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Alternative Use of Waste Material (Egg Shell) for Creation of Spirulina Alga (*Spirulina platensis*) as an Ecologically Sweet Method

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ABSTRACT
The study was conducted to find out the suitable concentration of egg shell powder for maximum growth of *Spirulina platensis*. In this work, Kosaric medium (KM) were used as control media while three different eggshell powder medium (ESPM) concentrations (25, 50, and 75%) were used to assess *Spirulina platensis* growth and culture performance. For a period of 20 days, each experiment was carried out in triplicates under fluorescent lighting in a situation of light: dark (12 : 12h). Every other day, measurements of the *S. platensis* treatments' cell weight and chlorophyll concentration were made. *S. platensis* was cultivated in KM media with eggshell in place of $NaHCO_3$, and the growth rate varied. The initial cell weight was 1.89mg/ L, reaching a maximum cell weight grown in KM, and 0.036 to 0.245mg/ L in medium contained 75% $NaHCO_3$ and 25% eggshell powder, 0.0042 to 0.0242g/ L in medium contained 50% $NaHCO_3$ and 50% egg shell powder, and 0.0039 to 0.033mg/ L in medium contained 25% $NaHCO_3$ and 75% egg shell powder. At the 16^{th} day of growth, the *S. platensis* had a chlorophyll a content of 0.034mg/ L, which had increased to 1.983mg/ L when grown in KM and 0.415mg/ L in medium made up of 75% $NaHCO_3$ and 25% egg shell powder. When compared to conventional KM, this medium concentration produced results that were satisfactory. This fluctuation in nutrient contents and the amount of egg shell powder likely resulted from variations in the nature of the medium. Therefore, it is inferred that the medium concentration that substituted 25% egg shell powder for $NaHCO_3$ is appropriate and beneficial for the growth of *S. platensis*.

INTRODUCTION

Bangladesh is the world's largest deltaic nation **(Tandra *et al.*, 2019; Mahmuda *et al.*, 2020; Rahman *et al.*, 2021)**. Additionally, as noted by **Baroi *et al.* (2019)**, **Islam *et al.* (2020)**, and **Mahmuda *et al.* (2020)**, the subsector of fisheries and aquaculture is essential in mitigating the adverse effects of protein shortage. Aquaculture is becoming a more significant method of fish production as a result of Bangladesh's diminishing natural fisheries resources and expanding human population **(Mahmud *et al.*, 2021;**

Nasrin *et al*., 2021; Rahman *et al.*, 2021; Noor *et al.*, 2024). The use of chemicals to regulate the health of aquatic animals has become more popular in aquaculture in Bangladesh **(Uddin *et al.*, 2020)**. Fish health benefits greatly from microalgae. In aquaculture, we can use microalgae rather than chemicals **(Uddin *et al.*, 2020)**. Algae were the first flora to seem on this planet. Billions of years ago, they transformed the carbon-dioxide-primarily based environment to an oxygen-rich atmosphere in which other existing paper work may want to evolve. Biotechnological techniques based on cyanobacteria were receiving growing interest owing to their capability to provide a numerous variety of chemical compounds and biologically energetic compounds, which include nutrients, carotenoid pigments, proteins, lipids and polysaccharides. Multicellular filamentous blue-green algae, known as spirulina, are becoming more and more popular as a protein and vitamin supplement to aquaculture diets and have gained great popularity in the fitness food business. It possesses extremely high macro- and micronutrient contents, can be harvested and processed with ease, and grows in water **(Jana *et al.*, 2014)**. The availability of nutrients, temperature, and light are the three most crucial factors in the large-scale generation of spirulina biomass. Within the range of 35-38°C, *Spirulina platensis* grows at its most effective temperature. Additionally, spirulina needs an extraordinarily high pH, which effectively prevents the growth of other algae in a traditional medium. To maintain the high pH and prevent fluctuation, enormous amounts of sodium bicarbonate must regularly be added to the culturing medium. *Spirulina platensis* is a multicellular, filamentous cyanobacterium that is motile, glides down its axis, lacks heterocysts, and is composed of blue-inexperienced threads of cylindrical cells (1 to 12m diameter) in unbranched helicoidal trichomes. Carbon is the primary nutrient required by *Spirulina* in alkaline lakes. This organism becomes the dominant species due to the presence of high concentrations of sodium carbonate. Studies have shown that the cost of nutrients, particularly the carbon supply, is the second most significant expense in the production of Spirulina biomass, following labor-intensive processes. Even while the microalgae used to produce biofuels require less land than cereal crops do, their cultivation, harvesting, and processing do not require less money **(Rahman *et al.*, 2022)**. Therefore, a less expensive method of generating microalgae is required. According to **Hossain *et al.* (2021),** microalgae transform animal wastes into food ingredients in addition to acting on agrochemical wastes. Because microalgae play a crucial role in both oxygen and carbon dioxide stability in the water, this *S. platensis* is extremely healthy beneficial for fish and shrimp development **(Rahman *et al.*, 2021)**. It now acts not only on agro-business but also on animal droppings, converting them into food substances. For this reason, spirulina contains around 60% of fully digestible protein, about 6-10% of lipids, micro-and macro-vitamins, and many other hints. It contains every essential amino acid, more carotenoids than any other complete meal, and an exceptional amount of minerals like iron, manganese, chromium, and vitamins A, K, B_1, B_2, and B_{12} **(Becker, 2007);** It is also abundant in vitamins, minerals, trace elements, enzymes, and

