

Article

Enhanced removal of nitrate and phosphate from wastewater by *Chlorella vulgaris*: Multi-objective optimization and CFD simulationMohammad Bagher Sabeti^{1,2}, Mohammad Amin Hejazi^{2,*}, Afzal Karimi³¹ Faculty of Chemical and Petroleum Engineering, University of Tabriz, Tabriz, Iran² Department of Food Biotechnology, Branch for Northwest & West Region, Agricultural Biotechnology Research Institute of Iran, Agricultural Education and Extension Organization (AREEO), Tabriz, Iran³ Department of Biotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

To enhance the efficiency of wastewater biotreatment with microalgae, the effects of physical parameters need to be investigated and optimized. In this regard, the individual and interactive effects of temperature, pH and aeration rate on the performance of biological removal of nitrate and phosphate by *Chlorella vulgaris* were studied by response surface methodology (RSM). Furthermore, a multi-objective optimization technique was applied to the response equations to simultaneously find optimal combinations of input parameters capable of removing the highest possible amount of nitrate and phosphate. The optimal calculated values were temperature of 26.3 °C, pH of 8 and aeration rate of 4.7 L·min⁻¹. Interestingly, under the optimum condition, approximately 85% of total nitrate and 77% of whole phosphate were removed after 48 h and 24 h, respectively, which were in excellent agreement with the predicted values. Finally, the effect of baffle on mixing performance and, as a result, on bioremoval efficiency was investigated in Stirred Tank Photobioreactor (STP) by means of Computational Fluid Dynamics (CFD). Flow behavior indicated substantial enhancement in mixing performance when the baffle was inserted into the tank. Obtained simulation results were validated experimentally. Under the optimum condition, due to proper mixing in baffled STP, nitrate and phosphate removal increased up to 93% and 86%, respectively, compared to unbaffled one.

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1. Introduction

The use of microalgae cultures for removing nutrients from wastewater with high amounts of nitrogen and phosphorus compounds has gained great interest recently. Nitrate and phosphate are the usual forms of nitrogen and phosphorus in the nature, respectively, and considered as the principal nutrients to be eliminated in order to control eutrophication [1]. The most common procedure of removing inorganic nitrate is denitrification, leading to the reduction of nitrate to nitrogen by bacteria. In the case of inorganic phosphate removal, the most common way is physicochemical dephosphatization. However, high operational costs of these two types of removal methods, due to the significant consuming of energy and chemicals, cause less interest in these common processes [2].

Algae based biological treatment is ecofriendly and offers the advantage of a cost effective approach of nutrient removal and biomass production [3]. With this technique the nutrients in the wastewater are removed through the photosynthesis of microalgae by uptake as biomass [4]. In addition to nutrient consuming, algae effectively

provides the needed oxygen for degrading toxic organic contaminants by aerobic bacteria [5]. Furthermore, waste grown microalgae have widely varying lipid contents which could be used as a possible feed-stock for biodiesel/biofuel production [6–10]. Due to the ability of microalgae to adapt their metabolism to a wide range of habitats, they could be cultivated in water of various sources such as fresh and seawater, domestic and industrial effluents [11]. The wastewater biotreatment with microalgae is related to the growth of the cells under different environmental conditions [12]. Understanding the physiological response of microalgae to the environmental conditions may help in providing the right culture conditions in order to improve the efficiency of the biological systems [11].

The freshwater unicellular green microalgae of the genus *Chlorella* are one of the most extensively used microalgae for nutrient removal. Based on literature review, this microalga has high potential in nutrient removal and could be incorporated as a step of wastewater treatment program [13–19].

Mixing is one of the most common and important operations in industrial processes that reduces non-uniformities in fluids by eliminating gradients of concentration, light, temperature and other properties [20]. There are some ways to improve the mixing efficiency of the reactor while reducing the size of the dead zones. Earlier studies have indicated

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that the use of suitable baffle configuration can act as a continuous static mixer and offers numerous advantages of increasing power input, improving mechanical stability, reduction of overall device dimensions, reduction of energy consumption, better process control and shorter residence time [20,21]. Computational Fluid Dynamics (CFD) is a powerful tool to visualize and investigate the fluid flow and improve our understanding of mixing phenomenon [21–23].

In the present study, the effect of temperature, pH and aeration rate on nitrate and phosphate removal by *Chlorella vulgaris* was investigated in a STP. To our best knowledge, most of the previous studies represent the individual effect of variables on the nutrient removal. This is while interactions between variables may have a greater effect on nitrate and phosphate removal than the direct effects of each factor. In order to evaluate the interaction between the variables, a multi-objective optimization study was conducted using response surface methodology (RSM) to maximize removal efficiency. Finally, the effect of the baffle on mixing efficiency and thus on removal was investigated in STB by means of the CFD.

2. Materials and Methods

2.1. Algal strain and culture medium for pre-culture

C. vulgaris 211-11C was provided from the culture collection of Agricultural Biotechnology Research Institute of Iran (ABRII). The strain was preserved in Bold's Basal (BB) medium, with a little change in composition, containing the following compounds in ($\text{g} \cdot \text{L}^{-1}$): KNO_3 , 1.011; KH_2PO_4 , 0.0136; NaCl , 0.5; MgSO_4 , 0.05; and CaCl_2 , 0.04. The concentration of trace elements in ($\text{mg} \cdot \text{L}^{-1}$) was: H_3BO_3 , 5.72; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 2.60; ZnSO_4 , 6.4; CuSO_4 , 3.66; Na_2MoO_4 , 0.042; CoCl_2 , 0.042; EDTA, 1.5; and FeSO_4 , 25. The initial pH of the medium was adjusted to 6.8 and sterilized at 121°C for 20 min before inoculation. The microalga was cultured in 250 ml Erlenmeyer flasks with the working volume of 100 ml in a shaking incubator at 25°C and $150 \text{ r} \cdot \text{min}^{-1}$ under light. The intensity of the continuous fluorescent light was $40 \mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, measured by a light meter (model LI-250A, LI-COR, USA). The microalga cells were used to inoculate the STP after 2 weeks of inoculation.

2.2. Synthetic wastewater

Wastewater contains large amount of materials like toxins and microbes. To achieve accurate investigation and prevent other microorganisms affecting microalga growth or nutrient removal, artificial wastewater was synthesized. The composition of artificial wastewater was the same as the pre-culture medium. However, only the nitrate and phosphate concentrations were fixed at $250 \text{ mg} \cdot \text{L}^{-1}$ and $22 \text{ mg} \cdot \text{L}^{-1}$, respectively, based on nitrate and phosphate levels in groundwater of the Great Park in Tabriz city.

