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BIOMASS AND LIPID PRODUCTIVITY BY TWO ALGAL STRAINS OF *Chlorella Sorokiniana* GROWN IN HYDROLYSATE OF WATER HYACINTH

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ABSTRACT

Hydrolysate prepared from water hyacinth biomass, containing a considerable amount of solubilised carbohydrate and nutrients, was utilised as a medium for the cultivation of two strains of *Chlorella sorokiniana*. These strains were isolated from an oxidation pond using two different media, i.e., BG-11 and Knop's media maintained at pH-9. Different light intensities, light-dark cycles, and various concentrations of external carbon sources (monosaccharides and inorganic carbon) were used to optimise the microalgal growth. It was observed that in the presence of organic carbon (glucose), biomass productivity increased significantly (~300 $mgL^{-1}day^{-1}$) as compared to that in the presence of only inorganic carbon (~100 $mgL^{-1}day^{-1}$). For the accumulation of stress products (lipids and carbohydrates), the microalgal strains were transferred to nutrient-amended media (N-amended and P-amended). The combined effects of glucose, inorganic carbon, and a 12h:12h light-dark cycle proved to be optimum for biomass productivity. For *Chlorella* sp. isolated from BG-11, maximum carbohydrate content (22%) was found in the P-amended medium, whereas high lipid content (17.3%) was estimated in the N-amended medium. However, for *Chlorella* sp. isolated from Knop's medium, both the lipid (17%) and carbohydrate accumulation (12.3%) were found maximum in the N- amended medium. Kinetic modelling of the lipid profile revealed that kinetic coefficients obtained for strain isolated from BG-11 media were statistically significant from each other.

Keywords: Hydrolysate; *Chlorella sorokiniana;* lipid; biomass productivity; nutrient amended media.

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1. INTRODUCTION

In recent thrust of searching renewable source for developing various chemicals and fuels, biomass takes a prominent role as a versatile feedstock for developing those chemicals and fuels simultaneously. Biomass feedstock is converted to various chemicals and energy using either thermochemical or biochemical route [1]. To use the biochemical route, one needs to produce media in which specialised microorganisms can grow to produce some of these chemicals or fuels [2]. To develop such medium, chemical and enzymatic treatment of biomass are being applied [3]. Such treatments solubilise the complex sugars present in the biomass to monomers and dimers and also some extent release nitrogen and phosphorus present in biomass. However, providing a constant supply of biomass is a challenge for most of the biomass based biochemical production facilities. Developing a dedicated feedstock increases the cost of the biomass and thus increases cost of the produced biochemicals. In this regard, biomass grown in waste and marginal land and biomass grown using wastewater can provide a cheap source for continuous supply of biomass. Among the various sources of waste biomass, algae and water hyacinth biomass are being chosen as the biomass of interest in this study.

Water hyacinth biomass is amenable to chemical treatment and may not require costly enzymatic hydrolysis for enhanced sugar recovery. Various acids and electrochemical treatments were used for treating the water hyacinth biomass [4,5]. Treatment conditions such as pressure, temperature, and concentration of hydrolysing agents affect sugar recovery which varies from 130-155 mg of sugar/g of treated biomass.

Hydrolysate prepared from biomass has been demonstrated to produce various value-added chemicals, including fuels using different microbial strains [6,7]. However, nutrient contents also play a vital role in the production of value-added products. Chowdhury and his co-workers [8- 10] showed that nutrient recovery from biomass and its utilisation along with waste nutrients increased biofuel production considerably. Produced biofuel has reduced energy

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demand and GHG emission. Hence, nutrient recovery from biomass should also be taken into account to evaluate the efficacy of a hydrolysis procedure.

Microalgae is one of the attractive routes of producing value added products by growing it on hydrolysate. Microalgae can accumulate various value-added products in the form of starch, protein, lipid, as well as, various pigments which have high market value. Types of value-added products depend on the algal species used, as well as, the various micronutrients present in the hydrolysate and the stress conditions applied. In this study, an attempt was undertaken to study the value-added products formation by algal strains isolated from a local oxidation pond used to treat municipal wastewater. Starch and lipid are stress induced products and during production of such products growth of microalgae is reduced considerably. Hence, a two-step strategy was applied where in the first stage, algae was grown in a nutrient sufficient condition (N, P in sufficient concentration) followed by a nutrient amended condition (either N or P or both are amended). It is assumed that due to introduction of two stage the overall biomass productivity and value-added products formation is increased. Under the nutrients sufficient condition, several compositions of the media were increased one at a time to understand their effects on the biomass productivity. Concentration of value added products in the form of lipid and its composition depends on the stress condition induced by nitrogen or phosphorus starvation or both. Hence, in this study, effects of nutrients starvation were studied in details. Either N or P starvation was applied to estimate their effects on lipid quantity and fatty acid composition. To understand further the kinetics of fatty acid elongation, rate limiting steps in fatty acid elongation and unsaturation, kinetic model was developed and implemented.

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2. MATERIALS AND METHODS

2.1 Screening and identification of algal strains.

For the isolation of algal species, a water sample was collected from a municipal wastewater stabilization pond situated near Lakkarghat, Rishikesh, Uttarakhand, India. The sample was serially diluted for screening and isolating the pure colonies of algal cells. Algal colonies were grown in two different media i.e., the modified BG-11 medium and Knop's medium maintained at pH-9 and a constant temperature (25 °C), supplemented with an antibiotic and antifungal cocktail. (The compositions of both the media are given in the supplementary information, Table S 1 and Table S 2). Genomic DNA of the isolated algal strains was extracted by using MB561 HiPurA™ Marine Algal DNA Purification kit. The selected algal strain was identified using 18S ribosomal DNA (rDNA) sequence. The 18S rDNA isolated algae (BG 11) was characterised, and procedural details are presented in Rani et al. [11]. Fragment of 18S rDNA region of the algal strain isolated from the Knop's medium was amplified by PCR. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out using NS1- GTAGTCATATGCTTGTCTC and NS4 - CTTCCGTCAATTCCTTTAAG primers. Sequencing was done using Sanger sequence chromatogram to generate reverse sequence data, forward sequence data, reverse complement, and G consensus data. The resulting data sequence was aligned using a combination of NCBI GenBank, and calculated the BLAST hits with respect to the query sequence. The evolutionary history was inferred by using UGENE software.

2.2 Biomass Growth

A synthetic growth medium was prepared with similar constituents of the hydrolysate obtained by hydrolysing the water hyacinth biomass with HNO_3 (Supplementary information, Table S 3). The isolated microalgae species were grown in the synthetic hydrolysate medium

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under different conditions (such as different light intensity, different carbon source and forms of monosaccharides, metal ion, etc.). The study was conducted in three different phases.

In the first phase, enhancement of microalgae growth was investigated by varying light intensity and medium supplementation. Each of these runs was carried out for 10 days under continuous illumination. Nitrate- N (75 mgL^{-1}) and phosphate-P (15 mgL^{-1}) were kept constant throughout the study. The growth pattern was checked every 24 hours (Table 1).

Table 1: Different parameters used in Phase 1

S. No.	*Medium supplement (mg/L)*	*Light intensity (µmol/sec/m^2)*
1.	Only Glucose (50)	140
2.	Only Glucose (100)	140
3.	Only Glucose (50)	221
4.	Inorganic-C source (75/day) + Glucose (50)	221
5.	Only Inorganic-C source (75/day)	221
6.	Inorganic-C (75/day) + Glucose (50) + $FeCl_3$ (10)	221

The second phase included the use of different forms of monosaccharides to increase the algal biomass in a 12 h:12 h light-dark cycle for 10 days. The growth pattern was monitored every 12 hours. Nitrate-N (75 mgL^{-1}) and phosphate-P (15 mgL^{-1}) concentrations were kept constant throughout the study. The light intensity was also kept constant at 221 $\mu molsec^{-1}m^{-2}$, which was found optimum in the 1st phase of the study. Details of the parameters used in this study are presented in Table 2. The experiments were carried out in duplicate.

