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Sea grape (*Caulerpa lentillifera*) cultivation in artificial seawater closed system

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ARTICLE INFO

Article history: Received 02 January 2020 Received in revised form 01 February 2020 Accepted 04 February 2020

Keywords: Green Macroalage Caulerpa lentillifera Sea grape Artificial seawater cultivation

ABSTRACT

In the present study, the daily relative growth rates of the green macroalga (sea grape, Caulerpa lentillifera) in artificial seawater closed system with cement ponds was evaluated. Cultivation in artificial seawater was investigated for the combined impact of three significant aspects: algal productivity, water quality and nutritional value. Sea grape was cultured for 30 days and important factors were analyzed weekly, and the results showed that the fastest growth rate (productivity rate) was recorded in the second week at 23.27 g.day⁻¹. After that, biomass yield was steadily decreased to 11.67 g.day-1 in the fourth week. The ranges of the experimental cultivation conditions were salinity (29.13 - 31.54 ppt), pH (8.42 - 9.41), air temperature (12.14 - 35.13°C), water temperature (21.02 - 24.64°C), light intensity (0 - 35,350.00 lux), electrical conductivity, EC (46.67 - 48.62 ms.cm⁻¹), total dissolved solids, TDS (25.22 - 26.14 g.l⁻¹) and alkalinity (74.00 - 106.67 mg.l⁻¹). During cultivation, the EC was high and the values were quite stable. The amount of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen ranged were 0.17 -7.20, 2.04 - 8.51 and 2.06 - 3.60 mg.l⁻¹, respectively. The amount of inorganic phosphorus (phosphate phosphorus) was between 0.58 - 0.91 mg.l⁻¹. The nutritional value of cultivated sea grape was performed by dry algal weight and the amount of protein, fat, moisture, ash, fiber and carbohydrate were 24.44 \pm 4.329, 2.07 \pm 0.13, 8.53 \pm 0.40, 38.99 \pm 0.30, 14.50 \pm 1.28 and 11.47%, respectively. This artificial seawater grown algae contained a high amount of protein (24.44%), and our study results show that artificial seawater cultivation presents positive production of nutritions.

1. Introduction

Seaweed production plays a vital role in providing a food source for both humans and marine and terrestrial animals, such as sea urchins and fishes. They are also the nutritional base of several food webs, animal fodder and chemical extracts. It is increasing from food-based products to functional health foods, skincare beauty products, pharmaceuticals and therapeutics (Bhuyar et al., 2020; Govindan et al., 2020). Seaweed is a healthy food that is popularly consumed in Pacific and Southeast Asian countries, especially in Thailand. Traditionally Thai people used seaweed in its raw or sun-dried form for food.

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18

Nomenclature and abbreviation			
NH ₃ -N Ammonia-Nitrogen			
NO2 ⁻ -N	Nitrite-Nitrogen		
NO ₃ ⁻ -N	Nitrate-Nitrogen		
PO4 ³⁻ -P	Phosphate-Phosphorus		
EC	Electrical Conductivity		
TDS	Total Dissolved Solids		

Caulerpa lentillifera (sea grape) is the most important seaweeds in Thailand. They are a genus of seaweeds in the green algae group. Algae biomass were applicable to get high photosynthetic yields, high oil content and to produce high concentrations of beneficial compounds such as proteins, carbohydrates, lipids, pigments, vitamins and minerals (Ramaraj et al., 2013; 2014; 2015). Sea grape is widely distributed in tropical areas, found in the Gulf of Thailand (Pugdeepan and Petchsut, 2008) as well as the Andaman coast (southern coastal areas of Thailand). This alga is a popular edible species rich in minerals and vitamins, including fatty acids, B vitamins, vitamin E, and minerals such as I, P, Zn, Ca, Mg, Se, Fe, Mn and Co; and several essential unsaturated fatty acids, which is eaten fresh or salted for later use.

