OPTIMIZATION OF PHYSICO CHEMICAL CHARACTRISTICS ON BIOMASS AND LIPID PRODUCTIVITY OF *TETRASELMIS STRAIATA* BUTCHER BBRR1 FOR BIOFUEL APPLICATIONS

Abstract

Fossil energy sources are petroleum (crude oil), coal, bitumens, natural gas, oil shales, and tar sands. During the last 200 years, developed countries have shifted their energy consumption toward fossil fuels. About 98% of carbon emissions result from fossil fuel combustion. Reducing the use of fossil fuels would considerably reduce the amount of CO_2 and other pollutants produced. The rapid depletion of fossil fuel resources and the increase in crude oil prices have made it inevitable to search for an alternative fuel as a substitute for diesel. Algae have been widely used for fuel production because of their high photosynthetic efficiency, high biomass production, and fast growth. Microalgae contain proteins, carbohydrates, and lipids; the lipids can be converted into biodiesel, carbohydrates into ethanol and H_2, and proteins into the raw material of biofertilizer. Biofuel from microalgae can be processed by using thermochemical and biochemical conversion. The present investigation deals with optimizing the growth and lipid production of *Tetraselmis straiata* BBRR1 at different laboratory conditions and formulating a Modified CFTRI medium for mass cultivation of the alga under open raceway ponds for Biofuel Applications.

Keywords: Fossil fuel, Microalgae, *Tetraselmis straiata*, Biomass, Biodiesel.

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I. INTRODUCTION

In modernization and development, humans always needed energy, which increased the dependency on the available fossil fuel sources. The exhaustive use of fossil fuel sources has raised severe concerns worldwide, not only about energy security but also about the negative impact on the environment. India's growing demand for petroleum-based fuel has created challenges for its energy security, as almost 90% of its crude oil requirement is imported from oil-producing countries. Out of industrial, agricultural and domestic sectors, the transport sector consumes nearly 80% of total energy from fossil fuels. According to 2012 - 13 estimates, the annual diesel consumption was 70 million tonnes at a 6 – 8% growth rate per annum (www.petroleum.nic.in/ pngstat.pdf assessed on 09/02/2014).

The rapid depletion of fossil fuel resources and increased crude oil prices have made it inevitable to search for alternative fuels as substitutes for diesel. Due to the similarity in diesel and biodiesel fuel properties, the latter is considered a substitute for diesel. Accordingly, the Government of India announced the Biofuels Policy in 2008 to promote biodiesel production and use to achieve a target of blending 20% biodiesel with diesel by 2017 (www.mnre.gov.in assessed on 21/02/2014).

1. **Fossil Fuel Demand:** Fossil energy sources are petroleum (crude oil), coal, bitumens, natural gas, oil shales, and tar sands. During the last 200 years, developed countries have shifted their energy consumption toward fossil fuels. About 98% of carbon emissions result from fossil fuel combustion. Reducing the use of fossil fuels would considerably reduce the amount of CO_2 and other pollutants produced. Climate change has emerged at the forefront of environmental problems and has received the most media attention and substantial international political discussion (Lindseth, 2004). The factor that distinguishes climate change from other environmental problems and generates more public interest is the multitude of social, political, and economic issues that are infused within the realm of climate change (Botkin and Keller, 2007).

2. **Biofuels:** Biofuel is energy recovered from organic matter known as biomass. Biomass can include forest products, agricultural residues, energy crops, animal residues or urban waste (Hinrichs and Kleinbach, 2006; Botkin and Keller, 2007). For the transportation sector, biofuels are usually limited to bioethanol and biodiesel because they are cleaner, easily transportable fuels that can operate on most existing automobile engine technology.

   Biodiesel is another liquid fuel made from renewable sources such as vegetable oils and used restaurant oils, which can serve as an alternative to petroleum diesel. Biodiesel is also non-toxic and biodegradable and burns with significantly fewer pollutants than its petroleum counterpart (USEPA, 2002). Biodiesel is commonly produced from restaurant waste and vegetable oils from plants like corn, soybeans, and sugarcane (USDOE, 2010).

   Biomass derived from photosynthesis includes a variety of organisms, such as plants, animals and microbes. As photosynthetic microorganisms, microalgae can use and, therefore, remove nitrogen and phosphorus in wastewater, sequester CO_2 in the air, and synthesize lipids, which can be converted into biodiesel. The declining supply of conventional fossil fuels and concern about global warming make microalgae-based

biodiesel a promising alternative. Although the potential and advantages of microalgae-based biodiesel over conventional biodiesel have been well recognized (Khan *et al*., 2009, Lim and Teong, 2010, Stephens *et al*., 2010), broad commercialization of microalgae biodiesel has not yet been realized, chiefly because of the techno-economic constraints, particularly in the areas of mass cultivation and downstream processing.

3. **Energy from Algae:** Algae are more efficient by converting that solar energy into chemical energy, the better it is from a biodiesel perspective, and they are among the most photosynthetically efficient plants on earth. Photosynthesis is an essential biochemical process in which plants, algae, and some bacteria convert the energy of sunlight into chemical energy. Microalgae are fast-growing beasts with a voracious appetite for CO_2. They can produce more oil per acre than any other feedstock used to make biodiesel, and they can be grown on land unsuitable for food crops (Demirbas, 2009).

Algae have been widely used for fuel production because of their high photosynthetic efficiency, high biomass production, and fast growth (Miao *et al*., 2004). Microalgae contain proteins, carbohydrates, and lipids; the lipids can be converted into biodiesel, carbohydrates into ethanol and H_2, and proteins into the raw material of biofertilizer. Biofuel from microalgae can be processed by using thermochemical and biochemical conversion. The thermochemical process can be divided into gasification, liquefaction, pyrolysis, and direct combustion; meanwhile, the biochemical process can be divided into anaerobic digestion, fermentation, and photobiological activity. Using a gasification process, the biomass produces CH_4, H_2, CO_2, and ammonia (Raja *et al*., 2013). Among the various potential renewable energy sources, biofuels are of most interest. Marine microalgae are the most promising oil sources for making biofuels, which can increase and convert solar energy to chemical energy via CO_2 fixation. The fatty acid profile of almost all the microalgal oil is suitable for biofuel synthesis (Sharmin *et al*., 2016).

Microalgae are traditionally considered a good source of fatty acids (Benemann, 1989; Borowitzka, 2013). The accumulation of fatty acids by microalgae is well-developed and presented elsewhere (Griffiths and Harrison, 2009; Rodolfi *et al*., 2009). Microalgae have a solid capacity to produce lipids, which can be easily converted to biodiesel. Transesterification using homogeneous and heterogeneous catalysts and in situ can produce biofuel from microalgae lipids (Chisti, 2007; Lam and Lee, 2012). The present investigation aims to optimize the growth and lipid production of *T. straiata* BBRR1 at different laboratory conditions and formulate a Modified CFTRI medium for mass cultivation of the alga under open raceway ponds for Biodiesel production.

II. MATERIALS AND METHODS

The following experiments were carried out for 21 days. At every three intervals, the following parameters: cell numbers, specific growth rate, doubling time, generation time, *a*, and Total lipid content were recorded.

1. **Effect of Different Strengths of f/2 Medium:** *Tetraselmis straiata* BBRR1 was inoculated in different strengths of f/2 medium such as 0.5 (f/2), 1.0 (f), 5.0 (5f) and 10.0 (10f) and record other parameters. The organism grown in f medium showed maximum growth. Therefore, it was chosen for further study.

2. **Effect of Different pH:** The algal culture was inoculated in the f medium at different initial pH: 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 (control), 8.5, 9.0, 9.5 and 10. The different pH of the medium was adjusted with 1 N NaOH and 1 N HCl before sterilization. The organism grown at the initial pH of 8.5 supported good growth. Therefore, this pH was chosen for the following studies.

3. **Effect of Different Salinities:** The test organism was grown in different salinities such as 10, 20, 30 (control), 35, 40, 50, 60, 70, 80 and 90 ppt. Distilled water and NaCl were used for the preparation of different salinities. The salinity of the sample was recorded by using the salinometer and Eutech instrument (CON2700). In this study, 35 ppt favored the organism for maximum growth and lipid production. This salinity was included in the following investigations.

4. **Effect of Different Concentrations of Sodium Nitrate:** *Tetraselmis straiata* BBRR1 was inoculated in the medium amended with different concentrations of Sodium nitrate such as 0.58 mM, 0.88 mM (control), 1.76 mM, 3.53 mM, 5.29 mM and 7.059 mM in minus Sodium nitrate basal medium. It revealed that 1.76 mM supported the maximum growth of this alga. Therefore, it was included in the following studies.

5. **Effect of Different Concentrations of Sodium Dihydrogen Phosphate:** The alga was grown in the medium amended with different concentrations of Sodium dihydrogen phosphate such as 0.02 mM, 0.04 mM (control), 0.08 mM, 0.016 mM, 0.025 mM, and 0.033 mM, amended in basal medium minus Sodium dihydrogen phosphate. The study revealed that 0.08 mM of Sodium dihydrogen phosphate supported the maximum parameters of the test alga. Hence, it was included in the following studies.

6. **Effect of Different Concentrations of Fe – EDTA:** Among the different concentrations of Fe-EDTA, such as 0.005 mM, 0.011 mM, 0.014 mM (control), 0.017 mM, 0.023 mM and 0.028 mM amended in the basal medium minus Fe – EDTA revealed that 0.023 mM of Fe – EDTA supported the maximum growth and lipid productivity of the organism. Therefore, it was included in the following study.

7. **Effect of Different Concentrations of Magnesium sulphate:** Different concentrations of Magnesium sulphate such as 0.415 mM, 0.830 mM, 1.246 mM, 1.66 mM, 2.076 mM, 2.492 mM, 2.907 mM, 3.323 mM, 3.738 mM, 4.153 mM were amended in the basal medium minus Magnesium sulphate were prepared and allowed the organism to grow. The medium amended with 4.153 mM of $MgSO_4$ supported good alga growth; hence, it was included.

8. **Effect of Modified CFTRI Medium on** *Tetraselmis straiata* **BBRR1 under Laboratory Condition:** In the present attempt, a Modified CFTRI medium (Venkataraman and Becker, 1985) was chosen to consider the cost-effectiveness of chemicals for mass cultivation of *T. straiata* in open raceway ponds.

- **Composition of Modified CFTRI Medium**

		mM
Urea	-	4.162
Superphosphate	-	0.085
Ferric chloride	-	0.024
Magnesium sulphate	-	1.246
Sodium bicarbonate	-	1.785
Bore well water	-	1000 mL
Sea salt	-	30 g
pH	-	8.0

The following experiments were conducted for a period of 30 days. At every five days of interval, the following parameters: Cell numbers, specific growth rate, doubling time, generation time, Chlorophyll *a*, Total carbohydrate, protein and lipid were recorded.

- **Optimization of Commercial Medium**

9. **Effect of Different Concentrations of Urea (CH_4N_2O):** Different concentrations of Urea viz.0.832 mM, 1.66 mM, 2.49 mM, 3.33 mM, 4.16 mM (control), 4.99 mM, 5.82 mM, 6.66 mM, 7.49 mM, 8.32 mM and 9.15 Mm were amended in the Modified CFTRI medium minus Urea. The test alga grown in 0.832 mM Urea showed maximum growth and lipid productivity. Therefore, it was included in the following experiments.

10. **Effect of Different Concentrations of Superphosphate (Ca (H_2OP_4) $2H_2O$):** The medium minus Superphosphate was amended with different concentrations of Superphosphate: 0.042 mM, 0.085 Mm (control), 0.128 mM, 0.170 mM, 0.213 mM, 0.256 mM, 0.299 mM, 0.341 mM, 0.384 mM and 0.427 Mm and inoculated the test alga, *T. straiata*. The alga grown in 0.170 mM of Superphosphate exhibited good growth characteristics, and therefore, it was included in the following experiments.

11. **Effect of Different Concentrations of Sodium bicarbonate:** Different concentrations of Sodium bicarbonate, such as 0.595 mM, 1.190 mM, 1.785 mM (control), 2.380 mM, 2.975 mM, 3.571 mM, 4.166 mM, 4.761 mM, 5.356 mM, 5.951 mM and 6.547 mM were amended in the basal medium minus Sodium bicarbonate and inoculated the test alga. The alga grown in the medium amended with 5.951 mM of Sodium bicarbonate showed enhanced growth parameters. Therefore, it was included in the following experiments.

12. **Effect of Different Concentrations of Ferric Chloride:** The basal medium minus Ferric chloride was amended with different concentrations of Ferric chloride, such as 0.012 mM, 0.024 mM (control), 0.036 mM, 0.049 mM, 0.061 mM and 0.073 mM. The alga grown above revealed that the medium amended with 0.061 mM of Ferric chloride showed maximum growth characteristics; hence, it was included.

13. **Effect of Different Concentrations of Magnesium Sulphate:** Different concentrations of Magnesium sulphate: 0.830 mM, 1.246 mM (control), 1.661 mM, 2.076 mM, 2.192 mM and 2.907 mM were amended in the basal medium minus Magnesium sulphate and inoculated the test organism. The alga grown in the medium amended with 2.076 mM

exhibited maximum growth parameters. Therefore, this parameter was also included in the medium.

14. **Low - Cost Medium for *Tetraselmis straiata* BBRR1:** Based on the above studies, the following ingredients were included and formulated. This low-cost medium was designated as Modified CFTRI ABRRI medium. This medium was used to cultivate *Tetraselmis straiata* BBRR1 in open raceway ponds massively.

15. **Comparative Studies on Growth and Lipid Production of *Tetraselmis straiata* BBRR1 in Modified CFTRI and Modified CFTRI – ABRR I media:** The alga *Tetraselmis straiata* BBRR1 inoculated in the above two different media was recorded for its growth by cell number and lipid productivity at every 5-day intervals for 30 days under laboratory conditions and compared.

III. RESULTS

Tetraselmis straiata Butcher BBRR1 was grown in different strengths of f/2 medium, different pH and salinity; as well as grown in different concentrations of Sodium nitrate, Sodium dihydrogen phosphate, Fe – EDTA and Magnesium sulphate under different laboratory conditions and recorded different parameters.

1. **Effect of Different Strengths of f/2 Medium:** Among the four different strengths of f/2 medium such as, 0.5 (f/2), 1.0 (f), 5.0 (5f) and 10 (10f) studied on the alga, *T. straiata* BBRR1 preferred f medium (1.0 f) for its maximum growth and lipid production. At this condition the alga showed maximum growth of 192×10^4 cells/ml and biomass of 0.58 g L^{-1} on 21st day which were 6% and 24% higher than control (f/2 medium) (Figure 1). The alga showed specific growth rate of 0.41, division rate of 0.59 and generation time of 1.69 when it was grown in f medium (Table, 1).

    - **Chlorophyll *a*:** Maximum Chlorophyll *a* content of 6.23 mg L^{-1} of the alga was also recorded in f medium on 21st day, which was 67% higher than control. The alga grown in f/2 medium showed only 2.04 mg L^{-1} of Chlorophyll *a* on 21st day.
    - **Total Lipids:** Among the different strengths of f/2 medium tested, the alga grown in f/2 medium showed a maximum total lipids of 117 mg L^{-1} on 21st day with 181×10^4 cells/ml, followed by 112 mg L^{-1} of total lipids with of 191×10^4 cells/ml in f medium. Maximum lipid productivity of 5.56 mg L^{-1} d^{-1} was recorded in f/2 medium which was 4% higher than the alga grown inf medium. The alga showed low lipid productivity of 1.63 mg L^{-1} d^{-1} when it was grown in 5f medium (Table,2) (Figure 2).

2. **Effect of Different pH:** Among the 11 different pH tested (basal f medium), the alga grown in the initial of pH 8.5 showed maximum 178×10^4 cells/ml on 21st day, whereas at pH 7.0 it was 127×10^4 cells/ml on 30th day (Figure 3). At pH 8.5, the alga showed specific growth rate of 0.47, division rate of 0.68 and generation time of 1.47 (Table, 3). The alga showed poor growth at pH 5.0.

- **Chlorophyll *a*:** The alga showed a maximum Chlorophyll *a* content of 2.84 mg L^{-1} at pH 8.5 on 21st day. Thereafter, the pigment level was gradually decreased up to 1.23 mg L^{-1} on 30th day. The alga grown in the initial pH 5.0 showed a minimum Chlorophyll *a* of 0.48 mg L^{-1} which was 52% less than the organism grown at pH 8.5.
- **Total Lipids:** The alga grown at pH 8.0 showed maximum total lipids of 156 mg L^{-1} on 24th day with 116 × 10^4 cells/ml followed by 151 and 112 mg L^{-1} of total lipids with 84 and 84 × 10^4 cells/ml at pH 7. 5 and 8.5 on 24th and 12th days, respectively. Maximum lipid productivity of 9.3 mg L^{-1} d^{-1} was recorded at pH 8.5 and low productivity of 3.6 mg L^{-1} d^{-1} at pH 6.5 (Table, 3) (Figure 4). The minimum amount of lipid content recorded at pH 10 was 66% less than control (pH 8. 0).

3. **Effect of Different Salinities:** Among the 10 different salinities studied, the alga grown in 35 ppt showed maximum growth of 139 × 10^4 cells/ml, whereas at 30 ppt 112 × 10^4 cells/ml was recorded on 21st day (Figure 5). At 35 ppt the alga showed specific growth rate of 0.57, division rate of 0.82 and generation time of 1.22 (Table, 4). The alga showed poor growth at 90 ppt.

