Capability of microalgae for local saline sewage treatment towards biodiesel production

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Abstract. Seawater flushing was introduced in Hong Kong since 1950's. High salinity has an inhibitory effect on nitrification and biological phosphorus uptake of microorganisms. Therefore, saline sewage has impact on traditional biological wastewater treatment. Saline conditions of domestic wastewater then pose opportunity to use algal technology in wastewater treatment. During the treatment (phycoremediation), biodiesel can be produced. This study aims to give an in-depth investigation and development on application of local microalgal strains on biodiesel production. *Dunaliella tertiolecta* was selected the appropriate algal species with high potential for phycoremediation then biodiesel production. *D.tertiolecta* was further investigated by optimizing its growth in different process condition in preliminary effluent as based medium. The optimized process condition were acclimated culture with medium initial cell number (5.0×10^5 cells mL^{-1}), under 5% CO_2 aerations in preliminary effluent adjust to 15 psu (denoting practical salinity unit). Results showed that lipid content increased from 30.2% to 42%, and biomass productivity reached 463.3 mg $L^{-1}day^{-1}$ by Fatty acid Methyl Ester (FAME) profile was found for biodiesel production in optimized stage. The treatment period of preliminary effluent was shortened from 15d in original design (un-acclimated culture, low initial cell number (5.0×10^5 cells mL^{-1}), without CO_2 aeration) to 4d.

1. Introduction

In the twenty-first century, the rapid growth of human development has increased large input of nitrogen and phosphorus into receiving water bodies and caused eutrophication, which resulting in red tide occurrence in coastal regions [1-7].

By means of different types of treatment processes and advanced technologies, pollutants, toxic materials and bacteria inside the sewage was removed to a level meeting the environmental standards. According to Hong Kong Drainage Services Department (DSD) sustainability report (2014-15), DSD is operating 297 sewage treatment facilities, including 70 sewage treatment works (STWs) and 277 sewage pumping stations. Among the treatment with these processes of treatment, the most capacity were Stonecutters Island, Sha tin and Tai Po in terms of treatment flow. Their treatment processes often up to secondary/biological treatment except for Stonecutter Island.

Among the STWs, most of the discharge standard of remaining nutrient level in terms of Total nitrogen (TN) and Biological Oxygen Demand-5 days (BOD_5) was 20 mg L^{-1} in the effluent. Part of

them even not required for nutrient removal in the standard. Therefore, the effluents from these STWs contain a certain amount of nutrient.

On the other hand, most of the STWs treat saline sewage except Shek Wu Hui STWs. As seawater flushing was introduced into the catchment areas of large-scale municipal treatment works in local area, for example, Tai Po and Sha Tin STWs in 1995 and 1996, respectively. Nevertheless, it was believed that the kinetic rates of nitrification and denitrification in secondary treatment were further affected by the variation (daily and seasonal) in salinity levels [8-10]. Some studies showing that good nutrient removal reached when salinity of influent were low (0.3 psu to 2 psu NaCl) but showed effect at high salinity levels of 5psu [11, 12]. Thus, the effect of interaction between salinity and pollutants in sewage increased the difficulty of saline wastewater treatment. Saline sewage has salt effect, lowering overall effective microbial metabolic rate and selectivity on nutrient removal. Therefore, microbial treatment capacity on per unit volume of saline wastewater is not high [8, 10-12].

Phycoremediation by using marine microalgae may be one of the efficiency and energy effective way to reduce these challenges [4, 13, 14]. Phycoremediation is a type of bioremediation by using microalgae metabolism [15, 16]. To improve treatment efficiency of STWs, wastewater can be acted as a nutrient source for microalgal biomass production. The biomass produced from the process can further produce bioenergy such as biodiesel. Microalgae convert nutrients or pollutants to organic materials (proteins, carbohydrates, fats) for growth through photosynthesis. Among these materials, triacylglycerols (TAG) and free fatty acid within microalgal cells are main raw materials for biodiesel production [17-22]. On the other hand, in microalgal biodiesel production, cultivation costs accounted for more than 70 percent of total costs, which is the major constrains of its industry development [22, 23]. Therefore, to complement both of saline wastewater treatment and microalgal biodiesel production, wastewater can used as nutrient sources for microalgae cultivation [14, 20, 24-28]. For example, in our previous study, *D.tertiolecta* was cultivated in the preliminary effluent successfully by acclimated cultivation and further biodiesel production [14, 28] .

In this study, preliminary effluent from Tai Po Sewage Treatment Works (TPSTW) and a local isolated microalgal species (*D.tertiolecta*) was used as a model to evaluate potential of phycoremediation associated with biodiesel production in local area. The selected species was adopted to cultivate in the wastewater based medium by acclimated cultivation in previous study[14, 28]. The following investigation then focuses on minimizing the cultivating days and maxizing the biomass production. Cultivating days indicate the day of the microalgal strain reach the maximum capacity and nutrient removal in specific conditions. The cultivation days is highly related to hydraulic retention time of microalgal wastewater treatment when the biomass productivity was important for biodiesel production.

Concentrated cultures require smaller cultivation areas, or reduced cultivation period, both have potential advantages for microalgal biomass production. Some studies stated that higher of algal density, better of growth and nutrient removal efficiency [29-33]. If the seeding cell density of selected species is not high enough, the microalgal growth will significantly affected by indigenous microorganisms and was not able to survive eventually from non-sterile wastewater [33]. However, in a relatively turbid medium, algal cells in wastewater may limit by light, carbon or nutrients rather than by plant, which called self-shading [31, 34]. Self-shading limits the operational maximum cell capacity. For example, *Chlorella* spp., in treating wastewater [35] have been investigated in high algal density for auto inhibitory effects [36]. A trade-off point between algal density, microalgal growth and treatment efficiency should consider in an algal system [37, 38].

2. Material & methods

2.1. Wastewater collection

The preliminary effluent (PE) in this study was collected directly from the Tai Po Sewage Treatment Works (TPSTW) in Hong Kong which studied in previous study [14]. PE was the discharge after screening treatment, solids which larger than 6 mm (diameter) were removed from raw wastewater.

The wastewater samples were taken in summer (June-September). Wastewater samples were stored in 10 liter buckets at 4°C and used within 2 hours after sampling before each experiment. The physicochemical characteristics of PE were shown in table 1. The BOD5-to-PO4 ratio is found relatively high. The PO4 to TP, BOD5: TP ratio and TN: TP ratios are found at lower range for domestic wastewater. The PE used in this study is then considered to be favorable for available nutrient removal with enhanced growth of microalgae.

Table 1. Physicochemical characteristics of preliminary effluent.

