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Potent Application of Microalgae *Scenedesmus Vacuolatus* in The Treatment of Waste Water

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Abstract
This work analyses the potential of using microalgae *Scenedesmus vacuolatus* for the wastewater treatment and biomass production. The chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen (TN) and total phosphorous (TP) was analysed, and the influence of light exposure was studied. Results show that the most adequate conditions for cultivating *Scenedesmus vacuolatus* in this effluent are the aerated cultures, exposed to an 8 hr period of daily light, at direct sunlight intensity. At these conditions a maximum of 1.5g of dry biomass per litre of culture was obtained, after 20 days, for a maximum reduction of wastewater. COD was 1043.1mg/L reduced to 56.6mg/L, BOD was 222.0 mg/L reduced to 8.7 mg/L, TN was 59.1 mg/L reduced 1.1 mg/L and TP was 46.3, reduced to 8.7mg/L respectively after 20days.The chemical precipitation was mainly as a result of the increased pH, which was biologically mediated by the photosynthesising algae. A significant nitrogen and phosphorus removal was also experienced, which implies that the microalgal wastewater treatment is appropriate both for phosphorus and nitrogen removal. Higher concentration of microalgae resulted in higher removal rates of TP, TN, COD, and BOD in all treatments. The study also reported that flocculation activity increases with higher cell concentrations of *Scenedesmus svacuolatus*.
Keywords: *Microalgae, wastewater treatment, COD, BOD, TN and TP.*

1. Introduction

The excessive consumption of fossil fuel is one of the largest problems for humans in the 21st century. In order to reduce the risk of energy shortage (Salameh, 2003)and global warming (Brennan & Owende, 2010) which were heightened by the consumption of fossil fuel, the development of biodiesel has been seriously considered and supported in recent years. The Energy Independence and Security Act (EISA) was passed by the federal government of the USA in 2007 to increase the production of renewable fuels to 36 billion gallons per year by 2022. Microalgae biomass, which is capable of meeting the large demand oftransport fuels, is a very promising source for biodiesel production(Chisti, 2010).
In recent decades, the intensity of agricultural and industrial activities, together with rapid urbanization, has generated large amounts of wastewaters (Qin, Su, Khu, & Tang, 2014). The continuous disposal of wastewaters without appropriate treatment to water sources has posed severe water pollution problems, especially in developing countries (Gonçalves, Pires, & Simões, 2017). These effluents contain a high concentration of nutrients such as nitrogen and phosphorus, which are the leading causes of eutrophication in natural waters (Glibert, 2017). This condition is favourable for the development of harmful algal blooms (HABs) resulted in the degradation of water quality and impairment of freshwater ecosystems (O’neil, Davis, Burford, & Gobler, 2012). Moreover, the occurrence of HABs can result in serious problems such as anoxic conditions, toxin productions, killing of fish, and altered biodiversity (Heisler et al., 2008). HABs are considered a public health risk due to produce a variety of toxic secondary metabolisms (Pham & Utsumi, 2018). For these reasons, many efforts have been done in order to mitigate eutrophication by reducing nitrogen and phosphorus concentration in wastewater effluents before discharging into water sources.
Physical and chemical methods have been developed for the removal of nutrients from wastewater, but these are costly and produce high sludge content (Boelee, Temmink, Janssen, Buisman, & Wijffels, 2011). Due to requiring large amounts of the nutrient, especially nitrogen and phosphorus for growth, many species of green microalgae such as *Chlorella* sp., *Scenedesmus* sp., and *Neochloris* sp. have been proposed as an alternative biological treatment to remove nitrogen and phosphorus from different sources of wastewater for many years.

Indeed, extensive studies have been carried out on the subject of microalgae cultivation using wastewater. High removal efficiencies of nitrogen and phosphorus (more than 80%) from wastewaters of different sources have already been recorded for several microalgae species (González, Cañizares, & Baena, 1997; Y. Li et al., 2011; Zhao et al., 2015).

