The Effect of CO_2 Injection on Macroalgae *Gelidium latifolium* Biomass Growth Rate and Carbohydrate Content

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Abstract

There are many species of macroalga grow in marine ecosystem and potentially as raw material for bioethanol resource. Bioethanol is a conversion result of carbohydrate, one of macroalgae biomass content. The exploration of macroalgae require information about growth rate ability to determine availability in the nature. This research analyze growth rate and carbohydrate content of marine macroalga Gelidium latifolium on cultivation using varied injection of carbon dioxide and aeration. The treatments were control (K), 2000 cc CO_2 injection and aeration (P1), 3000 cc CO_2 injection and aeration (P2), 2000 cc CO_2 injection without aeration (P3), and 3000 cc CO_2 injection without aeration (P4). Samples weight were 3 gram in early cultivation on laboratorium scale for 42 days observation. The results showed that the daily growth rate Gelidium latifolium during the study ranged from 0.02-1.06%. The highest daily growth rate was 1.06±0.14% (P2). Carbohydrate yield was 18.23% in early cultivation then 19.40% (K and P2), 20.40% (P1), 16.87% (K3), and 16.40% (P4) after cultivation. The high of carbohydrates value may not guarantee the sustainable Gelidium latifolium biomass utilization as raw material for bioethanol production because of the low growth rate, thus it is necessary to modified and encourage cultivation method effectively.

Keywords: *CO_2 injection, growth rate, carbohydrate, macroalgae, Gelidium latifolium*

Introduction

Indonesia has a diverse of macroalgae species that grow in coastal areas and support to develop bioenergy based on biomass such as bioethanol (Poespowati *et al.,* 2014). Macroalga are also accumulate polysaccharides which can be hydrolyzed to sugars and subsequently be fermented to ethanol (Goh and Lee, 2010; Adams *et al.,* 2011). *Gelidian* is one potential class of macroalgae due to contains 26.5% carbohydrate and rapid growth rate, supported by Wi *et al.* (2009) and Rajkumar *et al*. (2014). This presentation is quite high compared to average levels previously reported agarofit which is 15-40 % (Rashid *et al.,* 1999). *Gelidium* sp. has higher bioethanol yield than *Gracilarian* (Meinita *et al*., 2013) and several researches explain about *Gelidium* sp. potential as bioethanol resource (Jeong and Park, 2010; Kawaroe *et al*., 2014; 2015; Kim *et al*., 2015).

However, this species has not been widely cultivated and mostly taken directly from nature. Cultivation methods are necessary to be explored for domesticating the species from the nature to large scale artificial cultivation, like has been treated to several macroalga species such as *Gracilarian* (Alveal *et al*., 1997, Troell *et al*., 1997). Macroalgae convert the energy of sunlight through photosynthesis process, requires a carbon dioxide (CO_2) from the atmosphere and hydrogen molecule to build carbohydrates. It fix carbon dioxide levels higher than terrestrial biomass and allows restoring the larger carbon (Jeong and Park 2010, Lee 2011, Kawaroe *et al*. 2012). Carbon dioxide absorption by marine biomass is 36.8 tones/ha, which is five times higher than terestrial plants (Jeong and Park, 2010). Biomass resulting from photosynthesis of aquatic plants (8%) more efficient and higher than terrestrial plants (1.8-2.2%).

The exploration activities can utilize the exhaust gas (CO_2) for macroalgae cultivation, so that getting several benefits such reducing CO_2 content on the atmosphere and produce raw material for bioethanol production. Besides that, the abundant macroalga become suitable resource without causing negative ecological and food security impacts (Borines *et al.,* 2011; Borines *et al.,* 2013).

This research analyze growth rate and carbohydrate content of marine macroalga *G. latifolium* on cultivation using varied injection of

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carbon dioxide and aeration. The biomass growth rate may determine availability and potential of macroalgae as a raw material itself. Then, the carbohydrate content give the information about basic materials that will be processed become bioethanol.

