



## Original Research Article

### Study of Bicarbonate Tolerance by Micro Algae and Possible Application in CO<sub>2</sub> Sequestration

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#### A B S T R A C T

#### Keywords

*Chlorella vulgaris*,  
*Chlorococcum humicola*,  
*Spirulina platensis*

The present study deals with bio-carbonate tolerance of microalgae *Chlorella vulgaris*, *Chlorococcum humicola*, *Spirulina platensis*, *Scenedesmus quadricauda*, *Desmococcus olivaceus* and *Chroococcus turgidus* obtained from Algal culture collection of Vivekananda Institute of Algal Technology (VIAT), were cultivated in tap water and with added nutrients in a thermostatically controlled room at 24± 1°C and illuminated with cool white fluorescent lamps at an intensity of 2000 lux in a 12:12 light and dark regime. Experiments were conducted to study the growth rate of micro alga at different concentrations of NaHCO<sub>3</sub> and it was compared with control.

## Introduction

Microalgae are the most promising production facilities. They are capable of fixing several-fold more CO<sub>2</sub> per unit area than trees or crops. Such CO<sub>2</sub> fixation by photo- autotrophic algal cultures has the potential to diminish the release of CO<sub>2</sub> into the atmosphere, helping alleviate the trend toward global warming. To realize workable biological CO<sub>2</sub> fixation systems, selection of optimal microalgae species is vital. The selection of optimal micro- algae species depends on specific strategies employed for CO<sub>2</sub> sequestration.

Viewing microalgae farms or bioreactors as means to reduce the effects of a greenhouse

gas (CO<sub>2</sub>) changes the view of the economics of the process. Instead of requiring that microalgae derived fuel be cost competitive with fossil fuels, the process economics must be compared with those of other technologies proposed to deal with the problem of CO<sub>2</sub> pollution. However, development of alternative, environmentally safer energy production technologies will benefit society whether or not global climate change actually occurs[2]. Micro algal biomass production has great potential to contribute to world energy supplies and to control CO<sub>2</sub> emissions as the demand for energy increases. This technology makes productive use of arid and

semi-arid lands and highly saline water, resources that are not suitable for agriculture and other biomass technologies [1].

Although CO<sub>2</sub> is still released when fuels derived from algal biomass are burned, integration of micro algal farms for flue gas capture approximately doubles the amount of energy produced per unit of CO<sub>2</sub> released. Materials derived from micro algal biomass also can be used for other long-term uses, serving to sequester CO<sub>2</sub>. Flue gas has the potential to provide sufficient quantities of CO<sub>2</sub> for such large-scale microalgae farms [4].

The present work deals with Carbon Sequestration in Microalgae. Carbon sequestration is the process by which carbon sinks remove carbon dioxide (CO<sub>2</sub>) from the atmosphere. Microalgae serve as these carbon sinks and help in reducing the amount of carbon, the much feared gas of mankind due to its ability of increasing the global temperature.

Species of microalgae have both economical and energetic efficiency accompanied with high CO<sub>2</sub> fixing, solar energy conversion efficiency and thus becomes a natural choice for mitigating carbon from the atmosphere.

## Materials and Methods

### Cultivation of microalgae

*Chlorococcum sp.*, *Chlorella sp.*, *Spirulina sp.*, *Scenedesmus sp.*, and *Desmococcus sp.*, *Chroococcus sp.*, obtained from Algal culture collection of Vivekananda Institute of Algal Technology (VIAT), were cultivated in tap water and with added nutrients in a thermostatically controlled room at 24± 1°C and illuminated with cool white fluorescent lamps at an intensity of 2000 lux in a 12:12 light and dark regime.

### Light Intensity Measurement

Light intensity during the trials was measured using lux meter (Lutron LX – 101A).

### Microscopic Examination

The micro algal cultures were microscopically examined using Olympus (HB) microscope and photomicrographed using Nikon digital camera (Coolpix E8400).

### Medium Composition

**CFTRI -Medium was prepared using the following Composition** (Venkataraman, 1985).

**Chemicals - g/L** NaHCO<sub>3</sub> - 4.5

K<sub>2</sub>HPO<sub>4</sub> - 0.5

NaNO<sub>3</sub> - 1.5

K<sub>2</sub>SO<sub>4</sub> - 1.0

NaCl - 1.0

MgSO<sub>4</sub>.7H<sub>2</sub>O - 0.2

CaCl<sub>2</sub> - 0.04

FeSO<sub>4</sub> - 0.01

pH of the medium – 10

### Growth Measurement

Growth was measured by counting cells using a haemocytometer (Neubauer, improved) and the results were plotted in a semi-logarithmic graph. Growth rate (divisions/day) was arrived at using the formula.

$$\frac{\log N - \log N_0}{\log 2 \times t}$$

where,

N - No. of cells per ml at the end of log phase or mg weight/L

No - Initial count of cells per ml or mg weight/L

t -Days of log phase

For dry weight method, the algal cultures were pelleted by centrifugation at 7500 rpm (Remi cooling microfuge) for 15 minutes. Cells were washed with glass distilled water, again centrifuged and dried in an oven for 24 hours or until constant weight.

