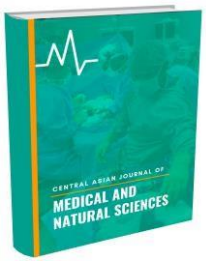

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Article

Study the Effect of Nitrogen Fertilizer on Cultivation and Growth of Algae *Chlorella vulgaris*

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Annotation: The study investigated the potential of *Chlorella vulgaris* to promote agricultural plant growth while inhibiting pathogenic microorganisms. *Chlorella* algae, purified from the Al-Gharraf River and cultured on Chu-10 medium, were examined under different conditions. The use of urea fertilizer led to varying *Chlorella* numbers across growth stages, with increases in early stages and a subsequent decrease. Additionally, three urea concentrations (3%, 5%, and 10%) were compared with a control treatment. Results showed *Chlorella* numbers reaching 50 cells per 100 mL on the seventh day and 400 cells per 100 mL on the fifth day. While the optimal cultivation conditions for *Chlorella vulgaris* were explored, revealing daylight, a temperature of 25°C as conducive to algae growth. The effective culture medium consisted of the Chu-10 medium. This research suggests the potential of *Chlorella* biomass for creating biopesticides and growth stimulators.

Keywords: Chlorella vulgaris, Chlorella, algae, nitrogen fertilizer, cultivation

1. Introduction

Chlorella vulgaris, renowned for its stimulating effect on agricultural plant growth and its capacity to inhibit the proliferation of pathogenic microorganisms, stands as one of the most commercially utilized algae species [1-8]. Achieving efficient chlorella biomass production necessitates the development of optimal cultivation methods. Various techniques, including utilizing industrial dairy waste, co-products, and food waste, have been explored for cultivating *Chlorella vulgaris* under heterotrophic and mixotrophic conditions [3], [4], [9], [10], [11], [12]. However, these methods, while effective on an industrial scale, pose challenges for small-scale cultivation endeavours.

Algae, ubiquitous in all ecosystems but most prevalent in aquatic environments, exhibit diverse sizes and shapes, from microscopic microalgae to macroalgae visible to the naked eye [13]. These autotrophic plants lack true roots, stems, or leaves, thriving in both fresh and saltwater environments due to the abundance of nutrients that facilitate their rapid growth [1]. *Chlorella*, a microbe ranging from 2 to 10 micrometers in size, shares structural similarities with higher plants, possessing essential organelles such as cell walls, mitochondria, and chloroplasts necessary for photosynthesis [7]. Notably, *Chlorella* cell wall serves as a boundary and provides mechanical and chemical defence against various substances, including bacteria and heavy metals [8], [10].

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While many algae species are adapted to saltwater environments, *Chlorella*, along with other species like *Euglena*, *Scenedesmus*, and *Microcystis*, can be found in septic environments [3]. Algal growth is stimulated by nutrients, with certain species becoming dominant under favourable conditions [14]. Chemical fertilizers, particularly nitrogen and phosphate, contribute to algal proliferation [15], with nitrogen and silica promoting eutrophication in aquatic systems [16].

The significance of nitrogen in agricultural settings, primarily derived from nitrogen fertilizers, is paramount [17]. Organic fertilization, including the use of urea, enhances soil quality and fosters aquatic life activity [9], [18]. However, inorganic nutrients like phosphorus, nitrogen, and silica contribute to algae blooming, originating from sources such as domestic sewage waste, animal waste, and agricultural runoff containing chemical fertilizers [5], [6]. The enrichment of aquatic environments with nitrogen through the process of nutrient addition to water accelerates eutrophication [15].

The purpose of the study was to evaluate how urea fertilizer affected on the number of *Chlorella vulgaris*, thereby contributing to our understanding of algae cultivation and its ecological implications, with various cultivation conditions to develop a simple, cost-effective method for *Chlorella* production.

2. Materials and Methods

Algological and biotechnological techniques were employed in the investigation. A real strain of *Chlorella vulgaris* was tested in the experiment (Figure 1).

