Oil and Gas Research

Research Article **Open Access**

Mass Cultivation from a Korean Raceway Pond System of Indigenous Microalgae as Potential Biofuel Feedstock

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Abstract

Naturally occurring freshwater microalgae were mass cultivated in continuous mode at a large-scale facility. From June 2014 to August 2015, biomass productivity, lipid content, and calorific value data were obtained from two 675.0 m^2 raceway ponds. The collected biomass had an overall average productivity of approximately 7.0 g dry weight/m^2/day and a lipid content of 12.2%. Ultimate analysis incorporated with thermal analysis indicated that the average calorific value was 17.5 MJ/kg. The dominant genera found were *Chlorella, Coelastrella, Acutodesmus*, and *Pseudopediastrum*. This pilot-scale study demonstrated the potential of microalgal biomass produced on a large-scale as a biofuel under Korean geoclimatic conditions.

Keywords: Biofuel; Calorific value; Elemental analysis; Mass cultivation; Microalgae

Introduction

The world's demand for renewable and sustainable energy resources is increasing exponentially because of changes in climate and energy shortage problems. This situation has given rise to the development of numerous new technologies such as biomass, geothermal, solar, tidal, and wind energies. Among these resources, microalgae are now considered one of the most attractive candidates for biofuel production due to their higher photosynthetic efficiency and oil yield compared to terrestrial sources [1,2]. A number of microalgae strains with desired characteristics for biofuel production have been found and/or developed [3,4]. However, the attempts to grow these microalgae in outdoor open pond systems have not always been successful due to rapid contamination with bacteria, predatory zooplanktons, and other algal species [5,6]. To overcome these challenges, indigenous microalgae strains have been cultivated for large scale production since these endemic wild types are well adapted to their local conditions and, therefore, they are able to outcompete other indigenous algal strains [7-9]. In this study, biomass productivity and characterization data obtained from commercial-scale microalgal cultivation from outdoor raceways over 1.25 years are presented.

Materials and Methods

Raceway (RW) pond system

A freshwater microalgal cultivation system was constructed in August 2013 at the Chilgok-gun agricultural Technology Center (36°02'N,128°22'E); Dongan-ri, Yakmok-myeon, Chilgok-gun, Gyeongsangbukdo, South Korea). Two identical 675.0 m^2 open raceway ponds (RW #1 and #3) were constructed (Figures 1 and 2). A semitransparent film cover was added to RW #1 to compare the RWs in terms of biomass yield and consumption of resources under unfavorable Korean weather conditions (monsoon and winter seasons). A 526.5 m^2 RW (uncovered, RW #2) also was built as an auxiliary pond for seed culture cultivation and harvest purposes. A coagulation-flotation system was added to the facility for large-scale microalgae harvesting.

Cultivation of microalgae

Initially, indigenous *Coelastrella* grown in small-scale raceways was inoculated into the 675.0 m^2 RWs and species succession was monitored both microscopically and molecularly. Commercial water soluble fertilizers, Eco-Sol (N-P-K: 25-9-18, Dongbu Farm Hannong, Ulsan, South Korea) and monopotassium phosphate (N-P-K: 0-52-34, Sang Rok Chemical, Daegu, South Korea), were added to each RW for a final concentration of 15.0-30.0 mg total nitrogen (TN)/L and 3.0-6.0 mg total phosphorus (TP)/L. A continuous mode of cultivation was carried out at a velocity of 25.0-30.0 cm/sec approximately two thirds of the algal culture was harvested from the pond and replaced with the same volume of underground water. The remaining culture was used as inoculum and appropriate amounts of nutrients were added. From October 2014 on, approximately 10.0 L/min carbon dioxide (CO_2) was aerated into the RWs through Venturi tubes to enhance biomass productivity.

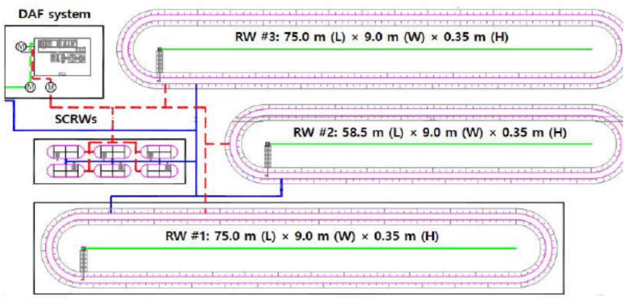

Figure 1: Schematic way of the raceway pond system.

