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ENVIRONMENTAL RESPONSE AND pH TOLERANCE OF INDUCED CO_2 IN *ULVA RIGIDA* C.AGARDH, 1823(CHLOROPHYTA) UNDER CONTROLLED CONDITIONS

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ABSTRACT

The increase in integrated multitrophic aquaculture (IMTA), where seaweed (particularly *Ulva rigida*C.Agardh, 1823) is used as a feedstock and a wastewater scrubber in South African IMTA systems, has necessitated research into seaweed growth rates, which hassubsequently increased production technologies.Seaweed growth can be increased by controlling the culture media.One of the means to control growth rate is through CO_2gas addition to culture media via aeration.This has the potential added benefit of usingwaste CO_2 production from an alternative source to decrease overall carbon dioxide emissions. The consequence of elevated CO_2 concentration on the pH of culture medium and the equivalent functional reactions in the seaweed were examined using *U. rigida* in flow-through systems.Toxicity investigation of Hydrogen ion concentrations werecarried out on *U. rigida* to examine their anatomy cum functional differences arising due to CO_2 exerted stress. Elevated CO_2 levels and the accompanying decrease in culture media pH (4.71 – 6.67) leadto a significant decrease in biomass with varied sporulation activities. In addition, *U. rigida* in flow-through systems showed a gradual degeneration in specific growth rate, from day 7, atvarying rates until the end of the experiment in the following sequence pH 7.20 > 8.20 > 7.50 > 7.80.The treatment set at pH 7.20 yielded the greatest specific biomassandthe greatest produce.The cultured input stocking rate of 5 $g.l^{-1}$ of seawater proved to be suitable for cultivation. ThepH toxicity reaction was significant in predicting the suitability of seaweed cultured under CO_2 induced concentrations.

Keywords: Increased CO_2concentrations, Ocean acidification, pH, physiochemical characteristics, seaweed, sequestration, *Ulva rigida*

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INTRODUCTION

With the high levels of greenhouse gases, such as CO_2, scientists are investigatingways of reducing atmospheric CO_2 concentrations(Suarez-Alvarez *et al*., 2012; NOAA,2019).The coastal ocean habitat and its underlying aquaticforests act as carbon sinksand are about 20 times more efficient in sequestrating carbon $acre^{-1}.year^{-1}$ than terrestrial forests (Suarez-Alvarez *et al*., 2012; Atiqur, 2017). The global oceans absorbed nearly $125x10^9$tons of carbon, which is about ¼ of the 500 $x10^9$tons of the atmospheric CO_2 produced from industrial processes (Intergovernmental Panel on Climate Change -IPCC, 2013).Atmospheric CO_2release is predicted to increase by 20 $x10^9tons.a^{-1}$ in 2100 due to modernization required byan increasing population, andmay later rise to 40 $x10^9tons.a^{-1}$ in 2200(Philip*et al*., 2013; Rapley, 2012; World Population Prospects (WPP), 2017). It has been estimated that 3D seaweed-containing ocean farms can sequester about 135 $x10^6$ tons of atmospheric CO_2 and$10x10^6$ tons of nitrogen with just 5% of US marine waters (Bjerregaard*et al*., 2016).High atmospheric CO_2 leads to decreased ocean pH through a process of ocean acidification, this resulting in a rise of HCO_3^-, which may affect photosynthetic processes inseaweeds (Raven and Falkowski 1999; Samesi*et al*., 2009; Ugoala*et al*., 2012; Aknaf *et al*., 2020).Given that seaweeds can sequester CO_2, the impact on decreasing ocean pH and its corresponding impact on growth rates are particularly important in commercially cultivated seaweeds either for bioremediation (Gao *et al.,* 1993) or as a harvestable resource (Israel *et al*., 2005).

Climate change and over exploitation of coastal seaweed harvestingare detrimental to a sustainable seaweedindustry(see Table 1) (Harley *et al.,* 2006; Kim *et al.,*2011; Price *et al.,* 2011; Atiqur, 2017).Suarez-Alvarez

et al., (2012) discussed the impacts of increased CO_2 concentrations and global climate on renewable resources such as seaweeds and investigated the physiology of the marine alga*Hypneaspinella* (C.Agardh) Kützing, 1847.Bioremediation by seaweeds has been predicted to be a significant phenomenonin carbon sequestration (Cure and Acock, 1986; Harley *et al*., 2012).Carbon concentrations in seaweed communities are rising due to anthropogenic emissions and increased upwelling of CO_3^{2-}, Na_2CO_3,.CN and HCN concentrations (Beardall*et al.,* 1998; Roleda*et al.,*2012Aknaf *et al*., 2020).

