Agric Res
DOI 10.1007/s40003-015-0168-4

FULL-LENGTH RESEARCH ARTICLE

Cost-Effective Cultivation of *Spirulina platensis* Using NPK Fertilizer

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Received: 14 November 2014 / Accepted: 2 March 2015

Abstract A cost-effective fertilizer-based optimum culture medium was formulated for the mass production of *Spirulina platensis* using NPK-10:26:26 complex fertilizer and growth results were compared with several standard culture media. The microalga was grown in an illuminated white LED with 50 μmol photons m^{-2} s^{-1} and 30 °C temperature under orbital shaking. The optimum NPK fertilizer medium was formulated by varying NPK-10:26:26 fertilizer and sodium bicarbonate loading for which maximum biomass growth rate was obtained. The newly formulated fertilizer medium contains NPK-10:26:26 (0.76 g l^{-1}), sodium bicarbonate (10.0 g l^{-1}), sodium chloride (1.0 g l^{-1}), and micro nutrients (1.0 ml l^{-1}). Maximum growth rate was recorded between 14 and 15 days and corresponding biomass concentration, chlorophyll, protein, and lipid were found to be 1.22, 8.92 g l^{-1}, 52.35, and 14.84 %, respectively. Above growth results using NPK fertilizer were superior over standard culture media, and 50.0 % cost saving was achieved using newly formulated NPK-10:26:26 fertilizer medium for *Spirulina* growth in comparison to the standard culture media. Uptake of nutrients (i.e., carbon, nitrogen, phosphorous, and potassium) and physicochemical parameters (i.e., pH and conductivity) were measured to monitor *Spirulina* growth.

Keywords *Spirulina platensis* · Batch cultivation · NPK-10:26:26 complex fertilizer · Biomass productivity · Chlorophyll · Protein · Lipid

Introduction

Spirulina platensis (a photoautotrophic unicellular blue-green microalga) is one of the cheapest sources of chlorophyll, protein, and high-valued cell compounds which are extensively used in food, pharmaceutical, and cosmetic industries [11, 17]. More recently, it was treated as an ideal food for astronauts by NASA. Although the cultivation of *Spirulina* was practiced mainly for the supplement of protein for human food and animal feed [1], however, it was cultivated to remove nitrogen and phosphorus contaminants from waste water [8, 22]. Moreover, *Spirulina* was used to remove heavy metals and textile dyes from waste waters [9]. Compared to other microalgae, *Spirulina* has the advantages of (i) easy cultivation using direct solar energy at a high specific growth rate, (ii) high tolerance limit toward alkaline pH and salinity, (iii) easy recovery of biomass by centrifugation, and (iv) healthy and easy to digest [21].

For the economic development, researches explored the several aspects of *Spirulina* cultivation considering the effects of environment, thermodynamic parameters, bioenergetics, cellular constituents, strain improvement, and cultivation process [14, 16, 21]. Usually, 25 % of total *Spirulina* production cost was related to the culture medium, therefore the success of large-scale production depends on the economic and effective development of the culture medium [3]. To reduce the cost of the culture media, researchers used urban effluents and animal waste as low-cost culture medium [13]. Materassi et al. [12] studied the growth of S*pirulina* using sea water. Raoof et al. [15]

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Published online: 28 March 2015

formulated low-cost medium for the mass production of S*pirulina* using single super phosphate. To improve *Spirulina* productivity, several studies were also carried out using mixed nitrogen sources involving urea and ammonium salts [4, 6, 19].

NPK-10:26:26 complex fertilizer is an admixture of diammonium phosphate, muriate of potash (i.e., potassium chloride), and minor quantities of urea. This NPK fertilizer was designed to supply three primary nutrients (i.e., nitrogen, phosphorous, and potassium) adequately which are required for biomass growth. The advantage of using NPK-10:26:26 complex fertilizer is mainly due to (i) reduced ammonia toxicity due to the controlled amount of urea and (ii) economical as compared to synthetic nutrients used in standard culture media. Moreover, it eliminates the need of addition of N, P, and K nutrients separately to the culture medium. As the degree of freedom to adjust the ratio of elemental N, P, and K in the culture medium is restricted using NPK-10:26:26 complex fertilizer, therefore the detailed experimental studies are need for efficient utilization of this fertilizer to maximize *Spirulina* growth.

In this study, the use of water soluble, low-cost NPK-10:26:26 complex fertilizer was explored for mass cultivation of *S. platensis*. Optimum culture medium was formulated to maximize *Spirulina* growth by varying loadings of NPK-10:26:26 complex fertilizer and $NaHCO_3$ using white light-emitting diode (LED) with main band 380–760 nm under fixed temperature, light intensity, and light–dark cycle. Growth study using NPK-10:26:26 complex fertilizer culture media was also compared with several standard culture media. Therefore, the aim of the present investigation was to formulate a commercial grade NPK-10:26:26 complex fertilizer medium for mass production of *Spirulina*, which would provide better or at par growth rates of *Spirulina* as compared to the standard culture medium.

