

PRODUCTION OF MICROALGAL BIOMASS AT DIFFERENT GROWTH PHASES TO USE AS BIOFUEL FEEDSTOCK

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Abstract

The growth of microalgae under optimized conditions was determined for assessing their growth rate and biomass production. In this study, the growth of both green algae (*Chlamydomonas noctigama* and *Chlorella vulgaris*) and cyanobacteria (*Anabaena variabilis* and *Nostoc spongiaeforme*) was measured as optical density. *Chlamydomonas noctigama* and *Chlorella vulgaris* showed the doubling time of 9.5 and 8.0 hours, respectively, whereas *Anabaena variabilis* and *Nostoc spongiaeforme* showed the doubling time of 14.8 and 16.6 hours, respectively. All the species exhibited the highest growth in terms of biomass at the phase in between stationary and death phases.

Keywords: Green algae, Cyanobacteria, Biofuel feedstock, Growth phase

Introduction

Concerns about the shortage of fossil fuels, increasing crude oil price, energy security, environment deterioration, and accelerated global warming have led to growing worldwide interests in renewable energy sources such as biofuels (Griffiths and Harrison 2009, Hanjalic *et al.* 2008). Bioethanol is a non-conventional fuel produced by the process of saccharification and fermentation from the bio-renewable sources, including sugars, starches, and ligno-cellulosic materials from solid wastes and plant biomass, including algal biomass, whereas biodiesel (monoalkyl esters) is one of such alternative fuel, obtained by the transesterification of triglyceride oil with monohydric alcohols (Pothiraj *et al.* 2015, Jasim and Maysam 2014, Blinova *et al.* 2015, Dvoretzky *et al.* 2015, Al-lwayzy *et al.* 2014, Christi 2007). Algal biomass, considered one of the most promising third generation biofuel feedstocks, was reported earlier by many investigators (Kumar *et al.* 2013, Agwa *et al.* 2012, Nigam and Singh 2011, Li *et al.* 2008).

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Microalgae, having massive, diversified characteristics, are emerging to be one of the most promising long-term, sustainable sources of biomass and fuel, food, feed, and other co-products (Strop 2014, Agwa *et al.* 2012, Milledge 2011). However, the potential for these natural resources as biofuel feedstock and other probable uses are assessed to some extent in relation to the techno-economic aspect (Quinn and Davis 2015). A few thousand algal species are reported to occur in Bangladesh's fresh water and marine environment in (Ahmed *et al.* 2008). Jones and Mayfield (2012) and Spolaore *et al.* (2006) reported a minimal commercial-scale cellulosic ethanol production because of the higher cost of production (almost twice than that ethanol production from corn). In view of the aforementioned issues, microalgae are gaining wide attention as an alternative renewable source of biomass for the production of bioethanol and biodiesel, which is grouped under 'third-generation biofuel' (Safi *et al.* 2014, Nigam and Singh 2011).

Algae can efficiently use CO₂ and are responsible for more than 40% of the global carbon fixation, with the majority of this productivity coming from marine microalgae. They are easy to grow and cultivate anywhere with fewer energy requirements and use very few nutrients following the appropriate culture designs and systems (Nwankwo and Agwa 2019, Sharma *et al.* 2011, Ugwu and Aoyagi 2012). Algal strains can produce biomass very rapidly, with some species doubling in as few as 6 hours and many exhibiting two doublings per day (Hannon *et al.* 2010). However, the ideal growth conditions for microalgal cultures are strain-specific and requires specific natural and supplement conditions where microalgae respond with physiological alterations to the environmental growth conditions, e.g., pH, light, temperature, aeration, nutrients, and accessible supplements (Nwankwo and Agwa 2019, Schenk *et al.* 2008). Higher productivity is usually considered an attribute to focus on the biochemical composition and growth characteristics of algal strains. High level of demand for clean, safe, and low-cost biomass production from selected strains requires to analysis on algal physiological response, i.e., growth under their optimum growth conditions with the possible potentials and challenges (Quinn and Davis 2015, Kim *et al.* 2014, Chia *et al.* 2013, González-Fernández *et al.* 2012, Pienkos and Darzins 2009).

