Experimental Validation of a Performance Model of a Biofiltration System

Juvyneil Echon Cartel

January 11, 2020

Experimental Validation of a Performance Model of a Biofiltration System

Juvyneil Echon Cartel

Chemical Engineering Department
Eastern Visayas State University
Tacloban City, Philippines 6500

juvyneil.cartel@evsu.edu.ph

***Abstract* - *Ulva lactuca* is a green alga, commonly named as "sea lettuce", which belongs to the Division *Chlorophyta*. It has been found to be a potential bio-filter that can assimilate nitrogen and phosphorus in seawater. This study has determined the feasibility of a flow system utilizing *U. lactuca* which was made possible by establishing a performance model of the system that could meet the ASEAN Marine Quality Criteria (AMQC) in terms of nitrogen and phosphorus effluent concentration. The performance model was validated through a series of laboratory experiments of three treatments. The biofiltration system has shown to have a fair reduction of nitrogen and phosphorus in the seawater with an average removal efficiency of 67% and 68% for nitrogen and phosphorus, respectively. Effluent nitrogen and phosphorus concentrations were observed to have considerable errors in relation to AMQC. However, biofiltration processes seemed to have decreasing decay ratio as time proceeds which means that AMQC may be sustained when the process time is extended, thereby giving more time for *U. lactuca* to recover from the stress experienced in its new environment. A three-stage biofiltration system design with increased acclimatization and residence time could improve the performance of the system.**

Keywords - biofiltration, Chlorophyta, performance model, sea lettuce, Ulva lactuca

I. INTRODUCTION

Wastewater discharged to bodies of water without proper treatment gives detrimental effects to marine life. An increasing number of worldwide reports on the eutrophication of benthic algae are causing serious economic and ecological problems [1]. In order to solve this problem, physical and chemical treatment has been widely used to treat wastewaters but the investment is high. For instance, the government of Yokohama, Japan is spending more than forty million yen per annum (equivalent to more than US$ 367,741.68 per annum) to remove the accumulation of *Ulva* spp. on the beach using physical and chemical treatment method [5]. As a result, some of the individuals or entrepreneurs do not follow effluent regulation law religiously. Utilization of an ample amount of green macro-algae in treating polluted seawater effluents (e.g. fishponds) such as *U. lactuca* is a good option to solve the problem since this has been proven by certain studies to be a good bio-indicator and bio-filter at a lower cost than the physical and chemical treatment method. Using this macro-algae as an alternative treatment method will help in maintaining ecological balance in the aquatic life [11].

Ulva lactuca Linnaeus is a green alga that belongs to Division Chlorophyta under the genus *Ulva* [23]. Its color is due to different pigments that the algae use to form chemical energy from the sunlight during the photosynthesis. *U. lactuca* lacks the complex array of tissues used for reproduction and water transport [17]. It does not have roots, stems, and leaves but is composed of a thallus (leaf-like), sometimes a foot, and a stem [26].

U. lactuca exhibits high growth rate, low epiphytism (frequently grows on top of or is supported by another plant), and low susceptibility and high resistance to environmental stress conditions [13]. Here in the Philippines, *U. lactuca* is distributed all over the archipelago and is common along the coasts of Mactan Island. *U. lactuca* occurs almost regularly at Mactan Island that reaches an average biomass of up to 0.5 kg dry weight per square meter causing a phenomenon called "green tide" [14].

Over-proliferation of *U. lactuca* is mainly caused by massive disposal of untreated wastes to seawater which contains high levels of nitrogen and phosphorus. Over-proliferation can contribute direct and indirect effect on the aquatic environment. They can block sea grass areas of light which causes a decline, eventually. Mats of algae that are decomposing can also cause depletion of the water column of dissolved oxygen. At some point, this condition may drive oxygen levels to hypoxic (deleteriously low oxygen level) or anoxic (zero oxygen level) which may cause massive fish kills even if the condition lasts for only a few hours [11, 16].