However, domestic wastewater is an adverse environment for microalgae. As numerous contaminants and predators exist in wastewater, microalgal cells can be preyed upon or disrupted. Liet al. tested the survivability of 11 species of high-lipid microalgae obtained from the algae bank and reported that none of these species could grow well in real secondary effluent. The isolation and selection of a suitable microalgal strain is the foundation of researching the coupled technology of wastewater treatment and biofuel production. The selected microalgal strain should be able to grow well in real domestic wastewater(without sterilization or adding any other nutrients) with a high lipid content and contaminant removal rate(K. Li et al., 2019).

I. METHODS AND MATERIAL

2.1 Microalga and medium

The *scenedesmus vacuolatus* used in this study was isolated from a wastewater treatment plant. This strain was kept in BG11 medium. Entrapment technique was used for immobilizing algal sample collected from the wastewater treatment plant. The algal species immobilized using the entrapment techniques was introduced into the wastewater sample that was collected from selected wastewater treatment plant. The efficiency of immobilized algal species was assessed, the physico-chemical parameters of the collected water sample was analysed before and after the introduction of immobilized algae. The efficiency of the immobilized algae in the treatment of the collected water sample from the wastewater treatment plant was calculated in percentage. The immobilization technique for microalgae described by Kobbai et al., for wastewater treatment was applied(Kobbai, Dewedar, Hammouda, Hameed, & May, 2000).

The growth medium BG11 contained the following:1,500 $mgL^{-1}NaNO_3$,40$mgL^{-1}K_2HPO_4 \cdot 3H_2O$, 75 $mgL^{-1}MgSO_4 \cdot 7H_2O$, 36 $mgL^{-1}CaCl_2 \cdot 2H_2O$, 6 mgL^{-1}citric acid, 6 mgL^{-1} ferric ammonium citrate, 1 mgL^{-1}EDTA,20 $mgL^{-1}Na_2CO_3$, and 1.0 mgL^{-1}A5+Co solution. TheA5+Co solution contained 2.86 $gL^{-1}H_3BO_3$, 1.81 $gL^{-1}MnCl_2 \cdot H_2O$, 222mgL^{-1} $ZnSO_4 \cdot 7H_2O$, 79 $mgL^{-1}CuSO_4 \cdot 5H_2O$, 390 $mgL^{-1}Na_2MoO_4 \cdot 2H_2O$, and 49 $mgL^{-1}Co(NO_3)_2 \cdot 6H_2O$. The stock conditions were: light intensity 55–60 lmol · photon · $m^{-2}s^{-1}$, light/dark periods of 14/10 h, relative humidity 75%, and temperature 25WC.Nitrate and phosphate were used as the nitrogen and phosphorus sources, respectively.

2.2 Experimental set-up and operation

2.2.1. Short-term experiments

The microalgae photosynthetic activity was determined by respirometric tests(Decostere et al., 2013). The oxygen production rate(OPR) was obtained by measuring the dissolved oxygen (DO) slope under well-defined experimental conditions in order to assess the photosynthetic activity of different sulphide concentrations in the microalgae culture.

2.2.2. Experimental set-up

The short-term experiments were carried out in a covered 500 mL flask with a magnetic stirrer to homogenise the microalgae culture inside a climatic chamber with air temperature setto 24 °C. 4 LED lamps (Seven ON LED 11 W) continuously illuminated the flask, supplying a light intensity of 300$\mu Em^{-2}s^{-1}$measured at the flask surface. In order to determine the OPR, an Orion TM-3 Star Plusportable oximeter (Thermo Scientific TM) was connected to a computer with BioCalibra ®software installed (Durán, García-Usach, Seco, Ferrer, & Ribes), which continuously registered dissolved oxygen (DO) concentration and temperature for data monitoring and storage.