Materials and Methods

Growth curves for *Chlamydomonas noctigama*, *Chlorella vulgaris*, *Anabaena variabilis* and *Nostoc spongiaeforme* were made using growth as µg chlorophyll per mL (chl *a* and chl *b* for green algae, and chl *a* for cyanobacteria) at respective optimum conditions to define the different phases of growth as described by Vonshak and Maske (1982).

Various optimum conditions considered as baseline for growth at different phases are given in Table 1 (Ali *et al.* 2016, Tarin *et al.* 2016).

Table 1. Optimum growth conditions for green algae and cyanobacteria.

| Microalgae | | Optimum growth conditions | | | | | |
|--------------------|------------------------------------|---------------------------|---|---------------------------------|-------------------|---------------------------|-----------------------|
| | | pH | Light intensity ($\mu\text{E m}^{-2} \text{s}^{-1}$) | Temp. ($^{\circ}\text{C}$) | Aeration (hrs) | Nutrient element conc. | Vitamin supplement |
| Green algae | <i>Chlamydomonas noctigama</i> | 6.5 | 110 | 25 | 72 | 2.0 \times Chu 10D | B1+B6 |
| | <i>Chlorella vulgaris</i> | 6.5 | 110 | 25 | 72 | 1.5 \times Chu 10D | B6 |
| Cyano- bacteria | <i>Anabaena variabilis</i> | 7.0 | 90 | 25 | 72 | 1.0 \times Chu 10D | Not required |
| | <i>Nostoc spongiaeforme</i> | 7.5 | 70 | 25 | 72 | 1.0 \times Chu 10D | Not required |

Growth rate and doubling time determination: Growth rate has been expressed in terms of the relative growth constant or specific growth constant (μ) (Fogg 1975):

$$\mu = \frac{\log_{10} N_t - \log_{10} N_0}{t}$$

where, t = Time in the hour

N_t = Biomass after t hour

N_0 = Biomass at "0" time

The maximum growth rate is defined as the maximum growth rate under light saturation at a specified temperature. The mean generation time or doubling time (g) has been calculated from a specific growth constant, μ :

$$\text{Doubling time, } g = 0.0301 / \mu \text{ (Fogg 1975)}$$

Estimating O.D. (Optical Density): Growth was also estimated by measuring the optical density at 750 nm using a Shimadzu digital spectrophotometer (model UV-120-01) as described by Rodolfi *et al.* (2009).

Production of green algae and cyanobacteria biomass at the 3 phases of growth under optimum conditions: All the respective optimum conditions (i.e., pH, light intensity, temperature, aeration, nutrient element concentration in medium and vitamin supplement) were provided to produce both green algae (*Chlamydomonas noctigama* and *Chlorella vulgaris*) and cyanobacteria (*Anabaena variabilis* and *Nostoc spongiaeforme*) biomass at

their 3 phases of growth (i.e., logarithmic phase, stationary phase, and in between of stationary and death phases) where unialgal cultures were used.

Harvesting and processing of microalgal biomass: The microalgae were harvested by centrifugation at 5000 rpm for 10 minutes using a Kokusan refrigerated centrifuge (model H-103N).

The wet algal biomass (harvested on petri dish) was dried in an oven at 60°C for at least 24 hours. Then the dried biomass was scrapped and stored in a plastic jar.

Result and Discussion

Estimation of growth: From the respective growth curves (Fig. 1) of microalgae used in the experiment, acceleration phase, logarithmic phase, deceleration phase, stationary phase and death phase was observed and the duration of different phases are presented in the following Table 2.