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Received December 14, 2015; **Accepted** December 28, 2015; **Published** January 28, 2016

Citation: Hong JW, Kim OH, Kim H, Jo SW, Cho HW, et al. (2016) Mass Cultivation from a Korean Raceway Pond System of Indigenous Microalgae as Potential Biofuel Feedstock. Oil Gas Res 2: 108. doi: 10.4172/2472-0518.1000108

Microalgal culture monitoring

The microalga cultures were sampled every three days and inspected at 1000X magnification on a Nikon Eclipse E100 Biological Microscope (Tokyo, Japan). In addition, optical density at 680 nm on an X-ma 1200 V spectrophotometer (Human, Seoul, South Korea) and dry mass were measured. Representative species of each season were axenically isolated and added to our laboratory culture collection. Molecular identification was carried out using NS1/NS8 and ITS1/ITS4 primer sets [10]. Consumption of TN and TP (Tables 1-4) was analyzed by using HS-TN(CA)-L and HS-TP-L water test kits (Humas, Daejeon, South Korea). In addition, dissolved oxygen, pH, and temperature in the RWs (Tables 2-4) were monitored every three hours with an automatic water quality meter (WQC-24, DKK-TOA, Tokyo, Japan).

Figure 2: (a) Outdoor race way pond that circulates over 236,000 kilolitres of algae culture medium and (b) 236,000-kiloliter raceway pond in a semi-transparent greenhouse.

Microalgal biomass harvest

When the microalgal culture reached late exponential or early stationary phase, biomass was harvested by chemical coagulation with 17% polyaluminum chloride (Kumsung E and C, Ansan, South Korea) followed by dissolved air flotation (Dongshin enTech, Yangsan, South Korea). The harvested biomass was stored at -20°C until utilization.

Microalga	Marker gene	Accession No.	Length (bp)	Closest match (GenBank accession No.)	Overlap (%)	Sequence similarity (%)	Taxonomic affinity
KNUA036	18S rRNA	KT883906	1771	*Micractinium* sp. KNUA034 (KM243325)	100	99	*Micractinium* sp.
	ITS	KT883910	721	*Micractinium* sp. KNUA034 (KM243327)	100	99	*Chlorella* sp.[a]
KNUA037	18S rRNA	KT883907	1768	*Coelastrella* sp. SAG 2471 (KM020087)	99	99	*Coelastrella* sp.
	ITS	KT883911	699	*Chlamydomonas moewusii* (JX290025)[b]	100	99	-
KNUA038	18S rRNA	KT883908	1767	*Acutodesmus obliquus* GS3e (AB917118)	99	100	*Acutodesmus* sp.
	ITS	KT883912	695	*Acutodesmus nygaardii* CCAP 276/50 (JQ082320)	100	99	*Acutodesmus* sp.
KNUA039	18S rRNA	KT883909	1765	*Pseudopediastrum integrum* Mj2008/86 (HM021309)	98	99	*Pseudopediastrum* sp.
	ITS	KT883913	707	*Pseudopediastrum integrum* Mj2008/86 (HM021309)	97	99	*Pseudopediastrum* sp.

[a] The key compensatory base changes in the ITS2 secondary structure confirmed that strain KNUA036 belonged to the genus Chlorella (data not shown).

[b] The second closest match was *Coelastrella* sp. shy-188 (AB762691).

Table 1: Results from BLAST searches using the 18S rRNA and ITS sequences of the dominant microalgae from the raceway ponds.

Season	Month	Productivity (g DW/m^2/day)	Lipid (%)	CV (MJ/kg)	Avg. Temp. (°C)	Nutrient consumption (mg/kg)	
						TN	TP
Summer 2014	June	7.1	7.5	13.2	25.5	8.4	4.2
	July	5.4	5.4	8.5	28.1	8.6	3.3
	August	4	6.8	11.9	25.9	6.9	3.2
	Avg.	5.5	6.6	11.2	26.5	7.9	3.5
Autumn 2014	September	1.1	6.8	10.7	25.9	9.5	4.5
	October	2.6	11.5	17.1	16.4	8.1	1.4
	November	3.4	13.9	20.1	13.7	10.7	1.4
	Avg.	2.4	10.7	16	18.7	9.4	2.4
Winter 2014-15	December	3.6	17	21.9	7.3	7.8	1
	January	4.2	16.1	21.1	9	7.5	1
	February	3.8	13.6	23.1	12.2	25.9	2.9
	Avg.	3.9	15.5	22	9.5	13.7	1.6
Spring 2015	March	4.3	16.1	23	14.7	29.5	4.8
	April	9.8	14.5	20.3	19.4	15.2	1.4
	May	10.7	13.2	17.8	21.5	11.1	1.5
	Avg.	8.2	14.6	20.4	18.5	18.6	2.6
Summer 2015	June	10.3	16.5	19.4	24.9	13.9	2.3
	July	8.8	14	19.3	28.6	14.9	2.4
	August	7.8	14.7	20.7	27	19.1	1.6
	Avg.	9	15.1	19.8	26.8	16	2.1
	Overall average	5.9	12.6	18	19.8	13.4	2.5

Table 2: Microalgal cultivation results from RW#1.

