Article

CO_2 bio-sequestration by *Chlorella vulgaris* and *Spirulina platensis* in response to different levels of salinity and CO_2

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Abstract
The limitation of fresh water and the exorbitant cost of land to grow such plants, algae are the most optimum organisms for CO_2 bio-sequestration and also microalgae cultures avail many characteristics that make them an arguable option for higher productivities as compared to higher plants. The aim of this study was the sequestrate CO_2 by *Spirulina platensis* and *Chlorella vulgaris* under the different levels of salinity and CO_2. The highest growth rate obtained during the first 4 days and higher biomass concentration at CO_2 concentration from0.03% to 10% respectively. The significant ($p<0.05$) biomass productivity, growth rate and CO_2 sequestration rate under different level of CO_2 and EC between *Spirulina platensis* and *Chlorella vulgaris* were detected. The ultimate CO_2 sequestration rate of *Spirulina platensis* and *Chlorella vulgaris* were 0.49 and 0.152 g/L/d in natural water respectively, although in artificial sea water 0.419 and 0.097 g/L/d were recorded at 10% CO_2 concentrations respectively.

Keywords green house gas; microalgae; Sea water; natural water; pure water.

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1 Introduction

Carbon dioxide (CO_2) has been known as one of the most important greenhouse gases. Global warming is caused by anthropogenic CO_2 emissions from fossil fuel utilization (Pachauri and Reisinger, 2007; Zhang and Liu, 2012). CO_2 emissions are expected to rise in the coming years because of energy needs increasing in all the world (Kessel, 2000).

So many attempts have been made to reduce atmospheric CO_2 including chemical absorption, physicochemical adsorption, membrane, cryogenics, chemical looping combustion and biotechnology techniques (e.g., terrestrial vegetation or hydroponic algae) (de Lary et al., 2012; Sayadi et al., 2011).

Algae are not only the most optimum organisms for CO_2 bioremediation but are also proved to be the most feasible option for the recycling or sequestration of CO_2 since they can simply fix carbon by photosynthesis. The most understudied method is CO_2 biofixation whereby autotrophic organisms and plants convert this CO_2

into organic carbon through photosynthesis producing large amounts of biomass (Stephan et al., 2001; Sayadi et al., 2011). Biofixation via microalgae has been known as a potential for CO_2 capture and storage and sequestration (Naoto and Masahiro, 1997; Masakazu and Masahiro, 1997). Environmental factors, particularly light, temperature, nutrient status, and salinity, affect photosynthesis and productivity biomass (Phycology, 2013). In order to assess the potential of a microalgal system for directly removing CO_2, biomass measurement or growth rate evaluations are necessary (Chang and Yang, 2003). Several studies have been done on microalgae CO_2 fixation capacity. For example, CO_2 of 1850 ppm was directly chosen for the culture of microalgae, with the corresponding bulk CO_2 removal rate of 63.9 mg/ha (Keffer and Kleinheinz, 2002). *Chlorella vulgaris* was cultivated in 3% CO_2 for 8 days and the mean rate 31.8 mg_{CO2}/m^3/day of CO_2 fixation was achieved while the efficiency of energy conversion to biomass was estimated as 4.3%. (Hirata et al., 1996). Sydney et al., (2010) performed analysis of growth parameters, media of cultivation, biomass composition and productivity and nutrients balance and reported the CO_2 fixation rate were 318.61 and 251.64 mg/ L/day for *Spirulina platensis* and *Chlorella vulgaris* respectively (Sydney et al., 2010). Kumar et al., (2014) studied the possibility of using *Chlorella sorokiniana* for CO_2 bio-sequestration from industrial flue gas and the result showed that the highest reduction in the CO_2 content of inlet flue gas was 4.1% (Kumar et al., 2014).

