Uttar Pradesh Journal of Zoology

Volume 45, Issue 10, Page 84-90, 2024; Article no.UPJOZ.3465
ISSN: 0256-971X (P)

Assessment of Cow Urine as a Nutrient Medium for Indoor Cultivation of *Spirulina* sp.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i104052

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3465

Original Research Article

Received: 18/02/2024
Accepted: 22/04/2024
Published: 26/04/2024

ABSTRACT

This study aims to explore the feasibility of utilizing cow urine as an economical alternative nutrient source for microalgae cultivation. By leveraging its mineral content and cost-effectiveness, this research investigates the potential to reduce production expenses associated with nutrient mediums, thus enhancing the value proposition of microalgae-derived products on a commercial scale. In order to assess its efficacy, cow urine was gathered, diluted, and introduced into our *Spirulina*-modified medium. This concoction was subjected to 8 hours of daily light exposure. Following an 18-day incubation period, we analysed the biomass quantity, specific growth rate, density, chlorophyll level, and total carotenoid content. In the Aquatic Biology laboratory at VNSGU, an experiment was conducted utilizing five distinct concentrations (0.2 ml, 1 ml, 2 ml, 3 ml, and 4 ml in 200 ml) over an 18-day period. The control flask (0 ml) did not contain cow urine. In our current investigation, *Spirulina* cultivated in a cow urine extract at a concentration of 0.2 ml/200 ml exhibited a dry weight of 0.034±0.0029 g, a specific growth rate of 0.0016±0.00015, and a

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carotenoid content of 4.683±0.015. The density of *Spirulina* peaked at 0.0052±0.00005 with a concentration of 1 ml/200 ml of cow urine extract. Across various concentrations of cow urine extract, including 2 ml/200 ml (8.022±0.016 mg/L), 0.2 ml/200 ml (2.191±0.021 mg/L), and 4 ml/200 ml (0.631±0.020 mg/L), *Spirulina* exhibited higher levels of chlorophyll a, b, and c compared to the control medium in indoor culture setups.

Keywords: Spirulina sp; cow urine; growth (Dry Weight); specific growth rate; density; chlorophyll and total carotenoid content.

1. INTRODUCTION

Spirulina is a type of blue-green algae that thrive in highly-alkaline aquatic environments. Under the microscope, *Spirulina* appears as small spiral-shaped structures. Given optimal conditions, it can grow rapidly. In ancient times, civilizations cultivated *Spirulina* in lakes and ponds. Nowadays, *Spirulina* producers cultivate it in carefully controlled aquatic environments to maintain its quality and safety [1]. *Spirulina* is known for its high protein content, necessary and non-essential amino acids, gamma-linolenic acid (GLA), chlorophyll, and phycocyanin, B12, iron, calcium, vitamin A in the form of beta-carotene [1]. Vonshak, 1997; Belay *et al.,* [2]. "Recent research endeavours focus on devising cost-effective methods for generating beneficial products from microalgae. Cow urine contains a variety of components, including 24 different types of salts, 2.5% urea, 2.5% enzymes, and approximately 95% water. Other constituents include ammonia, iron, phosphorus, potassium, carbonic acid, nitrogen, manganese, calcium, sulphur, amino acids, cytokines, lactose, and phosphate" [3,4].

2. METHODOLOGY

2.1 Culture medium and Modified Medium

Cow urine was added as a supplement to the modified medium. (pH-9.5).

2.2 Prepare the Medium

Conducted an in-depth investigation utilizing cow urine extract to examine its impact on the growth of *Spirulina* sp. An experiment was conducted using various concentrations of cow urine in 200 ml, detailed in Table 2. The control group was represented by 0 ml (without inoculation of cow urine) [5,6,7].

