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Monoculture of *Euglena Polymorpha* Dangeard on CHU#10 Medium and Bold's Basal Medium for the Best Growth of Various Crops

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Abstract. *Euglena polymorpha* is a freshwater microalga that is a common primary producer in aquatic ecosystems where it produces a large biomass with beneficial metabolites that have high commercial value. Euglena, a microalga, has drawn considerable interest due to its rich composition of many bioactive compounds, including dietary supplements, medicines, biofuels and growth-promoting agents for plants. Therefore, it has been widely applied in food, medicine and supplements, it is considered a potential source of biofuel. It is therefore, important to enhance its yield at a lower cost for its commercial viability. The culturing of *Euglena polymorpha* is beneficial for the growth of various crops. In the present study, *Euglena polymorpha* is cultured on BBM and Chu # 10 media for achieving the highest biomass production. This study showed that *Euglena polymorpha* cultured in BBM resulted in 34% higher biomass production than the Chu # 10, making the BBM the most suitable and economically viable candidate for the cultivation of *Euglena polymorpha* for its broad spectrum applications, including agricultural and industrial.

Keywords: freshwater microalga, *Euglena polymorpha*, agricultural application, CHU#10 and bold's basal media

Introduction

Freshwater algae are occupied all the natural environments where light and water present. Algae of aquatic habitats change the properties of water and generally used as bio indicators of water quality and ecosystem status (Bellinger and Sigee, 2010; Barinova *et al*., 2006; Rasiga *et al*., 1999). It is very important about algal diversity in inland waters because most of the algal species can be used as environmental indicators. Mostly species of freshwater microalgae do photosynthesis, and some are heterotrophic organisms and also have a tendency for cultivation as energy crops. They can produce many important commercial products such as oil, fats, sugar, active and bioactive compounds (Viso *et al*., 1987; Riechelt and Borowitska, 1984; Cooper *et al*., 1983; Duff *et al*., 1966).

Algae belongs to diverse variety of organisms range from unicellular to multicellular. Through the biological process of photosynthesis they fix the atmospheric CO_2 into their cell constituents (Meneterz, 2012). Filamentous green macro alga, that mostly present in freshwater environment. Micro algal cultivation can use in bioremediation process, assimilation of nutrients and organic pollutants (Henkanatte *et al*., 2015; Kumar *et al*., 2015).

Blue-green algae play an important role in maintenance of soil fertility, consequently increasing rice growth and yield as nitrogen fertilizers (Song *et al*., 2005). Microalgal species investigated so far for their water retention capacity. These microalgae are possibly beneficial for limiting moisture loss and preserving the freshness and moist texture of food products, particularly baked foods (Lafarga *et al*., 2019). Hormones (auxins, gibberellins), vitamins, amino acids are growth promoting substances.

Euglena polymorpha is a microalga that is well-known for its ability to generate a wide range of bioactive metabolites and for its resistance to various environmental stresses. *Euglena polymorpha* has been reported to boost the germination index of several crops, including lettuce and tomato by promoting cell expansion and root growth in cucumber cotyledons, showing similarities to phytohormones such as gibberellin, cytokinin and auxin (Butzke *et al*., 2024). Algal culturing has been great concern these days to evaluate the potential of various algal species to produce the various useful high valued products (Andersen, 2005). Nutritive medias

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directly regulate the potential of biosynthetic machinery of algal cell due to their nutrient profiling. BBM and Chu # 10 medium are most commonly used for algal culturing (Praba *et al*., 2016). The media rich in nutrient profiling have been proven the ideal choice for the culturing of high valuable algal strains like *Euglena polymorpha*. Gulhões *et al*., 2021 reported that BBM is more effective medium than other laboratory available nutrient mediums for the high productivity of algae. The nutrient profiling of BBM is said to be the reason for the high yield of biomass and production of highly valuable compounds of *Euglena polymorpha* when growing on BBM due to the presence of nitrates and phosphates (Yaakob *et al*., 2021). *Euglena polymorpha* are flagellated occur mostly in freshwater habitat, like ponds, streams, lakes and rivers. Only very few species grow on selective growth conditions are currently grow on commercial value.

Materials and Methods

Sampling. The algal samples were collected September and December from ponds of Nasir bagh Lahore as the environmental conditions are optimal in this period of time for the algal growth. The algal samples were collected in plastic bags, stored in labelled plastic bottles for further research work.

Identification. The algal samples washed with tap water. After washing small amount of algae were placed on the glass slide and observed under the microscope.

Materials required. Zipper bags; match sticks, gloves, newspaper, paper tape, scissor, aluminum foil, camel hair brush, cling film, cotton, tissue paper, permanent marker, distilled water and tissue paper.

Instruments/apparatus. Laminar air flow cabinet, autoclave, pH meter, hotplate, microscope, weighing balance.

Chemical required. Agar, 0.1N HCl, 0.1 NaOH and Alcohol.

