of PDWF was filtered through the 0.2 μm pores of a PTFE syringe filter (LABOGENE, Denmark) and diluted 1×10^3-fold with distilled water. The flow rate was regulated at 1.0 mL min^{-1} using 18 mM NaOH as a mobile phase, and the temperature was regulated at 25 ℃ with 15 μL injection volume. The standard curve was generated using a standard solution, which contained glucose, galactose, fructose, xylose, mannose, and fucose (Sigma Aldrich, USA). The results obtained were compared with retention time. Total nitrogen of PDWF was determined by the Kjeldahl method and total phosphorus was determined using ICP-MS analysis (described above) [15].

4. 3. Total carbohydrate determination

To determine the total carbohydrate content in DPDWF-supplemented microalgae culture medium, the phenol and sulfuric acid method was used [16]. Briefly, 1 mL of culture aliquot was centrifuged at 13,000 × *g* for 5 min, and 100 μL was reacted with 300 μL of concentrated-sulfuric acid and 100 μL of 5% (w/v) phenol solution. After incubation at 90 ℃ on a heating block (DAEIL TECH, Korea), the mixture was

tested at 490 nm (wavelength) using a Synergy microplate reader (BioTek, USA) and glucose was used for the standard reagent.

4. 4. Total lipid extraction and fatty acid methyl esters (FAMEs) analysis

To recover sufficient amounts of biomass, cultivation was performed in 6-L Erlenmeyer flasks with 4 L of algal culture (F/2 medium). The initial cell concentration was 2×10^5 cells mL^{-1} and aeration was provided at 1.23 L min^{-1}. After 10 d cultivation time, the culture was centrifuged at 8,000 × *g* for 5 min; then washed three times and freeze dried to recover the biomass.

The total lipid content was determined using a modified method of Blight & Dyer (1959) [17,18]. In brief, 20 mL of solution (methanol and chloroform, 2:1, v/v) was used to extract lipids from the dried biomass, with sonication for 30 min using a Power sonic 520 sonicator (HWASHIN, Korea). After incubation for 120 min at 60 ℃ in a BS-21 shaking water bath (JEIO TECH, Korea), 10 mL of deionized water was added to the mixture and centrifuged at 1,200 × *g* for 5 min. The chloroform-containing lower phase was collected and dried under flowing nitrogen gas. The remaining residue of each total lipid was weighed using an XP-205 electronic balance (METTLER TOLEDO, Switzerland).

FAMEs were analyzed using the method proposed by Breuer *et al*. (2013) [19]. In brief, 3 mL of methanol containing 0.5 N sodium hydroxide was added to the total lipid and incubated in the JSWB-06T Bath & Circulator (JSR, KOR) at 90 ℃ for 5 min. After 1 mL of 95% hexane (Sigma Aldrich, USA) was added to the mixture, it was incubated for 30 min at room temperature, and the upper layer (hexane phase)

was diluted and prepared in a vial for the gas chromatographic analysis.

The analysis was performed using a GC-2010 plus gas chromatograph with a flame ionization detector (FID) (Shimadzu, Japan). Both the injector and detector temperatures were 260 ℃ and the injection volume was 1.0 μL, with a split ratio of 100:1. The SP^{TM}-2560 Fused silica capillary column (100 m × 0.25 mm × 0.2 μm film thickness) was used for the chromatography, with helium gas as a carrier (1.03 mL min^{-1}). The initial oven temperature was 100 ℃ for 5 min, and a gradient was created from 100 to 240 ℃ at 4 ℃ min^{-1}, and then the temperature was held at 240 ℃ for 20 min. The FAMEs were identified by comparison of retention times with standard peaks (Sigma Aldrich, USA).

5. Statistical analysis

Statistical analysis was performed using one-way ANOVA and, subsequent *t*-tests using MS Excel 2007 (Microsoft, USA) and all the experiments were performed in triplicate. The differences with P value <0.05 were considered significant.