2.3 Sterilization and Culture Maintenance

"The growth media underwent sterilization through steam for 20 minutes at 121˚C and 15 pounds per square inch pressure in an autoclave. *Spirulina* sp culture was kept at ambient room temperature. Blue LED light was administered for 8 hours daily. Throughout the experiment, agitation was achieved by manually shaking the culture 3-4 times daily. All subculturing and inoculation procedures were conducted under aseptic conditions" [5,6,7,8,9].

2.4 Growth Measurement of *Spirulina*

"After 18 days, the concentration of *Spirulina* sp biomass was determined. Each culture medium was filtered using pre-weighted Whatman filter paper No. 1 and rinsed with acidified distilled water to remove all salts and nutrients. Subsequently, the filter paper was air-dried in an oven at 90˚C and then weighed using a precision balance. Dry weight was calculated based on the difference in weight before and after drying". Chaudhari *et al.,* [5,6,7] Pandey *et al.,* [8] Palanisamy *et al.,* [10] (Fig. 3).

2.5 Specific growth rate of *Spirulina* (Abu-Razaq *et al*., 1999) Kumaresan *et al.,* [11] Chaudhari et al., [5,6,7].

$$\mu \text{ (Cell weight day1)} = X2\text{-}X1/t$$

Where,

μ = Specific growth rate
In X1= Initial weight of *Spirulina* biomass
In X2= Final weight of *Spirulina* biomass

2.6 Density equation: Chaudhari *et al.,* [5,6,7].

$$p = \frac{m}{V}$$

Where,

p = Density
m= Mass
V= Volume

2.7 Pigment Content of *Spirulina*

Chlorophyll extraction from dried *Spirulina* involved crushing a measured amount with 10 ml

of 90% acetone in a pestle-mortar. The mixture was then refrigerated overnight for pigment extraction, with the tubes covered by carbon paper. After centrifugation for 10 minutes at 2500 rpm, the supernatant was collected. Readings were taken at 630 nm (A630), 645 nm (A645), 665 nm (A665), and 450 nm (A450) using a Shimadzu-UV-1800 spectrophotometer, with 90% acetone used as a blank. The concentrations of Chl-a, Chl-b, and Chl-c were determined using specific formula: Chaudhari *et al.,* [5,6,7]. APHA, [12] (Fig. 4).

$$Ca = 11.85\ (\mathrm{OD}664) - 1.54\ (\mathrm{OD}647) - 0.08(\mathrm{OD}630)$$

$$\mathrm{Cb} = 21.03\ (\mathrm{OD}647) - 5.43\ (\mathrm{OD}664) - 2.66\ (\mathrm{OD}630)$$

$$\mathrm{CC} = 24.52\ (\mathrm{OD}630) - 7.60(\mathrm{OD}647) - 1.67(\mathrm{OD}664)$$

The total carotenoid content (Cp): Ben-Amotz and Avron in 1983, Jeffrey *et al*., in 1997.

$$CP\left(\frac{\mu g}{L}\right) = 7.60\ (A480) - 1.49\ (A510)$$

2.8 Statistical Tools

The average value (mean ± SE) of three samples from each experimental culture flask was calculated. (Mean ± SE) were analyzed graphically. (Microsoft Excel) (Fig. 5-9).

Table 1. Chemical composition of the modified medium- Indoor culture

No	Chemical name	Concentration in stock solution (g/l)
1	Cooking soda	16
2	Potassium sulphate (K_2SO_4)	1
3	Sodium nitrate ($NaNO_3$)	2.5
4	di-Potassium hydrogen phosphate (K_2HPO_4)	0.6
5	Sodium chloride (NaCl)	1
6	Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)	0.01

Fig. 1. 1st day incubation of Spirulina in cow urine extract

Fig. 2. 18th day after growth of Spirulina in cow urine extract

Table 2. Preparation of media

No	Modified medium	Cow Urine (Different concentration)
A	200 ml	0 ml
B	200 ml	0.2 ml
C	200 ml	1 ml
D	200 ml	2 ml
E	200 ml	3 ml
F	200 ml	4 ml