2.3. Operation of STP

The STP was a water jacket glass vessel (BioFlo 110 Modular Fermentor), with a working volume of 10 L (Fig. 1). The STP culture medium was autoclaved at 121°C for 20 min. After STP sterilization, a certain volume of pre-cultures was centrifuged, and after disposing supernatant, inoculated into the STP inasmuch as OD_{680} (optical density at a wavelength of 680 nm) of the environment reached to 0.3. The culture was continuously stirred at $150 \text{ r} \cdot \text{min}^{-1}$ and aerated by filtered air at the specific rates based on experimental design. The pH value was controlled simultaneously by automatic CO_2 injection and adding of base ($0.1 \text{ mol} \cdot \text{L}^{-1} \text{ NaOH}$) and acid ($0.1 \text{ mol} \cdot \text{L}^{-1} \text{ H}_2\text{SO}_4$) with peristaltic pumps that were connected to the control system. The vessel was illuminated from four sides by eight fluorescent lamps (CONCENTRA SPOT R63, 60 W, OSRAM, France). The average light intensity was equal to $500 \mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the surface of STP.

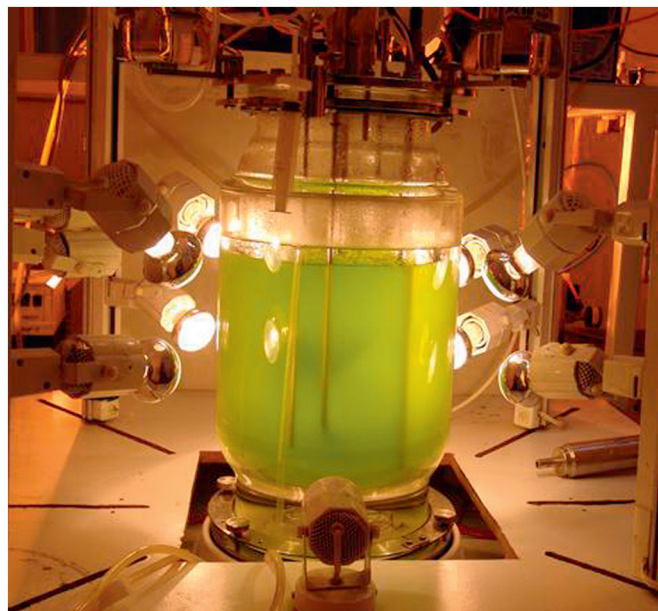


Fig. 1. The picture of used photobioreactor.

2.4. Experimental design

RSM is a statistical method employed to analyze and optimize problems which is widely used for experimental design [23]. Application of RSM provides opportunity to quantify the relationship between controllable input variables and the obtained response surfaces. The following second-order polynomial response equation was used to correlate the dependent and independent variables:

$$Y = b_0 + \sum_{i=1}^n b_i x_i + \sum_{i=1}^n b_{ii} x_i^2 + \sum_{j=1}^n \sum_{i=1}^n b_{ij} x_i x_j \quad (1)$$

where Y is the response, the x_i is the coded experimental level of the variables and b_0 is the interception term. The b_i determines the influence of the variable i in the response (regression coefficients for linear effects), the b_{ii} is the parameter that defines the shape of the curve (regression coefficients for squared effects) and b_{ij} refers to the effect of the interaction among variables i and j (regression coefficients for interaction effects).

Eq. (2) was used to transform the natural variables (X_i) into codified variables (x_i).

$$x_i = \frac{X_i - X_0}{\delta X} \quad (2)$$

where X_0 is the value of X_i at the center point and δX represents the step change.

In the present study a Central Composite Design (CCD) was used for the optimization of the nutrient removal process. The experimental ranges and the levels of the independent variables for the nitrate and phosphate removal process are given in Table 1. A total of 16 experiments, including eight cubic points, six axial points ($\alpha = 1.68$) and

Table 1
Experimental ranges and levels of the independent test variables

Variables	Ranges and levels				
	−1.68	−1	0	+1	+1.68
Temperature(X_1)/ $^\circ\text{C}$	16.6	20	25	30	33.4
pH (X_2)	5	6	7.5	9	10
Aeration rate(X_3)/ $\text{L} \cdot \text{min}^{-1}$	1.3	2	3	4	4.7

Table 2

The 3-factor Central Composite Design matrix and the experimental and predicted response values

Run	Temperature/°C	pH	Aeration rate/L·min ⁻¹	Nitrate removal percentage in 48 h/%		Phosphate removal percentage in 24 h/%	
				Experimental	Predicted	Experimental	Predicted
1	20	6	4	25.5	33.95	23.81	24.14
2	30	9	2	64.88	55.66	76.19	73.74
3	30	9	4	69.81	62.28	80.95	79.85
4	25	7.5	3	85.01	85.45	58.67	58.84
5	25	10	3	15.38	21.78	93.55	99.57
6	20	6	2	21.02	27.33	17.52	15.99
7	30	6	4	50.00	47.75	33.33	36.96
8	25	7.5	4.7	90.00	88.34	64.28	64.63
9	25	7.5	3	86.00	85.453	59.52	58.84
10	25	5	3	10.98	5.76	14.28	9.70
11	30	6	2	44.02	41.13	25.67	28.04
12	16.6	7.5	3	33.06	21.96	38.09	43.96
13	20	9	2	30.52	31.85	85.71	79.97
14	20	9	4	35.96	38.47	90.47	85.31
15	25	7.5	1.3	75.86	77.20	50.35	52.64
16	33.4	7.5	3	42.35	53.58	52.38	49.51

two replications at the center point were employed in this work (Table 2). To minimize the effect of unexplained variability on the response values the simulations were performed randomly. Experimental data were analyzed using the Minitab 15 software.

2.5. Analytical procedures

Samples were taken from STP each day for analyses. The cell concentration of the culture was determined by the measurement of optical density at the wavelength of 680 nm (i.e. OD₆₈₀) [24] using a spectrophotometer (Perkin Elmer LAMBDA 35 UV/Vis spectrophotometer). A linear relationship between OD₆₈₀ and dry cell mass (mg·ml⁻¹) was determined previously (Eq. (3)).

$$\text{Dry mass} = 0.3768 \times \text{OD}_{680}, R^2 = 0.997 \quad (3)$$

Nitrate and phosphate contents were measured according to the standard methods for the examination of waters and wastewaters [25]. Samples were centrifuged at 6000 r·min⁻¹ for 10 min and being filtered through 0.45 μm membranes to determine nitrate and phosphate concentration in the supernatants. All measurements were carried out in triplicate.