Due to limitation of fresh water and the exorbitant cost of land to grow such plants, algae are the most optimum organisms for CO2 bio-sequestration and also microalgae cultures also avail many characteristics that make them an arguable option for higher productivities as compared to higher plants (Sayadi et al., 2011). However most studies have focused on culturing microalgae in fresh water but according to water quality in the most part of Iran, in this paper the sequestrate CO_2 by *Spirulina platensis* and *Chlorella vulgaris* under the different levels of salinity and CO_2 were studied.

2 Materials and Methods

2.1 Microalgal cultures, medium

Pure culture of *Spirulina platensis* and *Chlorella vulgaris* were obtained from National Inland Water Aquaculture Institute Bandar-e Anzali, Iran. *Spirulina platensis* was cultured in modified Zarrouk medium (de Morais and Costa, 2007; Zarrouk, 1966) and *Chlorella vulgaris* cultivated in Bold's Basal Medium consisting of $NaNO_3$ (25 g/L), $CaCl_2{\cdot}2H_2O$ (2.5 g/L), $MgSO_4{\cdot}7H2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L), EDTA anhydrous (5 g/L), KOH (3.1 g/L), $FeSO_4{\cdot}7H_2O$ (0.05 g/L), H_2SO_4 (1 mL), H_3BO_3 (0.11 g/L), $ZnSO_4{\cdot}7H_2O$ (0.088 g/L), $MnCl_2{\cdot}4H_2O$ (0.014), MoO_3 (0.007 g/L), $CuSO_4{\cdot}5H_2O$ (0.016 g/L), $Co(NO_3)_2{\cdot}6H_2O$ (0.005 g/L) (Ramanan et al., 2009). Agitation and aeration were accomplished using air from a compressor (RESUN AC-9603-0.12MPa)

2.2 Experimental design

The microalgae *Spirulina platensis* and *Chlorella vulgaris* was incubated in 24flat plates glass bioreactor (40 Cm * 40Cm * 40 Cm) with 10 liter of working volume. Each strain was cultured under 3 different EC such as artificial seawater (EC34000 µS/cm), pure water (EC 3 µS/cm), natural water in the study area (EC 12000 µS/cm) and 4 different level of CO_2 (0.03%, 2%, 5% and 10%). It must be note that all experiments are conducted in triplicates. The cultures were maintaining under a 12h dark/light photoperiod with 3500 Lx of illumination for 8 days.

2.3 Preparation of Artificial sea water medium

In the present study artificial seawater preparation devised by Kester et al. (1967) was used. The recipe consists of two lists of mineral salts, the first of anhydrous salts that can be weighed out, the second of hydrous salts that should be added to the artificial seawater as a solution.

2.4 Cell counting and dry weight measurement

In the present study, direct microscopic cell count by Thoma haemocytometer was performed using optical microscope (Labomed). Microalgal dry weight (g/L) was measured by centrifuging 10 ml of each sample at 4500 RPM for 30 minutes and then washed by deionized water, finally dried at 105 °C for 40 minutes.

2.5 Calculation of biomass productivity

The productivity of Biomass (P.g / L/day) was calculated from the variation in Biomass density (x, g/L) according to the following equation (Kumar and Das, 2012)

$P_{overall}\,(g/L/d) = (x_t - x_0)/(t_t - t_0)$

where X was the initial biomass density at time and X_t was the Biomass density at any time.

2.6 Measurement of growth rate

Biomass was calculated from microalgae dry weight produced per liter. Specific growth rate (μ) was calculated from

$\mu\,(day^{-1}) = (\ln(x_t/x_0))/(t_t - t_0)$

where x_t and x_0 were the biomass (g/ L) on days t_t and t_0, respectively (Tang et al., 2011). In this report, we used biomass (g/L) to quantify *Chlorella vulgaris* and *Spirulina platensis* in the culture.