Glass ware. Test tubes, test tube stand, inoculation loop, forceps, test tube holder, syringe, flask (500 mL, 1 L), spirit lamp, measuring cylinder (250 mL, 500 mL, 1000 mL), dropper, petri plates, slides, sucker, stirrer, pipette (1 mL, 5 mL, 10 mL), reagent bottles (500 mL, 250 mL).

Methodology. Standard method for culturing was used according to the available literature (Benner *et al*., 2022; Munir *et al*., 2015; Borowitzka and Moheimani, 2013; Guillard and Ryther, 1962).

Media preparation. Different type of algal growth mediums are used for algal culturing. In this study, two media bold's basal medium (Bischoff and Bold, 1963) and CHU#10 medium (Chu, 1942) were prepared to culture *Euglena polymorpha* according to the recipe of each medium (Anderson, 2005).

Bold's basal medium preparation:

- For preparation of medium the given standard quantity of stock solutions (according to recipe) was added in to the flask. Then the solutions with the help of pipette (sucker) and raised its final volume up to 1 L with distilled water.
- Maintain the pH of working medium (according to recipe of medium). pH meter used to maintain the pH as 0.1N HCl and 0.1N NaOH used to adjust the pH.
- For the solidification of medium nutrient agar of 7.8 g was used for 1 L medium and for dissolving the agar it was heated on hot plate.
- After heating covered the mouth of conical flask with the help of cotton plug and placed in autoclave at 121 °C and 15psi for 20 min.
- After autoclaving, medium poured in to the sterilized petri plates under aseptic condition in Laminar air flow hood.
- After pouring the media in petri plater, covered it and allow the media cool. After cooling inoculate the algal sample in the media.

Chu#10 medium preparation:

- For preparation of medium took the given standard quantity of the stock solutions (according to recipe) in to the flask, when solutions were added with the help of pipette (sucker) and raised its final volume up to 1 L with distilled water.
- Maintain the pH of working medium (according to the recipe of medium). pH meter used to maintain the pH 0.1N HCl and 0.1N NaOH used to adjust the pH.
- For the solidification of medium nutrient agar used. Add 7.8 g agar for 1 L medium and heat the flask in hot plate until agar was completely dissolved.
- After heating covered the mouth of conical flask with the help of cotton plug and placed in autoclave at 121 °C and 15psi for 20 min.
- After autoclaving, medium poured in to the sterilized

petri plates under aseptic condition in Laminar air flow hood.

- After pouring the media in petri plates, covered it and allow the media cool. After cooling inoculate the algal sample in the medium.

Inoculation of the algal sample. After the solidification of agar, the petriplates were inoculated with algal samples by using the streaking technique. The initial cell density of the inoculum was adjusted at 2×10^6 cell/mL.

Streaking technique. Streaking is a useful technique for isolation of microalgae that grown on an agar surface (Anderson, 2005). First, algal specimen washed with distilled water. Agar plates were prepared with CHU# 10 and bold's basal media. The inoculation loop sterilized. Inoculate the inoculum with the help of loop by streaking technique in aseptic environment. Agar plates incubated at growth room at temperature 21 °C. After 22 days isolates the desired microalgae and re-streaked on the agar media to obtain the desired monoculture.

Optimum condition for growth. The inoculums were incubated in growth room at 25 °C temperature in 120 µ/mol/m^2/sec light intensity with 16 h: 8 h light and dark period.

Growth observation. The growth in both BBM and Chu # 10 media was recorded at different day interval.

Results and Discussion

BBM and Chu # 10 both showed the growth of *Euglena polymorpha*. BBM showed almost double growth (4.29±0.15) than Chu # 10 (2.83±0.75) in the 1st culture. Same trend was observed in the 2nd, 3rd and 4th culture with difference of 0.47 g, 0.15 g and 0.39 g, respectively. BBM showed more growth on 1st, 2nd, 3rd and 4th culture than Chu # 10 medium. But more growth was recorded in 5th culture on Chu # 10 medium, Chu # 10 showed (10.47±2.21) and BBM showed (7.29±1.15). Among all the cultures BBM showed more significant growth of *Euglena polymorpha* than the Chu # 10 which leads to high production of paramylon in the *Euglena polymorpha* growing on BBM as shown in the (Table 1).

Growth rate. The Fig. 1 shows the growth rate values indicating higher biomass production in BBM medium as compared to Chu # 10 medium.

Euglena polymorpha is a microalga capable of synthesizing several biomolecules, including proteins, vitamins and carbohydrates and serves as an optimal biomass source due to its lipid content, which is particularly advantageous for biodiesel extraction and conversion (Firmansyah *et al*., 2024; Chisti, 2007; Pulz and Gross, 2004). Therefore, the nutrient rich media is said to be an important factor responsible for the growth and species richness (Richmond, 2008). Two nutrient media have been used in this study for the cultivation of *Euglena polymorpha*. BBM has been found more suitable media in four culture batches out of five which high its probability up to 80% in case of culturing *Euglena polymorpha.* (Badr and Fouad, 2021) also reported the similar findings and showed that species richness and species growth directly depends on the selection of nutrient media and reported BBM most competing nutrient media for the culturing of freshwater unicellular algae including Euglenophycota. Cultivation of microalgae in the most suitable culture media has far more applications in terms of achieving high valuable compounds from algae. (Auyo *et al*., 2021) reported when growing *Euglena polymorpha* in the BBM, making it most suitable candidate for the production of biofuel. (Sayar and Eryalçýn, 2024) also reported the high biomass production of *Euglena polymorpha* on the BBM medium among all other mediums for the high production of various valuable products extracted from the microalgae. Yaakob *et al*., 2021 investigated that microalgae required the essential nutrients including phosphate and nitrates for the rapid growth of algae.

