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Enhancement of lipid yield in *Chlorella* sp. under different stress conditions for the production of biodiesel

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Abstract

Microalgae derived bio-lipids can be potentially converted to biofuel using appropriate technologies. However, optimum conditions for enhancing the growth of microalgae often lead to relatively low accumulation of bio-lipids. Alternatively, stress conditions favor the storage of lipids despite the low biomass yield. For commercial production of microalgae derived biofuel, the unit cost of production of biodiesel should be lower than that of petro diesel. The aim of the present study was to enhance the lipid yield of *Chlorella* sp. by introducing various stress conditions such as nitrogen deficiency, zinc deficiency and excessive salinity in the growth media. Conventional Bold's Basal Media (BBM) was used as the culture media in all the experimental conditions. Five treatments were used namely; normal Bold's basal media, three times concentration enhanced Bold's basal media, Nitrogen deficient media, Zinc deficient media and Salinity stress media. Light intensity was kept constant in all the experimental conditions.

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Air mixing was provided using spargers in all test conditions to keep the microalgae cultures in suspension. All the reactors were maintained in similar growth conditions in the first phase and the stress conditions were introduced in the second phase after 7 days. The microalgae biomass was harvested on 4th, 8th, 11th, 14th and 17th day of cultivation for the determination of lipids. A Chloroform: Methanol mixture with 1:1 ratio was used to extract lipids from dried biomass using Soxhlet extractor. A considerable increase in the lipid content was observed in all the test conditions compared to control samples (highest was 16.9%). Highest lipid content of 23.3% was observed in Zn stressed conditions. However, in terms of lipid productivity, salinity stress treatment produced the highest average lipid productivity of 0.008±0.005 $mgL^{-1}d^{-1}$. After certain period of time (14th day), lipid content of stressed samples decreased. Oleic acid and linoleic acid were found to be abundant in all of the treatments. The study suggests that imposing stressful conditions can result in a higher amount of lipid yield from the algae biomass with a higher rate of productivity that contains important fatty acids which can be a feasible opening for biofuel production.

Keywords: *Chlorella* Sp.; Stress Conditions; Lipid Production; Lipid productivity; Biodiesel

Introduction

Microalgae can produce their own food by utilizing inorganic raw materials from the surrounding environment. Years of research have resulted in a significant amount of interest worldwide due to their potential application in renewable and sustainable sources of biofuels, bioactive medicinal products, and food sources. Several microalgae species have been studied for their potential as value-added products with exceptional pharmacological and biological properties (Khan *et al*., 2018). With an ever-increasing global population, the demand for food and energy sources is increasing proportionally, but resources, particularly energy resources, are degrading inversely proportionally to the global demand. As a result, long-term solutions are required for these problems. On the other hand, the use of fossil fuels and improper disposal of end products have gradually annihilated the environment to the point where it is becoming increasingly toxic for all living beings to consume.

As a result, there is a growing demand for environmentally friendly energy and food resources. Phototrophic microalgae require light energy, carbon source and other inorganic compounds including nitrogen (N), phosphorus (P), iron (Fe) for their growth and maintenance. Restriction of nutrients can have an impact on the lipid composition of microalgae cells (Tsai *et al*., 2016). The accumulation of fatty acids and lipids in the microalgae body typically occurs during times of environmental stress (Sajjadi *et al*., 2018; Renuka *et al*., 2018). Green algae cells typically undergo metabolic acclimatization in response to nutrient stress, which always results in macromolecule cellular composition fluctuations. Nitrogen deficiency frequently leads to decreased protein content and increased carbohydrate or lipid storage (Lin , 2011). Phosphate restriction also has a significant impact on protein, lipid, and carbohydrate content. As a result, green alga's biochemical configuration is linked to its growth rate which reflects the physiological potential of primary productivity. Microalgae cells that are deprived of nutrients can change chemically. It can have an impact on the growth rate and photosynthetic efficiency. It has been demonstrated that changes in cellular composition improve their ability to cope with physiological parameters. Protein synthesis in chlorophytes is limited by nitrogen limitation because carbon is inserted into the formation of storage products such as carbohydrate and lipids (Minhas *et al*., 2016).

The response of microalgae in terms of particulate organic carbon, chlorophyll, and cell concentrations demonstrated that nitrogen is the better principal limiting nutrient than silicon (Delgadillo-Mirquez *et al*., 2016). In this study, nitrogen limitation retarded the growth of microalgae and led to accumulation of lipids due to algae's biosynthetic response to environmental changes. Li *et al.* (2008) reported that different microalgae species stimulate different levels of neutral lipid accumulation. Although microalgae can produce lipids and other nutrients that can be used to produce energy in the form of food or fuel, the amount they produce has not been found to be sufficient to meet global energy demands. As a result, various techniques and approaches to increasing the rate of production are being investigated.

