Thesis for the Degree of Master of Environmental Engineering

Microalgae Cultivation and Harvesting

by

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Microalgae Cultivation and Harvesting

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Abstract

In this research, we tried to use mixotrophic condition to study the effect of dissolved oxygen, pH, aeration, and organic carbon in the microalgal growth process, and try to find the effective harvesting methods. We realized that using acetate as additional carbon resources can enhance microalgal growth rate dramatically when we input ammonia acetate in the culture medium under automated pH controlling by using CO_2, the DO value was dropped to almost 0, at the same time the growth rate will arrive at the maximum 0.936 $g\ L^{-1}\ d^{-1}$. Whereas, the other groups showed the much lower growth rate, blank (0.017 $g\ L^{-1}\ d^{-1}$), DO controlling(0.019 $g\ L^{-1}\ d^{-1}$), aeration(0.127 $g\ L^{-1}\ d^{-1}$), pH control(0.102 $g\ L^{-1}\ d^{-1}$), aeration with pH controlling with acetate (0.662 $g\ L^{-1}\ d^{-1}$). From this fact, we conclude that using ammonia acetate can create the anoxic condition which can stimulate microalgae growth dramatically, but only removing dissolved oxygen by using N_2 has little impact on microalgae growth. Besides, in the microalgae harvesting tests, the PGA can be effective coagulator in microalgae harvesting processes. And with the solution cell concentration increased, the harvesting efficiency increased quickly at begining and keeps stable after the initial biomass concentration is more than 0.8 g/L.

1. Introduction

Microalgae is a kind of autotrophic plants with wide distribution both on land and aqueous system[30]. Besides, microalgae have valuable industrial applications. They are used as food for fish and shellfish in the aquaculture industry and for feedstock for biofuels, proteins, and natural pigments. The use of microalgae as a material from which biodisel can be produced has drawn considerable scientific attention because the algae grow quickly, are inexpensive, and are renewable [1-3]. The main drawback of microalgae cultivation is its intrinsic low biomass concentration, which results in high harvest and de-watering process costs [4].

Four microalgae cultivation modes are currently used: autotrophy, heterotrophy, photoheterotrophy, and mixotrophy. Autotrophy is the most common, and open pond and closed photo bioreactor systems are the two primary autotrophic cultivation methods [5,6]. In autotrophic cultivation, microalgae produce biomass and oxygen by using sunlight as the energy source, CO_2 as the inorganic carbon source, and nitrogen, phosphate, and other inorganic salts as nutrients [7]. In heterotrophic cultivation, microalgae can grow without light and growth depends on the metabolism of organics for carbon source and energy [8]. The main carbon source of heterotrophy has been glucose; however, various cheap substitutes have been tested for microalgae cultivation including cellulose hydrolysis products [9], glycerol [10], acetate [11], and sweet sorghum and yams [11]. For photoheterotrophy cultivation, microalgae can grow under light when organic compounds are the carbon source. For mixotrophy cultivation, microalgae use both light and organics as energy sources and CO_2 and organic carbon as carbon sources [12].

The mixotrophy process, which combines characteristics of autotrophy and heterotrophy, has several advantages because it eliminates light requirements, produces greater microalgae growth, and is cost effective for biomass harvesting and substrate degradation [13,14]. With mixotrophy, microalgae can simultaneously assimilate inorganic and organic substrates via concurrent respiratory and photosynthesis processes [15]. Several studies have shown the advantages of mixotrophy over autotrophy and heterotrophy. Cultivation of *Chlorella vulgaris* under mixotrophic conditions significantly increased biomass compared with autotrophy and heterotrophy [16]. Other microalgae (*Nannochloropsis sp., Neochloris oleoabundans, Nannochloropsis salina, Chlorella protothecoides*) cultivated under mixotrophy conditions showed increased biomass compared with autotrophy or heterotrophy [17-20].

Photobiological hydrogen production has been studied under mixotrophic conditions using various organic carbon compounds as the energy source with hydrogen produced anaerobically. Microalgae tested for the production of hydrogen included as *Chlamydomonas reinhardtii* (acetate), *Chlorella vulgaris* MSU01 (glucose and sodium pyruvate), *Anabaena* sp. strain CH_3 (fructose and glucose), and *Anabaena variabilis* SPU 003 (mannose) [21-24]. To enhance hydrogen production from green microalgae, anaerobic conditions should be maintained under illumination; however, this is problematic because of oxygen production from growing photosynthetic cells. Among the suggested solutions to induce anoxia in photobioreactors under illumination, Degrenne et al. [21] found that use of acetate resulted in anoxic conditions and also accelerated the growth rate of *C. reinhardtii*. They proposed stoichiometric equations for biomass production in autotrophic and mixotrophic conditions (Eqs. (1) and (2), respectively).

$$CO_2 + 0.575H_2O + 0.0063H_2SO_4 + 0.181NH_3 + 0.0213H_3PO_4 \rightarrow CH_{1.771}O_{0.472}N_{0.181}S_{0.0063}P_{0.0213} + 1.107O_2 \quad (1)$$

$$C_2H_4O_2 + 0.321NH_3 + 0.011H_2SO_4 + 0.037H_3PO_4 + 0.035O_2 \rightarrow 1.775CH_{1.771}O_{0.472}N_{0.181}S_{0.0063}P_{0.0213} + 0.225CO_2 + 0.976H_20 \quad (2)$$

These equations illustrate that when CO_2 is used as a carbon source for growth, O_2 is produced by photosynthesis in autotrophic conditions. However, the O_2 is quickly consumed as $C_2H_4O_2$ is the organic carbon source in mixotrophic conditions. Thus, the use of acetate in mixotrophic conditions results in low DO that is a favored condition for the production of hydrogen and also accelerates the production of biomass.

In the present study, we cultivated the microalgal strain *Scenedesmus acuminatus* in Jaworski's medium (JM) enriched with ammonium acetate. We investigated the influence of acetate on biomass production and the role of nutrients such as nitrate, phosphate, and ammonium. Unlike previous studies using *Scenedesmus* sp. [25-28], we attempted to increase biomass production in a short cultivation period under mixotrophy mode using acetate as the organic carbon source. We also tried to determine both the optimum amount of acetate and duration in a cultivation reactor.

2. Literature review

2.1. Microalgae cultivation and harvesting technologies

2.1.1. Microalgae cultivation

During industrial production, microalgae are mainly cultivated in aqueous systems, and two major alternatives are usually used in microalgae cultivation: photo-bioreactors (PBRs) and raceway pond systems(RWPs).

Raceway pond systems(RWPs) is a kind of shallow artificial systems which is the most widely utilized industrial plant for outdoor algae cultivation. This pond likes an automotive raceway circuit which makes the water flow continuously around the circuit, in which algae and nutrients are pumped around by a motorised paddle. Microalgae production in RWPs seems to be most promising, especially in the large scale.[29] However, the attempts to grow these microalgae in RWPs have not always been successful due to biological and environmental factors' impact in the RWPs, such as solar radiation, bacteria contamination, medium pH, and so on. [29]

Photo-bioreactors (PBRs) is a kind of device that can be used for cultivating phototrophic microorganisms by using an illumination source.[29] PBRs have similar structures with general bioreactors, which can work either outdoors or indoors. Compare with RWPs, it's easier to control the light intensity, temperature, and nutrients in PBRs, thus, this system can enhance microalgae growth rate and the harvested biomass purity level dramatically. Besides, by using PBRs, it's easier to recycle wastewater and flue gas carbon dioxide as nutrients and carbon resources for microalgae growth.

Whereas PBRs still have some weaknesses in practical utilization, the main obstacle should be overcame is the high cost. Even though higher biomass concentration and better control of culture parameters in PBRs, some studies have shown that the productivity and production cost in some RWPs are better than those in PBRs.[34]

In addition, during the microalgae cultivation process, there are several factors can cause a big impact on microalgae growth rate, such as pH, temperature, illumination intensity. In Qitao Gong's study[31][46], he showed that Chlorella Vulgaris can arrive at the highest growth rate during the cultivation pH range between 10 and 10.5. Besides, carbon resources can enhance microalgae growth efficiently, for example, Sunjin Kim(2013) found that when using organic carbon as the carbon source, Chlorella's growth rate would be more than two times high than groups uses inorganic sources.[32]

2.1.2. Microalgae harvesting

To improve the economics of microalgal production process, it's essential for us to find a harvesting process with low cost, because the harvesting step usually takes up 20–30% costs of the biomass production processes [35][10]. The traditional microalgal harvesting methods include centrifugation, flocculation, gravity filtration, etc.[38]. Among these methods, flocculation allows large quantities of culture to be treated and easy operation[37]. Whereas, harvesting methods usually involve technical or economic weakness, such as cultural medium contamination, high cost, or low harvesting efficiency.

Flocculation is the process that makes the suspended particles in water or liquid become more substantial, or to form flocs, to accelerate the aggregation of particles and achieve the purpose of solid-liquid separation. It is widely applied water treatment, foods, or chemistry industries, and the common flocculants used in flocculation are alumni, ferrous sulfate, polyacrylamide, and so on[38]. The operation of flocculation is no energy cost, and it's fast and simple. Whereas flocculants might cause the microalgae biomass or cultural medium contamination, and the expenditure of flocculants is high.[38]

Gravity sedimentation is the method by using the effect of gravity to precipitate the target or major impurities in the solution in the form of an amorphous solid phase and then carried out the separation operation. Simple and the inexpensive method. This operation is the simplest and the most economical way but time-consuming and hard to get a high concentration of the algae cake[38]. Usually, to enhance the efficiency of

gravity effect, we carry out flocculation steps before sedimentation processes [39] [40].

Flotation is the method by using buoyancy effect, the target objects in a liquid float in the upper layer. Then we can separate the desired materials. This method is usually utilized in pollutants abatement in wastewater[41]. During microalgae harvesting processes, flotation is commonly applied with flocculation or coagulation method, then by colliding or adhesive bubble-particles, the agminated microalgae would float in the upper layer of the cultural mediums[41][42]. The main strengths of flotation are that it's applicable for large-scale utilization, short operation time, and low cost[38]. However, flotation process usually requires flocculants usage which might damage microalgae cellular composition, and this method isn't feasible in marine condition[38].

Filtration is the operation to separate microalgae from cultural solutions under the action of propelling force or other external forces. This method has high recovery efficiency and can separate sensitive species available, but the filter membranes should be regularly cleaned or replaced, and the replacement of membranes should be costly[38].

Centrifugation is the method that requires a dominant centrifugal force that enables the suspended particles in the rotating body to settle or float so that some particles can be concentrated or separated from other particles. It can be used to harvest most of the microalgae species, and its the most efficient harvesting method[43]. Whereas, it's also the most expensive method to harvest microalgae for the high energy consumption. Besides, due to the shear forces, the microalgae cell might be damaged during the centrifugation process[44].

Polyglutamic acid is a water-soluble polyamino acid produced by microbial fermentation in nature. And it is a high molecular polymer in which amounts of glutamic acid units form peptide bonds through an α-amino group and a γ-carboxyl. Polyglutamic acid has a wide range of usage from food and medicine to water treatment. In this study, we mainly pay attention to the physical characters of polyglutamic acid which served as coagulator to gather microalgal cells to make it easy to be collected.

3. Materials and methods

3.1. Culture mediums and inoculum

S. acuminatus (LIMS-PS-1973) from Lake Andongho, Korea, was obtained from the Korea Marine Microalgae Culture Center (KMMCC, Pusan, Korea). *S. acuminatus* was routinely maintained at 20^0C in 250-mL Erlenmeyer flasks containing 100 mL of JM was kept in an incubator under continuous illumination (2,700 *lux*). Microalgae were grown on JM: $Ca(NO_3)_2 \cdot 4H_2O$ (20 mg/L), KH_2PO_4 (12.4 mg/L), $MgSO_4 \cdot 7H_2O$ (50 mg/L), $NaHCO_3$ (15.9 mg/L), $Na_2HPO_4 \cdot 12H_20$ (36 mg/L), $NaNO_3$ (80 mg/L), EDTA FeNa (2.25 mg/L), $EDTANa_2$ (2.25 mg/L), H_3BO_3 (2.48 mg/L), $MnCl_2 \cdot 4H_2O$ (1.39 mg/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.00 mg/L), cyanobalamin (0.04 mg/L), thiamine HCl (0.04 mg/L), and biotin (0.04 mg/L). Other culture media (modified JM) were prepared by increasing the quantities of all chemicals containing NO_3^- and PO_4^{3-} to determine the effects of high concentrations of NO_3^- and PO_4^{3-}. Microalgae were also cultivated under different concentrations of ammonium acetate (NH_4AC), which was prepared in JM. The seed culture was prepared using 250-mL Erlenmeyer flasks at a constant temperature of 23^0C in an illuminated incubator (model HB-201S; Han-Baek Scientific, Puchon, Korea). All media and cultivation apparatus were sterilized with steam at 121^0C, 0.11 MPa for 15 min.