Materials and Method

Materials

The strain NCIM-5143 of *S. platensis* was obtained from National Chemical Laboratory (NCL: Pune, India) culture collection center and it was grown in NCL medium with following composition (g l^{-1}): $NaHCO_3$-10.0, $NaNO_3$-2.5, NaCl-1.0, K_2HPO_4-0.5, K_2SO_4-1.0, $MgSO_4.7H_2O$-0.2, $CaCl_2.2H_2O$-0.04, $FeSO_4.7H_2O$-0.01. The growth of microalgae was studied using standard Zarrouk medium [24] with following composition (g l^{-1}): $NaHCO_3$-16.8, $NaNO_3$-2.5, K_2HPO_4-0.5, K_2SO_4-1.0, NaCl-1.0, $CaCl_2$-$2H_2O$-0.04, $MgSO_4{\cdot}7H_2O$-0.2, $FeSO_4{\cdot}7H_2O$-0.01 and 1.0 ml of A_5 solution (H_3BO_3-2.86, $MnCl_2.4H_2O$-1.81, $ZnSO_4.4H_2O$-0.222, Na_2MoO_4-0.018, $CuSO_4.5H_2O$-0.079 and all are in g l^{-1}). For growth comparison, Central Food Technological Research Institute (CFTRI: Mysore, India) medium was also used with following composition (g l^{-1}): $NaHCO_3$-4.5, $NaNO_3$-1.5, K_2HPO_4-0.5, K_2SO_4-1.0, NaCl-1.0, $MgSO_4{\cdot}7H_2O$-0.2, $CaCl_2{\cdot}2H_2O$-0.04, $FeSO_4{\cdot}7H_2O$-0.01. The differences in above three culture media are mainly due to carbon content and A_5 solution. Moreover, CFTRI medium has also less quantity of elemental nitrogen as compared to NCL and Zarrouk's medium. In this study, *Spirulina* was cultivated using NPK-10:26:26 complex grade fertilizer, which was obtained from the authorized dealers of Indian Farmers Fertilizers Cooperative Limited (IFFCO: Dhanbad, India). Typical composition of NPK-10:26:26 fertilizer used in this study was diammonium phosphate: 50.04 %; muriate of potash: 43.98 %; urea: 1.5 %; silica: 3.6 %; and moisture: 0.88 %. Above composition was also expressed in terms of elemental nitrogen, neutral ammonium citrate soluble phosphates (as P_2O_5), and water soluble K_2O, respectively, which was equivalent to as 10.0, 26.0, and 26.0 % respectively.

Inoculum Preparation and Microorganism Cultivation

To prepare inoculum of *Spirulina*, NCL medium was used and individual components of NCL medium were sterilized separately by autoclaving at 121 °C for 30 min. Culture medium was prepared in 500-ml Erlenmeyer flask with 250-ml stock standard solutions. *Spirulina* strain was transferred into liquid media and kept in an air flow chamber under laminar flow condition. Finally, inoculum was kept in an orbital shaker at 90 rpm and 30 °C. The culture was incubated under 50 µmol photons m^{-2} s^{-1} continuous illumination using white LEDs with photoperiod of 14/10 h light/dark cycle. The microorganism was filtered and washed with 9.0 % NaCl solution for complete removal of adsorbed salts after attending the exponential growth phase. Now, the cultivation of *Spirulina* was carried out using NCL, CFTRI, Zarrouk, and NPK-10:26:26 fertilizer medium with an initial dry biomass concentration of 50.0 mg l^{-1}. For mass cultivation of *Spirulina*, cultivation was carried out in 1000-ml Erlenmeyer flasks with 750 ml of culture medium under standard condition (i.e., temperature-30 °C; white LED illumination-50.0 µmol photons m^{-2} s^{-1}; orbital shaking speed-90 rpm; and photoperiod-14 h light/10 h dark).

Protein and Lipid Estimation

For protein estimation, it was extracted using Tris–HCl (0.1 M) buffer and allowed to react with Folin–Ciocalteu reagent for 30 min to develop color [10]. Corresponding absorbance was measured at 750 nm using double beam UV–Visible spectrophotometer and it was compared with

bovine serum albumin calibration standard. For lipid analysis, biomass was filtered and washed using phosphate buffer (pH 7.0). Residue was treated with chloroform/methanol (2:1 v/v) for lipid extraction and purified in methanol/water (2:1 v/v) containing 9.0 g l^{-1} NaCl to remove sugars, salts, and proteins. Lipid content was determined gravimetrically after concentrating lipid–solvent mixture in a rotary vacuum evaporator [7].

Chlorophyll Estimation

To determine chlorophyll, a typical 10 days old biomass sample was collected and chlorophyll was extracted as per standard procedure [18]. To select a better solvent for chlorophyll extraction, several new solvents (e.g., dimethyl formamide, *N*-methyl pyrrolidone, and propanol) were tested and compared with common solvents (e.g., acetone, methanol, ethanol, and dimethyl sulfoxide). Chlorophyll concentration was measured using double beam UV–Visible spectrophotometer with absorbance peak at 663 nm [18]. Equilibrium absorbance was measured after the attainment of equilibrium at a sufficiently high extraction time with varying biomass loading for different solvents. The solvent power to extract chlorophyll was determined by comparing the mass extinction coefficient of chlorophyll toward individual solvents. Details of chlorophyll absorbance versus biomass concentrations for different solvents are shown in Fig. 1a with correlation coefficients >0.99. Mass extinction coefficients (l g^{-1} cm^{-1}) for acetone, *N*-methyl pyrrolidone, dimethyl formamide, dimethyl sulfoxide–acetone, methanol, ethanol, and propanol were found to be 0.13, 0.15, 0.21, 0.22, 0.33, 0.38, and 0.66, respectively. It was observed that propane has the highest mass extinction coefficient with 0.66 l g^{-1} cm^{-1} (Fig. 1a) and it was selected for chlorophyll.

Analysis of N, P, K, and Total Carbon

To determine the uptake of elemental N, P, and K during biomass growth, culture medium (free from microalgae) was allowed to react with suitable reagents to develop the characteristic visible colors and corresponding absorbance was measured using double beam UV–Visible spectrophotometer. For ammoniacal nitrogen, culture medium was allowed to react with Nessler reagent which develops yellow–brown color. Similarly, nitrate nitrogen was estimated by the reaction with sodium salicylate in sulphuric acid medium using cadmium powder which formed yellow color salts of nitrosalicylic acid. To determine elemental P, culture medium was allowed to react with molybdate in acid solution forming a yellow-colored phosphomolybdate complex, which was then reduced by an amino acid, giving a characteristic molybdenum blue color. Absorbance peaks corresponding to the wavelengths of nitrate nitrogen, ammoniacal nitrogen, and phosphate phosphorous were obtained at 410, 425, and 690 nm, respectively. Potassium was measured by turbidimetric method using a HANNA nutrient analyzer (Model: HI 83225). To determine the unknown concentration of elemental N, P, and K in the culture medium, calibration plots were generated using known composition of Zarrouk and NPK-10:26:26 medium separately. The calibration plots for nitrate nitrogen (Zarrouk's medium) and ammoniacal nitrogen (NPK-10:26:26 medium) are shown in Fig. 1b, whereas the calibration plots for elemental phosphorous in Zarrouk's medium and NPK fertilizer culture medium are shown in Fig. 1c. Two separate calibration plots for elemental phosphorous were obtained using Zarrouk's and NPK fertilizer, which was mainly due to the presence of K_2HPO_4 in Zarrouk and $(NH_4)_2HPO_4$ in NPK fertilizer medium. The difference in absorbance values of the above phosphate compounds was mainly due to the strong hydrogen bonding between phosphate groups and ammonium hydrogen atom in $(NH_4)_2HPO_4$ crystals [23]. The calibration plots for elemental potassium in Zarrouk and NPK medium are also shown in Fig. 1d.

