Bangladesh J. Bot. **48**(3): 529-535, 2019 (September)

GROWTH PERFORMANCE ANALYSIS OF *SPIRULINA PLATENSIS* PRODUCTION BY SUBSTITUTING K_2SO_4-K OF KOSARIC MEDIUM WITH MOP-K

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Keywords: Spirulina platensis, K_2SO_4, MOP, Growth, Economic

Abstract

Five media were formulated with 0% (control, T_1), 25% (T_2), 50% (T_3), 75% (T_4) and 100% (T_5) inclusion of low-cost muriate of potash (MOP)-potassium (K) replacing high-cost reagent K_2SO_4-K and *Spirulina platensis* was cultured for 18 days. Cell dry weight, optical cell density and chlorophyll-a content of *S. platensis* cultured in five treatments were registered at every three-day interval and economic performance was calculated to observe the effect of K_2SO_4-K replacement with MOP-K. The cell biomass production and chlorophyll-a content of *S. platensis* cultured in 25 and 50% use of MOP-K instead of K_2SO_4-K (T_2 and T_3) did not represent any significant difference with the control treatment of 100% K_2SO_4-K (T_1). However, further addition of MOP-K in T_4 and T_5 significantly reduced the cell growth and pigment content of *S. platensis*. In addition, a significant reduction of production cost was calculated as more percentage of K_2SO_4-K was replaced with MOP-K.

Introduction

Spirulina spp. are multicellular, filamentous blue-green microalgae, which have been used since ancient times as a source of food due to its high nutritional value. *Spirulina* was found to be an excellent source of protein (up to 70%), along with high amount of fatty acids, essential amino acids, minerals, vitamins (especially B12), antioxidants, pigments (phycobili proteins and carotenoids) and polysaccharides (Belay *et al*. 1993). Furthermore, it is the richest algal source of gamma-linolenic acid (GLA), a precursor for the biologically-active compound (prostaglandins, PGE1) (Habib *et al.* 2008). *Spirulina* spp. are the most studied microalgae due to, not only for the nutritional value (Cost *et al.* 2001, Rafiqul *et al.* 2005) but also for their potential pharmaceutical specially antimicrobial properties (Hernandez-Corona *et al*. 2002, Hirahashi *et al*. 2002, Subhashini *et al.* 2004). *Spirulina* is used as a feed supplement for human, animals, and it is found to improve growth, feed efficiency, carcass quality and physiological response to the diseases in several aquatic species (Becker 2007). In addition, *Spirulina* has been found to remove nitrogen, phosphorus and heavy metals contaminants from wastewater (Lodi *et al*. 2003, Lodi *et al*. 2008). Moreover, it has huge potentiality to produce biofuel as a green energy source for future generations (Rahman *et al*. 2017).

The open pond monoculture of *Spirulina* in outdoor is easier to operate in tropical and subtropical areas (Grewe and Pulz 2012). For the successful mass outdoor production of *Spirulina,* the growth media in terms of required nutrients are considered as an important input and accounts for a major portion of the expenses (Vonshak 1997). Kosaric medium (KM) is the most commonly used for *Spirulina platensis* culture. However, the formulation of KM medium is very expensive and chemicals are not readily available. For the economical large scale, propagation of *Spirulina* emphasis should be given to reduce production costs. Therefore, development of low-cost produc-

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tion needs to be established for their large-scale biomass production for industrial purposes. The aim of the present investigation was to find out the cheaper alternative source of potassium (K) and their optimum replacement level by substitution of expensive K_2SO_4 of KM with readily available agricultural fertilizer muriate of potash (MOP) which would ensure similar growth performance of *S. platensis* as compared to the KM with reduced cost.

Materials and Methods

The pure strain of *S. platensis* was obtained from Bangladesh Agricultural University, Bangladesh. Kosaric medium (KM) was used as control media (T_1). Five formulated media were designed with 0% (control, T_1), 25 (T_2), 50 (T_3), 75 (T_4) and 100% (T_5) of MOP-K replacing K_2SO_4-K of KM media. The amount of MOP was adjusted to supply concentration of potassium equivalent to that provided by K_2SO_4 in KM (Table 1).

Table 1. Composition of Kosaric medium (KM) and the formulated media.

