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Effect of aeration flow rate on the growth of microalgae as a biofuel feedstock and wastewater treatment

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Abstract

The objectives of this study is to evaluate the effect of aeration inlet gas flow rate on the growth of microalgae as a sustainable biofuel feedstock and also the use of these cultured microalgae as a means of biological nutrient removal medium in wastewater. The final biomass yield of trial II (4.5 L/Min aeration flow rate) culture media with a value of 0.605 g/L was higher than trial I (9.0 L/Min aeration flow rate) and III (without aeration) with values of 0.418 g/L and 0.207 g/L respectively. The final Chlorophyll α content of the microalgae cultivated in trial II was higher (2.450 µg/mL) than trial I and III (0.906 µg/mL and 0.903 µg/mL) respectively. The concentration of total nitrogen and phosphorus from the fine screen chamber wastewater of Nicosia wastewater plant was reduced from 105.909 mg N/L to 1.847 mg N/L and 6.441 mg P/L to 0.805 mg P/L respectively. In conclusion, aeration culture media with 4.5 L/Min showed better growth outcome and which is therefore suitable for biofuel production and also microalgae could be used as a secondary treatment for wastewater containing high nutrient.

Keywords: Aeration, microalgae, wastewater, pH.

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1. Introduction

Microalgae growth is majorly controlled by turbulence and when it is supplied by means of air bubbling, mass transfer phenomena occurs between the culture medium and the air bubbles (Rodriguez-Maroto et al., 2005). Air bubbling is the most usual procedure to agitate cultures of micro and macroalgae, providing convenient mixing of the organisms and renewal of nutrients (e.g. carbon, nitrogen, and phosphate) around the surface of the cells. It is widely acknowledged that air bubbling provides CO_2 (inorganic carbon) to the cultures, in spite of its low concentration (300–400 ppm) in the atmosphere.

In recent years, the study of different aspects related to the behavior of microalgae has received renewed interest due to the wide field of application of these microorganisms (Valdes et al., 2012). Microalgae cultures have been basically developed as an important source of many products, such as animal, fish feed and feed ingredients (Abdel-Raouf et al., 2012), cosmetics (Wang et al., 2015), and antioxidant peptides production (Lauritano et al., 2016), and they have also been suggested as a very good candidate for fuel production (Show et al., 2017). For example, several papers have been published showing the possibility to obtain biodiesel fuel from the lipids accumulated in the microalgae cells (Tighiri & Erkurt, 2016; Pruvost et al., 2011; Mata et al., 2010).

Treated wastewater is high in nutrients such as phosphorus and nitrogen which if released to water channels can breed unwanted algae blooms and lead to eutrophication (Aslan et al., 2006). These nutrients can instead be used up by microalgae, which in return provide co-benefits of producing biofuels and other co-products (Mostafa et al., 2009). The objectives of this study is to evaluate the effect of aeration inlet gas flow rate on the growth of microalgae as a sustainable biofuel feedstock and also the use of these cultured microalgae as a means of biological nutrient removal medium in wastewater.

2. Methodology

2.1. Microalgae sampling and inoculation

The microalgae used for this study were collected from the natural lagoon of the old Nicosia wastewater treatment plant and they were inoculated at 5% ($V_{inoculation}/V_{BG\ 11\ media}$) in BG 11 (growth enrichment) culture medium. They were placed under Esco Class II Biosafety Cabinet photobioreactor in the laboratory, supplied with air blower (ADA AIR PUMP®, AP-2800) under continuous illumination of white fluorescent light of 45-50µmol photon $m^{-2}s^{-1}$ for two weeks before they were used for the wastewater treatment experiment. The microalgae growth parameters (such as optical density (OD), cell number and chlorophyll-*a* (Ch-*a*) and pH were analyzed. The BG 11 (growth enrichment) medium contained these following chemical components;

Macro nutrients (g/L)

$NaNO_3$	1.59
KH_2PO_4	0.40
$MgSO_4.7H_2O$	0.08
Na_2CO_3	0.02
$Ca(NO_3)_2.4H_2O$	0.02
EDTA	0.001
Citric acid	0.006
$FeCl_3$	0.002
Micronutrients (g/L)	
H_3BO_4	1.43
$MnSO_4.4H_2O$	0.91

$ZnSO_4.7H_2O$	0.11
$CuSO_4.5H_2O$	0.04
$Co(NO_3)_2.6H_2O$	0.03

Three 2L of BG 11 medium were produced and sterilized using autoclave at 1.5 MPa and 121°C for 15 mins (Tighiri & Erkurt, 2016).