$NaHCO_3$ was used as a carbon source for *Spirulina* growth, and the initial pH of culture medium was found to be between 8.3 and 9.3, which indicates the presence of both $NaHCO_3$ and Na_2CO_3 in the culture medium. Total carbon content in the culture medium was determined by titration method using 0.1 N H_2SO_4 with the use of phenolphthalein and methyl orange indicator.

Analysis of Variance

The growth results reported in this study were assessed by the analysis of variance (p). It was confirmed that the values of 'p' were *almost* less than 0.04 for the analysis of (i) chlorophyll, (ii) biomass growth rate (iii) uptake of elemental C, N, P, and K, and (iv) physicochemical parameters (i.e., pH and conductivity) during biomass cultivation for different culture media (i.e., Zarrouk, NCL, CFTRI, and NPK-10:26:26) for comparison. It was also mentioned that protein and lipid were analyzed at the end of experiments for above culture media, which was also confirmed by the values of 'p' with less than 0.05.

Calculation of Kinetic Parameters

Kinetic parameters for *Spirulina* cultivation were determined and these are (i) biomass productivity, P_X (mg l^{-1} d^{-1}), (ii) maximum specific growth rate, μ_{max} (d^{-1}), (iii) nitrogen-to-cell conversion factor, $Y_{X/N}$ (g, g^{-1}), and (iv) phosphorus-to-cell conversion factor, $Y_{X/P}$ (g, g^{-1}). P_X was calculated from the ratio of $(X_m - X_o)$ and the corresponding time to obtain X_m, where X_m and X_o are the maximum biomass concentration and the initial inoculum

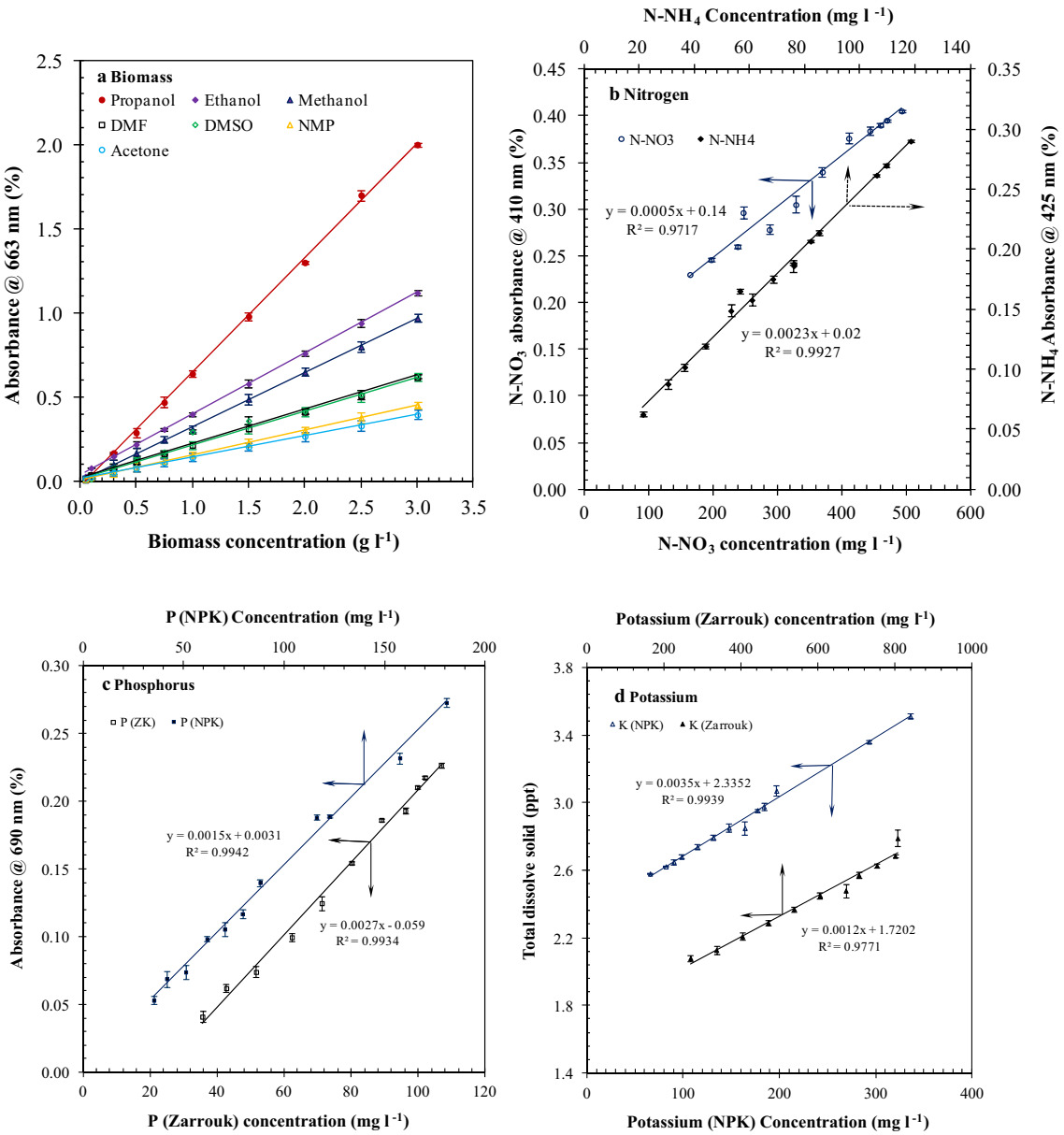

Fig. 1 Calibration plots for **a** biomass **b** nitrate and ammoniacal nitrogen **c** phosphorous in Zarrouk (K_2HPO_4) and NPK fertilizer [$(NH_4)_2HPO_4$] medium **d** potassium in Zarrouk and NPK culture medium

concentration, respectively, whereas $\mu_{\max}\left(=\frac{\ln(X_m/X_{m-1})}{t_m - t_{m-1}}\right)$ was calculated at X_m. $Y_{X/N}$ was determined from the ratio of biomass produced and the total nitrogen added to the culture medium. Similarly, $Y_{X/P}$ was calculated after considering phosphorous added in the culture medium.

