International Research Journal of Management Science & Technology

ISSN 2250 – 1959(0nline)
2348 – 9367 (Print)

An Internationally Indexed Peer Reviewed & Refereed Journal

www.IRJMST.com
www.isarasolutions.com

Published by iSaRa Solutions

Effect of Nitrogen Supplementation on Biomass Productivity and Biochemical Composition in Microalgae Consortium

Vijaya K. Jha
Final Year Student, M.Sc. (Biotechnology),
Mewar Institute of Management,
CCS University, Meerut

Correspondence: 3/433, First Floor, Sector-3, Vasundhara, Ghaziabad
Email: vijayakjha@gmail.com

Abstract
Energy security is the most challenging task of 21st century. But this task needs to get addressed dynamically. There is a need of systematic investigation on potential sources of bio energy and enhancing their yield using scientific methods. This study aims at understanding the effect of Nitrogen supplementation on growth and biomass productivity of microalgae. The effect of Nitrogen supplementation on chlorophyll-a, lipid and carbohydrate content in microalgae consortium has been observed. The effect of two different nutrient media on biomass composition of microalgae consortium at optimized urea concentration has also been observed for 21 days in control, BG-11 and BBM media. The results indicate that at 0.1% Urea treatment the BBM media favours the micro-algal growth compared to the BG-11 media. But, in absence of Urea, the BG-11 media favours growth over BBM media at later stages. Highest value of Chlorophyll-a concentration was reported in T_1 (0.1% Urea) followed by Control and T_2 (0.5% Urea) on 21st day of cultures.

Key words: Nitrogen supplementation, microalgae consortium, BBM, BG-11, Biomass productivity

Background of the Study

Estimates of the World energy reserves show that coal will last another 218 years, oils 41 years, and natural gas 63 years (Agarwal, 2007; cited by Bindra *et al.*, 2017).Energy crisis and global warming caused as a result of rapid Industrialization have raised the need for environmental friendly solutions that will satisfy the current energy consumption (Pandey and Kumar, 2017). According to Bindra *et al.*, (2017), Since petro-diesel burning causes increase in GHG's emissions such as carbon monoxide, hydrocarbons and smoke like pollutants causing climate change and health problems, hence they are the largest contributors of greenhouse gases (GHG's) emission in the atmosphere.High oil (Banse *et al.*, 2008) and gas (Gao *et al.*, 2012) prices encouraged alternatives energy sources like biofuels (Banse *et al.*, 2008) that are sustainable alternatives to fossil fuels and a viable means for environmental (Alam *et al.*, 2015) and economic feasibility (Gao *et al.*, 2012) and sustainability (Alam *et al.*, 2014).

According to Kajikawa and Takeda (2008), Biomass and Bio-fuels have gained growing interest as sustainable and renewable energy. Biofuel is an excellent alternative to traditional fossils fuels, as they can be produced in bulk from abundant supplies of renewable biomass (Singh and

Singh, 2014). According to Pandey and Kumar (2017), there exists four generations of biofuels in which first generation biofuels are made of mostly edible sugar and starch components, second generation biofuels (advance biofuels) contains various type of biomass, third generation Biofuels composition comprises of algae and the fourth-generation biofuels composition comprises of oils from genetically advanced algae and microbes. Efforts have been made by the scientists to increase the photosynthetic efficiency, lipid contents, etc., in plants/microalgae/cyanobacteria coupled with developments in enzymatic processing's, product recovery, strain improvements.

The cell growth and chemical composition of microalgae are critically affected by nitrogen supplementation. This ultimately determines the biomass productivity of the microalgae (Kim, Mujtaba & Lee, 2016). The enhancement of lipid and carbohydrate content through nitrogen supplementation is, therefore, a challenging area of research. Like autotrophic plants, most of the species of microalgae are capable of utilizing nitrogen sources in different forms ranging from nitrates to ammonium and the organic sources like urea (Becker, 1994). The energy required for assimilation of nitrogen sources is a crucial determinant of preference. The ammonium is the easiest source to get assimilated into amino acids is the most preferred nitrogen source of the microalgae. (Li *et al*., 2013).

Further, the source of nitrogen is also determinants of the change of biochemical composition within a particular species of microalgae (Norici et al., 2014). This provides evidence to support the assumption that the varying nitrogen sources result in a change of biochemical composition of the microalgae. Therefore, selecting the appropriate nitrogen source for a particular algal species is very much linked to the productivity of the target component of the biomass. The larger the productivity of the lipid the better the production of biodiesel and the larger the productivity of carbohydrate content the better is the production of bioethanol.

Objectives of Study:

- To study the effect of Nitrogen supplementation on growth and biomass productivity
- To study the effect of Nitrogen supplementation on chlorophyll-a, lipid and carbohydrate content in microalgae consortium
- To compare the effect of two different nutrient media on biomass composition of microalgae consortium at optimized urea concentration

Review of Literature:

The world is looking for alternate sources of energy which is eco-friendly and sustainable as the non-renewable sources of energy are depleting fast. The biofuels have the best prospects of providing alternate to the fossil fuels and to provide solutions to the problem of energy security, high crude oil prices and global warming (Dragone *et al*., 2010). The source of biofuels being microbial cells which are abundant in amount could be the best alternative to sustain the energy requirements of the globe (Bindra *et al*., 2017).

The organic matters present on earth provide biofuels in all the three states solid, liquid and gas (Dragone *et al*., 2010). Drawing the conclusion on the similar line Dutta *et al*., (2014) see huge potential in biofuels to meet the energy needs of the world in coming future. The previous scholarships have theoretically come to a conclusion that one kilogram of feedstock mass can result

in equal amount of biodiesel through efficient processing (Mata *et al.*, 2010).The other advantage biofuels have is that they are also renewable and clean sources of energy (Pandey and Kumar, 2017).

Depending on the biomass source, the biofuels have been categorized into four generations (Blinova *et al.*, 2015). The 1st generation or Conventional biofuels were derived from edible crop plants. This raised a concern of food security as the biomass was harnessed from arable land. It accelerated the price of food and fodder worldwide due to increase in demand of edible crop plants for biofuel production.

The 2nd generation biofuel were evolved from non-edible crop plants to address the concerns like food security, biofuel productivity (Bindra *et al*., 2017; Pandey and Kumar, 2017). This is why these fuels were considered better than the 1st generation biofuels. However the mass production of 2nd generation fuel is still a challenge because of the higher processing costs (Brennan *et al.*, 2010; cited by Dragone *et al*., 2010).

The most concerning area is the enzymatic pre-treatment of the woody biomass into the fermentable sugars (Brennan *et al.*, 2010; cited by Dragone *et al*., 2010).

The 3rd generation biofuels comprise of the fuels based on microalgal & cyanobacterial biomass. Microalgae are unicellular (Idenyi *et al*., 2016; Alam *et al*., 2014), prokaryotic or eukaryotic, photosynthetic thallophytes (Brennan *et al.*, 2010; cited by Dragone *et al*., 2010). They are found in abundance in fresh and marine water (Dutta *et al*., 2014; Viesturs and Melece, 2014; Alam *et al*., 2014).

Microalgae exists individually, or in chains or groups (Idenyi *et al*., 2016). This biomass is contemporarily being considered as the most ideal source of biofuel because of its photosynthetic efficiency (Saifullah et al, 2014; Li et al, 2008; Sibi *et al*., 2014), growth rate (Blinova *et al.*, 2015), high biomass productivity (Viesturs and Melece, 2014) (). According to Xin *et al.*, (2010). Microalgae are the promising alternative over fossil fuels in near future. Algae holds significant advantage over other oilseed crops because it does not require arable land it is more efficient in term of oil per acre.