Constituents	Composition (g/l)				
	T_1 (KM)	T_2	T_3	T_4	T_5
$NaHCO_3$	9 .0	9 .0	9 .0	9 .0	9 .0
K_2HPO_4	0.25	0.25	0.25	0.25	0.25
$NaNO_3$	1.25	1.25	1.25	1.25	1.25
K_2SO_4	0.50	0.375	0.25	0.125	0
$MgSO_4.7H_2O$	0.50	0.50	0.50	0.50	0.50
$CaCl_2$	0.10	0.10	0.10	0.10	0.10
$FeSO_4.\ 2H_2O$	0.005	0.005	0.005	0.005	0.005
Micronutrient* (ml/l)	0.5	0.5	0.5	0.5	0.5
Muriate of potash (MOP)	0	0.108	0.22	0.323	0.43

Micronutrient solution*: H_3BO_3, 2.86; $MnCl_2.\ 4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.\ 5H_2O$, 0.08; MoO_3, 0.01; $CoCl_2.\ 6H_2O$, 0.01(g/l). Except for K_2SO_4 all other components were same as like of Kosaric medium in all the treatments while T_1 was the control.

The stock culture was grown in the KM (Modified after Zarrouk's 1996). Artificial aeration was provided constantly, and pH of the culture was adjusted to 9.0 by adding the appropriate amount of 0.1 N HCl or 0.1 N NaOH. For the experimental culture, *S. platensis* was grown in 1.0 litre conical flask in five treatments with three replications. *S. platensis* was inoculated with 10% suspension (OD_{620} = 0.20) into each culture flask. The culture was conducted under white light at 12/12 hrs light-dark cycles (Phillips, FL-40, SD/38 daylight, Bangladesh) with constant aeration (Sobo, Aquarium pump SB- 348A) for 18 days. The physicochemical parameters of culture media such as temperature, DO, pH and light intensity were recorded periodically.

The dry weight and chlorophyll-a content of *S. platensis* were estimated following the method of Clesceri *et al.* (1989). Briefly, 50 ml culture suspension was filtered using Whatman GF/C filter papers (0.45 µm mesh size and 47 mm diameter) and weighed. The suspension was washed with 20 ml acidified water (pH = 4) in order to remove insoluble salts during filtration. All samples were dried in an oven at 70°C for 24 hrs and finally dry weight of *S. platensis* was calculated using following formula.

$$W = \frac{FFW - IFW}{\text{Amount of sample taken filtration (ml)}} \times 100$$

Where, W = Cell dry weight in g/l; FFW = Final filter weight in g; and IFW = Initial filter weight in g.

In addition, optical density (OD) of cells was recorded at 620 nm, using UV spectrophotometer (DR 5000).

For chlorophyll-a analysis, 10 ml filtered *S. platensis* sample was ground with a glass rod and mixed with 10 ml of 100% redistilled acetone. Then the samples were homogenized and centrifuged at 4000 rpm for 10 min. Finally, the chlorophyll-a content was calculated by recording OD at 664, 647 and 630 nm (spectrophotometer, DR 5000). Growth performance (dry cell weight, optical density) and chlorophyll-a content were determined at every three-day interval to observe the effect of K_2SO_4-K replacement with MOP-K. The data were analyzed statistically by one-way ANOVA. Tukey's post hoc test at 5% significance level test was applied in the case of significant differences using Statistix 10 statistical package.

Results and Discussion

The effect of cell biomass growth of microalgae on MOP-K inclusion instead of K_2SO_4-K is summarized in Fig. 1 and Table 2. Cell biomass of *S. platensis* increased with the progress of culture period, attained the highest at the 15th day, and then decreased in all treatments. Therefore, exponential phase of *S. platensis* culture continued until 15th day from the inoculation in the present study. At the end of the exponential phase, maximum cell biomass 0.92 ± 0.06 g/l and optical cell density (OD) 0.84 ± 0.12 mg/l were found in the treatment T_1 where 100% K_2SO_4-K was used. Exponential growth rate observed in the treatment T_2 and T_3 where 25 and 50% of K_2SO_4 of the KM was replaced with MOP-K was statistically at par with the control (T_1). More than 50% K replacement with MOP significantly reduced the cell growth of *S. platensis* in T_4 and T_5 while the lowest was recorded in which 100% replacement was applied. Similar to cell biomass, no significant difference of chlorophyll-a content was seen among T_1, T_2 and T_3 treatments ($p > 0.05$) (Fig. 2) with the highest at T_1 and the lowest at T_5 at the end of 15th day.

