Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(5): 27-31

Received: 04-07-2015
Accepted: 29-08-2015

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The influence of illumination and soil extract concentration on growth rate and protein content of *Cosmarium subtumidum* microalgae

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Abstract
In order to optimize *Cosmarium subtumidum* algae growth isolated from local from various geographic regions, this study was conducted at different concentrations of soil extract and under three light intensities as follows 1500, 2000, 2500 Lux with a photoperiod of 16 hours light : 8 hours dark. The maximum growth rate was 3.38, at the lowest doubling time of 2.14 and under light intensity of 2500 Lux. On the 35th day of *Cosmarium subtumidum* cultivation. Thus, the higher soil extract concentration and light intensity, the higher growth rate occurred. As for protein content, the highest record was 14.88% at a concentration of 8ml/50 of soil extract and under a light intensity of 1500 Lux. Thus, the higher soil extract concentration and the lower light intensity, the higher protein content was.

Keywords: Phytoplankton, green Alga, growth rate, doubling time, *Cosmarium subtumidum*

1. Introduction
Of the most important problems which the world suffer from are natural sources depletion and climate change linked to increasing emission of greenhouse gasses. Due to recent major problems with their exacerbated outcomes on energy, water and food, sustainable development has become a dire and strategic demand that most countries seek after. Besides, spiking prices of oil as a result of its potential depletion and high cost of extraction as well as global warming and atmospheric pollution related to oil consumption require hard working on searching for alternative sources of energy and food on one hand, and to develop new approaches to stop environmental pollution resulted from industrialization and population activities on the other hand. At present time, algae have been considered as a promising source of renewable algae as they can utilize solar energy and convert carbon dioxide into carbohydrates, proteins, and lipids. Comparatively, algae are more efficient than terrestrial plants. Thus, algae have been demonstrated as an important source of renewable biofuels since algae- conducted photosynthesis, carbon dioxide radiation can be fixed and environment would be protected from global warming thereafter [1-10]
Also the use of algae is very advantageous for polluted water treatment; they act as the bioindicators for the evaluation of water pollution in terms of type and degree [11]
Algae are considered a great food supplement for humans and animals. They contain high portion of carbohydrates, proteins, lipids, vitamins, mineral salts, antioxidants, pigments and other bioactive compounds [12-18]. It was demonstrated that algae have medical application as they can be utilized for their antimicrobial properties. Several anticancer and anti HIV drugs and medicines were designed from algae [14]. Moreover, many chemical compounds derived from algae have economic benefits and can be applied in many sectors such as agriculture, pharmaceutical manufacturing and other chemical industries [19].
Cultivation of algae has to be conducted at optimized conditions and has to be provided with specific growth factors such as nutrients, pH, temperature, light, adequate content of carbon dioxide and oxygen which related to algae species. These parameters influence on phytoplankton chemical composition. Due to the interactions amidst the previous mentioned parameters suggest the optimal conditions for the growth of algae with high productivity.
Marine and wet soil Phytoplankton are considered an essential environmental constituent. They act a major role in environment cycle. According to multiple applications of phytoplankton in sectors of medicine and industry, this study was conducted in purpose of:

1. Isolation of *Cosmarium subtumidum* algae from aqua environment.
2. Optimization of growth factors such as light intensity, and soil extract which influence on the growth rate and productivity of cultivated algae
3. Screening of the influence of some factors such as irradiance intensity and soil extract on the growth rate and productivity time of the study algae.
4. Identification of the ideal conditions for the growth of *Cosmarium subtumidum* algae

2. Materials and Methods

2.1 culture media

CHU Medium No. 10 is used for culturing blue green algae.
Its ingredients per Milligrams / Liter
Calcium nitrate 40.000
Magnesium sulphate 25.000
Dipotassium phosphate 5.000
Sodium carbonate 20.000
Sodium silicate 25.000
Iron Chloride 8.000
PDA medium
Its ingredients per Gm/L
Dextrose 20 g
Potato Extract 4 g
Agar 15 g
Bovine serum albumin,
Organic soil
Nutrient Agar
Its ingredients per Gm/L
Beef extract 10.0 g
NaCl 5.0 g
Peptone 10.0 g
Distilled water 1.0 L
Agar 20.0 g
Adjust pH to 7.0-7.5

