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Evaluation of Biomass Production and Wastewater Nutrient Removal Using Microalgae:Sustainable Strategy to CO_2 Bio-Fixation and Bioenergy Production Approach

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ABSTRACT

Nowadays, the replacement of renewable energies such as biofuels is one of the main priorities in environmental programming and investments. This study is based on sustainable strategy towards integrating algal biomass generation as a green feedstock with wastewater treatment, CO_2 bio-fixation, and bioenergy production. Therefore, the performance of *Trichormus variabilis* in biomass production together with ammonium and phosphate removal from an actual effluent obtained from a mixed wastewater streams has been investigated using two mixing methods under aeration and agitation conditions. Dilutions of 10 %, 20 %, 40 %, 60 %, 90 % and 100 % (v/v) were used for growth evaluation. The results showed that the bubbled air effectively enhances the biomass productivity. However, the agitation system was suggested to cultivate the algae in the wastewater due to the elimination of possible mechanical stimulation stress on cells. Moreover, high pH levels (pH>8) indicated a negative inhibitory effect on growth. Thus, unexpected inhibitory impacts were removed through providing the wastewater dilutions mixed with BG_{11} culture medium, which contains essential required nutrients, to support the algal growth in the wastewater, adjust pH and remove the mechanical stress induced by bubbling compressed air. The results with respect to investigating the effect of the inoculums and wastewater concentrations on the biomass production suggested that the highest biomass generates with 30 $mg.mL^{-1}$ inoculum in 40 % mixed wastewater diluted by the BG_{11} medium having the highest potential in CO_2 bio-fixation of 9.19±0.64 $g.L^{-1}$. The results of the wastewater analysis demonstrated the removal potential of ~43 % and ~75 % for NH_4^+ and PO_4^{3-}, respectively. The generated biomass after phycoremediation and CO_2 bio-fixation can be effectively utilized in different types of biofuel production.

1. INTRODUCTION

Population growth and increased demands for energy and water consumption together with air pollution and discharging the underground water reservoir have threatened natural resources. Therefore, this issue has led to researchers and investors try to develop alternative renewable and low carbon energy resources. Among different types of sustainable technologies, the biological systems have been considered as an appropriate, low cost and efficient option due to carbon fixation capability and eco-friendly energy production. But, providing water, nitrogen and some salts and metals for the growth of algal biomass is as a major cost challenge [1]. Particularly, when unbalanced competition between the water demands in domestic and industrial applications and insufficient available water [2] trigger to water scarcity. From the other side, the global freshwater withdrawals have been increased during the last decades [3]. It has been reported that ~2.2 million m^3 wastewater is generated in the world per year from different sectors [4], while the rich-nutrient wastewater can easily supply all the compounds required for the algal growth. Thus, to conserve the water resources for the next generation, the implementation of the water management system and sustainable technologies to recycle and reuse the wastewaters is vital. Some of microalgae species are well known as an efficient nutrient and heavy metal removal sources via phycoremediation process [5-7]. Hence, the