2.2.3. Experimental procedure

Seven different short-term experiments were performed in duplicate with microalgae culture collected from wastewater plant at different sulphide levels. To reach these concentrations, the microalgae culture from wastewater plant was diluted with the appropriate amount of An MBR effluent. Prior to each assay, the samples were kept in darkness to prevent the photosynthetic process from producing oxygen, and were bubbled with nitrogen for 3 min to remove any remaining dissolved oxygen.

2.3. Measurement of Wastewater Quality Parameters.

Wastewater quality parameters including total phosphate (TS), total nitrogen(TN), biochemical oxygen demand (BOD5), and chemical oxygen demand (COD) were analyzed according to the Standard Methods for Examination of Water and Wastewater (Rice, Baird, Eaton, & Clesceri, 2012).

2.4. Statistical Analysis: The differences between mean values of the specific growth rate, dry microalga biomass, and pollutant removal rates were tested for significance using a one-way analysis of variance (ANOVA). *P* values≤0.05 were considered significant differences. All results are presented in the form of mean values ± standard deviation from three samples.

II. RESULTS AND DISCUSSION

3.1. Characteristics of Wastewater

The wastewater used for the experiment was analyzed for evaluating physicochemical characteristics. The physico-chemical parameters of the wastewater, including pH, temperature, main nutrient species, as well as BOD5 and COD. The wastewater effluent collected from the Plant contained large amounts of nitrogen (47.3 ± 5.9 mg/L)in the form of ammonium (NH^{+4}) and nitrate (NO^{-3}) with small amounts of phosphate (${PO^{3-}}_4$) (0.9 ± 0.28 mg/L). Besides, the initial pH of the wastewater effluent was almost constant at pH 6.8. Ammonium is among the most common chemical forms of nitrogen that can be readily absorbed by most microalga species (Perez-Garcia, Escalante, De-Bashan, & Bashan, 2011). The concentration of phosphorus was also found sufficient to support algal growth. In this respect, the effluent collected from the Wastewater Treatment Plant could be used as a cheap source of nutrients for microalgal cultivation. The characterized of the raw wastewater was noted to be favorable for microalgae growth. A ratio of COD/TN/TP, i.e., 180/47/2, found with this wastewater, is suitable for nutrient removal with microalgae. The BOD5/TP, BOD5/${PO^{3-}}_4$, and ${PO^{3-}}_4$ /TP ratios were found to be reasonably high. The optimal inorganic N/P ratio for freshwater algae growth was suggestedto be in the range of 6.8–10 (Reynolds, Huszar, Kruk, Naselli-Flores, & Melo, 2002). In this study, theinorganic N/P ratio of the effluent was 21, much higher thanthe optimal ratio, indicating the wastewater as phosphoruslimitation media.

3.2. Growth of *Scenedesmus* sp. in Wastewater

The green algae *Scenedesmus* sp. was cultivated in the wastewater for 10days with different initial cell concentrations from 10 to50 mg/L. The microalgae grew well in the wastewater with the growth rate from 0.30 to 0.38/day. There was no significant difference in the growth rate as the initial microalgae concentration increased from 10 to 50 mg/L. In the present study, wastewater was used as a nutrient medium for the growth of microalgae, and the utilization of nutrients allows wastewater treatment. During the growth phase, microalgae consumed mineral nutrients and $CO_2$from wastewater to produce biomass and released O_2 in to the medium (Munoz & Guieysse, 2006).

3.3. Removal of Nitrogen

It has been observed from the study that with the increase in the number of days of treatment with the microalgae the concentration of TN in the wastewater reduced (**Figure 1**). The initial concentration of 59.1 mg/L dropped to 1.1 mg/L during the 20th day of treatment with the microalgae. This observation well agreed with previous studies that with the increase in the treatment days the TN removal efficiency of the microalgae increases (Chen et al., 2018; Delgadillo-Mirquez, Lopes, Taidi, & Pareau, 2016; Shen, Gao, & Li, 2017). Green algae can use a variety of nitrogen sources for growth, making it possible to use these algae for bioremediation to remove nitrogen from wastewater (Perez-Garcia et al., 2011; Wang et al., 2010). Microalgae can convert different inorganic nitrogen forms from wastewater to organic nitrogen (Zhao, Guo, Sun, Hu, & Liu, 2019). Previous studies have demonstrated that the major mechanisms of nitrogen removal in algae systems include nitrification or denitrification and biological uptake of nitrogen by dispersed biomass (Delgadillo-Mirquez et al., 2016; Dortch, 1990).

