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Effects of microalgae on nutrient removal from mariculture wastewater in Can Gio District, Ho Chi Minh City, Vietnam

Hiệu quả của vi tảo trong việc loại bỏ các chất dinh dưỡng gây ô nhiễm trong nước thải nuôi trồng thủy hải sản ở huyện Cần Giờ, thành phố Hồ Chí Minh, Việt Nam

Research Article

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Mariculture has currently brought greatly valuable products in many fields simultaneously released a large amount of wastewater contributing to water pollutions on account of its organic and inorganic constituents. Nowadays, with the development of environmental engineering, more and more approaches, especially friendly-environmental and highly effective wastewater biological methods, are being applied to tackle pollutions and minimize adverse effects of treatments to reach the sustainable development. This report focuses on the study of proliferation combined with elimination of polluting substances of marine algae species *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp.* in aquaculture wastewater sampled from Can Gio District, Ho Chi Minh City, Vietnam with levels of concentration during 14 days under normal marine algae culture conditions, and compared to that in Daigo's IMK media. The results shown that, the algae species all grew rapidly simultaneously gave high nutrients removal yields (COD, N, P) and created a considerable amount of biomass within a short period of culture. Particularly, *Platomonas sp.* and *Tetraselmis suiscica* could proliferate as well as give high treatment yields of organic substances (COD), PO_4^{3-}, NO_3^-, NH_4^+ and Total Nitrogen in concentrated wastewater. To sum up, this study showed the potential of using microalgae to reduce COD, nitrogen and phosphorus in mariculture wastewater.

Ngành nuôi trồng thủy hải sản trong những năm gần đây đã mang lại nhiều sản phẩm có giá trị trong nhiều lĩnh vực đồng thời thải ra một lượng lớn nước thải gây ô nhiễm nguồn nước bởi các thành phần vô cơ và hữu cơ có trong nước thải. Ngày nay, với sự phát triển của kỹ thuật môi trường, ngày càng nhiều cách tiếp cận, đặc biệt là các phương pháp sinh học hiệu quả cao và thân thiện với môi trường đang được ứng dụng để xử lý ô nhiễm và giảm thiểu hậu quả bất lợi sau xử lý, nhằm hướng tới sự phát triển bền vững. Bài báo cáo tập trung nghiên cứu khả năng sinh trưởng và phát triển của ba loài vi tảo biển Tetraselmis suiscica, Tetraselmis sp., Platymonas sp. kết hợp với việc loại bỏ các hợp chất gây ô nhiễm có trong nước thải từ ao nuôi tôm của huyện Cần Giờ, Việt Nam và so sánh đối chiếu với môi trường dinh dưỡng Daigo's IMK trong 14 ngày nuôi trồng. Kết quả cho thấy những vi tảo sinh trưởng rất nhanh nhờ vào việc sử dụng các chất ô nhiễm trong nước thải, đặc biệt hiệu suất xử lý COD, PO_4^{3-}, NO_3^-, NH_4^+ và nitơ tổng của Platomonas sp. và Tetraselmis suiscica rất cao thậm chí trong môi trường nước thải đậm đặc. Do đó, chúng tôi kết luận, vi tảo có tiềm năng rất lớn trong việc giảm nồng độ chất hữu cơ, phốt pho và nitơ trong nước thải nuôi trồng thủy hải sản.

Keywords: COD, mariculture, microalgae, nutrient removal, wastewater

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1. Introduction

Vietnam has favorable natural conditions for agricultural and industrial development especially aquaculture-shrimp industry bringing numerous profits particularly in Can Gio District, Ho Chi Minh City. However, large-scale shrimp farming is releasing much wastewater with a wide range of components called "nutrients" mainly biodegradable organic substances, phosphorus, nitrogen, algae species, causing pollution and requiring reasonable treatments. Moreover, people have currently faced an energy crisis and air pollution because of the future exhaustion of fossil fuel. Therefore, producing cleaner and cheaper energy from biomass is a potential for long-term uses.

