Educational Administration: Theory and Practice
2024, 30(4), 9003-9007
ISSN: 2148-2403
https://kuey.net/

Research Article

Growth Assessment of *Spirulina platensis* under Oxidative Stress

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Citation: Kushwah. J. S, *et al* (2024), Growth Assessment of *Spirulina platensis* under Oxidative Stress, *Educational Administration: Theory and Practice*, *3*(4), 9003-9007
Doi: 10.53555/kuey.v30i4.2975

ARTICLE INFO

ABSTRACT

Arthrospira platensis, the spiral, multicellular, filamentous, non-heterocystus, non-nitrogen-fixing photosynthetic cyanobacteria that make up spirulina, is an entirely biologically derived organism. Spirulina can tolerate varied fluctuations in H_2O_2 and requires an abundance of minerals. The current work investigates the possibility of enhanced growth and cultivation under stressful circumstances, like variations in concentration of H_2O_2.Different concentrations of H_2O_2 under which *S. platensis* untreated, 2, 4, 6, 8, and 10 mM have been grown for 20 days. *S. platensis* cells, grown under photoperiod of 12 hours light/dark provided by fluorescent lamps with the light intensity of 140 µmol photons $m^{-2}s^{-1)}$ in optimized Zarrouk's medium under controlled conditions. The dry biomass of algal (expressed as mgDW/ml) was moderately reduced under H_2O_2 stress with the exposure time and different H_2O_2 concentration. The present study observed that the biomass stimulated at lower oxidative stress concentration which ranges from 2mM (105mg DW/250ml culture) to 10mM(20mg DW/ml) but decreased at higher concentration as algal cells undergo disruption and results into inhibition of many cellular processes at high concentration.

Key words: Spirulina, Growth curve, Biomass, oxidative stress (H_2O_2)

Introduction

Spirulina, a type of multicellular, filamentous blue-green algae, is becoming more and more well- known in the health food sector and as an additional source of protein and vitamins for aquaculture diets. It is readily harvested and processed, grows in water, and has a very high level of macro and micronutrients. The people that live around Chad Lake in Africa have long utilized spirulina as nourishment. The predominant species of phytoplankton of the lake is *S. platensis*.(Ruma Arora *et al* 2020) The valuable filamentous cyanobacterium, *Arthrospira platensis* or *Spirulina platensis* as it was commonly named for a long time; is a concentrated source of many active compounds, consumed by humans as a functional super-food used as a supplement/ingredient to enhance the human body performance or improve a specific function (Elshouny *et al.,* 2017; Mostolizadeh *et al.,* 2020).*S. platensis* can produce beneficial compounds with therapeutic properties, characterized by fast digestion and absorption, in order to prevent its nutritional benefits. Agustini and Wijayanto,2020, which led some researchers to believe that *Spirulina* as an exceptional organism. Moreover, Jinhong Wu, *et al.,* 2021 reported that the dry weight of *Spirulina platensis* consists protein content 50-70 %, fiber 8-10 % and lipid 5-7 %. It also contains pigments (3-10 %), including phycoerythrin and phycocyanin, having potential properties such as anti-aging, antimicrobials, anticancer, antioxidant and anti- inflammatory (Kapoor and Huang, 2006; Mofeed and Mosleh, 2013; Elshouny *et al.,* 2017; Mofeed *et al.,* 2018; Deyab *et al.,* 2019; Mostolizadeh *et al.,* 2020) and various vitamins: B1, B2, B3, B6,B9, B12, vitamin E, vitamin D, vitamin C (Watanabe *et al.,* 2002), as well as vital unsaturated and saturated fatty acids. Moreover, the unsaturated fatty acids were known as vitamin F due to their significant role as bio-regulators and antioxidants and assumed to be responsible for many curative properties in the

treatment of pre- menstrual tension, arthritis and as an auxiliary in weight loss (Saki, *et al.,* 2015) as well as its role in treating atherosclerosis and hypercholesterolemia (Colla *et al.,* 2008; Cingi *et. al.,* 2008). The essential unsaturated fatty acid; Gamma-Linolenic acid (C18:3 - GLA) which known as ω–6 earned the attention of researchers due to its performance in inhibiting or at least controlling several diseases such as blood pressure, cholesterol level and inflammation (Khan, *et al.,* 2005; Colla *et al.,* 2008; Ali *et al.,* 2017) and play a vital role in the regulation of immune system (Kapoor and Huang, 2006),treating eczema (Kawamura *et al.,* 2011), rheumatoid arthritis (Zurier *et al.,* 1998), premenstrual syndrome (Saki *et al.,* 1995), besides affecting the health of the hair, nails, and skin (Otles and Pire, 2001).

