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# Enhancing *Spirulina* sp. Growth Performance through Rabbit Manure Supplement: A Comparative Study

**Chaudhari Ankit* and Manoj Kapila**

Department of Aquatic Biology, Veer Narmad South Gujarat University, Surat 395007, Gujarat, India

**Corresponding Author*

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**Abstract:** In this study the effect of rabbit manure supplementation on the growth of *Spirulina* sp was studied for 18 days. Rabbit manure was bought online and Sundried before powdering with a mixer grinder. 10 g of powdered rabbit manure was combined with 100 ml of tap water and steamed for 30 min in a water bath. *Spirulina* sp. were grown in *Spirulina* growth medium (200 ml) with different concentrations of rabbit manure extract (0.2 ml, 1 ml, 2 ml, 3 ml, and 4 ml). *Spirulina* growth medium without rabbit manure extract was used as a control. The experiment lasted 18 days, with the culture receiving adequate light for 8 h per day. Manual agitation was performed three to four times per day. This research aimed to assess the practicality of utilizing rabbit manure as a cost-efficient substitute nutrient for microalgae cultivation. Leveraging its rich mineral content and cost-effectiveness, the study aimed to cut down on expenses related to nutrient mediums, thereby improving the economic viability of microalgae-derived products for commercial purposes. This experiment marks the first exploration of *Spirulina* growth using different concentrations of rabbit manure extract. Positive outcomes were achieved through the utilization of varied concentrations of rabbit manure extract for cultivating *Spirulina* in indoor cultures.

**Keywords:** *Spirulina* sp, Rabbit manure extract, Growth (Dry Weight), Specific growth rate, Density, Chlorophyll, Total carotenoid content

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## Introduction

Over the past two decades, there has been a growing focus on microalgae biotechnology, particularly for the manufacturing of feed, health, and beauty products. Microalgae are globally recognized as a valuable reservoir of protein, carbohydrates, fibre, and enzymes, alongside containing numerous vitamins and minerals including vitamin A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium, and calcium. Four green microalgae species play a crucial role in the commercial production of food and feed for both humans and animals. These species include

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 |

Table 2: Preparation of media

| No. | **Modified medium** | **Rabbit manure (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 |

Cyanobacteria *Spirulina, Dunaliella salina*, *Haematococcus pluvialis*, and the *Chlorella vulgaris*. *Spirulina* holds a prominent position among microalgae in the market owing to its exceptional nutrient richness (Sukanya, 2023). The NPK ratio in rabbit manure extract is 2.4:1.4:0.6. For this purpose, we attempted to cultivate *Spirulina* using rabbit manure extract (Harrison, 2016).

## Materials and Methods

### *Culture media and experimental design*:

Rabbit manure was added to the modified medium. An experimental investigation was conducted, consisting of five different treatments: 0 ml (serving as control), 0.2 ml, 1 ml, 2 ml, 3 ml, and 4 ml, all within 200 ml of modified medium (pH of 9.5) (Tables 1, 2) (Chaudhari *et al.*, 2022).

### *Media preparation*:

A comprehensive study was carried out utilizing rabbit manure extract to evaluate the growth of *Spirulina*. After being dried, the rabbit manure was ground and sieved through a fine cloth to produce a fine powder. The powder was then combined with 100 ml of tap water and heated for 30 min on a water bath. Once cooled, the mixture underwent filtration through filter paper until achieving a clear solution. The resulting extract was then autoclaved and preserved as a 10% rabbit manure extract stock solution (Fig. 1) (Srivastava, 2017).

### *Culture incubation and upkeep*:

The growth medium is sterilized using an autoclave at 121°C for 20 min. Cultures of *Spirulina* sp. maintained at room temperature and


Fig. 1: Rabbit manure extract.

Fig. 2: 1st day incubation of *Spirulina* in rabbit manure extract.

Fig. 3: 18th day after growth of *Spirulina* in rabbit manure extract.

exposed to blue LED light for eight hours daily. Cultures were manually shaken three to four times a day during the experiment. Each step of the inoculation and subculturing procedures was carried out under sterile conditions (Pandey *et al.*, 2010; Shi *et al.*, 2016; Chaudhari *et al.*, 2022).

*Growth estimation of Spirulina*:

The amount of *Spirulina* sp. biomass was measured eighteen days later. Each culture medium was filtered using pre-weighed Whatman filter paper No. 1, washed with acidified distilled water to remove salts and supplements. The filter paper was allowed to dry in an oven set at 90°C, the dry weight was determined by comparing the weight before and after drying (Fig. 4) (Pandey *et al.*, 2010; Palanisamy *et al.*, 2021; Chaudhari *et al.*, 2022).