3.2. Experimental design

3.2.1. Microalgal cultivation

Photobioreactor system was prepared in a dark room to cultivate Senedesmus acuminatus (LIMS-PS-1973) under LEDs illumination, which were purchased from S-tech Inc. (Ilsan, Kyongi, Korea), and these LEDs were powered by DC power supplies (Whawoo Tech. Co., Ilsan, Korea). Each LED unit was installed on top of the reactor to ensure the light can illuminate from the top to the bottom of the reactor. We measured the light intensity by using a silicon photocell (model 0560, Testoterm GmbH & Co., Germany) and a quantum sensor (model MQ-306, Apogee Instruments, Ilsan, Korea). Also, the light intensity is 3540 lux, and the cultural temperature is 20±1 ℃. During the research, the cultural medium used is JW medium or the modified JW medium (with a higher concentration of nitrate and phosphate). The schematic diagram of photo-bioreactor illuminated by LEDs was shown in Fig. 1.

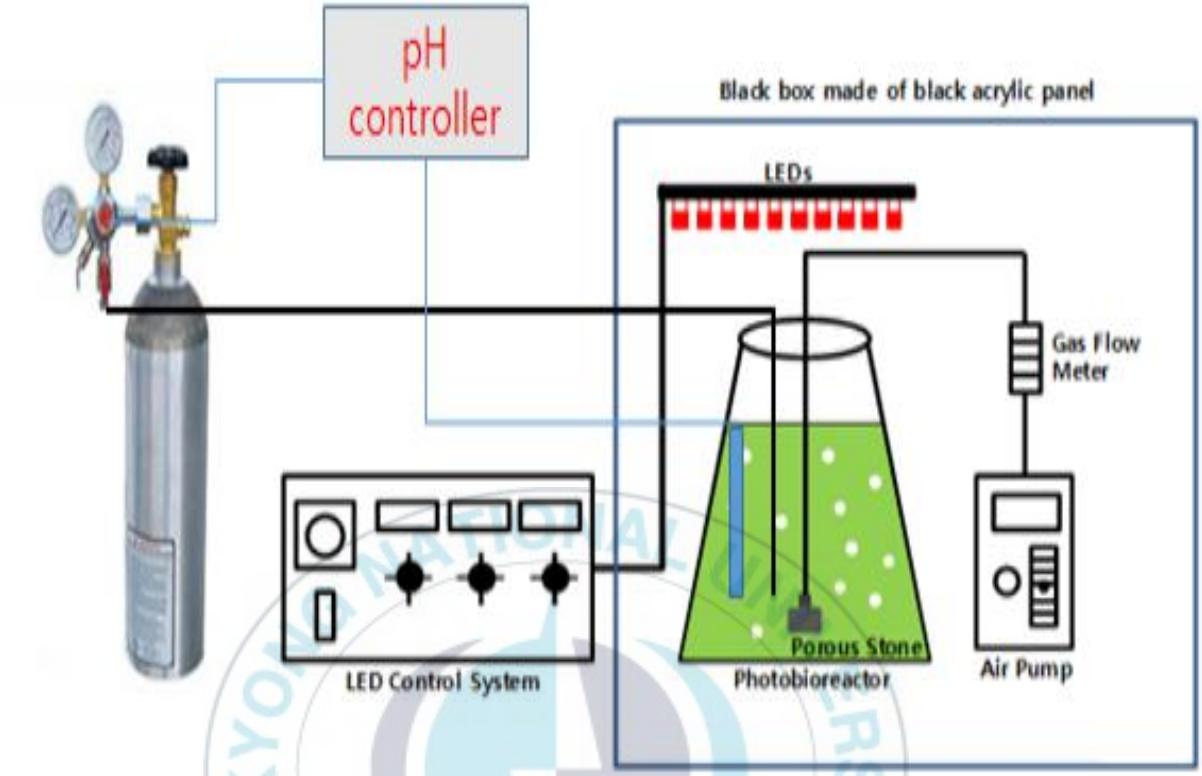

Fig. 1. Schematic diagram of photo-reactor illuminated by LEDsSchematic diagram of photo-reactor illuminated by LED

3.2.1.1. The impact of aeration on microalgal cultivation

To determine optimum aeration rate for autotrophic cultivation, cultures in 500 mL flasks were aerated continuously with three different flow rates of volume gas/ volume medium/ min (vvm) or no aeration. The aeration rates were 0.2 vvm, 0.72 vvm, and 1.28 vvm respectively. Each reactor was set under the same cultural condition described above. The details of the cultural parameters were showed in Table 1.

3.2.1.2. Microalgal cultivation with the different input of organic carbon quantity

To investigate the effect of using ammonium acetate as additional carbon resource on the growth of Scenedesmus, five 100 mL of flasks were inoculated with a dose of Scenedesmus and ammonium acetate. There was no air supply in the cultural systems, and the initial cultural acetate concentrations were adjusted to 0, 0.01, 0.02, 0.03, and 0.04 mM, respectively. And we adjusted the initial cell biomass concentration in the five groups to 0.057g/L, 0.055 g/L, 0.055 g/L, 0.059 g/L, and 0.055 g/L, respectively. The cultural solution used in this test is JW medium. The details of the cultural parameters were showed in Table 1.

Table 1. Test condition, maximum specific growth rate, maximum biomass productivity, maximum cell concentration under different cultivation settings.

Test set	vvm[1)]	IB[2)] (g/L)	Acetate[3)]	μ_{max} (d^{-1})	P_{max} (g L^{-1} d^{-1})	MB[4)] (g/L)	CT[5)]
Aeration	0	0.018	n/a	0.11	0.015	0.33	Auto[7)]
	0.2	0.017	n/a	0.31	0.22	3.62	Auto[7)]
	0.72	0.018	n/a	0.351	0.317	5.41	Auto[7)]
	1.28	0.017	n/a	0.352	0.319	5.38	Auto[7)]
Acetate doses	0	0.057	0	0.076	0.012	0.13	Mixo[6)]
	0	0.055	10	0.288	0.184	1.15	Mixo[6)]
	0	0.055	20	0.205	0.138	0.88	Mixo[6)]
	0	0.059	30	0.204	0.138	0.88	Mixo[6)]
	0	0.055	40	0.290	0.159	1.00	Mixo[6)]
pH control/ DO control	0	0.219	0	0.162	0.102	1.17	Auto[7)], CO_2 input for pH control
	0.34	0.208	0	0.179	0.127	1.53	Auto[7)], Aeration
	0	0.204	0	0.071	0.019	0.46	Auto[7)], DO less than 3 mg/L
	0	0.221	0	0.057	0.017	0.42	Auto[7)], Control test

[1)] Air flow rate, [2)] Initial biomass concentration, [3)] Acetate dosage (Mm), [4)] Maximum biomass concentration, [5)] Cultivation type, [6)] Mixotrophic, [7)] Autotrophic

3.2.1.3. The research of the difference of the impact of pH control, aeration, and organic carbon resource in microalgal cultivation processes

We set four same reactors with 3.0 L volume to cultivate Scenedesmus under the basic condition with different parameters. For the first reactor, we set an automated CO_2 regulator to adjust the solution pH value less than 7.52. The second reactor was installed an air pump to aerate the culture medium with air flow rate at 0.34 vvm. For the third reactor, we set an automated N_2 regulator to control the solution DO concentration less than 3.0 mg/L. The fourth reactor was the control without any aeration. The culture medium used in this test was JW medium, and the initial cell biomass concentration in the four reactors were 0.219 g/L, 0.208 g/L, 0.204 g/L, and 0.221 g/L, respectively. The details were shown in Table 1.

3.2.1.4. The research of the difference of the impact of pH control, aeration, and organic carbon resource in microalgal cultivation processes

Three 3,000 mL of beakers were used to set up three tests for Senedesmus cultivation, and JW medium was used as the initial cultural medium of this test. The first beaker was set with an automated pH controller by using CO_2 regulator to ensure the solution pH was under 7.50 (AC test). The second beaker was used to cultivate Scenedesmus by using JW medium under air flow rate at 0.51 vvm (AA test). The third beaker was used as a control containing only Scenedesmus and JW medium.

This study was divided into two periods, the period 1(0-240 h) exhibit the different impacts of pH controlling and aeration in microalgal growth processes, besides, we did fifty percent dilution by taking out 1500 mL algae solution from the second

reactor and input 1500 JW medium at time 156 h due to nitrites' insufficiency; the period 2(240-312 h) showed the different impacts of using acetate as additional carbon resource in reactor 1 and reactor 2. At the begin of period 2 we did fifty percent dilution by taking out 1,500 mL algae solution and input 1,500 mL modified JW medium with higher concentration of nitrates and phosphates for both of the aeration reactor and pH controlling reactor, then, we input 2.87g ammonium acetate into the first and second reactor at the same time of the begin of period 2. In addition, we did not change the cultivation condition for the control group during this study. The details of the cultural condition were showed in table. 2.

Table 2. Comparison of maximum specific growth rate and biomass productivity of Scenedesmus microalgae cultivated under different test conditions and cultivation periods.

Tests	μ_{max} (d^{-1})		P_{max} (g L^{-1} d^{-1})	MB[1)]	NRE (mg L^{-1} d^{-1})[2)]	PRE (mg L^{-1} d^{-1}) [3)]	Cultivation type
pH control (AA) pH<7.50	period 1: 0-240 hrs	0.25	0.162	1.76	8.19	0.32	Autotrophic
	period 2: 240-312 hrs	0.46	0.936	3.75	198.62	1.6	Mixotrophic, Ammonium acetate input after 50% dilution at 240 h
Aeration (AC) vvm=0.51	period 1: 0-240 hrs	0.28	0.222	1.80	11.19	0.60	Autotrophic, 50% dilution at 165 h
	Period 2: 240-312 hrs	0.41	0.662	2.80	15.50	4.3	Mixotrophic, Ammonium acetate input after 50% dilution at 240 h
Control		0.10	0.030	0.53	0.095	0.39	Autotrophic

1) Maximum biomass concentration, 2) Nitrate removal efficiency, 3) Phosphate removal efficiency

3.2.1.5. A study of Scenedesmus growth characteristics under Semi-continuous cultivation

In this research, a 2, 000 mL of the beaker was set as the photobioreactor to cultivate Scenedesmus by using JW medium as the initial cultural solution. An air pump was installed inside the reactor at the begin of the test to control the air flow rate at 0.89 vvm. And the initial cell biomass concentration was adjusted to 0.19 g/L in the reactor. This test was divided into three periods, the first period (0-504 h) exhibited cell's growth characteristics under the aeration condition and aeration condition with using ammonium acetate as additional carbon resource in conventional JW medium; The second period(504-1080 h) showed the cells growth characteristics under the cultural conditions with limited magnesium; The third period (1080-1360 h) exhibited the cells growth characteristics under the cultural condition without aeration, and used an automated pH controller to adjust the artistic medium pH lower than 7.7, in addition, we input ammonium acetate as additional carbon resource and magnesium sulphate in the reactor. The details of the cultural condition were showed in table 3.

Table 3. The research of the difference of the impact of pH, aeration, and organic carbon resource in microalgal different cultivation periods

Period 1 0-504 h	195.5	0.89	×	0.19	×	0.154	0.171	3.62	25.98	3.52	Mixo[6], start with JW medium
	195.5	0.89	×		×				29.07		Mixo[6], Nitrates input
	254	0.89	×		×				23.51		Mixo[6]), Nitrates input
	400	0.89	×		×				15.54	5.84	Mixo[6]), Nitrates and phosphate input
Period 2 504 -1080 h	504-697	0.89	×	3.22	√	0.069	0.141	3.78	2.43	5.22	Auto[7], Ammonium acetate, nitrates and phosphate input after 10% dilution without inputting magnesium
	697-838	0.89	×	1.70	√			2.02	5.76	5.2	Auto[7], Ammonium acetate, nitrates and phosphate input after 50% dilution without inputting magnesium
	838-1080	0.89	×	0.45	√			0.537	2.95	1.14	Auto[7], Ammonium acetate, nitrates and phosphate input after 90% dilution without inputting magnesium
	890-1080	0	7.7		√						Auto[7], stop aeration at 890h, install pH controller at 1034
Period 3 1080-1360 h	1080	0	7.7	0.49	√	0.250	0.512	6.28	70	6.1	Auto[7], Magnesium input
	1130-1360	0	7.7		√						Auto[7], Ammonium acetate, nitrates and phosphate input with inputting magnesium

[1] Air flow rate, [2] Initial biomass concentration, [3] Acetate dosage (mM), [4] Maximum biomass concentration, [5] Cultivation type, [6] Mixotrophic, [7] Autotrophic

3.2.2. Microalgal harvesting by using Poly-γ-glutamic acid(PGA)

In this part, we use the different amount of PGA to harvest Scenedesmus with different concentration of initial Scenedesmus solution in 100 ml beakers. There were five groups named H1, H2, H3, H4, and H5. Each beaker contained 100 microalgal solutions, and the initial biomass concentration of the five groups was 0.292g/L, 0.337g/L, 0.660g/L, 0.892g/L, and 1.121g/L, respectively. For group H1, five beakers were used, and we adjusted each beaker contained PGA 0.25, 0.50, 0.75, 1.00, 2.50 g/L, respectively; For group H2, we set five beakers, each carried the PGA solution in the concentration of 0.30, 0.50, 0.75, 1.00, 2.50 g/L; For group H3, five beakers with PGA concentration of 0.5, 1.0, 1.5, 2.0, 5.0 g/L were used; For group H4, six beakers were used, the PGA concentration of each beaker were 0.5, 1.0, 1.5, 2.0, 5.0, 10 g/L, respectively; For group H5, we used six beakers with adjusting the PGA concentration of 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 g/L. The details of the tests' parameters were showed in table 4. Then, we used a glass stirrer to mix the microalgae solution with PGA and waited about 5 minutes until the Scedesmus cells gathered to clots, after, we took out the clots from the beakers and measured the left microalgae biomass concentration in these beakers by using OD meter. Then we calculated the biomass harvesting efficiency by using the equation (4).