Table 1: Environmental consequences of climate change on seaweed production.

Indices	Effects
High CO_2 (µatm)	Raises photosynthesis and biomass,Alter species constituents, nutrient cycling and brakedown
Dissolved inorganic salt (‰)	Manage dispersal and diversity, Change causes mortality due to high intake and nutrient discrepancy
Sea level rise (mm)	Raises water depth and reduceslight penetration, Lowersphotosynthesis Lowersdispersal and reduces productivity
Temperature (°C)	Changein dispersal and abundance Influencesbiomass and seed sprout
Tidal datum (ft)	Influences light availability
Ultraviolet B radiation (nW/cm^2)	Restrictsenergy through chemosynthesis and photosynthesis Inhibits photosynthetic processes Raisesmetabolic processes, resistance to herbivores and pathogens Reducesbrake down, Atmospheric carbon removal

Source: Reviewed after Atiqur, 2017.

Seaweedscan grow in culture media with elevated CO_2, however, elevation of CO_2concentrations decreasesthe pH leading to ocean acidification and has been shown to lead to sporulation in green algae (Markelz*et al*., 2013).Seaweeds cultured under elevatedCO_2mediumsproduce additional protons for increased biomass production and photosynthetic activities (Cornelisen*et al*., 2007; Suarez-Alvarez *et al*., 2012).Most aquatic plants and seaweeds are reservoirs of aquatic carbon through utilization of CO_3^{2-}, HCO_3^- and CO_2 through rubisco diffusion for photosynthesisdepending on pH concentrations(Raven, 1991a; b; Aknaf *et al*., 2020).The impacts arising from variations in carbon dioxide concentrations and resulting hydrogen ionconcentrations are diverse (HongYan*et al*., 2008).Andria *et al*., (1999) reported that these changes, under normal seawater pH conditions, resulted in increased seaweed productionandwith correspondingly high nutrients requirements.Opposing this,Samesi*et al.* (2009) discovered high dissolved CO_2concentrations of 26 Mu mol kg^{-1} at 0.9 mbar gave rise to reduced water pH with <20 % insoluble calcium compound in aquatic algae for 120 hours.In closed systems CO_2 was a significant restricting factor in large-scale seaweed cultivation in achieving effective growth rates(Ugoala*et al.,* 2012).In marine water bodies with elevated carbon levels, pH may reduce to about 4 – 6, whilepH between 7.5 – 8.5 is required for optimalseaweed production (Duarte*et al*., 2017).Environmental responses as a result oflow pH concentrationsare a function of acidificationresponsible for several deleterious anatomical and physiological activities in most marine species, which have been revealed to both influence and decrease marine algal growth rates (Berge *et al*., 2010). pH is therefore important in photosynthetic activities and calcification rates (Buapet andSinutok, 2021).

All *Enteromorpha*spp. which have been subsumed in the genus *Ulva*, are among the 14 species of seaweedsthat are highlydiverse, occurringthroughout the coastal belt of South Africa (Figure 1) (Cyrus *et al.*, 2014). The aquaculture industry in South Africahas been well studied with *Ulva* spp.beingcultivated as feed on abalone farms, used as biofilters in aquaculture effluent, for biofuel andas a plant growth stimulants(Amosu, 2016).Researchers have proventhat extremely high atmospheric dissolved carbon may be tolerated during aquatic algae cultivation under semi controlled or controlled conditions (Andersen and Andersen, 2006; Gao*et al*., 2012).Currently, the literature is scant on the anatomical and physiological behaviors and the capacity to resist continuing reduced pH due to high CO_2levelsincultivated South African seaweeds – particularly *Ulva rigida* C.Agardh,1823 (Chlorophyta). The consequence of raised dosing regimens of CO_2levels on seaweed physiology are therefore important to ascertain the genuine pH at which biomass will be reticent in *Ulva*.Biomass production is the most important measure in commercial land-based seaweed farms.The aim of this experimentwas to determine how elevated CO_2 concentrations corresponds toreduced pH in *U. rigida*, this determined by spore production under controlled conditions and ocean acidification in nature.