2.2. Wastewater sampling and pre-treatment

The wastewater that was used in this study was collected from the New Nicosia Membrane Bioreactor (MBR) Wastewater Treatment Plant (WWTP) units in Nicosia, Turkish Republic of North Cyprus. The membrane bioreactor treatment plant system consists of primary treatment where the solid part of the wastewater is screened properly before feeding the water to the membrane treatment which consists of the biological phosphorus removal unit, aerobic unit, anaerobic unit, membrane filtraton unit and finally the disinfection unit (Tighiri and Erkurt, 2016). The wastewater sample was collected from Fine Screen Effluent (FSE) unit, and was filtered using 0.2 µm nylon microfilters to remove fine suspended particles.

2.3. *Experimental Setup*

The experiment was designed into two phases

Phase I (Microalgae culture under different aeration flow rate):

The culture of microalgae in BG 11 (growth enrichment) medium under different aeration flow rate was optimized in this phase in different culture conditions.

- I: culture medium with aeration of 9 L/Min inlet gas flow rate
- II: culture medium with aeration of 4.5 L/min inlet gas flow rate
- III: culture medium without aeration

Phase II (Nutrient Removal from Wastewater):

The microalgae suspension in the BG 11 (growth enrichment) media were adjusted to an absorbance of 1.5 at an optical density (OD) of 680nm as measured using a spectrophotometer (UV-2450, UV-VIS Spectrophotometer - Shimadzu) and were inoculated at 5% ($V_{inoculation}/V_{wastewater}$) into 1000mL of the wastewater treatment media in triplicate in Esco Class II Biosafety Cabinet photobioreactor in the laboratory and was supplied with aeration at 4.5 L/min inlet gas flow rate (ADA AIR PUMP®, AP-2800) under continuous illumination of white fluorescent light of 45-50 µmol photon $m^{-2}s^{-1}$. Batch treatment system was employed for this experiment and samples for analysis were collected once everyday for 5 days from the treatment media.

2.4. Algae growth and nutrient removal analysis

2.4.1. Growth evaluation

Growth of all trials was monitored for 14 days. Growth of the microalgae was evaluated based on these parameters; dry cell weight (DCW) Optical density (OD), chlorophyll-*a* (Chl-*a*) content, cell number and specific growth rate (µ).

Chlorophyll-a (Ch-a) content: Chlorophyll *a* was extracted and estimated using the procedure used by Chinnasamy et al. (2010). For this, 10mL of algae biomass was suspended in the medium and centrifuged at 6000 rpm for 30 mins. Biomass collected after centrifugation was again suspended in 5 mL of methanol. The methanol and algae biomass suspension was then immersed in the water bath for 60 mins at 60 ° C in order to extract the chlorophyll from the biomass. After the stipulated time,

the chlorophyll *a* concentration in the above suspension was spectrophotometrically determined using UV visible spectrophotometer (UV-2450, UV-VIS Spectrophotometer - Shimadzu). The absorbance value was then substituted in (1).

$$\text{Chl a}\left(\frac{\mu g}{mL}\right) = 16.29\left(A^{665.2} - A^{750}\right) - 8.54\left(A^{652} - A^{750}\right) \qquad (1)$$

Where A750, A665.2, A652 are referred to as the absorbance of algae biomass-chlorophyll suspension in methanol at 750, 665.2 and 652 nm, respectively.