   - **Chlorophyll *a*:** The alga grown at 35 ppt had maximum Chlorophyll *a* content of 1.70 mg L^{-1} on 21st day, followed by 1.42 and 1.34 mg L^{-1} at 30 and 20 ppt, respectively. The minimum amount of 0.95 mg L^{-1} Chlorophyll *a* recorded at 90 ppt on 21st day was 44% less than the alga grown at 35 ppt.
   - **Total Lipids:** Among the 10 different salinities tested the alga *T. straiata* preferred 35 ppt for its maximum growth and lipid production. At this condition the alga had maximum lipid content of 184 mg L^{-1} with 92 × 10^4 cells/ml on 12th day followed by 153 and 110 mg L^{-1} with 93 × 10^4 cells/ml and 86 × 10^4 cells/ml at 40 and 30 ppt, on 15th and 12th day, respectively. Minimum lipid content of 80 mg L^{-1} recorded at 80 ppt was 27% less than control (30 ppt) on 12th day (Fig. 6) Maximum lipid productivity of 15.37 mg L^{-1} d^{-1} was recorded at 8.5 ppt and minimum lipid productivity of 5.20 mg L^{-1} d^{-1} was recorded at 50 ppt (Table, 4).

4. **Effect of Different Concentrations of Sodium Nitrate ($NaNO_3$):** Among the 6 different concentrations of Sodium nitrate tested, the alga grown in 1.76 mM of Sodium nitrate showed a maximum of 146 × 10^4 cells/ml, whereas at 7.059 mM and 5.29 mM it showed 126 and 125 × 10^4 cells/ml, respectively, on 27th day (Figure 7). The alga grown in 1.76 mM of Sodium nitrate showed the specific growth rate of 0.17, division rate of 0.25 and generation time of 4.06 (Table, 5). Whereas the alga grown in 0.58 mM of Sodium nitrate showed poor growth. The alga showed maximum specific growth rate of 0.24, division per day of 0.34 and generation time of 2.95 at 7.059 mM of Sodium nitrate.

   - **Chlorophyll *a*:** The alga showed a maximum Chlorophyll *a* content of 1.17 mg L^{-1} at 1.76 mM, followed by 1.11, 1.01 and 0.94 mg L^{-1} at 7.059, 5.29 and 3.53 mM of Sodium nitrate, respectively, on 27th day. Minimum Chlorophyll *a* content of 0.60 mg L^{-1} was recorded at 0.58 mM of Sodium nitrate.
   - **Total Lipids:** The alga grown at 0.88 mM of Sodium nitrate contained a maximum total lipids of 65 mg L^{-1} on 21st day with 73 × 10^4 cells/ml followed by 48 and 37 mg L^{-1} at 5.29 mM and 7.059 mM with 61 and 126 × 10^4 cells/ml on 15th and 27th days, respectively (Fig. 8). Maximum lipid productivity of 3.22 mg L^{-1} d^{-1} was recorded at

5.29 mM Sodium nitrate and minimum lipid productivity of 1.30 mg L^{-1} d^{-1} was recorded at 3.53 mM Sodium nitrate (Table, 5).

5. **Effect of Different Concentrations of Sodium Di-Hydrogen Phosphate ($NaH_2PO_4.H_2O$):** Among the 6 different concentrations of Sodium di-hydrogen phosphate tested, the alga grown in 0.08 mM showed maximum growth of 67 × 10^4 cells/ml on 24th day, whereas at 0.033 and 0.04 mM they were 63 and 61 × 10^4 cells/ml, respectively on 24th day (Figure 9). At 0.08 mM of Sodium di-hydrogen phosphate the alga showed specific growth rate of 0.15, division rate of 0.22 and generation time of 4.65 (Table, 6). The alga showed poor growth of 51 × 10^4 cells/ml at 0.02 mM on 24th day, which was 16% less than control. Maximum specific growth rate of 0.29, division per day of 0.42 and generation time of 2.36 were recorded at 0.04 mM of Sodium di-hydrogen phosphate (control).

- **Chlorophyll *a*:** The alga synthesized maximum Chlorophyll *a* content of 0.52 mg L^{-1} on 24th day at 0.08 mM of Sodium di-hydrogen phosphate, followed by 0.51, 0.45 and 0.41 mg L^{-1} at 0.04 (control), 0.033 and 0.016 mM on 24th, 24th and 15th day, respectively, Whereas minimum Chlorophyll *a* content of 0.32 mg L^{-1} was recorded at 0.025 mM Sodium di-hydrogen phosphate on 24th day.
- **Total Lipids:** The alga grown at 0.04 mM of Sodium di-hydrogen phosphate had maximum total lipids of 80 mg L^{-1} with 45 × 10^4 cells/ml on 12th day (Figure 10). Maximum lipid productivity of 6.67 mg L^{-1} d^{-1} was recorded at 0.04 mM and minimum productivity of 2.39 mg L^{-1} d^{-1} recorded at 0.08 mM of Sodium di-hydrogen phosphate (Table, 6). A minimum lipid content recorded at 0.02 mM was 52% less than control (0.04 mM).

6. **Effect of Different Concentrations of Ferric – EDTA:** Among the 5 different concentrations of Fe - EDTA tested, the alga grown at 0.023 mM showed maximum growth of 153 × 10^4 cells/ml on 21st day, whereas at 0.017, 0.005 and 0.011 mM of Fe - EDTA the test organism exhibited 133, 133 and 127 × 10^4 cells/ml, respectively, on 21st day (Figure 11). At 0.023 mM of Fe - EDTA the alga showed the specific growth rate of 0.41, division rate of 0.60 and generation time of 1.67 (Table, 7). The alga showed poor growth at 0.011 mM. *Tetraselmis straiata* grown at 0.023 and 0.017 mM of Fe - EDTA showed the specific growth rate of 0.41 and 0.41, division per day of 0.60 and 0.59, generation time of 1.67 and 1.69, respectively.

- **Chlorophyll *a*:** The alga synthesized maximum Chlorophyll *a* content of 1.68 mg L^{-1} at 0.023 mM of Fe – EDTA, followed by 1.57, 1.54 and 1.49 mg L^{-1} at 0.017, 0.005 and 0.011 mM, respectively, on 21st day. Minimum Chlorophyll *a* content of 1.34 mg L^{-1} was recorded at 0.005 mM was 9% less than control (0.014 mM) on 18th day.
- **Total Lipids:** Among the 5 different concentrations of Fe - EDTA tested, the alga grown in 0.014 mM had maximum lipid content of 86 mg L^{-1} with 117 x 10^4 cells/ml on 18th day, followed by 67, 47, 42 and 42 mg L^{-1} with 112 × 10^4 cells/ml, 115 × 10^4 cells/ml, 115 × 10^4 cells/ml and 91 x 10^4 cells/ml at 0.011mM, 0.005 mM, 0.023 and 0.017 mM of Fe - EDTA on 18th , 18th , 15th and 15th days, respectively (Figure 12). The alga showed maximum lipid productivity of 4.79 mg L^{-1} d^{-1}, the specific

growth rate of 0.38, division per day of 0.55 and generation time of 1.81 at 0.014 mM of Fe – EDTA (Table, 7).

7. **Effect Of Different Concentrations of Magnesium Sulphate:** Among the 10 different concentrations of Magnesium sulphate tested, the alga grown at4.153 mM showed a maximum growth of 224 × 10^4 cells/ml on 21st day, whereas at 1.246 mM, 2.076 mM, 2.492 mM and 0.830 mM, it showed maximum of 218, 215, 207 and 206 × 10^4 cells/ml on 21st, 21st, 24th and 24th days, respectively (Figure 13). At 4.153 mM Magnesium sulphate the alga showed specific growth rate of 0.39, division rate of 0.57 and generation time of 1.76 (Table, 8). The alga showed poor growth at 0.415 mM Magnesium sulphate. Maximum specific growth rate of 0.66, division per day of 0.96, generation time of 1.05 were recorded at 1.246 mM of Magnesium sulphate.

   - **Chlorophyll *a*:** The alga had maximum Chlorophyll *a* content of 2.6 mg L^{-1} on 21st day at 4.153 mM Magnesium sulphate, followed by 2.5, 2.4 and 2.0 mg L^{-1} at 1.246, 2.076 and 0.830 mM Magnesium sulphate on 21st, 21st and 24th days, respectively. A minimum Chlorophyll *a* content of 1.3 mg L^{-1} recorded at 0.415 mM was 62% less than control on 30th day.
   - **Total Lipids:** Among the 10 different concentrations of Magnesium sulphate tested, the alga grown in 3.738 mM had maximum lipid content of 186 mg L^{-1} with 190 × 10^4 cells/ml on 18th day, followed by 134, 116 and 99 mg L^{-1} with 184 × 10^4 cells/ml, 134 × 10^4 cells/ml and 113 × 10^4 cells/ml at 2.076 mM, 4.153 mM, and 2.492 mM of Magnesium sulphate on 18th, 12th and 15th days, respectively (Fig.14). The alga showed maximum lipid productivity of 10.36 mg L^{-1} d^{-1}, specific growth rate of 0.44, division per day of 0.64 and generation time of 1.56 when it was grown in 3.323 mM Magnesium sulphate on 9th day (Table, 8).

8. **Effect of Modified CFTRI Medium on *Tetraselmis straiata* BBRR1 Under Laboratory Condition:** In order to minimize the cost of chemicals for the mass cultivation of *Tetraselmis straiata*, a Modified CFTRI medium was chosen in the present study. The following investigation was made to determine the optimum nutrient concentrations for achieving maximum biomass and lipid productivity of the test alga.

9. **Effect of Different Concentrations of Urea:** Among the 11 different concentrations of Urea tested, the Modified CFTRI medium amended with 0.832 mM Urea supported the maximum growth of 214 × 10^4 cells/ml of the alga on 20th day. Whereas at 1.66, 2.49 and 3.33 mM Urea showed 209, 115 and 65 × 10^4 cells/ml on 20th, 30th and 20th day, respectively (Figure 15). At 0.832 mM the alga showed specific growth rate of 0.32, division rate of 0.47 and generation time of 2.14 (Table, 9). The alga showed poor growth at 4.16 mM. Maximum specific growth rate of 0.33 and division per day of 0.48 generation time of 2.08 were recorded at 1.66 mM Urea.

   - **Chlorophyll *a*:** The alga synthesized maximum Chlorophyll *a* content of 7.25 mg L^{-1} on 20th day at 0.832 mM followed by 6.44, 4.76 and 2.45 mg L^{-1} at 1.66, 2.49 and 3.33 mM, respectively, on 20th day. Whereas a minimum Chlorophyll *a* content of 1.96 mg L^{-1} recorded at 4.16 mM was 59% less than control (4.16 mM) on 30th day.

- **Total Lipids:** Among the 11 different concentrations of Urea tested, the medium amended with1.66 mM of Urea supported the organism for maximum accumulation of 107 mg L^{-1} lipid content with 98 × 10^4 cells/ml on 25th day. The amount of total lipids recorded in the above condition was 15% more than control (4.16 mM Urea). Minimum lipid content of 55 mg L^{-1} recorded at 9.15 mM was 41% less than control on 25th day (Figure 16). The alga showed maximum lipid productivity of 6.32 mg L^{-1} d^{-1} at 3.33 mM Urea on 15th day (Table, 9).

10. **Effect of Different Concentrations of Super Phosphate:** Among the 11 different concentrations of Super phosphate tested, the medium amended with 0.170 mM supported maximum growth of 143 × 10^4 cells/ml on 20th day. Whereas the alga grown at 0.128, 0.085 and 0.042 mM showed 129, 129 and 117 × 10^4 cells/ml on 25th day (Figure 17). At 0.170 mM the alga showed specific growth rate of 0.44, division rate of 0.63 and generation time of 1.58(Table, 10). The alga showed poor growth at 0.213 mM. Maximum specific growth rate of 0.44 and division per day of 0.63 generation time of 1.58 were recorded at 0.170 mM of Super phosphate.

- **Chlorophyll *a*:** The alga had maximum Chlorophyll *a* of 4.30 mg L^{-1} on 20th day at 0.170 mM of Super phosphate, followed by 4.09, 3.90 and 3.68 mg L^{-1} at 0.085, 0.128 and 0.042 mM, respectively, on 25th day. Minimum Chlorophyll *a* content of 1.27 mg L^{-1} recorded at 0.341 mM was 69% less than control (0.085 mM) on 25th day.
- **Total Lipids:** The alga grown in 0.170 mM of Super phosphate showed maximum lipid content of 316 mg L^{-1} with 75 × 10^4 cells/ml on 15th day, followed by 202, 189, 159 and 137 mg L^{-1} with 94 × 10^4, 115 × 10^4, and 90 × 10^4 and 0.93 × 10^4 cells/ml at 0.299, 0.384, 0.341 and 0.128 mM on 15th, 30th, 15th and 15th days, respectively (Figure 18). A minimum lipid content of 106 mg L^{-1} recorded at 0.213 mM of Super phosphate was 16% less than control (0.085 mM Super phosphate) on 15th day. The alga showed maximum lipid productivity of 21.08 mg L^{-1} d^{-1}, specific growth rate of 0.44, division per day of 0.63 and generation time of 1.58 when it was grown in the medium amended with 0.170 mM of Super phosphate on 15th day (Table, 10).

11. **Effect of Different Concentrations of Sodium Bicarbonate:** Among the 11 different concentrations of Sodium bicarbonate tested, the medium amended with 5.95 mM supported the alga for its maximum growth of 184 × 10^4 cells/ml on 30th day, whereas at 3.57, 6.54 and 2.38 mM showed 174 × 10^4,153 × 10^4, and 126 × 10^4 cells/ml, respectively, on 30th day (Figure 19). At 5.95 mM of Sodium bicarbonate the alga showed specific growth rate of 0.34, division rate of 0.49 and generation time of 2.06 (Table,11).The alga showed poor growth at 2.97 mM of Sodium bicarbonate.

- **Chlorophyll *a*:** The alga synthesized maximum Chlorophyll *a* content of 6.75 mg L^{-1} on 30th day at 5.95 mM, followed by 6.57, 4.63 and 4.37 mg L^{-1} at 3.57, 6.54 and 2.38 mM on 30th day. The alga grown at 2.97 mM showed minimum Chlorophyll *a* content of 2.82 mg L^{-1} , which was 69% less than control on 30th day.
- **Total Lipids:** Among the 11 different concentrations of Sodium bicarbonate tested, the medium amended with 5.95 mM supported the alga for maximum lipid accumulation of 115 mg L^{-1} with 82 × 10^4 cells/ml on 15th day. The increment of lipid content of the above was 33% to that of control. The alga grown in 0.595 and 1.19

mM contained 102 and 100 mg L^{-1} of total lipids with 80 × 10^4 cells/ml and 88 × 10^4 cells/ml, respectively. The alga showed maximum lipid productivity of 6.82 mg L^{-1} d^{-1} at 0.595 mM on 15th day (Fig. 20). The test organism exhibited maximum specific growth rate of 0.14, division per day of 0.20 and generation time of 4.90 when the medium amended with 0.595 mM Sodium bicarbonate (Table, 11).

12. **Effect of Different Concentrations of Ferric Chloride:** Among the 6 different concentrations of Ferric chloride tested, in the alga grown in 0.061 mM showed maximum growth of 236 × 10^4 cells/ml on 20th day, whereas at 0.024, 0.073 and 0.012 mM, it showed 149 × 10^4, 134 × 10^4 and 127 × 10^4 cells/ml on 20th, 30th and 20th day, respectively (Figure 21). At 0.061 mM of Ferric chloride the alga showed specific growth rate of 0.45, division rate of 0.65 and generation time of 1.54 (Table, 12). The test alga showed poor growth at 0.036 mM. Maximum specific growth rate of 0.45, division rate of 0.65 and generation time of 1.54 were recorded at 0.061 mM Ferric chloride.

- **Chlorophyll *a*:** The alga had maximum Chlorophyll *a* of 2.75 mg L^{-1} on 20th day at 0.061 mM followed by 1.68 and 1.43 mg L^{-1} at 0.073, and 0.024 mM of Ferric chloride on 30th and 20th day, respectively. Minimum Chlorophyll *a* content of 1.15 mg L^{-1} recorded at 0.012 mM was 19% less than control on 20th day.
- **Total Lipids:** Among the 6 different concentrations of Ferric chloride tested, the alga grown at 0.061 mM showed maximum total lipids of 236 mg L^{-1} with 77 × 10^4 cells/ml on 15th day. A minimum lipid content of 98 mg L^{-1} recorded at 0.049 mM was 35% less than control (0.024 mM) on 15th day. The alga showed maximum lipid productivity of 15.72 mg L^{-1} d^{-1}, specific growth rate of 0.45, division per day of 0.65 and generation time of 1.54 when it was grown in the medium amended with 0.061 mM of Ferric chloride on 15th day(Table, 12) (Figure 22).

13. **Effect of Different Concentrations of Magnesium Sulphate:** Among the 8 different concentrations of Magnesium sulphate tested, the medium amended with 2.076 mM supported for maximum growth of 230 × 10^4 cells/ml on 20th day, followed by 2.492 mM, 1.660 mM, 2.907 mM, and 0.830 mM with 211, 195, 194 and 185 × 10^4 cells/ml, respectively, on 20th day (Figure 23). At 2.076 mM the alga showed specific growth rate of 0.17, division rate of 0.25 and generation time of 4.03 (Table, 13). The alga showed poor growth when the medium amended with 3.323 mM. Maximum specific growth rate of 0.27 and 0.39, division per day of 2.56 were recorded at 0.830 mM.

- **Chlorophyll *a*:** The alga had maximum Chlorophyll *a* content of 1.80 mg L^{-1} at 2.076 mM, followed by 1.50, 1.46 and 1.45 mg L^{-1} at 2.492, 1.660 and 2.907 mM, respectively, on 20th day. Minimum Chlorophyll *a* content of 1.23 mg L^{-1} recorded at 0.415 mM was 33% less than control (1.246 mM) on 20th day.
- **Total Lipids:** The alga grown in 2.076 mM of Magnesium sulphate had maximum lipid content of 120 mg L^{-1} with 89 × 10^4 cells/ml on 10th day, followed by 99, 95 and 94 mg L^{-1} with cell numbers of 95 × 10^4, 90 × 10^4 and 92 × 10^4 cells/ml at 2.492 mM, 0.415 mM, and 1.660 mM on 10th, 15th and 10th days, respectively (Figure 24). The alga showed maximum lipid productivity of 12 mg L^{-1} d^{-1}, specific growth rate of 0.17, division per day of 0.25 and generation time of 4.03 when it was grown in the medium amended with 2.076 mM of Magnesium sulphate on 10th day (Table, 13).

