



Enhancing the Growth of *Spirulina platensis* Using Sugarcane Molasses as a Low-Cost Carbon Source

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Abstract: *Spirulina platensis* is a type of green algae that grows in both saltwater and freshwater. It is highly nutritious and serves as an excellent source of protein for feed and food supplementation. The present study was carried out to utilize molasses as an organic additive for *Spirulina* cultivation. Typically, *Spirulina* is grown in conventional Zarrouk's medium, which requires high-quality NaHCO₃. To reduce the cost of the medium, *Spirulina* was cultivated using CFTRI medium supplemented with different concentrations of molasses (diluted and supernatant). The molasses, being rich in carbon, was expected to enhance *Spirulina* growth. Growth performance was evaluated using optical density (OD) and chlorophyll-a content. In this study, *Spirulina* showed good growth in both CFTRI medium and CFTRI medium supplemented with organic molasses.

Keywords: *Spirulina*, CFTRI medium, sugarcane molasses, organic medium.

1. INTRODUCTION:

Spirulina is a filamentous micro alga with high nutritive value. It is one of the most important cyan bacteria due to its wide range of industrial applications. *Spirulina* is a rich source of protein and also contains essential vitamins and pigments. It is considered a "super food" because it provides an array of nutrients, including protein levels comparable to those found in eggs. Due to its naturally bitter taste, people often mix *Spirulina* with yogurts, juices, and smoothies.

One tablespoon of *Spirulina* contains approximately 20 calories, 4 g protein, 1 g fat, 2 g carbohydrates, 0 g fiber, 0 g sugar, and various vitamins and minerals such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), copper, iron, and magnesium. These minerals support normal bodily functions, including muscle activity and heartbeat regulation. *Spirulina* also helps in protein synthesis and energy production. It contains a plant-based pigment, phycocyanin, which research shows may possess antioxidant, anti-inflammatory, and neuroprotective properties. It has also been reported to exhibit anticancer activity. Several antioxidants in *Spirulina* help reduce inflammation in the body. Phycocyanin, the blue-green pigment present in *Spirulina*, reduces inflammation, blocks tumor growth, and can induce cancer cell death. There is growing demand for natural pigments extracted from *Spirulina* due to their non-toxic and non-allergenic nature. Phycocyanin has even been used in producing phycocyanin-based ice cream. The proteins present in *Spirulina* help lower cholesterol levels, keeping arteries clear and reducing strain on the heart, thereby lowering the risk of heart disease and stroke. *Spirulina* also increases nitric oxide production, which helps relax blood vessels. Its rich vitamin and mineral content strengthens the immune system, promoting the production of white blood cells and antibodies that fight viruses and bacteria. *Spirulina* contains zeaxanthin, a plant pigment that may reduce the risk of cataracts and age-related vision loss. It is commonly available in powder or tablet form. The powdered form is often added to smoothies, salads, or soups, while tablets are taken as dietary supplements.

Sugarcane molasses is a viscous, dark, sugar-rich by-product of sugar extraction from sugarcane. Cane sugar is produced through successive evaporation, crystallization, and centrifugation steps. Molasses is generated during the sugar extraction and refining processes. Perez described different types of molasses: integral high-test molasses is produced from unclarified sugarcane juice; A molasses (first molasses) is an intermediate by-product resulting from the first sugar crystal extraction; B molasses (second molasses) is obtained after further processing; and C molasses is the final by-product of sugar factory operations. C molasses contains small amounts of sucrose and remains in liquid form, making it useful as a commercial feed ingredient. Typical molasses composition includes: sucrose (29–40%), water (17–



25%), glucose (4–14%), ash (7–15%), potassium (4–50.83%), calcium (0.8–15%), magnesium (1–14%), sodium (0.09–9%), protein (0.5–4.5%), sulfates (2.24–9.91%), amino acids (0.3–1.5%), non-nitrogenous acids (1.5–8%), wax, sterols and phosphatides (0.1–1%), biotin (0.1–2 ppm), and riboflavin (1–6 ppm).