*Spirulina specific growth and density*:

The specific growth rate (Abu-Razaq *et al.*, 1999) and density of *Spirulina* were determined using the following formula (Kumaresan *et al.*, 2020; Chaudhari *et al.*, 2022):

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

Where, μ = Specific growth rate, X1= Initial weight of *Spirulina* biomass, X2= Final weight of *Spirulina* biomass.

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

Where, $p$ = Density, $m$= Mass, $V$= Volume.

*Contents of pigments*:

A specific quantity of dried *Spirulina* was crushed in a pestle and mortar with 10 ml of 90% acetone in order to extract the chlorophyll. The mixture was then placed in the refrigerator for the entire night in order to extract the pigment, and the tubes were covered with carbon paper. After a 10 min centrifugation at 2500 rpm, the supernatant

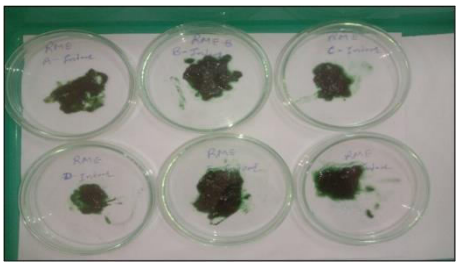

Fig. 4: 18th day after growth of *Spirulina* in rabbit manure extract.

Fig. 5: Chlorophyll (Rabbit manure extract).

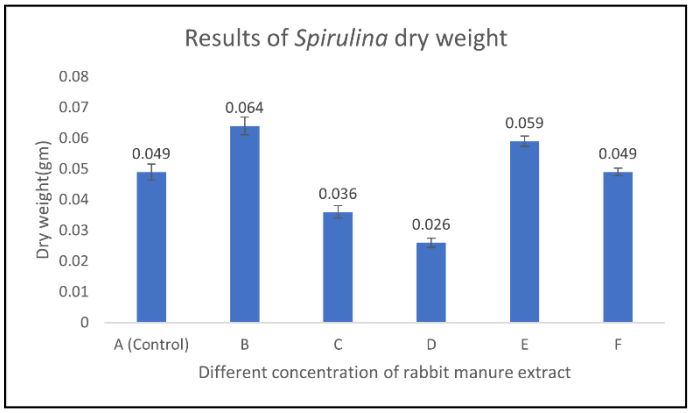


Fig. 6: *Spirulina* dry weight.

was collected. Using a Shimadzu-UV-1800 spectrophotometer, readings were taken at the following wavelengths: 630 nm (A630), 645 nm (A645), 665 nm (A665), and 450 nm (A450). The blank is prepared using 90% acetone. The concentrations of chlorophyll-a, chlorophyll-b and chlorophyll-c were calculated using formulas (Fig. 5) (APHA, 1988; Chaudhari *et al.*, 2022):

$C_a$ =11.85 (OD664) - 1.54 (OD647) - 0.08 (OD630)

$C_b$ = 21.03 (OD647) - 5.43 (OD664) - 2.66 (OD630)

$C_c$ = 24.52 (OD630) -7.60 (OD647) - 1.67 (OD664)

Following equation was used to determine the total carotenoid content (Cp) (Kafyra *et al.*, 2018; Chaudhari *et al.*, 2022):

Cp (µg/l) = 7.60 (A480) – 1.49 (A510)

Each experimental culture flask's mean value from three replicates was computed, and the results were shown as Mean ± SE. Based on this format, a graphical analysis was carried out (Figs. 6-10).

## Results and Discussion

In the present study, *Spirulina* cultivated in rabbit manure extract at a concentration of 0.2 ml per 200 ml exhibited a carotenoid content of 7.018±0.016 µg/l (Fig. 9), a specific growth rate of

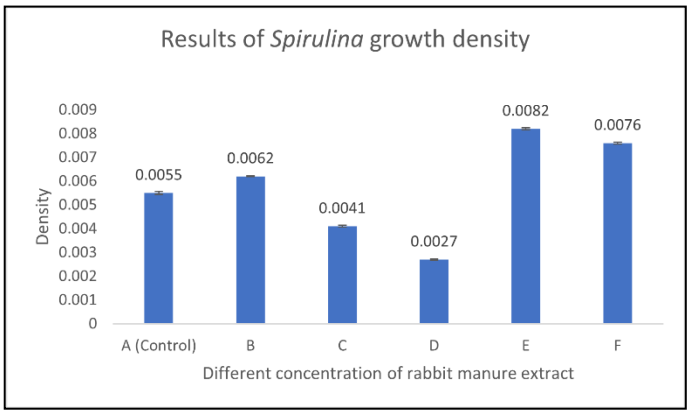


Fig. 7: *Spirulina* growth density.