Results and Discussion

Effect of NPK-10:26:26 Loadings Under White LED

Spirulina platensis growth was carried out by varying NPK-10:26:26 fertilizer loading under white LED by fixing $NaHCO_3$ concentration at 16.8 g l^{-1} under fixed temperature (i.e., 30 °C), light intensity (50 µmol photons m^{-2} s^{-1}), and light–dark cycle (14/10 h). Above fixed temperature, light intensity and light–dark cycle were considered as optimum parameters for *S. platensis* growth studies [8, 14, 15, 22, 24]. It was observed that *Spirulina* did not grow for the culture medium where NPK-10:26:26 fertilizer concentration was more than 1.5 g l^{-1}. Therefore, biomass cultivation was carried out with reduced concentration of NPK-10:26:26 fertilizer. Details of culture medium with varying NPK fertilizer loading with fixed $NaHCO_3$ concentration are given in Table 1 and these are designated by P1–P6 fertilizer medium. Satisfactory

growth of *Spirulina* was observed in P1–P4 fertilizer media and corresponding growth curves are shown in Fig. 2a. The results revealed that *Spirulina* growth increased with the increased fertilizer content in the culture medium up to the certain limit and thereafter growth decreased. The best *Spirulina* growth was obtained at 1.06 g l^{-1} NPK fertilizer concentration (i.e., P3 fertilizer medium) with maximum biomass concentration (i.e., 1.12 g l^{-1}) at the fifteenth day of biomass growth. It was also observed that NPK fertilizer concentrations at 0.76 g l^{-1} (i.e., P2 fertilizer medium) resulted in 0.81 g l^{-1} biomass concentration (Fig. 2a), which was next best run after P3 fertilizer medium. Details of growth responses (i.e., X_m, P_X and $\mu_{\max}$) for P1-P5 fertilizer medium are shown in Table 2. Depending on elemental N and P loading, $Y_{X/N}$ and $Y_{X/P}$ were also calculated for P1–P5 fertilizer media, and the details are also reported in Table 2. It was observed that best values of $Y_{X/N}$ and $Y_{X/P}$ were also obtained for P3 fertilizer medium. The chlorophyll, protein, and lipid content for P1–P5 fertilizer media were determined, and the results are listed in Table 2. It was noted that the accumulation of chlorophyll, lipid, and protein was in accordance with biomass growth. Under fixed temperature, light intensity, and light–dark cycle, it was observed that maximum biomass concentration was obtained at the fifteenth day of biomass growth using different culture media (i.e., Zarrouk, NCL, CFTRI, and NPK-10:26:26 ferilizer).

The detail uptake of elemental C, N, P, and K corresponding to biomass growth is shown in Fig. 2b–e, respectively. Elemental C uptake pattern for all P1–P5 culture media is in accordance with biomass growth. The maximum uptake of elemental carbon took place for P3 fertilizer medium and it was found to be ~30.0 % (Fig. 2b). The prime source of nitrogen in NPK-10:26:26 fertilizer is in the form of ammonium ions and urea. Ammonia transports easily without spending any extra energy in the presence of extracellular/intracellular gradient of pH across the plasma membrane, and assimilated with the help of glutamine synthetase enzyme releasing protons in the culture medium, whereas for nitrate-based medium (e.g., NCL, CFTRI, and Zarrouk medium), extra energy is required for the metabolism of nitrate, which occurs via the reduction of nitrate to nitrite and nitrite to ammonium ion. Elemental N uptake for P1–P5 fertilizer media was in Fig. 2c and maximum uptake took place for P3 fertilizer medium which is equivalent to ~81.0 % (Fig. 2c). Assuming the molecular formula of *Spirulina* as $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$, [5] nitrogen content based on biomass produced using P3 fertilizer medium was calculated as 106.0 mg l^{-1}, whereas actual nitrogen accumulation was found to be 98.0 mg l^{-1} (Fig. 2c). Similarly, elemental N uptake for P1, P2, P4, and P5 fertilizer media was calculated and an excellent agreement with the theoretical values was obtained with average absolute deviation less than 6.0 %. Phosphate is a major nutrient for microalgae growth, which produces nucleic acids and phospholipids. Elemental P uptake pattern for P1–P6 fertilizer media is shown in Fig. 2d, and elemental P consumption

Table 1 Experimental design of the culture medium studied for the growth of *Spirulina platensis* using NPK-10:26:26 complex fertilizer

Culture medium	$NaHCO_3$ (g l^{-1})	Salts[†] (g l^{-1})	NPK-10:26:26 (g l^{-1})	A_5 micronutrients[‡] (ml)
Varying NPK loading (fixed $NaHCO_3$ loading)				
P1	16.8	1.33	0.36	1.0
P2	16.8	1.33	0.76	1.0
P3	16.8	1.33	1.06	1.0
P4	16.8	1.33	1.26	1.0
P5	16.8	1.33	1.36	1.0
P6	16.8	1.33	1.46	1.0
Varying $NaHCO_3$ (fixed NPK loading)				
P2C1	22.0	1.33	0.76	1.0
P2C2	18.0	1.33	0.76	1.0
P2C3	10.0	1.33	0.76	1.0
P2C4	4.50	1.33	0.76	1.0
P3C1	22.0	1.33	1.06	1.0
P3C2	18.0	1.33	1.06	1.0
P3C3	10.0	1.33	1.06	1.0
P3C4	4.50	1.33	1.06	1.0

[†] NaCl: 1.0, MgSO4·7H2O: 0.2, EDTA: 0.08, CaCl2·2H2O: 0.04 and FeSO4·7H2O: 0.01 (g l^{-1})