The 4th generation biofuels are results of research and development advancements in Biotechnology and metabolic engineering. It is assumed that by the year 2050 the 4th generation biofuels will have major stake in the power sector (Pandey and Kumar, 2017).

Algae being photosynthetic have ability to fix atmospheric CO_2 into sugars, which are converted into fuel after biochemical processing (Blinova *et al.*, 2015). In microalgae, fatty acids convert into glucose, through glyoxylate pathway that bypasses Krebs cycle (Gonzalez-Fernandez and Ballesteros, 2012).

Microalgae are the promising candidates for commercial biodiesel production due to high productivity, lipid content (Hamed and Klock, 2014) and considered as a sustainable biodiesel feedstock (Chen *et al*., 2011; Saifullah *et al*., 2014). Biodiesel are obtained from bio-oils through trans-esterification process and considered as an alternative over Standard diesel fuel (Nigam *et al.*, 2011).Microalgal based carbohydrates are mainly consist of starch and lignin free cellulose, thus their saccharification process (conv. of Starch & other polysaccharides into fermentable sugars through microbial fermentation to produce ethanol, Wang *et al.*, 2011; cited by Ho *et al.*, 2013) doesn't require any energy intensive chemical pre-treatments (Ho *et al.*, 2013). Apart from that,

presence of hexose in microalgal carbohydrates eliminates further limitation as in pentose fermentation for bioethanol production (Ho *et al.,* 2013).

Microalgae biomass apart from energy source, offers various environmental benefits like Co_2 fixation & Carbon sequestration (reducing GHGs emission), phycoremediation, etc. Microalgae have higher photosynthetic efficiency, biomass production (Sibi *et al*., 2014; Li *et al.,* 2008), lipid productivity (Sharma *et al*., 2016) and reported to produce 30 times more energy than non-edible energy crops like Jatropha (Pandey and Kumar, 2017). A/c to Dragone *et al.,* (2010), microalgae are able to produce 15-300 times more oil than conventional crops (on an area basis) for biodiesel production. Apart from that, microalgae don't require arable land, requires less water for cultivation and can grow in wastewater. Studies that optimize overall carbohydrate and lipid content must be undertaken. High lipid and carbohydrate accumulation under nitrogen starvation results into low biomass productivity.

Therefore efforts must be made to ensure highest lipid productivity with highest lipid cell content as suggested by Li *et al.,*(2008). Bioethanol is a low value product, thus (Ho *et al.,* 2013) emphasized on the studies that yield best combination of carbohydrate and biomass production. In addition to this, further investigations to study the factors that affect carbohydrate profile (such as nutrient starvation, light intensity, temperature, inorganic carbon source, sodium chloride concentration, etc., Gonzalez-Fernandez and Ballesteros, 2012) must be undertaken.

Optimization of microalgal cell composition under controlled environmental conditions is an important factor for microalgal cultivation (Gonzalez-Fernandez and Ballesteros, 2012). It is important to note that all sugar forms are not fermentable, thus biomass characterization for carbohydrate is crucial for efficient bioethanol production (Gonzalez-Fernandez and Ballesteros, 2012).

Given plenty of sunlight, microalgae can photosynthesize enough organic matter, from carbon dioxide (CO2) and organic nutrients present in the water they are suspended in, to double their mass several times a day. Depending on the species, up to half their mass is made up of lipids – natural oils. These can be extracted and used as straight algal 'crude', or refined to higher-grade hydrocarbon products ranging from biodiesel to biojet fuel for aircraft. Strains of algae that produce more carbohydrate than oil can be fermented to make bioethanol and biobutanol.

Microalgal growth and biomass composition vary with media composition and cultivation conditions (Yeh and Chang, 2012). In a study on microalgal lipid production using different growth media, highest lipid productivity (mg/l/d) reported in Basal media followed by MBL and Modified Bristol media under phototrophic (CO_2) conditions. However, in comparison to different culture conditions i.e. Phototrophic (CO_2), Photoheterotrophic and Mixotrophic, the highest lipid productivity was recorded in Modified Bristol media (Mixotrophic), followed by Modified Bristol media (Photoheterotrophic) (Yeh and Chang (2012).

This review of literature provides the important insight that Algae biodiesel is a good replacement for standard crop biodiesels like soy and canola and up to 70% of algae biomass is usable oils. Algae does not compete for land and space with other agricultural crops and it can survive in water of high salt content and use water that was previously deemed unusable. Algae is a very efficient means of producing biodiesel and the oil production from algae farms is feasible and scalable. And, further research is necessary to unlock full potential of algae.

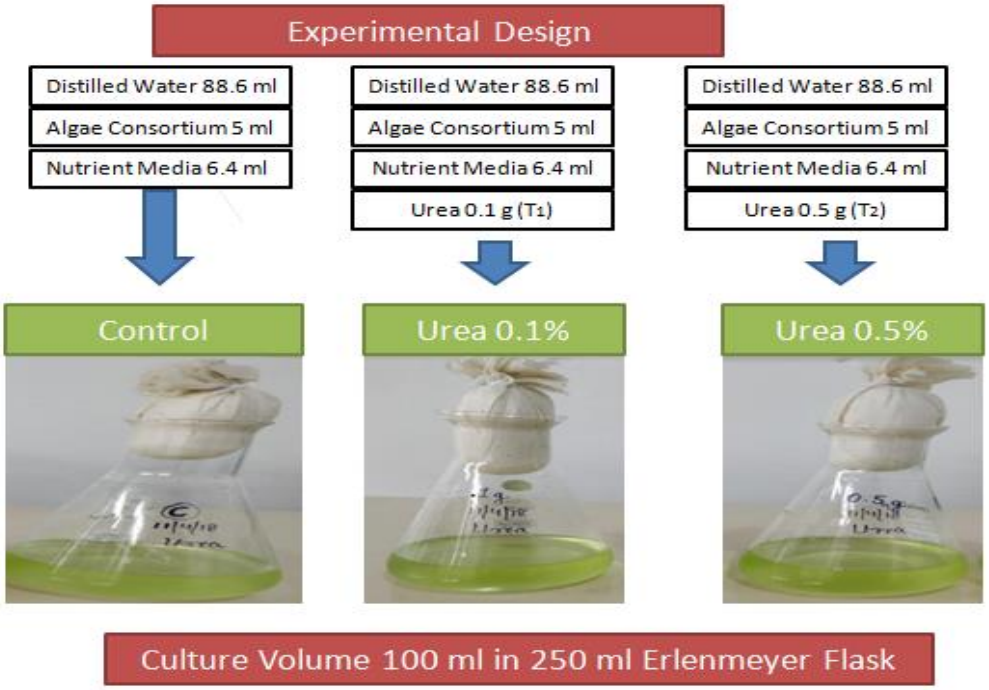
Characteristics of Algae biofuel

Algae biofuels contain no sulphur, are non-toxic and are biodegradable. A number of algal strains produce fuel with energy densities comparable to those of conventional fossil fuels. They are made from a renewable resource that is carbon neutral: the emissions that result from burning the fuel are balanced by the absorption of CO2 by the growing organisms. The microscopic algae can be harvested every 24 hours and can be grown in vertical towers which occupy one square meter of surface area. The Algae grown in just one of these towers can produce the energy equivalent to a 1000 square meter sunflower plantation. Extracting oils from the dried algae can be as simple as forcing them out with mechanical presses. Alternatively, cell membranes can be broken down with enzymes and chemicals, the oil then being extracted by dissolving it in a solvent – typically hexane, or even water where appropriate algal strains and membrane-eating enzymes are used. Other possible solvents include benzene, ether and CO2 liquefied under pressure. Alternatively, solvent use can be avoided with methods such as centrifuging and flocculation.

