Research Article

The Growth Performance of *Spirulina platensis* on Media Supplemented with Digested Poultry Droppings Slurry of Biogas Plant

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Submission Date: 14-06-2024; Revision Date: 13-08-2024; Accepted Date: 24-08-2024.

ABSTRACT

Background: The growth performance of *Spirulina platensis*, a type of blue-green algae known for its high nutritional value, on media supplemented with poultry droppings slurry from a biogas plant can be an intriguing study. Poultry droppings slurry, a by-product of biogas production, is rich in organic matter and nutrients, potentially serving as a cost-effective alternative to conventional nutrient sources for *Spirulina* cultivation. Using poultry-dropping slurry as a nutrient source for large-scale Spirulina cultivation potentially offer sustainable solutions for both waste management and the production of valuable biomass for various applications, including food, feed, and biofuel. **Objectives:** The present study was undertaken to utilize poultry droppings biodigested slurry from biogas plant as an organic additive for *S. platensis* cultivation. *S. platensis* was mass multiplied with the traditional Zarrouk media as a control and supplemented organically or additionally by the available poultry dropping spent of methane plant and Zarrouk media. **Materials and Methods:** Two treatments were carried out for this study. The control reactor consisted of Zarrouk's medium with inoculated *S. platensis* alone, and the experimental reactor contained poultry droppings supplemented with 9 g of Zarrouk's media and inoculated with *S. platensis* in duplications for about 35 days. The growth of *S. platensis* was measured using apparent turbidity and crude phycocyanin. Also, Direct Microscopic Count (DMC), crude protein, dry weight, biomass concentration, Electrical Conductivity (EC), pH, chlorophyll-a, and carbohydrate were additional metrics. **Results:** The findings showed that, compared to the control, the supplement treatment of poultry droppings bio slurry with Zarrouk's media had greater dry weights (2.01 g/L), growth rates (3.44 at 750 nm), protein levels (321 µg/mg), and phycocyanin (2.89 at 680 nm). **Conclusion:** These findings highlighted the potential of integrating waste-to-resource approaches in microalgae cultivation, contributing to sustainable solutions for both waste management and the production of valuable biomass for various applications. Further research is warranted to optimize cultivation parameters and scale up production for commercial applications.

Keywords: *Spirulina platensis*, Spent bio digested slurry, Poultry droppings, Zarrouk's media, Protein content, Phycocyanin, Biomass concentration, Electrical conductivity.

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www.ajbls.com

DOI: 10.5530/ajbls.2024.13.37

INTRODUCTION

S. platensis, a filamentous cyanobacterium, has garnered significant attention in recent years because of its excellent nutrient potential and valuable applications in various sectors, including food, feed, pharmaceuticals, and biofuels.[1,2] As the global demand for sustainable protein sources continues to rise, there is growing interest in exploring alternative nutrient sources for the

cultivation of Spirulina, aiming to reduce production costs and environmental impacts associated with conventional cultivation methods.[3]

Poultry droppings, a by-product of the poultry industry, represent a rich source of organic matter and nutrients. The utilization of poultry droppings as a nutrient source for microalgae cultivation has been investigated in recent years, showing promising results in terms of biomass production and nutrient content.[4,5] Moreover, poultry droppings can be further processed into slurry and utilized as a substrate in biogas plants for methane production, providing an additional avenue for waste valorisation and energy generation.[6]

Because of its great nutritional value, spirulina can be found in environments with freshwater, brackish water, and saltwater. It is known to grow widely in alkaline waters found in salty lakes and to thrive in warm, tropical climates.[7,8] Depending on the source, spirulina often has a high protein content of between 55 and 70% dry weight.[9] The two most important conditions for large-scale outdoor Spirulina cultures are high pH and warmth. Furthermore, the pH levels of 9.5 to 9.8 are rather high for spirulina, as they successfully prevent the majority of algae in the culture from becoming contaminated. To maintain the high pH and avoid swings in this respect, the culture medium needs to have high concentrations of sodium bicarbonate at all times. The micronutrients were given to the culture at half power after the spirulina were grown in drinking water using Zarrouk medium.[10]