[‡] A5 micro nutrients: 1 ml solution consisting of H3BO3−2.86 g l^{-1}, MnCl2.4H2O−1.81 g l^{-1}, ZnSO4.4H2O−0.222 g l^{-1}, Na2MoO4−0.018 g l^{-1}, CuSO4.5H2O−0.079 g l^{-1}

Table 2 Response of *Spirulina platensis* to the substitution of NPK-10:26:26 complex fertilizer at the 15th day of biomass growth

Culture medium	X_m (mg l^{-1})	P_X (mg l^{-1} d^{-1})	μ_{max} (d^{-1})	$Y_{X/N}$ (g g^{-1})	$Y_{X/P}$ (g g^{-1})	Chlorophyll (mg l^{-1})	Protein (%)	Lipid (%)
Varying NPK loading (fixed $NaHCO_3$ loading)								
P1	253.7	13.58	0.023	5.66	4.85	3.70	38.33	7.35
P2	806.5	49.33	0.072	9.66	8.50	6.82	40.41	8.82
P3	1120.0	71.33	0.098	10.09	8.74	8.75	41.15	9.34
P4	790.0	48.95	0.066	5.87	5.07	6.80	39.24	7.99
P5	410.0	24.00	0.037	2.65	2.29	4.60	38.14	7.20
Varying $NaHCO_3$ (fixed NPK loading)								
P2C1	797.6	49.84	0.078	9.84	8.40	6.87	38.78	7.66
P2C2	815.4	51.03	0.090	10.07	8.60	6.97	39.77	8.37
P2C3	1220.0	76.67	0.110	14.98	12.92	8.92	52.35	11.62
P2C4	570.0	34.67	0.058	6.87	5.84	5.54	43.17	10.78
P3C1	560.0	34.00	0.051	4.81	4.15	5.48	38.53	7.50
P3C2	962.0	60.80	0.075	8.60	7.45	6.65	39.12	7.90
P3C3	510.0	30.67	0.048	4.34	3.74	5.19	39.82	8.40
P3C4	480.0	28.67	0.043	4.06	3.50	5.01	40.78	9.08
Standard culture medium								
Zarrouk	1180.0	75.33	0.109	14.87	12.70	8.81	47.47	11.23
NCL	1078.0	68.53	0.095	2.50	11.55	8.50	50.67	11.84
CFTRI	800.0	50.00	0.083	3.04	8.43	6.88	48.05	10.23

X_m maximum biomass concentration (mg l^{-1}), P_X biomass productivity (mg l^{-1} d^{-1}), μ_{max} maximum specific biomass growth rate (d^{-1}), $Y_{X/N}$ yield of nitrogen to biomass (g g^{-1}), $Y_{X/P}$ yield of phosphorous to biomass (g g^{-1})

was in accordance with *Spirulina* growth. Theoretical elemental P consumption for P1–P5 media was calculated using molecular formula of *S. platensis* [5]. An excellent agreement was obtained with calculated and experimental values with average absolute percent deviation less than 7.0 %. This suggests that the culture medium pH is favorable for phosphorous removal due to biological assimilation of phosphorous only (i.e., biotic process). This also suggests that the chemical precipitation of phosphorous compounds (i.e., abiotic process) was absent during biomass growth. Bulk concentration pattern of elemental K uptake for P1–P5 medium was shown in Fig. 2e. It was noted that the concentration profile of K^+ ion gradually falls with the biomass growth. As the concentration gradient of K^+ was high at the beginning across plasma membrane, cellular uptake of K^+ ion took place at a faster rate for cell metabolism. The percent uptake of elemental K for P1–P5 media was almost same and found to be $\sim$8.0 % (Fig. 2e).

The variation of physicochemical parameters like pH and conductivity is also very helpful to monitor the cultivation of *Spirulina*, and the variation of these parameters for P1–P5 fertilizer media is shown in Fig. 2f, g, respectively. As seen from Fig. 2f, pH gradually increased in accordance with biomass growth for P1–P5 fertilizer medium, which can be explained by the consumption of both bicarbonate and ammoniacal nitrogen. Bicarbonates are transported through plasma membrane by increasing due to concentration gradient of sodium ions and bicarbonates are then assimilated in the cell, which is converted into CO_2 and carbonate. The released CO_2 was used in photosynthesis for biomass growth, whereas produced carbonate ions extruded through plasma membrane by increasing pH of the medium. The assimilation of ammonia from ammonium ion implies the release of hydrogen ions into culture medium, thus decrease in pH of the medium. Combined effect of bicarbonate and ammonium ions results the net increase in pH in the culture medium. The maximum increase in pH was occurred for P3 medium where maximum biomass growth was observed. Similarly, conductivity falls sharply with biomass growth for P1–P5 media and attains a minimum value in between 5–8 days, and thereafter increases steadily with the biomass growth. The initial fall of these parameters is mainly due rapid uptake of Na^+, K^+, HCO_3^-, NH_4^+, PO_4^{3-}, and Mg^{2+}. These ions are necessary for numerous enzyme activities, particularly for the synthesis of carbohydrates, proteins, lipids, and pigments. Initially, the high ionic concentration was maintained in the bulk culture medium as compared to the ionic concentration inside the cells. This resulted in high concentration gradient across the plasma membrane and was responsible for the falling trend of conductivity. The increase of conductivity takes place after 8 days (Fig. 2g) which was mainly due to the extrusion of ions