Algal fuel development

The production of microalgae biomass shows wide valuable uses, in the aquaculture, biotechnology, and food science, among others. However, microalgae show fluctuations in their chemical profile generated mainly by the culture conditions. Algal fuel development is at an early stage – and wide open to innovation. Although much basic research and development work is still being done by the government departments and the academia, commercial drive is evident in the way the pace of innovation is accelerating. New companies seem to enter the field on a weekly basis, bringing with them radical ideas for enhancing productivity, reducing cost and increasing scale. One strand of development is aimed at improving yield by selecting appropriate algal strains and in some cases modifying them by selective breeding or genetic manipulation.

Materials and Methodology:



All the chemicals/reagents used in the present study were of analytical grade. Distilled water was used to prepare microalgal nutrient media.

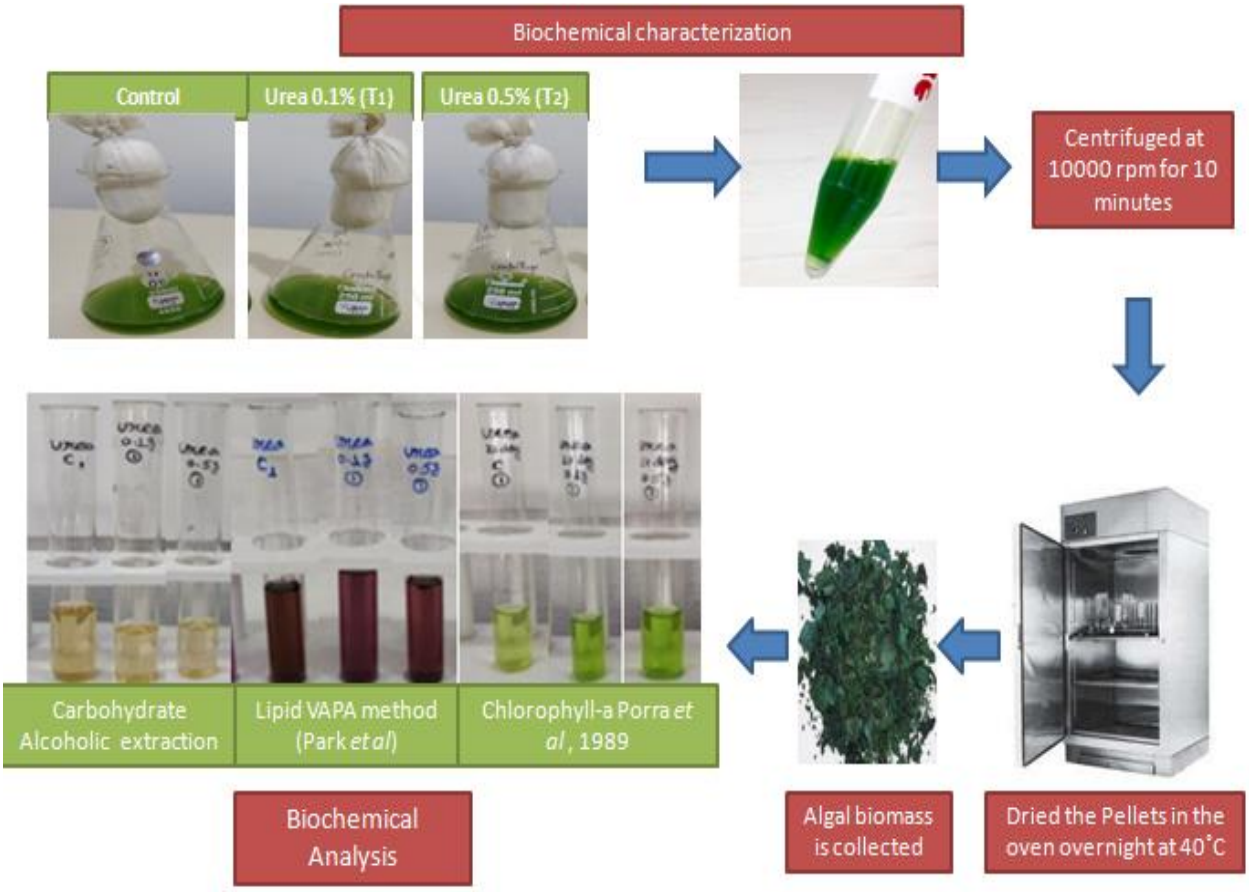


Fig 3.1: Schematic representation of Study design.

Procurement and Maintenance of cultures:

Microalgae culture (Consortia of *Chlorella sp.*, *Scenedesmus sp.*, and *Cosmarium*sp.) was procured from departmental Algal Biotechnology laboratory. Microalgae culture was maintained under fed batch system in 250 ml volumetric Erlenmeyer flasks (working volume 100 ml) under aseptic (Shaker *et al*., 2017; Kirrolia*et al*., 2011) and controlled conditions on a modified synthetic BBM medium (Nichols and Bold, 1965) (composition per liter as follows: $NaNO_3$: 0.25g, KH_2PO_4: 0.175g, H_3BO_3: 0.115g, K_2HPO_4: 0.075g, $MgSO_4.7H_2O$: 0.075g, KOH: 0.031g, $CaCl_2.2H_2O$: 0.025g, NaCl: 0.025g, Na_2EDTA: 0.01g, $FeSO_4.7H_2O$: 0.0049g, $MnCl_2.4H_2O$: 1.81mg, $NaMoO_4.5H_2O$: 0.390mg, $ZnSO_4.7H_2O$: 0.222mg, $CuSO_4.5H_2O$: 0.079mg, $CoCl_2.6H_2O$: 0.0404mg; maintained at pH 6.8) under 16/8-hr light/dark cycle and 3000 lux intensity at 25± 1 ˚C temperature (Shaker *et al*., 2017). The flasks containing microalgal cultures were closed with cotton plugs (allowing air diffusion) and allowed to shaken thrice a day on a rotatory shaker at 150 rpm (Rai*et al.*, 2015) to provide proper aeration and prevent adherence of the algal cells at the bottom of the flasks.

Optimization of Culture Conditions:

Microalgae were cultivated under 0.1% Urea (T_1), 0.5% Urea (T_2) and 1% Urea (T_3) for 21 days. Microalgae culture without Urea was served as control. Microalgae inoculation ratio was fixed at 1:20 (v/v). Further maintenance has been carried out as mentioned in section 3.2.

Estimation of Microalgal growth through Growth Curve:

Microalgal growth was observed upto 21 days (Agwa *et al*., 2016) by measuring absorbance of microalgal culture (3ml Volume) at 680 nm (Dhup*et al*., 2016). The graph between OD vs no. of days was plotted to obtained charactersic growth curve.

Determination of Biomass productivity:

Biomass productivity (mg/ml/day) from Microalgal cultures were determined as per Hempel *et al*., (2012) with slight modifications. Aliquot of 14 ml algal suspension was centrifuged at 10,000 for 5 min, dried at 40°C for 24 h to a constant weight and allowed to cool at room temperature and weighed.