3.4. Removal of Phosphorus

It has been observed from the study that with the increase in the number of days of treatment with the microalgae the concentration of TP in the wastewater reduced (**Figure 2**). The initial concentration of 46.3 mg/L dropped to 8.7 mg/L during the 20th day of treatment with the microalgae.P-PO_4^{-3} concentration in the medium reached to minimum on day 20th in all the treatments. Our study was found to be consistent with Khanzada, 2020 where the P-PO_4^{-3} was observed to reach to minimum in the 20th day (Khanzada, 2020). Rasoul-Amini et al., findings were also similar where it was observed that there was a faster removal efficiency for P-PO_4^{-3} (94.77%) than nitrogen (51.41%) within first 4 days using urban wastewater(Rasoul-Amini et al., 2014). Qu et al. also evaluated that P uptake was a relatively rapid process(Qu, Wu, & Shi, 2008). Su et al. also observed a faster 100 mg. L^{-1} P-PO_4^{-3} removal than N-NH_4^+(Su, Mennerich, & Urban, 2012).

3.5. Removal of BOD5 and COD

The COD concentration and the BOD concentration were found to decrease in the wastewater with the increase in the treatment days of the microalgae. The initial COD concentration of 1043.1 mg/L dropped to 56.6 mg/L during the 20th day of treatment with the microalgae. Similarly, the initial BOD concentration of 222 mg/L dropped to 8.7 mg/L during the 20th day of treatment with the microalgae. Our study was found to be at par with Mohammed et al, where it was observed that Chemical oxygen demand (COD) values started with 370mg/L, 270mg/L and 200mg/L for samples A, B and C respectively, and it reached after 14 days to 112 mg/L, 88 mg/L, and 120 mg/L. Biochemical oxygen demand (BOD) started with 241mg/L, 200mg/L, and 170 mg/L for samples A, B and C respectively, and it declined to 110 mg/L, 61 mg/L, and 112 mg/L (Mohammed, Ali, & Ali, 2016).

3. Tables, Figures and Equations

3.1 Tables and Figures

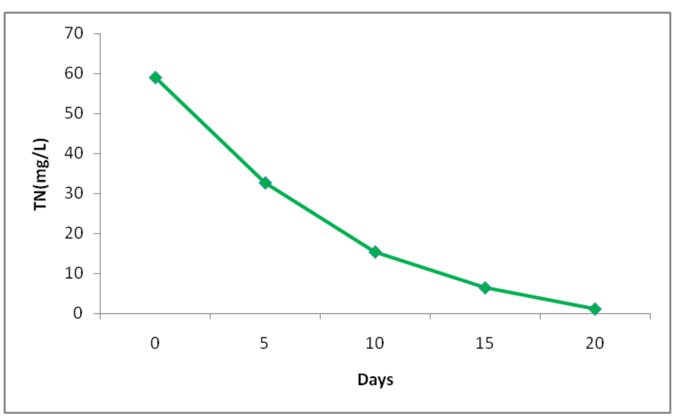

Figure 1 Figure representing the changes in the TN concentrations with respect to number of days of treatment with microalgae

