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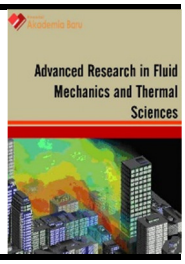

Interactive Effect of Temperature, pH and Light Intensity on Biodesalination of Seawater by *Synechococcus sp*. PCC 7002 and on the Cyanobacteria Growth

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ABSTRACT

Desalination allows to pipe viable water supply from ocean, the largest water resource, as potable water for drink and daily usage. The technology such as thermal distillation and membrane separation technology requires intense energy source. Almost all desalination plants using fossil fuel as their primary energy source, which leads to many challenges. Marine-euryhaline *Synechococcus* sp. strain PCC 7002, on the other hand, is one of the potential cyanobacteria that may contribute to the development and advancement in water treatment field. These cyanobacteria are able to generate a huge amount of biomass and producing fresh water (low-salt saline water). Thus, this leads the study to investigate the influence of process parameters on biodesalination by *Synechococcus* sp. PCC 7002 associate to growth rate of the cyanobacteria. The results showed that temperature, light intensity and pH play important role in sodium reduction. More than 50% of salinity reduction was obtained at optimum condition. Interactive effects between selected factors concluded that higher salinity removal was observed at temperature of 36^{o}C with the pH of 10 and light intensity of 3500 lux. However, the growth is contrast to biodesalination. Interaction effect between temperature-pH, suggested that the growth prefer near neutral condition regardless of the temperature. When temperature interacts with light intensity, inverse relation of these two factors resulted of higher growth rates. Further study is required to understand biodesalination by *Synechococcus* sp. and its growth. Nevertheless, the study has shown that biodesalination by *Synechococcus* sp. has the potential in biodesallination process and integration with current technology for potable water source can be further investigated.

Keywords:
Biodesalination; cyanobacteria; desalination technology; potable water; sea water

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1. Introduction

In Malaysia, fresh water mainly came from river, rain water or water reservoir. Recent year, the freshwater shortages issues arise due to the increasing of demand and limited of reserve margin especially during dry season [1, 2]. It became worst when the limited water resource is affected by environmental issue such as ammonia contamination in water supply [3, 4]. The problem also was due to low level of water in the river that leads to the high content of ammonia which is strictly not suitable to be consumed. Desalination is the new water treatment process that may maintain our good quality of freshwater in future. Furthermore, Gulf countries are relying on desalination for their water supply and to meet the growing water needs [5]. The fact that ocean water enclosed 97% of the world water sources [6], desalination process is one of the most beneficial, sustainable and efficient technology that might overcome the freshwater shortage issue [7]. Currently, desalination process applying thermal-driven (i.e. evaporation) [8] and filtration such as membrane technology by reverse osmosis process [9], have been widely used. However, this technologies require high energy consumption and unsustainable for future sustainability [10].

In general, salinity is the total amount of non-carbonate salt that may dissolve in the water. Salinity also can be measured by determine the sodium ion and chloride ion content in the salty sea water. Emerging technology of low-energy consumption of biological desalination process or also known as biodesalination process appears to be possible for immediate alternative with low and cost-efficient method [11]. This technology using cyanobacteria as an ion exchanger through manipulation of transport proteins in the cell membrane forming a low-salt reservoir [12]. Cyanobacteria are able to produce oxygen because it categorized in the phylum photosynthetic species. It may absorb photosynthetically active radiation as it is their main energy supplier [12]. Marine-euryhaline *Synechococcus* sp. PCC 7002 is one of the potential cyanobacteria that may contribute to the development and advancement in water treatment field. These kinds of cyanobacteria are able to generate a huge amount of biomass and producing fresh the low-salt water saline water. Temperature, pH and light intensity are among parameters studied by many with regard to the growth of the cyanobacteria, however the interactive effect between these parameters on growth as well as on biodesalination are very scarce. Thus, this study is aimed to investigate the interactive effect of temperature, pH and light intensity that influence biodesalination by *Synechococcus* sp. PCC 7002 associate to its growth.