chlorophyll. It serves a variety of functions in the food industry, including thickener, binder, disrupting agent, stabilizer, texture modifier, gelling and bulking agent. It is useful in the preservation of canned and frozen foods, in the creation of syrups, essences, and drinks, in confectionery and bakery, snacks, bakery, and mushroom permits **(Burrell *et al.*, 2003)**. Consequently, it is absolutely essential to maintain spirulina tradition in order to speed up the development of the aquaculture business. Our experiment's ultimate goal was to develop low-cost medium for sprulina manufacturing on a large scale. Therefore, egg shell powder can be used to make spirulina. The waste cloth made from these eggshells is used outside, where it decomposes and poses environmental dangers. However, it is generally made of calcium carbonate, a common calcium type. Different minerals and proteins make up the relaxation. Eggshell powder made from hen eggs has been used as a natural calcium supplement for many years. Eggshells contain about 40% calcium, with 381-401mg of calcium per gram. To get the 1,000mg of calcium per day that people need, half an egg shell might be sufficient. Egg shells also contain trace levels of strontium, fluoride, magnesium, and selenium in addition to calcium and protein. These elements, like calcium, might be important for bone health. Both benefits and drawbacks apply to egg shells. *Salmonella enteritidis* and other germs may be present in egg shells. Eggs should be boiled before eating the shell to reduce the chance of food poisoning. Natural calcium supplements may include disproportionately high levels of hazardous metals like lead, aluminum, cadmium, and mercury since egg shells naturally contain calcium. The purpose of this study was to determine the ideal egg shell powder concentration to replace sodium bicarbonate for maximum *Spirulina platensis* development.

MATERIALS AND METHODS

Field of study

The investigation was carried out in the Fish Nutrition Laboratory, Department of Aquaculture, and Water Quality Laboratory, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh-2202.

Assembling egg shell powder

For *S. platensis* cultivation, egg shell powder was chosen as the ingredient to replace $NaHCO_3$. Raw egg shells were gathered from the Abdul Jabbar Mor different food hotels at BAU in Mymensingh. These egg shells were cleaned with regular tap water and were then dried outside. Egg shells were subsequently processed into a fine powder using a grinder machine, sealed in a polythene bag, and stored in the lab for later use.

Spirulina (*S. platensis*) collection

Hygienic stock of *S. platensis* were taken from the stock of the Aquaculture department's laboratory at BAU in Mymensingh.

Upkeep of a pure spirulina stock culture

A pure stock culture of *S. platensis* was maintained in a laboratory environment using Kosaric Medium (KM). In accordance with the recommendations of **Bold and Wynne (1978)**, **Vymazal (1995)** and **Phang and Chu (1999)**, the development of *S. platensis* was monitored every other day and its purity was assessed under a microscope.

Preparation of Kosaric Medium (KM) with egg shell powder media

To prepare 25, 50, and 75% eggshell powder media, 2.25g, 4.50g, and 6.75g of eggshell powder were mixed with 9.0g/ L sodium bicarbonate and 0.20mL/ L commercially available micronutrient, respectively. Following that, 2.0L for each replication flasks containing three replications of each of these three distinct medium concentrations were used (Table 1). In parallel, a control culture of *S. platensis* was produced using Kosaric medium (KM) (Table 2). The mixture in the flasks was thoroughly well mixed before being autoclaved (Express Equipment, Dixon's Surgical Instrument Ltd.) at 120°C for 15 minutes with moist heat. Before growing microalgae, the medium was stored in a freezer for 24 hours after autoclaving to ensure they were contamination-free.

Table 1. Design of an experimental *Spirulina platensis* cultivation utilizing media with three different egg shell powder concentrations

Various media	Treatments	Replications	Media egg shell powder content (%)	Days that make up the culture
Egg shell powder media	T_1	3	25	20
	T_2	3	50	
	T_3	3	75	
Kosaric medium	T_4	3	-	

Table 2. Kosaric medium for *Spirulina platensis* cultivation (modified from Zarrouk, 1996) composition

SL. No.	Chemicals/ Compounds	g/L of concentration in stock solution
1.	$NaHCO_3$	9.0
2.	K_2HPO_4	0.250
3.	$NaNO_3$	1.250
4.	K_2SO_4	0.50
5.	NaCl	0.50
6.	$MgSO4.7H_2O$	0.10
7.	$CaCl_2$	0.02
8.	$FeSO_4.2H_2O$	0.005
9.	A_5 micronutrient solution	0.5mL/L
	a) A_5 micronutrient remedy	g/L
	i) H_3BO_4	2.86
	ii) $MnCl2.4H_2O$	1.81
	iii) $ZnSO_4.7H_2O$	0.22
	iv) $CuSO_4.5H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.6H_2O$	0.01

Spirulina (*S. platensis*) culture in KM and different concentrations egg shell powder media

S. platensis was grown in a 1.0L volumetric flask using four treatments at Kosaric medium (KM) as a control and three different concentrations (T_1, T_2, T_3), each with three replications. Each culture flask was injected with 20mL of spirulina to create a 10% spirulina suspension culture (optical density at 620nm = 0.20) (Habib, 1998). All of the flasks were kept in the animal nutrition laboratory under fluorescent lighting (TFC, FL-40 SD/38 daylight, Taiwan) on a light: dark (12 : 12h) schedule. Electric aerators (Daivo pumps) were used to continuously aeration in these culture flasks. Every alternate day, four subsamples from each flask were taken to measure the spirulina's dry cell weight, chlorophyll a content, and culture media characteristics.