2.2 Apparatus and Instruments

Centrifuge, temperature-programmed chamber, microscope, light bulbs of intensities 1500, 2000 and 2500 Lux, 5%Co_2,incubator,autoclave,UVlamp,spectrophotometer, 144 square divided counting chamber (Komorek Burkera), Petri dishes, 0.45µm filter paper, loops, forceps, 500ml poly-ethylene containers, 250 ml flasks, cotton and gauze

2.3 Sampling

Samples of fresh water were collected from Marqia River in Tartotous governorate using 500ml poly ethylene containers and brought to lab in purpose of the isolation of Cosmarium subtumidum algae.

2.4. Preparation of samples

2.4.1 *Cosmarium subtumidum* isolation

In order to get a pure isolate of Cosmarium subtumidum algae, aliquot of sampled river water was filtered using 0.45µm filter paper. The suspended was examined by microscope in order to identify the algae to be isolated from the study sample. In aseptic condition, the study samples were inoculated thereafter in autoclaved solid Chu 10 filled petri dishes [20]. The plates were moved to culturing unit which is equipped with illumination source of 2500 Lux the bulb with a photoperiod of 16 hours light: 8 hours dark [21]. After 4weeks, the grown algae colonies were microscopically examined and classified based on taxonomic key [22, 23]. Since an isolated colony of algae constitutes a pure isolate of colony, it was inoculated in a separate medium to obtain a pure culture of algae

2.4.2 Purification and cultivation of *Cosmarium subtumidum*

The identified colony of *Cosmarium subtumidum* algae was enriched in 250 ml flask contains 150 ml of Chu10 liquid media using a sterilized loop. Then the flasks were moved to isolating
25 ˚C temperature-programmed chamber with 16L/8D photoperiods. Lamps (2500 Lux) were used to illuminate the chamber. As to obtain a pure isolate of *Cosmarium subtumidum* algae from void from bacteria and fungi based on the described method of Weideman, 83 [24] algae isolate culture was centrifuged for two minutes at 3000 rpm and the result was rinsed with sterilized distilled water many times. In order to affirm that the isolate of the study algae was pure from bacteria and fungi, part of the mentioned isolate was cultured on PDA media and Co_2 incubated at 25 ˚C for 5 days. Besides, another loop was cultured on a nutrient agar medium and incubated at 37 ˚C for 48 hours at concentration of 5% of Co_2 at which algal growth is best. These processes were repeated several times in order to affirm the pure isolation of *Cosmarium subtumidum* algae. The result pure isolate was kept in nutrient CHU10 liquid medium until use.

2.4.3 Preparation of nutrient medium (soil extract)

1kg of organic soil taken from forest which is empty from chemical fertilizers, toxic substances or insecticides was added into 2 liters of distilled water, mixed well and autoclaved for half an hour. After that, the mixture was filtered and the filtrate was autoclaved again for 20 minutes to be kept in cold place until use. Using flasks, serial dilutions of soil extracts at concentration ratios of 0, 2, 3, 4, 5, 6, 7, 8 ml extract/50ml water. 5ml of pure isolate of *Cosmarium subtumidum* algae was added to each flask. The flasks were moved thereafter to 25 ˚C temperature-programmed chamber with different illumination intensities of 1500, 2000, 2500 Lux and 16L/8D photoperiods. The growth of the study algae was monitored along 35 day with continuous shaking of the flasks from time to time. To avoid to a self-shading effect of the cells and to maintain equal delivery of light, the positions of the flasks were changed every 12 hours. Considering cultured isolate of C. subtumidum algae on CHU 10 as a standard, previously mention cultures were repeated 3times for each concentration. Using 144 square divided counting chamber (Komorek Burkera), the abundance was measured and calculated based on the following equation:

$$N\,..\,mL^{-1} = 250\ .N_S.1000$$

Where N_s is the number of cells in one square
Growth rate and doubling time were calculated from the following equations [25]:

$$K = \frac{Log10Nt - Log10N0}{t}$$

where K= growth rate, Nt= cell number related to study period, $N_{0=}$ cell number at the beginning of the study, t =number of days

$$G = \frac{0.301}{K}24$$

Where G = doubling time

2.5 Estimation of Protein content

Protein content was quantitatively estimated by dry biomass of algae [26], Bovine serum albumin (BSA) was used as standard solution to prepared standard curve of the protein. The estimation of protein content was conducted on the 35th day of the culture.

3. Results and Discussion

It was revealed from the findings that algal cell number and growth rate increased with increasing light intensity Tabs 1, 2, 3. The maximum growth rate was 3.38, at the lowest doubling time of 2.14 and under light intensity of 2500 Lux. The average of grown cells was 2628.47 cell/ml at ratio of 1159.62 cell/ml. The highest growth rate was at least doubling time of 2.22. Using light intensity of 2000Lux, the number of cells decreased to reach 1977.43 cell/ml. Cell number decreased, *with* decreasing light intensity. Exceptionally, within lower doubling time of 2.28 and under light intensity of 1500 Lux spark in higher rate for growth and number of cells were 3.19 and 1647.57 cell/ml respectively. It's well known, that light intensity influences on the growth of algae through photosynthesis. While, the growth rate is maximum under ideal light intensity, it starts to decline whether with increase or decrease of light intensity. This is attributed to the fact that algae accommodate with stress condition of light using several self-responses such changes in content and type of pigment and in the metabolism rates [27]. Besides, the light accommodation is also associated with changes in cell volume, and membrane thickness. In other word, upon light stress, algae overcome the low light intensity by reducing the thickness of their membrane [28]. In 2011, by Stamenkovic and Hanelt demonstrated that *Cosmarium* strains can be categorized as algae adapted to rather high light intensities. Moreover, the mentioned algae secrets jelly substance which floats over the surface in flasks [29]. This phenomenon is proportional to the change in temperature and light intensity [29].

Table 1: *Cosmarium subtumidum* cell number under different light intensity and at different concentrations of soil extract

Soil extract concentration	2500 Lux		2000 Lux		1500 Lux	
	Cell number average	Highest cell number cell/ml	Cell number average	Highest cell number cell/ml	Cell number average	Highest cell number cell/ml
CHU10	1159.62	1960.07	1023.51	1670.14	891.57	1578.13
0	102.48	151.04	102.48	151.04	93.20	140.63
2ml/50	280.70	447.92	230.95	340.28	229.51	348.96
3ml/50	484.13	722.22	383.38	583.33	372.87	548.61
4ml/50	637.90	1017.36	467.11	708.33	472.57	708.33
5ml/50	705.95	1119.79	495.04	732.64	510.57	751.74
6ml/50	787.75	1355.90	619.39	1159.72	662.70	1119.79
7ml/50	949.06	2046.88	744.69	1470.49	700.60	1355.90
	1195.63	2628.47	930.01	1977.43	798.41	1647.57

Table 2: *Cosmarium subtumidum* growth rate under different light intensity and at different concentrations of soil extract

Soil extract concentration	2500 Lux		2000 Lux		1500 Lux	
	Growth rate	Highest growth rate	Growth rate	Highest growth rate	Growth rate	Highest growth rate
CHU10	2.73	3.23	2.69	3.15	2.64	3.12
0	1.81	2.10	1.81	2.10	1.77	2.06
2ml/50	2.20	2.58	2.13	2.46	2.13	2.47
3ml/50	2.50	2.82	2.34	2.70	2.35	2.67
4ml/50	2.52	2.95	2.41	2.79	2.42	2.79
5ml/50	2.55	3.00	2.51	2.83	2.45	2.82
6ml/50	2.58	3.08	2.49	3.02	2.53	3.00
7ml/50	2.62	3.27	2.54	3.12	2.53	3.09
8ml/50	2.69	3.38	2.61	3.25	2.56	3.17