Estimation of Lipid

Total Lipid content has been determined as per Park *et al.*, (2015). 5ml of Chloroform followed by equal volume of Sulphuric acid was added to the 0.005g dry microalgae sample. The tube was covered with aluminium foil and incubated in water bath at 100^0Cfor 10 minutes. The sample in a test tube was allowed to cool at room temperature followed by addition of 25 ml Vanillin-Phosphoric acid reagent (for 100 ml VA-PA reagent, add 0.12 gm Vanillin to 20 ml warm water and add 68 ml of 85% Phosphoric acid to 12 ml distilled water separately and mixed both the preparations). The resulting sample was allowed to stand for 2 hours at room temperature and the absorbance was recorded at 528 nm using UV-Vis Spectrophotometer (Elico-150 model).

Estimation of Carbohydrate

Total carbohydrate content from microalgae was determined as per Dubois *et al*., (1956) with slight modifications. 5ml of 70% ethanol was added to 0.05g of microalgae dry sample. The resulting sample was heated at 80^0C for 2 hours to evaporate the ethanol. Followed to this, ethanol was further added till 5ml mark. The sample was turned into dark green colour. The supernatant was collected separately and the precipitate obtained was again extracted with 5ml 70% ethanol. The obtained supernatant was mixed with previously obtained supernatant and make up the volume up to 100 ml with distilled water. The alcoholic extract was filtered with filter paper.

0.2 ml of alcoholic microlagal extract was mixed with 0.8ml of distilled water in a boiling tube. Followed to this, 5ml of 96% sulphuric acid was added and resulting sample was vortexed. Then, 1 ml phenol (5% w/v) was added, vortexed again and warmed at 30^0C temperature for 15-20 minutes. Orange-yellow colour was appeared. The resulting sample was allowed to stand for 5 min at room temperature and the intensity of Orange-yellow colour was recorded at 490 nm wavelength using UV-Vis Spectrophotometer (Elico-150 model).

Estimation of Chlorophyll-a content:

Estimation of Chlorophyll-a content from fresh microalgal suspension was done according to the method described by Porra *et al*., (1989) with slight modifications.5ml of microalgal suspension was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the pellet was suspended in 5ml methanol followed by heating at 60ºC in water bath for 30 minutes to ensure the complete extraction. The microalgal extract was allowed to cool and make up the final volume 5ml with methanol and centrifuged again. The absorbance of the supernatant obtained was measured at 652 nm and 665 nm with UV-visible spectrophotometer (Elico-150) using methanol as blank. Chlorophyll-*a* content was calculated using the following formula:

Results and Discussion:

$$\text{Chlorophyll a } (\mu g/ml) = 16.29 \times O.D._{665} - 8.54 \times$$

Effect of Urea on Growth and Biomass productivity:

Nitrogen concentration and appropriate medium are the important inputs for the microalgal growth (green algae) for producing value added products (like lipids), as reported by Yeh and Chang

(2012) in phototrophic cultivation of *C. vulgaris* (ESP-31 strain). Media composition and Nitrogen availability has prominent effect on microalgal growth and their biochemical composition. In the present investigation, microalgal growth has been carried out in BBM media supplemented with Urea as a Nitrogen source. The obtained results were compared with the microalgal cultures grown under BG-11 media at optimized Urea concentration.

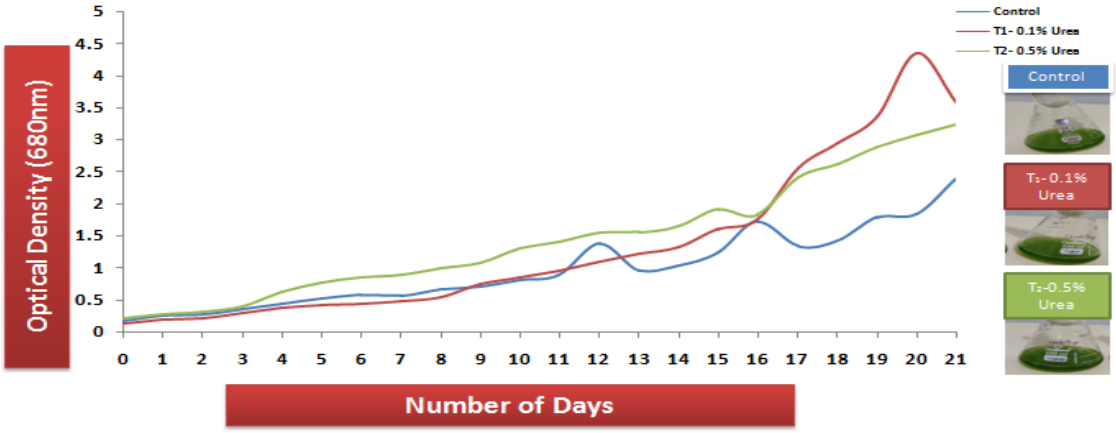


Fig. 4.1: Optical Density of Microalgal cultures cultivated at variable concentrations of Urea

Effect of Urea treatment on Growth, Biomass productivity and chlorophyll content has been discussed in the present section.The initial urea concentrations were adjusted to Control (without Urea), 0.1% (T_1) and 0.5% (T_2). Observing the trend of microalgal growth, there was not much significant variation in the growth up to three days in all the cultures. Thus upto three days, nitrogen is not a limiting factor for microalgal growth. Followed to this, maximum growth was reported in T_2 (0.5% Urea) followed by T_1 (0.1% Urea) as observed on 21st day **(Fig. 4.1)**.

From 3 to 9 days, higher growth was observed in Control as compared to T_1, followed to this a characteristic increase in growth was recorded in $T_1$and after 13th day, higher OD_{680} values was recorded in T_1 as compared to control cultures and from 16th day onwards, there was an exponential increase in growth of T_1 till 20th day. Comparing the periodic trend of microalgal growth in T_1 and T_2, it was observed that higher Urea concentrations limit microalgal growth after 16th day.

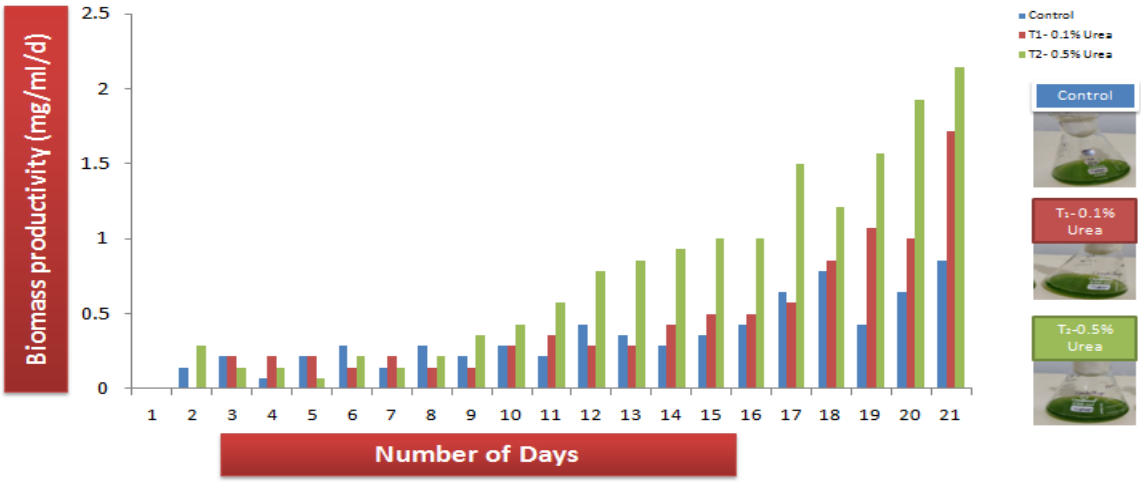


Fig. 4.2: Biomass Productivity (mg/ml/d) of Microalgal cultures cultivated at variable concentrations of Urea

Lower Urea concentrations favors microalgal growth, as observed by Arumugam *et al.*, (2014) in *Scenedesmus bijugatus*. In the present investigation, biomass productivity increases with Urea Concentration **(Fig 4.2)**.