Materials and Method

The research was conducted at Laboratory of Microalgae, Surfactant and Bioenergy Research Center (SBRC), Bogor Agricultural University. *G. latifolium* sample was taken from Ujung Kulon, Banten. Cultivation performed for 42 days in the seawater aquarium (8 liters) with controlled environment. Early seedling cultivation was 3 grams homogenized and cultivated in 15 aquariums. Each aquariums consisted of 3 bundles of sample that floating on the water. Additional nutrients were required to support macroalga growth in provided seawater media, liquid fertilizer was poured into media 3 times consist of TSP (15 ppm), urea and ZA (30 ppm).

Research treatments

Several treatments were differentiated by CO_2 injection and aeration, control (K) was normal treatment using aeration, P1 (aeration + CO_2 injection 2000cc/day), P2 (aeration + CO_2 injection 3000 cc), P3 (CO_2 injection 2000 cc/day) without aeration, and P4 (CO_2 injection 3000 cc/day) without aeration. Injection of CO_2 speed was 200 cc/minute and injected for 10 minutes every day in daylight time. Those concentrations referred to Kawaroe *et al.* (2012) research which injected 12.500 cc – 37.500 cc CO_2 in 130 L – 150 L. In this research, CO_2 injection were be diminished because the water on aquariums are less. Every treatment consisted of 3 repetitions (3 aquariums). The pure CO_2 was mixed with ambient air converted by compressor until reaching 50:50 ratio of the gas on a chamber. Then mixing CO_2 was injected to water on aquariums through the plastic pipes. The diagram below explain the flow of experiment.

Environmental parameters analyses

Calculation of dissolved carbon dioxide concentrations used the formula by Boyd, 1982. Water quality parameters was analyzed once every 3 days along with carbon dioxide injection. Temperature was monitored by thermometer and salinity was measured using a refractometer based on per mil (‰) unit while acidity (pH) was measured using a digital pH meter.

Growth rate analyses

The daily growth rate of macroalgae biomass was calculated based on the formula by Dawes *et al.,* 1993.

Carbohydrate analyses

Carbohydrate content was tested by acid hydrolysis process according to Luff Schrool method (SNI 01-2891-1992). The whole compound of carbohydrates were broken down into simple sugars (monosaccharides) by heating with HCl, then monosaccharide was analyzed by the Luff-Schoorl method. The principle of the analysis was Cu^{2+} reduction to Cu^{1+} by monosaccharides. Free monosaccharides will reduce alkali solution from metal into oxide or free form. The unreduced excess of Cu^{2+} was quantified by iodometric titration.

Result and Discussion

Environmental parameters

Daily temperature variation in *G. latifolium* cultivation was 25-27 ^{0}C during 42 days, fluctuated but there was no variation in different aquarium because the same placement position when exposed by sunlight. Macroalgae has specific temperature range and grow in tropical area at 20-30 ^{0}C but the optimum life temperature is 28 ^{0}C. It also explained by Raikar *et al*. (2001) that tropical species adapt easily when cultivated in similar temperature but macroalgae tolerate temperature fluctuation until 4 ºC (Stengel *et al.,* 2014). *G. latifolium* grow unwell in wide range temperature, thalli may be stuck and stop budding. Temperature and high humidity was not effective for macroalgae growth in cultivation.

Salinity range for effective macroalgae growth are 34-37 ‰ (Bird and Mc Lachlan, 1986) and 25-30 ‰ (Ding *et al.*, 2013). In this study, water salinity range was 32-34 ‰, it still in recommended range but categorized to high value. It was caused by evaporation rate of water medium in aquariums, shown by evaporated salt in aquarium walls. Indonesian *Gelidium* sp. habitually grow in hipersalinity condition such as 33 ‰ (Aslan 1998). Salinity is an important factor for photosynthesis, respiration, and growth of macroalgae (Li-hong *et al*. 2002). Lower salinity often inhibit growth of macroalgae, affect the branching patterns and change its chemical composition (Choi *et al*., 2006).

Beside temperature and salinity, acidity (pH) become important factor influencing the growth rate. Seawater pH values in each treatment varied from 6.3-8.8.