Spirulina cell weight estimation

According to the method of **Clesceri *et al*. (1989)**, a sartorius filter paper with 0.45µm mesh size and 47mm diameter was used to filter each sample that contained 20mL of spirulina suspension. Prior to filtering, the filter papers were weighed and dried for 24 or overnight at 70°C in an oven. To get rid of insoluble salts, the filtered samples were washed several times.

The filter papers were placed in a glass petri dish and were kept in the oven overnight at 70°C. For cooling, petri dish was placed in a sealed desiccator for 20 minutes, after that the filter paper was weighed. The following equation was used to calculate the dry weight of the algae on the filter paper:

$$\text{Dryweight (g/L)}, W = \frac{\text{FFW} - \text{IFW}}{\text{Amount of sample taken for filtration (ml)}} \times 1000$$

Where,

W = Cell dry weight in mg/L; FFW = Final filter paper weight in grams; and IFW = Initial filter paper weight in grams.

Estimation of spirulina's chlorophyll a

The chlorophyll a content of *S. platensis* was calculated from samples that were taken at 1 day intervals. With the help of filter sheets (sartorius filter paper with a 0.45µm mesh size and a 47mm diameter), 10mL of *S. platensis* sample was filtered using an electric vacuum filtration device. Along with filter paper, these filtered materials were put in test tubes and were ground with glass rods before being mixed with 10 milliliters of 100% redistilled acetone. Each test tube was wrapped in aluminum foil sheets to keep light from getting inside. The test tubes were wrapped and kept in a refrigerator (LMS Laboratory refrigerated) for the night. Following two minutes of homogenization, the refrigerated samples were centrifuged for ten minutes at 4000rpm. Upon centrifugation, the supernatant was extracted and sent for chlorophyll analysis. The optical densities of the samples were measured at 664, 647, and 630nm using a UV spectrophotometer (Milton Roy, Spectronic 1001 plus) and were computed **(Clesceri *et al*., 1989)**.

Additionally, a blank was run with 100% acetone. The following formula was used to determine the amount of chlorophyll a:

Chlorophyll a (mg/L) = 11.85 (OD 664) – 1.54 (OD 647) – 0.08 (OD 630)

S. platensis total biomass

Vonshak and Richmond (1988) provided the following formula, which was used to determine total biomass:

Total biomass = Chlorophyll a × 67

Specific growth rate (μ/day) of cultured spirulina on the basis of dry weight

SGR (μ/day) = In $(X_1-X_2)/t_1-t_2$

Where,

X_1 = Dry biomass concentration at the conclusion of the chosen time period; X_2 = concentration of dry weight biomass at the start of the chosen time interval; and t_1-t_2 = Time that has passed since the chosen point in the day.

Based on chlorophyll a, the specific growth rate (/day) of cultured spirulina

SGR (μ/day) = In $(X_1-X_2)/t_1-t_2$

Where, X_1 = Chlorophyll a at the conclusion of the chosen time period; X_2 = Chlorophyll a at the start of the chosen time period; and t_1-t_2 = Time that has passed since the chosen point in the day.

Cultured spirulina's specific growth rate (/day) based on total biomass

SGR (μ/day) = In $(X_1-X_2)/t_1-t_2$

Where,

X_1 = Total biomass at the end of selected time interval; X_2 = Total biomass at the beginning of selected time interval; and t_1-t_2 = Elapsed time between selected time in the day.

Statistical analysis

Analysis of variance (One way ANOVA with 5% level of significance) was performed on the suggested cell weight, chlorophyll a, crude protein, crude lipid, and ash of *S. platensis* cultivated in different mediums. The Duncan multiple range test (DMRT), which was developed by **Zar (1984)**, was used to determine the existance or non-existance of a significant difference between treatment approaches.

RESULTS AND DISCUSSION

Spirulina growth characteristics

Spirulina cell weight

The 14th day of spirulina culture revealed increased cell weight (mg/L) measurements than the prior days (Fig. 1). Spirulina cell weight grew from 0.036± 0.002 day (first day) up to 14th day (0.245 ±0.081mg/ L) of culture of 25% egg shell powder media, and subsequently dropped up to 20th day (0.157± 0.075mg/ L) of experiment. However, when grown on 25% egg shell powder media, the greatest cell weight of spirulina was discovered to be 0.245± 0.081mg/ L (Fig. 1). Spirulina cells weighed between 0.0042 and 0.001 in medium containing 50% egg shell powder. In contrast, it grew from 0.004 ± 0.001 on the eighth day up to 18th day (0.0242±0.005mg/ L) of culture of 50% egg shell powder media, and then reduced up to 14th day 5.554 ± 0.45mg/ L of experiment (Fig. 1). It subsequently declined in 0.0023 ± 0.001 on the 50% egg shell powder media (Fig. 1). Spirulina cells weighed between 0.0039 and 0.002 in the solution containing 75% egg shell powder on the first day. Then, it went up by 0.033± 0.004 on the fourth day of a culture using 50% egg shell powder, but it went down by 0.005 ±0.0002 on the twentieth day of a culture using 75% egg shell powder (Fig. 1). Spirulina-containing Kosaric media reached its highest cell weight of 1.89± 0.021mg/ L on the 14th day of the experiment before dropping to 0.398± 0.003 on the next day (Fig. 1).