The third phase was divided into two parts; 1st part: nutrient sufficient media (composition was derived from the optimum parameters obtained from the above two studies) was used to enhance the algal growth. Algae were grown in a 12h:12h light-dark phase for 6 days. The nutrient sufficient media contained glucose (50 mgL^{-1}), inorganic C (75 $mgL^{-1}day^{-1}$), nitrate-N (60 mgL^{-1}), NH_4-N (15 mgL^{-1}) and phosphate-P (15 mgL^{-1}). The second part emphasised the

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enhancement of lipid accumulation by transferring the algal biomass under the nutrient amended conditions. Two different media were prepared (i) **N-amended medium** containing 5 mgL^{-1} of NH_4-N, glucose (50 mgL^{-1}), and inorganic C-source (125 mgL^{-1}), (ii) **P-amended medium** containing 3 mgL^{-1} of PO_4-P, glucose (50 mgL^{-1}), and inorganic C- source (125 mgL^{-1}). The experiments were executed in duplicate, and the average value was used for the study.

Table 2: Different medium supplements and their concentrations used in Phase 2. (12 h :12 h light-dark cycle, Nitrate-N = 75 mg/L, Phosphate-P = 15 mg/L, Light intensity = 221 µmol/sec/m^2)

S. NO.	Medium supplement (mg/L)
1.	Only Glucose (5/day)
2.	Only Xylose (5/day)
3.	Glucose (5/day) + Inorganic-C (75/day)
4.	Xylose (5/day) + Inorganic-C (75/day)
5.	Only Inorganic-C source (75/day)

2.3. Analytical methods

2.3.1. Determination of biomass growth

To determine cell density, optical density of algal cells was measured at 750 nm using a UV-VIS spectrophotometer (DR 6000, HACH). Algal biomass concentration was also measured as TSS (Total suspended solids) using a standard gravimetric method [12].

2.3.2. FAME analysis

Transesterification of lipids extracted from a culture grown in the hydrolysates was executed using a modified transesterification process. Lipids were transesterified in a mixture of solvents containing 0.5 mL methanol and 2.5 mL HCl. The solvent mixture and biomass were heated at 100 °C for 60 min. After cooling it to room temperature, its fatty acid methyl esters (FAMEs)

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were retrieved using hexane. The hexane phase was then transferred to a glass vial for Gas Chromatography (Shimadzu GC- 2014) equipped with a flame ionization detector (FID) [10].

2.4. **Kinetic Modeling**

Two kinetic models were developed for two strains used in this study. One needs to develop two kinetic model as one model could not fit well with data for another strain. The fatty acid precursor and compositions were also different for each of the strain.

Chlorella sorokiniana (Knop's media):

$$\frac{dP_1}{dt} = -K_1$$

$$\frac{dP_2}{dt} = K_1 - K_2P_2$$

$$\frac{dP_3}{dt} = K_2P_2 - K_3P_3$$

$$\frac{dP_4}{dt} = K_3P_3 - \frac{K_4}{P_4}$$

$$\frac{dP_5}{dt} = \frac{K_4}{P_4}$$

Chlorella sokiniana (BG -11 media):

$$\frac{dP_2}{dt} = -K_2$$

$$\frac{dP_3}{dt} = -K_3P_3 - K_5P_3 + K_2$$

$$\frac{dP_4}{dt} = K_3P_3 - K_4/P_4$$

$$\frac{dP_5}{dt} = \frac{K_4}{P_4}$$

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$$\frac{dP_6}{dt} = K_5 P_3$$

Where P_1 stands for C14:0, P_2 stands for C16:0, P_3 stands for C18:0, P_4 stands for C18:1, P_5 stands for C18:2 and P6 stands for C20:0.

3. RESULTS AND DISCUSSION

3.1 Identification and characterisation of microalgae

As described in section 2.1 for the BG-11 medium, the algal species were characterised as *Chlorella sorokiniana*, and the details are given in Rani et al. [11]. For the algae strain grown in Knop's medium, gel electrophoresis results showed a discrete PCR amplicon band of 1250 bp. The fragment of the 18S rDNA was amplified using primers NS1 and NS4 (details are given in 2.1.) and then sequenced. The resulting sequences obtained were searched using BLAST program with the sequences available in the NCBI (National Centre for Biotechnology Information) database. Phylogenetic trees of the strains were constructed using UGENE software. Both the strains were found to be genetically similar to *Chlorella sorokiniana.*

3.2 Coupled effect of light intensity and carbon source on biomass productivity of *C. sorokiniana*

In this phase, microalgal growth was optimised using different parameters, as described in 2.5.1. Feng et al. [13] reported a decrease in biomass productivity at a high nitrate concentration. Nitrate in high concentration may be toxic to the microalgae species. Blair et al. [14] observed no toxic effect of nitrate on biomass productivity of *Chlorella vulgaris*. Whereas in the present study, nitrate was used as the sole source of nitrogen, and no such detrimental effect of nitrate on biomass productivity was observed. Six different combinations of the parameters used in this study are described in Table 1.

The biomass productivity of both the algal strains of *C. sorokiniana* was found to be dependent on the light intensity and carbon sources (Table 3a and 3b).

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For the algal strain isolated from the modified BG-11 medium: Higher biomass productivity of 39.8 $mgL^{-1}day^{-1}$ was found in the growth medium containing 100 mgL^{-1} of glucose (Table 3a). It was observed that low light intensity might affect the growth rate. Hence, for the subsequent studies, the light intensity was increased to 221 $\mu molsec^{-1}m^{-2}$ from 140 $\mu molsec^{-1}m^{-2}$. According to Sacristan de Alva et al. [15] adequate light intensity contributes to the enhanced cell growth as the light energy increases CO_2 assimilation and enhances the cell dry weight.

Table 3a: Change in cell density of *Chlorella sorokiniana* isolated from BG-11 (pH- 9) grown in synthetic hydrolysate under different light intensities and carbon sources.

S. No.	Medium supplement (mg/l)	Light intensity ($\mu mol/sec/m^2$)	Biomass productivity (mg/l/day)
1.	Only glucose (50)	140	31.49 ±0.03
2.	Only glucose (100)	140	39.81 ±0.03
3.	Only glucose (50)	221	56.77 ±0.02
4.	$Nahco_3$ (75/day) + glucose (50)	221	153.46 ±0.04
5.	Only $nahco_3$ (75/day)	221	43.55 ±0.03
6.	$Nahco_3$ (75/day) + glucose (50) + $FeCl_3$ (10)	221	24.13 ±0.01

Biomass productivity of *C. sorokiniana* increased with the increase in light intensity, and estimated biomass productivity was 56 $mgL^{-1}day^{-1}$ in a 50 mgL^{-1} of glucose dose. Optimum light stimulates the greater rate of synthesis of critical components required for the cell division of *Chlorella* strains [16].

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Supplementation of growth media with the inorganic carbon dose of 75mg/L/day increased the biomass productivity further to 153 $mgL^{-1}day^{-1}$. The growth rate and lipid production can be controlled via regulating the availability of essential nutrients and trace elements [17,18]. Various ions, such as iron and magnesium, are essential for cellular mechanisms, including photosynthesis, cell division, respiration, intracellular transportation, and protein synthesis in microalgae [19,20]. However, in the present study, supplementation of metal ion (iron) with organic and inorganic carbon has a detrimental effect on biomass productivity as it decreased to 24 $mgL^{-1}day^{-1}$ from 153 $mgL^{-1}day^{-1}$ (Table 3a). Wan et al. [21] reported that if the iron concentration in a medium is more than 55 mg/L, it can reduce the biomass growth of *Chlorella sorokiniana*. However, in our study, we observed a toxic effect of iron in a much lower dose as compared to the one reported by Wan et al. [21]. Besides iron, daily doses of inorganic carbon without the addition of organic carbon also reduced the biomass productivity to 43 $mgL^{-1}day^{-1}$ from 153 $mgL^{-1}day^{-1}$ (Table 3a). According to TSS measurements, the combined effects of the organic carbon and inorganic carbon in the presence of a high light intensity showed higher biomass productivity. Sun et al. [22] observed the positive effect of $NaHCO_3$ on the biomass productivity of algae. However, the addition of CO_2 along with $NaHCO_3$ further improved biomass productivity. Hence, it is most likely that a supply of CO_2 or organic carbon is the key behind the improved biomass productivity.

