Modern Environmental Science and Engineering (ISSN 2333-2581)
November 2015, Volume 1, No. 5, pp. 225-235
Doi: 10.15341/mese(2333-2581)/05.01.2015/003
Academic Star Publishing Company, 2015
www.academicstar.us

Effective Removal of Nitrogen and Phosphorus from Saline Sewage by *Dunaliella tertiolecta* through Acclimated Cultivation

Kam-Chau Wu, Kin-Chung Ho and Yiu-Hung Yau

Centerfor Research in Envirnomental Science, The Open University of Hong Kong, Hong Kong, China

Abstract: Conventional activated sludge processes used for biological treatment of saline sewage usually have low removal of nutrient especially at high salt concentrations. In meanwhile, saline sewage provides an opportunity for applying microalgal based sewage treatment by marine algal species. *D. tertiolecta* isolated from algal blooming sample in local costal water was grown in non-sterile saline and nutrient rich sewage effluent for a bioremediation study. To promote better growth and nutrient removal of *D. tertiolecta* in saline preliminary effluent (PE), acclimated cultivation was also investigated in this study.

With high nutrient removal efficiencies (i.e., orthophosphate, nitrate-nitrogen and ammonia-nitrogen), overall removal percentage of acclimated *D. tertiolecta* in PE was above 65% after 8d. The performance was relatively good concerning orthophosphate, which reached 70%±13.5% after 6d and 80±1.3% after 8d. With respect to nitrogen sources, the removal of nitrate reached 60 ±5.4% after 6d and 74±0.1% after 8d. The performance of acclimated cultures was improved significantly in comparison to un-acclimated cultures. Overall, acclimated *D. tertiolecta* was successfully cultivated in unsterilized saline PE. While having a high potential for nutrient removal (particularly for orthophosphate and nitrate) from sewage, microalgal sewage treatment is also believed to a useful tool for biomass production.

Key words: algal biomass, acclimated cultivation, *Dunaliella tertiolecta*, nutrient removal, saline sewage

1. Introduction

Environmental pollution caused by urban sewage is attracting increasing attention, and emission of pollutants, such as nutrients (e.g., ammonia, nitrate and phosphorus), can cause eutrophication, which results in the deterioration of water quality and ecosystems and leads to serious imbalances [7, 11, 31, 34].

In recent decades, inflow of saline urban sewage to treatment plants has increased considerably which represent as much as 5% of worldwide sewage treatment works [13]. Due to the use of seawater for toilet flushing in Hong Kong, sewage treatment plant often process sewage with a high salt content [7, 31]. For example, Tai Po sewage treatment work is a secondary sewage treatment facility. It serves a 93,000 m^3 of sewage per day which treats mixed domestic and industrial sewage. Among these undertreating sewage, there are about 80% urban sewage with a higher salt content (mean: 1% w/v) [31, 34]. The influent often characterized with a considerable amount of nutrient such as ammonia (~20 mg/L), nitrate (~6-13 mg/L) and phosphorus (~4-14 mg/L) [31, 34].

However, conventional activated sludge processes used for biological treatment of saline sewage usually have low nutrient removal. Plasmolysis of bacteria in activated sludge was induced particularly at high salt concentrations (>1% w/v) [2, 4, 9]. The salt content within saline sewage reduces the metabolite rate of

Corresponding author: Yiu-Hung Yau, Ph.D., MEngSt, BEng in Chemical Engineering, MSc in Environmental Management, Assistant Professor, research areas/interests: innovative environmental technologies, energy management, wastewater treatment, cleaner production technologies. E-mail: yhyau@ouhk.edu.hk.

microorganisms in activated sludge and further reduces treatment efficiency. Intrasungkha et al. (1999) showed that better biological nutrient removal achieved when salinity levels in sewage were low (0.03% to 0.2% NaCl) but showed difficulties when comparing with salinity levels of 0.5% [8]. Uygur & Karg (2004) studied that rate and extent of chemical oxygen demand, ammonia and orthophosphate removal decreased with increasing salt content in biological treatment [28].

Therefore, for conventional activated sludge processes of saline sewage, capacity per unit volume is not high [9, 31, 34]. Due to the difficulties of nutrient removal saline wastewater treatment, research concerning sewage treatment technology is then necessary [11, 31, 34].

In contrast, saline sewage provides an opportunity for cultivation of marine algal species. Microalgae can remove nutrients by assimilating inorganic substances, such as ammonia, nitrate and orthophosphate. During cultivation of microalgae, this assimilation is achieved through microbial metabolism within microalgal cells during growth [7, 11, 31 and 34]. Utilization of microalgae for sewage treatment can then serve as associate treatment unit for deep removal of nitrogen and phosphorus during saline sewage treatment [19]. After construction of a microalgal cultivation unit within sewage treatment process, effluent with low levels of nitrogen and phosphorus content can be achieved. Furthermore, microalgal biomass can also be produced during the sewage treatment [10, 21]. The microalgal biomass were serve as valuable resources for a wide range of commercial, environmental, pharmaceutical, food and fuel applications [1, 7, 10, 19, 21]. In order to reduce production cost, an economical algal cultivation method should be developed. It is then feasible to couple nutrient removal from sewage to algal biomass production.

