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Kinetic Study for Growth of *Phormedium Sp.* and *Chlorella Vulgaris*

N.N.El-Ibiari[1], OlaEl-Ardy[1], Olfat M.A.Salem[2] and A.M.Abdelrahman[2]

[1]Chemical Eng. & Pilot Plant Dept., National Research Centre, Egypt
[2]Botany and Microbiology Dept., Faculty of Science, Helwan Univ. Egypt

Abstract: Microalgae are efficient biological systems with a higher biomass production and faster growth rate than other energy crops. *Phormidium sp*. and *Chlorella vulgaris* were isolated from River Nile. The cultures were grown in BG11 medium and were performed in 24 h continuous light, 25±1°C, 25.7µEinstein light intensity, at pH 7 and 27 days incubation period for *Chlorella vulgaris* and 12/12 h light/dark cycle, 29±1°C, 33.87µEinstein light intensity, at pH 9 and 15 days incubation period for *Phormidium sp*. The cultures were carried out in a laboratory scale. From algae growth curves of *Phormidium sp*. and *Chlorella vulgaris* the maximum specific growth rate, µmax=0.135 d^{-1} (r^2=0.957), µmax= 0.165 d^{-1} (r^2=0.948) and doubling time t_d=5.1 and 4.2 days respectively. Light intensity and light duration were studied in both cases.
Key words: Phormidium sp., Chlorella vulgaris, maximum specific growth rate, doubling time, light intensity, light duration.

1. Introduction:

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multi-cellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta)[1,2].

A more in depth description of microalgae is presented by Richmond[3].Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed[3].

Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days[4]. Their growth rates and photosynthetic activities are widely higher than superior plants, representing more than 90% of the photosynthetic activity in the Earth[5].

While different strains and species of algae exhibit different growth rates[6-9], the following are the important factors that determine the growth rate of algae, in general:

1. Light is needed for the photosynthesis process.
2. Temperature - There is an ideal temperature range that is required for algae to grow.
3. Medium/Nutrients - Composition of the water is an important consideration.
4. Salinity – While algae may adapt to a wide range of salinity, many species do not tolerate a large sudden change in salinity.

5. pH - Algae typically need a pH between 7 and 9 to have an optimum growth rate.
6. Aeration - The algae need to have contact with air, for its CO2 requirements.
7. Alkalinity - High alkalinity promotes calcification and this encourages the rapid growth of calcifying algae such as red coralline algae and green Halimeda spp. High alkalinity combined with calcium dosing promotes the precipitation of phosphate and this limits algae growth.
8. Mixing - Mixing prevents sedimentation of algae and makes sure all cells are equally exposed to light.
9. Photoperiod - Light & dark cycles.

Thus the objectives of the present study were to determine the specific growth rate, the doubling time and to study the light intensity and time duration on biomass production for both Phormidium sp. and Chlorella vulgaris strains.

2. Experimental Work:

2.1. Stain Culture conditions:

Phormidium sp. and *Chlorella vulgaris* were isolated from River Nile - Helwan, Cairo, Egypt. The two strains were identified according to their morphological characteristics using light microscope, the references used were[10-11]. Then purification was done by using different treatments of antibiotic. The cultures were grown in BG11 medium [12] and were performed in 24 h continuous light, 25±1°C, 25.7µEinstein light intensity, at pH 7 and 27 days incubation period for Chlorella vulgaris and 12/12 h light/dark cycle, 29±1^{o}C, 33.87 µEinstein light intensity, at pH 9 and 15 days incubation period for Phormidium sp. All the experiments were carried out in triplicates.

2.2. Optimization of growth conditions:

2.2.1. Growth curve:

Three 500 ml sterile Erlenmeyer flasks each contain 200 ml BG11 sterile media (for each treatment) were inoculated with 1 ml algal culture (15 day ago sub cultured) and incubated under the following conditions, 12/12 light/dark cycle, 8.17µEinstein, 26 ±1°C and at pH 7. The culture growth measured every 72 h by optical density at 525 nm and by chlorophyll a extraction using Ritchie method [11].

2.2.2. Light Intensity:

Six light intensities regimes were tested as follow 4.86, 8.1, 13.2, 25.7, 27 and 33.87µEinstein. Three 500 ml sterile Erlenmeyer flasks each contain 200 ml BG11 sterile media (for each treatment) were inoculated with 1 ml algal culture (15 day ago sub cultured) and incubated under the following conditions, 24h complete light cycle for *Chlorella* and 12/12 light /dark cycle for *Phormidium sp* , 26±1°C, and at pH 7.

All flasks were incubated for 27 day for *Chlorella* and 15 day for *Phormedium sp* then the growth measured by optical density at 525 nm and by chlorophyll (a) extraction using Ritchie method [11].

