

Research Article

Effects of Rotten Vegetable-Based Low-Cost Media on the Growth and Morphology of an Astaxanthin-Producing Green Alga, *Monoraphidium Littorale*

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Growing microalgae on vegetable waste (rotten potatoes) media is not only a plausible technique to replace expensive commercial media but also a way of reducing feed costs for rearing zooplankton and fish larvae in aquaculture. As a way of starting an inevitable step up, *Monoraphidium littorale* was grown in 25% (T_1), 50% (T_2), and 75% (T_3) of digested rotten potato supernatant (DRPS) and at the same time in Bold Basal Medium (BBM) as a control (T_4) for 16 days. The highest cell density of *M. littorale* was recorded in T_1 , followed by T_4 , T_2 , and T_3 . A similar increasing trend of chlorophyll-a and optical density was also observed during the experimental period. The study also ascertained T_1 as a potential growth medium where *M. littorale* showed superior biomass production which was significantly higher ($P < 0.05$) than other treatments. It was also worth mentioning that the highest ($P < 0.05$) protein content was determined in T_1 at the end of the cultivation time. Similarly, a highly significant difference ($P < 0.05$) was also found in the lipid content of the microalgae grown in T_1 and T_4 . Furthermore, the morphology of the cells of *M. littorale* was found to be strongly affected by the DRPS concentrations and BBM. Thus, the lower concentration of the DRPS served as the best medium for enhancing the growth and biomass of *M. littorale*. Consequently, to expedite the sustainable progression of microalgal production, cost-effective culture techniques by using different wastes may be adopted.

1. Introduction

In aquaculture, microalgae play a significant role in nourishing zooplankton before they are supplemented to fish or other larvae because microalgal nutrients are transported through zooplankton to higher trophic levels [1]. Aside from that, microalgae are also being explored as a potent bulk-feed ingredient amenable for fingerlings and adult fishes [2]. Indicatively, microalgae can fix about 9% of solar energy to produce biomass more competently than terrestrial plants and are considered as a sustainable and potential repository of carotenoids, polyunsaturated fatty acids, and vitamins [3, 4]. Among different carotenoids available in nature, astaxanthin is deliberated as one of the most effective and potent antioxidants, which has multifaceted applications

in the aquaculture industry [5]. A good number of research reveal that astaxanthin from microalgal sources has great importance in the aquaculture industry by dint of its potency in boosting growth [6–8], reproductive performance [9, 10], and immunity [11, 12] and product quality development through giving hue to the skin and muscle [13–15] of various fish species. As a consequence, the global demand for natural astaxanthin has risen tremendously.

Among numerous astaxanthin-producing microalgae, *Monoraphidium* sp. is deliberated as one of the most important green alga, which has great prospects in aquaculture. Kaha et al. [16] reported that *Monoraphidium* sp. can produce higher content of astaxanthin under long-wave ultraviolet radiation. Moreover, Fujii et al. [17] revealed that *Monoraphidium* sp. GK12 has extensive potential for having

higher content of pantothenic acid and β -carotene as well as for producing astaxanthin, which further helps to enhance coloration as well as the survival of prawns successfully. Furthermore, due to high biomass productivity and lipid accumulation in the cell, *Monoraphidium* is also considered as a strong and potential candidate for biofuel production in a way of combating emerging energy crisis as well as for environmental benefits [18, 19]. By considering the aforementioned prospects of *Monoraphidium* sp., it is a telling option to initiate the mass production of this species to explore its potential in aquaculture industries especially for ameliorating the production of zooplankton and fish larvae. However, the emerging impediment to the commercialization of *Monoraphidium* sp. as well as other microalgae culture in aquaculture is indisputably the production costs. From this perspective, minimizing media costs by exploring low-cost media from agricultural waste may be an inevitable step up.

Bangladesh has clinched its envious position as sixth largest potato-producing country in the world [20]. Currently, while the annual average demand for potatoes was about 7 million tons, Bangladesh observed a huge surplus production of about 4 million tons, the lion's share of which was being wasted due to a lack of storage capacity [21]. This huge number of rotten potatoes may be supportive of developing waste media in boosting the growth of microalgae because of their prevailing organic and inorganic nutrients [22]. Nowadays, organic waste recycling is the most demanding and important technology to counteract the thriving crisis of nutrient scarcity. In this regard, microalgae are well-acquainted with their successful applications in biological nutrient recovery systems [23]. Microalgae are now successfully grown using different cost-effective medium including soil extracts and aquaculture sludge as a replacement for expensive media [24, 25]. As a consequence, considering the economic perspective, growing microalgae in digested rotten potato supernatant (DRPS) may have ample potential. On account of initiating the culture protocol of *Monoraphidium* sp., some studies demonstrated the growth and the morphology of *Monoraphidium* sp. at different physiological factors [26–29]. Very few significant research efforts have been conducted to explore the culture feasibility of *Monoraphidium* sp. in different waste media [30–32] for their improved growth and lipid accumulation. However, the effects of DRPS on the growth and morphology of *M. littorale* have not yet been studied elsewhere. Some previous studies demonstrated that DRPS had promising effects for enhancing the growth and biochemical composition of *Spirulina platensis* [22, 33]. In the present study, DRPS showed superior biomass production of *M. littorale*, which is far higher than other agricultural wastes including rotten tomatoes for *S. platensis* [34] and aquaculture waste for *Chlorella vulgaris* [35]. Following the usefulness, the knowledge of culturing *M. littorale* in DRPS may be effective for exploring its potentiality for large-scale cultivation of economically valuable biomass due to its possible cost reduction along with waste-remediation benefits which will be the most important catalyst in intensifying successful rearing of zooplankton and larvae of numerous commercially

TABLE 1: Composition of the chemicals in BBM for *M. littorale* culture.*

No.	Stocks of chemicals	g L ⁻¹
1	NaNO ₃	25.00
2	MgSO ₄ ·7H ₂ O	7.50
3	NaCl	2.50
4	K ₂ HPO ₄	7.50
5	KH ₂ PO ₄	17.50
6	CaCl ₂ ·2H ₂ O	2.50
	Trace elements	
	ZnSO ₄ ·7H ₂ O	4.42
7	MnCl ₂ ·4H ₂ O	1.44
	MoO ₃	0.71
	CuSO ₄ ·5H ₂ O	1.57
	Co (NO ₃) ₂ ·6H ₂ O	0.49
8	H ₃ BO ₃	11.40
	EDTA–KOH solution:	
9	EDTA Na ₂	50.00
	KOH	31.00
10	FeSO ₄ ·7H ₂ O with 1.0 mL concentrated H ₂ SO ₄	4.98

