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Title:	**Effect of Nitrogen, Phosphorus, and Potassium on the Biomass Production of *Spirogyra Hyaline***
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Effect of Nitrogen, Phosphorus, and Potassium on the Biomass Production of *Spirogyra Hyaline*

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ABSTRACT

Background. The current research investigated the effect of nitrogen, phosphorus, and potassium on the biomass production of *Spirogyra hyaline* Cleve.

Methods. Different nitrogen sources [(NH_4) SO_4, NH_4Cl, $NaNO_3$, Ca $(NO_3)_2$, and Co $(NO_3).6H_2O$] are used for *Spirogyra hyaline* biomass production. Particularly, 2.0% of Ca $(NO_3)_2$ significantly enhanced biomass (18.2 gL^{-1}), the volumetric rate of biomass production (4.7), specific rate of biomass production (2.47), high product yield coefficient (1.74), and specific rate of biomass production (1.54).

Results. Among all the phosphorus sources [Na_3PO_4, $(NH_4)_3PO_4$ and K_2HPO_4], K_2HPO_4 yielded the optimum biomass production (20.4 g L^{-1}), with 0.5 g L^{-1} resulted in maximum biomass production of 22.4 g L^{-1}. Moreover, from various potassium sources [KNO_3, KH_2PO_4, $K_2Cr_2O_7$, and $KNaC_4H_4O_6$], KH_2PO_4 resulted in significantly high volumetric rate of biomass production (2.1) and specific rate of biomass (3.65), with 0.5g L^{-1} showing the maximum biomass production of 18.6 g L^{-1}. Further increase in the concentration of nitrogen, phosphorus, and potassium significantly decreased the biomass production. Additionally, when tested in modified and control media, the algal biomass production was gradually increased in both media and reached optimum after 18 days of incubation (13.1 g L^{-1} and 10.3g L^{-1} respectively).

Conclusion. Therefore, it was concluded that standard media gave better results and was selected for optimum biomass production of *Spirogyra hyaline* Cleve.

Keywords: algal biomass production, nutrients, *Spirogyra*

Highlights

- Nitrogen is essential for plant growth, filamentous structure, and its availability can significantly impact biomass production of *Spirogyra*.
- Phosphorus plays a vital role in plant metabolism, and its interaction with nitrogen can affect biomass production.
- Potassium is involved in various physiological processes in plants, including photosynthesis and water relations.

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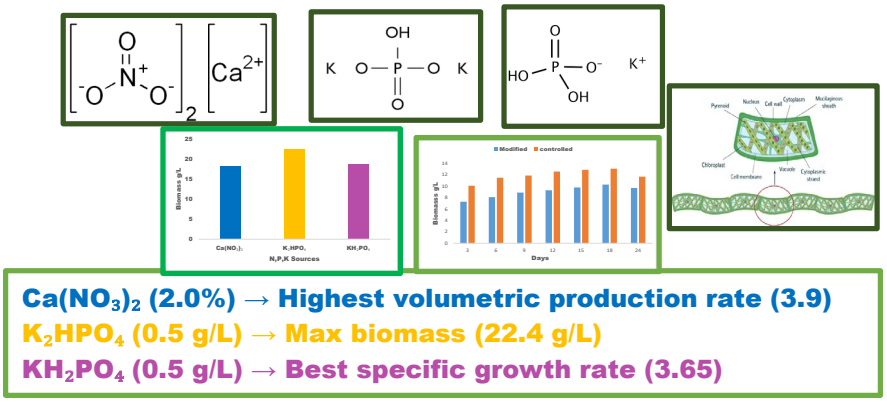
GRAPHICAL ABSTRACT



1. INTRODUCTION

Algae, with a wide range of morphological structures, are the most prevalent plants that produce spores. Based on their structure, researchers can classify organisms in this category as either unicellular or colony-forming [1]. Several algal species exhibit a growth form in which numerous individual filaments and thalli cluster together in aggregates. These dense, extensive mats can spread across the substrate for several meters, forming expansive, continuous growths that effectively resemble and function as "underwater grasslands" within their aquatic environment. Algae mostly inhabit aquatic environments; however, certain species may be found on solid surfaces with significant levels of moisture [2, 3]. Most algae are photoautotrophic organisms that exhibit a high level of photosynthesis. They generate almost 70% of the biological material and oxygen on Earth through the process of photosynthesis. Algae, from a taxonomic perspective, encompass cyanobacteria, diatoms, green algae, dinoflagellates, red algae, brown algae, and stone algae [4-6].

Algal production for cosmetic, medicinal, and nutritional reasons needs a sterile environment [2]. Algae biomass, which has a high capacity for accumulating biogenic components and light metals, may be controlled, artificially enhanced, and utilized in many sectors [7]. Macroalgae can be readily isolated from the culture media or their natural habitat. Biomass of macroalgae is readily and mechanically harvested utilizing uncomplicated methods [8]. There are various parameters (light intensity, nutrients, temperature and pH level) affect the growth of algal species and biomass production. These requirements must be standardized to achieve maximum algal growth and biomass production [9].

Spirogyra commonly grows in a filamentous structure and is found in freshwater habitats [10]. The potential of *Spirogyra* to manufacture biogas, bioethanol, and bio sorbents has been highlighted. Moreover, it can serve as a bioremediation agent for heavy metals and in coloring along with an ingredient in cattle feed [11]. It is also eaten as a food source in some Asian countries. These serve as a natural source for various bioactive chemicals, which participate in cytotoxic, antioxidant, antiviral, antibacterial, anti-inflammatory, and other activities. Recently, the *Spirogyra* algae, which is widely recognized for its anti-inflammatory and antioxidant properties, has also been

predicted to be therapeutic in diabetic patients [12, 13].

