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參展科別　地球與環境科學

作品名稱　**Removal of Nutrients by Chlorella Vulgaris Microalgae in Bandar Abbas Municipal Wastewater**

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Abstract

The entry of nutrients into the environment can cause the creation of eutrophication of aquatic ecosystems. One of the methods of removing nutrients from effluents is the use of algae. Algal purification is a new and inexpensive technology for this purpose. The present study investigated the rate of cell growth and nutrient removal of urban wastewater in Bandar Abbas in winter 2020 by the Chlorella vulgaris microalgae in the phycolab of Fisheries Research. Treatments with different dilutions (0%, 25%, 50% and 75%) were prepared; in addition, specific growth rate, cell density and removal efficiency of phosphate, nitrate, nitrite were examined during a 14 day period with initial constant density (1×10^6 cells / ml) of microalgae. The results indicated that 0% and 75% dilution had the highest and lowest cell densities (8.675×10^6 and 56.633×10^6), respectively; moreover, they had the specific growth rate (0.166 and 0.311). Furthermore, there was a significant difference between them ($P\geq 0.05$). The highest nitrate and nitrite removal efficiencies were -40.75 and -79.84 in effluent dilution of 50%; in addition, the lowest were 1.26 and -40.26 in dilution of 75% and 25% respectively. Phosphate had the highest removal efficiency at 0% dilution with a mean of -79.65 that showed a significant difference with the lowest at 25% dilution ($P\geq 0.05$). Therefore, high or low levels of nutrients can affect the removal efficiency and growth rate of microalgae.

Introduction

Water as one of the most essential resources of the earth, without which life would not be possible, has undergone a declining trend (Ghoreishi et al., 2016). Therefore, increasing water consumption and energy demand has raised many concerns about the significance of energy and wastewater treatment and reuse in order to return to the natural cycle. The increasing growth of industrial activities and the consequent non - compliance with environmental requirements have caused large amounts of pollutants to enter the environment in recent decades and have caused many problems (Safari et al., 2014). Although the strategic significance of safe water is now well known around the world, water resources encounter with serious risks. Growing pollution, industrialization and rapid economic development have endangered the quality of water resources in many parts of the world (Abdol Raouf et al., 2012,). One of the concerns of researchers and industry owners nowadays is to protect the environment from pollution, which is caused by modern technology and industry. Among these, one of the most important environmental pollutants is municipal wastewater. Wastewater that reaches the municipal treatment plant is the sum of wastewater that enters the sewage network from three different sources. These three sources are domestic wastewater, water leakage and industrial wastewater. Based on the definition of the collection of wastewater from three sources, it is called municipal wastewater or sanitary wastewater. Human beings' concerns arise when they know that physical and chemical treatment methods for such effluents fail to meet environmental standards. Since most physical and chemical methods are ineffective and uneconomical when the concentrations of heavy and toxic metals in contaminated environments are in the range of 10 to 100 ppm (Ahmadi Asb Chin and Jafari, 2013). Nowadays, one of the proposed solutions for sanitary wastewater treatment is the use of microalgae (Voltolina et al., 2006; Yalcin et al., 2004). Microalgae are included in a variety of environmental balance activities to control pollution in the environment. Not only do they act as the main agent for removing greenhouse gases from the Earth's atmosphere, but they can also be applied to treat wastewater and control environmental pollution. In order to overcome the shortcomings of conventional wastewater treatment methods, biological treatment by using microalgae has been extensively investigated in the last decade (Maity et al., 2014). Many studies have indicated that microalgae have a high potential for nitrogen and phosphorus removal. The main mechanisms for the removal of algal nutrients from wastewater consist intracellular uptake and removal of ammonia by pH (Aslan & Kapdan, 2006). In fact, high removal efficiency of nitrogen and phosphorus in about 80 - 100 % of wastewater in various sources (for examples: agricultural, industrial and urban) has been reported by microalgae. In addition, the use of microalgae to remove nutrients has several advantages as follows: 1. Nitrogen and phosphorus absorbed by microalgae can be recycled by producing fertilizer from microalgae biomass. 2. The resulting biomass can be used to produce bioenergy, food, animal feed and medicine. 3. Oxygen - containing effluent is discharged to water sources (Goncalve et al., 2017). Chlorella vulgaris microalgae is a single - celled green algae found extensively in the world's waters (Lee & Lee 2001; Li et al. , 2010). This is freshwater algae and can grow and reproduce in brackish to low salinity waters. This study was conducted in different dilutions of municipal wastewater in the creek of Gorsuzan in Bandar Abbas, which is near to the sea. Due to the fact that in the coastal areas and cities of the south and north of the Iran country, the sewage system is not properly controlled and monitored; moreover, eventually many of them are dumped into the sea or creeks and rivers leading to the sea, which has adverse environmental effects on the natural environment of seas and living organisms of these environments, low or high nutrients in effluents can play a role in inhibiting or increasing the growth of algae and also affect their efficiency in nutrient uptake (Kong et al., 2010). The purpose of this study was to determine the adsorption efficiency and growth rate of Chlorella vulgaris microalgae in the municipal wastewater of Gorsuzan estuary in Bandar Abbas with different concentrations.