Microscopic Cell Counting (cell $\times 10^4$/L): 1 mL of algae biomass was collected using micro-pipette and was dropped in the haemocytometer slide and was viewed under the microscopy for cell count. The OD_{680} was converted to Cell number (cell $\times$ 10^4/L), based on a linear relationship between the OD_{680} and Cell number, which was obtained after an extensive data analysis and is given by (2).

Cell number (cell $\times$ 10^4/L) = 18.412 $\times$ OD_{680} + 0.4977 (R^2 = 0.9812) (2)

Specific growth rate (μ - day^{-1}): was calculated by fitting the dry cell weight of microalgae for the 14 days of cultivation to an exponential function, as shown in (3) (Levasseur et al., 1993).

$$\mu = \frac{\ln(N_2/N_1)}{t_2 - t_1} \qquad (3)$$

Where, N_1 and N_2 are defined as the microscopic cell number at times t_1 and t_2, respectively.

2.4.2. Nutrient monitoring and removal analysis

Total Nitrogen (TN) Analysis: Ammonium (NH_4^+), Nitrite (NO_2^-) and Nitrate (NO_3^-) were monitored daily using Ion chromatography (IC- conductivity detector – Shimadzu (HIC-20A SUPER, Conductivity detector) at 1 mL/min loading flow rate for anions and cation. AS9-SC (4x50mm) column for anions and CS-12 (4 x 50mm) column for cation) and they were added up to give the total nitrogen, as the algae are growing until the final culture period to estimate the rate of nutrient removal from the wastewater.

Total Phosphorus (TP) analysis: Phosphorus was monitored daily spectrophotometrically using standard method (APHA-AWA-WEF, 2005). The OD_{880} were converted to Phosphorus (mg P/L), based on a linear relationship between the OD_{880} and Phosphorus (mg P/L) which was obtained after an extensive data analysis and is given by (4).

Phosphorus (mg P/L) = 1.847 $\times$ OD_{880} – 0.063 (R^2 = 0.98) (4)

Nutrient removal rate (N_{rx} – mgL^{-1}d^{-1}): this was calculated using (5).

$$N_{rx}(mgL^{-1}d^{-1}) = \frac{N_f - N_i}{t_f - t_i} \qquad (5)$$

Where, N_f and N_i are the final and initial nutrient concentration as TN or TP on t_f and *ti* respectively.

3. Result and Discussion

3.1. *Effect of aeration on microalgae growth*

Air bubbling is the most common procedure to agitate cultures of micro and macroalgae, which provides proper mixing of the organisms and nutrient renewal (e.g. carbon, nitrogen, and phosphate) around the cells surface. It is widely known that air bubbling supplies CO_2 (inorganic carbon) to algae cultures, despite its atmospheric low concentration of 300–400 ppm (Rodriguez-Mata et al., 2005).

The dry cell weight of the microalgae cultivated in various culture conditions were determined. In trial I (i.e. With aeration of 9 L/Min inlet gas flow rate), the algae biomass yield increased from day 0 and got to 0.418 g/L in day 10 but started decreasing from day 12 with a value of 0.308 g/L to 0.229 g/L in day 14 (figure 3.1) while that of trial II (i.e. With aeration of 4.5 L/Min inlet gas flow rate) increased from day 0 to day 14 with a value of 0.605 g/L and it also gave the best growth yield (figure 3.1). This result is in agreement with that of Choochote et al. (2012) whose result on effects of Urea and Light Intensity on the Growth of *Chlorella* sp. shows 18.37 g/L on day 5 of cultivation in the modified medium in a 5 L-fermenter at 100 rpm agitation and 3 L/Min aeration and also with that of Wu (2014) who reported a very good growth rate obtained at 33˚C, 300 µE/m2/s, and 0.5 L/min inlet gas with 10% CO_2. And that of trial III (i.e. without aeration) gave the least growth yield of 0.207 g/L (fig. 1). This result is in agreement with that of Rodriguez-Maroto et al. (2005), Persoone et al. (1980), and Ronda et al. (2012) who reported high yields in batch aeration culture condition.