Growth of *Spirulina* was found successful with composted biogas slurry has developed a low cost medium for Spirulina production in Thailand, using material such as seed kernel extracts of legumes.[8] Sludge leftover from the digestion process used to produce biogas is converted into biodigested slurry and it replaces chemical fertilizers to increase agricultural output; farmers refer to it as "block gold".[11] In comparison to composted manure and FYM, bio slurry includes more nutrients and micronutrients that are readily available to plants.[12,13]

In this study, we aim to investigate the growth performance of *S. platensis* on media supplemented with poultry-dropping slurry obtained from a biogas plant. Specifically, we will assess biomass yield, growth kinetics, crude protein, dry weight, biomass concentration, Electrical conductivity and biochemical composition cultivated under two different media (Zarrouk media alone and Zarrouk media supplemented with poultry slurry-30g concentration). Understanding the feasibility and efficacy of utilizing poultry droppings slurry as a nutrient source for Spirulina cultivation can contribute to the development of sustainable and cost-effective cultivation practices, with implications for both waste management and the production of valuable biomass.

MATERIALS AND METHODS

Cultivation Under Laboratory Condition

The freshwater cyanobacteria were collected as pure culture from V.O. Chidambaram College, Thoothukudi, Tamil Nadu, in a sterile plastic container. The cultivation was done in a 1000 mL conical flask of 100 mL sterile distilled water using Zarrouk's media, and pH was set at 9.0 initially using NaOH or HCl. The preparation of Zarrouk's medium according to the standard protocol[14] (Zarrouk, 1966). 10% of the mother culture of *S. platensis* was injected into the conical flask holding the medium. The medium was continuously aerated, and the conical flask was kept in a laboratory setting with a light source. After cultivating in a lab environment, it was fed by biodigested poultry droppings slurry from a biogas plant. Two treatments were carried out for this study period. The control reactor consisted of Zarrouk's medium alone, and the experimental reactor contained poultry droppings supplemented with 9 g concentrations in duplicates for about 35 days.

Collection and characterization of poultry slurry

Poultry droppings slurry was collected from a biogas plant in Snkarankoil, Tamil Nadu, and the nutrient composition, pH, and potential contaminants of the poultry slurry were characterized by following the methodology of Angelidaki and Ahring (1993).[15] (Tables 1 and 2).

Table 1: Characterization of poultry slurry.

Parameters analysed (%)	Poultry droppings
Total Solids (TS)	16.541±2.876
Total Volatile Solids (TVS)	80.260±2.651
Carbon	25.84±2.647
Nitrogen	5.38±2.765
Phosphorous	1.289±1.762
Potassium	0.256±0.982
Organic matter	07%
C:N ratio	25:1
pH	6.5

Preparation of Poultry Slurry Supplement

Using distilled water, 30 g of poultry slurry were diluted to 100 mL. The diluted slurry was then filtered to get rid of any particles or contaminants. From this, 30 mL was used as a working solution which may contain 0.3 g/mL

Table 2: Potential contaminants of poultry droppings.

Name of the organisms	Media employed	Microbial load (Cfu/mL)
E. coli	EMB agar	$2.9X10^3$
Salmonella sp	SS agar	$1.5X10^3$
Pseudomonas sp	Cetrimide agar	$2.6X10^4$

The concentration of the slurry in the initial dilution was,

$$\text{Concentration initial} = \frac{\text{Mass of solution}}{\text{Volume of solution}}$$

$$= \frac{30\ \text{g}}{100\ \text{ml}}$$

$$\text{Concentration}_{\text{initial}} = 0.3\ \text{g/mL}$$

Concentration in 30 mL working solution

When 30 mL of working solution used, the concentration remains the same. So in a 30 mL portion of the working solution contained 0.3 g/mL.

To measure the total mass of slurry in the 30 mL of working solution, it would be:

$$\text{Mass}_{\text{working}} = \text{Concentration initial} \times \text{Volume}_{\text{working}}$$

$$= 0.3\ \text{g/mL} \times 30\ \text{mL}$$

$$\text{Mass working} = 9\ \text{g}$$

Therefore, the concentration of the bio digested slurry in the 30 mL portion used for working remains 0.3 g/mL, and the total mass of slurry in that portion is 9 g.

Supplement Media Preparation

Zarrouk's medium was supplemented with 30 mL (9 g) of the prepared poultry slurry filtrate and mixed thoroughly to ensure uniform distribution of nutrients. *S. platensis* culture was inoculated into the prepared Zarrouk media supplemented with poultry slurry and in control at an initial cell density of OD 0.1-0.2 at 560 nm.