Spirogyra is a potential species for biomass cultivation. The development of *Spirogyra*, nevertheless, is largely dependent on the growth medium and nutrient availability [11, 14]. Nitrogen is essential for optimal algal growth; its absence in the medium may induce stressful conditions for the algae [15]. Similarly, an optimal level of phosphorus content is required in water for algal growth. This growth is closely associated with both nutrient assimilation and lipid accumulation [16]. Additionally, potassium is essential for green algae to survive and reproduce [17]. Hence, it needs nitrogen, potassium, and phosphate in appropriate amounts to grow well. The requirement for growth media used for *Spirogyra* depends on the species type. As a result, it is possible that different species may have different growth rates and respond differently to media composition [18].

In this context, the current research aimed to isolate and identify *Spirogyra hyaline* Cleve to produce a large amount of biomass. Additionally, the study was aimed at culturing the alga for biomass production and to investigate how various nutrients (phosphorus, nitrogen, and potassium) enhance algal biomass production.

2. MATERIALS AND METHODS

Specimens of green algae were collected from the bed of river Ravi, ponds, and slow running water. Fifty samples of algae were collected in plastic bottles by mesh net and brought to the laboratory of botany, Minhaj University Lahore. The samples were subjected to careful water wash to remove all dust and other particles and transferred into beakers for use in the experiments. The filamentous algal species was identified under light microscope and by herbarium. Standard media [19] was used for biomass production. Table 1 shows the chemical composition of the algae culture media. Specimens were kept in 1000 mL beakers filled with water as well as concentrations of salts were measured. A small quantity of urea was added in beakers for exceptional growth, and pH was adjusted to 7.5.

Table 1. Composition of Culture Media for Algal Growth

Ingredients	Amount g L^{-1}
$NaNO_3$	1.0
$MgSO_4$	0.513
NH_4Cl	0.050
KNO_3	0.2
$K_2Cr_2O_7$	0.57
K_2CrO_4	0.41
$KNaC_4H_4O_6$	0.39
KH_2PO4	0.04
Na_3PO_4	0.06
$CaNO_3$	0.058
$(NH_4)_3PO_4$	0.48
K_2HPO_4	0.59
$FeCl_2$	0.003
NaCl	0.053
$CoNO_3$	0.15
Glucose	10
Urea	1

The algae were cultured in glass beakers filled with 1000 mL of culturing medium following [19]. Approximately, 3g of algal biomass was inoculated in each beaker. The beakers were then placed under sunlight for 6 hours per day. The samples were weighed daily, and regular growth was observed for 18 days. The influence of various nitrogen, phosphorous, and potassium concentrations (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5 %.) on algae growth was studied. Nitrogen sources included potassium nitrate, ammonium chloride, sodium nitrate, calcium nitrate, and cobalt nitrate [20]. Phosphorus sources, including sodium phosphate, ammonium

phosphate, and dipotassium phosphate were studied [20]. Whereas, the potassium sources concentrations (0.1, 0.3, 0.4, 0.5, and 0.6 g L^{-1}) included potassium nitrate, potassium phosphate monobasic, potassium dichromate, potassium chromate, and potassium sodium tartrate [21]. The experiment was carried out in triplicates.

The following kinetic parameters were studied according to [22]:

μ= natural log.

$Y_{p/x}$ =product yield coefficient.

Q_x= volumetric rate of biomass production.

q_p=specific rate of biomass production.

3. RESULTS

3.1. Taxonomy and Characteristics

Fifty samples were collected and identified as *Spirogyra hyaline* Cleve. The isolated specie was taxonomically and morphologically identified [23]. According to [22], the taxonomical position of *Spirogyra Hyaline* Cleve is given below:

Spirogyra hyaline Cleve is a commonly occurring free-floating freshwater algae and is commonly known as 'blanket weed and water silk'. Its multicellular filamentous green structure is covered by a mucilaginous sheath. Each filament is unbranched, containing cylindrical cells connected from ends. A double layered cell wall is composed of cellulose and pectin. Cytoplasm is periphery with a vacuole in the center. One or more ribbon-shaped spirally-arranged chloroplasts are spread in the cytoplasm. Each chloroplast bears pyrenoids (Figure 2). Single nucleus is present in the center of the cytoplasm near the vacuole. Conjugation is scalariform and form tubes by both gametes.