However, compositions are very variable in sewage which was a complex mixture. For example, unbalanced nutrient level, presence of toxin, and competitors may have negative impact on the microalgal growth and nutrient removal efficiency. Acclimate cultivation in accordance with the local environment can facilitate the microalga growth of native species [29]. Normally, laboratories grow microalgal cultures with same medium for long term cultivation. In this case, no acclimation is needed because even the different generation the composition of the medium is similar [26]. As microalgae are dependent on essential nutrients for optimal growth and development, it may be hypothesized that microalgal species with different background are needed acclimated or adapted in a new medium, i.e., from commercial medium to sewage [11, 18]. The acclimated cells were found physiologically more active and took up more nutrients from the wastewater for their growth and metabolisms, resulting in were much higher than those obtained in the unacclimated system with similar algal density [11]. In Lau et al. (1996), *Chlorella vulgaris* was acclimated in wastewater for a period of 14 days before employed in treating primary settled wastewater [11]. Acclimation of *C. pyrenoidosa* is necessary for the application in Vitamin B2 wastewater treatment in Sun et al. (2013) [24]. The progressive acclimation method was adapted to enhance *Spirulina platensis* tolerance to swine farm raw wastewater by artificial selection [30].

Acclimation proved to be a valuable means to improve efficiency of wastewater nutrient removal by the microalgal species which were not indigenous in sewage. To promote better growth and nutrient removal of *D. tertiolecta* in local saline sewage, acclimated cultivation was then applied in this study.

D. tertiolecta is a marine algal species that exhibited a high growth rate and good adaptability to changing environments when cultivated in laboratory algal cultural medium in a previous study [31]. Moreover, biomass of *D. tertiolecta* was valuable for energy production [1, 31]. There is potential for developing cost-effective biomass production (i.e., sewage-based medium).

However, to our knowledge, adaptability and growth performance of this species in local saline sewage has not been examined. Different effluents from local saline sewage treatment plants are screened to find out the suitable one for microalgal growth in this study. To enhance the efficiency (e.g., shorten the cultivation time), acclimated cultivation was evaluated whether could enhance growth and nutrient removal of this species. The biomass production associated with nutrient removal during sewage-based cultivation is also worth to investigate.

2. Materials and Methods

2.1 Wastewater Collection

The types of saline sewage examined in this study were preliminary effluent (PE), primary settled effluent (PS), and secondary effluent (SE) without any pre-treatment. All of the effluents were collected from Tai Po Sewage Treatment Work (STW). The samples were stored in 10 L buckets at 4°C. The physicochemical characteristics of the wastewater, including the parameters pH, salinity, ammonia (NH_3-N), nitrate (NO_3-N), and orthophosphate (PO_4-P), were analysed. These effluents were used as a culture medium to screen for the most appropriate medium for microalgal cells. The physicochemical parameters of the sewage are shown in Table 1.

Table 1 Physicochemical Characteristics of Different Effluents and L1 Medium

	L1 medium	Preliminary effluent	Primary effluent	Secondary effluent
pH	6.83±0.02	5.81±0.12	6.38±0.1	6.72±0.1
Salinity (psu)	30±0.2	8±0.63	8±0.01	8±0.01
Dissolved oxygen (ppm)	8.64±0.1	3.9±0.88	0.31±0.1	8.3±0.6
Ortho-phosphate (mg/L)	4.61±0.1	14.7±0.1	13.4±0.2	4.7±0.14
Ammonia-Nitrogen (mg/L)	0±0.03	20±0.1	15±0.1	0.12±0.14
Nitrate-nitrogen (mg/L)	52.5 ±0.2	13±0.2	13±0.1	1±0.7
Chemical oxygen demand (mg/L)	0±0.01	276±2	643±1	15.6±2.8

2.2 Algal Culture and Growth Conditions

The algal strain used in present study was isolated from algal bloom samples from local coastal water. *D. tertiolecta* is a genus of algal family Dunaliellaceae. The strain was a motile, unicellular, rod- to ovoid-shaped (8-12 μm) (Chlorophyceae), which is commonly found in local marine waters. The stock culture was grown in L1 seawater medium (L1) [5] at 22±1°C, with a 12:12 light-dark cycle and a light intensity of 120 μE m-1s-1, which was provided by cool white fluorescent tubes in a growth chamber. Possible contamination of the algal culture was monitored via regular microscopic examination. The cultures were kept in the exponential growth phase by transferring the cells to new medium every week at a ratio of 1:10 v/v. Vegetative cells from cultures in the exponential growth phase were inoculated into L1 medium or freshly non-sterilized sewage based medium.

2.3 Cell Counting and Calculation of Specific Growth Rates

Cell density was measured at the same time every two days. Cells were counted under a light microscope using a Sedgwick-Rafter cell counter. The whole experiment was repeated three times, with triplicates in each run. Each data point on the curve represents the mean of triplicate results. The specific growth rate (μ) for the exponential growth phase was calculated using the following equation:

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

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

2.4 Initial Screening

A rapid preliminary survival test was performed to explore the adaptation of selected microalgal strains to each effluent. The selected microalgal strains were inoculated into different effluent media (i.e., PE, PS, and SE) (10 mL) at a ratio of 1:10 (v/v) for 7 d.