Algae are classified into macro-algae "seaweed" and micro-algae which are unicellular algae, mainly eukaryotic microalgae *Chlorophytes* (green algae) and prokaryotic cyanobacteria (blue-green algae) (Cai *et al.*, 2013). Micro-algae grow through transferring light, CO_2 and substances as "nutrients" in wastewater or even dead lands into energy-rich biomass $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007; Chen *et al.*, 2010) more rapidly than terrestrial plants (Pittman *et al.*, 2011; Rawat *et al.*, 2011) particularly double amount of biomass per day within 6h (Michael *et al.* 2010), which is feasibly employed for the production of biodiesel, bioethanol, biomethane and biohydrogen (Figure 1) (Schenk *et al.*, 2008, Chinnasamy *et al.*, 2010, Agwa *et al.*, 2012). Therefore, microalgae are commercialized as a green effective secondary wastewater treatment where pollutants are reduced significantly (Rawat *et al.*, 2011) by fixed biofilm or suspended growth operations (Logan and Ronald, 2011; Abdel-Raouf *et al.*, 2012) including raceway ponds since 1950s and photo-bioreactors with more advantages in well-controlled long culturing and producing larger quantities of biomass (Amit *et al.*, 2010), for example, 100,000kg/year with higher biomass concentration in broth, 4.00kg/m^3 compared to 0.14 of the former (Chisti, 2007).

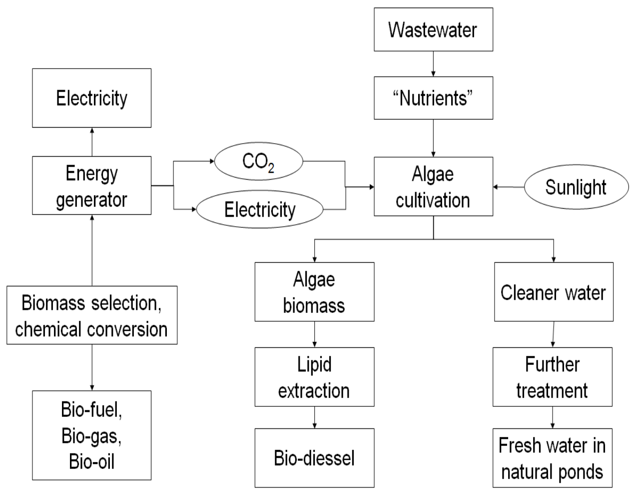

Figure 1. Diagram from waste to energy using algae
(Extracted from Pittman et al., 2011)

There have recently been studies about proliferation and nutrients removal efficiency of many algal species especially *Chlorella vulgaris* and *Chlorella sp.* with dramatic density increase, high quality and quantity biomass (Gouveia and Oliveira, 2009), high removal efficiency of ammonia and COD but slightly low removal rate of total phosphorus (Sriram and Seenivasan, 2012), for examples of *Chlorella vulgaris,* results of Valderrama *et al.* (2001) indicating 2×10^6 cells/ml of cell density, 28%, 71.6% and 61%, respectively in 4-day culture with diluted (10%) anaerobic industrial effluent; or studies of Li *et al.* (2011) presenting 80.9%, 93.9% and 90.8%, respectively in 14-day incubation with highly concentrated municipal wastewater. In addition, concentrations of metal ions typically Al, Ca, Mg, Mn, Fe were significantly reduced in wastewater from different points of treatment process of a municipal wastewater treatment plant by the algae according to Wang *et al.* (2009). The effects of culturing factors especially light/dark cycles on removal rate were also researched. For instance, Lee *et al.* (2001) showed that cell density and nitrate removal rate of *Chlorella kessleri* in the continuous illumination were higher than that under diurnal illuminating scheme while organic carbon was removed better in 12h light/12h dark cycles.

To support a theory that algae are considered to be as a strategy of sustainable development on behalf of their highly friendly-environmental, economical and effective fucntions in wastewater treatment, this report investigated the performances of nutrient removals in shrimp wastewaters containing mainly non-biodegradable organic substances and a small amount of heavy metals of *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp.* under well-controlled parameters optimal to proliferation of almost algae (Laura and Paolo, 2006), 25-35^{o}C, pH of 8.2-8.7, light intensities of 4000-5000lux in continuous illumination, salinities of about 18.6‰ in Daigo's IMK medium and in wastewater sampled from shrimp cultivation ponds at Can Gio District, Ho Chi Minh City, Vietnam.