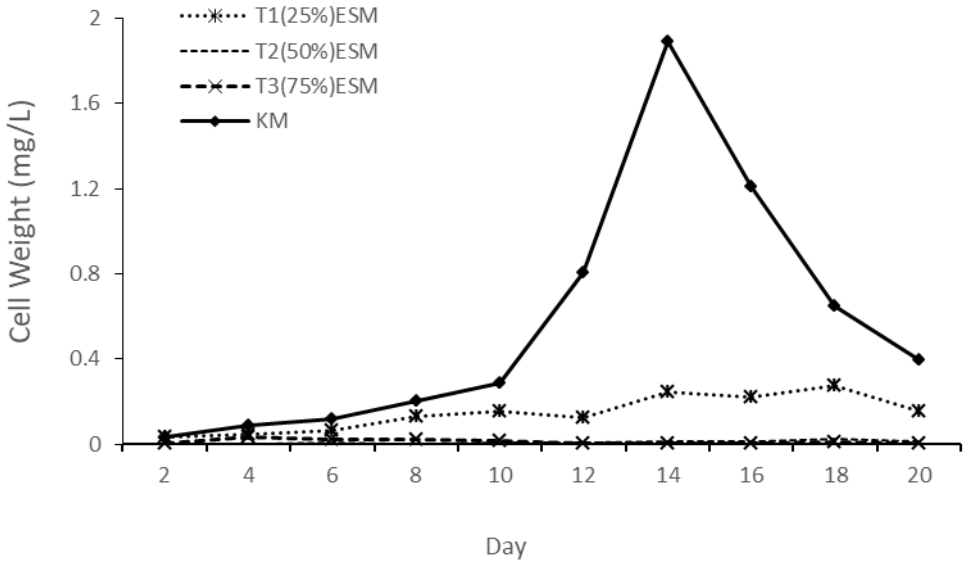

Fig. 1. *S. platensis* cultured in egg shell powder media and Kosaric medium showed mean values for cell weight (mg/L)

Spirulina's chlorophyll a

Spirulina's chlorophyll levels rose from the first day's value of 0.027±0.002 up to the 16th day's value of 0.415 0.108mg/ L of culture in 25% egg shell powder media before falling until the experiment's 20th day's value of 0.226 ± 0.066mg/ L (Fig. 2). On the first day, 0.0032–0.001 chlorophyll a of spirulina was discovered. Spirulina's chlorophyll a level increased during the course of the experiment, rising from 0.015 ± 0.001 on day 8 to 0.0217 ± 0.004mg/ L on day 18, before falling to 0.157 ± 0.075mg/ L on day 20 (Fig. 2). The concentration of 25% egg shell powder in the culture media increased up to the 14th day (0.245 ± 0.081mg/ L), and then declined up to the 20th day (0.0111 ± 0.003mg/ L) of the experiment (Fig. 2). Spirulina's chlorophyll a concentration was found to be 0.0031 ± 0.002 on the first day of the experiment, increased to 0.015 ± 0.001 on the sixth day and then to 0.016 ± 0.001mg/ L on the eighth day of the culture in 75% egg shell powder media, and then decreased from the 12th day to the 20th day (0.003 ± 0.0002) of the experiment in 75% egg shell powder media (Fig. 2). In the experiment in Kosaric medium, spirulina's chlorophyll a increased from 0.034 ± 0.002 day (first day) up to 14th day (1.983 ± 0.002mg/ L), and subsequently declined from 1.402 on the 16th day to 20th day (0.567 0.018mg/ L) (Fig. 2).

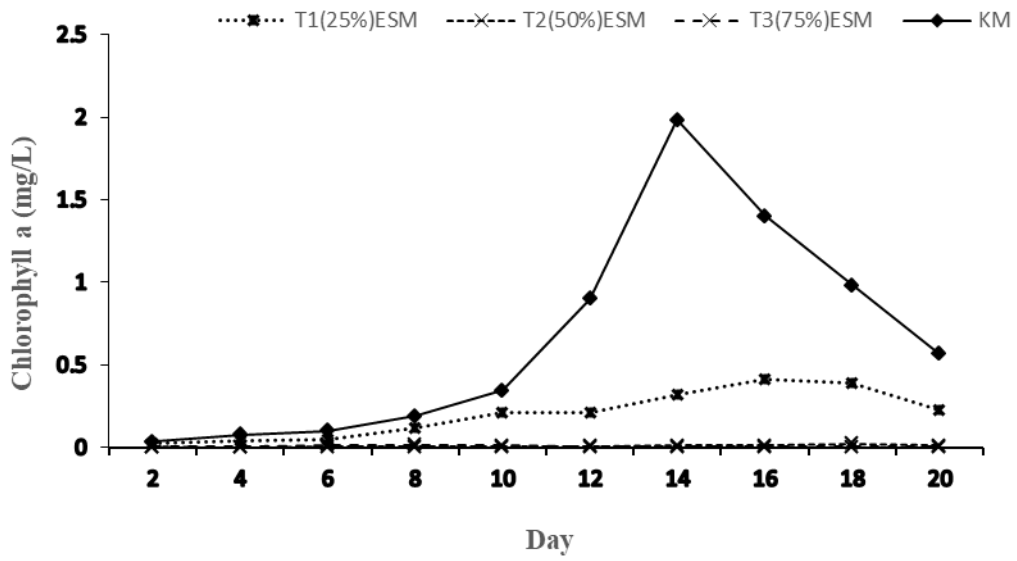

Fig. 2. *Spirulina platensis* cultured on egg shell powder media and Kosaric medium had mean amounts of chlorophyll a (mg/L)

