Article

Evaluation of three algal strains isolated for bioremediation of environmental pollutants

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Received 24 June 2019; Accepted 28 July 2019; Published 1 December 2019

Abstract

Response surface methodology was applied to optimize the removal of various pollutants by three newly isolated macroalgae strains from Miankaleh wetland. These strains were selected from 11 different isolates (including 8 micro and 3 macroalgal strains) based on their growth kinetic parameters. The effect of variables such as light intensity, CO_2 concentration and concentration of wastewater on the biosorption of nitrate, nitrite, phosphate as well as the rate of CO_2 sequestration were investigated using a Central Composite Design (CCD) method. Multiple regression analysis and analysis of variance (ANOVA) showed that all three species of algae were able to significantly remove the nutrient elements and sequester CO_2. A maximum nitrate removal of 91%, nitrite removal of 92%, phosphate removal of 95% and CO_2 sequestration of 30-60% was obtained using the biosorption kinetics under optimum conditions. Our results clearly confirm the ability of the studied strains in bioremediation of environmental pollutants. Moreover, the dynamics of phytoplankton populations in the Miankaleh wetland were surveyed using remote sensing information. The findings support the hypothesis that the high concentration of algal pigment in the wetland is correlated to the ability of the studied strains in bioremediation of environmental pollutants; a direct correlation exists between the prone algal biomass and the potential of carbon capture in the aquatic ecosystems.

Keywords biosorption; nutrient removal; response surface methodology; optimization; satellite imagery.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

"Threats to natural resources" is a broad topic having national and global dimensions. In the case of threats that cut across international boundaries, increasing greenhouse gase (GHGs) emissions are strongly correlated with global warming in recent decades (Zhang and Liu, 2012). This phenomenon has serious environmental consequences e.g. constantly-increasing temperature in the atmosphere and hydrosphere (Drake, 2014). CO_2 is one of the most important GHGs. Anthropogenic releases of CO_2 could imposes catastrophic changes in marine systems: increases in acidity, variation in ocean temperatures and related circulation changes and storm activities are among the effective factors which in turn determine the survival of organisms in aquatic environments (Podong, 2014; Sundar et al., 2014; Shabani et al., 2016; Asadian et al., 2018). An insatiable global appetite for energy is fed by increasing fossil fuel consumption, advancing of various technologies and instruments leading to increased CO_2 and halocarbons emissions, changes in land use and deforestation, disposal of toxic hospital and agricultural wastewater, etc. are some examples of human activities leading to global change (Cook et al., 2016). Therefore, many efforts have been made to reach a comprehensive plan of action to reduce GHGs and one of the most important methods is the biological fixation of the GHGs.

Photoautotrophic algal species take up CO_2 (directly, or indirectly as bicarbonate) as a carbon source to support photosynthetic activity and lead to biomass production (Lee, 2016). Numerous studies have discussed the positive effects of accumulated concentrations of CO_2 in promoting biomass production during algal cultivation. For example, it was previously reported that a concentration of 2% CO_2 led to an increase in growth of *Botryococcus braunii* (Ranga et al., 2007) and a similar response was observed in *Neochloris oleoabundans* supplemented by 5% CO_2 (Gouveia et al., 2009). It worth mentioning that any increase in algae biomass production can ultimately lead to increased lipid production and ultimately can result in an economically-feasible production of biofuels using algal feedstock (Sayadi et al., 2011; Talebi et al., 2013).

In the national dimension, natural resources such as freshwater reservoirs are directly threatened by human activities including intensive agricultural activities, urban/domestic development, industrialization and unplanned engineering infrastructures (Chiu et al., 2015). Large unmechanized procedures performed by farmers in the paddy fields of north Iran results in drainage systems heavily contaminated by a variety of hazardous compounds such as agricultural pesticides and chemical fertilizers, together known as agricultural wastewater (Rashed, 2013). Treatment of these compounds using algal cultivation is a promising biological method of bioremediation. Wastewaters contain organic and inorganic compounds along with nutrients such as nitrates, nitrites and phosphates that could be taken up by the algal cells during the cells' growth phase and increase the biomass production in algae cultivation systems. In addition, increased utilization of nitrates and phosphates can improve photosynthetic rates which translates into higher oxygen concentrations in wastewater systems (Delgadillo-Mirquez et al., 2016); the latter could lead to improved aerobic bacterial activity and better refinement of compounds in a coupled wastewater treatment strategy (Sutherland et al., 2015). On the other hand, nutrients in the wastewater are transformed to lipids and proteins in algal cells, which could be processed into different value-added products such as biofuels, forage for livestock or even health food for humans (Sayadi et al., 2011; Salama et al., 2017). Numerous studies have surveyed the biosorption potential of a range of species of green algae (Zeraatkar et al., 2016). Over five decades of research on algal-based wastewater treatment has provided valuable knowledge on the laboratory to industrial scale for pollution remediation. Biosorption and/or neutralization of the toxins have been repeatedly confirmed the high bioremediation potential of low-cost micro and macroalgae cultivation systems (Zeraatkar et al., 2016; Delrue et al., 2016).

On the other hand, the increasing availability of satellite ocean color data presents an opportunity to investigate climate change, ocean primary productivity, pollution of the environment and the power of phytoplankton in utilization of atmospheric CO_2 (Huot et al., 2007). The dynamic assessment and spatio-temporal variations of algal populations in wetlands using satellite imagery was developed at the end of the 21st century as a remote sensing solution providing an efficient way to produce an awareness about the biochemical, physical and biological processes in aquatic systems, at low cost (Van de Poll et al., 2013).

The present study was designed to evaluate the potential of three algal species isolated from the Miankaleh wetland in the southeast of the Caspian Sea, Iran on the biorefinery of agricultural wastewater. We also focused on the possible influence of operating parameters such as light intensity, CO_2 and wastewater concentrations on the performance of biosorption using Response Surface Methodology (RSM) (Zhang, 2010). Moreover, we studied the spatiotemporal variability of algal biomass in the studied area characterized by satellite-derived chlorophyll *a* (Chl*a*-sat) with the MODIS sensor on the Terra and Aqua satellites between 2010 and 2018 in the summer.

2 Study Area and Methodology

2.1 Sample collection and cultivation

In this study, the samples were collected from different locations in the Miankaleh wetland in the southeastern part of the Caspian Sea, Iran. The location of sampling sites is shown in Fig. 1. pH and temperature were recorded at the site of sampling. Water samples were placed in polyethylene bottles and then transferred to the laboratory immediately. The procedure of isolation and purification of algal stains was based on previously published studies (Talebi et al., 2013; Droop, 1954). The collected samples were subjected to purification by serial dilution followed by plating on agar in order to obtain pure algal cultures of the desired species. Later, the developed colonies were selected and transferred into new flasks. The purity of the culture was tested by repeated plating and by regular observation under a microscope. Moreover, antibiotic supplementation was used by addition of 500 µg ml^{-1} penicillin and chloramphenicol to achieve axenic cultures. All samples were cultivated in 3N-BBM medium in 250 ml *Erlenmeyer* flasks medium under the same growth condition as follow: continuous aeration rate of 1 vvm, illumination (80 µmol photon m^{-2} s^{-1}) at 22 ^{o}C. An illumination regime of 16 h light and 8 h dark was used.