Chlorella Sp. is one of the microalgae species that has been studied for its ability to accumulate a high amount of lipids under various stress conditions such as: light intensity; temperature; carbon dioxide; nutrient deficiency; salinity stress

and metal stress during cultivation (Zhu *et al*., 2015). The potential to produce biolipids through *Chlorella* Sp. has been found to be important due to the ability of *Chlorella* Sp to to grow under different conditions. In light of this, the current study aimed to enhance the lipid content of *Chlorella* Sp. by applying different conditions such as: nitrogen; Zn and salinity stress under laboratory conditions.

Materials and methods

Selection and culturing of microalgae strain

The model microalgae for the series of experiments were a culture of *Chlorella* Sp. (Obtained from the Environmental Microbiology Laboratory, Department of Civil Engineering, University of Jaffna). The chosen *Chlorella* Sp. mix culture was grown in clear 20 L plastic bottles and served as the base culture for the following experiments. The medium for algal growth was laboratory prepared Bold's Basal Medium (BBM) (Aragaw & Asmare, 2017). To prepare BBM, a standard procedure was followed (Aragaw & Asmare, 2017), and all of the stock solutions and trace elements used to prepare Bold's Basal Medium (BBM) were obtained from Sigma Aldrich, USA, Sri Lanka. All of the chemicals used were of the highest analytical grade. The prepared stock solutions and chemicals were kept in a refrigerator set to 4 ˚C (Anon *et al*., 1992). The pH of the finished BBM was adjusted to 6.6. All glassware was autoclaved at 121 ˚C for 30 minutes before use in all procedures.

Preparation of polybag Photo Bioreactor (PBR)

The photo bioreactors were built with thick, transparent polythene. The 150 mm wide, 500 mm long polythene was sealed at both the top and bottom ends to create a 400 mm x 150 mm working area polythene bag photo bioreactor. An opening in the bag's top was made to accommodate the air sparger, testing culture, and other treatments. It was hung in the metal setup, a lighting system that provides continuous 2000 lux white fluorescent light. The aquarium air pump was used to aerate the photo bioreactor, and porous air stones were used for mixing. The photo bioreactor had a total volume of 2.5 L.

Applying stress conditions on microalgae culture

To provide stress conditions, a mixed culture of microalgae (*Chlorella* Sp.) was grown as batch cultures using five (05) different treatment methods with

different amounts of growth medium and varying certain nutrients according to the labels. As tabulated in Table 1, the treatments were labeled as BB, BBx3, N, Z, and S.

Table 2: Treatments that have been used and their symbolic labelling

Treatment	Label
01. Normal BBM	BBM
02. 03 times strength increased BBM	BBMx3
03. Nitrogen stress	N
04. Zinc stress	Z
05. Salinity stress	S

As illustrated in Figure 1, each treatment had 05 replicates that were harvested at various points throughout the study. The algal biomass was extracted from the base culture using a refrigerated centrifuge (10000 rpm for 2 minutes at 4 ˚C). The harvested biomass was re-suspended in photo bioreactors that had been previously prepared (25 polythene pouches). The initial optical density was kept constant at 0.200 at 680 nm (OD_{680}).

Treatment 01 was designated as BB, and it included 05 replicates (BB-1, BB-2, BB-3, BB-4, and BB-5) containing microalgae biomass (*Chlorella* Sp. mix culture) and an appropriate amount of BBM. To prevent nutrient deficiency, the BBM was reinstalled in treatment 01 after 07 days from culture start (on the 8th day). This treatment served as the initial control set. The second treatment (Treatment 02) was called BBx3, and it contained three times the recommended amount of BBM. The same reinstalling procedure and replicates (BBx3-1, BBx3-2, BBx3-3, BBx3-4, and BBx3-5) were used as in the first treatment. The second control set was Treatment 02.

Treatment 03 was used to test microalgae growth and lipid production in N deficient medium. The treatment was labeled N, and there were 05 replicates (N-1, N-2, N-3, N-4 and N-5). In the absence of sodium nitrate, an appropriate amount of BBM was added to each PBR to simulate nitrogen stress conditions. To avoid any nutrient deficiency, sodium nitrate less BBM was added again on the eighth day of the culture in the same manner as before. Treatment 04 was

designed to put the Zinc (Zn) stress to the test. As a result, it was labeled Z, and 05 replicates were included (Z-1, Z-2, Z-3, Z-4 and Z-5). Except for the Zn present in BBM, all of the Zn bioreactors received an adequate amount of BBM. After 7 days, the same Zn-less BBM was reinstalled in the culture (on 8th day).