Spirulina exists in freshwater, brackish water and seawater environments. Its growth depends on environmental factors such as light, temperature, and pH. High pH and temperature are necessary for large-scale cultivation. A pH between 9.5 and 9.8 helps prevent contamination by other algae. Sodium bicarbonate must be present in the culture medium to maintain this high pH. Aeration also plays a crucial role in biomass production by increasing dissolved oxygen levels. Nutrient availability is another key factor influencing *Spirulina* growth and pigment production. Various media, including Zarrouk's medium, CFTRI medium, and JSPM medium, can be used for *Spirulina* cultivation. Optimization and reduction of production costs are essential for large-scale *Spirulina* cultivation. Low-cost sources such as inorganic fertilizers and sugarcane molasses are used to reduce costs and improve algal biomass. As organic carbon sources like glucose and acetate are expensive, sugarcane molasses—a cheap industrial by-product containing 50% total sugars—can be used as a low-cost medium supplement. Glucose provides higher energy content (2.8 kJ per mole) compared to acetate (0.8 kJ per mole). The main objective of the present work is to cultivate *Spirulina platensis* using CFTRI medium supplemented with organic molasses at various concentrations. *Spirulina* species are resistant to contamination, contain low fat, and are rich in protein. They can be cultivated on a large scale, and harvesting is relatively easy. This study aims to evaluate the growth of *Spirulina* based on commercial-grade fertilizers, enabling the formulation of a new, low-cost medium for *Spirulina* production. In this study, carbon-source optimization was carried out to enhance *Spirulina* growth, along with the estimation of chlorophyll-a content in *Spirulina* cultures. *Spirulina platensis* culture was obtained from Krishi Vigyan Kendra, Babhaleshwar, and Ahilyanagar.

2. MATERIALS AND METHODS:

Spirulina platensis culture was obtained from Krishi Vigyan Kendra, Babhaleshwar, Ahilyanagar, where it was originally grown in a production pond. The starter inoculums and experimental cultures were maintained in CFTRI medium. The pH of the culture was measured using a calibrated pH meter.

2.1 Culture Medium

The CFTRI medium was used for the cultivation of *Spirulina platensis*.

2.2 Composition of CFTRI Medium (per 5 L)

1. Sodium bicarbonate – 50 g
2. Suphala (N:P:K = 15:15:15) – 10 g
3. Magnesium sulfate – 2 g

2.3 Culture Conditions and Growth

Spirulina platensis culture was grown in an Erlenmeyer flask. The culture was maintained for 25 days, and growth was monitored spectrophotometrically at 550 nm. The pH of the medium was measured using a pH meter. *Spirulina platensis* grows well in alkaline conditions, and in this study, the pH was maintained within the range of 8.9 to 9.5.

2.4 Preparation of Organic Media

Composition and Characteristics of Sugarcane Molasses

Sugarcane molasses with a pH of 6.0 was obtained from Bhausahab Santaji Sahakari Sakharkarkhana, Sangamner. The molasses contained approximately 20% water, 8% fructose, 7% glucose, and several metal ions, including calcium, potassium, sodium, iron, magnesium, and copper.

Treatment of Molasses

The molasses was processed in two ways. In the first treatment, the molasses was centrifuged at 5000 rpm for 10 minutes, and the clear supernatant was collected. In the second treatment, the molasses was directly diluted with an equal volume of water. Various concentrations of both the diluted molasses and the supernatant were prepared and added to the culture medium for further experimentation.



Table -1. Treatment of molasses on *S. platensis*-

Organic Substrate	Concentration (ml)	CFTRI media (ml)+ <i>Spirulina</i> (ml)
Molasses(Supernatant)	5mg	45ml+5ml
	12.5mg	45ml+5ml
	25mg	45ml+5ml
	32.5mg	45ml+5ml
	50mg	45ml+5ml
Molasses(Dilute)	5mg	45ml+5ml
	12.5mg	45ml+5ml
	25mg	45ml+5ml
	32.5mg	45ml+5ml
	4ml	45ml+5ml

2.5 *Spirulina platensis* Growth Analysis

The following parameters were analyzed to determine the growth of *Spirulina platensis*:

- **Microscopy**

Microscopic examination of the culture was performed using a simple light microscope to observe the characteristic green-colored, spiral-shaped filaments.