Characteristics	Range	Average	Unit
pH	5.72-5.8	5.76	/
Dissolved oxygen	3.35-3.97	3.66	mg L^{-1}
Turbidity	679.5-707	693.3	NTU
Total dissolved solid	6.33-6.83	6.58	mg L^{-1}
Total suspended solid	330-460	395	
Salinity	5.8-8	6.9	psu
Total nitrogen (TN)	55-60	57.5	mg L^{-1}
Ammonium-Nitrogen (NH_4^+)	19-28	23.5	
Nitrate- Nitrogen (NO_3^-)	5.5-13	9.25	
Total phosphorus (TP)	13.6-20.1	16.85	
Ortho-Phosphate (PO_4^{3-})	11.8-18.7	15.25	
Biological Oxygen	240-302	271	
Demand(BOD_5)	255-350		
Chemical Oxygen	6.61-8.95	302.5	
Demand(COD)	0.87-0.93		
N:P ratio*	15.0-17.6	7.78	/
PO_4:TP ratio	16.1-20.3	0.9	
BOD_5:TP ratio		16.3	
BOD_5: PO_4 ratio		18.2	
Metallic ions			
Cadmium	<0.01	<0.01	
Chromium	<0.01	<0.01	
Copper	0.06-0.12	0.09	mg L^{-1}
Iron	0.05-0.17	0.11	
Lead	<0.01	<0.01	
Zinc	<0.01	<0.01	

*Atomic ratio calculated by TN and TP

2.2. Algal culture and growth conditions

The microalgal strain was isolated from local algal blooming samples from coastal water in this study. *D. tertiolecta* is motile and unicellular (8-12 μm) which is a genus of *Dunaliellaceae*. It can commonly found in Hong Kong costal area. *D. tertiolecta* cells are cultivated in L1 medium prepared using autoclaved seawater. The cultures were transferred to new medium weekly at 1:10 v/v in order to keeping in the exponential growth phase. Microalgal cells in the exponential growth phase were inoculated into artificial medium (L1) or preliminary effluent. The microalgae were cultivated in 800ml preliminary effluent in conical flasks with 12:12 light dark cycle. 125 μmol photons $m^{-2}day^{-1}$ was provided by cool white fluorescent tubes in a convison growth chamber. Constant temperature at 24°C was provided for the cultivation.

2.3. Experimental set-up

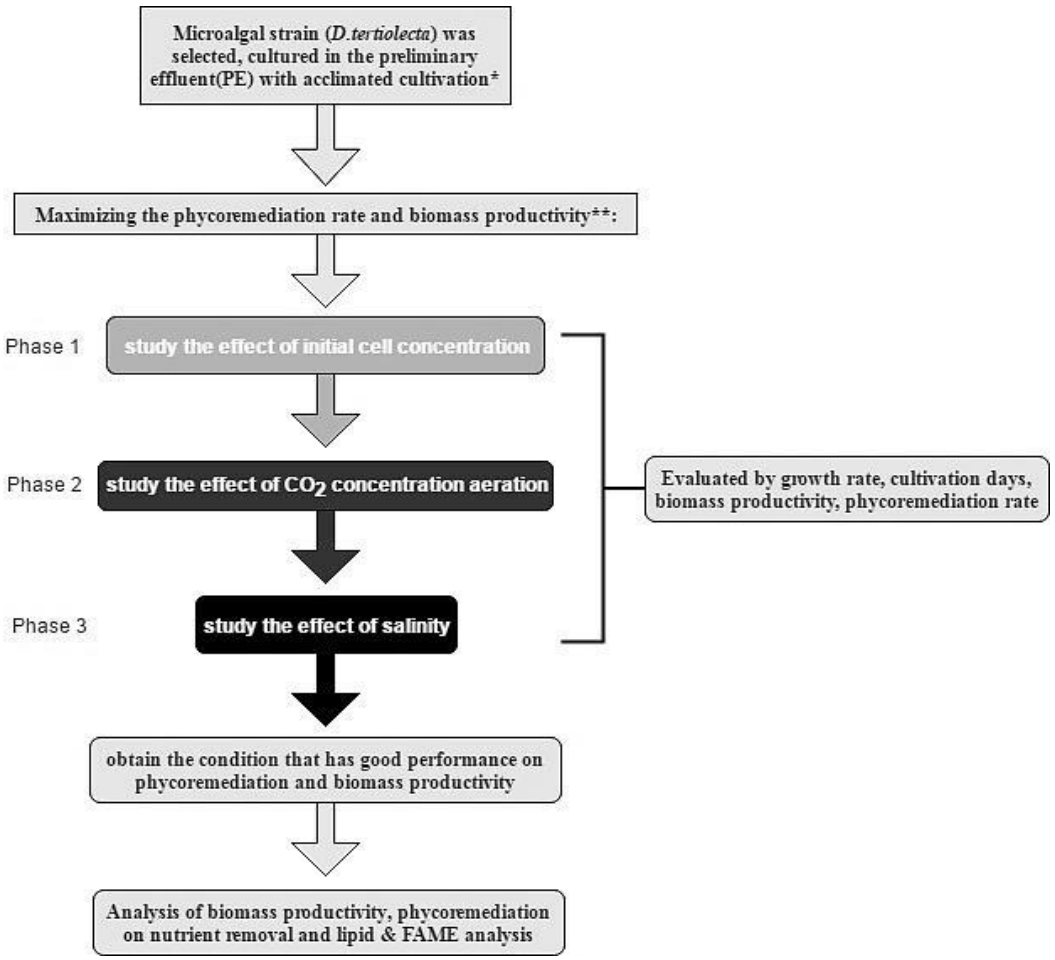

Figure 1. Flow diagram of experimental processes involved in the present study of microalgae cultivation in domestic wastewater [14, 28] present study.

Table 2. Related cell density and biomass concentration with different cell density of inoculums.

Different initial cell density					
Related concentration	Low	Median	Concentrated	Hyper-concentrated	Ultra- concentrated
Cell density (cells mL^{-1})	1×10^5	5×10^5	1×10^6	5×10^6	1×10^7
Biomass concentration (g dry weight L^{-1})	0.15	0.75	1.5	7.5	14.5

The experiments were carried out in three phase (figure 1). Each experiment was done for triplicate. In the first phase, different cell density of inoculums ranged from 1×10^5 to 1×10^7 cells mL^{-1} presenting dry weight of biomass range from 0.15 to 14.5 g dry weight L^{-1} (table 2). Each set was namely as 'low', 'median', 'concentrated', 'hyper-concentrated' and 'ultrahigh concentrated' cultures, respectively. An algal biomass larger than 1.5 dry weight g L^{-1} was regarded as hyper-concentrated cultures. Ultrahigh celldensity culture describes cultures with cell density above 10 g dry weight L^{-1}. The control was settled as without any inoculums. The different cell densities of inoculum in the culture were prepared by concentrated by centrifuge (Eppendorf® 5804) at 3000rpm for 2 mins, and sub-cultured to related

medium. In the second phase, gas provided as different concentrations of CO_2 mixed with ambient air was prepared with a volumetric percentage of CO_2 (provided by Hong Kong oxygen®) to give CO_2 concentrations of air (0.04%), 1%, 5%, 10%, or 100%. Microalgal cultures were aerated continuously at the bottom atrate of 100 mL min^{-1} (i.e. 0.125 vvm, volume gas /volume/min). In this phase, the culture without any aeration was acted as control. In the third phase, the saline sewage was adjust salinity by adding sodium chloride from Sigma (USA) in (8 psu, 10 psu, 12 psu, 15 psu and 30 psu) equivalent to 0.1 M to 0.25 M NaCl while the original salinity of PE was 6 psu.