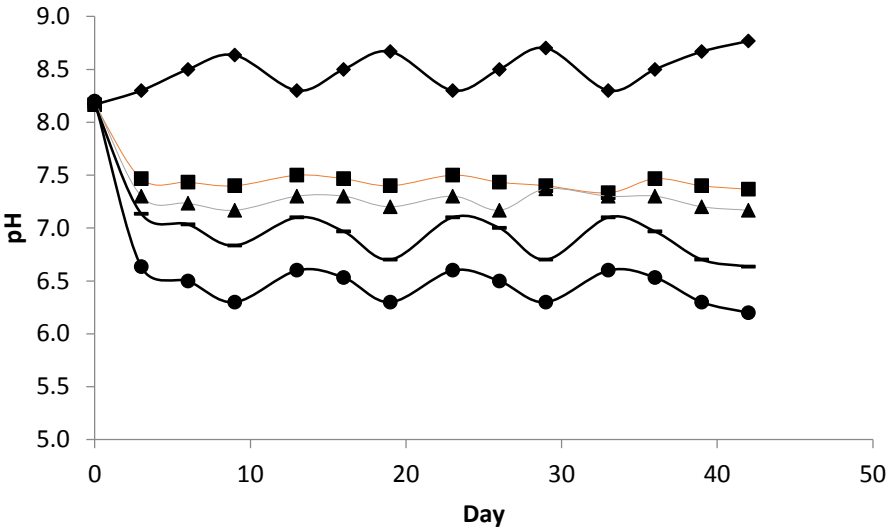

Figure 1. Acidity condition in cultivation

Note. = K; = P1; = P2; = P3; = P4

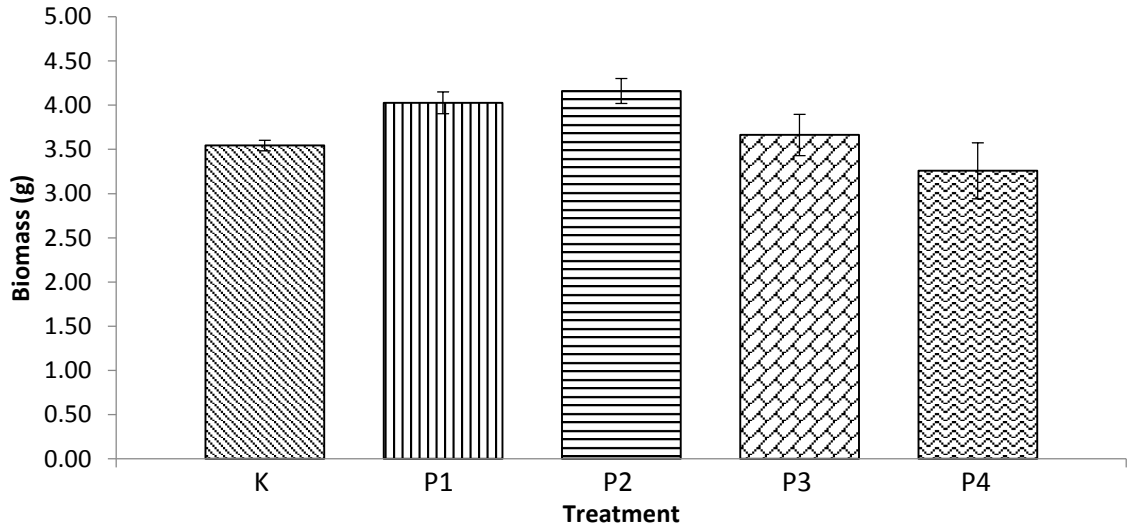

Figure 2. Wet biomass average

Note. = K; = P1; = P2; = P3; = P4

The optimum pH for macroalgae is 8-9 normally seawater pH, but it also grow in pH 7 (Zatnika and Angkasa, 1994). The length and concentration of CO_2 injection influenced to pH condition with positive correlation. Aeration normalized seawater, inputting outside gases especially oxygen may reduce acidity and CO_2 concentration in seawater. Acidity condition is intimately associated with CO_2 (Mackereth *et al.,* 1989). Hydrogen ion is produced by CO_2 and water reaction which produce carbonic acid. Lowered pH cause a significant reduction in germination and physiological stress of thalii (Roleda *et al*., 2012).