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phycoremediation provides a great opportunity to benefit from the nutrient removal ability of microorganisms in order to the economic nutrient removal and water recycling. The massive generated algal biomass during phycoremediation can be efficiently coupled with CO_2 bio-fixation and generation of valuable industrial bioproducts. During CO_2 fixation, the algal cells convert CO_2 into the lipid and other hydrocarbons [8]. This can be also considered as a key solution to provide the required biomass for bioenergy production. There are several studies about the integration of wastewater treatment and biofuel production using the microalgal systems [9-14]. Applying the different marine and freshwater microalgal biomass in domestic [15-18], industrial [19] and mixed agricultural [20, 21] wastewater treatment and the removal efficiency of various nutrients such as nitrogen and phosphorus [22, 23] and metals such as cadmium (Cd), chrome (Cr), nickel (Ni), zinc (Zn), copper (Cu) and mercury (Hg) [24-27] have been investigated. Several microorganisms are used in the phycoremediation including *Spirulina platensis* [28], *Synechocystis salina* [29], *Phormidium bohneri* [30], *Tolypothrix ceytonica* [31], *Cyanosarcina fontana* and *Anabaena oryzae* [32], *Oscillatoria* and *Phormidium* [33]. Among the blue-green algaes, *Trichormus variabilis* (*T. variabilis*, Syn: *Anabaena variabilis* [34]) is a subject of interest because of its capability in both phycoremediation and valuable biomass production. Different types of bioenergy production from *T. variabilis* biomass including liquid [35, 36] and gaseous [37, 38] biofuels have been reported. On the other hand, the phycoremediation potential using this species has been only examined on limited nutrients and heavy metals removal like Cr, Cd, Ni, Pb, Zn, Ca, Mg, SO_4 and phenolic compounds [39-42]. There is a major lack of research on ammonium and phosphate removal from real wastewater streams using this species. The only ammonium removal efficiency reported for *T. variabilis* has been estimated by artificial wastewater supplemented by a low concentration of ammonium ions (0.5 $mg.L^{-1}$) [37]. It should be mentioned that one of the most important problems during the wastewater treatment process is the high ammonium and phosphate content which causes environmental degradation. During the first and the second stages of the wastewater treatment process, sedimentation and decomposition of organic materials are performed. But, a high amount of N and P, which are the main factors in nitrification and pollution of water and soil, remains in the wastewater effluent. Hence, PO_4^{3-}, NH_4^+ and NO_3^-, organic and inorganic hard toxins and heavy metals and inorganic salts, respectively, need to be removed in the third to five stages of the treatment. The cost of covering these three extra stages is ~4 to 16-fold more than that of the first 2 stages of the treatment [33]. For that reason, in this study, the growth performance of *T. variabilis* together with NH_4^+ and PO_4^{3-} removal from an actual effluent obtained from a mixed industrial and municipal wastewater streams were evaluated. Different methods were used to overcome the unknown inhibitory growth compounds in the wastewater. The main objectives of this study can be expressed as (i) study the ammonium and phosphate removal potential from an actual mixed wastewater using *Trichoemus variabilis*, (ii) investigation the effects of wastewater concentration, pH adjustment and aeration on the biomass production via the phycoremediation, and (iii) presenting sustainable strategy in order to integrate the wastewater treatment, CO_2 bio-fixation and biomass production for the bioenergy.

2. MATERIAL AND METHODS

2.1. Cultivation of algae biomass

The biomass of *Trichormus variabilis* was cultivated in BG_{11} culture medium (Table 1) [43]. The samples were grown in the tubes and flasks aerated by bubbling compressed air or agitating in an orbital shaker (IKA labortechnik KS250, Germany) with a speed of 150 rpm at 25 ^oC and under 65.5 $\mu Em^{-2}s^{-1}$illuminations. Samples were grown in an automatic growth chamber to provide a uniform aeration system via bubbling compressed air (Figure 1).

Table 1. BG_{11} culture medium (per litter).

No.	Components	Value
1	$NaNO_3$	1.5 g
2	K_2HPO_4	40 mg
3	$MgSO_4.7H_2O$,	75 mg
4	$CaCl_2.2H_2O$	36 mg
5	Citric acid	6 mg
6	Ammonium ferric citrate green	6 mg
7	$EDTANa_2$	1 mg
8	Na_2CO_3	20 mg
9	H_3BO_3	2.86 mg
10	$MnCl_2.4H_2O$	1.81 mg
11	$ZnSO_4.7H_2O$	0.22 mg
12	$Na_2MoO_4.2H_2O$	0.39 mg
13	$CuSO_4.5H_2O$	0.08 mg
14	$Co(NO_3)_2.6H_2O$	0.05 mg

2.2. Growth parameters and analysis

According to literature, the biomass weight, pH and concentration of the wastewater are the most important parameters in analyzing the microalgae growth [8]. Furthermore, optical density (OD) is considered as a proxy for biomass accumulation [44]. Biomass productivity, OD and pH were measured using fresh weight ($g.L^{-1}$), spectrophotometer (Amersham Ultrospec 1100 Pro UV Vis) at a wavelength of 680nm and pH-meter (pH Electrodes P11, SenTek, UK), respectively. In order to study the effect of primary inoculums and

dilution rate of the wastewaters on *T. variabilis* biomass production and pH changes, a set of experiment was conducted based on two factors of (A): wastewater concentration and (B): three replicates of inoculums. Historical data were analyzed via response surface methodology (RSM) in Design Expert Software using Quadratic Polynomial model. Final optimization was given by considering the inoculums (0-1g) and wastewater concentration (0-100%) in the range of experimental data (as described in section 3.4) to maximize biomass production.

