STUDIES ON EFFECT OF BICARBONATE IONS AND PH ON PHYSIOLOGY AND MOISTURE CONTENT OF *Gracilaria changii*

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STUDIES ON EFFECT OF BICARBONATE IONS AND PH ON PHYSIOLOGY AND MOISTURE CONTENT OF *Gracilaria changii*

ABSTRACT

Ocean acidification is the after-effect changes in seawater chemistry caused by rising atmospheric CO_2 levels and the resulting repercussions on marine life. In less than a decade, this phenomenon has become one of the most severe and crucial concerns confronting the ocean research community and marine resource managers alike. Seaweed plays a vital role in the coastal carbon cycle and contributes remarkably to sea-farming activities. This research aims to determine the effect of different dissolved inorganic carbon (DIC) concentrations on seaweed and their specific growth rates (SGR). Also, this study investigated the potential of red seaweed, *G. changii*, as a carbon sequester. Based on our preliminary findings, *G. changii* can survive in acidic conditions at least at pH 6 but showed better growth performance at pH 8. In terms of SGR, all seaweeds have shown various adaptation responses every week in all bicarbonate concentrations, which showed that increased CO_2 concentration might enhance, inhibit, or not affect growth.

Keywords: Ocean acidification, bicarbonate concentrations, *Gracilaria changii,* seaweeds, pH.

KAJIAN MENGENAI KESAN ION BIKARBONAT DAN PH TERHADAP FISIOLOGI DAN KANDUNGAN KELEMBAPAN *Gracilaria changii*

ABSTRAK

Pengasidan lautan ialah perubahan yang terkesan dalam kimia air laut disebabkan oleh peningkatan paras CO_2 atmosfera dan memberi impak kepada hidupan marin. Fenomena ini telah menjadi salah satu kebimbangan yang serius kepada komuniti penyelidikan laut dan pengurusan sumber marin dalam tempoh kurang daripada satu dekad. Rumpai laut memainkan peranan penting dalam kitaran karbon pantai dan menjadi penyumbang terbesar kepada aktiviti penternakan laut. Penyelidikan ini bertujuan untuk mengkaji kesan kepekatan karbon tak organik terlarut (KTOT) yang berbeza terhadap rumpai laut dan kadar pertumbuhan spesifik (KPS). Selain itu, kajian ini melihat potensi rumpai laut merah, *G. changii*, sebagai peyerap karbon. Berdasarkan penemuan awal, *G. changii* boleh bertahan dalam keadaan berasid sekurang-kurangnya pada pH 6 tetapi menunjukkan prestasi pertumbuhan yang lebih baik pada pH 8. Dari segi KPS, semua rumpai laut telah menunjukkan pelbagai tindak balas penyesuaian setiap minggu dalam semua kepekatan bikarbonat, yang menunjukkan bahawa peningkatan kepekatan CO_2 mungkin meningkatkan, menghalang, atau tidak menjejaskan pertumbuhan.

Kata kunci: Pengasidan laut, kepekatan bikarbonat, *Gracilaria changii,* rumpai laut, pH.

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LIST OF SYMBOL AND ABBREVIATION

%	:	Percentage
℃	:	Degree Celcius
ANOVA	:	Analysis of Variance
$CaCO_3$	:	Calcium carbonate
CCM	:	Carbon dioxide Concentrating Mechanism
CO_2	:	Carbon dioxide
DIC	:	Dissolved Inorganic Carbon
g	:	gram
G. changii	:	*Gracilaria changii*
g/m^2	:	gram per square meter
HCl	:	Hydrochloric acid
HCO_3	:	Bicarbonate
IMTA	:	Integrated Multi-Trophic Aquaculture
kg	:	kilogram
L	:	liter
LED	:	Light-emitting diode
M	:	Molar
m	:	meter
mg/kg	:	milligram per kilogram
mmol/L	:	millimol per liter
$NaHCO_3$	:	Sodium bicarbonate
NaOH	:	Sodium hydroxide
O_2	:	Oxygen gas
OA	:	Ocean Acidification
ppm	:	parts per million
ppt	:	parts per thousand

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1. Introduction

Ocean acidification (OA) refers to changes in the ocean's carbonate chemistry resulting from increased anthropogenically produced CO_2 absorption (Hurd, Hepburn, Currie, Raven, & Hunter, 2009). The expansion of the aquaculture population in coastal waters has resulted in more dissolved inorganic carbon (DIC) being released into aquatic ecosystems. Through respiratory processes taking place within inner bays with sluggish flow velocity, intensive-culture animals such as fish and oysters operate as carbon dioxide generators (Morris & Humphreys, 2019). This somehow, contributes to the elevated level of bicarbonate and hydrogen ion concentrations in seawater, along with a lower carbonate content, which subsequently results in a lower surface ocean pH (Han et al., 2021). Phytoplankton and macroalgae play important ecological functions in coastal and open oceans as primary producers, contributing fixed carbon to the vast marine food chain, recycling nutrients, and controlling ocean temperatures.

The consequences of OA on calcifying organisms like crustaceans and algae have been a prominent research topic. In saltwater with an elevated pH, seaweed photosynthetic CO_2 fixation rates may be slowed, especially in the presence of high biomass and limited seawater exchange (Han et al., 2021). Increased CO_2 concentrations in saltwater will diminish carbonate saturation states, thus, reducing the ability of calcifiers to maintain and produce new carbonate skeletons. The consequent OA will result in a pH reduction of 0.4–0.5 units below current levels (Wittmann & Pörtner, 2013). OA potentially affect the metabolism and growth rates of all algae, both non-calcareous and calcareous. Numerous experiments to determine the biological impacts of ocean acidification on algae are somewhat challenging to conduct because changes in pH affect carbon speciation in seawater, which subsequently affects photosynthesis (Hurd et al., 2009). Moreover, respiration, calcification and photosynthesis will all affect the pH of the ocean medium.

Seaweed provides various ecosystem services, including bioremediation for coastal pollution, localised control of ocean acidification, climate change mitigation, and habitat for other marine animals. *Gracilaria changii* is one of Malaysia's most abundant agarophytic red seaweeds, which can be found in both Malaysia and Thailand. This agar-producing seaweed can be found in a wide variety of marine environments with varying pH, temperature, and salinity. In the food industry, seaweed is utilised as a thickening and gelling agent. Seasonal variations in agar content and gel strength, as well as spore generation, were tracked in a natural population of *Gracilaria changii* abundantly growing in mangroves and seawater, which is subjected to a variety of harsh climatic circumstances, especially in the low light condition in the murky waters of a mangrove swamp.

The rising of CO_2 levels in the atmosphere is hypothesized to have a significant impact on *G. changii* development throughout the middle and later stages of mariculture in addition to the changed speciation of dissolved inorganic carbon in seawater and the decreased pH level via the carbonate buffer system and the differing abilities of algae to utilise CO_2 and HCO_3. Therefore, research without biases that can be carefully duplicated and assess the effects of increased CO_2 has to be designed to understand both ocean carbonate chemistry and physiological mechanisms connected to carbon metabolism is required. Therefore, under laboratory conditions, the effect of various pHs ranging from pH 5 to 8 towards the seaweed growth rate was investigated.

Bicarbonate ions are an essential element for the ocean's life. The increasing bicarbonate level could be a possible option to reduce OA. This is because when bicarbonate levels increase, it is a sign that the pH level results in alkalinity. Scientifically, if a base is added to seawater, these bicarbonate ions will donate hydrogen ions to neutralize the base. If the CO_2 level in seawater is high, then alkalinity addition from bicarbonate ions may help ameliorate the impacts of ocean acidification. In the environment, the source of bicarbonate can come from cement waste spilling from the factory into the seawater. However, the growth rate, pH fluctuations, moisture content and enzyme activity related to carbon-use macroalgae, especially *G. changii*, remain unknown upon the effect of different sodium bicarbonate levels. This research aims to promote the ability of seaweed as a carbon dioxide absorber to mitigate ocean acidification. Thus, a study to investigate the effects of different concentrations of bicarbonate water on the growth rate, pH and moisture content of cultivated *Gracilaria changii* was established. However, the interactions between mariculture and the seawater carbonate system are complex. Thus, a species-based approach to analysing their overall effects is necessary.