Wet Biomass

The observation indicates that the average wet biomass of each treatment was varied after cultivation. The highest biomass was shown by P2 (4.16±0.14 g), followed by P1 (4.03±0.12 g), P3 (3.66±0.23 g), K (3.54±0.06 g) and P4 (3.26±0.23g). Macroalgae needed sunlight to synthesis CO_2 and

dissolved nutrients in the water, it was absorbed by thalli include holdfast, blade, and stipe. Carbon dioxide injection supplied carbon source for assimilating become carbohydrates yield. Aeration also increased absorption ability of macroalgae and facilitate water movement. Aeration stone helped to break up the air bubbles in the water and diffused to macroalgae body. This factor caused absorption effectivity in P1 and P2 which have higher biomass in the end of cultivation. The amount of CO_2 was also important for photosynthesis process, but the addition of CO_2 without aeration was not effective because of uncompleted mixing in the water medium. Control of biomass was less than CO_2 injection and aeration additional treatment.

Daily and relative growth rate

Daily growth rate of *G. latifolium* during the study ranged from 0.02% to 1.06% based on treatment response in the observation. Daily growth rate of P4 was fluctuated, in the 2nd week decreased from 0.15±0.54% to 0.02±0.85% but in the 3rd week increased from 0.12±0.58%. This decreasing was caused by softening part of thalli thus reduced the weight of the wet macroalgae in cultivation. The thalli was cut to avoid the spreading to other parts. Carbon dioxide injection without aeration was caused acidity of seawater as medium and the decreasing of pH also occurs in P3. In contrary, acidity of P1 and P2 were normal effected by aeration that leading of air recirculation on aquariums.

Daily growth rate during 42 days of cultivation may be regressed to determine growth rate in the following days. Here is the regression of *G. latifolium* daily growth rate in cultivation.

The value of x is growth rate, while t mean length of cultivation time. Regression of control and treatment show the different values. Highest daily growth rate of all treatment occurred in P2 (1.06±0.14%) at 4th week, while the lowest occurred in P4 (0.02±0.85%) in 2nd week. At the end of cultivation, all of daily growth rate were decrease because reaching stationary phase. Daily growth rate indicates the percentage ratio between early seedling and the last biomass of macroalgae per time unit.

Table 1. Linear regression equation of *G. latifoloum* daily growth rate

Treatment	Regression
K (aeration)	x = 0.463 - 0.0007 t
P1 (CO_2 2000 cc + aeration)	x = 0.913 - 0.0040 t
P2 (CO_2 3000 cc + aeration)	x = 1.02 - 0.0049 t
P3 (CO_2 2000 cc)	x = 0.698 - 0.0040 t
P4 (CO_2 3000 cc)	x = 0.322 - 0.0050 t

Floating monoline cultivation is an effective methods for macroalgae maintaining during the study according to Aslan (1998), growth rate of macroalgae in floating method is 2.00-3.00%, bottom off method 1.66-1.75%, and basic method is 0.30-0.53%. The effective range of macroalgae growth rate is 2-3%. In this study, *G. latifolium* belonged to low growth rate because less than 2% until the end of cultivation. The habitat of this species are generally found on rocky substrate in the low intertidal to high sub tidal areas (Kim *et al*., 2011) and the size are relatively small ranged 6-18 cm. The relative growth rate is shown in Figure 4.

Relative growth rate at the end of cultivation in different treatment from the highest to the lowest were 32.66% (P2), 29.43% (P1), 19.82% (P3), 16.64% (K), 7.91% (P4). Relatives growth rate showed the relationship between early and the last biomass of macroalgae cultivation.

Utilization of carbon dioxide in the cultivation

Carbon dioxide is a gas whose can be dissolved in seawater and binds with H_2O molecules to form carbonic acid which decomposes into bicarbonate ions. Acidity increased after carbon dioxide injection into water. Aeration affected to CO_2 solubility level and macroalgae response in research treatments. The following diagram shows dissolved CO_2 in each treatment (except Control).