Ⅲ. RESULTS AND DISCUSSION

1. Monosaccharide composition and amounts of total nitrogen (TN) and total phosphorus (TP)

To analyze the amount of organic carbon sources in raw PDWF, the monosaccharide composition was determined using an HPAEC-PAD system (Dionex, USA). As shown in Figure 2, although other monosaccharides were not detected, about 9.78 g L^{-1} of glucose was detected in PDWF. According to a previous report, glucose is considered the most commonly used carbon source for heterotrophic or mixotrophic conditions. Higher growth rate and respiration can be achieved using a glucose supplement, rather than other carbon sources, due to its higher energy content per mole [20]. Moreover, glucose more easily promotes heterotrophic growth in many microbial species (compared to other substrates such as sugars, sugar alcohols, sugar phosphates, and organic acids) and is closely related to the carbon assimilation pathways in *Chlorella* sp [20,21]. Therefore, *Chlorella* sp. was selected for the mixotrophic culture experiments. The amounts of PDWF containing TN and TP were also determined by Kjeldahl method and ICP-MS analysis, respectively. Because renal failure patients have problems removing waste products such as urea and phosphorus from their blood, it was predicted that the obtained PDWF would contain great amounts of TN and TP. As expected, high concentrations of TN (185.4 mg L^{-1}) and TP (20.5 mg L^{-1}) were obtained from raw PDWF. Previous studies reported that microalgae retain 7–20% (w/w) of nitrogen and 1% (w/w) of phosphorus in their cells for synthesizing all structural and functional proteins and

nuclei (respectively), and these elements are considered essential nutrients for the cultivation of microalgae [22-24]. From the results, it is considered that the use of dried-PDWF as a carbon source may be a good choice because it not only contains abundant glucose, but also includes high concentrations of nitrogen and phosphorus.