2. Methodology

2.1 Microalgae Strain and Media

Synechococccus sp. PCC 7002 strain was purchased from American Type Culture Collection (ATCC) and grown in ATCC medium 957 at 37^{o}C with aeration of filtered compressed air at room light. Sea water was collected from Kapar, Selangor, Malaysia.

2.2 Design of Experiment and Analysis

Faced Centered Central Composite Design (FCCCD) under response surface methodology (RSM) was used to design the experiment. Three factors were selected and each factor was varied at three levels (-1, 0, +1). Table 1 shows level and code of selected variables. Design Expert (V8.0.7.1, State-Ease Inc., Minneapolis, USA) was used for the experimental design, data analysis, quadratic model buildings, and 3D graph response surface and contour plotting. The experiment was conducted in 250 mL Erlenmeyer flask with working volume of 150 mL in water bath to control the temperature

and was grown for 7 days. The pH of the culture was adjusted using acid (HCl) and base (NaOH). Cool white florescent lamp was used as source of light to the culture and the light intensity is measured using Lux meter. The initial salinity of seawater was recorded for each sample on day 0 (just before inoculation) and the final salinity was recorded 7 days after the inoculation in triplicate using handheld salinity refractometer. The percent of salinity reduction was calculated using Equation (1).

$$Sodium\ Reduction\ (\%) = \frac{Initial\ amount\ of\ salinity - final\ amount\ salinity}{Initial\ amount\ of\ salinity} \times 100\% \quad (1)$$

Table 1
Level and code of variables for FCCCD design

Variable	Symbol	Coded level		
		-1	0	+1
Temperature (^{o}C)	A	30	37	44
pH	B	7	8.5	10
Light (Lux)	C	2500	3000	3500

About 3 mL of sample was withdrawn every 24 hours from the cyanobacteria culture. Cells density was measured using spectrophotometer at wavelength of 600 nm. The data of optical density (OD) of the cells were then fitted nonlinearly using nonlinear regression software, CurveExpert Professional software, version 2.4.0. The logistic equation model (Equation 2) was selected to fit the data [13]. The maximum growth rate (μ_{max}) obtained from Equation (2) were then analyzed by ANOVA in Design Expert (V8.0.7.1) software.

$$X(t) = \frac{X_{max}}{1+\left(\frac{X_{max}}{X_o}+1\right)e^{-\mu_{max}t}} \quad (2)$$

3. Results

3.1 Sodium Reduction of Sea water

In this study, FCCCD design under RSM was used to analyse the interactive effect of temperature, pH and light intensity with the aim for maximum salinity reduction and growth at optimum condition. *Synechococccus* sp. PCC 7002 was grown under different conditions of temperature (30^{o}C to 44^{o}C), pH (7 to 10) and light intensity (2500 lux to 3500 lux). Table 2 shows the design matrix which consists of 15 total runs. The salinity reading of seawater collected from Kapar, Selangor, Malaysia was between 28 to 30 ppt. Biodesalination by *Synechococccus* sp. PCC 7002 is presented as percent of salinity reduction. Table 2 shows the actual (experimental) and predicted values of percent salinity reduction as the response calculated from Equation (1) and Equation (3), respectively. The predicted values were predicted by the statistical model as given in Equation (3). Maximum specific growth rates were also determined using Logistic model of Equation (2) and tabulated in Table 2 with the respective predicted value obtained from statistical model of Equation (4).

$$Y_1 = 50.08 - 0.81A + 2.95B + 8.02C + 8.45AB + 3.38AC - 12.33A^2 - 3.45C^2 \quad (3)$$

$$(Y_2 + 0.24)^{0.22} = 0.83 - 0.009A - 0.22B - 0.057C - 0.026AB - 0.23\ AC + 0.027BC + 0.15A^2 - 0.18B^2 + 0.10C^2 \quad (4)$$

where Y_1 and Y_2 are the predicted response of salinity reduction (%) and maximum specific growth rate (day^{-1}), while *A, B* and *C* are the temperature, pH and light, respectively.