Figure 1. Automatic bubbling growth chamber.

2.3. Wastewater treatment

A mixed wastewater effluent was used. The effluent was centrifuged for 10 min in 15000 RCF to remove solid particles and then the liquid was separated and sterilized by autoclaving at 120^{o}C for 20 min. Inoculums having 0.5 and 1 g biomass was used in 30 mL of each sample prepared in 3 replicates of different concentration (including 10-100 %v/v). The treatment process was performed under the same growth temperature and light condition through applying compressed air for bubbling and orbital shaking for agitation.

2.4. Nutrient removal analysis

Ammonium and phosphate were measured via two LCK test kits of 303 and 348 (Hach Lange, Manchester, UK). Thermostat User Manual (Hach-Lange LT 200, Manchester, UK) and DR3900 spectrophotometer (Hach Company, Loveland, US) were utilized to preheat and read the values, respectively. Briefly, for measuring the ammonium content, at first, 0.2 mL of sample was added into the cuvette test of LCK 303, then, after 15 min in ambient temperature, the value was read by inserting cuvette into the spectrophotometer. The phosphate content was measured using 0.5 mL of sample in a cuvette test of LCK 348 and incubating at 100 ^{o}C for 60 min. The value was read after adding 0.2 mL of reagent.

2.5. CO_2 bio-fixation calculation

There is a close relation between the CO_2 bio-fixation mechanisms and algae growth. Biomass measurement is essential for direct calculating of CO_2. Previous experiments have been illustrated that ~45 % of biomass consists of carbon [45]. Consequently, the amount of recycled CO_2 was estimated based on the generated biomass during phycoremediation process as shown in Equation (1) [46].

$$\text{Amount of } CO_2 \text{ consumed (g)} = 1.88 \times \text{Biomass produced(g)} \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Biomass growth in the culture medium

The biomass was cultivated in a BG_{11} medium as a control system in both bubbling and shaking system (see Figures 2a,b). The growth rate of *T. variabilis* in nitrogen acquisition medium was compared in these two systems (see Figures 2c,d). The algal biomass generates faster when it grows in the aeration (OD_{680} 0.13±0.08 per day) rather than agitation (OD_{680} 0.06±0.03 per day) system. The results of studying the culture turbidity indicated that the algal growth rate enhances by 2-fold under bubbling condition. Therefore, bubbling compressed air into the medium could significantly accelerate and intensificate algal mass production. The same results were observed in *T. variabilis* and the other species such as *Chlorella vulgaris* and *Spirulina platensis* [47, 48] which are indicated a significant increase in OD after 72 hr bubbling air. It was demonstrated that the aeration positively improves the dissolved oxygen level in the medium and influences the proper CO_2 consumption and nutrient absorption in order to gain higher algal specific growth rate [48, 49].

3.2. Biomass production in the wastewater coupled with the bubbling system

According to the advantages of higher biomass generation rate, the wastewater treatment was conducted first under the bubbling condition with the compressed air using the aerated system. The biomass was inoculated into the wastewater effluent which had been prepared in a concentration of 0, 10, 20, 40, 60, 90 and 100 (%v/v). The concentration of 0 was considered as a control system (C) inoculated by the biomass without the wastewater. Initial attempts to grow the algal cells were failed via losing the algal biomass in all

samples except the control ones (see Figure 3a). Pre-treatment and post-treatment labels are illustrated the samples at day 0 and day 14, respectively. The pH measurement results before and after the treatment process showed that the pH in concentrations of 20 % to 100 % significantly increases up to 9.3 as the highest pH level. The pH level was not significantly raised up in the control system and a concentration of 10 % (see Figure 3b). It was suggested that an unsuccessful growth of the biomass can be due to the over stress induced by out of range pH, lacking some essential nutrients required for the growth in the wastewater or the inhibitory effect of the mechanical stimulation of bubbling system on the *T. variabilis* cells which is not adapted to the new stressful growth condition.