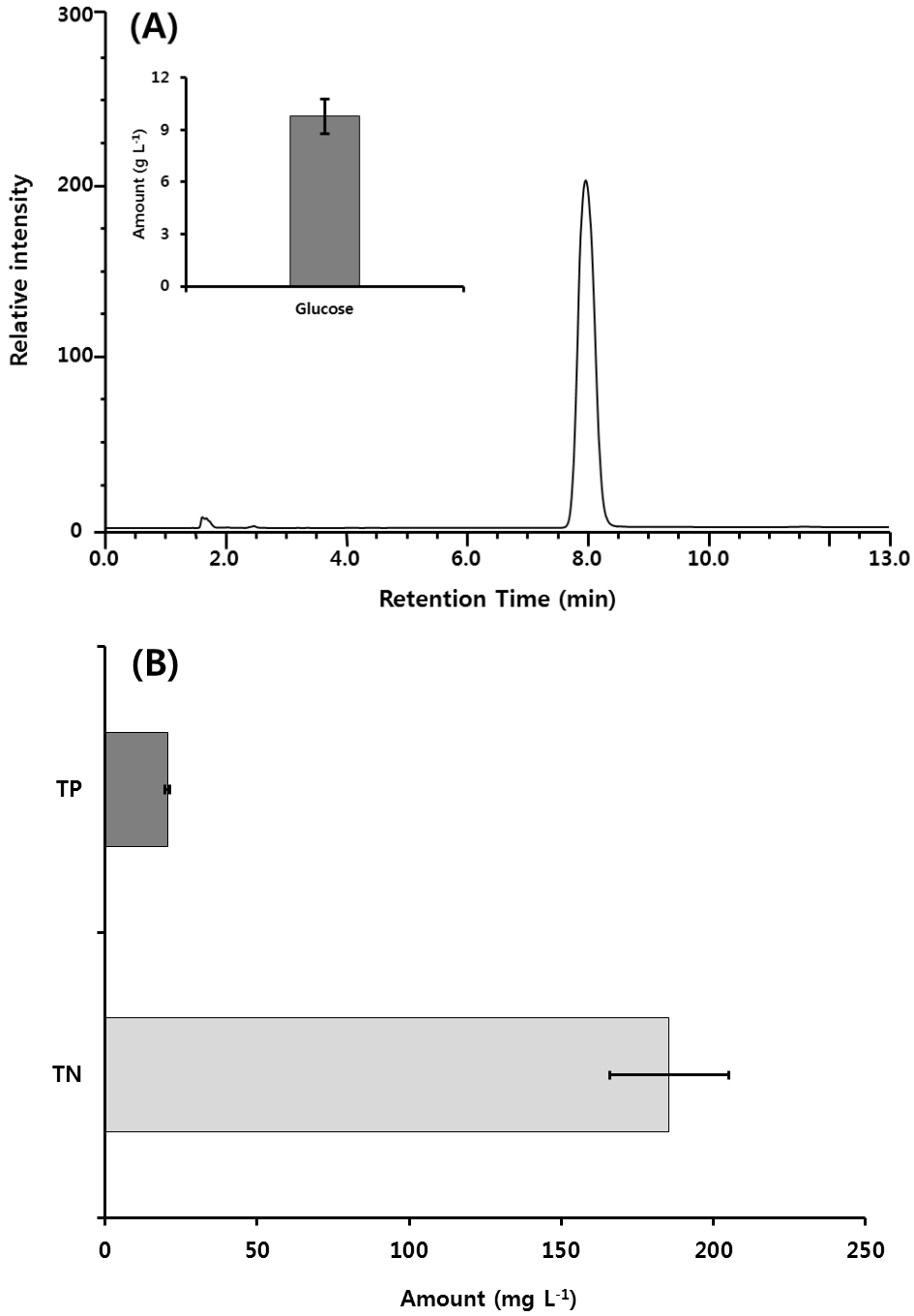

Fig. 2. Monosaccharide composition (A), and total nitrogen and total phosphorus concentrations (B), of raw peritoneal dialysis waste fluid (PDWF). Error bars represent average mean ± standard deviation (SD).

2. Inorganic element composition of peritoneal dialysis waste fluid (PDWF)

To determine the composition of PDWF, inorganic elements were detected using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipment (Agilent Technology, USA). As shown Table 1, PDWF contains a variety of elements including about 2902 mg L^{-1} of sodium, 84 mg L^{-1} of potassium, 65 mg L^{-1} of calcium, 17 mg L^{-1} of magnesium (the main components), and a number of trace elements. PDWF also contains nutrients required for algal growth such as iron (0.0141 mg L^{-1}), copper (0.0129 mg L^{-1}), zinc (0.0112 mg L^{-1}), cobalt (0.0002 mg L^{-1}), manganese (0.0005 mg L^{-1}), and molybdenum (0.0015 mg L^{-1}). According to Guillard (1975), F/2 medium contains 11.7 μmol L^{-1} of iron (0.6500 mg L^{-1}), 0.04 μmol L^{-1} of copper (0.0025 mg L^{-1}), 0.08 μmol L^{-1} of zinc (0.0050 mg L^{-1}), 0.05 μmol L^{-1} of cobalt (0.0025 mg L^{-1}), 0.9 μmol L^{-1} of manganese (0.0500 mg L^{-1}), and 0.03 μmol L^{-1} of molybdenum (0.0025 mg L^{-1}) as trace metals. Compared to F/2 medium, PDWF had high concentrations of copper (5.16-fold) and zinc (2.24-fold), and low concentrations of iron, cobalt, and manganese [13]. From these results, it is suggested that imbalance between the trace elements might affect the growth of microalgae if raw PDWF were used directly as the algal medium.

Table 1. Inorganic element concentration found in sterilized peritoneal dialysis waste fluid (PDWF) by inorganic component ICP-MS analysis

Element	Isotopic mass	Amount (mg L^{-1})	Tuning mode
Li	7	0.0016 ± 0.0001	No Gas
B	11	0.1102 ± 0.0022	No Gas
Na	23	2902.2681 ± 15.0472	No Gas
Mg	24	17.0477 ± 0.2157	No Gas
Al	27	0.0055 ± 0.0012	H_2
K	39	83.8223 ± 0.3024	No Gas
Ca	40	65.153 ± 1.1214	H_2
Sc	45	0.0030 ± 0.0001	H_2
Ti	47	0.0090 ± 0.0003	No Gas
V	51	0.0010 ± 0.0001	No Gas
Cr	52	0.0015 ± 0.0001	He
Mn	55	0.0005 ± 0.0002	No Gas
Fe	56	0.0141 ± 0.0004	He
Co	59	0.0002 ± 0.0001	No Gas
Ni	60	0.0008 ± 0.0001	No Gas
Cu	63	0.0129 ± 0.0001	He
Zn	66	0.0112 ± 0.0001	No Gas
As	75	0.0142 ± 0.0015	He
Rb	85	0.0605 ± 0.0007	No Gas
Sr	88	0.0227 ± 0.0001	No Gas
Mo	95	0.0015 ± 0.0001	No Gas
Ba	138	0.0008 ± 0.0002	No Gas

Uncertainties represent average mean ± standard deviation (SD) for triplicate experiments.