Spirulina biomass

Spirulina (*S. platensis*) total biomass (mg/L) grew from 1.809± 0.072 on the first day to 26.063 ± 0.151mg/ L on the 18th day of the culture of 25% egg shell powder media, and then dropped up to the 20th day (15.142 ± 0.12mg/ L) of the experiment (Fig. 3). Spirulina's total biomass was found to be 0.2144 x 0.012 on the first day, and it increased from 0.227 x 0.003 on the eighth day to 1.453 x 0.024mg/ L on the 18th day of the experiment in the culture of 50% egg shell powder media before declining up to 0.737 x 0.033mg/ L on the twentieth day (Fig. 3). Spirulina's total biomass was found to be 0.207±0.024 on the first day, and it increased from 1.005 ± 0.001 on the sixth day to 2.01±0.03mg/ L on day 10 of the experiment's culture of 75% egg shell powder media before declining up until day 20 (0.201±0.004mg/ L) (Fig. 3). During the experiment, the total biomass of spirulina grown in Kosaric medium grew from 2.278 ± 0.023 (first day) to 14 days (132.861 ± 0.034 mg/L), and then declined from 93.934 ± 0.023 on the 16th day to 20 days (37.989 ± 0.22 mg/L) (Fig. 3).

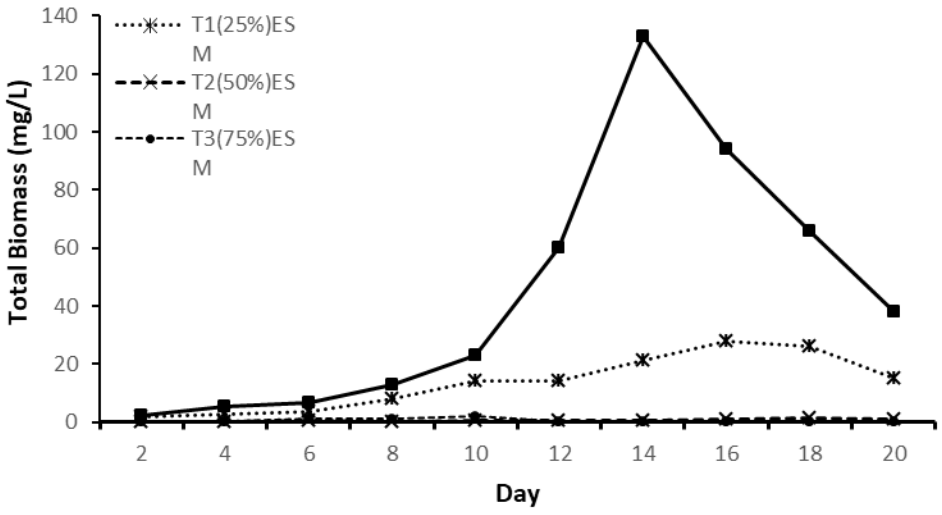

Fig. 3. Average values for *Spirulina platensis* cultivated on egg shell powder media and Kosaric medium in terms of total biomass (mg/L)

Optical density of the spirulina-containing medium

In a culture of 25% egg shell powder media, spirulina's optical density increased from the first day, at 0.046 ±0.002, up to the 18th day, at 0.32 ±0.006mg/ L, and subsequently fell until the 20th day, at 0.262 0.011mg/ L (Fig. 4). On the first day of 50% egg shell powder media, spirulina optical density was discovered to be 0.623 ±0.002. In a culture of 50% egg shell powder media, it was elevated from 0.065 ± 0.001 on the eighth

day to 0.181 ± 0.004mg/ L on the 18th day, and subsequently dropped up to 0.155 ± 0.002mg/ L on the 20th day of the experiment (Fig. 4). In the experiment's culture of 75% egg shell powder media, the OD of spirulina fell from 0.0573 ±0.003 day (first day) to 0.008 ±0.001mg/ L on the 20th day (Fig. 4). In the experiment, the optical density of the spirulina-containing Kosaric medium grew from 0.0463 ±0.036g/ L on the first day to 0.933 ±0.09 on the 14th, but it declined from 0.5216 ± 0.01 on the 16th to 0.3036 0.01 on the 20th (Fig. 4).