Arumugam *et al.*, (2014) observed increase in biomass growth (DW g/l) of S. *bijugatus*) with increase in Urea concentration from 5 mM to 10 mM. On comparing the microalgal growth at 0.1% Urea treatment in BBM and BG-11 medium, it was clearly observed that BBM media favors microalgal growth **(Fig. 4.3)**.

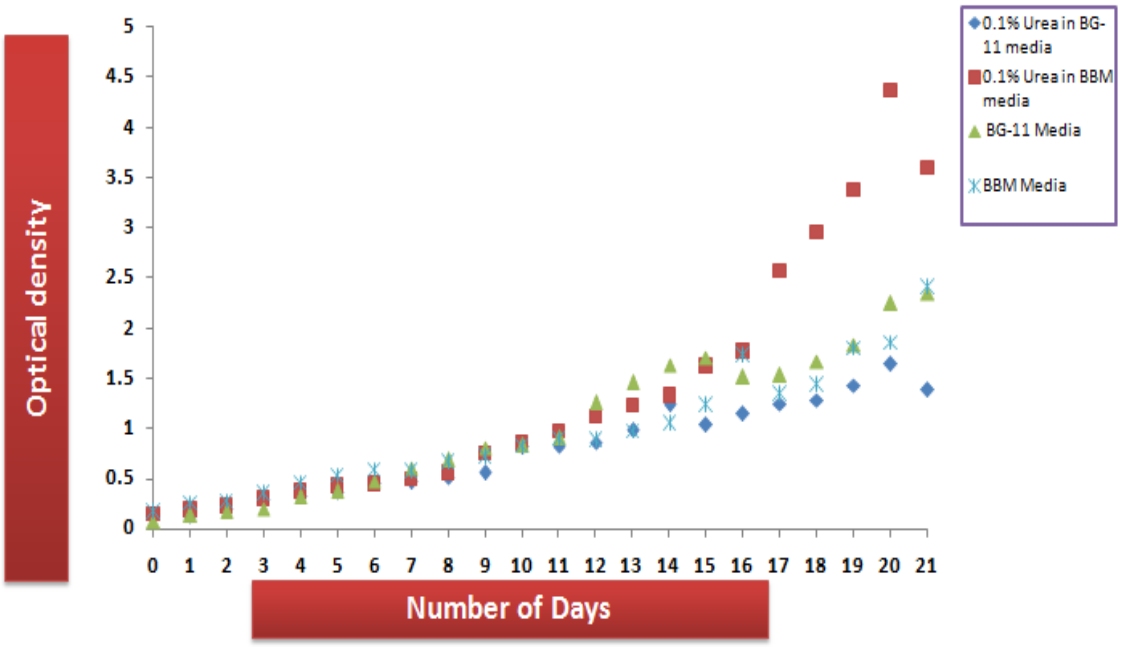


Fig. 4.3: Comparison of media composition in Microalgae consortium

Up to 8th day, there was not much significant variation in growth pattern in both the medium cultures. Followed to that, microalgal cultures in BBM media showed sharp increase in growth as compared to BG-11 media. However, on comparing the growth of control cultures in both the media (i.e. without Urea treatment) the BG-11 media favours growth over BBM media at later stages. This could be attributed to the preferential effect of Urea towards Ferrous sulphate (BBM media) as compared to Ferric ammonium citrate (BG-11 media). Rizwan *et al.*, (2017) studied the preferential effect of different Iron sources on microalgal growth in marine microalgae *Dunaliella tertiolecta.*Yeh and Chang, 2012) also reported the effect of media composition and cultivation conditions on microalgal growth. The present study has laid preliminary findings and further studies must be undertaken to explore the relative mechanisms associated with the present observation.

Effect of Urea on Chlorophyll-a, Lipid and Carbohydrate Content:

Chlorophyll-a Content (mg/l) was also influenced by the Urea concentrations and highest values were reported in T_1 (0.1% Urea) followed by Control and T_2 (0.5% Urea) on 21st day of cultures **(Fig. 4.4)**.

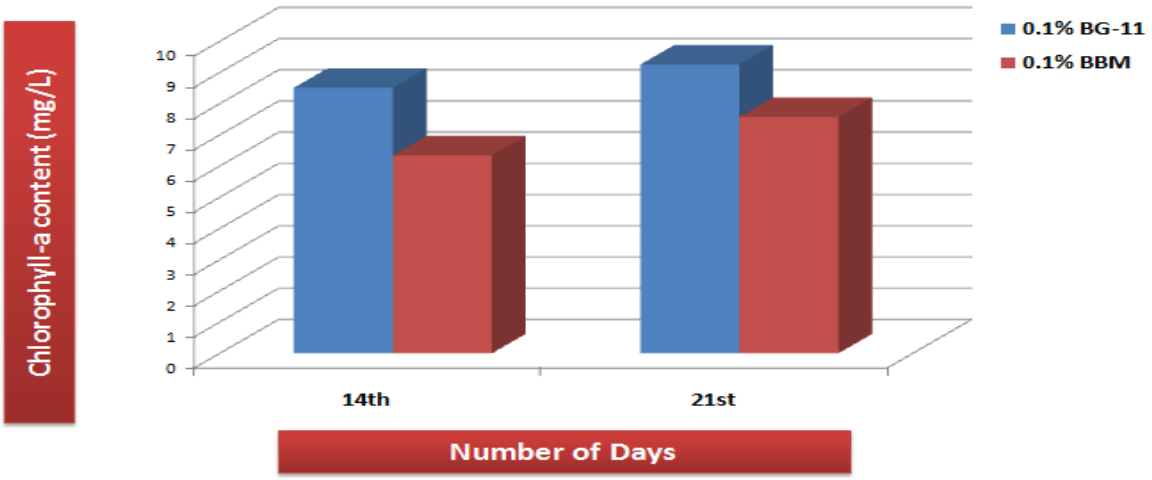


Figure 4.4: Effect of media composition on chlorophyll-a content in Microalgae consortium

On comparing the chlorophyll-a content of 21st day observation with 14th day, it was found that there was not much variation in chlorophyll-a content in T_1 (8.514±0.252) and T_2 (8.886±0.131) cultures on 14th day with overall higher reported values as compared to 21st day observation (6.346±0.157 in T_1; 2.372±0.397 in T_2). This could be attributed to the ammonia toxicity with increase in Urea concentrations.

Nitrogen has influential effect on Lipid and Carbohydrate content. Highest lipid values were observed in T_1 (0.1% Urea) followed by control cultures (without Urea) and T2 (0.5% Urea). At higher concentrations of Urea (<0.1 g/L Urea) Goswami and Kalita (2011) recorded gradual decrease in biomass, cell number and lipid productivity microalgae *Scendesmus dimorphus* and Scendesmus *quadricauda*. Carbohydrate content decreases with increase in Urea concentration (Control>T_1>T_2) **(Fig. 4.5)**.