1.2. Hypothesis

1. *G. changii* have different survival rates at four different pH levels – pH 5,6,7 and 8.
2. *G. changii* have different survival rates at bicarbonate concentration 2 mmol/L, 4 mmol/L, 8 mmol/L and 16 mmol/L.

1.3 Objectives

1. To evaluate the growth and morphology of *Gracilaria changii* in response to different pH levels.
2. To analyse the effects of different bicarbonate concentrations on the growth and moisture content of cultivated *Gracilaria changii.*

CHAPTER 2: LITERATURE REVIEW

2.1. Ocean acidification (OA)

OA results from rising CO_2 levels in the atmosphere altering the ocean's carbonate chemistry. The total dissolved inorganic carbon (DIC) and bicarbonate ion (HCO_3) accessible for marine calcification grows due to CO_2 absorption by the surface ocean. However, seawater's pH and carbonate ion concentration decrease (de Putron et al., 2011). The seawater is slightly basic. The natural cycle of carbon dioxide uptake from the atmosphere causes a continual drop in the pH of the ocean (Tee et al., 2015). The burning of fossil fuels is one of the primary causes of OA due to the transition of pH from neutral to acidic conditions. Furthermore, extremely high pH frequently occurs in coastal nutrient-enriched systems exposed to human waste and agricultural waste. In contrast, coastal eutrophication and increased atmospheric carbon dioxide levels may result from low pH and sea acidification due to intense industrial processes. Extreme pH levels affect the distribution, growth and abundance of marine photosynthetic organisms.

Concerns about aquaculture's negative influence on the environment are growing (Mawi et al., 2020). The impacts of CO_2-induced ocean acidification on marine calcifiers are expected to be negative, leading to the calcified components being dissolved and calcification rates dropping. This phenomenon would directly impact many marine creatures' ability to manufacture biogenic carbonate because the carbonate ions and calcium ions ($CaCO_3$) are the basic building blocks of carbonate skeletons and shells. This also can cause existing carbonate shells, skeletons, and other structures to dissolve in extreme circumstances. These negative impacts are unusual among calcifiers, for which there has been relatively little research on the impacts of ocean acidification (Taylor et al., 2015).

On the other hand, crustaceans and molluscs rely on their calcified exoskeleton for various essential activities. Reducing calcium carbonate shells in shellfish and other aquatic life highlighted ocean acidification. Individual marine species' physiological responses may differ, affecting their interactions at the ecosystem level. An estimated 30–40% of the carbon dioxide produced into the atmosphere by human activities dissolves in seas, rivers, and lakes. As a result, aquatic living responds to ocean acidification differently under different pH settings, primarily reflected in carbon fixation and nitrogen metabolism and the growth rate and nutritional content.

2.2. Marine life' responses to ocean acidifications

2.2.1 Marine calcifiers' responses to ocean acidifications

The oceans are becoming increasingly acidic due to anthropogenic increases in atmospheric carbon dioxide (CO_2), lowering their saturation with calcium carbonate ($CaCO_3$). Future CO_2-induced declines in the $CaCO_3$ saturation state of seawater raise concerns about the effects on marine animals that use this mineral to build their shells and skeletons (Hurd et al., 2019). A recent study has shown some solid findings on how marine calcifiers may fare in the future as the water becomes increasingly acidic. For example, some urchins and snails' shells oysters and clams are partially disintegrated at extremely high CO_2 circumstances in the lab. On the other hand, crustaceans such as prawns, shrimps, crabs and lobsters appeared to improve their shell-building capacity. In contrast, other species seem to remain unaffected. In a research conducted by J. Ries and his team in 2009, they set up a miniature of test tanks in which the atmospheric CO_2 levels were increased in the tiny captive oceans to artificially increased acidity to mimic the ocean acidification. They try to look at the rate of shell growth in a variety of species, from crustaceans to algae, from tropical to temperate environments by raising a few species of coralline algae and corals, which serve as the basis for key habitats, as well as marine living that feed the seafood industry in the test tanks. (Ries et al., 2009).

As a result, the shells of some species, such as conchs, deteriorate substantially at the highest CO_2 levels utilised, as predicted. For example, tropical pencil urchin spines have disintegrated into nubs. As CO_2 levels climbed, oysters, clams and scallops have developed fewer shells. On the other hand, two species of calcifying algae performed better at 600 ppm CO_2 compared to current CO_2 levels, but then performed even worse at even greater CO_2 levels. The biggest shock came from the crustaceans. As CO_2 levels rose, all three species studied, including American lobster, giant prawn and the blue crab, contradicted predictions and developed heavier shells. Ries and his team concluded that temperate urchins, crustaceans, red coralline algae, and mussels are less vulnerable to acidified saltwater than hard clams and conchs, which have less-protective shells. Ries et. al., (2019) claimed that while all of the test organisms continued to make new shells throughout the experiment, some lost a net amount of shells as older and more massive portions of their shells dissolved at the highest CO_2 circumstances.

2.2.2 Seaweed responses to ocean acidifications

Ocean acidification has become a threat to marine ecosystems, leading to lower pH levels interfering with many species' life processes and eventually impacting commercially important animal groups such as molluscs and crustaceans. As these two kinds of species grow, they deposit shell material. However, the shell production is inhibited by a decreased pH caused by increased amounts of dissolved CO_2 in seawater. This phenomenon will eventually lower the pH level of seawater due to the formation of carbonic acid that dissociates into bicarbonate and hydrogen ions. Seaweed respiration rate is usually high at night, but the amount of oxygen consumed and CO_2 emitted in the dark does not usually offset the amount of O_2 produced and CO_2 absorbed in the light during the day. Because shellfish constantly breathe CO_2 while seaweeds absorb it, this exchange of inorganic carbon could be another mutually advantageous component to consider as part of an ecosystem management strategy with Integrated Multi-Trophic Aquaculture (IMTA), especially when the seawater gets more acidic over time.

According to research, seaweed farming leads to a net positive increase in pH and oxygen in the immediate region of the farm activities, notably during the exponential growth phase of the seaweed life cycle. Seaweeds act as carbon dioxide sink that can offset some of the consequences of climate change and ocean acidification to a degree proportionate to the scale of culture. Seaweeds are the closest to plant-based agriculture of all the aquaculture crops being investigated for large-scale production since they both rely on photosynthesis to thrive. (Langton et al., 2019).

2.3 Environmental roles of seaweed

Seaweeds have historically been used as food, both for humans and livestock, as well as medicine. Seaweeds are also a source of unique chemical extracts used as fertiliser and soil conditioners. In recent years, the traditional wild harvesting of seaweeds has been supplemented by cultivation, which employs a limited number of species that are well adapted to their environment and intended use (Langton et al., 2019). Over time, the use of seaweeds has expanded to include bio-based fuels and new specialty biochemicals. The potential production scale is changing, especially as governments and entrepreneurs consider the large-scale farms required for biofuel production (Roesijadi et al., 2011). Aside from these industrial applications, seaweeds are increasingly recognised for their ecosystem services.

Seaweed cultivation does not require freshwater, land, or nutrient resources, allowing them to be conserved, unlike crops that require irrigation, fertilisation, and tillable soil. As primary producers, seaweeds absorb inorganic nutrients and carbon dioxide, thus converting them into organic matter via photosynthesis. In the environment ecosystem, seaweed has been promoted to reduce ocean acidification and mitigate climate change by acting as a carbon dioxide excellent absorber (Duarte et al., 2017). Furthermore, their ability to extract nutrients and scrub off some toxic chemicals from the water may be used as tools for remediating both coastal eutrophication and pollution (Marinho et al., 2015). As a result, these characteristics may provide some protection against harmful algal blooms.