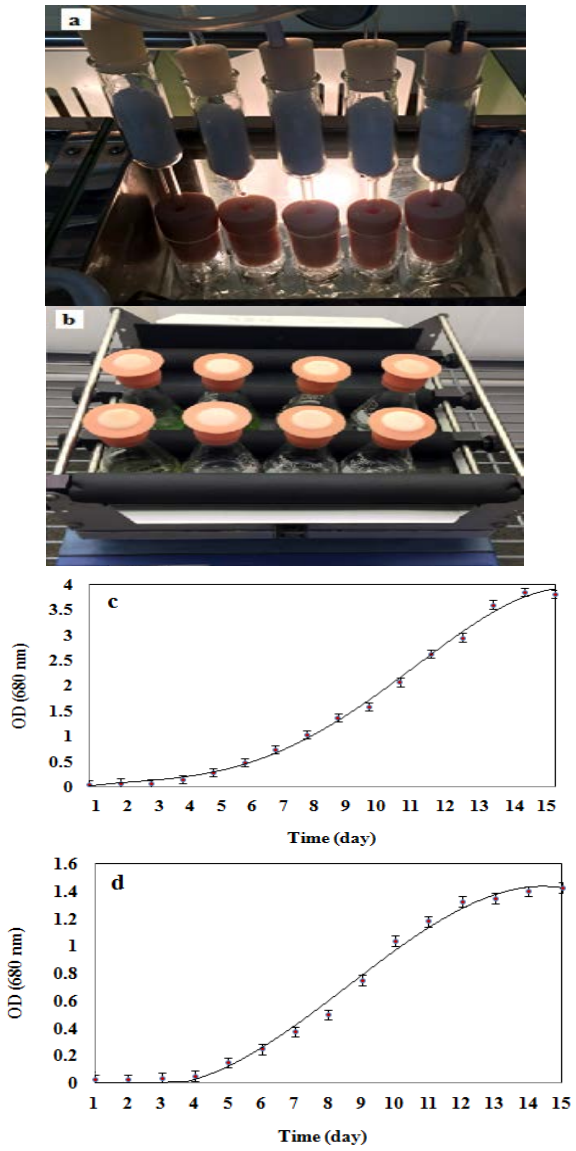

Figure 2. *T. variabilis* growth in BG_{11} medium: (a) the aerated biomass by bubbling compressed air, (b) the agitated biomass by the orbital shaking, the algal growth rate under, (c) the aeration system and (d) agitating system.

The pH effect was previously studied on the growth as chlorophyll (*chl a*) of *T.variabilis* [47]. The results showed that the cells are severely affected at low and high pH. The 1.9-fold decline in the chlorophyll content was recorded at pH 8.5, while the best *T.variabilis* cells growth was reported at pH 7.0 [44, 47]. Hence, the wastewater samples were diluted at different concentrations in the growth medium adjusted in the proper pH, but again no growth was observed. This is likely due to the presence of some growth inhibitory materials in the wastewater which might over react during the bubbling air or due to the mechanical stress of bubbling compressed air on the cells. It was reported that the high rate of aeration (0.8-1.6 $L.min^{-1}$) can negatively decrease the heavy metal removal efficiency [50]. Therefore, the treatment process was resumed using the growth medium and adjusting pH together with removing mechanical stimulation of the bubbling system in order to prevent all unexpected environmental negative effects on the cells.

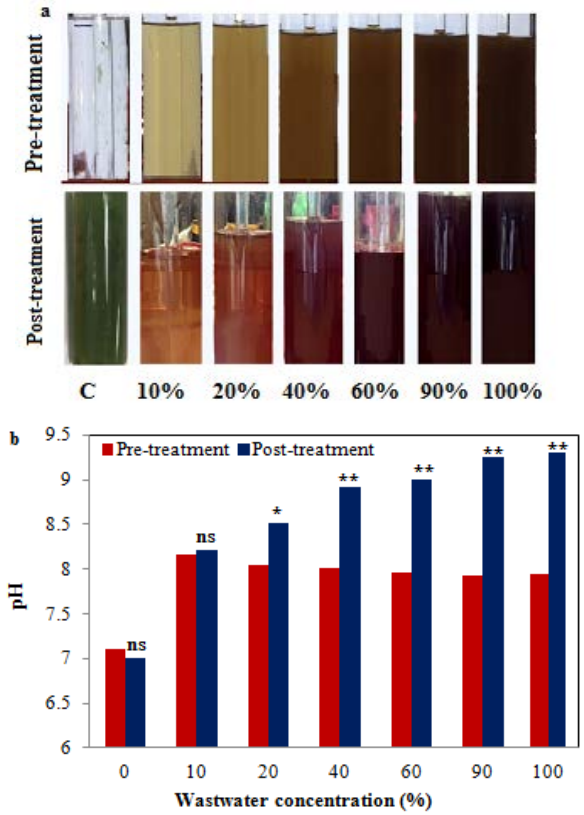

Figure 3. Inoculation of *T. variabilis* cells into a different concentration of the wastewater: (a) failure growth in all concentrations as shown in post-treatment samples, (b) the range of pH changes before and after the process.