Literature review

Many studies have been carried out in this field that have had effective results in the field of microalgae biomass production and nutrient removal of municipal effluents and treatment plants, including Qin et al (2016) who investigated microalgae cultivation in dairy wastewater to remove nutrients and produce biodiesel raw materials by cultivating chlorella with discontinuous system of municipal wastewater. The results indicated that the use of chlorella microalgae removed 3.99% of nitrogen and 2.95% of phosphorus in the tested treatments. In addition, McGinn et al. (2012) removed 90% of ammonia and 90% of phosphate by culturing Scenedesmus algae in the effluent after the UV stage of the wastewater treatment plant. Another study was conducted by Gholizadeh and Nosrati (2019) on the urban effluent of one of the treatment plants in Tehran. Moreover, the results indicated that the potential of municipal and Vinasse wastewater was effective for replacing Zarrouk culture medium. This microalgae also removed nitrogen, phosphate and COD equal to 63, 97, 73, respectively. Therefore, spirulina microalgae had a high ability to remove nutrients from the effluent. Another study was conducted in by Abolhassani et al. (2016), who investigated the possibility of biomass production and removal of phosphate and nitrate from municipal effluent by culturing the Chlorella vulgaris alga. The results showed that the Chlorella vulgaris was able to grow well in the effluent and caused 100% phosphate removal in the effluent treatment with zero dilution and 99.37 % nitrate in the 50% dilution treatment. Furthermore, the highest production biomass (0.25 g / l), chlorophyll a (3.34 mg / l) and the number of cells (1.4×10^6 cells / ml) were observed in the effluent treatment with zero dilution. Based on the results of this study, it seems that this algae in wastewater treatment with zero dilution can be used to remove phosphate and nitrate and also produce algal biomass in urban wastewater treatment systems before entering the natural environment. In addition, municipal wastewater treatment plant can be used as a suitable cultural environment for mass production of this algae. In a study by Su et al. (2012), the effect of living (algal density) and non - living (light cycle, mixing speed, and nutrient capacity) factors on treatment efficiency, biomass production, and deposition ability by mixing culture of algae was examined. Dark conditions had poor efficiency in nutrient removal. There was no significant difference in nutrient removal and biomass deposition capability between continuous and intermittent lighting; nevertheless, biomass production was higher in continuous lighting conditions . The reactor with a stirrer speed of 300 rpm had the best ability to remove N. The results showed higher nitrogen removal rates and higher phosphorus removal rates for reactors with algae inoculation concentrations for low durability wastewater. However, wastewater with higher durability had lower nutrient removal efficiency, and lower biomass deposition capacity.

Materials and methods of experiment

Place of research

This research was carried out in the winter of 2020 in the phycolab laboratory of the aquaculture department of the Persian Gulf and Oman Sea Ecology Research Institute in Bandar Abbas.