For the algal strain isolated from Knop's medium: This strain showed higher biomass productivity as compared to the previous strain (BG-11). The highest biomass productivity of 160 $mgL^{-1}day^{-1}$ was recorded in the growth medium containing 50 mgL^{-1} of glucose under the light intensity of 140 $\mu molsec^{-1}m^{-2}$. In a 100 mgL^{-1} of glucose dose, the biomass productivity decreased to a 106 $mgL^{-1}day^{-1}$ from a 160 $mgL^{-1}day^{-1}$ (Table 3b).

Light intensity was increased from 140 $\mu molsec^{-1}m^{-2}$ to 221 $\mu molsec^{-1}m^{-2}$ for improving biomass productivity. As with 50 mgL^{-1} of glucose dose, the estimated biomass productivity

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was 163 $mgL^{-1}day^{-1}$. An increase in light intensity could not improve biomass productivity further. However, the addition of inorganic carbon source along with organic carbon (glucose) enhanced biomass productivity by 10 mgL^{-1} (biomass productivity: 170 $mgL^{-1}day^{-1}$). On the other hand, daily doses of only inorganic carbon (75 $mgL^{-1}day^{-1}$) in the growth media resulted in biomass productivity of 90 $mgL^{-1}day^{-1}$ (Table 3b). Hence, both the carbon sources (inorganic and organic) are required for enhanced biomass productivity. This observation indicates that organic carbon helps the algal cells to reduce their dependency on light for inorganic carbon assimilation and channelise the harvested light energy in the form of ATP and NADPH for cell growth [23]. Lin and Wu [24] reported that glucose enhanced the acetyl CoA/malonyl CoA for cell synthesis. These observations also suggest that *Chlorella sp.* utilises the energy from light in the non-glucose added mode and uses the organic carbon source available for cell anabolism to obtain a remarkably higher growth rate [25]. As carbon uptake is the main mechanism for energy conversion and utilisation by microalgae, the consumption rate of carbon would be directly affected by nutrient supply, and further control microalgal biomass production [26,27].

In addition to the carbon source, various trace elements also affect cell metabolism. Various ions, such as Ca^{2+}, Fe^{3+}, Cu^{2+} are the key factors involved in the physiological metabolism of algae and increased the biomass yield and oil content at a low concentration [20, 28]. However, in the present study (Table 3b), the addition of ferric ion showed a detrimental effect on biomass productivity (74 $mgL^{-1}day^{-1}$ of biomass production).

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Table 3b- Change in cell density of *Chlorella sorokiniana* isolated from Knop's medium (at pH- 9) grown in synthetic hydrolysate under different light intensities and carbon sources.

S. No.	Medium supplement (mg/l)	Light intensity (µmol/sec/m^2)	Biomass productivity (mg/l/day)
1.	Only glucose (50)	140	106.26 ±0.01
2.	Only glucose (100)	140	160.31 ±0.03
3.	Only glucose (50)	221	163.57 ±0.01
4.	Na_2co_3 (75/day) + glucose (50)	221	170.14 ±0.01
5.	Only na_2co_3 (75/day)	221	90.65 ±0.04
6.	Na2CO3 (75/day) + glucose (50) + $FeCl_3$ (10)	221	74.72 ±0.01

The most common growth mode for microalgae is autotrophic cultivation using CO_2 and light, while in the heterotrophic mode, algae only utilise organic compounds without light illumination [26, 29]. However, according to the results observed in the present study, the combined effects of light intensity, and organic and inorganic carbon resulted in better algal biomass productivity for both the algal strains. Lin and Wu [24] also reported that the specific growth rate of *C. vulgaris* under mixotrophic growth condition was significantly higher than the sum of those from photoautotrophic and heterotrophic growth, illustrating synergistic growth mechanisms. Mixotrophy comprises a dual system in which the autotrophic process utilises light energy and converts the light energy into chemical energy via photosynthesis, and the heterotrophic process facilitates catabolism of organic compounds to obtain energy essential for cell division [25, 27]. On the other hand, carbon dioxide evolved from cellular

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respiration becomes available for reutilisation in photosynthesis under light conditions [26]. External CO_2 supply is therefore reduced.

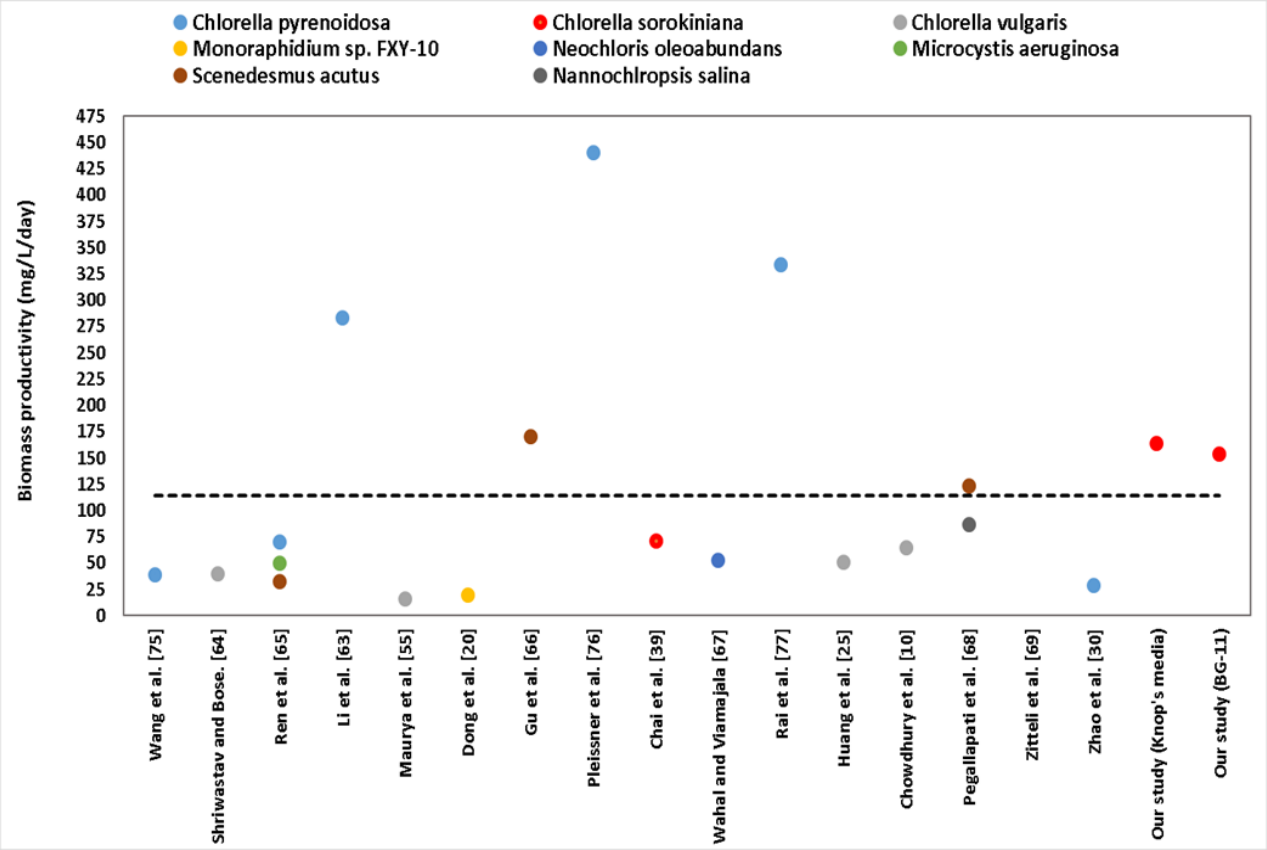

Figure 1: Comparison of biomass productivity of different algal species with the present study *(Dashed Line represents the average value).*

A comparative study of biomass productivity was carried out to evaluate the attractiveness of biomass productivity of the present strain as compared to the other strains reported in the literature (Fig. 1). The biomass productivity in this study was higher than the average biomass productivity of previous studies, which states that our study showed better results. If we evaluate only the biomass productivity of *Chlorella sp*., our study shows improved biomass production. It ultimately demonstrates that hydrolysate medium is an attractive growth medium for algal biomass production. One more advantage of using hydrolysate medium is that it is cheap as compared to the other growth medium and is produced from the waste source.