2.4. Gracilaria changii

2.4.1. **Introduction to *Gracilaria changii***

Agarophytes or macroalgae are seaweeds that produce agar and can be found in all types of marine ecosystems with a broad spectrum of seawater temperature, salinity and pH. Many red seaweeds produce agar that acts as a cell wall polymer. It is frequently used in the food industry as a culture media in biological research, thickening and gelling agent, agar-based nanoparticles and biodegradable carrier for drug delivery systems, wound dressing and foods packaging (Alba & Kontogiorgos, 2018). Based on species-specific physiological traits such as nutrient uptake kinetics and economic value, several known seaweed taxa and cultivars have been investigated as possible biofilter organisms (Mawi et al., 2020). Red seaweed, also known as *G. changii* thrives on intertidal mudflats, where it attaches itself to shells, mangrove tree roots, plastic waste, fish cages and stones like an epiphyte. In terms of the number of species and global distribution, Gracilaria is the most important genus in the Gracilariacea family (Maslie, 2010).

There are 285 Gracilaria species worldwide, with 170 taxonomically recognised as stated by Guiry and Guiry (Lee et al., 2019). The carposporophyte develop toward the thallus periphery, crucially divided tetrasporangia and a pseudoparanchymatous morphology in which the medulla cells are more or less isodiametric than filamentous are all shared characteristics across the family members. According to Lim and Phang, the agarophytic genus Gracilaria comprises twenty species that grow in mangroves, sandy mudflats, and rocky beaches (Yow et al., 2011). In Malaysia, *G. changii* is one of the most abundant agarophytic seaweeds species that grows mainly in the mangrove lands (Andriani et al., 2016). Gracilaria agar has a larger amount of sulfated sugar, which can be improved by alkaline or enzymatic hydrolysis with sodium hydroxide and sulfohydrolase, respectively, during agar preparation.

Figure 2.1: Red seaweed (*G. changii*) thalli

2.4.2. Nutrient content

In terms of nutrient content, *G. changii* had a more significant percentage of unsaturated fatty acids (74%), primarily omega-3 and omega-6 fatty acids, and a lower percentage of saturated fatty acids (26%), primarily palmitic acid, as well as relatively high calcium and iron levels. The primary amino acid components are glutamic acid, arginine, glycine and alanine. Natural compounds extracted from the red algae could be used as anti-inflammatory and gastric anti-ulcerogenous therapies as food and traditional drugs that have been used since the old days (Shu et al., 2013). Currently, only this algae's colloidal agar content and local delicacies of this algae are harvested commercially. These algae are widely used to treat different diseases, including inflammation and gastric disorders (Shu et al., 2013). *G. changii* nutritional contents, in combination with their physicochemical features, have the potential to become a valuable food additive (Chan &

Matanjun, 2017). The biomass of *G. changii* populations in two mangrove sites in Selangor and Malaysia's western coast, ranged from 58 to 98 g/m^2 of dry weight. The growing use of seaweed as a source of biopharmaceuticals, nutraceuticals, and biofuels has boosted global demand for seaweed resources are why red seaweed was chosen for this experiment. *Gracilaria changii* appears to be a good candidate for potential commercialisation due to its high-quality agar and adaptability to harsh mangrove conditions (Yeong et al., 2014)

2.4.3. Growth and development

One of the elements required for photosynthesis is carbon dioxide (Arbit, Omar, Tuwo, & Soekendarsi, 2018). *G. changii* is a photosynthetic plant that takes up carbon, phosphorus and nitrogen during photosynthesis. As it grows, seaweed can remove massive volumes of excess nitrogen and carbon dioxide. The growth and development of *G. changii* may be influenced by rainfall, salinity, sunshine duration, temperature and nutrient levels in the water. The salinity in the mangroves where *G. changii* grows is between 27 ppt and 35 ppt. Due to water movement, the sandy or muddy bottom relief changes, and some of these thallus fragments become exposed to the light and begin to grow. They will sprout and branch until their size and shape cause water frictional drag to rise. Some parts will sink, eventually being covered by sand resulting in new thalli formation (Maslie, 2010). Although the advanced aquaculture system is not a novel concept, there are fewer examples of aquatic animal polyculture with seaweeds such as corals and crustaceans. As part of an integrated aquaculture system, a seaweed production or biofilter system is being developed to lessen the environmental impact of marine wastewater in coastal habitats.

2.5. The importance of bicarbonate ions in seawater

Carbon is one of the most critical elements in photosynthesis, vital for plant growth and development. Despite its critical role in biological metabolism, excessive or insufficient carbon levels are toxic and hinder the growth of the organisms. There is 0.041 percent of carbon content in the Earth's atmosphere (Vietti & Fastook, 1976). Therefore, terrestrial higher plants can successfully utilise carbon (CO_2) from the surrounding environment. On the other hand, photosynthesis in aquatic plants occurs in a more complex carbon source environment with lower carbon concentrations and more diverse carbon types. Bicarbonate is the most abundant group of dissolved inorganic carbon in natural seawater at pH 8.2, with a concentration of 2 mmol/L, followed by carbonate and carbon dioxide (Zou & Gao, 2002). Most carbon dioxide dissolves in saltwater is converted to bicarbonate and hydrogen ions. The reduction in pH is due to an increase in hydrogen ions. Furthermore, part of the hydrogen reacts with carbonate to generate additional bicarbonate, lowering the carbonate content in saltwater. Carbon dioxide is partially transformed to carbonate ions (CO_3^{2-}), bicarbonate (HCO^{3-}), hydrogen ions (H^+), and carbonic acid upon coming into direct contact with water.

$$\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO^{3-} \leftrightarrow 2\ H^+ + CO_3^{2-}}$$

Seawater has a substantially higher CO_2 assimilation capacity than fresh water. This is because bicarbonate and carbonate ions have been continuously dumped into the sea for ages (Taylor et al., 2015). Carbonate interacts with CO_2 to create bicarbonate, resulting in increased CO_2 absorption and decreased CO_3^{2-} concentration in the ocean. Dissolved inorganic carbon (DIC) refers to all CO_2-derived chemical species in water, including carbonic acid, carbon dioxide, carbonate ions and bicarbonate. CO_2 uptake by photosynthesis and CO_2 release during respiration impacted aquatic dissolved inorganic carbon (DIC) concentrations (Jiang et al., 2014). The number of free protons in saltwater and the pH value are determined by this carbonic acid-carbonate balance. Because oxygen is used during aerobic respiration, free oxygen diminishes as DIC rises. A vast amount of research in marine macroalgae has focused on gaseous CO_2 application instead of bicarbonate salts ($NaHCO_3$) on growth and biological processes. The use of gaseous CO_2 can accomplish the stated goal. However, in commercial farming operations where CO_2 supplementation is limited due to the cost of a gas bottle and complex gas mixing equipment, an alternative technique of delivering carbon nutrition via carbonate or bicarbonate could be used (Shu et al., 2013).

2.6 Water quality analysis towards the survival rate of seaweed

2.6.1. Acclimatisation

Like other organisms, *G. changii* must undergo an acclimatisation process before culturing in artificial seawater. Acclimatisation is the process by which an individual organism adjusts to changes in its environment such as, pH, altitude, temperature, photoperiod, or humidity that allows it to retain performance under various situations. Acclimatisation takes place over around 3-4 days for *G. changii*. Therefore, any physiological changes need to be monitored daily to confirm the adaptability towards the synthetic seawater. Moving the agar from lower to higher salinities had little effect on gel strengths, indicating that agar structure is not significantly altered as an acclimation response in a short time frame. Previous research by Daugherty and Bird in 1988 suggested that some interaction between temperature and salinity in terms of optimal growth had a strong effect on Gracilaria productivity. The effect of salinity on agar yield was significant, and the interaction of salinity and temperature on agar yield suggests that these two factors can influence the overall agar content. Review sites with moderate temperature fluctuations in the 22-32°C range, as well as moderate freshwater input or constant oceanic salinities of 30-32 ppt, should be ideal. Such conditions result in year-round high growth and high gel strength. Seaweed productivity and physiological functions may also be affected by changes in seawater pH and the availability of dissolved inorganic carbon (Daugherty & Bird, 1988).