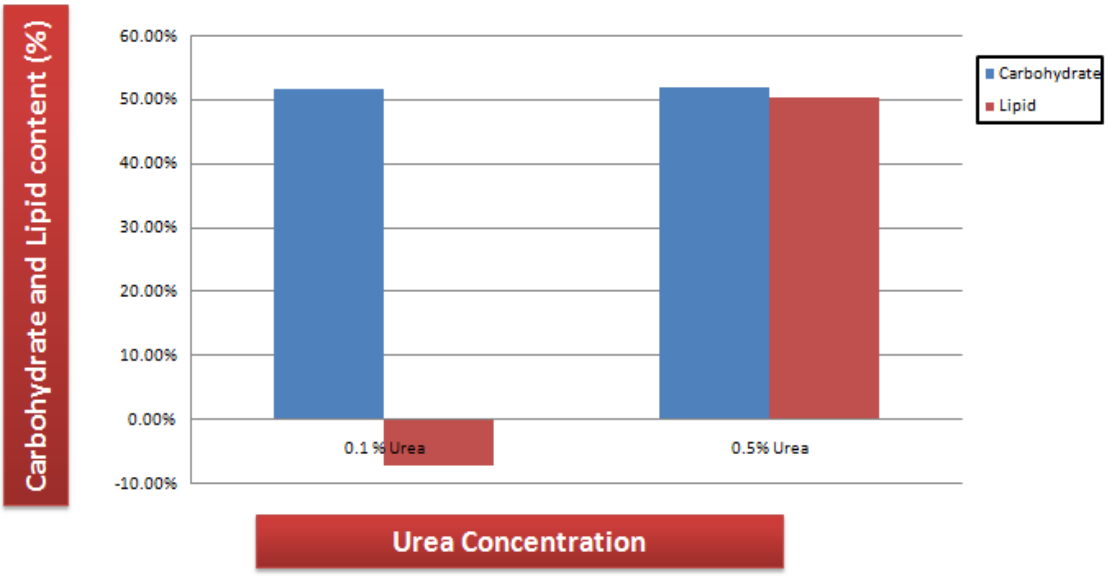


Fig. 4.5: Variable concentration of Urea on Lipid and Carbohydrate content on Microalgae consortium with respect to Control Culture

Overall, with respect to control cultures 51.8% and 76.87% decrease in Carbohydrate content was recorded in T_1 and T_2 respectively. Microalgal cultures grown in BBM media (FS) showed

30.78% higher lipid content as compared to the microalgal cultures in BG-11 media (FAC) at 1% Urea concentration. The presence of ammonium sulphate moiety also affects the metabolism of microalgae as reported by Rizwan *et al.*, (2017) and noted that, regardless of ferrous or ferric groups, the lipid content has not promoted by ammonium sulphate moiety.

Summary and Conclusion:

The first objective of the study was to experimentally determine the effect of Nitrogen supplementation on growth and biomass productivity of microalgae. The results of the study in BG-11 and BBM media indicate that when urea is taken as the nitrogen supplement the BBM media favours the micro-algal growth at 0.1% Urea treatment. The BG-11 media favours growth over BBM media at later stages in the control culture (i.e. without giving urea treatment).

The second objective of the study was to measure the effect of nitrogen supplementation on chlorophyll-a, lipid and carbohydrate content in microalgae consortium. The results indicate that Chlorophyll-a Content (mg/l) was also influenced by the Urea concentrations and highest values were reported in T_1 (0.1% Urea) followed by Control and T_2 (0.5% Urea) on 21st day of cultures. Nitrogen has influential effect on Lipid and Carbohydrate content. Highest lipid values were observed in T_1 (0.1% Urea) followed by control cultures (without Urea) and T_2 (0.5% Urea). The Carbohydrate content decreases with increase in Urea concentration (Control>T_1>T_2).

The third objective of the study was to compare the effect of two different nutrient media on biomass composition of microalgae consortium at optimized urea concentration. The results indicate that the carbohydrate content decreases significantly in control cultures. As far as lipid content is concerned the micro-algal cultures grown in BBM media (FS) showed 30.78% higher values as compared to the BG-11 media (FAC) at 1% Urea concentration.

The findings of the present study recommend further investigation pertaining to characterize the effect of nutrients supplementation in different native and exotic microalgal species with reference to the metabolite accumulation (i.e. lipid and carbohydrate content) under variable growth condition to optimize the value added product generations apart from biofuels.

References:

Agarwal, A. K. (2007). Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in energy and combustion science*, *33*(3), 233-271.

Agwa, O. K., & Abu, G. O. (2016). Influence of Various Nitrogen Sources on Biomass and Lipid Production by Chlorella vulgaris. British Biotechnology Journal, 15(2), 1-13.

Akansu, S. O., Dulger, Z., Kahraman, N., &Veziroğlu, T. N. (2004).Internal combustion engines fueled by natural gas—hydrogen mixtures. *International journal of hydrogen energy*, *29*(14), 1527-1539.

Alam, F., Mobin, S., & Chowdhury, H. (2015).Third generation biofuel from algae. *Procedia Engineering*, 105: 763-768.

Banse, M., van Meijl, H., &Woltjer, G. (2008, June). The impact of first and second generation biofuels on global agricultural production, trade and land use.In *GTAP Conference Paper, June*.

Becker, EW. (1994). *Microalgae biotechnology and microbiology*. Cambridge University Press, New York, pp 18

Bhuiya, M. M. K., Rasul, M. G., Khan, M. M. K., Ashwath, N., Azad, A. K., &Hazrat, M. A. (2014). Second generation biodiesel: potential alternative to-edible oil-derived biodiesel. *Energy Procedia*, *61*, 1969-1972.

Bindra, S., Sharma, R., Khan, A., & Kulshrestha, S. (2017). Renewable energy Sources in different Generations of Bio-fuels with special Emphasis on Microalgae Derived Biodiesel as Sustainable industrial fuel Model. *Biosciences Biotechnology Research Asia*, *14*(1), 259-274.

Blinová, L., Bartošová, A., &Gerulová, K. (2015).Cultivation of microalgae (Chlorella vulgaris) for biodiesel production. *Research Papers Faculty of Materials Science and Technology Slovak University of Technology*, *23*(36), 87-95.

Borowitzka, M. A., & Moheimani, N. R. (2013).Sustainable biofuels from algae. Mitigation and Adaptation Strategies for Global Change, 18(1): 13-25.

Brennan, L, Owende P. Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews.2010; 14: 557-577.

Chen, Y. H., & Walker, T. H. (2011).Biomass and lipid production of heterotrophic microalgae Chlorella protothecoides by using biodiesel-derived crude glycerol. *Biotechnology letters*, *33*(10), 1973.

Chong, W. T., Naghavi, M. S., Poh, S. C., Mahlia, T. M. I., & Pan, K. C. (2011). Techno-economic analysis of a wind–solar hybrid renewable energy system with rainwater collection feature for urban high-rise application. *Applied Energy*, *88*(11), 4067-4077.

Dhup, S., Kannan, D. C., &Dhawan, V. (2016). Understanding Urea Assimilation and its Effect on Lipid Production and Fatty Acid Composition of Scenedesmus Sp.

Dittmar, M. (2012). Nuclear energy: Status and future limitations. *Energy*, *37*(1), 35-40.

Dragone, G., Fernandes, B. D., Vicente, A. A., & Teixeira, J. A. (2010).Third generation biofuels from microalgae. Current research, technology and education topics in applied microbiology and microbial biotechnology, 2, 1355-1366.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

Dutta, K., Daverey, A., & Lin, J. G. (2014). Evolution retrospective for alternative fuels: First to fourth generation. *Renewable energy*, *69*, 114-122.

Gao, Y., Gregor, C., Liang, Y., Tang, D., & Tweed, C. (2012). Algae biodiesel-a feasibility report. *Chemistry Central Journal*, *6*(1), S1.