Control and reference cultures were generated by inoculating the cells into natural seawater (NS) and L1 medium, respectively. In addition, the presence of original in situ *D. tertiolecta* cells was also examined by incubating sewage without inoculated cells. All experiments were performed in triplicate and incubated under the same conditions as described previously. Cell viability was examined under a 100× optical microscope. Samples with more than 5.0E+05 live cells/mL were scored as survive.

2.5 Growth Trends of D. tertiolecta Among Different Effluents

To further examine which effluents were suitable for *D. tertiolecta* growth, microalgal growth in the effluents was determined. The types of sewage used were primarily the PE and the SE. NS and L1 were used as controls. *D. tertiolecta* was inoculated into flasks (250 mL) with different media, as described above. The initial cell density of the selected strain was set at 5.0E+03 cells/mL for all investigated media.

2.6 Acclimated Cultivation

In this study, the inoculums were acclimated by growing the cells in the chosen sewage based medium for five generations, and the experimental cultures were started with a low inoculum, close to 5.0E+03 cells/mL. After 10 days, when the cultures had reached the end of exponential growth but were not yet in the stationary phase, all cultures were harvested and the cell yields were evaluated. In addition, to determine whether the cells used as inoculum had been sufficiently acclimated, the process was repeated for three cycles.

2.7 Sample Preparation

Aliquots of the cultures were collected to assess the extent of microalgal-mediated nutrient removal during growth. Fifteen-milliliter samples were filtered through a 1 μm GF/F filters and frozen until the time of analysis. The filtrates were then appropriately diluted and analyzed.

2.8 Wastewater Analysis

The physicochemical characteristics of the wastewater, including pH, salinity, NH_3-N, NO_3-N, PO_4-P, and chemical oxygen demand (COD), were analysed. Salinity was checked with Atago Master-S/Mill M series refractometer. The residual free nitrate, ammonium and ortho-phosphate concentrations in the culture were determined using commercially available, spectrometry-based assay systems according to the manufacturer's instructions. The assays were performed using a DR 2800 portable spectrophotometer (Hach Co., Loveland, CO, USA) coupled with a DRB200 dual block reactor (Hach Co., Loveland, CO, USA).

Nutrient removal efficiencies were obtained according to Eq.:

$$R_i = (S_{i0}-S_{it})/S_{i0} \times 100\%$$

where: R_i represents removal efficiency of substrate i (nitrate, ammonia, or orthophosphate); S_{i0} and Sit are defined as the mean values of substrate;

i concentration at initial time t_0 and time t_i, respectively.

2.9 Statistical Analysis

Statistical analysis of data was done by following the methods suggested in SPSS 17.0 Guide to data analysis. All the experiments were carried out in triplicate and average values are reported. Results were analysed using software SPSS 17.0. ANOVA analysis and Tukey's post hoc analysis were used to determine the significance of difference wherever applicable.

3. Results and Discussion

3.1 Survival Test of D. tertiolecta in Different Effluents

Survival test was performed to explore the adaptation of *D. tertiolecta* to the effluents (Table 2) [7]. The presence of algal cells was observed in all effluents tested up to 7 d, except for the PS. The cell density was much higher in PE and L1 medium than in

SE and natural seawater. The cells appear to adapt better to PE without pretreatment. Because no live D. tertiolecta cells could be observed in the control group (i.e., effluent without inoculum) (data not shown), all of the *D. tertiolecta* cells observed in this study were believed to have arisen from inocula rather than from the effluents.

Domestic saline sewage provides most of nutrients required by microalgae (Table 1). Either PS or PE show considerable amount of inorganic nitrogen and phosphorus which are up to three orders of magnitude level then L1 medium. Moreover, the COD measurement showed abundant organic substance (including organic nitrogen and phosphorus) such as various trace elements, which providing additional nutrient sources for microalgal growth [6]. Therefore, the effluents such as PE have high potential for supporting microalgal growth.

Although the nutrient level of primary effluent showed sufficient amount for microalgal growth (Table 1), there no live cell after incubation. The co-existing micro-organisms in the primary effluent would possibly lead to considerable effects on the growth of *D. tertiolecta*. Each of them competes with the others to gain access to the resources. Some of them may be aggressive grazer which probably led to no live cell within PS [7]. On the other hand, the relatively low dissolved oxygen concentration of PS (Table 1) may provide insufficient oxygen for photo-respiration which also affecting the microalgal growth.

Table 2 Survival of *D. tertiolecta* in Effluent from Tai Po STWs

Growth medium	*D.Tertiolecta* inoculation	Result		
		Sample 1	Sample 2	Sample 3
L1 medium	Inoculation in 1:10 (V:V) from exponential phase	+++	+++	+++
Preliminary effluent		++	++	++
Primary effluent		-	-	-
Secondary effluent		+	+	+
Natural seawater		+	+	+

According to the results, PE and SE were then selected for further experiments. These experiments were conducted to determine the most suitable effluent for the growth of *D. tertiolecta* cells.

3.2 In-depth Screening of Different Effluents

The further experiment performed in-depth screening for a suitable sewage-based medium for *D. tertiolecta*. Fig. 1 shows microalgal growth conditions in the two effluents (i.e., PE and SE) and control groups. In control group (i.e., L1 medium), no lag phase was observed, and exponential phase occurred on days 0-6, followed by stationary phase. In natural seawater (i.e., the negative control), no lag phase was observed, and exponential phase lasted for only 4 days. The cell density increased from days 0 to 4, although no nutrients were added to the natural seawater (NS). This growth might occur due to the intracellular storage of nutrients in *D. tertiolecta* cells or due to the presence of trace nutrients within seawater itself [14, 22]. These nutrients could support the initial growth of *D. tertiolecta* on days 0-4. After depleting the available nutrients, the growth rate of *D. tertiolecta* dropped, and the culture entered stationary phase.

