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Influence of Water Depth on Microalgal Production, Biomass Harvest, and Energy Consumption in High Rate Algal Pond Using Municipal Wastewater S

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The high rate algal ponds (HRAP) powered and mixed by a paddlewheel have been widely used for over 50 years to culture microalgae for the production of various products. Since light incidence is limited to the surface, water depth can affect microalgal growth in HRAP. To investigate the effect of water depth on microalgal growth, a mixed microalgal culture constituting three major strains of microalgae including *Chlorella* sp., *Scenedesmus* sp., and *Stigeoclonium* sp. (CSS), was grown at different water depths (20, 30, and 40 cm) in the HRAP, respectively. The HRAP with 20 cm of water depth had about 38% higher biomass productivity per unit area (6.16 ± 0.33 $g \cdot m^{-2} \cdot d^{-1}$) and required lower nutrients and energy consumption than the other water depths. Specifically, the algal biomass of HRAP under 20 cm of water depth had higher settleability through larger floc size (83.6% settleability within 5 min). These results indicate that water depth can affect the harvesting process as well as cultivation of microalgae. Therefore, we conclude that water depth is an important parameter in HRAP design for mass cultivation of microalgae.

Keywords: Biomass, mass cultivation, microalgae, microalgal cultivation parameter, high rate algal ponds (HRAP)

Introduction

Microalgal biomass is a highly promising and sustainable resource for the production of energy and varying commodities owing to their higher growth rate and oil productivity than other oil crops, and the use of low-quality water, including sewage. It has been well-explained by previous studies about the advantages of microalgae compared with terrestrial plants regarding biomass and oil production in various experimental facilities [1–3]. Thus, microalgal biomass is considered as a sustainable and future feedstock for bioenergy production [4]. The microalgal mass cultivation for bioenergy has been studied for over 60 years, and high rate algae pond (HRAP) along with open pond systems are widely used to obtain algal biomass [5]. The HRAPs operated by a paddlewheel have been used for large-cultivation of microalgae since the 1950s. Most HRAPs are made of a closed loop and channels with constant width and depth. They are normally low-cost and durable and do not require high energy for mixing (about 4 W m^{-3}) compared with photobioreactor systems. Above all, owing to high flexibility and convenient scale-up cultivation of microalgae, many studies about HRAP-based algal cultivation have been performed over the years [6–9]. However, industrial-scale cultivation of microalgae is not currently viable owing to nutrient and harvesting costs [1, 2]. Mostly, HRAPs are limited as they have large area requirement, lower gas-liquid mass transfer, difficulties of culture contamination and temperature control, and lower biomass productivity. In particular, light utilization is generally limited because HRAPs have only one surface of light incidence. On an industrial scale, light intensity is one of the key factors and it plays a very essential role in photosynthesis and the growth of microalgae. Furthermore, algal growth is highly affected by the water depth, because light penetration into the culture medium is significantly inhibited by the increase of water depth in HRAPs. Accordingly, the light intensity should be augmented to penetrate the culture medium [10, 11]. Generally, the water depth used for mass cultivation of microalgae in HRAPs varies from 10 to 50 cm [12]. Therefore, it can affect algal biomass production, the harvesting process, and operation costs [13]. The HRAP system for microalgal biomass production has structural weaknesses, the disadvantage of which needs to be overcome.

In this study, we verified the effect of biomass production and settleability for harvest in HRAPs according to different water depths. Additionally, we explored the optimal water depth for economical operation of HRAPs by comparing nutrient utilization and energy consumption.

Materials and Methods

Study Site and High Rate Algal Pond System

To operate the HRAP system, we used a facility located at the Daejeon Municipal Wastewater Treatment Facility (latitude: 36°22′48.89′′N; longitude: 127°24′27.71′′E), which treats the municipal wastewater of Daejeon Metropolitan city, obtained after secondly settling treatment (effluent water). We modified the HRAP system using 60 L municipal wastewater as previously described by Kim *et al.* [1]. To examine the effect of water depth in the HRAP, about 1 m^2 of HRAPs were designed, and the total width and length of ponds were 90 and 130 cm, respectively. The HRAPs had one paddlewheel for mixing, and the diameter and width of the paddlewheel were 90 and 30 cm, respectively. Above 45 cm from the bottom of the pond, the paddlewheel shaft was installed, and the blade edge clearance was 5 cm from the bottom (Fig. 1). An optimum water velocity (approximately 0.3 m/sec) was made after mixing with the aid of the paddlewheel, from previous reports [1].