The highest CO_2 dissolved was 37.25 mgL^{-1} in P4 at 33rd day, while the lowest 19.95 mgL^{-1} in P1 at 42nd day. Injection length reacted to CO_2 solubility values because determine input concentration. Control treatment was not measured using NaOH titration, the amount of carbon dioxide was undetected and mostly absorbed by the thalli. In addition, carbon dioxide is transformed into another form such as bicarbonate ion (HCO_3^-) (Zeebe and Wolf, 2001). The solubility values were fluctuated after 3 times injection and seawater replacement in aquariums. All macroalgae diffuse CO_2 directly to the chloroplasts where fixed by RuBisCO in photosynthesis (Lobban and Harrison, 1997), but in most cases, diffusion uptake of CO_2 is insufficient to support maximal rates of photosynthesis (Kubler *et al.,* 1999). Elevated concentration of CO_2 may provide advantages to macroalgae that actively pump CO_2 and HCO_3^- into their cells using energetically carbon injection (Cornwall *et al*., 2012).

Partial pressure is a factor affecting solubility of the gas (Effendi, 2003) and reduced when photosynthetic and heating activity. Solubility of CO_2 in P1, P2, P3, and P4 exceeded normal limits in waters, therefore the insoluble residual gas was diffused and accumulated in plastic bag that