*Z.H. Scientific and Chemicals Mart, Bangladesh, is the supplier of the above-mentioned chemicals.

important fishes, crustaceans, and mollusks in the aquaculture industry. Hence, the aim of the current study was to evaluate the effects of low-cost media (DRPS) on the growth and morphology of *M. littorale*. Moreover, the development of the culture of *M. littorale* on an industrial scale may be a golden opportunity for achieving 17th sustainable development goals (SDGs) directly and indirectly, especially the SDG-6 “Clean Water and Sanitation,” SDG-7 “Affordable and Clean Energy,” and SDG-13 “Climate action.”

2. Materials and Methods

2.1. Analyses of Proximate Composition of Rotten Potato. The proximate composition of rotten potatoes, including moisture, crude protein, crude lipids, ash, and nitrogen-free extract (NFE), was examined in triplicates by following the standard method of Horwitz [36].

2.2. Isolation of Microalgae and Media Preparation. The microalga, *M. littorale*, was isolated from freshwater ponds located beside the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. Isolation was carried out by repeated streaked agar plating and serial dilution method. Stock cultures of *M. littorale* were maintained in Bold Basal Medium (BBM) at a temperature of $25 \pm 2^\circ\text{C}$, a light intensity of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a photoperiod of 12:12 hr, L:D. With regard to preparing BBM as a culture medium, 10 mL from the mentioned amount of ingredients from each of 1 to 6 and 1 mL from each of 7 to 10 (Table 1) stock solutions were taken in a 1 L conical flask and distilled water was added to make the volume 1.0 L. Then, the flask was well mixed and sterilized at

TABLE 2: Composition of the micronutrient solution.*

No.	Stocks of chemicals	g L ⁻¹
1	H ₃ BO ₄	2.86
2	MnCl ₂ ·4H ₂ O	1.81
3	ZnSO ₄ ·7H ₂ O	0.22
4	CuSO ₄ ·5H ₂ O	0.08
5	MoO ₃	0.01
6	CoCl ₂ ·6H ₂ O	0.01

*Z.H. Scientific and Chemicals Mart, Bangladesh, is the supplier of the above-mentioned chemicals.

121°C for 15 min with moist heat by autoclave (Model SS-V35HD, WINCOM, China).

Rotten potatoes, used in this experiment, were collected from the KR market, Bangladesh Agricultural University Campus, Mymensingh, Bangladesh. For the preparation of DRPS, 200 g of rotten potatoes were subjected to digestion in previously cleaned 8.0 L glass jars containing 6.0 L of distilled water for 22 days under continuous aeration. After getting a light reddish-colored supernatant, screening was done by a net, having a 30 μm mesh size to remove particulate materials. Then, 9.0 g L⁻¹ sodium bicarbonate (NaHCO₃) along with 0.20 mL L⁻¹ micronutrient solution was added to the extract. For preparing the micronutrient solution, the nutrients from no. 1 to 6 mentioned in Table 2 were taken in a 1.0 L conical flask, distilled water was added to make the volume 1.0 L and finally sterilized at 121°C for 15 min. After the preparation of DRPS, the three DRPS levels (25%, 50%, and 75%) with replications were made to observe the growth of *M. littorale* (Figure 1).

2.3. Growth Conditions of *M. littorale*. The growth of *M. littorale* was observed in 25% (T_1), 50% (T_2), and 75% (T_3) of DRPS and in BBM (T_4) (Figure 2). The experiment was conducted in triplicates using an initial inoculum for all cultures of about 45,914 cells mL⁻¹ from the culture in the logarithmic phase maintained in BBM. This study was performed in 1,000 mL Erlenmeyer flask containing 700 mL of culture media under a light intensity of 60 μmol m⁻² s⁻¹ at a photoperiod of 12 : 12 hr, L : D with continuous aeration for 16 days. Cell numbers were counted using a Sedgwick-Rafter Chamber, immediately after inoculation and on every alternate day for up to 16 days. After 16 days of culture period, 100 mL of microalgal solution from each replication was transferred to 250 mL conical flask for the morphological study of *M. littorale*. During the experimental period, cell numbers of *M. littorale* at different shapes were estimated on every 10 days from 20 to 70 days at early, mid, and late stationary phases. The average number of cell divisions per day (K) for the 10-day growing period was calculated using the following equation:

$$K = \ln\left(\frac{C_t}{C_0}\right) \left(\frac{1}{t \ln 2}\right), \quad (1)$$

where C_t and C_0 are the cell concentrations at times t and 0, respectively [37].



FIGURE 1: Preparation of three concentrations of digested rotten potato supernatant ($T_1 = 25\%$, $T_2 = 50\%$, and $T_3 = 75\%$).



FIGURE 2: Growth condition of *M. littorale* at three concentrations of DRPS ($T_1 = 25\%$, $T_2 = 50\%$, and $T_3 = 75\%$) and BBM (T_4) on day 16 of the culture period.

The physicochemical parameters of the culture media including pH, temperature, dissolved oxygen, electric conductivity, and total dissolved solids were measured using pH Meter (HI98107, HANNA), Dissolved Oxygen Meter (DO-5509, LUTRON, Taiwan), and pH/EC/TDS Multiparameter Meter (HI98194, HANNA, USA), respectively, during the experimental period. All of the measured aforementioned physicochemical parameters were found within the acceptable range of *M. littorale* culture [26].