14. **Low - Cost Medium for *Tetraselmis straiata* BBRR1:** Based on the above studies a Modified CFTRI ABRR I medium (optimized) was formulated and used for the mass cultivation of *Tetraselmis straiata* in open raceway ponds. Composition of the Modified CFTRI ABRR I medium: Urea – 0.832 mM, Super phosphate – 0.170 mM, Sodium bicarbonate – 5.951 mM, Ferric Chloride – 0.061 mM, Magnesium Sulphate – 2.076 mM, Sea salt – 35 g L^{-1}, pH – 8.5.

15. **Comparative Study on Growth and Lipid Production of *Tetraselmis straiata* BBRR1 in Modified CFTRI and Modified CFTRI - ABRRI Media:** Among the two different media tested, the Modified CFTRI – ABRR I medium supported the alga for maximum growth of 238 × 10^4 cells/ml on 25th day. Whereas in the Modified CFTRI medium (control) it showed 172 × 10^4 cells/ml on 25th day (Figure 25,26). It was 28% less than the alga grown in Modified CFTRI ABRR I medium. The alga grown in the Modified CFTRI – ABRR I medium showed specific growth rate of 0.60, division rate of 0.86 and generation time of 1.16 (Table, 14).

- **Chlorophyll *a*:** The alga synthesized maximum Chlorophyll *a* content of 4.09 mg L^{-1} in Modified CFTRI – ABRR I medium on 25th day. Whereas a minimum Chlorophyll *a* content of 2.35 mg L^{-1} recorded at Modified CFTRI medium was 42% less than Modified CFTRI ABRR I medium on 25th day (Fig.27).
- **Total Lipids:** Among the two different media tested, the Modified CFTRI – ABRR I medium supported the alga for a maximum accumulation of 202 mg L^{-1} of lipid content with 152 × 10^4 cells/ml on 15th day. The amount of lipid content recorded in the above condition was 60% more than control (Modified CFTRI medium) (Figure 28). The alga showed maximum lipid productivity of 14.34 mg L^{-1} d^{-1} in the Modified CFTRI – ABRR I medium on 15th day (Table, 14).

IV. DISCUSSION

This study envisaged researching the feasibility and sustainability of microalgae as a kind of biofuel feedstock to meet the energy crisis and commercialization. The high oil yield and less land use are the significant advantages of microalgae for biofuel production. Microalgae cultivation is the basis of biofuel development; genetic engineering must be developed to break through the microalgae oil content problem and growth rate to establish a new sustainable renewable energy (Hannon *et al*., 2010; Lam and Lee, 2012).

Physical parameters and medium constitutions are the essential factors influencing microalgae growth and lipid accumulation. Microalgae are regarded as an alternative feedstock for biodiesel production owing to their fast growth rate and high lipid productivity. Mainly, to improve the lipid productivity of microalgae, it is a pre-requisite to select an appropriate strategy for inducing the cellular lipid accumulation during the cultivation, but not decrease the biomass production (Hu *et al*., 2008; Lee and Seong, 2015; Kim *et al*., 2015; Kim *et al*., 2016). Microalgae growth and increasing lipid contents are dependent on light, water, temperature, nutrient concentrations, salinity and pH (Scott *et al*., 2010; Brennan and Owende, 2010; Mutanda *et al*., 2011; Huang *et al*., 2013; Kim *et al*., 2016).

Among the four strengths of the basal f/2 medium investigated, *Tetraselmis straiata* BBRR1 preferred f medium 1.0 (f) for maximum growth and lipid productivity.

The alga showed a maximum specific growth rate of 0.41 g day^{-1}. Alsull and Omar (2012) and Arkronrat *et al.* (2016) reported the top typical growth rates of 0.16 and 0.13 g day^{-1} , respectively, on *Tetraselmis* sp., which were less than the test alga, *T. straiata* BBRR1. *Tetraselmis* sp. grown in f/2, K, and L1 media showed specific growth rates of 0.14, 0.19 and 0.24 g day^{-1} (Huerlimann *et al*., 2010). The present alga, *T. straiata*, had a maximum chlorophyll content of 6.23 mg L^{-1} in f medium. This value followed the observation made on *Tetraselmis* sp. by Melina *et al*. (2016). Whereas, Imamogluthe *et al*. (2015) reported a maximum Chlorophyll – concentration of 7.93 mg L^{-1} in *T. straiata*.

Algal cells change their lipid content depending on the nutrient conditions (Kim *et al*., 2016). Huerlimann *et al*. (2010) reported a maximum lipid content of 11.8%/dry biomass in *Tetraselmis* sp. when it was grown in f/2 medium. In the present study, the alga developed in f/2 medium, f/2 and f medium had high lipid contents of 25.07% and 19.42% /dry biomass, respectively.

pH plays a vital role in determining the growth rate of microalgae. Among the 11 different pH investigated, the test alga *Tetraselmis straiata* BBRR1 preferred pH 8.5 for maximum growth. Khatoon *et al*. (2014) stated that the alga *Tetraselmis* sp. showed a maximum specific growth rate of 0.352 at pH 8.5. Bartley *et al*. (2014) reported that *Nanochloropsis* salina exhibited the highest specific growth rate of 0.19 at pH 8.0. *T. suecica* showed a continued growth at pH 8.0, as reported by Moheimani. (2013). Our research also suggested that a pH of 8.5 could maximize the specific growth rate of 0.47 in *T. straiata*. Alberte (1991) reported that the concentrations of Chlorophyll *a* were maximum of 2.48 and 1.2 mg L^{-1}, respectively, in *Tetraselmis* sp. at pH 8.5. According to Moheimani (2013), a pH 7.0 was ideal for lipid accumulation in *T. suecica*. Ratledge (2002) and Courchesne *et al*. (2009) described that nutrient starvation and other stress factors may cause enhanced lipid accumulation in microalgae with reduced cell division. The test alga, *T.straiata*, had a maximum lipid content of 156 mg L^{-1} at pH 8.0 with a reduced growth rate compared to pH 8.5.

Salinity stress affects various physiological and biochemical mechanisms related to growth, and it can lead to an increase in the lipid content of microalgae due to its essential role in causing changes in fatty acid metabolism (Kalita *et al*., 2011). *Tetraselmis* sp. grown at 30 ppt salinity had shown significantly high cell density and lipid content, as reported by Khatoon *et al*. (2014). Purba and Siburain (2012) stated that *Tetraselmis* sp. and *Dunaliella tertiolecta* produced high lipids at high salinity. *Tetraselmis* sp. accumulated much total lipid in 35 and 40 ppt. However, low salinity (i.e., below 20 ppt) decreased the algal growth. Hu (2004) reported that increasing concentrations of Sodium chloride enhanced the total lipids of the algae.

The test alga, *T.straiata*, survived in all the range of salinities chosen from 10 ppt to 90 ppt. However, 35 ppt supported *T.straiata* for maximum growth of 139×10^4 cells/ml and a specific growth rate of 0.57. The cell number and growth rate were decreased up to 49.65% and 34.28%, respectively, at 90 ppt when compared to 30 ppt (control). The value of the maximum specific growth rate recorded at 35 ppt in the present attempt was in accordance with Khatoon *et al*. (2014). The alga grown at 35 ppt also had maximum Chlorophyll *a* of 1.70 mg L^{-1}. At the same time, high salinity at 90 ppt decreased the amount of Chlorophyll by 33.16%. According to Sigaud and Aidar (1993), *T. gracilis* had a maximum growth rate and

chlorophyll content at 40 ppt. According to Yao *et al*. (2013), there was an inconsistency in the growth of *Tetraselmis* sp. towards salinity.

The rate of nitrate consumption was directly proportional to the cell number produced in *Tetraselmis suecica* (Garcia *et al*., 2013). Among the 6 concentrations of Sodium nitrate investigated in the present attempt on *T.straiata*, 1.76 mM supported a maximum cell numberof 146 × 10^4 cells/ml, a specific growth rate of 0.17 and a division rate of 0.25. *Tetraselmis straiata* BBRR1 grown in the enriched nitrogen concentration of 1.76 mM Sodium nitrate had a maximum chlorophyll content of 1.17 mg L^{-1}, The lower concentration of 0.58 mM decreased the Chlorophyll up to 36%. The results of the present study also revealed that the concentration of Chlorophyll *a* was found to be directly proportional to Sodium nitrate up to 1.76 mM. Li *et al*. (2008, 2015) suggested that the nitrogen pools of Chlorophyll molecules were consumed to support the algal growth rate after nitrogen exhaustion. Therefore, the Chlorophyll content of alga is low, with a lower nitrogen concentration in the medium.

Nitrogen is an essential constituent of all structural and functional proteins in the algal cells (Hu, 2004). Chlamydomonas sp. grown in nitrogen starvation showed a rapid lipid accumulation, as reported by Tan *et al*. (2016). In the present attempt, the alga grown at the initial low concentration of 0.88 mM Sodium nitrate showed maximum lipid accumulation of 65 mg L^{-1} on the 21st day compared to the alga grown at a higher nitrate concentration. The above results are in accordance with the observations made by Reitan *et al*. (1994), Sheehan *et al*. (1998) and Griffiths and Harrison (2009).

Phosphate plays a fundamental role in microalgal growth (Hu, 2004). In the present investigation, among the six different concentrations of Sodium di-hydrogen phosphate tested, 0.08 mM supported the alga *T. straiata* for maximum growth of 67 × 10^4 cells/ml and a specific growth rate of 0.15. The cell numbers of the test alga were decreased by up to 16% at the low concentrations of 0.02 mM and 0.025 mM when compared to control (0.04 mM). Jayappriyan *et al*. (2010) investigated that high concentrations of phosphate inhibited the growth of *Dunaliella salina* and *Nanochloropsis oculata*. In the present study, concentrations above 1.76 mM of Sodium dihydrogen phosphate inhibited the growth of *Tetraselmis straiata*. The alga *Tetraselmis straiata* also synthesized a maximum Chlorophyll content of 0.52 mg L^{-1} at 0.08 mM. Sharma *et al*. (2012) and Juneja *et al*. (2013) reported that phosphate is a key constituent of phospholipids and lipid production. In the present study, the alga grown in less phosphate concentration, such as 0.04 mM of Sodium dihydrogen phosphate, enhanced the total lipids of 80 mg L^{-1}. Similarly, Purba and Siburian (2012) reported that the alga accumulated maximum lipid content at phosphate starvation conditions.

Retardation of algal growth, reduction of photosynthetic activity and Chlorophyll content were recorded in the absence of iron, as observed by Haque *et al*. (2012) and Kean *et al*. (2015). Ferric–EDTA is important for algal metabolism, being a part of cytochromes. It is utilized for nitrogen assimilation, fixation, photosynthesis, and DNA synthesis (Hardie *et al*., 1983). Concas *et al*. (2014) reported that the growth rate and lipid content of *Chlorella* vulgaris were increased at higher concentrations of iron. In our experiments, among the 5 different concentrations of Fe – EDTA tested, 0.023 mM supported the alga, *T. straiata*, for maximum growth of 153 × 10^4 cells/ml and a specific growth rate of 0.41. Whereas *Nanochloropsis salina* had a maximum growth rate at a low iron concentration, as

stated by Adetola (2011).

Higher iron concentrations could stimulate biomass production in microalgae, as stated by Liu *et al*. (2007). In the present study, the *Tetraselmis* strain showed a maximum specific growth rate at the high concentration of Fe – EDTA at 0.023 mM. Whereas maximum lipid productivity of 4.79 mg L^{-1} d^{-1} was recorded at 0.014 mM of Fe – EDTA, indicating that the alga preferred a low concentration of Fe-EDTA for high accumulation of total lipids in contrast to biomass productivity. Similarly, the lipid productivity in *Nanochloropsis oculata* was increased at lower concentrations of Fe – EDTA, as reported by Dou *et al*. (2013).

Magnesium is an important component of Chlorophyll biosynthesis. Algal growth rate was increased at high concentrations of Magnesium sulphate (Schwenk *et al*., 2010). Haque *et al*. (2012) also reported that the high concentration of Magnesium had stimulated high biomass production, similar to the observation made on *Tetraselmis* sp. by Yao *et al*. (2012). The test alga, *Tetraselmis straiata* BBRR1, preferred 4.153 mM and 3.738 mM of Magnesium sulphate for maximum growth and lipid productivity of 224 × 10^4 cells/ml and 10.36 mg L^{-1} d^{-1}, respectively. The alga showed maximum synthesis of Chlorophyll *a* of 2.6 mg L^{-1} at 4.153 mM of Magnesium sulphate.

A variety of different media are being used for microalgae cultivation, especially marine salts and agricultural fertilizers, due to chemically defined nutrients being relatively expensive to formulate and also the development of large-scale algal cultivation (Barakoni *et al*., 2015). It is clearly indicated that less expensive nutrient sources are desperately needed for microalgal cultivation. Amin *et al*. (2013) and Sarpal *et al*. (2015) reported that the algal biomass and the lipid productivity, particularly triglycerides, are achieved by varying composition of nutrient medium, particularly different sources of nitrogen (Urea fertilizer) and phosphorus (Superphosphate). Different nitrogen sources such as Ammonia, Nitrate, Nitrite and Urea are used for microalgal cultivation (Anderson, 2005). Organic nitrogen sources such as Urea are a good source of nitrogen, and it is cheaper than nitrate used for algal culturing (Danesi *et al*., 2002; Harben and Theune, 2006). According to Arumugam *et al*. (2012), increasing the concentration of Urea leads to higher growth and biomass productivity in algae. In the present findings, among the 11 different concentrations of Urea investigated, 0.832 mM Urea supported the alga *T. straiata* for maximum growth of 214 × 10^4 cells/ml on the 20^{th} day. Similarly, the alga synthesized maximum Chlorophyll *a* of 7.25 mg L^{-1} at 0.832 mM. Saumya *et al*. (2016) reported that *Scenedesmus* sp. had a maximum lipid productivity of 4.1 mg L^{-1} d^{-1} at 2.5 mM of Urea. *T. straiata* accumulated a high lipid content of 107 mg L^{-1} at 1.66 mM of Urea on the 25^{th} day.

Increased Urea concentration in the medium above the tolerance level leads to inhibit the algal growth due to its toxicity (Xu *et al*., 2011). Torre *et al*. (2003) reported that Urea concentrations had a strong influence on cell division. Urea quickly converted into Ammonia; the problem of Ammonia toxicity is due to high concentrations of Urea. This finding was similar to the present observation made on *T. straiata*. The test alga showed poor growth above 4.16 mM of Urea in the basal medium.

Phosphorus plays a vital role in microalgae because it is a building block of nucleic acids, phospholipids and carbohydrates (Rai and Gaur, 2001). Sarpal *et al*. (2015) investigated the use of Superphosphate fertilizer for biomass and lipid productivity in

microalgae as a cheaper source of phosphorous. In the present attempt, among the 11 different concentrations of Superphosphate investigated, the alga *Tetraselmis straiata* grown in the medium amended with 0.170 mM showed maximum growth of 143×10^4 cells/ml and Chlorophyll of 4.30 mg L^{-1} on the 20^{th} day. The above results were in accordance with the observations made by Jamaluddin *et al*. (2015). They reported that the microalgal growth rate was increased at the phosphate concentration increased in the medium. The test alga showed a maximum of 3.47 divisions/ day at 0.128 mM of Superphosphate. Wang and Lan (2010) and Pingzhong *et al*. (2012) stated that the highest lipid productivity was recorded under low phosphate concentrations. This statement was also justified in the present attempt made on *Tetraselmis straiata*, which showed maximum lipid productivity of 21.08 mg L^{-1} d^{-1} at 0.170 mM (less concentration of Superphosphate). In addition, Khozin Goldberg and Cohen (2006) and Xin *et al*. (2010) reported that lipid accumulation was high at the low concentration of phosphorous in *Monodus subterraneous* and *Scenedesmus* sp.

Tetraselmis suecica was able to tolerant at high concentration of Sodium bicarbonate (White *et al*., 2013). Similarly, in the present study, among the 11 different concentrations of Sodium bicarbonate investigated, the medium amended with 5.95 mM supported the alga *Tetraselmis straiata* for its maximum growth of 184×10^4 cells/ml on the 30^{th} day. At 5.95 mM of Sodium bicarbonate, the alga showed a specific growth rate of 0.34, similar to observations made on *Chaetoceros gracilis* (Pimolrat *et al*., 2010). The alga showed poor growth at 2.97 mM of Sodium bicarbonate. In contrast, 11.90 mM of Sodium bicarbonate supported maximum cell numbers of *Tetraselmis suecica* and *Nanochlorpsis salina*, as reported by White *et al*. (2013). Zhou *et al*. (2016) stated that different concentrations of Sodium bicarbonate are significantly affected by the specific growth rates of the algae.

The test alga synthesized maximum Chlorophyll *a* of 6.75 mg L^{-1} on the 30^{th} day at 5.95 mM of Sodium bicarbonate. The medium amended with 5.95 mM of Sodium bicarbonate supported the alga for a maximum carbohydrate of 76 mg L^{-1} with 184×10^4 cells/ml on the 30^{th} day, which was 71% more than the control (5.356 mM). Similarly, *Tetraselmis straiata* grown in the medium amended with Sodium bicarbonate at 5.95 mM had a maximum protein content of 234 mg L^{-1} with 184×10^4 cells/ml on the 30^{th} day, which was 91% more than the control. White *et al*. (2013) reported that adding Sodium bicarbonate showed a positive effect on the pigment levels in *Tetraselmis suecica* and *Nanochloropsis* sp. In the present attempt, the medium amended with Sodium bicarbonate of 5.95 mM supported the alga for maximum lipid accumulation of 115 mg L^{-1} with 82×10^4 cells/ml on 15^{th} day. The increment of the lipid content of the above was 33% higher than the control. The alga showed maximum lipid productivity of 6.82 mg L^{-1} d^{-1} at 0.595 mM on the 15^{th} day. Our results were in accordance with the observations made on *Tetraselmis suecica* and *Nanochloropsis* sp. (White *et al*., 2013).