Table 4. The parameters of microalgal harvesting test by using PGA

Group	Initial biomass concentration (g/L)	PGA concentration (g/L)	Removal rate (%)
1	0.292	0.25	38.00
		0.50	57.91
		0.75	79.80
		1.00	77.82
		2.50	76.68
2	0.337	0.30	40.19
		0.50	57.32
		0.75	74.45
		1.00	84.99
		2.50	77.74
3	0.660	0.5	63.35
		1.0	74.13
		1.5	89.63
		2.0	92.66
		5	89.63

Group	Initial biomass concentration (g/L)	PGA concentration (g/L)	Removeal rate (%)
4	0.899	0.5	65.92
		1.0	86.87
		1.5	90.93
		2.0	91.52
		5.0	82.07
		10.0	68.52
5	1.121	1.0	89.16
		1.5	92.97
		2.0	94.30
		3.0	94.87
		4.0	93.28
		6.0	91.54

3.3. Analytical methods

3.3.1. Sampling

Each time we take out 5 ml of samples from the cultural medium below the water surface in the bioreactor, and a 0.45-μm Teflon syringe filter was used to filter all samples before the chemical measurement. Solution pH, ORP, and DO were measured using a pH/conductivity meter after filtration.

3.3.2. Growth rate and biomass

The growth curve is a curve describing the growth of a single batch of microalgae by combining the light absorbance and dry weight of the microalgal solution. Through the growth curve, we can directly monitor the dry weight of microalgae and facilitate the progress of experiments. The light absorbance was measured by using ultraviolet spectroscopy under the light at 680 nm. The OD680 values were converted to dry cell weight (DCW) concentration (g/L), based on a linear relationship between OD680 and dry cell weight, which was obtained after multiple data analysis. Maximum biomass productivity (Pmax, $g\ L^{-1}\ d^{-1}$) was calculated from Eq. (1), where C_0 is the initial biomass concentration (g/L) at t_0 (day) and C_t was the biomass concentration (g/L) at the end of the cultivation period (te).

$$P_{\max} = \frac{(C_t - C_0)}{(t_e - t_0)} \qquad (1)$$

Specific growth rate (μmax, d^{-1}) was calculated from Eq. (2), where C_1 (g/L) and C_2 (g/L) are the biomass concentration at the beginning (t_1) and at the end (t_2) of the exponential growth phase.

$$\mu_{max} = \frac{(\ln C_2 - \ln C_1)}{(t_2 - t_1)} \quad (2)$$

3.3.3. Nutrients removal efficiency

The concentrations of phosphorus, nitrate, and ammonium during cultivation were measured by a DR 900 Colorimeter (HACH, USA). A 0.45-μm Teflon syringe filter was used to filter all samples before measurement. Solution pH, oxidation reduction potential (ORP), and dissolved oxygen (DO) were measured using a PC510 bench pH/conductivity meter (Shimadzu, Kyoto, Japan) after filtration. And the nutrients removal rate (X, mg/day) was calculated from Eq. (3), where C_b is the nutrients concentration at time b and Ca is the nutrients concentration at the time a.

$$X = \frac{(C_b - C_a)}{(t_b - t_a)} \quad , \quad (3)$$

3.3.4. pH, DO, ORP detection

To have a better understanding of the microalgae growth condition, we measure pH, DO, ORP third per day by using the pH, ORP meter(EUTECH, Singapore), and DO meter (Thermo Meter, Singapore). Each value was measured 2 times to calculate the average value.

3.3.5. Microalgae harvesting efficiency

The microalgae harvesting efficiency E was calculated by the Eq. (4), where the C_0 is the initial microalgae biomass concentration (g/L), The C_t is the microalgae biomass concentration after being added to the PGA.

$$E = \frac{(C_0 - C_t)}{C_0} \quad (4)$$

4. Results and discussion

4.1. Biomass productivity under basic cultural conditions

The trail test's growth characteristics, specific growth rate, maximum biomass productivity, and maximum concentration at each experimental condition are summarized in Table 1.

4.1.1. The impact of aeration rate on microalgal cultivation

The microalgae growth stagnation phase was exhibited about 40 h and then arrived at the exponential growth periods during the next 280 h (Fig. 2). Biomass productivity (Pmax) and Specific growth rate (μmax) are summarized in Table 1. And the Pmax (0.319 $g\ L^{-1}\ d^{-1}$) and μmax (0.352 d^{-1}) were obtained from 1.28 vvm aeration test. In addition, the Pmax and μmax in 0.78 vvm test were 0.317 $g\ L^{-1}\ d^{-1}$ and 0.351 d^{-1} respectively, which is similar as the result in the 1.28 vvm test. As for 0.22 vvm test, both specific growth rate and maximum biomass productivity were obviously lower than those in 0.72 vvm test, but they are clearly higher than those in the control test. Thus, we conclude that air aeration has sufficient impact on the growth of microalgae, but it's not necessary for air flow rate higher than 0.72 vvm. Similar results were reported for the cultivation of Anabaena azollae in our previous research, where 0.54 vvm was most effective for the cells growth and greater air flow rate at 1.08 vvm resulted in similar growth rate as 0.54 vvm (Hong et al., 2017).

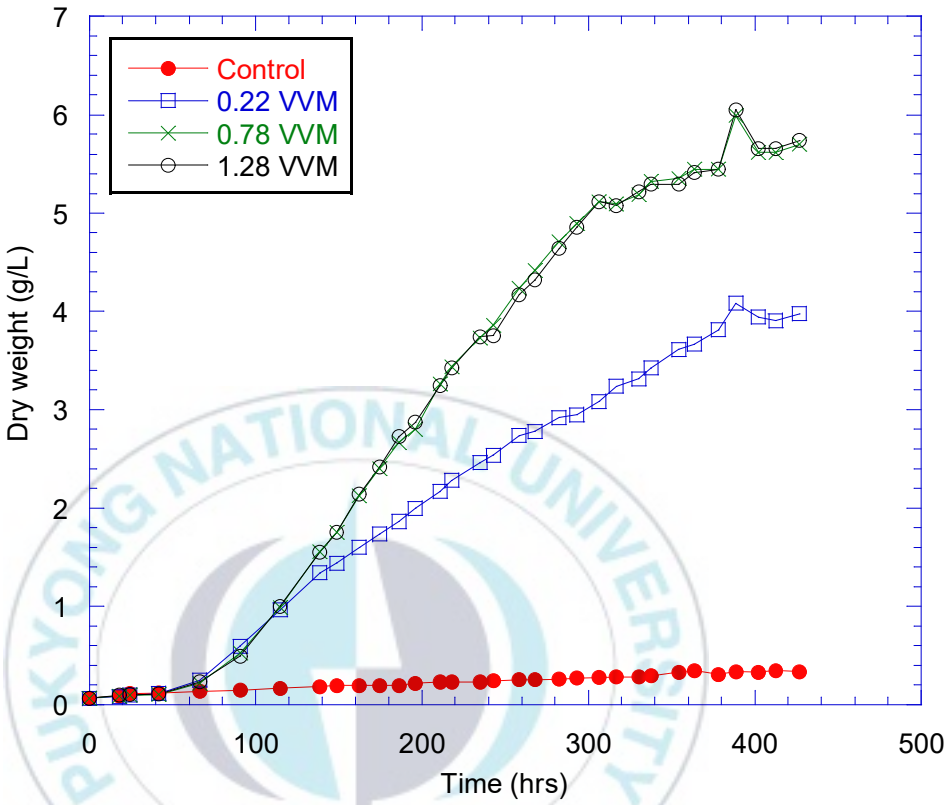

Fig. 2. Growth curves of Senedesmus in a photobioreactor grown under different air flow rates. All photobioreactors were illuminated by white LEDs at the light intensity of 38 µmol m^{-2} s^{-1}.

4.1.2. Microalgal cultivation with the different input of organic carbon quantity

Cells exhibited stagnation phase about 10 h, and then there was notable growth for acetate-grown cells during the next 140 h (Fig. 3). μ_{max} and Pmax are summarized in Table 1. The greatest μ_{max} (0.288 d^{-1}) and Pmax (0.184 $g\ L^{-1}\ d^{-1}$) were obtained for 10 mM acetate test. Other tests showed similar μmax and Pmax, but μmax of acetate tests was more than two times greater than that of the acetate-free test. For Pmax, acetate tests showed 10 times greater value than that of the acetate-free test. Therefore, 10 mM acetate in the culture medium was the optimum concentration for cell growth among the acetate concentration tested. Besides, the maximum biomass concentration of 1.15 g/L was obtained in the 10 mM acetate test, which is about 10 times higher than that in the acetate-free test. Similarly, the previous study has shown that Chlamydomonas was successfully cultured under a mixotrophic condition at acetate concentration of 17 mM (Degrenne et al., 2010; Jurado-Oller et al., 2015).

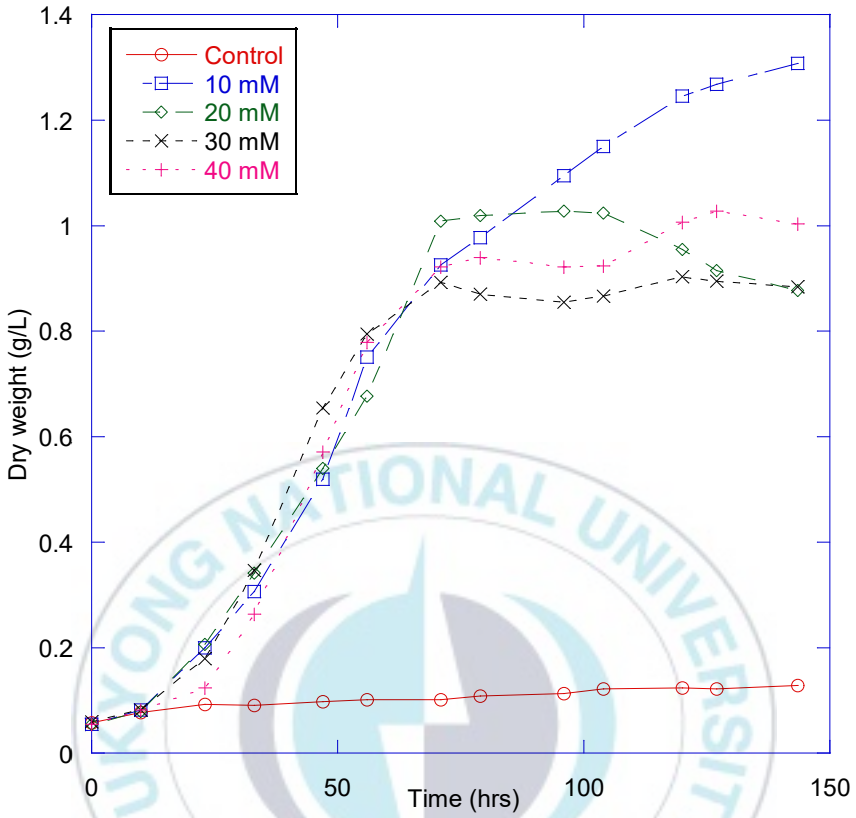

Fig. 3. Growth curves of Senedesmus in a photobioreactor grown under different acetate concentrations.

4.1.3 Investing the different impact of dissolved oxygen, solution pH by aerating with CO_2, N_2, and air in the cultural mediums

Biomass concentration, DO, pH, and ORP are shown in Fig. 4. 5,6,7, and growth parameters are summarized in Table 1. Aeration test showed the greatest μmax (0.179 d^{-1}) and Pmax (0.127 $g\ L^{-1}\ d^{-1}$). The values were lower than those of other aeration tests. It might because of the difference of reactor size. pH control test using CO_2 input showed slightly lower μmax (0.162 d^{-1}) and Pmax (0.102 $g\ L^{-1}\ d^{-1}$) than the aeration test. DO controlling test and control showed the lowest but similar μmax (0.071 d^{-1}, 0.057 d^{-1}) and Pmax(0.019 $g\ L^{-1}\ d^{-1}$, 0.017 $g\ L^{-1}\ d^{-1}$), respectively.

Dissolved oxygen is an important parameter for the mixotrophic cultivation, and it is produced by cells during the photosynthetic process, the process was shown in the Eq. (5). As Scenedesmus growth, dissolved oxygen concentration increased quickly until the maximum DO value of 16 mg/L at the pH controlling reactor. On the contrast, the DO value keeps almost constant in the aeration reactor about 7.5 mg/L, that is because of the aeration equipment not only strip some dissolved oxygen out of algae solution but also supply oxygen from the air into the reactor. Thus, there was a balance between the stripped oxygen and the inputted oxygen, which lead to the constant dissolved oxygen concentration in the aeration reactor. To verify the effect of DO on the growth of cells, a dissolved oxygen controlling equipment was installed on the third reactor to adjust DO value less than 3 mg/L by aerating N_2 gas in the reactor, and the result shows that only removing dissolved oxygen does not affect the growth of cells at autotrophic cultivation condition. For the control test, DO concentration increased quickly at the begin of the test due to the increase of cells at the beginning of cultivation and then slowly decreased as cells concentration stop increasing.

$$6CO_2 + 6H_2O \xrightarrow{\text{light energy}} C_6H_{12}O_6 + 6O_2 \qquad \text{Eq. (5)}$$

Even though, DO plays an important role in microalgae cultivation processes. Because dissolved oxygen is produced by microalgae during the photosynthetic process when CO_2 is served as carbon source for microalgal growth under light illumination. But DO can be quickly removed during the assimilation of organic carbon (Degrenne et al., 2010; Qiao et al., 2012). Thus, the high DO value in cultural mediums presents that the photosynthetic process dominates, and the low DO value in the algal solution exhibit the assimilation process is prevalent. Therefore, study the DO concentration changes can help us have a better understanding of the microalgal growth and find the optimum cultivation methods.