2.4. Determination of Optical Density and Chlorophyll-a Content. In case of estimating optical density (OD) and chlorophyll-a content, 15 mL of *M. littorale* sample from each replication was filtered with Whatman filter paper (0.45 μm mesh size and 47 mm diameter), kept in 20 mL tubes mixed with 10 mL of 100% acetone that was further ground with a glass rod, and kept in a refrigerator overnight. The refrigerated samples were homogenized for 2 min followed by centrifugation at 4,000 rpm for 10 min. Eventually, the OD of the samples was determined at 620 nm by using a UV spectrophotometer. For determination of chlorophyll-a content, OD of samples was further taken at 664, 647, and 630 nm by using a UV spectrophotometer against blank (T60UV-Visible Spectrophotometer, PG Instruments Ltd.), and chlorophyll-a content was calculated using the following equation [38]:

$$\text{Chlorophyll-a (mg L}^{-1}\text{)} = 11.85 (\text{OD } 664) - 1.54 (\text{OD } 647) - 0.08 (\text{OD } 630). \quad (2)$$

2.5. Protein and Lipid Determination. About 0.221 g of sample was obtained to estimate the crude protein content. The

TABLE 3: Proximate composition of rotten potato (% dry matter basis).*

Variables	Composition (%)
Moisture	9.78
Crude protein	10.65
Crude lipid	9.20
Ash	15.75
Crude fiber	18.89
NFE	35.73

*NFE, nitrogen free extract = 100 - (moisture + crude protein + crude lipids + ash + crude fiber) [22].

standard micro-Kjeldahl nitrogen method was used to determine the crude protein content utilizing a Behrosetlnkje M digesting device and a Behr SI steam distillation apparatus (both Labor-Technik GmbH, Dusseldorf, Germany). Prior to

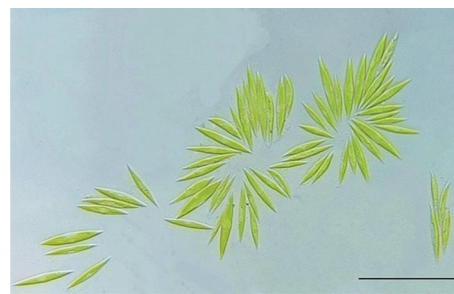


FIGURE 3: Isolated cells of a green alga *M. littorale*. Scale bar: 10 μm .

titrating with 0.2 N HCl, the distillate containing ammonia was trapped in a 4% boric acid solution. By multiplying the nitrogen content with a factor of 5.85, crude protein was calculated using the following equation:

$$\begin{aligned} \text{Percent (\%)} \text{ of crude protein} &= \text{Nitrogen (\%)} \times \text{conversion factor (5.85 for plant - based sample)} \\ \text{\% of nitrogen} &= (\text{Milliequivalent of } N_2 \times \text{strength of HCl} \times \text{titrant used (mL)}) / \text{weight of sample (g)} \times 100, \end{aligned} \quad (3)$$

where milliequivalent of $N_2 = 0.014$ and strength of HCl = 0.2 N.

Again, for estimating the crude lipid content, about 0.231 g of sample was taken. For the analysis of the lipid content, mentioned samples were dried in the oven at 105°C and then extracted the fat with acetone in a Soxhlet Extractor for about 4 hr. The crude lipid content of the sample was estimated by using the following equation:

$$\text{Percent (\%)} \text{ of crude lipid content} = \left\{ \frac{(D-B)}{A} \right\} \times 100, \quad (4)$$

where

- D is the weight of crude lipid,
- B is the empty beaker weight, and
- A is the weight of sample.

2.6. Morphological Observations. When the experiment was performed to examine morphological responses, an alternation of three morphotypes was observed using a microscope (B-510BT OPTIKA, Italy). The morphological study of *M. littorale* was lasted for 70 days.

2.7. Statistical Analysis. The data are expressed as mean \pm standard deviation (SD) of three replicates. Mean daily division rates, total biomass, crude lipid content, crude protein content, and morphology data in four treatments were subjected to one-way ANOVA (SPSS 25). Significant differences among means were determined using Duncan's multiple range test (DMRT) for paired comparison [39]. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Proximate Composition of Rotten Potato. The proximate composition of rotten potato is presented in Table 3. On a

dry matter basis, the crude protein, crude lipid, ash, and fiber content in rotten potato were 10.65, 9.20, 15.75, and 18.89%, respectively.

3.2. Isolation and Identification of *M. littorale*. During the isolation period, after repeated streak agar plate technique, a verdant growth of unidentified microalgal species was observed in the agar plate, which was further serially diluted in test tubes for obtaining the axenic culture. Moreover, a morphological study of cultured cells was performed under the microscope (B-510BT OPTIKA, Italy), which revealed that isolated microalga was morphologically similar in accordance with the Selenastraceae family and exhibited close proximity with *M. littorale* with a fusiform, straight shape, and tapering toward both end apices (Figure 3).

3.3. The Effects of DRPS and BBM on the Growth and Biomass Production of *M. littorale*

3.3.1. Effects on Cell Density, Biomass Production, and Mean Daily Division Rate of *M. littorale*. The growth of cell densities of *M. littorale* in different treatments with culture days is shown in Figure 4(a). In T_1 and T_4 , the cell density of *M. littorale* rose with a discernible difference from day 1 to 6, then got increased until day 12 with exceeding growth, and dropped progressively while culture time was prolonged. The highest cell density of *M. littorale* was found in T_1 (114.485×10^5 cells mL^{-1}) on day 12 and the cell density persisted at 77.425×10^5 cells mL^{-1} up to day 16. Between T_1 and T_4 , the peak cell densities did not substantially tend to fluctuate. Under both conditions, an initial lag period of 2 days after the culture inoculation was noted.