2.2.3. Light Duration:

Four regimes of light duration were tested as follow 24 h complete light, 16/8 light/dark, 12/12 light/dark, 8/16 light/dark cycle. Three 500 ml sterile Erlenmeyer flasks each contain 200 ml BG11 sterile media were inoculated with 1 ml algal culture (15 day ago sub cultured) and incubated under the following conditions, 8.1µEinstein, 26±1°C temperature ,and at pH 7.

All flasks were incubated for 27 days for *Chlorella* and 15 day for *Phormidium sp* then the growth measured by optical density at 525 nm and by chlorophyll a extraction using Ritchie method [13].

3. Results and discussion:

3.1. Algae growth:

Several phases of cell growth are observed in batch culture, lag phase (specific growth rate $\mu \approx 0$) in which cells adapt to the new environment, no or very little growth, acceleration phase ($\mu < \mu max$) in which growth starts ,growth phase ($\mu \approx \mu max$) in which growth achieves its maximum rate, decline phase ($\mu < \mu max$) in which growth slows due to nutrient exhaustion or build-up of inhibitory products ,stationary phase ($\mu = 0$) in which growth ceases, then the last phase is the death phase ($\mu < 0$) in which cells lose viability and lyses.

During the growth and decline phases, rate of cell growth is described by equation: $r_x = \mu x$, where r_x is the volumetric rate of biomass production with (kg $m^{-3}s^{-1}$), x is viable cell concentration (kg m^{-3}) and μ is the specific growth rate (T^{-1}).

In a closed system where growth is the only process affecting cell concentration, $r_x = dx/dt$ after integration $x=x_0e^{\mu t}$ where x_0 is the viable cell concentration at time zero.

Cell growth rates are often expressed in terms of the doubling time td. Starting with a cell concentration of x_0, the concentration at $t = t_d$ is $2x_0$.

Substituting in equation $2x_0 = x_0 e^{\mu t}$, taking the natural logarithm $t_d = \ln 2/\mu$.

Light is the energy source driving photosynthesis and it is one of the most important factors determining the growth of photosynthesis algae. Figure (1) plots the chlorophyll (a) content µg/ml versus time for *Phormidium sp*. It shows that the chlorophyll increases since it starts and reached maximum after 15 days. No lag phase is evident. According to equation

$\ln x = \ln x_0 + \mu t$,where x is viable chlorophyll (a) content µg/ml and μ is the specific growth rate T^{-1} .A plot of ln x versus time gives a straight line with slope μ. Because the relationship of equation is strictly valid only if μ is unchanging, a plot of ln x versus t is often used to assess whether the specific growth rate is constant during the growth phase. So we must determine which data points belong to the exponential growth phase. In figure (1) the final four points appear to belong to the decline and death phases of the culture. Fitting a straight line to the remaining data figure (2) gives a slope of 0.135. Therefore, $\mu = 0.135\ d^{-1}$ ($r^2 = 0.957$). Cell growth rate is often expressed in terms of doubling time td which was calculated as follows:

$T_d = Ln2/\ \mu max = 5.1 day$

Figure (3) plots the optical density nm (OD) versus time for Chlorella vulgaris strain. It shows that the OD increases and reached maximum after 15 days. Applying the same technique as in case of *Phormidium sp*, $\mu = 0.165\ d^{-1}$ ($r^2 = 0.948$) figure (4). And the doubling time was 4.2day.

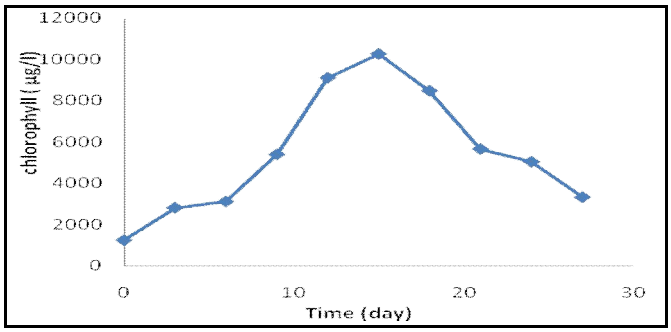

Figure 1: Effect of different incubation periods on the growth (Chlorophyll content) of *Phormidium sp*