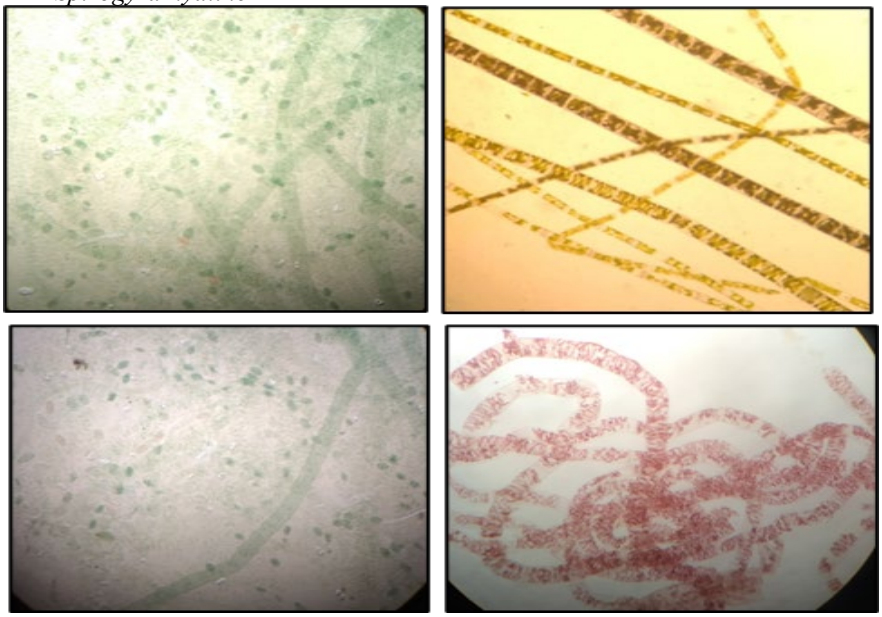


Figure 2. Microscopic View of *Spirogyra hyaline* Cleve

3.2. Effect of Different Nitrogen Sources on the Biomass Production

The impact of nitrogen sources on the production of *Spirogyra hyaline* Cleve biomass is explained in Figure 3. The sources, such as $(NH_4)SO_4$, NH_4Cl, $NaNO_3$, $Ca(NO_3)_2$, $Co(NO_3).6H_2O$ were tested for the growth of *Spirogyra hyaline* Cleve. Among all the nitrogen sources tested, calcium nitrate [$Ca(NO_3)_2$] was the most effective, yielding the optimum production of algal biomass at 18.2 g L^{-1}. The other sources resulted in comparatively lower biomass production, with cobalt nitrate [$Co(NO_3)_2 \cdot 6H_2O$] yielding the least biomass (2.8 g L^{-1}) for *Spirogyra hyaline* Cleve.

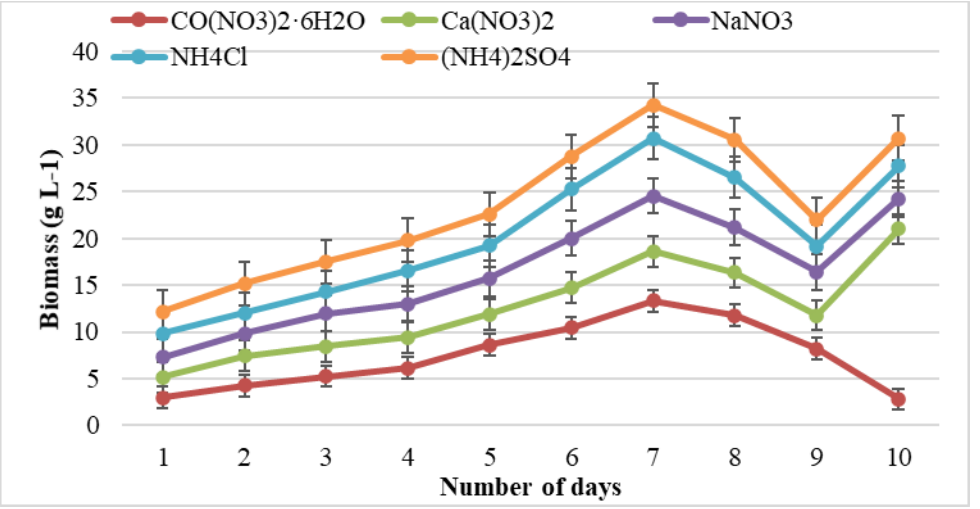


Figure 3. Effect of Various Nitrogen Sources on Algal Biomass Production (at pH 7) with Time

Table 2. Kinetic Evaluation of Different Nitrogen Sources

Chemicals	μ	$Y_{p/x}$	Q_x	q_p
$(NH_4)SO_4$	0.262	0.82	1.3	0.215
NH_4Cl	0.832	1.14	2.3	0.949
$NaNO_3$	1.163	1.42	3.2	1.651
$Ca(NO_3)_2$	1.360	1.82	3.9	2.476
$CO(NO_3).6H_2O$	0.262	0.56	1.3	0.146

The error bars in Figure 3 above represent the standard deviation of measurements, indicating variability in the data. The results showed that $CO(NO_3)_2.6H_2O$ exhibited the highest biomass production on day 7, followed by NH_4Cl. Whereas, on the 10th day, $Ca(NO_3)_2$ produced the highest (18.2 g L^{-1}), with $CO(NO_3)_2 \cdot 6H_2O$ showing the least effect on biomass production (2.8 g L^{-1}). A notable decline in biomass production was observed between days 7 and 9 for all nitrogen sources, with a recovery phase in most sources towards day 10. These trends suggest that the nitrogen source significantly impacts the growth kinetics and biomass yield of algae over time.

The kinetic parametric study also supported these results, indicating the volumetric rate of biomass production (3.9) and

yield of biomass production (1.82 g L^{-1}) were significantly high as the calcium nitrate was added in the medium. While, other sources gave insignificant results (Table 2).

3.3. Effect of Different Concentrations of $Ca(NO_3)_2$ on the Biomass Production

The influence of different concentrations of $Ca(NO_3)_2$ on the biomass generation of *Spirogyra hyaline* Cleve is illustrated in Figure 4. The tested concentrations included 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5 %. The biomass increased with an increase in the concentration of $Ca(NO_3)_2$ and reached (17.3g L^{-1}) at 2.0 % $Ca(NO_3)_2$. Further increase in the concentration of nitrogen sources had no significant effect on the algal biomass production rather it decreased. However, 0.5 % gave least production of biomass (4.3 g L^{-1}).

