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The Optimum N:P Ratio of Kitchen Wastewater and Oil-Extracted Fermented Soybean Water for Cultivation of *Spirulina platensis*: Pigment Content and Biomass Production

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ABSTRACT

The optimum N: P ratio of kitchen wastewater (Kw) and oil-extracted fermented soybean water (Sw) for cultivation of *S. platensis* (Nordstedt) Geiteler was evaluated. A Complete Randomized Design (CRD) was carried out with 22 treatments and 3 replicates: Zarrouk media (Zm), 10 dilutions of Kw and 11 dilutions of Sw. The water quality, biomass production and pigment content of *S. platensis* were determined every 5 days for a period of 15 days. The chemical properties of cultivated wastewater had pH 6.84-9.50. The optimum levels of N: P ratio (N: P ~ 6: 1) for algal cultured in the 5% Sw and Zm, with biomass production of 0.90 gL^{-1} and 0.84 g L^{-1} (dry weight), respectively. The highest levels of β-carotene and C-phycocyanin of *S. platensis*, when cultured in 10%Sw with the optimum N: P ratio (N: P~5.7: 1.0), were achieved 0.37 mg g^{-1} and 21.27 mg g^{-1}, respectively. Implications for using *S. platensis* for β -carotene and C-phycocyanin production when cultured with 10% Sw are discussed.

Key Words: Biomass production; Kitchen wastewater; Oil-extracted fermented soybean water; Pigment content; β -Carotene; C-phycocyanin; *S. platensis*

INTRODUCTION

Among the microalgae, *S. platensis*, a mixotrophic unicellular algae belonges to division Cyanophyta, can be easily and inexpensively harvested by filtration from the cultured medium, because of its relatively large size. *S. platensis* has even been used as nourishment by humans in Mexico and Africa for a long time (Bold & Wynne, 1985). It has been the subject of research for three decades and production has been carried out on various scales and degrees of sophistication. It has gained world wide acclaim as a good source of protein, β-carotene, phycocyanin, vitamin and minerals as a healthy food for humans and farm animals (Switzer, 1982; Venkataraman, 1983). C-phycocyanin is one of the major biliproteins of *S. platensis*, with antioxidant and radical scavenging properties. It is also known to exhibit anti-inflammatory and anti-cancer properties in humans and animals (Schlfsser, 1982). Enhancing high biomass production of *S. platensis* in mass culture is important.

Most algae prefer to use NH_3-N rather than NO_3-N (Khalaf & Zeinab, 2007) with the optimum nitrogen concentrations for algal growth in the range 1.3 - 6.5 mg L^{-1}, while the optimum N: P ratio for *S. platensis* culture is 6-8: 1 mg L^{-1} (Reynold, 1986). Culturing *S. platensis* for animal feed purposes using inorganic culture media is relatively expensive. Low-cost alternatives, such as oil-extracted fermented soybean water (Sw) and kitchen wastewater (Kw), should be evaluated as more cost-effective media for producing this important nutritional product. The research on cultivation of *S. platensis* using livestock wastewater and urban effluents was conducted (Rangel-Yagui *et al*., 2004). Kw has received relatively little attention but it can make a significant contribution to water pollution, especially from dining facilities in large institutions such as universities. It is rich in nutrients (nitrogen, phosphorous, etc.) but contains relatively few toxic components. However, to avoid eutrofication of the aquatic ecosystem and water pollution, the nutrients present in Kw need to be removed. If Kw can be used for culturing algae, the dried product should be suitable as a feed for some animals, where human food-grade product is not required. For example, tilapia larvae my thrive, whereas adult tilapia would require additional nutrients not found in algae alone.

Soybean is an important crop that can be used directly as an animal feed. However, it may also be used, after fermentation to break down the structure to produce oil-extracted fermented soybean water (Sw), as a culture

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medium for the production of *S. platensis* that can then be harvested for use as a food for many organisms, including tilapia larvae.