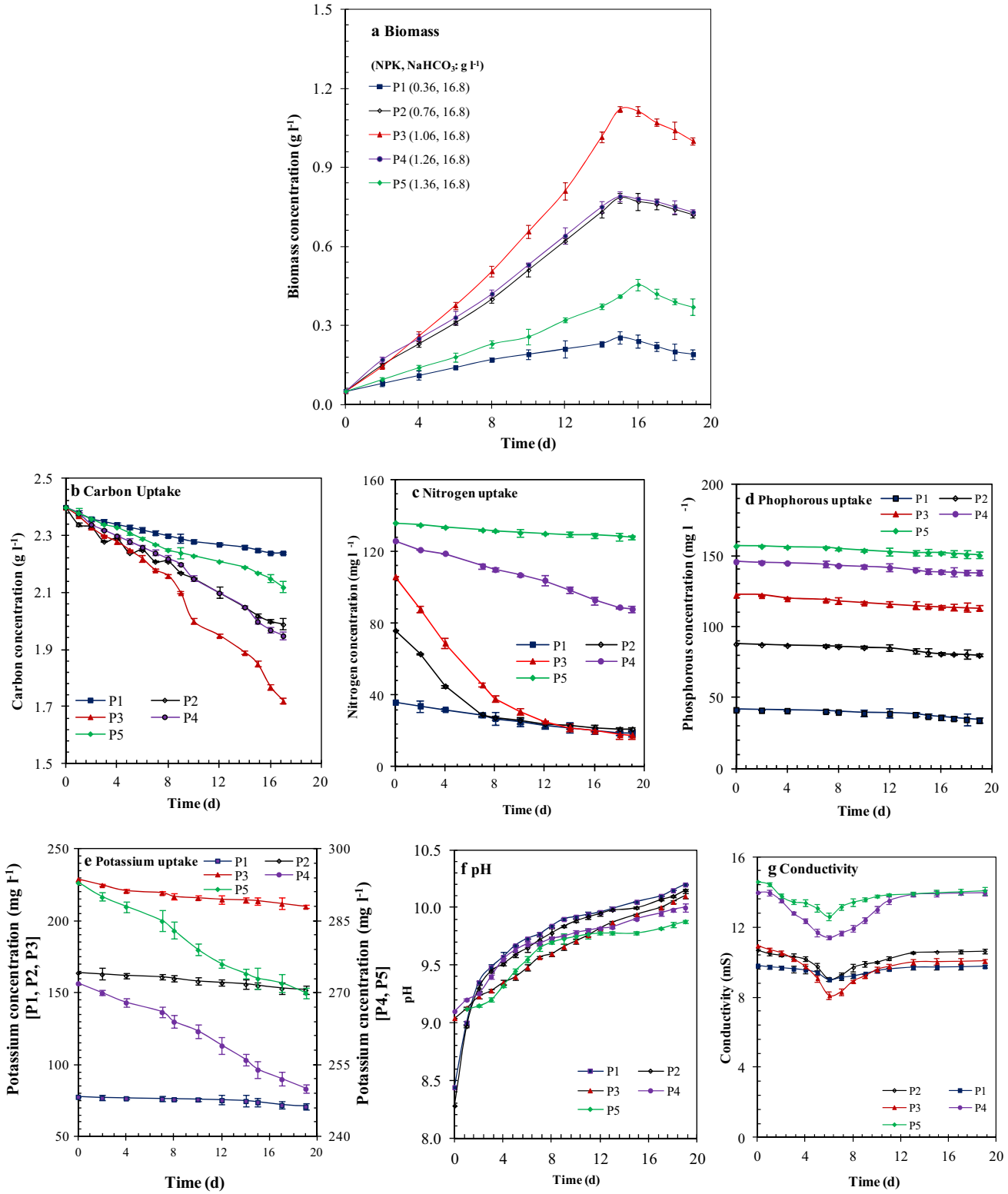

Fig. 2 Time behavior of **a** biomass growth **b** uptake of elemental carbon **c** uptake of elemental nitrogen **d** uptake of elemental phosphorous **e** uptake of elemental potassium **f** variation of pH and **g** variation of conductivity for *Spirulina platensis* cultivation under different NPK-10:26:26 fertilizer loading for fixed $NaHCO_3$ concentration (i.e., 16.8 g l^{-1})