Season[a]	Month	Productivity (g DW/m^2/day)	Lipid (%)	CV (MJ/kg)	Avg. Temp. (°C)
Summer 2014	June	6	6.6	12.7	25.1
	July	8.7	5.8	9.1	28.9
	August	4.5	6.6	10.6	26.3
	Avg.	6.4	6.3	10.8	26.7
Autumn 2014	September	8.9	6.5	10.4	24.2
	October	10	7	11	16.9
	November	4.5	12.2	17.6	7.7
	Avg.	7.8	8.6	13	16.3
Spring 2015	March	7.8	13	20.7	13.9
	April	8.5	13.6	19.4	18.5
	May	10.3	13.3	18.8	23.5
	Avg.	8.9	13.3	19.6	18.6
Summer 2015	June	15.1	15.8	19.4	25.7
	July	10.4	13.9	19.1	27.4
	August	17.1	14.4	18.6	28
	Avg.	14.2	14.7	19	27
Overall average		9.2	10.8	15.8	21.8

[a] The open raceway pond was shut down for the winter months.

Table 3: Microalgal cultivation results from RW#3.

Season	Month	Productivity (g DW/m^2/day)	Lipid (%)	CV (MJ/kg)	Avg. Temp. (°C)	Nutrient consumption (mg/kg)	
						TN	TP
Summer 2014	June	6.5	7.1	12.9	25.3	7.6	4.3
	July	7	5.6	8.8	28.5	7.4	3.4
	August	4.2	6.7	11.2	26.1	8.3	3.2
	Avg.	5.9	6.5	11	26.6	7.8	3.6
Autumn 2014	September	5	6.7	10.6	25.1	9.1	4.1
	October	6.3	9.3	14	16.6	9.1	2.1
	November	3.9	13	18.8	10.7	13.1	1.5
	Avg.	5.1	9.7	14.5	17.5	10.4	2.6
Winter 2014-15	December	3.6	17	21.9	7.3	7.8	1
	January	4.2	16.1	21.1	9	7.5	1
	February	3.8	13.6	23.1	12.2	25.9	2.9
	Avg.	3.9	15.5	22	9.5	13.7	1.6
Spring 2015	March	6	14.5	21.8	14.3	22.7	3.8
	April	9.1	14.1	19.8	19	14.6	2
	May	10.5	13.2	18.3	22.5	13.2	2.1
	Avg.	8.6	13.9	20	18.6	16.9	2.6
Summer 2015	June	12.7	16.2	19.4	25.3	14.3	2.5
	July	9.6	14	19.2	28	14.4	2.3
	August	12.5	14.5	19.7	27.5	18.1	1.8
	Avg.	11.6	14.9	19.4	26.9	15.6	2.2
Overall average		7	12.2	17.5	19.6	13.1	2.5

Table 4: Average cultivation results from RW#1 and RW#3 (from June 2014 to August 2015).

Characterization of microalgal biomass

The biomass samples were freeze-dried, pulverized with a mortar and pestle, and sieved through ASTM No. 230 mesh (opening=63 μm). Total lipid content was determined by the sulfo-phospho-vanillin colorimetric method [11]. Ultimate analysis was conducted to determine the carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents using a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Milan, Italy). Proximate analysis was carried out to measure ash content using a DTG-60A thermal analyzer (Shimadzu, Kyoto, Japan). The oxygen (O) content was calculated by subtracting the ash and CHNS contents from the total and gross calorific value (GCV, hereafter CV) was estimated by the following equation developed by Given et al. [12]: $CV=0.3278C+1.419H+0.09257S-0.1379O+0.637$ (MJ/kg).

Results and Discussion

Dominant microalgal species

In this study, various species were allowed to dominate naturally and reach their stable states to avoid competition and increase productivity [13,14]. Since South Korea experiences four distinct seasons, the changes in the dominant species could be attributed to the

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daylight time and temperature variations of each season. As presented in Figure 3, *Coelastrella* dominated in the summer of 2014, *Chlorella* and *Acutodesmus* were most common in the winter and spring of 2014, and then *Pseudopediastrum* became dominant in the summer of 2015. It seemed that the changes in CO_2 allowed for the *Pseudopediastrum* to outcompete the *Coelastrella*.