The current study was carried out to assess the optimum N: P ratio for cultivation of *S. platensis* using kitchen wastewater, Kw and oil-extracted fermented soybean water, Sw. Biomass production, β-carotene and C-phycocyanin were also evaluated.

MATERIALS AND METHODS

Preparation of *S. platensis* Stock Culture

***S. platensis* cultured in modified zarrouk's medium (stock Zm).** Modified Zm composed of commercial grade chemicals (Promya, 2000): 2 g L^{-1} of $NaHCO_3$ (Qingdao Co., LTD, China), 1 g L^{-1} of NaCl (Purity Salt Industry, Co., LTD, Thailand), 1 g L^{-1} of $MgSO_4$ (UTIDS Enterprise Co., LTD, Thailand), 0.5 g L^{-1} of $NaNO_3$ (Qingdao Co., LTD, China) and 1 g L^{-1} of N:P:K (16:16:16, YARA International ASA Co., Ltd, Norway), adjusted to pH 10 $\pm$ 0.5 using NaOH. Stock Zm (Fig. 1a): *S. platensis* was cultured in modified Zm in a 5 L bottle and allowed to grow for 2 weeks until the optical density (OD) at 560 nm reached 1 (OD_{560nm}=1).

***S. platensis* cultured in kitchen wastewater (Kw).** One hundred liters of Kw, one day of food preparation and dish washing processes at the cafeteria, Department of Biology, Faculty of Science, Chiang Mai University, was collected and allowed to ferment for 3 weeks (Fig. 1b). Fermented Kw was then filtered through an 80 micron plankton net filter. The filtrate was analyzed for pH, total N (TN) by the Macro Kieldahl method and total P (TP) by persulfate digestion and followed by stannous chloride method (APHA & WPCF, 1998; Traichaiyaporn, 2000). Stock Kw (Fig. 2a): *S. platensis* from stock Zm was cultured in fermented Kw in 50 L glass tanks and allowed to grow for 2 weeks until the OD at 560 nm reached 1 ($OD_{560\ nm\ =\ 1}$). Stock Kw would then be used as "raw *S. platensis*" for the experiments.

Laboratory cultures of *S. platensis*. Modified Zm, Kw and soybean water (Sw) were used as culture media. Sw was oil-extracted soybean meal mixed with tap water (ratio= 1:9) Fig. 1c. The soybean suspension was left for 3 weeks to ferment and then filtered through an 80 micron plankton net filter. The filtrate together with modified Zm and Kw were analyzed for pH, TN and TP using methods (APHA & WPCF, 1998; Traichaiyaporn, 2000). The experiment was laid down in a Complete Randomized Design (CRD) using 22 treatments with 3 replication: modified Zm as control, 10 dilutions of Kw and 11 dilutions of Sw. All treatments were adjusted the N: P ratio to be about 6-8:1 (Reynolds, 1986). The dilutions were filtered again and analysed for physico-chemical water quality of pH, TN and TP using methods (APHA & WPCF, 1998; Traichaiyaporn, 2000). *S. platensis* from stock Kw were cultured in modified Zm, Kw and Sw in 5 L bottles (Fig. 2b). Each bottle had the initial OD of 0.30 (OD_{560nm} = 0.30) before adding the inoculum of *S. platensis*. All cultures were continuous aerated and left for 15 days. Samples (100 mL of each) were collected every 5 days from each bottle to determine algal biomass production and analyzed for their pH, TN and TP. Algal biomass production was determined by filtration through a 120 μm plankton net and was tray-dried at 70^{o}C for 5 h. Dried *S. platensis* was then ground into powder. The samples of dried *S. platensis* powder (Fig. 2c) were analyzed for β-carotene and C-phycocyanin (Berns *et al*., 1963; Simonne *et al*., 1996).