During this cultivation process, the ORP range of the algal solution is between 216 mV and 260 mV, and the pH range of the algae solution is between 7.0 and 8.1. The details show in the Fig. 5 and Fig. 7.

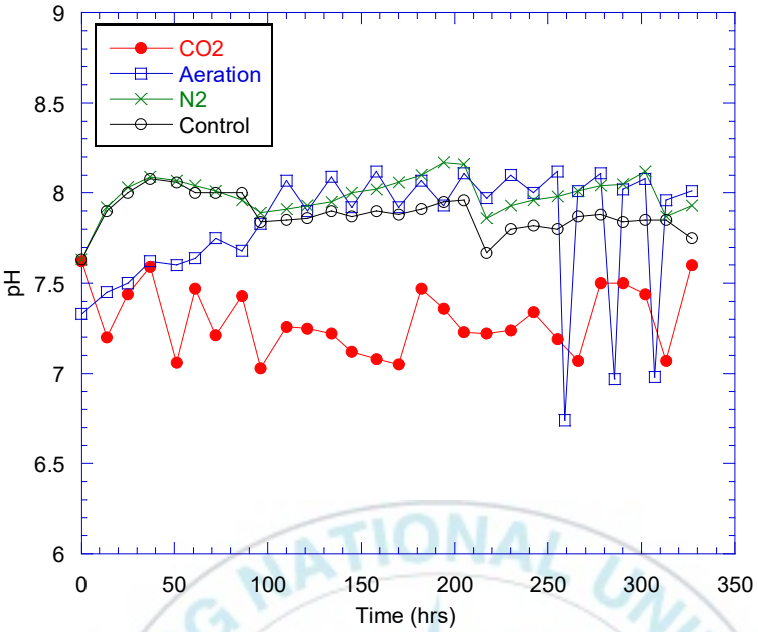

Fig. 4. pH changes curves of Scenedesmus cultivation under pH control, aeration, and DO control conditions.

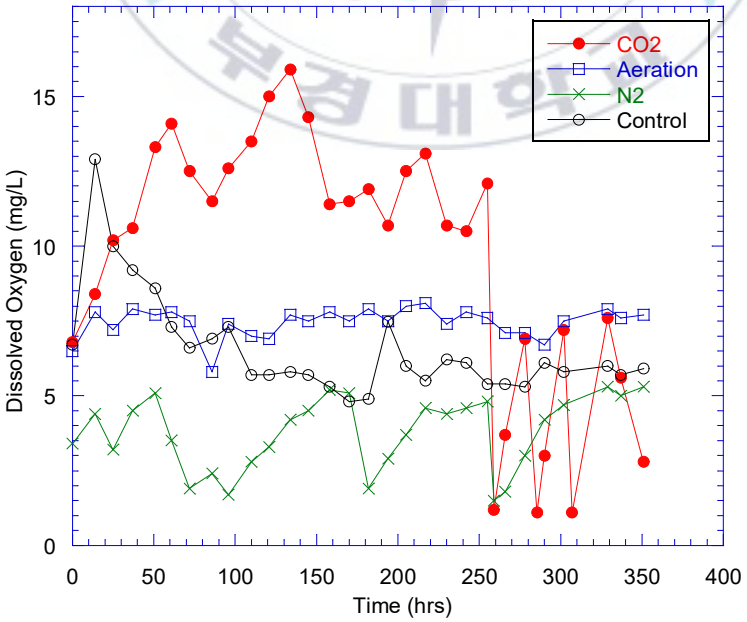

Fig. 5. DO changes curves of Scenedesmus cultivation under pH control, aeration, and DO controlling conditions

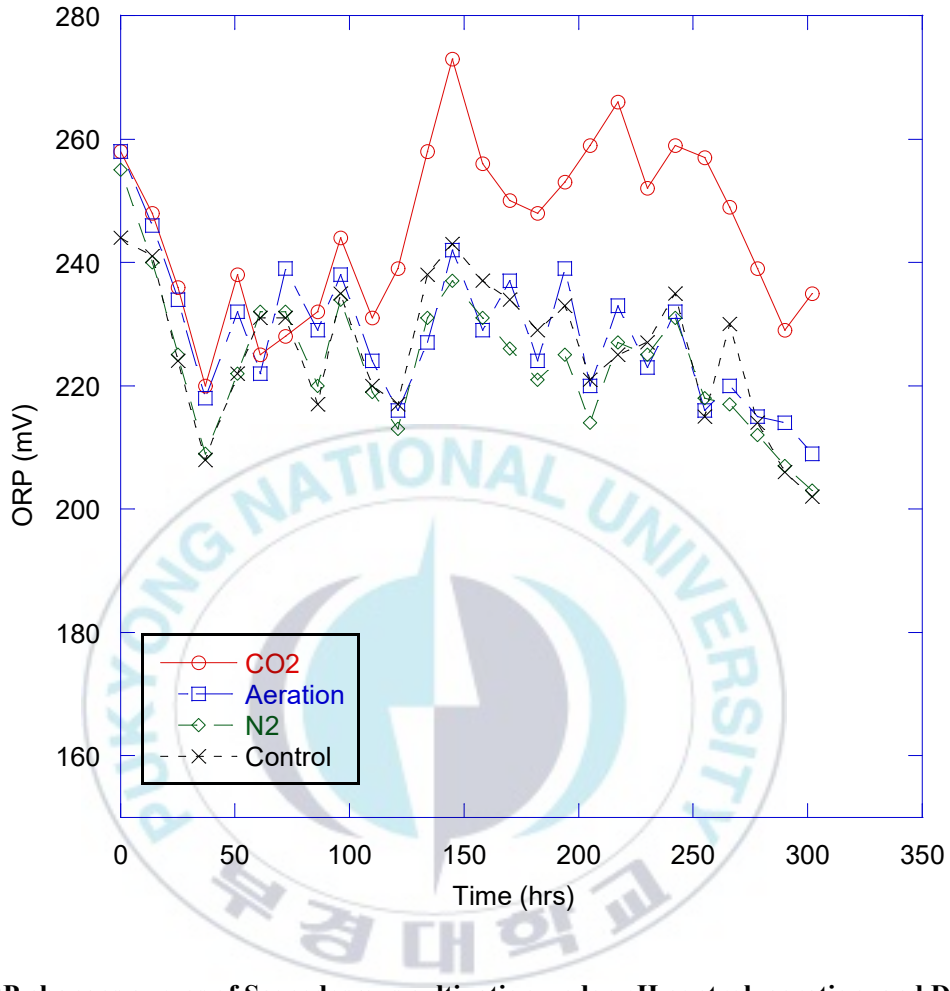

Fig. 6. ORP changes curves of Scenedesmus cultivation under pH control, aeration, and DO controlling conditions.

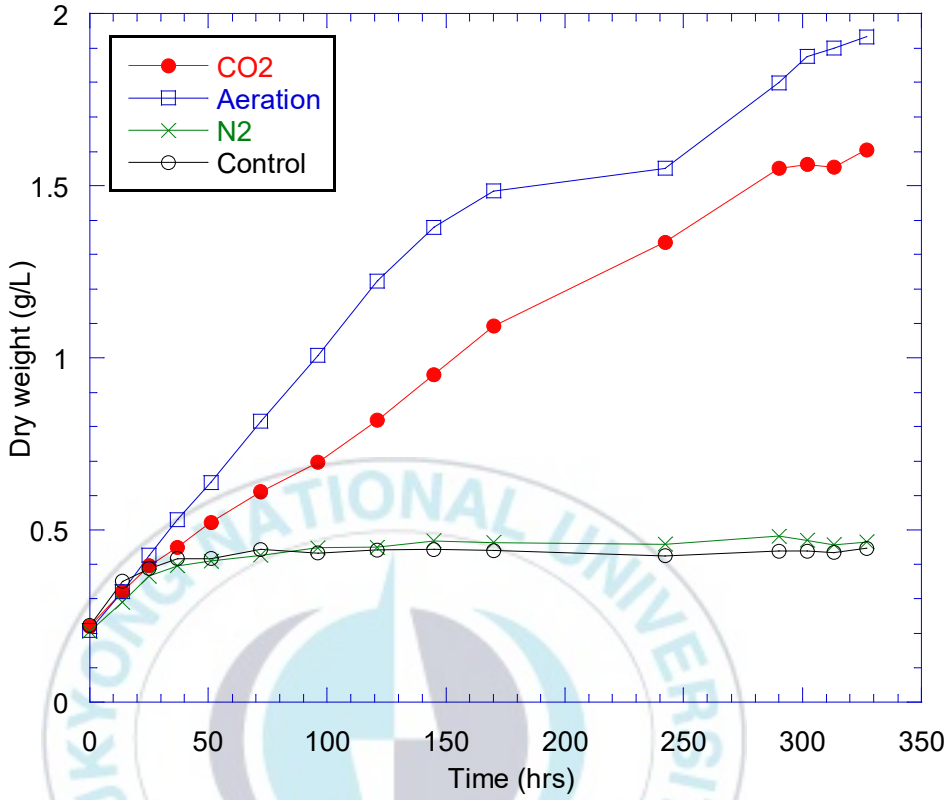

Fig .7. Growth curves of Senedesmus in photobioreactors grown under pH control, aeration, and DO controlling conditions.

4.2. Microalgal growth characteristics and nutrients removal efficiency under the specific cultivation conditions

4.2.1. The research of the difference of the impact of pH control, aeration, and organic carbon resource in microalgal cultivation processes

4.2.1.1. Biomass concentration and growth parameters

During the two cultivation periods of the three reactors, the control test showed the lowset μmax and Pmax was (0.102 d^{-1}) and (0.030 $g\ L^{-1}\ d^{-1}$), respectively. On the contrast, the other two tests showed faster microalgae growth rate compared to the control test on both the period 1 and period 2. Besides, after we input ammonium acetate to the first two group (AC, and AA) at beginning of period 2, the AC group showed the fastest growth rate, and the DO value decreased to almost 0 (explained later). The details of the growth parameters are displayed in table 2.

Among the two tests during the first period without inputting ammonium acetate except for the control test, AA showed higher μmax (0.28 d^{-1}) and Pmax (0.222 $g\ L^{-1}\ d^{-1}$) during the period 1, and AC test showed the lower μmax (0.25 d^{-1}) and Pmax (0.162 $g\ L^{-1}\ d^{-1}$) during this period compared with AA. When ammonium acetate was inputted in the AC and AA reactor at the begin of period 2, the microalgae in both of the two reactors grows faster than in the previous cultivation stage. And the growth data for AC and AA are(μmax, 0.46 d^{-1}, Pmax 0.936 $g\ L^{-1}\ d^{-1}$) and (μmax, 0.41 d^{-1}, Pmax 0.662 $g\ L^{-1}\ d^{-1}$), respectively. Previous studies for Senedesmus obliquus showed that μmax ranged from 0.22 to 0.33 d^{-1} and Pmax from 0.056 to 0.293 $g\ L^{-1}\ d^{-1}$ under autotrophic

cultivation with CO_2 input (Tang et al., 2011). Our results were slightly lower for μmax but much greater for Pmax than literature values.

4.2.1.2. pH, DO, ORP changes under the two periods

During the Senedesmus growth processes, cultural solution pH usually increases due to the usage of HCO_3^- by the photosynthetic reaction. From the Fig.8, we realized that the pH value in the AC reactor and the control increased dramatically during the first 30 hrs. For the control test, the pH value reached at 10.2. But the pH value arrived at lower than 9.3 in the AC reactor, and the pH value in the AC reactor increased slower than in the control, that is because of the air bubbles in the AA reactor supply CO_2 to the cultural solution.