3.3. Biomass production in the wastewater coupled with an agitation system

In the next attempt, in order to cover full supplementary nutrients required for the algal growth, the BG_{11}

medium was used to uphold the algal growth. Samples were prepared in 4 concentrations of wastewater (ww) including 10 %, 20 %, 40 %, and 60 % and 3 replicates inoculated by 0.5g (low inoculums: L) and 1g (high inoculums: H) of the algal biomass per 30 mL medium as shown in Figure 4a. The pH was adjusted on 7.1 for all the samples which is the optimum range for the growth of *T. variabilis* in the BG_{11} medium. Flasks were agitated via orbital shaking to remove the bubbling system. The samples were transferred into the falcon tubes and centrifuged after 2 weeks. The green color of the algal biomass settled at the bottom of tubes has been preserved by it (see Figure 4b). A distinct difference was observed between the samples with lower and higher primary inoculation (Figure 4c). The highest algal biomass production (342.3±23.9 $mg.mL^{-1}$) was recorded at 40 %. The results indicated that the aeration via bubbling system significantly enhances the biomass production in the BG_{11} culture medium (as described in section 3.1), however, it seems that the agitation is more efficient for the cell growth in the wastewater stream. It is speculated that continues mechanical stimulation raised by bubbling compressed air has induced additional stress to the cells which are encountered with the stressful wastewater medium. Further research works are required to investigate the aeration effect on different metabolisms of *T. variabilis* during the wastewater treatment.

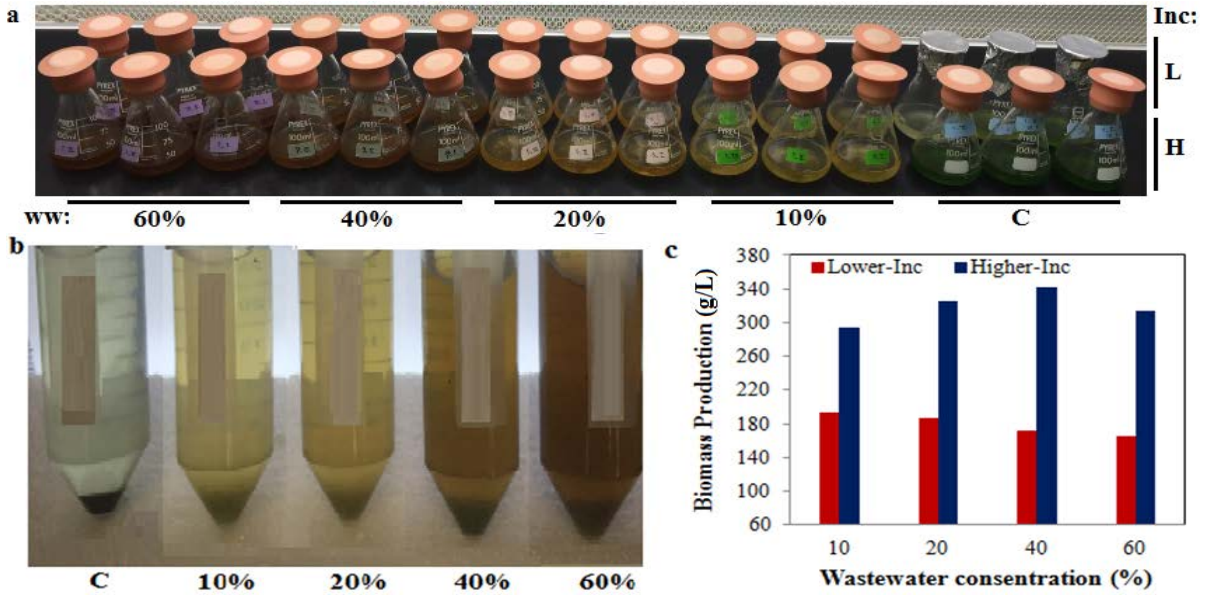

Figure 4. *T. variabilis* grown in the wastewater under the agitation system: (a) inoculation of the samples by different concentrations of wastewater in 2 levels of primary inoculums, (b) the settled alive cells at the bottom of tubes after centrifuging, (c) biomass production in different wastewater concentrations.