Preparation and cultivation of Chlorella vulgaris algae

Pure stock of Chlorella vulgaris was prepared from phycolab Laboratory of Aquaculture Research Institute of Persian Gulf and Oman Sea Ecology, Bandar Abbas. The algae stock was cultured in a 2 liter Erlenmeyer flask containing filtered seawater under controlled conditions with light period (brightness: dark) 13:11, constant temperature of 26 ± 1 ° C, salinity of 25 ppt and light of 5000 - 3500 lux with ambient Gillard culture in order to conduct the test (Piri & Ordog,1978; Miller et al, 1997).

Sewage collection

In order to carry out this experiment, a sample of Bandar Abbas effluent was prepared in the winter of 2020 from the creek of Gorsuzan in Bandar Abbas and then transferred to the Persian Gulf and Oman Sea Ecology Research Institute. The collected effluent was filtered in the laboratory through a plankton mesh to separate large particles and suspended solids. It was then filtered by a Whatman filter and autoclaved for 20 minutes at 121 ° C and a pressure of 1.5 atmospheres (Martinez et al. 2000).

Design of the experiment

In order to perform the experiment, each treatment was cultured with 3 replications in 2 liter Erlenmeyer for 14 days in vitro (Miller et al., 1978). Treatments in this experiment include effluents with dilution of 0% (seawater), 25% (one part effluent and three parts filtered water), 50% (two parts effluent and two parts filtered water) , 75% (three parts effluent and one part of filtered water), in which the specific growth rate and cell density (every other day) and the amount of its nutrients (phosphate, nitrate, nitrite) were measured during a 14 - day test period (beginning and end of the period) according to the standard method.(Boyd & Green 2012; Oliveira et al., 2002). The effluent was added with the mentioned dilutions to each of the Erlenmeyer flask , the rest of the volume of the container was inoculated with filtered and sterilized sea water with the desired salinity along with algal stock with an initial constant density (cell / ml) of 1×10^6 (Gao et al, 2014).

Cell density and specific growth rate (SGR)

During the experiment, the number of algae was counted and recorded every other day to evaluate the cell density (Song et al., 2013) and the specific growth rate (calculated by the method proposed by Ikeda and Omori in 1984 and using the following formula) of Chlorella algae by using a homocytometric slide for 14 days.

SGR = (LnN2-LnN1) / Δt

In this formula, N2 is the number of algal cells at the end of the period and N1 is the number of algal cells at the end of the period; in addition, t is the duration of the experiment.

- Physical and chemical faZctors of water

Chlorella microalgae was investigated with an initial constant density (cell / ml) of 1×10^6 and controlled culturing conditions with light period (light: dark) 13:11, constant temperature of 26 ± 1 ° C, salinity of 25 ppT and brightness of 5000 - 3500 lux . Then, at the beginning of the period after cultivation of all treatments and at the end of the period, a certain volume of water was removed from each Erlenmeyer and filtered using a vacuum device, filtration system and filter paper of 0.45 microns and kept in the freezer until analysis.

(Strickland and Parson, 1972). The samples were then sent to the laboratory for nutrient analysis.

- Measurement of water - soluble phosphate

The filtered water of the samples was studied in order to measure the amount of phosphate by adding the necessary reagents for the formation of ammonium phosphomolybdate complex and the adsorption of the samples at 882 nm by spectrophotometry with an accuracy of 1 µg / l. Moreover, phosphate levels of the samples were measured (Strickland and Parson, 1972).

- Measurement of nitrite and nitrate (NO2, NO3)

To measure the nitrate content of the samples, first the nitrate was converted to nitrite by cadmium reduction method and then the reagents were read by adding a spectrophotometer at 540 nm. The read number was related to the total nitrite and the amount of reduced nitrate. To measure nitrite, all previous steps were performed without nitrate reduction. The obtained number was less than the previous number ; in addition, the amount of nitrate was calculated (ROPME, 1999).

- Nutrient removal rate (Removal efficiency)

To calculate the removal and separation of municipal wastewater nutrients at the beginning and end of the cultivation period, the amount of nitrite, nitrate and phosphate was measured by standard laboratory methods (ROPME, 1999; Strickland & Parson) and then was calculated by the following formula (Han et al., 2015).

