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Producing biomass *Chlorella sp.* and *Moina sp.* from domestic wastewater

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Abstract

This study was conducted in two lab-scaled high rate algae cultivation ponds operating at hydraulic retention time (HRT) of 1.5 days and 1.8 days to cultivate *Chlorella sp*. in combination with continuous ponds raising *Moina sp.* to evaluate the effectiveness of biomass production. The study result showed that the flow rate of the algae pond having HRT 1.5 days is 133 mL/mins, while that of 1.8 days HRT is 111 mL/mins. The result indicated that the Chlorophyll content in the effluent of the 1.5 day HRT algae pond was 1.92 times higher than that of the 1.8 day HRT algae pond and significant difference (5%). The quality of treated waste-water was improved when the effluent of algae ponds (without algal biomass removal) went through the *Moina sp.* raising ponds. Therefore, we can take advantage of domestic wastewater to feed *Chlorella sp.* in the high rate cultivation pond and to minimize pollution matter containing in the wastewater, and at the same time *Chlorella sp.* could be used as feeding to *Moina sp.* and then *Moina sp*. applicable for fish cultivation.

Keywords: *domestic wastewater, Chlorella sp., high rate algae cultivation ponds, Moina sp.*

1. Introduction

In Vietnam, rapid urbanization and population growth has created huge pressure on infrastructure system. According to the water sector review report (ADB, 2009), water supply service covers over 70% of the whole country in Vietnam while the drainage system covers about 40 - 50%. The drainage coverage rate ranges from 70% in large urban areas to only 10 - 20% in small urban areas. However, most of domestic wastewater in urban areas is not treated centrally but by households' septic tanks and discharged directly into open sources such as rivers, lakes and streams. This practice occurs especially in rural areas where people live decentralization, and sewage network is seldom built due to economic matter. The result of water quality monitoring of major canals, lakes and rivers in some areas showed that concentration of organic pollutants is 1.5 to 3.0 times, or even 10 - 20 times higher than the standards set forth in the QCVN 14:2008/BTNMT - National technical regulation on domestic wastewater (VEA, 2010). The situation has existed for years leading to serious consequences for local people and environment. The study is aimed to set up a lab-scaled model of applying decentralization domestic wastewater treatment units to take advantage of the value of the nutrients from domestic waste-water and promote the use of the treatment to help solve the problem in these rural areas.

Using domestic wastewater to produce *Chlorella sp.* is a new direction due to good characteristics of algae such us fast growth, withstanding of environmental changes and capacity to grow in wastewater with high nutritional value and organic content. The biological activities in algae ponds remove organic matter and nutrients from the wastewater and convert them into nutrients for algae cells through photosynthesis processes. Algae capable of treating and recycling nutrients in wastewater are *Spirulina sp.*, *Chlorella sp.*, etc. (Wang *et al*., 2009; Tran Chan Bac, 2013). In regard of *Chlorella sp.*, the algae with high protein content but very small size and hard to collect, they could be applied to raise *Moina sp.* - a great food to feed small fish due to its high nutrient value (Tran Suong Ngoc *et al*., 2010; Rottmann *et al*., 1992).

Using stabilization ponds to treat domestic wastewater requires much land; therefore, this treatment is applicable in rural areas, where low-cost land is available. Oswald (1962) recorded economic advantage of combining protein production with waste treatment. A stabilization pond that can be used for the treatment must be shallow (less than 0.5 m deep) to let oxygen available during daytime for photosynthetic action of the algae developing in the pond. In case of a highly oxidational pond, oxygen supplying needs to operate at a few days residence time. The algae biomass then could remove and utilizing them in feed should be attractive (Oswald and Gotaas, 1957).

This study was conducted to evaluate the production of biomass from raising *Chlorella sp.* in a lab-scaled high rate cultivation algae pond model to feed *Moina sp.*; and at the same time to evaluate efficiency in treating wastewater. This model is considered as a precondition for establishing a technological process of raising *Moina sp.* to collect biomass for feeding purpose.