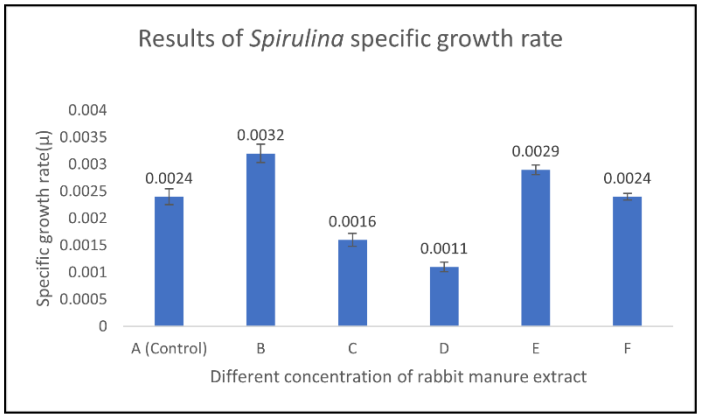


Fig. 8: *Spirulina* specific growth rate.

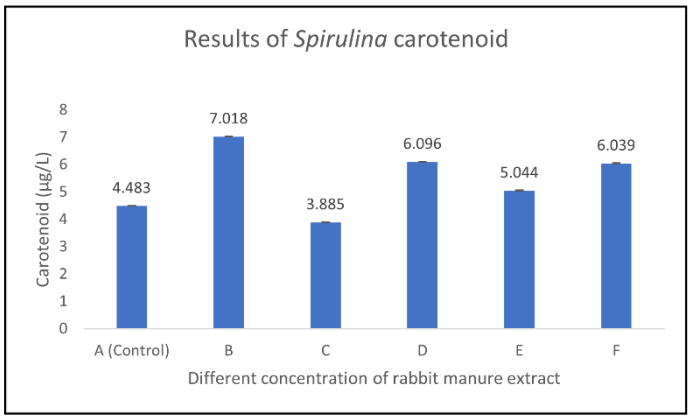


Fig. 9: *Spirulina* carotenoid.

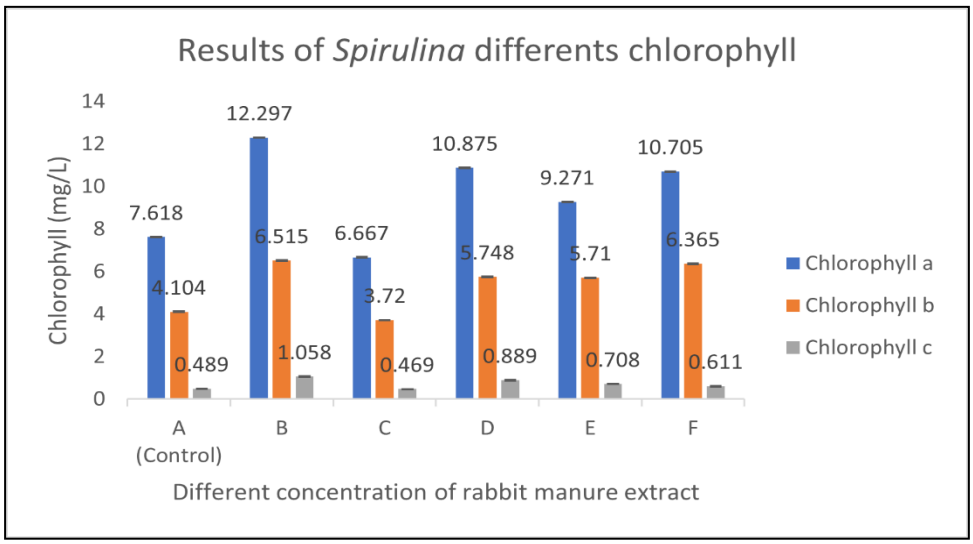


Fig. 10: *Spirulina* differents chlorophyll.

0.0032±0.00017 (Fig. 8), and a dry weight of 0.064±0.0029 g (Fig. 6). *Spirulina* density peaked at 0.0082±0.00005 at a concentration of 3 ml per 200 ml of rabbit manure extract (Fig. 7). *Spirulina* grown in rabbit manure extract contained chlorophyll-a 12.297±0.011 mg/l, chlorophyll-b 6.515±0.026 mg/l, and chlorophyll-c 1.058±0.027 mg/l was at a concentration of 0.2 ml/200 ml (Fig. 10) which, in terms of indoor culture, was higher than the control medium.

## Conclusion

In this study it was found that a concentration of 0.2 ml of rabbit manure extract per 200 ml of medium was effective in promoting significant growth in biomass. Density, carotenoid and Chlorophyll levels were significantly higher in the rabbit manure extract group at different concentrations compared to the control group.

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