Removal efficiency%= (Ci-C0)/C0 ×100

In this formula, Ci represents the concentration at time ti ; in addition, C0 represents the initial concentration.

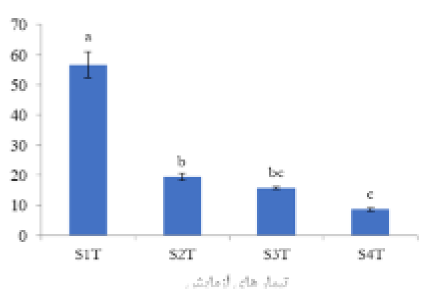
Statistical Analysis

The obtained data was entered in Excel software ; in addition, descriptive results are provided in the form of figures. Statistical analysis of the results was applied in SPSS software (15) using parametric tests (one - way analysis and variance) and Tukey post - hoc test to compare data.

Results and discussion

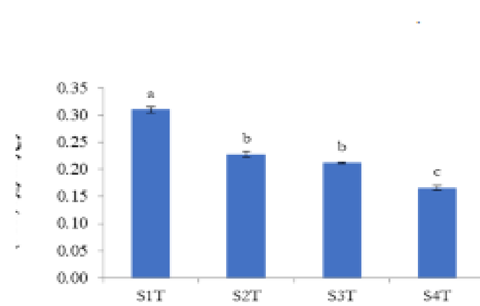
The results of one - way analysis of variance ANOVA at 95% confidence level indicated that the highest cell density was in S1T treatment (0% dilution) with a mean of 56.633 ± 4.29; moreover, the lowest cell density was in S4T treatment with a mean of 0.51 ± 675.8 that showed a significant difference ($P \geq 0.05$). However, there was no significant difference between S2T and S3T treatments with a mean of 1. 24 ± 367.19 and 0.29 ± 0.733.15, respectively ($P \geq 0.05$) (Figure 1, Part A). The results of one - way analysis of variance ANOVA in the study of specific growth rate showed that the highest growth rate was in S1T treatment with a mean of 0.311 ± 0.006 ; in addition, the lowest cell density was in S4T treatment with a mean of 0.166 ± 0.004 which a significant difference was observed between them ($P \geq 0.05$). However, there was no significant difference between the S2T and S3T treatments with an mean of 0.228 ± 0.005 and 0.212 ± 0.001, respectively, at the 95% confidence level ($P \geq 0.05$) (Figure 1, Part B).

Final cell density



(A)

Specific growth rate



(B)

Figure 1: *A. Final cell density during culture (cell density × 10^6). B. Specific growth rate during the growing season (se ± mean)*

The obtained results of one-way analysis of variance of the data in the present study to evaluate the efficiency of nutrient removal showed that the efficiency of algae in nutrient removal at different dilutions was significantly different ($P \leq 0.05$). The efficiency of algae in nitrate removal was the highest in S3T treatment (50% dilution) with a mean of - 40.75 ± 1.34 ; moreover, S4T treatment (75% dilution) with a mean of 1.26 ± 0.01 showed the lowest efficiency. The difference between them was significant ($P \leq 0.05$). In nitrite removal, algae efficiency was the highest in S3T treatment (50% dilution) with a mean of - 79.84 ± 2.10 and had a significant difference with the lowest removal efficiency in S2T treatment (25% dilution) with a mean of 40.26 ± 23.78 ($P \leq 0.05$). The results of analysis of variance of the percentage of algae removal efficiency in phosphate material showed that the highest efficiency was in S1T treatment (0% dilution) with a mean of - 79.65 ± 4.40 and the lowest in S2T treatment (dilution 25%) with an average of 40.4 ± 95.79 ; in addition, a significant difference was observed between them ($P \leq 0.05$) (Table 1).

