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Full Length Article

Biofiltering and Uptake of Dissolved Nutrients by *Ulva armoricana* (Chlorophyta) in a Land-based Aquaculture System

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Abstract

An on-land flow-through cultivation system was designed for the macroalgal species *Ulva armoricana* (Chlorophyta) to reduce the environmental impact of aquaculture effluent in coastal ecosystems as part of an integrated aquaculture system. The macroalgae was cultured in various enriched media at a stocking density of 500 kg wet weight/pond. Overall, *U. armoricana* was able to remove a greater percentage of inorganic nitrogen in the double fertilizer ratio. The total dissolved phosphate was higher in standard seawater. *U. armoricana* showed preference for bioaccumulation, with ranges as follows: zinc (9.908 – 32.942 $mg.kg^{-1}$); copper (1.893 – 5.927 $mg.kg^{-1}$); cadmium (0.254 – 1.500 $mg.kg^{-1}$); and lead (none detected). Apart from the presence of cadmium (Cd), the algal biomass produced at the end of the experiment was of a relatively good quality with limited heavy metal contamination so that *U. armoricana* could be successfully used as a plant stimulant but not as part of a feed formulation for livestock and for the food industry. This study showed that *U. armoricana* can effectively be used as a biological filter for dissolved nutrient uptake from aquaculture effluents. The prospect of better management practices, based on the utilization of *Ulva* mariculture designs, bodes well for the aquaculture industry.

Keywords: Dissolved nutrients; Fertilizer; Heavy metals; Integrated multitrophic aquaculture (IMTA); Macroalgae; *Ulva armoricana*

Introduction

Global aquaculture production continues to improve at about 10% annually, outpacing terrestrial livestock production and capture fisheries (FAO, 2010). However, the rapid development of intensive aquaculture along coastal areas throughout the world has raised increasing concerns on environmental degradation and specifically the impact of nutrient loading if these industrial production practices are not sustainably managed using the best available technology (BAT) (Haylor and Bland 2001; Pauly *et al.,* 2003; Zhou *et al*., 2006; Troell, 2009; Ihsan, 2012). Waste products from aquaculture activities consist mainly of CO_2, nitrogen, phosphorus, and heavy metals.

Aquaculture waste can result in pollution that contributes to the degradation of the environment through (organic and inorganic inputs) agro-allied and industrial activities that can lead to a substantial increase of organic matter and nutrient loading into adjacent water bodies. Modern integrated aquaculture systems like (non-fed aquaculture) macroalgae-based aquaculture contribute to eco-monitoring by playing a significant role in coastal wastewater filtration and bioaccumulation (Costa-Pierce *et al.,* 2011; Klinger and Naylor, 2012; Boxman, 2013; Redmond *et al.,* 2014). This is due largely to the ability of macroalgae to achieve high biomass and have a significant potential as nutrient bioremediators (Msuya and Neori, 2002; Tyler and McGlathery, 2006; Marinho-Soriano *et al.,* 2009; Winberg *et al*., 2011).

In aquatic environments, nitrogen and phosphorus (major aquaculture contaminants), are the two most important nutrients that usually limit biomass production of macroalgae (Smith and Smith, 1998; GESAMP, 2001; UNEP and Gems Water, 2006). Nitrogenous compounds (NH_4^+, NO_3^-, and NO_2^-) have been indicted as a source of pollution in aquaculture effluent due to discharge of untreated non-point aquaculture run-off, animal waste and failed technology practices. According to estimates, 78 kg N and 9.5 kg P per ton of fish are released into water bodies per year. This is because about 72% N and 70% P constituent of feed are not utilized in the fish physiology (Ackefors and Enell, 1994; Chopin *et al.,* 1999).