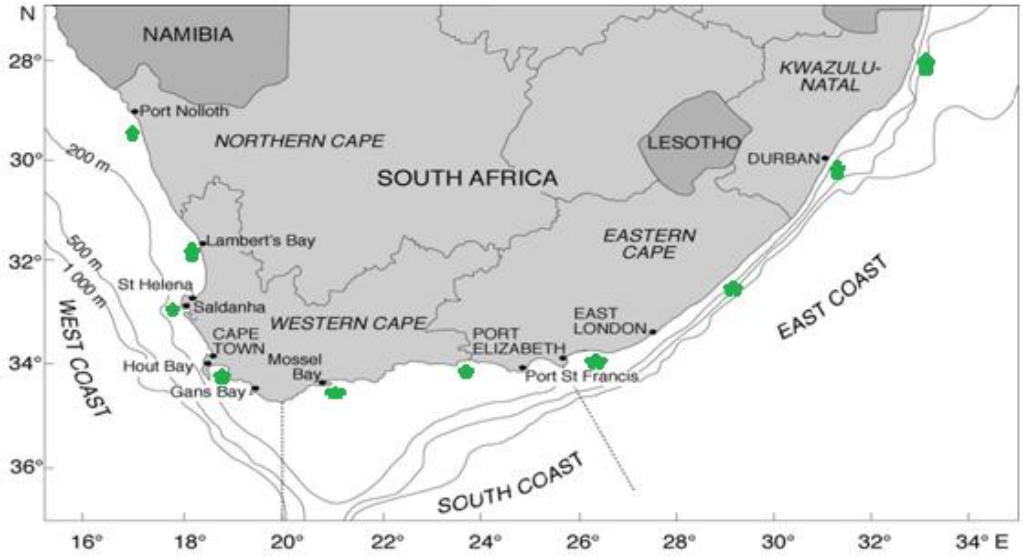

Figure 1. Locations where *Ulva* species occur along the South African coast.

MATERIALS AND METHODS

The experimental design was in two phases: (A) closed culture and;(B) a flow-through culturesystem.

(A) Closed culture system: (i) Fresh samples of *U. rigida* were collected during low tide at Simon's town (34° 14' S, 18° 26' E). After collection, epiphyte free *U. rigida*were randomly selectedto be stocked for *in situ*research.A stainless steelcork borer was used to prepare 1 cm samples of thalli discs for the cold room culture (Figure 2a).At the Sea Point research aquarium in Cape Town, ocean siphoned,filteredseawater was collected. Preparation of enriched seawater (PES),using standard Provosoliprotocol with additives of different concentrations, was used for the solution (Provasoli, 1968). Discs(6)were cultivated in500 ml of 1-L autoclave(sterilized) conicalglass flasks, sealed with cellophane wrap, and provided with independent inlets(Figure 2a). The flasks were divided into three treatments: one set contained only filtered seawater (n=18); one set was filled with Provosoli solution + filtered seawater (n = 18); and one set acted as the control (n=3), which contained onlyfiltered seawater without CO_2 dosage bubble (Figure 2b). The flasks with thallus discs were constantlydosedwith filtered air at graduated intensities (numbers of bubbles /second (s^{-1}): 0 s^{-1}, 1 s^{-1}, 2 s^{-1}, 3 s^{-1}, 4s^{-1}, 5 s^{-1} and 6 s^{-1}at (1 bars) 10 l.min^{-1} CO_2 for a period of 7 days) to investigate the sporulation behaviour of the graduatedCO_2dosages. The growth media were replaced each day.

Phase (ii) was set up with constant low and high CO_2 dosage systems for 48 hours comprising (6) thallus discsin the culture flasks(Figure 2c).Seven bubbles.$s^{-1}$was used in the low bubbling regimes, whilehigh bubbling regimes were characterized with continuous dosing to give corresponding oscillation of the *Ulva*thallus discs. In both experimentsthree replicates were set up for each treatment. Fluorescent white tubes were used to provide light (Sylvania Daylight F36W, Germany) at light:dark 16:8h photoperiod. Irradiance wasdetermined with a skye quantum sensor (Skye Instruments Ltd, UK). The cold room was kept constant at 15±1°C.Physicochemical variables were measured as follows: Dissolved Oxygen (DO)- a Waterproof CyberScan Series 300 (Eutech Instruments Pte Ltd, Singapore); pH – model 8414, Hangzhou rock biological technology Co. LTD; Salinity - Rrefractometer (S/Mill-E 0 ~ 100 ‰, ATAGO, Japan).