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3.3. Effect of monosaccharide supplementation to the hydrolysate growth medium on *C. sorokiniana* biomass productivity

In the previous experiment, various doses of organic and inorganic carbon were added to understand their effects on biomass productivity. The hydrolysate prepared from waste biomass contains organic carbon in the form of C5 and C6 sugars. The hydrolysate also contains various forms of nitrogen, especially ammonium nitrogen. Hence, the effects of C5 sugars (xylose) and ammonium- N were evaluated based on biomass productivity of the two algal strains.

Ammonium is a growth substrate for many oleaginous microalgae [30]. Microalgae usually prefer ammonium over other nitrogen sources for intracellular metabolic activities [31]. In general, when nitrogen is supplied as nitrate, conversion of nitrate to ammonium consumes a considerable amount of energy as compared to the uptake of ammonium [10]. Hence, uptake of nitrogen as nitrate is energy intensive and may reduce algal growth. Also, Rani et al. [11] observed higher uptake of ammonium nitrogen by *Chlorella sorokiniana* as compared to nitrate. So, in further study, the growth medium was supplemented with two different nitrogen sources, i.e., Nitrate-N (60 mgL^{-1}) and Ammonium-N (15 mgL^{-1}). Phosphate-P was kept at 15 mgL^{-1}. Sufficient P supply ensures unhindered ATP production, which ultimately ensures algal growth [32, 33, 34].

Microalgae have a much higher growth potential when they use organic carbon for growth [35]. The uses of exogenous carbon sources with either heterotrophic (without light) or mixotrophic (with light) culture modes have been reported to increase the biomass of several microalgal species [36, 37, 38]. The biomass growth rate and lipid content of *Chlorella* strains can be raised by supplementing the culture medium with organic carbon sources [39]. Various organic carbon sources, including polysaccharides, disaccharides, monosaccharides, starch and ammonium acetate, have been used for microalgal cultivation, but systematic comparison data between primary monosaccharides are limited. Hence, in this study, effect of two different

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monosaccharides i.e. glucose (C6 sugar) and xylose (C5 sugar) were analysed with respect to the optical density of media along with light/dark treatments to develop a cultivation mode that enhances biomass production. Monosaccharides and inorganic carbon sources were added every 24 hours to the growth medium (with a concentration of 5 mg $L^{-1}day^{-1}$ and 75 $mgL^{-1}day^{-1}$, respectively, details are given in Table 2). The light intensity was also kept constant at 221 $\mu molsec^{-1}m^{-2}$, which was optimum according to the 1st phase of the study as explained in 3.2.

For the microalgae isolated from BG-11: The highest biomass productivity (38 mg $L^{-1}day^{-1}$) was observed in the growth medium supplemented with glucose along with an inorganic carbon source (Table 4a). Biomass productivity in the growth medium supplemented with xylose was found to be low (13 $mgL^{-1}day^{-1}$). In the absence of monosaccharides (control), the cultures displayed the typical green colour. Whereas, supplementation of xylose in the growth medium resulted in the discolouration of the culture, which could be due to the decline in the microalgal biomass. However, supplementation with glucose turned the microalgal culture to a deep green colour as compared to the control, which showed the increase in the algal biomass and chlorophyll content (data not shown).

Table 4a: Change in cell density of *Chlorella sorokiniana* isolated from BG-11 medium pH 9 when grown in synthetic hydrolysate supplemented with different monosaccharides under 12 h:12 h of light: dark cycle (Light Intensity- 221 µmol/sec/m^2).

S. No.	Medium supplement (mg/l)	Biomass productivity (mg/l/day)
1.	Glucose (5/day) + $nahco_3$ (75/day)	38.36 ±0.02
2.	Xylose (5/day) + $nahco_3$ (75/day)	13.42 ±0.05
3.	Only $nahco_3$ (75/day)	27.13 ±0.01

Some researchers like Ribeiro et al. [40] and Derner et al. [41] reported that the mixotrophic action of *Chlorella* sp. is possible due to the photoperiod cycles, as the light

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availability is directly related to the inorganic carbon consumption. Similarly, in this study, the 12h:12h light-dark cycle is responsible for the uptake of $NaHCO_3$. In the mixotrophy, organic carbon, such as glucose, provides auxiliary energy in the form of Acetyl-CoA, NADPH and other intermediates for the cellular biosynthesis [22, 40].

Algae isolated from Knop's medium: The highest biomass productivity (187 $mgL^{-1}day^{-1}$) was found in the growth medium supplemented with glucose (10 $mgL^{-1}day^{-1}$) along with inorganic carbon source (Table 4b), whereas in the growth medium supplemented with xylose, the biomass productivity was found to be less (134 $mgL^{-1}day^{-1}$). Biomass productivity was estimated to be 150 $mgL^{-1}day^{-1}$ when the growth media was supplemented with inorganic carbon (Na_2CO_3) only. The biomass productivity under inorganic carbon supplement was higher than the one reported in the presence of xylose+Na_2CO_3 and lower than that observed under the glucose+Na_2CO_3 supplement. Hence, from the different mixtures of organic and inorganic carbon, it was observed that C5 sugar reduced the growth of the algal strain, whereas the C6 sugar increased the biomass productivity (Table 4b). Our results are corroborated by the findings of Chai et al. [39], who investigated the effect of glucose, galactose, fructose, and xylose on the growth of *Chlorella sorokiniana* UTEX 1230. They reported that supplementation with fructose promoted *C. sorokiniana* UTEX 1230 growth to a much lesser extent as compared to glucose. Supplementation with galactose had no effect, and supplementation with xylose inhibited the growth. According to the results, xylose supplementation had no effect on the growth of *C. sorokiniana* UTEX 1230 grown in dark, whereas xylose significantly inhibited algal growth under light conditions [39].

Table 4b: Change in cell density of *Chlorella sorokiniana* isolated from knop's medium pH- 9 when grown in synthetic hydrolysate supplemented with different monosaccharides under 12 h:12 h of light: dark cycle (Light Intensity- 221 µmol/sec/m^2).

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S. No.	Medium supplement (mg/l)	Biomass productivity (mg/l/day)
1.	Only glucose (10/day)	160.28 ±0.04
2.	Only xylose (10/day)	92.15 ±0.09
3.	Glucose (10/day) + na_2co_3 (75/day)	187.08 ±0.06
4.	Xylose (10/day) + na_2co_3 (75/day)	134.61 ±0.03
5.	Only na_2co_3 (75/day)	150.43 ±0.02
6.	Glucose (200)	204.32 ±0.04

As compared to the strain isolated from the BG-11 medium, algae isolated from Knop's medium showed improved biomass productivity. After observing such an encouraging result, a one-time dose of glucose was also tried to check its role on biomass productivity. An initial dose of 200 mgL^{-1} glucose increased the biomass productivity of the strain to 204 mg L^{-1}. For a low glucose dose of 10 mg $L^{-1}day^{-1}$, the lower dry weight production suggested that lower contents of glucose might have been assimilated and converted to energy directly leading to cell growth. A low glucose dose could not improve the production of storage compounds that can increase biomass dry weight. Thus, both glucose and inorganic source contributed to the algal growth and likely play a cummulative effect. Whereas, xylose had a negative impact on algal growth.