2.4. Cell Counting and calculation of specific growth rates

A benchtop instrument was developed by The Countess™. It uses the standard trypan blue technique for cell number determination. Digital image was captured and a sophisticated image analysis program determines the cell count within the medium [39]. Cell density was determined every two days at the same time. Each point on graph shows average of triplicate results. Growth rate (μ) in exponential growth phase was calculated by following equation:

$$\mu = \frac{\ln N_0 - \ln N_1}{t_0 - t_1}$$

Where N_0 and N_1 are the cell concentration at time t_0 and t_1.

2.5. Biomass concentration determination

The dry weight of microalgae was measured though filtering 10 mL culture suspension through pre-weighed GF/C glassfiber filters (Whatman GF/F, pore size 0.7 μm). Vacuum pressure was kept at 35 to 55 mm Hg during filtration [40]. After rinsing with natural seawater which filtered with 0.45 μm nylon membrane, the filters were dried at 75°C for 24 hrs until there was no further lot of weight.

2.6. Wastewater analysis

10 ml samples were collected from each trial in the experiments. The physicochemical characteristics of the wastewater including, salinity, pH ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), and chemical oxygen demand (COD), were analyzed. Salinity was checked with Atago Master-S/Mill refractometer. Residual free NO_3^-, NH_4^+ and PO_4^{3-} concentrations in culture were determined by using commercial spectrometry-based assay systems according to manufacturer's instruction menu. Assays were done by using DRB 200 reactor and DR 2800 spectrophotometer (Hach Co., Loveland, CO, USA).Nutrient removal efficiencies were obtained according to the following equation:

$$R_{\%} = \frac{S_0 - S_t}{t(d)}$$

where: $R_{\%}$ represents removal efficiency of substrate i (NO_3^-, NH_4^+, or PO_4^{3-}); S_0 and S_t are defined as the mean values of substrate; i concentration at initial time t_0 and time t_i.

2.7. Lipid content determination

Modified method from Bligh and Dyer, 1959 [40] were used for total lipids extraction. Microalgal lipids were extracted by chloroform–methanol (1:1, v/v) Chloroform and aqueous methanol layers then separated by addition of 5% NaCl solution to give a final solvent ratio of chloroform: methanol: 5% NaCl solution of 2:2:1. The chloroform layer was collected and dryness by nitrogen flow. The total lipids were determined gravimetrically.

2.8. Fatty acid methyl ester determination

Fatty acids profile was examined (in triplicate) by the modified transesterification method described by Lepage and Roy [41]. Fifty-milligram samples were mixed with the transesterification reagent (i.e. 100.0 ml methanol containing 2.2 ml of sulphuric acid with 98% purity) and then stirred for 2 hours at 90°C under reflux. Afterwards the reaction product is extracted with hexane/water mixture (1/1v/v %). Fatty acids profile was determined by gas chromatography using Gas Chromatograph (Perkin Elmer

GC 600) equipped with flame ionization detector (FID) and a fused silica capillary column OmegawaxTM 250 (30 m×0.25 mm×0.25 μm film thickness). The injections volume was 1 μl of triplicate in each extraction. FAME was identified by comparing retention times with standards (Sigma-Aldrich R, USA). Analysis programs were: injector temperature and detector temperature were 250 and 260°C respectively. For thermal program, 150°C was held for 16 min firstly, 2°C min^{-1} was increased to 180°C for 25 mins. 210°C was reached up of 5°C min^{-1} and held for more than 25 min. 1.3 mL min^{-1} helium was used as carrier gas. Make up gas was nitrogen gas as 30 ml min^{-1}, flow of hydrogen gas was provided at 30 ml min^{-1}. Synthetic air was provided at 300 ml min^{-1}.

2.9. Statistical analysis

The measurements of nutrient concentration were averaged in three samples of influent and effluent. Every data point on graph represented average of triplicate results. Standard deviations (+SD) were plotted as error bars in the curve.

3. Results and discussion

3.1. Effect of seeding cell density on phycoremediation and biomass productivity

Variation in seeding concentration influenced microalgal growth and further nutrient removal efficiencies. Figures 2 and 3 show the change in cell density, biomass productivity and growth rates at various seeding cell density. From the results, biomass productivity and growth rates of low, median, concentrated and hyper-concentrated cultures were positive value while ultra-concentrated was negatively value (death rate). The highest of biomass productivity was achieved by median (74.7 mg L^{-1} day^{-1}) when the second is low (64 mg L^{-1} day^{-1}) in PE after cultivate for 14 d. The control, without inoculum, showed no biomass productivity in terms of microalgae.

Table 3. Nutrient removal rate and removal percentage in different seeding cell density.

Seeding cell density	Ortho-phosphate		Nitrate		Ammonium	
	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)
Low	1.56 ±0.2	86.4 ±7.2	3.81 ±0.9	92.6 ±0.7	4.46±1.22	95.9 ±5.1
Median	2.52 ±0.14	87.1 ±8.3	4.04 ±1.5	87.6 ±7	5.40±0.85	96.4 ±3.2
Concentrated	2.74 ±0.11	87.8±2.04	4.69 ±2.6	85.2 ±8.6	4.54±1.33	97.6 ±1.8
Hyper concentrated	2.55 ±0	80.3 ±8.3	4.48 ±1.8	98.1 ±0.6	3.31±0.94	94.6 ±5.6
Ultra concentrated	3.13 ± 0.1	63.9 ±9.6	2.50 ±0.5	85.2 ±0	4.95±1.41	99.4 ±0.4
Control	/	2.7 ±0	/	-1.25 ±0	/	N.D.

*The removal rate and removal percentage were calculated from the nutrient removed at the end of experiment (14 d).