3. Effect of different concentrations of PDWF on the biomass production of *Chlorella* sp.

To evaluate the direct use of raw PDWF as an algal medium, algal biomass was determined for medium containing from 0 to 100% (v/v, with seawater) raw PDWF, after 15 d cultivation time. As shown in Fig. 3, biomass production initially showed a PDWF-concentration-dependent increase (0–75%), but then decreased at 100%. It is considered that the reason for biomass inhibition at 100% PDWF was lack of iron and imbalance between trace metals (Table 1). Moreover, because seawater contains abundant iron and other trace elements, and PDWF contains abundant nitrogen and phosphorus, it is suggested that the best growth might be achieved with PDWF concentrations near 75%. The results showed that production of algal biomass could be achieved using raw PDWF in seawater. However, because lower biomass was obtained in PDWF with seawater medium, than with F/2 algal medium (Fig. 4), and because PDWF contains large amounts of nutrients and organic carbon, it is hypothesized that F/2 medium supplemented with dried-PDWF (DPDWF) might show the greatest biomass production and lipid accumulation under the condition of light-induced mixotrophic culture. Also, due to its much reduced volume DPDWF (rather than raw PDWF), might more easily be applied to production of algal biomass at industrial scale, Therefore, we dried the raw PDWF and added it to algal medium to evaluate the effectiveness of DPDWF for this purpose.

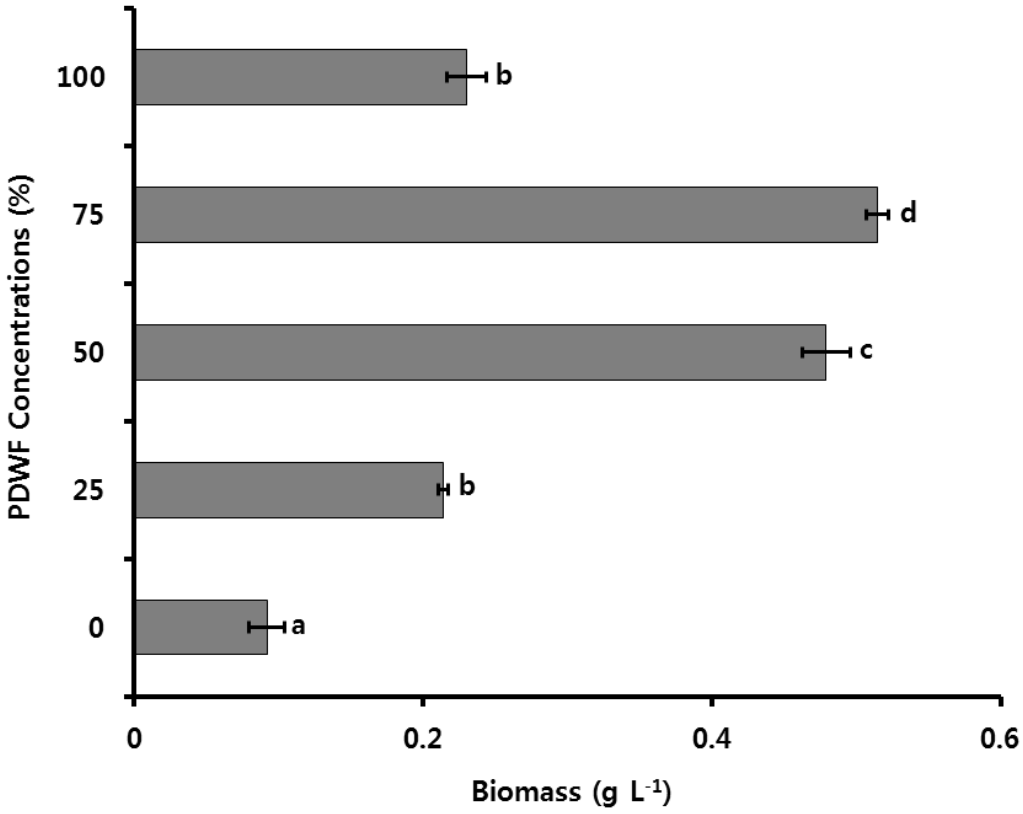

Fig. 3. Effect of different raw peritoneal dialysis waste fluid (PDWF) concentrations on the biomass (g L^{-1}) of *Chlorella* sp. Error bars represent average mean ± standard deviation (SD).