**pH and conductivity measurement**

For all the trials, pH was measured using digital pH meter (Elico LI 120) and conductivity using digital Conductivity meter (Equiptronics EQ -660A) respectively. Experiments were conducted to study the growth rate of micro alga at different concentrations of NaHCO<sub>3</sub> and it was compared with control.

**Results and Discussion**

Generally, phototrophic micro algal growth requires a supply of carbon dioxide as a carbon source. CO<sub>2</sub> supply contributes to control the pH of the culture [8]. According

to previous studies, the supply of carbon to micro algal mass culture systems is one of the principal difficulties and limitations that must be solved [1,7,9]. The principal point of all considerations relating to the CO<sub>2</sub> budget is that, on the one hand, CO<sub>2</sub> must not reach the upper concentration that produces inhibition and, on the other hand, must never fall below the minimum concentration that limits growth [9]. These maximum (inhibition) and minimum (limitation) concentrations vary from one species to another and are not yet adequately known. [8, 6].

Hence feasibility study was conducted by growing the micro algae with NaHCO<sub>3</sub>. Analytical data revealed that *Chlorococcum sp.*, was able to grow well in 4.5g/L concentration in medium and in tap water 0.5g/L concentration.(Table 2a and 2b)*Chroococcus sp.*, and *Chlorella sp.*, was able to grow well in 2g/L concentration in medium and in tap water. (Table1a,6a and 1b and 6b) The other algae *Spirulina sp.*, *Scenedesmus sp.* and *Desmococcus sp.*, were not able to grow in different concentrations of NaHCO<sub>3</sub>.(Table3a,4a,5a and 3b,4b,5b) *Chlorococcum sp.*, *Chroococcus sp.*, and *Chlorella sp.*, can be employed in CO<sub>2</sub> sequestration at industrial Scale.

**Table.1a -Chlorella vulgaris**

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	180	140	180	100	7.1
3	21.9.11	160	240	300	110	7.2
4	27.9.11	260	350	380	140	7.3
After 22days in 1ml		F.W	0.750g	0.992g	0.564g	0.573g
		D.W	0.371g	0.248g	0.226g	0.298g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	

TEST TUBE

1. Control-20ml(19ml CFTRI Medium+1ml culture)
2. 0.5g -20ml(18.9ml CFTRI Medium+1ml culture+0.1mlstock)
3. 2g -20ml(18.6ml CFTRI Medium +1ml culture+0.4mlstock)
4. 5g-20ml(18.1ml CFTRI Medium +1ml culture+0.9mlstock)

**Table.2a** *Chlorococcum humicola*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	180	292	298	300	7.2
3	21.9.11	280	400	240	384	7.3
4	27.9.11	450	520	350	540	7.4
After 22days in 1ml		F.W	0.551g	0.652g	0.389g	0.751g
		D.W	0.041g	0.029g	0.006g	0.192g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		450	520	350	540	
GROWTH DIVISION/DAY		0.1587	0.1682	0.1422	0.1706	

**Table.3a** *Spirulina platensis*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				Ph
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	160	140	120	220	7.1
3	21.9.11	360	280	160	184	7.2
4	27.9.11	450	350	260	340	7.3
After 22days in 1ml		F.W	0.660g	0.562g	0.464g	0.581g
		D.W	0.300g	0.220g	0.230g	0.252g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		450	350	260	340	
GROWTH DIVISION/DAY		0.1587	0.1422	0.1227	0.1403	

**Table.4a** *Scenedesmus quadricauda*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	180	160	140	100	7.1
3	21.9.11	160	240	240	100	7.1
4	27.9.11	220	350	340	180	7.2
After 22days in 1ml		F.W	0.251g	0.389g	0.379g	0.238g
		D.W	0.031g	0.050g	0.052g	0.027g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		220	350	340	180	
GROWTH DIVISION/DAY		0.1117	0.1422	0.1403	0.0986	