Culture maintenance

The cultures were incubated in a growth chamber set to 25-30ºC under continuous illumination. Aeration was provided using sterile CO_2 supplementation. The pH was maintained between 8.0-9.0 using $NaHCO_3$ or Na_2CO_3 buffer.

Monitoring and sampling

Spirulina growth was monitored by measuring OD at 560 nm. The samples were analyzed at regular intervals for biomass analysis and characterization.

Harvesting

Spirulina biomass was harvested during the exponential growth phase, typically after every 7 days of cultivation for a period of 35 days. Harvested cells were centrifuged at 5000 rpm for 10-15 min. Harvested cells were washed with sterile water to remove residual medium components.

Biochemical analysis

Spirulina biomass was analyzed for biochemical composition using standard methods like Bradford assay for protein,[16] Anthrone assay for carbohydrates,[17] Direct Microscopic Method (DMC) for Population Growth,[18] Dry Weight[18] Electrical conductivity,[19] Chlorophyll-a estimation[20] and Extraction of Crude Phycocyanin and estimation using spectrophotometric analysis.[21,22]

pH measurement

Measurement of pH was carried using a pH meter. *S. platensis* grows at pH range of 8.5 to 11 as it is maintained at alkaline point.

Temperature

The measurement of temperature was carried out using the thermometer. The *S. platensis* grows in optimum temperature at 30-35ºC.

Analytical Statistics

Using Microsoft Excel, the average (mean) and standard deviation of all the experimental data were determined and presented as mean±SD in the table. ANOVA can be used to compare the mean growth performance of Spirulina across control and supplemented media (Table 5). ANOVA can help determine if there are significant differences in growth among the groups. The experimental data were analysed using Student's t-test analysis as well (Table 6).

RESULTS

Before the utilization of poultry slurry as a supplement nutrient along with Zarrouk's media, the parameters were analysed using standard methodology, and the analysed results were given below (Table 1).

The protein content of *S. platensis* was high in experimental bioreactor (321 µg\mg) and was comparatively low in control bioreactor (119 µg\mg). The result was shown in Figure 1.

The results of the carbohydrate content of *S. platensis* indicated higher growth results, as shown in Figure 2. In the treatment, the highest growth was seen on the

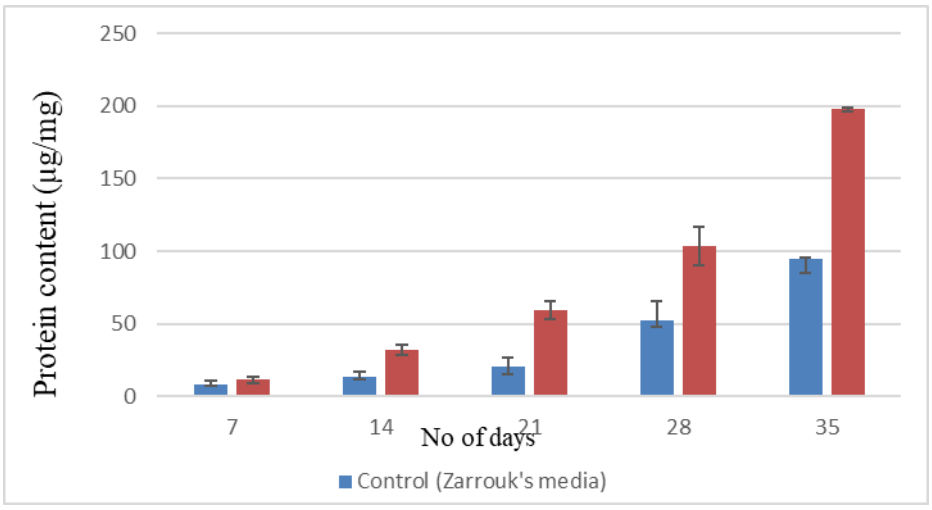

Figure 1: Protein content (µg/mg) in control and experimental reactor (mean±SD).