2. Materials and Methods

The study was conducted at Technical Lab of Department of Environmental Engineering, College of Environment and Natural Resources, Can Tho University, from June 2015 to December 2015.

2.1 Research equipment

2.1.1 Experimental design

The lab-scale experiments were conducted on glass models of high rate cultivation algae ponds that were introduced by Moraine *et al.* (1979). The ponds raising *Moina sp.* were designed by composite tank that were continuously connected by PVC pipe.

The experiments were outside set up with two treatments and each treatment included one high rate cultivation *Chlorella sp.* pond and one pond for *Moina sp.* raising. The design parameters were showed in Table 1.

Table 1. The model design parameters

Parameters	***Chlorella sp.* pond**	***Moina sp.* pond**
Length × width × height (m)	0.8 × 0.6 × 0.4	(0.58 × 0.40 × 0.23) × 2
Operation water level (m)	0.3	0.16
Operation vol. (L)	144	74.2
Surface area (m^2)	0.48	0.46
Ratio of area / volume (m^2/m^3)	3.33	6.2
Length of water flow (m)	5	-

In addition, the models had auxiliary equip-ment such as:

- The Marriott tank (by composite 200 L) to supply water to the model in steady flow;
- The stirring unit in the *Chlorella sp.* pond to create the flow within the pond that prevents the algae from settling to the bottom of the pond keeping the algae floated to eat nutrients; prevents temperature separated inside water body and avoids anaerobic situation at the pond bottom.
- The submersible water pump in the *Moina sp.* pond to provide air for the pond and well mixing of nutrients inside the pond.

2.1.2 Research subjects

a) <u>Domestic wastewater</u>

The domestic wastewater was collected at the sewage pit located at 124 lane, 3/2 street, Xuan Khanh ward, Ninh Kieu district, Can Tho city. The time of collection was 9:00 every morning, coincidently with the time of wastewater discharge by daily activities of households as cleaning, food processing, etc. that represent for domestic wastewater.

b) <u>Algae breed</u>

Chlorella sp. breed was obtained from the laboratory of College of Aquaculture and Fisheries - Can Tho University. The living condition of algae is 20‰ of water salinity, so we need to lower the water salinity to 0‰ by creating adapted environment for algae with supplying biogas wastewater and every day the water salinity was reduced to 5‰. After 6 days, the water salinity was at 0‰, continues breeding algae to 125 L, and then divided algae equally into two ponds to produce algae in high rate cultivation condition.

In the process of breeding, $NaHCO_3$ and $NaNO_3$ need to be added to maintain the pH and to provide protein source for algae activities.

To check if *Chlorella sp.* was able to become dominant in the pond, we took samples of water from the pond to observe algae by a microscope. Figure 1 shows that *Chlorella sp.* dominated in the water samples.

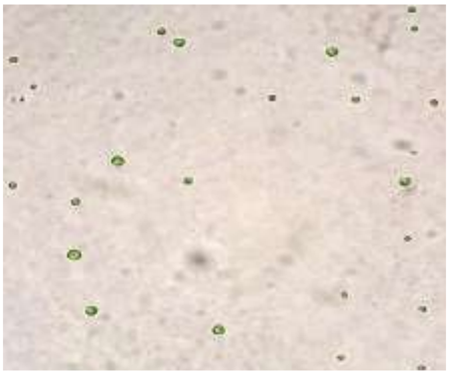

Figure 1. Snapshot of *Chlorella sp.*in the X40 objective lens

c) <u>*Moina sp.*</u>

According to Rottmann *et al.* (1992), under optimum conditions, *Moina sp.* reproduce at only 4 - 7 days of age, with a brood size of 4 - 22 per female, broods are produced every 1.5 - 2.0 days. After bought from aquatic shop, the *Moina sp.* were raised by tap water for 7 days and fed with *Chlorella sp*. that help *Moina sp*. adapt to living environment and increasing their biomass. The initial *Moina sp.* applied to each treatment was 85 g.

The surface of the *Moina sp*. raising pond was divided into 16 parts equally by using wires. The *Moina sp*. biomass was harvested in 4 parts (i.e. approximately 25% of the pond area) in every morning. The 4 parts were randomly chosen, but 2 parts should be nearby pond edge and the other in the middle. The *Moina sp.* was collected by net for their fresh weight and dry weight.