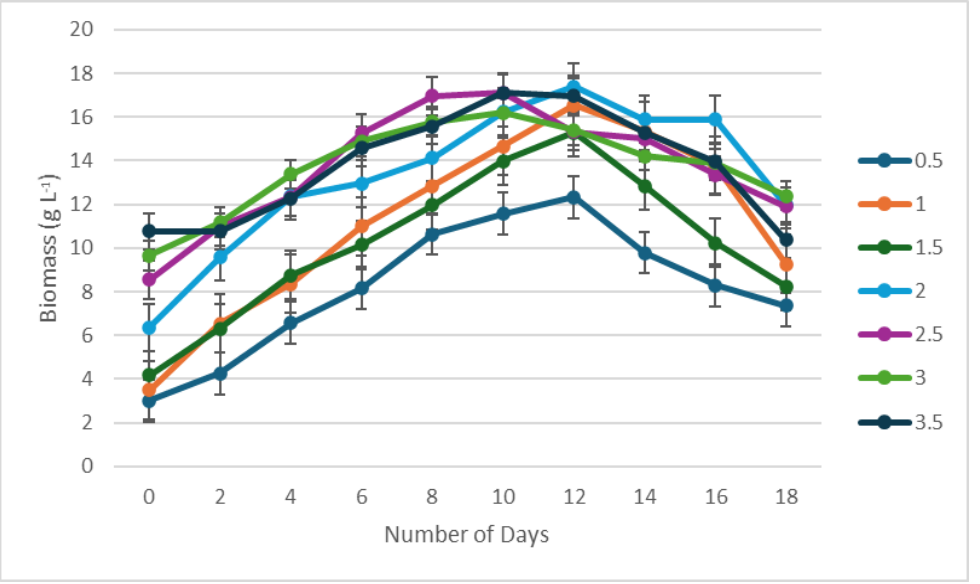


Figure 4. Effect of Different $Ca(NO_3)_2$ Concentrations on Algal Biomass Production (pH 7) Over 18 Days

The data shows a general trend of increasing biomass with time, peaking between 8 and 12 days, followed by a decline across all concentrations. Increased concentrations (2.5 to 3.5 %) supported greater biomass production, reaching a peak around 16-18 gL^{-1}. Error bars indicate standard deviations, demonstrating variation in biomass measurements. The data suggests that $Ca(NO_3)_2$ concentration significantly influences biomass accumulation.

The kinetic parametric study indicates that the volumetric rate of biomass production (4.7) and yield of biomass production (4.4) were highly significant as the $Ca(NO_3)_2$ at 2.0 % level was added in the medium. Further increase in the concentration of $Ca(NO_3)_2$ showed no significant effect on the algal biomass production.

Table 3. Kinetic Evaluation of Different of $Ca(NO_3)_2$ Concentrations

Concentrations	μ	$Y_{p/x}$	Q_x	q_p
0.5	0.530	1.42	1.7	0.764
1.0	0.832	1.02	2.3	0.849
1.5	1.098	0.973	3	1.069
2.0	1.547	1.74	4.7	1.547
2.5	1.252	0.648	3.5	0.811
3.0	0.916	1.44	2.5	4.031
3.5	0.832	0.337	2.3	0.280

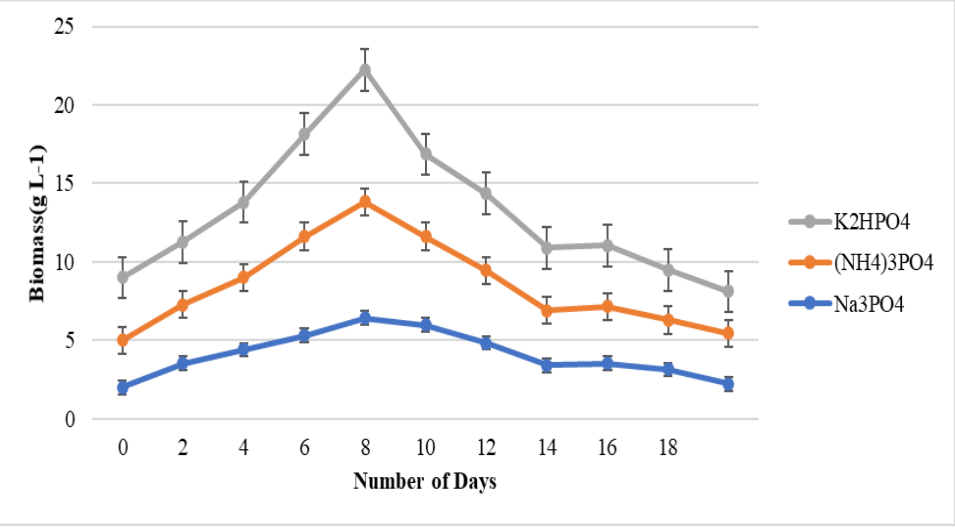


Figure 5. Effect of Different Phosphorus Sources on Algal Biomass Production

3.4. Effect of Different Phosphorus Concentrations on Algal Biomass Production

The effect of different phosphorus sources, such as Na_3PO_4, $(NH_4)_3PO_4$, and K_2HPO_4 on the biomass production of *Spirogyra hyaline* Cleve is explained in Figure 5. The production of biomass was maximum (20.4 g L^{-1}) when K_2HPO_4 (0.5g L^{-1}) was added in the growth medium. The other sources, such as Na_3PO_4 and $(NH_4)_3PO_4$ produced 14.5 g L^{-1} and 12.4 g $L^{-1}$of biomass, respectively. The results depicted that phosphorus along with potassium have noteworthy effect on biomass production of *Spirogyra hyaline* Cleve.