2.6.2. The effect of pH towards the carbon uptake by *G. changii*

To reflect new environmental conditions, it is likely that new growth, thus novel agar synthesis is required. Changing the pH of coastal water alters the chemistry of inorganic carbon, causing abiotic stress in photosynthetic marine organisms. Sulphated agar is found in red algae's cell wall, protecting them from environmental stresses. *G. changii* are expected to have the ability to utilise bicarbonate as an external inorganic carbon source for photosynthesis since the extracellular carbonic anhydrase activity catalysed HCO_3 dehydration, which was intimately linked to bicarbonate use. The inorganic carbon content of saltwater might easily saturate the seaweeds' photosynthesis. Significantly low pH conditions in ocean acidification will affect seaweed performance and cause abiotic stress to the organisms. Although, seawater has a natural bicarbonate buffering system, pH fluctuations in the coastal ecosystem can occur daily, ranging from 7.8 to 9.1, with a significantly lower pH at night (Lee et al., 2019). Lower diffusion rates of atmospheric carbon dioxide into highly alkaline seawater, poorer operation of ion pumps for essential ion uptake, reallocation of energy to maintain a lower cellular pH, and conversion of bicarbonate to carbon dioxide via the carbon-concentrating mechanism (CCM) could all contribute to the decrease in growth rate (Zhou et al., 2016).

2.7 Biotechnology interventions

Currently, aquaculture industries are struggling with numerous challenges, particularly in natural resource degradation. Regardless of the fact that water covers almost three quarters of the planet, there is a limited supply of fresh water (Dervash et al., 2020). Aquatic pollution is one of the main challenges in the vast field of environmental issues in the modern day. Biotechnological interventions offer a wide range of options in the form of cutting-edge remediation procedures to prevent the growing concern of water pollution. It comprises all novel developments that enhance and mediate biological entities' activity. Therefore, biotechnological tools like bioremediation are very beneficial to preserve ocean pH regime to reduce ocean acidification (Vangronsveld et al., 2009). Through the use of biological organisms with an increasing emphasis on seaweeds, bioremediation is a sustainable treatment approach that lessens the impact of this pollution on the marine environment. Recent research has revealed that the species Gracilaria (Rhodophyta sp.) is better suited for bioremediation potential in intensive aquaculture due to its capacity to rapidly absorb more nutrients (Mawi et al., 2020). Due to the nature of seaweed that requires low nutritional needs, this organism is an effective and affordable biofilter. Seaweed photosynthesis and growth depend on inorganic carbon, light, and nutrients, which also interact to control the rate of seaweed production. The removal of DIC from the surrounding saltwater by seaweed photosynthesis alters the seawater's carbonate system, causing pH to rise and the availability of CO_2 and HCO_3 to fall. According to statistical research, seaweed have a greater absorption rate than other microbial biosorbents, ranging from 15.3 to 84.6%. Therefore, seaweed aquaculture has the potential to absorb anthropogenic carbon dioxide emissions that contribute to ocean acidification and beneficial not just to the economy but also to the ocean's health (Roleda & Hurd, 2019). However, at pH 9.0, the majority of DIC is present as carbonate, which cannot be used for photosynthesis (Björk et al., 2004).

CHAPTER 3: MATERIALS AND METHODS

3.1 Experimental design

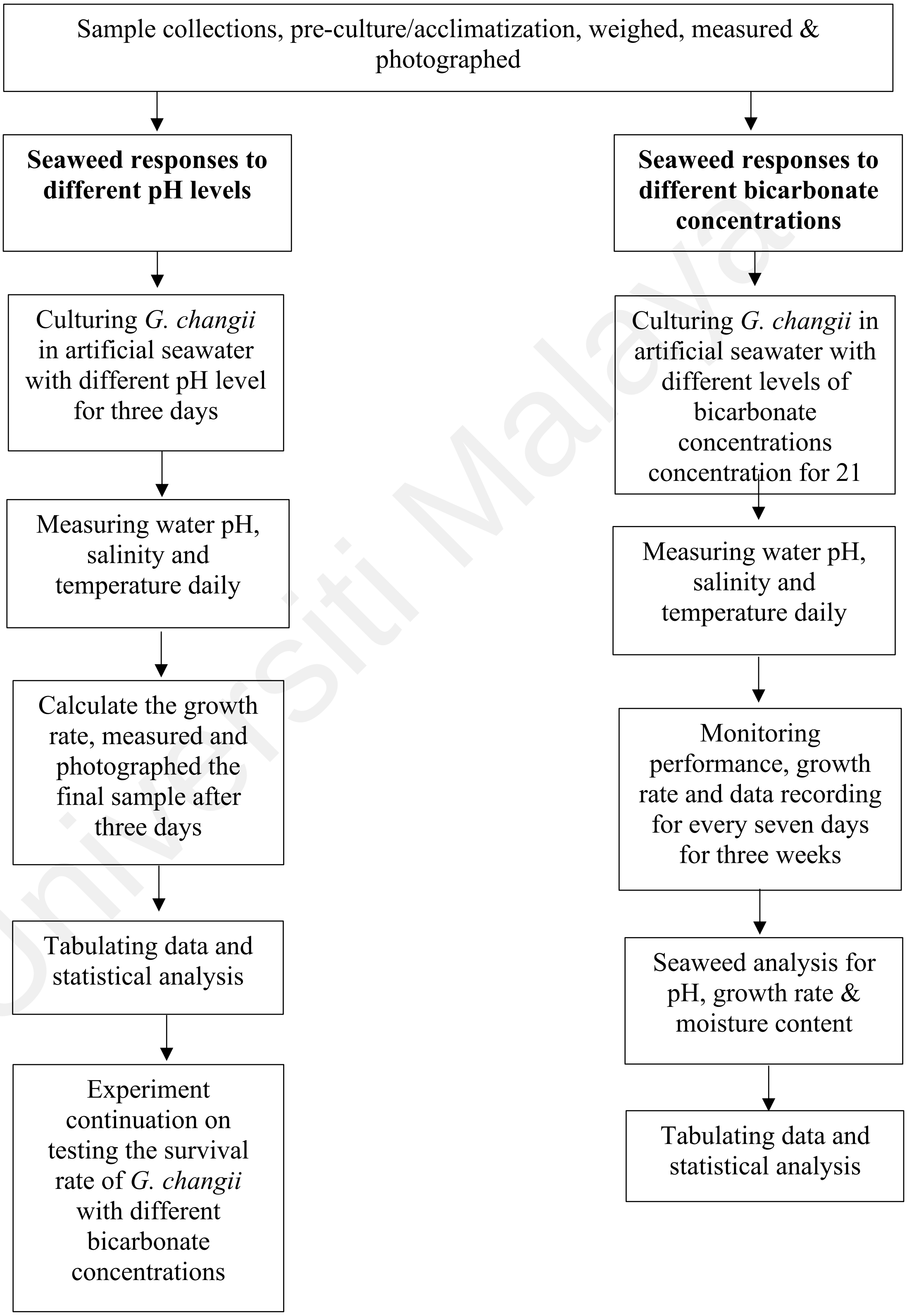

Figure 3.1: Experimental design

3.2 Sample collection and pre-culture/acclimatisation

A total of 6 kg samples of *G. changii* were collected from a seawater mudflat in Morib Beach, Banting, Malaysia (2°45'10"N 101°26'26"E) during early March 2021. These seaweeds were submerged at the time of collection in the murky water with significant turbidity cast in shadow by the mangrove tree. The seaweeds were put in a plastic bag with fresh seawater and brought back to the lab immediately. All thalli were hand-cleaned under running tap water to remove any visible mud, sand and epiphytes. A soft brush was used to scrub off the remaining epiphytes growing on the seaweed thalli. After being cleaned, only healthy thalli were chosen and cultured in eight tanks of 10-L aquariums filled with artificial seawater of 30 ppt salinity with formulation as shown below. A refractometer was used to confirm the level of salinity of the seawater. The samples were illuminated with LED light in blue-violet (435 nm) and red (680 nm) wavelengths to support photosynthesis (Zetlight E200S) throughout the incubation period. The experiment area was covered with canvas to limit the sunlight from outside. The pH was adjusted using 1.0M HCL and 1.0M NaOH. The water quality, including salinity, temperature and pH, were monitored daily. The pre-cultured samples were then taken out after a week and photographed, measured and weighed before starting the next phase.