Identification of dominant microalgae

The dominant microalgae were isolated and named as strains KNUA036, 037, 038, and 039. All the isolates were further identified by small subunit 18S ribosomal RNA (rRNA) and internal transcribed spacer (ITS) sequence data analyses (Table 1). For strain KNUA036, molecular characterization by 18S rRNA and ITS sequences indicated that the isolate belonged to the genus *Micractinium*, but the key compensatory base changes (CBS) in the ITS2 secondary structure confirmed that the isolate was a member of the genus *Chlorella* [15]. Due to its extremely simple morphology, no conclusion could be drawn by the morphological features of strain KNUA036. Strain KNUA037 was identified as a member of the genus *Coelastrella* by 18S rRNA and morphological characterization results. Even though its closet match inferred from the ITS sequence was *Chlamydomonas moewusii*, strain KNUA037 did not have any common features of the genus *Chlamydomonas* such as an eyespot and flagella. This may be due to the lack of sequence data in GenBank for *Coelastrella* ITS genes, so no identification could be made with this. It was found that strains KNUA038 and 039 belonged to the genera *Acutodesmus* and *Pseudopediastrum*, respectively. Their molecular and morphological identification results were in agreement. DNA sequences obtained in this study were deposited in the database of the National Center for Biotechnology Information (NCBI) under accession numbers KT883906-KT883913 (Table 1). Numerous studies have shown that *Chlorella* and *Scenedesmus* (some of them have recently been reclassified as *Acutodesmus* [16] are able to synthesize $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ fatty acids, which can be used as biodiesel [17]. Therefore, strains KNUA036 and 038 could have potential to serve as a biodiesel feedstock. Not much is known about the possibility of deriving biofuels from *Coelastrella* and *Pseudopediastrum*. Further researches using the pure cultures of these dominant microalgae should be followed.

Microalgal productivity and biomass characterization

From June 2014 to August 2015, the microalgal biomass had an overall average productivity of approximately 7.0 g dry weight (DW)/m^2/day, a lipid content of 12.2%, and a CV of 17.5 MJ/kg (Table 4). Year-round cultivation, including during the winter season, was possible only in RW #1. The average biomass productivity of RW #3 was higher than that of RW #1. Nonetheless, the average lipid content and CV of RW #1 were slightly higher than those of RW #3 (Figure 4 and Table 4). This is probably due to the growth phases at the time of harvest [18]. In many cases, more actively growing cells were harvested in RW #3 compared to RW #1 to avoid biomass reduction and contamination since aging algal cultures tend to enter the death phase quickly. The highest monthly productivity was achieved in RW #3 in August 2015, but the highest monthly lipid content and CV were found in RW #1 in December 2014 and in RW #1 in February 2015, respectively. The long rainy weather in August 2014 led to substantial reductions in biomass productivity in both RWs. In addition, rotifers caused two sudden algal population crashes in RW #1 leading to the lowest monthly productivity measured of 1.1 g DW m^2/day in September 2014. However, under favorable weather conditions in the spring and summer of 2015, the productivities of RW#1 and RW#3 were approximately 8.2-9.0 g DW/m^2/day and 14.2-15.1 g DW/m^2/day, respectively. The average CV from June 2014 to September 2014 in both RWs was only 10.9 MJ/kg, which is lower than that of terrestrial energy crops (17.0-20.0 MJ/kg) [19-21]. Nevertheless, after the addition of extra CO_2, the value (19.7

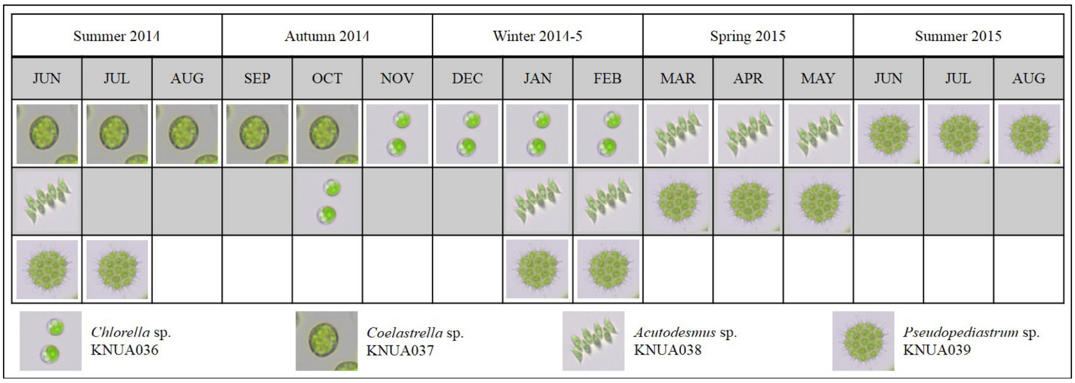

Figure 3: Seasonal Succession of dominant microalgal Species in the race way ponds.