β-Carotene and C-phycocyanin analysis. The β-carotene concentration (mg g^{-1}, dry weight) in *S. platenisis* was determined by HPLC (Mightysil RP-18 GP, 5 µm, 150 x 4.6 mm ID column, Kanto Reagent Co., Ltd, Japan). The algal sample (0.75 g, dry weight) was homogenised in a homogeniser with hexane as extracting solvent, then filtered and the filtrate collected and evaporated to dryness. The residue was dissolved in methanol: chloroform (4:1) before injection. The mobile phase flow rate and the measured wavelength were 1 mL min^{-1} and 456 nm, respectively. Authentic β-carotene (Sigma) was run through the same procedure as described above (Simonne *et al*., 1996). The C-phycocyanin concentration (mg g^{-1}, dry weight) in *S. platenisis* was determined by HPLC (Agilent Technologies, USA) using a reverse phase ZORBAX Eclipse XDB-C-18 (5 µm, 150 x 4.6 mm) column. Approximately 1 g (dry weight) of *S. platensis* was suspended in 10 mL of phosphate buffer solution (PBS), pH 7.2 and maintained in the dark at 4^{0}C for 16 h. The crude extract was then centrifuged at 10,000 rpm for 15 min at 4^{0}C to separate cell debris. The volume was adjusted to 10 mL with PBS. Then other components in the supernatant were precipitated by addition of solid ammonium sulfate (25% w/v final composition). The resulting C-phycocyanin-containing solution was evaporated to dryness. The solid residue was dissolved in 10 mL of a methanol:water mixture (1:1) prior to analysis by HPLC. The mobile phase was methanol: water (1:1), the flow rate was 0.50 mL/min and the detector wavelength was 214 nm. Authentic C-Phycocyanin (Sigma Aldrich, USA) was run through the same procedure as described for the *S. platensis* samples (Berns *et al*., 1963).

Statistical analysis. Data were presented as mean values ± standard deviation. Comparison of mean values were made by one way-analysis of variance (ANOVA), followed by Duncan's multiple range (DMRT) test at a significance level of $p<0.05$ (SPSS Inc., Chicaco, USA, Ver.15).

RESULTS AND DISCUSSION

The optimum N: P ratio for cultivation of *S. platensis* with Kw and Sw and biomass production (dry weight) of *S. platensis* was compared among culture media as shown in Table I, Fig. 3 and 4. Initial N: P of Zm was 6.9:1 after 15 days, the N: P was 1.2:1. Initial N: P of Kw and Sw ranged from 6:1 to 6.2:1 and 8.2:1 to 8.3:1 after 15 days, the N: P of

Table I. Statistical summary (mean ± SD) of water quality values of Zm, Kw and Sw media and biomass production of *Spirulana platensis* culture for 15 days