**Table.5a** *Desmococcus olivaceus*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	240	180	120	100	7.1
3	21.9.11	320	280	100	160	7.2
4	27.9.11	440	360	190	260	7.4
After 22days in 1ml		F.W	0.651g	0.582g	0.130g	0.441g
		D.W	0.341g	0.290g	0.106g	0.211g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		440	360	190	260	
GROWTH DIVISION/DAY		0.1572	0.1440	0.1021	0.1227	

**Table.6a** *Chroococcus turgidus*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.9.11	40	40	40	40	7.1
2	19.9.11	170	140	180	160	7.1
3	21.9.11	260	280	300	240	7.2
4	27.9.11	450	300	450	350	7.3
After 22days in 1ml		F.W	0.860g	0.840g	0.920g	0.860g
		D.W	0.368g	0.300g	0.410g	0.370g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		450	300	450	350	
GROWTH DIVISION/DAY		0.1587	0.1321	0.1587	0.1422	

TEST TUBE-

1. Control-20ml(19ml Tap water+1ml culture)
2. 0.5g -20ml(18.9ml Tap water +1ml culture+0.1mlstock)
3. 2 g -20ml(18.6ml Tap water +1ml culture+0.4mlstock)
4. 4.5g-20ml(18.1ml Tap water +1ml culture+0.9mlstock)

**Table.1b** *Chlorella vulgaris*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.10.11	40	40	40	40	7.1
2	14.10.11	136	80	200	84	7.1
3	18.10.11	160	172	200	100	7.2
4	27.10.11	280	180	320	80	7.3
After 22days in 1ml	F.W	0.760g	0.962g	0.564g	0.573g	
	D.W	0.371g	0.248g	0.226g	0.298g	
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		280	180	320	80	
GROWTH DIVISION/DAY		0.1276	0.0986	0.1364	0.0455	

**Table.2b** *Chlorococcum humicola*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.10.11	40	40	40	40	7.1
2	14.10.11	200	360	140	132	7.2
3	18.10.11	224	400	140	136	7.3
4	27.10.11	480	500	480	480	7.5
After 22days in 1ml		F.W	0.651g	0.545g	0.487g	0.741g
		D.W	0.051g	0.009g	0.007g	0.092g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		480	500	480	480	
GROWTH DIVISION/DAY		0.1629	0.1656	0.1629	0.1629	

**Table.3b** *Spirulina platensis*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.10.11	40	40	40	40	7.1
2	14.10.11	96	212	120	80	7.1
3	18.10.11	360	340	220	80	7.2
4	27.10.11	520	488	280	220	7.4
After 22days in 1ml		F.W	0.770g	0.972g	0.574g	0.563g
		D.W	0.391g	0.250g	0.236g	0.288g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		520	488	280	220	
GROWTH DIVISION/DAY		0.1682	0.1640	0.1276	0.118	

**Table.4b** *Scenedesmus quadricauda*

S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.10.11	40	40	40	40	7.1
2	14.10.11	260	168	120	80	7.2
3	18.10.11	620	256	220	220	7.3
4	27.10.11	680	300	280	280	7.6
After 22days in 1ml		F.W	0.431g	0.545g	0.544g	0.351g
		D.W	0.065g	0.095g	0.067g	0.057g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		680	300	280	280	
GROWTH DIVISION/DAY		0.1858	0.1321	0.1276	0.1276	

**Table.5b** *Desmococcus olivaceus*

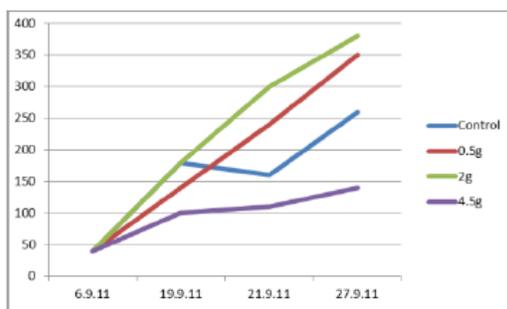
S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH
		CONTROL	Strength of NaHCO <sub>3</sub>			
			0.5g	2 g	4.5g	
1	6.10.11	40	40	40	40	7.1
2	14.10.11	360	320	188	280	7.2
3	18.10.11	420	400	160	360	7.3
4	27.10.11	560	440	240	360	7.4
After 22days in 1ml		F.W	0.818g	0.739g	0.158g	0.618g
		D.W	0.493g	0.389g	0.151g	0.308g
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40	
FINAL X10 <sup>4</sup> cells/ml		560	440	240	360	
GROWTH DIVISION/DAY		0.1730	0.1573	0.1175	0.1441	