Table 1*: Nutrient removal percentage: nitrate, nitrite and phosphate during cultivation*

Treatment	Nitrite removal efficiency (RE ± se)	Nitrate removal efficiency (RE ± se)	Phosphate removal efficiency (RE ± se)
S1T (dilution 0%)	- 76/43 ± 2 . 19 ^{b}	-1.97 ± 0.28 ^{bc}	- 79.65 ± 2 . 73 ^{a}
S2T (dilution 25%)	- 40.26 ± 23 . 78 ^{a}	- 4.56 ± 0 . 57^{b}	79 . 95 ± 4 /.40 ^{c}
S3T (dilution 50%)	-79.84 ± 2/10 ^{b}	- 40.75 ± 1 . 34^{a}	48 . 16 ± 5 . 47 ^{b}
S4T (dilution 75%)	-42.22 ± 1 / 43^{b}	1 . 26 ± 0 . 01^{c}	41 . 14 ± 2 . 66 ^{b}

The dramatic growth of algae in nutrient - rich waters is a general phenomenon that plays an important role in the elimination of a variety of minerals and substances resulting from the metabolic activities of living organisms (Geetha et al., 1994). The removal of nutrients by microalgae cells is consistent with their cell density and metabolic activities. Among these, the uptake of nitrogen by microalgae during metabolic activities is one of the main processes. Wastewater is a suitable context for metabolism and growth of microalgae due to its high content of nutrients (nitrogen and phosphorus) (Aravantinou et al., 2013). The amount of open matter recycled by the microalgae is converted to biomass. A simple comparison of initial and final nutrient concentrations can lead to the evaluation of algae as food recycling or their efficiency in removing these substances from the effluent (Voltolina et al., 2004), which algae grows and multiply by absorbing nutrients and eventually reduce nitrogen and phosphorus in the effluent. Therefore, several studies have been conducted in this field, which show the consumption and removal of nutrients by algae and their growth and increase in biomass in sewage and wastewater (Gonçalves et al., 2017).

On the other hand, low or high nutrient content of effluents can play a role in inhibiting or increasing the growth of algae and also affect their efficiency in nutrient uptake (Kong et al., 2010). The findings of the present study showed that the Chlorella vulgaris has grown in all effluents, but the higher the dilution, the lower the cell growth rate and specific growth rate. The highest cell density was at 0% dilution with a mean of 56.63×10^6 in which no effluent was used. After which 25% dilution had the highest growth of 19.367×10^6, which had a significant difference. The lowest cell density was at 75% dilution with a mean of 8.675×10^6 , which showed a significant difference with other dilutions ($P \leq 0.05$). Therefore, the results of growth and low growth rate in higher dilutions may be due to high levels of nitrate and phosphate and have a deterrent effect. Low or high nutrient in the effluent can be an effective factor in reducing or increasing the growth of algae. A study by Abolhassani et al. (2016) who examined the possibility of biomass production and removal of phosphate and nitrate from municipal effluent by culture of Chlorella vulgaris showed that Chlorella vulgaris grew well in the effluent ; moreover, the highest amount of biomass produced (0.25 g / l), chlorophyll a (3.34 mg / l) and number of cells (1.4×10^6 cells / ml) were observed in the effluent treatment with zero dilution. Based on the results of this study, it seems that this algae in wastewater treatment with zero dilution can be used to remove phosphate and nitrate and also produce algal biomass in urban wastewater treatment systems before entering the natural environment. Other research results in this field by Gholizadeh and Nosrati (2019) using Spirulina microalgae to grow algae and using municipal wastewater nutrients in one of Tehran's treatment plants and the efficiency of nutrient removal by this microalgae showed the potential of urban waste and Vinasse was effective to replace the culture medium of Zarrouk . In addition, the effluent can be used as a suitable culture medium for mass production of this algae. Meanwhile, with another part of the experiment that was carried out in this research, by measuring the amount of nitrite, nitrate and phosphate at the beginning and end of the period, it showed that the removal efficiency of nitrite , nitrate phosphate were - 79.84 and - 40.75, respectively in the effluent dilution of 50% that had a significant difference with the lowest percentage of nitrite and nitrate removal efficiency of 40.26 and - 1.97% in effluent dilution of 25% and 0%, respectively. The results indicate the effective efficiency of algae in removing nitrogen in the higher dilutions of the effluent. However, this trend was gradually decreasing in the effluent with higher dilution, which could be due to the effect of increasing and decreasing the growth and removal of nutrients with low or high concentrations of nutrients in the effluent (2010, Kong et al. 2010).