Figure 5 shows that K_2HPO_4 produced highest biomass on day 8 before gradually decreasing. $(NH_4)_3PO_4$ produced lower biomass yield, peaking at approximately 12 gL^{-1} on day 8, with a gradual decline afterwards. Error bars indicate standard deviations, highlighting variability in the biomass measurements. These results suggest that K_2HPO_4 is the most effective phosphate source for promoting biomass growth.

The kinetic parametric study indicates that the volumetric rate of biomass production and yield of biomass production were significantly high as the K_2HPO_4 was added in the medium (Table 4). The product yield

coefficient of K_2HPO_4 was 5.73, volumetric rate was 3.7, and specific rate of biomass was 7.49, while others gave insignificant results.

Table 4. Kinetic Analysis of Different Sources of Phosphorus

Salts	μ	$Y_{p/x}$	Q_x	q_p
Na_3PO_4	0.765	3.93	2.15	3.01
$(NH_4)_3 PO_4$	0.693	3.5	2	2.42
K_2HPO_4	1.308	5.73	3.7	7.49

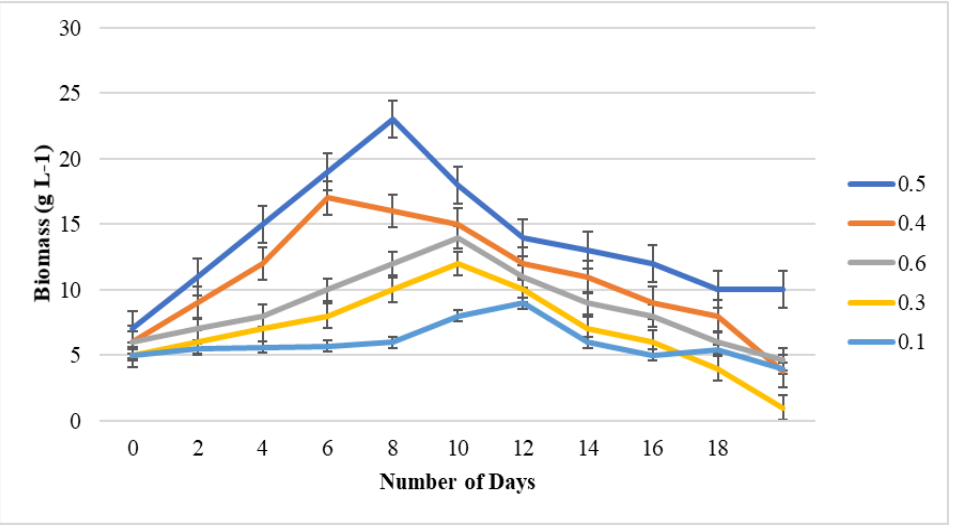


Figure 6. Effect of Different K_2HPO_4 Concentrations on the Biomass Production Over Time

3.5. Effect of Different K_2HPO_4 Concentrations on the Biomass Production

Among all the selected phosphorus sources, K_2HPO_4 turned out to be the best phosphorus source. So, it was necessary to determine at what concentration K_2HPO_4 was easily available to algae as the best phosphorus source. Figure 6 shows the impact of various concentrations of K_2HPO_4, such as 0.1, 0.3, 0.4, 0.5, and 0.6 g L^{-1} on the biomass production of *Spirogyra hyaline* Cleve. Biomass increased with an increase in the concentration of K_2HPO_4 and reached maximum (22.4 g L^{-1}) at 0.5 g L^{-1}. Further increase in the concentration reduced the biomass production. The other concentration, such as 0.1, 0.3, 0.4, 0.5, and 0.6g L^{-1} produced 10.8 g L^{-1}, 12.8 g L^{-1}, and 13.8

Error bars in the Figure 6 represent standard deviation. The graph shows an increase in biomass production with the increased concentrations of K_2HPO_4 and reached a maximum (22.4 g L^{-1}) at 0.5 g L^{-1} on 8th day. However, further increase in concentration reduced the biomass production. Lower concentrations produced lower biomass with the least production at 0.1 g L^{-1}.

The study of kinetic parameters indicated a significantly high volumetric rate of biomass production and yield of biomass production at 0.5 g L^{-1} concentration of

K_2HPO_4 (Table 5). The product yield of K_2HPO_4 at 0.5 g L^{-1} concentration was 6.73, volumetric rate of biomass was 3.3, and specific rate was 8.02. On the other hand, other concentrations gave insignificant results.

Table 5. Kinetic Analysis of Different K_2HPO_4 Concentrations

Concentration	μ	$Y_{p/x}$	Q_x	q_p
0.1	0.438	3.2	1.55	1.41
0.3	1.081	4.93	2.95	5.32
0.4	0.916	4.46	2.5	4.08
0.5	1.193	6.73	3.3	8.02
0.6	1.16	5.13	3.2	5.95