(B) ***Flow-through culture system:*** The flow-through culture systems were where water was delivered at a consistent flow rate (~ 3 L.min^{-1} at the Sea Point research aquarium) into each individual rearing tank.The designated pH was controlled with the introduction of pCO_2 gas via an air stone that was controlled via a Tunze system (Tunze pH controller 7070/2- The Age of Aquariums, Germany).Tanks were constantly dosed with CO_2.Vibrator propeller pumps were used to induce mixing and movement using a SUNSUN New Design 24W Dual Propeller Circulation Pump (JVP-202A/B, Guangzhou Weierma Electrical Co., Ltd, Zhejiang, China).Seawater within the rearingtanks was at pH 8.20 with three *culture system*replicates as a control. Water chemistry was determined with (YSI Incorporated, US) multi-parameter equipment.

Culture in flow-through media: The second experiment was performed in three 111.1 L (85.3 cm X 28 cm X 46.5 cm) tanks, stocked with seaweed at 5 g. l^{-1} specifically designed for flow-through culture of *Ulva* (Figure 3).The source of growth medium and nutrients were provided from the unfiltered seawater at the aquarium, which was the same as that used during the stock maintenance

period with pH at 8.20. Seawater within the tanks was maintained at several pH levels (namely, 7.20, 7.50, 7.80 and a control of 8.20), with three replicates each in the different treatments). The pH levels chosen were those that were predicted by (IPCC) models for South Africa (Niang *et al*., 2014; Barange *et al*., 2018). Illumination was provided ($100\mu mol.m^{-2}.s^{-1}$) and controlled by fluorescent tubes attached to a controller to provide a 16:8h photoperiod.A pH electrode measured the seawater pH. If the seawater pH moved above that of the set value (on the controller) a magnetic valve was automatically opened and CO_2 from the cylinder was bubbled into the seawater via the air stone.This then mixed with the surrounding water with the help of the circulating pumps; the magnetic valve continually opened and closed until the set pH value was acquired.The one-way valve was placed in the system to prevent water moving from the tank into the magnetic valve. O_2 was continually pumped into the system via another air stone.

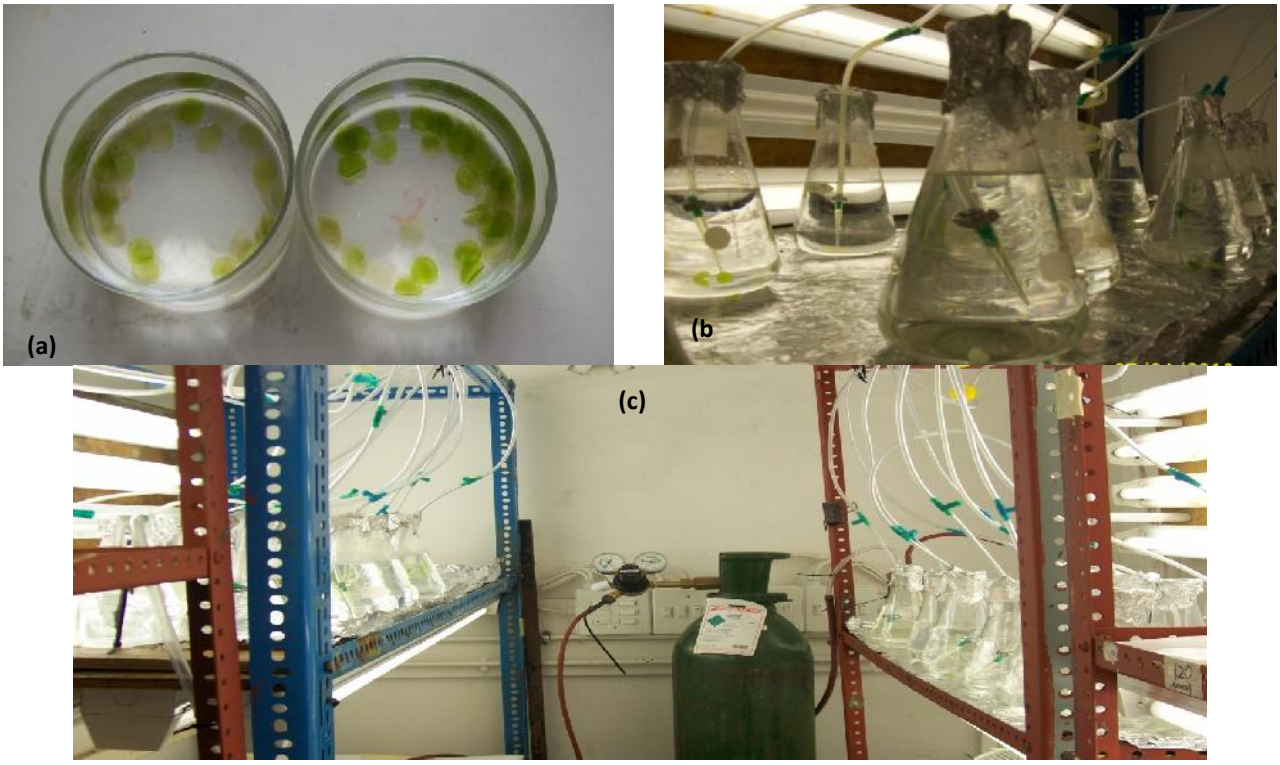

Figure 2: (a) Thallus discs, (b) *Ulva*thallus discs placed in different flasks with different bubbling regimes and (c) CO_2 induced replicates of enriched and non-enriched media.