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In the past few decades, increasing emphasis have been placed on developing sustainable approaches to coastal aquaculture development of large-scale Integrated Multitrophic Aquaculture (IMTA) seaweed farming (Robertson-Andersson, 2007; Smith *et al.*, 2010; Redmond *et al.*, 2014). The integrated culture system provides mutual benefits for the cultured organisms and improves water quality of the aquaculture system. Macroalgae take up inorganic nutrients for growth and can thus alleviate the seasonal nutrient depletion from aquaculture (Chopin *et al.*, 2001; Neori *et al.*, 2004). Several aquaculture research and development efforts have shown the efficiency and benefits of integrating macroalgae in on-land treatment systems for treating aquaculture waste effluents before being discharged into open water bodies (Winberg *et al.*, 2011; Dittert *et al*., 2012; Renzi *et al.*, 2014).

Macroalgae have found applications in the removal of nutrients from effluent waters of sewage, industry and aquaculture. More recently it has been demonstrated that using different dissolved CO_2 concentrations in seawater, has the potential to improve nutrient uptake, a possible solution to the problems associated with coastal eutrophication around the world (Zou and Gao, 2009). Furthermore, research findings have also demonstrated that the incorporation of co-cultured organisms from different trophic levels is the basis for sustainable and safe aquaculture practices (Chopin *et al.*, 2001; Neori *et al.*, 2004). This is so because in the polyculture of integrated fauna and macroalgal mariculture, the wastes from one consumer become a resource for the other in the mutually beneficial system. This integrated approach gives nutrient bioremediation efficacy, mutual benefits to co-cultured organisms, and results in a more stable aquaculture environment (Neori *et al.*, 2000; Chopin *et al.*, 2001).

Tissue metal contents are also potential hazard prediction indices for organisms and the environment when natural concentrations are higher than the maximum standard recommended by monitoring agencies (Ayers and Westcot, 1994; Almela *et al.*, 2002, 2006; Smith, 2009; Sánchez-Bayo *et al.*, 2011). Macroalgae naturally take up elements like Na, K, Ca, Mg, Cl, I and Br from the surrounding water bodies. The major metallic pollutants implicated in culture systems and coastal waters are Pb, Cr, Hg, U, Se, Zn, As, Cd, Au, Ag, Cu and Ni among which Cd is readily absorbed in a combined state with sulphur, chlorine and oxygen and stored in the algal thalli (Komjarova, 2009; Dittert *et al*., 2012; Renzi *et al.*, 2014). Green macroalgae (Chlorophyta) are known to be a significant biological indicator of heavy metal contamination in marine ecosystems (Nelson *et al.*, 2010). Various studies have demonstrated the use of green macroalgae from the genus *Ulva* as a bio-filter/monitor of coastal contamination because of their relatively simple morphology, high tissue bioaccumulation, and widespread distribution (Alkhalifa *et al*., 2012; Zoll and Schijf, 2012; Renzi *et al.*, 2014).

In IMTA, bio-filtration processes easily remove considerable amounts of pollutants contained in the outflowing water, resulting in a reduced permissible discharge into open water bodies. The development of such systems requires the removal of solid compounds and dissolved metabolites contained in the outlet water of the systems. The specific justification of this research has evolved from aquaculture's environmental consequences, and the nutrient enrichment of the outlet water systems associated with more general aquaculture practices. Aquaculture practices generally lead to high nutrient loading that can facilitate changes in the natural dynamics of water bodies and can lead to oxygen depletion, green tide (harmful algal blooms) events, eutrophication, fish kills, low productivity, increased risks of infectious diseases, and deterioration of the groundwater with serious consequences for human health, the environment and economic development (Van Alstyne *et al.*, 2007; Nelson *et al.*, 2010; DEC, 2014; Redmond *et al.*, 2014). In this study, we investigated the nutrient uptake potential, efficiency and bioaccumulation potential of the green macroalga *Ulva armoricana* in an outdoor, on-land flow-through paddle wheel system.