Nitrogen and phosphorus are major nutrients of macroalgae. Reductive metabolism to amino group-NH_2 requires more energy as well as the presence of the enzyme nitrate reductase which makes ammonia (as nitrogen) preferable for the growth of macroalgae. Ammonium concentration in seawater ranges from 2 to 94.2 μM while 7 to 80 μM in fishponds [18, 20]. A nitrogen to phosphorus ratio of 15.5:1 by mole (7:1 by weight) is typically required by a living matter [21, 22]. Generally, if the nitrogen to phosphorus ratio is greater than 10 (by weight), phosphorus is said to limit macroalgae growth since it is available in the form of orthophosphate ions almost entirely in the sea. Otherwise, nitrogen limits growth [10]. For *Ulva* spp., nitrogen is usually the growth-limiting factor [13].

U. lactuca has been found to be a potential bio-filter that can absorb nitrogen and phosphorus in seawater where it could reach up to 126.6 μmol NH_4^+/g(dry weight)-h ([24]. Meaning, utilizing *U. lactuca* properly would help in maintaining ecological balance in the aquatic life. The study can help promote the utilization of *U. lactuca* for industrial use such as food, feed, biogas production, an anti-microbial agent, and most especially, as a growth-limiting factor [4, 8, 9, 13].

The goal of the project is to determine the feasibility of a continuous-flow treatment system utilizing *U. lactuca*. The specific objectives of the project are: (1) to make a performance model of the system; (2) validate the performance model of the system through a series of experiments; and (3) determine the simultaneous ammonium and phosphate uptake of *U. lactuca* in a polluted seawater employing the continuous flow system at the effluent of a fishpond at known algal biomass concentration and volumetric flow rate.

II. Materials And Methods

A. Collection of water and macro algae

The seawater was taken in coastal water of Portofino Beach Resort, Brgy. Angasil, Lapu-lapu City, Cebu. Seawater is said to be unpolluted when its nitrogen and phosphorus concentration fall below the ASEAN Water Quality Criteria which is 5 µM and 0.48 µM, respectively. *U. lactuca*, on the other hand was collected in Brgy. Kalawisan, Lapu-lapu City, Cebu [2].

B. Experimental Set up

The seawater that was used as a culture medium in nutrient uptake experiments was filtered using an ordinary filter to remove physical contaminants (e.g. suspended solids).

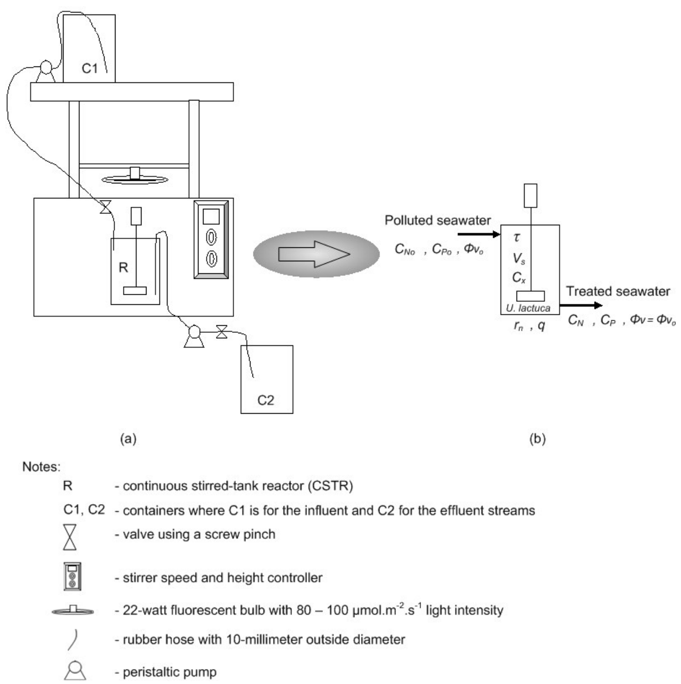

Fig. 1 Experimental set-up layout (a) and conceptual diagram (b) of the continuous-flow aquatic treatment system.