Treatment	0 day					5 days					10 days					15 days				
	pH	TN	TP	N: P	algae dry weight	pH	TN	TP	N: P	algae dry weight	pH	TN	TP	N : P	algae dry weight	pH	TN	TP	N: P	algae dry weight
		(mg L^{-1})	(mg L^{-1})		(g L^{-1})		(mg L^{-1})	(mg L^{-1})		(g L^{-1})		(mg L^{-1})	(mg L^{-1})		(g L^{-1})		(mg L^{-1})	(mg L^{-1})		(g L^{-1})
Zm	8.73	29.80±1.02	4.35±0.39	6.9:1	0.30	9.50	19.60±0.98	1.95±0.01	10:1	0.55	9.32	10.72±1.13	1.77±0.02	6.1:1	0.84	9.38	1.83±0.67	1.65±0.20	1.2:1	0.51
100%Kw	8.95	26.36±0.47	4.25±0.13	6.2:1	0.30	9.13	16.64±0.98	1.91±0.02	8.5:1	0.43	9.48	6.45±0.14	1.78±0.02	3.6:1	0.82	8.51	0.71±0.02	1.62±0.14	0.4:1	0.49
90%Kw	8.96	23.59±0.64	3.83±0.12	6.2:1	0.30	8.97	14.98±0.89	1.76±0.02	8.5:1	0.50	9.10	6.04±0.08	1.60±0.01	3.8:1	0.73	8.91	0.64±0.02	1.45±0.13	0.4:1	0.41
80%Kw	8.99	20.97±0.57	3.40±0.10	6.2:1	0.30	9.00	13.31±0.78	1.56±0.01	8.5:1	0.47	9.28	5.63±0.09	1.43±0.01	3.:1	0.66	8.98	0.57±0.03	1.30±0.11	0.4:1	0.34
70%Kw	8.72	18.34±0.50	2.98±1.00	6.2:1	0.30	8.80	11.65±0.68	1.37±0.01	8.5:1	0.42	9.14	3.26±0.06	1.26±0.01	2.6:1	0.19	8.42	0.50±0.03	1.13±0.10	0.5:1	0.17
60%Kw	8.67	15.72±0.43	2.55±0.08	6.2:1	0.30	8.70	9.99±0.59	1.17±0.01	8.5:1	0.45	9.06	2.96±0.14	1.07±0.01	2.8:1	0.21	8.42	0.43±0.04	0.97±0.08	0.5:1	0.15
50%Kw	8.57	13.10±0.36	2.13±0.07	6.2:1	0.30	8.67	8.32±0.49	0.98±0.01	8.5:1	0.41	9.00	2.58±0.09	0.91±0.01	2.8:1	0.12	8.34	0.36±0.05	0.81±0.07	0.4:1	0.07
40%Kw	8.49	10.48±0.28	1.70±0.05	6.2:1	0.30	8.57	6.68±0.39	0.78±0.01	8.5:1	0.38	8.82	2.12±1.02	0.76±0.08	2.8:1	0.08	8.39	0.29±0.06	0.65±0.06	0.4:1	0.05
30%Kw	8.35	7.61±0.32	1.28±0.04	6:1	0.30	8.43	4.99±0.29	0.59±0.01	8.5:1	0.26	8.71	1.52±1.00	0.56±0.04	2.7:1	0.06	8.28	0.21±0.02	0.49±0.04	0.5:1	0.03
20%Kw	8.29	5.29±0.14	0.85±0.03	6.2:1	0.30	8.33	3.33±2.10	0.39±0.003	8.5:1	0.15	8.72	1.18±0.23	0.37±0.03	3.2:1	0.04	8.35	0.14±0.02	0.32±0.03	0.4:1	0.02
10%Kw	8.10	2.62±0.70	0.43±0.02	6.2:1	0.30	8.20	1.66±0.10	0.20±0.002	8.5:1	0.16	8.50	0.45±0.04	0.18±0.04	2.5:1	0.03	8.13	0.07±0.01	0.16±0.01	0.4:1	0.01
100%Sw	7.00	75.01±2.47	9.09±0.16	8.3:1	0.30	8.42	63.19±1.36	8.04±0.22	7.8:1	0.15	8.42	43.33±1.51	7.26±0.31	5.97:1	0.10	8.47	31.37±0.51	4.53±0.28	6.93:1	0.02
90%Sw	6.84	67.51±2.22	8.18±0.15	8.3:1	0.30	8.45	56.87±1.23	7.24±0.20	7.8:1	0.16	8.46	38.43±1.03	6.89±0.57	5.60:1	0.12	8.50	28.19±0.49	4.08±0.25	6.92:1	0.03
80%Sw	7.08	60.00±1.98	7.27±0.13	8.3:1	0.30	8.55	50.62±1.15	6.43±0.18	7.8:1	0.15	8.49	35.86±0.04	5.85±0.42	6.61:1	0.23	8.42	25.07±0.42	3.63±0.22	6.93:1	0.06
70%Sw	7.15	52.51±1.73	6.36±0.12	8.3:1	0.30	8.68	44.23±0.96	5.63±0.15	7.8:1	0.16	8.54	30.30±1.0	5.13±0.06	5.91:1	0.30	8.54	21.93±0.37	3.17±0.19	6.93:1	0.09
60%Sw	7.24	45.01±1.49	5.45±1.00	8.3:1	0.30	8.75	37.91±0.82	4.83±0.13	7.8:1	0.16	8.55	24.57±1.48	4.42±0.07	5.56:1	0.33	8.55	18.80±1.13	2.72±0.17	6.94:1	0.13
50%Sw	7.33	37.51±1.23	4.54±0.08	8.3:1	0.30	8.83	31.59±0.68	4.02±0.11	7.8:1	0.16	8.61	22.50±1.14	3.92±0.63	5.83:1	0.39	8.61	15.67±0.26	2.27±0.14	6.93:1	0.16
40%Sw	7.44	30.00±0.99	3.64±0.07	8.3:1	0.30	8.85	25.28±0.55	3.22±0.09	7.8:1	0.25	8.66	18.27±0.06	2.89±0.04	6.32:1	0.51	8.54	12.53±0.21	1.81±0.11	6.93:1	0.22
30%Sw	7.72	22.50±0.74	2.73±0.05	8.3:1	0.30	8.97	18.96±0.41	2.41±0.07	7.8:1	0.31	8.67	13.70±0.44	2.23±0.06	6.15:1	0.61	8.55	9.40±0.16	1.36±0.08	6.93:1	0.22
20%Sw	7.75	14.99±0.49	1.82±0.04	8.3:1	0.30	9.25	12.64±0.27	1.61±0.04	7.8:1	0.41	8.67	9.08±0.41	1.52±0.08	5.98:1	0.21	9.09	6.27±0.10	0.91±0.06	6.93:1	0.26
10%Sw	7.78	7.50±0.25	0.91±0.02	8.2:1	0.30	9.32	6.30±0.14	0.80±0.02	7.8:1	0.47	8.89	4.33±0.19	0.76±0.04	5.70:1	0.80	8.89	3.13±0.05	0.45±0.03	6.93:1	0.32
5%Sw	7.80	3.75±0.17	0.45±0.01	8.3:1	0.30	9.06	3.16±0.07	0.40±0.01	7.8:1	0.35	9.22	2.17±0.16	0.36±0.03	6.0:1	0.90	9.09	1.57±0.03	0.23±0.01	6.93:1	0.38