Table 3: *Cosmarium subtumidum* doubling time average under different light intensity and at different concentrations of soil extract

Soil extract concentration	2500 Lux		2000 Lux		1500 Lux	
	Doubling time average	Lowest doubling time	Doubling time average	Lowest doubling time	Doubling time average	Lowest doubling time
CHU10	7.35	2.24	7.37	2.29	11.82	2.31
0	12.94	3.45	12.94	3.45	13.02	3.51
2ml/50	6.44	2.80	7.99	2.93	6.52	2.92
3ml/50	3.15	2.56	6.23	2.67	4.04	2.70
4ml/50	4.86	2.45	5.41	2.59	4.66	2.59
5ml/50	5.28	2.41	3.13	2.55	4.93	2.57
6ml/50	6.02	2.34	7.57	2.39	5.31	2.41
7ml/50	7.46	2.21	7.55	2.31	5.35	2.34
8ml/50	7.28	2.14	11.93	2.22	11.96	2.28

3.2 Soil extract concentration

It was found from the findings that different growth rates were related to different concentrations of soil extract whereas increasing growth rate, cell number, and doubling time decline in accompany with increasing soil extract concentrations. Thus, on the 35th day of Cosmarium subtumidum cultivation highest record for cell was 2628.47 cell/ml at a concentration of 8ml/50 of soil extract and under 2500 Lux light intensity in comparison with the sample blank of which highest cell number was 1960.07 cell/ml on the 23rd day of cultivation. This can be attributed to the fact that soil extract provides essential organic and inorganic substances. The study findings

matched those reported by previous researches. Godines *et al.* found that the best rate for the growth of *Chaetoceros mulleri* and *Tetraselmis suecica* in a medium consist of organic fertilizers and low contents of added nutrient [30]. As shown in Figs1, 2, 3 it was noticed increasing cell number with increasing soil extract concentration without affecting on algal cell morphology and its activity for longer period than that of cell blank. Besides, exponential growth rate remained till the 35th day of high concentration of soil extract upon cultivation. But slow decrease in individual cell number was revealed after fixed phase and when lower concentration of soil extract was used. This can be attributed to the fact that decline in the levels of nutrients in culture medium. While it was noticed that upon using CHU10 as an artificial medium on the 23rd of cultivation, the growth phase stopped with sharp decline in individual cell number after stationary phase. Comparatively, it can be concluded that soil extract was preferred to be used as an alternative medium for expensive artificial media [31, 32]

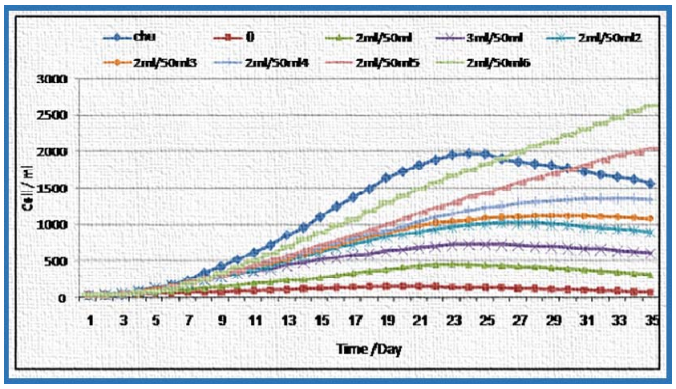

Fig 1: *Cosmarium subtumidum* cell number under light intensity of 2500 Lux and at different concentrations of soil extract