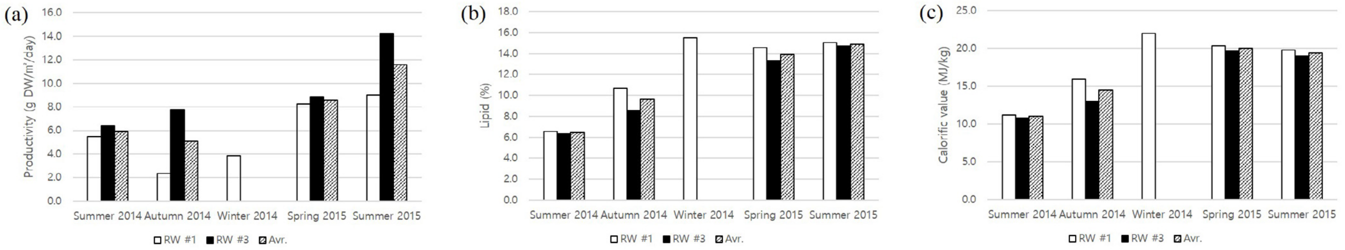

Figure 4: (a) Productivity, (b) Lipid content and (c) CV of each raceway pond.

Season	Month	Biomass production (kg)	CO_2 utilized by biomass (kg)[a]
Summer 2014	June	183.4	335.6
	July	193.3	353.7
	August	109.2	199.8
	Sum	485.9	889.1
Autumn 2014	September	115.6	211.6
	October	170	311.1
	November	162.3	297
	Sum	447.9	819.6
Winter 2014-15	December	42.7	78.2
	January	69.2	126.6
	February	54.2	99.2
	Sum	166.1	303.9
Spring 2015	March	180.5	330.3
	April	221	404.5
	May	289.5	529.9
	Sum	691.1	1264.7
Summer 2015	June	329.2	602.4
	July	279.9	512.2
	August	212.7	389.2
	Sum	821.8	1503.8
Total		2612.7	4781.2

[a] Slade and Bauen

Table 5: Microalgal biomass production and CO_2 fixation (from June 2014 to August 2015).

MJ/kg) became close to the CVs of the terrestrial biomass resources. Therefore, it can be concluded that when a higher concentration of CO_2 is available, higher productivity, lipid content, and CV are attainable. Since industrial flue gas could serve as a CO_2 source at no or a very little cost [22], the additional CO_2 supply was not considered as an expense in this study. In addition, after extraction of C14 to C18 fatty acids, the resulting microalgae biomass could be used as energy pellets or organic fertilizer.

Nutrient consumption and CO_2 fixation

The nutrient consumption was largely dependent on the biological (dominant species) and operational (culturing period) parameters. The overall average consumption of TN and TP for each run was approximately 13.1 mg/kg and 2.5 mg/kg, respectively (Table 5), suggesting that N/P-rich wastewater could be used as a growth medium [23,24]. It has been reported that 1.0 kg of dry algal biomass utilizes 1.83 kg CO_2 [25]. In this study, a total of 2612.7 kg of dry microalgal biomass, which is equivalent to 4781.2 kg CO_2, was produced. South Korea introduced carbon emission trading on January 12, 2015 and carbon is currently traded at the price of US$ 9.7 per ton of CO_2 equivalent on the market. This opens up new opportunities for the microalgae-based industry to profit from carbon trading.

Conclusions

This study demonstrated the potential of commercial-scale microalgal biomass production for biofuels under Korean geoclimatic conditions. Naturally occurring microalgae were allowed to dominate in the RW ponds to establish more reliable cultures. The species composition along with nutrient availability strongly affected the biomass productivity and characteristics. In this study, the CV was calculated to understand the potential of microalgal biomass as a biofuel feedstock and the overall microalgal biomass CV (17.5 MJ/kg) approached the CV found for terrestrial energy crops in other studies. Although more innovative work is still needed to enhance the biomass and lipid productivity, the present work showed that microalgae hold great promise as a potential biofuel source, more so than crop plants.

Acknowledgments

This work was supported by the Advanced Biomass R&D Center (ABC) of the Global Frontier Project funded by the Ministry of Science, ICT and Future Planning (2015M3A6A2065698), South Korea. This project was also supported by the Freshwater Microalgae-based Bioenergy Research and Development Project from Chilgok-gun, Gyeongsangbuk-do, South Korea. Hong JW and Kim OH contributed equally to this work.

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