2.3 Implementing experiments

Before feeding to algae pond, domestic wastewater was sediment in 30 minutes to help reduce the settled solids of wastewater and increase the diffusion of light into the algae ponds. According to Chongrak (2007), experiments were conducted with two different hydraulic retention time (HRT) treatments of 1.5 days and 1.8 days. Feeding wastewater by 12/24 (from 6:00 to 18:00 every day), the flow rate applied for treatment of HRT 1.5 days and 1.8 days were 133 mL/minutes, and 111 mL/minutes, respectively. The Marriott was used to stabilize the feeding flow rate to algae ponds. All effluent from algae ponds was continuously fed to the *Moina sp*. raising pond.

Both water samples of input and output were collected to analyses polluted parameters. The parameters of the inflow samples were collected in 3 continuous days that inform on background data of wastewater. After operating for 10 days, the outflow from both *Chlorella sp*. pond and *Moina sp*. ponds were collected and analyzed for biomass and water quality parameters. All the analyzed procedure followed the standard process prescribed by the current Vietnamese standards.

Table 2. Analyzed method of samples

Parameters	Analyzed methods
SS	TCVN 6625:2000
VSS	Standard Methods 2540 E
BOD_5	TCVN 6001-1:2008
COD	TCVN 6491:1999
TKN	TCVN 6638:2000
TP	TCVN 6202:2008
Chlorophyll-a	SMEWW PP 10200

In parallel, the pond operation parameters such as pH, DO, light intensive, and temperature from inside and outside of the ponds were hourly recorded through three continuous days by pH meter of Orion 230A (USA), DO meter of WTW *330i (Germany)*, temperature meter of Taylor T441 (USA), and light meter of Lutron XL107 (Taiwan).

Figure 2 Arrangement of experiment model

3. Results and Discussion

3.1 Experimental conditions

3.1.1 Wastewater characteristics

The domestic wastewater had dark grey, turbines, odor, oil and settled solid. The settled solid matter could limit the light diffusion into water body, which has negative effect to photosynthesis conditions of algae community. In parallel, the settled solid matter also attached to algae part causing algae sediment to the pond bottom and the death of algae. Thus, wastewater needs to be sediment in 30 minutes to help reduce settled solid and oil before feeding to algae ponds. The results from parameters of the wastewater samples are shown in Table 3.

Table 3. Pollutant parameters in wastewater

Para-meters	Unit	Polluted concentration (n = 3)	QCVN 14:2008/ BTNMT
pH	-	7.26 ± 0.17	5 - 9
DO	mg/L	1.68 ± 0.76	-
COD	mg/L	145.67 ± 22.72	-
BOD_5	mg/L	72.00 ± 4.00	30
SS	mg/L	132.17 ± 5.03	50
TKN	mg/L	29.89 ± 2.80	-
TP	mg/L	2.46 ± 0.23	6
$N\text{-}NO_3^-$	mg/L	0.06 ± 0.05	30
$N\text{-}NH_4^+$	mg/L	25.22 ± 2.80	5

Note: QCVN 14:2008/BTNMT - National Technical Regulation on Domestic Waste-water (column A)

The analysis results showed that:

- pH value was 7.04 ± 0.04, which is suitable for both the activity range of the symbiosis bacteria and that of *Chlorella sp.*

- The concentration of polluted parameters was low, especially the recorded value of $N\text{-}NH_4^+$ was high but low $N\text{-}NO_3^-$ indicated that domestic wastewater has just released and has not yet got strong effect by the denitrification process.

- The concentration of organic matter reflected the ratio BOD_5/COD ~ 0.5 that was suitable for application of biological treatment methods.

- The ratio of N/P ~ 12.0 that is a bit higher than the suitable range of 6.8 to 10.0 for *Chlorella sp.* growth (Wang, 2009).