Strain, Water Sources and Semi-Continuous HRAP

To compare the growth of microalgae, an indigenous microalgal consortium containing *Chlorella* sp., *Scenedesmus* sp., and *Stigeoclonium* sp. (CSS) was isolated from Daejeon municipal wastewater [1]. The small scale with different water depths of 20, 30, and 40 cm (working volume: 0.2, 0.3, and 0.4 ton) were run as batch culture mode for 20 days and semi-continuous culture mode for 20 days with 4-day hydraulic retention times (HRTs). The HRAP system was operated in a greenhouse, and the detected average natural illumination was 630 μmol·m^{-2}·sec^{-1} during the study period (August 2014). The treated effluent obtained from the wastewater treatment plant (Korea) is used with solid fertilizer as nutrient source. The initial total nitrogen (TN) and total phosphorous (TP) were adjusted to about 40 and 7 ppm, respectively.

Analysis of Dry Cell Weight (DCW) and Chlorophyll *a* Content

The DCW was measured according to the standard methods [14]. The algal DCW was determined by filtering 50 ml of algal cell culture through dried GF/C filters (Whatman, England). After washing with deionized water, the filters containing cell pellets were re-dried (at 105°C for 1 h), and precisely weighed using a microbalance. The concentration of chlorophyll *a* was determined by the spectrophotometric method as described previously [15]. Based on the measured optical density values, chlorophyll *a* was calculated by the following equation:

$$\text{Chlorophyll } a \text{ (mg m}^{-3}) = (11.85 \times (E_{664} - E_{750}) - 1.54 \times (E_{647} - E_{750}) - 0.08\,(E_{630} - E_{750})) \times V_e/L \times V_f$$

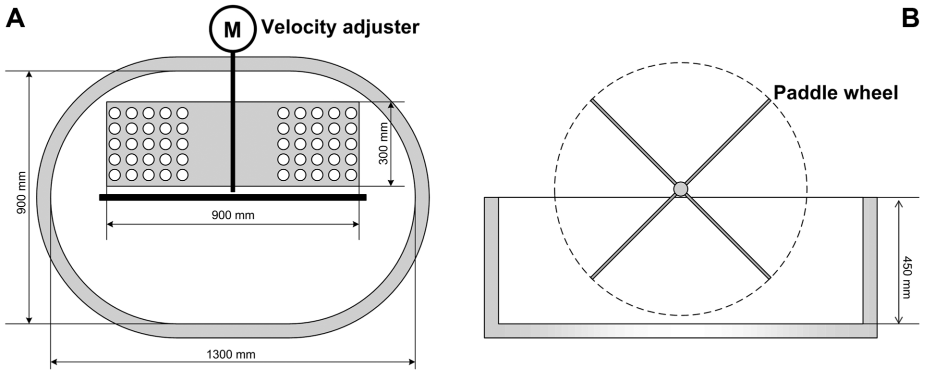

Fig. 1. Scheme of the high rate algal pond.

where

L = Cuvette light-path in centimeter

V_e = Extraction volume in milliliter

V_f = Filtered volume in liter

E_x = optical density at x nm of wavelength

Total Nitrogen and Total Phosphorus Analysis

For the chemical analysis, samples were filtered through membrane filters (0.2-µm pore size; Sartorius, Germany) before analysis. The TN content was measured by the chromotropic acid method [14]. The TP was measured by the persulfate acid digestion method for wastewater and seawater [14].

Settleability Analysis of Algal Biomass

The microalgal consortium settleability was analyzed by the sludge volume index (SVI) test, according to the proposed standard method [14].

Light Intensity and Energy Consumption

The light intensity was measured with a spherical underwater quantum sensor, LI-193 (LI-COR, USA). The light intensity on the water surface was set at 100 µmol·m^{-2}·sec^{-1}, and the light penetration according to water depth was calculated. The energy applied to the motor was measured using a digital inverter, VS mini drive (Yaskawa, Japan).

Results and Discussion

In this study, the mixed microalgal culture constituted three major algal strains, including *Chlorella* sp., *Scenedesmus* sp., and *Stigeoclonium* sp (CSS). A mixed species assemblage such as the mixed microalgal culture CSS exhibited improved algal biomass and culture stability compared with a single culture [16, 17]. In particular, the mixed microalgal culture CSS had advantages of high nutrient removal performance and settling efficiency. Therefore, this can provide potential as a biomass resource for bioenergy or value-added products, making wastewater a sensible nutrient resource [1].