- **Optical Density (Growth Measurement)**

Optical density (OD) was measured at 550 nm using a spectrophotometer.

Initially, the culture displayed an OD of approximately 0.2, and it gradually increased throughout the incubation period.

- **pH Measurement**

The pH of the culture medium was measured using a pH meter. *Spirulina platensis* typically grows in alkaline conditions, with an optimal pH range between 8.5 and 11.

- **Temperature**

The *Spirulina platensis* culture was maintained at room temperature throughout the experiment.

2.6 Estimation of Chlorophyll-a

Procedure

1. 5 mL of algal suspension was centrifuged at 3500 rpm for 5 minutes.
2. The resulting pellet was homogenized with 5 mL of 80% methanol.
3. The mixture was incubated in a water bath at 70°C.
4. It was then centrifuged again at 3500 rpm for 5 minutes.
5. The clear supernatant was collected, and absorbance was measured at 665 nm.
6. Chlorophyll-a content was calculated using the formula:

$$\text{Chlorophyll-a (mg/L)} = \text{OD at 665 nm} \times 13.9$$

(Here, 13.9 is a constant derived from the absorption coefficient.)

3. RESULTS

3.1 Microscopy:

Microscopic analysis of *S. platensis* showed that the initial culture was green in color and consisted of long, loosely coiled filaments. Similar morphological characteristics were observed in the cultures grown using both types of organic substrates (molasses).