Ferric chloride is one of the important iron sources for microalgal growth due to the ferric ion is involved in the primary enzymatic reactions, photochemistry in photosystem II and Chlorophyll synthesis for microalgae (Behrenfeld *et al*., 2006; Wang and Lan, 2010). Among the 6 different concentrations of Ferric chloride tested, 0.061 mM supported the test alga, *T. straiata*, for maximum growth of 236×10^4 cells/ml on the 21^{st} day. Also, the alga synthesized a maximum content of Chlorophyll of 2.75 mg L^{-1} at 0.061 mM on the 20^{th} day. However, the test alga showed poor growth at 0.036 mM of Ferric chloride, which was 30% less than the control (0.024 mM of Ferric chloride). Similarly, a lack of iron can reduce

microalgal growth rates, as investigated by Oijen *et al*. (2004). *Tetraselmis straiata* had a maximum lipid content of 236 mg L^{-1} with 77 × 10^4 cells/ml at 0.061 mM of Ferric chloride on the 15th day. Liu *et al*. (2008) reported that increased total lipids of *Chlorella* sp. were recorded at high iron concentrations.

Among the eight different concentrations of Magnesium sulphate tested in the amended medium, the alga *T. straiata* showed maximum growth of 230 × 10^4 cells/ml and had Chlorophyll *a* of 1.80 mg L^{-1} in the medium amended with 2.076 mM on the 20th day. The test alga showed a maximum specific growth rate of 0.27 and 0.39, division per day of 2.56 at 0.830 mM. Similarly, our results have supported the work of Ulloa *et al*. (2011) and Schwenk (2012). The addition of micronutrients, particularly Magnesium, did not affect the growth rate of *Tetraselmis suecica*, and the growth rate was high at the increased concentrations of Magnesium sulphate. *T. straiata* preferred at 2.076 mM of Magnesium sulphate for maximum lipid content of 120 mg L^{-1} with 89 × 10^4 cells/ml on the 10th day.

The development of microalgal cultivation depends upon the optimization of the medium due to cost-effectiveness for large-scale production, as indicated by Wang and Lan (2011) and Wang *et al*. (2012). Rodalfi *et al*. (2009), Kamal and Nabris (2011), Cheng *et al*. (2013) and Zhang *et al*. (2013) described the significance of biomass and lipid productivity of microalgae in mass cultivation. Among the two different media tested, the Modified CFTRI – ABRR I medium formulated in the present attempt supported the test alga *T. straiata* for maximum growth of 238 × 10^4 cells/ml on the 25th day. At the same time, the Modified CFTRI medium (control) showed 172 × 10^4 cells/ml on the 25th day, which was 28% less than the Modified CFTRI ABRR I medium. A high concentration of Urea leads to higher growth and biomass productivity in algae due to its strong influence on cell division (Arumugam *et al*., 2012). In the present results, poor growth was recorded in the Modified CFTRI medium due to increased Urea concentration in the medium above the level of 4.16 mM, which leads to inhibiting the algal growth due to its toxicity (as ammonium) (Xu *et al*., 2011).

The test alga, *T. straiata* showed a maximum accumulation of 202 mg L^{-1} of total lipids with 152 × 10^4 cells/ml on the 15th day in the Modified CFTRI – ABRR I medium. The lipid content recorded in the above medium was 60% higher than the control (Modified CFTRI medium). The alga showed maximum lipid productivity of 14.34 mg L^{-1} d^{-1} in the Modified CFTRI – ABRR I medium. Increased total lipids of *Chlorella* sp. were recorded at high nutrient concentrations (Liu *et al*., 2008).

V. CONCLUSION

Investigations made on *T. straiata* BBRR1 elucidate the following findings. The test alga *T. straiata* BBRR1 grown in four different strengths of f/2 medium revealed that the alga preferred f medium for maximum growth and lipid productivity. Among the 11 different pH chosen, 8.5 could be ideal for the organism to maximize the specific growth rate of 0.47. The test alga of *T. straiata* had a maximum lipid content of 156 mg L^{-1} at pH 8.0 with a reduced growth rate compared to pH 8.5. The test alga survived in all ranges of salinities chosen from 10 ppt to 90 ppt. However, 35 ppt supported *T. straiata* for a maximum growth of 139 × 10^4 cells/ml and a specific growth rate of 0.57. However, the cell number and growth rate decreased to 49.65% and 34.28%, respectively, at 90 ppt compared to 30 ppt (control).

Sodium nitrate at 1.76 mM supported the test alga, *T. straiata*, for a maximum cell number of 146 × 10^4 cells/ml, a specific growth rate of 0.17 and a division rate of 0.25. However, a low concentration of 0.88 mM of Sodium nitrate enhanced the lipid accumulation up to 65 mg L^{-1} on the 21st day. Among the six concentrations of Sodium di-hydrogen phosphate tested, 0.08 mM supported the alga *T. straiata* for maximum growth of 67 × 10^4 cells/ml and a specific growth rate of 0.15. A low Sodium di-hydrogen phosphate concentration of 0.04 mM also enhanced the total lipids of *T. straiata* up to 80 mg L^{-1} on the 12th day.

Among the five different concentrations of Fe - EDTA tested, 0.023 mM supported the alga for maximum growth of 153 × 10^4 cells/ml and a specific growth rate of 0.41. Whereas maximum lipid productivity of 4.79 mg L^{-1} d^{-1} was recorded at a low concentration of 0.014 mM of Fe – EDTA. *Tetraselmis straiata* BBRR1 preferred 4.153 mM and 3.738 mM of Magnesium sulphate for maximum growth and lipid productivity of 224 × 10^4 cells/ml and 10.36 mg L^{-1} d^{-1}, respectively. The alga showed the complete synthesis of Chlorophyll *a* of 2.6 mg L^{-1}, similar to growth at 4.153 mM Magnesium sulphate.

The addition of Urea at 0.832 mM supported *T. straiata* for maximum growth of 214 × 10^4 cells/ml and synthesized maximum Chlorophyll with *a* content of 7.25 mg L^{-1} on the 20th day. *T. straiata* had a maximum total lipid content of 107 mg L^{-1} at 1.66 mM of Urea on the 25th day. Among the 11 concentrations of Superphosphate investigated, the alga *Tetraselmis straiata* showed maximum growth of 143 × 10^4 cells/ml and Chlorophyll of 4.30 mg L^{-1} in the medium amended with 0.170 mM on the 20th day. The alga grown in Superphosphate of 0.170 mM showed a maximum lipid content of 316 mg L^{-1} with 75 × 10^4 cells/ml on the 15th day.

The alga grown in the medium amended with Sodium bicarbonate of 5.95 mM showed maximum growth of 184 × 10^4 cells/ml on the 30th day and a specific growth rate of 0.34. Sodium bicarbonate at 5.95 mM also supported the alga for maximum lipid accumulation of 115 mg L^{-1} with 82 × 10^4 cells/ml on the 15th day. The increment of the lipid content of the above was 33% to that of the control.

Ferric chloride at 0.061 mM supported the test alga for maximum growth of 236 × 10^4 cells/ml on the 21st day. *T. straiata* had a maximum lipid content of 236 mg L^{-1} with 77 × 10^4 cells/ml at 0.061 mM of Ferric chloride on the 15th day. Among the eight concentrations of Magnesium sulphate tested, the alga *T. straiata* showed maximum growth of 230 × 10^4 cells/ml and Chlorophyll of 1.80 mg L^{-1} at 2.076 mM on the 20th day. *T. straiata* preferred 2.076 mM of Magnesium sulphate for a maximum lipid content of 120 mg L^{-1} with 89 × 10^4 cells/ml on the 10th day.

The Modified CFTRI – ABRR I medium formulated in the present attempt supported the test alga for maximum growth of 238 × 10^4 cells/ml on the 25th day. The alga grown in the Modified CFTRI medium (control) showed 172 × 10^4 cells/ml on the 25th day, 28% less than the former medium. *T. straiata* had a maximum total lipid of 202 mg L^{-1} with 152 × 10^4 cells/ml on the 15th day when grown in the Modified CFTRI – ABRR I medium. The lipid content of the above was 60% more than control.

As a result, it revealed that the marine microalga, *Tetraselmis straiata* BBRR1, could be a suitable candidate for achieving biofuel to meet future energy demand.

VI. FIGURES AND TABLES

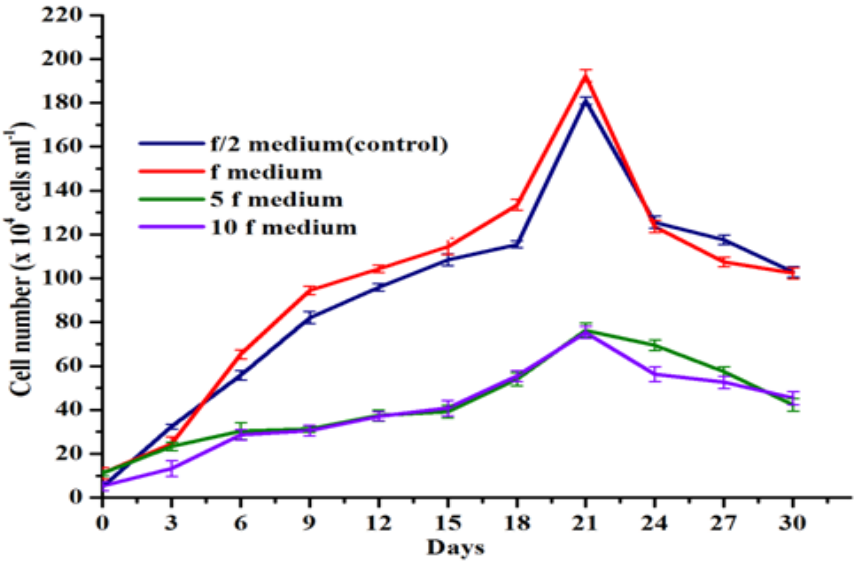

Figure 1: Effect of different strengths of f/2 medium on cell number of *T. straiata* BBRR1 at different intervals.

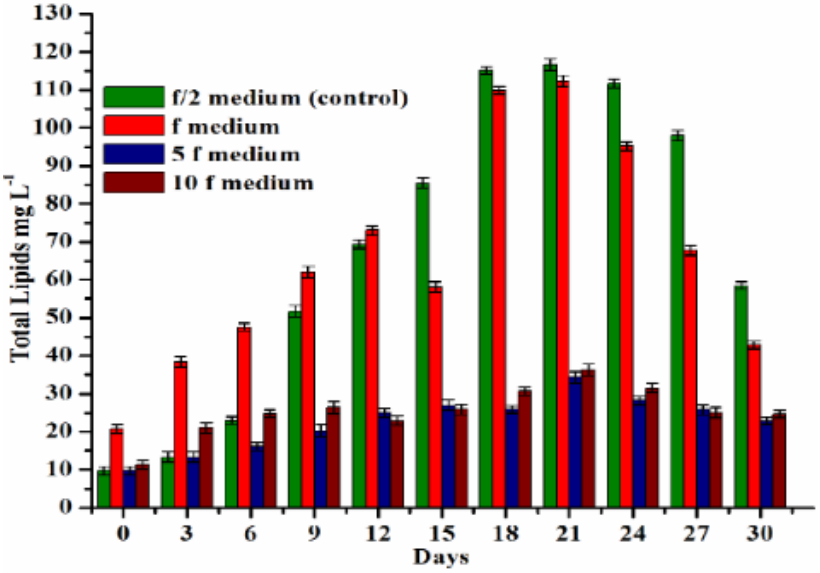

Figure 2: Effect of different strengths of f/2 medium on total lipids of T. straiata BBRR1 at different intervals.

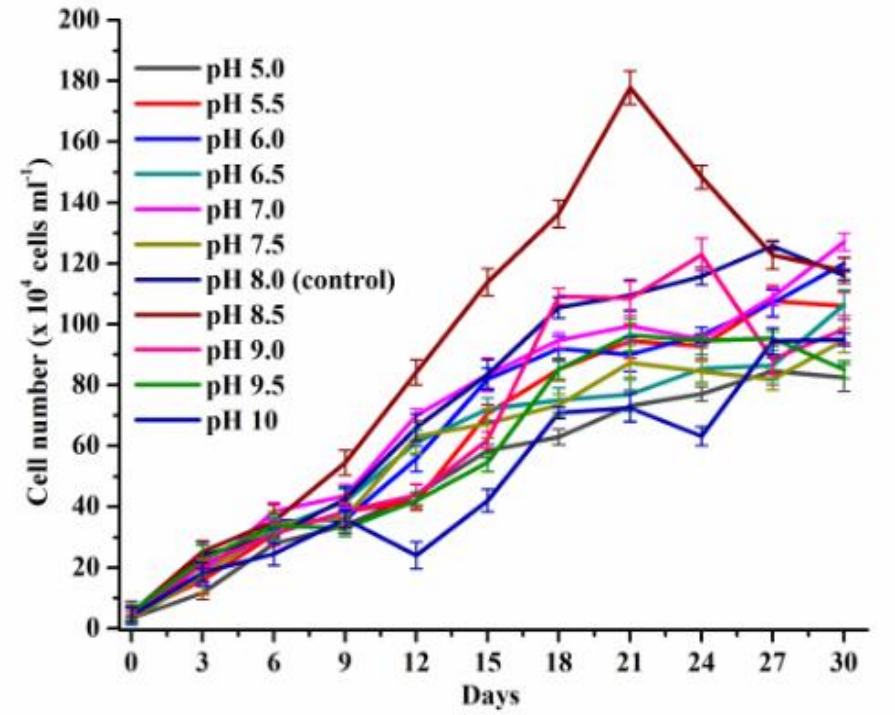

Figure 3: Effect of different pH on cell number of T. straiata BBRR1 at different intervals.

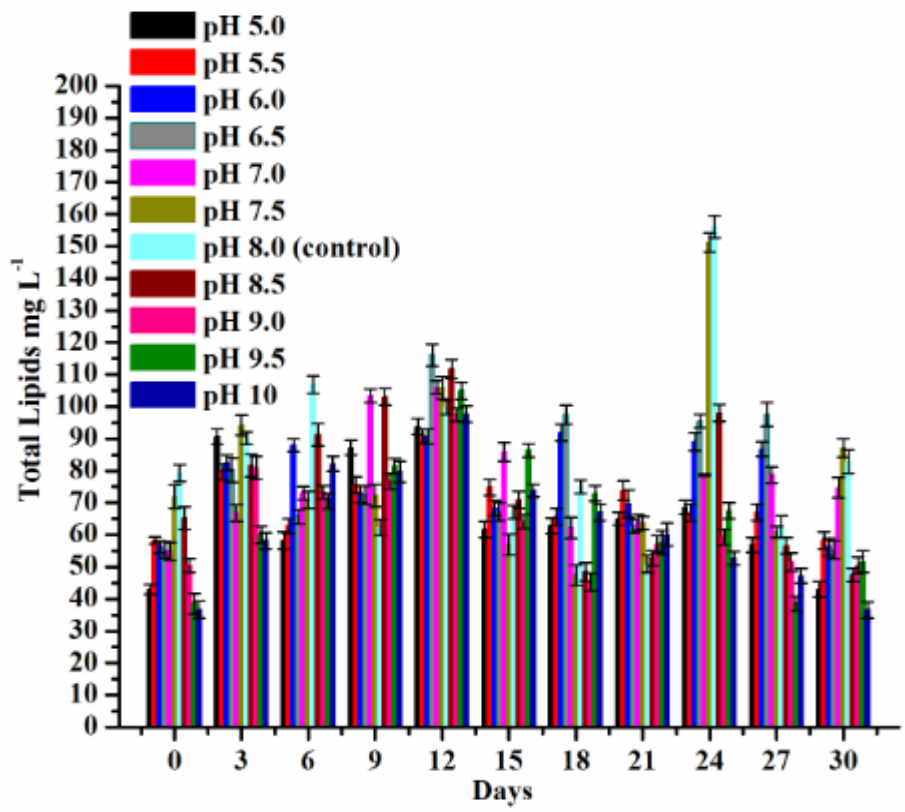

Figure 4: Effect of different pH on total lipids of T. straiata BBRR1 at different intervals.

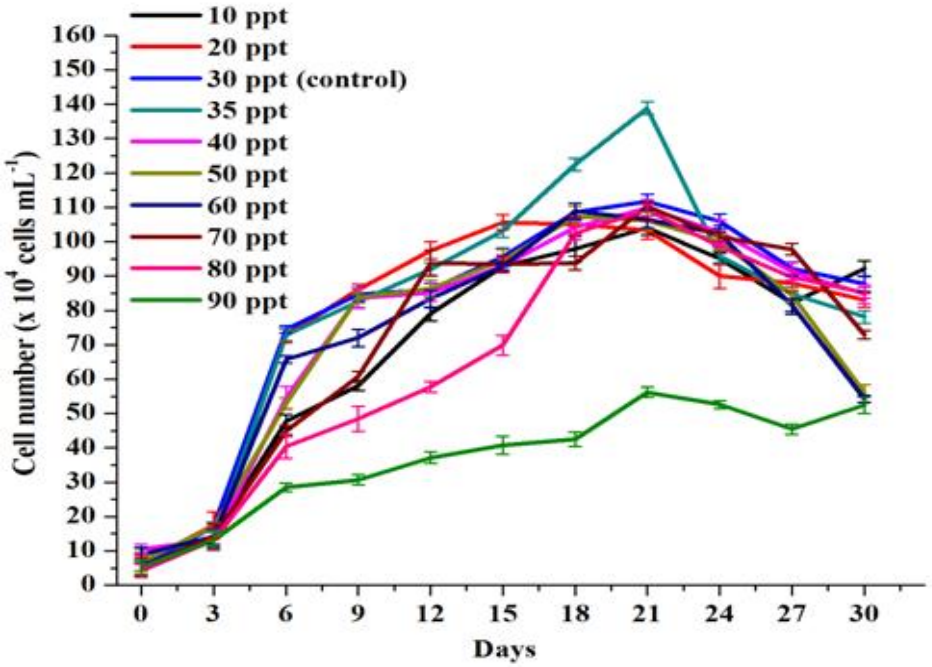

Figure 5: Effect of different Salinities on cell number of *T. straiata* BBRR1 at different intervals.