Materials and Methods

Ulva Materials

Ulva armoricana used in this experiment was sampled from the I & J Cultured Abalone farm (34º34′60 S; 19º21′0 E) and were transported to the research farm at Benguela Abalone Group (32°54'24" S; 17°59'17" E) on the West Coast of South Africa. Samples were rinsed with filtered seawater and gently scrubbed to remove sediments and any epiphytes. The specimens were then stabilized in a culture for 3-4 days (acclimatization) under a continuous flow of seawater pumped from the ocean (mean nutrient concentrations were 0.6 µM NH_4^+, 0.5 µM NO_3^-, NO_2^- and 0.7 µM PO_4^{3-}) and kept at 20°C in concrete paddle ponds.

Experimental Systems

Macroalgae production experiments were carried out during winter in four 32 m X 8 m (180 m^3) concrete paddle ponds and filled to approximately 0.55 m depth with unfiltered seawater in a flow-through system (Fig. 1). Ponds received two volume exchanges per day. The experimental treatments were as follows:

- 0 X base pond with standard seawater (control).
- 2 X nutrients added to improve growth (double fertilizer ratio).
- 4 X nutrients added to improve growth (quadruple fertilizer ratio).

Initial *Ulva* biomass of 500 kg wet weight was stocked in each pond and growth rates were measured after 21 days.

The algae were fertilized (7 days before the experiment in order to allow assimilation) with a mixture of (10:16:0) Maxipos® and ammonium sulphite at 100 g/kg providing both nitrogen and phosphorous respectively (algae need N & P in a ratio: 16 atoms of N for every 1 atom of P - Greenfield *et al.,* 2012). Fertilization was carried out in the evenings with the incoming water turned off and the paddle wheel remaining in motion. The mean physico-chemical parameters measured during the experiment include temperature (17°C), pH (6.53), and dissolved oxygen (8.07 mg L^{-1}).

Water Sampling and Analysis

Water samples were collected at 10:00 and every hour thereafter for 24 h to determine the inorganic nutrients concentrations. Four inorganic nutrients were measured 12 times at different intervals and included Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-) and phosphorus (PO_4^{3-}). Analysis of the various inorganic nutrients Ammonium, Nitrate, Nitrite and Phosphorus were determined using a Spectroquant® Pharo 300M. The detailed chemical analysis methods were done photometrically based on the manufacturer's manual – Merck KGaA, (Germany) www.merck-chemicals.com/test-kits, www.merck-chemicals.com/photometry. The amount of light (µE m-² s-1) was also recorded as irradiance levels and were measured using a Biospherical Instruments probe (QSP200). Algal tissue metal content was determined every 21 days for 3 months. The heavy metals tested for included cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb), using an Atomic Absorption Spectrophotometer (AAS), Unicam Atomic Absorption – M Series), Unicam Limited, U.K.

Statistical Analysis

The design of the experiment was completely randomized with three replications. Apart from light and temperature data collected every hour, other inorganic nutrients were sampled every three hours for 24 h. For heavy metals, % N and % P significance differences were used to juxtapose with standards. Data are presented as means ± standard deviation (SD). All data were analyzed using GraphPad Prism5.