2.6. Simulation model and numerical details

Two STPs with baffle and without baffle configuration were modeled. The diameter and height of the STPs were 20 cm and 26 cm, respectively. A 45° pitched blade impeller with 3 blades ($D = 11$ cm), mounted on a 2 cm diameter shaft placed at the centerline of the reactors and located at 19 cm from the headplate of the vessels, was used for agitation. The impeller rotational speed was fixed at 120 r·min⁻¹. The air particles were supplied through a 0.05 m diameter ring sparger with 7 holes ($d = 1$ mm) located over and under the sparger (Fig. 2). In baffled STP, four baffles with 23 cm length, 1.7 cm width, and 1 mm thickness were set at 3 mm away from the tank wall. Geometry and mesh were generated by using GAMBIT 2.4.6 software. Tetrahedral unstructured cells were used in the simulation of the baffled and unbaffled STPs. In baffled one for ensuring mesh independency, three mesh sizes including (a) 423071, (b) 790832 and (c) 972126 cells were examined using the mean velocity magnitude at the STP. Difference between mean velocity magnitude of cases (a) and (b) was 5.2% while this difference between cases (b) and (c) was just 0.6%. Therefore, case (b) was chosen for baffled STP. Also in unbaffled STP three mesh sizes including (a) 128288, (b) 187487 and (c) 220256 cells were examined using the mean velocity magnitude at the STP. Difference between mean velocity magnitude of cases (a) and (b) was 4.7% while this difference between cases (b) and (c) was just 0.4%. Therefore, case (b) was chosen for

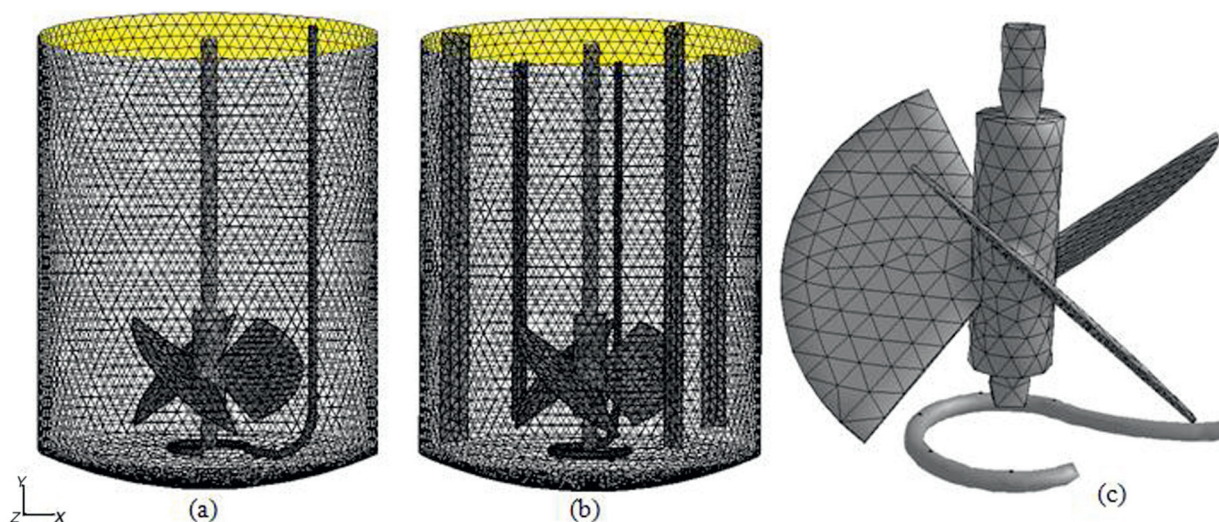


Fig. 2. Reactor grid. (a) unbaffled STP; (b) baffled STP; (c) impeller and sparger.

unbaffled STP. Fig. 2 demonstrates the geometry configuration and discretized domain of the STPs, impeller and sparger.

Numerical solution was carried out using CFD code Fluent 6.3.26. For precise modeling of impeller effect on the hydrodynamic behavior, the multiple reference frame (MRF) technique was employed. In this method, the impeller became stationary with respect to the rotating frame. Therefore, solution can be performed without the need for a complex and time-consuming dynamic mesh technique [23]. For impeller, shaft, baffles and all parts of the reactor wall except the top part, wall boundary condition was used; also no-slip shear condition was applied for these surfaces. The top boundary of the reactor was defined as symmetry boundary condition, thus zero normal gradients and no-penetration conditions were applied for all variables. The Green–Gauss cell Based Gradient option was used for gradient calculations at cell interfaces. Second-order upwind discretization was employed in the solving process for all governing equations except pressure for which the PRESTO scheme was utilized for discretization. Moreover, the PISO algorithm was used for the velocity–pressure coupling. The unsteady state solver was utilized to solve for all flow variables. In this work, single-phase Reynolds averaged Navier–Stokes equations with a standard $k-\epsilon$ turbulence model were solved using finite volume discretization. Under these assumptions, the continuity equation (Eq. (4)) and momentum conservation equation (Eq. (5)) were used in CFD modeling and are as follows:

$$\frac{\partial(\rho)}{\partial t} + \nabla(\rho \bar{V}) = 0 \tag{4}$$

$$\frac{\partial}{\partial t}(\rho \nabla) + \nabla(\rho \bar{V} \bar{V} + \rho \bar{v} \bar{v}) = -\nabla \bar{P} - \nabla \bar{\tau} \tag{5}$$

where ρ is the density, V is the velocity of fluid, P is the pressure, τ is the viscous stress tensor and v is the fluctuating flow velocity. DPM based on the Euler–Lagrange approach was employed to simulate air particle injection in vessels [26]. The dispersion of particles due to turbulence was predicted using the stochastic tracking model, which includes the effect of instantaneous turbulent velocity fluctuations on the particle trajectory. FLUENT predicts the trajectory of a discrete phase particle by integrating the force balance on the particle. This force can be written as (Eq. (6)) (for y direction):

$$\frac{dV_p}{dt} = F_D (V - V_p) + \frac{g_y (\rho_p - \rho)}{\rho_p} \tag{6}$$

$$F_D = \frac{18\mu C_D Re}{\rho_p d_p^2 24} \tag{7}$$

where $F_D(V - V_p)$ is the drag force per unit particle mass, V_p is the particle velocity, μ is the molecular viscosity of the fluid, ρ_p is the density of the particle, g_y is the gravitational acceleration in y direction, d_p is the particle diameter and C_D is the drag coefficient [26]. The air particles as discrete phase were injected to the STPs through the sparger holes with a constant diameter. A time step of 0.01 s is applied for both time integration and particle time step size with 10 iterations of continuous phase per an iteration of DPM. The characteristics of discrete phase and water as primary phase were shown in Table 3.