The specific growth rates of the microalgae cultivated in the various aeration media were determined using the exponential phase (which was day 2 and 10 for 4.5 L/Min inlet gas flow rate and day 2 and 10 for 9.0 L/Min inlet gas flow rate and day 2 and 10 for media without aeration). The specific growth rate of microalgae for 4.5 L/Min inlet gas flow rate was 0.263 day^{-1}, for 9.0 L/Min inlet gas flow rate was 0.238 day^{-1} and for media without aeration was 0.186 day^{-1} (Table 2). From the result, a higher μ was obtained in 4.5 L/Min inlet gas flow rate which is in line with a previous report such as Vidyarathna et al. (2014) whose report showed higher specific growth rate of *Prymnesium parvum* at low aeration.

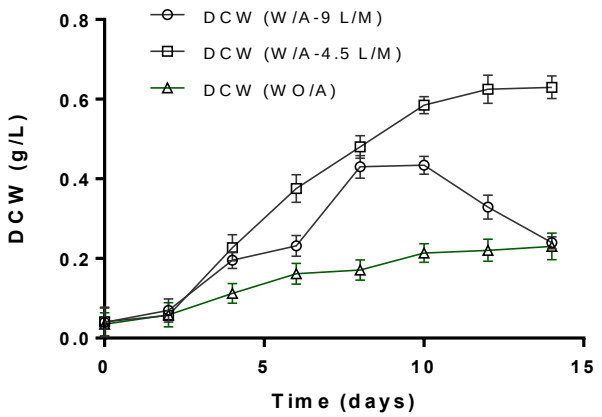

Figure 1. Dry cell weight (DCW) of algae with aeration at 9 L/Min inlet air flow rate (W/A - 9 L/M), algae with aeration at 4.5 L/Min inlet air flow rate (W/A - 4.5 L/M) and without aeration (WO/A)

Table 2. Specific Growth Rate (SGR) of dry cell weight of microalgae with different aeration system

Treatment media	SGR (μ - day^{-1})
Trial I (9 L/Min)	0.238
Trial II (4.5 L/min)	0.263
Trial III (W/A)	0.186

The Chlorophyll α content of the microalgae cultivated in the various culture conditions were determined. In trial I (i.e. With aeration of 9 L/Min inlet gas flow rate), the Chlorophyll α content increased from day 0 and got to 1.583 µg/mL in day 10 but started decreasing from day 12 with a value of 1.111 µg/mL to 0.906 µg/mL in day 14 (fig. 2) while that of trial II (i.e. With aeration of 4.5 L/Min inlet gas flow rate) increased from day 0 to day 14 with a value of 2.450 µg/mL and it also gave the best Chlorophyll α content (fig. 2) and that of trial III (i.e. without aeration) gave the least Chlorophyll α content of 0.903 µg/mL (fig. 2).

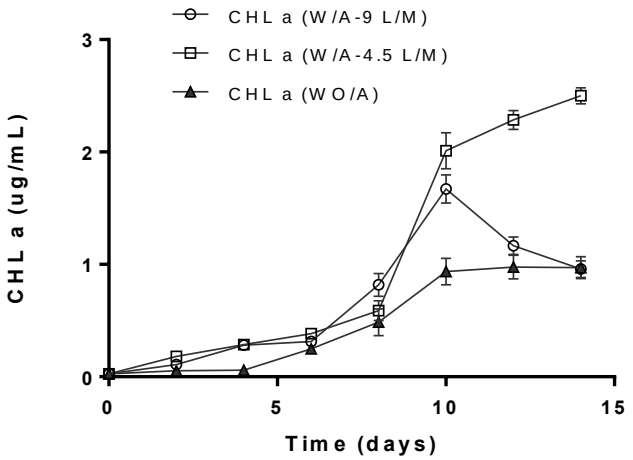

Figure 2. Chlorophyll α (µg/mL) of algae with aeration at 9 L/Min inlet air flow rate (W/A - 9 L/M), algae with aeration at 4.5 L/Min inlet air flow rate (W/A - 4.5 L/M) and without aeration (WO/A).