In T_2 , *M. littorale* grew slowly with a relatively poor maximum cell density of 45.96×10^5 cells mL^{-1} , which was markedly lower than T_1 and T_4 . Whereas in T_3 , *M. littorale* cells were observed to be aggregated at the bottom of the flask just after

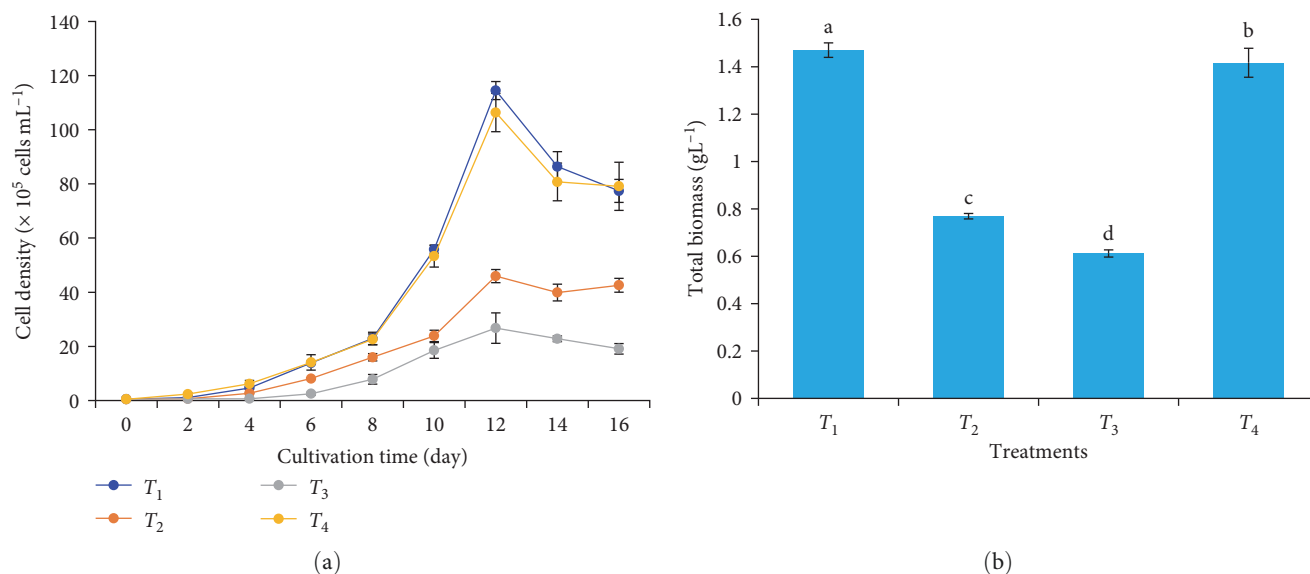


FIGURE 4: (a) Cell density of *M. littorale* ($\times 10^5$ cells mL^{-1}) during the culture in three different DRPS concentrations and BBM; (b) total biomass of *M. littorale* on 16th day in different concentrations of DRPS and BBM. (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM). Each point and vertical line represent mean \pm SD for three replicates. Means with different letters are significantly different from one another (DMRT, $P < 0.05$).

inoculation, and cell growth was hardly noticed until day 4. After 4 days of the culture period, cells started to grow very slowly and reached a peak of 26.75×10^5 cells mL^{-1} on day 12.

M. littorale yielded a remarkably different amount of total biomass (determined on a dry weight basis) at varied DRPS concentrations and BBM at the end of the experimental period (Figure 4(b)). Considerably greater biomass output ($P < 0.05$) was recorded in T_1 (1.47 g L^{-1}) compared with that in T_4 (1.42 g L^{-1}) demonstrating higher biomass production with lower DRPS concentration (25%). In addition, it was apparent that the biomass was drastically affected by an increase in DRPS concentrations resulting in the reduction of total biomass production significantly ($P < 0.05$) in T_2 (0.769 g L^{-1}) and T_3 (0.61 g L^{-1}).

During the course of the experiment, it was also notable that DRPS and BBM had a marked impact on the cell condition of *M. littorale*. Cell conditions were monitored under a microscope, where expanded and disrupted cells were predominantly found in T_3 and then T_2 from day 2 and very healthy cells were observed in the case of T_1 and T_4 (Figure 5).

In addition, the mean daily division rates plotted as a function of different concentrations of DRPS and BBM are shown in Figure 6. The DRPS and BBM had a pronounced effect on the growth rate of *M. littorale*. The best growth rate of *M. littorale* was found in both T_1 and T_4 with identical division rate of 0.33 ± 0.02 divisions day^{-1} , which was significantly higher ($P < 0.05$) than in T_2 (0.274 ± 0.02 divisions day^{-1}) and T_3 (0.256 ± 0.02 divisions day^{-1}). Despite there being no significant difference between the growth rate of *M. littorale* in T_2 and T_3 on day 10, T_2 had a significantly higher ($P < 0.05$) cell density than T_3 on day 12.

3.3.2. Effects on the Chlorophyll-a Content and Optical Density. The chlorophyll-a content and OD both followed a similar trend as observed in the growth profile in terms of cell density and growth rate of *M. littorale*. The chlorophyll-a contents of *M. littorale* had no obvious changes in the first 2 days in the case of all treatments. The chlorophyll-a content sharply increased from 0.812 mg L^{-1} to 9.367 mg L^{-1} and 0.088 mg L^{-1} to 8.809 mg L^{-1} in T_1 and T_4 , respectively, on day 12; afterward, the chlorophyll-a content of *M. littorale* continued to decline. While for T_2 and T_3 , elevated DRPS concentrations led to a significant drop in the chlorophyll-a content which caused a reduced growth of *M. littorale* considerably poorer than T_1 and T_4 (Figure 7(a)). The OD of *M. littorale* was increased with decreasing DRPS concentration where a considerably higher value was observed in T_1 (0.208 ± 0.01) and T_4 (0.196 ± 0.01) than in T_2 (0.084 ± 0.004) and T_3 (0.071 ± 0.007) (Figure 7(b)). In T_2 and T_3 , the lower value of OD along with lower growth was distinguished.

3.4. Effects on Crude Protein and Crude Lipid Content of *M. littorale*. The influence of different concentrations of DRPS and BBM on the crude protein and crude lipid accumulation of *M. littorale* during the experimental period was also analyzed. Figure 8 shows how the crude protein content and lipid content evolved with the changes in the culture media. The maximum protein content was found in T_1 (39.08%) at the end of the cultivation time, which was quite significant ($P < 0.05$) than T_4 resulting in 38.02% of protein content. In T_2 and T_3 , protein accumulation did not appear to have a significant difference and the corresponding crude protein content was 32.98% and 32.45%, respectively.