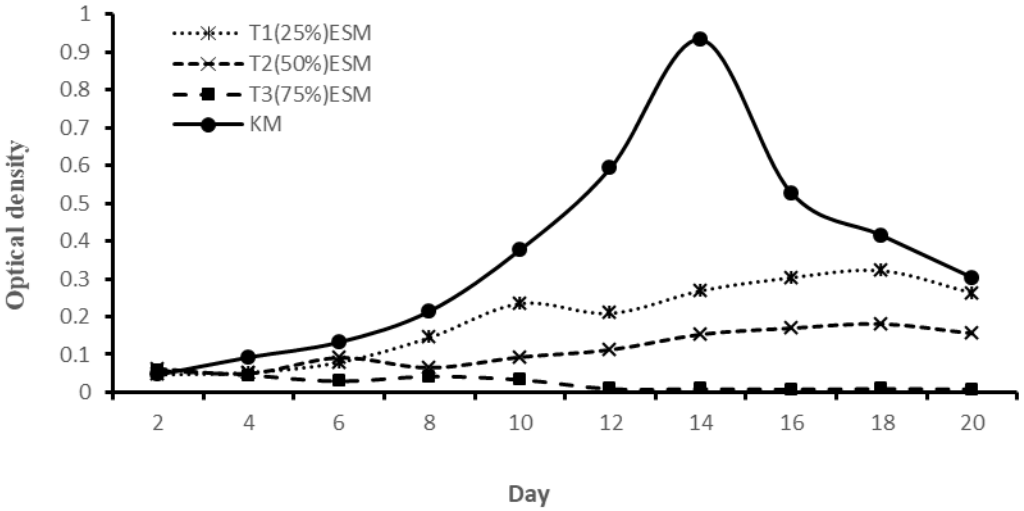

Fig. 4. Mean optical density values for egg shell powder media and kosaric medium that contained *S. platensis*

Comparison of spirulina (*Spirulina platensis*) growth metrics on the 14th day of culture

Spirulina cell mass

Spirulina grown on Kosaric medium had the highest cell mass (mg/L) ever discovered (Table 3). When compared to spirulina grown in 50% and 75% ESPM, the cell weight of the latter was significantly different ($P < 0.05$) (Table 3). However, there was no discernible difference between the weight of spirulina cells grown in 50% and 75% ESPM ($P > 0.05$).

Spirulina's chlorophyll a

Spirulina was substantially ($P< 0.05$) higher in chlorophyll a (mg/L) when grown in Kosaric medium and 25% (ESPM) than when grown in 50 and 75% (ESPM) (Table 3). The chlorophyll a of spirulina cultivated in Kosaric medium and 25% ESPM, did not differ significantly from the same spirulina cultured in 50 and 75% ESPM.

Table 3. *Spirulina platensis* cultured in egg shell powder media (ESPM) and Kosaric medium were compared on the 14th day of culture, just before stationary phase, for cell weight, chlorophyll a content, and total biomass

Parameter	T_1 (25% ESPM)	T_2 (50% ESPM)	T_3 (75% ESPM)	T_4 (KM)
Optical density	0.268± 0.002[b]	0.153± 0.005[a]	0.0086± 0.001[b]	0.933± 0.091[a]
Cell weight (mg/L)	0.245± 0.08[b]	0.0125± 0.004[a]	0.005± 0.002[b]	1.89± 0.021[a]
Chlorophyll a (mg/L)	0.318± 0.072[b]	0.0109± 0.003[a]	0.003± 0.002[b]	1.983± 0.002[a]
Total biomass (mg/L)*	21.306± 0.012[c]	0.73±0.06[b]	0.201± 0.003[c]	132.861± 0.034[a]

At a 5% level of probability, figures in common letters do not considerably diverge.

Spirulina's overall biomass

In comparison to spirulina cultivated in 50 and 75% ESPM, total biomass (mg/L) of spirulina cultured in Kosaric medium and 25% ESPM was considerably ($P < 0.05$) greater (Table 3). The total biomass of spirulina cultivated in 50 and 75% ESPM was not significantly different.

***Spirulina platensis* growth metrics are correlated with one another**

S. platensis cell weight and chlorophyll a concentration of spirulina produced in the concentration of three different egg shell powder media (ESPM) and Kosaric medium during the investigation showed a highly significant ($P< 0.05$) direct association (r=0.9893) (Fig. 5). Similar to how spirulina produced in egg shell powder media (ESPM) and Kosaric medium's, chlorophyll a levels were high ($P< 0.01$) and directly associated (r=0.822), in addition *S. platensis* total biomass was also high and directly associated (Fig. 6). Again, it was discovered that the cell weight of spirulina grown in various ESPM and Kosaric medium was high ($P < 0.05$) and directly linked with the total biomass of spirulina (r = 0.9489) (Fig. 7).

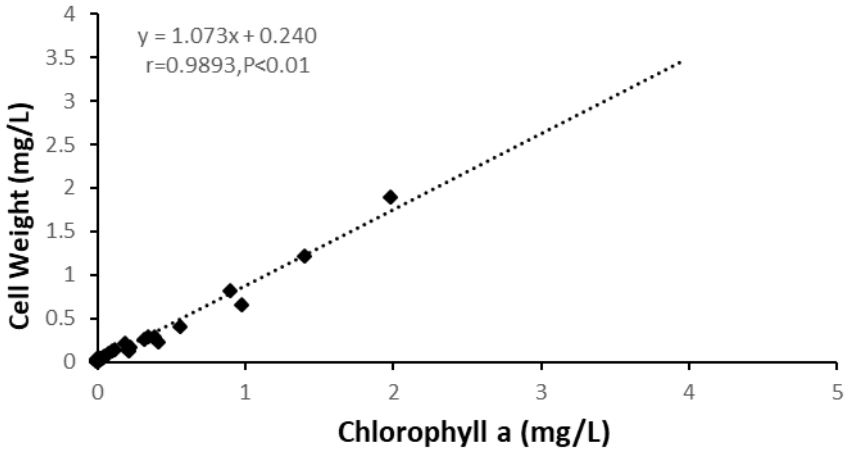

Fig. 5. Correlation coefficient (r) between the cell weight (mg/L) of *Spirulina platensis* and the chlorophyll a concentration (mg/L) in both the Kosaric and egg shell powder media