Biomass Productivity

For the industrial application, we evaluated the effect of water depth on cultivation of microalgae in HRAPs, and different water depths (20, 30, and 40 cm) were used for the pilot-scale HRAP in the Daejeon municipal treatment facility. An optimum horizontal water velocity (approximately 0.3 m/sec) was obtained after mixing with the aid of paddlewheel. Prior to semi-continuous operation, the HRAP was initially operated in a batch mode for 20 days to enable stabilization and biomass growth of CSS. The biomass production in HRAPs at 20, 30, and 40 cm water depths was 0.67 ± 0.03, 0.35 ± 0.01, and 0.27 ± 0.02 g/l for 20 days of batch cultivation, respectively (Fig. 2A). Moreover, the volumetric biomass productivity (VBP) was reduced significantly at 30.79 ± 1.64, 14.61 ± 0.51, and 10.53 ± 1.21 mg·l^{-1}·d^{-1} for the 20, 30, and 40 cm water depths, respectively. The area biomass productivity (ABP) was reduced significantly at 6.16 ± 0.33, 4.38 ± 0.15, and 4.21 ± 0.48 g·m^{-2}·d^{-1} for the 20, 30, and 40 cm water depths, respectively (Table 1). As results, the biomass growth increased with decreasing water depth. Furthermore, after 20 days of cultivation time, the VBP and ABP in the HRAP with 20 cm of water depth were 30.79 ± 1.64 mg·l^{-1}·d^{-1} and 6.16 ± 0.33 g·m^{-2}·d^{-1}, respectively. The VBP was twice higher than the other treatments and the ABP also was about 38% higher than the others (Table 1). The results indicate that a lower water depth is favorable for biomass productivity.

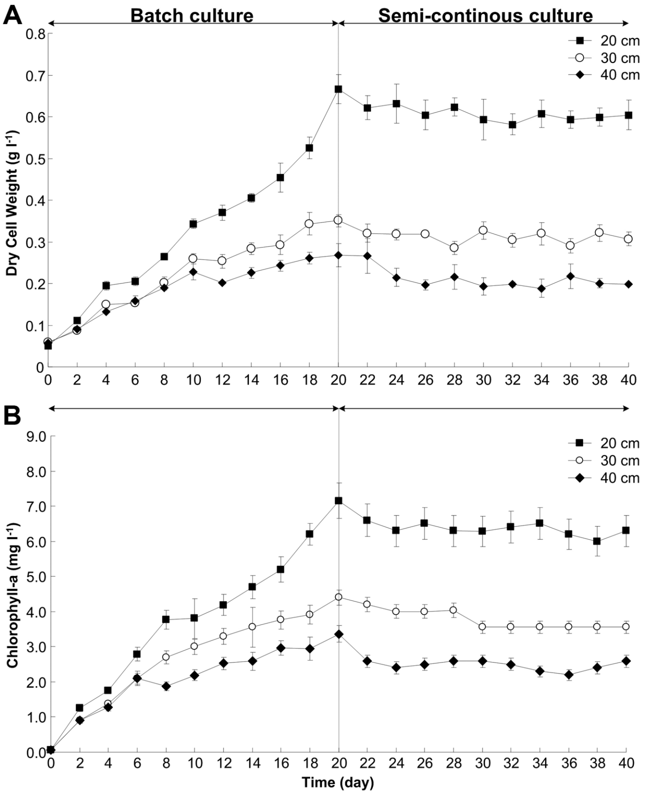

Fig. 2. Time course of dry cell weight (**A**) and chlorophyll *a* (**B**) concentrations of HRAPs under different water depths. ■, 20 cm; ○, 30 cm; ◆, 40 cm.

For continuous evaluation, semi-continuous cultivation was subsequently performed for 20 days right after batch cultivation. In the semi-continuous culture mode, 4 days of hydraulic retention time (HRT) was regulated by removing 50, 75, and 100 L of microalgal cultures while adding an equal volume of treated wastewater and solid fertilizer (TN

Table 1. Summary of microalgal cultivation in HRAPs under different water depths.