Results

Our findings show that nutrient availability followed the fertilizer ratio (Fig. 2-7; Table 1). Availability of Ammonium (NH_4^+) showed a diurnal variation with the different treatments, the highest being observed during the day (0.18 mg L^{-1}) in the quadruple fertilizer ratio (12:00 pm) and reducing with time, its lowest (0.04 mg L^{-1}) value recorded at 6:00 pm (seawater), 6:00 pm and 4:00 am (double fertilizer ratio), and 10:00 pm (quadruple fertilizer ratio) respectively. Nitrate (NO_3^-) was highest in the quadruple fertilizer ratio (8 mg L^{-1}), with the lowest value (0.11 mg L^{-1}) occurring at 10:00 pm and 10:00 pm in the double fertilizer ratios. Nitrite (NO_2^-) was stable in the treatments and ranged from 0.01 – 0.02 mg L^{-1}, but was highest in the seawater control at 0.03 mg L^{-1} at 12.00 pm. Phosphorus (PO_4^{3-}) availability in the different treatments increased with day time and attained a peak (0.44 mg L^{-1}) at 2:00 pm (quadruple fertilizer ratio), while the lowest value (0.06 mg L^{-1}) was observed in the seawater control at 10:00 am. Temperature in this study ranged between 14.1 – 20.7°C and was a function of the availability of day light, which showed a gradual decrease in photoperiod (0 – 1900 µE m^{-2} s^{-1}) with time (16:8 h light: dark). With regards to heavy metals, *U. armoricana* showed a preference for bioaccumulation, which ranged as follows: zinc (9.908 – 32.942 $mg.kg^{-1}$); copper (1.893 – 5.927 $mg.kg^{-1}$); cadmium (0.254 – 1.500 $mg.kg^{-1}$); and lead (none detectable). The results also showed that *U. armoricana*'s assimilation affinity decreased as follows: double > quadruple > 0 (Table 1). Apart from cadmium, heavy metal contamination levels in cultured *U. armoricana* showed safe uptake mechanisms in all fertilizer ratios compared to various local and international standards.

Discussion

Aquaculture effluents are rich in NH_4^+ that could come from feed and nutrients in the inlet water. Although NH_4^+ concentrations > 2.0 mg L^{-1} can be detrimental (Lazur 2007), aquaculture effluents are highly suitable as a nutrient source for *Ulva* species. Values of NH_4^+ in this study were comparatively low. NH_4^+ in winter is typically low due to the lower mean temperatures (Fig. 6 and 7) and pH. These results are consistent with those reported by Robertson-Andersson (2007). Nitrate-nitrogen concentrations above 3 mg L^{-1} and any detectable amounts of total P (above 0.025 mg L^{-1}) may be indicative of pollution from fertilizers, manures or other nutrient-rich wastes (Cole *et al.,* 2014). Nitrogen and phosphorus are nutrients that may cause increased growth of aquatic plants and algae. Dissolved inorganic phosphorus (DIP) obtained in this study corroborated the outcome of a related experimental investigation by Robertson-Andersson (2007). Nitrites from feed are not toxic to seaweed. Several authors have reported assimilation rates of NH_4^+ in the range of 50−90 µmol N g^{-1} DW h^{-1} among different *Ulva* species, and these species have been verified as successful biofilters of aquaculture wastewaters (Hernández *et al.,* 2002; Neori *et al*., 2003; Copertino *et al.,* 2009; Cahill *et al*., 2010). Nitrite results from enriched nutrients and there is evidence of nitrate uptake during the day (Potgieter, 2005; Robertson-Andersson, 2007). The inorganic nutrients observed in this study were below the South African water quality guidelines for Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-), and phosphorus (PO_4^{3-}) (DWAF, 1996).

Table 1: Heavy metals and nutrient composition in *U. armoricana* grown in the various experimental treatments

Heavy metals/ nutrient	Experimental Treatments (Mean ± SD)			Standards	
	Seawater (0 fertilizer)	Double fertilizer ratio	Quadruple fertilizer ratio	*SA permissible limit ($mg.kg^{-1}$) (lettuce)	**FAO/WHO permissible Limit ($mg.kg^{-1}$)
Cd	0.639±0.023^a	1.166±0.360^b	0.8451±0.566^b	0.1	0.2
Cu	4.619±1.193^a	5.676±1.367^a	4.687±1.148^a	30.0	0.1
Pb	ND	ND	ND	0.5	0.3
Zn	18.640±4.814^a	20.244±2.011^a	22.158±8.991^a	40.0	0.015 - 0.030***
% N	2.122±0.862a	3.220±0.494^b	2.350±1.039^b	-	GMP
% P	1.789±0.082^a	1.711±0.318^a	1.700±0.269^a	-	2200

Means in the same row with the same superscript are not significantly different ($p > 0.05$), *South Africa Government Gazette, 9 September, 1994, metals in foodstuffs, cosmetics and disinfectants act, (Act no. 54 of 1972), **FAO/WHO (2001) standard for seaweed/vegetable, ***Australia recommended leaf nutrient concentrations, GMP = Good manufacturing practices (GMP) must be followed (hygiene, low temperature, and disinfection) as in packaging gas. ND = none detected