At the end of experiment (14 d), almost complete removal was showed in each initial density (table 3). For orthophosphate, ultra-concentrated can remove higher than 80% in first four days and thus the phycoremediation rate was highest while the median, concentrated and hype concentrated show similar phycoremediation rate and reach 2.5-2.7 mg $L^{-1}day^{-1}$. Both algal and bacterial cells were able to assimilate phosphorus from preliminary effluent. It is suggested that phosphorus precipitation may also be contributed for phosphate removal [4]. Removal rate of nitrate in each seeding cell density was

remarkable and reached 90% and reach nearly 4.5 mg $L^{-1}day^{-1}$ except for hyper-concentrated. Nitrate

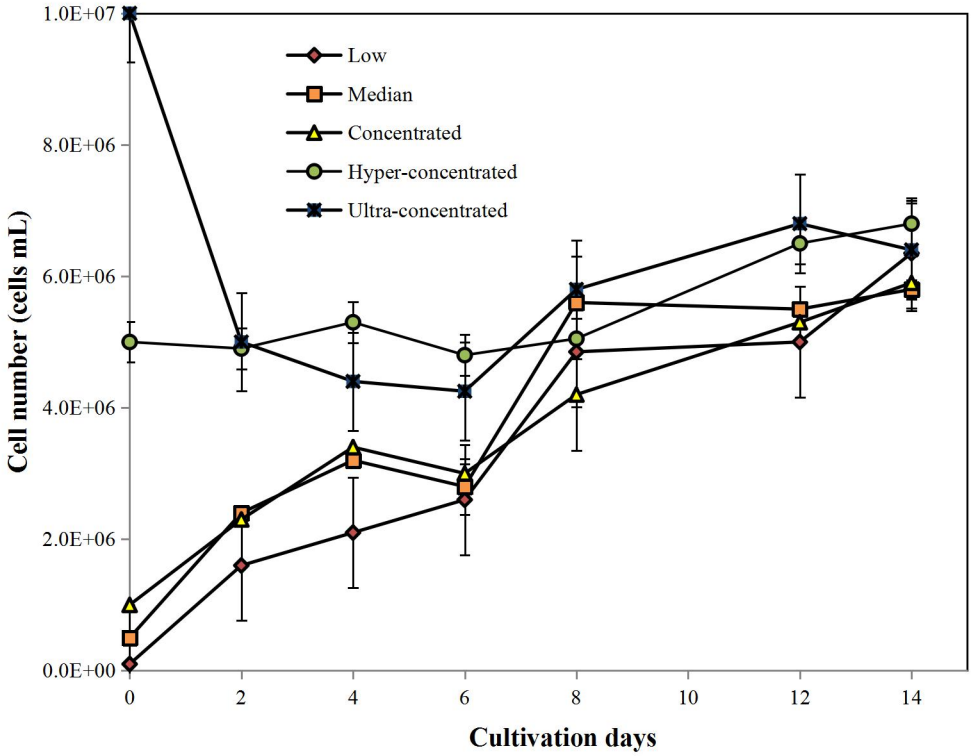

Figure 2. Variation of cell concentration with different seeding cell density.

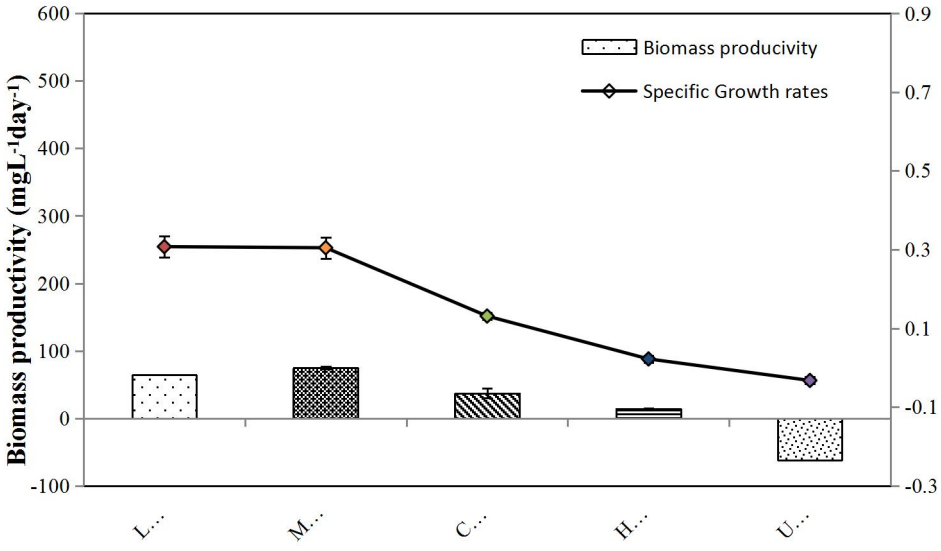

Figure 3. Biomass productivity and specific growth rate depending upon seeding cell concentration.

* Negative value indicate the decay rate of microalga cell concentration

** The specific growth rates and biomass productivity were calculated from the biomass produced and cell number at the end of experiment (14d)

is essential nitrogen sources rather than ammonium for *D.tertiolecta* growth [14]. Therefore, it shows relatively good removal performance of nitrate in PE. Concentration shows the best removal and higher than ultrahigh concentrated in ammonium). About 4.48 and 4.69 mg L-1 ammonium were removed by hyper- and concentrated culture each day. The degree of ammonia stripping was showed in control (without any algae) that indicated a similar alkalization. About 5% ammonium was lost in control in first 4 cultivation days (data not shown). The removal percentage decrease with an average of about 1% ammonium was loss throughout the experiment. It is then suggested that ammonia stripping has been considered as one of the mechanism to remove ammonium from PE.

In other similar studies, for example, Lau et al 1995 [31] studied relationship between inoculum concentration and nutrient removal efficiency in primary settled effluent. They stated that super-concentrated inoculum of C.vulgaris (1.2×10^7 cells mL^{-1}) did not promote any self-shading that will limit removal of nutrient. Super-concentrated culture was also considered to optimal level for nutrient removal.

Previous studies stated that higher of inoculum concentration, better of growth and nutrient removal efficiency [31, 42]. Impacts of cultivation such as accumulation of auto-inhibitors and self-shading may not present in initial phase of low to concentrated inoculums. Some study also claimed that higher of inoculum concentrations reduce toxic effect of ammonium to microalgae. Competition between microalgae with other microorganisms was also reduced when cultivating in the wastewater [43]. *S. obliquus* showed high nitrogen removal for 1.9 g L^{-1} inoculum compared to lower density inoculum of 0.5 g L^{-1} [29]. They also stated that removal rate of ammonium and phosphorous were related to microalgal concentration and obvious light limitation is independent of due to self-shading. However, growth of *D.tertiolecta* shows better in median and low initial inoculums in first 5 d than others in this study. Limitation of gas exchange or availability of inorganic carbon such as hyper-concentrated, ultra-concentrated could be one of the key limiting factors [44, 45]. Accumulation of auto-inhibitory substances (e.g. chlorellin) [46] in very dense algal cultures may also affect microalgal growth. Different concentrated culture showed similar nutrient removal performance. The cultures showed a pattern of an exponential removal while those of high, hyper-concentrated and ultrahigh concentrated ones were more hyperbolic.

In the view of microalgal biomass productivity, median concentrated cultures show considerable phycoremediation. Therefore, median concentration was chosen for the following experiment.

3.2. Effect of CO_2 concentration on phycoremediation and biomass productivity

Table 4. Nutrient removal rate and removal percentage in different CO_2 concentration.