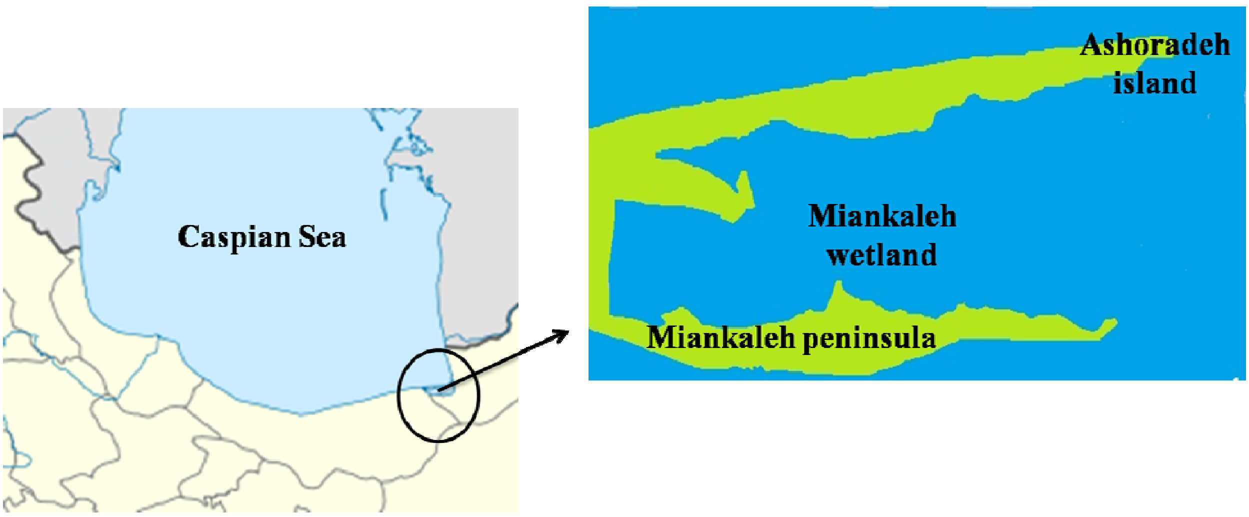

Fig. 1 The location of sampling sites in Miankaleh wetland in the southeastern part of the Caspian Sea, Iran.

2.2 Growth kinetic parameters

Within the first 40 days of cultivation, growth kinetic parameters of strains were measured in triplicate. This facilitated further screening of potential strains for bioremediation applications. The parameters analyzed included:

(1) Biomass productivity (BP) or the amount of produced dry biomass (g l^{-1} day^{-1}), was measured gravimetrically as follow:

$$BP= ((dW_i-dW_0))/ (t_i-t_0) \quad \text{Eq. (1)}$$

where dW_i: dry biomass on the i^{th} day; dW_0: initial dry biomass; t_i: i^{th} day; t_0: initial time

(2) Total lipid content (LC) as percentage of the total lipid per 100 /gr dried biomass (% dwt). LC was determined according to method described by Talebi et al. (2013) with some modification; 50 mg of dried algal sample was vortexed with 3 ml of chloroform/methanol (1:2) for 10 minute followed by centrifugation at 4000 rpm for 15 minutes. The supernatant was collected and sediments in the tube is mixed with 2 ml of chloroform/methanol (1:1) for three times and centrifuged similar to the previous step. The supernatant was collected and filtered through Whatman filter paper to remove impurities. The chloroform and alcoholic residues were evaporated by keeping the sample at (65°C) in hot air oven. Finally, weighed sediments were used to measure the total lipid content.

(3) Lipid productivity (LP) was also measured with the following equation:

$$LP= (LC \times BP \times 1000)/ 100 \quad \text{Eq. (2)}$$

where LC is lipid content and BP is biomass productivity.

2.3 Morphological identification of the isolated strains

To identify the algal strains, morphological characteristics were studied. The morphology of single cells was studied microscopically (NikonEclipse FN1). Characters such as cell size, cell shape, length and width of vegetative cells, the presence or absence as well as shape of flagella, filaments and gas vesicles in addition to cells' potential aggregation into colonies were thoroughly recorded. Identification of algal strains was done using the descriptions provided in the manuals of Sohrabipour and Rabii (1999), and Sterrer (1986). The morphological characteristics of macroalgae including cell size, thallus color, height and cell shape were considered to determine the taxonomy of the studied strains.

2.4 Optimizations of culture condition and bioremediation by RSM

In this study, three species selected from the isolates were aseptically grown in 3N-BBM culture medium and different growth conditions, such as different concentrations of CO_2 (380, 5190 and 10000 ppm), different light intensity (113, 170 and 225 µmol photon m^{-2} s^{-1}) and wastewater (100% culture media, 100% wastewater and 1:1 dilution of medium and wastewater). 5 liter Erlenmeyer flasks containing one liter of the medium were used for algae cultivation. White LED lamps provided different light intensities. Light intensity was evaluated with a lux meter every day. Illumination regime of 16 h light and 8 h dark was used. Temperature (25 °C) and pH (8.5) were constant in all cultures. Aeration was carried out with CO_2-containing tanks. The concentration of CO_2 was measured by titration method; CO_2 was bubbled 2-3 times every day for 20 minutes and after enrichment of the culture medium at the desired concentration the aeration gas was stopped. The pH was measured before and after the CO_2 injection, and the pH was adjusted to 8.5 using appropriate buffers.

Three levels of the variables in coded units are given in Table 1. RSM was used to evaluate the influencing factors and also to optimize the culture condition for maximum biorefinery capacity. Biosorption of various pollutants such as nitrate, nitrite, phosphate, as well as rate of CO_2 sequestration was concurrently surveyed. Central Composite Design (CCD) method is used to reduce execution cost and time during the experiment. Using Design-Expert software version 7.0 (Stat-Ease Inc., Minneapolis, USA) an experimental plan containing 30 experiments was designed (Table 2). Data processing was done to obtain the effects and surfaces of the responses. The software was used for regression and graphical analysis of the obtained data. The adequacy of the RSM was evaluated by calculation of the determination coefficient (R^2). Finally, the optimum condition for the highest bioremediation was calculated.

Table 1 Independent variables and encoded levels in the experimental design.

Factors	unit	Range and levels		
		-1	0	1
CO_2	ppm	380	5190	10000
Light intensity	#	113	170	225
Wastewater	-	Culture media	1:1	Wastewater
Macroalgae	-	1	2	3

μmol photon $m^{-2} s^{-1}$

Table 2 Experimental design and the number of runs with Design Expert Software.

Run	**Medium**	**CO_2 (ppm)**	**Light intensity(μmol photon $m^{-2} s^{-1}$)**	**Algal strains**	**Biomass (mg dw / L day)**
1	3N-BBM	10000	113	1	303.33
2	Waste water	380	225	1	370
3	3N-BBM	380	225	1	326.66
4	3N-BBM	10000	225	1	316.66
5	Waste water	10000	225	1	320
6	Waste water	10000	113	1	300
7	3N-BBM	380	113	1	283.33
8	1:1	5190	170	1	496.66
9	Waste water	5190	225	1	500
10	1:1	5190	170	2	526.66
11	1:1	5190	170	2	441.17
12	1:1	380	170	2	473.33
13	1:1	5190	113	2	408.82
14	Waste water	10000	225	2	525
15	1:1	5190	170	2	419.44
16	1:1	5190	170	2	369.04
17	Waste water	5190	170	2	370
18	1:1	5190	170	2	405.26
19	1:1	5190	170	2	419.44
20	3N-BBM	5190	170	2	394.73
21	1:1	5190	225	2	442.85
22	1:1	5190	170	3	384.37
23	Waste water	10000	113	3	270
24	3N-BBM	10000	113	3	276.66
25	Waste water	380	113	3	284.37
26	3N-BBM	10000	225	3	293.33
27	Waste water	380	225	3	309.37
28	Waste water	10000	225	3	270.58
29	3N-BBM	380	113	3	263.33
30	3N-BBM	380	225	3	237.5

2.5 Analytical methods

In this study, the following methods were used to measure the concentration of pollutants remaining in the growth medium after harvesting of the algal cells. The concentration of nitrate ion was measured according to the method described by Kalimuthu et al. (2015) and Chandra (2002). In brief, the procedure is as follows: 3 ml of culture medium was centrifuged for 15 min at 5000 rpm. The supernatant was used for the analysis. Optical density was measured using a spectrophotometer (MODEL UV 1800, Shimadzu company) at 410 nm and the nitrate concentration was determined using a calibration curve. This curve was drawn up by preparing a standard nitrate solution (potassium nitrate) at concentrations of 0 to 10 mg / L. The concentration of nitrate ion (mg / l) in the medium was determined from the standard curve.

Determination of nitrite ion was carried out by the method reported by Wang et al (1998). The description of this method is as follows: 3 ml of culture medium was centrifuged for 15 min at 5000 rpm. 2.5 ml of the supernatant was diluted with ammonium chloride to achieve final volume of 10 ml. 0.2 ml of sulfanilamide (1%) was added to 5 ml of this solution and after 5 minutes, 0.2 ml of N-1- naphthylethylenediamine (0.02 M) was added. Formation of the purple color was measured spectroscopically at 543 nm. The nitrite concentration was determined using a calibration curve. The calibration curve was determined using nine different levels of standard nitrite solution from 0.01 to 0.4 mg / l.