2. Materials and Methods

2.1. Sampling and storage

After being collected from planned points in wastewater ponds using polyethylene 5liter bottles with plastic lid, samples are transported to the laboratory under cool conditions without sunlight and stored at 5±3^{o}C (ISO 19458:2006).

Purified species *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp.* provided by Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam were stored at 5±3^{o}C.

2.2. Algae culture condition

Daigo's IMK medium was prepared from the following chemicals: $NaNO_3$ 200mg/L, $CaCl_2.2H_2O$ 50mg/L, K_2HPO_4 5mg/L, Na_2HPO_4 1.4mg/L, NH_4Cl 2.68mg/L, Fe-EDTA 5.2mg/L, Mn-EDTA 332µg/L, Na_2EDTA 5.2mg/L, $ZnSO_4.7H_2O$ 23µg/L, $CoSO_4.7H_2O$ 14µg/L, $Na_2MoO_4.2H_2O$ 7.3µg/L, $CuSO_4.5H_2O$ 2.5µg/L, H_2SeO_3 1.7µg/L, $MnCl_2.4H_2O$ 180µg/L, thiamin.HCl 200µg/L, biotin 1.5µg/L, vitamin B12 1.5µg/L, and appropriate amount of NaCl to obtain the same salinity as wastewater, approximately 3.5%NaCl.

Algae were inoculated at 15% (V_{algae}/V_{media}) in 250 mL erlenmeyer flasks containing 100mL Daigo's IMK medium, and incubated in stationary conditions at 30±2°C, 4000-

5000lux to obtain the primary generations, which were then multiplied in 1 litre to reach enough cells, roughly 1.5-2.0×10^6 cells/ml to find the optimal wavelength and make the cell density curve (absorbance, cells/ml) for each species by using the spectrometer (UV/VIS Genesys 20 Thermo spectronic), combined with the haemocytometer and microscope.

After achieving sufficient cells, they were cultivated in 5 litre plastic bottles containing wastewater (WW) with different concentrations (25%, 50%, 75%, 100%) in the same conditions as that of Daigo's IMK culture to compare, evaluate the treatment yields, biomass weight and determine best suitable wastewater concentrations for each species' proliferation.

2.3. Determination of algal growth

Samples were taken from the culture media every day to measure optical density (OD) at the optimal wavelength by spectrometer and haemocytometer. Each recorded OD was corrected by subtracting that of the corresponding blank sample.

2.4. Algae harvesting

Algae were harvested mechanically on the day when the cell number is maximum by centrifugation, most rapid and reliable method (Logan and Ronald, 2011). After being taken 100ml, samples were dried at 50^0C and centrifuged at 6000rpm in 10minutes, then tubes and samples were weighed, and the biomass was calculated by this equation:

$$m_{biomass} = m_{samples+tubes} - m_{centrifuge\ tubes}$$

2.5. Analysis of wastewaters

All the experiments were carried out in triplicate and average values of absorbance were recorded.

All wastewater samples were filtered using glass microfiber filters (Whatman, USA) to remove native bacteria and large particles. Ammonium ($N\text{-}NH_4^+$), nitrate ($N\text{-}NO_3^-$), phosphorus ($P\text{-}PO_4^{3-}$), total nitrogen (TN) and COD were determined by colorimetry, Kjeldahl and titration, following Standard Methods 5220-COD, 4500-Nitrogen, NH_3, 4500.E-Phosphorus. These parameters were determined in input of each batch (wastewater sampled from Can Gio District) and in output of each batch to make the comparisons and calculate the removal efficiency of each species.

3. Results

3.1. Determination of optimal wavelength and cell density curve

Results of scanning the absorbance shows although different algal species gave different maxima peak, their wavelength was in visible region, their density curve had the format of straight line according to Lambert-Beer law.