U. lactuca samples were placed in a 20-liter bioreactor filled with filtered unpolluted seawater and acclimatized for four days to eliminate the lag phase of macroalgal growth during the experiment. The reactor was aerated gently using a portable aerator, which also ensured mixing in the tank. A light was set at 12:12 light to dark cycle with an intensity of 80 to 100 µmol photons.m^{-2}.s^{-1}. Initial nitrogen to phosphorus ratio of 10:1 (by mole) was used to ensure that there was no phosphorus limitation that would happen in the system. Normal seawater salinity of 30-35%, ambient temperature of 26 to30ºC, and seawater pH range of 7 to 8 were monitored throughout the experiment [24, 27].

C. The Model

The performance model was based on the kinetic study of Taboada *et al.* [24] and data of Neori *et al.* [19] that could design a residence time, τ and eventually set a volumetric flow rate, *Φv* (Equation 1) that would lead to the desired uptake and nutrient levels in seawater at the given initial ammonium and phosphate concentration, and known initial algal biomass concentration that conforms the ASEAN Water Quality Criteria [2]. The performance model was validated through series of experiments using synthetic polluted seawater. Equation 2 is the performance model used in order to validate the performance of the designed aquatic treatment system. Volumetric flow rate can be estimated by dividing the working volume of the reactor with the residence time [7, 15].

$$\tau = \frac{(C_{So} - C_S)}{C_x\,(r_S{}')} \tag{1}$$

$$\tau = \frac{(C_{So} - C_S)}{C_x \left\{ \frac{\left[71.25\,\frac{\mu mole}{g(DW)}.h\right] C_S}{14.69\ \mu M + C_S} + (0.51\,h^{-1})C_S \right\}} \tag{2}$$

Where τ is the residence time (in hours), *V* is the volume of the reactor (in liters), *Φv* is the volumetric flow rate in (liters per hour), C_x is the final biomass concentration (in grams-dry weight per liter), $r_S{}'$ specific uptake rate (micromoles per gram-dry weight and hour), while C_{So} and C_S are the influent and effluent ammonium or phosphate concentration.

The established model was then validated by conducting a series of experiments at varied conditions of initial ammonium concentration, phosphate concentration, and volumetric flow rates. Validation was made by measuring the removal efficiency of the biofiltration system. Necessary adjustments to residence times were made in order to meet the ASEAN Marine Quality Criteria.

The established model was then validated by conducting a series of experiments at varied conditions of initial ammonium concentration, phosphate concentration, and volumetric flow rates. Validation was made by measuring the removal efficiency of the biofiltration system. Necessary adjustments to residence times were made in order to meet the ASEAN Marine Quality Criteria.

D. Experimental Design

Two liters of seawater medium was maintained in the reactor. Each run was conducted and monitored for five days to ensure a steady-state condition. One control set-up containing same ammonium and phosphate concentration was used in the experimental run in the absence of *U. lactuca*. Other set-ups were done in duplicate. Three treatments were made: (1) 20:2:25; (2) 60:6:10; and (3) 100:10:8 [C_{No} (µM): C_{Po} (µM): Φv (ml/min)].

Two-liter CSTR (continuous stirred-tank reactor) with 12-rpm stirrer speed was contained with 0.90 gram dry-weight algal biomass (or an algal biomass concentration of 0.45 gram dry-weight per liter). The reactor was fed with unpolluted seawater enriched with ammonium and phosphate at varying concentrations from 10 to 100 µM ammonium and 1 to 10 µM phosphate [3]. The estimated volumetric flow rate of each ammonium and phosphate concentrations was applied. Influent ammonium and phosphate concentration were monitored every 12 hours in each experimental run for possible degradation while the effluent ammonium and phosphate concentration were monitored every four hours for the first day, every six hours in the second and third day, and every eight hours in the fourth and fifth day. Influent and effluent concentrations monitoring were done until a steady-state condition was achieved which is expected to occur within five days. The initial and final algal biomass concentration was determined in each experimental run [3, 6]. Light intensity was monitored once in every run. The salinity of seawater and seawater level inside the reactor were monitored once a day while the monitoring of pH and temperature was done after the ammonium and phosphate analyses [6]. Specific nutrient uptake, nutrient uptake rate, and removal efficiency were also determined in each experimental run [6].