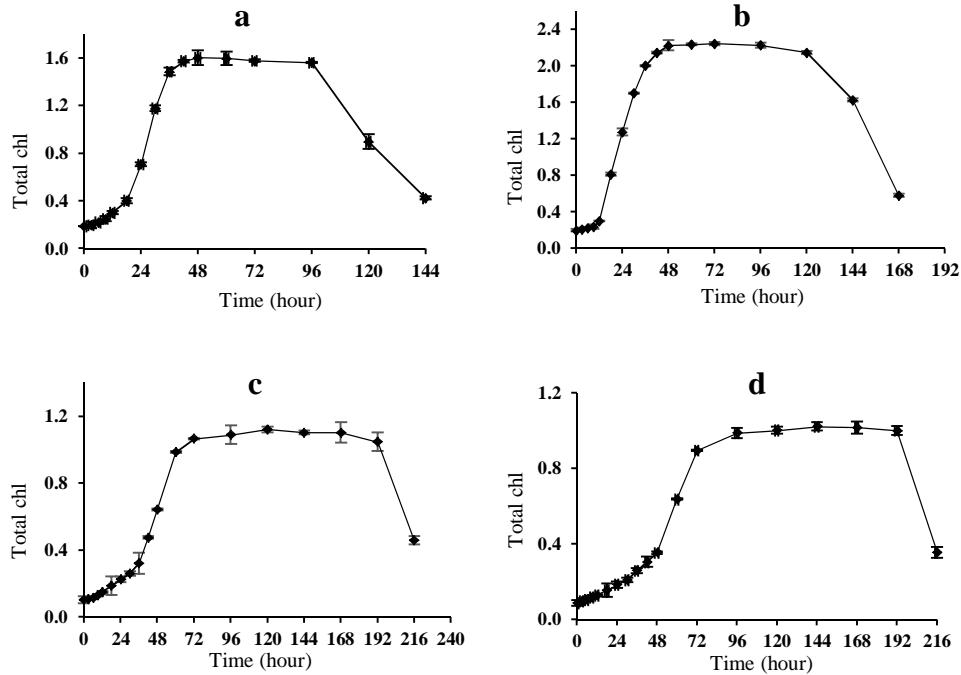


Fig. 1. Growth curve of *Chlamydomonas noctigama* (a), *Chlorella vulgaris* (b), *Anabaena variabilis* (c) and *Nostoc spongiaeforme* (d).

There was no lag phase or initial phase in the growth curve, which may be for the reason that fresh algal strain was taken for the experiment. Cyanobacteria took more time to show the respective phase than green alga (Table 2).

Table 2. Growth phases of microalgae.

| Microalgae | Growth phase with Duration (hrs) | | | | |
|--------------------------------|----------------------------------|-------------------|--------------------|------------------|-------------|
| | Acceleration phase | Logarithmic phase | Deceleration phase | Stationary phase | Death phase |
| <i>Chlamydomonas noctigama</i> | 0-18 | 18-36 | 36-42 | 42-96 | 96- |
| <i>Chlorella vulgaris</i> | 0-12 | 12-36 | 36-48 | 48-120 | 120- |
| <i>Anabaena variabilis</i> | 0-36 | 36-60 | 60-72 | 72-192 | 192- |
| <i>Nostoc spongiaeforme</i> | 0-48 | 48-72 | 72-96 | 96-192 | 192- |

Before the log-growth phase, all the microalga started to be divided towards growth by 18, 12, 36, and 48 hrs, respectively. Moreover, both the green alga had the logarithmic growth within 36 hrs, whereas *A. variabilis* increased by 60 hrs and *N. spongiaeforme* showed growth exponentially by 72 hrs. Following the maximum growth phase, *Chlamydomonas noctigama* appeared at the deceleration phase within 42 hrs and *Chlorella vulgaris* showed that within 48 hrs where the cyanobacteria (*A. variabilis* and *N. spongiaeforme*) took the slowing down phase in 72 and 96 hrs, correspondingly. The periods in between stationary and death phase were found as 42-96, 48-120, 72-192 and 96-192 hrs for *Chlamydomonas noctigama*, *Chlorella vulgaris*, *A. variabilis* and *N. spongiaeforme*, separately after which the isolates started to be dead.