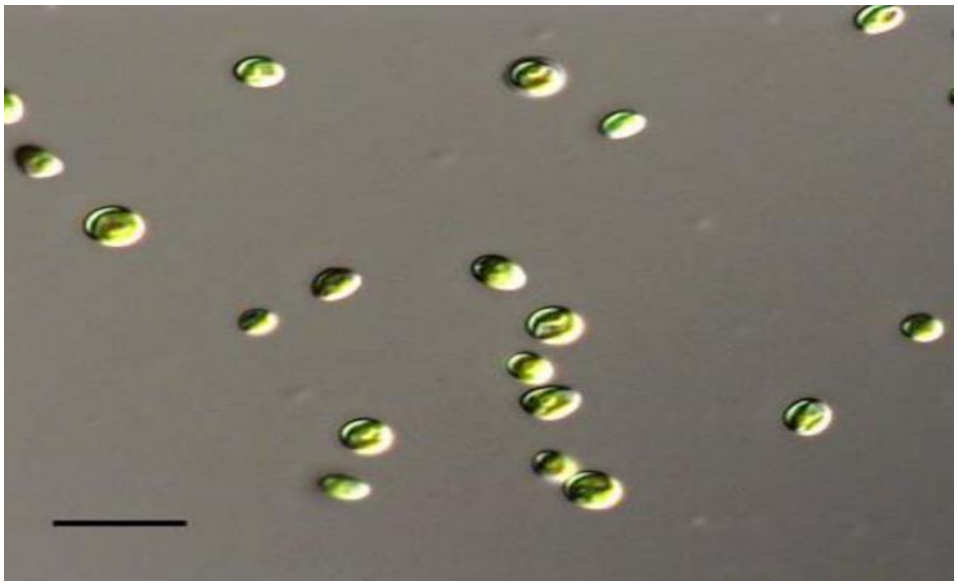

Figure 1. *Chlorella vulgaris* cells, scale bar: 10 μm

2.1. Sample collection

Surface water samples of the Gharraf River were collected from different regions in clean plastic bottles with an area of 500 cm^3 and brought to the laboratory. Part of the sample was fixed with formalin at a concentration of 4% for the purpose of microscopy, while the other part was left unfixed for the purpose of culture.

2.2. Medium for cultivation

Chu-10 medium was placed in the necessary conditions, heated to 25°C with stirring, under daylight, under a phytolamp, and under lighting equipment during the light and dark phases of 16:8 hours in experiments to determine the influence of urea fertilizer and ideal culture conditions. Two measures were used to evaluate the impact of conditions: the

optical density of the suspension (measured with a photospectrometer) and the concentration of cells in 1 milliliter of suspension (measured with a chamber camera). Observations of algae growth were conducted by microscope. Al-Aarajy (1996) [12] used and modified the Ch-10 culture medium for the creation of algal isolates (Table 1).

Table 1. Lists the elements of the Chu-10 culture medium that were utilized in the current investigation to produce algae

The concentration is in 100 ml of distilled water	Salts
2.5	$MgSo_4.7H_2o$
5.76	$Ca(NO_3)2.4H_2O$
1.585	$NaHCO_3$
0.262	Na_2SIO_3
0.146	$FeCL_3$
0.318	Na_2.EDTA
3.583	$CaCL_2.2H_2O$
1	K_2HPO_4
0.0045	$MnCL_2.4H_2O$
0.0007	$MoNa_2O_4$
0.0057	$ZnSO_4.5H_2O$
0.002	$CuSo_4.5H_2O$
0.072	H_3BO_3
7.4	PH

2.3. Algae isolation and purification

The agar plate planning approach was employed in order to create Unialgal cultures, and these cultures were created following a series of dilutions [19]. The algae were purified of spores in accordance with [20].

2.4. Diagnosis of algae

The type of algae utilized in the study was identified using the source listed below [14]. The location where the algae were isolated and their categorization are indicated below.

Division: Chlorophyta

Class: Trebouxiophyceae

Order: Chlorellales

Family: Chlorellaceae

Genus: *Chlorella*

2.5. Preparation of urea fertilizer solutions

By dissolving urea fertilizer, NH_2CONH_2, in deionized water, standard solutions with concentrations (1000 mg/l) of urea fertilizer were made. The concentrations were made by producing the appropriate dilution as the concentrations were prepared (3-5–10%) of the fertilizer.