III. Results and Discussion

A. Nitrogen Concentration, Removal Efficiency, and Uptake

Figures 2, 3, and 4 illustrate the effluent profile of nitrogen at a given influent ammonium (as nitrogen) concentration. The set value for effluent nitrogen concentration in Experiments 1, 2, and 3 is 5 µM to meet the ASEAN marine quality criteria. It came to show that as the influent concentration increases, the margin of error also increases (Table I). The trend for removal efficiency for nitrogen was almost the same which has shown fair reduction which is maximum during Experiment 2 at 75.06% which is just close to Experiment 3 at 74.06% (Table II). The trend was also consistent with the specific nitrogen uptake rates which were high during Experiment 3 at 36.44 µmol /g(DW).h (Table II).

TABLE I

Influent and Effluent Nitrogen and Phosphorus at Different Residence Times

Run#	Residence Time (min)	Influent Concentration (µM)		Effluent Concentration (µM)	
		N	P	N	P
1	78.66	20.42±0.17	2.12±0.04	5±2.22	0.48±0.19
2	192.08	60.65±0.39	6.25±0.11	5±4.74	0.48±0.56
3	248.70	100.52±0.17	10.46±0.14	5±9.76	0.48±1.12

TABLE II

Specific Uptake Rates and Removal Efficiency at Different Residence Times

Run#	Specific Uptake Rates (µmol/g(DW).h)		Removal (%)	
	N	P	N	P
1	17.97	2.71	52.20	59.91
2	30.30	3.63	75.06	71.62
3	36.44	4.92	74.06	72.39

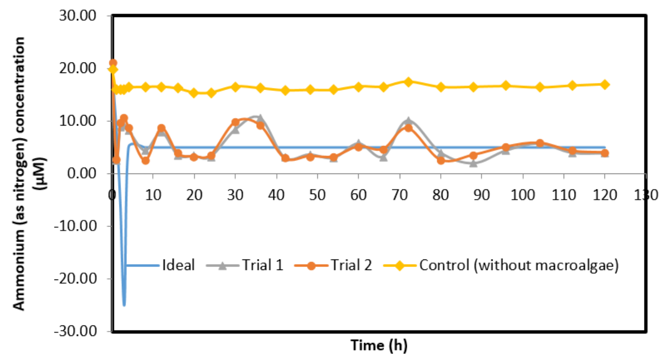

Fig. 2 Effluent nitrogen concentration profiles of Experimental Run#1 at an influent ammonium (as nitrogen) concentration of 20.42±0.17 µM and τ = 78.66 min

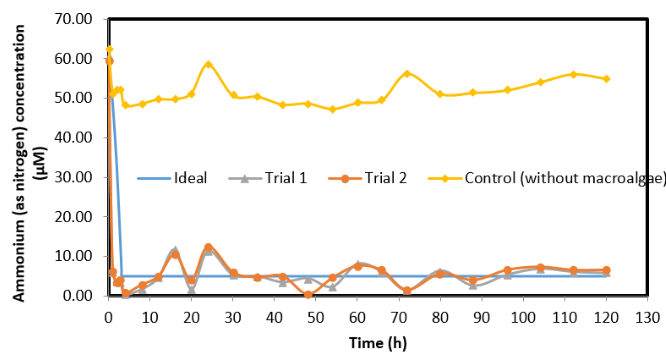

Fig. 3 Effluent nitrogen concentration profiles of Experimental Run#2 at an influent ammonium (as nitrogen) concentration of 60.65±0.39 µM and τ = 192.08 min.