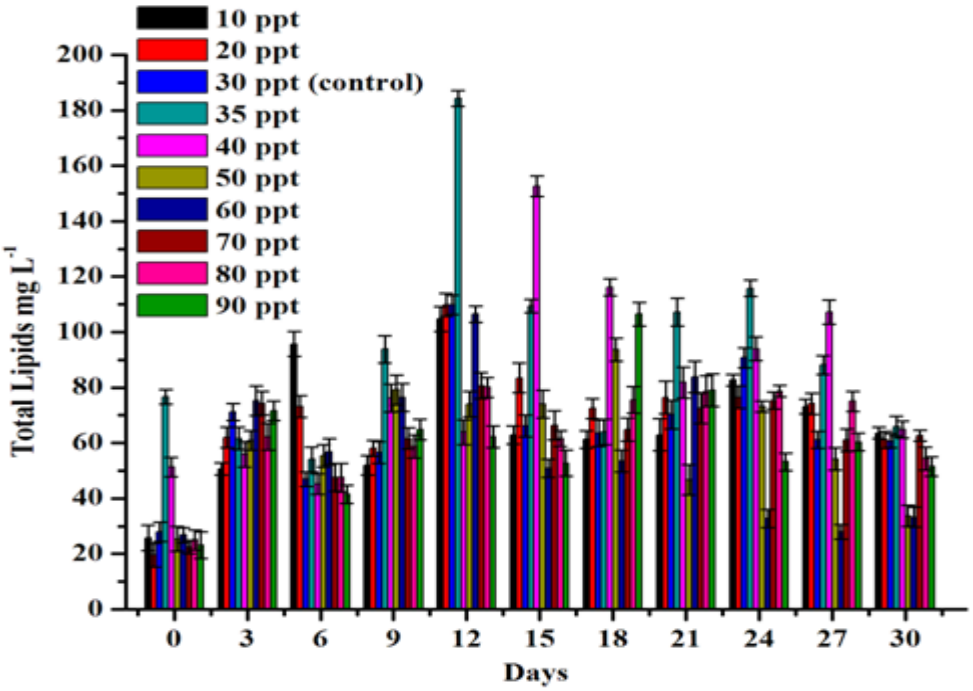

Figure 6: Effect of different Salinities on total lipids of *T. straiata* BBRR1 at different intervals.

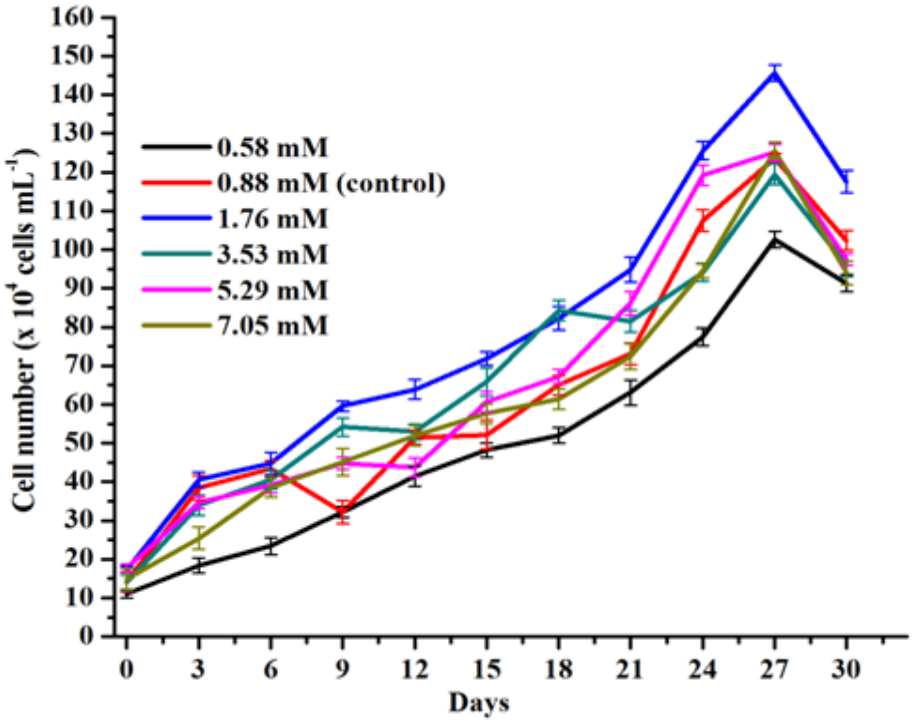

Figure 7: Effect of different concentrations of Sodium nitrate on cell number of *T. straiata* BBRR1 at different intervals.

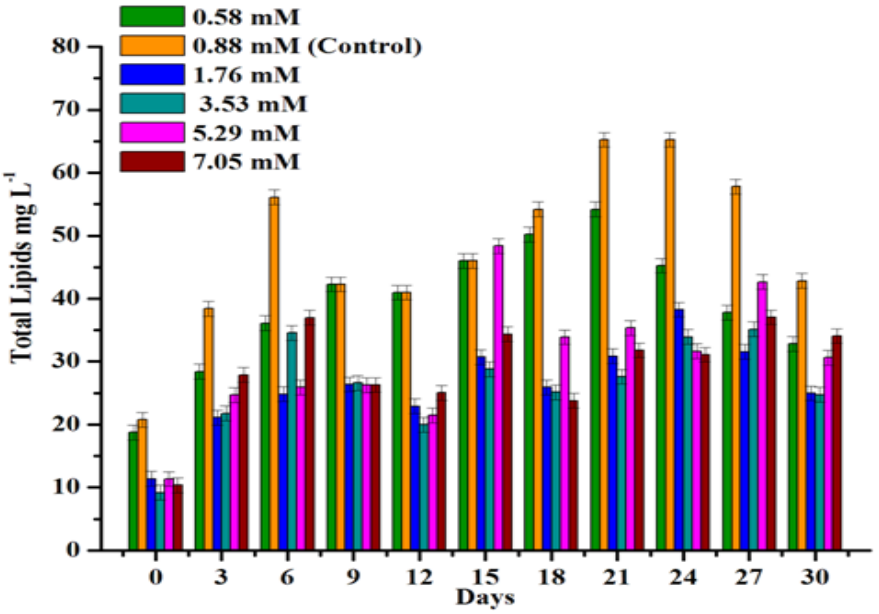

Figure 8: Effect of different concentrations of Sodium nitrate on total lipids of *T. straiata* BBRR1 at different intervals.

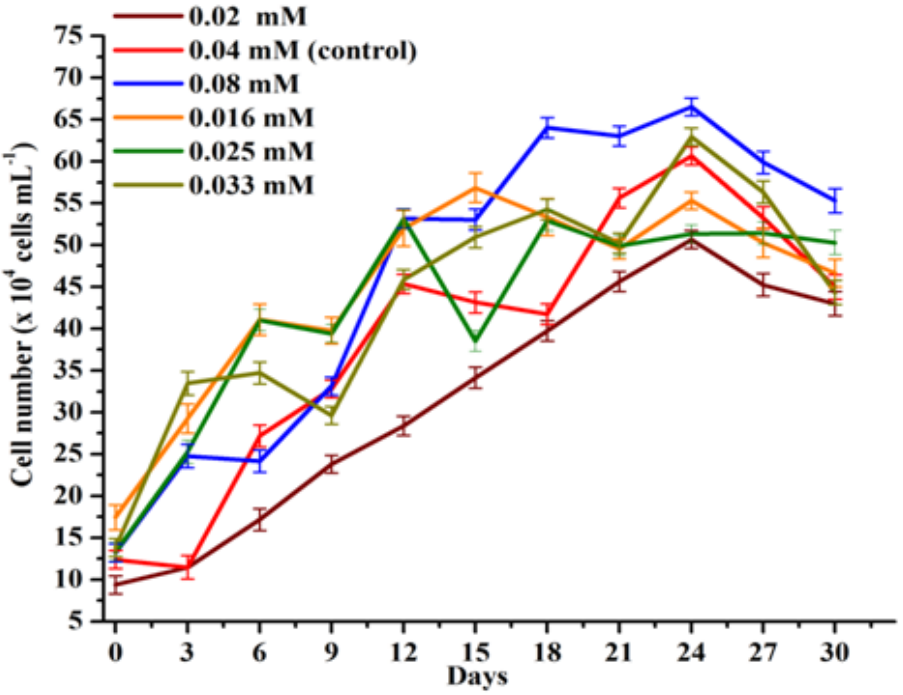

Figure 9: Effect of different concentrations of Sodium dihydrogen phosphate on cell number of *T. straiata* BBRR1 at different intervals.

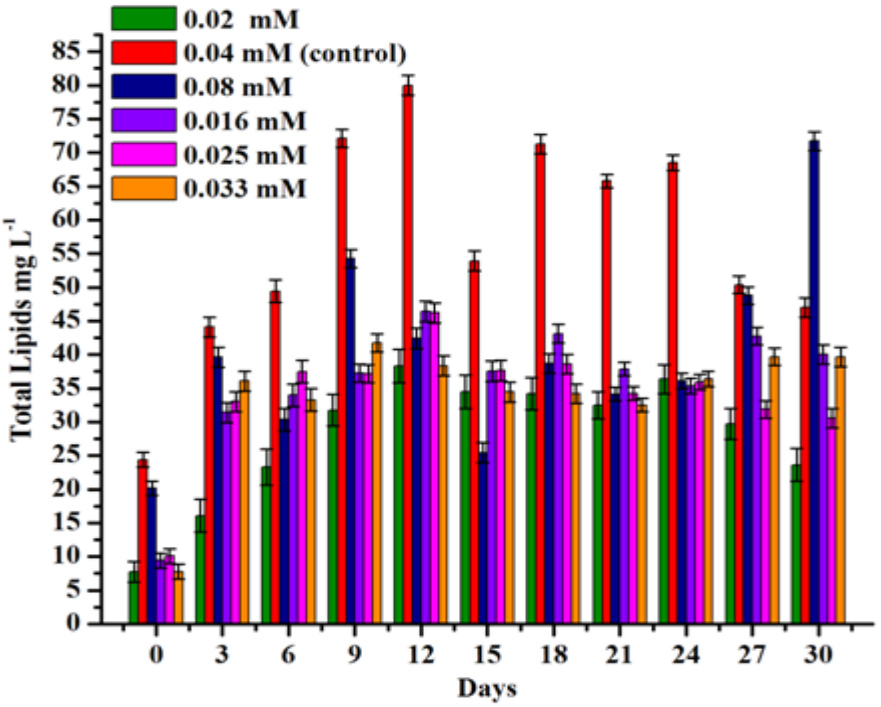

Figure 10: Effect of different concentrations of Sodium dihydrogen phosphate on total lipids of *T. straiata* BBRR1 at different intervals.

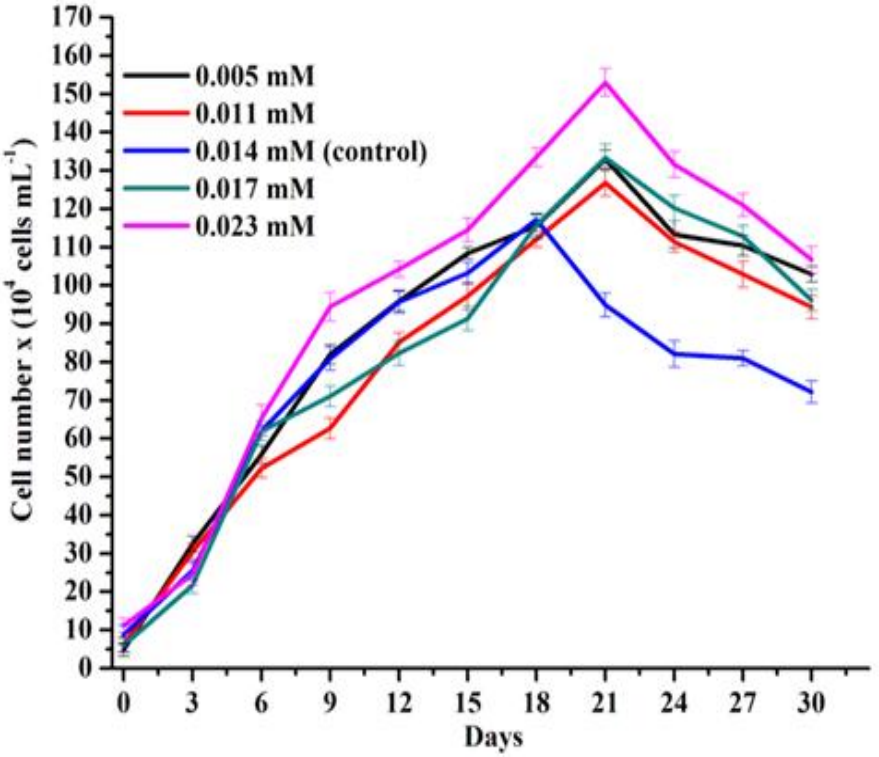

Figure 11: Effect of different concentrations of Fe - EDTA on cell number of *T. straiata* BBRR1 at Different intervals.

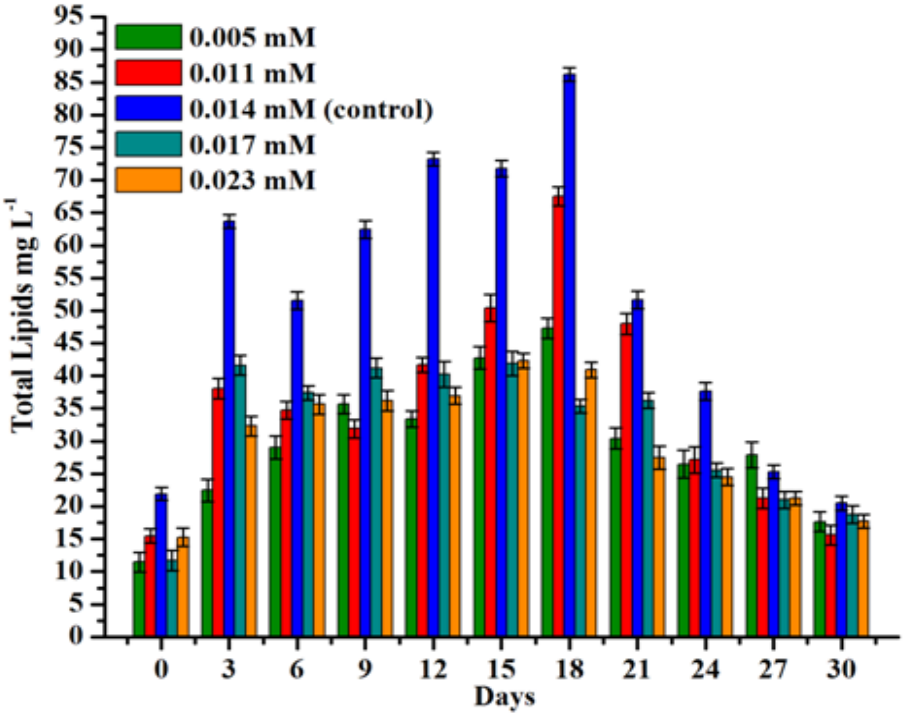

Figure 12: Effect of different concentrations of Fe - EDTA on total lipids of *T. straiata* BBRR1 at different intervals.

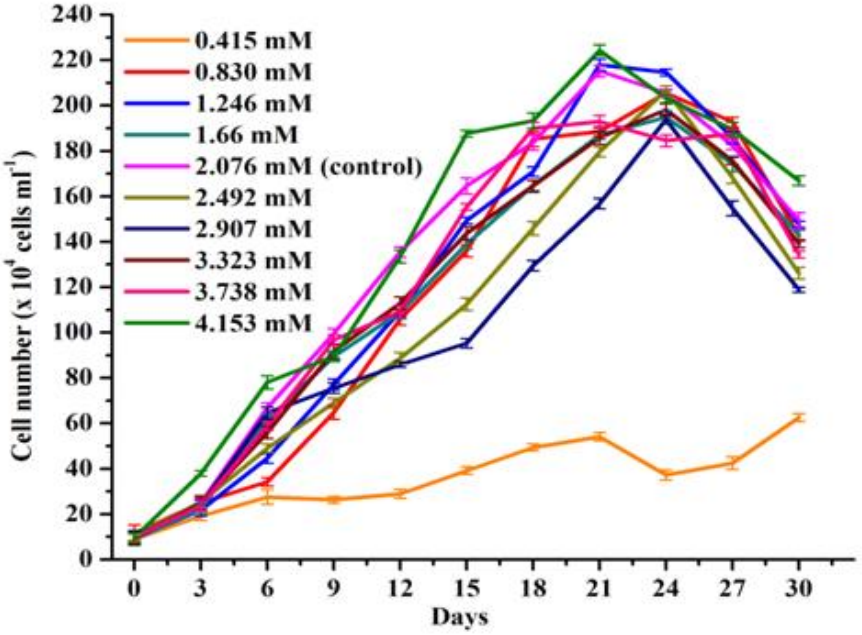

Figure 13: Effect of different concentrations of Magnesium sulphate on cell number of *T. straiata* BBRR1 at different intervals.