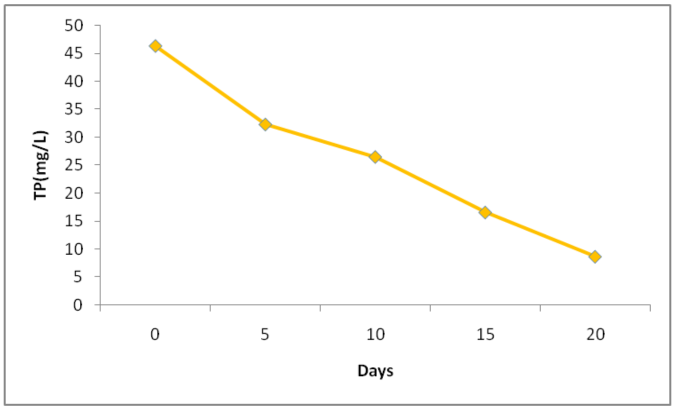

Figure 2 Figure representing the changes in the TP concentrations with respect to number of days of treatment with microalgae

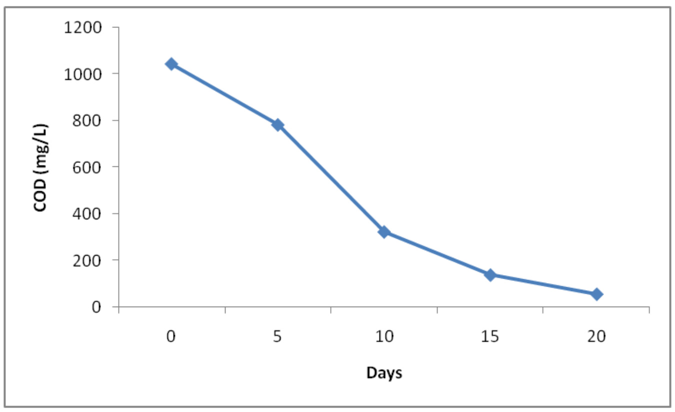

Figure 3 Figure representing the changes in the COD concentrations with respect to number of days of treatment with microalgae

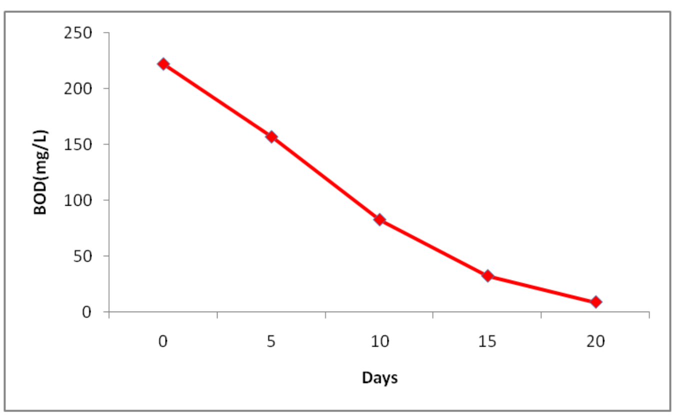

Figure 4 Figure representing the changes in the BOD concentrations with respect to number of days of treatment with microalgae

Table 1 Table representing the removal efficiency of TN, TP, COD and BOD5 with various initial concentrations of microalgae

Initial Microalgae Concentration	Removal efficiency (%)			
	TN	TP	COD	BOD5
Control	13.2±1.6	24.5±2.2	28.7±2.6	18.4±1.8
10mg/L	58.1±2.4	76.4±2.1	72.4±2.1	66.7±1.7
20mg/L	61.2±2.2	83.5±1.9	75.6±2.8	68.2±2.1
30mg/L	68.5±2.8	87.9±2.3	79.2±1.9	76.1±2.3
40mg/L	74.5±2.9	92.3±1.8	87.5±2.7	83.1±2.2
50mg/L	83.5±3.2	96.1±2.4	90.1±2.4	85.4±2.4

Table 1 shows the removal efficiency at the end of the experiment. Compared with the control, the treatments with higher initial microalgae concentration had greater removal. Previous studies have reported that the green algae *C. vulgaris* and *S. obliquus*could be used to remove $PO^{3-}{}_4$ from the difference of wastewaters (Delgadillo-Mirquez et al., 2016; Ruiz-Marin, Mendoza-Espinosa, & Stephenson, 2010; Shen et al., 2017). Other studies suggested that the immobilized microalgae are more effective than free form for nutrient removal in wastewater treatment (Nguyen, Tran, et al., 2019; Shen et al., 2017; Zhao et al., 2019).

