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COMPARATIVE ANALYSIS OF BIO-CULTURING OF FRESH WATER ALGAE, *SPIROGYRA COMMUNIS* (HASSALL) KÜTZING AND *HYDRODICTYON RETICULATUM* L.

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Keywords: Comparative analysis, Bio-culturing, Freshwater algae

Abstract

For optimization of cultural conditions for algal biomass production of two local filamentous freshwater algae, namely *Hydrodictyon reticulatum*, and *Spirogyra communis* were cultured. Among all these tested media, *H. reticulattum* gave maximum biomass (18.6 g/l) in Bristol medium whereas, *S. communis* gave the biomass of 10.5 g/l in Bristol soil media. Then the effect of different carbon sources (lactose, glucose, cellulose and starch) supplement in Bristol media was evaluated for biomass production. Among all the sources cellulose at 1 g/l was found to be significant for optimal mass production of (15.81 g/l) and (18.6 g/l) of *S. communis* and *H. reticulatum*, respectively. Both the algal species gave insignificant results in all other carbon sources. The effect of different nitrogen sources (ammonium nitrate, ammonium sulphate, ammonium chloride, potassium nitrate and urea) was tested for biomass production. Urea at 0.1 g/l was found to be best (20.7 g/1000 ml) for optimal growth of *H. reticulatum* as compared to (16.86 g/1000 ml) of *S. communis.* However, cellulose as carbon source and urea as nitrogen source were optimized for significant growth of both the algal species. The comparison between the growth rates of both specimens was evaluated. Both the species gave maximum growth up to 15 days of incubation and then the growth started decreasing gradually. It is indicated that the volumetric growth of *H. reticulatum* is significant in the selected media as compared to *S. communis.*

Introduction

The production of algal biomass has been a worth praising achievement in the field of industrial microbiology. The algae commenced several commercial applications in the field of nutrition, medicine, pharmaceutical, cosmetics, waste removal, biofuel and as fertilizer. But algae still remain neglected although they contain all the important constituent that proved to be worth praising for future development and enhanced economy of country. It is worthwhile to isolate, and optimize the culture conditions for algal biomass production (Beardall and Ravan 2004, Barsanti and Gualtieri 2006, Schenk *et al.* 2008, Khola and Gazala 2012). For culturing the algal biomass various biotic and abiotic factors are involved with the *in vitro* mass production of algae. Number of culturing strategies has been advanced through which high yield algal cultures may be executed (Huber and Dale 2009).

Essentially, an excessive yield culture entirely depends upon a good nutrition source. Adequate supply of nutrients in culture media might enable to flourish the algal culture (Munir *et al*. 2015). Carbon, nitrogen, phosphorous and sulphur are important elements constituting algal cells. Other essential trace elements include iron, magnesium and sulphur (Bala 2006, Cao *et al*. 2016). These, elements are important to develop balanced media for optimal micro-algal cultivation and carbon and nitrogen are the most important nutrients, which contribute to the biomass production (Zulkifly 2013).

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Mixed effect of nitrogen and carbon complements the algal increase but as the concentration increases the impact on biomass production decreases. Because high degree of nitrogen hindered the mobile, growth and can cause the breathing, which might also be enhancer for biomass manufacturing (Ogbonna *et al.* 2000, Kim *et al*. 2002).

However, the present study is concerned with the collection and isolation of filamentous green and optimization of cultural condition such as selection of growth media, effect of carbon and nitrogen sources on the mass production of selected filamentous algae.

Materials and Methods

The research work was conducted during 2016 - 2017 in Research laboratory, Department of Botany, Government. College of Science, Lahore. The study was confined for the optimization of different cultural conditions for the production of algal biomass. Different algal specimens were collected in a plastic bottle from different areas of Lahore. The samples were then washed properly with tap water using 0.25 µm pore size sieve. The collected species were then taxonomically identified according to Prescott (2007). Among all the identified specimens these two specimens were selected for further experimental analysis. The identified samples as shown in Fig. 1 were then cultured on different media.

