



Research Article

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Impact of Ag²⁺ stress on growth and phytochemical production by *Spirulina platensis*

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ABSTRACT

The biocidal effect of silver is particularly well known and the term, oligodynamic activity was coined for this phenomenon. Silver is highly toxic to bacteria, and that toxicity seems to be accentuated when silver is delivered by a nanoparticle. In context of this the effect of Ag²⁺ ion was investigated on *S. platensis*. pH (=9.0±0.2) and temperature (32°C) was optimized. Under these conditions, Ag²⁺ ion stress helped in growth at concentration of 1.0mg/ml followed by 0.5mg/ml as compared to control samples. The biomass production, protein synthesis, carbohydrate and chlorophyll contents were also determined. The specific phytochemical tests were confirmed presence of glycosides, terpenoids, phenolics, carbohydrates, amino acid, steroids, flavanoids, alkaloid and phenolics. Despite of biocidal activity of silver at higher concentrations this study suggests that Ag²⁺ at concentration of 1.0mg/ml may be added in the culture medium of *S. platensis* for optimum growth and production of value added products.

Key words: *Spirulina platensis*, Biomass, pH, temperature, phytochemical.

INTRODUCTION

Spirulina platensis is a fast growing microalgae, it is multicellular and filamentous cyanobacteria. The cell wall of spirulina is surrounded by network consisting of peptidoglycan, teichuronic acid, teichoic acid polysaccharides and proteins. These cell wall components can bind to metal ions and can be effective biosorbent due to the presence of large surface area and high binding affinity [1].

Pollution of ecosystem by heavy metal is a real threat to environment. Metal toxicity affects all forms of life though the degree of toxicity varies for different organisms. Microorganisms including bacteria, algae, fungi, yeast serves as constructive models for studying harmful effects of metal at cellular level [2] and can also be used to toxic metals from contaminated sites through accumulation [3].

The biocidal effect of silver is particularly well known and the term, oligodynamic activity was coined for this phenomenon. Silver ions have an affinity to sulfhydryl groups in enzyme systems, through which they interfere with the transmembranous energy transfer and electron transport of bacterial microorganisms. Silver ions also block the respiratory chain of microorganisms. Silver ions induce errors in DNA transcription processes, which may cause disturbance of normal functionality of nuclear acids. Silver affects the primary process of photosynthesis in aquatic

organisms and act as potent inhibitor of energy transfer and electron transport in cyanobacteria. The most common overall toxicity sequence to algae is Hg>Cu>Cd>Ag>Pb [4].

Silver (Ag) is a chemical element with an atomic weight of 47. It is rare (67th in abundance among the elements). Ionic silver is one of the most toxic metals known to aquatic organisms in laboratory testing [5]. Well-documented examples also exist where silver contamination in water and mud corresponds strongly with ecological damage to the environment [6].

Biological systems including fungi and algae, have demonstrated the ability to uptake silver ions from contaminated aqueous solutions [7]. Among lower organism algae have tremendous role in bioremediation of toxic and precious metals and their bioconversion to different non-toxic forms [8]. Cyanobacteria also have the capability to accumulate, detoxify, or metabolize such substances, to some extent. In addition, cyanobacteria are effective biological metal sorbents, representing an important sink for metals in aquatic environment.

The present study is done with an aim to investigate the effect of silver ions on growth of *S. platensis* and available bioactive compounds.

EXPERIMENTAL SECTION

Sample collection and maintenance: Pure *S. platensis* was obtained from Department of Biotechnology, SOS Jiwaji University, Gwalior (M.P.) India, was maintained in Zarrouk's medium [9] in culture room at 25^oC and illuminated fluorescent tubes light (50 $\mu\text{mol}/\text{m}^2\text{Sec}^2$ at surface of the vessels with 18/6 light–dark rhythm).

Culture Medium: Zarrouk's medium was supplemented with silver sulphate (Merk Pvt. Ltd) at concentrations of 0.5mg/ml and 1.0 mg/ml.

Preliminary phytochemical screening: The preliminary phytochemical screening of algal extract was determined as per previous work [10] and the methods described by Trease and Evans [11], [12].