Several researchers have evaluated the effect of monosaccharide supplementation on the biomass productivity of various strains of *Chlorella* species. Woodworth et al. [42] found that the biomass productivity of *C. vulgaris* cultured under mixotrophic condition (with glucose supplementation of 20 gL^{-1}) was higher as compared to autotrophic and heterotrophic cultures. Similarly, Li et al. [43] investigated the impact of glucose on *C. protothecoides* and applied a two-step process in which glucose was added in the first step and then removed in the second. Glucose addition in the process was found to influence biomass productivity and lipid

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accumulation, chlorophyll content, protein, and starch levels. Hawkins et al. [44] and Chai et al. [39] reported that *C. sorokiniana* utilised glucose as the primary monosaccharide as compared to other sugars such as xylose, galactose, and fructose. Glucose, being the primary metabolic fuel, is more stable than other monosaccharides and less susceptible to the formation of nonspecific glycol conjugates [39]. A perusal of these literature, it was observed that the effect of glucose on biomass productivity of several *Chlorella sp*. matched with our results obtained in the present investigation. So, among C5 and C6 sugar, C6 sugar (Glucose) was found to be the better monosaccharide for the growth of *Chlorella sorokiniana* in terms of biomass productivity.

3.4. Effects of different parameters on *C. sorokiniana* lipid productivity and FAME composition

After optimisation of different cultivation conditions, the microalgal biomass was grown in a 3 L photobioreactor using the best parameters for growth obtained from the previous study, i.e., the combination of glucose (50 mgL^{-1}) and inorganic C-source (75 mgL^{-1}) in 221 $\mu molm^{-2}sec^{-1}$ light intensity. As N, P content was high in the growth medium; it is not possible to produce value-added products in the form of lipid and carbohydrate. However, increasing lipid accumulation by subjecting them to suboptimal conditions negatively affected lipid productivity due to the low growth [45]. Hence, in this study, after a growth in nutrients sufficient condition (6 days), the algal biomass was transferred to the nutrient amended medium for the value-added product formation (7 days). A similar strategy was also adopted previously by various researchers [46, 47]. As described in section 2.3.3., the nutrient sufficient media in this study consists of glucose (50 mgL^{-1}), Inorganic C-source (75 $mgL^{-1}day^{-1}$), Nitrate-N (60 mgL^{-1}), NH_4-N (15 mgL^{-1}) and phosphate -P (15 mgL^{-1}). The study was conducted under 12h:12h light-dark phase.

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The biomass productivity in the photobioreactor under the nutrient sufficient condition was found to be 73 $mgL^{-1}day^{-1}$ for the algae isolated from the BG-11 (Table 5), which was lower than the one obtained during our previous study (Table 3 a and 3 b). Nitrogen and phosphorus content was measured on the 7th day, and the nitrate-N decreased to 0.082 mgL^{-1} from 60 mgL^{-1}, Ammonium-N content was found to be 0.019 from the initial amount of 15 mg L^{-1}, Phosphate-P content decreased to 0.837 mgL^{-1} from 15 mg L^{-1}. For the strain isolated using the Knop's medium, the biomass productivity was found to be 267 mg $L^{-1}day^{-1}$ (Table 5). The nitrogen and phosphorus contents were measured on the 7th day, and the nitrate-N decreased to 1.66 mgL^{-1} from 60 mgL^{-1}, Ammonium-N content decreased to 0 from the initial amount of 15 mg L^{-1}, Phosphate-P content decreased to 1.2 mg L^{-1} from 15 mg L^{-1}.

Table 5: Change in cell density of *C. sorokiniana* isolated from BG-11 and Knop's media (pH- 9) grown in synthetic hydrolysate growth medium with supplementation of N and P to accumulate large amount of lipid.

S. No.	Growth media	Biomass productivity (mg/l/day)	
		BG-11	**Knop's media**
1.	Nutrient sufficient medium	73.08 ±0.02	267.11 ±0.03
2.	N-medium	58.52 ±0.01	94.37 ±0.02
3.	P-medium	82.97 ±0.01	178.12 ±0.03

The second part of this study emphasises the enhancement of lipid accumulation by transferring the algal biomass into the nutrient amended conditions. Two different media were prepared (details are given in section 2.3.3). The first nutrient amended medium (referred as N amended) contains an initial amount of 5 mg L^{-1} ammonium-N with no phosphate-P in the medium. The other nutrient amended medium (referred as P- amended) had an initial amount of 3 mg L^{-1}

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phosphate-P without any nitrogen source. The carbon sources were regularly added (5 mgL^{-1} of glucose and 125 mgL^{-1} $NaHCO_3$).

The biomass productivity was found to be higher in the P-amended medium for both the algal strains. *C. sorokiniana* (BG-11) registered an 82.9 $mgL^{-1}day^{-1}$ biomass productivity (Table 5), and the other strain (the Knop's medium) showed biomass productivity of 178 $mgL^{-1}day^{-1}$ (Table 5). Whereas in the N- amended medium, biomass productivity was found to be 58.5 $mgL^{-1}day^{-1}$ (Table 5) and 94.3 $mgL^{-1}day^{-1}$ for the algal strains of BG-11 and Knop's media respectively.

Contrary to biomass productivity, the lipid accumulation was higher in the N- amended medium than the P-amended medium for both the algal strains (Table 6 and Table 7). **For, the algal strain isolated from BG-11**, lipid accumulation increased to the highest (17.2%) on the 5th day in the N-amended medium. In the P-amended medium, the highest (9.9%) lipid production was observed on the 3rd day (Table 6).

Table 6: FAME composition of *Chlorella sorokiniana* isolated from BG-11 pH- 9 cultured in nutrient amended media. (3,5 and 7 denotes the culture time in day, and N and P represent the N-amended medium and P- amended medium respectively).

FAME component (%)	0-day	3-N	3-P	5-N	5-P	7-N	7-P
C16:0	3.062 ±0.005	4.822 ±0.004	3.990 ±0.001	6.738 ±0.008	3.325 ±0.005	2.682 ±0.001	2.019 ±0.004
C18:1	2.7 ±0.3	2.9 ±0.1	0.7 ±0.3	2.5±0.1	1.0±0.2	2.9±0.2	0.4±0.1

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C18:2	2.3±0.1	1.2 ±0.2	3.5±0.2	5.0±0.2	3.1±0.2	3.3±0.3	1.23 ±0.09
C18:0	0.42 ±0.04	2.2±0.2	1.1 ±0.1	2.11 ±0.04	0.66±0.09	0.31±0.04	0.32 ±0.03
C20:0	0	0.45 ±0.04	0.59 ±0.03	0.9 ±0.1	0.183 ±0.006	0.144 ±0.006	1.0 ±0.2
Total	8.7±0.5	11.6 ±0.5	9.9 ±0.7	17.2 ±0.4	8.3 ±0.5	9.3±0.6	5.0 ±0.4

Lipid productivity is one of the indicators for selecting an algal strain for biodiesel production. Hence, lipid productivity for both the strains was estimated. It was observed that for the BG-11 medium, lipid productivity (taking into account biomass productivity in the nutrient sufficient condition) was 20 and 13 $mgL^{-1}day^{-1}$ for the N and P- amended media, respectively. On the other hand, the algal strain isolated from the Knop's medium registered lipid productivity of 30 and 33 $mgL^{-1}day^{-1}$ for the N and P-amended media, respectively. Hence, although lipid production was low in the Knop's medium strain, however, because of high biomass productivity in the P- amended medium, the strain showed higher lipid productivity than the other strain.