To determine the residual phosphate ion in the medium, 4 ml of ammonium molybdate (0.05 M) was dissolved in 100 ml of the supernatant and 0.5 ml of stannous chloride was added to the solution. After 10 minutes of incubation, absorption at 690 nm was measured by the spectrophotometer. The residual phosphate ion concentration was determined using a calibration curve using nine different concentration of standard phosphate solution from 0.1 to 0.9 mg / l. Standard phosphate solution was prepared by dissolving 219.5 mg KH_2PO_4 in 1liter of distilled water that contains 50 mg/L of phosphate (Tanada et al., 2003).

CO_2 sequestration was determined using titration method according to Black et al (1954) and Fidel (2015). A few drops of phenolphthalein were added to 10 ml of supernatant and titrated with NaOH (0.02 M) until the color of the solution change to pink and the alkalinity reached to pH=8.3. Finally, the volume of consumed sodium hydroxide was recorded. The concentration of dissolved CO_2 in the medium was determined using Eq. 3.

$$CO_2\ (\text{mg/ml}) = (A \times N \times 44000)/\ (\text{The initial volume of the sample}) \qquad \text{Eq. (3)}$$

where A represents the volume of consumed NaOH (ml) and N represents the normality of sodium hydroxide solution.

2.6 Remote sensing of phytoplankton blooms in the Miankaleh wetland

Studies on the dynamics of phytoplankton population in the Miankaleh wetland (southeast of the Caspian Sea) were performed using remote sensing information. The satellite imagery obtained from worldview tool (https://worldview.earthdata.nasa.gov) captured with the MODIS sensor on the Terra and Aqua satellites between 2010 and 2018 in the summer. All the images were evaluated in these years and the average of chlorophyll *a* concentration were measured to estimate the potential of the CO_2 bio-sequestration in the region. The sensor and imagery resolution is 1 km, and the temporal resolution is daily. This sensor can be used to measure chlorophyll *a* concentration in the studied area (Fu et al., 2018).

3 Results and Discussion

3.1 Selection of suitable algal strains

The results of BP, LC and LP for all 11 strains are presented in Table 3. To minimize the effect of incubation period and condition on biomass as well as lipid production, all cultures were inoculated in 3N-BBM medium and harvested after reaching the stationary phase. The highest BP was observed in isolate number 9. Also, the highest levels of LC and LP were observed in isolate No 10. Based on the data presented in Table 3, three macroalgal strains; number 9, 10 and 11, were selected for further investigations. The growth parameters LP, LC and BP were considered as the most important criteria for selection of suitable strains.

During the present study, we used BP, LC and LP as the important criteria for screening productive algal strains. These characteristics have been repeatedly used in published studies; for example Hempel et al. (2012) have identified BP as the main influencing factor for the capability of the algal feedstock for further applications. BP and LP were introduced by Talebi et al. (2013) as adequate criteria to estimate the potential of different microalgal isolates for industrial applications. Since they calculated a strong correlation (R^2 = 0.93) between BP and LP, the productive strains in the present study were selected based on the highest BP (Table 3). However, other influencing parameters such as adaptability to environmental conditions, ease of cultivation and harvesting, release of possible toxic metabolites etc., should also be considered for the final screening of potential algal strains for biorefinery approaches.

Table 3 Growth kinetic parameters measured for 11 isolates algal strains grown in 3N-BBM culture medium.

Isolates	**Dry weight ($mg\ l^{-1}$)**	**Biomass Productivity ($mg\ l^{-1}\ day^{-1}$)**	**Lipid content (%)**	**lipid productivity ($mg\ l^{-1}\ day^{-1}$)**
1	183	6.10	23	1.403
2	176	5.86	17	0.996
3	185	6.16	10	0.616
4	164	5.46	20	1.092
5	193	6.43	25	1.607
6	187	6.23	22	1.370
7	170	5.66	15	0.849
8	191	6.36	22	1.399
9	211	7.03	34	2.390
10	205	6.83	39	2.663
11	200	6.66	31	2.064

3.2 Phenotypic analysis of selected strains

Morphological characteristics of the three top biomass producer strains were studied using microscopic analysis. Three macroalgae strains namely *Chaetomorpha antennina*, *Ulva intestinalis* and *Bryopsis pennata*, were identified (Fig. 2) based on their morphological key characters. For example, *C. antennina* (strain number 9), has cylindrical cells and non-branching filaments with a large number of chloroplasts. This genus has parietal chloroplasts and multiple pyrenoids, also, the filaments are arranged in a row with a smooth wall. However, straight and rigid filaments in company with rhizoids that firmly attach the algae to the substrates, form a typical development of *Chaetomorpha* spp. in culture. Rhizoids are also very compact (Deng et al., 2013). The characteristics of *U. intestinalis* (strain number 10) were rounded rectangles in short longitudinal rows. Studies showed that *Ulva* spp. have thalli that are light green. The shape of cells in this genus is regular/irregular round. *U. intestinalis*is usually non-branching and the presence of one pyrenoid in each cell and the chloroplasts at the apical end are also features of this species (Kong et al., 2011). *B. pennata* (strain number 11), has a central axis with lateral branches around it. The tip of the thallus is rounded in the main and lateral branches. This species has been previously described by its massive and branchy thallus in tuft-like, dark green color. Moreover, it has a single utricle cell in a coenocytic structure (Ciancia et al., 2012).

Morphological features such as size and situation of the cells, development of uniseriate/biseriate filaments and unilateral/bilateral branching were considered for the identification. Moreover, details of ecological characterization such as distribution and environment, physiological properties and nutrient requirements match the 3 selected algae to the close description. Comprehensive evaluation of all the mentioned criteria and comparative assessment with previous studies has been recently employed by the authors (Kabirnataj et al., 2018).

3.3 Optimization of medium components to maximize the biomass production

According to the Table 1, three operational parameters were varied in three levels to optimize the BP as well as the biorefinery capacity of the studied strains. Three algal species were analyzed after reaching the end of the stationary phase. The results (Table 2) showed that final dry weight was a function of light intensity, inoculum type, concentration of available nutrients and CO_2. BP was influenced by the supplemented nutrients and all studied strains showed a similar response to this variable. Synthetic culture medium and wastewater in a ratio of 1:1 had the best effect on the improvement of BP in algal cultivation (Fig. 2a). The physico-chemical properties of the agricultural wastewater were measured and summarized in Table 4.

Table 4 The physico-chemical properties of the agricultural wastewater.

Parameter	Concentration mg / l
pH	8.05
DO	6.5
BOD	11.35
COD	56.25
TS	2480
Nitrate	65.2
Nitrite	3.1
Phosphate	17.1

When the effect of different CO_2 concentrations and light intensity on final dry weight are examined (Fig. 2b), it can be seen that at low and moderate light intensities (113 and 170 μmol photon m^{-2} s^{-1}) the final dry weight did not represent a strong correlation with different concentration of CO_2; a very moderate effect of CO_2 concentrations on final dry weight was observed throughout the range of light intensity tested. The maximum BP was observed at around 10000 ppm CO_2 and 225 μmol photon m^{-2} s^{-1} light intensity.

The final equation in terms of actual factors was determined to be:

$1.0/(\text{Dry Weight}) = +3.32567E\text{-}003 - 3.96803E\text{-}005\times\text{medium} - 4.90241E\text{-}009\times CO_2 - 3.94192E\text{-}008\times\text{light intensity} + 2.89942E\text{-}004\times\text{algae} + 3.61116E\text{-}007\times\text{medium}^2$

The model F-value is equal to 9.72, which indicates that the used model is significant. There is only 0.01% chance that this large F-Value for the model could occur due to noise. The Lack of Fit F-value of 2.72 implies the Lack of Fit is not significant relative to the pure error.