Growth rate and doubling time determination: The growth rate and doubling time of *Chlamydomonas noctigama*, *Chlorella vulgaris*, *Anabaena variabilis* and *Nostoc spongiaeforme* are given below in Table 3.

Table 3. Growth rate and doubling time of *Chlamydomonas noctigama*, *Chlorella vulgaris*, *Anabaena variabilis* and *Nostoc spongiaeforme*.

| Microalgae | Growth rate (μ), (hr^{-1}) | Doubling Time (g), (hrs) |
|--------------------------------|---|--------------------------|
| <i>Chlamydomonas noctigama</i> | 0.0316 | 9.5 |
| <i>Chlorella vulgaris</i> | 0.0037 | 8.0 |
| <i>Anabaena variabilis</i> | 0.0020 | 14.8 |
| <i>Nostoc spongiaeforme</i> | 0.0181 | 16.6 |

The doubling time (g) of *Chlamydomonas noctigama* was 9.5 hrs which was similar to the finding of Hemaiswarya *et al.* (2013). Again, *Chlorella vulgaris* exhibited a doubling time of 8 hrs. In contrast, Maxwell *et al.* (1994) showed that during the exponential phase of growth *Chlorella vulgaris* had a doubling time of 8.6 hrs at a temperature of 27°C. The reason behind the less time for doubling shown by *Chlorella vulgaris* may be the trend of fast-growing with the optimum environmental conditions. *Anabaena variabilis* exhibited a doubling time of 14.8 hrs while different strains of *Anabaena* showed a doubling time of 18-24 hrs observed by Prasanna *et al.* (2006). Meeks *et al.* (1983) observed that the doubling time of *Anabaena* 7120 and *A. cylindrical* in nitrogen free BG11 medium was 21.5 and 18.2 hrs, respectively. Besides, *Nostoc spongiaeforme* had a doubling time of 16.6 hrs which was less than that reported by Rodriguez *et al.* (1986) for *Nostoc* sp.

Production of Chlamydomonas noctigama, Chlorella vulgaris, Anabaena variabilis, and Nostoc spongiaeforme biomass at the 3 phases of growth under optimum conditions: The biomass of microalgae (*Chlamydomonas noctigama*, *Chlorella vulgaris*, *Anabaena variabilis* and *Nostoc spongiaeforme*) (as mg/L) at 3 different phases of their growth under respective optimum conditions were presented in Fig. 2. The test of significance of different treatment means was computed by Duncan's New Multiple Range Test (DMRT) at a 5% level. The result was statistically significant at the 5% level.

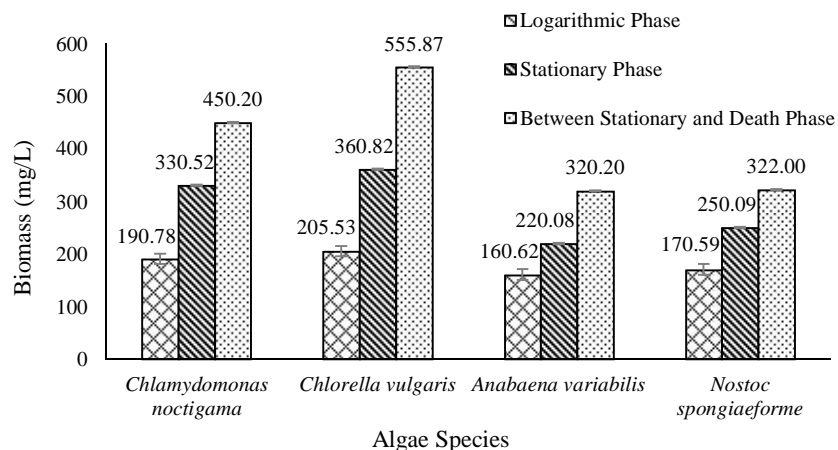


Fig. 2. Microalgal biomass (as mg/L) at 3 different growth phases under respective optimum conditions.