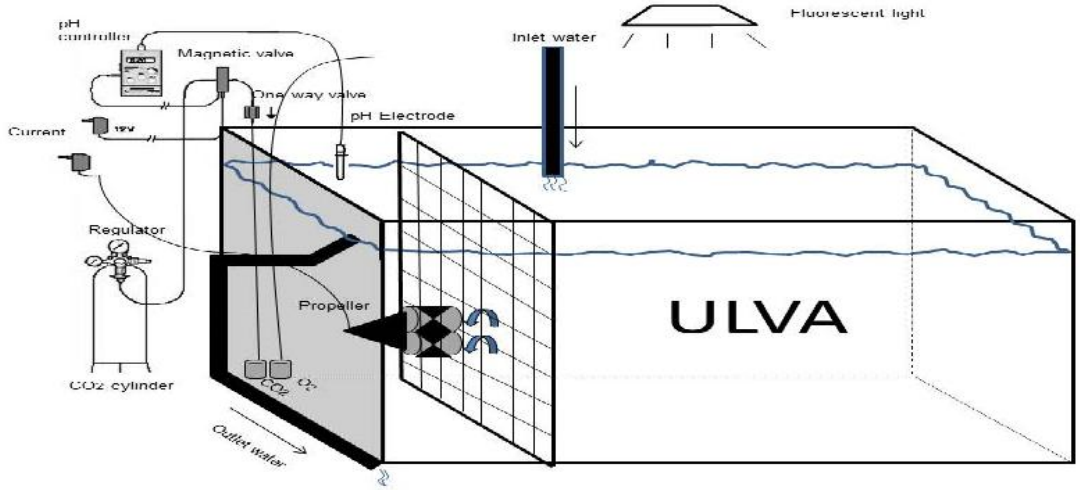

Figure 3: Flow-through experimental design.

Specific growth rate data acquisition: The seaweed was harvested every 3 days for 16 days, blotted dry and weighed for restocking.*Ulva* biomass was determined using (SGR, %):

SGR = (wt – wo) 100/t… (Syazili*et al,* 2021; Samad *et al,* 2022)

Here worepresents the initial weight of *Ulva*,wtthe harvested weight andt (g. l^{-1} day^{-1}) days as expressed from Lersten and Voth, (1960); Duke *et al*, (1986).

Data analysis: Data are expressed as means ± standard deviation (SD). A two-way analysis of variance (ANOVA), using the Graph Pad Prism V analytical tool, was used to compare the various treatments.Differences observed in treatments were analyzed using Least Significant Difference (LSD) and the DNMR test (Duncan, 1955). Differences among treatments were considered significant at $p < 0.05$.

RESULTS

Closed experiment: Regardless of nutrient and fresh seawater inclusion,or the days of the experiment, the greater the CO_2 levels, the higher the spore formation (Table 2). The highCO_2concentration gave rise to low pH(acidity), resulting in thalli disintegration, the process known as sporulation. Survival was therefore a function of pH.In the control sporulation occurred only after day 6 of the experiment.

Table 2: Degree of sporulation in *U. rigida* under different CO_2 consecutive numbers of bubbles per second.

Day	Media	CO_2 consecutive numbers of bubbles (s^{-1})						
		0	1	2	3	4	5	6
1	SW		×		×	×	××	××
	EW				×	×	×	××
2	SW		×	×	×	×	××	××
	EW		×	×	×	×	×	××
3	SW		×	××	××	××	××	××
	EW		×	×	×	×	××	××
4	SW		×	×	××	××	××	×××
	EW		×	×	×	×	××	×××
5	SW		×	×	××	××	×××	n
	EW		×	××	××	××	×××	n
6	SW	×	×	××	××	×××	n	n
	EW	×	××	××	××	×××	n	n
7	SW	×	××	××	n	n	n	n
	EW	×	××	××	n	n	n	n

× = minor sporulation (one disc or spherical sporulate), ×× = moderate sporulation (more than one disc or sporulate), ××× = significant sporulation (5/6 discs turning pale), n = no algae in media; thalli entirely disintegrated.Empty cells represent no noticeable sporulation.