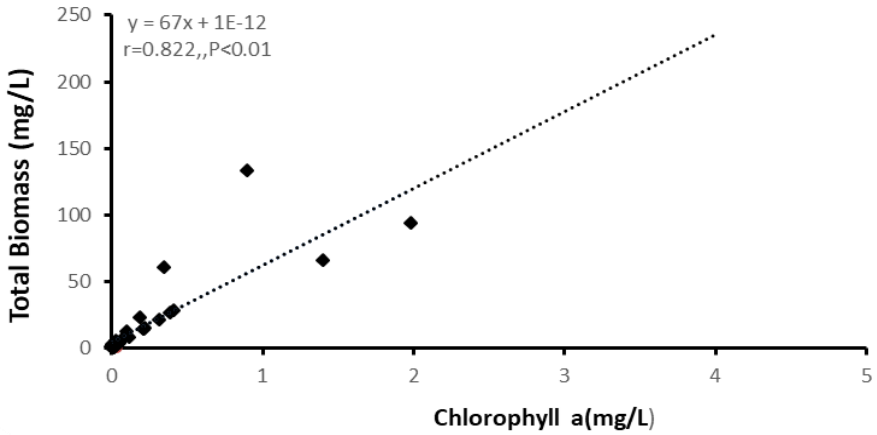

Fig. 6. Correlation coefficient with total biomass (mg/L) and chlorophyll a (mg/L) of *Spirulina platensis* cultured in egg shell powder media (ESPM) and Kosaric medium

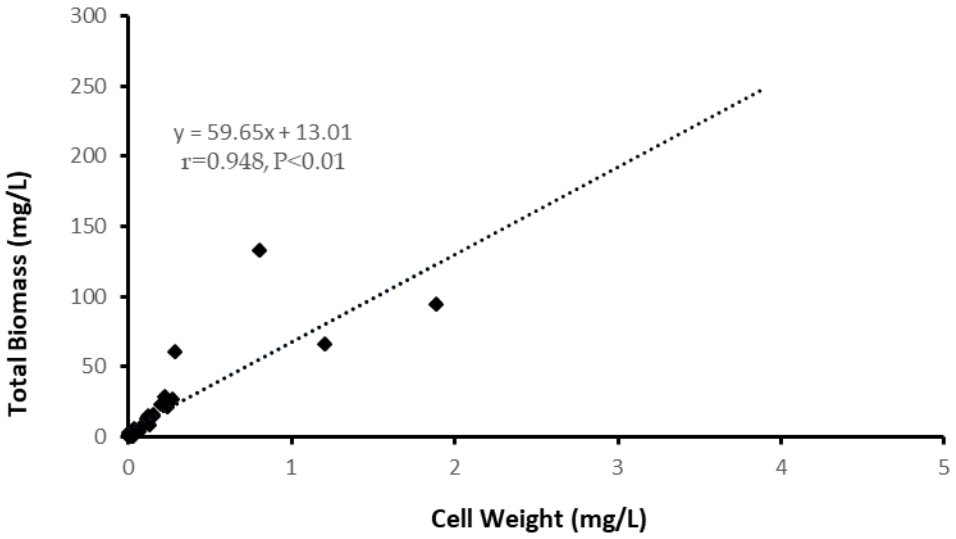

Fig. 7. Correlation coefficient (r) of the total biomass (mg/L) and cell weight (mg/L) of *S. platensis* cultured on Kosaric and egg shell powder media

Optical density of the spirulina-containing medium

In comparison with two other media (50% ESPM and 75% ESPM), the optical density of the 25% egg shell powder media (ESPM) and the Kosaric medium with spirulina (*S. platensis*) was substantially greater ($P< 0.05$) (Table 3). During the investigation, there were no significant differences between the optical densities of 25% ESPM and Kosaric medium, or between 50 and 75% ESPM ($P > 0.05$).

Specific growth rates (SGRs) *Spirulina platensis*

SGR in relation to Spirulina platensis cell weight

The specific growth rate (SGR) of spirulina cultivated in Kosaric medium and 25% egg shell powder media (ESPM) was substantially ($P< 0.05$) higher than that of spirulina cultured in the supernatant of 50 and 75% (ESPM) (Table 4). SGR is measured in relation to cell weight. There was a notable significant difference between SGRs of cell weight of Kosaric medium and 75% shell powder media (ESPM), but there was no significant ($P> 0.05$) difference between SGRs of cell weight of spirulina produced in 50 and 75% ESPM.

SGR in relation to *Spirulina platensis*'s Chlorophyll a

When compared to spirulina cultivated in egg shell powder media (ESPM) of 50 and 75%, the SGR of spirulina cultured in Kosaric medium and 25% ESPM was considerably ($P< 0.05$) different (Table 4). SGRs, on the basis of chlorophyll a, did not significantly differ when spirulina was grown in egg shell powder media (ESPM) of 50 and 75%, and a similar result was obtained when spirulina was grown in ESPM of 50 and

75%. However, there was a notable significant difference between SGRs of chlorophyll a of Kosaric medium and 25% ESPM.

SGR in relation to total biomass of *Spirulina platensis*

The SGR of spirulina cultivated in Kosaric medium and 25% egg shell powder media (ESPM) differed considerably ($P< 0.05$) from that of spirulina produced in 50 and 75% egg shell powder media (ESPM) in terms of total biomass (Table 4). SGRs were not significantly different ($P< 0.05$) based on the total biomass of spirulina grown in 50 and 75% egg shell powder media (ESPM), but there was a notable significant difference between SGRs based on the total biomass of Kosaric medium and 25% ESPM.