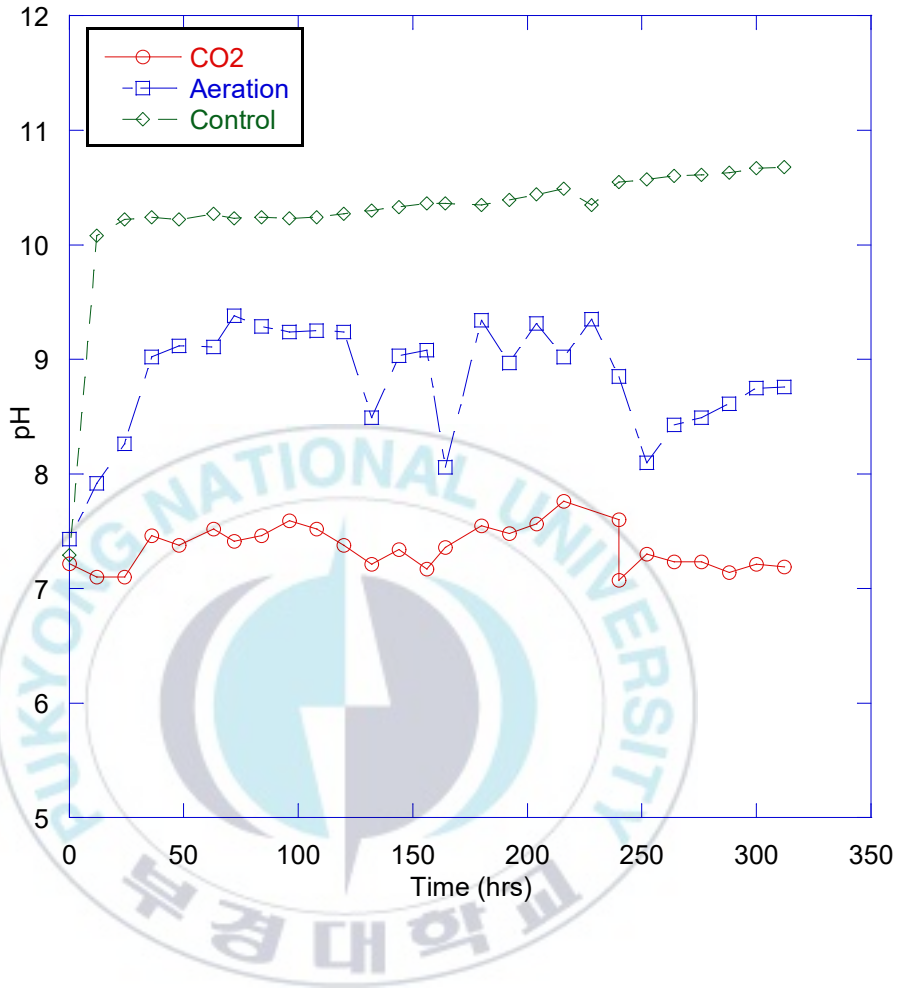

Fig. 8. pH changes curves of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

Dissolved oxygen can be produced by the cell's photosynthesis processes, from the Fig. 9. we realized that the DO value in the reactors increased quickly at the begin as the cell concentration increased besides the aeration reactor. For the control test, at the time 24 h, the DO concentration reached the maximum (15.5 mg/L) and began to decrease, which keeps in line with the fact that the cell concentration increased quickly at the first 24 hours, and then keeps stable. The DO value in the AA group remain almost constant, that is because even though AA reactor showed highly cell concentration growth, the air bubbles in this reactor might remove the redundant oxygen in the reactor. Thus the DO concentration in the AA reactor kept the balance. Besides, the DO in the AC reactor always stay in the highest concentration during the period 1, that's because the cells in the AC reactor kept high growth rate among the period 1. After we input ammonium acetate into the AC and AA reactor, we realized that the DO value decreased quickly to almost 0 in the AC reactor, that is because even though CO_2 can be used as carbon resource for photosynthesis and produce O2, O2 can be quickly consumed under the mixotrophic condition to synthesize biomass. The Eqs. (5) and (6) explains this process. However, the DO value in the AA reactor still keeps almost constant, that's due to the aeration makes the DO balance.

$$CO_2 + 0.575H_2O + 0.0063H_2SO_4 + 0.181NH_3 + 0.0213H_3PO_4 \rightarrow CH_{1.771}O_{0.472}N_{0.181}S_{0.0063}P_{0.0213} + 1.107O_2 \quad (5)$$

$$C_2H_4O_2 + 0.321NH_3 + 0.011H_2SO_4 + 0.037H_3PO_4 + 0.035O_2 \rightarrow 1.775CH_{1.771}O_{0.472}N_{0.181}S_{0.0063}P_{0.0213} + 0.225CO_2 + 0.976H_20 \quad (6)$$

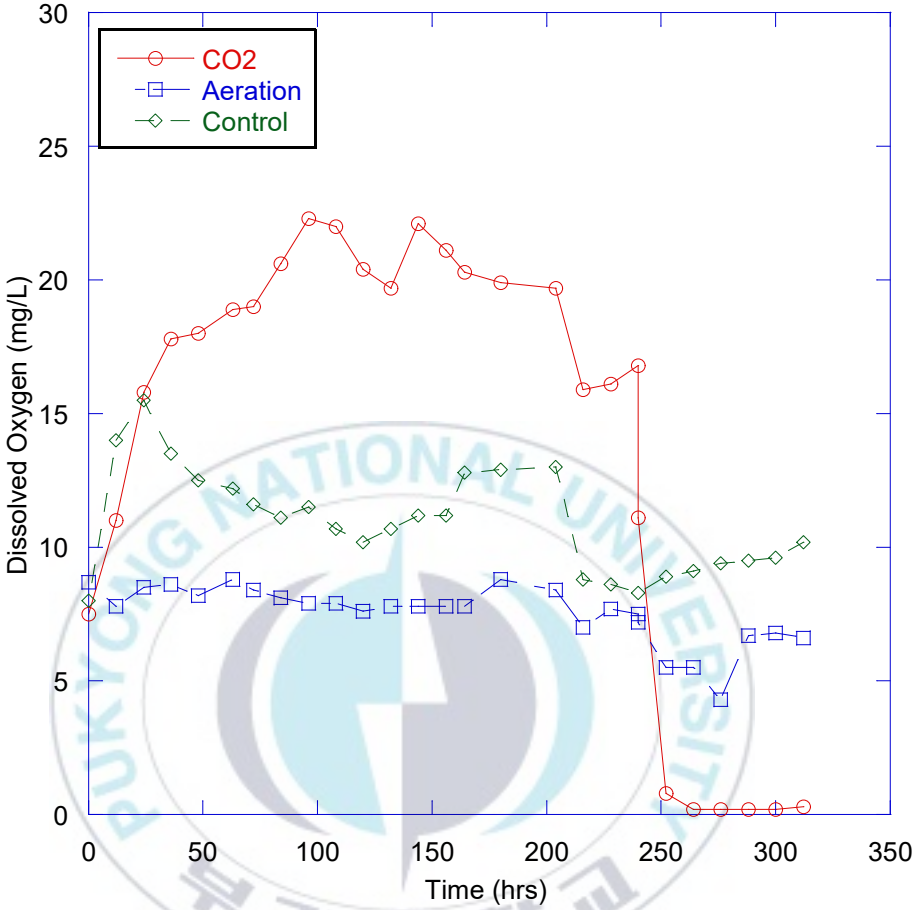

Fig. 9. DO changes curves of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

ORP values for three tests are shown in Fig. 10. During the period 1, the ORP value keeps constant as the DO value, and the ORP in the AA reactor is higher than AC and control reactor as the DO value, for the AA and control test, the ORP value maintained around 160 mV, and the ORP in the AC reactor maintained around 210 mV. However, during the period 2, when the DO value decreased to almost 0 in the AC reactor, the ORP value in the AC test also dropped to around -500 mV. In conclusion, the fluctuation of ORP keeps in line with the DO value.

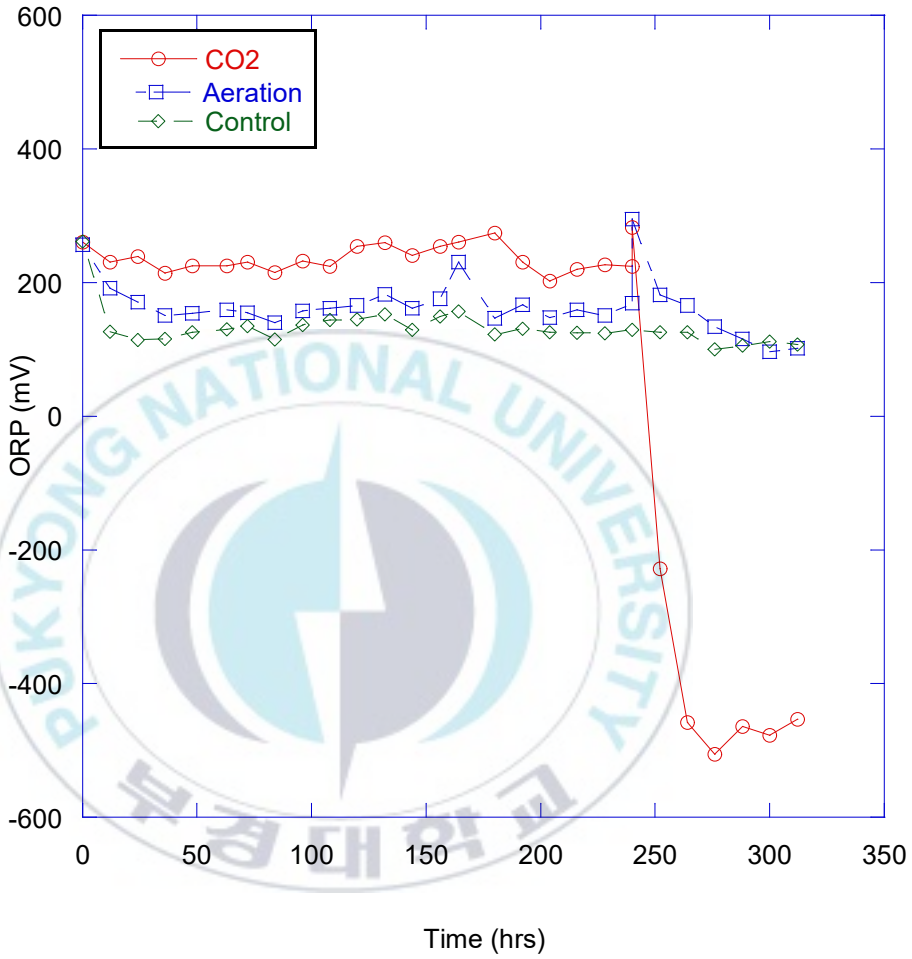

Fig. 10. ORP changes curves of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

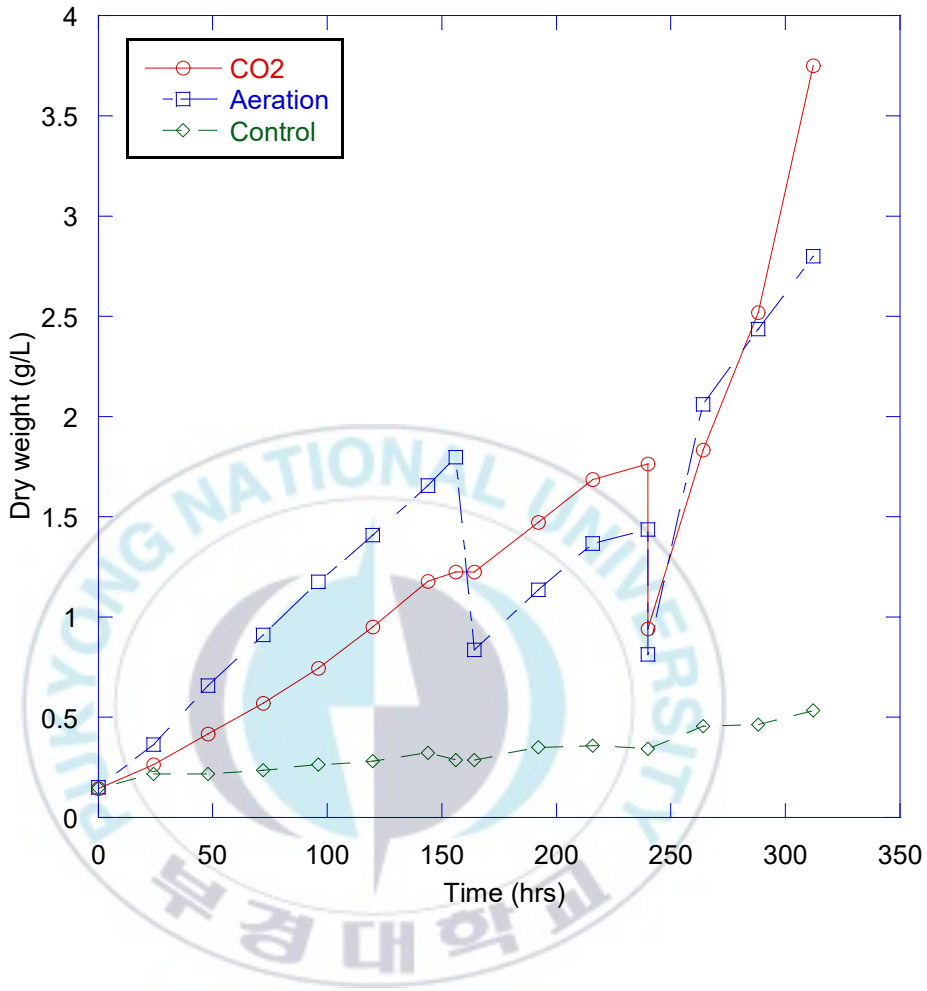

Fig. 11. Growth curves of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

4.2.1.3. NO_3^-, PO_4^{3-}, concentrations

The concentration of NO_3^- and PO_4^{3-} is shown in Fig.12 and Fig. 13, respectively, and removal parameters are summarized in Table 2. The initial cultural medium is JW medium and the concentration of NO_3^- and PO_4^{3-} were 86.0 mg/L and 18.2 mg/L, respectively. At the time 164 h, we did fifty percent dilution by taking out 1.5 L algae solution and input 1.5 L JW medium. At the begin of period 2, we used modified JW medium with high concentration of NO_3^- and PO_4^{3-} to do the fifty percent dilution, then the NO_3^- and PO_4^{3-} concentration in the AC and AA at the begin of period 2 were 434 mg/L and 416 mg/L, respectively. In addition, we did not input additional nitrates and phosphates in the control reactor.