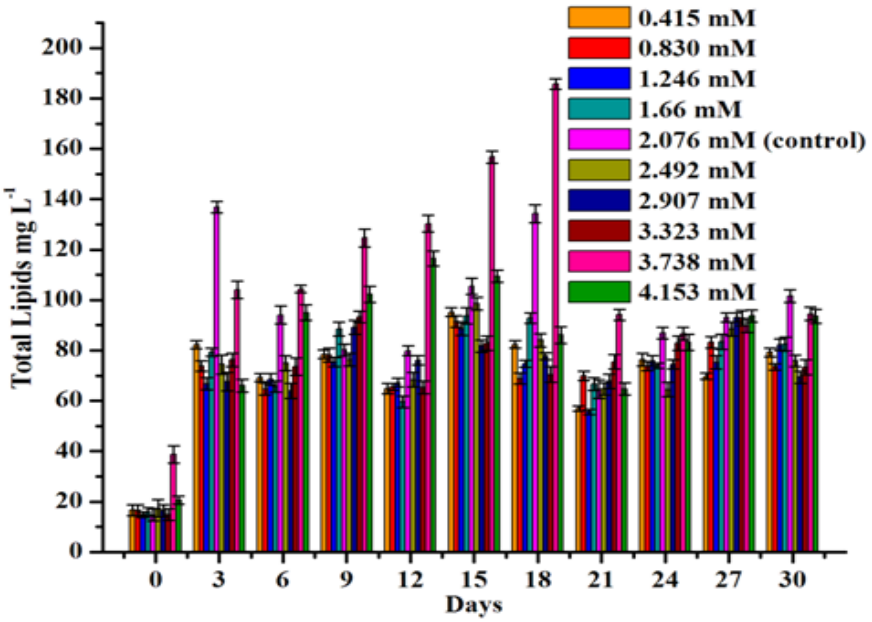

Figure 14: Effect of different concentrations of Magnesium sulphate on total lipids content of *T. straiata* BBRR1 at different intervals

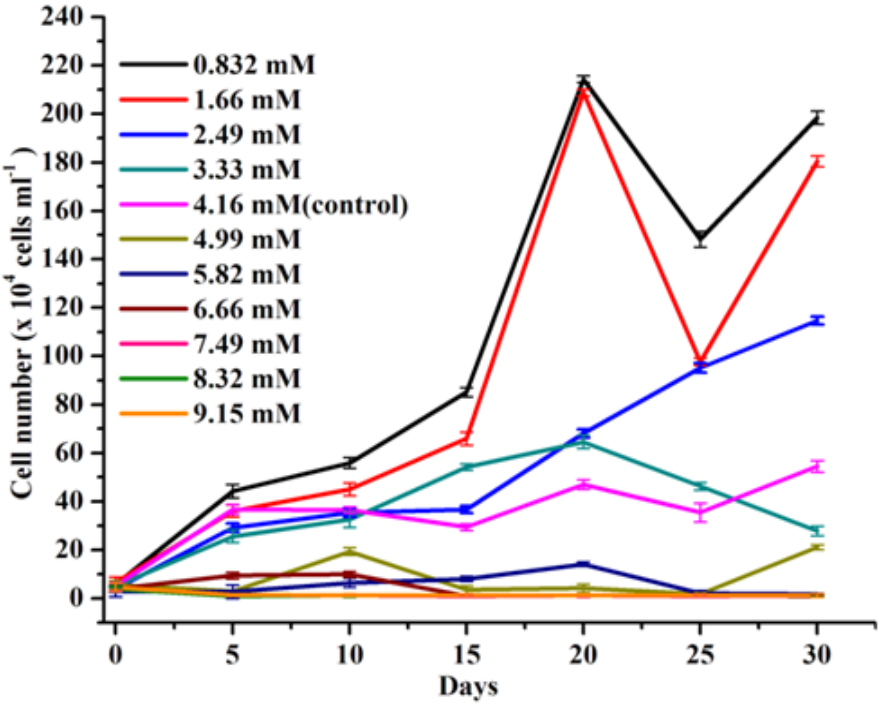

Figure 15: Effect of different concentrations of Urea on cell number of *T. straiata* BBRR1 at different intervals.

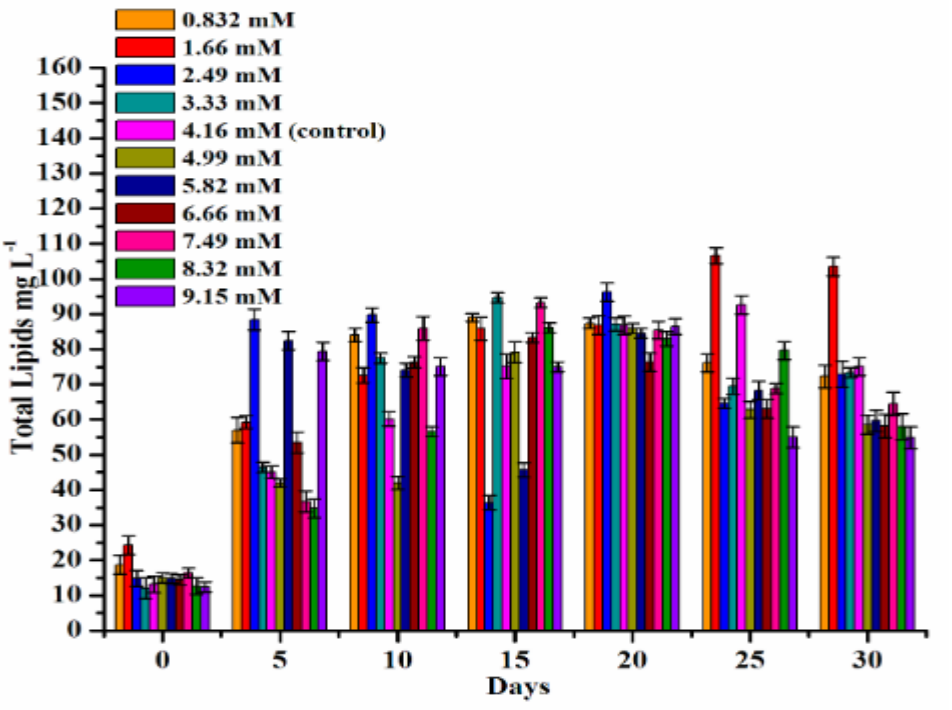

Figure 16: Effect of different concentrations of Urea on total lipids of T.straiata BBRR1 at different intervals.

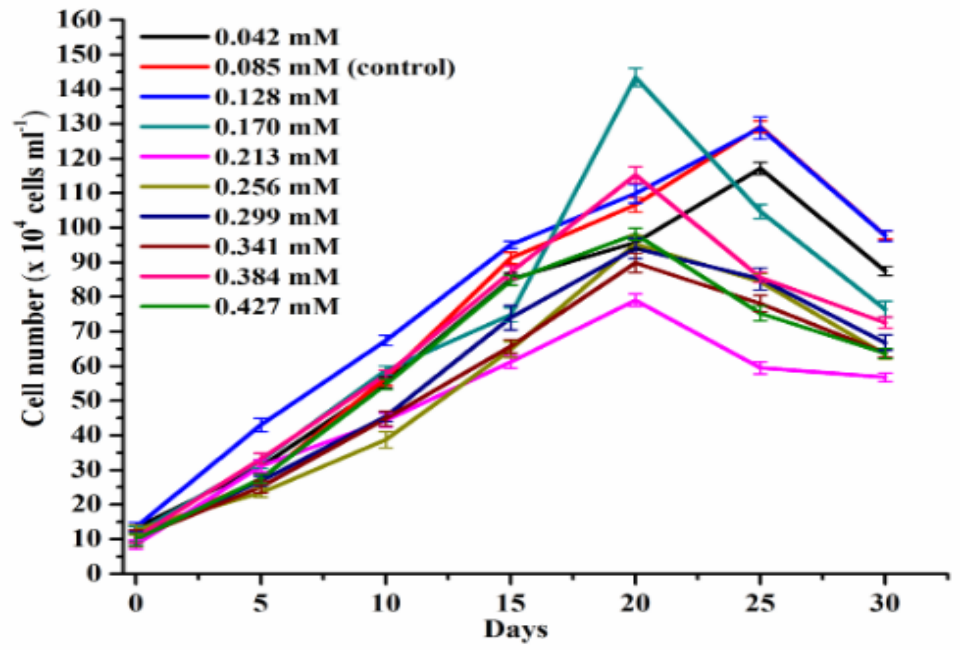

Figure 17: Effect of Different Concentrations of Super Phosphate on Cell number of T. straiata BBRR1 at Different intervals.

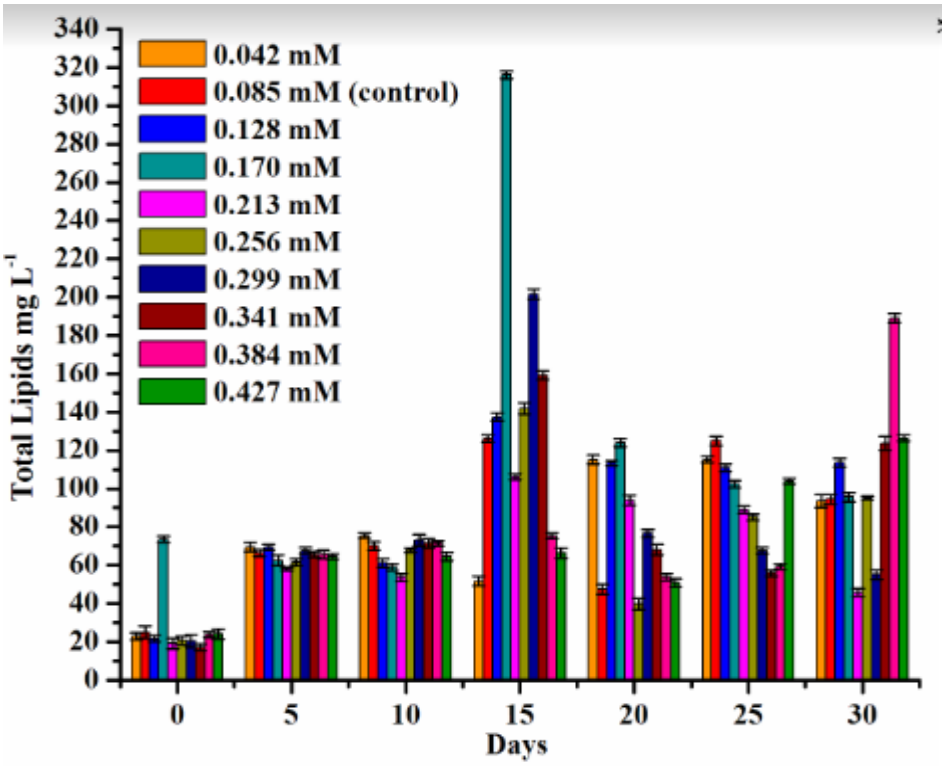

Figure 18: Effect of different concentrations of Super phosphate on total lipids of T. straiata BBRR1 at different intervals.

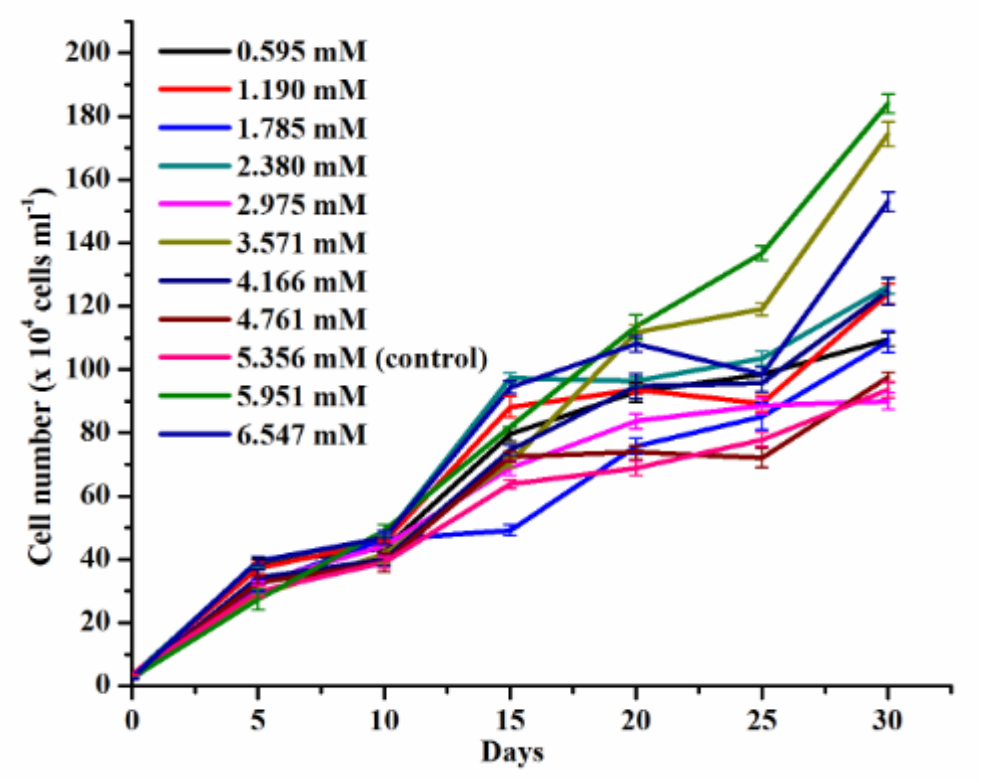

Figure 19: Effect of Different Concentrations of Sodium Bicarbonate on Cell number of T. straiata BBRR1 at Different intervals.

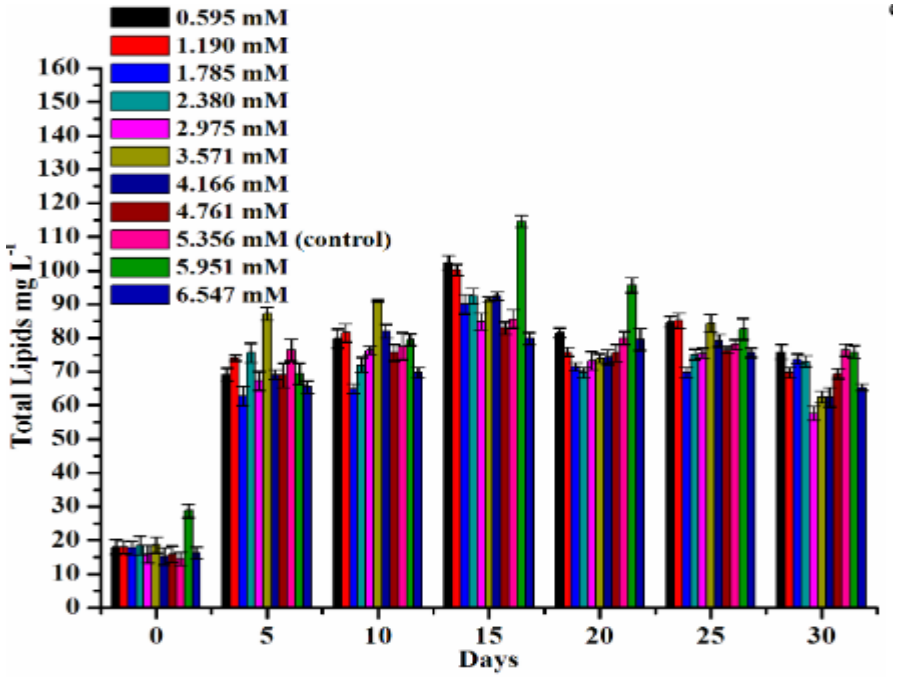

Figure 20: Effect of different concentrations of Sodium bicarbonate on total lipids of T. straiata BBRR1 at different intervals.

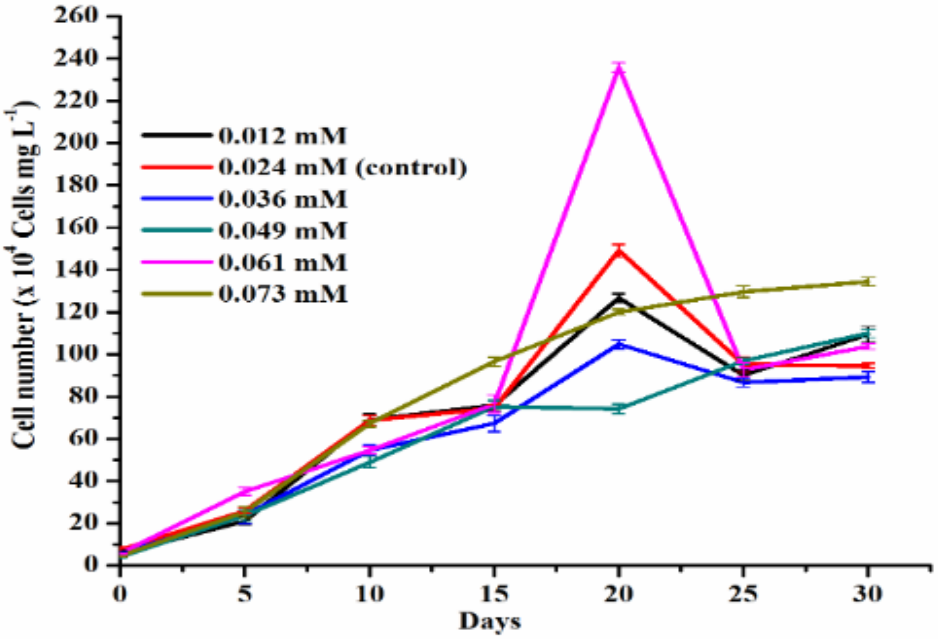

Figure 21: Effect of Different Concentrations of Ferric Chloride on Cell number of T. straiata BBRR1 at Different intervals.