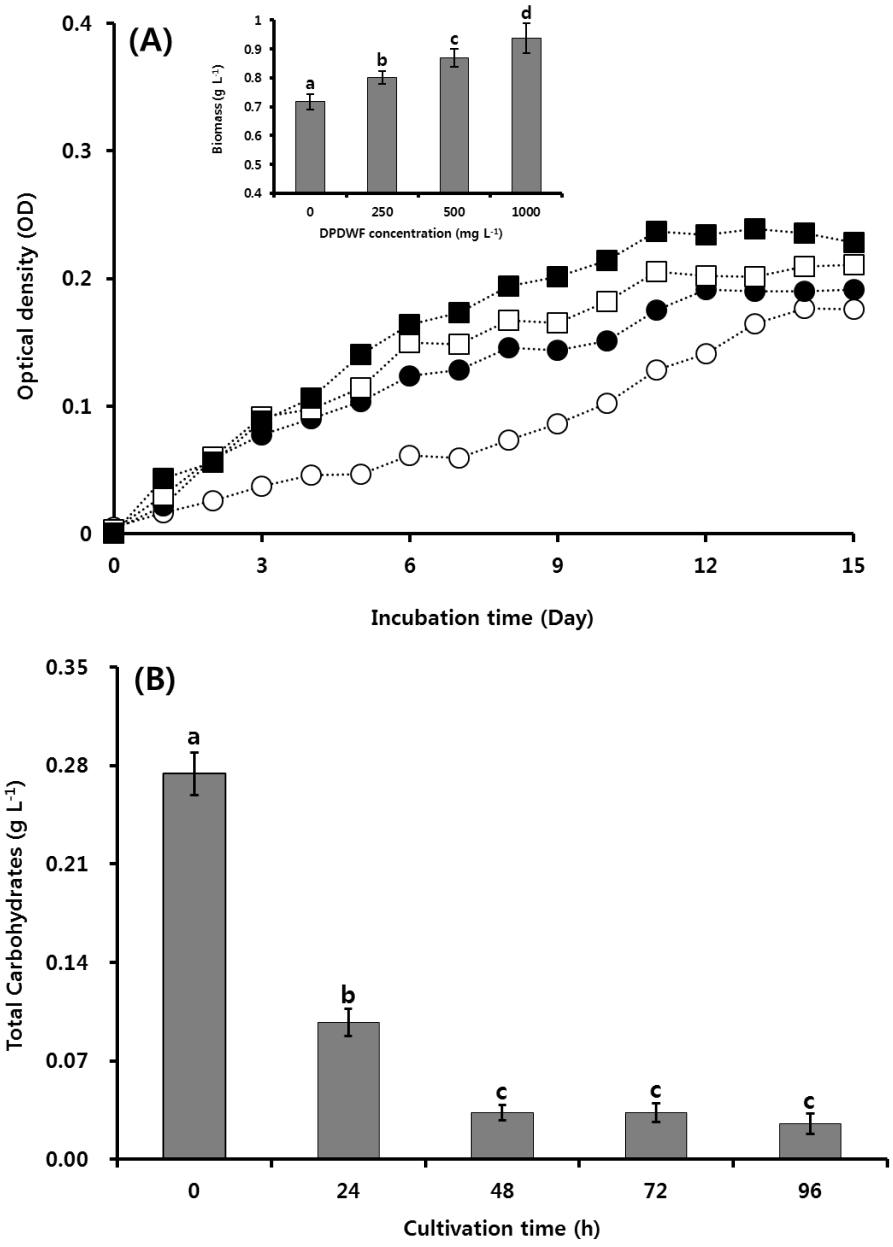

Fig. 4. Effect of 0 (○), 250 (●), 500 (□), and 1000 (■) mg L^{-1} of dried peritoneal dialysis waste fluid (DPDWF) concentrations on the daily growth and biomass of microalga *Chlorella* sp. (A), and changes of total carbohydrate content in 1000 mg L^{-1} of DPDWF containing algal medium (B). Error bars represent average mean ± standard deviation (SD).

4. Growth and biomass production of *Chlorella* sp. under DPDWF supplemented mixotrophic culture and uptake of glucose

As shown Figure 4A, the daily growth and biomass of *Chlorella* sp. significantly increased in a DPDWF-concentration-dependent manner. The highest biomass was obtained with DPDWF supplementation of 1000 mg L^{-1}, and was about 1.31-fold higher than that without DPDWF (0 mg L^{-1}). According to previous reports, marine *Chlorella* sp. exhibited higher growth rates under a mixotrophic culture condition than under photoautotrophic or heterotrophic conditions, and exhibited greater respiration than during photosynthesis [25,26]. Similarly, *Chlorella* sp. exhibited higher biomass production under DPDWF-supplemented mixotrophic condition than under the photoautotrophic condition. Boyle & Morgan (2009), reported that glucose has more energy (about 2.8 kJ mol^{-1}) compared to acetate and other candidate substrates [27]. Therefore, it was hypothesized that the marine *Chlorella* sp. CKC2 would readily take up the glucose contained in DPDWF, and that this might increase its growth.