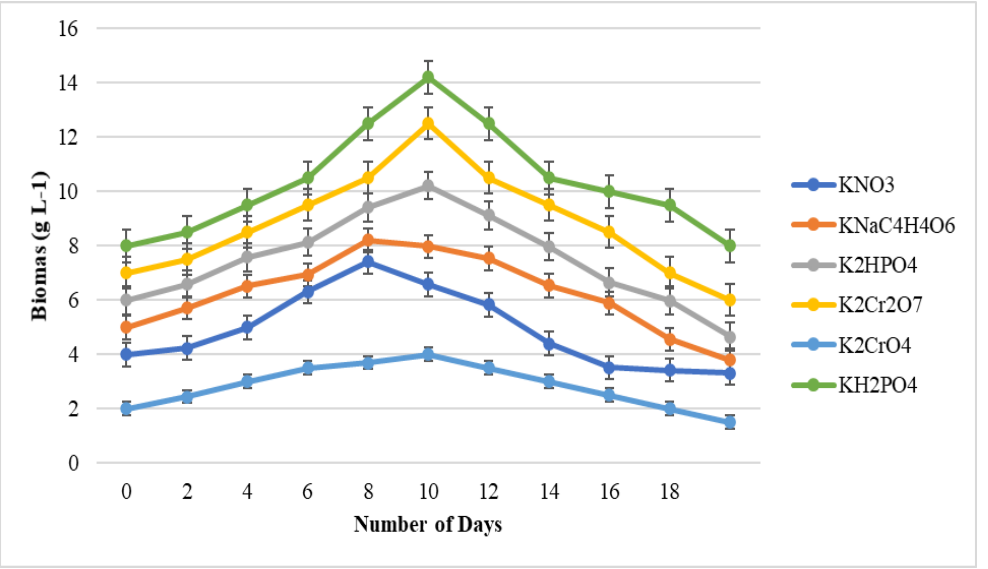


Figure 7. Effect of Different Potassium Sources on the Biomass Production Over 18 Days

3.6. Effect of Different Potassium Sources on the Biomass Production

The impact of various potassium sources, such as KNO_3, KH_2PO_4, $K_2Cr_2O_7$, K_2CrO_4, as well as $KNaC_4$ and $KNaC_4H_4O_6$ on the biomass production of *Spirogyra hyaline* Cleve is explained in Figure 7. The production of biomass was maximum (14.8 g L^{-1}) when KH_2PO_4 was added in the growth medium. The other sources KNO_3,$K_2Cr_2O_7$, K_2CrO_4, K_2HPO_4, and $KNaC_4H_4O_6$ resulted in 12.5g L^{-1}, 9.5 g L^{-1}, 5.6 g L^{-1},10.6 g L^{-1} and 7.9 g L^{-1}biomass, respectively.

The results in Figure 7 show that KH_2PO_4 produced highest biomass on the 10^{th} day, followed by $K_2Cr_2O_7$, K_2HPO_4, and KNaC4H4O6. The biomass production gradually decreased after 10 days, depicting that further addition of these sources reduced the biomass yield. Error bars represent data variability in the measurements.

The study of kinetic parameters indicates that the volumetric rate of biomass production and yield of biomass production were significantly high as the KH_2PO_4 was added in the medium. The product yield coefficient of KH_2PO_4 was 4.93, volumetric

rate of biomass was 2.1 and specific rate of biomass was 3.65, while other sources gave insignificant results.

Table 6. Kinetic Analysis of Different Sources of Potassium

Salts	μ	$Y_{p/x}$	Q_x	q_p
KNO_3	0.471	4.2	1.6	1.97
KH_2PO_4	0.741	4.93	2.1	3.65
$K_2Cr_2O_7$	0.182	3.16	1.2	0.57
K_2CrO_4	0.139	1.86	1.15	0.25
$KNaC_4H_4O_6$	0.405	3.53	1.5	1.42

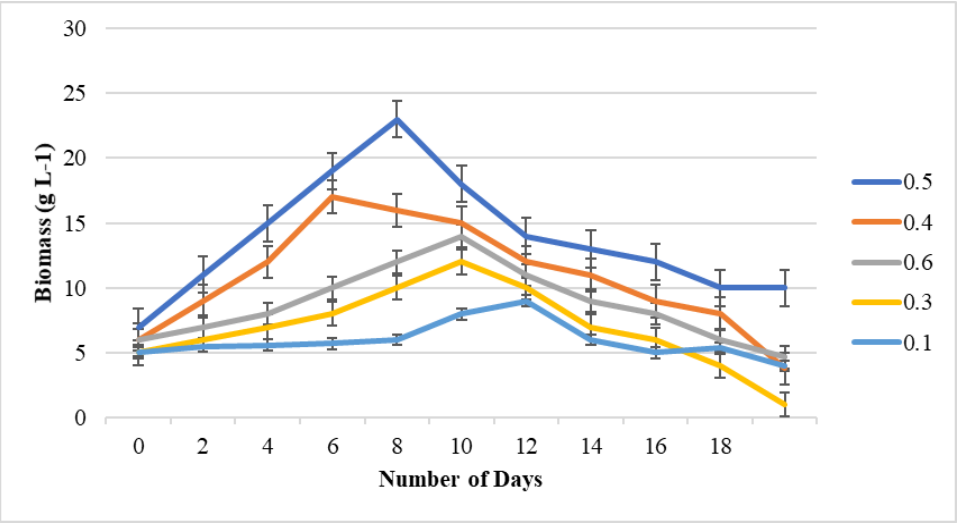


Figure 8. Effect of Different KH_2PO_4 Concentrations on Biomass Production Over 18 Days

3.7. SaEffect of Different KH_2PO_4 Concentration on Biomass Production

The effect of different concentration of KH_2PO_4, such as 0.1, 0.3, 0.4, 0.5, and 0.6 g L^{-1} on the biomass production of *Spirogyra hyaline* Cleve is illustrated in Figure 8. The production of biomass was augmented when the concentration of KH_2PO_4 increased and reached (18.6 g L^{-1}) at 0.5 g L^{-1} of KH_2PO_4. The other concentrations, 0.1, 0.3, 0.4, and 0.6 g L^{-1} produced 11.2 g L^{-1}, 11.8 g L^{-1}, 15.3 g L^{-1} and 13.8 g L^{-1} of biomass, respectively.