Salinity stress has been shown in previous studies to improve total lipid content and saturated portions of fatty acids (Church *et al*., 2017), therefore the 5th and final treatment was added an extra amount of NaCl (2M) to a PBR containing an appropriate amount of BBM. S was the name given to treatment 05, which had 05 replicates (S-1, S-2, S-3, S-4 and S-5). On the eighth day, each salinity stress replicate received the same extra amount of NaCl (2M) and the appropriate amount of BBM. The 25 PBR were arranged in a random manner in the pre-made metal setup as mentioned in Figure 1. Random sampling was used to determine microalgae growth in the treatments using OD680 and a UV visible spectrometer (Lovibond XD, 7500). Daily samples were collected for analysis.

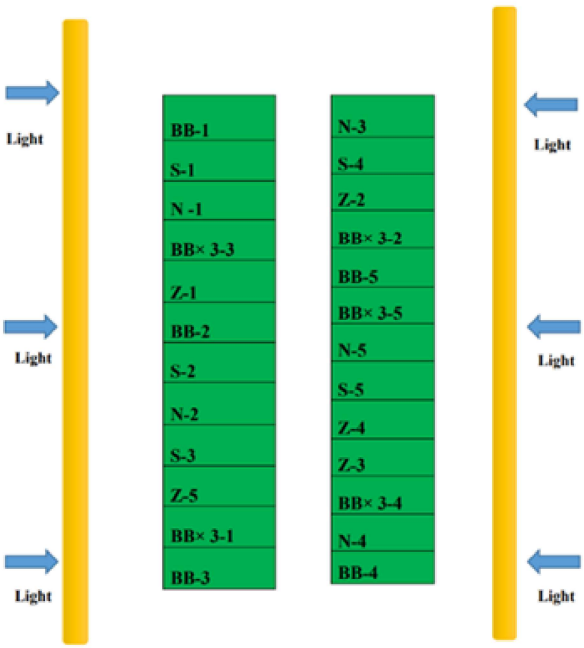

Figure 1: Placement of each photo bioreactor in experimental setup

Microalgae biomass harvesting and preparation

To obtain algal precipitation, Chlorella Sp. culture samples were centrifuged (under 10000 rpm at 4 ^{o}C for 2 minutes) (pellet). Wet algal biomass was obtained through centrifugation. Wet algal biomass was collected in petri dish after supernatant was removed from centrifuge tubes and dried in an oven at 60^{o}C until dry biomass was obtained. Lipid was extracted from dry biomass. A random sample from each treatment was taken. One sample pouch was randomly selected from each treatment on each day of harvesting (As there were 5 treatments, 5 samples were harvested). Harvesting was done on the 4th, 8th, 11th, 14th and 17th day since the start of the experiment.

Determination of biomass productivity

Samples of 50 mL from each treatment were collected and filtered through pre-weighed 45 m Whatman glass fiber filter papers. It was then rinsed three times with 20 mL deionized water. The sample was then dried at 105℃ until it reached a constant weight. Using dry weight measurements, the biomass productivity was calculated using the following equation. (Ambat *et al*., 2019)

$$Biomass\ productivity\ (gL^{-1}d^{-1}) = {Biomass\ concentration\ (gL^{-1})}/{No.of\ days}$$

Equation 2

Lipid extraction from dry microalgae biomass

For lipid extraction, a Büchi B 811 Soxhlet (BÜCHI Universal Extraction System, Switzerland) apparatus was used. The continuous flow method was used (Ramluckan *et al*., 2014), and the extraction took 3 hours. According to the preliminary test results, the solvent system was chloroform: methanol (1:1, v/v). Following extraction, the lipid percentage was calculated using the equation;

$$Lipid\ percentage = \frac{M_L}{M_B} X\ 100$$ Equation 2

Where M_L = Mass of lipid extracted (g), M_B = Mass of Biomass weighted (Dry) (g).

The lipid productivity was calculated using following equation from (Ambat *et al*., 2019)

$$Lipid\ productivity\ (mgL^{-1}d^{-1}) = biomass\ productivity\ (mg\ ^{-1}d^{-1}) \times Lipid\ content\ (\%)$$

Equation 3

Trans-esterification of extracted lipids

Toluene (2 mL) was used to dissolve the extracted lipids. The contents were then transferred to a screw-capped glass test tube lined with Teflon (20 mL capacity). A 7% BF_3-methanol reagent of 2 mL was added. BF_3 is used as an acid catalyst (Ichihara and Fukubayashi, 2010). The Teflon-lined screw-cap was used to seal the glass. The tube was heated in a heating block, oven, or hot water bath for 45 minutes at 100 °C. Every 10 minutes, the tube was gently shaken (Note: evaporation of solvent from tubes indicates inadequate seals. If this happens, discard the solution and repeat the methylation procedure). After removing the tube from the heating block (oven or hot water bath), it was allowed to cool to room temperature. There was 5 mL distilled water, 2 mL hexane, and 1 g sodium sulphate added. The tube was sealed and shaken. Fatty acid methyl esters (FAMEs) were collected in hexane solution to a small seal glass vial after 10 minutes. Filled with nitrogen and secured the cap. The sample was immediately analyzed on GC (Lohman *et al*., 2013 and Griffiths *et al*., 2010).