Table 3
Primary phase and discrete phase properties

Characteristic	Value	Characteristic	Value
Air particle diameter	0.4 cm	Water density	998.2 kg·m ⁻³
Particle density	1.225 kg·m ⁻³	Water viscosity	1.003 × 10 ⁻³ kg·m ⁻¹ ·s ⁻¹
Particle injection speed	9 m·s ⁻¹	Gravitational acceleration (g_y)	−9.8 m·s ⁻²
Particle flow rate	6 × 10 ⁻⁵ kg·s ⁻¹	Drag coefficient (C_D)	Spherical drag law [26]

3. Results and Discussion

3.1. Construction of the models

The experimental results and predicted values for nitrate and phosphate removal percentage are presented in Table 2. Based on these results, the following second-order polynomial response equations were used to correlate the dependent and independent variables (the non-significant terms was removed when necessary (P -value > 0.05)):

$$Y_1 = 85.4530 + 9.4025X_1 + 4.7628X_2 + 3.3117X_3 - 16.8571 X_1^2 - 25.3424X_2^2 - 0.9472X_3^2 + 2.5X_1X_2 \tag{8}$$

$$Y_2 = 53.8387 + 2.7710X_1 + 44.9349X_2 + 5.9963X_3 - 12.1057 X_1^2 - 4.2009X_2^2 - 0.2009X_3^2 - 129300X_1X_2 + 0.5387X_1X_3 - 1.9866X_2X_3 \tag{9}$$

where Y_1 is the response variable of nitrate removal percentage in 48 h, Y_2 is the response variable of phosphate removal percentage in 24 h; and X_1 , X_2 and X_3 indicate the uncoded experimental levels of temperature, pH and aeration rate, respectively. Due to the low concentration of phosphate compared to nitrate, removal of this contaminant was conducted entirely at 48 h almost in all experiments. That is why, for accurate investigation and to provide an appropriate model, phosphate removal percentage in 24 h was considered as a response.

Tables 4 and 5 show the results of the quadratic response surface model fitting in the form of analysis of variance (ANOVA) for nitrate and phosphate removal percentage. ANOVA is required to test the significance and adequacy of the model. The F -test of the regression model produced low P -value, referring that the model was of high significance. Moreover, the regression model had a high value of determination coefficient ($R_1^2 = 92.73\%$, $R_2^2 = 97.93\%$). The R^2 -value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. This implies that 92.73% and 97.93% of the variations are explained by the independent variables for nitrate and phosphate removal percentages, respectively.

Table 4
ANOVA for fit of nitrate removal percentage

Source of variations	Sum of squares	Degree of freedom	Adjusted mean square	F -value	P -value
Regression	9745.9	9	1082.88	8.51	0.008
Residuals	763.8	6	127.30		
Total	10509.8				

Note: $R^2 = 92.73\%$.

Table 5
ANOVA for fit of phosphate removal percentage

Source of variations	Sum of squares	Degree of freedom	Adjusted mean square	F -value	P -value
Regression	10335.3	9	1148.37	31.46	0.000
Residuals	219.0	6	36.5		
Total	10554.3				

Note: $R^2 = 97.93\%$.

3.2. Nitrate and phosphate removal

The experimental and predicted response values for nitrate and phosphate removal were shown in Table 2. The highest nitrate removal percentage (90% in 48 h) was achieved in the temperature of 25, pH of 7.5 and aeration rate of $4.68 \text{ L} \cdot \text{min}^{-1}$. Also, the best result for removal of phosphate (93.55% in 24 h) occurred at the temperature of 25°C , pH of 10 and aeration rate of $3 \text{ L} \cdot \text{min}^{-1}$. Table 6 shows the nitrogen and phosphorus removal efficiencies from several wastewater sources by different strains of *Chlorella*. As it is obvious the remarkable results, almost for all experiments, were obtained in terms of nitrate and phosphate removal percentage compared to other studies (Tables 2 and 6).

3.3. Effect of temperature and pH on nitrate removal efficiency

The 2D contour plots, described by the regression model, were drawn to explain the effects of the independent factors and the interactive effects of each factor on the responses. Fig. 3 illustrates the effect of temperature and pH on nitrate removal percentage for an aeration rate of $3 \text{ L} \cdot \text{min}^{-1}$. As it is obvious from this figure the highest removal percentage of nitrate was obtained near the mean values of temperature and pH ($T = 25^\circ\text{C}$, pH 7.5), where removal of nitrate fell off progressively with higher or lower levels of pH and temperature. Nitrogen source removal by microalga in wastewater treatment processes is mainly reached by assimilation to algal cells. The nitrogen assimilation can be optimized by maximizing algal growth with the aid of high treatment efficiency [27]. Temperature has a direct effect on metabolic activity (i.e., assimilation, photosynthesis rates and respiration), and consequently on the growth rate of microalga [28]. Enzymatic production as an adaptive mechanism for maintaining rates of photosynthesis and respiration increases under the optimal growth temperature [11].

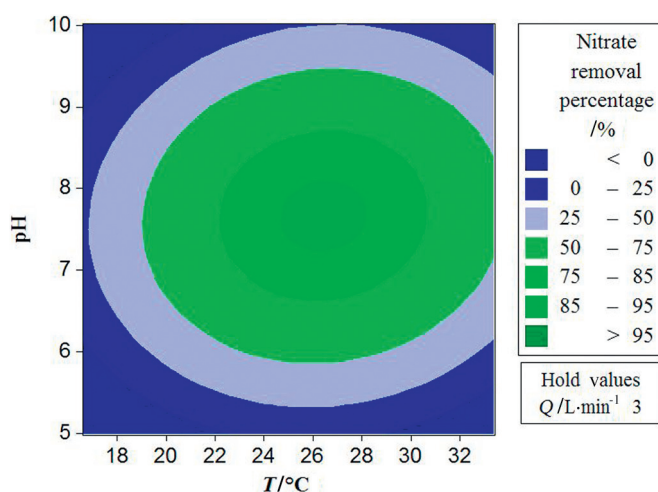


Fig. 3. The contour plot of the nitrate removal percentage (%) as the function of temperature ($^\circ\text{C}$) and pH.