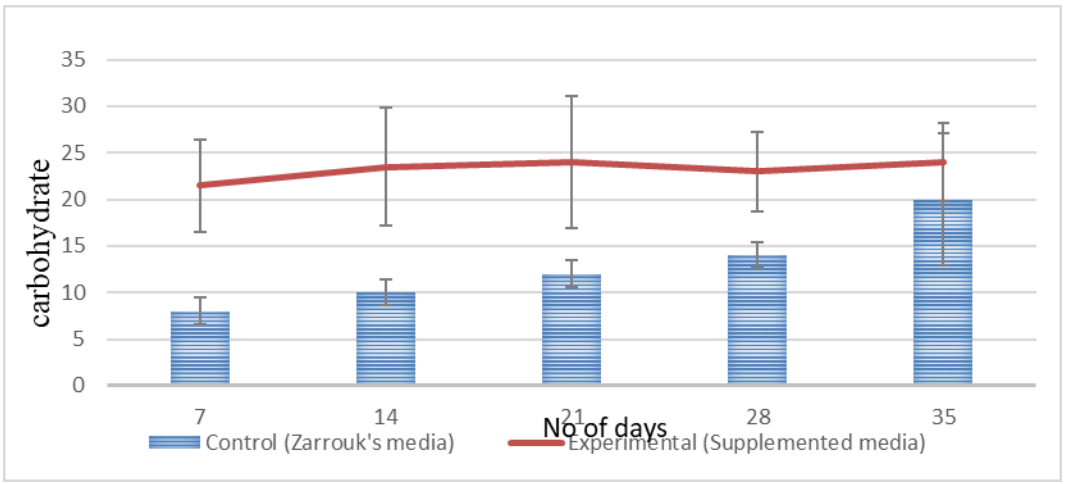

Figure 2: Carbohydrate content in both control and experimental reactor (mean±SD).

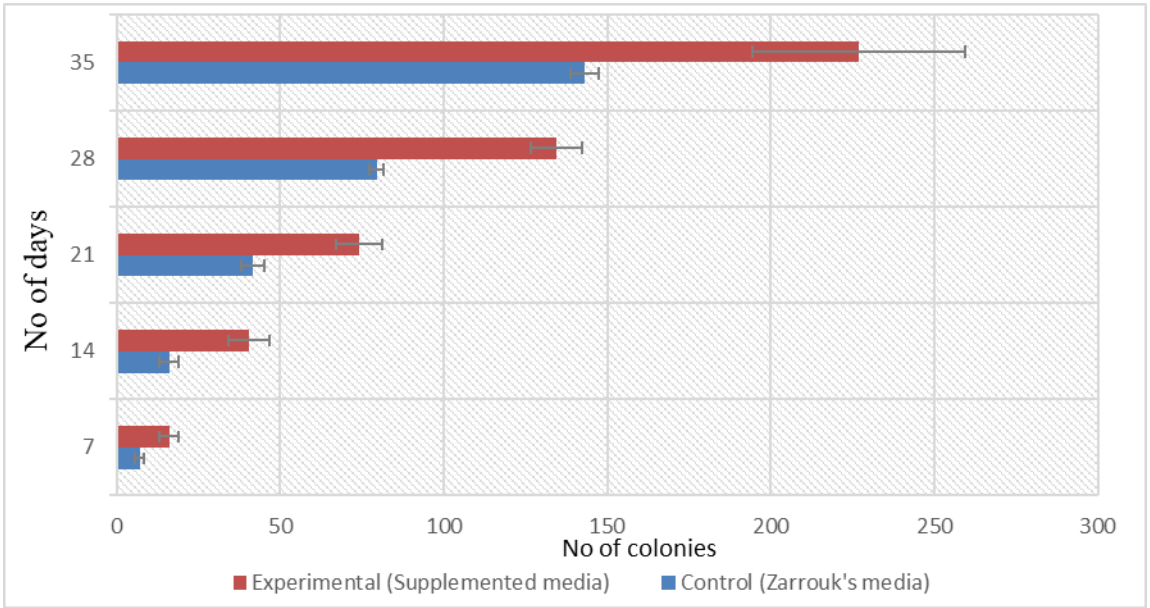

Figure 3: Direct Microscopic Count (DMC) in both control and experimental reactor (mean±SD).