It is an essential source of magnesium that helps lower blood pressure and prevents heart failure. Mainly, it helps against cancer and thyroid disease. Sea grape was used to treat water quality and also used for aquatic feed. It is one of the popular seaweeds produced for commercial cultivation in Thailand (Pimolrat et al., 2019). There are several advantages of sea grape cultivation. They are more accessible to culture and have opportunities to export usually, and products containing edible quality seaweed are fresh and safe for consumers. However, they are expensive, and the freshness of the algae is compromised when transported some distance from the production area. Sea grape cultivation in a closed system that controls various factors could be promoted growth and the possibility of sea grapes algae cultivation in the northern region of Thailand, which is far away from the production area (sea zone).

This macro green alga can be cultivated by closed system cultivation or in raceway ponds and bioreactors. The temperature and contaminants can be easily controlled in these cultivation processes, improving algal growth. However, growing sea grape can be cultivated in a closed system that hard to culture with seawater (Sudtongkong, 2008). Artificial sea salt that is full of the mineral compounds found in natural seawater was used to prepare for raising marine aquatic animals or seaweed. Artificial seawater closed system was used to cultivate marine algae. The advantages of the closed system are that environmental factors such as salinity, water temperature and pH can be controlled.

Nevertheless, wastewater treatment needs to be effectively managed to avoid releasing marine water into the environment. A significant increase in water salinity has an effect on freshwater livings in aquatic habitats. Therefore, this research was conducted to develop a sea grape cultivation process using artificial seawater in a closed system. The water quality parameters were measured using smart sensors and standard practical methods. Furthermore, the approximate quality of sea grape was analyzed. Therefore, this study emphasizes the possibility of inland sea grape cultivation using artificial seawater.

2. Materials and methods

2.1. Sea grape preparation

Sea grape (*Caulerpa lentillifera*) cultivation was carried out in artificial seawater closed system at the Faculty of Fisheries and Aquatic Resources, Maejo University, Chiang Mai, Thailand. The stock algae were collected from Chaolay Sea grape Co. Ltd., Takua Thung, Phang-nga province, southern Thailand. They were kept in artificial seawater salinity 30.00 ppt fiberglass tank then transferred to the laboratory in northern Thailand.

2.2. Study on Sea grape cultivation growth

Sea grape was cultured in triplicate with 3.00 m^3 cement tanks of artificial seawater. They were spread on 0.50 * 0.50 meters of panel flame using PVC pipes. A one-centimeter mesh size net was used to support the algae filament. They were spread all over the panel flames with 500.00 grams fresh weight per panel and having 6 panels per tank. The algal panels were hung 10 cm below water surface and added fertilizer 16-16-16 in the ratio of 5 g(m³)⁻¹ of water and provide oxygen aeration. The algal cultures were grown for 30 days. The growth of algae was analyzed weekly by weighing and assessing the growth of algae during the experiment. The production of sea grape was analyzed using the following formula:

Productivity rate $(g.day^{-1}) = \frac{Harvest fresh weight (g) - Initial fresh weight (g)}{Cultivation time (day)}$

2.3. Water quality parameters measurement

Some water quality parameters were measured using a smart sensor from SPsmartplants Co., Ltd. to record water and air temperature (°C), acidity (pH), salinity (ppt), electrical conductivity, EC (ms.cm⁻¹), total dissolved solids, TDS (g.l⁻¹) and light intensity (lux). The sensor recorded the information every minute. All parameters were recorded and stored in a central database; also, it could be able to retrieve the information at any time for further analysis.

In addition, the chemical properties of the water were analyzed according to standard methods (APHA, 1998), notably, alkalinity, inorganic nitrogen content such as ammonia nitrogen (NH₃-N) (Phenate method), nitrite-nitrogen (NO₂⁻-N) (Coupling method) and nitrate-nitrogen (NO₃⁻-N) (Cadmium reduction method), inorganic phosphorus; phosphate phosphorus (PO₄³⁻-P) (Stannous chloride method).