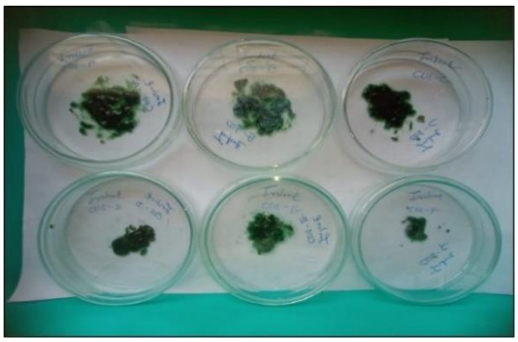

Fig. 3. 18th day after growth of Spirulina in cow urine extract

Fig. 4. Result of chlorophyll (Cow urine extract)

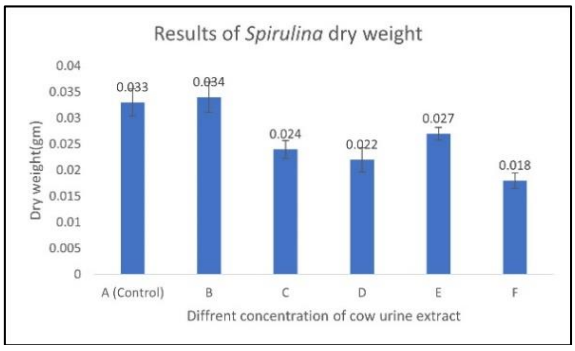

Fig. 5. Results of Spirulina dry weight

3. RESULTS AND DISCUSSION

3.1 Results of Descriptive Statics of Study Variables

In the cultivation of *Spirulina* using cow urine extract at a concentration of 0.2 ml/200 ml, it displayed a dry weight of 0.034±0.0029 g (Fig. 5), a specific growth rate of 0.0016±0.00015 (Fig. 7), and a carotenoid content of 4.683±0.015 µg/L (Fig. 8). The highest density of *Spirulina*, reaching 0.0052±0.00005 (Fig. 6), was observed at a concentration of 1 ml/200 ml of cow urine

extract. In experiments involving different concentrations of cow urine extract, such as 2 ml/200 ml (8.022±0.016 mg/L), 0.2 ml/200 ml (2.191±0.021 mg/L), and 4 ml/200 ml (0.631±0.020 mg/L), *Spirulina* displayed elevated levels of chlorophyll a, b, and c compared to the control medium in indoor culture conditions (Fig. 9).