The commercial production of *Spirulina* used growth medium with sodium bicarbonate as a carbon source (Kebede, 1997) which counts for at least 60% of all nutrient costs. Because environmental stress causes oxidative damage at the cellular level, it has an impact on *S. platensis* mass cultivation. To overcome the challenges of oxidative damage *S. platensis* developed enzymatic and non-enzymatic antioxidants (Cameron *et al.,* 2010). Early interest in *S. platensis* focused mainly on its potential as a source of protein and vitamins but recently, more attention has been paid to its potential pharmacological properties, which include reports of the ability of preparations of this micro alga to prevent and inhibit cancers, to decrease blood cholesterol levels, to decrease blood glucose levels, free radical scavenging, stimulate the immunological system, to reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effects of radiation (Guan and Guo, 2002; Xue *et al*., 2007; Ismail *et al.,* 2009; Muthuraman *et al*., 2009; Nielsen *et al*., 2010).

Material and methods

Sample Collection:

Spirulina sample was procured from the Department of Biotechnology, SOS, Jiwaji University, Gwalior (MP), India and further its slants were maintained in Zarrouk's agar media at 4^{O}C.

Inoculum preparation:

Microalgae was cultivated in 4 L Erlenmeyer flasks and fluorescent lights were used to create a 12-hour light/dark cycle with 140 µmol photons.m-2.s-1 of intensity and 30 ± 1ºC of temperature. 10% (v/v) inoculum was utilized as a starter culture and it was Zarrouk's medium were used to cultivate S. *platensis*, which was then enriched with H2O2 (30%, w/v) at stock concentrations of 0, 2, 4, 6, 8, and 10 mM. In 250 ml sterilized Zarrouk media-filled Erlenmeyer flasks, the spirulina was grown. 10% (v/v) or 25ml of the prepared inoculums (0.07 O.D.) were added to the flasks. Before the inoculation, the medium's pH was adjusted with 1 M NaOH, and it was then grown at room temperature. Cool white fluorescent lights were used continuously for 12 hours to light the cultured flasks. All the flask were cultured in three replicates.

Growth evaluation:

The growth of *S. platensis* can be specifically determined by measuring optical density. Growth of *Spirulina* under normal and stress conditions would be assessed spectrophotometrically by measurement of absorbance at 560 nm (Fatma *et al*., 1994).

Harvesting and Dry weight measurement

Algal samples varying in H2O2 content were vacuum-filtered via a 0.45 µm filter membrane, and the residual salts were eliminated from the algal surface by repeatedly washing with distilled water. Algal cells were dried at 100°C for 30 min and weighed (Abd El-Baky *et al*., 2003, Ali and Amber, 2010).

Results

Effect on growth of *S. platensis* at different concentration of H_2O_2

The impact of different H2O2 concentrations (0, 2, 4, 6 ,8 and 10 mM) on the productivity of *S. platensis* ,was conducted based on the results of the previous experiment, revealing that, all concentrations of H2O2suppressed the biomass of *S. platensis* and hence reduce the dry weight (Jelan Mofeed, 2019) **.**Showing in figure 1 and 2 *S. platensis* H_2O_2 stress conditions affected algal growth ,it was found that biomass stimulated at lower concentration 2mM (105mg DW/250ml culture),4mM (87mg DW/250ml), 6mM (60mg DW/250ml), 8mM (48mg DW/250ml) ,10mM(20mg DW/ml) at higher concentration as compared to untreated culture. After fourth and sixth days of exposure, a dramatic decrease in biomass was recorded at 2-

10 mM H_2O_2. after 3, 6, 12 and 15 days respectively and the cells did not start to bleach. In general, the results revealed that *S. platensis* tolerated up to 8 mM H_2O_2. Bleaching process was noticed at 10mM concentration concomitantly the cells exhibited much softer consistence under the highest H_2O_2 level.

Figure 1. Growth curve of *S. platensis* cultivated under different H2O2 concentration (Untreated,2, 4,6, 8 and 10Mm salt concentration) during 20 days incubation period.