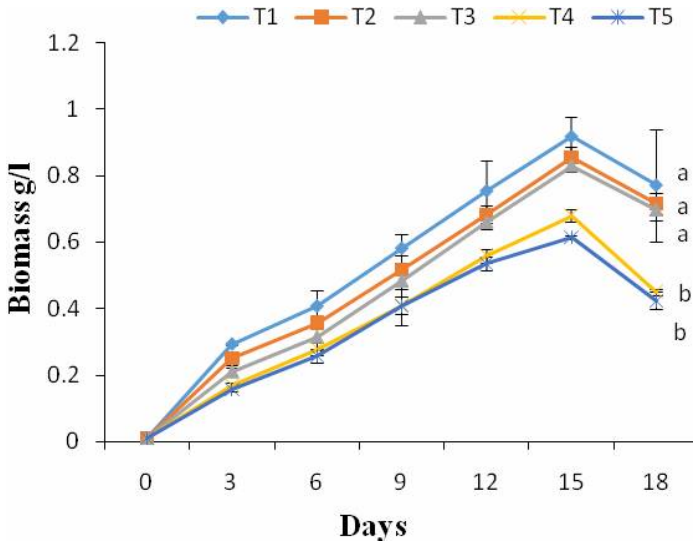

Fig. 1. Effect of cell biomass (g/l) of *S. platensis* in different levels of K_2SO_4-K replacement with MOP-K. Values are mean ± standard deviation (SD) (n = 3). Different letters at the end of trend line represent significant differences among the treatments.

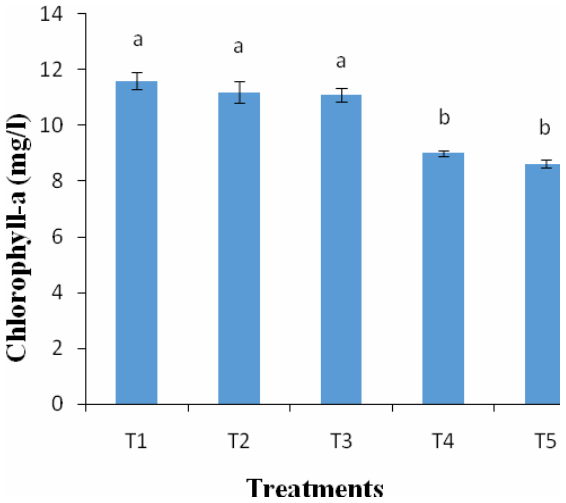

Fig. 2. Mean chlorophyll-a content (mg/l) of different treatments at the end of 15th days experiment period. Values are mean ± SD, n = 3. Different letters denote significant difference ($p < 0.05$).

Table 2. Effect of optical cell density (OD) (mg/l) of *S. platensis* on K_2SO_4-K replacement with MOP-K.

Culture period (Day)	T_1	T_2	T_3	T_4	T_5
3rd	0.36 ± 0.10^a	0.33 ± 0.10^a	0.34 ± 0.12^a	0.30 ± 0.10^a	0.31 ± 0.11^a
6th	0.44 ± 0.10^a	0.43 ± 0.13^a	0.43 ± 0.10^a	0.39 ± 0.10^b	0.40 ± 0.11^b
9th	0.68 ± 0.12^a	0.54 ± 0.12^b	0.65 ± 0.12^a	0.52 ± 0.05^b	0.53 ± 0.12^b
12th	0.75 ± 0.11^a	0.70 ± 0.10^a	0.72 ± 0.15^a	0.61 ± 0.10^b	0.60 ± 0.05^b
15th	0.84 ± 0.12^a	0.78 ± 0.10^a	0.79 ± 0.10^a	0.66 ± 0.11^b	0.65 ± 0.10^b
18th	0.67 ± 0.11^a	0.59 ± 0.10^b	0.60 ± 0.13^b	0.63 ± 0.10^b	0.59 ± 0.10^b

Values are expressed as mean ± SD, n = 3. Values with different superscript letters are significantly different.

Kumari *et al.*(2015) found maximum specific growth rate of *S. platensis* in NPK fertilizer medium with MOP-K, urea, and diammonium phosphate compared with commercial Zarrouk medium. About similar growth performance of *S. platensis* was found between synthetic (SM) (1.84 dry weight/l) and fertilizer media (FM) (1.81 dry weight/l) by Gami *et al.* (2011). On the other hand, Raoof *et al.* (2006) reported statistically comparable yields of *S. platensis* in standard Zarrouk's medium (SM) and other cost-effective formulated media containing commercial grade single super phosphate, sodium nitrate, muriate of potash, sodium chloride, magnesium sulfate, calcium chloride, and sodium bicarbonate. Madkour *et al.* (2012) conducted an experiment where microalga *S. platensis* was grown in modified Zarrouk's medium by replacing laboratory grade K_2SO_4 with MOP-K along with other nutrients and reported the progressive effect of MOP in the chlorophyll-a content of *S. platensis.* However, in this experiment, only 50% replacement of K_2SO_4 with MOP-K was found to be effective for the good production output.

The growth of *S. platensis* was reported to have great influence on different media compositions and several environmental conditions (Temperature, pH, DO and light intensity)

(Saranraj and Sivasakthi 2014). The average physico-chemical parameters over the 18 days of culture period at different treatments of *S. platensis* culture are presented in Table 3. For *S. platensis* culture 25 - 35ºC temperature and 2000 - 2500 lux/m^2/sec light intensity are considered the optimum for growth and cell multiplication (Chowdhury 2005, Rahman 2005). Moreover, pH, 8.5 - 10.0 and DO, 3.1 - 5.5 mg/l are the suitable ranges found for *S. platensis* (Nuruzzaman 2005, Joshi *et al.* 2013). However, it is evident that all the physicochemical parameters were within the optimum level and not varied significantly ($p > 0.05$) among the treatments. Therefore, varied source of K did not affect the culture environment of *S. platensis* in this study.