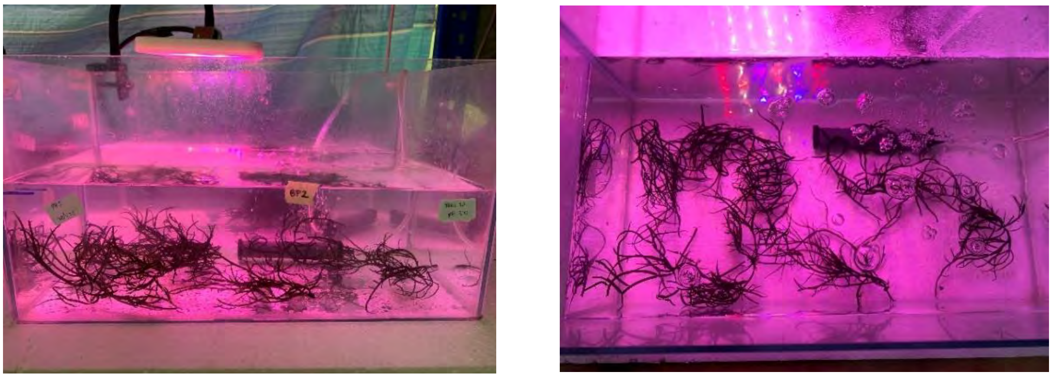

Figure 3.2: The acclimatisation of *Gracilaria changii* in 10-L aquarium of saltwater at salinity 30 ppt illuminated with LED light - side & top view of the aquarium tank.

3.3 Culturing *G. changii* in artificial seawater with different pH levels

Eight tanks of aquarium (0.4 x 0.2 x 0.2 m) equipped with aeration and LED light (Zetlight E200S) were used in this experiment (Figure 1). The tanks were filled with 10L of saltwater with 30 ppt salinity. The experiment consists of four treatments with different levels of pH; T1 (pH 8), T2 (pH 7), T3 (pH 6) and T4 (pH 5) were arranged in duplicate next to one another as shown in Figure 3. All treatments were equipped with two replicates, and each tank was allocated with three healthy seaweed at an average weight of ±10 g wet weight (W_t). The experiment was conducted for three days at 12h:12h (light: dark photoperiod). The experiment area was covered with canvas to limit the sunlight from outside. The pH was adjusted using 1.0M HCl and 1.0M NaOH. Each aquarium was supplemented with two drops of nitrogen supplement (Seachem Fluorish Nitrogen) for seaweed nutrient requirements. The water quality, including salinity, temperature and pH, was monitored every day for three days. The weight of seaweed was taken before the experiment, after 24 and 72 hours.

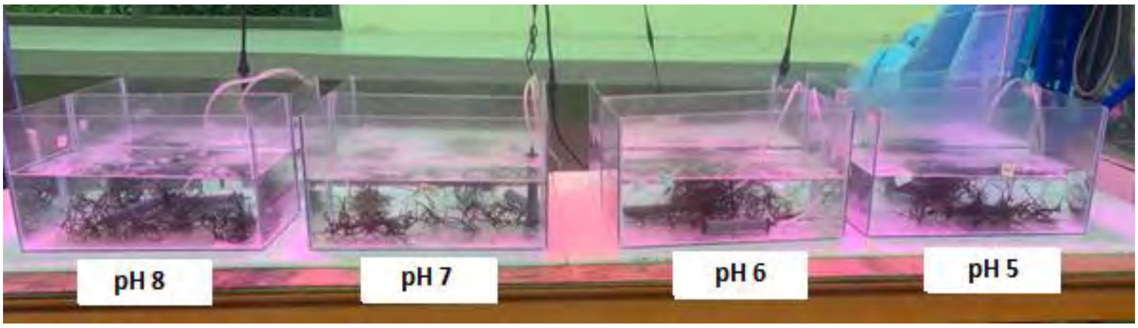

Figure 3.3: The cultivation of *Gracilaria changii* in 10-L aquarium of saltwater at salinity 30 ppt illuminated with LED light treated with different pH levels ranging from pH 5 to pH 8.

3.4 Culturing *G. changii* in artificial seawater with different bicarbonate concentration

New artificial seawater was prepared under the laboratory conditions with the salinity level of 30 ppt and pH 8 filled in the tanks of aquariums illuminated with LED light (Zetlight E200S). Then, all aquariums were supplemented with sodium bicarbonate, $NaHCO_3$ (Sigma-Aldrich) used as the culture medium. $NaHCO_3$ was set by doubling the concentration at four different levels, 2 mmol/L, 4 mmol/L, 8mmol/L and 16mmol/L. The 2 mmol/L concentration was being set without supplemental $NaHCO_3$ and was used as the control in this experiment. Each concentration was filled in two aquariums, respectively. Calcium bicarbonate does not exist as a solid and must be directly created in the water phase. This was done by mixing sodium bicarbonate and calcium chloride salts, both were purchased in solid form. The added sodium and chloride were offset by reducing the amount of sodium chloride added to make up the total salinity. Using a different set of samples, five healthy samples of acclimatised or pre cultured *G. changii* weighing approximately 3g each was placed in each tank. At the same time, the temperature and bicarbonate concentration was maintained throughout three weeks. The whole experiment is being set up in duplicate. The condition, colour, growth form, size and weight of algae were constantly monitored every seven days and all data were recorded. After three weeks, all samples were taken from each aquarium to analyse specific growth rate, pH and moisture content.

3.4.1 Measurement of the growth rate of *G. changii*

All final samples from each tank will be taken out and dried with tissue paper to remove excess water before weighing on an electronic weighing balance for final weight. The specific growth rate (SGR) was expressed as % day^{-1} and calculated from the following formula proposed by Yu and Yang (Yu & Yang, 2008).

$$\textbf{SGR} = \left[\left(\frac{W_t}{W_0}\right)^{\frac{1}{t}}\right] \textbf{ x 100\%} \qquad \textbf{(3.1)}$$

Where,

W_t is the final fresh weight of the sample (g)

Wo is the initial fresh weight (g)

t is the experimental period (days).

3.4.2 Moisture content

The seaweed samples were oven-dried overnight at 40°C until they reached constant weight to analyse the moisture content. The dried samples were weighed on an electronic weighing balance and the value obtained was recorded. Moisture content was calculated as the following formula:

$$\textbf{Moisture content} = \left[\frac{W_{wet} - W_{dry}}{W_{wet}}\right] \textbf{ x 100\%} \qquad \textbf{(3.2)}$$

Where,

W_{wet} is the fresh weight of the sample (g)

W_{dry} is the dry weight of the sample (g)

3.5 Statistical analysis

The replication was expressed in terms of mean ± standard error. The software IBM SPSS Statistics Version 26 (from SPSS Inc, USA) was used to perform data analysis. Test conducted to determine the significant difference in mean between two groups. In addition, one-way analysis of variance (ANOVA) with a 95% significance level was used to determine the significant pH increment of saltwater and SGR of *Gracilaria changii* while a one-sample t-test to analyse the moisture content of the seaweed (Rabiei et al., 2014).

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Growth performance and pH levels of seaweed cultured under different levels of pH treatments

In this study, the salinity and temperature were maintained at 30 to 31 ppt and 25.6°C – 27.2°C throughout three weeks, respectively. The pH levels were not required to be maintain since the rate of pH fluctuation were recorded to determine the seaweed's availability to optimize the seawater's pH level. The results of the mean growth performance ± SE and the total weight gain of seaweed cultivated at various pH levels within three days are shown in Table 1. After one hour of cultivation, the weight for seaweed was not taken as there was no significant change in seaweed in the short period. The initial weight of seaweed in all treatments is the same and does not differ statistically. However, compared with other treatments, the weight of seaweed in T4 decreased immensely after 24 hours of cultivation. The increasing growth performance was observed in seaweed from T4 persists towards the end of three days of the experiment. On the contrary, after three days of the trial, the seaweed in T1, T2 and T3 indicate an increment in body weight. The growth rate of *Gracilaria changii* is generally high, thus, the significant results can be observed within three days (Lee et al., 2019). This can eventually conclude that seaweed can thrive at pH levels 8, 7, and 6 but not at pH 5.