CO_2 concentration	Ortho-phosphate		Ammonium		Nitrate		pH
	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)	
Air	3.21 +0.77	86.6 ±6.6	1.87 ±0.16	94 ±0.5	3.2 ±0.67	61.5 ±2.7	7.6-8.6
CO_2 (1%)	3.01 +0.72	66.7 ±0.1	1.77 ±0.15	86.6 ±0.1	1.88 ±0.4	50.7 ±7.1	6.4-7.1
CO_2 (5%)	3.54 +0.74	96.3 ±0.53	1.92 ±0.17	94 ±0.5	3.29 ±0.69	91.7 ±2.2	5.9-7.8
CO_2 (10%)	3.2 +0.67	62.7 ±1	1.03 ±0.08	71.5 ±0.1	2.41 ±0.51	46.4 ±0.5	5.1-6.2
CO_2 (100%)	2.17 + 0.45	33.3 ±8.1	0.9 ±0.07	44.1 ±2.7	0.33 ±0.07	-15.2 ±3.9	5.1-4.8
Control	/	92.3 ±4.1	/	72.4 ±3.4	/	61.5 ±4.9	6.9-7.8

*The removal rate and removal percentage were calculated from the nutrient removed at the end of experiment (6 d).

From the results (table 4), amount of CO_2 dissolved in water varies with pH, (i.e. pH decrease resulting from addition of CO_2). The optimum growth and metabolism of various microalgae was also affected by pH value. In air aeration, the pH had not shown any drop. For the groups aerated with CO_2

enriched air, initial pH value rapidly dropped in day 1. With the assimilation of CO_2 by microalgae, pH value increased due to CO_2 acclimation rate was higher than the supply rate. Nevertheless, when CO_2 supplied rate was higher than the acclimation rate, pH value would be dropped. The low pH also leads the cell death or lower growth rate and further decrease nutrient acclimation. This maybe one of the reason, biomass productivity and growth rate were low in high concentration of CO_2 aeration [47, 48].

As microalgae fix the carbon dioxide, hydroxyl ions will then accumulate which often increasing the pH value. During pH values larger than 9, inorganic carbon form carbonate (CO_3^{2-}) which microalgae cannot assimilate. Lower the availability of CO_2 then limit algal growth [49, 50]. Microalgal species favour neutral pH while some species such as *S.platensis* are tolerant to high pH(e.g. pH 9) [51] or lower pH 4 such as *C.littorale* [52]. There is relationship between pH and carbon dioxide concentration in cultivation, underlying balance among such the form as CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}.

For the results of microalgal growth (figures 4 and 5), it was expected that the microalgae could not be survival under 100% CO_2 concentration [53]. At the aeration of 5% CO_2, *D.tertiolecta* increased most rapidly at growth rate of 0.593 d^{-1} and markedly fell to be 0.293 d^{-1} and 0.148 d^{-1} when cultures were provided with 10% and 100% CO_2. These results also showed in Tang's study [47]. In their experiments, it showed that highest growth rate can be reached in the range of 2-6% CO_2 in air while 1 and 10% showed lower growth rates. These results are consistent with studies that optimal condition for *D.tertiolecta* owards highest growth rate was 6% CO_2 [54, 55].

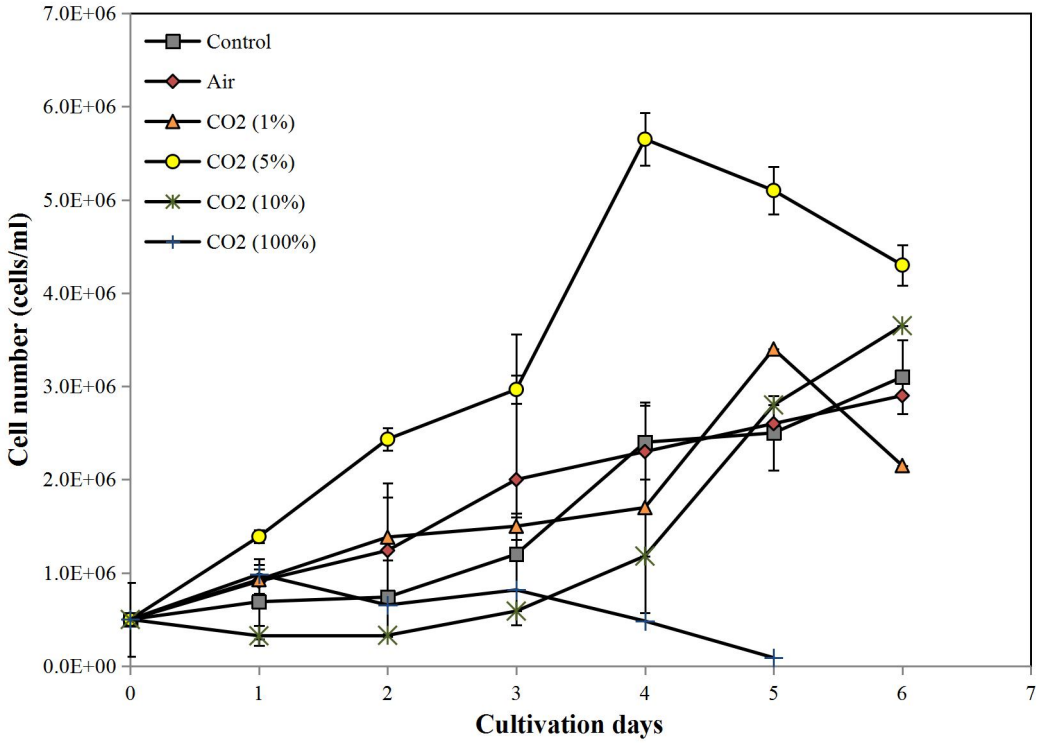

Figure 4. Variation of cell concentration with different CO_2 concentration aeration.

In nitrate removal, the best was CO_2 (5%) and reached 3.29 mg $L^{-1}day^{-1}$ and second high was air and reached 3.20 mg $L^{-1}day^{-1}$. Moreover, removal of ammonium was better in the 5% CO_2 and air compared to others treatments which reached 1.92 mg $L^{-1}day^{-1}$ and 1.87 mg $L^{-1}day^{-1}$ respectively. However, the ammonium removal of 5% was also relatively low when compare to previous experiment even it show the best growth in this study. In contrast, the nitrate removals of 5% CO_2

show much higher value (3.29 mg L^{-1} day^{-1}) than the ammonium one (1.92 mg L^{-1} day^{-1}). This is because the strain could use ammonium than nitrate as the nitrogen sources as previous discussion. Ammonium removal efficiency in culture using air aeration was higher than carbon dioxide enriched air aeration except 5% aeration in our study. This can be explained by pH differences triggered ammonium stripping. Some studies reported that the volatile NH_3 of total NH_4-N is a function between pH and temperature [56, 57]. Therefore, it is suggested that depletion of NH_4^+-N was not attributed to nitrogen uptake by algae only, but also volatilization of ammonia. Moreover, as BOD within PE was relatively high, the heterotrophic microorganisms such as bacteria may also contribute to the ammonium removal. For phosphorus removal, it were stated that sedimentation and direct cellular absorption by microorganisms under aerobic conditions. More than 90% of phosphorus removal was showed in culture with CO_2 aeration and air aeration control even the pH value was not high in this experiment. As pH value of the culture increased to 8.7, which was high enough to cause phosphorus to precipitate [58, 59]. Therefore, the orthophosphate within the CO_2-enriched air aeration was assimilation mainly though phycoremediation but not sedimentation. Increasing CO_2 levels in air provided the higher growth rates which were demonstrated in many studies. However, a balance should be established between acclimation of CO_2 and pH value during the phycoremediation. 5% CO_2 was found to be the optimum percentage for selected strains on phycoremediation in this study either the growth and nutrient removal also show a relatively good performance.