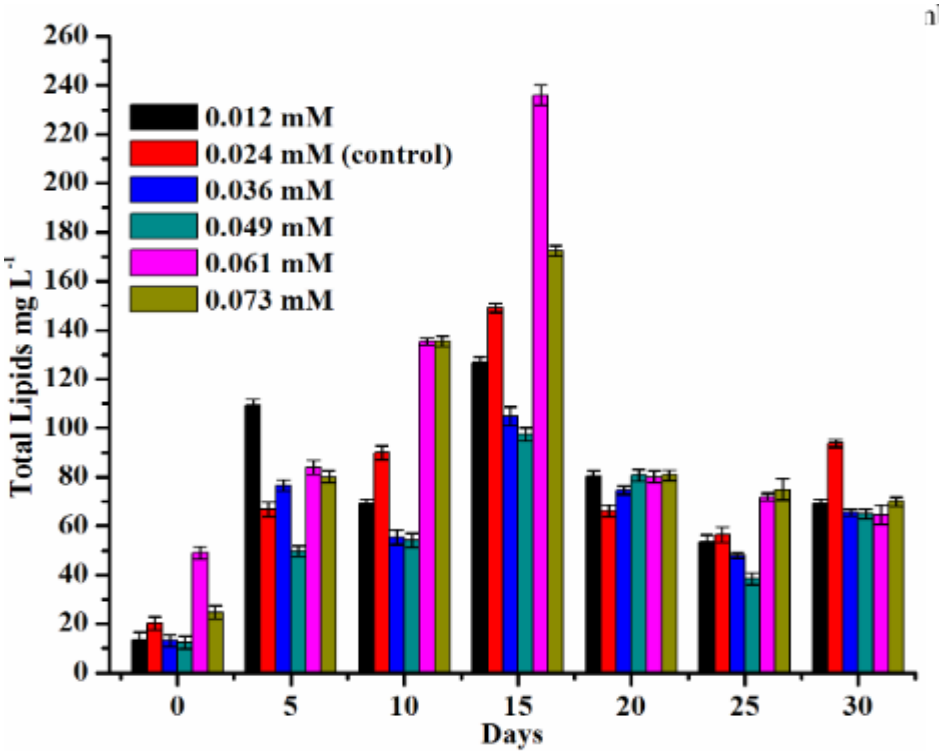

Figure 22: Effect of different concentrations of Ferric chloride on total lipids of T. straiata BBRR1 at different intervals.

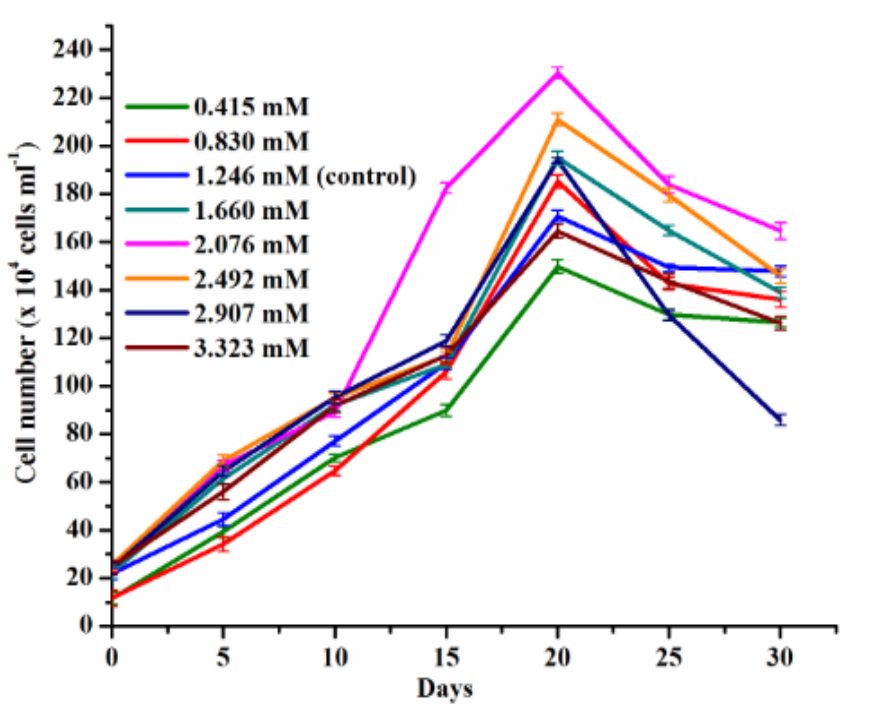

Figure 23: Effect of different concentrations of Magnesium sulphate on cell number of T. straiata BBRR1 at different intervals.

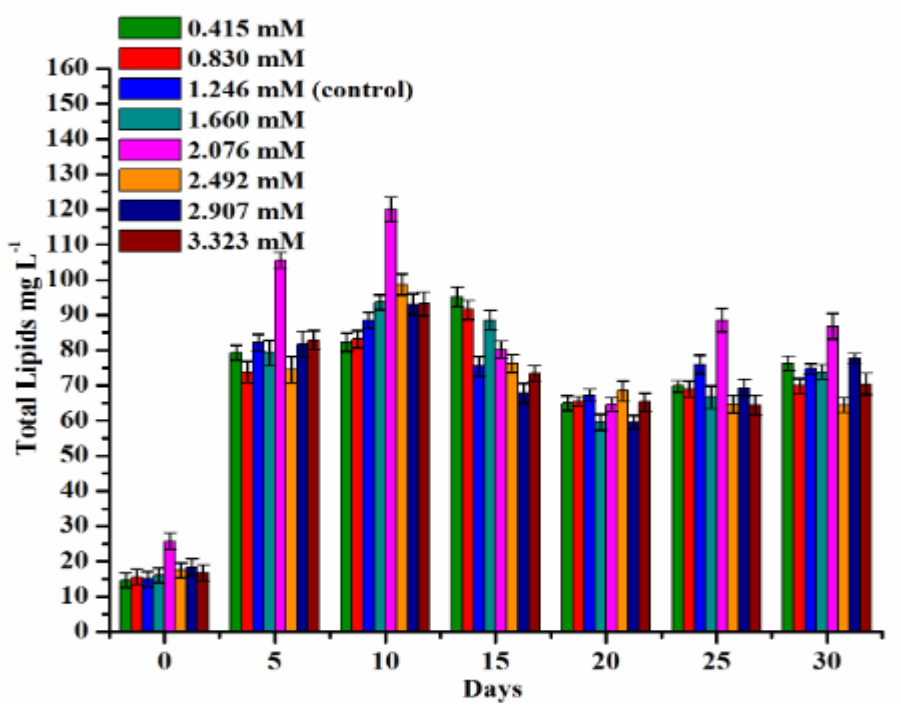

Figure 24: Effect of different concentrations of Magnesium sulphate on total lipids of T. straiata BBRR1 at different intervals.

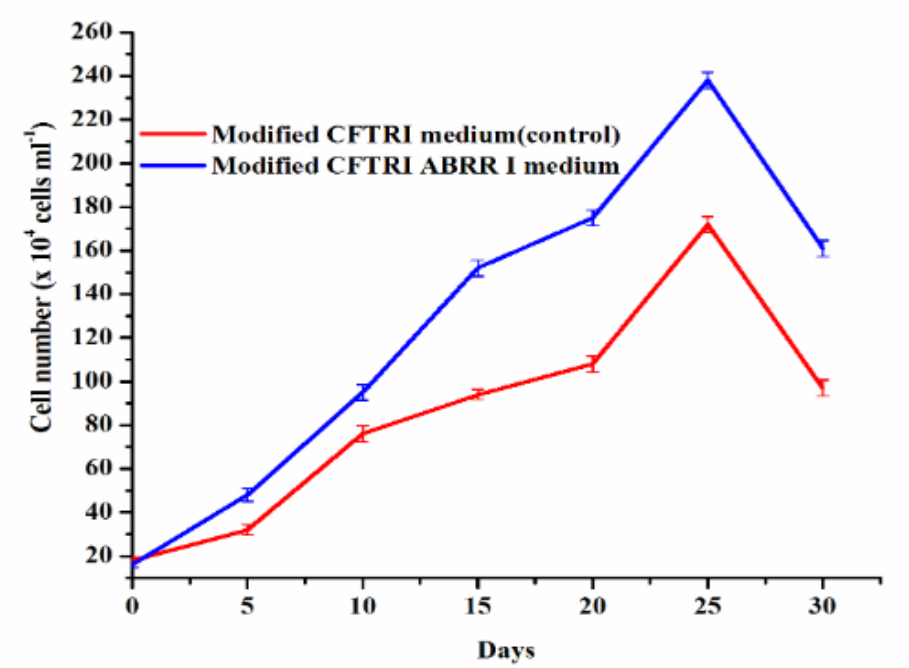

Figure 25: Effect of two different media on cell number of T. straiata BBRR1 at different intervals.

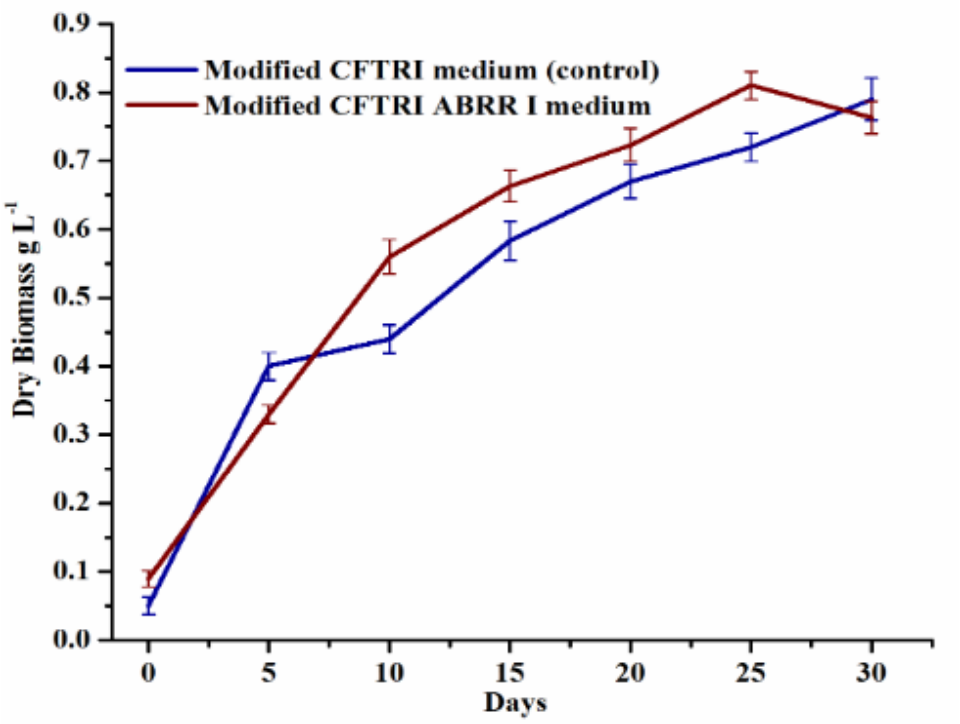

Figure 26: Effect of two different media on dry biomass of T. straiata BBRR1 at different intervals.

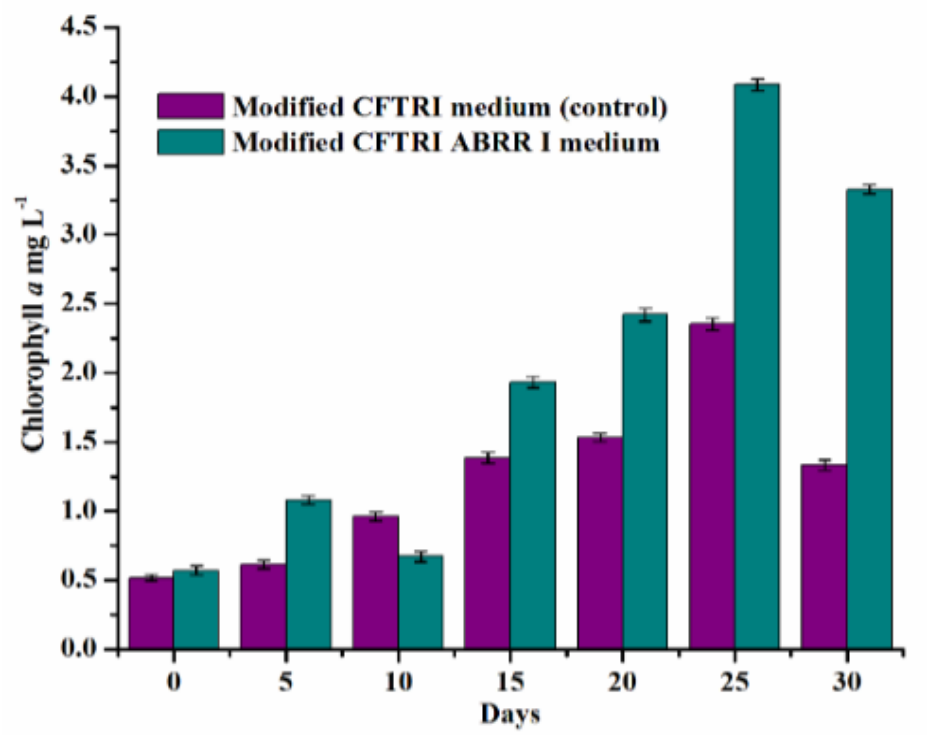

Figure 27: Effect of two different media on Chlorophyll a content of T. Straiata BBRR1 at different intervals.

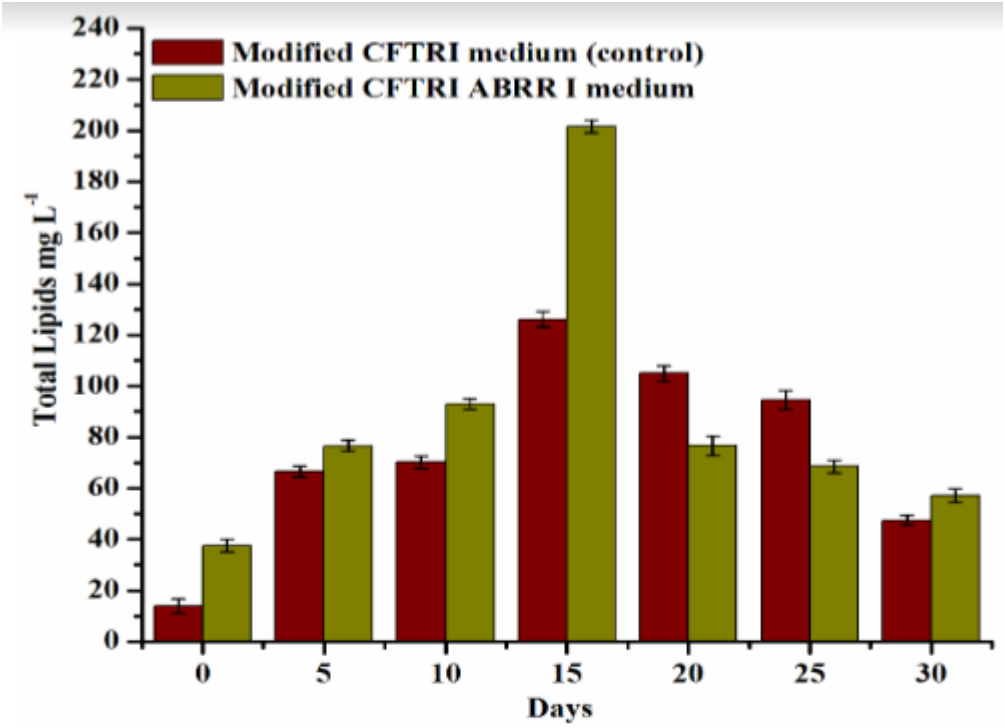

Figure 28: Effect of two different media on total lipids content of T. straiata BBRR1 at different intervals.

Table 1: Specific growth rate, Division rate and Generation time of different strengths of f/2 medium

Different strengths of f/2 medium	Growth rate (K')	Div. day^{-1}	Gen' t (d)
0.5 x (f/2 medium)	0.39	0.56	1.79
1.0 x (f medium)	0.41	0.59	1.69
5.0 x (5f medium)	0.16	0.24	4.25
10 x (10 f medium)	0.16	0.23	4.31

Table 2: Biomass, lipid productivity and percentage lipid content of different strengths of f/2 medium

Different strengths of f/2 medium	Biomass productivity (g L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})	% Lipid content / Dry biomass
0.5 x (f/2 medium)	0.022± 0.005	5.56 ± 0.004	25.07 ± 1.04
1.0 x (f medium)	0.027 ± 0.004	5.33 ± 0.006	19.32 ± 1.10
5.0 x (5 f medium)	0.019 ± 0.006	1.63 ± 0.003	8.50 ± 1.13
10 x (10 f medium)	0.003 ± 0.003	1.73 ± 0.002	56.54 ± 1.15

Table 3: Effect of different pH on Specific growth rate, Division per day, Generation time and Lipid Productivity of *Tetraselmis straiata* BBRR1

pH	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
5	0.35	0.50	2.01	7.8 ± 0.006
5.5	0.28	0.40	2.49	9.1 ± 0.008
6.0	0.28	0.40	2.49	5.1 ± 0.007
6.5	0.24	0.35	2.83	3.6 ± 0.005
7.0	0.29	0.42	2.36	8.8 ± 0.003
7.5	0.23	0.33	3.05	6.3 ± 0.004
8.0 (control)	0.19	0.28	3.58	6.5 ± 0.003
8.5	0.47	0.68	1.47	9.3 ± 0.003
9.0	0.32	0.46	2.15	8.1 ± 0.006
9.5	0.23	0.34	2.98	8.7 ± 0.002
10.0	0.21	0.31	3.22	8.1 ± 0.004

Table 4: Effect of different Salinities on Specific growth rate, Division per day, Generation time and Lipid Productivity of *Tetraselmis straiata* BBRR1

Salinity (ppt)	Specific Growth rate (K')	Div.day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
10	0.40	0.58	1.73	8.73 ± 0.007

20	0.51	0.73	1.37	9.15 ± 0.008
30 (control)	0.36	0.52	1.92	9.15 ± 0.004
35	0.57	0.82	1.22	15.37 ± 0.007
40	0.46	0.66	1.52	10.18 ± 0.006
50	0.35	0.50	1.99	5.20 ± 0.005
60	0.42	0.60	1.66	8.87 ± 0.008
70	0.43	0.62	1.61	6.72 ± 0.007
80	0.46	0.67	1.50	6.67 ± 0.007
90	0.24	0.34	2.93	5.92 ± 0.007

Table 5: Effect of different concentrations of $NaNO_3$ on Specific growth rate, Division per day, Generation time and Lipid Productivity of *Tetraselmis straiata* BBRR1