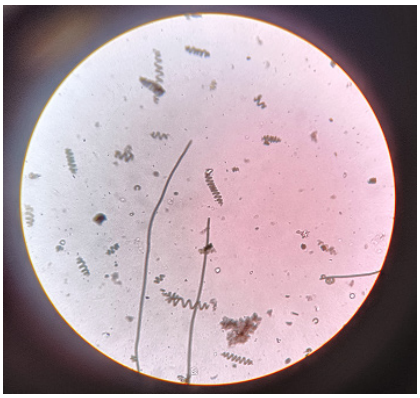

Plate 1: *S. platensis* in control (Phase contrast microscopic view)

Plate 2: *S. platensis* in supplemented media (Phase contrast microscopic view)

21st day, which contained 20 µg of carbohydrate at 25 mg of concentration.

Microscopic analysis of *S. platensis* culture in initial days was green colored and long, loosely to tightly coil. The direct microscopic count of *S. platensis* in the experimental context revealed a continuous growth in its number from 0 to 35 days was highest 35th day analysis and beyond the limit of 200, it was brought under TNTC after 35 days of inoculation followed by control (Plates 1 and 2).

Regarding population growth, the optical density observation revealed that the Biomass Concentration (BMC) and apparent turbidity (3.65) were higher in the experimental bioreactor than the control.

The results of dry weight were shown in Figure 2, where the dry biomass of *S. platensis* was highest in the bioreactor containing 30g bioslurry supplemented with Zarrouk's medium (3.28 mg/L) and lowest in the control (1.63 mg/L).

The electrical conductivity of the supplemented experimental set up results was shown in Figure 6. In the treatment analyzed, the highest growth of EC was measured on the 35th day, about 1.5×10^{4} mho^{-1}.

Table 3 showed the results of chlorophyll a and phycocyanin at 663 and 680 nm. Chlorophyll-a result insisted on maximum chlorophyll-a growth in the 35th day, containing 9.605 µg/mL in the experimental reactor where poultry droppings spent slurry as a supplement and 3.140 µg/mL in the control reactor. The spectronic phycocyanin results showed maximum phycocyanin accumulation in the 35th day, both in the experimental and control bioreactors.

The temperature and pH results were presented in Table 4. Maximum temperature was observed on the very first day, 14th day, and 35th day. In the experimental reactor, the pH rose from its initial day to its final day. The low pH was observed on the 1st day (9.1) and reached its maximum on the 35th day (10.9). Likewise, in control, the maximum pH occurred in the 35th day of observation (10.1), respectively.

Table 4: Temperature and pH of both control and experimental reactor.

No of Days	Substrates			
	Zarrouk's Medium (Control)		Bio slurry Supplemented	
	Temperature (°C)	pH	Temperature (°C)	pH
0 day	38	9.1	38	9.1
7th day	37	9.4	37	9.6
14th day	38	9.6	38	9.9
21st day	37	9.5	37	10.3
28th day	38	9.9	37	10.7
35thday	37	10.1	38	10.9

Table 3: Concentration of Chlorophyll and Phycocyanin in control and experimental settings.

No of days	Chlorophyll-a (µg/mL) OD value (663 nm)-control	Chlorophyll-a (µg/mL) OD value (663 nm)-Experimental reactor	Phycocyanin (µg/mL) OD value(680nm) control	Phycocyanin (µg/mL) OD value (680 nm)-Experimental reactor
7	0.209±0.045	1.046±0.092	0.12±0.098	0.27±0.068
14	1.065±0.062	2.093±0.169	0.19±0.078	0.51±0.091
21	2.921±0.164	3.140±0.093	0.71±0.085	1.06±0.074
28	3.140±0.129	5.213±0.167	1.21±0.083	1.89±0.047
35	3.140±1.043	9.605±1.029	1.41±0.028	2.78±0.048

Values in mean±SD.