Several other studies have also been conducted on lipid production using various species of algae [10, 48, 49, 50, 51, 52]. Some of these studies showed very high biomass productivity and lipid content, whereas some studies showed either low biomass productivity or low lipid content [10, 48, 49]. Among the previous studies, lipid productivity and content reported by Shi

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et al. [49] was the most striking. Shi et al. [49] studied a marine algae *Micractinium inermum*, which could grow in phototrophic, mixotrophic and heterotrophic conditions and showed lipid productivity as high as 0.68 $gL^{-1}day^{-1}$ in the heterotrophic condition. Similarly, a *Chlorella vulgaris* strain studied by a research group at the University of Minnesota showed that the strain could grow on three modes autotrophic, mixotrophic and heterotrophic. The largest lipid productivity, however, was observed in the mixotrophic mode (0.23 $gL^{-1}day^{-1}$), and the largest lipid content was observed when the culture was fed solely by glucose (40% lipid content) [53]. In autotrophic and heterotrophic modes, the lipid content varied from 8- 25%. Similarly, Gladue and Maxey [54] observed that in the presence of acetate *Chlorella vulgaris* could produce 0.027 $gL^{-1}day^{-1}$ lipid. Maurya et al. [55] used hydrolysate produced from algal biomass as a feed for producing algal biomass (*Chlorella vulgaris*). In the present study, supplementation of algal hydrolysate increased the lipid content in the algal cells (depending on the concentration of algal hydrolysate in the synthetic media), and lipid content varied from 23% to 32%. However, lipid productivity (0.007 $gL^{-1}day^{-1}$) was very low compared to the other studies. To evaluate the attractiveness of the present strains, lipid productivity of other species reported in the literature was collected and presented in Fig. 2. Average productivity of 100 $mgL^{-1}day^{-1}$ was estimated from lipid productivity obtained from the literature . However, the high productivity was because of a few species of *Scenedesmus* and *Micractinium.* These species showed extremely high lipid productivity (500- 2000 mg $L^{-1}day^{-1}$) [56]. However, if one considered only the species of *Chlorella*, the average lipid productivity was 49 mg $L^{-1}day^{-1}$, whereas species of *Nannochloropsis* registered lipid productivity of 120 mg $L^{-1}day^{-1}$. Various species of *Botryococcus* showed an average lipid productivity of 32 mg $L^{-1}day^{-1}$. Hence, the strains used in this study showed below- average lipid productivity as compared to the average lipid productivity observed in the literature (Fig. 2). However, most of the species showed lipid productivity ranging between 5- 50 mg $L^{-1}day^{-1}$. Hence, to understand the attractiveness of the

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present strain as a biodiesel feedstock, a frequency distribution of the lipid productivity was plotted (data are not shown). A frequency distribution revealed that the most common lipid productivity obtained from the literature was between 20 to 40 mg $L^{-1}day^{-1}$. Hence, the analysis showed that the present strains showed lipid productivity, which is frequently encountered for most of the species, and these species showed a higher than average biomass productivity.

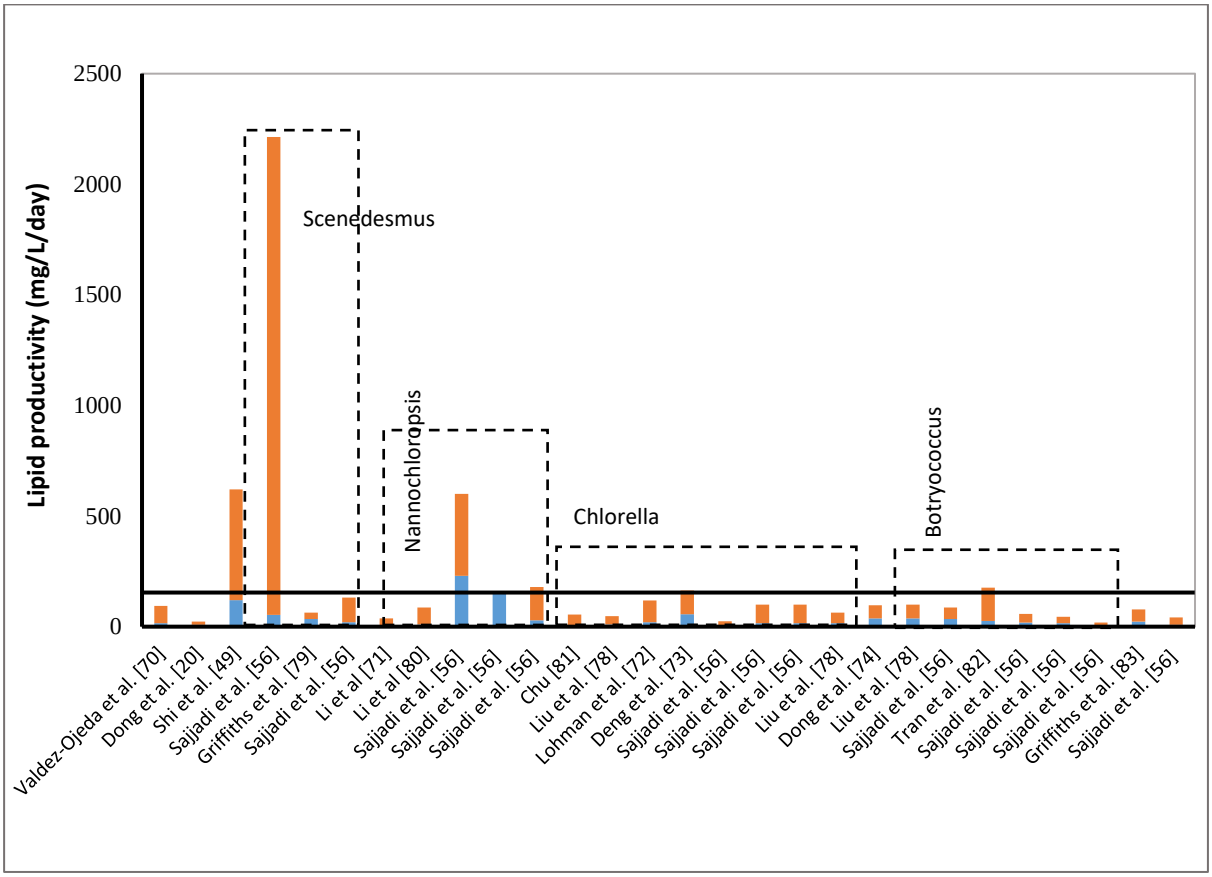

Figure 2: Lipid productivity of various species reported in the literature. *Two data bars (blue and orange) showed lowest and highest productivity of a particular species as given in that reference.*

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Beside lipid productivity and lipid content, lipid composition, and individual fatty acid components also affect the biodiesel quality. Hence, fatty acid profiles of individual strains were also evaluated in detail.

In the case of algal strain isolated using BG 11 medium: In the N-amended medium (5th day 5N), the saturated fatty acids (SFA) comprised of 9.8%, and total FAME content was 11.56%. At the end of the 5th day the total FAME content increased by 5% as compared to the Day-0. Polyunsaturated fatty acid (PUFA) increased by 3% as compared to the day 0 (Table S4, Supplementary Information). Lipid content declined after 5-days as only 9.3% and 5% of lipid production was estimated on the 7th day in the N-amended medium (7-N) and P-amended medium (7-P), respectively (Fig. 3). The SFA comprised of C16:0 (Palmitic acid) C18:0 (Stearic acid) and C20:0 (Eicosanoic acid). C16:0 constituted the significant fraction of SFA. C18:1 and C18:2 were the major fractions of unsaturated fatty acid.

Contrary to lipid accumulation, the highest carbohydrate content was recorded in the P-amended medium at the end of the study (22%).