*Ulva*underhigh CO_2bubbling showedsporulation earlier and disintegrated within fewer days. *Ulva*discs in the seawater culture treatment sporulated faster in the enriched seawater between 1 – 4 days under high levels of CO_2bubbling.About two days prior to the suspension of the experiment, very high sporulation occurred regardless of the treatments.The 48 hours of high and low dose of CO_2, salinity proved to correspond to normal seawater for Simon'sTownregardless of the bubbling regime.Figures 4, 5 and 6 shows that DO and pH show a diurnal difference for both bubbling regime with time.

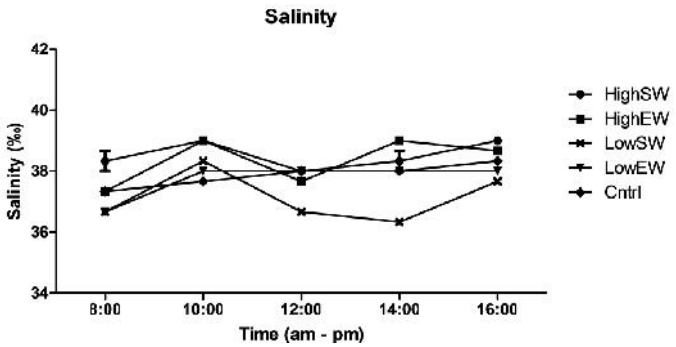

Figure 4: Salinity time graph of CO_2concentration in *U. rigida* under the various treatments.

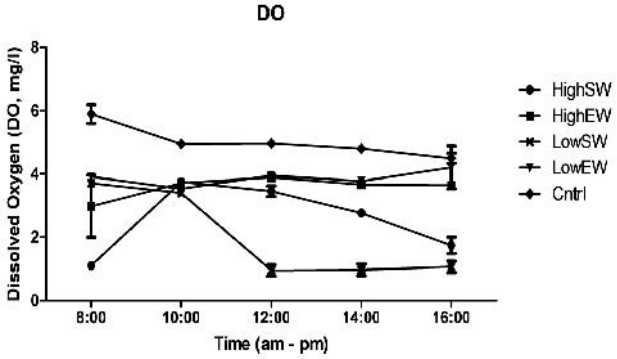

Figure 5: Dissolved oxygen time graph of Co_2 concentration in *U. rigida* under the various treatments.

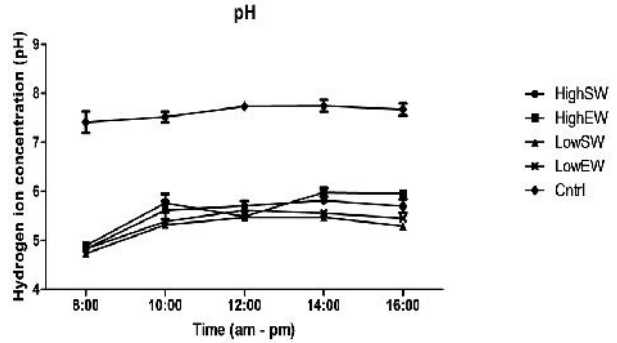

Figure 6: pH time graph of Co_2 concentration in *U. rigida* under the various treatments.

Legend key: High bubbling regimes for non-enriched seawater = HighSW, High bubbling regimes for enriched seawater = HighEW, Low bubbling regimes for non-enriched seawater = LowSW, Low bubbling regimes for enriched seawater = LowEW, Control =Cntrl.

The observed salt concentration values in this research varied (36.6 – 39). Low salinity (36.6 ‰) corresponded with low bubbling, while high salinity (39 ‰) corresponded with high bubbling.The changes in salinity are thought to be due to increased evaporation induced by the bubbling action. In non-enriched seawater, lowDO occurred during 8h00 and 16h00 (Figure 4) and reduced the pH (7.07), which was low compared to pH 8.14 of normal seawater.With DO revealing a significant diurnal form attributed to photosynthesis activities at different times of the day (ANOVA: F = 7.041, p = 0.0001).The experimental treatments revealed a slightly acidic value pH (4.73 - 6.67), despite and with spore formation doesn't require nutrient (Figure 6).