In treatment groups (i.e., PE and SE), no observable lag phase occurred. The cultures only exhibited exponential phase (days 2-12) and stationary phase (after day 12) in the PE. In the SE, exponential phase occurred on days 0-4, but the cell number dropped after day 4, indicating that the algae could not maintain growth after 4 days. The reason for this lack of growth may be similar to the reason discussed above for the negative control; SE cannot provide sufficient nutrients (Table 1) and supporting factors for microalgal growth.

The maximum growth rates and cell numbers of *D. tertiolecta* in PE were close to those observed in L1 medium. The PE can supply enough nutrients, such as nitrogen and phosphorous, for autotrophic growth (Table 1). However, the growth conditions of the PE were still undesirable, as indicated by the observed instability in comparison to L1 medium (days 4-8). The

maximum cell capacity (MCD) was only 1.1E+06 cells/mL, while in L1 medium, the value was 3.2E+06 cells/mL. Many factors may affect microalgal growth in sewage. Since microalgal growth also depends on the types and consortium of other microorganisms, the nutrient balance and the amounts of nutrients within the sewage-based medium [11].

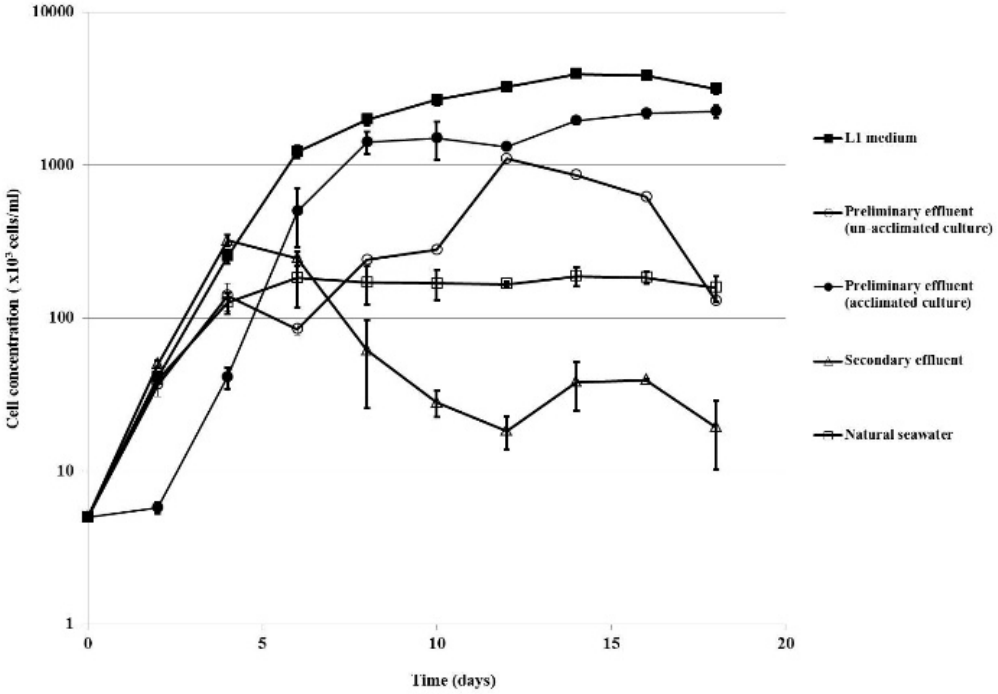

Fig. 1 Growth curves of *D. tertiolecta* cultivated in the effluents from the Tai Po STW (preliminary effluent with un-acclimated and acclimated cultures), in L1 medium and in natural seawater.

3.3 Growth of Acclimated and Un-acclimated Cultures

The growth patterns and growth rates of *D. tertiolecta* (un-acclimated and acclimated cells in PE and cells in L1 medium) are shown in Figs. 1 and 2. Since acclimated cells were cultivated in PE before the experiment, they could adapt well to the environment of PE. According to Fig. 2, daily growth rate of the acclimated cells peaked from days 1-8 (growth rate = 0.92) and was steady from day 8 until day 18. This growth pattern was similar to the growth pattern observed in L1 medium. The daily growth rate of un-acclimated cells peaked on day 12 (daily growth rate = 0.45) and then rapidly decreased from day 12 to day 14. The exponential phase was longer for un-acclimated cells than for acclimated cells, which stayed in this phase for 7 days. Moreover, the growth rate of the acclimated culture was not significantly different from that of cells grown in L1 medium, as indicated by the statistical analysis. This finding indicates that the acclimated culture can reach maximum capacity in a relatively short time and behaves more similarly to the culture grown in L1 medium than the un-acclimated culture. The maximum cell density (MCD) (2.2 E+06 cells/mL) of the acclimated culture was also higher than that of un-acclimated cells, for which this value was only 1.0 E+06 cells/mL.