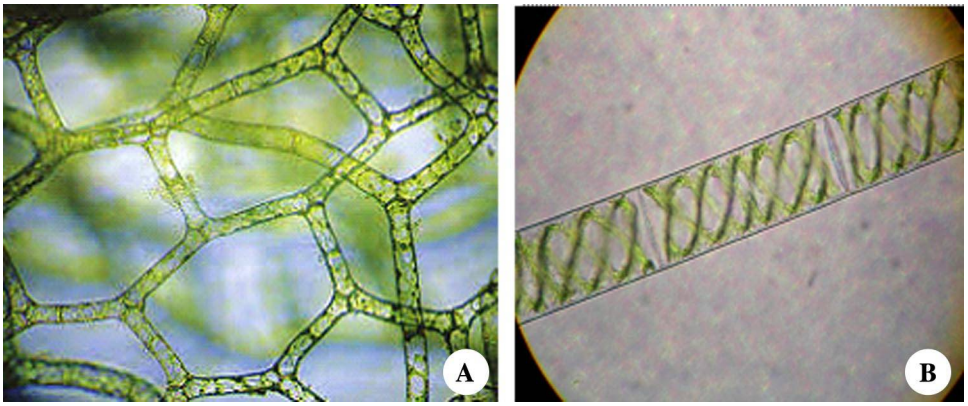

Fig. 1. Selected algal species for biomass production. A. *Hydrodictyon reticulatum,* B. *Spirogyra communis.*

Sl. No.	Species	Family	Locality
1	*Hydrodictyon reticulatum* L.	Hydrodictyaceae	Head Baloki
2	*Spirogyra communis* (Hassall) Kützing	Zygnemataceae	Ravi siphon

For culturing these specimens, different culture media *viz*. A - Z media (Prescott 2007) M1, Bristol's media (Alam *et al*. 2015) M2 and Bristol's soil media (Bristol media along with 10 g soil) M3 were used for the biomass production. For culturing the algae in laboratory different media were used for their bulk biomass production. The media used were M1, M2 and M3 media and the experiment was carried out in 1000 ml glass beaker containing 1 litre of one of the above-mentioned media. The media were prepared by following the standard protocol. After that 3 g of algae was added in the media and pH of the media was adjusted up to 8.00. The beakers were then

exposed to sunlight for 6 hrs every day for optimum growth of algal species. The same protocol was followed for all the respective media and each experiment was run in triplicate.

The biomass productivity P (mg/l/day) was calculated by following equation:

$$P = \frac{W_2 - W_1}{t}$$

where, W_1 is the initial biomass concentration, W_2 is the biomass concentration at the last day of cultivation, t is the cultivation time.

Effect of different carbon sources (sucrose, glucose, cellulose, maltose and lactose) and their concentrations (0.4 - 3.0%) on algal growth was also tested with selected Bristol growth media (Prabakaran and Raavindran 2012). Same as the effect of different nitrogen sources (ammonium sulphate, ammonium nitrate, potassium nitrate, urea and ammonium chloride) and their various concentrations (0.1 - 1.0%) on algal growth were studied using the selected Bristol growth medium (Nigam *et al*. 2011). The experiment was performed for 20 days. At initial pH of medium was adjusted to 8.0.

The data were further analysed by ANOVA with three-way complete randomized designs (Steel and Torrie 1984) to know significant differences between various means and also within the blocks of treatments and the types of microorganisms.

Results and Discussion

The work was done to study the biomass production, effect of different nutritional sources, and cultural media on algal growth. While culturing algae the main purpose was to select the cheapest methods and easily available sources and also culturing was conducted by keeping in mind the commercial and economical perspective.