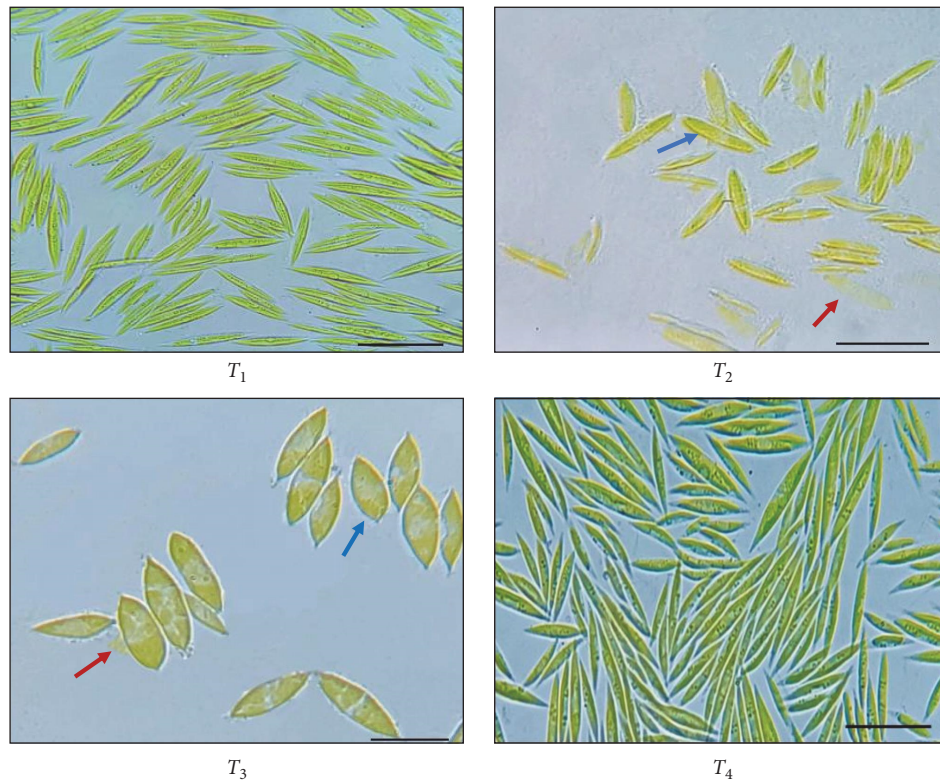


FIGURE 5: Cell conditions in different concentrations of DRPS and BBM: T_1 and T_4 are depicting healthy cells where T_2 and T_3 are exhibiting expanded (blue arrow) and disrupted cells (red arrow) of *M. littorale* during the experimental period. Each scale bar: $10\ \mu\text{m}$. (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM).

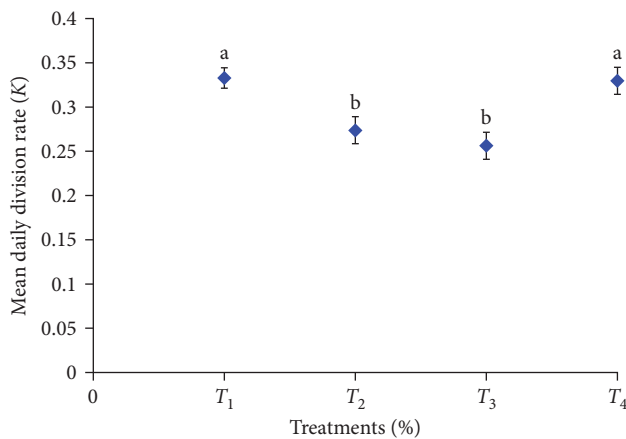


FIGURE 6: The influence of different DRPS concentrations and BBM (control) on the mean daily division rate of *M. littorale*. Means with different letters are significantly different from one another (DMRT, $P < 0.05$). (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM).

The highest crude lipid content (26.623%) was found in T_1 . There was no significant difference between the lipid content found in T_2 (16.93%) and T_3 (16.34%). It was also worth mentioning that a statistically significant difference ($P < 0.05$) was found among in the lipid content of T_1 and T_4 where two times lower lipid content was observed in T_4 (12.697%) than T_1 .

3.5. Effect of Different Concentrations of DRPS and BBM on the Morphology of *M. littorale*. Morphological observation of *M. littorale* cultivated under the three DRPS concentrations and BBM was performed on days 20, 30, 40, 50, 60, and 70 using a light microscope which revealed the high phenotypic plasticity of this species. The morphology of *M. littorale* was variable and strongly affected by DRPS concentrations and BBM. At optimum conditions, cells of *M. littorale* were mostly fusiform like. During the late logarithmic phase, with a change in unfavorable conditions, cells began to change their shape as well as the number of oval and spherical cells. First, fusiform cells were observed to produce 2–4 fusiform progeny during the early exponential phase (Figure 9(a)–9(c)). Then, fusiform cells were noticed to produce 4–8 oval progeny in the late exponential phase (Figure 10(d)). In the stationary phase, fusiform, oval, and spherical cells produced spherical progeny (Figure 9(e)–9(k)). In addition to the morphological changes, aggregation of spherical cells in the late stationary phase was also observed (Figure 9(l)).

3.5.1. Effect of Different Concentrations of DRPS and BBM on the Morphology of *M. littorale* on 20th and 30th Days of the Culture Period. The cell morphology of *M. littorale* was markedly affected ($P < 0.05$) by various concentrations of DRPS and BBM (Figure 10). In T_1 and T_4 , the early stationary phase cultures consisted of 98% and 100% fusiform-like cells on day 20, whereas 78% and 96.7% of fusiform cells were