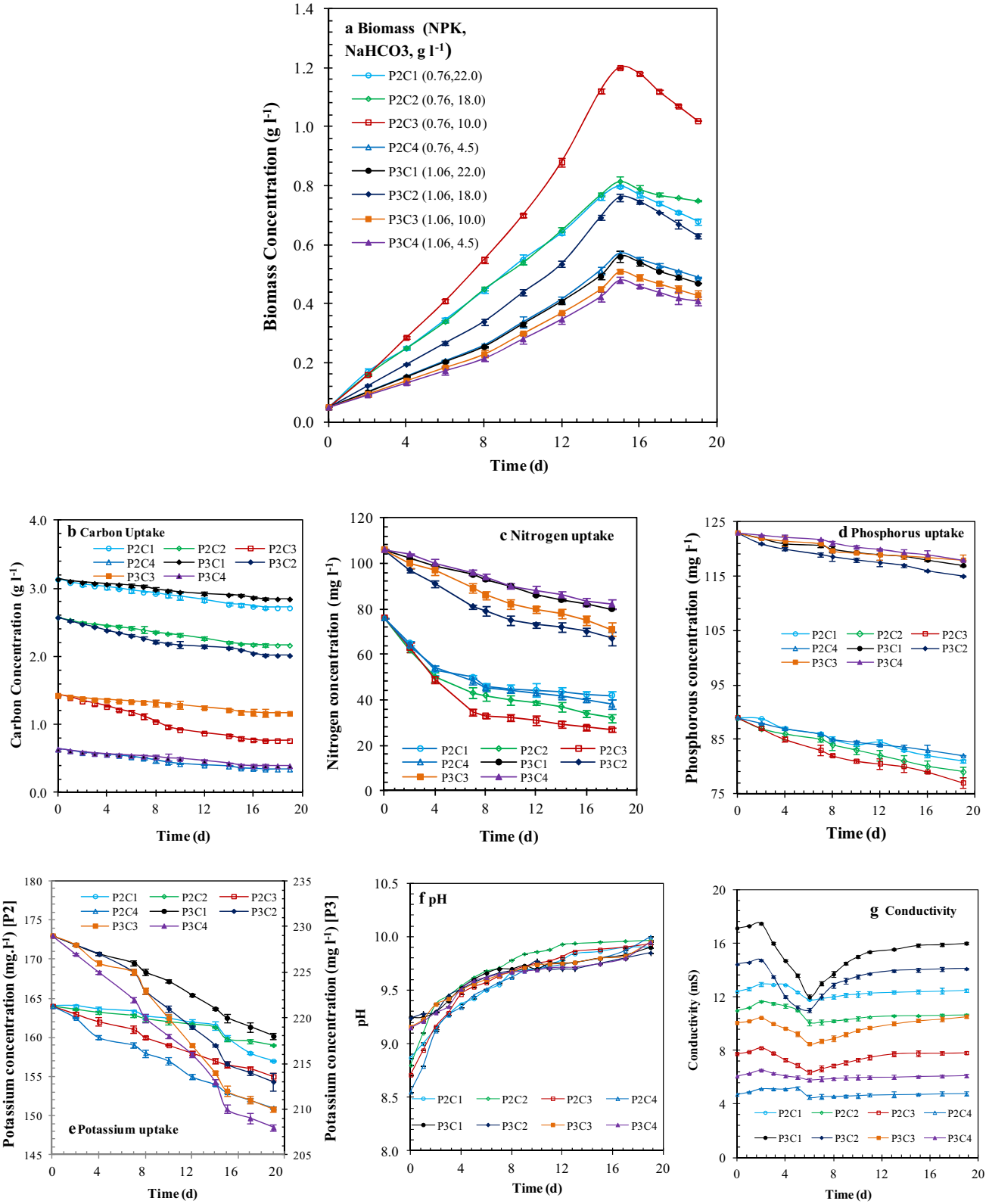

Fig. 3 Time behavior of **a** biomass growth **b** uptake of elemental carbon **c** uptake of elemental nitrogen **d** uptake of elemental phosphorous **e** uptake of elemental potassium **f** variation of pH and **g** variation of conductivity for *Spirulina platensis* cultivation under different $NaHCO_3$ loading for fixed NPK-10:26:26 fertilizer concentration (i.e., P2: 0.76 g l^{-1} and P3: 1.06 g l^{-1})

from cells, particularly sodium ions for which Na^+/H^+ antiporters are the major mechanism for sodium extrusion [2].

Effect of $NaHCO_3$ Loading Under White LED

To determine the optimum $NaHCO_3$ loading, NPK fertilizer loading was kept constant and $NaHCO_3$ loading was varied from 4.5–22 g l^{-1} for *Spirulina* growth. Details of fertilizer media are given in Table 1 and these were designated by P2C1–P2C4 and P3C1–P3C4 fertilizer media. Biomass growth using this culture medium under standard operating condition is shown in Fig. 3a. The detail of growth kinetic parameters and the accumulation of chlorophyll, lipid, and protein in P1–P5 fertilizer media are given in Table 2. Comparing growth results, maximum growth responses (i.e., X_m, P_X, μ_{max}, $Y_{X/N}$, and $Y_{X/P}$) and the accumulation of chlorophyll, protein, and lipid were obtained for P2C3 fertilizer medium where $NaHCO_3$ and NPK-10:26:26 complex fertilizer loadings were found to be 10.0 and 0.76 g l^{-1}, respectively. Similarly, comparing growth results using P3 and P2C3 fertilizer medium, superior growth result was obtained for P2C3 (Table 2). Corresponding to Fig. 3a, elemental C, N, P, and K uptake are presented in Fig. 3b–e. Similarly, the variation of pH and conductivity is also reported in Fig. 3f, g. Growth results presented in Fig. 3a revealed that excess $NaHCO_3$ has a strong inhibitory effect on biomass growth and this was mainly due to high salinity stress resulting from high intracellular Na^+ ion concentration across the plasma membrane, high initial alkalinity of the culture medium, and structural damage of the reaction center in photosystem II and photosystem I [25].

Growth Comparison Using Optimum NPK-10:26:26 Fertilizer and Standard Culture Media

Growth studies involving different standard media (e.g., Zarrouk, NCL, and CFTRI: Table 1) were carried out and the results (i.e., biomass growth: Fig. 4a and uptake of elemental C, N, P, and K: Figs. 4b–e, pH: Fig. 4f and conductivity: Fig. 4g) were compared with the optimum NPK-10:26:26 fertilizer culture medium (i.e., P2C3 fertilizer medium). Among the standard culture media, Zarrouk medium resulted into maximum biomass growth as compared to NCL and CFTRI culture medium (Fig. 4a). The culture medium composition of Zarrouk and NCL was identical except the concentration of $NaHCO_3$. In Zarrouk's medium, $NaHCO_3$ concentration is 16.8 g l^{-1}, whereas initial $NaHCO_3$ concentration was 10.0 g l^{-1} in NCL medium. Similarly, $NaHCO_3$ and $NaNO_3$ concentration in CFTRI medium was comparatively less than Zarrouk and NCL medium. Moreover, the uptake of C, N, and P was comparatively higher for Zarrouk medium over NCL and CFTRI medium (Fig. 4b–d), which resulted into the increased biomass growth in Zarrouk's medium as compared to NCL and CFTRI medium. Therefore, the reduced growth for CFTRI and NCL was mainly due to lower concentration of nitrate nitrogen and bicarbonate in the culture medium respectively. It was noted that the cultivation using NPK culture medium (i.e., P2C3 fertilizer medium) helped to increase biomass production and the accumulation of chlorophyll, protein, and lipid as compared to Zarrouk culture medium (Table 2). In standard culture media, metabolism of nitrate occurs via the reduction of nitrate to nitrite and nitrite to ammonium ion with subsequent assimilation of ammonium ions into the carbon skeletons resulted into amino acids and proteins in the cell, whereas direct assimilation of ammonium took place in fertilizer medium using controlled amount of $NaHCO_3$ and NPK-10:26:26 fertilizer. Comparing the biomass growth using P2C3 fertilizer medium and standard culture media, superior result was obtained using P2C3 fertilizer medium (Fig. 4a), which demonstrates the efficacy of fertilizer-based culture medium. It was also observed that the initial limitation of elemental N in NPK fertilizer culture medium helped to accumulate extra lipid particularly in P3 and P2C3 fertilizer medium. This was mainly due the decreased cellular nitrogen content of thylakoid membrane which activates acyl hydrolase and stimulates phospholipid hydrolysis. Moreover, nitrogen limitation activates diacylglycerol acyltransferase and converts acyl-CoA to tri-glycerides [20]. Finally, the cost of nutrients involved in NPK fertilizer and Zarrouk's culture medium was calculated and the cost of nutrients per kilogram of dry biomass for P2C3 NPK fertilizer medium was found to be US$ 71.0 kg^{-1}, whereas cost of nutrients using for Zarrouk culture medium was US$ 144.0. Almost 50.0 % reduction in unit cost of biomass was obtained mainly due to the reduced quantity of the low-priced NPK-10:26:26 fertilizer and enhanced biomass growth using optimum fertilizer medium.