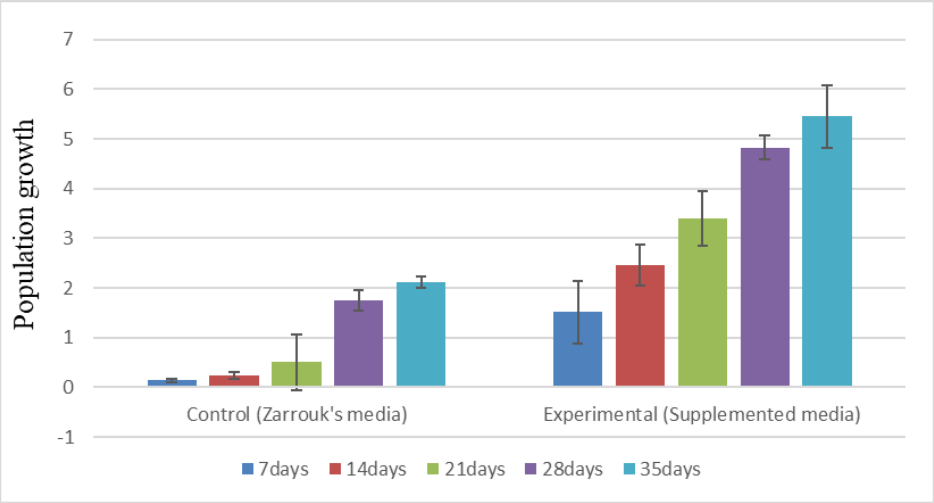

Figure 4: Population growth in control and experimental reactor (mean±SD).

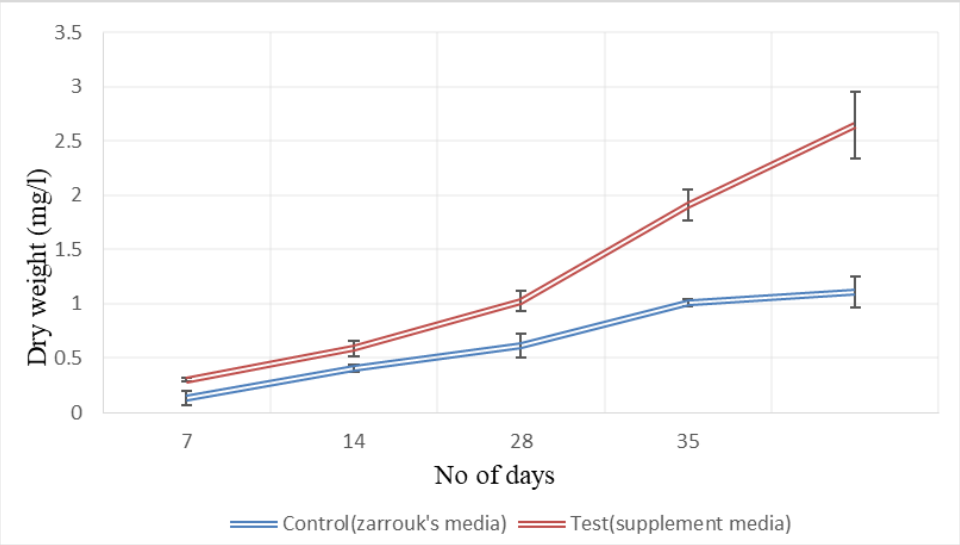

Figure 5: Dry weight (mg/L) of *S. platensis* in control and test media (mean±SD).

Table 5: ANOVA to compare the BMC between the experimental bioreactor and the control bioreactor.

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	5	5				10
∑X	5	15.9				20.9
Mean	1	3.18				2.09
∑X2	7.6446	61.6152				69.2598
Std.Dev.	0.8131	1.6623				1.6858

Result Details				
Source	SS	df	MS	
Between-treatments	11.881	1	11.881	F = 6.93892
Within-treatments	13.6978	8	1.7122	
Total	25.5788	9		

The *f*-ratio value is 6.93892. The *p*-value is. 029983. The result is significant at *p*<.05.

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Ho: Dry weight, protein content, and population growth in the experiment and control groups do not differ significantly.

DISCUSSION

Spirulina, a type of filamentous cyanobacterium, can be enhanced in its growth by adding several types of biological waste. Studies have demonstrated that Spirulina may be efficiently cultivated utilizing aquaculture wastewater, which contains abundant nutrients such as nitrogen and phosphorus that are crucial for its development.[23] This methodology not only facilitates the production of useful biomass but also aids in the treatment of wastewater, thereby making it a sustainable method. In this study, the bio-digested slurry was mixed as supplemented nutrient for spirulina growth.

Table 6: Students '*t*' test analysis of dry weight, protein content and population growth of biogas spent slurry supplemented media for *S. platensis* cultivation.