Table 2
Design of experiment of three factors culture conditions at three levels with responses

Run	Temperature (˚C)	pH	Light intensity (Lux)	Salinity reduction (%)		μ_{max}	
				Actual	Predicted	Actual	Predicted
1	37	10	3000	53.06	52.46	-0.222	-0.221
2	44	7	3500	34.37	34.08	0.174	0.194
3	30	7	2500	37.02	36.42	0.584	0.546
4	37	8.5	3000	52.72	49.30	0.317	0.228
5	37	8.5	3000	52.15	49.30	0.031	0.228
6	44	8.5	3000	35.18	35.85	0.654	0.588
7	37	8.5	3000	52.15	49.30	0.032	0.228
8	30	8.5	3000	36.45	37.70	0.733	0.822
9	37	8.5	3000	52.15	49.30	0.177	0.228
10	44	10	2500	34.37	34.08	0.372	0.398
11	37	8.5	3500	45.02	49.30	0.515	0.228
12	37	8.5	3000	52.72	57.76	0.322	0.327
13	30	10	3500	35.29	34.79	0.408	0.377
14	37	8.5	2500	36.68	37.72	0.740	0.747
15	37	7	3000	47.17	46.79	0.295	0.299

Analysis of variance (ANOVA) analysed statistical significance of experimental data as shown in Tables 3 and 4 for biodesalination and growth, respectively. The Model *F*-values of 13.66 and 5.23 imply that both models (biodesalination and growth) are significant. There is only a 0.13% (biodesalinaton) and 4.16% (growth) chances that a "Model *F*-Value" these large could occur due to noise. Values of "Prob > *F*" less than 0.0500 indicate model terms are significant. In the case of biodesalination *C*, *AB*, A^2 are significant model terms, while in the case of growth, *B*, *AC*, A^2, B^2 are the significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. In the case of biodesalination, *BC* and B^2 model terms were excluded to improve the model hierarchy and the response surface reduced quadratic model was applied to analyse the significant of the model term as shown in Table 3.

Table 3
ANOVA for the response surface reduced quadratic model for biodesalination

Source	Sum of square	df	Mean Square	F value	*p*- value Prob>F	
Model	878.02	7	125.43	13.66	0.0013	significant
A-temperature	3.91	1	3.91	0.43	0.5348	
B-pH	17.38	1	17.38	1.89	0.2112	
C-Llight intensity	128.69	1	128.69	14.02	0.0072	
AB	95.29	1	95.29	10.38	0.0146	
AC	15.24	1	15.24	1.66	0.2386	
A^2	439.49	1	439.49	47.87	0.0002	
C^2	34.34	1	34.34	3.74	0.0944	
Residual	64.26	7	9.18			
Lack of Fit	21.67	3	7.22	0.68	0.6095	not significant
Pure Error	42.59	4	10.65			
Cor Total	942.28	14				

R^2 = 0.9318; adjusted-R^2 = 0.8636; Pred R-Squared = 0.5637

Table 4
ANOVA for the response surface reduced quadratic model for growth

Source	Sum of square	df	Mean Square	F value	*p*- value Prob>F	
Model	0.25	9	0.028	5.23	0.0416	significant
A-temperature	1.671E-004	1	1.671E-004	0.031	0.8669	
B-pH	0.097	1	0.097	18.04	0.0081	
C-Llight intensity	6.512E-003	1	6.512E-003	1.21	0.3210	
AB	9.302E-004	1	9.302E-004	0.17	0.6946	
AC	0.068	1	0.068	12.70	0.0162	
BC	9.835E-004	1	9.835E-004	0.18	0.6865	
A^2	0.058	1	0.058	10.75	0.0220	
B^2	0.089	1	0.089	16.55	0.0097	
C^2	0.027	1	0.027	5.09	0.0737	
Residual	0.027	5	5.372E-003			
Lack of Fit	1.209E-004	1	1.209E-004	0.018	0.8995	not significant
Pure Error	0.027	4	6.685E-003			
Cor Total	0.28	14				

