Assessment Of Cow Urine as A Nutrient Medium for Indoor Cultivation of *Spirulina* sp

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 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 (Mahato *et al.,* 2023). *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 (Mahato *et al.,* 2023; Vonshak, 1997; Belay *et al.,* 1993). 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 (Bhadauria, 2002; Randhawa *et al*., 2015).

2.RESEARCH METHODOLOGY

2.1 Culture medium and Modified Medium

Table No: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

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) (Chaudhari *et al.,* 2022;2023;2024).

Table No: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

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 (Chaudhari *et al.,* 2022;2023;2024, Pandey *et al.,* 2010; Shi *et al.,* 2016;).

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.,* 2022;2023;2024, Pandey *et al.,* 2010; Palanisamy *et al.,* 2021) (Fig-3).

2.5 Specific growth rate of *Spirulina* (Abu-Razaq *et al.,* 1999) (Kumaresan *et al.,* 2020; Chaudhari et al., 2022; 2023; 2024):

μ (Cell weight day1) = X2-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.,* 2022; 2023; 2024).

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

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.,* 2022;2023;2024, APHA, 1988) (Fig-4).

$$Ca = 11.85\ (\mathrm{OD664}) - 1.54\ (\mathrm{OD647}) - 0.08(\mathrm{OD630})$$
$$\mathrm{Cb} = 21.03\ (\mathrm{OD647}) - 5.43\ (\mathrm{OD664}) - 2.66\ (\mathrm{OD630})$$
$$\mathrm{CC} = 24.52\ (\mathrm{OD630}) - 7.60(\mathrm{OD647}) - 1.67(\mathrm{OD664})$$

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

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

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

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

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

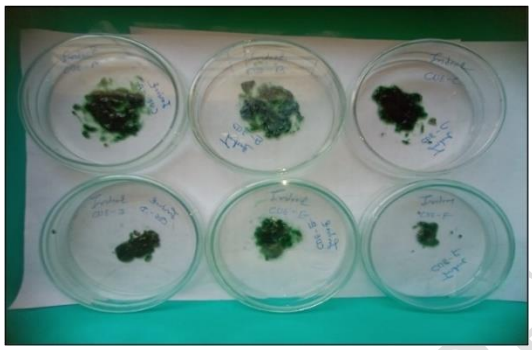

Fig. 3 18th day after growth of *Spirulina* in cow urine extract

Fig. 4 Result of chlorophyll (Cow urine extract)