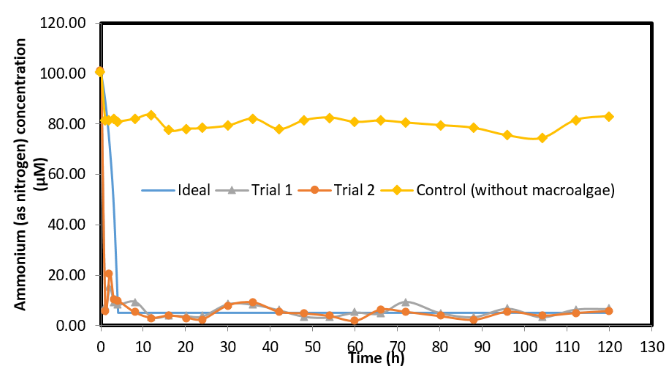

Fig. 4 Effluent nitrogen concentration profiles of Experimental Run#3 at an influent ammonium (as nitrogen) concentration of 100.52±0.17 µM and τ = 248.70 min.

B. Phosphorus Concentration, Removal Efficiency, and Uptake

Figures 5, 6, and 7 illustrate the effluent profile of phosphorus at a given influent phosphate (as phosphorus) concentration. The set value for effluent phosphorus concentration in Experiments 1, 2, and 3 is 0.48 µM to meet the ASEAN marine quality criteria. It came out that the margin of error at 95% confidence interval was consistent with the trend of the margin of error for effluent nitrogen concentration which is highest during Experiment 3 at 1.12 µM (Table I).

The trend for removal efficiency for phosphorus was similar to that of the nitrogen which is highest during Experiment 3 at 72.39% (Table II). The trend was also consistent with the specific phosphorus uptake rate which highest at 4.92 in µmol /g(DW).h (Table II).

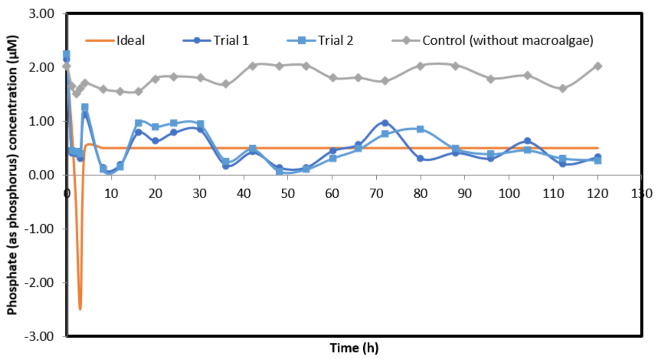

Fig. 5 Effluent phosphorus concentration profiles of the Experimental Run#1 at an influent phosphate (as phosphorus) concentration of 2.12±0.04 µM and τ = 78.66 min.

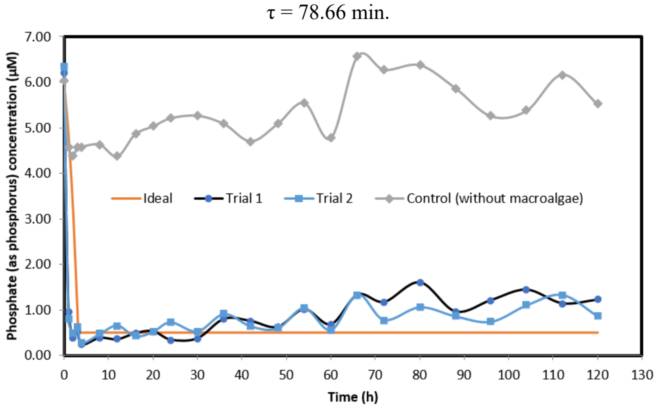

Fig. 6 Effluent phosphorus concentration profiles of the Experimental Run#2 at an influent phosphate (as phosphorus) concentration of 6.25±0.11 µM and τ = 192.08 min.