Tetraselmis suiscica: y = 23.731x + 0.9502 (R^2 = 0.9921) at λ = 420 nm,

Tetraselmis sp.: y = 20.477x + 0.2906 (R^2 = 0.9938) at λ = 420nm,

Platymonas sp.: y = 24.733x + 0.3616 (R^2 = 0.9924) at λ = 680nm.

3.2. Selection of suitable dilution of wastewater for the algae growth

From Figure 2, 3, 4 and Table 1, the cell number and biomass of *Tetraselmis suiscica, Tetraselmis sp, Platymonas sp* surged similarly after a week culture from about 2×10^6 cells/ml in relatively concentrated wastewaters (50%, 75%, even 100%) and reached the peak of around 10-12 million cells/ml on the beginning days of the second weeks (days of 3, 4 in the figures), particularly 11.2×10^6 cells/ml with the weight of 0.8612 g/ml on the days of 4 in 75%WW, 11.06×10^6 cells/ml with the weight of 0.2684 g/ml on the days of 4 in 50%WW, 10.95×10^6 cells/ml with the weight of 0.4578 g/ml on the days of 5 in 75%WW, respectively, but then decreased gradually in the ending days of the batch culture when nutrients run steadily out of and more dead cells increased. Therefore, this type of wastewater is appropriate for the proliferation of these algae of interest, the optima wastewater of 75% for *Tetraselmis suiscica and Platymonas sp, 50% for Tetraselmis sp.* even though their growth and weight of biomass in the optimal wastewater is moderately less than those in Daigo's IMK.

Table 1. Biomass of algae species on the day of highest cell number (optimal days)

Wastewater dilutions	*Tetraselmis suiscica*	*Tetraselmis sp.*	*Platymonas sp*
25%WW	0.5248	0.1059	0.2015
50%WW	0.7458	0.2684	0.4492
75%WW	0.8612	0.2472	0.4578
100%WW	0.6983	0.1046	0.2368
Daigo's IMK	1.0188	0.7375	0.5078

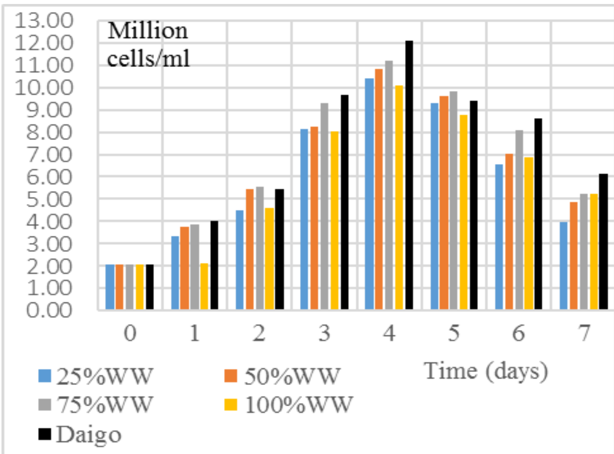

Figure 2. Changes in cell number of *Tetraselmis suiscica* during the batch culture