Determination of the effect of temperature and pH on *S. platensis*: To determine the effect of temperature, 250ml Erlenmeyer conical flasks were used each containing 50ml of the growth medium. Each flask was inoculated with 10ml (0.05mg) of the pure culture of *S. platensis*. Each temperature regime was in triplicate. The cultures were incubated in incubator shaker at temperature (26, 28, 30, 32, 36 and 38^oC). Similarly, the effect of pH was determined by maintaining pH (8.0, 8.4, 8.8, 9.2, 9.6 and 10.0) of medium. The experiments were performed in triplicates.

Biomass analysis: Biomass concentration in the culture suspension was determined as cell dry weight by the method of Vonshak et al. [13].

Growth measurement: Growth was monitored by measuring an increase in chlorophyll-a, carbohydrate content and protein content in the presence of different iron concentrations and was expressed in terms of biomass produced per mg cell dry wt.

Estimation of protein content: The growth of *S. platensis* was also monitored by measuring an increase in the total protein content ($\mu\text{g}/\text{ml}$) by the method of Lowry et al. [14] and modified by Herbert et al [15]. The absorbance was measured at 650nm and represented in **Figure 4**.

Carbohydrate estimation: The total carbohydrate content was estimated as per method Dubois et al. [16]. The intensity of the characteristic straw colour was determined by reading absorbance at 492nm and the total carbohydrate content ($\mu\text{g}/\text{mg}$ protein) was calculated from the glucose standard.

Chlorophyll-a estimation: Chlorophyll-a was estimated as per Mackinney [17]. The absorbance of the cell – free metabolic extract was taken at 663nm against methanol as a blank. Quantification of Chlorophyll-a ($\mu\text{g}/\text{ml}$) was done by using the absorption coefficient of 12.63.

RESULTS AND DISCUSSION

The growth conditions for *S. platensis* were optimized in Zarrouk's medium. In term of biomass production (mg/ml) temperature =32^oC and pH= (9.0 \pm 0.2) was found optimum for growth under illuminated fluorescent tubes light (50 $\mu\text{mol}/\text{m}^2\text{Sec}^2$ at surface of the vessels with 18/6 light–dark rhythm (Figure 1, 2 and 3). The metals in form of ions

play a critical role in the development of cells, biosynthesis and metabolic activity. In accordance of this, the effect of Ag^{2+} stress was investigated in term of proteins synthesis, carbohydrate and chlorophyll content. Although, the higher concentrations of silver were toxic to *S. platensis*; but Ag^{2+} at 1.0mg/ml followed by 0.5mg/ml enhanced the growth and ultimately leads production of protein, carbohydrate and chlorophyll.