Removal rate (mg L^{-1} d^{-1}) of NO_3^- is shown in Fig. 12 and summarized in Table 2. Removal rate of NO_3^- during the period 1 is the following order AC(8.19 C), AA(11.19 g L^{-1} d^{-1}), control(0.095 g L^{-1} d^{-1}), thus, we conclude that the AA test has the best performance in nitrates removing, the removal rate ratio of nitrates in this period is 82: 118: 1. After we input the ammonium acetate at the begin of period 2, the nitrates removal rate increased dramatically especially the AC test, the values were AC(198.62 g L^{-1} d^{-1}), AA(15.50 g L^{-1} d^{-1}), respectively, and the nitrates removal rate ratio in this period is 1996: 163: 1 of the three tests.

The changes in phosphorus in the semi-continuous reaction experiments are shown in Fig. 13. In the control, phosphorus was slowly and continuously removed with the removal rate at 0.39 g L^{-1} d^{-1}. In the case of air injection experiment and pH controlling test, the phosphorus removal rates were 0.60 g L^{-1} d^{-1} and 0.31 g L^{-1} d^{-1}, respectively. It showed the similar result as the control test. In addition, with the inputting of

ammonium acetate in the aeration and pH controlling test, the phosphorus removal rate increased, and the removal value was 4.3 $g\ L^{-1}\ d^{-1}$ and 1.6 $g\ L^{-1}\ d^{-1}$, respectively. Tan et al. (2016) reported the removal rate of PO_4^{3-} were 7.3 (Chlorella sorokiniana), 10.6 (Chlorella vulgaris), 6.4 (Chlorella vulgaris ESP-6), and 5.9 $mg\ L^{-1}\ d^{-1}$ (Desmodesmus sp. F51), which were cultivated in biogas slurry. These values are similar to those obtained from the Aeration test.

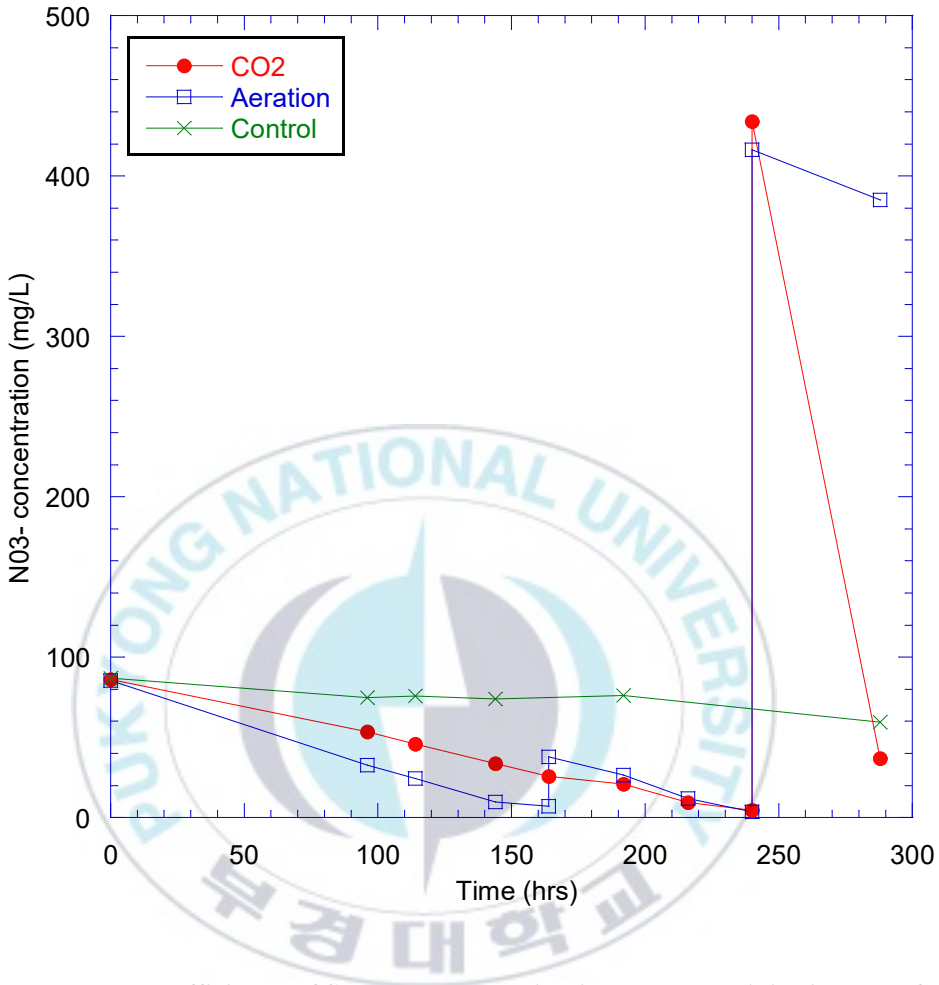

Fig. 12. Nitrates removal efficiency of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

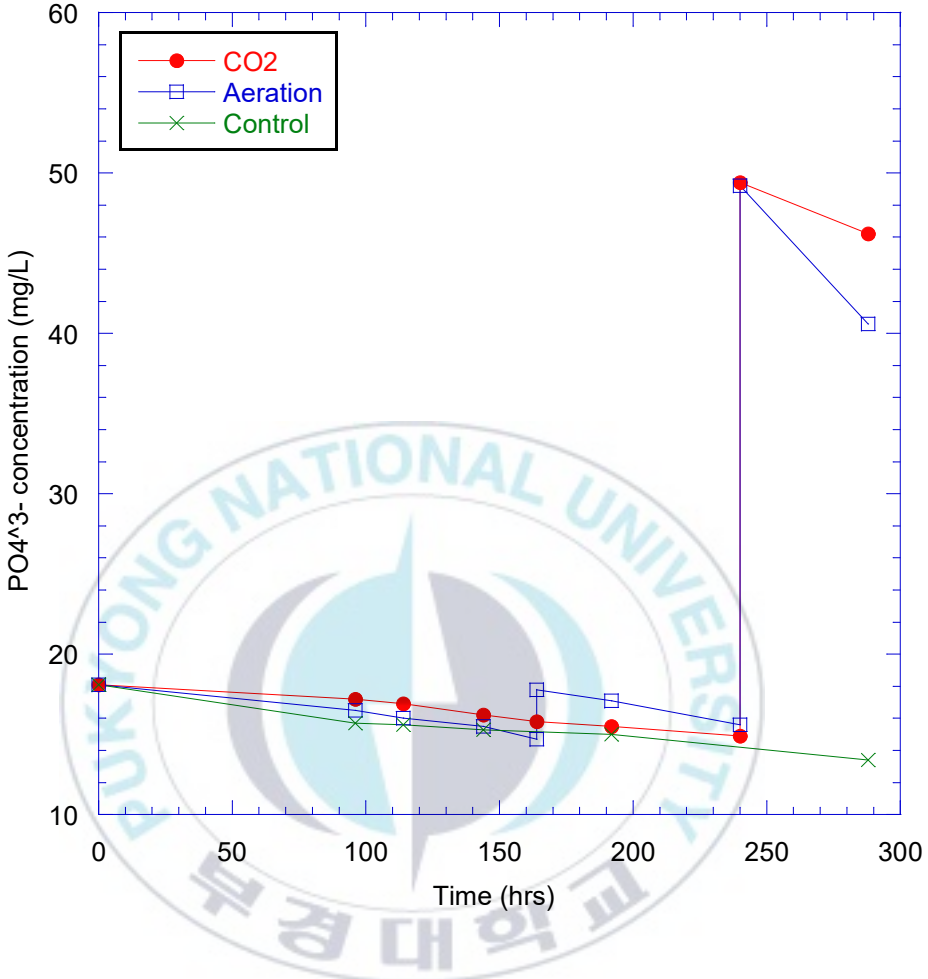

Fig. 13. Phosphates removal efficiency of Scenedesmus cultivation under the joint impact of aeration, carbon resources, and pH controlling condition.

4.2.2. A study of Scenedesmus growth characteristics under Semi-continuous cultivation

4.2.2.1. pH, DO, ORP changes under different culturing conditions

The details of test condition and results are showed in Table 3.

From the Fig. 14, 15, and 16, we realized that during the period 1 the pH value increased quickly to almost 9 in 300 hours, then kept stable, that is the same reason as we showed in test 3.2.1.5 that the microalgae using HCO_3^- by the photosynthetic reaction to decrease the concentration of hydrogen, and the aeration supplied additional CO_2 which made the pH balance in the reactor. Besides, the DO concentration(range from 9.0 to 10.0 mg/L) and the ORP (range from 220 to 240 mV) kept in a stable value during the period 1.

During the period 2, whenever we did dilution at time 504h and 697h, the pH decreased to a lower value and then come back to the previous condition, but when we did dilution at time 838 h, the pH value decreased first but did not increase to the previous value, that is because the cell concentration was too low at that period and could not use enough HCO_3- to decreased the hydrion concentration, and at the time 1034 h, we installed the pH controller to adjust pH value lower than 7.7, then the pH value kept stable around 7.6 until the end of this test. And the DO concentration kept stable about 9.5 before we remove the aeration equipment, Besides, during the period 2, after we input ammonium acetate into the reactor, the DO concentration decreased quickly to 4 and then come back to the previous value, that is because of the same reason as we explain in the 3.2.1.5 test. And the change of ORP value kept in line with the vibration of the DO value. As for the period 3, we realized that whenever we

input ammonium acetate in to the reactor, the DO concentration decreased dramatically to almost 0 and then come back to a high value, and the ORP value decreased to almost -50 mV and then come back to about 250 mV, in addition, the ORP fluctuation period kept step with the DO value's vibration.

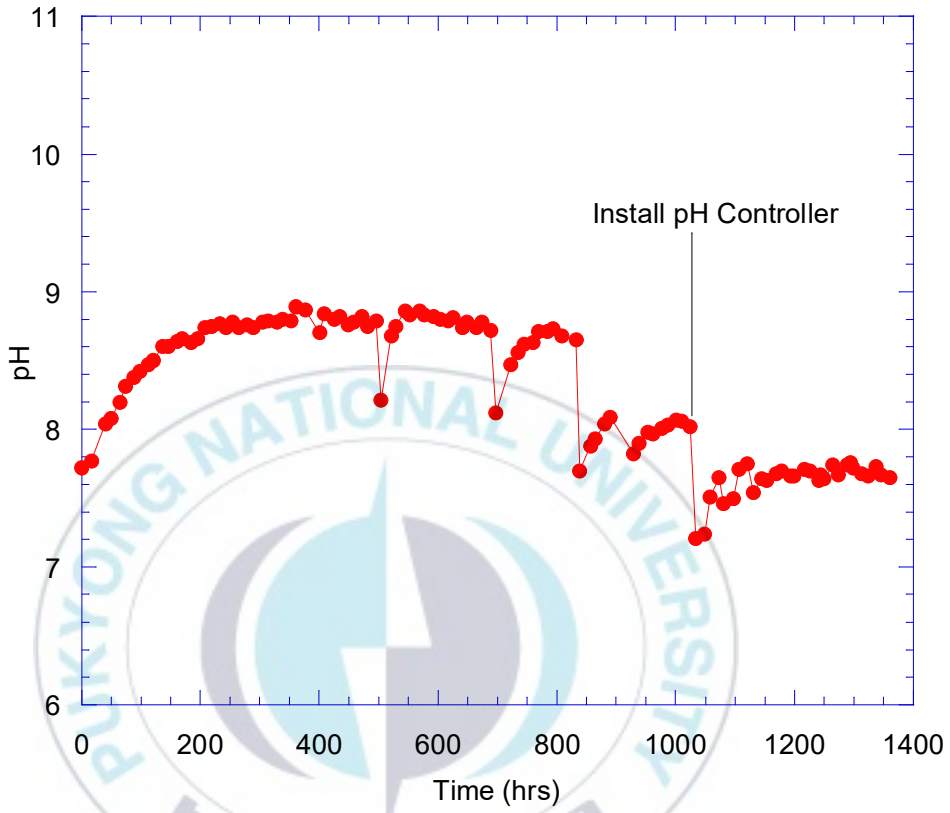

Fig. 14. pH changes curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate

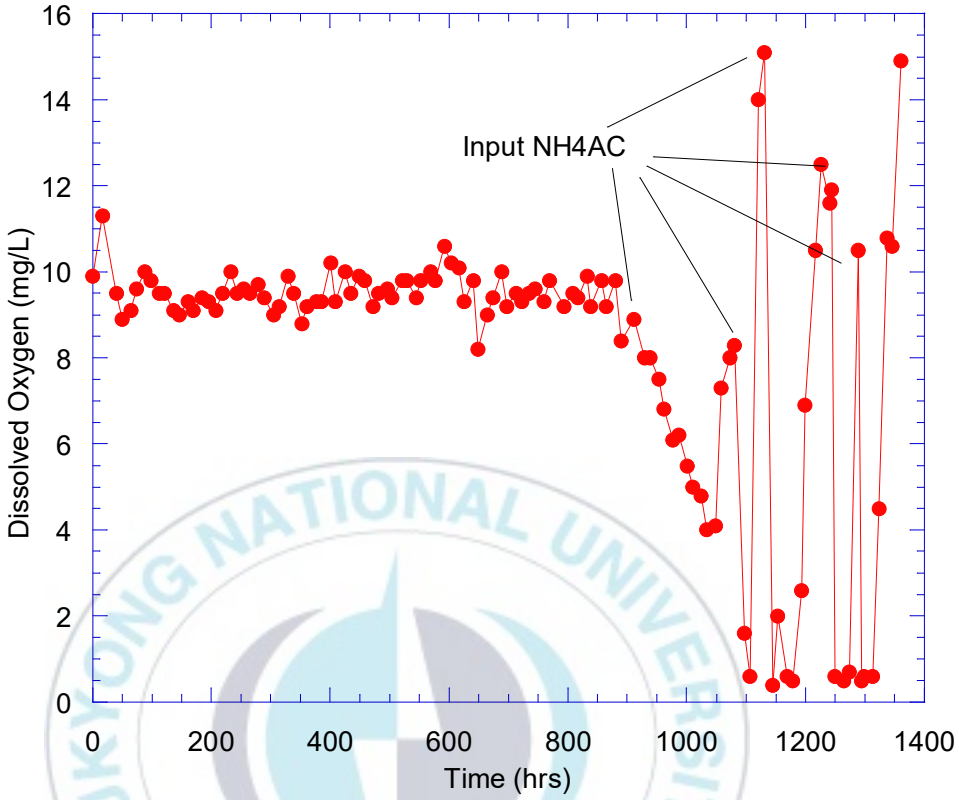

Fig. 15. DO changes curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate.