2.7 Carbon sequestration rate

CO_2 sequestration (R_{CO2}, g/L/d) by each organism was calculated from the following formula:

$R_{CO2}\,(g_{CO2}\,L^{-1}\,d^{-1}) = C_c\,P\,(m_{CO2}\,m_C^{-1})$

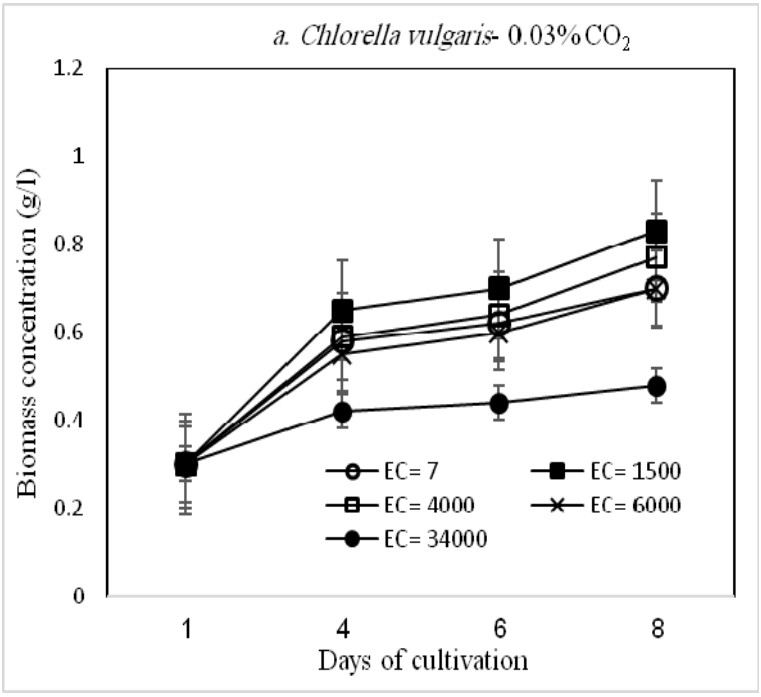
where C_c is the carbon content of the microalgae cell (%, w/w) measured with elemental analyzer; m_{CO2} is the molecular weight of CO_2; and mc is the molecular weight of carbon