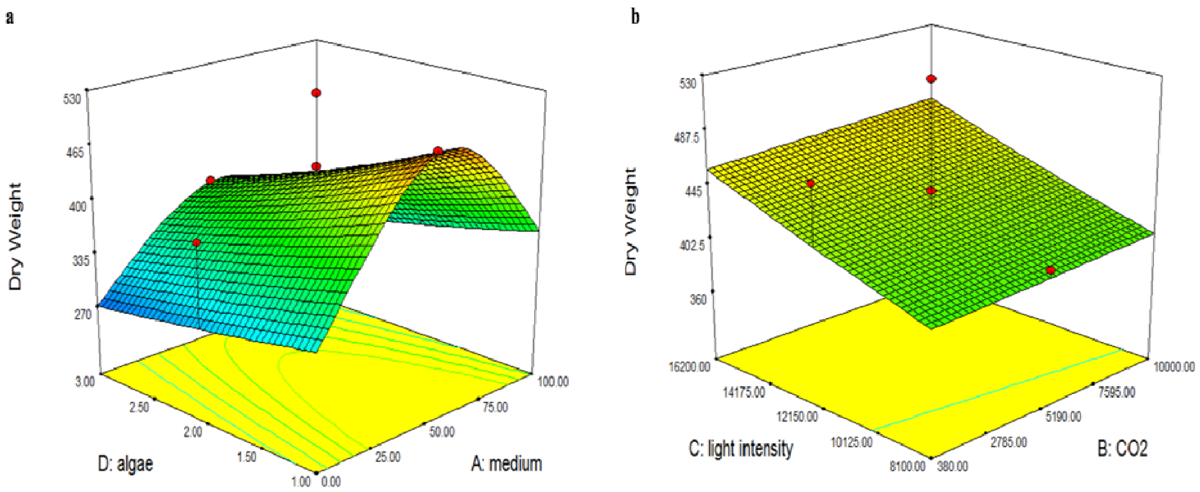

Fig. 2 Response surface 3D plots for evaluation of medium components to maximize the biomass production. a: algal strain vs. culture medium; b: light intensity (lux) vs. CO_2 concentration (ppm).

Bioremediation of environmental pollutants was considered as one of the most important goals of this study. To achieve the highest performance, it is essential to optimize the medium components to maximize the biomass production using three operational parameters (light intensity, CO_2 concentration and concentration of available nutrient). One of the important parameters in the economically-feasible production of any algal strains is providing a cheap and at the same time, rich, source of required nutrients. This factor especially in large-scale cultivation, impose strong influences on the productivity and environmental aspects, so it is important to understand how this response can be optimized. This topic is discussed in numerous previously published studies in the field; Singh and Das (2014) showed that utilizing rich sewage sources (urban, animal and agriculture) is useful for supplying the nutrients (nitrogen and phosphorus) required for algae cultivation. Such an approach helps to reduce the final costs of algae biomass production and also leads to the elimination of environmental pollutants. In another study, Nelson et al. (2007) showed that the growth of algae in agricultural wastewater helps to improve their growth due to the optimal absorption of nutrients. Also, these algae are suitable for the production of biofuels because, in addition to their high growth rates, their production costs are also reduced.

The synergistic effect of mixed culture medium in the present study was in line with already published reports; Jiang et al. (2011) observed that a 1:1 ratio of municipal wastewater and seawater with f/2 medium could increase the biomass concentration of *Nannochloropsis* sp. up to 31% (212 mg L^{-1}) compared to a control (161 mg L^{-1}). Also, the relationship between parameters is very important. The correlation of these parameters on growth stimulation of algal cells was investigated by Mejia Rendon (2014). This study showed that increasing CO_2 concentration and white light treatment, increased biomass production in algae. It was also reported in a study done by Rooijakkers (2016), that increasing light intensity up to 200 µmol photon m^{-2} s^{-1} could increase photosynthesis activity and biomass production in aquatic freshwater plants. In general, algae are capable of producing high biomass under normal and high concentrations of CO_2 (Singh and Singh, 2014). It was reported that the addition of CO_2 in intensive culture of three green algal species, *Chaetomorpha linum*, *Cladophora coelothrix* and *Cladopha rapatentiramea* enhance the productivity of targeted algal strains and this was then followed by enhanced adsorption of nitrogenous compounds from the culture media (De Paula Silva et al., 2013).

The presented model for optimization of medium components to maximize the biomass production also introduces the influencing factors on biomass production. Factors such as CO_2 concentration, light intensity and algal strains positively affect the final dry weight. Based on the strong correlation between BP and LP (Griffiths and Harrison, 2009), it is expected that the physico-chemical growth properties which could result inthe higher BP would enhance the potential of biorefinery capacity as well as biofuel production.

3.4 Optimization of culture condition to increase the biorefinery capacity

3.4.1 Nitrate and Nitrite removal

Table 5 summarizes the potential of algal cultivation to carry out bioremoval of environmental pollutants in presence of different treatments (three operational parameters in three levels). The results showed that cultures grown at 5190 ppm of CO_2 were most effective in removing nitrate (Fig. 3a). Also, strain number 2 (*C. antennina*) showed a higher capacity for nitrate removal compared to other strains (Fig. 3a).

Also, the results showed that increasing light intensity to 225 µmol photon $m^{-2}s^{-1}$ in interaction with other treatments played an important role in improving nitrate removal (Fig. 3b). In summary, the maximum nitrate removal is seen at around 5190 ppm CO_2, and 170 µmol photon m^{-2} s^{-1} light intensity.

The final equation in terms of actual factors was:

Nitrate removal =+20.51209 -0.32637× medium +6.85194E-003× CO_2 +2.17014E- 003× light intensity +28.43831 × algae -6.36256E-006×medium ×CO_2 +1.85039E- 006×medium×light intensity -

0.016432×medium ×algae -7.59993E-008×CO_2×light intensity -9.77742E-006×CO_2×algae -6.28222E-005×light intensity ×algae +3.57008E- 003×$medium^2$ -5.86035E-007×CO_2^2 -3.74822E-008×light $intensity^2$ -7.22200× $algae^2$

The Model F-value is equal to 6.72, which indicates that the used model is significant. There is only 0.04% chance that calculated F-Value could occur due to noise. The "Lack of Fit F-value" of 3.48 implies there is a 9.06% chance for "Lack of Fit F-value". Other statistical information is included: Standard deviation = 4.64, R^2 = 0.8625, mean = 66.56, adjusted R^2 = 0.7342, coefficient of variation % = 6.96. This model introduces the influencing factors and their interactions on nitrate removal.

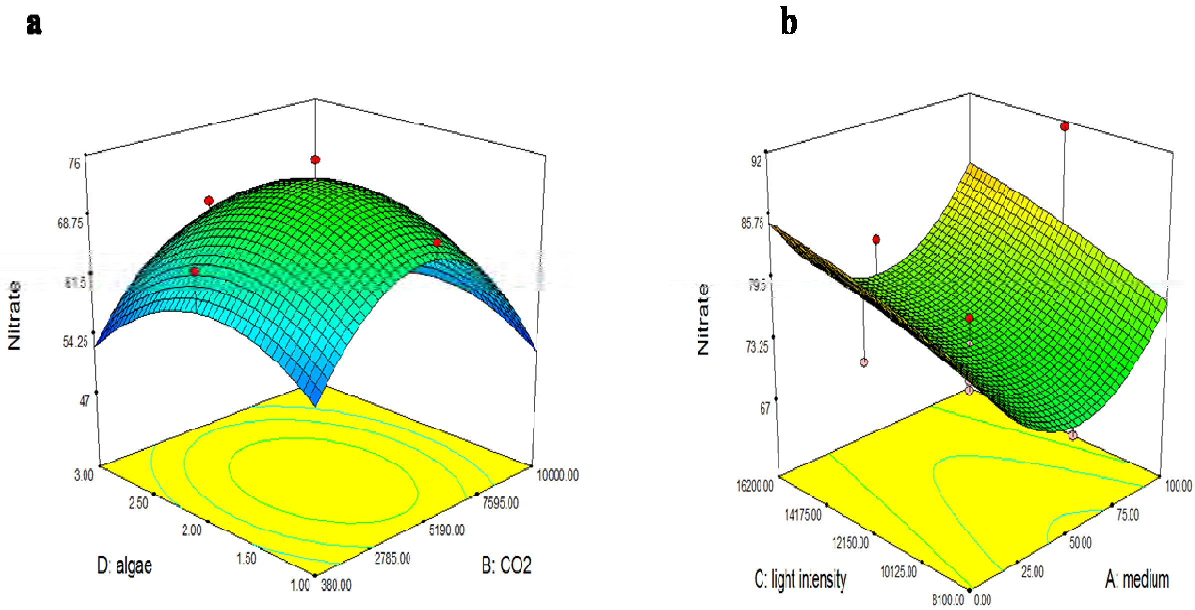

Fig. 3 Response surface 3D plots for optimization of culture condition to nitrate removal. a: algal strain vs. CO_2 concentration (ppm); b: light intensity (lux) vs. culture medium.