Gullison, R. E., Frumhoff, P. C., Canadell, J. G., Field, C. B., Nepstad, D.C., Hayhoe, K., Avissar, R. A., Curran, L. M., Friedlingstein, P., Jones, C. D., and Nobre, C., Environment. Tropical forests and climate policy.*Science.*2007, 316: **985-986.**

Hamed, S. M., &Klöck, G. (2014). Improvement of medium composition and utilization of mixotrophic cultivation for green and blue green microalgae towards biodiesel production. Advances in Microbiology, 4(03), 167.

Hamed, S. M., &Klöck, G. (2014).Improvement of medium composition and utilization of mixotrophic cultivation for green and blue green microalgae towards biodiesel production. *Advances in Microbiology*, *4*(03), 167.

Hamedi, S., Mahdavi, M. A., & Gheshlaghi, R. (2016). Improved lipid and biomass productivities in Chlorella vulgaris by differing the inoculation medium from the production medium. Biofuel Research Journal, 3(2), 410-416.

Hempel, N., Petrick, I., &Behrendt, F. (2012). Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. Journal of applied phycology, 24(6), 1407-1418.

Henrard, A. A., da Rosa, G. M., Moraes, L., de Morais, M. G., & Costa, J. A. V. (2015). The cultivation of microalgae Cyanobium sp. and Chlorella sp. in different culture media and stirring setting. *African Journal of Microbiology Research*, *9*(21), 1431-1439.

Huang, D., Zhou, H., & Lin, L. (2012). Biodiesel: an alternative to conventional fuel. *Energy Procedia*, *16*, 1874-1885.

Idenyi, J. N. Ebenyi L. N., Ogah, O. Nwali, B.U. and Ogbanshi, M.E.(2016). Effect of Different Growth Media on the Cell Densities of Freshwater Microalgae Isolates.(2016). *IOSR Journal of Pharmacy and Biological Sciences.*11(3): 24-28.

Kajikawa, Y., & Takeda, Y. (2008). Structure of research on biomass and bio-fuels: A citation-based approach. *Technological Forecasting and Social Change*, *75*(9), 1349-1359.

Kaur,R.,Mahajan,A.,Bhatia,A.,Mahavidyalaya,M.R.H,&Jalandhar.(2017). Effect of Two Different Nitrogen Sources on Lipid Accumulation in Microalgae Chlorella Pyrenoidosa.International Journal of Trend in Research and Development.5(4): 2394-9333.

Keeling, C. D. (1973).Industrial production of carbon dioxide from fossil fuels and limestone. *Tellus*, *25*(2), 174-198.

Kim, G., Mujtaba, G. & Lee, K. (2016). Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte Tetraselmis sp. for lipid production, Algae, 31(3), 257-266 http://dx.doi.org/10.4490/algae.2016.31.8.18

Kirrolia, A., Bishnoi, N. R., & Singh, N. (2011). Salinity as a factor affecting the physiological and biochemical traits of Scenedesmus quadricauda. Journal of Algal Biomass Utilization, 2(4), 28-34.

Li, T., Zheng, Y., Yu, L. & Chen, S. (2013). High productivity cultivation of a heat-resistant microalga *Chlorella sorokiniana* for biofuel production. Bioresour Technol, 131, 60–67.

Li, Y., Horsman, M., Wu, N., Lan, C. Q., & Dubois-Calero, N. (2008). Biofuels from microalgae. *Biotechnology progress*, *24*(4), 815-820.

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: a review. *Renewable and sustainable energy reviews*, *14*(1), 217-232.

Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. Progress in energy and combustion science, 37(1), 52-6.

Norici, A., Dalsass, A. & Giordano, M. (2002). Role of phosphoenolpyruvate carboxylase in anaplerosis in the green microalga *Dunaliella salina* cultured under different nitrogen regimes. Physiol Plant, 116, 186–191.

Pandey, R. and Kumar, G. (2017). A Comprehensive Review on Generations of Biofuels: Current Trends, Development and Scope.*International Journal on Emerging Technologies.***8**(1): 561-565.

Park, J., Jeong, H. J., Yoon, E. Y., & Moon, S. J. (2016). Easy and rapid quantification of lipid contents ofmarine dinoflagellates using the sulpho-phospho-vanillin method. Algae, 31(4), 391-401.

Phukan, M. M., Chutia, R. S., Konwar, B. K., &Kataki, R. (2011). Microalgae Chlorella as a potential bio-energy feedstock. Applied Energy, 88(10), 3307-3312.

Porra, R. J., Thompson, W. A., &Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. BiochimicaetBiophysicaActa (BBA)-Bioenergetics, 975(3), 384-394.

Radway, J. C., Wilde, E. W., Whitaker, M. J., &Weissman, J. C. (2001). Screening of algal strains for metal removal capabilities. Journal of Applied Phycology, 13(5), 451-455.

Rodolfi, L., ChiniZittelli, 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. *BiotechnolBioeng.*, 102: **100-112.**

Rushdy, (2016). Effect of copper on growth, bioactive metabolites, antioxidant enzymes and photosynthesis-related gene transcription in Chlorella vulgaris.

Saifullah, A. Z. A., Karim, M. A., & Ahmad-Yazid, A. (2014). Microalgae: an alternative source of renewable energy. *Am. J. Eng. Res*, *3*(3): 330-338.

Sameera, V., Sameera, C., & Ravi Teja, Y. (2011). Current strategies involved in biofuel production from plants and algae. *J Microbial BiochemTechnol*, *1*, 002.

Shaker, S., Morowvat, M. H., &Ghasemi, Y. (2017). Effects of Sulfur, Iron and Manganese Starvation on Growth, β-carotene Production and Lipid Profile of Dunaliellasalina. Journal of Young Pharmacists, 9(1).

Sharma, A. K., Sahoo, P. K., Singhal, S., & Patel, A. (2016). Impact of various media and organic carbon sources on biofuel production potential from Chlorella spp. *3 Biotech*, *6*(2), 116.

Sibi, G., Anuraag, T. S., &Bafila, G. (2014).Copper stress on cellular contents and fatty acid profiles in Chlorella species. *Online Journal of Biological Sciences*, *14*(3): 209.

Sims, R. E., Rogner, H. H., & Gregory, K. (2003). Carbon emission and mitigation cost comparisons between fossil fuel, nuclear and renewable energy resources for electricity generation. *Energy policy*, *31*(13), 1315-1326.

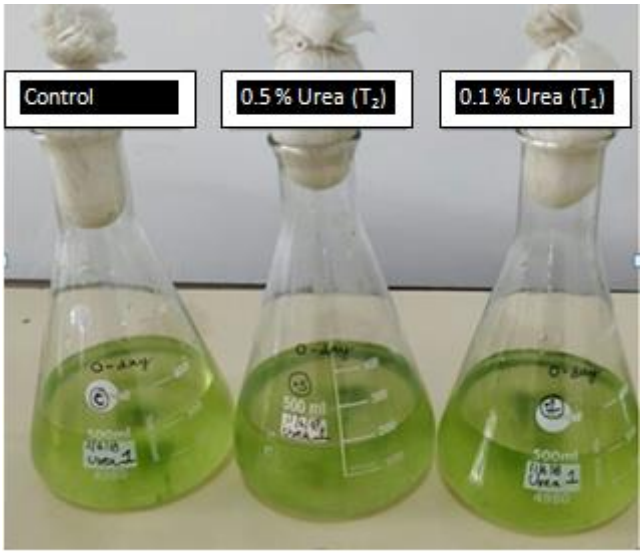
Singh, A. K., & Singh, M. P. (2014).Importance of algae as a potential source of biofuel. *Cell. Mol. Biol*, *60*(5), 106-109.