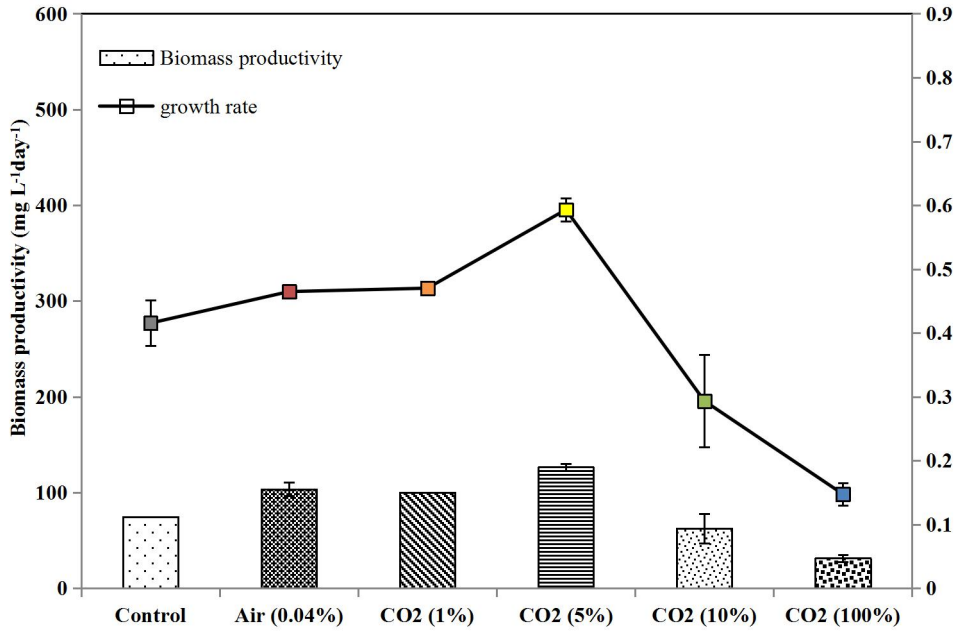

Figure 5. Specific growth rate and biomass productivity depending upon CO_2 concentration aeration.
*The specific growth rates and biomass productivity were calculated from the biomass produced and cell number at the end of experiment (6d)

3.3. Effect of salinity on phycoremediation and biomass productivity

In this phase, algal inoculums were cultivated with different salinity of preliminary effluent to real applicate the salinity adjustment as one of the major optimized process condition (figures 6 and 7). The additions of salt provide niche opportunity for the selected microalgal strains and show more superiority in 12-15 psu for *D.tertiolecta* growth. The maximum capacity in biomass concentration was also reached in 15 psu which was 1.09 g dry weight L^{-1} in day 3, while the lowest maximum capacity was 6 psu in day 4 (0.58 g dry weight L^{-1}). Optimum salinity for nutrient removal in PE was

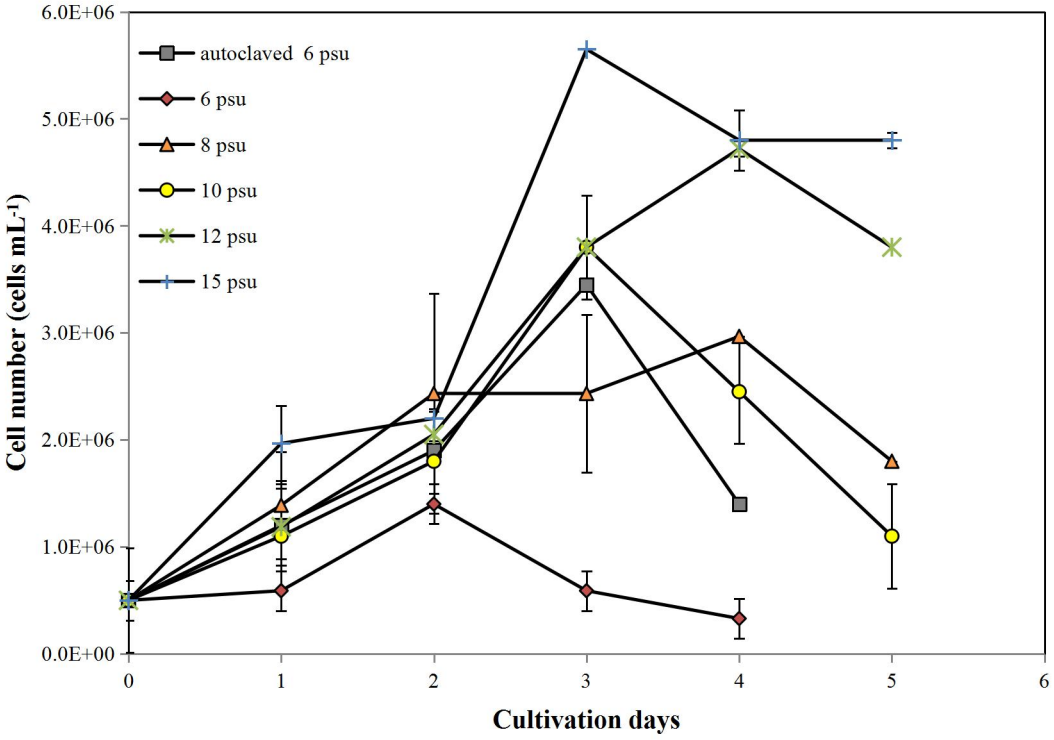

Figure 6. Variation of cell concentration with different salinity.