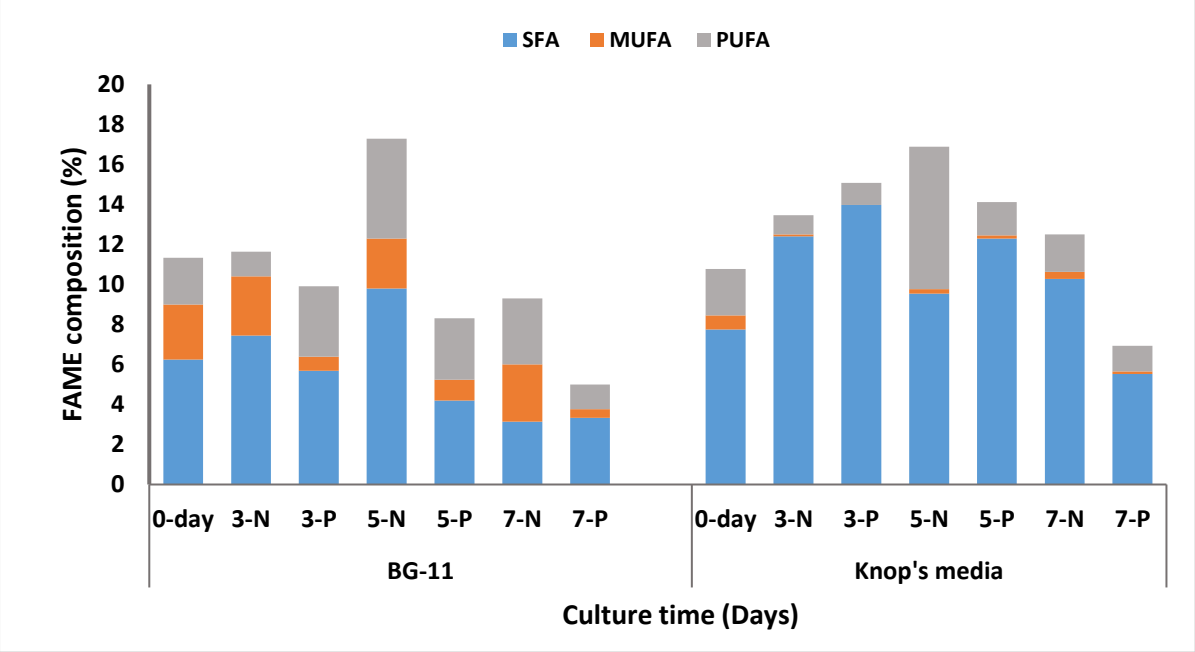

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Figure 3. FAME composition of *Chlorella sorokiniana* isolated from BG-11 and Knop's media (at pH- 9) cultured in nutrient amended media.

In the case of algal strain isolated using the Knop's medium: Lipid accumulation on the initial day (referred to as 0-day) was 10.87% (Fig. 3) in which 72% represents the SFA. After that, the lipid accumulation increased in both the media. On the 3rd day, (referred as 3-N and 3-P) accumulated lipid was 13.43% and 15.09% in the N- amended medium and P- amended medium, respectively (Table 7). 92% of the total FAME composition consisted of SFA (Table S 5, supplementary information). The highest lipid content was observed on the 5th day with 16.88% and 14.07% in the N- amended medium (referred as 5-N) and P- amended medium (referred as 5-P), respectively (Fig. 3). In the N- amended medium (5th day), the content of PUFA was 42% of the total FAME composition and was found to be the highest as compared to the other days. Whereas, SFA content was estimated to be 56% of the total FAME, and the rest was monounsaturated fatty acid (MUFA). However, in the P- amended medium, 88% of the total FAME was SFA, and the rest were MUFA and PUFA. Lipid content declined after 5 days as only 12.51% and 6.92% of lipid was estimated on 7th day in the N medium (7-N) and P medium (7-P), respectively (Table 7). In the N - amended medium (5th day), the total FAME content was the highest as compared to other days but the composition of SFA alone was less. Whereas in the P- amended media, the SFA alone comprised the maximum FAME composition, whereas MUFA and PUFA were only about 1-2%. At the end of the study, the carbohydrate content was estimated to be higher in the N- amended medium (12.7 %) than the P- amended medium (3.13%). This result was found to be contrary to the algae isolated from the BG-11 medium.

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Table 7: FAME composition of *Chlorella sorokiniana* isolated from Knop's medium pH-9 in nutrient amended media. (3,5 and 7 denotes the culture time in days, and N and P represent the N-amended medium and P- amended medium respectively).

FAME COMPONENT (%)	0-DAY	3-N	3-P	5-N	5-P	7-N	7-P
C14:0	0.32 ± 0.03	1.1 ± 0.8	1.01 ± 0.09	0.54 ± 0.05	1.1 ± 0.2	0.6 ± 0.2	0.31 ± 0.01
C16:0	5.1 ± 0.7	7.8 ± 0.7	10 ± 1	7 ± 1	8 ± 1	7.6 ± 0.7	3.8 ± 0.3
C18:1	0.78 ± 0.04	0.057 ± 0.008	0	0.22 ± 0.02	0.16 ± 0.01	0.36 ± 0.04	0.13 ± 0.02
C18:2	2.3 ± 0.2	1.0 ± 0.4	1.1 ± 0.3	7 ± 14	1.66 ± 0.09	1.87 ± 0.08	1.27 ± 0.02
C18:0	2.3 ± 0.4	3.48 ± 0.03	3.4 ± 0.4	2.1 ± 0.6	2.9 ± 0.5	2.1 ± 0.2	1.43 ± 0.08
TOTAL	10 ± 1	13 ± 1	15 ± 2	17 ± 3	14 ± 2	12 ± 1	6.9 ± 0.4

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According to the biodiesel standard EN 14214, IS 15607 and ASTM D-6751, the linolenic methyl ester (produced from C18:3 FA) content of biodiesel must be less than 12%, and the polyunsaturated methyl ester (at least four double bonds) was less than 1% [57]. In the present study, for both the algal strains, the proportions of linolenic acid were 0, which satisfied the standard of biodiesel. As compared to the present strains, Yu et al. [58] reported that marine *Phaeodactylum tricornutum* showed enhanced lipid productivity under nitrogen deprived conditions. Whereas, *Isochrysis zhangjiangensis* showed enhanced lipid productivity under phosphorus deficient condition, which is in accord with our observation. Fu et al. [59] observed that phosphorus supplementation enhanced the synthesis of saturated fatty acids while shifting the fatty acid pathways. Zhu et al. [60] and Singh et al. [61] also discussed in their work that all nitrogen limited conditions led to an increasing proportion of C18:1. In the present study, similar results were observed for the algal strain isolated from Knop's medium when grown in nutrient amended medium especially P- amended medium. The major fraction of the saturated fatty acid was C16:0, while a minor fraction of C14:0 was also observed. However, for the other strain (BG-11), no C14:0 fatty acid was detected (Table 6 and Table 7). A considerable portion of C18:0 was also detected in the strain isolated from Knop's medium, making it more suitable for producing biodiesel as compared to the other strain (BG-11).

From a review of this study, it was observed that the constituents of FAME were different in the two strains, and the most striking difference was found with respect to the saturated and unsaturated fatty acids. To better understand the inherent difference in the fatty acid production, kinetic modeling of the fatty acid profile of these strains was carried out. Before presenting a kinetic model, one needs to know the various fatty acids, which are being used to produce neutral lipids and free fatty acids. In this study, C14:0 (myristic acid) was the first fatty acid produced, which later elongated to C16:0 and C18:0. These saturated fatty acids were produced from shorter chain fatty acids by delta elongase enzyme (Fig. 4). The saturated fatty acids were

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later converted to unsaturated fatty acids, and delta desaturase enzyme was used to catalyse the conversion. However, depending on the position of unsaturated bond, different desaturase enzymes act on particular fatty acids. For simulating such a conversion, it was assumed that the kinetics was first order (Section 2.4). The algal strain isolated from the BG-11 medium produced C16:0, C18:0, C 20:0 as SFA and C18:1, C18:2 as the unsaturated fatty acid. Hence, C16:0 worked as the precursor for the production of other fatty acids, and the rate of production of C16:0 was assumed as a zero-order kinetics. Other rate constants were modeled as the first order (details of the model are provided in material and methods section). The algal strain isolated from the Knop's medium C14:0 appeared to act as the precursor for producing other higher-order chain fatty acids.