To confirm the organic carbon (glucose) uptake of *Chlorella* sp., we determined daily the amount of total carbohydrate (TC) in mixotrophic culture medium supplemented with 1000 mg L^{-1} of DPDWF. As shown in Figure 4B, a significant amount of TC was removed after 24 h and 48 h of cultivation time. Also, the daily growth of *Chlorella* sp. was shown to be greater after 1 d of incubation than for culture without DPDWF (Fig. 4A). From these results, it is suggested that the greater growth and biomass production observed was the result of organic carbon uptake from DPDWF by *Chlorella* sp.

5. Effect of DPDWF on the photosynthetic pigment accumulation of *Chlorella* sp.

In mixotrophic culture conditions, it has been reported that the decreased accumulation of chlorophyll content can be observed in many microalgal species (compared to photoautotrophic culture conditions) because the presence of organic carbon sources can change the photosynthetic metabolism of microalgae [25,28-30]. Therefore, we analyzed the accumulation of photosynthetic pigments, including chlorophyll *a* and chlorophyll *b*. As shown in Table 2, decreased levels of chlorophyll *a* and chlorophyll *b* were observed in a DPDWF-concentration-dependent manner, during the exponential growth phase (after 6 d). The highest chlorophyll *a* and *b* content were exhibited under a photoautotrophic condition (0 mg L^{-1}), and about 1.32-fold decreased chlorophyll *a* + chlorophyll *b* were exhibited when DPDWF supplement at 1000 mg L^{-1} was added, under a mixotrophic condition. Previous reports showed that yellow microalgal biomass could be obtained under a glucose-supplemented heterotrophic growth condition [9,25]. However, under a mixotrophic growth condition, microalgae exhibited green biomass and decreased chlorophyll levels [25]. Similarly, although we obtained decreased levels of chlorophyll *a* and chlorophyll *b*, green biomass was obtained from the DPDWF-supplemented mixotrophic culture. According to Stadnichuk *et al* (1998), glucose can inhibit algal chlorophyll biosynthesis via inhibition of coproporphyrin III, which is considered a precursor of chlorophyll *a* [31]. Although more studies are required, it is considered that the reason for decreased chlorophyll levels in *Chlorella* sp. may be the inhibition of coproporphyrin III by the DPDWF-containing glucose supplement.

Table 2. Effect of dried-peritoneal dialysis waste fluid (DPDWF) supplemented mixotrophic cultivation on chlorophyll *a*, and chlorophyll *b* (μg g^{-1}) accumulation by *Chlorella* sp. after six days of cultivation

Pigments	DPDWF concentrations (mg L^{-1})			
	0	250	500	1000
Chlorophyll *a*	8.510 ± 0.369	7.103 ± 0.358	7.029 ± 0.201	6.861 ± 0.163
Chlorophyll *b*	4.515 ± 0.297	3.064 ± 0.163	3.048 ± 0.304	3.007 ± 0.089
Chl *a* + Chl *b*	13.025	10.167	10.077	9.868

Uncertainties represent average mean ± standard deviation (SD) for triplicate experiments.

6. Effect of DPDWF on the lipid accumulation of *Chlorella* sp.

To verify the potential of DPDWF for use as a supplement for microalgae-derived biodiesel production, we determined the total lipid content and FAME compositions of *Chlorella* sp. CKC2 after 15 d of cultivation. Previous studies showed that the lipid accumulation of various microalgal species could be significantly increased when supplemented with organic carbon sources under mixotrophic and heterotrophic culture conditions [25,28,29]. *Chlorella* species, in particular, can accumulate higher lipid content when their media are supplemented with glucose or other organic carbon sources [32,33]. Similar results were obtained in this study. As shown in Figure 5, total lipid content was increased in a DPDWF-concentration-dependent manner. The highest lipid content was obtained with supplementation of 1000 mg L^{-1} DPDWF, and the increase was 1.31-fold, compared to the un-supplemented medium. According to previous reports, a number of microalgal species accumulate higher lipid content under mixotrophic, rather than photoautotrophic, conditions [34]. There was also a rapid decrease in the amount of thylakoid membranes, and degeneration of chloroplasts, when large amounts of lipid droplets accumulated in microalgal cells under heterotrophic conditions, when supplemented with organic carbon [35,36]. Other studies reported that these phenomenon were associated with lipogenesis and enhancement of the acetyl CoA/malonyl CoA pool, which is considered a central carbon donor for the synthesis of fatty acids in algae [36,37]. Therefore, it is proposed that the glucose uptake by algal cells in cultures supplemented with DPDWF, led to the high lipid accumulation.