Water depth (cm)	Working volume (L)	Batch culture		Semi-continuous culture		Nitrogen removal efficiency (%)	Phosphorous removal efficiency (%)	Settleability (%, 30 min)	Microaglal floc size (μm)
		Volumetric productivity ($mg{\cdot}l^{-1}{\cdot}d^{-1}$)	Area productivity ($g{\cdot}m^{-2}{\cdot}d^{-1}$)	Volumetric productivity ($mg{\cdot}l^{-1}{\cdot}d^{-1}$)	Area productivity ($g{\cdot}m^{-2}{\cdot}d^{-1}$)				
20	200	30.79 ± 1.64	6.16 ± 0.33	30.28 ± 1.60	6.06 ± 0.32	82.5	89.7	96.4	150–600
30	300	14.61 ± 0.51	4.38 ± 0.15	15.55 ± 0.86	4.67 ± 0.26	43.4	36	87	100–300
40	400	10.53 ± 1.21	4.21 ± 0.48	10.45 ± 0.98	4.18 ± 0.39	18.6	32.3	68	50–100

40 ppm, and TP 7 ppm), every day, respectively. Additionally, the biomass production in HRAPs with 20, 30, and 40 cm of water depth was maintained at 0.61 ± 0.03, 0.31 ± 0.02, and 0.21 ± 0.02 g/l for semi-continuous cultivation, respectively (Fig. 2A). The VBP was reduced significantly at 30.28 ± 1.60, 15.55 ± 0.86, and 10.45 ± 0.98 $mg{\cdot}l^{-1}{\cdot}d^{-1}$ for the 20, 30, and 40 cm water depths, respectively. The ABP was reduced significantly at 6.06 ± 0.32, 4.67 ± 0.26, and 4.18 ± 0.39 $g{\cdot}m^{-2}{\cdot}d^{-1}$ for the 20, 30, and 40 cm water depths, respectively (Table 1). Surprisingly, the HRAP systems for semi-continuous mode have maintained good microalgal productivity. Moreover, after 20 days of semi-continuous cultivation, the VBP and ABP in the HRAP with 20 cm of water depth were 30.28 ± 1.60 $mg{\cdot}l^{-1}{\cdot}d^{-1}$ and 6.06 ± 0.32 $g{\cdot}m^{-2}{\cdot}d^{-1}$, respectively. The results of batch and semi-continuous modes showed that the microalgal biomass production in the HRAP with 20 cm of water depth was much higher than that of the other treatments.

The Chlorophyll *a* results also showed a similar trend of increase with decreasing water depth both for batch and semi-continuous cultivations. Chlorophyll *a* in the HRAP with 20 cm of water depth consistently was the highest. It was 7.15 ± 0.50 mg/l at the end of batch cultivation and retained average 6.34 ± 0.44 mg/l (Fig. 2B).

The HRAP system is relatively more appropriate for wastewater treatment than traditional waste stabilization ponds because disadvantages like nutrient limitation arising from quality changes of the effluent are overcome in the HRAP system. Furthermore, from an economic point of view, the recovered algal biomass is applicable for plant fertilizer, protein-rich feed, and different types of biofuel by varying bioprocesses [18]. Although biofuel production from microalgae is not feasible yet owing to their lower economic value, the combined process with wastewater is considered as a viable process economically [19, 20]. For the optimization of economic values from wastewater treatment and algal biomass production, the core subject is enhancing algal biomass productivity within a short treatment time [21].

For the optimization of algal biomass productivity, light is an essential factor and it has been widely recognized that the depth of culture medium is one of the important operation features for affecting the penetration of light in HRAPs [21, 22]. The reported optimal medium depths of HRAPs are from 15 to 100 cm [23–25], and other studies also have explained the depth of the HRAP must be maintained as shallow as possible to optimize the penetration of light intensity [26, 27]. Although many studies about pond depth have been performed to verify its effects on biomass productivity in common pond-based systems, the recent review or research articles do not suggest definite guidelines for regulation of depths in HRAPs for the optimization of algal mass cultivation.