Gas chromatography analysis

FAMEs were determined by injecting 1 µL into a GC (Agilent technologies) system outfitted with a Flame Ionization Detector and a fused silica capillary column (100 m length, 0.25mm diameter, and 0.20 m film). At a flow rate of 20 mL/min, nitrogen was used as the carrier gas. The split was 30:1. Operating parameters for the GC: The initial column oven temperature was maintained at 140 ^{o}C for 5 minutes before increasing to 240^{o}C for 10 minutes at a rate of 4 ^{o}C/min and remaining at that temperature for 10 minutes. The injection port temperature was kept at 260 ^{o}C, and the detector temperature was kept at 260 ^{o}C (Ambat *et al*., 2019).

Results and discussion

Microalgae growth behavior

The growth of *Chlorella* Sp. under five different treatments is shown in Figure 2. The *Chlorella* Sp. mix culture exhibited substantial growth in all treatments based on the variation of optical density over 17 days. The algal strains exhibited a two-day lag phase in each of the treatments (Ambat *et al*., 2019). However, among other treatments, the Zinc stress media (Treatment 04) exhibits a distinct increase in growth. This finding contradicts with a previous study (Hamed *et al*., 2017), in which the author reported that zinc stress inhibits growth and induces oxidative stress.

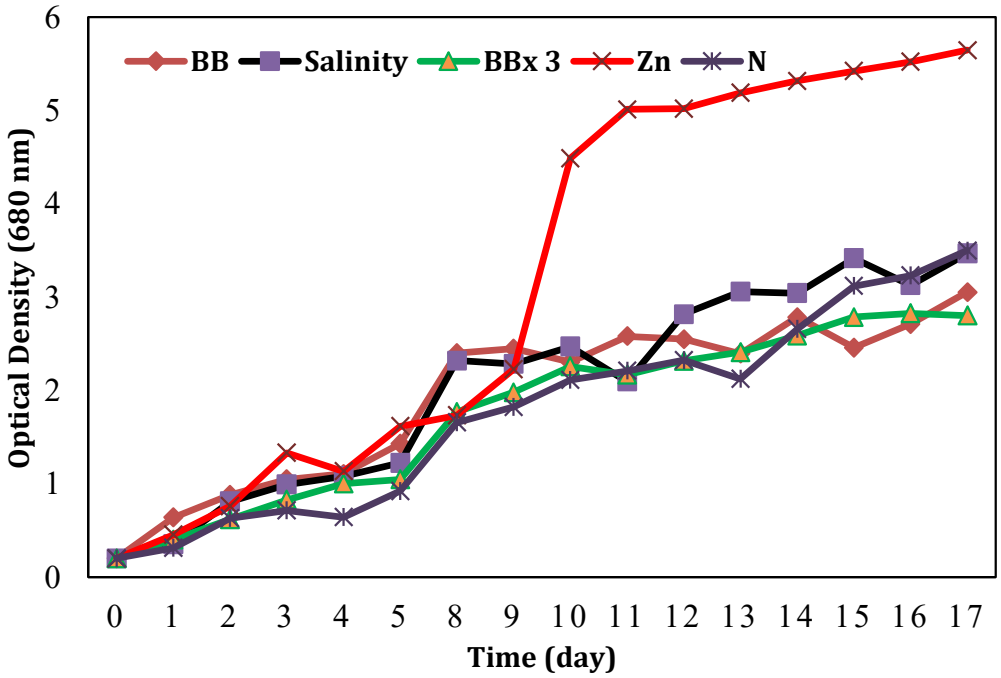

Figure 2: Growth of Chlorella Sp. microalgae in all treatment conditions

For the treatment with the appropriate amount of BBM, the optical density increased rapidly on the first day and gradually increased from the second to the fifth day. From the fifth to the eighth day, the optical density increased rapidly again, and from the eighth day onwards, the parameter had a pattern of rapidly increasing and decreasing.