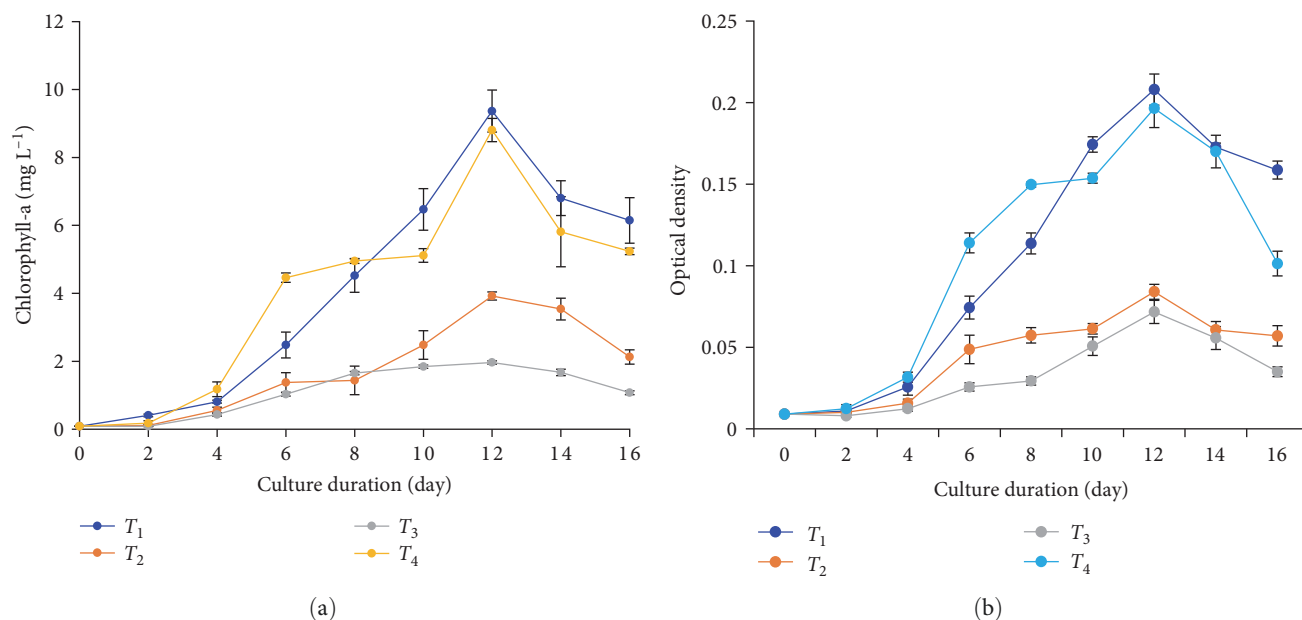


FIGURE 7: Growth curve of *M. littorale* in terms of (a) chlorophyll-a content and (b) optical density at different DRPS conditions and BBM (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM).

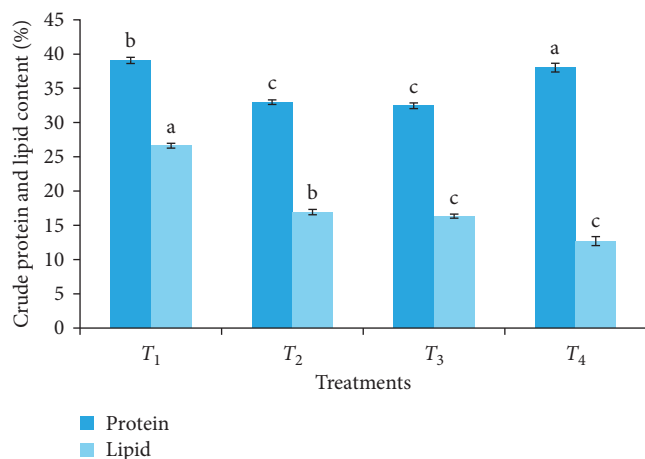


FIGURE 8: Crude protein and lipid content (in dry weight basis) obtained from *M. littorale* grown under different concentrations of DRPS and BBM (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM). Means with different letters are significantly different from one another (DMRT, $P < 0.05$).

observed on day 30. In T_2 and T_3 , the number of fusiform-like cells was significantly lower ($P < 0.05$) on 20th and 30th days. In T_3 , shape changes of cells occurred within 8 days after inoculation where most of the cells were oval (35.8%) and spherical (18.6%) on 20th day, and only 34.6% of fusiform cells were observed on 30th day. Morphological changes of the *M. littorale* cells on 20th and 30th days have been shown in Figure 11.

3.5.2. Effect of Different Concentrations of DRPS and BBM on the Morphology of *M. littorale* on 40th and 50th Days of the Culture Period. Fusiform-like cells on 40th and 50th days accounted for 80.49% and 68.6%, respectively, in T_4 and

63.73% and 40.5%, respectively, in T_1 . Fusiform-like cell numbers were drastically decreased ($P < 0.05$) in T_2 and T_3 on 40th and 50th days (Figure 12). In T_2 , spherical cells increased 1.45-fold from 38% to 55.43%, and in T_3 , spherical cells increased 1.31-fold from 48.47% to 63.67% on 50th day. The highest percentage of spherical cells was recorded in T_3 on 50th day. The morphological shapes of the cells changed with the increasing concentration of DRPS and culture ages. Morphological changes of *M. littorale* cells on 40th and 50th days have been shown in Figure 13.

3.5.3. Effect of Different Concentrations of DRPS and BBM on the Morphology of *M. littorale* on 60th and 70th Days of the Culture Period. As shown in Figure 14, the percentage of the spherical cells was markedly variable ($P < 0.05$) in different DRPS concentrations and BBM. The spherical morphotype showed the greatest percentage and fusiform cells exhibited the lowest percentage in three concentrations of DRPS and BBM on 60th and 70th days. In T_2 , fusiform cells decreased from 10.8% to 5%, whereas spherical cells increased from 71.6% to 90.1% from 60th to 70th days, respectively. In contrast, about 91.67% of the cells became spherical in T_3 on day 70, and the cells became clustered. During the late stationary phase, the fusiform cells in T_1 and T_4 were 20.93% and 30.3%, respectively, which were higher than the cultures grown in T_2 (5%) and T_3 (2%) on 70th day. Morphological changes of the *M. littorale* cells on 60th and 70th days have been shown in Figure 15.