3.4. RSM data analysis

The biomass production results in the wastewater under the agitation condition demonstrated different growth rates in response to the different primary inoculums and wastewater concentrations. Here, the effect of these two parameters on the biomass production is investigated by considering the Quadratic Polynomial model in RMS. Based on the designed model, in terms of actual factors, final equations of A: wastewater concentration (%) and B: inoculums (g) are presented in Equations (2) and (3).

$$R1=(-3.38E-0.003)A^2+(0.12)AB+(3.16)B+(0.17)A+ 0.79 \quad (2)$$

$$R2=(-9.07)A^2-(0.02)AB+(0.99)B+(0.09)A+6.65 \quad (3)$$

where R1 and R2 are recorded responses for biomass production and pH, respectively. Experiments design including factors and responses are presented in Table 2. The results of comparing two different levels of inoculations illustrated that the lower primary inoculums do not have a successful function in higher concentrations of the wastewater. The maximum biomass production (10.83±2.82 g) was recorded at 40 % of the wastewater concentration. The pH level was in the range of 6.4±0.6<pH< 8.26±06.

Figure 5 shows contour plots of the biomass production and pH changes as responses of interaction between two factors of A and B.

Increasing both the primary inoculum and wastewater concentration led to enhance the biomass production and pH level (see Figure 5). But, this improvement stopes at the specific level at the wastewater concentration of ~40 % in which the higher concentrations cause lower biomass generation. Because of using actual mixed wastewater in this study, the combination of various chemical industrial and domestic wastes was not chemically defined. Thus, an

inhibitory compound in the wastewater can restrict the cells growth at the higher concentrations. The result of ANOVA is presented in Table 3. The model is significant. Moreover, A, B, AB, A^2 are significant model terms. It should be noted that the recorded data were used to predict the inoculums optimum range and wastewater concentration. According to the optimized solution provided by the software, the best option with the desirability of 95.8 % was suggested in the primary inoculums of 1 g per 30 mL medium (~30mg.mL^{-1}) and wastewater concentration of 43.75 %.

Table 2. Design of experiments, factors, and responses.

Run	Factor A	Factor B	Response1	Response2
	Concentration (%)	Inoculum (g)[1]	Biomass (g)[1]	pH
1	0	1	2.67	7.275
2	0	1	2.592	8.63
3	0	1	2.259	7.198
4	0	0.5	1.11	6.92
5	0	0.5	3.198	6.915
6	0	0.5	1.119	6.408
7	10	1	9.81	8.093
8	10	1	8.856	8.458
9	10	1	7.752	8.226
10	10	0.5	5.262	8.23
11	10	0.5	4.5	8.333
12	10	0.5	7.566	8.1
13	20	1	8.7	8.691
14	20	1	7.551	8.67
15	20	1	7.707	8.668
16	20	0.5	3.834	8.58
17	20	0.5	6.15	8.682
18	20	0.5	6.84	8.648
19	40	1	10.506	8.501
20	40	1	9.462	8.612
21	40	1	10.839	8.68
22	40	0.5	4.338	8.48
23	40	0.5	4.623	8.64
24	40	0.5	5.859	8.64
25	60	1	8.745	8.49
26	60	1	8.76	8.691
27	60	1	10.755	8.64
28	60	0.5	5.166	8.507
29	60	0.5	5.256	8.753
30	60	0.5	5.001	8.656

[1] Weight was expressed in terms of g per 30 mL medium.