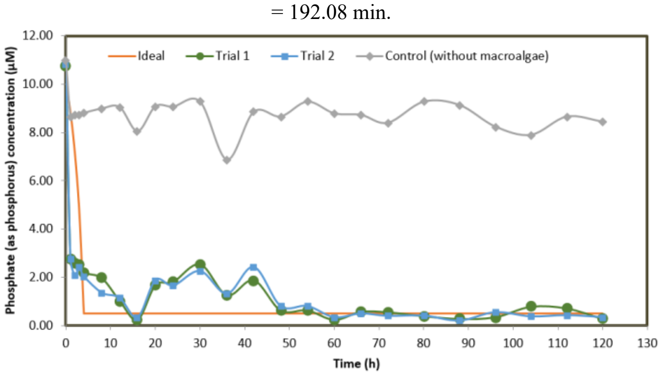

Fig. 7 Effluent phosphorus concentration profiles of the Experimental Run#3 at an influent phosphate (as phosphorus) concentration of 10.46±0.14 µM and τ = 248.70 min.

B. Biomass Concentration Profiles

The initial and final concentration (after 120 hours) biomass concentration were determined from the experiment while the biomass concentration from a time equal to 1 h to 112 h were calculated using the following equation [18]:

$$\mu = \left[\frac{\ln\left(C_x/C_{xo}\right)}{t}\right].100 \tag{3}$$

Where C_{xo} is the initial biomass concentration (in g(DW)/L), C_x (in g(DW)/L) is the final biomass concentration at a time, t (in h) and μ is the specific growth rate (in %h^{-1}). Based on the results, it came out that the specific growth of the *U. lactuca* came out to be low which 0.04 % h^{-1} (average) was for Experiment 1 and 0.17 % h^{-1} (average) for Experiments 2 and 3. With these findings, the growth of *U. lactuca* for five days seemed to be negligible which is advantageous in making the system as much as possible, ideal. These specific growth rates are lower than what has reported by Neori *et al.* [18] of 0.75 % h^{-1} done in a nutrient-rich fishpond effluent. This result indicates environmental stress experienced by *U. lactuca* on its new environment at synthetic conditions.

C. The Validity of the Performance Model

The trends for reduction of nitrogen and phosphorus profiles suggest that there is a direct relationship of the removal efficiency, specific uptake rates, and the margin of error of the effluent phosphorus concentration with the residence time which is consistent with the nitrogen reduction profiles. It can also be deduced from Figures 2 to 7 that though there are significant errors observed in the five-day experimental runs, there is a great chance that if this is extended for more days, the ASEAN Marine Quality Criteria (AMQC) for effluent nitrogen and phosphorus concentrations will be met. This is evident in the reduction of the decay ratio observed in the process. In addition, the extension of the experimental run may favor *U. lactuca* in the adaptation process in its new environment which in effect, could give higher specific uptake rate.

Moreover, it was observed from the obtained results, through the effluent nitrogen and phosphorus concentration profiles, that the performance model has a considerable margin of errors that it cannot meet the ASEAN marine quality criteria (AMQC). The result also suggests that the performance model is validated further through field tests to eliminate or reduce possible environmental stress that *U. lactuca* could experience and eventually meet the desired criteria.

C. Other parameters

Table III shows the values observed in the three experimental runs. It came out that all the parameters were within the acceptable values.