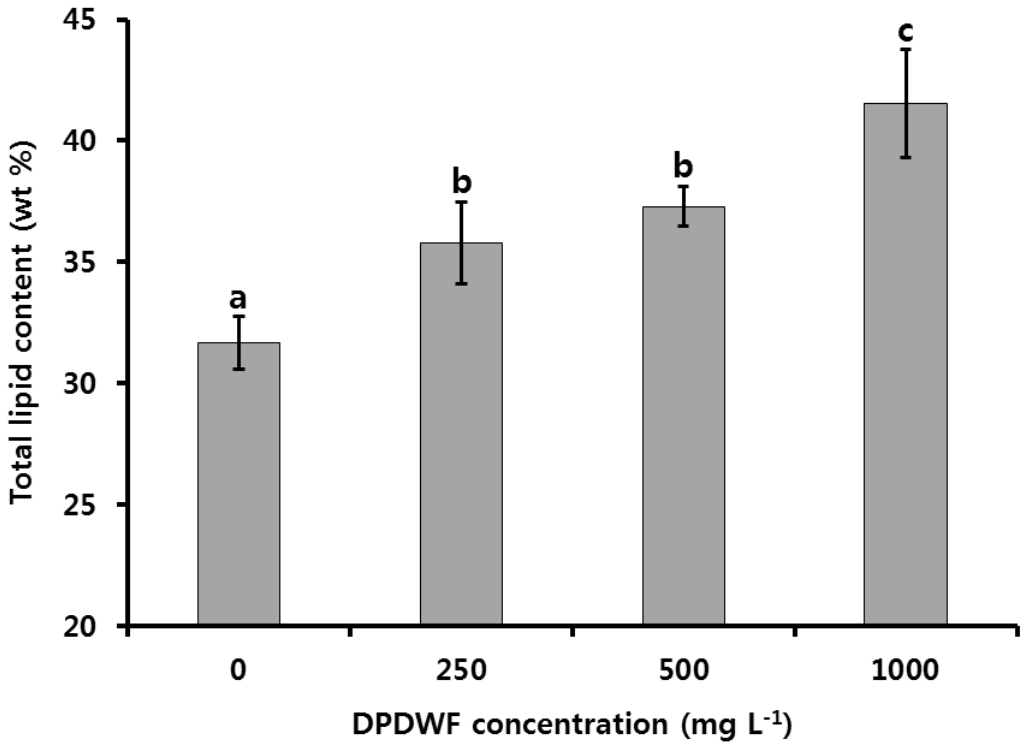

Fig. 5. Effect of different dried peritoneal dialysis waste fluid (DPDWF) concentrations on the changes of total lipid content accumulation of microalga *Chlorella* sp. Error bars represent average mean ± standard deviation (SD).

7. Effect of DPDWF on the fatty acid methyl ester (FAME) composition of *Chlorella* sp.

FAME compositions of algal biomass under different concentrations of DPDWF-supplemented cultures were determined to evaluate the biodiesel properties of the algal oil. As shown in Table 3, the main fatty acid products were C16:0, C18:2(n-6) *cis*, and C18:3(n-3); and they accounted for about 84–85% of total fatty acids. As the DPDWF concentration was increased, higher levels of C18:2(n-6) *cis* and lower levels of C18:3(n-3), were obtained in a concentration-dependent manner. Although the degree of unsaturation (DU) was slightly increased in the DPDWF-supplemented culture (compared to 0 mg L^{-1}), there were no significant changes of iodine value (IV), saponification value (SV), or cetane number (CN). DU is closely related to the oxidative stability of biodiesel, and IV is defined as the amount of iodine (grams) required to completely saturate 100 g of oil [38,39]. IV is related to DU and the number of double bonds in diesel, and if it is higher than standard, this can affect engine deposition [39,40]. The CN is considered an important parameter for evaluating biodiesel properties because it is highly related to engine performance, emission of nitrous oxide, and combustion efficiency of diesel fuel [41,42]. SV is defined as the amount of potassium hydroxide required (mg) to completely saponify 1 g of diesel [43]. SV is required to calculate CN and it is not specified in biodiesel standards [40]. Therefore, it is considered that DPDWF-supplemented mixotrophic cultivation did not significantly affect biodiesel properties, including oxidative stability and engine performance, when compared to products of photoautotrophic cultivation.