Figure 8 shows that increased concentration yielded higher biomass production, such that 0.5 g L^{-1} produced maximum biomass on the 8th day, followed by a gradual decrease. Whereas, 0.4 g L^{-1} yielded higher biomass on 6th day, followed by 0.6 and 0.3 g L^{-1} on 10th day, and 0.1 g L^{-1} on 12th day of culture. Biomass production gradually declined after 12 days of culture with 0.3 g L^{-1} producing the least biomass output.

The kinetic parametric study indicates that the volumetric rate of biomass production and yield of biomass production was significantly high at 0.5 g L^{-1} of KH_2PO_4

(Table 7). The product yield coefficient of KH_2PO_4 in 0.5 g L^{-1} concentration was 6.2, volumetric rate of biomass was 3.1, and specific rate of biomass was 7.01, while other concentrations gave insignificant results.

Table 7. Kinetic Evaluation of Different KH_2PO_4 Concentrations

Concentration	μ	$Y_{p/x}$	Q_x	q_p
0.1	0.875	3.73	2.4	3.26
0.3	0.471	3.93	1.6	1.85
0.4	1.011	5.1	2.75	5.15
0.5	1,131	6.2	3.1	7.01
0.6	0.587	4.6	1.8	2.71

3.8. Rate of Algal Biomass Production in Control and Modified Media

The rate of *Spirogyra hyaline* Cleve biomass production in control media and modified media is illustrated in Figure 9. The production of algal biomass increased in both media and reached 13.1 g L^{-1} and 10.3g L^{-1} in modified and controlled media, respectively after 12 days of incubation. Further increase in the time period significantly decreased growth.

Figure 9 shows an increase in biomass production in modified media till the 10th day of culture. Whereas, controlled media showed higher biomass production on 12th day of culture. After that, biomass yield gradually decreased.

The kinetic parametric study indicates that the yield of the biomass increased with increased time duration (Table 8). The product yield coefficient of modified media was optimum at 6.73 after 18 days of incubation. The volumetric rate of biomass was 3.7 and specific rate was 8.02. The product yield coefficient of control media was optimum at 1.86 after 18 days of incubation. The volumetric rate of biomass was 1.15 and the specific rate was 0.25. The modified media gave better results as compared to the control media.

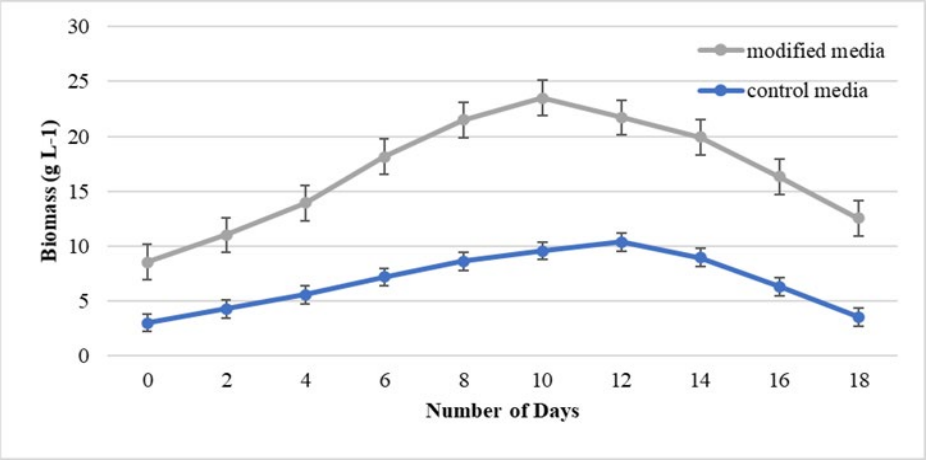


Figure 9. Difference in the Rate of the Biomass Production of *Spirogyra hyaline* Cleve in Controlled and Modified Media Over 18 Days

Table 8. Kinetic Analysis of the Rate of Biomass Production

Media	μ	$Y_{p/x}$	Q_x	q_p
Modified media	1.193	6.73	3.3	8.02
Control media	0.139	1.86	1.15	0.25

4. DISCUSSION

The findings of the study recognized the *Spirogyra hyaline* Cleve species both morphologically and taxonomically. The findings explained that nitrogen sources affect *Spirogyra hyaline* Cleve's biomass output. The sources included Ca $(NO_3)_2$, NH_4Cl, Co (NO_3), (NH_4) SO_4, and $NaNO_3$ The source that produced the most algal biomass (18.2 g L^{-1}) irrespective of all the sources examined was calcium nitrate ($Ca(NO_3)_2$). It can possibly happen due to the fact that calcium nitrate has both nitrogen and calcium components which are required by algae to grow. Nitrogen, in particular, can be a limiting nutrient for algae biomass production [16].