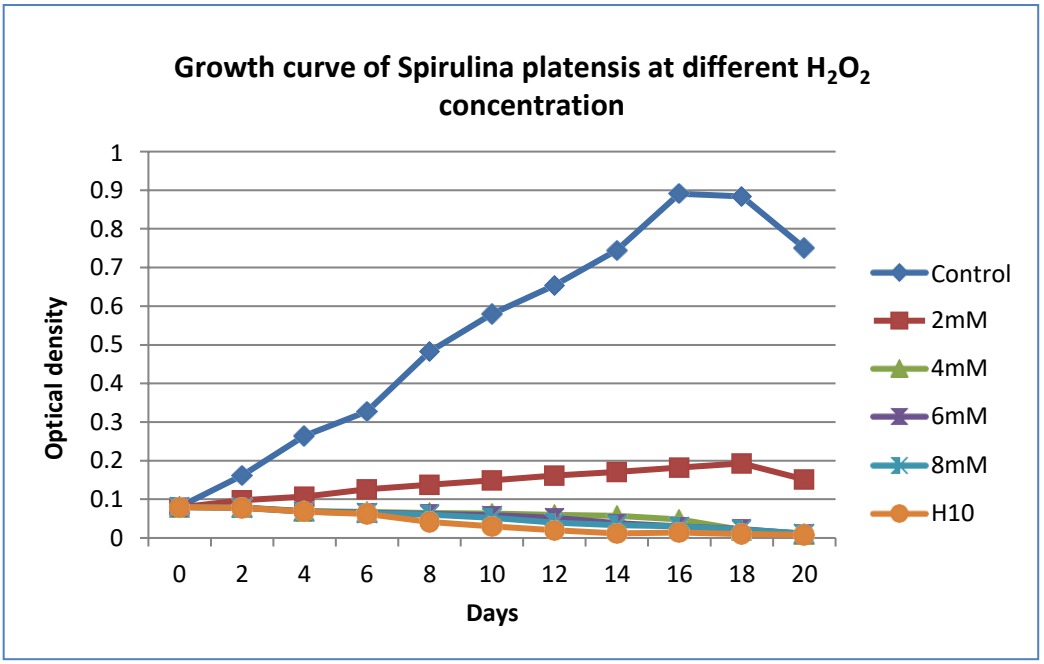
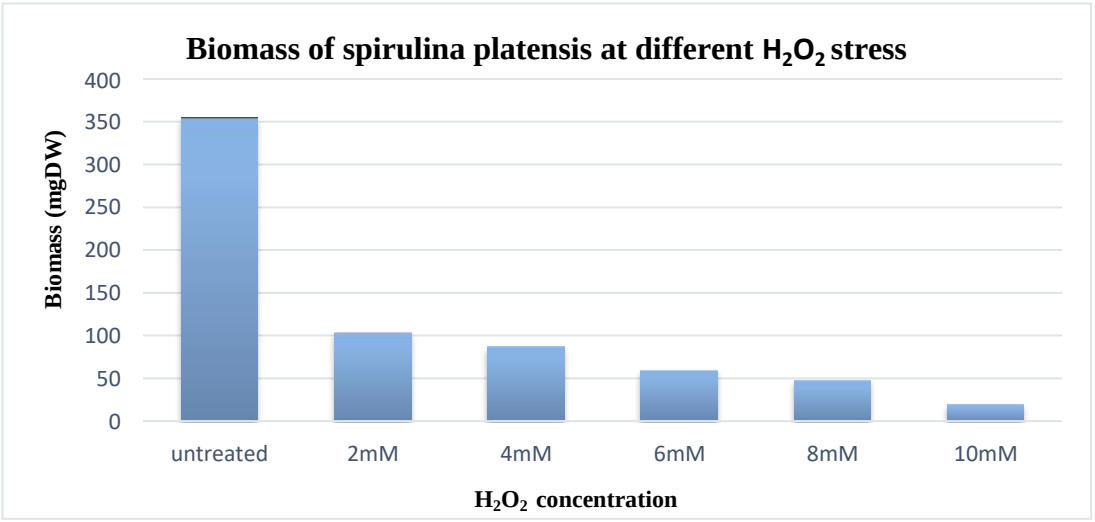


Figure 2 Represented as dry weight (mgDW/250ml culture) of *Spirulina platensis* at different *H2O2*, stress



Discussion

Free radicals are essential for both the aging process and the development of illness. Our first line of defense against the harm caused by free radicals is antioxidants, which are also essential for preserving our best possible health and wellness. As exposure to free radicals increases, the need for antioxidants becomes even more important. Exposure to free radicals can be increased by cigarette smoke, pollution, drugs, disease, stress, and even physical activity. Oxidative stress can be caused by a wide range of events, therefore

determining one's own vulnerability becomes crucial. Antioxidant supplementation is becoming widely acknowledged as a critical component of a balanced, wholesome diet and a healthy lifestyle that enhances free radical protection. Oxidative stress may progress to oxidative damage involving cellular proteins (contractile, structural, MDA, SOD, and GPX of rats muscle and enzymatic), lipids, DNA, and other molecules in ways that might lead to abnormal cellular function (Lakatta, 1980; Davydov and Shvets, 2001; Nayanatara *et al*., 2005).Since 1974, Spirulina platensis, which is high in proteins, vitamins, fatty acids, carbs, fibers, and other essential nutrients, was ranked by the United Nations World Food as one of the most nutritious food sources for the future. The cultivation circumstances have a significant impact on the development and dry weight of *S. platensis*. Specifically, the biomass production is supported by 24L illumination regimes, which allow the plant to reach its maximum dry weight of 1.81g/L at 33^0C.

The present study revealed that light significantly influenced growth and metabolic activities of *S. platensis*; where it considered as a catalyst in cell building processes through controlling the photosynthesis.

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