***Flow-through experiment**:* In the flow-through experiment, water chemistry revealed conducive physico-chemical parameters for *U. rigida* (Table 3). Researchwith *Ulva*spp. showed the survival rate between 15 – 20 °C and60 µmol.m^{-2}. s^{-1} as experienced by Jiang *et al*., (2013).Dissolved Oxygen values across experiments were similar despite the addition of CO_2.The temperature of the growth media increased from 12°C at the beginning of experiment with a gradual increase of 0.10 °C to attain the maximum 16.9 °C at the end of cultivation.The pressure recorded in the study ranged from 101.85 to 103.54 KPa.The salinity was between 34.52 to 35.27 ppt but varied significantly over time and levels (ANOVA: p < 0.0001, F = 17.93).The salinity increased slightly in different treatments for the 3rdday. The observed TDS was34125 to 34710 TDS mg l^{-1}.

Table 3: Some physicochemical parameters of *Ulva rigida* cultured in flow-through systems. Entries on the same row with the same superscript are not statistically different at p > 0.05.

	pH regulations			
	Trnt.1(8.20)	**Trnt.2(7.20)**	**Trnt. 3(7.50)**	**Trnt.4(7.80)**
pH	8.22 ± 0.10^a	7.39 ±0.13^b	7.61 ±0.15^c	7.74 ±0.14^c
A_T (µmol kg^{-1})	2159 ± 32^a	2127 ± 85^a	2160 ± 22^a	2169 ± 21^a
Temperature (°C)	13.0± 0.7^a	13.0±0.7^a	15.0±1.1^b	15.1±1.2^b
Salinity (‰)	34.8 ±0.2^a	34.8±0.2^a	35.1±0.1^a	35.1±0.1^a
pCO_2 (µatm)	232 ± 71^a	1952 ± 673^b	1192 ± 384^b	860 ± 278^c
Ca^{2+} (mmol l^{-1})	10.4 ± 0.3^a	10.9 ± 0.3^a	11.3 ± 0.3^a	11.1 ± 0.1^a
Mg^{2+} (mmol l^{-1})	46.9 ± 1.1^a	45.7 ± 1.9^a	52.5 ± 3.4^b	50.8 ± 1.1^b
Pressure (KPa)	102.91±0.420^a	102.95±0.418^a	102.42±0.318^a	102.43±0.310^a
DO (mg l^{-1})	8.32± 0.344^a	8.44±0.340^a	7.37±0.707^b	7.65±0.378^b
SPC	52.89± 0.253^a	52.87±0.251^a	53.24±0.147^a	53.23±0.142^a
TDS (mg l^{-1})	34382±160^a	34386±164.6^a	33600±5597^b	34607±96^a

Trnt. = Treatment

Biomass of *U. rigida* at different pH concentrations recorded over the period of cultivation in the flow-through system were as follows**:***U. rigida* cultured in the flow-through system showed increased yields after 1 week (Figure 7). The gradual degeneration and reduction in biomass started within a few days of

cultivation, especially in pH 7.80. However, pH 7.20 produced the highest yield in this order: week 4 > 10> 13> 16> 7. The biomass and yield of *U. rigida* revealed a progressive distributionwithin sixteen days in the same seawater at the different treatments (Figure 7).

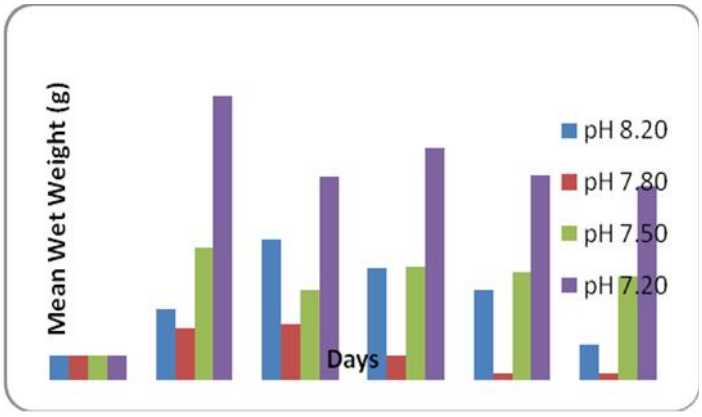

Figure 7: Biomass of *Ulva rigida* at the different pHconcentrations over the duration of the experiment.