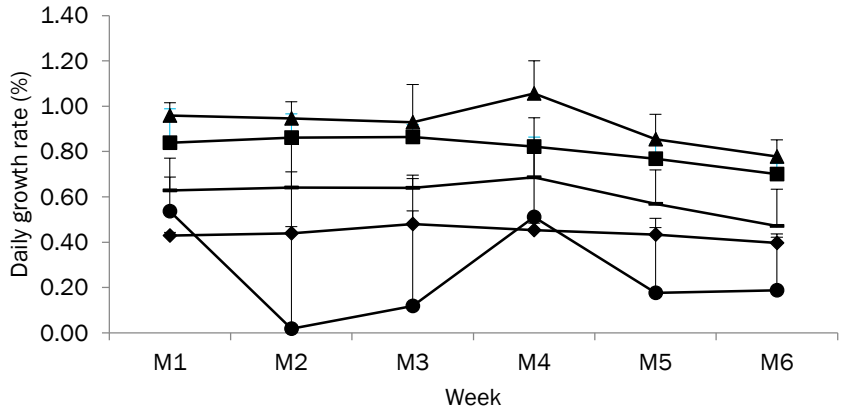

Figure 3. Daily growth rate

Note. = K; = P1; = P2; = P3; = P4

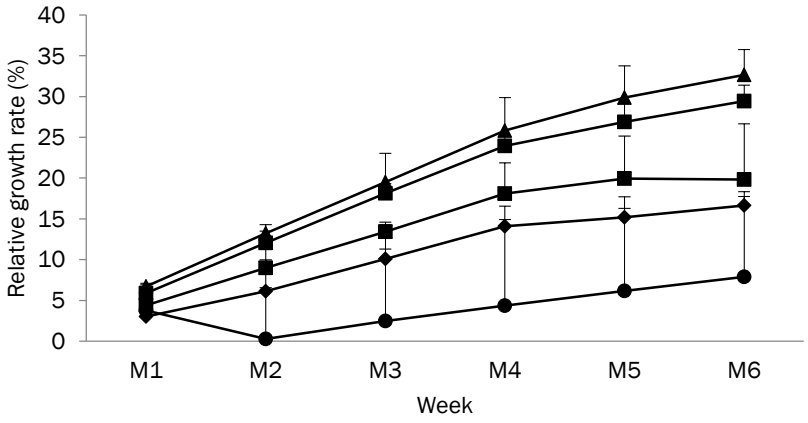

Figure 4. Relative growth rate

Note. = K; = P1; = P2; = P3; = P4

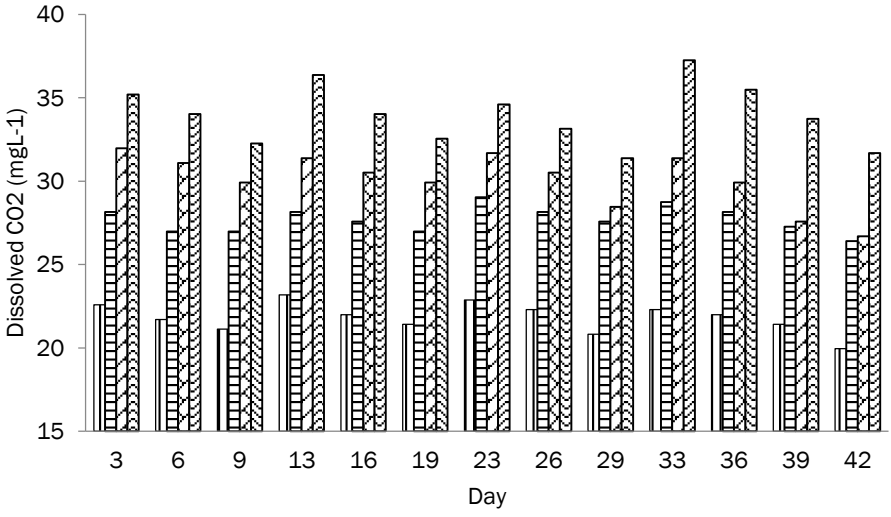

Figure 5. Dissolved CO_2 in cultivation

Note. K; = P1; = P2; = P3; = P4

attached on aquariums. The highest value was 12.27% at P4 in 42nd day, while the lowest was 9.53% at P3 in 13rd day. The residual gas average of P3 was 10.16% and P4 was 11.73%. Increasing CO_2 may reduce pH then enhances the ability of macroalgae to absorb and grow rapidly (Pulido *et al.,* 2011). In the other hand, the high levels of these gas may cause changes in algae physiology and metabolism which includes reduction in stomatal density, RuBisCo levels, chlorophyll, and lower photorespiration (Papazi *et al.,* 2008).

Carbohydrate content

Carbohydrates represent a broad group of substances which include the sugars, starches, gums and celluloses (Chow and Halver, 2015) composed during cultivation into biomass. The addition of CO_2 and subsequent pH of culture water had no prominent effects on the biochemical composition of the biomass as quantified especially carbohydrate content. The carbohydrate content ranged after cultivation was relatively stable over the 42 days growth trial except P3 and P4.

A similar result occurred in other study where CO_2 injection in several treatments caused an increase of biomass production but didn't increase carbohydrate on biomass itself (Cole, *et al*., 2013). Otherwise, since metabolism of carbohydrates in plants grow at high CO_2, it would be a factor that associated with the decrease Chl (chlorophyll) and RuBisCo on the leaf (Makino and Mae, 1999). Environmental parameters degradation such as elevated of CO_2 concentration allow the changes of cell division rate, growth patterns, blade anatomy (Masle, 2000), cell structure and function (Sharma *et al.,* 2014) . Besides that, it may be easier to be infected by invasion organism from outside. In this research, one of the threats organism was fungi which caused spoilage and attack one of bundle macroalgae in P4 treatment.

Table 2. Carbohydrate content of *G. latifolium* before and after cultivation

Time	Treatment	Carbohydrate yield
Before cultivation	-	18.23%
After cultivation	K (aeration)	19.40%
	P1 (CO_2 2000 cc + aeration)	20.40%
	P2 (CO_2 3000 cc + aeration)	19.40%
	P3 (CO_2 2000 cc)	16.87%
	P4 (CO_2 3000 cc)	16.40%

Conclusion

Carbon dioxide injection in macroalgae cultivation increase biomass effectively with aeration treatment to normalize water quality. This result is not be accompanied with carbohydrate content accumulation on its biomass. *G.latifolium* growth rate is categorized to low biomass growth rate because its value less than 2%. The better method have to be developed to improve better outcome. However this data may be preliminary data for further studies conducted on macroalgae exploration and cultivation for bioethanol resource.

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