The cell numbers of the microalgae cultivated in the various culture conditions were determined. In trial I (i.e. With aeration of 9 L/Min inlet gas flow rate), the cell number increased from day 0 and got to 2.96 cell/L X 10^4 in day 10 but started decreasing from day 12 with a value of 2.45 cell/L X 10^4 to 2.201 cell/L X 10^4 in day 14 (fig. 3) while that of trial II (i.e. With aeration of 4.5 L/Min inlet gas flow rate) increased from day 0 to day 14 with a value of 5.45 cell/L X 10^4 and it also gave the microalgae cell number value (fig. 3) and that of trial III (i.e. without aeration) gave the same value of 2.45 cell/L X 10^4 with that of trial I at day 10 (fig. 3).

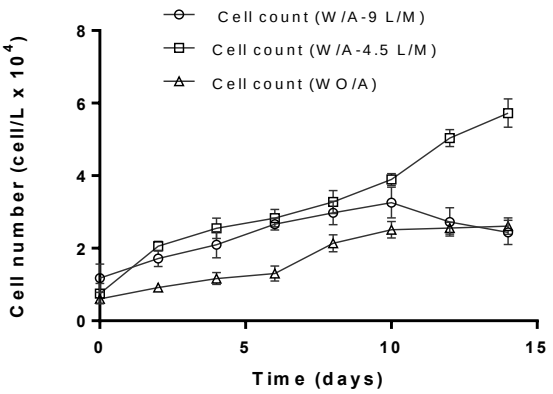

Figure 3. Cell numbers (cell/L X 10^4) of algae with aeration at 9 L/Min inlet air flow rate (W/A - 9 L/M), algae with aeration at 4.5 L/Min inlet air flow rate (W/A - 4.5 L/M) and without aeration (WO/A).

3.2. Effect of aeration on pH stability

The pH value for culture media with aeration was quite in a steady state than the culture media without aeration (fig. 4). This must have resulted in higher growth in culture media with aeration than in culture media without aeration but the pH of trial I went up a bit from 8.2 in day 10 to 8.43 in day 14.

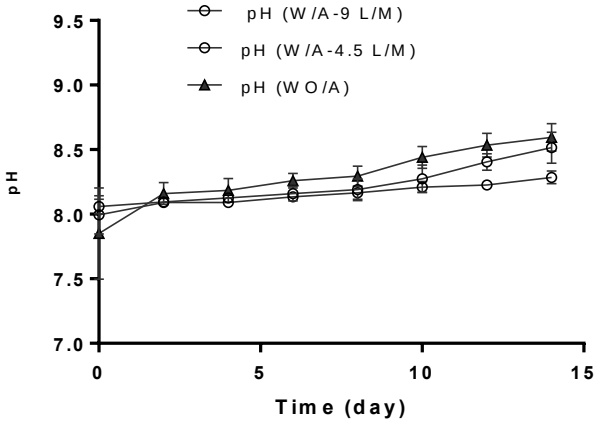

Figure 4. pH of algae with aeration at 9 L/Min inlet air flow rate (W/A - 9 L/M), algae with aeration at 4.5 L/Min inlet air flow rate (W/A - 4.5 L/M) and without aeration (WO/A).

3.3. Wastewater nutrient removal

Treatment of wastewater with algae resulted in better removal of nutrients than the conventional activated sludge system (Ruiz-Martinez et al., 2012). Algae treatment is applied in some treatment processes, after an activated sludge system, as a tertiary treatment used to comply with discharged standards (Rasoul-Amini et al., 2014). It may be beneficial to completely replace or simultaneously integrate the activated sludge system with algae based treatment system for simultaneous nutrients and energy production rather than only being used as a tertiary treatment. Hence this research tends to look at both possibilities (Tighiri & Erkurt, 2016).