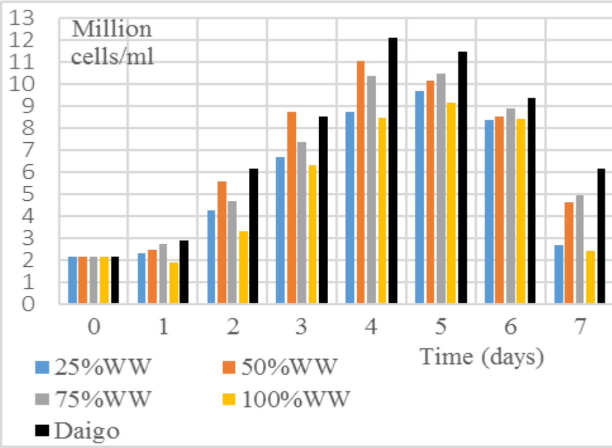

Figure 3. Changes in cell number of *Tetraselmis sp.* during the batch culture

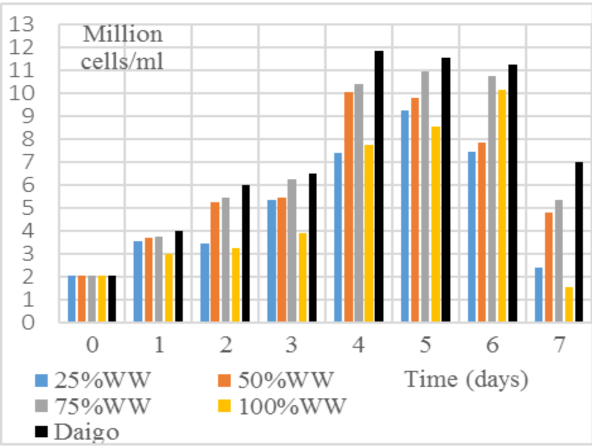

Figure 4. Changes in cell number of *Platymonas sp.* during the batch culture

3.3. The function of algae species in removals of COD, total nitrogen, nitrate, ammonia and phosphorus

Based on duplicates of determination of concentrations (mg/l) of COD, nitrate, ammonium ion and total phosphorus, these components were in wastewater with a high amount even in the most diluted sample (Table 2).

Table 2. Concentrations of COD, nitrate, ammonium ion and total phosphorus before treatment

Wastewater dilutions %	COD mg/l	$N\text{-}NO_3^-$ mg/l	$N\text{-}NH_4^-$ mg/l	$P\text{-}PO_4^{3-}$ mg/l
25	320	0.713	18.9	1.996
50	480	1.206	20.5	3.954
75	576	1.703	26.1	4.059
100	672	2.184	28.7	5.058

As can be seen from Table 3, levels of COD, nitrate, ammonium ion and phosphorus were steadily reduced by single *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp* after a week incubation in wastewaters with different dilutions, 25%, 50%, 75% and 100%, then significantly in the beginnings of the second week and eventually rose slightly in the ends of the batch culture when "nutrients" wastewater pollutants was absent in very small amount- whilst wastes of algae as well as the cells of other microorganisms harming to algae increased, thus dead algae cells is more than alive cells.

Equivalent to the reduction of pollutants' concentrations, treatment yields increased when concentrations decreased apart from some cases, yields remained stable in the eighth, ninth days of the batch (marked as days 4, 5, 6 in Figures). Yields of COD removal of *Platymonas sp* in Figure 5 as the example, the yield reached the plateau of nearly 86.67%, equivalent to 64 mg/l in wastewater dilution of 50%.

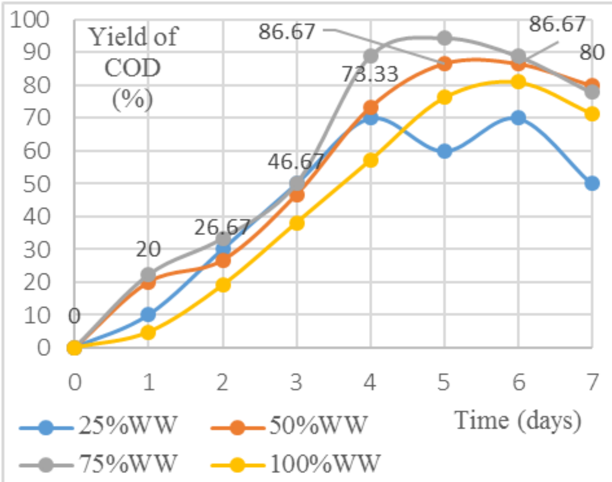

Figure 5. Yields of COD removal of *Platymonas sp.* during the batch culture

In the one hand, when the wastewater was much diluted, to 25%, the yields were smaller than those in more concentrated wastewaters, which was possibly owing to the insufficient supply of nutrient available in wastewater for algae proliferation, particularly in cases of ammonia removals of *Tetraselmis sp* in Figure 6, the maximum yield was only 35% equivalent to 12.3mg/l in wastewater dilution of 25% whereas the yield obtained up to nearly 70%, equivalent to 6-7mg/l in the dilution of dilution of 50%.