Table 3. Effect of average physicochemical parameters on K_2SO_4-K replacement with MOP-K.

Treatments	Temperature (ºC)	pH	DO (mg/l)	Light intensity (Lux/m^2/sec)
T_1	25.17 ± 0.05^a	9.52 ± 0.03^a	3.78 ± 0.22^a	2221 ± 0.41^a
T_2	25.15 ± 0.03^a	9.48 ± 0.04^a	3.97 ± 0.13^a	2220 ± 0.82^a
T_3	25.14 ± 0.06^a	9.51 ± 0.05^a	3.99 ± 0.14^a	2221 ± 0.47^a
T_4	25.12 ± 0.05^a	9.50 ± 0.04^a	4.03 ± 0.24^a	2225 ± 0.24^a
T_5	25.19 ± 0.49^a	9.50 ± 0.05^a	3.64 ± 0.18^a	2221 ± 1.03^a

Values are expressed as mean ± SD, n = 3. Values with same superscript letters are not significantly different.

Table 4. Average economic analysis of K_2SO_4-K replacement with MOP-K at different treatments.

Treatments	Cost of *S. platensis* production ($/Kg)		
	K_2SO_4	MOP-K	Total Cost
T_1	9.78	0	9.78 ± 0.08^a
T_2	7.94	0.04	7.98 ± 0.05^b
T_3	5.42	0.10	5.52 ± 0.04^c
T_4	3.31	0.17	3.48 ± 0.11^d
T_5	0	0.25	0.25 ± 0.01^e

The variation in cell biomass and pigment content in the present study was not affected by the culture conditions in different treatments. However, the variation could be due to the different source and composition of potassium nutrient. Potassium is considered as one of the macronutrients for the freshwater phytoplankton growth. It acts as a co-factor in many enzymatic reactions and responsible for protein synthesis and several physiological functions (Talling 2010). Potassium-based fertilizers especially MOP-K are widely applied to enhance the growth and yield of terrestrial plants (Gour *et al.* 2018). Here, *S. platensis* successfully utilized 50% K supplied by MOP but perhaps in case of more replacement the growth requirements were not completely met by the potassium chloride (KCl) of MOP. The different composition of K_2SO_4 especially because of having sulfur (S) and oxygen (O) molecules could have some influence on the growth of *S. platensis*. Sulfur is the constituent of an important amino acid called methionine, vitamins and certain lipid of the plant. Therefore, in case of higher replacement of K_2SO_4 with KCl of MOP may limit the growth due to lack of S or O molecules.

Among several constraints of *S. platensis* production at large scale, the high production cost is a major concern (Raoof 2002). KM gives better growth but it contains a higher amount of reagent grade chemicals that lead to significant increase in cost. On the other hand, availability of chemicals used in KM medium is infeasible for the mass culture operation. For the scale-up

economical production, either the expensive chemicals of commercial media need to be altered with cheap ingredients or alternative cheap media with all essential nutrients need to be developed (Materassi *et al*. 1984). In the present study, the modification of KM medium by the replacement of K of commercial grade chemicals K_2SO_4 with fertilizer grade MOP-K was found to be highly economical.

Control KM with 100% K_2SO_4-K showed significantly higher ($p < 0.05$) production cost than all other treatments (Table 4). The more percentage of K_2SO_4-K replaced with MOP-K the more significant reduction of production cost was calculated. The cost was lowest in case of 100% replacement of K_2SO_4-K with MOP-K. The cost of commercial grade K_2SO_4-K used in KM is very high (17.75 $/Kg) compared to the fertilizer MOP-K (0.35 $/Kg). Moreover, MOP-K is locally available which ensures its ease supply for the large-scale culture of *Spirulina* comparing to K_2SO_4. So, MOP-K can be used for algal production in large scale as it will cut the production cost of *Spirulina* considerably.

The present results clearly indicate that 50% replacement of K_2SO_4-K with MOP-K is statistically at par with 100% K_2SO_4-K used in KM when evaluated in terms of production and chlorophyll-a content. Further, cost analysis clearly indicates the low-cost potentiality of using MOP-K fertilizer. Therefore, 50% replacement of MOP-K instead of K_2SO_4-K may give a good yield and could be used as an economical option for the biomass production of *S. platensis*.

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(Manuscript received on 13 June, 2018; revised on 9 January, 2019)