To investigate this issue further and in more detail, growth curve of microorganism within 7 days in three different operating conditions in terms of temperature is compared in Fig. 4. As it can be understood from this figure, there was a significant difference in the growth of microalgae under different temperature conditions. Microalga at 25°C had shorter lag phase (less than 24 h) and has the highest growth rate compared to temperature values of 16.6°C and 33.4°C due to suitable growth condition. Algal cultures grown outdoors are usually subjected to environmental stress such as low temperature. The influence of low temperature on photoinhibition was studied in outdoor cultures by

Table 6

Nitrogen and phosphorus removal efficiencies from wastewater with *Chlorella* microalga strains

Microalga	Wastewater	Initial concentration/ $\text{mg} \cdot \text{L}^{-1}$			Removal/%			Experimental set-up	Ref.
		NO_3^-	NH_4^+	TP	NO_3^-	NH_4^+	TP		
<i>Chlorella</i> sp.	Municipal wastewater	82.5 ± 2	212 ± 7		93.9%		80.9%	Coil BR; $V = 25 \text{ L}$; $T = (25 \pm 2)^\circ\text{C}$; $\text{LI} = 50 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; CT = 14 days; initial pH not defined, without control; without aeration	[13]
<i>Chlorella vulgaris</i>	Synthetic wastewater	20	4		97%		96%	Column BR; $V = 2 \text{ L}$; $T = 30^\circ\text{C}$; $\text{LI} = 40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; CT = 14 days; initial pH = 6, without control; aeration rate = $1000 \text{ ml} \cdot \text{min}^{-1}$	[14]
<i>Chlorella vulgaris</i>	Simulated domestic wastewater	≈ 30	≈ 6		100%		95%	Column BR; $V = 5 \text{ L}$; $T = (23 \pm 2)^\circ\text{C}$; $\text{LI} = 174 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; CT = 2 days; initial pH = 7.5, without control; aeration rate = $35 \text{ ml} \cdot \text{min}^{-1}$	[15]
<i>Chlorella pyrenoidosa</i>	Piggery wastewater	1388	158		Over 90%		Over 30%	Conical flasks; $V = 500 \text{ ml}$; $T = (25\text{--}27)^\circ\text{C}$; $\text{LI} = 63 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ under continuous light; CT = 10 days; initial pH = 8 without control. Aeration rate = $300 \text{ ml} \cdot \text{min}^{-1}$	[16]
<i>Chlorella vulgaris</i>	Industrial wastes	32			33%			Conical flasks; $V = 50 \text{ ml}$; $T = 30^\circ\text{C}$; $\text{LI} = 40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; LDR = 16 h:8 h; initial pH = 5 without control; without aeration	[17]
<i>Chlorella kessleri</i>	Synthetic wastewater	168.1			19%			Conic BR; $V = 100 \text{ ml}$; $T = 30^\circ\text{C}$; $\text{LI} = 45 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; LDR = 12 h:12 h; CT = 3 days; initial pH = not defined, without control; without aeration	[18]
<i>Chlorella pyrenoidosa</i>	Soybean processing wastewater	52.1	56.3		89.1%		70.3%	Conic BR; $V = 500 \text{ ml}$; $T = (27 \pm 1)^\circ\text{C}$; $\text{LI} = 40.5 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; LDR = 14 h:10 h; CT = 5 days; initial pH = 6.5 without control; without aeration	[19]
<i>Chlorella vulgaris</i>	Synthetic wastewater	250	22		85% In 48 h		77% In 24 h	Stirred tank BR; $V = 8 \text{ L}$; $T = 26.3^\circ\text{C}$; $\text{LI} = 500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ under continuous light; CT = 7 days; pH = 8, with control condition; aeration rate = $4.7 \text{ L} \cdot \text{min}^{-1}$; Above conditions	This work at optimum point in unbaffled STP
<i>Chlorella vulgaris</i>	Synthetic wastewater	250	22		93% In 48 h		86% In 24 h		This work at optimum point in baffled STP

BR Bioreactor, V volume, T temperature, LI light intensity, LDR light/dark ratio, CT culture time.

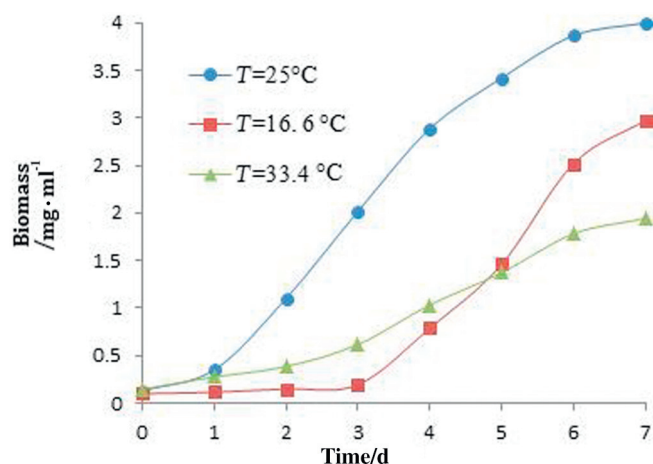


Fig. 4. The growth curve of microalgae at different temperatures (pH = 7.5, aeration rate = 3 L·min⁻¹).

Vonshak *et al.* [29]. It was found that, almost all the photosynthetic parameters decrease when the cultures were exposed to sub-optimal temperature, just for a short time. In another research, exposure of a high temperature adapted algal strain to 10 °C resulted in a 50% decline in chlorophyll-a in 15 h [30]. Furthermore, low temperatures cause cellular accumulation of polyols and amino acids or amino acid derivatives as metabolic defense mechanisms against cold which may cause energy waste by the cell [11,31]. Also, increasing temperature outside the optimum leads to degradation of the protein synthesis and consequently results in decreased growth rate of algae [32]. This unfavorable condition for *C. vulgaris* growth reduces the amount of nitrate consumption by it and thereby decreases the rate of removal of this pollutant ion from the culture. Temperature would be influential on the water ionic equilibrium, pH and gas (oxygen and CO₂) solubility, in addition to intracellular enzymatic activities [31,33]. These results clearly demonstrate that controlling the temperature inside the reactor will be essential to the cell growth and nutrient removal. Growth of *C. vulgaris* over a wide range of temperatures showed the ability of this microorganism to withstand low and high temperature stress (Fig. 4). The optimum temperature range was reported 25 °C–35 °C for growth of *Chlorella* strains [34–36]. However, the optimal temperatures for growth and nutrient conversion differ from each other, therefore the optimal temperature for the culture depends on the aim to be achieved.