DISCUSSION

Closed experiment: Information is still scant on the influence of increased CO_2concentrationson *Ulva*production.Our research revealed that CO_2concentrationof culturemedia and the resulting pH changes (8.0 and 8.5) influence the levels of sporulation in*U. rigida* cultivation, which were similar to those determined by Nordby, (1977) for *Ulva mutabilis*Dominguez and Loret (2019) who discovered that a pH concentration range between 6.5 - 7.5 lead to high levels of sporulation in *Enteromorpha* spp. which is more acidic than in this study. Lower pH, due to elevated CO_2 concentrations in this study, led to high levels of *Ulva* disintegration (Björk*et al*., 1993).Toxicity behaviors in *Ulva pertusa*Kjellman, 1897 at similar pH to this study were alsoexperienced by Han and Choi, (2005) and Han *et al*., (2007). *Ulva*spp. regularly sporulate as an imminent sign of bio-physiological tension (Beach *et al*., 1995; Kalita and Titlyanov, 2003; Kalita and Titlyanov, 2011), suggesting that pH is important in the growth of this species.Our findings confirmed the consequenceof high CO_2on growth production as an effect of low pH,similar to that of Harley *et al*., (2012) andRoleda*et al*., (2012).Low pH(4.73- 6.67) results in sporulation but not when grown in enriched nutrient media.Andria *et al.,* (1999) found that at pH 7.9 – 8.4 *Gracilariagaditana* nom. prov. had high nutrient requirements.A similar result was found with *U.pertusa* (Han and Choi, 2005) and *Ulva lactuca* L. 1753 (Robertson-Andersson, 2007). The low pH (4.73) concentration observed at elevated CO_2(8h00 – early morning) dosage, as observed in the normal process of photosyntheticactivities, where similar to those found for *Ulva prolifera*O. F. Müller 1778and *Ulva linza* L. 1753, but these seaweedswere additionally exposed to Cd stress (Jiang *et al*., 2013).

Seaweedstake up CO_2across biochemical pathways using C_3in rubisco, usually reticent by DO along photorespirationpathway (Raven, 1989; 1997).Choi *et al*., (2010) and Boyd, (1998) found that biomass reduction due to low salt concentrations (below25 ‰)causedsporulation that influenced CO_2 increase.Our observations(salt content 36.6 - 39 ‰) agreed more with Dickson *et al*. (1982) for *U. lactuca*who postulated that the physiological response of sporulationtallied with the impact that the salt concentration variation has in decreasing the internal turgor pressure (Lobban and Harrison 1994).

Flow-through system: Seaweed survival in a culture-flow through system is controlled by the water quality, which corresponds to the biophysiological processes(Troell *et al*., 2003; Robertson-Andersson, 2007). *Ulva rigida*cultured in this system showeddecreased yields after 1 week.The gradual degeneration was noticed after day 4.Nikolaisen*et al*. (2011),while mass cultivatingseveral green algae,found this decrease, which lead to sporulation and reduction in biomass within a few days of culture, this despite pH 8.2 being within the optimum pH for algae cultivation.According to research, stocking density well above1kg.m^{-2}is suboptimal because itdecreases biomass and production (Neori*et al*., 1991; Nikolaisen*et al*., 2011); about1 kg.m^{-2} stocking density for*U. lactuca*is favorable(Ryther*et al*.,1984).Prue, (2009) found that stocking densities around 5-20g for *Ulva*l^{-1}coupled with enriched nutrients at 87 g.l^{-1}concentration of marine water, wouldproduce a weekly significant biomass.The stocking density of*Ulva* in this study was 5g. l^{-1}, which was well within the suggestedrange and thus the observed sporulation and subsequent degeneration were likely not due to the stocking density.The biomass and yield of *U. rigida* ranged between 467 – 1433 g in sixteen days

without enriched nutrients.The *U. rigida*biomass observed during our research differed to that of *U.lactuca*in flow-through culture systemby Neori*et al*., (1991), but were similar to the results obtained by Corey *et al*.(2014).However, some findings with*Ulva* cultivation have shown an array of disparities in the cultivation conditions.For example, Neori*et al*. (2004) noted that as cultivation volumes increased, total yield was reduced along with growth rates.This supports our findings.

Conclusion: In flow- through system, despite sporulation, *U. rigida*can tolerate a sustained low pH of 7.2.This implies that in large-scale cultivation systems CO_2 could be added and pH maintained at 7.2.In addition, the application of soluble fertilizer could achieve a better seaweed growth and help prevent sporulation.It will be useful to conduct future experiments on rates of fertilizer application at varied low pH.

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Authors' contribution: This work was carried out in collaboration between all authors as team work.AAO, DVR-A, LA and JJB conceived and designed the study. AAO, DVR-A and JLK executed the experiment and analyzed the compiled information. Authors AAO and DVR-A worked with author GWM in data analysis and interpretation. Authors AAO, DVR-A and GWM interpreted the data, critically revised the manuscript for important intellectual contents. All authors approved the final manuscript.

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