Light Efficiency on Water Depth

The high biomass productivity at a low-level water depth like 20 cm is due to the water permeability of the sunlight. Light penetration in the water with increasing water depth dramatically decreased in the HRAPs (Fig. 3). At 5 cm of water depth, light penetration decreased by 40%. Light barely penetrated between 20 and 30 cm water depths and never arrived at >30 cm of water depth in the HRAPs. At depths >30 cm, the light could not reach the bottom of the HRAPs (Fig. 3). As a result, a high water level means that the growth of microalgae is suppressed. Thus, whereas microalgae were able to use light everywhere within 20 cm of water depth, light available to microalgae was significantly limited above >20 cm of water depth. In other words, the euphotic zone that can undergo photosynthesis in the HRAP was 20 cm of water depth. The light must be necessary for autotrophic growth of microalgae. The light utilization decreased according to increase of water depth. Light intensity plays a core role in microalgal biomass productivity in HRAPs because the photosynthetic activity of algal cells light-dependently occurred in mass cultivation system. Therefore, the effective regulation of light intensity in the HRAP is fundamental for economic algal biomass production

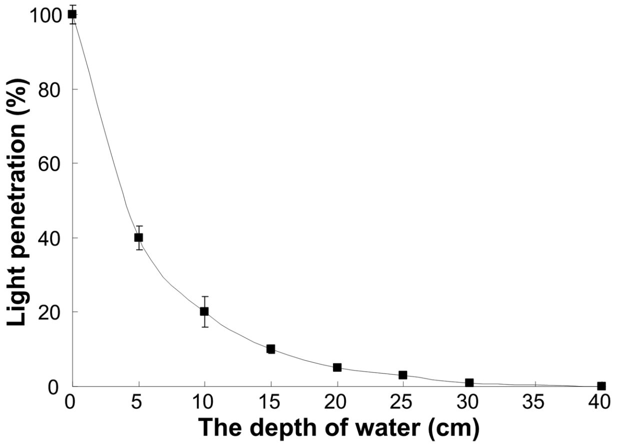

Fig. 3. Light penetration by water depth.

[28]. Since the penetration of light in the high-density microalgal HRAP significantly attenuated forming a steep slope near the bottom area, low algal culture medium was exposed to low light intensity, whereas the surface area was exposed to relatively high light intensity [29]. However, very shallow water depth in the HRAP reduces the area biomass productivity of microalgae. Furthermore, it is difficult to engineer at water depths of <15 cm for cultivation of microalgae [13]. *Pleurochrysis carterae* had the highest ABP at a water depth of 21 cm in HRAPs in summer [30]. Therefore, for light penetration and microalgal growth, the optimal depth was considered as 20 cm in HRAPs.

Nutrient Removal Efficiency

The nutrient removal efficiency of HRAPs was evaluated by analysis of the TN and TP by standard methods. As a result of batch culture, the TN concentrations were 7.9 ± 0.53, 25.1 ± 2.57, and 38 ± 4.24 ppm in the HRAPs for the 20, 30, and 40 cm water depths, respectively. The TP concentrations were 0.8 ± 0.04, 4.6 ± 0.47, and 5.2 ± 0.26 ppm for the 20, 30, and 40 cm water depths, respectively (Fig. 4). During batch cultivation, the TN removal efficiencies were about 82.5%, 43.4%, and 18.6%, and the TP removal efficiencies were approximately 89.7%, 36.0%, and 32.3%, respectively (Table 1). The amount of nutrients including nitrogen and phosphorous is one of the most important factors because microalgae require those nutrients for their growth. The reduction of nutrients in the medium corresponds to the growth of microalgae. Therefore, for the 20 cm depth in the HRAP, both biomass productivity and nutrient removal efficiency were higher than for the other treatments.

The average TN concentrations of the HRAPs were maintained at 10.7 ± 2.2, 23.3 ± 2.2, and 29.8 ± 2.0 ppm under 20, 30, and 40 cm of water depths for semi-continuous mode, respectively, corresponding to average TN removal efficiencies of 76.3 ± 4.0%, 47.5 ± 2.3%, and 36.3 ± 3.2%. Moreover, the average TP concentrations of the HRAP were maintained 3.6 ± 0.2, 7.0 ± 0.4, and 7.0 ± 0.4 ppm under different water depths for semi-continuous mode, respectively, corresponding to average TP removal efficiencies of 51.5 ± 3.9% under 20 cm water depth. In this result, TN and TP concentrations showed a similar trend as for batch cultivation, and both the lowest concentrations of TN and TP were maintained under 20 cm of water depth in the HRAP. The amount of nutrients in HRAP culture was maintained at less than about 15 ppm (TN) and about