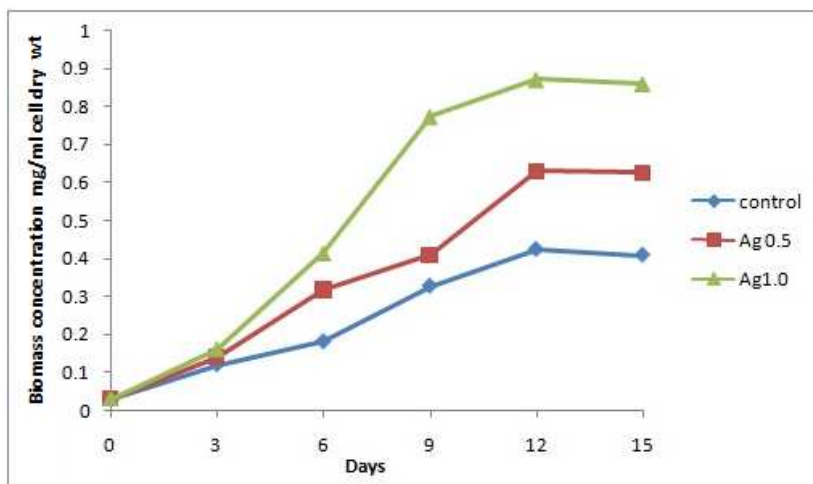


Figure 1: Effect of silver on growth of *S. platensis*

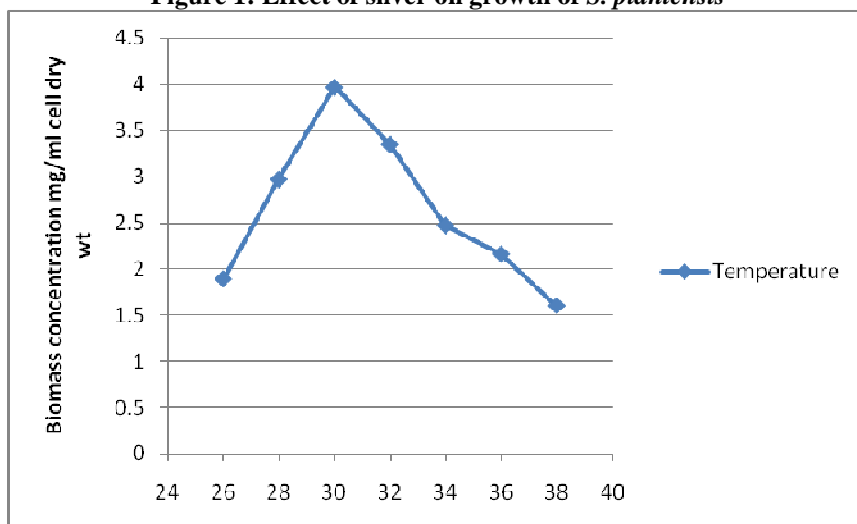


Figure 2: Effect of temperature on biomass production

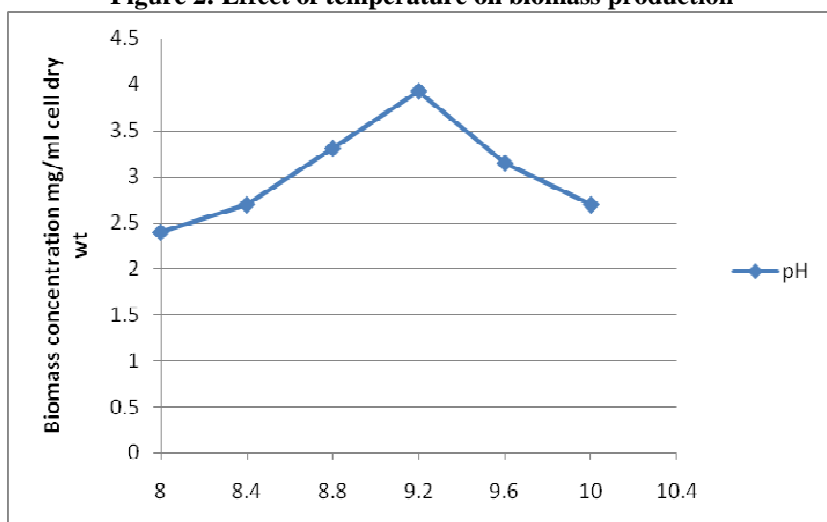


Figure 3: Effect of pH on biomass production

The protein content in *S. platensis* was found maximum when culture medium was supplemented with Ag^{2+} at 1.0mg/ml followed by 0.5 mg/ml; compared to control at the 9th day of incubation under optimized culture conditions (Figure 4). The carbohydrate level in *S. Platensis* also found increased at 6th day of incubation and then it was decreased at 1.0mg/ml of Ag^{2+} followed by 0.5mg/ml (Figure 5). Similarly, chlorophyll-a content was too increased in the presence of Ag^{2+} at 1.0mg/ml followed by 0.5mg/ml and found maximum in 9th day of incubation (Figure 6).