For the treatment of salinity, the behavior is nearly identical to that of BB treatment. However, it increased slightly on the first day, and from the ninth to the sixteenth day, the salinity variation is opposite to the BB treatment. When considering the treatment of three times the concentration of BBM, there is a slight increase from the first day to the fifth day, and a rapid increase between the fifth and eighth days. Then it gradually increases and decreases until it reaches a constant value. The variation in the treatment with nitrogen stress is almost identical to the variation in salinity, with a small deviation up to the twelfth day, when it rapidly increased. Previous research confirmed that nitrogen deficiency can reduce biomass growth rate, but nitrogen addition at specific time intervals can keep biomass growth at an optimal level (Zhu *et al*., 2015). The ability of *Chlorella* Sp. to thrive in various conditions throughout these experiments in various treatments suggests that *Chlorella* Sp. has the potential to be used as a better option for nutrient removal in a variety of wastewaters.

Biomass production

The observed weight of biomass under all the treatments is shown in Figure 3. Almost in all the treatments, 3rd day of harvesting resulted in twice the weight of biomass which were harvested in the 2nd harvesting day possible due to the addition of nutrients again in the 8th day of the experiment. An exponential biomass growth was observed in the BBx3 treatments because of the presence of nutrients 3 times more than the standard Bold's basal medium (BBM). The recorded highest biomass yield from Zinc stress treatment and Nitrogen stress treatment came on 3rd and 4th harvesting days respectively (11th and 14th days of the experiment). All other treatments recorded their highest biomass yield on the final day of the harvesting which indicates that deficiency of Zinc and Nitrogen have an effect on the production biomass in *Chlorella* Sp. microalgae. A study by Chandra *et al*. (2019) states that Zinc is an essential micronutrient for microalgae growth, therefore, the lack of Zinc results in a weaker growth in microalgae. A recent study suggests that the deficiency of Nitrogen suppresses the growth of *Chlorella* Sp. microalgae (Ratomski *et al*., 2021). All these observations are in line with the findings of the present study. The prediction of cell density by $OD_{680}$was not always in good agreement with the actual cell

density measurements. This is presumable due to the formation of microalgae flock in the bioreactor despite the gas mixing.

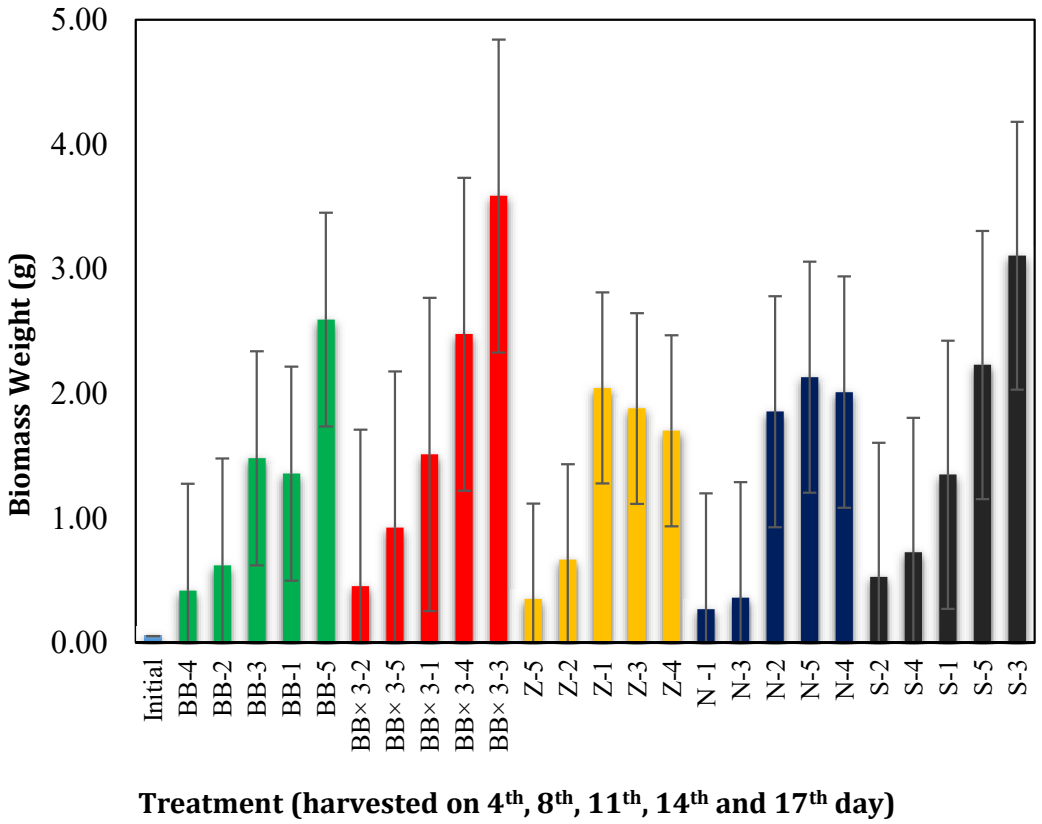

Figure 3: Dry biomass yield of different treatments at different harvesting days (4th, 8th, 11th, 14th and 17th day). The labels in the x-axis of the chart indicate the randomly collected pouch number of each treatment.