The adaptation of microalgal cells to a unique sewage environment occurs through a process known as physiological acclimation [11]. However, acclimated cells did not represent a stressful condition when cultivating in sewage environment. The cells

need not develop a specific transport system for uptake essential nutrients [20]. Use of PE effluent has potential as a cost-effective approach for the mass cultivation of *D. tertiolecta* cells after acclimated cultivation when comparing to L1 medium.

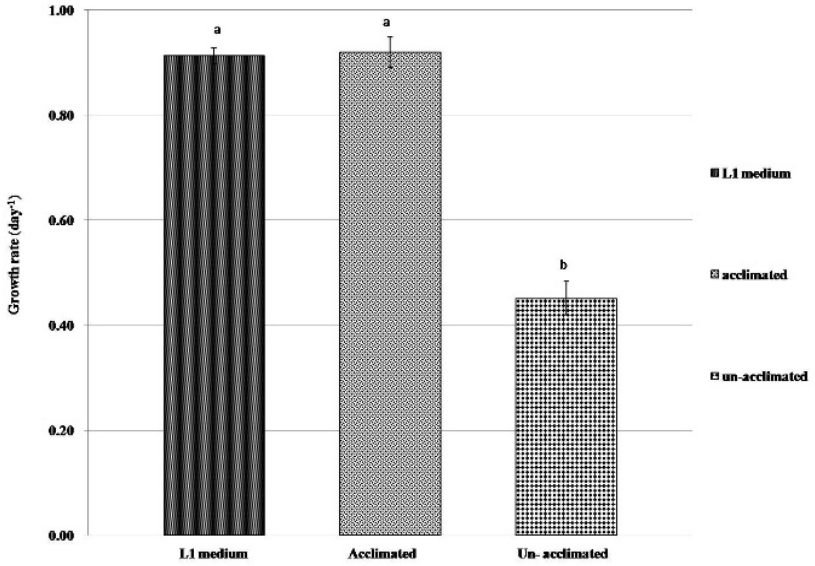

Fig. 2 Comparison of *D. Tertiolecta* growth rate and biomass productivity in un-acclimated cultures, acclimated cultures and L1 medium. Different letters indicate significant differences ($p < 0.05$) between the treatment groups and the L1 medium control.

3.4 Nutrient Removal by Acclimated Cultures

The results concerning nutrient removal by acclimated cells are shown in Figs. 3-5. As described above, PE can supply enough nutrients, such as nitrogen and phosphorous, for autotrophic growth.

Most of the major contaminants were removed to a significant extent after incubation for 15 days. During the first five days, approximately 70% of phosphate could be removed by acclimated microalgae. At the end of experiment, more than 90% of the phosphate was removed. Figs. 4 and 5 also show the removal of N sources (i.e., ammonia and nitrate). Acclimated cells could remove 70% of the nitrate and 65.0% of the ammonia (NH_3-N) during the first 8 days. The efficiency of nitrate removal was better than that of ammonia. Syrett [25] reported that microalgae preferred nitrogen species in the following order: ammonia > nitrate > simple organic-N, such as urea and simple amino acids. However, the same result was not obtained in this study. Some studies claimed that inorganic nitrogen in the form of nitrate (NO_3) rather than ammonium (NH_4^+) was required for maximal biomass production. This requirement may due to the specific regulation of uptake mechanisms in D. tertiolecta [1, 15, 33].

Based on the results shown in Figs. 3-5, the nutrient removal rate (i.e., ammonia and orthophosphate) of acclimated cells was significantly higher than that of un-acclimated cells. No significant differences in nitrate removal were observed. Thus, the acclimated culture exhibited a relatively better performance in terms of nutrient removal than the un-acclimated culture. Lau et al. claimed that acclimation could improve the efficiency of wastewater nutrient removal by microalgal species [11]. However, in many cases, the specific physiological mechanisms underlying the acclimation process are unknown [18, 24].

Nutrient removal efficiencies in domestic preliminary effluent by *D. tertiolecta* and other microalgal species at the stationary growth are compared in Table 3. As mentioned above, *D.*

tertiolecta removes phosphorus rapidly within 8 d of culture with a removal efficiency of 80%, even higher than final removal efficiency achieved by some other microalgal species on PS effluent, such as *C. protothecoides* and *C. minutissima*. Nevertheless, Lee et al. (2015) also apply local unsterile saline sewage which was secondary effluent. They reported that *Chlorogonium sp.* show better nutrient removal than this study within shorten cultivation days (6d). Franchino et al. (2013) also reported removal rates of phosphorus by *C. vulgaris*, *N. oleoabundans* and *S. obliquus* in autotrophic conditions were about 96%, which were higher compared to rates observed in this study [3]. However, the actual removal amounts of phosphorus were only 0.28 mg-P per day in Lee study when 0.28-0.36 mg-P per day in Franchino's study respectively, which lower values compared to those observed in this study (1.47 mg-P per day).