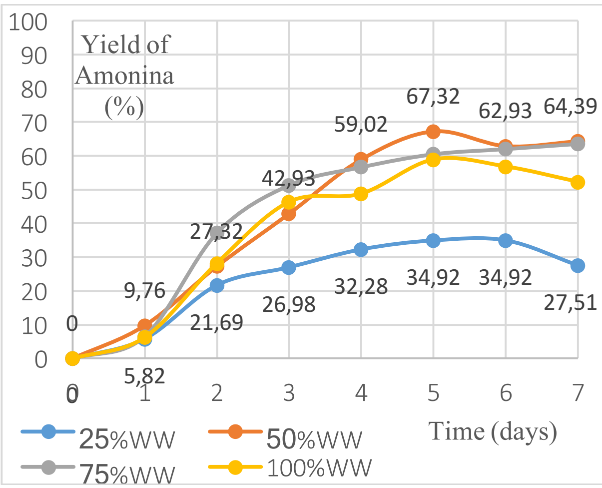

Figure 6. Yields of ammonia removal of *Tetraselmis sp.* during the batch culture

Table 3. Changes in concentrations of COD, nitrate, ammonia and total phosphorous as well as yields of removals of these substances during the batch culture

		Day	1		2		4		6		End	
		WW %	**C mg/l**	**Y %**	**C mg/l**	**Y %**	**C mg/l**	**Y %**	**C mg/l**	**Y %**	**C mg/l**	**Y %**
Tetra-	**C**	25	288	10	256	20	192	40	128	60	192	40
selmis	**O**	50	448	6.67	384	20	160	66.67	96	80	128	73.33
suis-	**D**	75	416	27.78	416	27.78	128	77.78	96	83.33	160	72.22
cica		100	544	19.05	448	33.33	256	61.9	160	76.19	192	71.43
	N-	25	0.684	4.07	0.559	21.6	0.469	34.22	0.233	67.32	0.235	67.04
	NO_3^-	50	1.110	7.96	0.993	17.66	0.801	33.58	0.268	77.78	0.456	62.19
		75	1.421	16.56	1.154	32.24	0.841	50.62	0.218	87.2	0.306	82.03
		100	2.027	7.19	1.785	18.27	1.329	39.15	1.035	52.61	1.049	51.97
	N-	25	18.1	4.23	16.4	13.23	10.4	44.97	11.5	39.15	12.9	31.75
	NH_4^+	50	18.7	8.78	15.7	23.41	11.7	42.93	7.6	62.93	7.9	61.46
		75	23.7	9.2	18.8	27.97	13.9	46.74	9.3	64.37	10.5	59.77
		100	26.5	7.67	20.5	28.57	13.3	53.66	11.6	59.58	12.1	57.84
	P-	25	1.368	31.46	1.117	44.04	0.938	53.01	0.465	76.7	0.470	76.45
	PO_4^{3-}	50	2.220	43.85	1.986	49.77	1.602	59.48	0.536	86.44	0.912	76.93
		75	2.843	29.96	2.308	43.14	1.681	58.59	0.436	89.26	0.612	84.92
		100	4.054	19.85	3.570	29.42	2.659	47.43	2.070	59.07	2.099	58.5
Tetra-	**COD**	25	288	10	224	30	128	60	96	70	160	5
selmis		50	448	6.67	320	33.33	32	93.33	96	80	128	73.33
sp.		75	512	11.11	416	27.78	96	83.33	160	72.22	224	61.11
		100	640	4.76	608	9.52	352	47.62	288	57.14	320	52.38
	N-	25	0.678	4.91	0.613	14.03	0.212	70.27	0.247	65.36	0.222	68.86
	NO_3^-	50	1.141	5.39	1.035	14.18	0.099	91.79	0.193	84	0.252	79.1
		75	1.54	9.57	1.354	20.49	0.469	72.46	0.425	75.04	0.868	49.03
		100	2.056	5.86	1.789	18.09	0.314	85.62	0.634	70.97	1.047	52.06
	N-	25	17.8	5.82	14.8	21.69	12.8	32.28	12.3	34.92	13.7	27.51
	NH_4^+	50	18.5	9.76	14.9	27.32	8.4	59.02	7.6	62.93	7.3	64.39
		75	24.4	6.51	16.4	37.16	11.3	56.7	9.9	62.07	9.5	63.6
		100	26.9	6.27	20.6	28.22	14.7	48.78	12.4	56.79	13.7	52.26
	P-	25	1.743	12.68	1.608	19.44	0.856	57.11	0.456	77.15	0.442	77.86
	PO_4^{3-}	50	3.215	18.69	2.994	24.28	1.705	56.88	0.486	87.71	0.852	78.45
		75	3.654	9.98	3.415	15.87	1.865	54.05	0.582	85.66	0.844	79.21
		100	4.514	10.76	3.858	23.72	2.715	46.32	2.325	54.03	2.385	52.85
Platy-	**COD**	25	224	10	224	30	96	70	96	70	160	50
monas		50	352	20	320	26.67	128	73.33	64	86.67	96	80
sp.		75	384	22.22	416	33.33	64	88.89	64	88.89	128	77.78
		100	544	4.76	608	19.05	288	57.14	128	80.95	192	71.43
	N-	25	0.669	6.17	0.54	24.26	0.404	43.34	0.18	74.75	0.212	70.27
	NO_3^-	50	1.158	3.98	0.953	20.98	0.843	30.1	0.224	81.43	0.394	67.33
		75	1.618	4.99	1.265	25.72	0.978	42.57	0.364	78.63	0.425	75.04
		100	2.054	5.95	1.63	25.37	1.133	48.12	0.847	61.22	0.744	65.93
	N-	25	17.7	6.35	14.5	23.28	9.7	48.68	9.4	50.26	9.8	48.15
	NH_4^+	50	19.3	5.85	14.6	28.78	8.9	56.59	8.5	58.54	8.3	59.51
		75	24.6	5.75	16.8	35.63	8.5	67.43	7.9	69.73	8.9	65.9
		100	25.3	11.85	18.3	36.24	11.6	59.58	10.3	64.11	9.1	68.29
	P-	25	1.835	8.07	1.725	13.58	0.965	51.65	0.510	74.45	0.498	75.05
	PO_4^{3-}	50	3.651	7.66	2.972	24.84	1.705	56.88	0.548	86.14	0.853	78.43
		75	3.826	5.74	2.889	28.82	1.615	60.21	0.459	88.69	0.928	77.14
		100	4.729	6.50	3.581	29.20	2.058	59.31	2.187	56.76	2.247	55.58