- ***Nitrogen removal***

The concentration of Nitrogen and nutrient removal rate per day during different periods of time and treatments are shown in Fig. 5. The achieved concentration after treatment in the wastewater treatment media was 1.847 mg N/L. The nutrient removal rate per day for various wastewater treatment media was 26.22 mg N $L^{-1}d^{-1}$. Rasoul-Amini *et al.* (2014) reported 30.30 mg N/L as achieved concentration after treatment of wastewater using *Chlorella sp.* for 14 days. Ji *et al.* (2013) reported that the maximum specific consumption rate of TN (16.8 mg-N g-$cell^{-1}$) was observed with *C. vulgaris.*

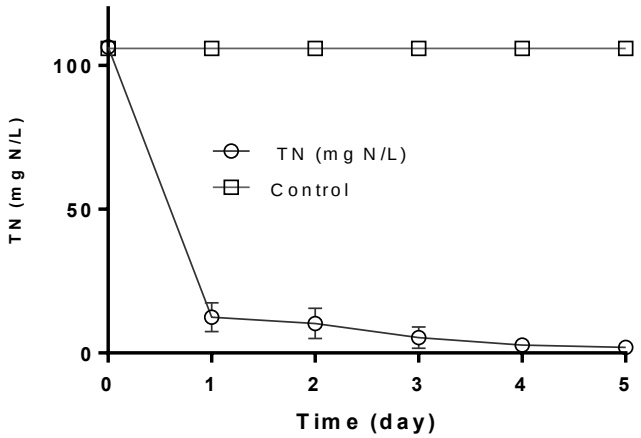

Figure 5. Microalgae uptake of total nitrogen

- ***Phosphorus removal***

The concentration of phosphorus and nutrient removal rate per day during different periods of time and treatments are shown in Fig. 6. The achieved concentrations after treatment in the wastewater treatment media was 0.805 mg P/L. The nutrient removal rate per day for various wastewater treatment media are 1.12 mg P $L^{-1}d^{-1}$. Rasoul-Amini *et al.* (2014) reported 1.95 mg P/L as achieved concentration after treatment of wastewater using *Chlorella sp.* for 14 days. Ji *et al.* (2013) reported that the maximum specific consumption rate of TP (3.1 mg-P g-$cell^{-1}$) was observed with *S. obliquus.*

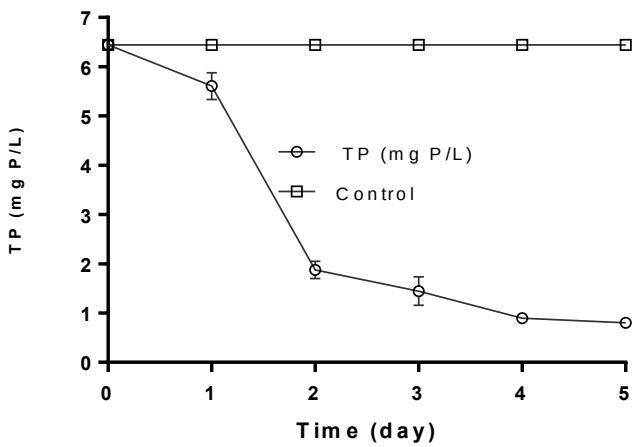

Figure 6. Microalgae uptake of total nitrogen in wastewater

4. Conclusion

In conclusion, aeration culture media with 4.5 L/Min showed better growth outcome and which is therefore suitable for biofuel production and also microalgae could be used as a secondary treatment for wastewater containing high nutrients.

References

Abdel-Raouf, N., Al-Homaidan, A. A. & Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences, 19*(3), 257-275.

American Public Health Association, (2005). *Standard methods for the examination of water and wastewater* (21th edition). US: American Public Health Association.

Aslan, S. & Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering, 28*(1), 64-70.

Chinnasamy, S., Bhatnagar, A., Hunt, R. W. & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology, 101*(9), 3097-3105.

Choochote, W., Paiboonsin, K., Ruangpan, S. & Pharuang, A. (2010). *Effects of urea and light intensity on the growth of chlorella sp.* Retrieved from http://www.science.kmitl.ac.the/downloads/proceeding on 1 April 2017.