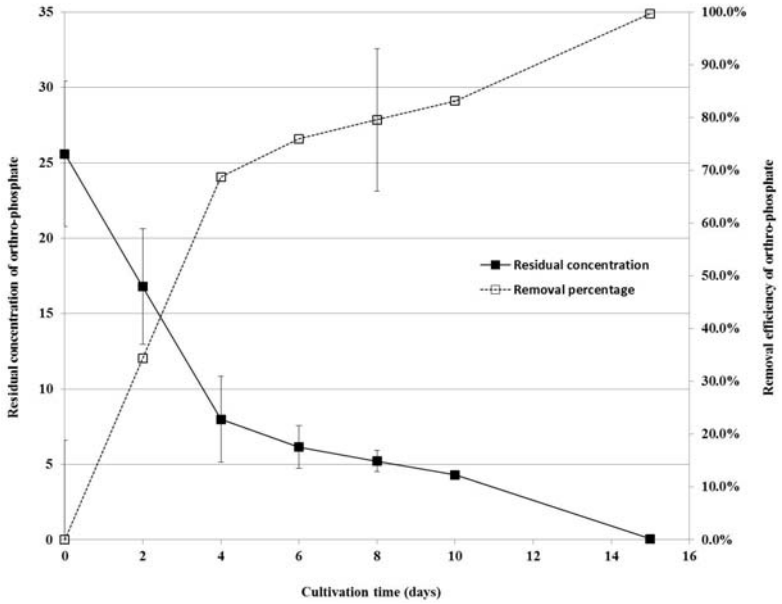

Fig. 3 Changes in the ortho-phosphate concentration and removal efficiency of *D. Tertiolecta* cultivated using acclimated cultivation. The error bars represent the standard deviation of triplicate (n = 3) experiments.

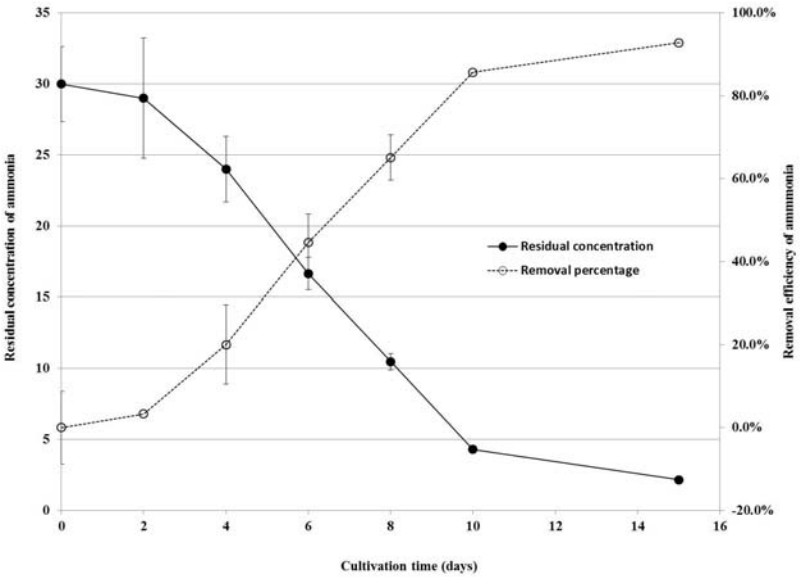

Fig. 4 Changes in the ammonia concentration and removal efficiency of *D. tertiolecta* cultivated using acclimated cultivation. The error bars represent the standard deviation of triplicate (n = 3) experiments.

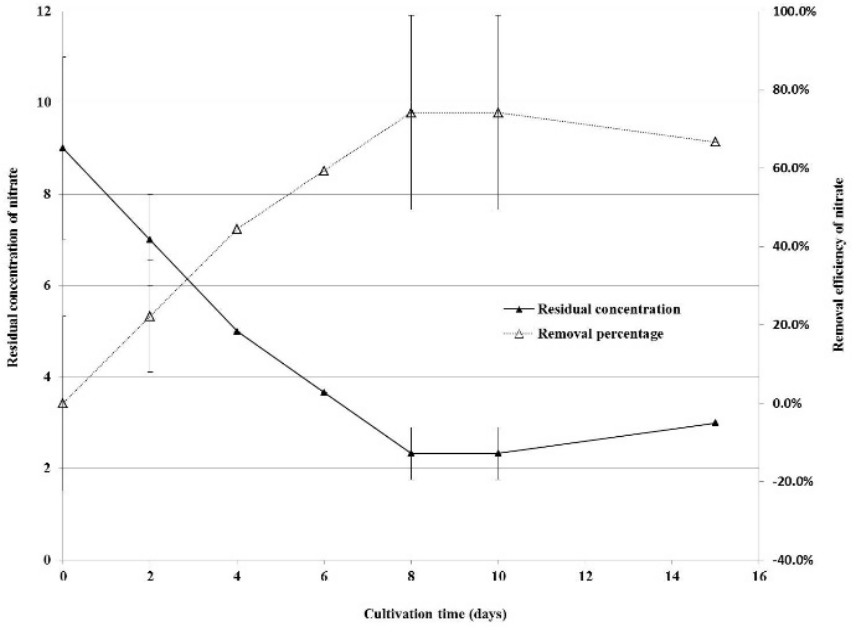

Fig. 5 Changes in the nitrate concentration and removal efficiency of *D. Tertiolecta* cultivated using acclimated cultivation. The error bars represent the standard deviation of triplicate (n = 3) experiments.