2.6. Adding urea fertilizer

Using three replicates for each concentration and an incubation temperature of 227 °C, we choose isolates by introducing 0.10 of the pure liquid culture as inoculums to a volumetric flask (250 ml) containing a culture medium fortified with urea fertilizer (10-5-3) of the fertilizer. A sample was planted without any urea fertilizer added to it because it is a control sample, and the flask was shaken every day during the illumination period (12 hours of light to 1 hour of darkness).

2.7. Growth rate calculation

The counting chamber method was used to compute or count the algae. Algal cells are counted in this procedure using a slide known as a Petroff-Hauser slide, which is divided into large and small squares after a known-size sample is taken and placed in the slide. The following equation was used to get the algal growth rate:

$$m = \ln(N_2/N_1)\ t_2\text{-}t_1 \quad \ldots\ldots\ldots\ldots\ (1)$$

where N_2 and N_1 are the cell numbers at periods t_1 and t_2 [38].

3. Results and Discussion

The concentration of nutrients has a significant impact on the growth and reproduction of algae, as nitrogen and phosphorous compounds are included in the composition of cell components like amino acids, proteins, acids, enzymes, and energy, making nutrients one of the most significant environmental factors affecting algae, according to [21].

Algal blooming is caused by the availability of nutrients in the environment, the most significant of which are nitrogen and phosphorus, which are added naturally or by human action, such as the addition of fertilizers or the removal of wastewater and industrial waste [22].

One of the fundamental nutrients found in the aquatic environment in relation to primary products is nitrate, and it comes from two different sources: external (external loading) from human species and their use of fertilizers and internal (internal loading) as a result of the decomposition of organisms (algae, bacteria, and other organisms) after her death [23].

Nitrate is a crucial component in determining the growth of algae, and nitrogen compounds are one of the fundamental elements in all living things. The types of bacterial groups and agricultural flows both affect nitrate concentrations in water [24]. Additionally, agricultural operations that use nitrogen sources contribute to raising their concentration in certain ecosystems [25].

The findings of the present study demonstrate the existence of studies by Al-Rikabi (2003) [31], which found that the growth of algae on the medium of animal extracts was good because it was rich in most nutrients for its growth, and noted that algae's access to sewage water was good and close to their growth on the base medium. It also accords with Al-Hasnawi (2015) [32] and the research conducted by [26], in which he noted that the

growth of microalgae in sewage water was both well-defined and comparable to that of the base media.

It had been found that *Chlorella vulgaris* optical density increases considerably when Chu-10 medium and various urea fertilizer amounts are combined (Figure 2). As a result, the best culture media variation for producing vast amounts of an algal suspension is this one. At a wavelength of 670 nm, the optical density of the suspension was used to calculate the microalgae productivity. On the fifth day of culture, *Chlorella* reaches its maximum productivity.

The highest biomass increase was noted in the experiment on estimating the ideal temperature for algal cultivation when the suspension was at 25° C. According to a prior study on *Chlorella vulgaris*, high temperatures between 20 and 28°C increased the development and death of the algae cells [27].

The productivity of an algal suspension increased under cultivation, as demonstrated by the results of our experiment and data from the literature [28], [29]. Therefore, it is imperative to establish favorable temperature conditions up to 25° C. It was discovered through research on the effects of various lighting conditions on *Chlorella* cultivation that keeping natural illumination—daylight—is ideal for enabling the microalgae to reach their optimum growth on the fifth day of cultivation. A phytolamp-illuminated cultivation similarly shown a minor rise in biomass.

When the growth rate of the algal population was calculated under culture conditions, it was found that the Chu-10 medium combined with varying concentrations of urea fertilizer produced the highest growth rate. Algae that were cultivated at 25° C with stirring also demonstrated a good growth rate.

Different urea fertilizer concentrations were added to the medium, and the effect of the fertilizer on the amount of algae was seen. Additionally, due to the nitrogen content of the urea fertilizer, which is estimated to be 46%, more algae cells were seen on the first, third, and fifth days [17].