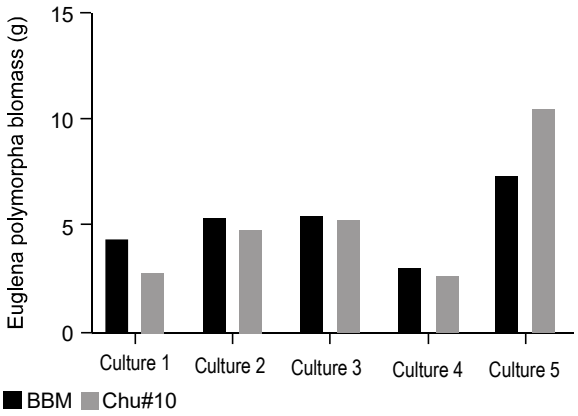


Fig. 1. Biomass (g) of *Euglena polymorpha* on BBM and Chu # 10 medium by streaking technique.

Table 1. Comparison between growth rate (g) on CHU#10 and bold's basal media

Culture no.	Media	Petri plates empty (g)	Petri plates + media (g)	After streaking (g)	Per week (growth rate) (g)
1st	CHU#10	96.34±2.49	145.37±3.04	145.53±3.29	2.83±0.75
	BBM	97.29±5.4	153.25±4.90	153.43±5.16	4.29±0.15
2nd	CHU#10	98.28±0.40	154.08±4.56	153.98±4.64	4.89±0.50
	BBM	97.22±0.45	160.39±3. .09	160.33±3.03	5.36±0.36
3rd	CHU#10	98.56±0.78	159.10±1.57	154.32±3.31	5.30±0.47
	BBM	98.24±0.79	138.38±0.72	138.34±0.73	5.45±0.70
4th	CHU#10	97.55±5.05	143.56±8.49	143.55±8.49	2.61±0.20
	BBM	84.42±8.2	128.31±2.59	128.29±2.59	3.00±0.36
5th	CHU#10	97.80±0.44	163.08±3.18	163.08±3.18	10.47±2.21
	BBM	98.69±0.44	158.04±2.81	157.98±2.81	7.29±1.15

Thus, nutrient profiling of BBM is found to be the driving force behind high yield of biomass of *Euglena polymorpha* when growing on BBM. Gulhões *et al*., 2021 reported that BBM is more effective medium than other laboratory available nutrient mediums for the high productivity of algae, but optimization of BBM by replacing the nitrogen source of BBM by urea PA could be a better substitute of the cost (up to 68 %) for the large industrial scale production of *Euglena polymorpha* for obtaining the high valuable compounds at relatively lower cost.

The experiment was performed in triplicate for all cultures growing on BBM and Chu # 10 medium. Standard deviation and standard error were calculated by using Microsoft excel 2019. 2-way ANOVA was performed to evaluate the growth significance of *Euglena polymorpha on* both medium BBM and Chu # 10. The ANOVA analysis depicted that the *Euglena polymorpha* grow significantly ($P<0.0001$) at high rate than the Chu # 10.

Conclusion

Euglena polymorpha are photosynthetic eukaryotic algae, having a capacity to perform roles at a time *i.e.* enhance crop productivity and maintains a soil integrity and fertility. They performed important role in prevention the colonization of the pathogenic micro-organisms. In this study the relative growth of *Euglena polymorpha* was evaluated by growing different culture batches on both BBM and Chu # 10 medium. BBM appeared as more suitable nutrient media due to its nutrient profiling which pays off in high biomass yield of *Euglena polymorpha*. Microalgae production has been grown to a large industry due to its tremendous applications in all fields of life which pushes it into profitable industry which can be achieved by optimization of the nutritive media and replacing with the cost effective ones. Further studies are required to fill the gaps between the high quality and low cost productions of microalgae for their high valuable products.

General Characters. *Euglena polymorpha* belongs to phylum Euglenophycota, family Euglenophyceae is unicellular its cells are metabolic, its shape ovoid to sub-cylindrical, end points narrowed to shore, blunt tip, periplast consist of spiral striations and chloroplast contains many disc like laciniate margins. *Euglena polymorpha* having 1 pyrenoid and paramylon bodies (ovoid grains). *Euglena polymorpha* cells diameter 20-26 µ and their body 80-90 µ long (Prescott, 1962).

Conflict of Interest. The authors declare that they have no conflict of interest.

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