No of days	*Dry weight		*Protein content		*Population growth	
	Control	Experimental	Control	Experimental	Control	Experimental
7th day	0.09	0.78	09	26	08	46
14th day	0.43	1.03	15	58	18	92
21st day	0.69	1.98	24	96	44	195
28th day	1.03	2.09	56	175	81	380
35th day	1.21	3.94	101	343	146	704
Total	3.45	9.82	205	698	297	1417
Mean	0.6900	1.9640	41.00	139.60	59.40	283.40
Varience	0.16272	1.238984	1162.8	12817.84	2511.84	57408.64
Standard deviation	0.4510	1.2445	38.12	126.58	56.03	267.88
T	2.1521		1.6678		1.8302	

t's table value at the 5% significance level; Degrees of freedom=8; t=2.306

* The experimental bioreactor and control bioreactor differ significantly ($p<0.05$). As a result, the quantity of cow dung added to Zarrouk's media has an impact on the population growth, protein content, and dry weight.

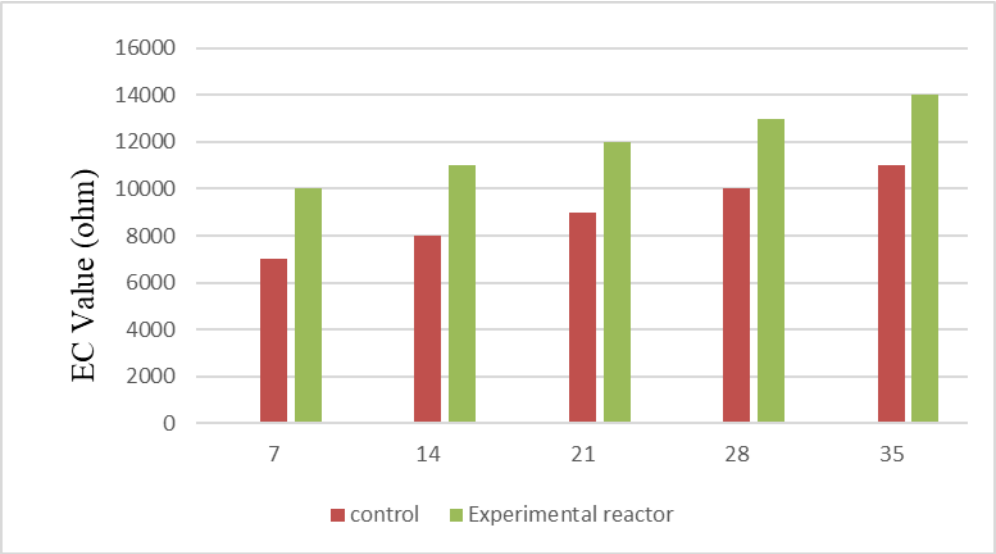

Figure 6: Electrical conductivity analysis of Spirulina platensis in both control and experimental reactor.

For instance, *Spirulina* sp. LEB 18 cultures have been used to treat aquaculture wastewater by supplementing it with different percentages of Zarrouk's synthetic culture medium. The study found that supplementing with 25% of the Zarrouk medium resulted in the highest concentrations of protein, phycocyanin, polyunsaturated fatty acids, and γ-linolenic acid in the biomass. Moreover, this combination also achieved the highest removal rates of sulfate, phosphate, bromine, and COD from the wastewater.[23] In the current study also, showed the high protein, carbohydrate, BMC, and other parameters than control Zarrouk's media.

Additionally, Spirulina has been cultivated using other types of wastewaters such as fishpond, industrial, and Mari culture waters. It has shown adaptability and a high specific growth rate, especially when grown in fishpond wastewater.[24] Another study indicated that agricultural and vegetable wastes could be used as an alternative medium for Spirulina cultivation, resulting in better growth rates and higher pigment production compared to control mediums.[25] This is coincided with the present study also.

Aktar *et al*., (1996)[26] utilized Rice Husk Ash (RHA) and $NaHCO_3$ as carbon sources in Spirulina production. They discovered that adding RHA every two days promoted Spirulina growth. In this study, the inclusion of bio slurry as supplemental feed increased Spirulina growth, with the highest growth achieved after 30g bio slurry supplementation. Roof *et al* (2006)[27] conducted similar research and developed a novel media for

mass production of Spirulina. Minkov *et al.*, (2003)[28] investigated the growth pattern of *S. platensis* in conventional and modified media based on sea water chemicals, and his comparison tests with other nutrient sources gave good findings.