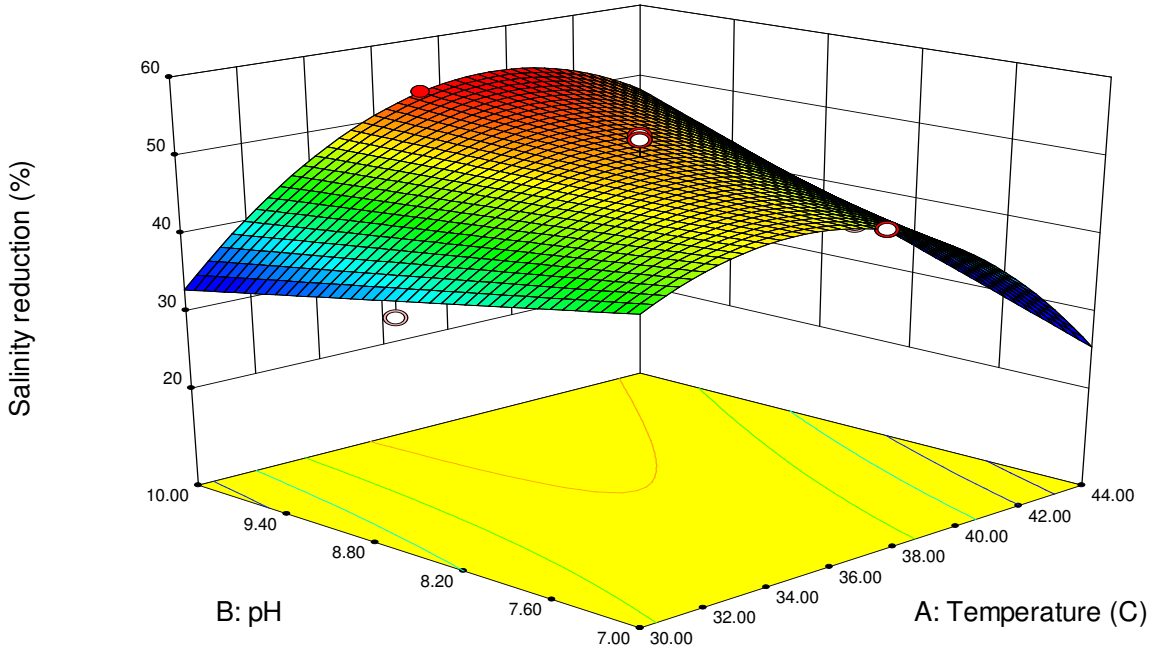
R^2 = 0.9040; *adjusted-*R^2 = 0.7312; *Pred R-Squared* = 0.7744

Meanwhile for the growth rate model, all terms are included for the analysis. However, power transformation was applied to the growth rate model to meet the assumptions that make the ANOVA valid. Table 4 shows the ANOVA for response surface reduced quadratic model statistically analyse growth of the cyanobacteria.