Table II. Statistical summary (mean ± SD) for analysis of pigment contents of *S. platensis* culture in the Zm, Kw and Sw for 10 days

Treatment	Zm and Kw Wastewater		Treatment	(Sw) wastewater	
	N:P	β- carotene	Phycocyanin	N:P	β- carotene	c-phycocyanin
	(10 days)	(mg g^{-1})	(mg g^{-1})	(10 days)	(mg g^{-1})	(mg g^{-1})
Zm	6.1:1	0.27 ± 0.02	6.94 ± 1.69	-	-	-
100%Kw	3.6:1	0.26 ± 0.03	18.44 ± 1.09	100%Sw	5.97:1	0.18 ± 0.07	8.27 ± 0.86
90%Kw	3.8:1	0.24 ± 0.03	14.87 ± 1.62	90%Sw	5.60:1	0.17 ± 0.02	7.33 ± 0.62
80%Kw	3.9:1	0.21 ± 0.03	12.89 ± 1.82	80%Sw	6.61:1	0.14 ± 0.03	7.43 ± 1.40
70%Kw	2.6:1	0.18 ± 0.02	10.94 ± 1.80	70%Sw	5.91:1	0.12 ± 0.02	6.47 ± 2.28
60%Kw	2.8:1	0.15 ± 0.02	9.57 ± 1.34	60%Sw	5.56:1	0.14 ± 0.01	7.15 ± 2.15
50%Kw	2.8:1	0.13 ± 0.02	8.40 ± 0.72	50%Sw	5.83:1	0.15 ± 0.04	8.22 ± 1.34
40%Kw	2.8:1	0.10 ± 0.02	6.56 ± 0.75	40%Sw	6.32:1	0.14 ± 0.04	7.96 ± 1.95
30%Kw	2.7:1	0.07 ± 0.02	4.02 ± 1.70	30%Sw	6.15:1	0.22 ± 0.07	9.45 ± 1.42
20%Kw	3.2:1	0.04 ± 0.02	2.76 ± 0.87	20%Sw	5.98:1	0.17 ± 0.05	8.47 ± 0.85
10%Kw	2.5:1	0.02 ± 0.01	1.42 ± 0.42	10%Sw	5.70:1	0.37 ± 0.06	21.27 ± 2.59
-	-		-	5%Sw	6.0:1	0.20 ± 0.02	10.17 ± 0.93