2.4. Nutritional value of sea grape measurement

Sea grape filament was dried in an incubator at 55.00-60.00°C until dried after that; biomass was blended thoroughly with a blender. Subsequently, they were sifted with a 150-micron algae sieve for analysis. The nutritional value of dried algae, including moisture, ash, protein, fat, fiber and carbohydrates, was estimated using AOAC methods (AOAC, 1990). The crude protein content was determined by measuring nitrogen using the Kjeldahl method;

the crude lipid content was measured by ether extraction using Soxhlet extractor; ash contents, a known amount of dry sample was burnt in the muffle furnace at 550.00°C for 4 hours; crude fiber, an amount of sample after digestion in H₂SO₄ and NaOH solution and the residue calcined (AOAC, 1990).

2.5. Statistical analysis

Algal growth and some water quality data were subjected to one-way ANOVA. All percentage data were arcsine transformed prior to analysis. The significant difference and the group means were analysed with Tukey's multiple range tests. All statistical analyses were carried out using SPSS/PC for Windows ver. 15.1.

3. Results and discussion

3.1. Sea grape productivity rate

The sea grape productivity rate cultured in artificial seawater the closed system increased almost double time within a month; the results were presented in Table 1. In the first phase (2 weeks

Table 1

Sea grape cultivation growth cultured in an artificial seawater system.

cultivation), the productivity rate was increased rapidly. Algal filament length was also increased. However, after two weeks, algal growth gradually decreased (Fig. 1-3). The algal production in the first two weeks increased (Fig. 1, 3). The algal productivity rate was highest after two weeks of cultivation (23.27 g.day⁻¹). After four weeks of cultivation, the algal productivity rate decreased to 11.67 g.day⁻¹. Due to the fluctuating climate, some algal filaments died.

The watercolour of the ponds turned a dark green color. Some bacterial pathogen infections can occur. It affected algal dying and the watercolor in some ponds was turned to be orange color from many reasons affected to decrease the algal growth in the 3^{rd} and 4^{th} weeks. The algal productivity rate was higher than other research; Pimonrat et al. (2019) found that the sea grape grew with natural seawater at 1.67 g.day⁻¹ yields when cultured for 30 days. Sudthongkong (2008) compared the coastal algal and earth pond cultivations for 14 days and found that sea grape cultivation in the coastal algal and earth pond cultivations had an average yield of 4.5 ± 1.6 and 3.2 ± 1.1 g.day⁻¹, respectively.

C	Time				
Growin parameters	Week 1	Week 2	Week 3	Week 4	
Algal productivity (kg)	$0.56 + 0.04^{a}$	0.83 ± 0.05^{b}	0.82 ± 0.08^{b}	0.85 ± 0.09^{b}	
Algal length (cm)	1.20 ± 0.19^{a}	1.24 ± 0.18^{a}	1.41 <u>+</u> 0.17 ^b	1.42 <u>+</u> 0.18 ^b	
Algal productivity rate (g.day ⁻¹)	8.81 ± 1.80^{a}	23.27 <u>+</u> 5.36 ^c	15.08 <u>+</u> 0.73 ^b	11.67 <u>+</u> 2.20 ^b	

Each value represents mean \pm SD. Values in the same row with different superscript letters are significantly different (p < 0.05).



Fig. 1. Sea grape productivity cultured in an artificial seawater system.

3.2. Water quality parameter analysis

Water quality parameters monitored in the algal cultured tanks using artificial seawater are shown in Table 2. Air and water temperatures ranged 12.14 - 35.13 and 21.04 - 24.64°C, respectively. The air and water temperatures in the cultured tanks were sometimes slightly below the range considered to be optimal



Fig. 2. Length of algal filament cultured in an artificial seawater system.



Fig. 3. The rate of algal productivity culture in an artificial seawater system.

due to the algae being cultured in winter. The average light intensity was 0-34,483 lux. The average salinity was 29.13-31.54 ppt. The average pH value was 8.42-9.41. Algal grown cell density has a significant influence on electrical conductivity. The electrical

conductivity was considered high in the range of 47.00-48.50 (ms.cm⁻¹) and the total dissolved solids (TDS) were relatively stable, in the range of 25.22 - 26.14 g.l⁻¹. There was no significant difference (p > 0.05) in water quality among the treatments.