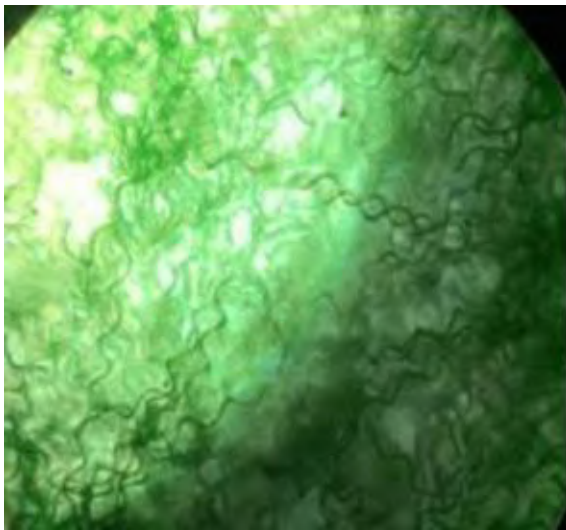


Fig. 1. Growth of *Spirulina platensis* in CFTRI medium

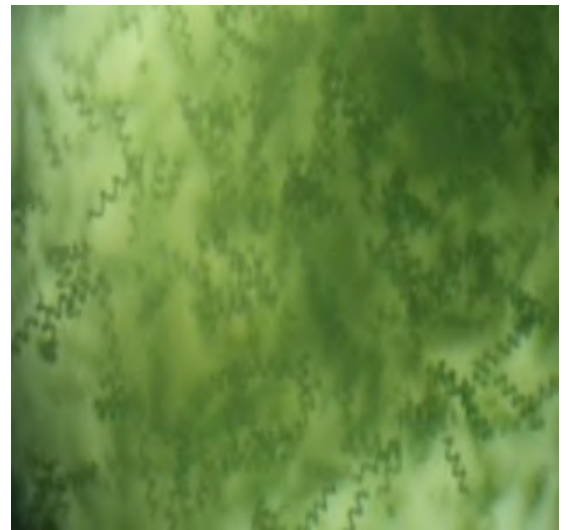


Fig. 2. Growth of *Spirulina platensis* in molasses (supernatant)-supplemented

Growth Analysis –

Effect of Molasses (Supernatant) on the Growth of *Spirulina platensis* -

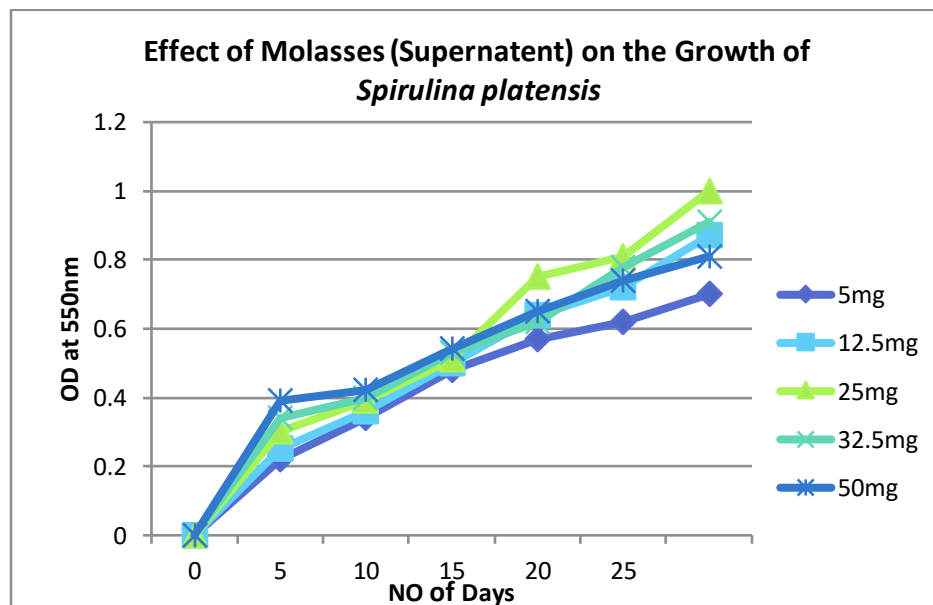


Fig.3- Growth of *Spirulina platensis* in molasses (supernatant)

The maximum OD value was observed in the 25 mg treatment of molasses (supernatant), with an OD of 1.0 on the 25th day. The minimum OD value was recorded in the 5 mg treatment, with an OD of 0.70 on the 25th day.



Table 2. Estimation of Chlorophyll- a in *S. platensis* growth on molasses (Supernatant) –

S. No.	Concentrations (mg)	OD value (663nm) 22thday	Chlorophyll-a (µg/ml) 22th day
1	5mg	0.20	2.78
2	12.5mg	0.19	2.641
3	25mg	0.18	2.502
4	32.5mg	0.38	5.282
5	50mg	0.10	1.56

The maximum chlorophyll-a content of 5.282 µg/mL was observed on the 22nd day at a concentration of 32.5 mg.

Effect of Molasses (Diluted) on the Growth of *Spirulina platensis* -

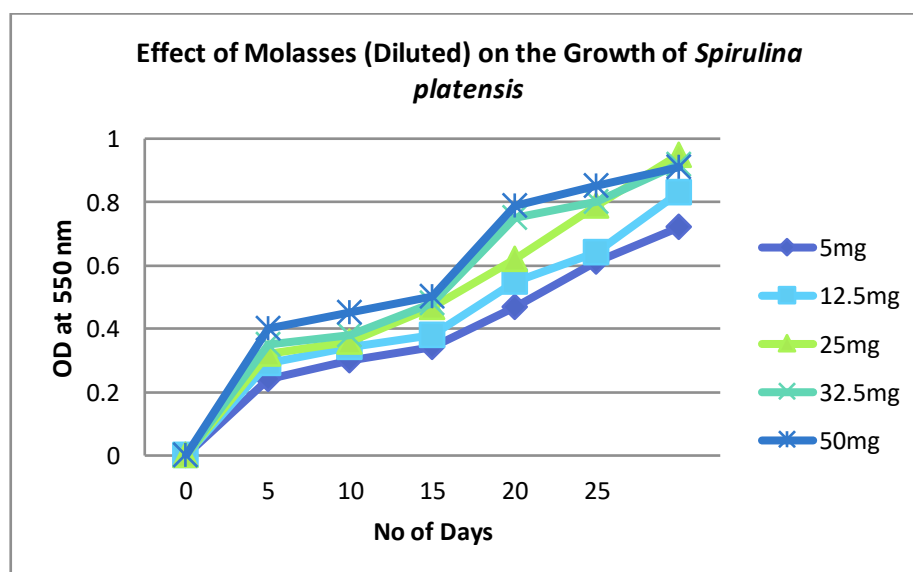


Fig.4 Growth of *S. platensis* in molasses (Diluted)

The results for molasses (diluted) are presented in Table 4. Growth was observed from the 1st day to the 25th day. The maximum OD value of 0.95 on the 25th day was recorded in the 25 mg treatment of diluted molasses, while the minimum OD value of 0.72 on the 25th day was observed in the 5 mg diluted molasses treatment.