The pH of the environment influences many of the biochemical processes related to algal growth and metabolism, comprising uptake of nutrient ions and the accessibility of CO₂ for photosynthesis. The importance of pH in ecology and controlling the growth rate of algae has been indicated in a number of studies [36]. The results of these studies showed a limitation of algal growth and photosynthesis at high pH levels. At alkaline pH levels, the availability of CO₂ decreases that causes reduction in photosynthesis and growth of algal [37]. Similar to alkaline pH, acidic conditions can change nutrient uptake and thus affect algal growth [38]. Fig. 3 illustrates that the nitrate removal efficiency decreased when the pH value was beyond the range of 7–8 and the pH above 9 and below 6 was not appropriate for nitrate removal due to undesirable growth condition. This is a direct result of the biological metabolism reduction due to the decrease in CO₂ and HCO₃⁻, the forms of inorganic carbon that are more easily assimilated by microalgae [5,39]. Also, the enzymatic reaction rate is pH dependent. Any deviation from optimum pH level either higher or lower may result in cellular function impairment and energy losses for maintaining the cell function [37,40]. Although some algae are capable of growing well in high pH values, the optimal pH of many freshwater algae is about 8, thus in pH above or below 8 algal productivity decreases [41,42]. Growth curve of microalgae within 7 days at different pH values is shown in Fig. 5. As can be seen, the microalgae growth rate in the pH value of 7.5 is greater

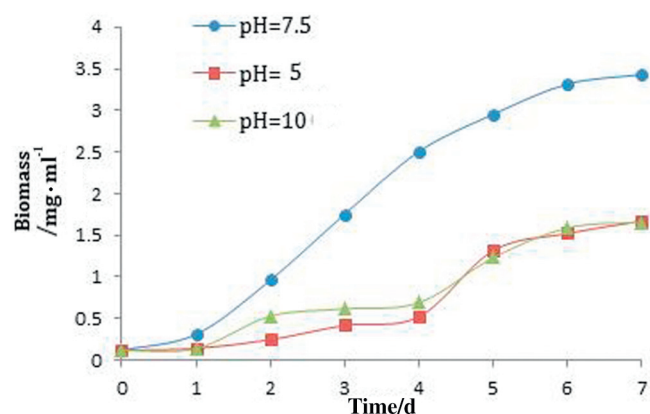


Fig. 5. The growth curve of microalgae at different pH values (T = 25 °C, aeration rate = 3 L·min⁻¹).

than other values so the better biological assimilation as well as faster nitrate removal occurred in this condition (Fig. 3). One of the most significant points of this study is the ability of *C. vulgaris* to grow in pH above 10 and less than 6 (Fig. 5). According to literature, growth of most marine algae at pH higher than 10 and less than 6 due to poor conditions and stressors (inappropriate pH) was practically impossible [37]. The capability of this microalgae to adapt its metabolism to varying pH values shows a high ability of *C. vulgaris* to grow and eliminate contaminants in alkaline and acidic environments.

3.4. Effect of temperature and pH on phosphate removal efficiency

Effects of temperature and pH on the phosphate removal process at the constant aeration rate of 3 L·min⁻¹ are shown in Fig. 6. This figure shows that temperature weakly affected the phosphate removal compared to pH. The best temperature value for phosphate removal was achieved near mean values (T ≈ 25 °C). Increase in pH values contributed to increasing phosphate removal whereas, mean values were better for biological assimilation. Phosphate removal in addition to biological assimilation can be achieved by chemical precipitation with available cations in the water to form metal phosphates. The high pH values led to phosphate precipitation and reduced the concentration of this nutrient in the medium [27]. Nevertheless, to maximize the phosphate removal, it is important to optimize the growth conditions for the microalgae since the assimilation of phosphate generally depends on the microalgae growth.

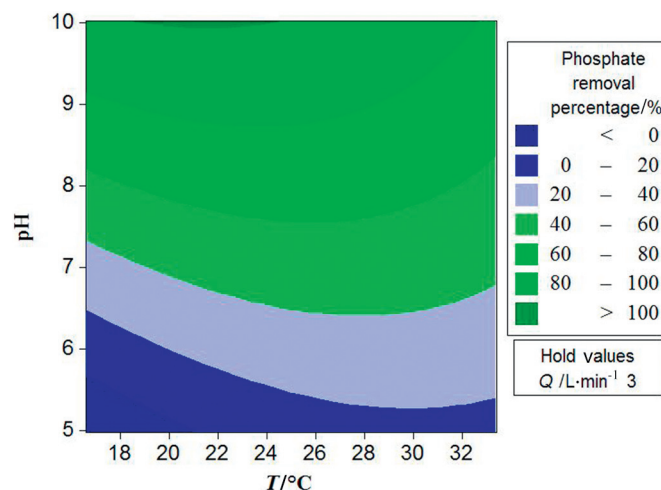


Fig. 6. The contour plot of the phosphate removal percentage as the function of temperature and pH.

3.5. Effect of aeration rate on nitrate and phosphate removal efficiency

Percentage of nitrate and phosphate removal as a function of aeration rate and temperature for constant pH of 7.5 was depicted in Figs. 7 and 8, respectively. As it is clear from these figures, both nitrate and phosphate removal efficiency increased with the increase of aeration rate. Aeration in biological systems could be advantageous to the growth and efficiency of microbial cells by enhancing the mass transfer characteristics. The photosynthetic performance can be improved significantly by increasing the gas flow rate causing to shorter light and dark cycle, efficient release of O_2 and residual gas mixture [43,44]. To investigate this issue better and more accurate, growth curve of the microalga within 7 days at different operating conditions in terms of aeration rate is shown in Fig. 9. As can be seen, increasing aeration intensity had a direct influence on the growth of microalga. As a result, by increasing aeration rate, growth of the microalga and consequently nitrate and phosphate assimilation rate increased.

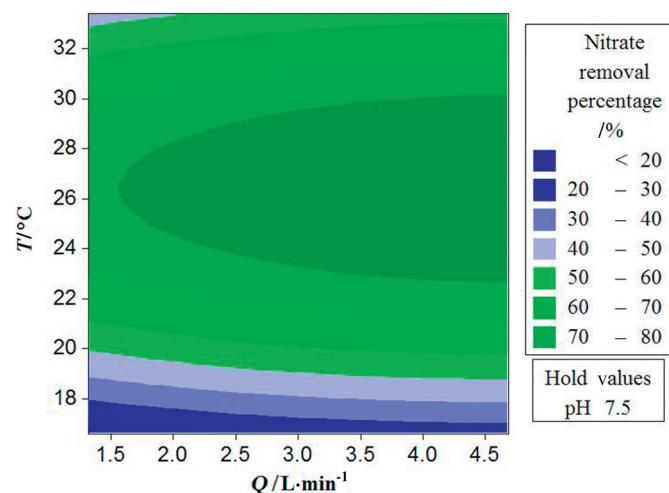


Fig. 7. The contour plot of the nitrate removal percentage as the function of temperature and aeration rate.