Table 2

Water quality parameters in cultured tanks of sea grape in an artificial seawater system (Mean ± SE).

Parameters	Time				
Parameters	Week 1	Week 2	Week 3	Week 4	
Air temperature (°C)	15.12 - 35.03	14.76 - 35.13	12.25 - 32.63	12.14 - 26.11	
Water temperature (°C)	21.53 - 24.26	21.37 - 23.96	21.04 - 23.25	21.39 - 24.64	
pH	8.42 - 8.77	8.55 - 9.20	8.42 - 9.22	8.61 - 9.41	
Light intensity (Lux)	0 - 30,606.00	0 - 35,351.00	0 - 29,411.00	0 - 34,483.00	
Salinity (ppt)	30.55 - 31.29	30.10 - 30.45	30.56 - 31.12	31.26 - 31.54	
Conductivity (mS.cm ⁻¹)	47.26 - 47.93	46.67 - 47.14	47.23 - 47.99	48.22 - 48.62	
Total dissolved solid (g.l ⁻¹)	25.42 - 25.81	25.22 - 25.46	25.56 - 25.89	26.00 - 26.14	
Alkalinity (mg.l ⁻¹)	79.00 - 81.00	74.00 - 84.00	88.00 - 92.00	101.67 - 106.67	
NH ₃ -N (mg.l ⁻¹)	0.22 - 0.26	6.66 - 7.20	5.99 - 6.66	1.74 - 1.91	
NO ⁻ 2 -N (mg.l ⁻¹)	2.20 - 2.23	2.44 - 2.45	7.22 - 7.34	7.90 - 8.51	
NO ⁻ ₃ -N (mg.l ⁻¹)	3.41 - 3.60	3.20 - 3.22	2.96 - 3.06	2.09 - 2.67	
$PO_4^{3-}-P(mg.l^{-1})$	0.79 - 0.80	0.58 - 0.64	0.89 - 0.90	0.70 - 0.71	

Most of the water quality parameters were suitable for culturing sea grape. However, the light intensity was quite high because natural sunlight was used, whereas Pugdeepan and Petchsut (2008) which reported *C. lentillifera* seedlings grown under controlled conditions in the laboratory at the salinity of 20, 30 and 40 ppt, the light intensity of approximately 7.20 ± 0.70 , 12.80 ± 1.80 and $21.30 \pm 1.20 \ \mu \text{Em}^2 \text{s}^{-1}$ (or 500, 1,000 and 1,500 lux, respectively) and air temperature as 20, 25 and 30°C. Sea grape can grow at a salinity of 30 to 40 ppt (Guo et al., 2014; Chen et al., 2019).

The optimal growth of this algae was in 30.00 ppt salinity, and the optimal temperature was 25.00°C. The optimum light intensity for algal growth was $7.2 \pm 0.7 \ \mu\text{E m}^{-2}\text{s}^{-1}$ (500 lux), as well as the report of Pariyawatee et al. (2003), who studied the optimal environmental factors suitable for the C. lentillifera J. Agardh growth in order to use sea grape to treat shrimp cultured wastewater. It was found that sea grape optimal growth in saline water was in the range of 25 - 30 ppt and cultured in seawater added fertilizer, nitrogen: phosphorus ratio equal to 8: 1, while nitrate is a nitrogen source. The optimal fertilizer concentration was 4 mg.l⁻¹ and initial stock algal density 1 g.l⁻¹ was the highest growth. Ammonia (NH₃) concentration in sea grape cultured water should be controlled to be not less than 0.05 mg.l⁻¹. Orthophosphate concentration should be not less than 0.01 mg.l⁻¹. The ideal pH is between 6.30 - 8.90, which is the range of pH that algae can be used (inorganic phosphate form) (Guo et al., 2014).