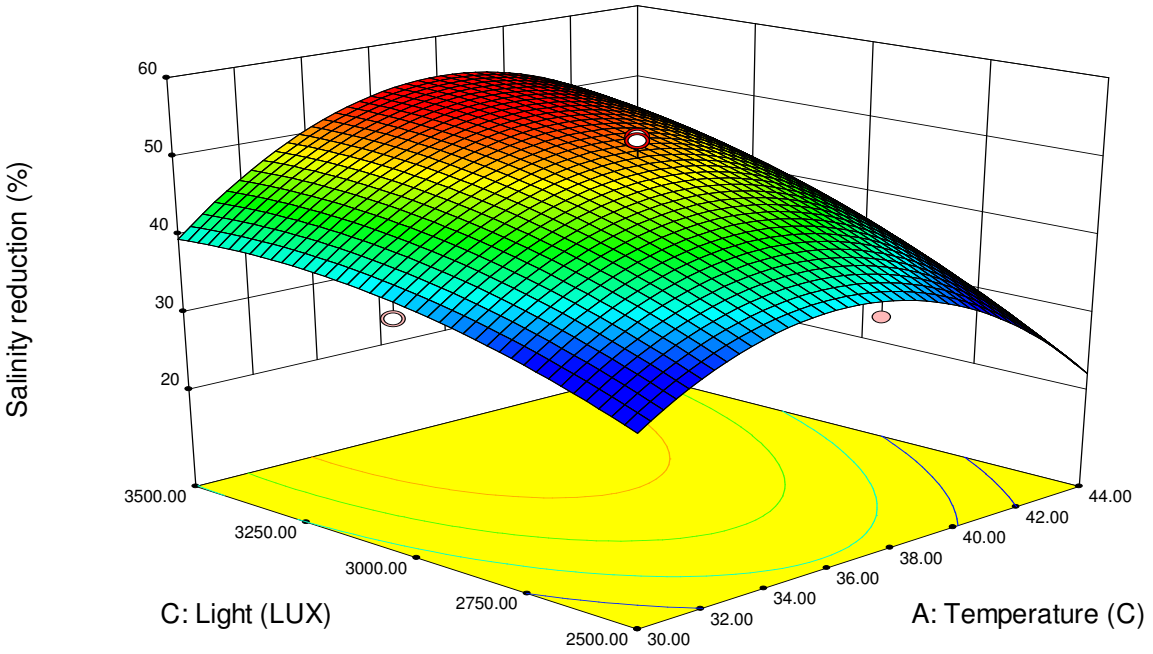
Figure 1(a) shows 2-dimensional model of two-interactive factor between temperature and pH. The graph clearly shown the desalination reached maximum when culture temperature is centred on 36^{o}C at pH 10. Similar to light-temperature interaction also showed the same effect towards desalination where maximum reduction was observed at temperature of 36^{o}C at 3500 lux.

However, growth rate shown a contrast relationship (Figure 2(a)), the specific growth rate of *Synechococccus* sp. PCC 7002 is higher as pH approach neutral value regardless of the temperature. Interestingly, when temperature interacts with light intensity, inverse relation of these two factors resulted of higher growth rates, while light – pH interaction showing maximum growth at 2500 lux and at pH 7.6.

Temperature one of the major factors that controls photosynthetic rates [14], and other microalgae activities. Photosynthetic organisms can efficiently perform photosynthesis at a range of temperatures associate to optimal growth temperature [14]. It has been reported that *Synechococccus* sp. PCC 7002 grows optimally at temperature of 38^{o}C [15, 16] and some has suggested best grown between 20 to 30^{o}C and ceased at 40 and 50^{o}C [17]. Generally, cyanobacteria grown well near neutral to alkaline pH and the growth significantly reduced at pH 9 and higher[18] [17, 19]. At pH from 6.3 to 10.3, HCO_3^- predominates in the solution, while at pH below 6.3 and above 10.3, CO_2 and CO_2^{3-} are respectively the dominant forms [20, 21]. HCO_3^- transport into the cell is generally coupled to Na^+ gradient or ATPS hydrolysis [22], thus in this case reduced the sodium in the culture medium containing seawater. The range of light intensity provide to the culture in this study is below the saturation irradiation (18,500 lux). Thus, interaction with light is less significance compare to other.