As the constituents of the media were different, their effects on biomass were also different. Fig. 2 indicates the effect of different cultured media on algal biomass growth. Three cultured media M1, M2, M3 were used for algal biomass production. Among all the media Bristol's soil medium M3 gave 18.6 g/l and 10.5 g/l as maximum growth for both *H. reticulatum* and *S. communis* whereas Bristol's medium gave 10.6 g/l and 8.4 g/l as intermediate growth for both algae. These results showed that the Bristol's soil medium was best suitable medium for maximum yield of both *H. reticulatum* and *S. communis*. As the chemical constituent of these medium contain both the macro and micro elements which were mainly necessary for the algae to grow and the soil used gave the proper habitat to algae, so the sample grew preferably in it. So, this medium was selected for the further studies.

Carbon acts as main growth factor that directly or indirectly effects the algal growth. Fig. 3 indicated the effect of different carbon sources on algal biomass growth. Four different carbon sources (sucrose, glucose, cellulose, maltose and lactose) were used for algal mass culturing. Among all the carbon sources cellulose proved to be best and showed increase in biomass up to10.7 g/l of *H. reticulatum* and 10.3 g/l of *S. communis* within 20 days. While glucose from the other sources gave 9.4 g/l of *H. reticulatum* and 7.5 g/l of *S. communis*, lactose gave 8.3 g/l of *H. reticulatum* and 6.9 g/l of *Spirogyra* and maltose gave 8.1 g/l of *H. reticulatum* and 7.4 g/l of *S.communis.* These outcomes indicated that each species has different carbon source requirement for its proper growth as nourishment (Negoro *et al.* 1991, Zarina *et al*. 2009). The research explores that there may be present a cellulose degrading bacteria that firstly degrade the cellulose to cellobiose and then into glucose subunits that may directly influence the algal biomass to grow.

In this way the carbon source was available in two different forma at two different stages. So this source was selected for further studies.

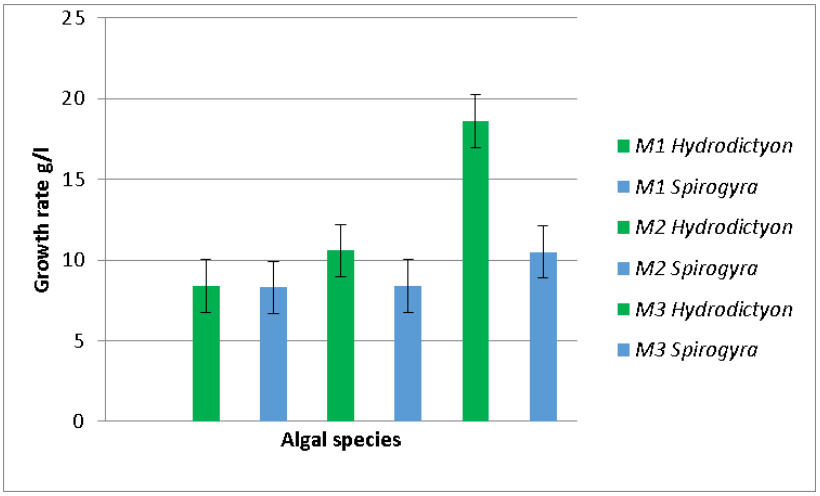

Fig. 2. Influence of diverse culture media on algal biomass production (DMRT, $p < 0.05$). Bars indicate ± S.E. (n =3)