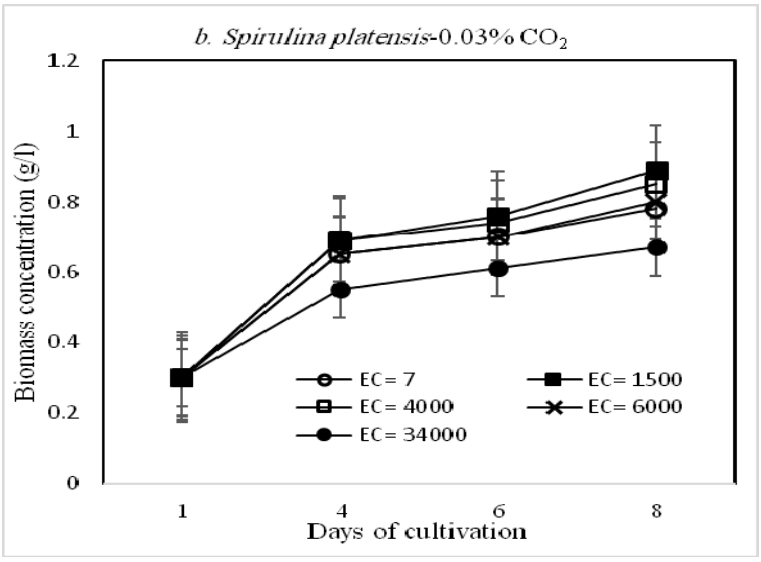
2.8 Statistical analysis

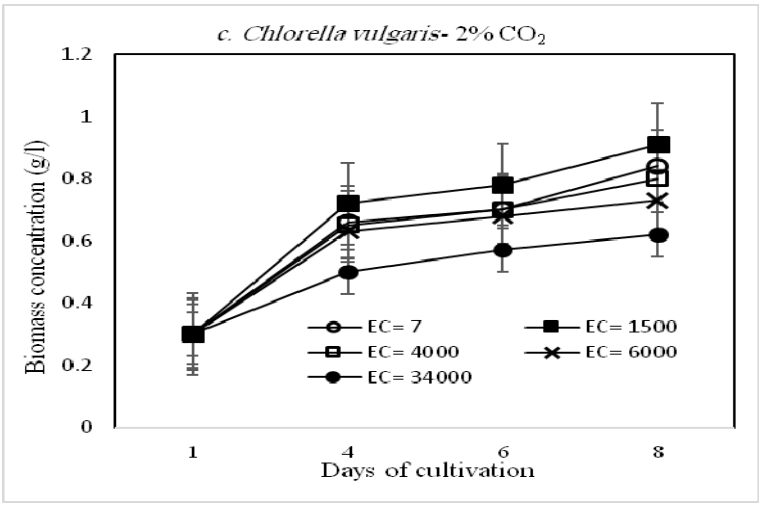
In the present study, the raw data were stored in Ms Excel and then the relationship among biomass production, specific growth rate and bio-sequestration rate of CO2 with the different level of salinity and CO_2 treatments were interpreted by ANOVA analysis using SPSS (version 17) software.

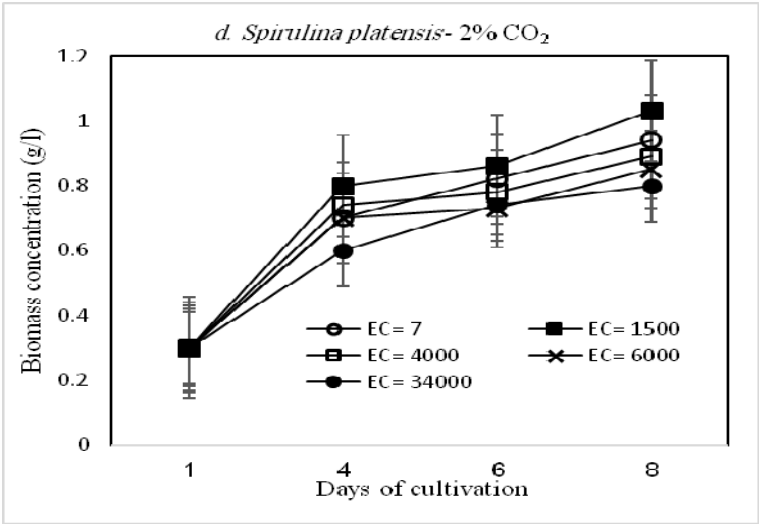
3 Results and Discussion

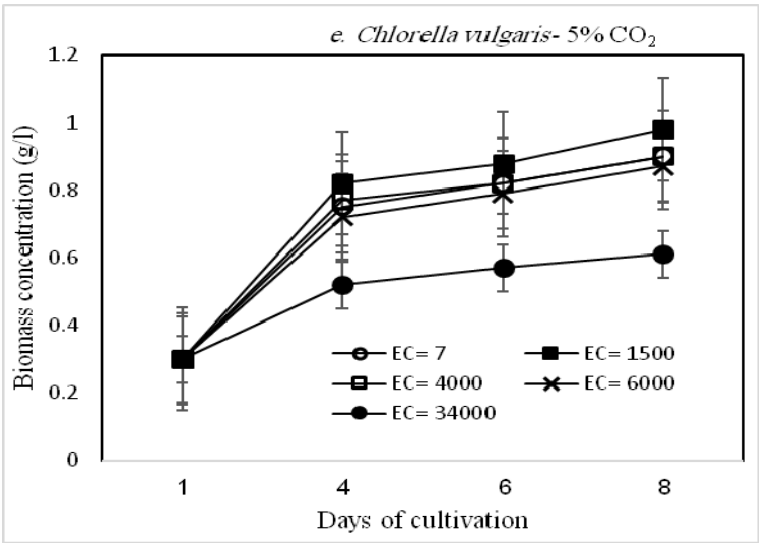
Fig. 1 (a-f) shows the effect of the different CO_2 concentrations on *Spirulina platensis* and *Chlorella vulgaris.* in the 24 photobioreactor containing 3 different EC culture and 4 different level of CO_2. In general aspect *Spirulina platensis* and *Chlorella vulgaris* were found to grow better at higher CO_2 concentration. Microalgae are very common to be used as carbon sequestration. Microalgae easily grown at high level of CO_2 and their cell organism containing chlorophyll a and b where high photosynthetic efficiency to convert CO_2 to O_2 (Singh and Singh, 2014). As shown in Fig. 1 both microalgae have shown higher biomass concentration at CO_2 concentration from0.03% to 10% respectively however some researchers showed that the CO_2 concentration higher than 5% had negative effects on microalgae growth (Chiu et al., 2008; de Morais and Costa 2007a,b; Yoo et al., 2010). In the present study both microalgae showed a good growth under 10% CO_2 concentration, the same has happened in the study done by Tang et al. (2011) which showed both *Scenedesmus obliquus* and *Chlorella pyrenoidosa* grew well under the CO_2 concentration ranging from 5% to 30%.