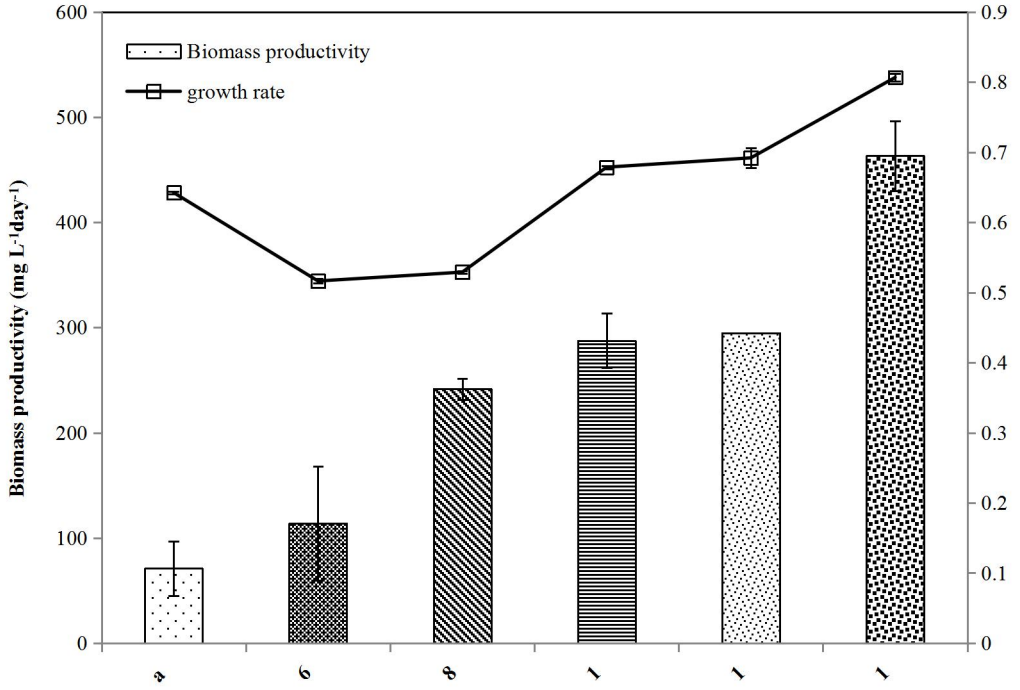

Figure 7. Biomass productivity and specific growth rate depending upon salinity.
*The specific growth rates and biomass productivity were calculated from cell number and the biomass produced at the end of experiment.

Table 5. Nutrient removal rate and removal percentage in different salinity.

Salinity	Total Phosphorus		Ortho-phosphate	
	Removal rate (mg L^{-1} day^{-1})	Removal percentage (%)	Removal rate (mg L^{-1} day^{-1})	Removal percentage (%)
Autoclaved 6 psu	1.64 ±0.03	72.9 ±9.1	2.42 ±0.19	97.0 ±3.54
6 psu	1.08 ±0.08	59.7 ±1.5	2.2 ±0.11	83.3 ±5.6
8 psu	1.8 ±0.14	90.4 ±0.1	1.28 ±0.46	96.3 ±0.07
10 psu	4.98 ±0.27	90.0 ±2.2	3.15 ±0.21	96.5 ±0.37
12 psu	4.52 ±1.32	65.7 ±3.5	2.35 ±0.1	94.7 ±0.52
15 psu	7.19 ±2.5	86.2 ±0.1	3.02 ±0.31	97.1 ±0.28

Total nitrogen		Ammonium		Nitrate	
Removal rate (mg L^{-1} day^{-1})	Removal percentage (%)	Removal rate (mg $L^{-1}$$day^{-1}$)	Removal percentage (%)	Removal rate (mgL^{-1} day^{-1})	Removal percentage (%)
6.25 ±1.12	69.4 ±0.3	6.75 ±0.66	54.5 ±1.45	2.63 ±0.53	71.9 ±4.4
6.01 ± 0.1	66.6 ±0.1	3.75 ±0.1	37.7 ±3.04	2.55 ±0.19	91.4 ±3.6
5.01 ±0.82	26.5 ±0	2.63 ±0.53	15.52 ±0.1	3.53 ±0.11	87.5 ±0.24
6.25 ±0.35	71.4 ±2.8	8.25 ±1.32	70.96 ±1.39	2.36 ±0.16	93.9 ±4.1
6.75±1.9	82.1 ±0.5	7.01±1	56.95 ±4.69	3.75 ±0.35	76.8 ±2.5
6.38 ±0.53	83.7 ±0.47%	7.88 ±0.13	79.07 ±3.95	3.02 ±0.31	98.4 ±2.15

*The removal rate and removal percentage were calculated from the nutrient removed at the end of experiment (5 d).

found in 10 psu & 15 psu when the P-related nutrients were almost 80% in first three days and reached 3.17 mg L^{-1} day^{-1} and 3.58 mg L^{-1} day^{-1} (table 5). Rao et al concluded that phosphate removal was because used by freshwater microalga *B. braunii*, which salinity concentration did not influence the removal [60]. This conform to this study that most of phosphorus in preliminary effluent was used for the synthesis biomass of *D.tertiolecta*. For N-related nutrient, the nitrate removal reached 80% in first three days when ammonium also showed considerable amount (6-8.5 mg $L^{-1}$$day^{-1}$).

Compositions are very variable in wastewater. Even though acclimation, initial cell number and CO_2 enriched air aeration improve growth of microalgae and efficiency of phycoremediation, interaction between microalgae and other microorganisms during wastewater treatment may affect the stability [20, 55, 61]. Although sterilization such as UV radiation, autoclaved provide a solution for this problem, high energy and cost usage are required and further affect the treatment process [20, 35]. Therefore, phycoremediation in real application may need to be consistently enriched using low cost and energy consumption method to reduce microbial activity that impacts the microalgal growth. Microorganisms have their own ecological niche [62, 63]. Salinity, for instance, is a condition that limits the growths and reproductions of many aquatic microorganisms, different organisms tolerate different ranges of salinity [64]. This range is one dimension of an organism's ecological niche. To further improve the growth of selected microalgal species for phycoremediation, optimal niche opportunity can be provided. For example, adjust the salinity to an optimal value for the selected microalgal species.

3.4. Effects of salinity on total Lipid content and FAME composition

Figures 8 and 9 show the comparison of different salinity on lipid production. Under unfavorable environments, algal species produce a large amount of storage lipid as triglyceride [64-66]. However, it may not the case in current study. The lipid content of *D. tertiolecta* increased from 6 psu to 15 psu ranging from 32.2% to 43.5%. As the growth rate and biomass productivity were also better in higher salinity, therefore, increased lipid content following the salinity was not due to salt stress. The reasons behind may base on optimum niche was reached and further to attain a relativity good yield of lipid [67].