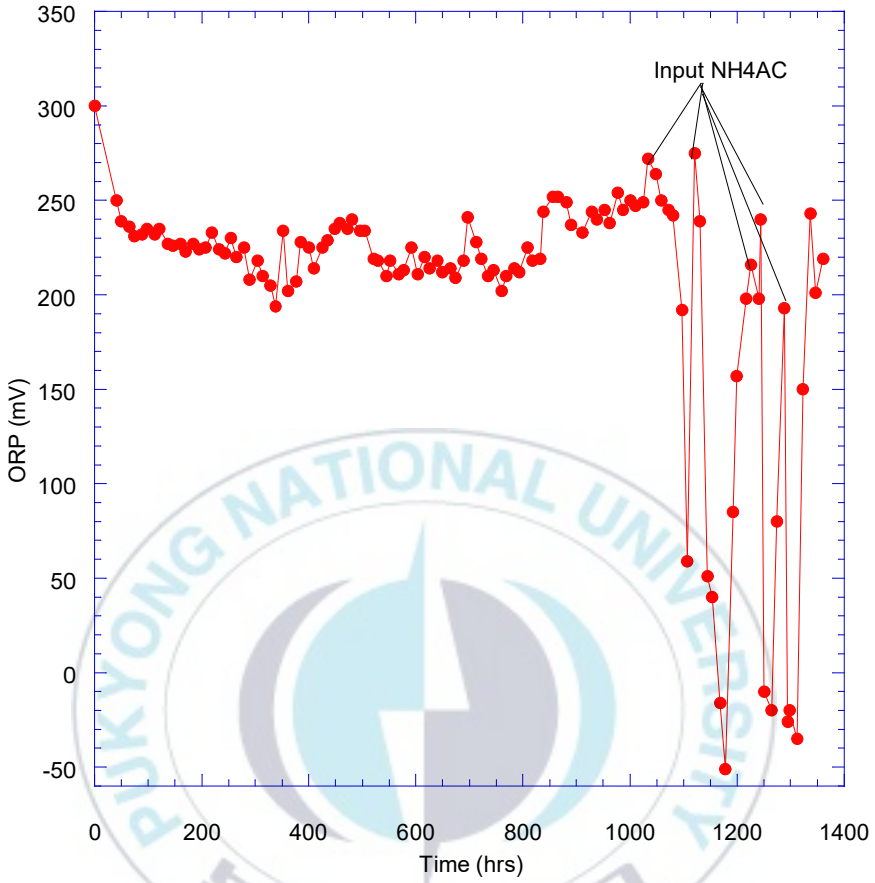

Fig. 16. ORP changes curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate.

4.2.2.2. Microalgal growth parameters and cultural conditions

From the Fig. 17, we realized that Scenedesmus grows steady and quickly during the period 1. But the growth rate(0.069 d^{-1}, 0.141 d^{-1}) and the maximum cell concentration (3.78, 2.02, 0.537 g/L) in period 2 are obviously lower than those in period 1(0.154 d^{-1}, 0.171 $g\ L^{-1}\ d^{-1}$, 3.62 g/L), even though we inputted ammonium acetate and kept the same cultural medium as the period 1. The only difference is the culture medium used in period 2 lacked magnesium, and we monitored that the microalgae solution colour was light yellow then, and some studies cited that lacking magnesium in would inhibit the cell chlorophyll's production and photosynthesis process, which indicated that magnesium plays an essential role in microalgal cultivation. For the period 3, we input magnesium and ammonium acetate at the begin of period 3. Apparently, the microalgae growth rate (0.250 d^{-1}, 0.512 d^{-1}) and maximum cell concentration (6.28 g/L) were higher than those in period 1. The detail data were shown in Table 3.

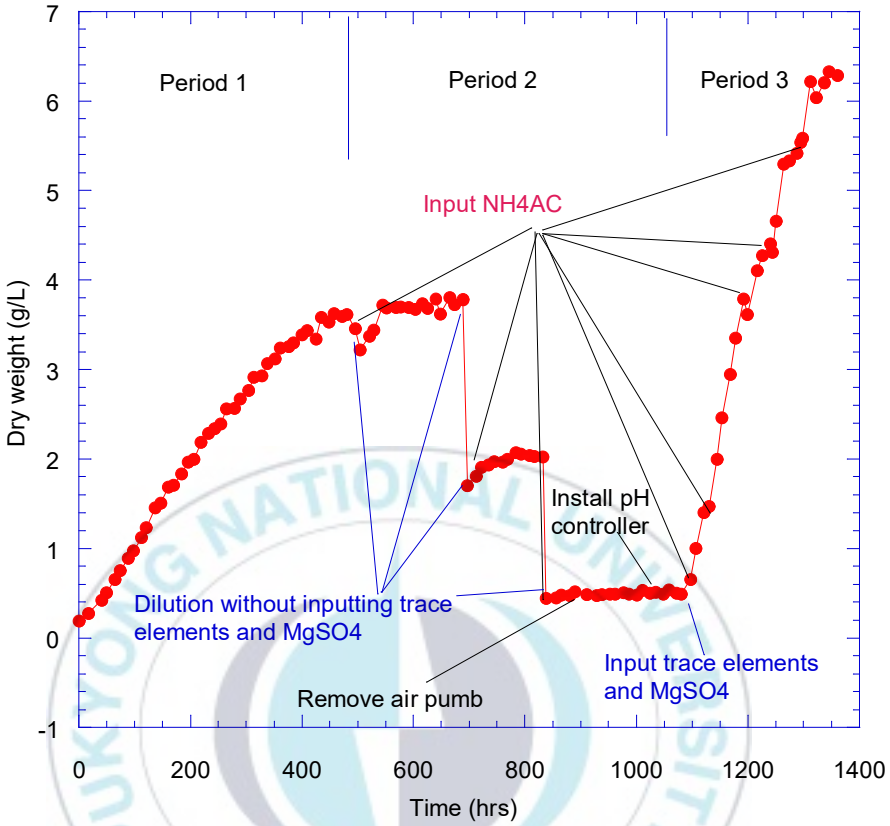

Fig. 17. Growth curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate.

4.2.2.3. NO_3^-, PO_4^{3-}, concentrations

The concentration of NO_3^- and PO_4^{3-} are showed in Fig.18 and Fig. 19, respectively, and nutrition removal rate is summarized in Table 3. The initial concentration of the NO_3^- and PO_4^{3-} in the culture medium were 206 mg/L and 57 mg/L, respectively. The average nitrates removal rate in period 1, 2, and 3 are 23.53 $g\ L^{-1}\ d^{-1}$, 3.71 $g\ L^{-1}\ d^{-1}$, and 70 $g\ L^{-1}\ d^{-1}$, respectively. And the average phosphorus removal rate in the three periods are 4.68 $g\ L^{-1}\ d^{-1}$, 3.85 $g\ L^{-1}\ d^{-1}$, and 6.1 $g\ L^{-1}\ d^{-1}$, respectively. The nutrition removal rate keeps in line with the microalgae biomass productivity, the figure in the period 3 exhibits the highest nitrates removal rate, which is 2.97 times higher than it in period 1, and the biomass productivity in period 3 is three times higher than it in period 1. Besides, the nutrients concentration changed less in period 2, and the biomass concentration increased the least during the period 2. In conclusion, we stand the points that by increasing the microalgae biomass productivity, the solution nutrients removal efficiency can be enhanced obviously.

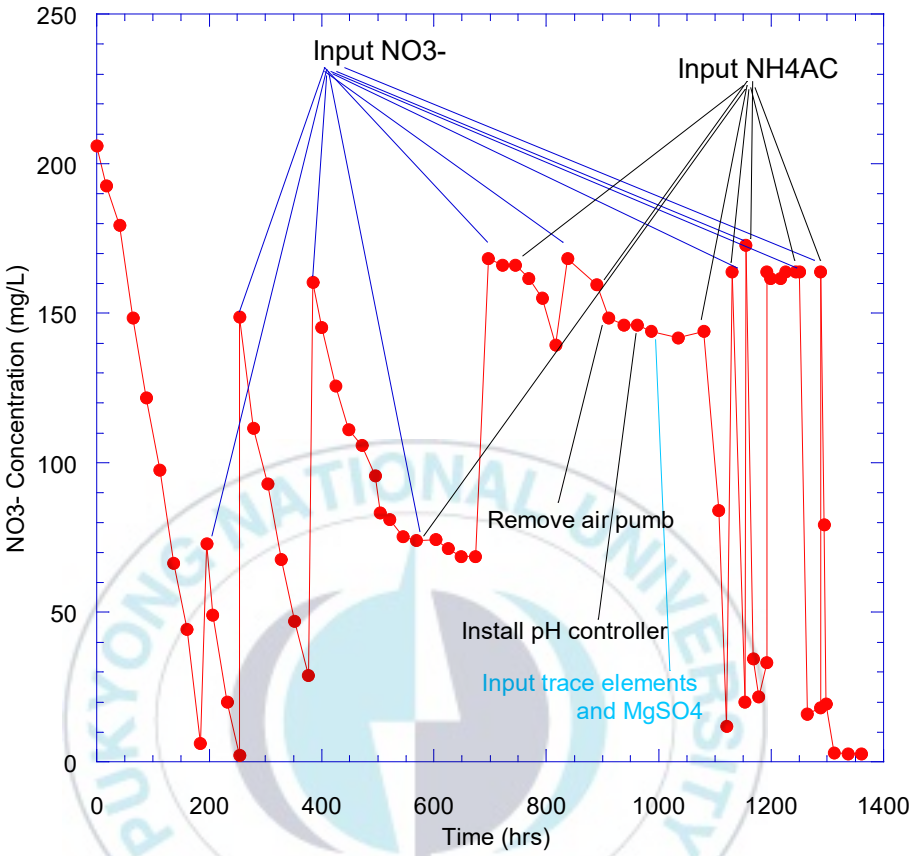

Fig. 18. Nitrates removal rates curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate.

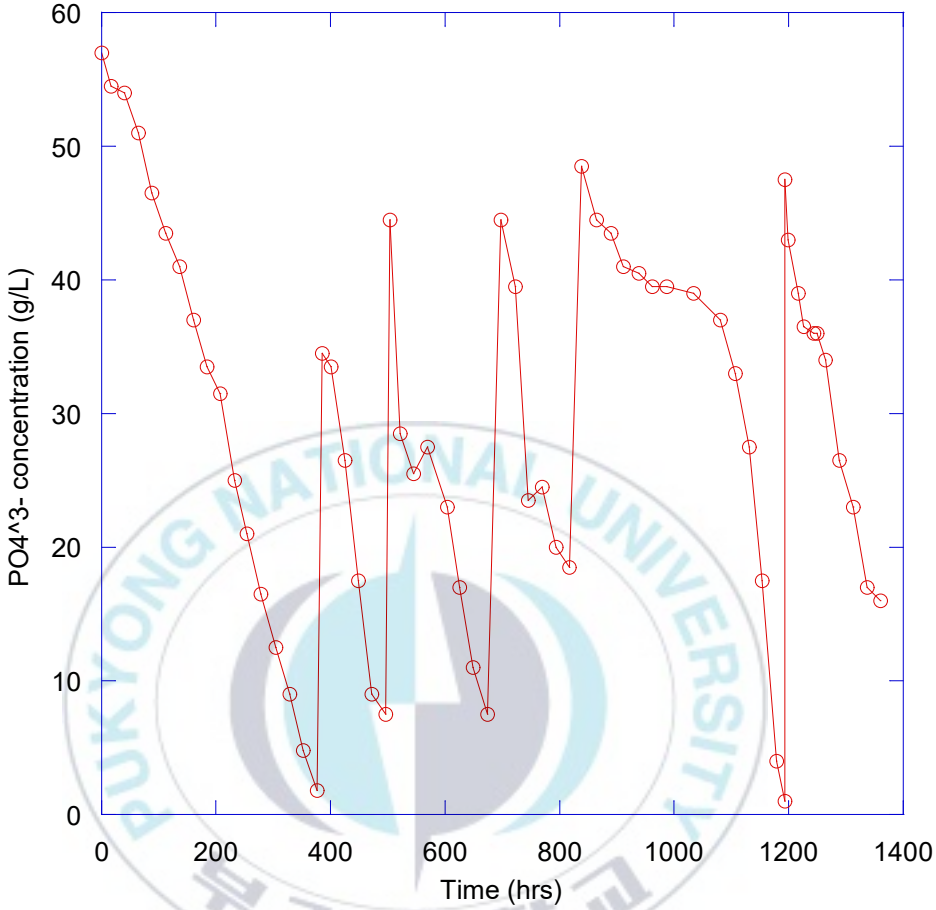

Fig. 19. Phosphates removal rates curves of Scenedesmus cultivation under the same reactor with different cultural conditions, such as pH controlling, aeration, and inputting ammonium acetate.