The result showed significant differences in the amount of biomass (as mg/L) obtained from the algal species (*Chlamydomonas noctigama*, *Chlorella vulgaris*, *Anabaena*

variabilis and *Nostoc spongiaeforme*) at 3 different phases of growth under the respective optimum conditions.

About *Chlamydomonas noctigama*, the highest biomass (450.20 mg/L) at the phase between stationary and death phase and the lowest biomass (190.78 mg/L) at the logarithmic phase under the optimum conditions were found statistically different and also from the biomass at the stationary phase (330.52 mg/L) under the optimum conditions.

In the case of *Chlorella vulgaris*, the highest biomass (555.87 mg/L) at the phase between stationary and death phase and the lowest biomass (205.53 mg/L) at the logarithmic phase under the optimum conditions were found statistically different from each other and also from the biomass obtained at the stationary phase (360.82 mg/L) under the optimum conditions.

Regarding *Anabaena variabilis*, the highest biomass (320.20 mg/L) at the phase between stationary and death phase and the lowest biomass (160.62 mg/L) at the logarithmic phase under the optimum conditions were found statistically different from each other and also from the biomass obtained at the stationary phase (220.08 mg/L) under the optimum conditions.

In the case of *Nostoc spongiaeforme*, the highest biomass (322.00 mg/L) at the phase between stationary and death phase and the lowest biomass (170.59 mg/L) at the logarithmic phase under the optimum conditions were found statistically different and also from the biomass at the stationary phase (250.09 mg/L) under the optimum conditions.

Both the green alga (*Chlamydomonas noctigama* and *Chlorella vulgaris*) and cyanobacteria (*Anabaena variabilis* and *Nostoc spongiaeforme*) showed the highest growth in terms of biomass at the phase between stationary and death phase. This phase of growth may provide the final concentration of biomass. It might be because the biomass parameter remains constant during this phase, according to Vonshak and Maske (1982). The depletion of some essential nutrients in the medium becomes limited inhibiting the growth and results into the death, in this regard.

Among all the microalgae (both green algae and cyanobacteria) in this experiment, green alga *Chlorella vulgaris* had the highest growth in terms of biomass (555.87 mg/L) at the phase between stationary and death phase under the optimum growth conditions considered. This result showed the similarity to the study by Yatirajula *et al.* (2019), where the growth phase was observed after the stationary phase though the duration was

varied due to the respective optimum conditions. It can be recommended to use biofuel feedstock for the biofuel, e.g. bioethanol and biodiesel, instead of conventional energy source (fossil fuel, coal, petroleum, natural gas, etc.) that are usually known as non-renewable sources of energy. The acceptability of the green alga, *Chlorella vulgaris*, as a potential biofuel feedstock is found worldwide, as mentioned by Ru *et al.* (2020), Varaprasad *et al.* (2020), Purkan *et al.* (2019), Ramírez-López *et al.* (2019), Sakarika and Kornaros (2019), Papapolymerou *et al.* (2018), Suthar and Verma (2018), Daliry *et al.*, (2017), Lam *et al.* (2017), Rajanren and Ismail (2016), Villagrancia *et al.* (2016), El-Sayed *et al.* (2015), Safi *et al.* (2014), Jasim and Maysam (2014), Mallick *et al.* (2011), Phukan *et al.* (2011), though the amount of biomass produced can vary due to the production scale, operational design, and optimum growth conditions taken under consideration.

Conclusion

All the randomly isolated and selected freshwater microalgae produced variable amounts of biomass with variable growth rates at different phases of their life cycles. The algal strains can be used as a source of biomass for biofuel and other valuable products if growing at the phase between stationary and death phase under the set of optimum conditions. Further research for analysis and large-scale biomass production by considering the highest amount with the growth phase is required.

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