Nitrite removal was observed when the cultures were grown under different treatments and their interactions with each other. The results (Table 5) of this experiment showed that the increase in light intensity in interaction to other treatments has a high effect on nitrite removal (Fig. 4a). Also, the results showed the use of sewage in reaction with light intensity treatment can help to improve nitrite removal (Fig. 4a). CO_2 concentration was another key parameter in efficiency of nitrite removal. In our study, maximum nitrite removal was carried out at 5190 ppm concentration (Fig. 4b). Also, *C. antennina* (strain Nu. 2) showed higher ability in nitrite removal compared to other species (Fig. 4b). In summary, the maximum nitrite removal was recorded at around 5190 ppm CO_2, and 170 µmol photon m^{-2} s^{-1} light intensity. The following model introduces the influencing factors and their interactions on nitrite removal.

The final equation in terms of actual factors was:

Nitrite=-57.06905 +0.27955 × medium +8.99600E-003× CO_2 +0.013618× light intensity +33.21989 × algae +9.84083E-006× medium × CO_2-2.74624E-005× medium × light intensity +0.039960×medium × algae -2.38306E-007× CO_2× light intensity - 4.06735E-004 × CO_2 × alga +4.97438E-005× light intensity × algae -1.08227E-003× $medium^2$ -5.79000E-007× CO_2^2 -4.33511E-007× light $intensity^2$ -8.93328× $algae^2$

The Model F-value is equal to 9.99, which indicates that the used model is significant (0.01% chance for the noise). The Lack of Fit F-value of 6.27 implies the Lack of Fit is significant. Other statistical information is included: Standard deviation = 7.12, R^2 = 0.9031, mean = 69.92, adjusted R^2 = 0.8127, coefficient of variation % = 10.18.

Table 5 Percentage of pollutants removal and CO_2 fixation in different treatments according to the Table 2.

Run	Nitrate (%)	Nitrite (%)	Phosphate (%)	CO_2 (%)
1	62.7	52.63	69.05	19.48
2	69.63	45.16	75.43	28.21
3	67.26	78.94	74.51	18.94
4	64.62	63.15	75.68	23.79
5	59.5	51.61	74.26	20.53
6	53.68	71.61	89.47	18.95
7	59.43	52.63	56.95	14.31
8	69.93	85	93.92	30.14
9	82.36	69.35	70.76	47.09
10	75.42	86.42	90.63	29.97
11	69.39	92.14	92.15	33.19
12	66.61	80	78.73	25.89
13	68.23	74.28	75.94	29.12
14	67.39	52.25	95.41	60.84
15	72.41	85	91.1	34.89
16	68.93	92.14	91.1	33.02
17	91.41	82.9	76.02	37.6
18	72.64	92.14	91.1	33.53
19	67.92	92.14	91.1	29.97
20	75.49	89.47	78.93	38.28
21	79.59	89.28	71.89	51.33
22	66.46	69.28	75.69	28.61
23	51.99	54.83	71.34	14.11
24	58.86	57.89	63.06	12.7
25	54.29	61.29	70.17	18.94
26	60.42	47.36	67.1	12
27	63.34	58.06	64.32	18.94
28	62.76	54.83	53.8	21.06
29	56.97	42.1	50.06	12
30	67.3	73.68	71.91	16.63

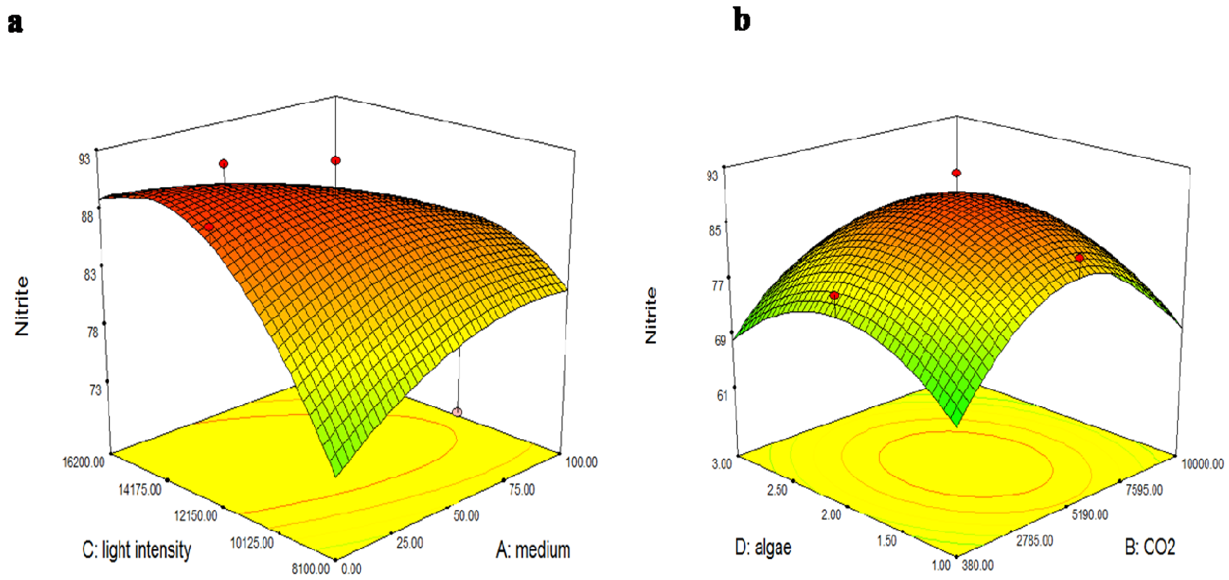

Fig. 4 Response surface 3D plots foroptimization of culture condition to nitrite removal. a: light intensity (lux) vs. culture medium; b: algal strain vs. CO_2 concentration (ppm).

Absorption of nitrogenous substances by algae is a response in medium containing high CO_2 (Suárez-Álvarez et al., 2012). CO_2 supplementation for different algae strains easily increase uptake and assimilation of nitrate ion because the function of nitrate reductase enzyme is enhanced under the condition of elevated CO_2 (Xu et al., 2010; Zou, 2005). On the other hand, excessive concentrations of CO_2 reduce nitrate uptake, which can be due to reduced pH in the medium (Azov and Goldman, 1982; Markou et al., 2014). It has also been observed that high concentrations of CO_2 could lead to reduced rates of nitrate uptake in some macroalgae species (García-Sánchez et al., 1994; Andria et al., 1999). During the present study, nitrate uptake did decrease significantly in extreme concentrations of CO_2 (10000 ppm).

Our results also showed that the increase in light intensity plays an important role in the removal of nitrate. Light stimulates algal growth and this in turn leads to increased uptake of nitrate, nitrite and phosphate from the medium. A direct correlation between biomass productivity and bioremoval capacity of the members of Cladophoraceae family has been comprehensively discussed (Bootsma et al., 2004; Ross, 2018; Szabo, 2012). Optimized growth condition not only improves the biosorption capacity, but also it could leads to improved CO_2 uptake (Wood, 1975).

In general, the process of nitrate removal is light-dependent; in other words, elevated light intensity could improve the photosynthetic activity; the resultant enhanced equilibrium of cell energy facilitates nitrate uptake by photoautotrophic algal cells. However, extreme intensities of light might lead to photo-stress and the latter could negatively affect growth and the rate of nitrate bioremoval.