Table 3 Microalgal Strains Used for Nutrient Removal

Species	Type of sewage	NO_3^- removal (%)	NH_4^+ removal (%)	PO_4^{3-} removal (%)	Cultivation days	Ref
C. minutissima	PS from STW Tertiary effluentfrom STW	65.4 41	>99 59.9	44.8 85.7	12	15
C. protothecoides	Unsterilized PS from STW	96.3	97	70	5	27
Chlorella sp.	Influent before PS from STW	/	82.4	83.2	9	29
C. vulgaris *Neochloris oleoabundans,* *S. obliquus*	Agro-zootechnical digestate (1:10)	11% -ve -ve	99.0 99.0 83.7	96.0 96.9 96.1	11	3
S. obilquus	Sterilized SEfrom STW	/	99	98	8	16
Chlorogonium sp.	Unsterilized saline SE from STW	85.39	88.35	91.8	6	12
D. tertiolecta	Unsterilized saline PE from STW	74.2	65	80	8	present study

The initial concentration of nitrate and ammonia was similar among the comparison ranging from 7.0-9.3 mg/L of nitrate and 20.4-27.4 mg/L of ammonia. For the comparison on nitrogen removal such as nitrate and ammonia, *D. tertiolecta* show better removal in nitrate but less removal in ammonia. As discussed above, *D. tertiolecta* tend to assimilate nitrate as nitrogen source. This leads to higher removal than other microalgal species, for example, *B. braunii*, *C. minutissima*, *C. vulgaris*, *N. oleoabundans* and *S. obliquus*. Parts of these species even show negative value (*N. oleoabundans* and *S. obliquus*). Nevertheless, this is because most of the microalgal species prefer ammonia as nitrogen source under not too high level [10, 11, 15, 21]. Moreover, nitrate removal efficiency in terms of percentage of Lee et al. (2015) show higher value than the present study. The actual amount was 1 mg-NO_3 per day while 1.2 mg-NO_3 per day in present study. In ammonia removal, *D. tertiolecta* show a relatively insufficient removal (65%) when comparing with the others which show less removal on nitrate. The actual amount was 1.625 mg-NH_4^+ per day when the other

such as *C. vulgaris*, *C. protothecoides*, *N. oleoabundans* and *S. obliquus* showed 6.9-8.6 mg-NH_4^+ per day.

These results indicate that *D. tertiolecta* is a promising microalgal species for efficient phosphorus and nitrogen especially nitrate removal from sewage under acclimated cultivation.

4. Conclusion

This study demonstrated that *D. tertiolecta* grows relatively well in PE from local sewage treatment plant. The results indicated that microalgal biomass can be produced from cultivating in raw PE. Furthermore, acclimation of *D. tertiolecta* in PE during cultivation proved to be a valuable means of improving microalgal growth that were not indigenous in the sewage.

Moreover, acclimated *D. tertiolecta* has a high potential for nutrient removal from raw PE, particularly for removal of ortho-phosphate and nitrate. This organism can improve sewage purification efficiency during acclimated cultivation. Further research concerning the utilization of *D. tertiolecta* for more efficient sewage treatment is needed. The potential use of this organism for biomass production should also be explored.

Acknowledgement

The first author would like to acknowledge Dr. Y. H Yau and others who provide advices and technical support to his research during the postgraduate studies. The authors also thank the anonymous referees for reviewing the manuscript and the comments suggested.

References

[1] M. Chen, H. Tang, H. Ma, T. C. Holland, K. Y. Ng and S. O. Salley, Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta, *Bioresour. Technol.* 102 (2011) (2) 1649-1655.

[2] C. Cortés-Lorenzo, M. Rodríguez-Díaz, C. López-Lopez, M. Sánchez-Peinado, B. Rode-las and J. González-López, Effect of salinity on enzymatic activities in a submerged fixed bed biofilm reactor for municipal sewage treatment, *Bioresour. Technol.* 121 (2012) 312-319.

[3] M. Franchino, E. Comino, F. Bona and V. A. Riggio, Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate, *Chemosphere* 92 (2013) (6) 738-744.

[4] S. P. Gregory, R. J. Shields, D. J. Fletcher, P. Gatland and P. J. Dyson, Bacterial community responses to increasing ammonia concentrations in model recirculating vertical flow saline biofilters, *Ecol. Eng.* 36 (2010) 1485-1491.

[5] R. R. L. Guillard and P. E. Hargraves, Stichochrysis immobilis is a diatom, not a chrysophyte, *Phycologia* 32 (1993) (3) 234-236.

[6] P. J. Harrison and J. A. Berges, *Marine Culture Media: Algal Culturing Techniques*, 2005, pp. 21-34.

[7] K. C. Ho, S. J. L. Xu, K. C. Wu and F. W. F. Lee, Effective growth of dinoflagellate prorocentrum minimum by cultivating the cells using municipal wastewater as nutrient source, *Water. Sci. Technol.* 68 (2013) (5) 1100-1106.

[8] N. Intrasungkha, J. Keller and L. L. Blackall, Biological nutrient removal efficiency in treatment of saline wastewater, *Water Sci Technol.* 39 (1999) 183-190.

[9] D. F. Kincannon and A. F. Gaudy, Response of biological waste treatment systems to changes in salt concentrations, *Biotechnol. Bioeng.* 10 (1968) (4) 483-496.

[10] Q. X. Kong, L. Li, B. Martinez, P. Chen and R. Ruan, Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production, *Appl. Biochem. Biotechnol.* 160 (2010) (1) 9-18.

[11] P. S. Lau, N. F. Y. Tam and Y. S. Wong, Wastewater nutrients removal by Chlorella vulgaris: Optimization through acclimation, *Environ. Sci. Technol.* 17 (1996) (2) 183-189.