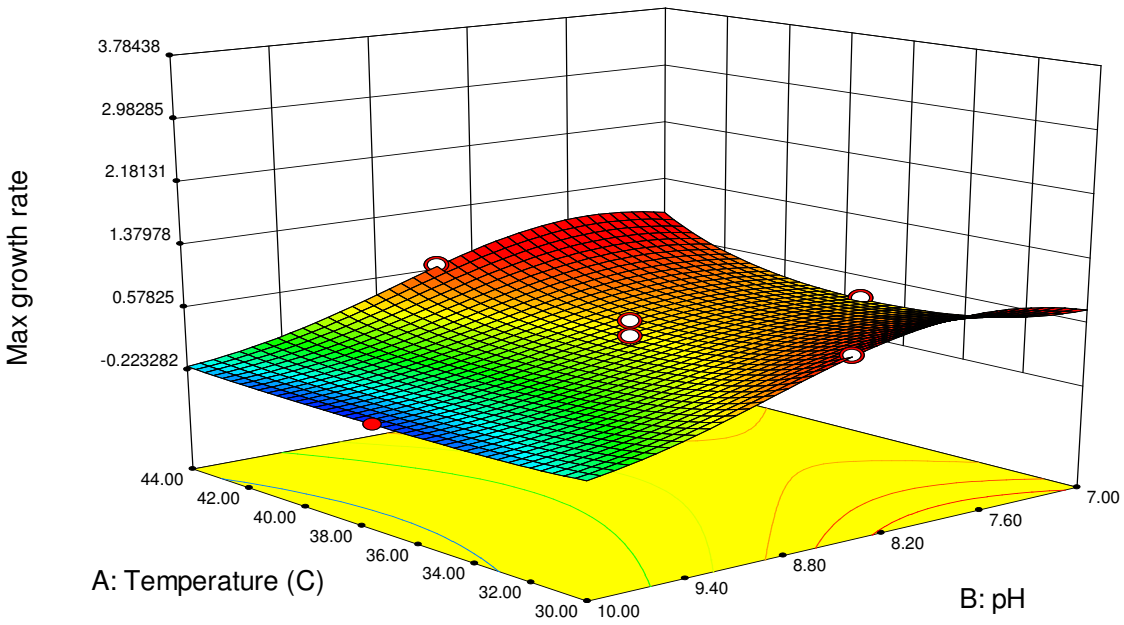


(a)



(b)