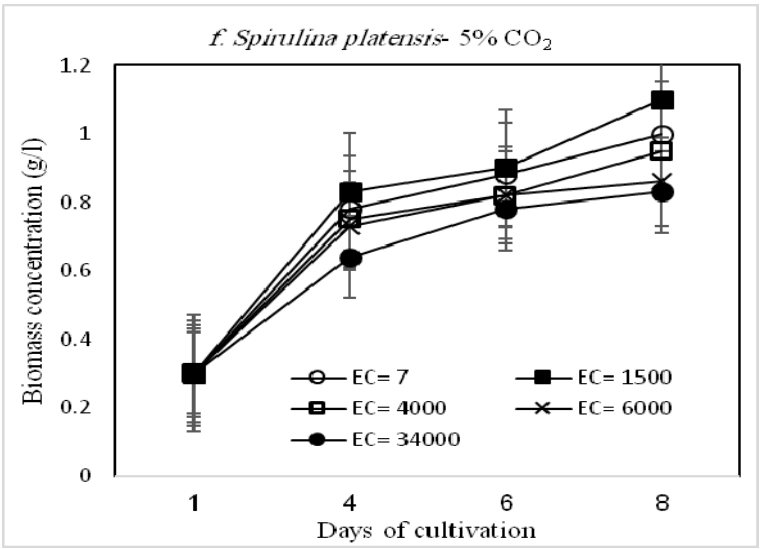

a. Chlorella vulgaris- 0.03% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation


b. Spirulina platensis-0.03% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation


c. Chlorella vulgaris- 2% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation


d. Spirulina platensis- 2% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation


e. Chlorella vulgaris- 5% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation


f. Spirulina platensis- 5% CO2
Biomass concentration (g/l)
1.2
1
0.8
0.6
0.4
0.2
0
EC= 7
EC= 1500
EC= 4000
EC= 6000
EC= 34000
1
4
6
8
Days of cultivation