[12] K. Y. Lee, T. W. Ng, G. Li, T. An, K. K. Kwan, K. M. Chan and P. K. Wong, Simultaneous nutrient removal, optimized CO_2 mitigation and biofuel feedstock production by Chlorogonium sp. grown in secondary treated non-sterile saline sewage effluent, *J. Hazard. Mater.* 297 (2015) 241-250.

[13] O. Lefebvre, S. Quentin, M. Torrijos, J. J. Godon, J. P. Delgenes and R. Moletta, Impact of increasing NaCl concentrations on the performance and community composition of two anaerobic reactors, *Appl. Microbiol. Biotechnol.* 75 (2007) 61-69.

[14] Y. Li, D. Han, G. Hu, M. Sommerfeld and Q. Hu, Inhibition of starch synthesis results in overproduction of lipids in Chlamydomonas reinhardtii, *Biotechnol. Bioeng.* 107 (2010) (2) 258-268.

[15] F. A. Malla, S. A. Khan, G. K. Sharma, N. Gupta and G. Abraham, Phycoremediation potential of Chlorella minutissima on primary and tertiary treated wastewater for nutrient removal and biodiesel production, *Eco. Eng.* 75 (2015) 343-349.

[16] M. E. Martinez, S. Sánchez, J. M. Jiménez, F. Yousfi and L. Muñoz, Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus, *Bioresour. Technol.* 73 (2000) 263-272.

[17] C. J. Molloy and P. J. Syrett, Interrelationships between uptake of urea and uptake of ammonium by microalgae, *J. Exp. Mar. Biol. Eco.* 118 (1988) (2) 85-95.

[18] O. Osundeko, A. P. Dean, H. Davies and J. K. Pittman, Acclimation of microalgae to wastewater environments involves increased oxidative stress tolerance activity, *Plant Cell Physiol.* 55 (2014) (10) 1848-1857.

[19] J. B. K. Park, R. J. Craggs and A. N. Shilton, Wastewater treatment high rate algal ponds for biofuel production, *Bioresour. Technol.* 102 (2011) (1) 35-42.

[20] O. Perez-Garcia, F. M. Escalante, L. E. De-Bashan and Y. Bashan, Heterotrophic cultures of microalgae: Metabolism and potential products, *Water. Res.* 45 (2011) (1) 11-36.

[21] I. Rawat, R. R. Kumar, T. Mutanda and F. Bux, Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, *Appl. Energ.* 88 (2011) (10) 3411-3424.

[22] L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini and M. R. Tredici, Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, *Biotechnol. Bioeng.* 102 (2009) (1) 100-112.

[23] E. B. Sydney, T. E. Da Silva., A. Tokarski, A. C. Novak, J. C. De Carvalho, A. L. Woiciecohwski and C. R. Soccol, Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage, *Appl. Energ.* 88 (2011) (10) 3291-3294.

[24] X. Sun, C. Wang, Z. Li, W. Wang, Y. Tong and J. Wei, Microalgal cultivation in wastewater from the fermentation effluent in Riboflavin (B2) manufacturing for biodiesel production, *Bioresour. Technol.* 143 (2013) 499-504.

[25] P. J. Syrett, Nitrogen metabolism of microalgae, *Can. J Fish Aquat Sci.* 210 (1981) 182-210.

[26] D. Voltolina, M. Nieves, G. Navarro, T. Oliva and D. Peraza, The importance of acclimation for the evaluation of alternative media for microalgae growth, *Aquacult Eng.* 19 (1998) (1) 7-15.

[27] E. R. Tercero, E. Sforza, M. Morandini and A. Bertucco, Cultivation of Chlorella protothecoides with urban wastewater in continuous photobioreactor: Biomass productivity and nutrient removal, *Appl. Biochem. Biotechnol.* 172 (2014) (3) 1470-1485.

[28] A. Uygur and F. Karg, Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor, *Enzyme. Microb. Technol.* 34 (2004) 313-318.

[29] L. Wang, M. Min, Y. Li, P. Chen, Y. Chen and Y. Liu, Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant, *Appl. Biochem. Biotechnol.*162 (2010) 1174-1186.

[30] T. Y. Wang, H. C. Liu and Y. Lee, Use of anthropic acclimated spirulina platensis (arthrospira platensis) bio-adsorption in the treatment of swine farm wastewater, *Int J Agric Biol.* 15 (2013) 107-112.

[31] K. C. Wu, K. C. Ho, F. W. F. Lee, J. L. S. Xu and Y. H. Yau, The exploration of marine microalgae as an environmental friendly and sustainable source for renewable energy production, in: *Proceedings of New & Renewable Energy Development – Response to Global Climate Change cum Cross-Strait Scientists Forum on Energy Efficiency and Management*, Hong Kong, 2011.

[32] E. B. Young and J. Beardall, Photosynthetic function in Dunaliella tertiolecta (Chlorophyta) during a nitrogen starvation and recovery cycle, *J Phycol.* 39 (2003) (5) 897-905.

[33] S. Yu, W. Leung, K. Ho, P. Greenfield and W. Eckenfelder, The impact of sea water flushing on biological nitrification-denitrification activated sludge sewage treatment process, *Water Sci. Technol.* 46 (2002) (11-12) 209-216.