Kw and Sw ranged from 0.4:1 to 0.5:1 and 6.92:1 to 6.94:1 (Table I). *S. platensis* cultivation has been shown to be a promising approach to nitrogen and phosphorus removal from wastewater, giving a decrease in N: P after 15 days (Chuntapa *et al*., 2003). Each bottle had the initial OD of 0.30 (OD_{560nm} = 0.30), after adding the inoculum of *S. platensis* from Stock culture or initial biomass production (dry weight) was 0.30 g L^{-1}. After 15 days, the biomass production ranged from 0.01 to 0.51 g L^{-1}, the maximum biomass production was achieved in 5% Sw and Zm (N: P ~ 6:1) at ten days (Table I & Fig. 3). These are the same as in the earlier report that the optimum nitrogen concentrations for algal growth were 1.3-6.5 mg L^{-1} and the optimum N: P ratio for cultivation *S. platensis* was 6-8: 1 (Reynold, 1986). Cultivation of *S. platensis* in wastewater, with an average C: N: P ratio of 24: 6.14: 1, could support large amounts of *S. platensis* produced in pond-culture with an average specific growth rate of 0.51 g L^{-1} day^{-1}, which is comparable to the 0.54 g L^{-1} day^{-1} of *S. platensis* cultivated in an inorganic medium (Siew-Moi *et al*., 2000). Initial pH's ranged from 6.84 to 8.96 after 15 days; however the pH's rose to 8.13 and 9.38, respectively (Table I) consistent with the usual behavior of blue green algal cultures. For commercial purposes, the culture media employing *S. platensis* must have a high pH (8.5-12), which is particularly selective for this organism, an important factor in preventing contamination of the reactor by bacteria, algae and protozoa (Walach *et al*, 1987).

β-carotene and C-phycocyanin levels in *S. platensis*, cultured with 10% Sw were 0.37 mg g^{-1} and 21.27 mg g^{-1}, respectively, whereas at the optimum N: P ratio (5.7:1) the algal production was significantly higher than with the other treatments, with $p < 0.05$ (Table II). It should be noted that the present study utilizes a Zm with substantially fewer nutrients compared to the standard Zarrouk's medium, so substantially lower concentrations of β-carotene and C-phycocyanin were anticipated. When *S. platensis* was cultured outdoors with standard Zarrouk's medium, levels of β-carotene and C-phycocyanin of 1.5 mg g^{-1} and 60.70 mg g^{-1}, respectively, were obtained (Carlos *et al.*, 2003).

CONCLUSION

The highest levels of biomass production of *S.*

Fig. 1. *S. platensis* (a) Stock Zm (b) 3-week fermented Kw (▽) and (c) Sw (⊗)

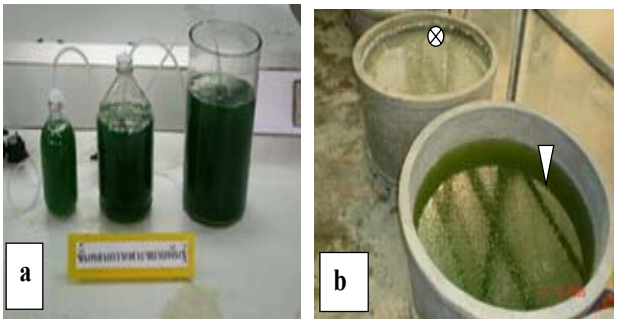


Fig. 2. *S. platensis* (a) Stock Kw) and (b) *S. platensis* cultured in laboratory (c) Dried *S. platensis* and powder