Viesturs, D., & Melece, L. (2014). Advantages and disadvantages of biofuels: observations in Latvia. In *Engineering for rural development: Conference proceedings*(Vol. 13, pp. 210-215).

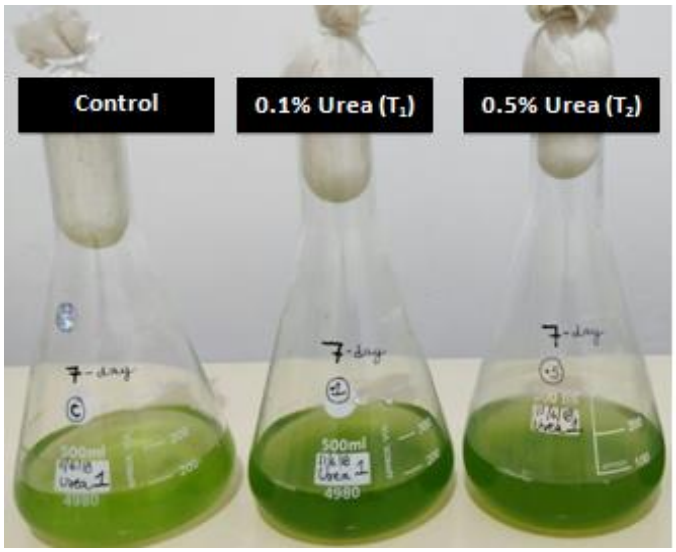
Yeh, K. L., & Chang, J. S. (2012).Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31.Bioresource technology, 105, 120-127.

Xin, L., Hong-ying, H., Ke, G., & Ying-xue, S. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresource technology, 101(14), 5494- 5500.

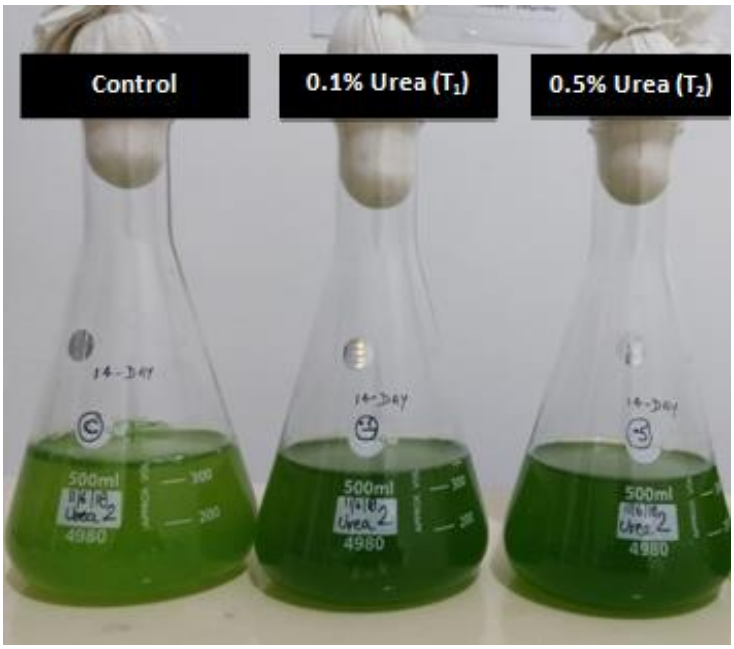
Picture Gallery



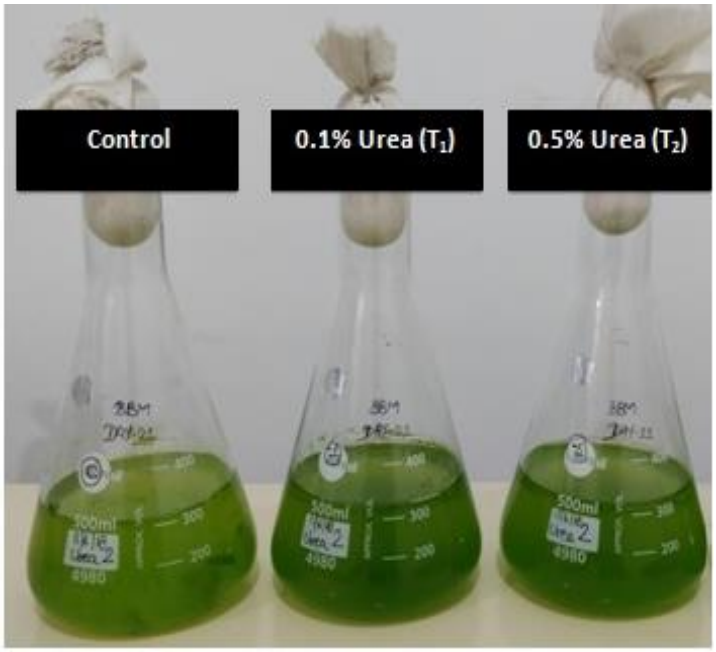
(Urea concentration at 0- Day)



(Urea concentration at 7- Day)

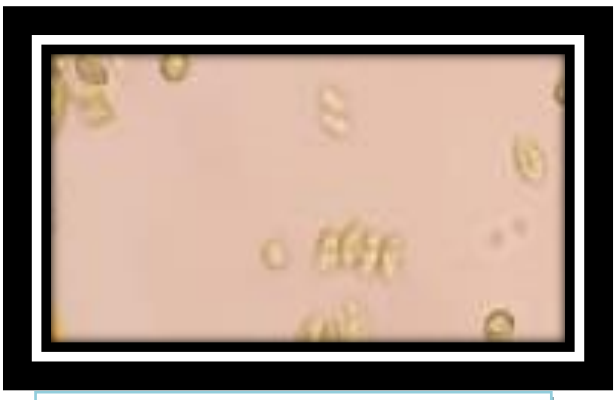


(Urea concentration at 14- Day)



(Urea concentration at 21- Day)

Chlorella species



Scendesmus species

Cosmarium species

ISARA INSTITUTE OF MANAGEMENT & PROFESSIONAL STUDIES

RESEARCH GATEWAY

AROGYAM ONLINE

PARIVARTAN PSYCHOLOGY CENTER

भारतीय भाषा, शिक्षा, साहित्य एवं शोध

ISSN 2321 – 9726

WWW.BHARTIYASHODH.COM

INTERNATIONAL RESEARCH JOURNAL OF MANAGEMENT SCIENCE & TECHNOLOGY

ISSN – 2250 – 1959 (0) 2348 – 9367 (P)

WWW.IRJMST.COM

INTERNATIONAL RESEARCH JOURNAL OF COMMERCE, ARTS AND SCIENCE

ISSN 2319 – 9202

WWW.CASIRJ.COM

INTERNATIONAL RESEARCH JOURNAL OF MANAGEMENT SOCIOLOGY & HUMANITIES

ISSN 2277 – 9809 (0) 2348 - 9359 (P)

WWW.IRJMSH.COM

INTERNATIONAL RESEARCH JOURNAL OF SCIENCE ENGINEERING AND TECHNOLOGY

ISSN 2454-3195 (online)

WWW.RJSET.COM

INTEGRATED RESEARCH JOURNAL OF MANAGEMENT, SCIENCE AND INNOVATION

ISSN 2582-5445

WWW.IRJMSI.COM

JOURNAL OF LEGAL STUDIES,POLITICS AND ECONOMICS RESEARCH

WWW.JLPER.COM