Table 3. Effect of different DPDWF concentrations on the changes of fatty acid methyl ester (FAME) compositions and biodiesel properties, including degree of unsaturation (DU), iodine value (IV), saponification value (SV) and cetane number (CN) of the oceanic microalga *Chlorella* sp.

FAME	DPDWF concentration (mg L^{-1})			
	0	250	500	1000
C13:0	0.79 ± 0.05	0.78 ± 0.11	0.79 ± 0.08	0.95 ± 0.15
C16:0	24.08 ± 1.02	23.29 ± 2.16	23.51 ± 2.51	23.90 ± 3.05
C16:1	1.21 ± 0.19	1.34 ± 0.25	1.24 ± 0.16	1.40 ± 0.13
C18:0	2.59 ± 0.21	2.74 ± 0.33	2.62 ± 0.19	2.48 ± 0.22
C18:1(n-9),cis	2.86 ± 0.35	2.65 ± 0.05	2.65 ± 0.11	2.54 ± 0.08
C18:2(n-6),cis	20.51 ± 2.88	21.72 ± 2.33	22.17 ± 1.59	22.89 ± 3.12
C20:0	6.56 ± 0.89	6.96 ± 0.31	6.76 ± 0.15	6.58 ± 0.48
C18:3(n-3)	40.27 ± 1.25	39.31 ± 3.56	39.06 ± 3.99	38.06 ± 2.08
C22:0	0.41 ± 0.01	0.45 ± 0.02	0.45 ± 0.01	0.42 ± 0.06
C22:1(n-9)	0.72 ± 0.02	0.76 ± 0.09	0.75 ± 0.12	0.78 ± 0.03
Σ SFA[a]	34.43	34.22	34.13	34.33
Σ MUFA[b]	4.79	4.75	4.64	4.72
Σ PUFA[c]	60.78	61.03	61.23	60.95
DU	126.35	126.81	127.1	126.62
IV	144.39	143.95	143.98	142.69
SV	193.71	193.48	193.55	193.76
CN	41.99	42.12	42.10	42.36

Uncertainties represent average mean ± standard deviation (SD) for triplicate experiments.

[a]Saturated fatty acid, [b]Monounsaturated fatty acid, [c]Polyunsaturated fatty acid.

8. Feasibility of PDWF

Mixotrophic cultivation using PDWF supplement in the media has high potential for industrial applications. First, it contains a large amount of glucose as an organic carbon source. Other potential sources, such as municipal wastewater and animal urine, usually contain low levels of organic carbon sources. Therefore, PDWF can be used to produce large amounts of algal oil via mixotrophic cultivation. Second, PDWF has no bad smell, in contrast with other wastewater sources. Third, PDWF could easily be obtained from hospitals and dialysis-treated renal failure patients, and it contains high amounts of nitrogen, phosphorus, and other trace elements. Therefore, it could be used directly as a nutrient source for algal cultivation (with supplement of several elements) concurrent with purification of wastewater. However, because hospital-derived PDWF may contain dangerous substances (e.g., infectious agents), guidelines for safe collection of PDWF from hospitals and homes are required before PDWF can be used as an algal nutrient source. Also, further studies involving cultivation at a larger scale, heterotrophic cultivation, and screening for other useful algal species are required prior to industrial applications of this approach.

Ⅳ. CONCLUSION

This study was carried out to evaluate the feasibility of using peritoneal dialysis waste fluid (PDWF) for the cultivation of oceanic microalga *Chlorella* sp. CKC2. The PDWF contains sufficient nutrients (including nitrogen, phosphorus, and other trace elements) as well as a large amount of glucose as an organic carbon source. Both algal biomass production and lipid accumulation were increased under DPDWF-supplemented mixotrophic culture, and no significant changes in the biodiesel properties (including CN and IV) were observed. From these results, it is suggested that DPDWF could be a useful additive for algal cultivation in the sustainable and eco-compatible production of microalgal biodiesel. However, further studies about safe collection and large scale cultivation methods are required to provide an industrial process.

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