Fig. 1: Flow-through, paddle-wheel raceways are the preferred method for growing *Ulva*

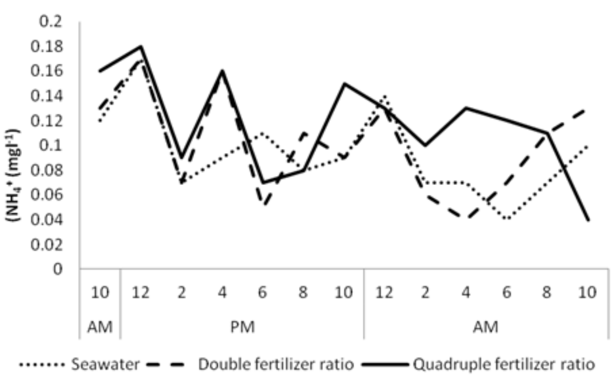

Fig. 2: Ammonium (NH_4^+) time graph for different fertilizer ratios

Light intensity is well correlated with temperature, which is largely subject to diurnal and seasonal changes both in irradiance and photoperiodic systems. This finding differs from those of Lüning (1993) and Kirk (1994) who showed that light intensity correlated with day length and not temperature. Most outdoor culture systems research on *Ulva* showed that the alga could readily be cultured at 15 – 20°C and at 400 – 1000 $\mu Es^{-1}m^{-2}$ (Winberg *et al.*, 2011; Corey *et al.*, 2012, 2014). These values are similar to the irradiance and photoperiod ranges found in the present study using *U. armoricana*. This study showed that double fertilized *U. armoricana* had high Cd, Cu and Zn values, but that values for Cu, Pb and Zn were lower than the permissible South African limits for lettuce (Table 1). Concentrations of Cd in all treatments were, however, higher than SA limits for lettuce. This may be due to the rate of fertilizer application. Only low/trace levels of Pb were