$NaNO_3$ (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.58	0.14	0.20	4.91	1.68 ± 0.011
0.88 (control)	0.15	0.22	4.52	3.11 ± 0.008
1.76	0.17	0.25	4.06	1.59 ± 0.010
3.53	0.17	0.24	4.14	1.30 ± 0.007
5.29	0.17	0.25	4.03	3.22 ± 0.006
7.05	0.24	0.34	2.95	1.37 ± 0.005

Table 6: Effect of different concentrations of NaH_2PO_4 on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

NaH_2PO_4 (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.02	0.25	0.35	2.83	3.19 ± 0.005
0.04(control)	0.29	0.42	2.36	6.67 ± 0.009
0.08	0.15	0.22	4.65	2.39 ± 0.010
0.016	0.22	0.31	3.21	3.87 ± 0.008
0.025	0.17	0.25	3.97	3.85 ± 0.009
0.033	0.16	0.23	4.42	4.64 ± 0.010

Table 7: Effect of different concentrations of Fe – EDTA on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

Fe – EDTA (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.005	0.27	0.39	2.54	2.63 ± 0.006
0.011	0.28	0.40	2.50	3.75 ± 0.005
0.014 (control)	0.38	0.55	1.81	4.79 ± 0.010
0.017	0.41	0.59	1.69	2.79 ± 0.009
0.023	0.41	0.60	1.67	2.82 ± 0.008

Table 8: Effect of different concentrations of $MgSO_4$ on Specific growth rate, Division

per day, Generation time and Lipid Productivity of *Tetraselmis straiata* BBRR1

$MgSO_4$ (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.415	0.14	0.20	5.08	6.35 ± 0.005
0.830	0.46	0.66	1.52	6.10 ± 0.004
1.246	0.66	0.96	1.05	5.91 ± 0.006
1.660	0.48	0.69	1.44	6.24 ± 0.005
2.076 (control)	0.58	0.83	1.20	7.46 ± 0.007
2.492	0.45	0.65	1.53	6.58 ± 0.005
2.907	0.45	0.64	1.56	3.88 ± 0.004
3.323	0.44	0.64	1.56	10.36 ± 0.006
3.738	0.54	0.78	1.28	10.32 ± 0.007
4.153	0.39	0.57	1.76	9.70 ± 0.004

Table 9: Effect of different concentrations of Urea on Specific growth rate, Division per day, Generation time and Lipid Productivity of *Tetraselmis straiata* BBRR1

Urea (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.832	0.32	0.47	2.14	5.93 ± 0.015
1.66	0.39	0.56	1.80	4.26 ± 0.045
2.49	0.16	0.23	4.41	4.81 ± 0018
3.33	0.17	0.24	4.12	6.32 ± 0.015
(Control) 4.16	0.07	0.11	9.35	3.70 ± 0.018
4.99	0.01	0.01	97.30	4.29 ± 0.015
5.82	0.01	0.02	53.46	4.23 ± 0.019
6.66	0.04	0.06	17.63	5.56 ± 0.023
7.49	0.04	0.06	15.75	6.21 ± 0.020
8.32	0.07	0.10	10.11	5.74 ± 0.018
9.15	0.04	0.06	16.44	4.32 ± 0.016

Table 10: Effect of different concentrations of Super phosphate on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

Super phosphate (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.042	0.25	0.36	2.75	5.76 ± 0.010
(Control) 0.085	0.32	0.45	2.20	8.41 ± 0.005
0.128	0.20	0.29	3.47	9.17 ± 0.015
0.170	0.44	0.63	1.58	21.08 ± 0.025
0.213	0.25	0.37	2.73	7.08 ± 0.010
0.256	0.41	0.59	1.71	9.45 ± 0.012
0.299	0.35	0.51	1.97	13.44 ± 0.015

0.341	0.36	0.51	1.94	10.61 ± 0.012
0.384	0.35	0.50	2.00	6.30 ± 0.023
0.427	0.36	0.51	1.94	4.21 ± 0.016

Table 11: Effect of different concentrations of Sodium bicarbonate on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

Sodium bicarbonate (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.595	0.14	0.20	4.90	6.82 ± 0.010
1.190	0.17	0.24	4.15	6.68 ± 0018
1.785	0.17	0.25	4.06	6.00 ± 0.008
2.380	0.16	0.23	4.31	6.16 ± 0.010
2.975	0.14	0.20	5.04	5.66 ± 0.015
3.571	0.31	0.44	2.26	6.10 ± 0.012
4.166	0.18	0.28	3.81	6.16 ± 0.017
4.761	0.15	0.22	4.65	5.53 ± 0.013
(Control)5.35 6	0.16	0.23	4.43	5.71 ± 0.015
5.951	0.34	0.49	2.06	7.64 ± 0.018
6.547	0.19	0.28	3.58	5.32 ± 0.010

Table 12: Effect of different concentrations of Ferric chloride on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T.straiata* BBRR1

Ferric chloride (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.012	0.40	0.58	1.73	8.45 ± 0.014
(Control)0.024	0.38	0.55	1.81	9.94 ± 0010
0.036	0.29	0.42	2.38	6.99 ± 0.006
0.049	0.23	0.33	2.99	6.50 ± 0.015
0.061	0.45	0.65	1.54	15.72 ± 0.016
0.073	0.27	0.38	2.61	11.49 ± 0.010

Table 13: Effect of different concentrations of Magnesium Sulphate on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

Magnesium Sulphate (mM)	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
0.415	0.19	0.27	3.64	6.35 ± 0.016
0.830	0.27	0.39	2.56	6.10 ± 0.010
(Control)1.246	0.19	0.28	3.62	8.86 ± 0.006
1.660	0.16	0.23	4.36	9.37 ± 0.013
2.076	0.17	0.25	4.03	12.00 ± 0.018
2.492	0.15	0.22	4.53	9.87 ± 0.014
2.907	0.15	0.22	4.62	9.31 ± 0.010

3.323	0.15	0.21	4.71	9.32 ± 0.015

Table 14: Effect of Modified CFTRI and Modified CFTRI ABRR I on Specific growth rate, Division per day, Generation time and Lipid Productivity of *T. straiata* BBRR1

Medium	Specific Growth rate (K')	Div. day^{-1}	Gen' t (d)	Lipid Productivity (mg L^{-1} d^{-1})
Modified CFTRI	0.38	0.55	1.81	8.41 ± 0.013
Modified CFTRI ABRR I	0.60	0.86	1.16	13.44 ± 0.015

REFERENCES

[1] Alsull, M. and Omar, W.M.W. 2012. Responses of *Tetraselmis* sp. and *Nannochloropsis* sp. isolated from Penang National Park coastal waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. In International Conference on Chemical, Ecology and Environmental Sciences (ICEES 2012).

[2] Anderson, D.M., Stock, C.A., Keafer, B.A., Nelson, A.B., Thompson, B., McGillicuddy, D.J., Keller, M., Matrai, P.A. and Martin, J. 2005. Alexandrium fundyense cyst dynamics in the Gulf of Maine. Deep-Sea Res., II-Top. Stud. Oceanogr. **52:**2522–2542.

[3] Arkronrat, W., Deemark, P. and Oniam, V. 2016. Growth performance and proximate composition of mixed cultures of marine microalgae (*Nannochloropsis* sp. and *Tetraselmis* sp.) with monocultures. Songklanakarin.J. Sci. Tech. **38:** 326-345.

[4] Arumugam, M., Agarwal, A., Arya, M.C. and Zakwan, A. 2013. Influence of nitrogen sources on biomass productivity of microalgae *Scenedesmus bijugatus.* Biores. Technol. **131:** 246–249.

[5] Aylott M. 2011. European EnAlgae partnership to unlock the potential of algal bioenergy. NNFCC. http://www.nnfcc.co.uk/news/energetic-algae-project-to-investigate-potential-of-algae-energyand-fuels. Last accessed in October 2014.

[6] Barakoni, R., Awal, S. and Christie, A. 2015. Growth performance of the marine microalgae Pavlova salina and *Dunaliella tertiolecta* using different commercially available fertilizers in natural seawater and inland saline ground water. J. Algal Biom Utln. **6:** 15-25.

[7] Bartley, R., Bainbridge, Z.T., Lewis, S.E., Kroon, F.J., Wilkinson, S.N., Brodie, J.E. and Silburn, D.M. 2014. Relating sediment impacts on coral reefs to watershed sources, processes and management: A review. Sci. Total Environ. **468:**1138^{-1} 153.

[8] Behrenfeld, M.J. and Boss, E. 2006. Beam attenuation and chlorophyll concentration as alternative optical indices of phytoplankton biomass. J. Mar. Res. **64:**431-451.

[9] Benemann, J. 1989. The Future of Microalgal Biotechnology. Algal and Cyanobacterial Biotechnology. Longman Scientific and Technical, Harlow, UK, 317 - 337.

[10] Botkin D.B., and Keller, E.A. 2007. Environmental science: Earth as a living planet (6th ed.). Hoboken, N.J. John Wiley and Sons.

[11] Brennan, L. and Owende,P. 2010. Biofuels from microalgae – A review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energ. Rev.**14:** 557-577.

[12] Chisti, Y. 2007. Biodiesel from microalgae. Biotechnol adv.**25:** 294-306.

[13] 1Concas, A., Steriti, A., Pisu, M. and Cao, G. 2014. Comprehensive modeling and investigation of the effect of iron on the growth rate and lipid accumulation of *Chlorella vulgaris* cultured in batch photobioreactors. Biores. Technol. **153:**340-350.

[14] Courchesne, N.M.D., Parisien, A., Wang, B. and Lan, C.Q. 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. J. of biotech.**141:**31-41.

[15] Danesi,1 E.D.G., Rangel-Yagui, C.D.O., De Carvalho, J.C.M. and Sato, S. 2002. An investigation of effect of replacing nitrate by urea in the growth and production of Chlorophyll by Spirulina platensis. Biom. Bioene.**23:** 261-269.

[16] Gaur, J.P. and Rai, L.C. 2001. Heavy metal tolerance in algae. In Algal adaptation to environmental stresses (pp. 363-388). Springer Berlin Heidelberg.

[17] Harben, P. and Theune, C. 2006. Nitrogen and nitrates. Industrial minerals and rocks: Commodities,

markets, and uses. 7th ed. Society for Mining, Metallurgy, and Exploration, Inc., Englewood, CO, pp.671-678.

[18] Hardie, L.P., Balkwill, D.L. and Stevens, S.E. 1983. Effects of iron starvation on the physiology of the cyanobacterium Agmenellum quadruplicatum. Appl. Environ.Microbial.**45:** 999^{-1} 006.

[19] Hu, G., Heitmann, J.A. and Rojas, O.J. 2008. Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. Bio Res.**3:**270-294.

[20] Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. J. Plant. **54:** 621-639.

[21] Huerlimann, R., De Nys, R. and Heimann, K. 2010. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotech. Bioeng.**107:**245-257.

[22] Jayappriyan, K.R., Rajkumar, R., Kannan, P.R., Divya, S. and Rengasamy, R. 2010. Significance of 18S rDNA specific primers in the identification of genus *Dunaliella*. J. Exp. Sci.**1:** 33-42.

[23] Juneja, A., Ceballos, R.M. and Murthy, G.S. 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. Energies, **6:**4607-4638.

[24] Khan, S.A., Rashmi Hussain, M.Z. Prasad, S. and Banerjee, U.C. 2009. Prospects of biodiesel production from microalgae in India. Renew. Sustain. Eneg. Rev., **13:** 2361–2372.

[25] Khatoon, H., Rahman, N.A., Banerjee, S., Harun, N., Suleiman, S.S., Zakaria, N.H., Lananan, F., Hamid, S.H.A. and Endut, A. 2014. Effects of different salinities and pH on the growth and proximate composition of *Nannochloropsis* sp. and *Tetraselmis* sp. isolated from South China Sea cultured under control and natural condition. Inter. Biodeter. Biodegrad.**95:**11^{-1} 8.

[26] Khozin-Goldberg, I. and Cohen, Z. 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water Eustigmatophyte Monodus subterraneus. Phytochemistry.**67:** 696-701.

[27] Kim, Z.H., Park, H., Hong, S.J., Lim, S.M. and Lee, C.G. 2016. Development of a floating photobioreactor with internal partitions for efficient utilization of ocean wave into improved mass transfer and algal culture mixing. Bioprocess and biosystems engineering, **39:** 13-23.

[28] Lam, M.K. and Lee, K.T. 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol. Adv. **30:** 673-690.

[29] Li, Q., Du, W. and Liu, D. 2008. Perspectives of microbial oils for biodiesel production. Appl. Microbial. Biotechnol. **80: 7**49-756.

[30] Lindseth, G. and Aall, C. 2004. Kommuner og klima. Envanskelig kombinasjon. – En sporreundersøkelse om klima- ogenergy planleggingi norske kommuner og fylkeskommuner, Rapport 04/04, Oslo: ProSus.

[31] Liu, Z.Y., Wang, G.C. and Zhou, B.C. 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Biores. Technol. **99:** 4717-4722.

[32] Moheimani, N.R. 2013. Inorganic carbon and pH effect on growth and lipid productivity of *Tetraselmis suecica* and *Chlorella* sp (Chlorophyta) grown outdoors in bag photobioreactors. J. Appl. Phycol, **25:**387-398.

[33] Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A. and Bux, F. 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. Biores. Technol.**102:**.57-70.

[34] Nianjun Xu, Xuecheng, Z., Xiao, F., Lijun, H. and Chengkui, Z. 2001. Effects of nitrogen source and concentration on growth rate and fatty acid composition of Ellipsoidion sp. (Eustigmatophyta). J. Appl. Phycol.**13:** 463-469.

[35] Pimolrat, P., Direkbusarakom, S., Chinajariyawong, C. and Powtongsook, S. 2010. The effect of sodium bicarbonate concentrations on growth and biochemical composition of Chaetoceros gracilis Schutt. Kasetsart University Fisheries Research Bulletin, **34:** 40-47.

[36] Purba, E. and Siburian, K. 2012. The determination of salinity and nutrition (NaH_2PO_4) profile in Nannochloropsis oculata cultivation to gain maximum lipid. Reaktor.**14:** 135^{-1} 42.

[37] Raja, A., Vipin, C. and Aiyappan, A. 2013. Biological importance of marine algae an overview. Int. J. Curr. Microbiol. App. Sci.**2:** 222-227.

[38] Reitan, K.I., Rainuzzo, J.R. and Olsen, Y. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycology. **30:**972-979.

[39] Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. **102:** 100^{-1} 12.

[40] Schwenk, R.W., Holloway, G.P., Luiken, J.J., Bonen, A. and Glatz, J.F. 2010. Fatty acid transport across the cell membrane: regulation by fatty acid transporters. Prostaglandins, Leukotrienes and Essential Fatty

Acids (PLEFA), **82:** 49^{-1} 54.
[41] Sharmin, T., Hasan, M., Md, C., Aftabuddin, S., Rahman, M.A. and Khan, M. 2016. Growth, Fatty Acid, and Lipid Composition of Marine Microalgae Skeletonema costatum Available in Bangladesh Coast: Consideration as Biodiesel Feedstock. J. Mar. Biol.**8:** 33-51.
[42] Sheehan, J., Camobreco, V., Duffield, J., Graboski, M. and Shapouri, H. 1998. Life cycle inventory of biodiesel and petroleum diesel for use in an urban bus. Final report (No. NREL/SR--580-24089). National Renewable Energy Lab., Golden, CO (US).
[43] Sheehan, J., Dunahay, T., Benemann, J. and Roessler, P. 1998. A look back at the US Department of Energy's aquatic species program: biodiesel from algae. Nat. Renew. Ene. Lab. 328.
[44] Stephens, E., Ross, I.L., King, Z., Mussgnug, J.H., Kruse, O., Posten, C., Borowitzka, M. and Hankamer, B. 2010. An economic and technical evaluation of microalgal biofuels. Nature Biotechnol. **28:** 126–128.
[45] Tan, A. 2016. Le Joy Luck Club. Leduc. s Éditions.
[46] U.S. Department of Energy. 2010. Alternative fuels and advanced vehicles data center. Retrieved from http://www.afdc.energy.gov/afdc/ fuels/ biodiesel_ what_is.html.
[47] U.S. Environmental Protection Agency. 2002. A comprehensive analysis of biodiesel impacts on exhaust emissions: Draft technical report. USEPA 420-P-02-001.Washington, D.C. U.S. Gov. Print. Office.
[48] Wang, B. and Lan, C. 2011. Optimizing the lipid production of the green alga *Neochloris oleoabundans* using Box-Behnken experimental design. Can. J. Chem. Eng.**89:** 932–939.
[49] Wang, B., Lan, C. and Horsman, M. 2012. Closed photobioreactors for production of microalgal biomasses. Biotechnol. Adv.**30:** 904 - 912.
[50] White, D.A. Pagarette, A., Rooks, P. and Ali.S.T.2013.The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. J. Appl. Phycol.**25:**153–165.
[51] Xu, L., Brilman, D.W.F., Withag, J.A.M., Brem, G. and Kersten, S. 2011. Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis. Biores. Technol. **102:**5113–5122.
[52] Yao, C.H., Jiang-Ning, Cao, X.P. and Xue, S. 2013. Salinity manipulation as an effective method for enhanced starch production in the marine microalga *Tetraselmis subcordiformis*. Biores. Technol. **146:** 663-671.
[53] Young, E., Beardall, J. and Giordano, M, 2001. Inorganic carbon acquisition by *Dunaliella tertiolecta* (Chlorophyta) involves external carbonic anhydrase and direct HCO_3 utilization insensitive to the anion exchange inhibitor DIDS. Eur. J. Phycol. **36:**81–88.
[54] Zheng, Y., Chi, Z., Lucker, B. and Chen, S. 2012. Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production. Biores. Technol. **103:**484–488.
[55] Zhi-Yuan., Guang-C, Wang, A and Bai-Cheng, Z.2008.Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Biores. Technol. **99:** 4717–4722.
[56] (www.mnre.gov.in annual report assessed on 21/02/2014).
[57] (www.petroleum.nic.in/pngstat.pdf assessed on 09/02/2014).