3.1.2 The recorded results of the experimental conditions

During 3 days of collecting water samples, the parameters of light intensity, temperature inside and outside of the intensive algae ponds, pH and DO of the water in the algae ponds were monitored continuously every hour to record the operating conditions of the models.

a) <u>The intensity of the light</u>

The measurement results from 6 hours to 18 hours showed that the light intensity varied according to the time of measurement because the experiments were conducted in a rainy season and solar radiation was affected by clouds. The intensity of light in the two algae ponds was similar to each other, but it was lower than the light intensity in the external environment. The highest light intensity appeared from 9:00 to 12:00, but the lowest light intensity appeared at 18:00. The light intensity within three continuous days reached the highest value at 95,267 ± 43.829 Lux and lowest value at 14 ± 7 Lux. According to Nguyen Thanh Phuong *et al.* (2003), the suitable light intensity range for activity of *Chlorella sp.* was from 4,000 to 30,000 Lux; therefore, the ponds in our experiments were covered by textile from 9:00 to 14:00 to limit light access to the water body to offer a good condition for algae growth. The light intensity also affects temperature inside the water body, which makes impact on photosynthesis process and then on DO and pH value of the water body.

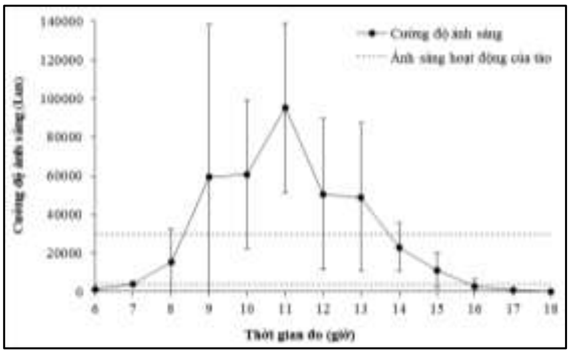

Figure 3. Changed of the light intensive at the day time

b) <u>Temperature</u>

The heat supplier to the algae pond comes from the sunlight and the inner biological activities of the pond. The temperature was recorded at the experimental site place and inside of the pond, 24/24 hours within 3 continuous days. The measurement temperature outside and inside the algae ponds showed that when receiving sunlight, the atmosphere temperature rose faster than the water temperature, and when there was no light, it would fall faster. This phenomenon happened due to water absorbed and released the heat more slowly than the air. The lowest temperature at night was 27.17 ± 1.44°C close to the optimum temperature 28 - 35°C of *Chlorella sp.* (Tran Chan Bac, 2013).

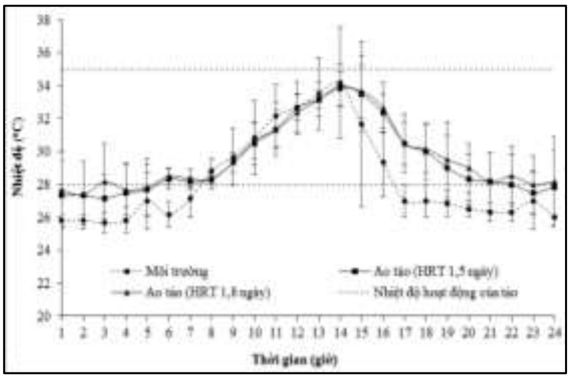

Figure 4. Changed of temperature due to daily

time

c) <u>pH</u>

According to Nguyen Thanh Phuong *et al.* (2003), the suitable pH range for activity of *Chlorella sp.* is from 6.0 to 8.5. Additionally, Tran Thi Thuy (2008) reported that pH of 8.0 is optimum value for growing *Chlorella sp.*, but it could also grow at pH from 9.0 to 10.0. The pH values from our study ranged from 8.2 to 9.3 and from 7.9 to 9.3, respectively, to the treatment of HRT 1.5 days and 1.8 days, which is suitable for *Chlorella sp.* growth. The values had a tendency to increase gradually from 2:00 - 16:00 and then gradually decrease until the next morning; this is because during sunlight time, the algae photosynthesis CO_2 in water making pH increases. When there was no light, the algae emitted CO_2 and this made pH decrease. The pH of the aerated wastewater algae ponds was always higher than that of the non-aerated wastewater algae ponds. This possibly helped the algae grow better. The highest pH value and lowest pH one in the two algae ponds were still in good condition for *Chlorella sp.* growth.