**Table.6b** *Chroococcus turgidus*

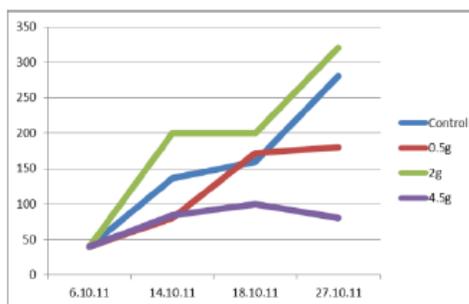
S.NO	DATE	CELL COUNT X10 <sup>4</sup> cells/ml				pH	
		CONTROL	Strength of NaHCO <sub>3</sub>				
			0.5g	2 g	4.5g		
1	6.10.11	40	40	40	40	7.1	
2	14.10.11	80	120	200	180	7.1	
3	18.10.11	340	200	370	260	7.2	
4	27.10.11	470	380	500	440	7.3	
After 22days in 1ml		F.W	0.890g	0.850g	0.997g	0.873g	
		D.W	0.440g	0.400g	0.493g	0.422g	
INITIAL X10 <sup>4</sup> cells/ml		40	40	40	40		
FINAL X10 <sup>4</sup> cells/ml		470	380	500	440		
GROWTH DIVISION/DAY		0.1615	0.1476	0.1656	0.1572		

1. *Chlorella vulgaris*

CFTRI MEDIUM

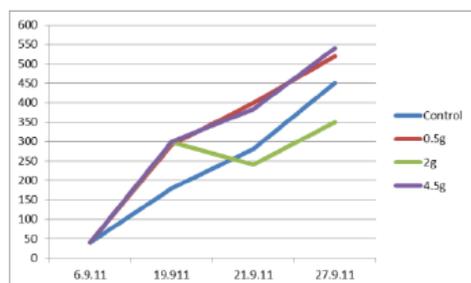


TAP WATER

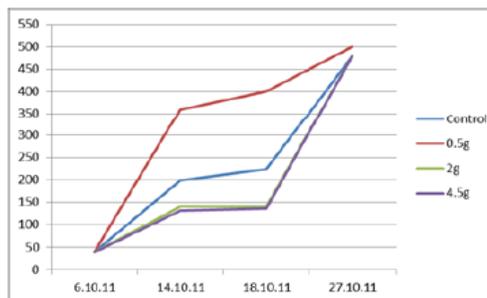


2. *Chlorococcum humicola*

CFTRI MEDIUM

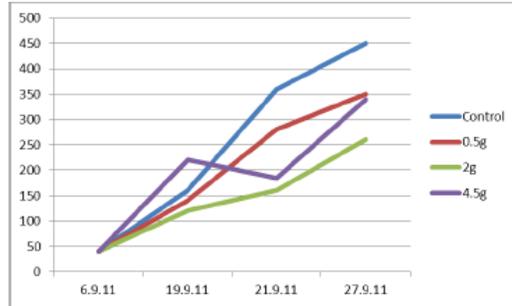


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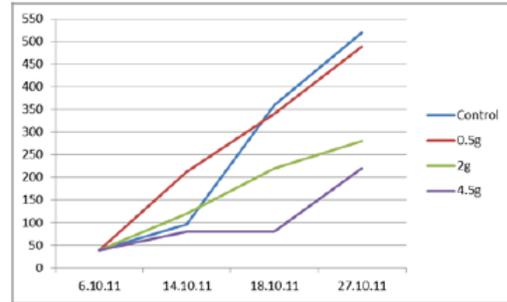


3. *Spirulina platensis*

CFTRI MEDIUM

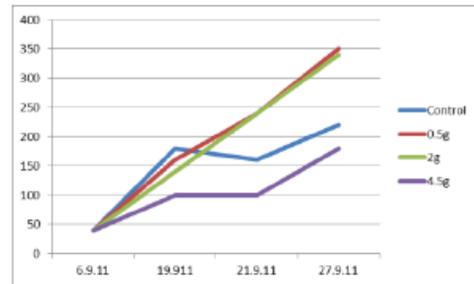


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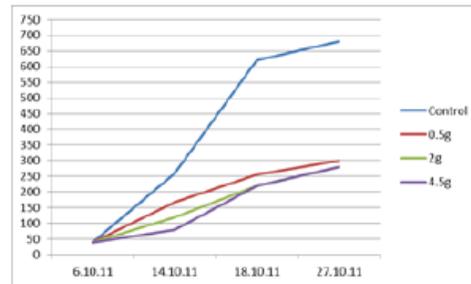