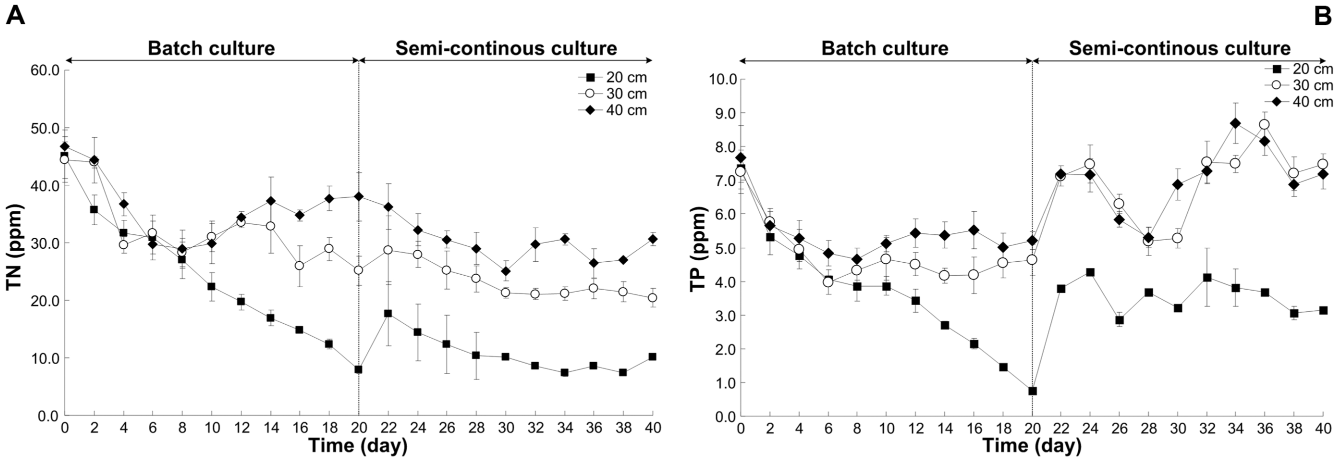

Fig. 4. Time course of total nitrogen (TN) (**A**) and total phosphorus (TP) (**B**) concentrations in the HRAPs.
■, 20 cm; ○, 30 cm; ◆, 40 cm.

4 ppm (TP) during semi-continuous mode under 20 cm water depth. These were at least twice less than for the other treatments.

To reduce cultivation costs, low-priced nutrient resources such as wastewater or solid fertilizer were used as nutrient sources for microalgal cultivation. The amount of nutrients is an essential factor because the nutrient supplementation into the medium requires a high cost. The high amounts of nutrients are generally required with increasing water depth, resulting in increase of the cultivation costs. Because the flow rate and water depth highly fluctuate the nutrient distribution in raceway ponds, these parameters should be considered to maintain optimal algal culture condition [31]. Furthermore, the dead zone where the flow is stagnant is usually augmented by increasing the water depth in HRAPs [32]. Therefore, the low water depth can enhance uptake of nutrients by microalgae in HRAPs, and it can also reduce the cultivation costs.

Settleability Analysis of Algal Biomass

The biomass harvesting process requires considerable cost (approximately 20−30% of total biomass production) [33, 34]. Thus, cost-effective methods including flocculation, sedimentation, centrifugation, and filtration have been developed over the decades. Auto-flocculation has an advantage owing to low energy consumption and reduced cost for the harvesting process compared with conventional centrifuge or filtration methods [35]. Hence, the settleability of microalgal biomass by auto-flocculation was investigated after the cultivation process. The settleabilities in HRAPs with 10, 20, and 30 cm of water depths were about 83.6%, 60.9%, and 36.0% after 5 min and 96.4%, 87.0%, and 68.0% after 30 min, respectively (Fig. 5). The microalgal biomass in the HRAP at 20 cm of water depth had the highest settleability, and most of the biomass efficiently precipitated within 30 min. It has been reported that the mixed microalgal culture CSS has a special feature in high settleability capacity [1]. Interestingly, the floc size of CSS was decreased in a water depth-dependent manner in this study (Fig. S1, Table 1). Furthermore, the larger floc size in the HRAP with low water depth enhanced the settleability. Therefore, we concluded that the 20 cm of water depth can enhance the harvesting efficiency. In a previous report, the symbiotic bacteria in microalgal culture played a significant role in flocculation and floc-size of the algal culture, and it also generated efficient sedimentation of the algal biomass [35]. Based on the reports, it is speculated that the phycosphere bacteria in CSS may be grown together with growth of microalgae, leading to an increase of floc size and improved sedimentation of microalgae. On the other hand, the deeper water depth has the more diluted algal suspension due to the larger culture volumes, resulting in harvesting cost increment, and it is hard to engineer owing to the larger culture volumes [13]. The water depths of >30 cm demand larger quantities of liquid and it can adversely affect harvesting as well as the growth of microalgae.