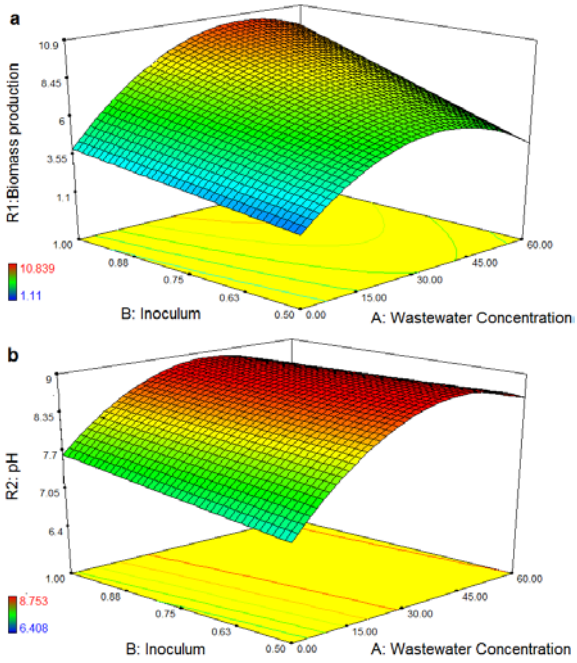

Figure 5. Contour plots for (a) biomass production and (b) pH level changes in response to different wastewater concentrations and inocolum.

Table 3. Variance analysis for the response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value * Prob > F
Model	182.050	4	45.512	19.579	< 0.0001
A:astewater Concentration	23.635	1	23.635	10.168	0.0038
B:Inoculums	70.274	1	70.274	30.232	< 0.0001
AB	12.527	1	12.527	5.389	0.0287
A^2	44.424	1	44.424	19.111	0.0002
Residual	58.112	25	2.324		
Lack of Fit	37.123	5	7.424	7.074	0.0006
Pure Error	20.989	20	1.049		
Cor Total	240.162	29			

* Values of "Prob > F" less than 0.05 indicate that the model terms are significant.

3.5. Phycoremediation with the potential for the CO_2 bio-fixation and bioenergy production

In this study, a real removal potential of *T. variabilis* was investigated using the actual mixed wastewater. Thus, the various growth inhibitory factors, which were eliminated in the previous study, were considered here. The phycoremediation potential of the cells was investigated via analyzing the ammonium and phosphate removal (Table 2). The results of remediation showed that the highest NH_4^+ and PO_4^{3-} removal efficiency were recorded for 42.64 % and 75.22 %, correspondingly. Investigating NH_4^+ and PO_4^{3-} removal

through the real wastewater stream lead to an actual image of *T. variabilis* nutrient removal potential which provides a path with scaling up the process in the industrial application for further research.

Table 4. The wastewater analysis and nutrient removal efficiency.

Wastewater concentration (%)	Pre-treatment ($mg.L^{-1}$)		Post-treatment ($mg.L^{-1}$)		Removal Efficiency (%)	
	NH_4^+	PO_4^-	NH_4^+	PO_4^-	NH_4^+	PO_4^-
20	324	39.2	229	20.5	29.32	47.83
40	746	59.2	455	21.51	39.07	63.67
60	1060	87.2	608	21.6	42.64	75.22

The optimum environmental factors positively improve biomass productivity. The higher biomass production consequently requires more nitrogen and phosphorus macro-elements for cellular construction which causes higher ammonium and phosphate removal. For instance, it was illustrated that exceeding temperature beyond optimum levels leads to cell losses as well as the removal efficiency decline [8]. Therefore, the parameters such as temperature, light, culture medium, gas mixture and the presence of supplementary nutrients can control the nutrient removal via direct impact on the algal cells growth. Nitrogen and phosphorous are two main components necessary for the cell buildup. The mechanisms of ammonium and phosphate removal in the microalgae systems were reviewed suitably [51]. In the different types of wastewater, particularly in domestic streams, N and P sources are found in the forms of ammonium, nitrate, and orthophosphates which can be consumed by the cells to develop the cellular constituents. It was shown that direct N-removal carry out via assimilation or uptake of nitrogen by the algal cells [52]. Furthermore, P is taken up as the orthophosphates and can be stored as the polyphosphate granules. Nonetheless, in the lack of inorganic orthophosphates, organic P can be taken up and converted to the orthophosphate at the cell surface via the phosphate enzymes function [53]. Therefore, the algal cells act as a factory during the phycoremediation process to uptake macronutrients and convert them into the vital component required for different biological metabolisms. In addition to N and P, for growth and construction of necessary carbon components, the algal cells consume their required carbon source in the form of CO_2. Taken advantages of the high growth rate of microalgae, the CO_2 removal efficiency of these microorganisms are considerably higher than the plants [46]. In this study, based on the highest biomass production during the phycoremediation of the mixed wastewater stream, the highest CO_2 bio-fixation was recorded at 9.19±0.64 $g.L^{-1}$. This would be equal to the annual recycling amount of 335.55±23.47 $kgCO_2.m^{-3}.year^{-1}$. Furthermore, valuable biomass can be harvested for different types of biofuel production. The potential of bioenergy production using this species is summarized in Table 5.