4. *Scenedesmus quadricauda*

CFTRI MEDIUM

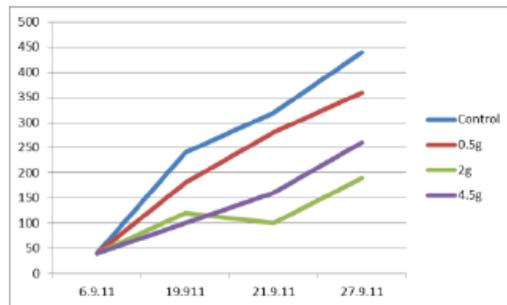


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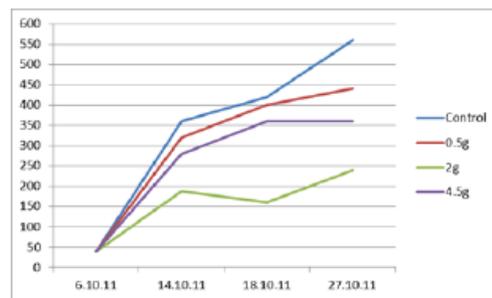


5. *Desmococcus olivaceus*

CFTRI MEDIUM

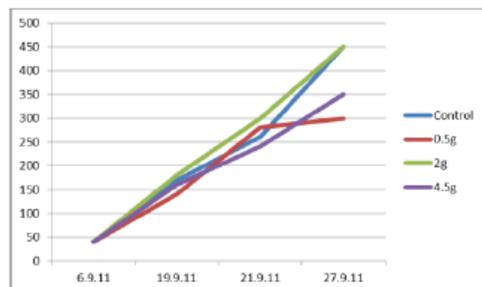


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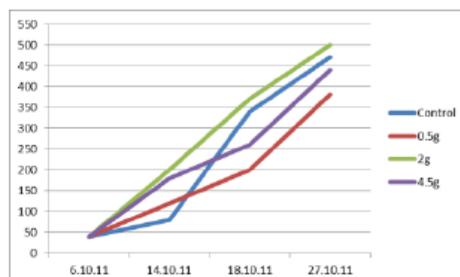


## 6. *Chroococcus turgidus*

CFTRI MEDIUM



TAP WATER



## References

1. Beneman J. R, Tillett D. M and J. C. Weissman J. C. 1987. Micro-algae Biotechnology. *Trends in Biotechnology*, Vol. 5, No. 2, pp.47-53.
2. Biofr. 2009. The Promise and Challenges of Microalgal-Derived Biofuels, [http://www.afdc.energy.gov/afdc/pdfs/microalgal\\_biofuels\\_darzins.pdf](http://www.afdc.energy.gov/afdc/pdfs/microalgal_biofuels_darzins.pdf)
3. Brown, L. M.1996. Uptake of Carbon Dioxide from Flue Gas by Microalgae," *Energy Conversion and Management*, Vol. 37, No. 6-8, pp. 1363-1367
4. Chelf, P., Brown L.M and Wyman, C. E. 1993. Aquatic Bio-mass Resources and Carbon Dioxide," *Biomass and Bio-energy*, Vol. 4, No. 3, pp. 175-183. doi:10.1016/0961-9534(93)90057-B
5. Fike, J. H., Parrish D. J, Alwang J. and J. S. Cundiff, J. S. 2007.Challenges for Deploying Dedicated, Large-Scale, Bio-energy Systems in the USA. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, Vol. 2, No. 64, pp. 1-28.
6. Lee Y. K. and Hing, H. K.1989. Supplying CO<sub>2</sub> to Photosynthetic Algal Cultures by Diffusion through Gas Permeable Membranes," *Applied Microbiology and Biotechnology*. Vol. 31, No. 3, pp.298-301. doi:10.1007/BF00258413
7. Oswald, J. A.1988.Large-Scale Algal Culture Systems (Engineering Aspects), In: L. J. Borowitzka and M. A. Borowitzka, Eds., *Microalgal biotechnology*,Cambridge University Press, Cambridge, pp. 357-395.
8. Rados S., Vaclav B and Frantisek D.1975. CO<sub>2</sub> Balance in Industrial Cultivation of Algae. *Archives of Hydrobiology*, Vol. 46, No. 12, , pp. 297-310.
9. Tapie P. and Bernard A. 1988. Microalgae Production Technical and Economic Evaluations," *Biotechnology and Bioengineering*, Vol. 32 A., No. 7, 1988, pp. 873-885. doi:10.1002/bit.260320705.