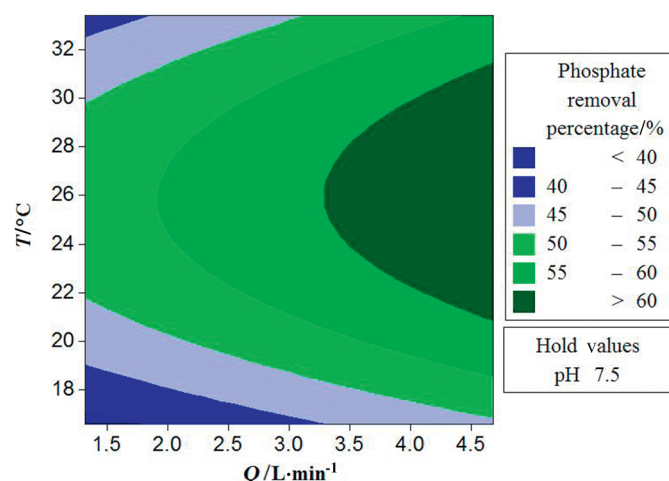


Fig. 8. The contour plot of the phosphate removal percentage as the function of temperature and pH.

3.6. Determination of optimal condition for nitrate and phosphate removal process

The multi-objective optimization was applied to the response equations to achieve the highest amount of nitrate and phosphate removal simultaneously. The process parameters were optimized using

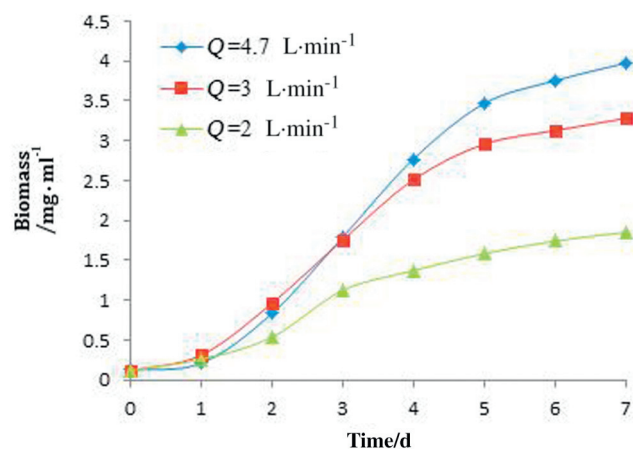


Fig. 9. The growth curve of microalgae at different aeration rates ($T = 20\text{ }^{\circ}\text{C}$, $\text{pH} = 9$).

desirability-based approach response surface methodology. The optimum values of the process variables for the maximum nutrient removal efficiency are shown in Table 7. After verification through a further experimental study with the predicted values, the result indicates good agreements between predicted and observed values. The values of nitrate and phosphate removal percentage were 85.3% and 76.6%, respectively, which were in excellent agreement with the values predicted by the model. It implies that the strategy to optimize the process conditions and to obtain the maximal nitrate and phosphate removal efficiency by RSM was successful in this study.

Table 7
Optimum operating condition of the process variables and related predicted and observed results

Temperature/ $^{\circ}\text{C}$	pH	Aeration rate/ $\text{L}\cdot\text{min}^{-1}$	Nitrate removal percentage in 48 h/%		Phosphate removal percentage in 24 h/%	
			Predicted	Observed	Predicted	Observed
26.3	8	4.7	87.90	85.3	74.14	76.6

3.7. Flow behavior

Distribution of velocity magnitude in the tank is an important factor representing proper agitation. Fig. 10 demonstrates path lines of particles released from the central cross section of the baffled/unbaffled STPs and colored by velocity magnitude. To keep the figure clear, just a limited number of path lines were shown in Fig. 10. Velocity magnitude was higher near the impeller blades than the far distance surrounding area, which was due to the effect of impeller motion on the flow. As it can be observed, there was a higher traffic area around the impeller in baffled STP (Fig. 10(a)) compared to unbaffled one (Fig. 10(b)). Almost all of the path lines passed through this area were induced by the impeller, which is an indicative of proper mixing of fluid flows in the baffled tank.

Fig. 11 illustrates air particle residence time through the whole STP after 3 s of injection. Again, to keep the figure clear, just a limited number of particles were shown. As mentioned before, air bubbles were injected to the STP through the sparger holes. Fig. 11 depicts how the air particles were distributed in the photobioreactor through injection operation. It can be seen that most of the bubbles in the unbaffled tank rise with low dispersion. Further, lack of air particles in the far distance of the impeller and shaft was due to undesired mixing pattern (Fig. 11(b)). Uniform distribution of bubbles required a proper mixing pattern in the vessels. The higher uniformity of bubble distribution in baffled STP (Fig. 11(a)) compared to unbaffled one, ensured that