Higher removal rates were also found in the treatments with higher initial microalgae concentrations. There is a relationship between initial concentrations of microalgae with the removal efficiencies of TP, TN, COD and BOD5. Higher concentration of microalgae resulted in higher removal rates in all treatments. Nguyen et al. (Nguyen, Tran, et al., 2019) investigated the ability to removal of COD and BOD5 in seafood wastewater; results indicated that the removal rates of COD and BOD5 were 88% and 81%, respectively, under sunlight mode and 81% and 74% under fluorescent illumination, respectively, as clear formation of flocculation was observed. Previous studies reported that the presence of microalgae in the cultivation medium had increased the consumption of organic as well as inorganic matters (Y. Li et al., 2011; Nguyen, Tran, et al., 2019; Woertz, Feffer, Lundquist, & Nelson, 2009).

Table 2 Table representing the flocculation activityof TN, TP, COD and BOD5 with various initial concentrations of microalgae

Initial Microalgae Concentration	Flocculation activity (%)
Control	12.1±1.4
10mg/L	53.4±2.3
20mg/L	63.5±1.9

30mg/L	69.2±2.2
40mg/L	75.4±2.5
50mg/L	86.7±2.1

Our results showed that flocculation activity increases with higher cell concentrations. Significant buildup of flocculation activity (75.4% and 86.7%) was observed at thehighest initial microalgae concentrations of 40 mg/L and 50 mg/L. Nguyen et al. (Nguyen, Le, et al., 2019) reported that the clear formation offlocculation occurred when *Chlorella vulgaris* in seafoodwastewater with the initial concentration above 20 mg/L.Results of the present study are consistent with previousobservations that higher initial biomass in the medium abetter formation of flocculation because cell-cell encountersare more frequent, leading to better aggregation(Grima, Belarbi, Fernández, Medina, & Chisti, 2003). Microalgalbioflocculation is an efficient low-costtechnology for microalgal harvesting and wastewatertreatment. In general, the bioflocculationprocess is assisted with microorganisms (including bacteria,fungi, and yeasts) or their polymer substances(Ummalyma et al., 2017; Zhao et al., 2019). Since no chemical is added in this process, the bioflocculationhas been considered as a sustainable and greentechnique for algal biomass harvesting (Zhao et al., 2019). The formationof algal flocculation and its application in wastewatertreatment has been reviewed in detail (Gerde, Yao, Lio, Wen, & Wang, 2014; Zhao et al., 2019). Inthe present study, autoflocculation occurred when cultured*Scenedesmus*sp. in the wastewater for 10 days.Microalgalautoflocculation was found to be associatedwith increase in culture pH levels, due to CO_2 consumptionby algal photosynthetic activity. Under thesealkaline conditions, some metal ions precipitated togetherwith the algal biomass(Sukenik & Shelef, 1984). Guo et al. (Guo et al., 2013) reported thatcell wall-associated polysaccharides mediated self-flocculationof the microalga *S. obliquus*. Future study is neededin this area to understand the mechanism of self-flocculationof microalgal cells and increase application inwastewater treatment technology.

4. Conclusions

The present results demonstrated the use of wastewater from a fertilizer plant in the cultivation and harvesting of *Scenedesmus*sp. Our results indicated that wastewater from fertilizer plants could be used as a cost-effective growth medium for algal biomass. The nutrient removal efficiency by *Scenedesmus*sp. isolated from the wastewater evidenced that microalgae are potential in removing nitrogen and phosphorous as well as COD and BOD5 from a highly concentrated nutrient-rich such as fertilizer plant wastewater. Higher removal rates were also found to be associated with treatments with higher initial microalgae concentrations.The present study highlights the autoflocculation of microalgae could be used as a more practical alternative eco-friendly approach for wastewater treatment using microalgae to eliminate eutrophication.

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