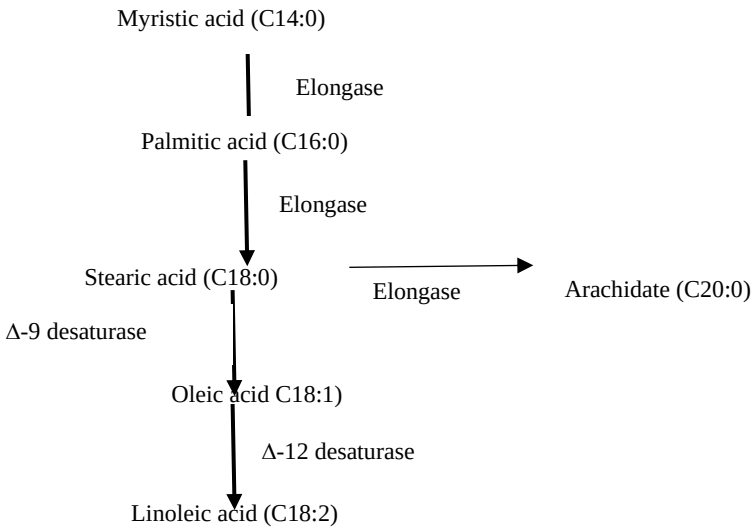

Figure 4: Pathway involved in the present study for the lipid formation.

A perusal of the kinetic coefficients obtained from the models, no definite trend was observed. Some of the kinetic coefficients were higher in the algal strains isolated from BG 11 media, as compared to the algal strain isolated from Knop's media. Maximum kinetic coefficient for BG 11 and Knop's media was for the conversion of C16:0 to C18:0 and C18:0 to C18:1 (K_3). Prema facie it seems that the conversion was bit higher in BG-11 media as compared to Knop's media.

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Similarly, some of the kinetic coefficients are quite different in the N amended medium as compared to the P – amended medium. Therefore, to understand the statistical difference among the kinetic coefficients, one way ANOVA followed by Tukey's test for multiple comparison of means were undertaken. A persual of the ANOVA, it was observed that kinetic coefficients for the strain isolated from Knop's media were statistically significant (p=0.05). Later Tukey's test revealed that K_3 was statistically significant as compared to other kinetic coefficients (Table 8). However, no such difference was observed between kinetic coefficients obtained using the N- amended and P – amended media. Similarly, no such statistical significance was observed between kinetic coefficients obtained for the strains isolated from Knop's and BG-11 media. Hence, the statistical analysis revealed that kinetics of lipid production for the strains are the same, eventhough there was some difference observed in lipid quantity and lipid profile between the two strains. It can also be deduced that as the C18:0 production and conversion to C18:1 were quite fast, hence, the rate limiting steps for lipid production was most likely production of C:14 and C:16 and their conversion (for algal strain isolated from Knop's media).

Table 8: Kinetic coefficients of interconversion of various fatty acids.

KINETIC	**KNOP MEDIUM**		**BG 11 MEDIUM**	
PARAMETERS (D^{-1})	N amended	P amended	N-amended	P amended
K_1	0.28±0.02**	0.34 ± 0.04**	NA	NA
K_2	0.15 ± 0.05	0.27 ± 0.02	0.25 ± 0.01**	0.14 ± 0.01**
K_3	0.7 ± 0.5	0.5 ± 0.1	1.0 ± 0.3	0.9 ± 0.2
K_4	4 E-05 ± 1E-05*	7E-06 ± 2E-06 *	2.77E-04 ± 5.06E-06*	5.1E-05 ± 0.4 E-05*
K_5	NA	NA	0.19 ± 0.05	0.4 ± 0.1

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To better understand the lipid production, biochemical processes responsible for lipid production were also examined. Lipid production is a stress- induced phenomenon. Under the nitrogen depleted condition, adenosine monophosphate (AMP) can be decomposed into IMP and $NH4^{+}$ ions, which are catalysed by AMP deaminase as a way to release ammonia. The decrease of AMP, a co-substrate for isocitrate dehydrogenase catalysing the transformation of isocitrate to ⍺-ketoglutarate, will disturb the Kreb's cycle and, as a result, the mitochondrion accumulates isocitrate that remains imbalanced with citrate. Excess non-metabolized citrate is then transported to the cytoplasm via the citrate/malate shuttle to be converted to acetyl- CoA by ATP-citrate lyase (ACL), which is an additional pathway in oleaginous microorganisms. Under conditions of nitrogen exhaustion, the flow of carbon in oleaginous microorganisms is transferred towards the accumulation of citric acid, which is later transformed to acetyl-CoA, a precursor for fatty acid synthesis. The present study appears to support these observations as nitrogen amended media seemed to trigger the lipid accumulation in both the strains.

When the availability of nitrogen is restricted, the synthesis of proteins and nucleic acids is curtailed. Carbon used for protein and nucleic acid synthesis used for lipids synthesis, and its concentration increases. Therefore, enhancing the activity of acetyl-CoA synthetase (ACS) and reducing the activity of isocitrate dehydrogenase (ICDH), resulted in more acetyl-CoA to participate in the lipid biosynthetic pathway, which was suggested to be beneficial for lipid accumulation [22]. Acetyl-CoA carboxylase (ACCase) converts acetyl-CoA into malonyl-CoA, the first and rate-limiting step of fatty acid biosynthesis [22]. Phosphoenolpyruvate can be converted into oxaloacetate by phosphoenolpyruvate carboxylase (PEPC), which decreases the flow of carbon towards lipid biosynthesis in microalgae. Accordingly, inhibition of PEPC may be beneficial for lipid production.

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In the P-amended medium, phosphorus was stored in the form of Poly-P, providing energy to participate in the synthesis of cellular materials, which enhanced cell growth and lipid accumulation, similar to ATP provided in the anabolic processes under nitrogen starvation [59, 62]. According to the previous literature, the following potential mechanisms may be involved during the presence of phosphorus: (1) nitrogen limitation causes upregulation of malic enzyme generating NADPH, which leads to lipid accumulation [63]; (2) upregulated glutamate dehydrogenase promotes the tricarboxylic acid cycle that offers intermediates and energy for lipid biosynthesis [31]; and (3) abundant phosphorus intake causes the downregulation of ADP-glucose pyrophosphorylase activity of *Chlorella sp.*, inhibiting starch synthesis, and regulating carbon assimilation towards the lipid synthesis pathway [60].

In the present study, nitrogen deficiency certainly inhibited the TCA cycle for energy production, and a part of the organic carbon participated in the lipid and starch production. It was observed that presence of phosphorus affects the fatty acid synthesis and conversion of saturated fatty acids to unsaturated fatty acids.

4. CONCLUSIONS

The combined effects of glucose, inorganic carbon, and 12h:12h light-dark cycle were the optimum combination of parameters for the high biomass productivity. Both strains showed different biomass and lipid productivity. For *Chlorella* sp. isolated from BG-11, maximum carbohydrate content (22%) was observed in the P-amended medium, and high lipid content (17.3%) was observed in the N-amended medium. Whereas, in the case of *Chlorella* sp. isolated from the Knop's medium, both high lipid (17%) and carbohydrate (12.7%) accumulation was found in the N-amended medium. Biodiesel produced from both the strains had a suitable quality, which satisfies the biodiesel standards specified by various international regulating agencies. Biomass productivity of both the strains exceeded the average productivity of algal species previously reported in the literature.

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