Co $(NO_3).6H_2O$ yielded the minimum amount of biomass (2.8 g L^{-1}). The possible reason of this could be that cobalt has a heavy metal-related developmental toxicity. The results of [16, 24] supported these findings concluding that nitrate was a predetermining factor on the development of algae. On the other hand [25, 26] demonstrated that the level of nitrate had no effect on algal growth, thereby deviating the findings. The concentrations are as follows: 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5%. With an increase in Ca $(NO_3)_2$ level, biomass growth rose and reached its maximum (17.3g L^{-1}) at 2.0 % $Ca(NO_3)_2$. No further increase in biomass was observed with the increase in nitrogen source. The reason for this might be because the growth medium's elevated levels of N and C exhibit bias against the absorption of other nutrients, such as P and K. [27] found that *Chlorella kessleri* algae consumed more nitrogen when exposed to continuous light, which is consistent with our results. It is evident that light may encourage nitrogen intake when there is a discrepancy between the observed and predicted amounts of nitrogen under constant illumination. According to [27], algae produce the nitrogen-containing molecules adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) when exposed to light. The current study did not evaluate ATP or NADPH levels; nonetheless, *Spirogyra* sp. green algae may enhance the absorption of nitrogen. In relation to the nitrate level, it may be said that within 10 to 12 days, noticeable alterations were seen as nitrate increased.

The addition of K_2HPO_4 to the growth medium yielded maximum biomass generation (20.4 g L^{-1}) since it provided both potassium and phosphate content, which are critical for the healthy development of *Spirogyra hyaline* Cleve. The other sources, such as Na_3PO_4 and $(NH_4)3PO_4$, yielded biomass weight of 14.5 g L^{-1} and 12.4 g L^{-1}, respectively. Therefore, the current findings depicted that potassium and phosphorus significantly affect the biomass output of *Spirogyra hyaline* Cleve [28-30] demonstrated that phosphate levels greatly impact the development of algae, thereby confirming the results. Similarly, [31] found that phosphorus is essential for algal development and blooms in lakes and oceans. Whereas, [32] showed that manganese and phosphorus together had a mild impact on algal development. It can be asserted that *Spirogyra* species uses phosphorus to manufacture phosphorous-containing substances, such as ATP and NADPH. Abiotic

phosphorous deposition and biotic phosphorous absorption into biomass are two ways to extract phosphorus [17].

The results also depicted that with increasing concentration of K_2HPO4, biomass rose and reached maximum (22.4 g L^{-1}) at 0.5 g L^{-1}. It is because phosphorus is essential for cellular development and reproduction, facilitating sunlight transformation into useful energy [33]. Similar findings have shown that the biomass of *Spirogyra* algae may float on the surface of the water by forming oxygen bubbles. Such bubbles of oxygen, however, are unable to reach the biomass of Spirogyra algae at the ocean's surface for photosynthesis when the water level is too deep. An excessively low water level might potentially affect the production of *Spirogyra* algal biomass. Consequently, in the cultivation of *Spirogyra* algae, water level is also critical [34].

Moreover, the inclusion of KH_2PO_4 to the growth medium also caused a higher biomass output (14.8 g L^{-1}). It is again due to the crucial role of phosphate and potassium in the development of *Spirogyra hyaline* Cleve [35]. The biomass production from the other sources, which included KNO_3, K_2CrO_4, $K2Cr_2O_7$, and $KNaC_4H_4O_6$, was 12.6 g L^{-1}, 5.6 g L^{-1}, 9.5 g L^{-1}, and 10.6 g L^{-1}, in that order. However, K_2CrO_4 generated a negligible biomass output (1.1 g L-1). It may be because potassium and Cr become poisonous together, which limits biomass production. According to [36], chromium, a heavy metal, has a harmful inhibitory impact on algal development and cell counts, causing the filamentous types to lose weight. Therefore, potassium and chromium together produced the least amount of biomass in *Spirogyra hyaline* cleve. At 0.5 g L^{-1} of KH_2PO_4, the generation of biomass reached its maximum (18.6 g L^{-1}) as the amount of KH_2PO_4 rose. It might be because *Spirogyra hyaline* Cleve development required greater levels of potassium ions [8]. This might be because potassium has a limited capacity and can absorb only a certain quantity at most. The concentration of KH_2PO_4 has been absorbed and has an inhibitory impact with further increases [37]. After 18 days of incubation, the amount of algal biomass rose in both conditions and reached 1.1 g L^{-1} and 22.4 g L^{-1}, respectively. Additional time extensions resulted in a dramatic decline in growth. The reason for this might be because the nutrients are depleted after twenty days of incubation [38]. Findings of a similar kind support our results, which indicate that *Spirogyra* filaments develop well in the media-based constituents that have been supplied. In contrast to *Spirogyra*, the other algal species produced a negligible amount of biomass; this might be because they grew more slowly in the given culture [39].

4.1. Conclusion

The study concluded that nitrogen, phosphorus, and potassium are crucial for *Spirogyra hyaline* Cleve growth. The $Ca(NO_3)_2$ at 2.0% level, KH_2PO_4 at 0.5g L^{-1}, and K_2HPO_4 at 0.5g L^{-1} levels were best for the growth of *Spirogyra hyaline* Cleve. Further increase in the concentrations showed toxic inhibitory effect on the growth of *Spirogyra hyaline* Cleve. Kinetic parametric (volumetric rate of biomass production, specific growth rate, high product yield coefficient) also supported the above results. The modified selected media gave better results as compared to control media. The production of algal biomass can be further improved by optimizing the other micronutrients sources, such as Zn, Cu, and Fe etc. can be drawn by synthesizing current scientific understanding with the specific results of the experiment.

Author Contribution

Nadia Jabeen: conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft, supervision**. RimshaArshad**: Investigation, data curation, visualization, writing – review & editing. **Muhammad Hammad Ashraf:** methodology, validation, statistical analysis, writing – review & editing. **Ishrat Fatima:** resources, supervision, project administration, writing – review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

The data supporting the findings of this study are available within the article

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