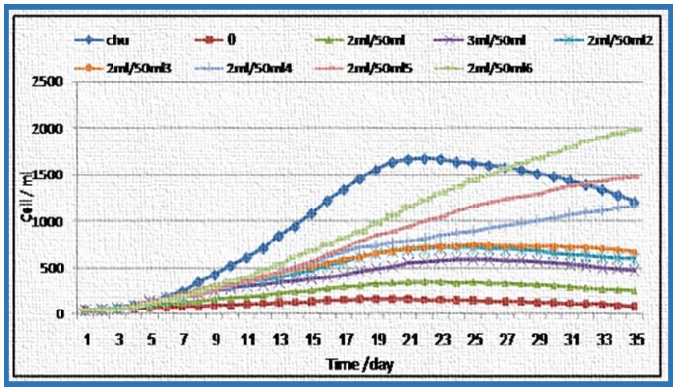

Fig 2: *Cosmarium subtumidum* cell number under different light intensity of 2000 Lux and at different concentrations of soil extract

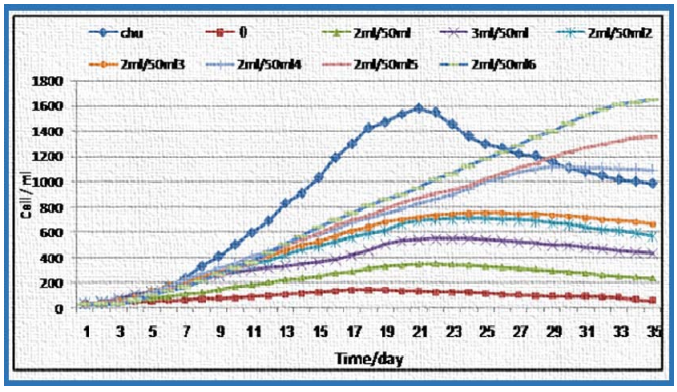

Fig 3: *Cosmarium subtumidum* cell number under different light intensity of 1500 Lux and at different concentrations of soil extract

3.3 protein content changes

The higher soil extract concentration and the lower light intensity, the higher algal protein content was produced as sown in tab 4. The highest record was 14.88% at a concentration of 8ml/50 of soil extract and under a light intensity of 1500 Lux. Then, under light intensities of 2000 and 2500 Lux the protein content started to decreased to reach 14.61 and 13.83% respectively. Generally, it was noticed that protein content decreased as the concentration of soil extract decreased that is 3.98, 3.95, 3.14 were recorded for protein content at concentration of 2ml/50 of soil extract and under light intensities of 1500, 2000, 2500 Lux respectively. This can be attributed to the fact that upon stationary stage when nutrients are depleted from the medium, algae become consumable to their stored content [33, 34]. Also, it was noticed that low light intensity led to increasing protein content [35]

	2500 Lux	2000 Lux	1500 Lux
CHU10	8.16%	8.81%	9.12%
0	3.14%	3.19%	3.26%
2ml/50	3.81%	3.95%	3.98%
3ml/50	4.24%	6.21%	7.26%
4ml/50	5.47%	6.04%	8.22%
5ml/50	6.38%	6.98%	8.46%
6ml/50	9.53%	10.02%	10.24%
7ml/50	11.86%	12.81%	13.17%
8ml/50	13.83%	14.61%	14.88%

N.B: Each value in the table represents an average of three replicate measurement the rates % are expressed for dry weight

4. Conclusion

1. In purpose of obtaining multi-application biomass, artificial media should be replaced with cheap natural media that can be utilized in phytoplankton nutrition.
2. In contrary to synthetic culture nutrients, as Culture conditions should mimic the natural conditions of media for phytoplankton nutrients which promote longer lifespan of phytoplankton associated with consistent morphology and activity of individual cells.
3. Growth rate, doubling time and protein content are exponentially related to the concentration of soil extract and light intensity.

5. Acknowledgments

The Authors acknowledge the help of Tishreen University and college of science for the financial and technical support.

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