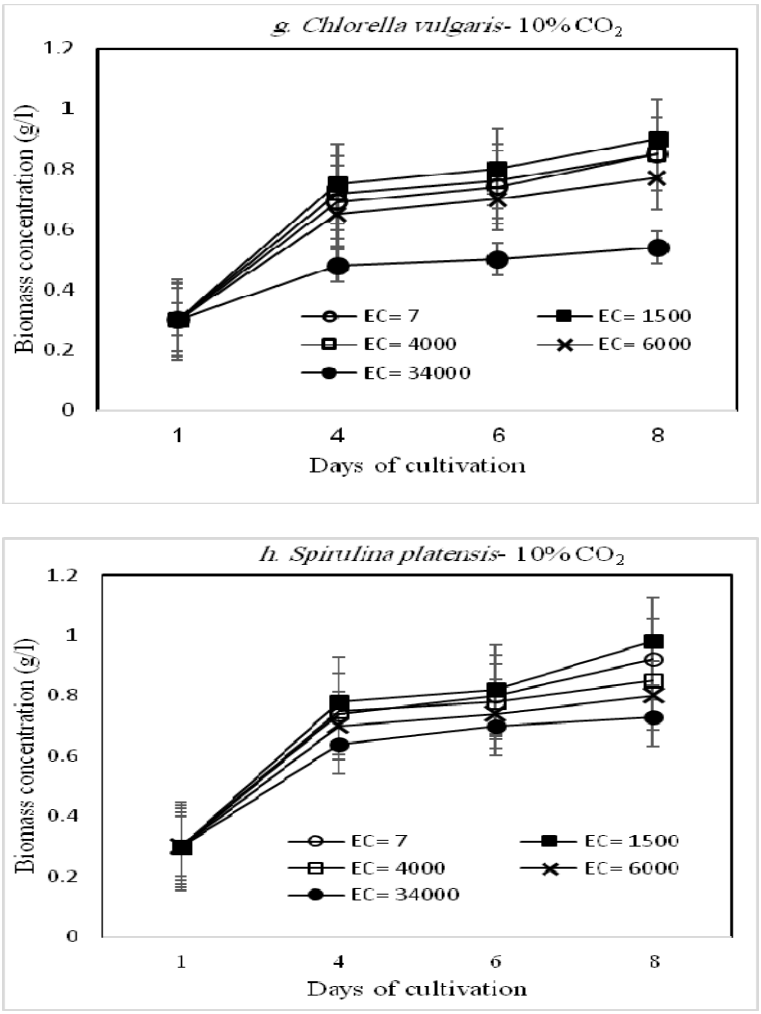


Fig. 1 Biomass production of *Chlorella vulgaris* and *Spirulina platensis* under different level of salinity and CO_2 by passing time.

Generally the highest growth rate has been obtained during the first 4 days. Fig. 1 shows, during the first 4 days the highest biomass production was observed in natural water medium while at the same CO_2 concentration the lowest (0.28 g/L) belongs to *Chlorella Sp.* cultured in artificial sea water (Fig. 1b). The highest biomass growth rate for *S.obliquus* and *C.pyrenoidosa* was 0.4 and 0.2 g/L, respectively at the first 4 days (Tang et al., 2011).

Spirulina platensis is grown better in the artificial sea water than *Chlorella vulgaris* ($p<0.05$) which is obtained by other researchers (Devanathan and Ramanathan, 2013). Although no significant ($p>0.05$) biomass productivity, growth rate and CO_2 sequestration rate under different level of CO_2 and EC were detected however significant different biomass productivity, growth rate and CO_2 sequestration rate and the algae ($p<0.05$) were observed.

The results of mean ±SD of biomass, specific growth rate and CO_2 sequestration for the cultures under different level of CO_2 and EC are presented in Table 1.

In the actual experimental run, *Chlorella sp*. showed sequestration rate in the order of 0.093, 0.123, 0.131 and 0.144 g/L/d for input CO_2 concentrations of 0.03%, 2%, 5% and 10% in pure water, 0.041, 0.084, 0.087, 0.097 g/L/d in artificial sea water and 0.111, 0.133, 0.143 and 0.152 g/L/d in natural water, respectively. In the

study done by Kumar et al., (2014) the growth rates without carbon injection of *Chlorella sorokiniana* was 0.42 (d^{-1}).

Table 1 Mean biomass productivity (p), growth rate (μ) and CO_2 sequestration rate (R) of *Spirulina platensis* and *Chlorella vulgaris* under different level of CO_2 and EC**.**