4. Discussion

For the enduring upliftment of the bio-based economy, the inclusion of nutrients from the accessible waste resources is indispensable for attaining the economic liability in microalgal mass cultivation. Assessment of microalgal growth

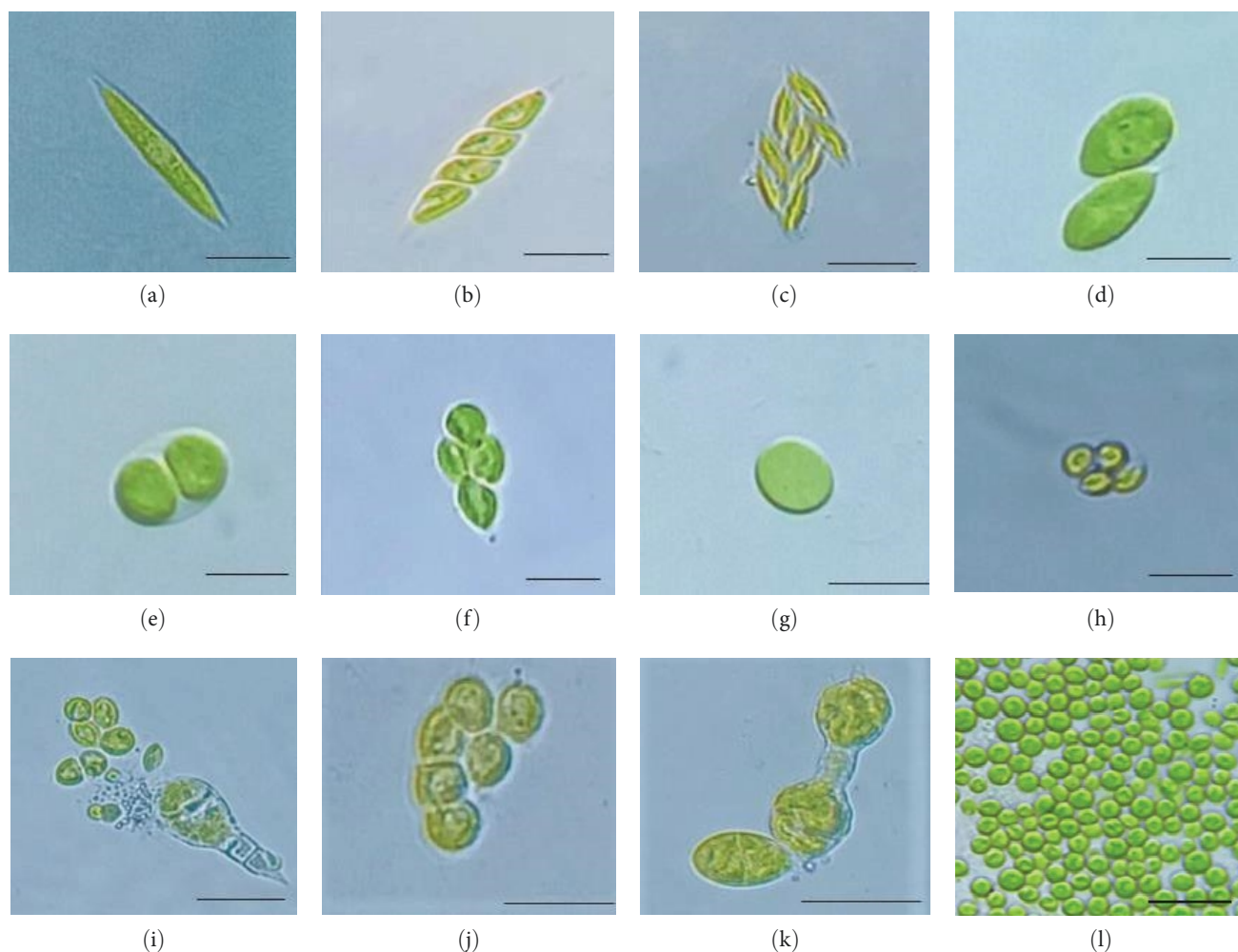


FIGURE 9: Microscopic observation of the morphological plasticity in *M. littorale*: (a) fusiform-like cells; (b) four fusiform progeny cells dividing inside a fusiform mother cell; (c) eight fusiform progeny cells dividing inside a fusiform mother cell; (d) two oval cells dividing inside a fusiform cell; (e) two spherical cells dividing inside the oval mother cell; (f) four spherical cells dividing inside the oval mother cell; (g) spherical cell; (h) four spherical cells inside the spherical mother cell; (i) releasing of spherical cells from a fusiform cell in late stationary phase; (j) releasing of spherical cells from the oval cell; (k) spherical cells from fusiform cells; (l) aggregation of spherical cells in late stationary phase. Each scale bar: $10\mu\text{m}$.

improvement via vegetable waste supplementation is one of the most reliable approaches to cost-effective cultivation mode. Therefore, it is important to explore the beneficial functions of the green alga, *M. littorale*, as well as its growth potential in supernatant of rotten potatoes for the future upliftment of aquaculture globally by conducting meticulous research. Subsequently, in this study, an evaluation of the mass culture of *M. littorale* performed under DRPS treatments showed improved biomass growth at lower concentration of DRPS. The highest cell density of *M. littorale* was recorded in T_1 and T_4 respectively on 12th day of the culture period which values were lower than other reported strains of *Monoraphidium* (*Monoraphidium* sp. CCALA; 11×10^8 cells mL^{-1}) [40], (*Monoraphidium* sp. HDMA-20; 204×10^5 cells mL^{-1}) [41], and higher than *M. littorale* (5.45×10^6 cells mL^{-1}) [42], and *Monoraphidium contortum* (2.88×10^4 cells mL^{-1}) [43]. During the experimental period, it was notable that *M. littorale*

alga was highly sensitive to higher DRPS concentrations. The reason behind this lower growth at a higher concentration of DRPS was likely to be occurred owing to the excessive availability of nutrients in the culture media. Moreover, another astaxanthin-producing green algal strain, *Scenedesmus* sp. KT-U, was also found to be shown the best growth rate in vegetable waste extract media [44]. In contrast, Tan et al. [45] unveiled the potential of utilizing organic fruit waste medium where *Chlorella vulgaris* and *Haematococcus pluvialis* yielded similar higher biomass concentrations ($4.133\text{--}4.533$ g L^{-1}) in 20% tropical fruit waste and 10% mango waste medium, respectively. In addition, during the course of the experiment, significantly higher chlorophyll-a content, OD, and total biomass were observed in T_1 , which indicated that a lower concentration of DRPS did not impede the photosynthetic yield of *M. littorale*. In view of the above-mentioned facts, it appears that culture media made from rotten potatoes especially the