Table 4. *Spirulina platensis* cultured in egg shell powder media (ESPM) and Kosaric medium was found to exhibit specific growth rates (SGRs) based on cell weight, chlorophyll a, and total biomass

Parameter	**T_1 (25% of (ESPM),**	**T_2 (50% of (ESPM),**	**T_3 (75% of (ESPM),**	**T_4 (KM)**
SGR of cell weight	0.32 ± 0.002^c	0.0125 ± 0.002^b	0.005 ± 0.003^c	0.31 ± 0.002^a
SGR of chlorophyll a	0.31 ± 0.002^c	0.022 ± 0.03^b	0.002 ± 0.003^c	0.29 ± 0.004^a
SGR of total biomass	0.27 ± 0.021^c	0.21 ± 0.033^b	0.207 ± 0.043^c	0.81 ± 0.024^a

N.B. Figures in the same row with similar letters do not substantially differ at the 5% level of significance.

Below, the findings of the current study were compared with those of other academics working in related domains. Three different supernatant concentrations (25, 50, and 75%) of egg shell powder medium were used to cultivate *Spirulina platensis*, with KM serving as the control. 0.036 to 0.245mg/ L in 25% egg shell powder medium, 0.0042 to 0.0242mg/ L in 50%, 0.0039 to 0.033mg/ L in 75% egg shell powder media, and 0.0367 to 1.89mg/ L in KM were found to be the cell weights of *S. platensis*. *Spirulina platensis* grew more effectively in 25% egg shell powder media than in 50 and 75% egg shell powder media. This fluctuation may be brought on by variations in nutrient contents and medium composition. *Spirulina platensis* has the best growing performance in controlled KM. It might have happened because the nutrients were suitable and readily available for the species' growth.

Compared to 25 and 50% egg shell powder media, the growth performance of *Spirulina platensis* in the medium at 75% concentration was inferior. The media's lower concentration and higher nutrient dilution may be to blame for this. Because of the nutrient content, the egg shell powder concentrations of 25 and 50% are acceptable and

beneficial for the growth of *Spirulina platensis*. Higher dilution was followed by lower nutrient concentration and higher growth performance, according to a comparative research of *Spirulina platensis* growth performance in varied media concentrations.

In contrast to other media where light intensity, aeration, and temperature played key roles to the growth system. **Satter (2017)** found that the cell weight and chlorophyll a content of *S. platensis* were significantly ($P< 0.05$) greater in 4.0g/ L digested poultry waste. In the current investigation, the beginning cell weight was 1.89mg/ L, which increased to a maximum cell weight of 0.0039 to 0.033mg/ L in media containing 75% egg shell powder and 0.0042 to 0.0242mg/ L in media containing 50% egg shell powder. *S. platensis* was infected with a chlorophyll a concentration of 0.034mg/ L, which increased to 1.983mg/ L when it was grown in KM and 0.415mg/ L in 25% egg shell powder media at day 16 of culture. The conclusions of **Phang *et al*. (2000)**, **Habib *et al.* (2003)**, **Satter (2017)** and **Habib *et al.* (2019)** are more or less comparable to those of this study. In the current investigation, three different doses of egg shell powder media were utilized to cultivate *Spirulina platensis*. The maximum optical density in 25% egg shell powder media was seen on day 18 of culture (0.320.006) when compared to KM, which is consistent with the findings of **Habib *et al.* (1997, 2003)**. **Satter (2017)** discovered that the supernatant of 4g/ L digested poultry waste (DPW) produced superior growth performances than other two alternative media, although it was somewhat lower than the growth of spirulina in KM. But when grown in egg shell powder media and KM, it had nearly identical growth capabilities. According to the discussion above, 25% egg shell powder media showed higher growth performance for *Spirulina platensis* than 50 and 75% egg shell powder media. This fluctuation may be brought on by variations in nutrient contents and medium composition. The present study's physical characteristics, chemical parameters, and technological tools can be stated to have been more or less comparable to those employed by previous researchers. However, in certain instances, differences in the media's nutritional content, technological capabilities, ambient conditions, and other factors led to variations in the results.

CONCLUSION

In this study, *Spirulina platensis* growth performance was found to vary with media concentration variations. According to the study's recommendations, spirulina grows well in the supernatant of 25% egg shell powder media, which is equivalent to spirulina growth in Kosaric medium. Spirulina can also be cultivated in media containing 50% egg shell powder, however the yield is not as good as with 25%. If egg shells aren't thrown around, the environment might not be polluted. Egg shells may be utilized for the commercial cultivation of spirulina and may be sold as live food for the management and good production of fish health. Organizations from both the public and private sectors ought to volunteer to train staff members on spirulina culture.

AUTHOR CONTRIBUTION

Md. Ahsan Bin Habib and Md. Hamidur Rahman conceived the idea. Md. Hamidur Rahman and Meherun Nisa Jinia performed the experiment. Md. Sazzad Hossain, Meherun Nisa Jinia and Md. Hamidur Rahman analyzed the data, and wrote the manuscript. Sheikh Rasel and Zannatul Ferdous participated in the research, discussed and commented on earlier drafts. All authors have read and approved the final manuscript.

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