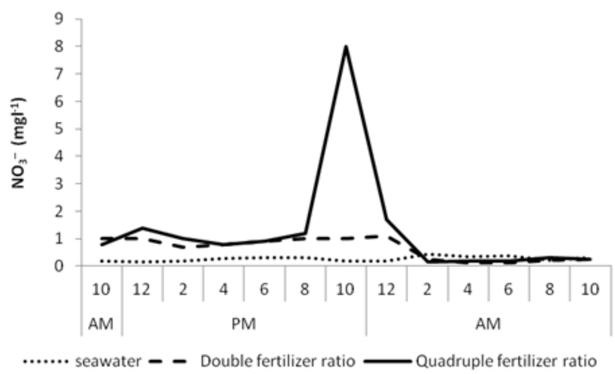

Fig. 3: Nitrate (NO_3^-) time graph for the different fertilizer ratios

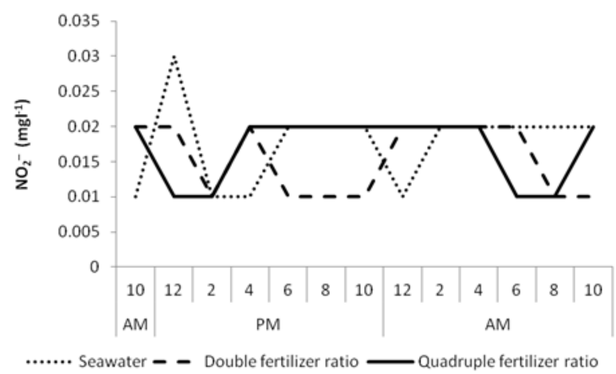

Fig. 4: Nitrite (NO_2^-) time graph for the different fertilizer ratios

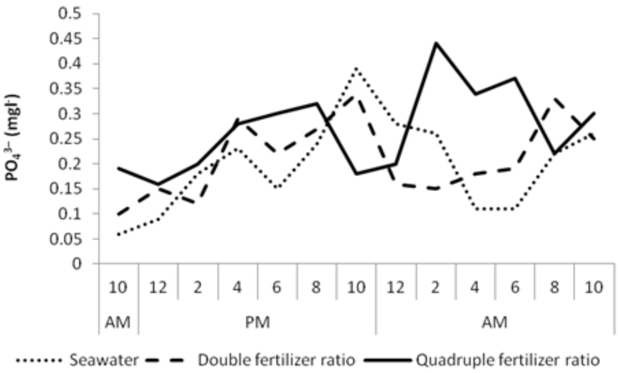

Fig. 5: Phosphorus (PO_4^{3-}) time graph for the different fertilizer ratios

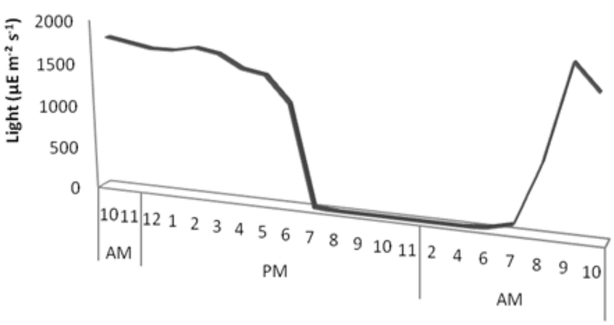

Fig. 6: The mean amount of light available over a 24 h period for *U. armoricana* biomass production in the culture ponds from this study

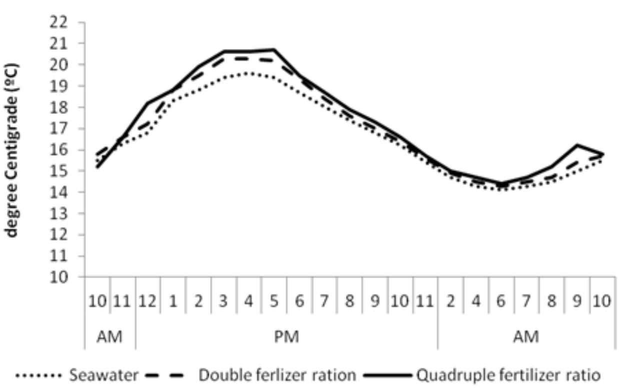

Fig. 7: The mean temperature over a 24 hour period in the culture ponds from this study

found in seawater and in fertilized *U. armoricana*. Apart from Pb that was not detected in all treatments, the other heavy metals had values higher than the FAO/WHO (2001) standard for seaweed/vegetable. The main observation here seems to be that cultured *U. armoricana* at Benguela Abalone Group tended to have higher levels of heavy metals than *Ulva* from the unfertilized/seawater tanks. This result is contrary to the findings of Shuuluka (2011).

The Cd values in this research were higher than the maximum recommended level for Cd in the FAO/WHO (2001) standard for seaweed/vegetable, the South African limits for lettuce, the French limits for edible seaweeds (<0.5 $\mu g g^{-1}$ dw, Besada *et al.*, 2009) and the Australian and New Zealand limits for edible seaweeds (0.2 $\mu g g^{-1}$ dw, Almela *et al*., 2002, 2006; Besada *et al*., 2009). The high Cd concentrations in the current study could well have originated from the unfiltered seawater and/or the fertilizer (Shuuluka 2011). Irrespective of the source, our Cd values negate the use of these seaweeds for human consumption.

Conclusion

As human health is directly affected by ingestion of vegetables, the biomonitoring of trace elements in macroalgae needs to be continually monitored because these algae are the main sources of food for humans in many parts of the world. It is therefore of great importance that South Africa implements a continuous update of its seaweed safety monitoring by formulating a standard guideline and permissible limits of nutrients in macroalgae that must be strictly adhered to by all industries.

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