Conclusions

The present investigation was carried out with the aim to formulate a simple and inexpensive culture medium for mass production of *S. platensis*. The newly formulated fertilizer-based culture medium using NPK-10:26:26 complex fertilizer provides enhanced growth results over standard culture medium (i.e. Zarrouk, NCL, and CFTRI) when culture media were exposed to 50 µmol photons m^{-2} s^{-1} white LED, 14 h/10 h light–dark cycle, and 30 °C temperature under 90 rpm orbital shaking. The optimum loadings of NPK fertilizer, sodium bicarbonate, and sodium

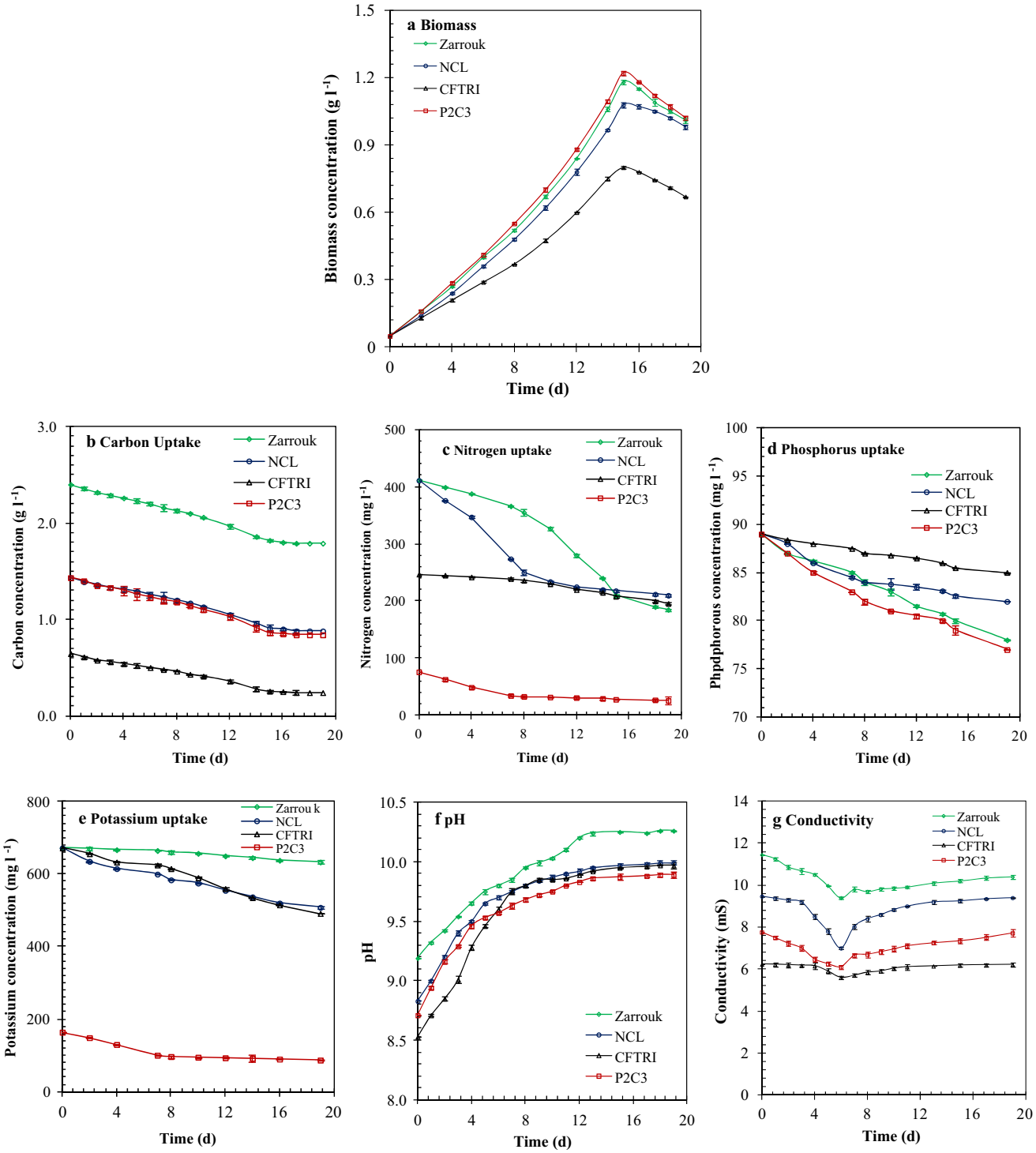

Fig. 4 Time behavior of **a** biomass growth **b** uptake of elemental carbon **c** uptake of elemental nitrogen **d** uptake of elemental phosphorous **e** uptake of elemental potassium **f** variation of pH and **g** variation of conductivity for the cultivation of *Spirulina platensis* using Zarrouk, NCL, CFTRI, and optimum NPK-10:26:26 fertilizer medium (i.e., P2C3 fertilizer medium)

chloride in the newly formulated were found to be 0.76, 10.0, and 1.0 g l^{-1}, respectively and corresponding maximum dry biomass concentration and the accumulation of chlorophyll, protein, and lipid accumulation were found to be 1.22 g l^{-1}, 8.92 g l^{-1}, 52.35 %, and 11.62 %, respectively, at the fifteenth day of biomass growth. Enhanced biomass productivity and the accumulation of chlorophyll, protein, and lipid were obtained using optimum NPK

culture medium as compared to Zarrouk's culture medium. Elemental C, N, P, and K concentrations were measured to find the limiting nutrients for the growth of *S. platensis*, whereas several physicochemical parameters (e.g., pH and conductivity) were measured to monitor biomass growth. An *almost* 50.0 % reduction of nutrient cost was achieved using newly formulated NPK-10:26:26 complex fertilizer medium for *Spirulina* growth in comparison to standard Zarrouk's culture medium.

Acknowledgments Partial financial support from the Indian School of Mines, Dhanbad [through research project grant FRS (28)2010-11/PE] is gratefully acknowledged.

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