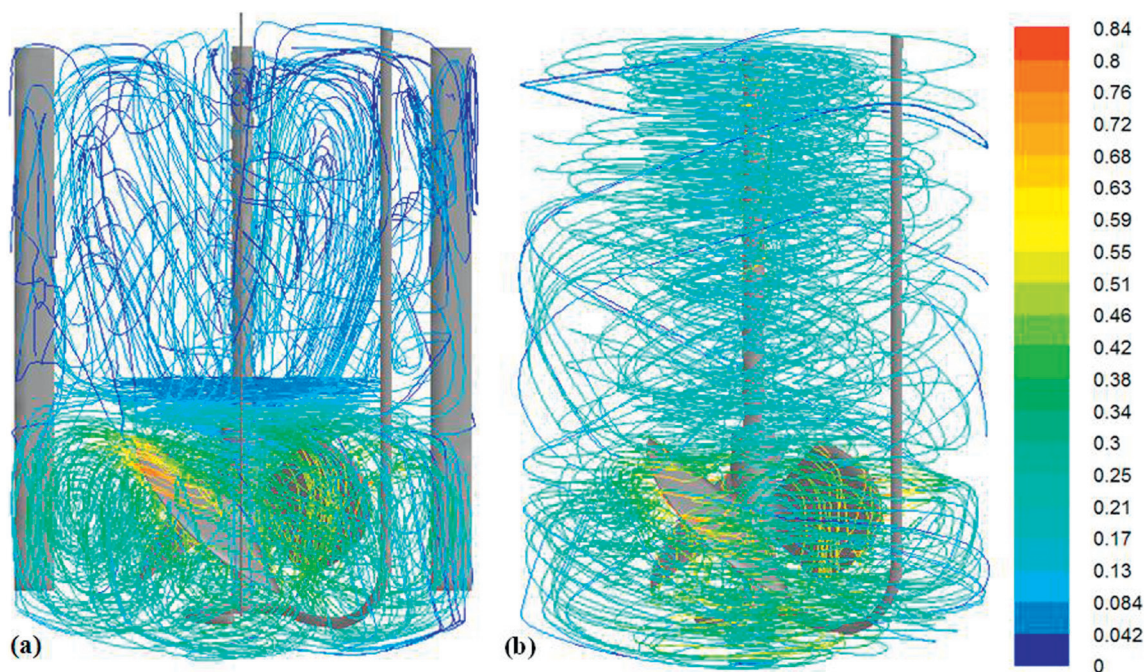


Fig. 10. Path lines of particles colored by velocity magnitude. (a) Baffled STP; (b) unbaffled STP.

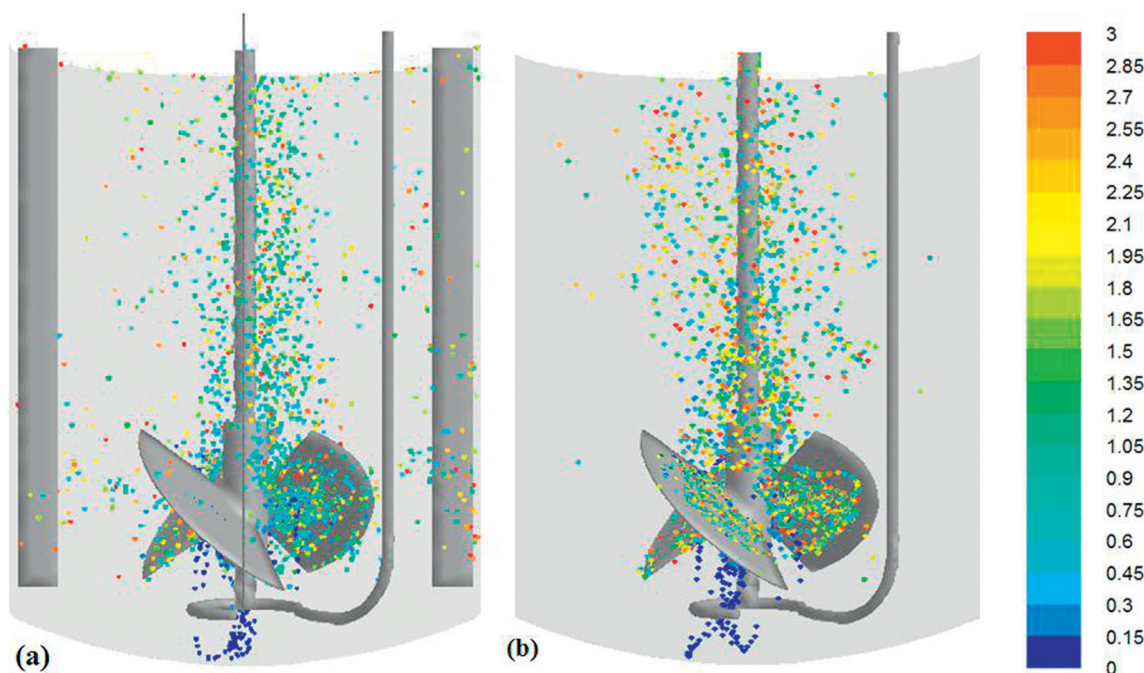


Fig. 11. Particle residence time: (a) baffled STP, (b) unbaffled STP.

the mixing process was more efficient and successful in this system. Moreover, desirable distribution of bubble particle and presence of particles with different residence time almost in the whole domain of the baffled photobioreactor, proved the existence of recirculation zones and proper mixing pattern in baffled STP.

3.8. Experimental validation of CFD simulation

Efficient mixing provides a uniform dispersion of microalgae within the culture medium, thus eliminating gradients of light, nutrient

concentration (N source and P source) and temperature. Comparison of microalgae growth and then nutrient removal in baffled and unbaffled STP was used to evaluate the validity of simulation. Under the optimum condition achieved from multi-objective optimization (temperature of 26.3 °C, pH of 8 and aeration rate of 4.7 L·min⁻¹), 93% of nitrate and 86% of phosphate were removed after 48 h and 24 h, respectively, in baffled STP (Table 8). According to these results, due to mixing improvement nitrate and phosphate removal increased 8% and 9%, respectively, in baffled STP compared to unbaffled one.

Table 8

Nitrate and phosphate removal percentage and biomass production in optimum conditions in both baffled and unbaffled STPs

	Nitrate removal percentage in 48 h/%	Phosphate removal percentage in 24 h/%	Biomass in 7 days/mg·ml ⁻¹
STP with baffle	93	86	4.32
STP without baffle	85	77	3.91

Note: Optimum condition (temperature 26.3 °C, pH of 8 and aeration rate of 4.7 L·min⁻¹).

4. Conclusions

The effect of temperature, pH and aeration rate on removal of nitrate and phosphate from synthetic wastewater by *C. vulgaris* microalga in STP was investigated in this work. Based on experimental results, an empirical relationship between the response and independent variables was obtained and expressed by the second order polynomial equations. In addition, the interactive effects of significant medium variables on the removal efficiency were established using contour plots of the model-predicted responses. Analysis of variance showed a high coefficient of determination values ($R_1^2 = 0.9273$ and $R_2^2 = 0.9793$), thus ensuring a satisfactory adjustment of the second-order regression model with the experimental data. The optimum levels of process variables were as follows: temperature 26.3 °C, pH 8 and aeration rate 4.7 L·min⁻¹. The actual experimental results for removal of nitrate and phosphate were 85% and 77%, respectively, under optimum condition which compared well with the predicted values. The experimental and predictive values were closely related showing that the models correctly predicted the response variables. Lastly, the effects of baffle on mixing efficiency and distribution of air particles in STP were investigated by means of CFD. Flow pattern showed a proper distribution of air particles and extraordinary mixing improvement when the STP was equipped with baffle. In baffled STP because of more appropriate mixing compared to unbaffled condition, nitrate and phosphate removal was elevated by 8% and 9%, respectively.

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