S. platensis phycocyanin crude extract was spectrophotometrically measured. The concentration of crude phycocyanin was high in the supplemental reactor (2.32) and low in the control reactor (0.41). Zhang (2004)[29] performed similar separation and purification work at 680nm wavelength. Although microalgal technology is a very attractive field, the industry still is in infancy. In addition to microalgae, *S. platensis* is a very promising cyanobacterium that is becoming more and more in demand globally for its valuable chemical components.[30,31]

In the present study, the treatment involving the supplementation of biodigested poultry droppings from biogas plant recorded considerable efficiency when compared to Zarrouk's media in terms of biomass and in all parameters analysed comparatively with Zarrouk's media alone as control. They recorded higher profits because the higher market price of organically grown *S. platensis* is much higher than inorganically. Organically grown crops and products have more demand in the present-day market. The consumers were also benefitted by organic foods.

These results imply that using spirulina for waste treatment can be an economical and environmentally beneficial choice, while also producing high-value compounds that can be used for various applications, including as a food supplement and for biodiesel production.

CONCLUSION

Finally, the research showed that *S. platensis* can thrive on media supplemented with slurry made from chicken droppings that was collected from a biogas facility. According to our research, it is possible and yields encouraging outcomes to use poultry dropping slurry for spirulina cultivation. In comparison to control media, we saw favourable growth performance metrics, such as higher biomass yield and growth rate. Furthermore, the study of the biochemical composition demonstrated that the Spirulina biomass grown on slurry media containing poultry droppings maintained its nutritional quality, exhibiting similar amounts of protein, lipids, and carbs to those grown on traditional nutrient sources. Moreover, no notable pollutants were found in the Spirulina biomass, indicating that poultry dropping slurry is a suitable and secure source of nutrients for Spirulina production. These results demonstrate how incorporating waste-to-resource methods into microalgae farming can lead to sustainable waste management solutions as well as the production of valuable biomass for a range of uses. To maximize culture parameters and scale up output for commercial uses, more study is necessary.

FUTURE RECOMMENDATIONS

Future recommendations for the growth of spirulina supplemented with poultry droppings must prioritize addressing potential obstacles, guaranteeing sustainability, and optimizing growth conditions. It is possible to perform in-depth examinations of the nutritional makeup of chicken droppings in order to comprehend their unpredictability and possible influence on the growth of spirulina. To make sure spirulina gets enough of vital nutrients including nitrogen, phosphorus, potassium, and trace minerals, techniques for nutrient enrichment or supplementation can be investigated. Research investigations such as co-cultivation or sequential cultivation with other microbes to more effectively and synergistically exploit the nutrients found in poultry droppings. Pre-treatment techniques, such as microbial sterilization, pH correction, or composting, could be created to reduce any pollutants and pathogens found in chicken droppings.

Analysation of various pre-treatment techniques that can improve the usefulness of chicken droppings as a source of nutrients for spirulina growing while maintaining the quality and safety of the final product. Examining whether it would be possible to co-locate spirulina farms and chicken farms in order to minimize transportation expenses and improve waste management procedures. It is necessary to carry out thorough environmental impact evaluations in order to determine whether spirulina farming enhanced with chicken droppings is sustainable. From laboratory or pilot-scale trials, scale up spirulina cultivation supplemented with chicken droppings to commercial production levels.

By putting these suggestions into practice, stakeholders can support the development of long-term and financially feasible strategies for using chicken droppings as a source of nutrients for spirulina cultivation, supporting the ideas of the circular economy and addressing the environmental issues related to poultry farming.

ACKNOWLEDGEMENT

My heartfelt gratitude to Shri. A.P.C.V. Chockalingam (Secretary), Principals of both V.O. Chidambaram College and A.P.C. Mahalaxmi College, Tuticorin and Research supervisor Dr. D. Radhika, Head and Associate Professor, PG and Research Department of Zoology, Tuticorin for their support and guidance to carry out this work.

AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

In the present study the poultry droppings slurry from the biogas plant was used as an organic additiuve for S.platensis cultivation with Zarrouk media along with a control (Zarrouk media alone) in dublications for about 35 days. The results revealed that, compared to control the supplemented media had great impact on the growth of *S. platensis*.

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Cite this article: Kumari VJ, Radhika D, Veerabahu C. The Growth Performance of *Spirulina platensis* on Media Supplemented with Digested Poultry Droppings Slurry of Biogas Plant. Asian J Biol Life Sci. 2024;13(2):287-96.