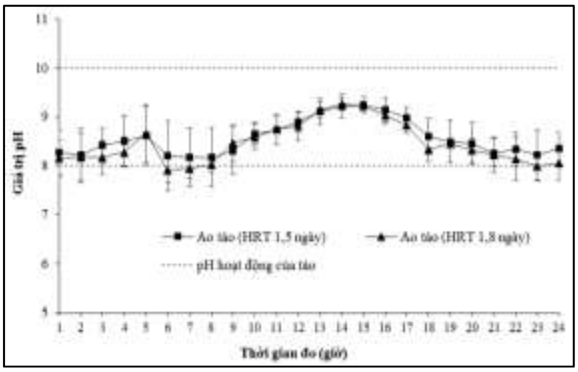

Figure 5. Changed of pH due to daily time

d) <u>DO</u>

At the time of high light, the algae photosynthesis strongly, and the DO concentration increased more. These happened because a part of $N\text{-}NH_4^+$ in the wastewater was converted into $N\text{-}NO_3^-$ along with the process of symbiotic algae and the ongoing-bacteria in the pond. In the experiments, the recorded DO values were not significant different between two ponds. The DO values were low in the early morning, gradually ascended in the afternoon, and started falling when the light intensity decreased.

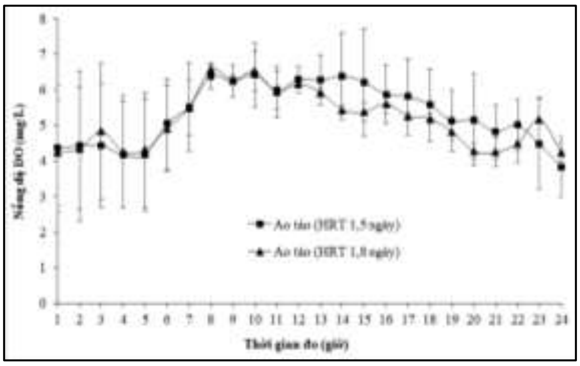

Figure 6. Change of DO due to daily time

3.2 Algae biomass

After the algae pond operated for 10 days, wastewater samples were collected at 6:00 and 12:00 (the two periods of time having the lowest and highest light of the day) and mixed them together to assess algae biomass by 3 indicator parameters: SS, VSS, and Chlorophyll.

The SS concentration in the wastewater input was rather high 132.17 ± 5.03 mg/L. After going through the algae ponds, the SS concentration in the wastewater output decreased a little due to more algae were created from the photosynthesis process. This result proved that the algae grew in the intensive ponds. The ANOVA analysis results and the F test indicated that the concentration of SS between the treatments did not differ significantly at 5%.

The Chlorophyll density obtained in the treatment of HRT 1.5 days and of HRT 1.8 days were 416.33 ± 117.23 mg/m^3 and 217.33 ± 21.94 mg/m^3. This result could be due to high N and P content in the loading flow from the treatment of HRT 1.5 days, so it would help algae easily absorb and develop. The ANOVA analysis results and the F test showed that the Chlorophyll density from the treatment of HRT of 1.5 days and 1.8 days had significant difference at 5%.

Table 4. SS, VSS and Chlorophyll in wastewater before and after treatment

Parameters	Input	Output from algae pond		Output from *Moina sp.*	
		HRT 1.5 days	*HRT 1.8 days*	*HRT 1.5 days*	*HRT 1.8 days*
SS (mg/L)	132.17 ± 5.03	94.73 ± 3.44	81.00 ± 5.29	60.67 ± 6.28	65.50 ± 5.54
VSS (mg/L)	94.47 ± 4.53	74.50 ± 3.97	66.67 ± 2.31	-	-
Chlorophyll (mg/m^3)	-	416.33 ± 117.23	217.33 ± 21.94	-	-

3.3 *Moina sp.* biomass

The *Moina sp.* was collected in the early morning every day at 25% area of the pond. The Moina weight was defined by fresh weight, and the dried weight (desiccate the *Moina sp.* at 105^{o}C up to unchanged weight). The initial weight of *Moina sp*. was 85 g, after raising them within 7 days, the *Moina sp.* could adapt and grow well. The *Moina sp*. were collected every day within 7 continuous days to define the gained biomass, then all *Moina sp.* were collected at the day 8th to define total biomass.