Ji, M. K., Abou-Shanab, R. A., Kim, S. H., Salama, E. S., Lee, S. H., Kabra, A. N. & Jeon, B. H. (2013). Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO 2 for nutrient removal and biomass production. *Ecological Engineering, 58,* 142-148.

Lauritano, C., Andersen, J.H., Hansen, E., Albrigtsen, M., Escalera, L. Esposito, F., Helland, K., Hanssen K., Romano, G. & Lanora, A. (2016). Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. *Frontiers in Marine Science,* 3(68), 1-12.

Levasseur, M., Thompson, P. A. & Harrison, P. J. (1993). Physiological acclimation of marine phytoplankton to different nitrogen sources. *Journal of Psychology, 29*(5), 587-595.

Mata, T. M., Martins, A. A. & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews, 14*(1), 217-232.

Mostafa, S. S., Shalaby, E. A. & Mahmoud, G. I. (2012). Cultivating microalgae in domestic wastewater for biodiesel production. *Notulae Scientia Biologicae, 4*(1), 56-65.

Persoone, G., Morales, J., Verlet, H. & De-Pauw, N. (1980). Air-lift pumps and the effect of mixing on algal growth. *Algal Biomass, 11*, 505-522.

Pruvost, J., Van Vooren, G., Le Gouic, B., Couzinet-Mossion, A. & Legrand, J. (2011). Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. *Bioresource Technology, 102*(1), 150-158.

Rasoul-Amini, S., Montazeri-Najafabady, N., Shaker, S., Safari, A., Kazemi, A., Mousavi, P. & Ghasemi, Y. (2014). Removal of nitrogen and phosphorus from wastewater using microalgae free cells in bath culture system. *Biocatalysts and Agricultural Biotechnology, 3*(2), 126-131.

Rodriguez-Maroto, J. M., Jimenez, C., Aguilera, J. & Niell, F. X. (2005). Air bubbling results in carbon loss during microalgae cultivation in bicarbonate-enriched media: Experimental data and process modeling. *Aquacultural Engineering, 32*(3), 493-508.

Ronda, S. R., Bokka, C. S., Ketineni, C., Rijal, B. & Allu, P. R. (2012). Aeration effect on Spirulina platensis growth and γ-linolenic acid production. *Brazilian Journal of Microbiology, 43*(1), 12-20.

Ruiz-Martinez, A., Garcia, N. M., Romero, I., Seco, A. & Ferrer, J. (2012). Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. *Bioresource Technology, 126,* 247-253.

Show, P. L., Tang, M. S., Nagarajan, D., Ling, T. C., Ooi, C. W. & Chang, J. S. (2017). A holistic approach to managing microalgae for biofuel applications. *International Journal of Molecular Sciences, 18*(1), 215.

Tighiri, H. O., & Erkurt, E. A. (2016). Integrated microalgae cultivation, nutrient removal and product utilization bio-processes. *Desalination and Water Treatment, 57*(47), 22329-22340.

Tighiri, O. H. & Erkurt, A. E. (2017). Effect of aeration flow rate on the growth of microalgae as a biofuel feedstock and wastewater treatment. *New Trends and Issues Proceedings on Humanities and Social Sciences*. [Online]. 4(4), pp 195-204. Available from: www.prosoc.eu

Valdes, F. J., Hernandez, M. R., Catala, L. & Marcilla, A. (2012). Estimation of CO 2 stripping/CO 2 microalgae consumption ratios in a bubble column photobioreactor using the analysis of the pH profiles. *Application to Nannochloropsis Oculata Microalgae Culture. Bioresource Tchnology, 119,* 1-6.

Vidyarathna, N. K., Fiori, E., Lundgren, V. M. & Graneli, E. (2014). The effects of aeration on growth and toxicity of Prymnesium parvum grown with and without algal prey. *Harmful Algae, 39,* 55-63.

Wang, H. M. D., Chen, C. C., Huynh, P. & Chang, J. S. (2015). Exploring the potential of using algae in cosmetics. *Bioresource Technology, 184*, 355-362.