As seen in the final equation for nitrate removal presented here, the interaction between two treatments (including medium and CO_2) plays an important role in nitrate uptake. In a similar investigation, Park and Craggs (2011) observed that adding CO_2 to a medium containing wastewater leads to an increase in adsorption of nitrogenous compounds. The possible reasons for stimulation of nitrate reductase under elevated CO_2 were previously described by Hofmann et al (2013). They found that the combination of light and CO_2 can be effective on the process: a reduced plastoquinone pool under high light conditions and elevated CO_2 stimulate nitrate reductase enzyme and absorption of nitrate. Accordingly, the interaction between CO_2 and light intensity was also evaluated positively in our study.

Different algal strains showed different bioremoval capacity. In the present study, it was observed that the *C. antennina* removed environmental pollutants such as nitrite more efficiently. In a study done by Menéndez et al. (2002) the related species, *Chaetomorpha linum* significantly absorbs nutrients such as nitrates and nitrites and other nitrogenous compounds.

3.4.2 Optimization of medium components for phosphate removal

Our results (Table 5) showed that increasing light intensity between 115 to 200 µmol photon $m^{-2} s^{-1}$ has led to improved phosphate removal (Fig. 5a). In general, phosphorus plays an important role in the photosynthesis and the conversion of light energy to biochemical compounds through the electron donor mechanism. In our experiment, another important factor in removing phosphate is CO_2 concentration (to 10000 ppm); higher concentration of CO_2 accelerated the rate of phosphate bioremoval in all 3 macroalgae cultivation systems. It should be noted that *C. antennina* (strain 2) showed higher capacity in phosphate bioremoval, too (Fig. 5b); according to the Table 5, this strain is able to remove phosphate upto 95%. The following model introduces the influencing factors and their interactions on phosphate removal. The maximum phosphate removal was observed at around 10000 ppm CO_2, and 225 µmol photon $m^{-2} s^{-1}$ light intensity. The final equation in terms of actual factors was:

Phosphate = -64.54298 +0.66534× medium +8.85665E-004 × CO_2 +0.017077 × light intensity +30.59598× algae -3.22428E-009× medium × CO_2 -2.22732E-005 ×medium ×light intensity -0.036140× medium ×algae -1.24683E-007 × CO_2 ×light intensity -3.62199E-004 ×CO_2 ×algae -8.70676E-005×light intensity ×algae -2.57581E-003 ×medium^2 +1.98345E- 007× CO_2^2 -6.09634E-007×light intensity^2 -7.78591×algae^2

The Model F-value is equal to 4.49, which indicates that the used model is significant. There is only 0.33% chance that Model F-Value this large could occur due to noise. The Lack of Fit F-value of 330.89 implies the Lack of Fit is significant. Other statistical information is included: Standard deviation = 7.48, R^2 = 0.8074, mean = 76.39, adjusted R^2 = 0.6274, coefficient of variation % = 9.80.

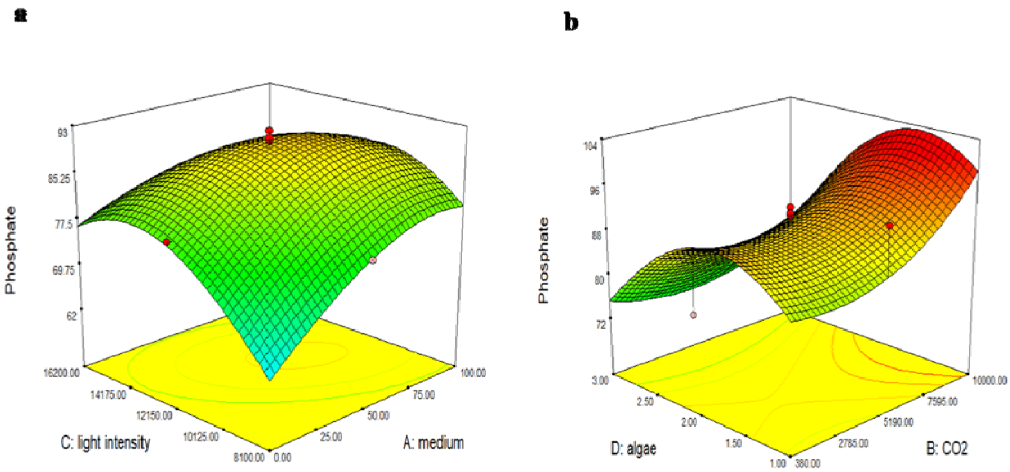

Fig. 5 Response surface 3D plots foroptimization of culture condition to phosphate removal. a: light intensity (lux) vs. culture medium; b: algal strain vs. CO_2 concentration (ppm).

Phosphate is one of the limiting factors for the growth of algal communities. Excessive concentration of this nutrient is considered as one of the factors caused algal blooms in aquatic systems. *C. antennina* was also more successful in bioremoval of phosphorus in comparison to the other strains. Various studies have shown that *Chaetomorpha* sp. is capable to absorb phosphorus compounds significantly (Ribeiro et al., 2017). In a study, it was found that the macroalgae *C. linum* absorbed nutrients (such as phosphate) from primary and secondary wastewaters. In summary, macroalgae-based wastewater treatment could serve as an integrated approach for concurrently nitrogen and phosphorus biosorption (Champagne, 2017). The potential of strain 2 (*C.antennina*) herein confirms this opportuenty to coupled nutrient bioremoval using a productive algal strain.

The interactions between different parameters, their effect on phosphate removal as well as related enhancement of photosynthetic activity and growth rate in algae have been the subject of various studies; Scherfig (1973) and Al Ketife et al. (2017) evaluated the effects and interactions between light and CO_2 to remove nutrients such as phosphates. Their results showed that these parameters have significant effects on phosphate removal. However, it is also necessary to evaluate the effect of other factors such as temperature, pH, etc., on phosphate bioremoval; since each of these factors can be effective on the mechanism of phosphorus metabolism (such as phosphate transportation and phosphate hydrolysis) (Lee and Lee, 2001).

3.4.3 Optimization of medium components for CO_2 sequestration

During the present study, this potential of algal cells was optimized in laboratory scale. The results (Table 5) of this study showed that addition of agricultural wastewater as well as increasing light intensity play an important role in improving the potential of CO_2 sequestration (Fig. 6). Moreover, among the three macroalgae species, *C. antennina* sequestrates more CO_2 (Fig. 6b). The following model introduces the influencing factors and their interactions on CO_2 sequestration. The maximum CO_2 sequestration was recorded at around 10000 ppm CO_2, and 225 μmol photon m^{-2} s^{-1} light intensity. Also, in the present study, a significant positive correlation between light intensity as well as wastewater concentration for promoted CO_2 sequestration was observed (Fig. 6).

The final equation in terms of actual factors:

$1.0/\mathrm{Sqrt}(CO_2)$ = +0.39042 +4.21237E-004 ×medium -8.27027E-007 ×light intensity- 0.15109 ×algae +5.49730E-009 ×medium ×CO_2 -2.43086E-008 ×medium ×light intensity -1.47469E-004 ×medium ×algae +1.10011E-010 ×CO_2×light intensity +1.48611E-006 ×CO_2×algae +8.59583E-007 ×light intensity ×algae - 1.24189E-006 ×medium^2 +1.70532E-009 ×CO_2^2 -1.83783E-010 ×light intensity^2 +0.038433 ×algae^2

The Model F-value is equal to 7.69, which indicates that the used model is significant. There is only 0.02% chance that Model F-Value might be occurred due to the noise. The Lack of Fit F-value of 21.74 implies the Lack of Fit is significant. Other statistical information is included: Standard deviation = 0.021, R^2 = 0.8777, mean = 0.21, adjusted R^2 = 0.7636, coefficient of variation % = 10.42.