4.3. The potential of PGA served in microalgal harvesting

The details of test settings are showed in table 4, and the Fig. 20, 21, 22, 23, 24 showed the harvesting efficiency tendency. With the initial biomass concentration increased, the harvesting efficiency increased dramatically when the initial biomass concentration is lower than 0.660 g/L, but when the initial biomass concentration is higher than 0.660 g/L, the PGA harvesting efficiency keeps stable. The maximum harvesting efficiency is 94.87% when the initial biomass concentration in cultural solution is 1.121 g/L. Besides, from the Fig. 25, we can observe the connection between the initial biomass concentration and the optimum PGA concentration used for the harvesting process, and the equation ($y = 2.4484x + 0.1298$, $R^2 = 0.9383$) meets this relationship. In addition, we realized that with the initial biomass concentration increased, the optimal harvesting efficiency by using PGA increased dramatically at first, then slow down and kept stable. Besides, the highest harvesting efficiency in our research is 94.86%, when the initial biomass concentration is 1.12 g/L, and the PGA concentration we used is 3.0g/L then. The details were showed in Fig. 26.

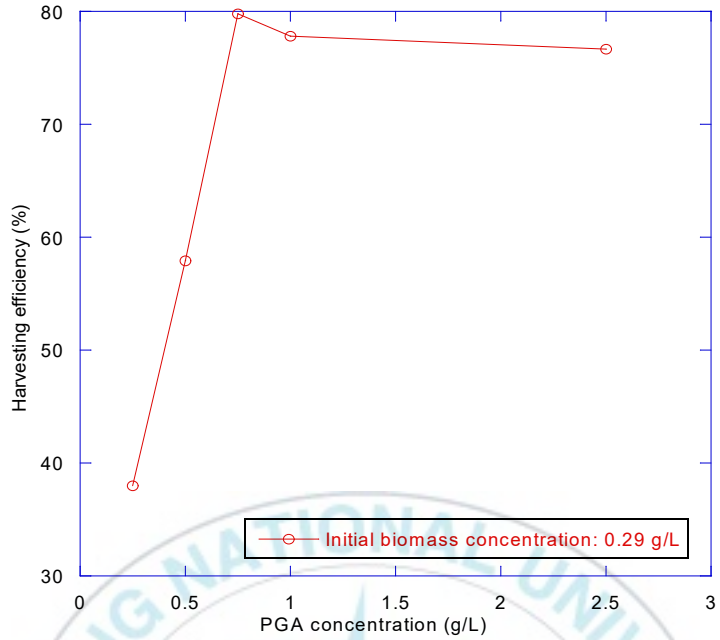

Fig. 20. The Scenedesmus harvesting efficiency under the initial biomass concentration at 0.29 g/L.

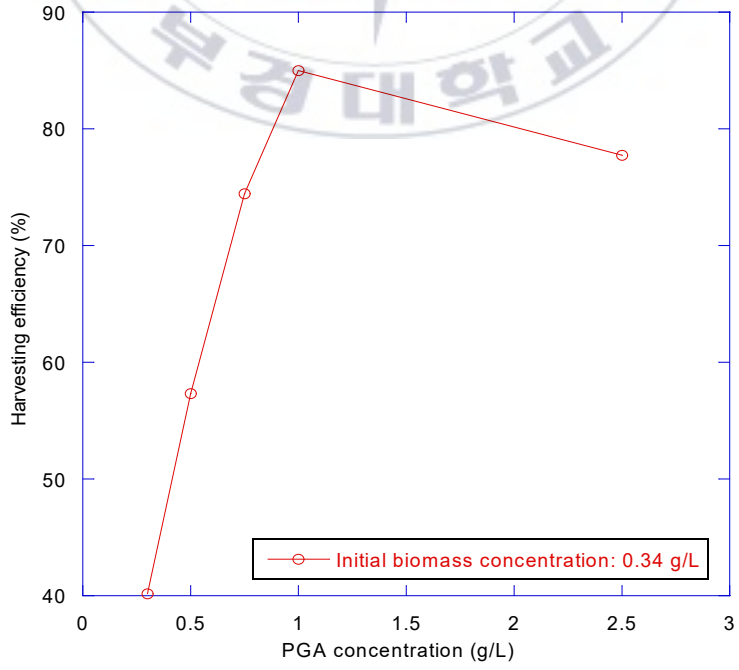

Fig. 21.The Scenedesmus harvesting efficiency under the initial biomass concentration at 0.34 g/L.

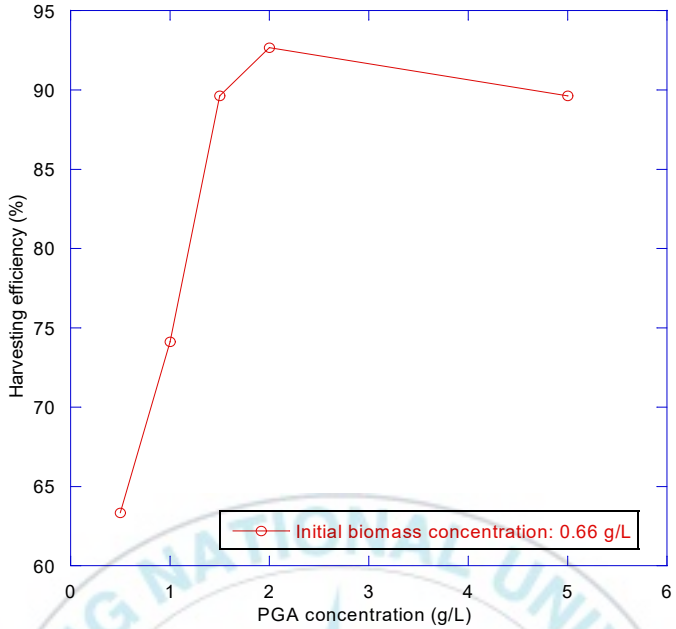

Fig. 22. The Scenedesmus harvesting efficiency under the initial biomass concentration at 0.66 g/L.

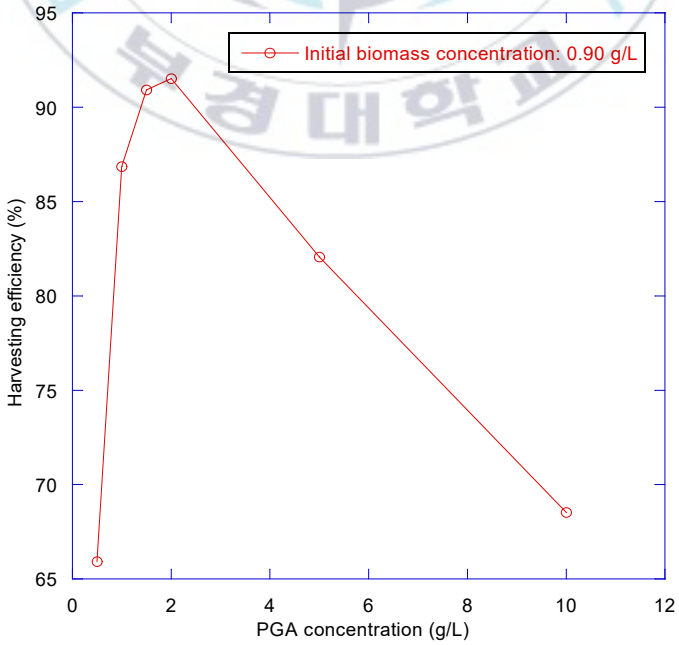

Fig. 23. The Scenedesmus harvesting efficiency under the initial biomass concentration at 0.90 g/L.

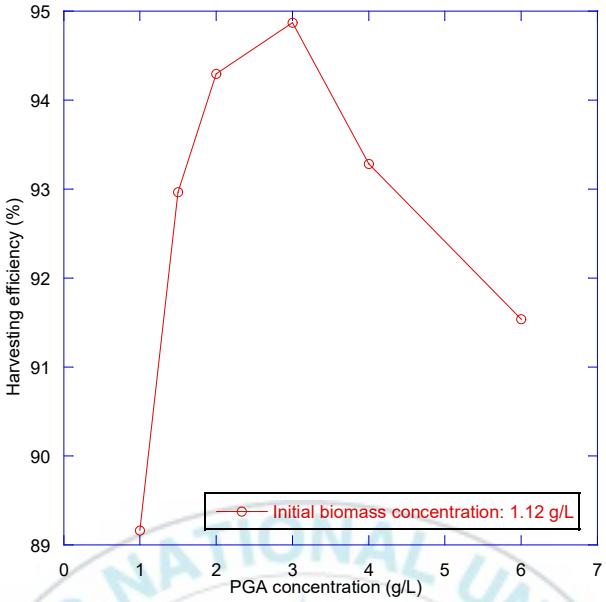

Fig. 24. The Scenedesmus harvesting efficiency under the initial biomass concentration at 1.12 g/L.

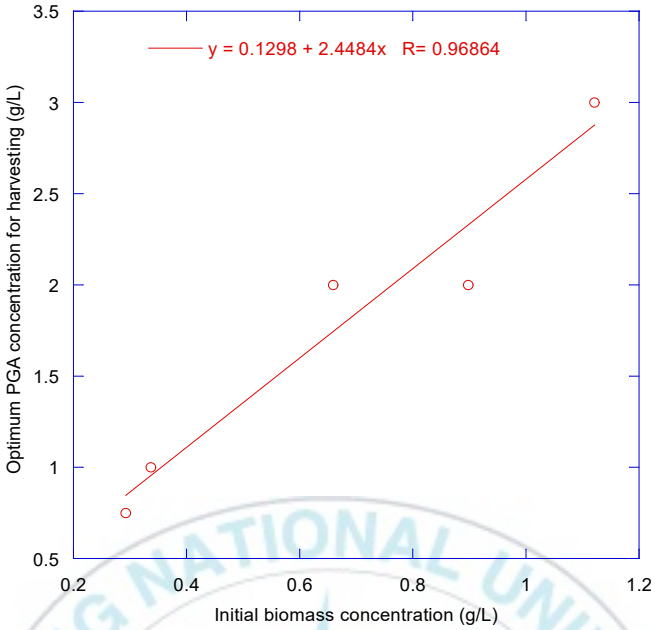

Fig. 25.The relationship curves of the initial biomass concentration and the optimum PGA concentrations.

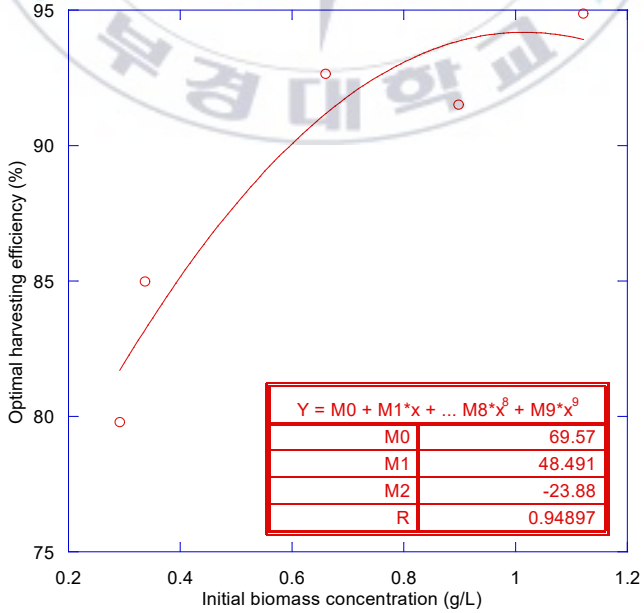

Fig. 26.The relationship curves of the initial biomass concentration and the optimum harvesting efficiency by using PGA coagulators.

5. Conclusion

The growth characteristics of Scenedesmus was invested through the mixotrophic and autotrophic cultural conditions. A combined effect of pH controlling, dissolved oxygen, pH, aeration, and organic carbon resources were studied in this study. And the various conclusion of this study are as follows:

1. Either of aeration or pH controlling has an active effect on microalgae cultivation, and aeration tests showed higher efficiency compared with the pH controlling test.

2. Controlling the dissolved oxygen concentration on the culture medium has little impact to enhance the microalgae growth rate compared with the control.

3. Ammonium acetate has profoundly effect to improve the cell growth rate, and the 10 mM concentration showed the most economical and best stimulation in microalgae cultivation compared with the 20 mM, 30 mM, and 40 mM concentration.

4. The joint cultivation of organic carbon with pH controlling and with aeration can enhance microalgae growth dramatically, and the pH-controlling test with ammonium acetate as additive showed the highest cell growth rate.

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