3.3. Nutritional value of sea grape analysis

The nutritional analysis of the sea grape is presented in Table 3. The nutritional value of sea grape from this study compared with other researches was not significantly different from the other similar research.

The sea grape cultured in this closed system had a high percentage protein of 24.44%, which is higher than the other researches. Due to the high protein content of sea grape algae, it was found that when harvesting time increased, the protein content in algae also increased. Darmawati et al. (2016) found that at 28 days and 42 days of harvesting algae, the protein percentage was 9.60% and 13.80%, respectively. The protein percentage of sea grape from most studies is between 10-13%. The protein content in green algae was usually higher than red and brown algae, where the protein content in the cells of seaweed derives from the absorption of nitrogen contained in water. According to Thongdet (2010), the percentage of protein in sea grape when cultured for two weeks in culture media with different nitrogen-phosphorus ratios (10:1, 23:1 and 33:1), were 13.67 ± 0.68 , 16.07 ± 1.66 and $19.65 \pm 1.11\%$, respectively.

From the experiment, it can be concluded that if the nitrogen content in the water is high, the protein content in algae will also increase accordingly. It was found that the inorganic nitrogen concentration was higher than the inorganic phosphorus concentration to the ratio of 18:1. Sinurat and Fadjriah (2019) found that the protein content of sea grape contained many essential and non-essential amino acids, exceptionally high aspartic acid and glutamic acid contents. The ash content in this alga is higher than in the spinach plant. The high ash content value indicated the presence of many kinds of minerals (Matanjun et al., 2009; Kumar et al., 2011; Nagappan and Vairappan, 2014). The consumption of sea grape is good for health. Because of the high amount of fatty acids, especially the unsaturated fatty acid and PUFA/SFA ratio, it can reduce the risk of human coronary heart disease. Sea grape has the potential to be used as a food or food supplement (Nagappan and Vairappan, 2014; Manas et al., 2017).

	This study	Ratana-arporn and Chirapart	Sudthongkong (2008)	Nguyen et al. (2011)	Darmawati et al. (2016)	Sinurat and Fadiriah (2019)
		(2006)	(2000)	(2011)	ce un (2010)	1 uuji uu (2017)
Moisture	8.53 ± 0.40	25.31 ± 1.15	9.57	-	43.65	5.40
Ash	38.99 ± 0.30	24.21 ± 1.7	17.18	22.20 ± 0.27	28.56	41.85
Lipid	2.07 ± 0.13	0.86 ± 0.10	5.26	1.57 ± 0.02	0.13	0.85
Protein	24.44 ± 4.33	12.49 ± 0.3	18.50	9.26 ± 0.03	9.60	14.40
Fiber	14.50 ± 1.28	3.17 ± 0.21	-	2.97 ± 0.01	10.83	8.50
Carbohydrate	11.47	59.27	49.49 (include fiber)	64.00 ± 0.11	7.58	32.95

 Table 3

 Approximate composition (%DW) of the sea grape.

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4. Conclusion

The sea grape (*Caulerpa lentillifera*) cultivation is applied cultured in artificial seawater closed system. The growth rate or productivity rate of the sea grapes was high in the first phase (i.e., 2 weeks growth period) at 23.27 g.day⁻¹, and in the second phase (3 to 4 weeks), algal production decreased to 11.67 g.day⁻¹. From the water temperature decreasing result, it was unsuitable for algae growth and some crops of algae died. Therefore, the optimal water temperature range of 27 - 30°C should be maintained. The nutritional value of cultivated sea grape was examined. This study is highlighting that sea grape contains valuable nutrition and a high amount of protein (i.e., 24.44%). Therefore, sea grape could be considered as healthy food, applicable for pharmaceutical and medicinal purposes. Our results also revealed that it is easier to scale up or produce sea grape in large-scale cultivation, adopting our study experimental culture conditions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by grants from the National Innovation Agency (NIA), Thailand. The authors thank Chaolay Seagrape Co. Ltd. for providing the stock of *Caulerpa lentillifera* algae. They also thank the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, for the use of their research facilities.

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