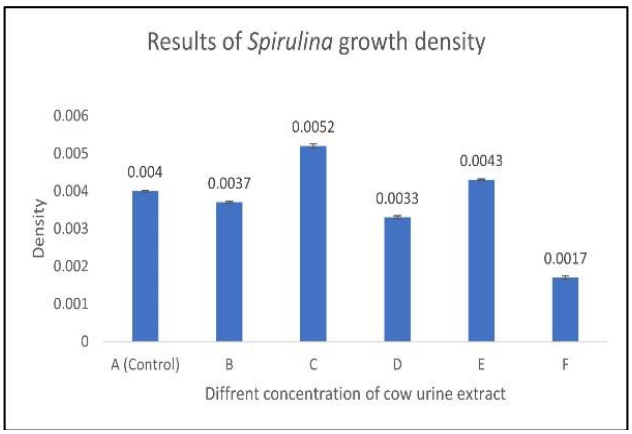

Fig. 6. Results of Spirulina growth density

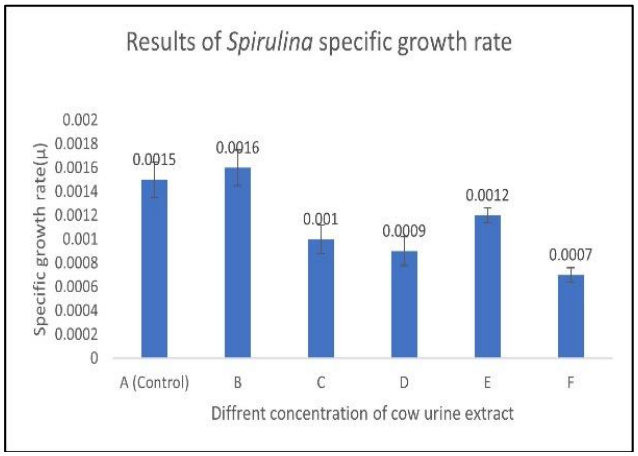

Fig. 7. Results of Spirulina specific growth rate

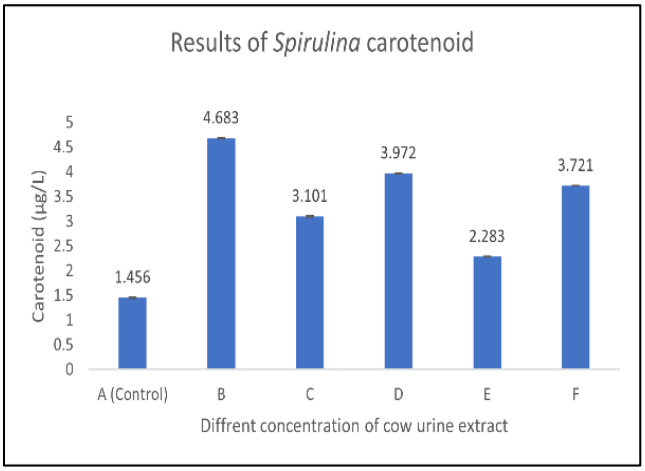

Fig. 8. Results of Spirulina carotenoid

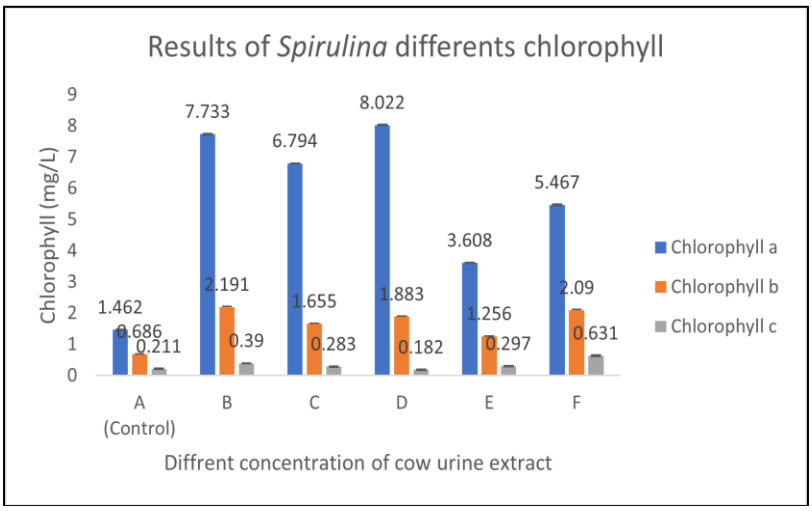

Fig. 9. Results of Spirulina differents chlorophyll

4. CONCLUSIONS

The objective of enhancing *Spirulina* growth through the utilization of a cost-effective cow urine extract supplement in a modified medium has been largely achieved. Notably, the concentration of 0.2 ml of cow urine extract per 200 ml of medium proved effective in promoting substantial biomass growth. Across various concentrations of cow urine extract, the density, chlorophyll, and carotenoid levels exhibited significant increases compared to the control group.

5. ACKNOWLEDGEMENT

I extend my heartfelt appreciation to Dr. Kapila Manoj, the Head of the Aquatic Biology Department at Veer Narmad South Gujarat University, for his invaluable mentorship throughout this research and for graciously granting access to laboratory resources.

6. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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