A gradual decrease of biomass was observed in Zinc stress treatment (Z) on 14th and 17thday compared to the 11th day of experiment due to lack of nutrients in the medium. The previous studies suggest that increasing Zinc concentrations can reduce the biomass production in microalgae *Chlorella* Sp. (Kondzior and Butarewicz, 2018). Therefore, the decrease of biomass did not seem to be induced by the lack of Zinc in the treatment. The peak value for the harvested biomass under zinc stress has been identified on the 11th day of the experiment.

In the nitrogen stress treatment, the highest biomass weight was obtained on the 14th day of the experiment which was slightly higher than the peak of zinc stressed treatments. However, it was still lower than the other 3 treatments namely; BB, BBx3, S (Salinity stress). The salinity stressed treatment showed a continuous increase in the biomass production with the increased salinity. Therefore, it shows the *Chlorella* Sp. mixed culture is well tolerant against the salinity stress. The highest average biomass concentration of 0.715 g/L with a standard deviation of ±0.349 was obtained in BBx3 treatment as it contained three times nutrients compared to normal Bold's basal media.

Variation of lipid percentage according to the treatment and harvesting date

A well distinguished difference was observed in the lipid production of Zinc stressed treatments from the first day to final day of the biomass harvesting (Figure 3). The least lipid percentage was observed in zinc stress treatment (4th day) which was higher than the peak lipid percentage obtained in BBx3 treatments. The highest lipid percentage of all treatments was obtained on the 14th day of experiments with Zinc stress medium whereas the lowest was recorded on the 8th day of experiment with BB medium. The percentage of lipid was ranging around 10% throughout all harvesting days in the BBx3 treatment. The lipid percentage of both nitrogen stress and salinity stress treatments were consistently increasing till the 14thday of experiment and showed a significant drop on the final day. This is due to the cell disruption triggered by lack of nutrients. The results of the previous studies also confirmed the primitive role of salinity stress in triggering the lipid production in microalgae species. Microalgae accumulate lipids according to the growth conditions and environmental stress (Gour *et al*., 2019)

As shown in Figure 4, the decline of nitrogen in media reduces the biomass production after 14 days. But it is still a substantial yield due to addition of nutrients on the 8th day of the experiment. The lipid production rate of the nitrogen stress media increases with the continued starvation that agrees with the finding of literature where they state nitrogen starvation could induce neutral lipid accumulation in *Chlorella* Sp*.* (Zhu *et al*., 2016). Microalgae use as biodiesel feedstock should ideally show high biomass productivity and efficient biosynthesis of lipids, i.e., it is not the single parameter (lipid content or growth

rate) but the volumetric lipid productivity that should be the main criterion for choice of feedstock for biodiesel production (Zhu *et al*., 2016