In the other hand, when the wastewater was much concentrated, to 100% (the origin), the yields were smaller than those in more diluted wastewaters, which was feasibly on account of the excess of nutrients beyond the tolerance of algae. This was especially observed in the cases of phosphorus removals of all studied algae. For instance, results of *Tetraselmis suiscica* in Figure 7, the maximum yield was under 60%, equivalent to 2.1mg/l in wastewater dilution of 100% whereas the yield obtained up to nearly 90%, equivalent to 0.4 mg/l in the dilution of dilution of 75%.

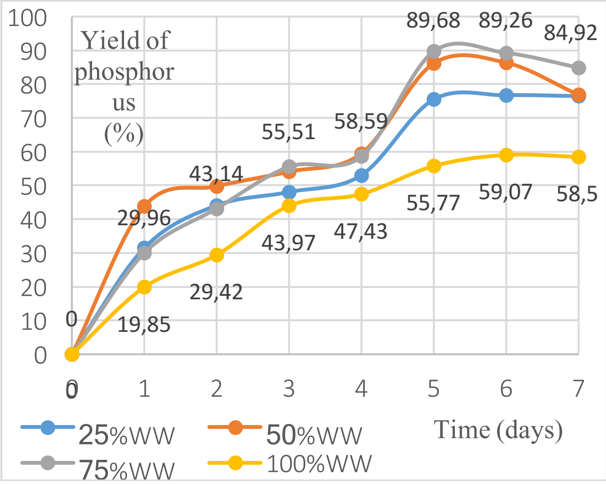

Figure 7. Yields of phosphorus removal of *Tetraselmis suiscica* during the batch culture

From the results of changes in total nitrogen determined in the first day of the batch and the optimal days of each species when the growth rate is maximum (table 4), it is concluded that these algae had the same high performances (over 70%) in eliminating nitrogen from wastewater even in the cases of concentrated wastewaters, particularly 83.9%, 81.8%, 76.2% in the decreasing order of *Platymonas sp, Tetraselmis suiscica, Tetraselmis sp.*, respectively in the environment of 75%WW.