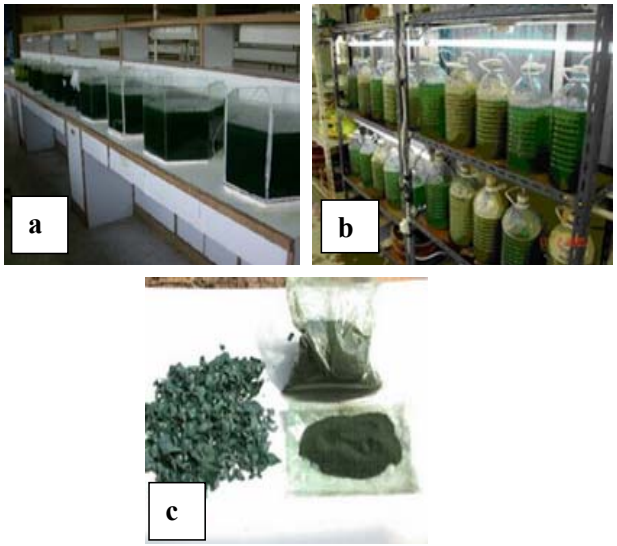


Fig. 3. Changes of TN, TP and *S. platensis* biomass (dry weight) of Zm and Kw in laboratory cultured over a 15 days period

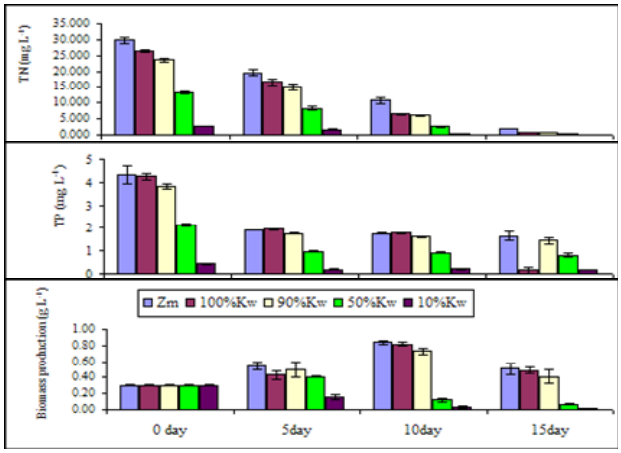
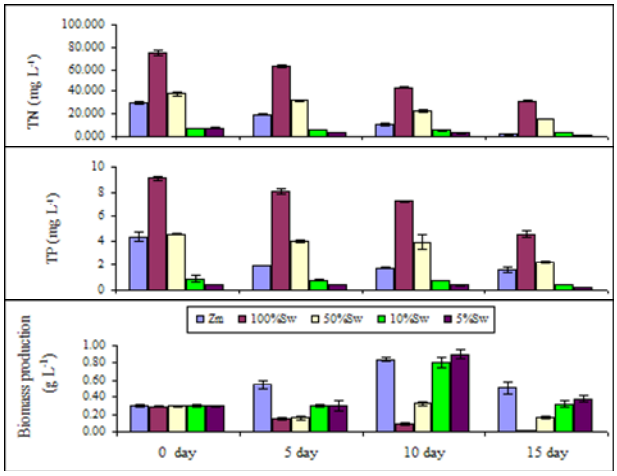


Fig. 4. Changes of TN, TP and *S. platensis* biomass (dry weight) of Sw in laboratory cultured over a 15 days period



platensis were achieved using 5% Sw (0.90 g L^{-1}) and Zm (0.80 g L^{-1}) with an N: P ratio of 6:1 and 6.1:1, respectively, at 10 days of culturing. The highest levels of β-carotene and C-phycocyanin in *S. platensis*, were achieved in cultures with 10% Sw (0.37 mg g ^{1} & 21.27 mg g^{-1}, respectively). TN and TP levels were dramatically reduced during cultivation under these optimal growth conditions, indicating successful removal of two primary nutrients responsible for water pollution, through incorporation into a useful product, *S. platensis*.

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