Species	CO_2 Concentration	Pure water (3 μS/cm)			Natural water (1500 μS/cm)			Artificial sea water (34000 μS/cm)		
		P (g/L/d)	μ (day^{-1})	R (g/L/d)	P (g/L/d)	μ (day^{-1})	R (g/L/d)	P (g/L/d)	μ (day^{-1})	R (g/L/d)
Chlorella vulgaris	0.03%	0.057	0.15	0.093	0.068	0.131	0.111	0.025	0.019	0.041
	2%	0.075	0.157	0.123	0.081	0.16	0.133	0.028	0.084	0.045
	5%	0.08	0.162	0.131	0.088	0.171	0.143	0.030	0.087	0.049
	10%	0.088	0.17	0.144	0.093	0.176	0.152	0.034	0.097	0.055
Spirulina platensis	0.03%	0.065	0.15	0.33	0.083	0.25	0.41	0.063	0.15	0.31
	2%	0.08	0.143	0.4	0.091	0.154	0.46	0.074	0.133	0.37
	5%	0.088	0.15	0.438	0.098	0.156	0.487	0.079	0.141	0.394
	10%	0.09	0.153	0.45	0.098	0.157	0.49	0.084	0.147	0.419

Chlorella vulgaris presented the highest biomass productivity when cultivated in natural water (EC 1200) at 10% CO_2. The highest specific growth rate (0.176 d^{-1}) and biomass productivity (0.093 g/L/d) and the highest carbon bio-sequestration rate (0.152 g/L/d) was achieved during 8 days. *Chlorella vulgaris ARC1* could fix 18.3 mg and 38.4 mg $_{CO2}$L/day at ambient (0.036%) and elevated CO_2 (6%) (Chinnasamy et al., 2009) while Sydney et al., (2010) showed CO_2 sequestration rate of *Chlorella vulgaris* was 251.64 mg/L/day.

The ultimate CO_2 sequestration rate of *Spirulina platensis* was 0.33, 0.4, 0.438 and 0.45 g/L/d at CO_2 concentrations of 0.03%, 2%, 5% and 10% in pure water, 0.31, 0.37, 0.394 and 0.419 in artificial sea water(EC=34000) and 0.41, 0.46, 0.487 and 0.49 in natural water (EC=1200). Generally, the cultures with CO_2 aeration displayed better microalgae growth compared to the control run with no CO_2 aeration. *Spirulina platensis* showed the highest productivity of 0.098 g/L/ d when cultivated in natural water under 10% CO_2 and highest growth rate of 0.157 day^{-1}. As it was observed *Spirulina platensis* has the highest biosequstration rate of 0.489 g/L/d during 8 days. In an experiment performed on *Spirulina platensis* by de Morais and Costa (2007b) at CO_2 concentration of 6% and 12%, the specific growth rate of 0.27 and 0.33 d^{-1} was reported. According to table 1, the growth rate of the both species seemed to increase with increasing CO_2 concentration. The highest CO_2 sequestration by *Chlorella* species and *Spirulina platensis* were observed at input 10%CO_2 concentration (Rammana et al., 2010) and also Sung et al., (1999) grown *Chlorella strain KR-1* under different level of CO_2 and reported different productivity 10%, 1.1 g/L/d; 30%, 0.8 g/L/d; 50%, 0.6 g/L/d and 70%, 0.1 g/L/d. Hanaga et al., (1992) reported that *Scenedesmus* and *Chlorella* presented a productivity of 0.15 g/L/d at 10% CO_2 and 0.18 g/L/d at 40% CO_2. Lam and Lee (2013) grew *chlorella vulgaris* under 0.03 CO_2 and reported a productivity and growth rate of 0.031 g/L/d and 0.156 day^{-1}. Although some studies reported that, the optimum CO_2 concentration is 5%. (Chiu et al., 2008; de Morais and Costa 2007a,b; Yoo et al., 2010).

4 Conclusions

Spirulina platensis is grown better in the artificial sea water than *Chlorella vulgaris* ($p<0.05$). The ultimate CO_2 sequestration rate of *Spirulina platensis* and *Chlorella vulgaris* were 0.49 and 0.152 g/L/d in natural water respectively, although in artificial sea water 0.419 and 0.097 g/L/d were recorded at 10% CO_2 concentrations respectively. Regarding the lack of enough fresh water resources in arid and semi-arid area, in where the cultivation of plants is also limited due to shortage of water resources, culturing algae is one of the best suggested solutions.

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