However, in phosphate, the results of the present study showed that the highest removal efficiency was - 79.65 in zero dilution, where no effluent was used. In addition, the only nutrient was Gillard culture medium. Which was used in all treatments in addition to effluent, which was probably the only source of phosphate in zero dilution of this culture medium and was used. Nevertheless, in treatments where dilution of effluents increased, the more dilution was, the lower the removal efficiency. In addition, there was also a significant difference with zero and 25% dilution treatment. Numerous studies that have been carried out, for example, Abolhassani et. al. (2016) investigated the possibility of biomass production and removal of phosphate and nitrate from municipal effluent by culturing algae Chlorella vulgaris caused 100% phosphate removal in zero dilution effluent treatment and 99.37% nitrate with 50% dilution. Other researches can be referred to the results of Qin et al.'s study (2016), which examined the cultivation of microalgae in dairy wastewater to remove nutrients and produce biodiesel raw materials by cultivating chlorella. The use of this microalgae removed 99.3% of nitrogen and 95.2% of phosphorus in the tested treatments. In addition, Mcginn et al. (2012) achieved the removal of 90% of ammonia and 90% of phosphate by culturing the Scenedesmus algae in the effluent after the UV stage of the wastewater treatment plant. All the results of various researches as well as the current research show the proper growth of microalgae, especially microalgae that have an effective role in wastewater treatment . Moreover, they have been able to have acceptable removal of organic and mineral nutrients in appropriate dilutions and not above the effluent . Furthermore, they can be used in biodiesel production even with proper biomass production and proper growth rate during the culture period. Gholizadeh and Nosrati (2019) confirmed the removal of nitrogen, phosphate and COD from the effluent environment with an experiment performed by spirulina microalgae on the effluent. Therefore, spirulina microalgae also had a high ability to remove nutrients in the effluent. Su et al. (2012) examined the effect of living (algal density) and non - living (light cycle, mixing rate, and nutrient capacity) factors on biomass production efficiency and disposition ability by culturing a mixture of algae. The results of that study showed the higher nitrogen and phosphorus removal rates for reactors with algae inoculation concentrations for low - durability wastewater. However, wastewater with higher durability had lower nutrient removal efficiency and lower biomass deposition capacity. Therefore, the amount of effluent dilution due to the concentration of nutrients and the type of microalgae can have a positive or negative effect on growth, biomass production and the efficiency of effluent nutrient removal. As observed in this experiment, the higher the dilution of the effluent was, the lower the cell growth and the efficiency of nutrient removal was. However, in the treatments with moderate dilutions of the effluent, the growth and removal efficiency were more appropriate than very low or very high dilutions.

References

- Abdel-Raouf, N., Al-Homaidan, A.A., Ibraheem, I.B.M. 2012. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences. 19: 257-275.
- Abolhassani , M. H., Hosseini, S. A., Qorbani, R. & Vinceh, A. (2016). Feasibility study of biomass production and removal of phosphate and nitrate from municipal wastewater by culture of Chlorella vulgaris. *Journal of Aquaculture Development,* 10 (2), 8-1.
- Ahmadi Asb Chin, S. &Jafari, N.(2013). Comparison of biological isolation of cadmium metal from effluent by Bacillus and Fucus Serratus algae. *Journal of Environmental Science and Technology,* 15 (2), 119-126.
- Aravantinou, A.F., Theodorakopoulos, M.A. and Manariotis, I.D., 2013. Selection of microalgae for wastewater treatment and potential lipids production. *Bioresource technology*, *147*, pp.130-134.
- Aslan S, Kapdan IK. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological Engineering. 2006;28(1):64-70.
- Boyd, C.E. & Green, B.W.,2002. Coastal water quality monitoring in shrimp farming areas, an example from honduras. *Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. Work in Progress for Public Discussion. Published by the Consortium,* **29**.
- Geetha, P.K., Martinez, M.E., Proulx, D, 1994. Rubber effluent treatment in a high-rate algal pond system. In: Phang S. M., Lee, Y. K., Borowitzka, M. & Whitton, B. (Eds.), Proceeding of

the 1st Asia-Pacific Conference on algal Biotechnology. University of Malaya, Kuala Lumpur, 306-312.