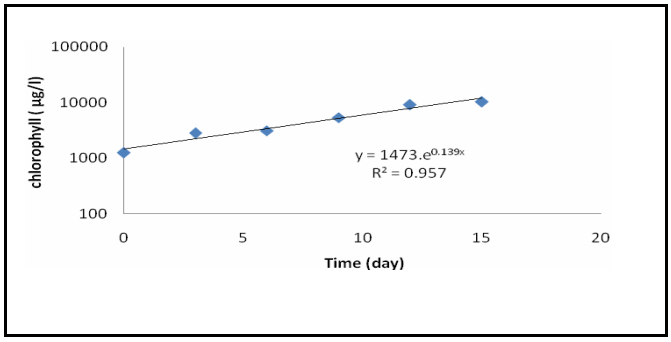

Figure 2: Chlorophyll of *Phormidium sp*. versus time

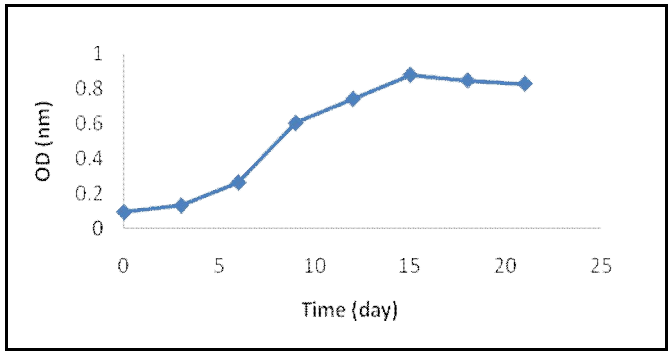

Figure 3: Effect of different incubation periods on the growth (optical density) of *Chlorella vulgaris*

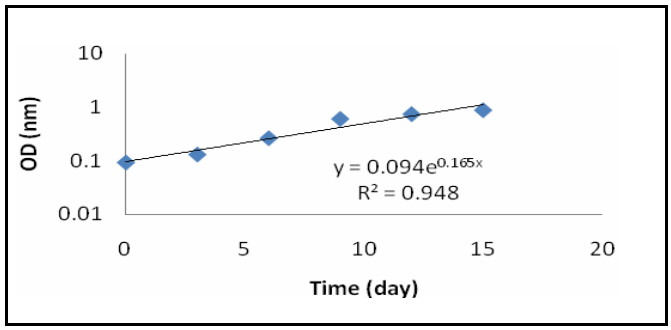

Figure 4: Optical density of *Chlorella vulgaris* versus time

3.2. Effect of Light Intensity:

The experiments of *Phormidium sp.* and *Chlorella vulgaris* growth under different light intensity were carried out with the specification that has been previously indicated. The results are shown in Tables (1-2). As shown from tables, in case of *Phormidium sp*. as light intensity increased the growth rate decreased which indicate that this strain prefer shaded place with very low light intensity, thus when light intensity dose increased this mate-forming cynao-bacterium fall down at the bottom of the flask to move away from this high dose of light intensity. While in case of *Chlorella vulgaris* as the light intensity increases the growth increases till 1880 µEinstein then it decreases.

Table (1) Effect of different light intensity on chlorophyll of *Phormidium sp*

Light Intensity µEinstein	Mean Chlorophyll µg/ml
4.86	12.519
8.10	10.492
13.2	3.804
25.7	2.644
27	3.452
33.8	3.128

Table (2) Effect of different light intensity on optical density of *Chlorella vulgaris*

Light Intensity µEinstein	Mean Optical Density
4.86	0.522
8.10	0.56
13.2	0.704
25.7	0.87
27	0.828
33.8	0.76

3.3. Effect of Light Duration:

The experiments of Phormidium sp. and Chlorella vulgaris growth under different light duration were carried out with the specification that has been previously indicated. The results are shown in tables (3-4). As shown from tables, in case of Phormidium sp. the optimum light duration was (12/12 L/D cycle), while in case of Chlorella vulgaris the optimum light duration was 24 hr light.

Table 3 : Effect of different light duration on chlorophyll of *Phormidium sp*

Light Duration, hours	Mean Chlorophyll, µg/ml
8	16.82
12	19.048
16	11.887
24	11.14

Table 4 : Effect of different light duration on optical density of *Chlorella vulgaris*

Light Duration, hours	Mean Optical Density
8	0.481
12	0.566
16	0.62
24	0.785

4. Conclusion:

Two strains of microalgae; Chlorella vulgaris and Phormidium sp were grown autotrophically and the effect of light intensity and light duration on chlorophyll and optical density were studied. It was found that in case of Phormidium sp. as light intensity increased the growth rate decreased while in case of Chlorella vulgaris as the light intensity increases the growth increases till 1880 µEinstein then it decreases. The optimum light duration was (12/12 L/D cycle) in case of Phormidium sp., while in case of Chlorella vulgaris the optimum light duration was 24 hr light. From algae growth of Phormidium sp. and Chlorella vulgaris the maximum specific growth rate was $\mu max=0.135\ d^{-1}$(r2=0.957), $\mu max= 0.165\ d^{-1}$ (r2=0.948) and doubling time td=5.1 and 4.2 days respectively

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