In Table 5, the results of collected *Moina sp*. within 7 continuous days showed that the biomass tends to decrease by time due to every day collection, so the *Moina sp*. had no enough time to create new generation. That the weight of *Moina sp.* collected was higher than their initial weight indicated that biomass of *Chlorella sp*. could be used to feed to *Moina sp.* However, the total weight of *Moina sp*. was less because the depth of water layer in the pond is not high enough, which resulted in change of temperature of water body between day and night time.

3.4 The water treatment efficiency

The above-mentioned results showed that domestic wastewater could use to grow *Chlorella sp*. biomass, and then this biomass can be used for *Moina sp*. raising. Besides, our study showed that the quality of water output from both the algea pond and the *Moina sp*. pond was improved significantly (Table 6). The ANOVA analysis results and the F test indicated that the concentration of water quality between the two treatments did not differ significantly at 5%.

Table 5. The results of collected *Moina sp.*

Day	Treatment of HRT 1.5 days		Treatment of HRT 1.8 days	
	Fresh weight (g)	***Dried weight (g)***	***Fresh weight (g)***	***Dried weight (g)***
1	16.46	3.37	17.40	3.76
2	14.41	2.95	12.24	2.65
3	11.48	2.35	13.93	3.01
4	9.38	1.92	7.82	1.69
5	6.46	1.32	7.11	1.54
6	7.11	1.46	6.93	1.48
7	5.47	1.12	6.27	1.34
8	21.37	4.37	19.92	4.26
Total	**90.14**	**18.86**	**91.62**	**19.73**

Table 6. Concentration of water quality after treated through algae and *Moina sp.* pond

Parameters	Output from algae pond		Output from *Moina sp.*		QCVN 14:2008
	HRT 1.5 days	***HRT 1.8 days***	***HRT 1.5 days***	***HRT 1.8 days***	
pH	7.26 ± 0.17	9.27 ± 0.34	9.09 ± 0.41	9.57 ± 0.37	5 - 9
SS (mg/L)	94.73 ± 3.44	81.00 ± 5.29	60.67 ± 62.77	65.50 ± 55.43	50
DO (mg/L)	1.68 ± 0.76	6.73 ± 0.67	6.98 ± 0.74	6.08 ± 0.29	-
BOD_5 (mg/L)	16.00 ± 1.00	15.33 ± 0.58	14.67 ± 1.15	15.67 ± 1.53	-
COD (mg/L)	34.67 ± 2.08	34.00 ± 1.00	35.00 ± 1.00	35.67 ± 0.58	30
TKN (mg/L)	19.43 ± 5.61	20.45 ± 6.80	8.78 ± 5.09	9.34 ± 3.08	-
TP (mg/L)	1.07 ± 0.30	1.43 ± 0.30	0.31 ± 0.30	0.38 ± 0.27	6

Note: QCVN 14:2008/BTNMT - National Technical Regulation on Domestic Wastewater (column A)

6. Conclusions

The study on applying decentralization domestic wastewater treatment units to make use of the nutrients from domestic wastewater at a lab-scaled model showed a promising result to promote such a model in rural areas where environment pollution is partly due to daily practice of discharging domestic wastewater into open sources. The study indicated that biomass of *Chlorella sp*. from the treatment of HRT 1.5 days was significantly higher than 1.92 times compared to the treatment of HRT 1.8 days (5%) and raising *Chlorella sp.* can improve the quality of domestic wastewater, but there is no significant difference between treatments of HRT 1.5 days and 1.8 days. In addition, *Moina sp*. could be used as a biological way to improve water quality from algae, and then *Moina sp.* can be applied as food for fish raising.

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