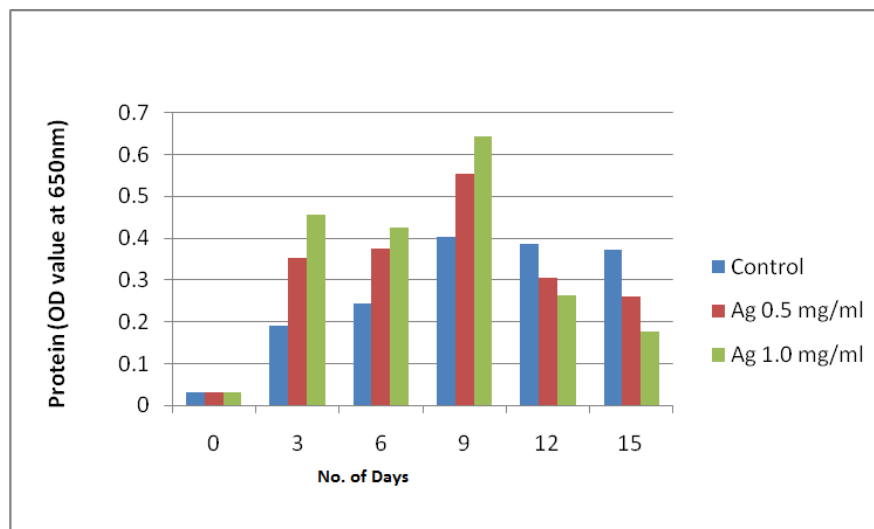


Figure 4: Effect of silver on protein production by *S. platensis*

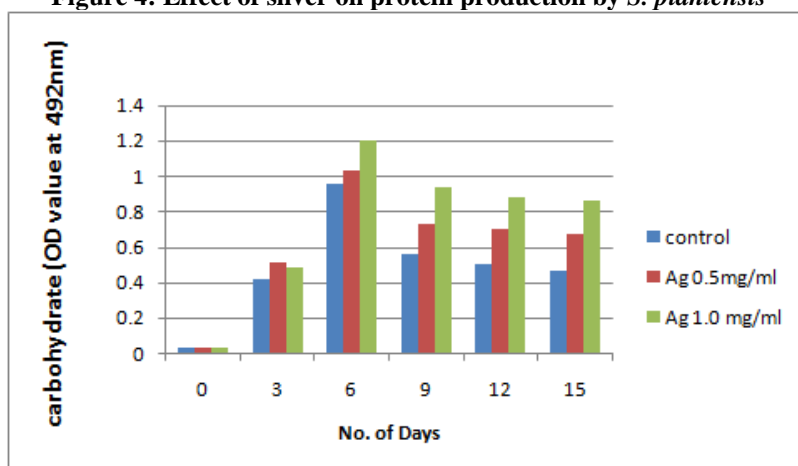


Figure 5: Effect of silver on carbohydrate production by *S. platensis*

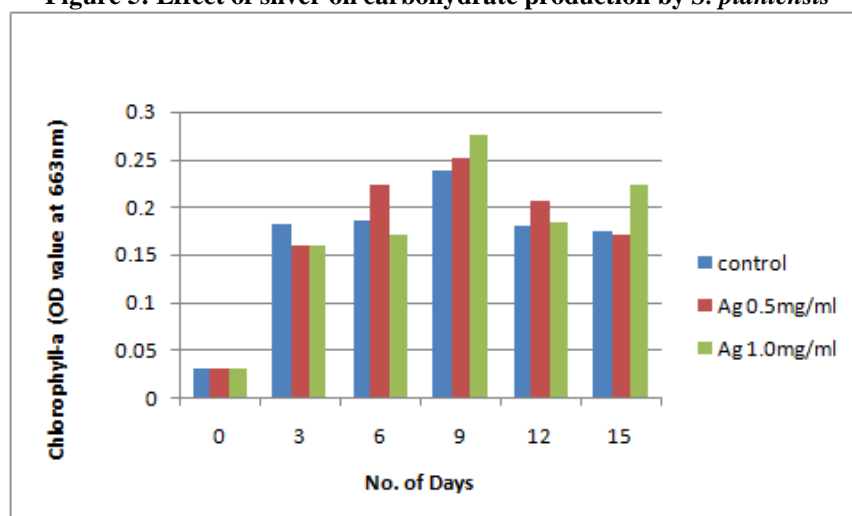


Figure 6: Effect of silver on Chlorophyll-a production by *S. platensis*

Accordance of with experiments outcome the phytochemical screening was also performed using specific qualitative tests to identify various available bioactive compounds in crude extract of *S. platensis* under Ag^{2+} stress. Glycosides, terpenoids, phenolics, carbohydrates, amino acid, steroids, flavanoids, alkaloid and phenolics compounds were detected in crude extract but tannin, hydrolysable tannins and saponins were found absent (Table 1).

Therefore, this finding suggests that Ag^{2+} ions in 1.0mg/ml concentration may be added in the culture medium of *S. platensis* for optimum growth and production of value added products.

Table1. Preliminary phytochemical screening of crude extract of *S. platensis*

Name of Test	Name of Compound	<i>Spirulina platensis</i>
Fehling's and Molisch's test	Carbohydrate	++
Xanthoprotic test	Protein	++
Killer killanis test	Glycosides	++
Salwoski test	Steroids	--
Zinc hydrochloride reduction test	Flavonoids	++
Mayer's and Hager's test	Alkoloids	++
Gelatin Test	Tannins	--
Killer killanis test	Hydrolysable tannin	--
Ninhydrin test	Amino Acid	++
Salkowski test	Terpenoids	++
Gelatin Test	Phenolics	++
Frothing test	Saponins	--

- = Negative (absent); ++ = Positive (present)

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