JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 907-910

Studies on the Growth of *Arthrospira platensis (Spirulina)* as Influenced by Inorganic (Zarrouk's) and Organic Medium

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(Received: 04 March 2010; accepted: 10 April 2010)

***Spirulina plantensis*, now named *Arthrospira plantensis*, is a microscopic and filamentous cyanobacterium (blue-green alga) has high commercial value. In order to grow the *A.Platensis*, Inorganic (Zarrouk's) and Organic media were formulated using organics such as Woodash, CO_2, Cow dung, Sea salt, Soil extract. Parameters such as OD, pH, Chlorophyll content and dry weight were estimated for both medium and it showed that *A.Platensis* grown in inorganic medium has high growth rates.**

Key words: Spirulina, Zarrouk's medium, Organic medium.

Arthrospira platensis (Spirulina) is a spirally coiled, multicellular, filamentous blue-green algae & oxygenic photosynthetic organism has gained importance and international demand for its high value phytonutrients and the various pigments produced by this organism, phycocyanin and chlorophyll (Becker.,1994). A major portion of commercial chlorophyll is used in the food industry, pharmaceutical and cosmetic industries. Spirulina is currently mass produced as wherein the growth medium utilized forms an important input and accounts for a major share of the costs involved in production of Spirulina. The first synthetic medium formulated for cultivation of Spirulina was Zarrouk's medium (Zarrouk, 1966). Subsequently, many media were developed using seawater, sewage water, industrial effluents, CO2 as medium (Vetayasuporn, 2004; Tredici et al., 1986). The present study was to formulate an organic medium for mass production of Spirulina and to compare the growth rates of Spirulina as compared to the inorganic Zarrouk's medium.

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MATERIAL AND METHODS

Mother inoculum for cultivation of Spirulina was collected from ICAR, New Delhi. It is grown in 1 and 5 L flasks in batch cultures in Zarrouk's medium (Zarrouk,1966).

Cultivation by using Inorganic medium (Zarrouk's)

Zarrouk's medium (Zarrouk,1966) was prepared based on the composition/litre $NaHCO_3$ - 16.8g, K_2HPO_4- 0.50g, $NaNO_3$- 2.50g, NaCl- 1.0g, $MgSO_4.7H_2O$- 0.20g, $FeSO_4.7H_2O$- 0.01g, H_2SO_4- 1.0g, $CaCl_2.2H_2O$- 0.04g, A_5M *- 1.0 ml. (A_5M * composition/liter H_3BO_3-2.86g, $MnCl_2.4H_2O$-1.80g, $ZnSO_4.7H_2O$-0.22g,MoO_3-0.01g,$CuSO_4.5H_2O$-0.08g).

The mother inoculum of Spirulina with a volume of 20 litres was taken and it is added to the 100 litres of medium in the small open pond for continues supply of sunlight. Medium was maintained at an alkaline pH of 8.5-11 and temperature at 26°-35° C. Stirring was done at a regular interval of 5 hrs for a period of 20 days for equal distribution of culture and medium. To determine the contamination and growth rate regular microscopic examination was done at a regular interval of 24 hrs.

Cultivation by using Organic medium

Organic medium was prepared based on the availability of organic materials in remote places. Where composition/litre was CO_2-8.8g, Wood ash-20g, Soil extract-2g, Sea salt-2g, Cow dung-20.0g.The mother inoculum of Spirulina was added and culture was maintained as above.

Parameters analyzed during the growth of Spirulina

In continues culture system optical density measurement was the practical way of estimating the rate of algal growth. Optical density was determined using spectrophotometer at 540 nm, pH, Microscopy were monitored daily, For the estimation of growth rate, estimation of chlorophyll & dry weight were monitored at 76 hrs intervals (Goksan et *al*., 2007)

Chlorophyll estimation

About 10 ml of sample was taken and it was centrifuged at 5000 rpm for 5 – 10 minutes. Supernatant was discarded and wash twice with distilled water, a 4 ml of methanol was added to the pellet and again it was centrifuged at 5000 rpm for 10 minutes. This supernatant was incubated in a boiling water bath at 60° c for 10 minutes and continues the same procedure again until the supernatant become white in colour. The tube was maintained in dark. The tubes were then cooled and absorbance was read at 663 nm with methanol as blank. Amount of Chlorophyll was calculated by using the following formula, (Dere., 1998)

$$\text{Amount of Chlorophyll (mg/l)} = \text{Absorbance at 663 nm X 12.63 X } \frac{\text{Volume of Methanol}}{\text{Volume of sample}}$$

Estimation of dry weight

Weigh the Petri dish and to this add known quantity of sample. Note the weight of the sample and sample with dish. Place the dish in hot air oven at 105°C for 2 – 4 hrs

After 4 hrs, cool the dish in dessicator and weight of the dish was noticed.

$$\% \text{ Dry weight} = \frac{\text{A-B}}{\text{F-A}} \times 100$$

Where
A= Empty dish weight
B= Sample + dish weight
F= Final sample + dish weight

RESULTS AND DISCUSSION

The changes obtained in different parameters Viz., optical density and pH at 24 hrs interval, chlorophyll and dry weight on 4th, 8th, 12th, 10th and 20th day are analysed for organic and inorganic medium.

OD helps to determine the growth of the *A.platensis.* The optical density for *A.platensis* is determined everyday by spectrophotometer at 540 nm. The OD values were high at inorganic (Zarrouk's) medium than organic medium on 20th day of observation as shown in the fig1. On first 5 days the OD values are similar in both organic and inorganic medium. Later it increase in inorganic medium, because of the nutrients uptake. In organic medium, there is the slow nutrients uptake because Spirulina enters the new organic environment. The growth rate is good in the organic medium. Similar results were obtained from the Vonshak and Richmond (1988), Mostert, Grobbelaar (1987) suggested that nitrogen in the form of $Ca(NO_{3)\,2}$ were essential components for high growth of Spirulina, because it acts as a double source of nitrate to increase the yield. Morais, Costa (2007) reported that the growth of these algae is influenced by CO_2 and nutrients.

A. platensis grows well at alkaline condition the optimum pH required for its growth ranges from 8.5-11. For inorganic (Zarrouk's) medium the initial pH was 9.34 and on the final day it increased to 10.02 as shown in the fig 2. For organic medium the initial pH was 9.30, which also increased to 10.29. Venkataraman and Becker (1985) and Vonshak & Richmond (1988)

nutrients, including CO_2 at the cell surface which removes excessive Oxygen from the medium to the atmosphere, there by improving the light regime & facilitating improved of solar irradiance.

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