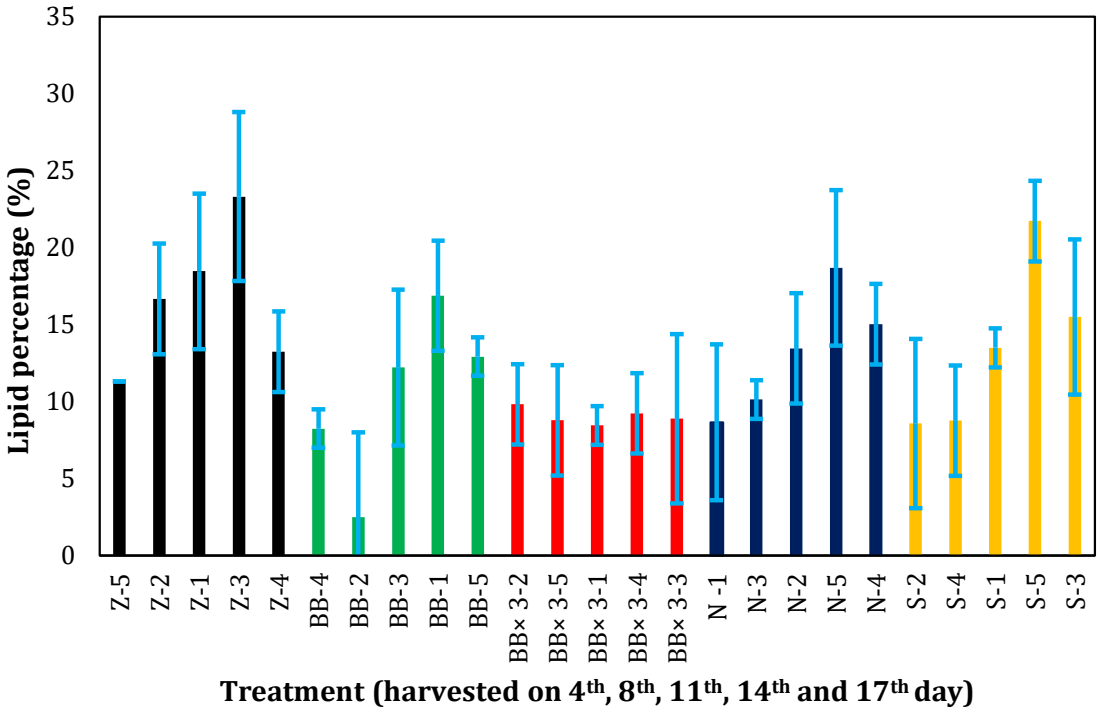

Figure 4: Lipid percentage in the treatments on different harvesting days. (Labels on the bars indicate the randomly collected pouch number in each treatment. Replicates from left to right are 4th, 8th, 11th, 14th and 17th harvesting days respectively.)

Generally, all the kinds of lipids in microalgae cannot be converted into fatty acid methyl esters (FAME), for example, fatty acids without O-linkage won't be converted into FAME. All types of fatty acids with *O*-ester linkages and Free Fatty Acids (FFA) can be converted almost quantitatively into the corresponding methyl esters in one-step reactions (Ichihara and Fukubayashi, 2010). Therefore, the conversion of microalgae lipids into FAME is a measure of microalgae lipids that are convertible into FAMEs. The available fatty acids are an important factor in biofuel production as well as in promoting value added products in industries such as food and pharmaceuticals. The fatty acids which were present in the 05 different treatments are listed below in Table 2.

Table 3: Major fatty acids and their percentages in different treatments obtained by FAME analysis

Name of the Fatty Acid	Major fatty acid Percentages					Standard Deviation
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	
Caproic acid (C6:0)	6.76	6.97	2.55	0.64	1.32	±3.02
Undecanoic acid (C11:0)	3.51	3.42	5.28	4.01	0.00	±1.96
Oleic acid (C18:1n9c)	38.32	36.20	39.87	45.38	43.83	±3.82
Linoleic acid (C18:2n6t)	20.24	2.50	34.52	22.05	4.21	±13.38
cis-11-Eicosenoic acid (C20:1)	0.00	7.70	0.00	0.00	0.00	±3.44
Heneicosanoic acid (C21:0)	0.75	22.86	6.77	11.05	22.67	±9.79
cis-11,14-Eicosadienoic acid (C20:2)	7.55	5.99	2.09	2.86	12.37	±4.12
Cis-8,11,14-Eicosatrienoic acid (C20:3n6)	6.64	0.00	0.00	0.00	0.00	±2.97
Arachidonic acid (C20:4n6)	7.30	1.79	1.35	1.64	3.99	±2.86

A total of 22 fatty acids have been identified and 8 of them were present in all the treatments at least in a minute percentage. Oleic acids have recorded the highest percentage in all the treatments where it hits the peak in nitrogen stressed treatment (45.38%). According to the literature, the Oleic acid is present in a variety of food resources around a percentage of 50% of the total fatty acid concentration and hasn't found any significant level of toxicity (Acid *et al*., 1987). Moreover, in a previous paper (Shim *et al*., 2018), the authors state it can be used as a biofuel by following deoxygenation, and the biofuel produced by Oleic acid using the CoMo-SG catalyst exhibited

a calorific value (10,119 cal/g) that was similar to that of commercial diesel (10,180 cal/g). The percentage of Oleic acid (45.38%) in this study is comparatively higher than that reported by Zhu *et al*. (2015) where they obtained 12 – 35% of lipids under Nitrogen stress with *Chlorella zofingiensis*. Oleic acid is considered ideal for biodiesel because it has better cold flow properties without losses to oxidative degradation. (Zhu *et al*., 2015). The Linoleic acid showed significant percentages over 20% in 03 treatments whereas the highest percentage of 34.52% was observed in Zinc stress treatment. Earlier studies revealed that linoleic acid is the highly consumed poly unsaturated fatty acid in human diet (Whelan and Fritsche, 2013) and it is also convertible into ethanol thus into biofuel. Because of the higher percentages of significant fatty acids, the stressing conditions can be helpful in producing biofuel efficiently.