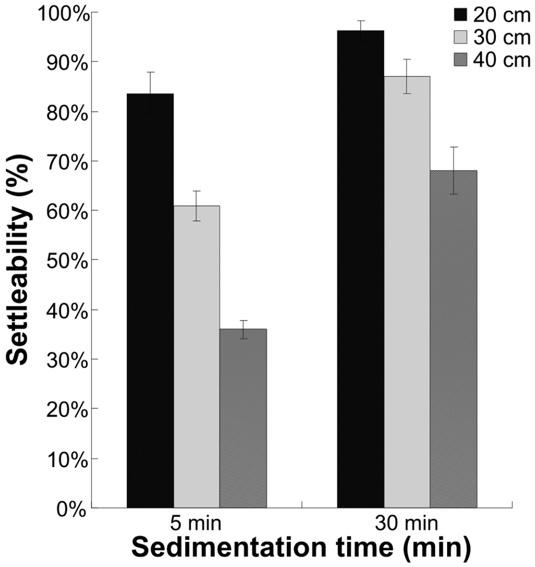

Fig. 5. Settleability efficiency of microalgal biomass in HRAPs. ■, 20 cm; ○, 30 cm; ◆, 40 cm.

Energy Consumption Analysis

Energy consumption is also considered an important factor because it influences the operation costs in HRAPs. Hence, energy consumption of the wheel was measured in HRAPs under different water depths (Fig. 6). As a result, energy consumption increased along with increasing water depth. The energy consumption at 30 cm water depth was about 13−31% higher with increment of the motor frequency in comparison with that at 20 cm. In addition, the energy consumption of 40 cm water depth showed about 33−50% higher values than those of 20 cm. Therefore, the deeper water depth increased the energy consumption to operate the paddlewheel in the HRAPs, and resulted in increasing total costs for cultivation of the microalgae. The power consumption of the HRAP was also augmented with increasing water depth, indicating that regulation of the water depth is very important in terms of energy costs. Thus, a low water depth is highly beneficial for energy

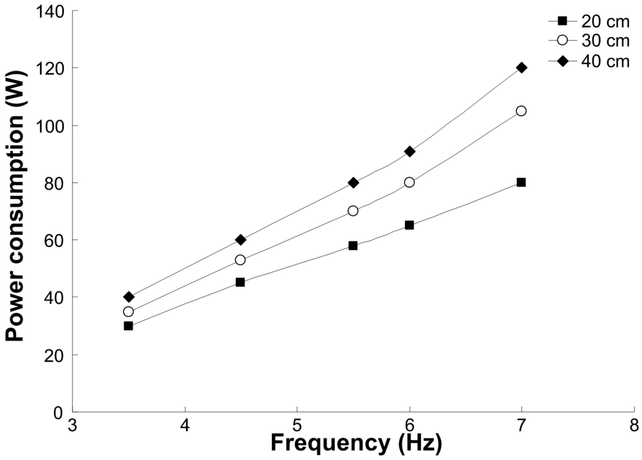

Fig. 6. Power consumption of paddlewheel under different water depths.

■, 20 cm; ○, 30 cm; ◆, 40 cm.

costs. The lowest power consumption represented from 20 cm in the HRAP [8]. However, a much lower water depth reduced biomass production owing to a lack of total culture volume. Accordingly, the lower water depth not only reduced energy demand, but was also considered as a downstream process because the yield of algal biomass decreased significantly [5].

In this study, the water depth highly affected the growth and settleability of microalgae in HRAPs. The optimal depth of culture medium was determined at 20 cm, based on the nutrient removal, growth, harvesting, and energy consumption. Both the biomass productivity and nutrient removal efficiency showed the highest values at 20 cm. Furthermore, it had advantages in terms of the harvesting process and reducing total costs for operation, due to their high settleability and relatively low energy consumption. Based on the results, we concluded that the water depth is considered as an important factor for microalgal mass cultivation in HRAPs, and it must be considered to design and operate HRAPs.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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