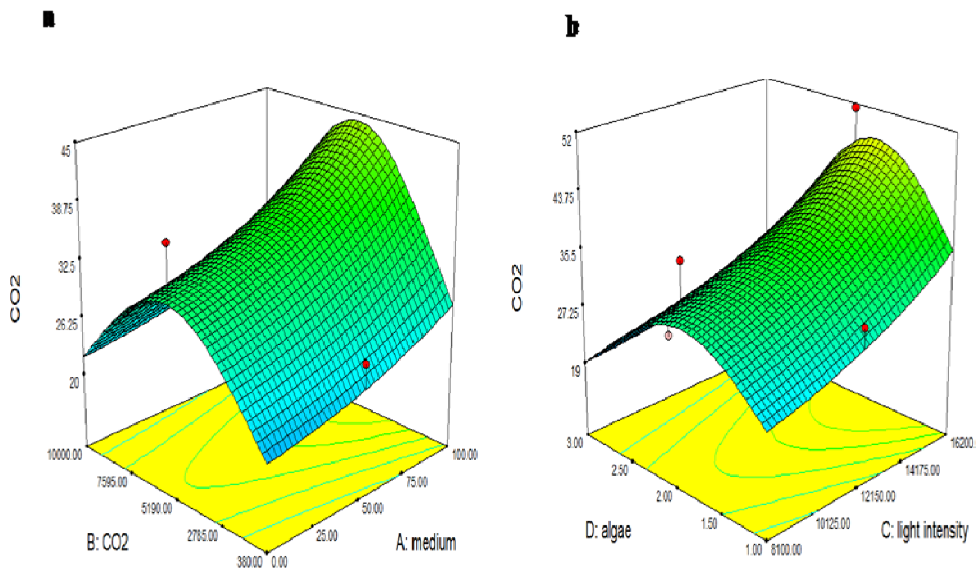

Fig. 6 Response surface 3D plots for optimization of culture condition to CO_2 sequestration. a: CO_2 concentration (ppm) vs. culture medium; b: algal strain vs. light intensity (lux).

The rate of CO_2 biofixation is influenced by different parameters including light intensity, inoculum type, concentration of available nutrients. Several studies showed the role of nutrients in CO_2 fixation. Hessen et al (2004) determined that reducing nutrients leads to reduction in CO_2 fixation. In another study, Zhao and Su (2014) referred to the role of nutrients and light intensity in CO_2 fixation. Among the three macroalgae species, *C.antennina* sequestrates more CO_2 (Fig. 6b). In various studies it has been observed that, light intensity, cell density and light penetration directly influence the photosynthesis, biomass production and CO_2 sequestration (Zhao and Su, 2014; Moreira and Pires, 2016). Jacob-Lopes et al (2008) evaluated the interaction of light intensity and CO_2 concentrations for CO_2 sequestration. The results showed that optimization of light intensity and CO_2 concentration are effective in increasing CO_2 sequestration compared to initial inoculum. Various studies have evaluated the effects of light intensity and the supply of adequate nutrients. Providing proper conditions could lead to increase photosynthesis, resultant enhanced algal biomass productivity as well as more biofuel production (Kumar et al., 2010; Mondal et al., 2017).

In our study, a significant positive correlation between light intensity as well as wastewater concentration for promoted CO_2 sequestration was observed. Similar trends have been repeatedly published in the literature; for example, in a study by Guo et al. (2018), interaction of algae grown in Shanghai's plant wastewater and different light intensities was evaluated. The results showed that under light intensity of 250 µmol m^{-2} s^{-1} the highest percentage of carbon removal (63%) was observed. In another study the activity of algae grown in wastewater under different light conditions (50 to 80 µmol m^{-2} s^{-1}) along with variations in the pH of the culture medium (8 to 9) was evaluated. The results showed that under light intensity 80 µmol m^{-2} s^{-1} and media containing wastewater, algae are able to remove CO_2 by up to 50% (Bhakta et al., 2015).

3.5 Spatiotemporal variations and chl-*a* concentration

The spatiotemporal variability of phytoplankton biomass in the studied area was investigated by satellite-derived chlorophyll *a* (Chl*a*-sat) data. The result showed that chl*a*-sat concentration varied from 12 to 17 mg / m^3 from 2010 to 2018. The observed variability could be explained by factors influencing the growth of phytoplankton namely the emission of the gas and nutrients contamination into the ecosystem; the elevated CO_2 dissolution in seawater could increase carbon biofixation, result in enhanced growth of algal population (Fig. 7). In the area of the Miankaleh wetland, a fossil fuel power plant, Neka power plant, is located and provides more than 6% of the total electricity generation in Iran. However, due to the use of mazut, a low quality fuel oil, this fossil fuel power plant releases a lot of environmental gas pollution namely CO_2 into the Miankaleh wetland which negatively affects the sustainability of the ecosystem.

It was also observed that chl*a* concentration is higher in summer. The reason might be the higher supply of the nutrients thorough agricultural wastewaters into the Miankale Wetland. Moreover, higher temperature and elevated light intensity could help to increase the biomass of algal communities during the warm season.

According to the predicted concentration of chl*a* obtained from satellite imagary (Chla-Sat), the amount of fresh phytoplanktonic biomass in the total area of the water section of Miankaleh wetland (45000 hectare, 1 meter depth), was estimated approximately76500 ton wet biomass. If the sequestration rate of 1.83 grams of CO_2 per 1gram of algal biomass was adopted (Rosenberg et al., 2011), 140 kilotons CO_2 could be sequestrated in this wetland during the phytoplanktonic photosynthesis in a growth season. Although, this level of bio-sequestration might be different depending on the season, richness of the wetland by nutrients and provided CO_2 in the atmosphere. All these parameters in a correlation with type of algal population directly influence the final capacity of CO_2 bio-sequestration.

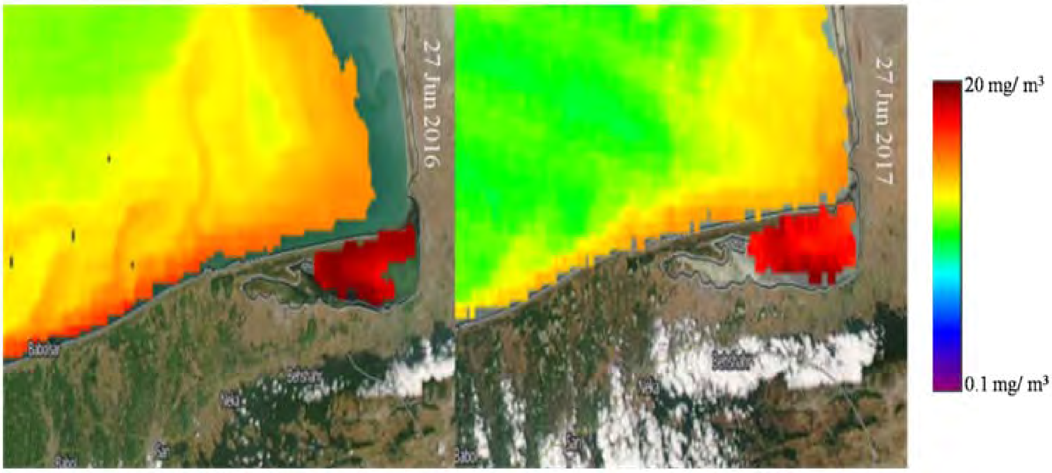

Fig. 7 Determination of chlorophyll a concentration in the studied area using MODIS Terra and Aqua.

Phytoplankton populations, during the photosynthesis process, absorb dissolved CO_2 in the water and release oxygen. Subsequently, the increased biomass of phytoplankton and their photosynthetic pigments, such as chlorophyll a (chl*a*), absorb sunlight in the visible range. Analysis of the upper layers of water to determine the chl*a* concentration can be done using remote sensing. This is based on the change in optical properties such as water color (Wang et al., 2018). The MODIS chl*a* layer provides chl*a* concentration in mg / m^3 in aquatic systems. It used as an indicator of phytoplankton biomass which later can be used as an indicator of changes in productivity, global climate change, carbon cycleand release of pollutants in the ecosystem. Changes in the biomass of phytoplankton indicate that there is a direct correlation between the increase of biomass and carbon capture in the form of carbon dioxide.