Biomass productivity and lipid productivity

The highest average biomass productivity 0.060±0.017 $gL^{-1}d^{-1}$ was observed in BBx3 treatment where it had an extra amount of nutrients compared to other four treatments which were responsible for biomass production (Figure 5). The lowest biomass productivity 0.044±0.021 $gL^{-1}d^{-1}$ was recorded in Nitrogen stress media because the nutrients such as N, P, K play a major role in producing the biomass in microalgae (Mahmood and Khudhair, 2017). The coefficient of variation for the average biomass productivity values for BB, BBx3, Zn stress, Nitrogen stress and Salinity stress are 3.75, 3.57, 2.76, 2.06, and 3.90 respectively, which are on the lower side meaning that the results show more consistency.

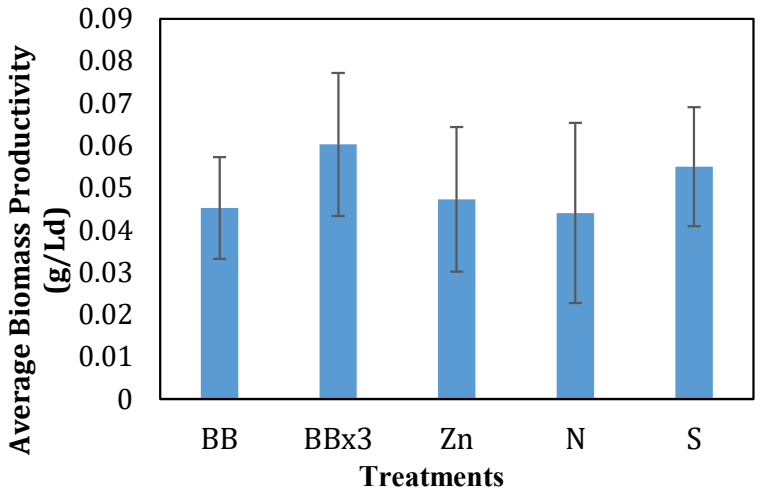

Figure 5: Average biomass productivity of Chlorella sp. microalgae in different treatments

As shown in Figure 6, the highest average lipid productivity 0.008±0.005 $mgL^{-1}d^{-1}$ was achieved in Zn stress treatment because of the high stressful conditions which force microalgae to store their food in the form of lipids to sustain in unfavourable conditions. Although, BB and BBx3 treatments recorded almost similar results 0.005±0.003 $mgL^{-1}d^{-1}$ and 0.005±0.110 $mgL^{-1}d^{-1}$ respectively suggesting the favourable conditions produce less amount of lipids compared to extreme conditions. An important observation in the present study is that the lipid content and the lipid productivity cannot be achieved in a same treatment. Thus, our study provides considerable insights into the enhancement of lipid content with respect to stress period.

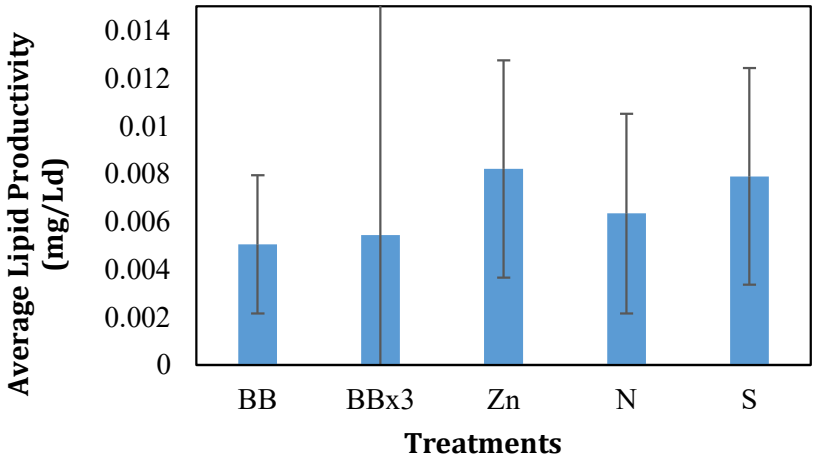

Figure 6: Average biomass productivity of Chlorella sp. microalgae in different treatments

Conclusion

The present study confirmed that the lipids in *Chlorella* Sp. can be enhanced by introducing various stress conditions in the culture media. It was clearly demonstrated that the biomass productivity and lipid productivity can be achieved in two consecutive phases in the same bioreactor configuration. Enhancement of biomass productivity required optimum addition of nutrients. Whereas, stressful conditions were required to enhance the lipid content and lipid productivity, Zn-stressed biomass exhibited the highest lipid percentage of 23.3% and the highest average lipid productivity of 0.008±0.005 mg lipids $L^{-1}d^{-1}$. The extracted lipids from the biomass yield contained important fatty acids such as Oleic acid and Linoleic acid in all treatments. All the treatments confirmed a substantial amount of biomass yield and lipid yield around 14^{th} day of the experiment. Therefore, it can be suggested to perform the cultivation in stressful conditions for 14 days to get maximum efficiency.

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