The reason for the lack of growth on the ninth day is that all the nutrients in the medium have been consumed. It is also noted from the results that, as fertilizer concentration increases, a variation in the number of algae (*Chlorella*) occurs. This finding is consistent with the study by [30], which demonstrated the variation in algae numbers. The lowest value for the number of Chlorella algae was 50 cells per 100 mL on the seventh day, while the highest value was 400 cells per 100 mL on the fifth day at a concentration of 10% (Table 2, Figure 1).

Table 2. Numbers of *Chlorella vulgaris* algae at various urea fertilizer concentrations

Concentration/Time	One day	Three day	Five day	Seven day	Nine day
3%	190	200	250	80	0
5%	220	250	300	100	0
10%	300	350	400	110	0
control	120	150	200	50	0

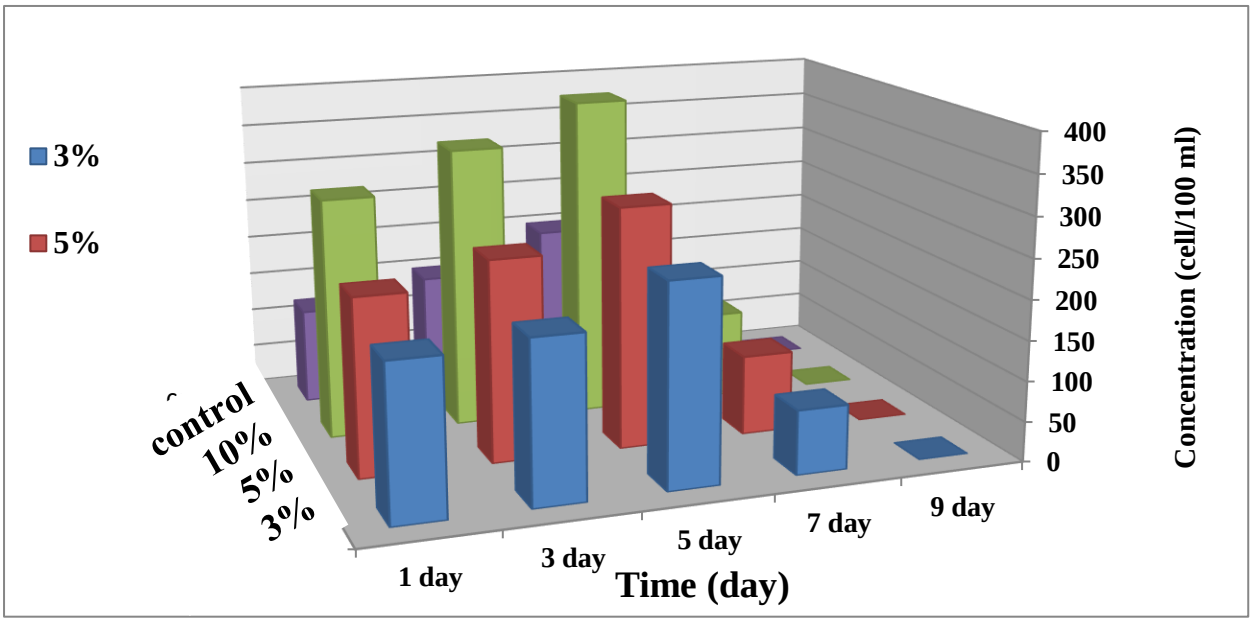

Figure 2. Comparison of the number of *Chlorella vulgaris* algae with control and different urea fertilizer concentrations

4. Conclusion

1) Urea fertilizer promotes good algal cell development due to its high nitrogen content, which is an essential food source for algae.
2) Locating focused culturing media for identifying algae. Therefore, it is possible to accelerate the development rate of algae using the suggested technique of cultivating *Chlorella vulgaris* at a temperature of 25°C in daylight without the need for costly equipment. Using *Chlorella vulgaris* biomass, this technique can be applied to the production of biopesticides and growth stimulants.
3) Farmers should avoid using urea fertilizer extensively to prevent the eutrophication phenomenon, which is brought on by an increase in the quantity and diversity of algae.
4) Establishing open farms with a lot of space for the goal of producing large quantities of algae for research.

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