'Brown cloud' and 'black carbon' are the causes of global concerns, but their presence and impacts are particularly strong in Asia. Changes in rainfall patterns, rising air temperatures, decreases in albedo and decreased crop productivity are the main negative impacts of brown cloud and black carbon. These 2 phenomena are created by a range of airborne particles and pollutants from incomplete combustion of fossil fuels, biofuel and biomass. The smoke plume usually appears to rise out of coastal power plants, factories and small fires. The small fires originate from local farmers preparing the land for spring planting. Smoke can be thick enough near the source to be easily visible from space. Numerous images were captured by the MODIS instrument on NASA's Aqua satellite showing thick layers of dust over the Caspian Sea. The smoke diffuses shadows on the Caspian Sea surface and could lead to algal blooms. Given adequate sunlight, temperature and nutrients supplied by agricultural run-off rich in fertilizers, phytoplankton populations can turn into blooms large enough to be visible from space.

Uncontrolled growth of algae in the water, eutrophication, brings negative consequences for aquatic life; however if the stimulated growth of algal cells is controlled, it could positively reduce the environmental pollutants in the region. The confirmed capability of the studied strains in laboratory conditions for sequestration of CO_2 could be employed to reduce CO_2 concentration in the Caspian Sea. Moreover, the sequestrated CO_2 could be used for biofuels production. On the other hand, the biorefinery of the nutrients improves the quality of the water through elimination of environmental pollutants. This operational process is previously performed in large-scale. For example Wang et al (2015) reviewed the algal capacity in CO_2 sequestration near the industrial plants. They reported that using combined technology of biological sequestration of CO_2, more than 75% of emitted CO_2 could be recycled. The injected CO_2 in algal cultures led to over 30 times increase in the biomass of algae. In an attempt to mitigate global ocean acidification, as a consequence of rising atmospheric CO_2, Mongin et al. (2016) successfully surveyed the potential of a reef-scale cultivation of multicellular algae in carbon removal in the Heron Island reef. The issue of CO_2 mitigation coupled with commercial seaweed production in Asian-Pacific region has been the topic of numerous investigations. A potential of 2.87 million tons CO_2 sequestration in algae farms of Asian Pacific countries was reported by Sondak et al. (2017). Australia's largest coal-fired power stations use piped pre-emission smokestacks to convert CO_2 into hydrocarbons within the algal ponds. CO_2 and other greenhouse gases, such as N_2O, from local emitters are sourced at the production site and no additional emissions are released by transportation (Rhodes, 2012).

To date, various studies have been carried out on the monitoring of chl*a* content using MODIS-based satellite imagery in the Caspian Sea (Moradi, 2014; Salman et al., 2013; Jamalomidi, 2013). In a study done by Salman et al (2013), it was shown that the chl*a* concentration in the southern part of the Caspian Sea was 1.4 to 4.8 mg / m^3 in October 2008. Naghdi et al. (2018) showed that there is a significant relationship between cyanobacteria and chlorophyll *a* in the southern Caspian Sea using MODIS satellite imagery. Bianchi et al (2013) expressed that increasing biomass concentration in an environment could lead to an increase in carbon fixation. In general, the analysis of satellite images associated with Chl*a* content (specifically in the Miankaleh wetland in 9 years) provides an overview to evaluate the spatial-temporal variations of phytoplankton and algal biomass productivity and resultant carbon biofixation.

We estimate the spatiotemporal and biomass variations in the algal population in the studied area. According with the recent activities of the Neka power plant in the vicinity of the Miankale Wetland, there is a high probability of increasing greenhouse gases concentration such as CO_2 in the wetland. This can be a factor in increasing biomass during the period of study. Neka power plant generates 2214 megawatt electricity power. However, due to the burning of mazut, this fossil fuel power plant releases a lot of environmental pollution in the Miankaleh wetland. These environmental pollutants can help to improve the biomass of the predominant algae in the Miankaleh Wetland through the supply of carbon and nutrients to stimulate the growth of algal communities. On the other hand, by exploring new facilities, performing new plan of power generation, applying worldwide strategies and developing renewable fuels, they are to diminish the environmental effects of power generation and lead to a reduction of emission of CO_2 from 2017. The new strategy of Iran's power sector planned to enhance the contribution of natural gas as an alternative fuel. It was led to a reduction of heavier fuels burning in the fossil fuel power plants. Moreover, this strategy aims to increase the contribution of combined cycle power plants to increase thermal efficiency and diminish the external impact of emissions. By performing this plan in Iran's power sector, the average emissions of CO_2 will reduce by 20%.

Reliable monitoring of coastal waters is not possible without using remote sensing data. It is obvious that the infrequent monitoring using routine measurement tools cannot provide the spatial and temporal coverage needed for monitoring such dynamic environments like coastal waters. As Gholamalifard et al. (2013) used specific optical properties of the optically active substances in developing regional algorithms for retrieval of water characteristics in the southern Caspian Sea, herein, we analyzed the satellite images associated with Chl*a* content and provide an overview for evaluation of the algal biomass. Our hypothesis was based on 2 facts: 1) Given adequate sunlight and nutrients, phytoplankton populations can swell into blooms large enough to be visible from space 2) increasing biomass concentration could lead to an increase in carbon fixation in an environment. So, the findings support the hypothesis that the high concentration of algal pigment in the wetland is correlated with the ability of the studied strains in bioremediation of environmental pollutants; Our results in the laboratory confirmed that the studied algal strains could successfully sequestrate CO_2.

As shown in Section 3.4.3, all three predominant macroalgae in the Miankaleh wetland (especially *C. antennina*) showed the ability to sequestrate CO_2 with an appropriate rate in laboratory conditions. Therefore, the existence of these algal species in the Miankaleh wetland could play an important role in reducing concentrations of CO_2 and other greenhouse gases**,** preventing further contamination as well as global warming (Venkatesalu et al., 2012). This can be an operational process to help decisions made at the Paris climate conference 2015. So, cultivation of macroalgae to sequestrate carbon, conversion of biomass to biofuels and burning natural gases in the power plants are among affordable program to reduce greenhouse gas emissions in the Miankaleh wetland.

Even the critics that express concern about scale up of experimental achievements and the limited market of algae production, assessing the current potential, believe that very good perspectives exist either to produce biofuels or other valuable products such as pharmaceutical or cosmetics in the future (Patricio et al., 2017; Ibrahim et al., 2016). This potential application of algal strains as solar biofactories of valuable chemicals was recently reviewed (Norena-Caro and Benton, 2018). In summary, the reviewed results highlight that only a kilometer-scale cultivation of algal strains can positively influence the global carbon cycle, depending on future global carbon emissions. Algal feedstock can act as a newly-emerged carbon donor to other ecosystems and by this means significantly improve the global carbon sequestration.

4 Conclusions

Surplus usage of nutrients in agricultural wastewater could lead to dangerous disruption to aquatic ecosystems such as algal blooms. In this study, the effects of light intensity, CO_2 concentration and different loadings of agricultural wastewater on the biosorption capacity of three algal strains were investigated. The local isolates usually represent enough adaptation to abiotic stresses and also show good ability for bioremoval of pollutants and CO_2. Our study showed that the interactions between high concentration of CO_2 (5190 up) and light intensity (177 µmol photon $m^{-2} s^{-1}$ up) could increase the BP, pollutants removal and CO_2 sequestration. Also, the results showed that *C. antennina*is a successful strain in bioremediation and CO_2 biofixation in comparison to other studied stains. In the present study, algal strains were able to absorb 70 to 90% of nitrate, nitrite and phosphate. Therefore, the cultivation of algae in agricultural wastewater not only absorb nutrients and increase the biomass productivity, but also help to absorb greenhouse gases (such as CO_2) and reduce the effects of climate changes, more sustainable than any other physico-chemical approaches. According to the obtained results, studied macroalgal strains were able to sequestrate 30% to 60% of the provided CO_2. Moreover, the produced algal biomass can be used in biofuel production and as biofertilizer by recycling nitrates, nitrites and phosphate absorbed by algal strains. The results obtained provide an overview of the satellite evolution of the temporal variability of chl*a* and the ability of the algal strain in biosorption of environmental pollutants. This approach could be sustainably served as an indicators of biochemical, physical and biological processes in the aquatic systems.

Acknowledgement

The authors wish to thank the Iran Biotechnology Initiative Council, Vice-presidency for Science and Technology of Iran, for financial assistance. This study was financially supported by grant NO: 950701 of the Biotechnology development council of the Islamic republic of Iran. A part of the work was supported by the department of biotechnology, Sari University.

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