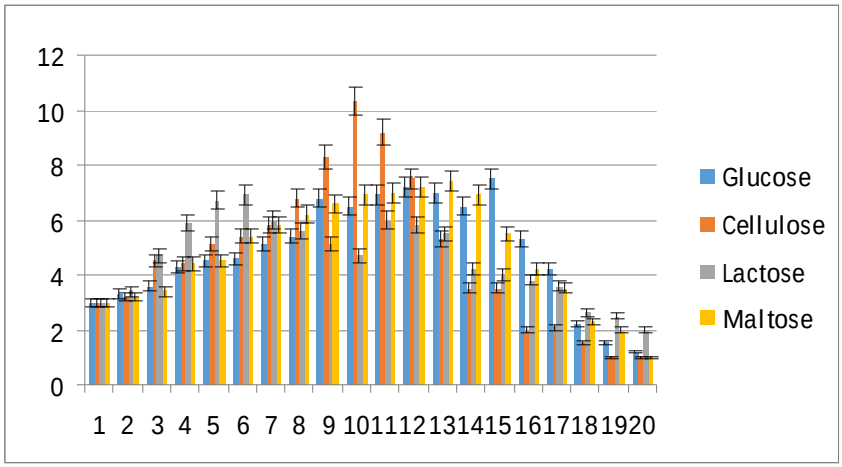

Fig. 3. Influence of diverse carbon sources on *H. reticulatum* biomass production (DMRT, $p < 0.05$). Bars indicate ± S.E. (n = 3).

The selected cellulose was then employed to check that at what concentration it may be beneficial for algae to increase its biomass. The Fig. 4 indicated the effect of variable concentration of cellulose on algal biomass. Four different concentrations of cellulose 0.5, 1, 1.5 and 2 g were used on algal growth. The results specified that the 1 g cellulose gave the best algal biomass 18.7 g/l of *H. reticulatum* and 15.81 g/l of *S. communis* whereas 1.5 g cellulose gave the algal mass 8.1g/l of *H. reticulatum* and 8.9 g/l of *S. communis*, on the contrary use of 2 g gave the algal mass of 11.5 g/l of *H. reticulatum* and 8.7 g/l of *S. communis*. These results suggested that 1

g cellulose in 1000 ml of cultured medium is best for algal biomass production. Because cellulose was easily available and consumed by algae as compared to the other sources applied and the respective algae require cellulose as its carbon source (Lee and Poulickova 2004).

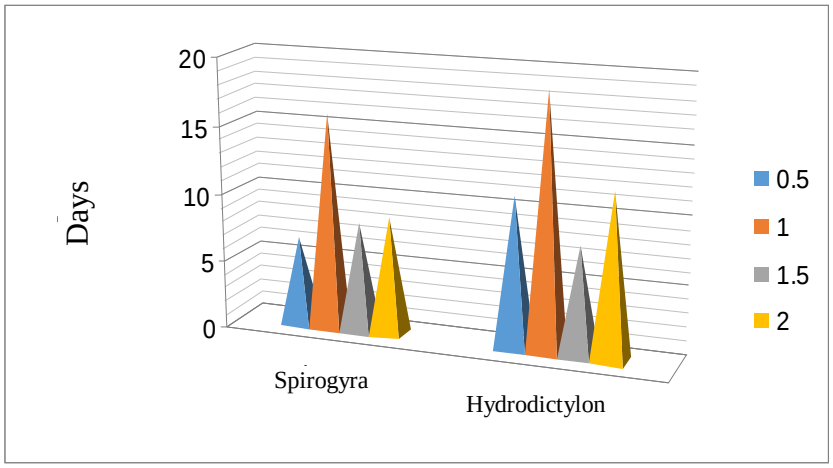

Fig. 4. Effect of different concentrations of cellulose on algal growth.

The Fig. 5 specified the effects of different nitrogen sources on algal biomass production. Four different nitrogen sources (ammonium sulphate, ammonium nitrate, potassium nitrate, urea and ammonium chloride) were used for this purpose. The data from results showed that among all nitrogen sources urea gave the best results of 12.5 g/l of *H. reticulatum* and 8.6 g/l of *S. communis*. Whereas ammonium sulphate gave 4.3 g/l of *H. reticulatum* and 3.9 g/l of *S. communis* and ammonium chloride similarly gave 8.0 g/l of *H. reticulatum* and 4.6 g/l of *S. communis*. Potassium nitrate and ammonium nitrate gave 4.3, 3.0 g/l of *H. reticulatum* and 3.8, 3.7 g/l of *S. communis*. Thus, there is a high probability of loss of ammonia due to the high pH around 8.5 - 9.5. That's why urea proves to be the best nitrogen source for algae to increase its biomass. Therefore, it seems that the nitrogen consumption by algal cells was the largest in medium with urea and not ammonium, as the cell growth rate supports this. These data showed that urea is the best suited for algal mass production, and it was further tested by Yoshihara *et al*. (1996).