- Ghoreishi B, Aslani H, Dolatkhah A, Abdoli Seilabi A, Mosaferi M. Evaluation of Microbial Quality in Biosolids Generated from Municipal Wastewater Treatment Plants. Iranian Journal of Health and Environment. 2016;9(1):81-90 (in Persian).
- Goncalves AL, Pires JC, Simoes M. A review on the use of microalgal consortia for wastewater treatment. Algal Research. 2017;24:403-15.
- Gonçalves, A.L., Pires, J.C. and Simões, M., 2017. A review on the use of microalgal consortia for wastewater treatment. *Algal Research*, *24*, pp.403-415.
- Kong, Q.X., L. Li, B. Martinez, P. Chen., R. Ruan, 2010. Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production. Applied Biochemistry and Biotechnology, 160, 9-18.
- Lee, K., Lee, C.G, 2001. Effect of light/dark cycles on wastewater treatments by microalgae. Biotechnology and Bioprocess Engineering, 6, 194–199.
- Li, X., Hu, H.Y., Yang, J., 2010. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, Scenedesmus sp. LX1, growing in secondary effluent. New Biotechnol, 27, 59-63.
- Maity JP, Bundschuh J, Chen C-Y, Bhattacharya P. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives–A mini review. Energy. 2014;78:104-13.
- McGinn PJ, Dickinson KE, Park KC, Whitney CG, MacQuarrie SP, Black FJ, et al. Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode. Algal Research. 2012;1(2):155-65.
- Oliveira, V.P., Morais Freire, F.A. & Soriano, E.M.,2012. Influence of depth on the growth of the seaweed Gracilaria birdiae (Rhodophyta) in a shrimp pond. *Brazilian Journal of Aquatic Science and Technology,* **16,** 33-39.
- Qolizadeh, M. & Nosrati, M. (2019). Algae treatment of a mixture of municipal and Vinasse effluent using Spirulina platensis microalgae. *Iranian Journal of Environmental Health Scientific Research Quarterly,* 12 (3), 436-424. \
- ROPME, 1999. Manual of oceanographic observation on pollutant analysis methods in 111.1.6.6 Nutrients. Regional Organization for the Protection of the Marine Environmant. 3th ed., Kuwait

- Safari M, Ahmady-Asbchin S, Soltani N. The potential of cyanobacterium Schizothrix vaginata ISC108 in biodegradation of crude oil. Iranian Journal of Health and Environment. 2014;7(3):363-74 (in Persian).
- Strickland, J., D., H., Parson, T., R., 1972. A practical handbook of seawater analysis Information Canada, Ottava (ICD), 310 p.
- Su, Y., Mennerich, A. and Urban, B., 2012. Coupled nutrient removal and biomass production with mixed algal culture: impact of biotic and abiotic factors. *Bioresource Technology*, *118*, pp.469-476.
- Voltolina, D., Gmez-Villa, H., Correa, G., 2004. Biomass production and nutrient removal in semicontinuous cultures of Scenedesmus sp. (Chlorophyceae) in artificial wastewater, under a simulated day-night cycle. Vie Milieu, 54, 21-25.
- Yalcin, T., Naz, M., Turkmen, M., 2006. Utilization of different nitrogen sources by cultures of Scenedesmus acuminatus. Turkish Journal of Fisheries and Aquatic Sciences, 6, 123-127.

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Experimental design and method are sound, but somewhat lacking innovation. Experiments can be further extended to become more complete in terms of variability of experimental parameters and conclusions. Some sensitivity experiments and discussion could be very helpful for further application!