Figure 4 shows the result of pH during the experimental period. In T1, the pH reading significantly increases after 24h. However, the reading reduced significantly after 48 h and 72 h. On the other hand, the pH value increases significantly from the initial day to the final day (after 72h) in T2, T3 and T4. According to the findings, it can be concluded that seaweed can enhance the pH level from acidic to alkaline. This is due to their ability to soak up the CO_2 through photosynthesis and subsequently increase the pH level within a short period.

In terms of seaweed morphology, from the observation, there are no significant changes in physiology and colouration shown by the seaweed from T1, T2 and T3. However, there is a noticeable change observed in the weight and colour of seaweed from T4. Upon monitoring for 72 hours, the seaweed has begun to wilt and turn pinkish, thus, supporting the data presented in Table 2 that shows the significant decrease in seaweed weight recorded in T4. Because of inputs from lab-scale experiments, the pH of seawater would fluctuate more in coastal seas where seaweeds are abundantly found. However, because ocean acidification lowers pH regimes, moving the pH range to a lower one, increased CO_2 intake can still impact seaweed in the aquarium tanks.

Table 1: Growth performance and total weight gain of seaweed cultured under different levels of pH treatments in three days of experimental periods.

Variables	**Period**	**Treatments**			
		T1 (pH 8)	**T2 (pH 7)**	**T3 (pH 6)**	**T4 (pH 5)**
Growth performance (g)	Initial	10.07 ± 0.08^{a}	9.89 ± 0.16^{a}	10.22 ± 0.06^{a}	10.49 ± 0.24^{a}
	After 24H	10.34 ± 0.08^{b}	10.21 ± 0.17^{b}	10.31 ± 0.06^{b}	8.47 ± 0.22^{a}
	After 72H	10.65 ± 0.09^{b}	10.46 ± 0.24^{b}	10.53 ± 0.11^{b}	8.32 ± 0.20^{a}
Total weight gain, $W_f - W_i$ (g)	After 72H	0.58	0.57	0.31	-2.17

^{a,b} Within a row indicates there is a significant difference obtained in the data with p-value less than 0.05 (n= 4). The results represent mean ± standard error of mean (SEM).

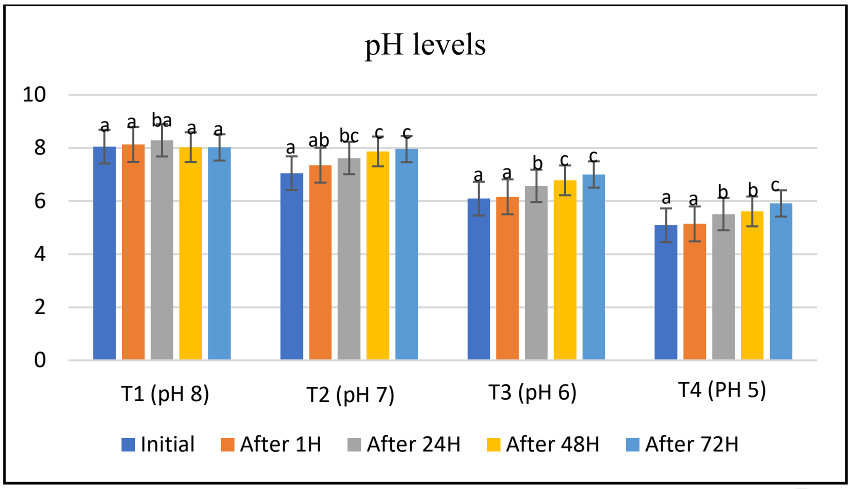

Figure 4.1: pH levels of seaweed cultured under different levels of pH treatments within three days of experimental periods

4.2 Seaweed tested with different concentration of bicarbonate levels

4.2.1. The specific growth rate of *Gracilaria changii* treated with different concentrations of bicarbonate in three weeks

In this study, the salinity and temperature were maintained at 30 to 31 ppt and 25.6°C – 27.2°C throughout three weeks, respectively. Table 3 shows the mean growth performance ± standard error and weight gain of *G. changii* treated with different concentrations of bicarbonate in three weeks. Upon the first week of monitoring, the results obtained from Day 7 show a slight decrease in growth for seaweed treated with 8 mmol/L and 16 mmol/L but does not show any significant difference in all treatments. During the second week, all seaweed shows positive growth with a remarkable weight increment recorded throughout that particular week and there is a significant difference ($p < 0.05$) for the seaweed treated with 2 mmol/L and 16 mmol/L of bicarbonate. Finally, on the last monitoring day, all seaweeds show an inclining growth pattern but no significant difference.

Table 2: The growth performance and weight gain of *G. changii* treated with different bicarbonate concentrations in three weeks.

Variables	**Day**	**Treatments**			
		2 mmol/L	**4 mmol/L**	**8 mmol/L**	**16 mmol/L**
Growth performance (g w/w)	Day 0	15.66 ± 0.14	15.33 ± 0.12	15.20 ± 0.31	14.93 ± 0.10
	Day 7	15.89 ± 0.3	15.44 ± 0.14	15.03 ± 0.27	14.85 ± 0.25
	Day 14	16.34 ± 0.27*	15.88 ± 0.12	16.43 ± 0.24	15.27 ± 0.24*
	Day 21	15.83 ± 0.17	15.42 ± 0.08	15.62 ± 0.23	15.14 ± 0.15
Total weight gain, $W_f - W_i$ (g)	After 21 days	0.17	0.09	0.42	0.21

*Within a row indicates significant difference obtained in the data with p-value less than 0.05 (n= 4). The results represent mean ± standard error of mean (SEM).

The results of the specific growth rate (SGR) of seaweed cultivated at various bicarbonate concentrations are shown in Figure 5. Results were estimated as mean and standard error. The mean of specific growth rate for *G. changii* treated with 2 mmol/L, 4mmol/L, 8 mmol/L and 16 mmol/L bicarbonate concentration are 0.156 ± 0.052% day^{-1}, 0.077 ± 0.025% day^{-1}, -0.064 ± 0.097% day^{-1} and -0.030 ± 0.048% day^{-1}, respectively. Upon observation conducted throughout the three weeks of experimental periods, the SGR graph shows the same pattern for all concentrations.

Seaweed treated with bicarbonate levels of 8 mmol/L and 16 mmol/L showed a negative SGR value of -0.161% day^{-1} and -0.077% day^{-1}, respectively, during the first week of cultivations. This indicates a reduction in the growth rate of the seaweed throughout the first week. However, the SGR of *G. changii* has recorded a remarkable increment and reached its peak between the first and second week of monitoring for all treatments. The increased growth was attributed to improved N-assimilation. DIC acquisition uses passive diffusion to absorb CO_2, which is more energy-efficient than active transport to absorb HCO_3 that uses energy (Raven et al., 2011). Seaweeds can enhance their uptake of CO_2 in comparison to HCO_3 while growing in environments with low light as a means of lowering their energy expenses (Celis-Pla et al., 2015).

On the other hand, the SGR value of *G. changii* dropped during the second and third weeks of monitoring for all treatments. This indicates that the species studied showed a decrease in growth rate, probably as a result of decreased CO_2 (Fernández et al., 2015). The impacts of rising CO_2 levels on seaweeds are mostly determined by the degree of carbon restriction in natural systems. Even at low CO_2 concentrations, such growth suppression was linked to a reduction of photosynthetic activity. Such non-responsiveness occurs due to the presence of CCMs that use HCO_3^- as a source of energy.

Seaweeds require an exogenous inorganic carbon supply to promote photosynthesis and growth. Under current atmospheric circumstances, the photosynthesis of seaweeds would be severely limited if it relied solely on CO2 diffusion from the medium to the fixation site via the carbon-assimilating enzyme Rubisco. Despite the presence of CO_2-concentrating mechanisms (CCMs) that allow the algae to efficiently exploit the bulk bicarbonate (HCO_3^-) pool in saltwater, photosynthesis in the examined species can be fully or less saturated with the current ambient dissolved inorganic carbon (Ci) composition. However, the response of macroalgal growth rate to elevated pCO_2 in seawater is species-specific (Zou & Gao, 2010).