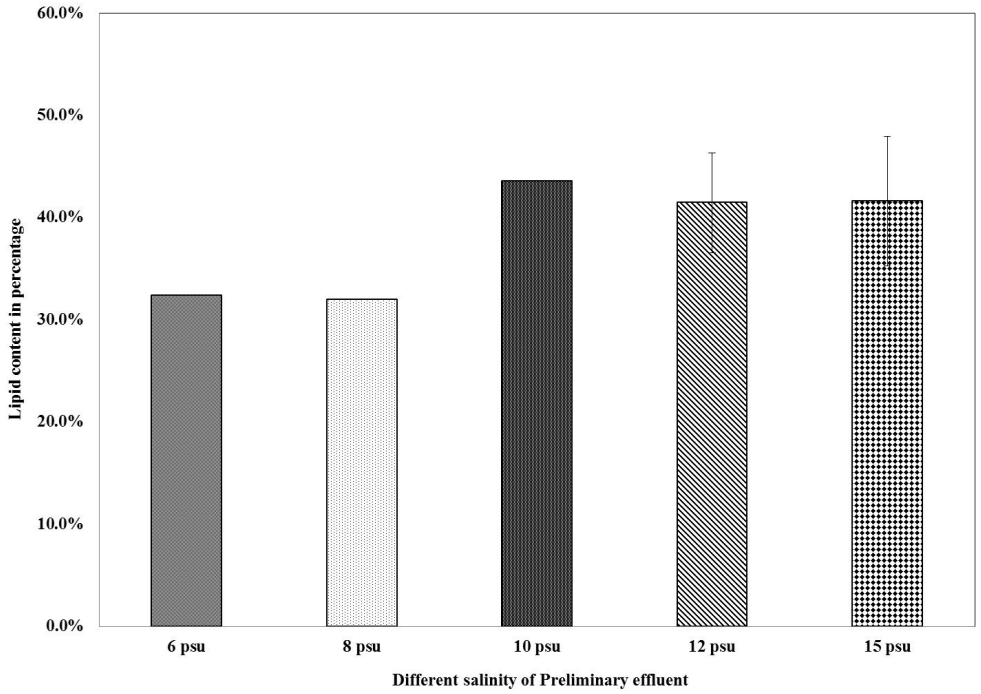

Figure 8. Lipid content of D.tertiolecta on correlated cultivation day under different salinity.

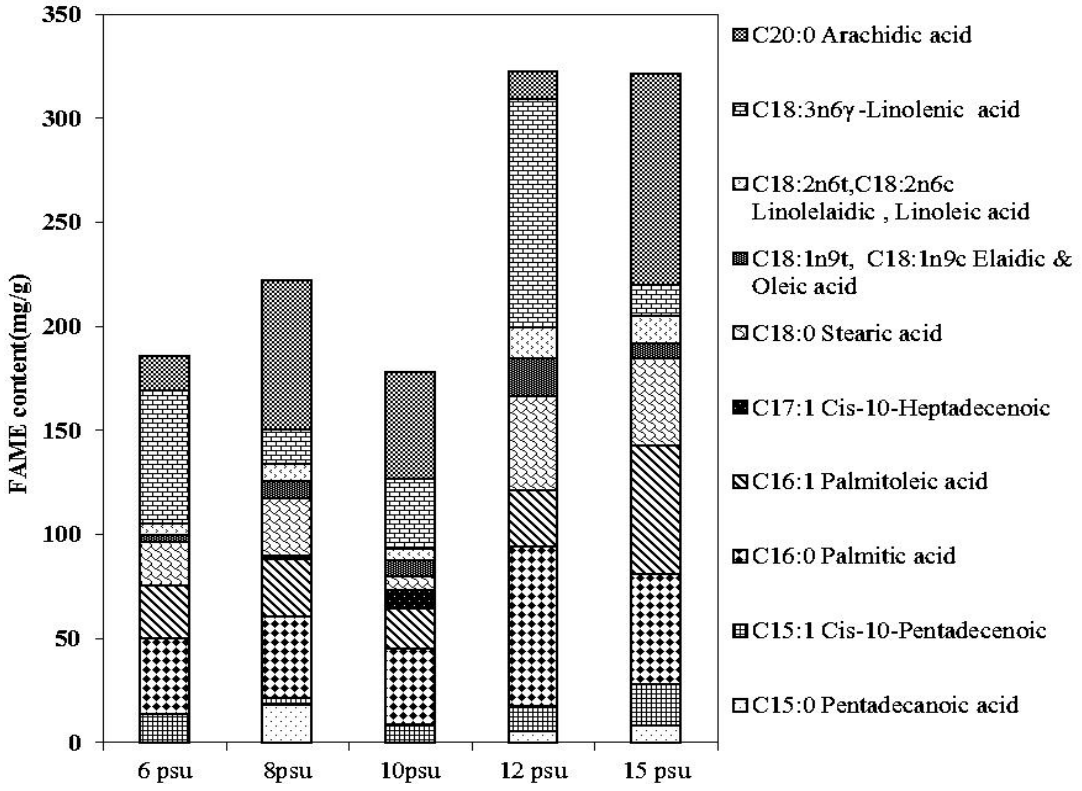

Figure 9. Fatty acid profiles (mg/g) of D.tertiolecta grown under different salinity.

A study state that salinity affect the growth of *Chlamydomonas mexicana* cultivating in municipal wastewater and lipid content in microalgal biomass due to the accumulation of glycerol in responding

osmotic pressure [68]. Their results showed that inorganic carbon transfer to glycerol during photosynthesis in *C. mexicana* improving lipid production with increasing NaCl concentrations in wastewater.

Salinity is an intricate biotic factor affecting physiological and biochemical metabolism associated with microalgal growth. Salinity can also lead to improvement in the FAME content of microalgae in changing in fatty acid metabolism [69, 70].

FAME produced from *D.tertiolecta* in different psu contains fatty acid profiles comprising mainly C_{16} and C_{18} fatty acids (figure 3). Fatty acid composition was studied at maximum capacity (end of exponential phase) to reduce the variation in the distribution of fatty acids. The 15 psu conditions also show highest FAME productivity while 6 psu show the least. This was also similar to the growth pattern of each salinity condition in terms of biomass production. Salinity does not seem to affect significantly overall structure but amount of production. The current results show that key fatty acid compositions of *D.tertiolecta* cultivated in different salinity preliminary effluent were Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Linolelaidic acid (C18:2n6t), Linoleic acid (C18:2n6) and γ -Linolenic acid (C18:3n6) alongside several minor fatty acid components with carbon number which is lower than C_{16} and higher than C_{18}. Although the major profiles among different salinity were not different, amounts of each fatty acid were variable. In briefly summary, the C_{16} and C_{18} groups still contain for about 97% of the total FAME. Therefore, microalgal lipid produced from *D.tertiolecta* cultivating in enhanced salinity of PE based medium has high potential for producing biodiesel.

4. Conclusion

It was found that in acclimates culture with 5.0×10^5 cells mL^{-1} initial cell densities under 5% CO_2 aeration when culturing with 15 psu salinity of preliminary effluent show the best improvement in phycoremediation of *D.tertiolecta* of preliminary effluent. It show 0.463 g L^{-1} day^{-1} biomass production with 41.6 % total lipid content, and remove most of the nutrient in three cultivation days by the combination of optimized cultivation method. Phycoremediation show the potential insert into the current sewage treatment plant to act as one of treatment unit in local area.

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