Table 4. Changes in total nitrogen (TN) and yields of TN removals during the batch culture

WW	Input (mg/l)	*Tetraselmis suiscica*		*Tetraselmis sp.*		*Platymonas sp*	
		Output	H%	Output	H%	Output	H%
25%	7.36	4.36	40.8	4.41	40.1	2.41	67.3
50%	10.89	2.78	74.5	1.97	81.9	3.15	71.1
75%	12.01	2.19	81.8	2.86	76.2	1.93	83.9
100%	13.31	4.25	68.1	4.24	68.1	4.24	68.1

4. Conclusion

The same as other microorganism, micro algae have a tremendous growth in almost environments even in various types of wastewater, such as effluents from citric acid and ethanol production (Valderrama *et al.*, 2002), wastewater from municipal wastewater treatment plants (Wang *et al.*, 2010) with a plethora of components in wide ranges of concentration owing to abilities of using and transferring polluting substances into nutrients essential for building their bio-materials, for example, using phosphates in wastewater to synthesize ATP, phospholipases and DNA, utilizing nitrogen compounds to produce acid amines and proteins.

In this report, *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp.* virtually proliferated quickly and reduce significantly substances polluting mariculture effluents from shrimp ponds at Can Gio District, Ho Chi Minh City, Vietnam. Although cell density declined in the end of the batch after maximum days in the middle of the batch, it was still higher than the cell number in the beginnings, approximately 4–5 million cells/ml. Similarly, there was a general trend in changes in yields of removal of COD, total nitrogen (Kjeldahl nitrogen), nitrate, ammonium ion and phosphorus, the frequencies rocketed to highest values on optimal days in the middle of the batch, particularly 80-90%, 70-80%, over 70%, 60-70% and 70-80% respectively, and then fell down in the ends but remained around 50% in appropriate wastewater dilutions.

Amongst studied algae species, *Tetraselmis suiscica and Platymonas sp.* could develop well and give more biomass in more concentrated wastewater than *Tetraselmis sp.*, 75%WW compared to 50%WW, especially *Tetraselmis suiscica* with the highest biomass weight, 0.8612 in wastewater of 75%, and 1.0188 g/100ml in Daigo's IMK. On behalf of performances of reducing pollutants in the optimal wastewater dilutions and on maximum days, *Tetraselmis sp. and Platymonas sp.* gave higher treatment yields of COD, 93.33% and 88.89%; *Tetraselmis sp.* gave the highest treatment yields of nitrate, 91.79%; *Platymonas sp.* gave the highest treatment yields of ammonia, 74.71%. All of them eliminated well total nitrogen and total phosphorus with the efficiency of 80% and 85%, respectively, which demonstrates that in suitable kinds of wastewater, these algae remove phosphorus better than *Chlorella vulgaris,* which gives slightly small yields in total phosphorus removal (Valderrama *et al.*, 2001; Li *et al.*, 2011; Sriram and Seenivasan, 2012).

In the scope of study in small scale, *Tetraselmis suiscica, Tetraselmis sp., Platymonas sp.* shown their potentials in reducing significantly COD, nitrogen and phosphorus from wastewater of shrimp cultivation, from 672 mg/l to 100-200 mg/l of COD, from 2.18 mg/l to 0.5-1.0 mg/l of nitrate, from 28.7 mg/l to 9.0-10.0 mg/l of ammonium, from 5.06 mg/l to 2.1-2.2 mg/l of phosphate, therefore hopefully they will be applied in wastewater treatment process in municipal wastewater treatment plants.

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