TABLE III
PROCESS VALUES OF OTHER PARAMETERS

Parameters	Mean	Unit	Margin of Error
pH1	7.80		0.040
pH2	7.79		0.030
pH3	7.82		0.030
Temperature1	26.04	°C	0.070
Temperature2	26.09	°C	0.100
Temperature3	26.04	°C	0.070
Salinity1	33.00	%	0.000
Salinity2	33.00	%	0.000
Salinity3	33.00	%	0.000
Light intensity1	89.13	μmol photons $m^{-2}.s^{-1}$	13.670
Light intensity2	90.31	μmol photons $m^{-2}.s^{-1}$	7.920
Light intensity3	92.58	μmol photons $m^{-2}.s^{-1}$	6.610
Impeller speed1	12.00	rpm	0.180
Impeller speed2	12.04	rpm	0.220
Impeller speed3	11.91	rpm	0.180
Volumetric flowrate1	25.03	ml/min	0.009
Volumetric flowrate2	10.01	ml/min	0.006
Volumetric flowrate3	7.98	ml/min	0.006

IV. CONCLUSIONS AND RECOMMENDATIONS

The continuous-flow treatment system utilizing *U. lactuca* where the performance model was used in designing the experimental conditions (e.g. flow rate, volume) has shown to have considerable errors in its effluent N and P concentration in relation to the ASEAN Marine Quality Criteria (AMQC) which was caused by low specific uptake rates that in effect, showed low removal efficiency. However, an extension of the biofiltration process may favor *U. lactuca* in the adaptation process in its new environment as observed, which in effect, could give higher specific uptake rates and eventually, higher removal efficiency. Environmental stress experienced by *U. lactuca* is evident when its growth rate came out to be lower by approximately 77% compared to that reported by Neori *et al.* [18] on a biofiltration process done in a nutrient-rich fishpond effluent. Residence time also plays a vital role in improving the removal efficiency of the system for it can be manipulated. Hence, designing a three-stage biofiltration system while increasing the acclimatization and residence time could improve the removal efficiency of the system and eventually, meet the ASEAN Marine Quality Criteria.

ACKNOWLEDGMENT

This research project was supported by the Department of Science and Technology (DOST), Philippines in collaboration with Eastern Visayas State University and University of San Carlos.