The selected urea was then utilized to study that at what focus it might be advantageous for algae to increase its biomass. The Fig. 6 shows that by using variable concentrations of urea only 10 mg urea was best suited for biomass production of 20.7 g/l of *H. reticulatum* and 16.86 g/l of *S. communis*. While 20 mg gave the algal mass of 6.5 g/l of *H. reticulatum* and 5.3 g/l of *S. communis* while 30 mg gave the algal mass of 8.9 g/l of *H. reticulatum* and 4.4 g/l of *S. communis* and 40 mg gave the algal mass of 4.3 g/l of *H. reticulatum* and 3.9 g/l of *S. communis*. Results indicated that only 10 mg concentration of urea gave the maximum growth as nitrogen in urea form was the available source for algae and algal biomass consume easily nitrogen in urea form. Same result was observed by Cheirsilp and Torpee (2012). The 20 mg, urea gave the best algal biomass as nitrogen in form of urea and which found to be the best nitrogen source for algal growth (Yang *et al*. 2000, Teoh *et al*. 2013).

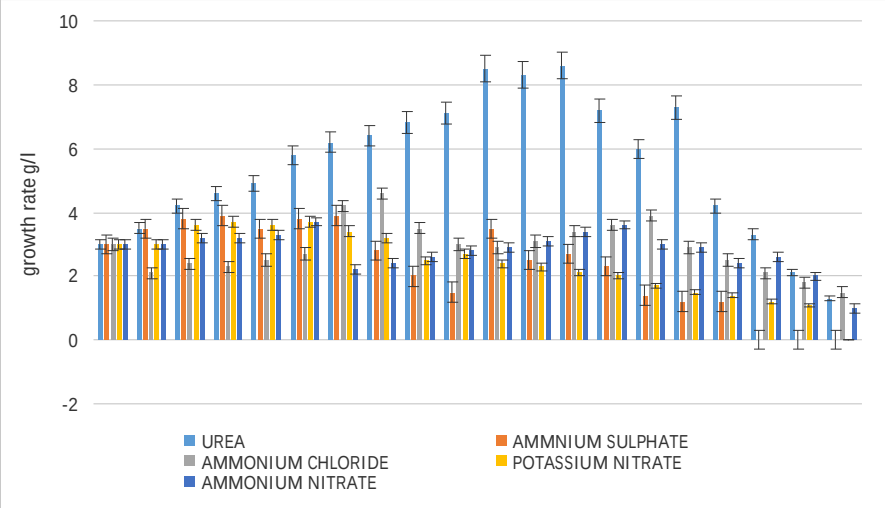

Fig. 5. Effect of different nitrogen sources on *S. communis* biomass production (DMRT, $p < 0.05$). Bars indicate ± S.E. (n = 3).