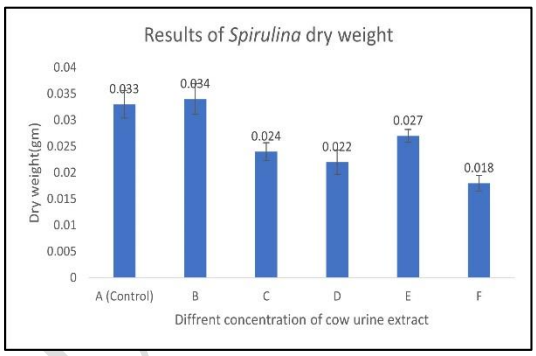

Fig.5 Results of *Spirulina* dry weight

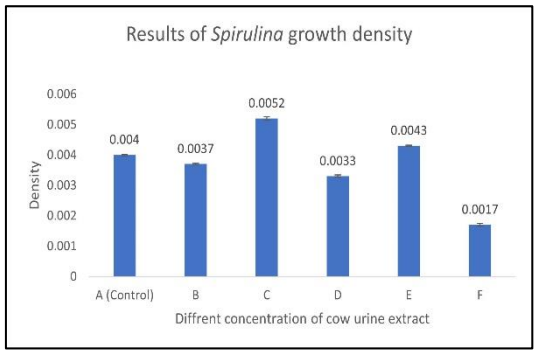

Fig. 6 Results of *Spirulina* growth density

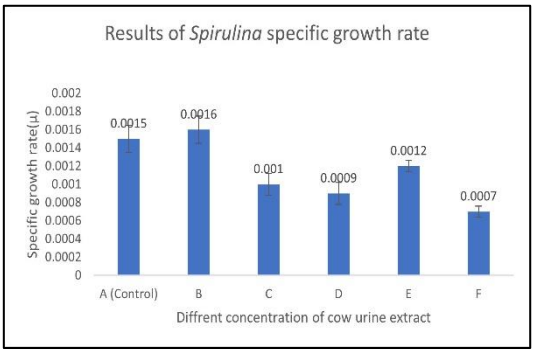

Fig.7 Results of *Spirulina* specific growth rate

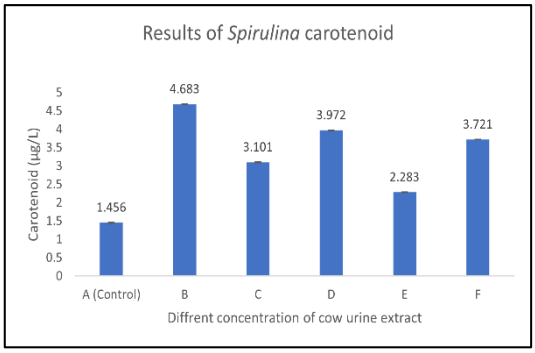

Fig.8 Results of *Spirulina* carotenoid

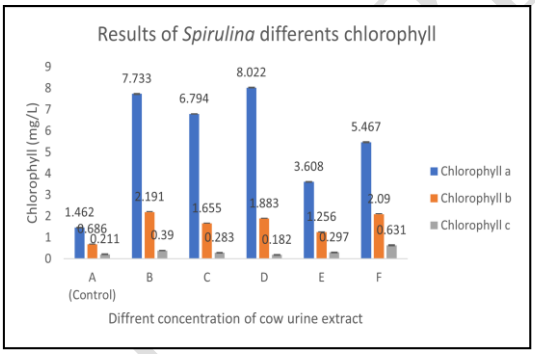

Fig.9 Results of *Spirulina* differents chlorophyll

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.

6.References

i. APHA. (1998) Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, American Water Works Association and Water Environmental Federation, Washington DC.

ii. Belay, A., Ota, Y., Miyakawa, K., & Shimamatsu, H. (1993). Current knowledge on potential health benefits of Spirulina. *Journal of Applied Phycology*, *5*(2), 235–241. https://doi.org/10.1007/bf00004024

iii. Bhadauria, H. (2002). Cow urine-a magical therapy. *Int J Cow Sci*, *1*, 32-6.

iv. Chaudhari A, Manoj K and Patel V. (2022) Study on the use of poultry manure extract in the growth of Spirulina maxima with reference to indoor culture. World Wide J Multidisciplinary Res Develop. 8(8): 109–113.

v. Chaudhari, A., & Kapil, M. (2024). "Production of *Spirulina* sp. in a Modified Medium with Rice Bran Extract in a Controlled Indoor Environment." NATURALISTA CAMPANO, 28*(2)*, 134-140.
vi. Chaudhari, A., & Manoj, K. (2023). "Investigation on the utilization of horse manure extract as an additive in the growth of spirulina SP." *Journal of Survey in Fisheries Sciences*, *10*(1), 1493–1498. https://doi.org/10.53555/sfs.v10i1.2373
vii. Feng, D., & Wu, Z. (2006). Culture of *Spirulina platensis* in human urine for biomass production and O2 evolution. *Journal of Zhejiang University-SCIENCE B*, *7*(1), 34–37.
viii. Kumaresan G, Sivakumar K and Rajan LFS. (2020) Effect of abiotic factors on the growth of Spirulina platensis strains. Plant Arch. 20(suppl. 2): 4259-4263.
ix. Mahato, A. K., Gani, O., Yadav, N., Sher-e-Bangla Agricultural University, Sher-e-Bangla Agricultural University, & Sher-e-Bangla Agricultural University. (2023). Production of spirulina (Arthrospira platensis) using different culture media. *International Journal of Research Publication and Reviews*, *4*(9), 1408–1413.
x. Murugan, T., & Radhamadhavan. (2010). Media optimization for the enhanced growth and yield of *Spirulina platensis* biomass and determination of generation time. *Journal of the Medical Sciences*, *3*, 34–39. *Spirulina platensis*
xi. Palanisamy KM, Paramasivam P, Jayakumar S, Maniam GP, Rahim MHA and Nagarajan G. (2021) Economical cultivation system of microalgae Spirulina platensis for lipid production. IOP Conference Series. Earth Environ Sci 641(1): 012022.
xii. Pandey JP, Pathak N and Tiwari A. (2010) Standardization of pH and light intensity for the biomass production of Spirulina platensis. J Algal Biomass Utilization 1(2): 93-102.
xiii. Randhawa, G. K., & Sharma, R. (2015). Chemotherapeutic potential of cow urine: A review. *PubMed*, *4*(2), 180–186. https://doi.org/10.5455/jice.20150222100032
xiv. Shi W, Li S, Li G, Wang W, Chen Q, Li Y and Ling X. (2016) Investigation of main factors affecting the growth rate of Spirulina. Optik 127(16): 6688-6694.
xv. Srivastava P. (2017) Nutraceutical Spirulina: Commercial cultivation using rural technology in India. Aavishkar Publishers. India.