The various cultivation factors affect the algal biomass composition [6] which directly influence the biofuel production. The biochemical composition of *T. variabilis* under free-nitrogen growth condition was reported elsewhere [54]. The highest lipid content of this species as the main factor in biodiesel production under ultrasound treatment at 200 W is equal to ~47 % [35]. Applying supercritical fluid and lyophilisation [36, 55] with an average productivity of ~2.3 $g.L^{-1}$ [55] was investigated by bio-ethanol production potential.

Table 5. Bioenergy production potential using *T. variabilis.*

Products	Method/treatment	Yield	Ref
Lipid	N- depleted medium	10.5 %	[54]
	Ultrasound	46.9 %	[35]
Bio-ethanol	Supercritical fluid	2.28 $g.L^{-1}$	[36]
	Supercritical fluid followed by fermentation	24.1 %	[55]
	Lyophilization followed by fermentation.	13.6 %	
Biogas	Anaerobic cellulolytic substrate, methanogenic *Archaea*, and the genera *Methanoculleus* and *Methanosarcina.*	64 %	[38]
	Immobilised methanogenic bacteria and *Rhodobacter capsulatus* on polymeric matrix in anaerobic bioreactor.	450 $mL.g^{-1}$ biomass	[56]
Bio-hydrogen	N- depleted BG medium + 10 mM glucose.	49 $\mu mol.mg\ chl\ a^{-1}.h^{-1}$	[57]
	N- depleted BG medium + 45 $mL.min^{-1}$ Ar.	0.9 $mL.g\ cell^{-1}.h^{-1}$	[58]
	N-aquisition BG medium + 100 % Ar.	~6 $mg.g\ cell^{-1}.h^{-1}$	[59]
	AA medium in Hydrophilic cuprammonium rayon hollow fibers PBR, 100 % Ar.	17-20 $mg\ H_2\ g\ cell^{-1}\ h^{-1}$	[37]
	AA medium + Ar + 5 % CO_2, 77 mM Tween 85	0.44 $mL.mg\ cell^{-1}.h^{-1}$	[60]

Besides, *T. variabilis* biogas production was surveyed with the highest methane production yield of 64 % during anaerobic digesters [38] and in combination with immobilizing technologies [56]. Hydrogen is the most common form of the bioenergy production using this species with an average production rate of $185\mu mol.m^{-2}.s^{-1}$ [49]. Promising results for the bioenergy production potential by means of *T. variabilis* and this cyanobacteria provides an opportunity to develop an

integrated process for the wastewater treatment, CO_2 bio-fixation, and bioenergy production.

4. CONCLUSIONS

The ammonium and phosphate removal efficiency of *Trichormus variabilis* grown in actual mixed wastewater stream was investigated under fast and slow growth rate by applying bubbling and agitation system, respectively. The results illustrated that bubbled air can effectively enhance biomass productivity by 2-fold, but it is not successful in the wastewater treatment process due to the high pH level. This failure in the growth was compensated by adjusting pH and providing the required nutrient to support the algal biomass growth in the wastewater and also removing mechanical stimulation of bubbling system to prevent all unexpected environmental negative effects on the cells. The results demonstrated successful NH_4^+ and PO_4^{3-} removal with the values of ~43 % and ~75 %, respectively. Study the effects of the primary inoculums and wastewater concentration indicates that the highest biomass can be generated at 40 % wastewater diluted by the BG_{11} medium with the highest CO_2 bio-fixation of 9.19 ± 0.64 $g.L^{-1}$. In general, the agitation system will prolong the growth, while it provides an opportunity for the cells for resisting against the stressful growth condition with enough time in order to absorb ammonium and phosphate from the medium. Therefore, *T. variabilis* provide a promising alternative solution for removing a considerable amount of ammonium and phosphate and recycling CO_2. It can couple with bioenergy production and positively affect the operation cost of the industrial wastewater treatment process.

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