REFERENCES

[1] Anderson RJ, Monteiro PMS, Levitt GJ. 1996. The effect of localized eutrophication on competition between *U. lactuca* (*Ulva*cease Chlorophyta and a commercial resource of Gracilaria verrucosa (Gracilariaceae, Rhodophyta), Hydrobiologia 326/327:291-296.
[2] ASEAN Secretariat. 2004. Marine Water Quality Criteria for the ASEAN Region, 6th Meeting of the ASEAN Working Group on Coastal and Marine Environment, 22 – 23 June 2004, Jakarta, Indonesia.
[3] Buapet P, Hiranpan R, Ritchie RJ, Prathep A. 2008. Effect of nutrient inputs on growth, chlorophyll, and tissue nutrient concentration of *Ulva* reticulata from a tropical habitat, Science Asia 34: 245–252.
[4] Bruhn A., Dahl J., Nielsen A.B., Nikolaisen L., Rasmussen M.B., Markager S., Olesen B., Arias C., Jensen, P.D. (2011). Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion, Bioresource Technology 102 (2011) 2595–2604.
[5] Carayo CC, Culango KC, Estrera D, Largo DB, Danilo DT. 2004. Correlation of inorganic nutrients to the standing crop of "Green Tide" algae during the peak growth season in Easten Mactan Island, Central Philippines, The Phillippine Scientist 41:36-46.
[6] Eaton AD, Clesceri LS, Rice EW, Greenberg AE. 2005. Standard Methods for the Examination of Water and Wastewater. Port City Press, Baltimore, Maryland, USA, pp 4:49-50, 108, 146-147.
[7] Fogler H. 1999. Elements of Chemical Reaction Engineering, 3rd edition, Prentice Hall, New Jersey, USA, pp 16-22.
[8] Geertz-Hansen O, Sand-Jensen K. 1992. Growth rates and photon yield of growth in natural populations of a marine macroalga *U. lactuca*, Marine Ecology Progress Series 81: 179-183.
[9] Harlin MM, Miller TM, Thursby GB. (1979). Ammonium Uptake by Glacilaria sp. (Florideophyceae) in Closed System Fish Culture, Proc. Int. Seaweed Symp. 9: 285- 292.
[10] Horne AJ, Goldman CR. 1994. Limnology 2nd Ed. McGraw-Hill Book Co., Singapore, pp 210-245.
[11] Jones AB. 1999. Environmental Management of Aquaculture Effluent: Development of Biological Indicators and Biological Filters, Thesis, Department of Botany, the University of Queensland, Australia.
[12] Kargi F, Shuler M. 2002. Bioprocess Engineering: Basic Concepts, 2nd ed., Prentice –Hall Inc., New Jersey, pp 57-77, 155-175.
[13] Kuhn H, Melgo JL, Palomar MJ. 2000. The Effects of Different Nitrate and Phosphorus Concentration on the Growth Rate of Two Species of *Ulva* under Low Light Intensity, Thesis: Kuh 96/SL 0170. Marine Biology Department, University of San Carlos, Cebu City, Cebu, Philippines.
[14] Largo D, Sembrano J, Hiraoka M, Ohno M. 2004. Taxonomic and ecological profile of 'green tide' species of *Ulva* (*Ulva*les, Chlorophyta) in central Philippines, Hydrobiologia 512:247-253.
[15] Levenspiel O. 1999. Chemical Reaction Engineering, 3rd edition, John Wiley and Sons, Inc., New York, pp 90-111.
[16] Licker MD. 2003. McGraw Hill Dictionary of Scientific and Technical Terms, 6th ed., New York, p 2024.
[17] Lobban CS, Harrison PJ. 1994. The Physiological Ecology of Seaweeds. Cambridge University Press, New York, pp 75-110.
[18] Neori A, Cohen I, Gordin H.1991. *Ulva lactuca* Biofilters for Marine Fishpond Effluents:Growth Rate, Yield, and C:N Ratio. Botanica Marina 34:483-489.
[19] Neori A, Krom KD, Ellner SP, Boyd CE, Popper D, Rabinovitch R, Davison PJ, Dvir O, Zuber D, Ucko M, Angel D, Gordin H.1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. Aquaculture 141:183-199.
[20] Parker HS. 1981. Influence of Relative Water Motion on the Growth, Ammonium Uptake and Carbon and Nitrogen Composition of *U. lactuca* (Chlorophyta). Mar. Biol. 63: 309-318.
[21] Pedersen MF. 1994. Transient ammonium uptake in the macroalga *U. lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique, J. Phycol. 30:980-986.
[22] Pedersen MF, Borum J. 1997. Nutrient control of estuarine macroalgae:growth strategy and the balance between nitrogen requirements and uptake, Marine Ecology Progress Series 161: 155-163.
[23] Silva M, Vieira L, Almeida AP, Kijjoa A (2013) The Marine Macroalgae of the Genus *Ulva*: Chemistry, Biological Activities and Potential Applications. Oceanography 1: 101. doi:10.4172/2332-2632.1000101.
[24] Taboada EB, Maglente WM, Tan PU. 2007. Ammonium Uptake by the Green Macroalgae (*U. lactuca*) species, Proceedings of Asia-Pacific

Biochemical Engineering Conference (APBioCheC), November 4-7, 2007, Taipei, Taiwan.

[25] Torrefiel IC, Mislos KJ, Apalisok AR, Dy DT. 2004. Comparative NH_4^+ uptake of Caulerpa lentillifera (Chlorophyta, Caulerpales) and Gracilariopsis Heteroclada (Rhodophyta, Gracilariales): competition between a cultured alga versus an unwanted species, The Phillippine Scientist 41:36-46.

[26] Trono GC, Jr.. 1997. Field Guide and Atlas of the Seaweed Resources of the Philippines, Bookmark Inc., Makati City, Philippines, pp 23-27.

[27] Vermaat JE, Sand-Jensen K. 1987.Survival, metabolism, and growth of *U. lactuca* under winter conditions: a laboratory study of bottlenecks in the life cycle, Marine Biology 95:55-61.