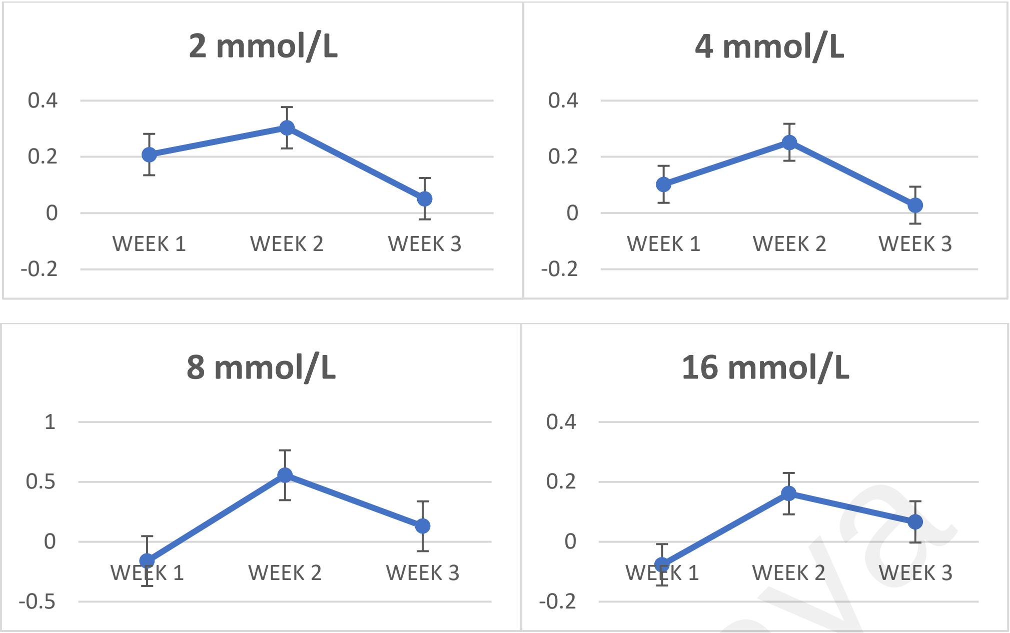

Figure 4.2: Specific growth rate of *G. changii* in three weeks.

In terms of morphology, there is a strong connection on how nitrogen and carbon in saltwater can influence the morphology of the seaweed over time (Roleda & Hurd, 2019). From this study, we observed that the seaweed showed degradation of the thallus fragments towards the end of the experiment. This occurrence, also known as thallus fragmentation, has contributed to weight loss and reduced growth rate of seaweed. In some cases, thallus fragmentation is actually showing a sign that the seaweed has reached reproductive maturation (Liu et al., 2017). However, this is possible in this study due to lower photosynthetic rates at high pH. Other than that, lower diffusion rates of atmospheric carbon dioxide into highly alkaline seawater, poorer operation of ion pumps for essential ion uptake, reallocation of energy to maintain a lower cellular pH, and conversion of bicarbonate to carbon dioxide through a carbon-concentrating mechanism (CCM) could all contribute to the decrease in growth rate (Riebesell, 2004).

Initially, we observed that there was a discolouration of the seaweed morphology. The seaweed discolouration occurs due to the decreasing availability of nitrogen sources in its surroundings (Sasuga et al., 2018). We supplied two drops of nitrogen-supplemented nutrients in the tank once a week because both carbon and nitrogen are necessary for seaweed growth. The concentration of DIC in saltwater can influence the amount of nitrogen taken up by seaweed. Towards the end of the experimental periods, positive results showed in the colouration of the seaweed which turned the seaweed from brown to dark red. This indicates that the seaweed absorbed sufficient nutrients from the saltwater with the help of water motion circulating in the aquariums.

4.2.2. The pH changes of *Gracilaria changii* in 21 days

Figure 6 shows the trend of pH of *G. changii* in three weeks. In this study, the salinity and temperature were maintained at 30 to 31 ppt and 25.6°C – 27.2°C throughout three weeks, respectively. The pH of all treatments during the experiment were maintained within 0.1 units of the treatment target of pH 8 throughout the experiment. The mean of pH recorded for saltwater (cultivated with *G. changii*) treated with 2 mmol/L, 4mmol/L, 8 mmol/L and 16 mmol/L bicarbonate concentration throughout the three weeks of the experiment are pH 8.3 ± 0.1, pH 8.4 ± 0.1, pH 8.4 ± 0.1 and pH 8.4 ± 0.1, respectively. Upon observation conducted throughout the three weeks of experimental periods, the pH graph shows almost the same pattern for all concentrations and does not show any significant difference for all treatments.

During the first week of observations, a pH fluctuation was recorded for all treatments. The highest pH fluctuation was observed at bicarbonate levels of 2 mmol/L with an increment of 0.45 units (pH 7.93 at day 0 and pH 8.38 at day 7) while the lowest pH increment was observed at bicarbonate levels of 8 mmol/L with an increment of 0.37 units (pH 8.1 at day 0 and pH 8.47 at day 7). This occurs due to the seaweed's ability to absorb dissolved inorganic carbon (DIC) that can absorb dissolved inorganic carbon (DIC), which allows them to alter local seawater chemistry, resulting in carbon, pH, and oxygen gradients in their proximity. Seaweed can operate as carbon sinks by taking up both bicarbonate and CO_2 as carbon sources for photosynthesis to support its growth, reducing acidity and boosting dissolved oxygen in the surrounding area (Murie & Bourdeau, 2020). Photosynthetic fixation of dissolved CO_2 in seaweed results in a decrease in DIC and pCO_2 resulting in a rise in pH level (Jiang et al., 2014). Thus, we can conclude that seaweed has the ability to absorb carbon from the water and creates oxygen, perhaps, eliminating enough CO_2 to alleviate acidification.

During the second week of observations, a slight pH increment was observed for all treatments but not as great as the first week. The highest pH fluctuation was observed at bicarbonate levels of 2 mmol/L with an increment of 0.13 units (pH 8.38 at day 7 and pH 8.51 at day 14) while the lowest pH increment was observed at bicarbonate levels of 16 mmol/L with an increment of 0.07 units (pH 8.48 at day 7 and pH 8.55 at day 14). At this level, although seaweed respiration releases CO_2 into the seawater, the rate of photosynthesis surpasses the respiration rate. Subsequently, the released of CO_2 is balanced by CO_2 uptake during photosynthesis.

During the last week of observations, only saltwater treated with 4 mmol/L of bicarbonate concentration showed a slight decrement by 0.05 units (pH 8.57 at day 14 and pH 8.52 at day 21). For saltwater with bicarbonate concentrations of 2 mmol/L, 8 mmol/L and 16 mmol/L, an increment was recorded with 0.02 units, 0.07 units and 0.09 units, respectively. The highest pH recorded throughout these three weeks of the experiment was 8.64. At pH 9.0 above, most DIC is available as carbonate, which cannot be used in photosynthesis (Björk et al., 2004). As a result, carbon may inhibit the growth of some seaweeds in highly productive environments. However, since it is species-specific based approach, this will depend on the mechanisms of DIC uptake and their carbon requirements for growth (van der Loos et al., 2019).