Table 3. Estimation of Chlorophyll-a in *S. platensis* growth on molasses (diluted)-

S. No.	Concentration (mg)	OD value (665nm) On day.	Chlorophyll-a (µg/ml) on 22th day
1	5	0.16	2.363
2	12.5	0.19	2.641
3	25	0.20	2.78
4	32.5	0.13	1.807
5	50	0.11	1.529

The maximum chlorophyll-a content of 2.78 µg/mL was observed on the 22nd day at a concentration of 25 mg.

4. Discussion

The cultivation of *S. platensis* was carried out using sugarcane molasses as an organic carbon source in CFTRI medium. Different culture media, such as Zarrouk, JPJM, and BG11, are commonly used for *S. platensis* growth, but these media are costly. For large-scale production, reducing the production cost is essential, and the use of low-cost media is an effective strategy. Sugarcane molasses is an inexpensive industrial by-product that is easily available. In this study, the highest growth of *S. platensis* was observed in CFTRI medium supplemented with sugarcane molasses.

Different concentrations of molasses (diluted and supernatant) were tested in CFTRI medium. The supernatant was obtained by centrifuging molasses at 5000 rpm for 10 minutes, while in another treatment; molasses was directly diluted with an equal volume of water.

The *S. platensis* culture was grown in the laboratory at 30–35°C with sunlight exposure (in shaded conditions). The culture was maintained for 25 days, and growth was measured spectrophotometrically at 550 nm. Growth performance was evaluated in terms of microscopy, optical density (OD), chlorophyll-a content, and pH. Microscopic examination in the initial days showed green-colored, long, loosely coiled filaments. Similar observations were made for cultures



grown in both types of organic substrates. Illumination using sunlight and aeration for mixing were provided throughout the cultivation. The growth of *S. platensis* depends on temperature and light.

Maximum OD was observed in the 25 mg treatment of molasses. Optical density generally increased with increasing molasses concentration from 5 mg to 50 mg. Initially, the culture showed an OD of approximately 0.2, which increased gradually during incubation. The temperature was monitored using a thermometer and maintained at the optimal range of 30–35°C. The pH was measured using a pH meter, and *S. platensis* grew well in the alkaline range of 8.5–11.

Chlorophyll-a content was also estimated. Five milliliters of algal suspension were centrifuged at 3500 rpm for 5 minutes. The pellet was homogenized with 5 mL of 80% methanol and incubated in a water bath at 70°C for 2 minutes. It was then centrifuged again at 3500 rpm for 5 minutes. The clear supernatant was measured at 665 nm using a spectrophotometer, with methanol as a blank. Chlorophyll-a content was calculated using the formula:

$$\text{Chlorophyll-a content } (\mu\text{g/mL}) = \text{OD at 665 nm} \times 13.9$$

(Maximum chlorophyll-a content was observed on the 22nd day.)

In this study, the addition of organic molasses as a substrate resulted in enhanced growth of *S. platensis*. Organically grown Spirulina has a higher market value than conventionally grown Spirulina due to increased demand for organic products.

5. Conclusion

The carbon source is a critical factor for the high growth of *Spirulina platensis*. Sugarcane molasses is an inexpensive and easily available carbon source. Appropriate concentrations of molasses combined with sufficient light exposure significantly enhanced the growth of Spirulina. Growth was evaluated in terms of microscopy, optical density, and chlorophyll-a content. Adequate sunlight and aeration were provided for mixing the culture. The growth of *S. platensis* depends on sunlight and temperature. In this study, CFTRI medium supplemented with organic molasses resulted in improved growth. Currently, organically grown Spirulina has higher market demand and value compared to conventionally grown cultures.

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