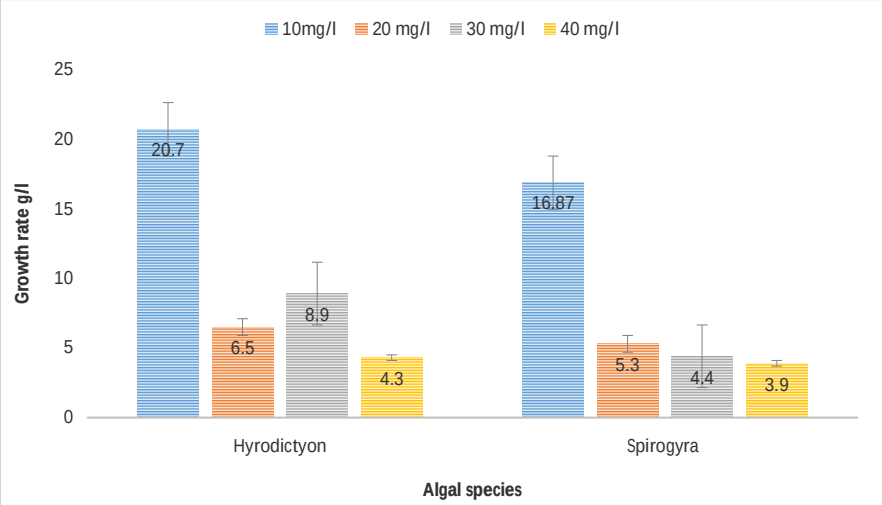

Fig. 6. Effect of different concentrations of urea on biomass production of algae (DMRT, $p < 0.05$). Bars indicate ± S.E. (n = 3).

To study the growth rate and mass production of *H. reticulatum* and *S. communis*, these specimens were cultured for 20 days in the Bristol soil media using the cellulose as carbon and urea as a nitrogen source at pH 8 and the temperature was maintained up to 25 ± 2ºC (Fig. 7). Results showed that algal growth increased day by day till the 15 days, afterwards there was minor increase, or it was stationary and after 18 days the decline rate started. It was observed that *H. reticulatum* achieved its maximum growth in approximately 15 days.

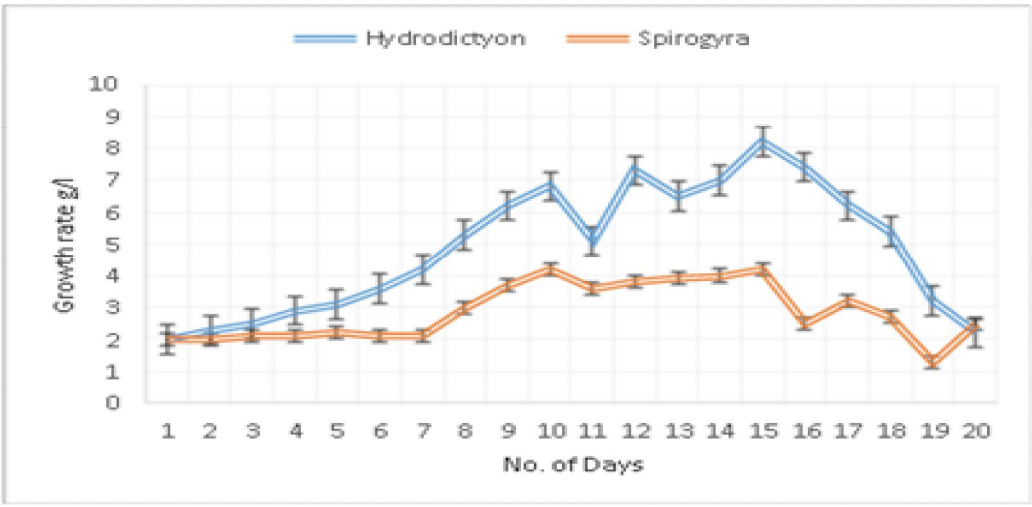

Fig. 7. Comparison between the growth rates of algal biomass (DMRT, $p < 0.05$). Bars indicate ± S.E. ($n = 3$).

Keeping in mind the increasing demand of algae as an alternative to food, medicine, color and dietary supplement, it is necessary to harvest algae in bulk mass. As algae is temperature dependent, a minor fluctuation in temperature results in spoilage of algae. So, it is necessary to culture algae in laboratory and increase its biomass. For biomass production specific growth media containing the major carbon and nitrogen source were used, so that at any time and at any season algae will be obtained easily.

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