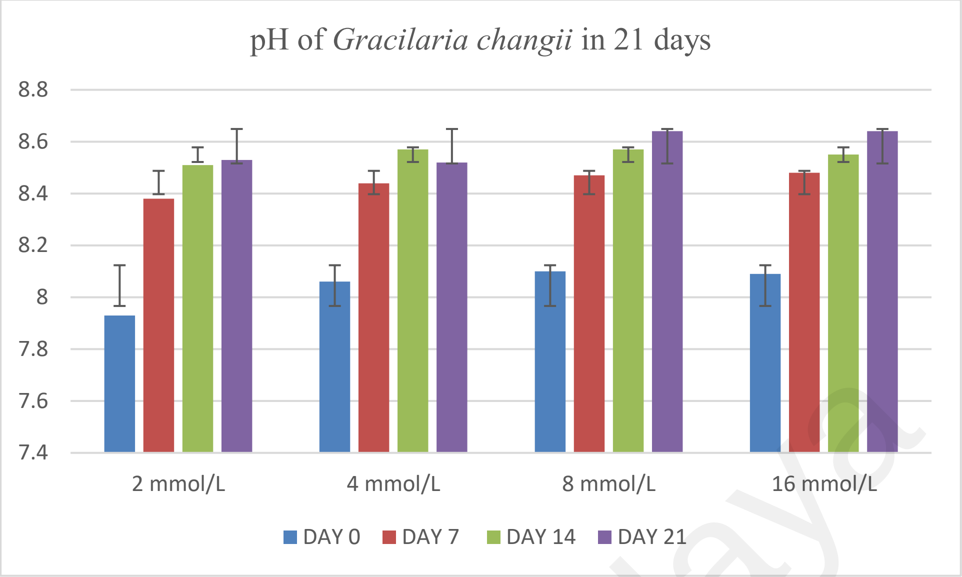

Figure 4.3: The pH of *G. changii* treated with different bicarbonate concentrations within three weeks.

4.3 Moisture content

By definition, dry mass is a sample mass after all the free water has been removed from it by oven-drying, a value found by subtracting the moisture content from the wet mass. Depending on the species, fresh seaweed has a high moisture content of up to 85% (Gupta et al., 2011). In this study, a one-sample t-test was used to determine whether the mean moisture content of *G. changii* treated with different concentration of bicarbonate is different from the moisture content of *G. changii* that thrives in their natural habitat. The average value of the dry mass of alga samples of the *G. changii* species obtained here was 85.25% with a standard deviation of 0.47%.

These results provided a good indicator of the biomass to estimate the quantity of sample to be collected according to the mass required for a given analysis. The moisture content was expressed as a percentage by the weight of the sample. Seaweed physiology would be affected by increased CO_2 levels in the water. Therefore, one of the most fundamental and analytical procedures which need to be performed is moisture content determination. Moisture is a quality factor often specified in the compositional standard (Syad et al., 2013). In order to make seaweed for food consumption purpose, the amount of water content is critical because it impacts sensory quality, microbiological stability, physical properties, and shelf life (Silva et al., 2008). Therefore, these studies provide an excellent biomass indicator for estimating the quantity of sample to be collected as a function of the mass necessary for a certain study, as well as for estimating the stability and microbiological safety of *G. changii* used as feeds. The data collected also can be used for further analysis to determine their nutritional compositions.

Table 3: Moisture content of *G. changii* treated with different bicarbonate concentrations.

TREATMENTS	WET MASS (g)	DRY MASS (g)	MOISTURE CONTENT (%)
2 mmol/L	15.83	2.28	85.6
4 mmol/L	15.42	2.34	84.8
8 mmol/L	15.62	2.36	84.9
16 mmol/L	15.14	2.17	85.7

Table 4: One-sample t-Test statistic of moisture content.

Variables	Treatments				One-sample statistics
	2 mmol/l	4 mmol/l	8 mmol/l	16 mmol/l	
Moisture content (%)	85.6	84.8	84.9	85.7	85.25 ± 0.47

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study concluded that seaweed growth rate would be affected by the decrement of pH level and CO_3^{2-} concentration which elevated the amount of pCO_2 in saltwater which supported our initial hypotheses that stated rising CO_2 levels in the atmosphere have a significant impact on *G. changii* development. Upon cultivating *G. changii* with different bicarbonate concentrations, it shows that bicarbonate enrichment in seawater can affect seaweed growth in direct or indirect ways, either positively, neutrally, or adversely. Bicarbonate concentrations were observed to be increased over time, which made the saltwater become more alkali than the normal pH of seawater (pH 8.2) and the seaweed growth rate was reduced. On the other hand, pH fluctuations of saltwater cultivated with *G. changii* under treatments of various bicarbonate levels resulting in this study give a promising hope to reduce ocean acidifications.

5.2 Value-adding & recommendations for future research

A thorough understanding of the role of cultured seaweeds in the marine ecosystem is required to promote not only the economic value of the goods produced, but also the ecosystem services provided by marine farming activities. Furthermore, this will lead to a better understanding of how an ecosystem approach to aquaculture incorporates the role and need for the goods and services these macroalgae provide. Thus, from the results obtained in this experiment, further study can be proposed by cultivating *Gracilaria changii* with crustaceans such as crabs or shrimp as in Integrated multi-trophic aquaculture (IMTA) ecosystems to analyse the effectiveness of carbon-concentrating mechanisms in seawater. Another recommendation is to prolong the experimental durations to evaluate the pH compensation point of *G. changii* regulated in the saltwater of different bicarbonate concentrations and to study its nutritional composition of *G. changii* for human consumptions.

5.3 Limitations of this study

There are a few problems arise during the experimental periods of this lab-scale seaweed cultivation. Starting from a week after harvesting, the seaweeds were affected by the "ice-ice" disease. This disease is common in seaweed farming which mainly cause by the bacteria *Vibrio-Aeromonas* and *Cytophaga-Flavobacteria* due to the inadaptability of seaweed against the changes in salinity, ocean temperature, and light intensity (Hurtado et al., 2006). This is, somehow, has created stress on the seaweeds, causing them to create a "moist organic substance" that attracts bacteria in the water and causes the tissues to whiten and harden. "Ice-ice" disease normally infected at the end of the thallus filaments and it can spread to the neighbouring seaweed if it is not being removed (Zainuddin et al., 2019).

During the acclimatisation stage, one of the problems encountered was epiphyte growth on the seaweed thalli. Although all seaweeds were cleaned and had their epiphyte removed, somehow, the epiphytes were observed growing on the seaweed during the acclimatisation period. These epiphytes, however, compete with the *Gracilaria changii* for light, substrate, and nutrition which subsequently leads to the reduction on the rates of their photosynthesis and growth (Titlyanov & Titlyanova, 2010). On the last day of acclimatisation period, weeding has been done once again to remove the epiphytes from absorbing the nutrient from the cultivated seaweed before starting the first set of experiment. Another approach that has been done was maintaining a certain light intensity that supports cultivated plant growth while reducing epiphytic algae growth during the experimental periods.

Upon conducting the experiment, the experimental periods given is supposedly sufficient for this research. However, there were new rules applied by the faculty that requires an approval from dean to access the laboratory according to a specific date and time. Furthermore, the time allocated for the student to access the laboratory has been shortened from 9am to 1pm daily. Two analysis proposed in the proposal defence for DIC in saltwater and proximate analysis for seaweed were not being conducted due to the time constraint.

CHAPTER 6: SIGNIFICANCE OF THE RESEARCH

Oceans absorb a large percentage of the CO_2 released into the atmosphere due to anthropogenic, which may have adverse implications for shell-forming organisms. Since 1850, the oceans have absorbed between a third and half of the CO_2 people have emitted into the atmosphere. The rate of climate change has slowed as a result of this. When CO_2 dissolves in seawater, the pH of the water decreases. Since about 1850, the acidity of the ocean has increased by 26%, a pace of change roughly 10 times higher than any other time in the previous 55 million years (citation please). Seaweed farming, the fastest-growing sector of world food production, offers several climate change mitigation and adaptation options. Seaweed can absorb carbon dioxide from the ocean, which means reducing ocean acidification and climate change impacts. To convert carbon dioxide (CO_2) into seaweed biomass, seaweed, like terrestrial plants, uses photosynthesis. Carbon sequestration is the term for this process. Because seaweed grows so rapidly, it can absorb CO_2 at an incredible rate (Lee et al., 2019). When CO_2 is locked up in seaweed biomass, it can be harvested for consumption, sink to the seafloor, or be stored underground, where all of the extra CO_2 originated. For the past two decades, scientists have been evaluating seaweed's carbon sequestration capacity. That is why we proposed this study because seaweed may also absorb carbon dioxide from the ocean, lowering ocean acidification and mitigating the effects of climate change, but what is the threshold for seaweed to survive in different level of carbon? This research is designed to evaluate the growth and quality of red seaweed in response to different pH levels

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