Fig. 1. Effect of (a) temperature and pH, and (b) light intensity and temperature on the salinity reduction by *Synechococcus* sp. PCC 7002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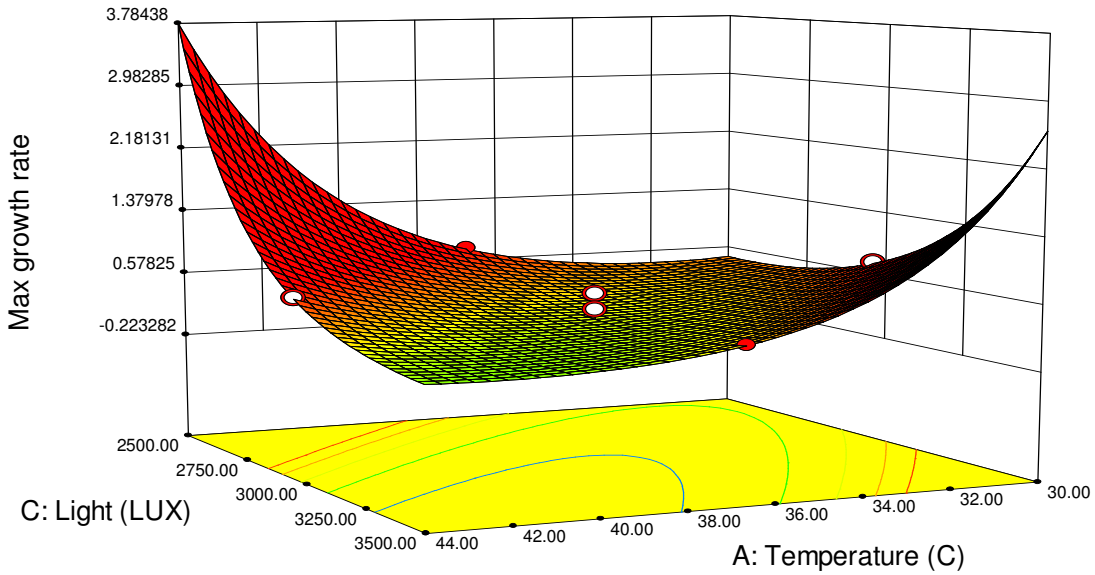
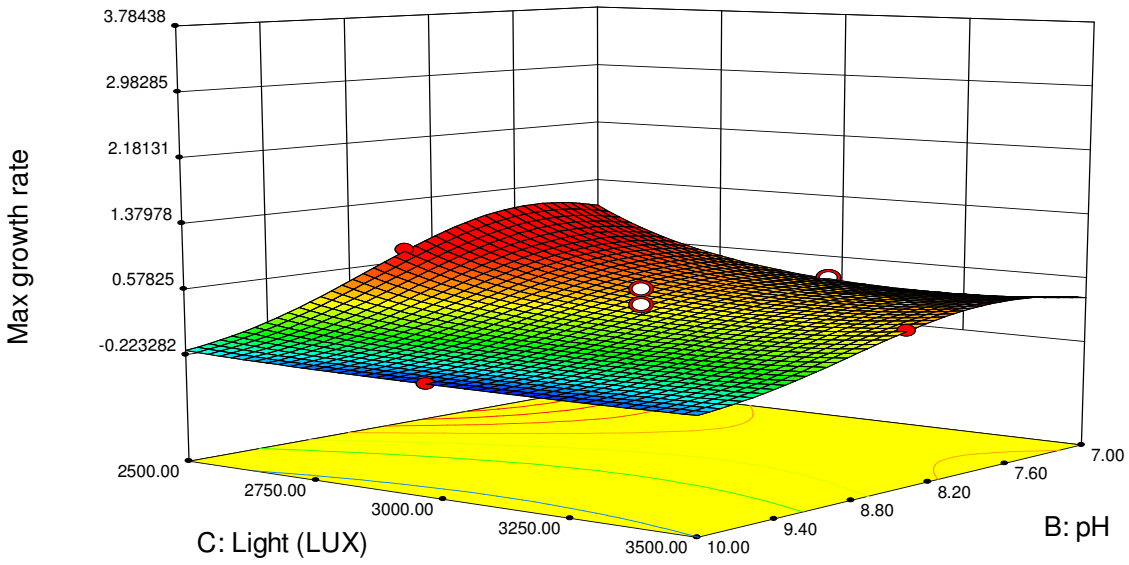
2.98285
2.18131
1.37978
0.57825
-0.223282
44.00
42.00
40.00
38.00
36.00
34.00
32.00
30.00
A: Temperature (C)
10.00
9.40
8.80
8.20
7.60
7.00
B: pH

(a)


Max growth rate
3.78438
2.98285
2.18131
1.37978
0.57825
-0.223282
2500.00
2750.00
3000.00
3250.00
3500.00
C: Light (LUX)
44.00
42.00
40.00
38.00
36.00
34.00
32.00
30.00
A: Temperature (C)

(b)



(c)

Fig. 2. Effect of (a) temperature and pH, (b) temperature and light intensity, and (c) pH and light intensity on the growth of *Synechococcus* sp. PCC 7002

4. Conclusions

In this study, biodesalination and the growth rate of *Synechococcus sp*. PCC 7002 was investigated and evaluated using Central Composite Design under Response Surface Methodology. Three factors which were temperature, pH and light intensity were studied and the design matrix of 15 experimental runs was conducted. The result showed that the highest removal of salinity was observed at temperature of 37^{o}C, pH of 10 and light intensity of 3500 lux and removal over 50% was achieved. However, the growth rate of the cyanobacteria strain was contrast to the biodesalination. The interactive effect between the three factors gave maximum growth rate at pH 7.6 regardless to the temperature (range from 30^{o}C to 40^{o}C). However, temperature interaction with light intensity resulted of maximum growth at inverse relation of both factors. Different cyanobacteria possibly may have different optimum growth condition. However, temperature, pH and light intensity were proven to be the factor that influenced the activities of this species.

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