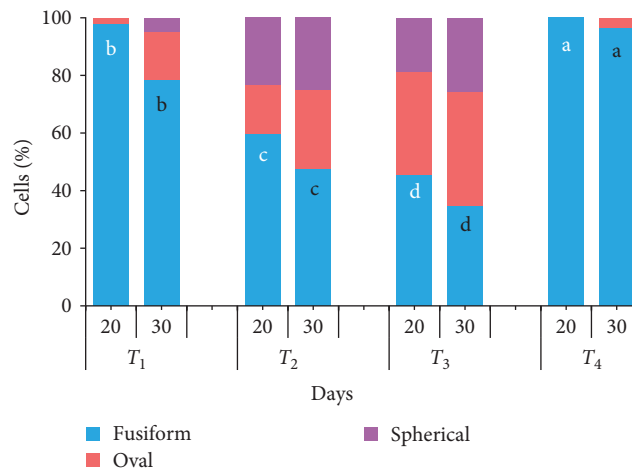


FIGURE 10: Morphology of *M. littorale* grown in different DRPS concentrations and BBM ($T_1 = 25\%$ concentration of DRPS; $T_2 = 50\%$ concentration of DRPS; $T_3 = 75\%$ concentration of DRPS; $T_4 = \text{BBM}$) on 20th and 30th days. Means with different letters (bold white letters for 20th day and black letters for 30th day) are significantly different from one another (DMRT, $P < 0.05$).

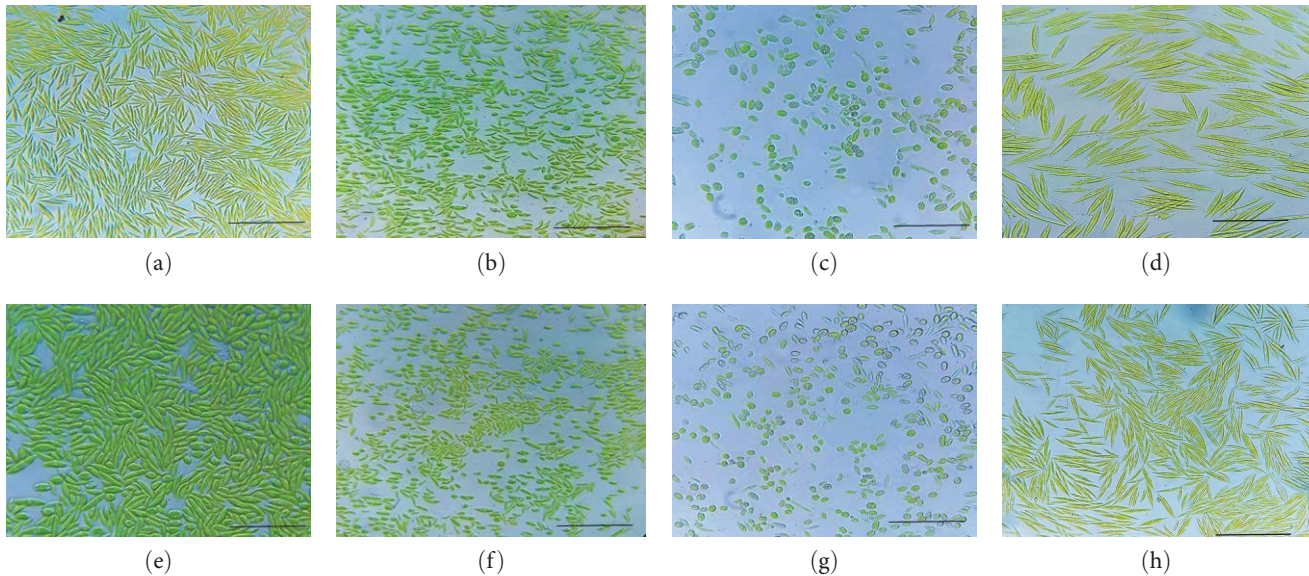


FIGURE 11: Morphological changes of *M. littorale* at the early stationary phase where (a–d) depict phenotypic changes of cells in the four treatments ($a = T_1$, 25% of DRPS; $b = T_2$, 50% of DRPS; $c = T_3$, 75% of DRPS; $d = T_4$, BBM) on 20th day and (e–h) depict phenotypic changes of cells in the four treatments ($e = T_1$, 25% of DRPS; $f = T_2$, 50% of DRPS; $g = T_3$, 75% of DRPS; $h = T_4$, BBM) on 30th day of the culture period. (Each scale bar: 10 μm ; 40 \times magnification; B-510BT OPTIKA, Italy).

lower concentration of DRPS (25%) can make a colossal impact on the growth of *M. littorale* at the marginal stage.

The biomass content of *M. littorale* based on the dry weight in T_1 , T_2 , T_3 , and T_4 was recorded as 1.47, 0.77, 0.61, and 1.42 g L^{-1} , respectively, which clarified a simultaneous declining trend of total biomass in the higher concentration of DRPS. Probably, the downfall in growth was endorsed by the diminution of chloroplasts and voluminous damage to the photosynthetic machinery [46]. The biomass content found in BBM (1.42 g L^{-1}) of newly isolated microalga, *M. littorale*, was higher than the strains *Monoraphidium* KMNS (0.65 g L^{-1}) [47] and *M. contortum* (0.095 g L^{-1}) [43] and lower than the strain

Monoraphidium sp. QLY-1 (5.54 g L^{-1}) [19]. Moreover, Mishra and Mohanty [30] reported that while *Monoraphidium* sp. KMC4 was grown in raw domestic sewage wastewater, superior biomass production (1.47 \pm 0.08 g L^{-1}) was obtained, which was similar to our recorded maximum biomass value (1.47 g L^{-1}) of *M. littorale* in T_1 . Furthermore, Zhang et al. [48] reported that *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 showed optimal biomass production of 0.42 and 0.55 g L^{-1} , respectively, under anaerobically digested effluent from kitchen waste which was quite lower than the present findings. In addition, Giwa et al. [49] demonstrated that *C. vulgaris* showed maximum biomass production in

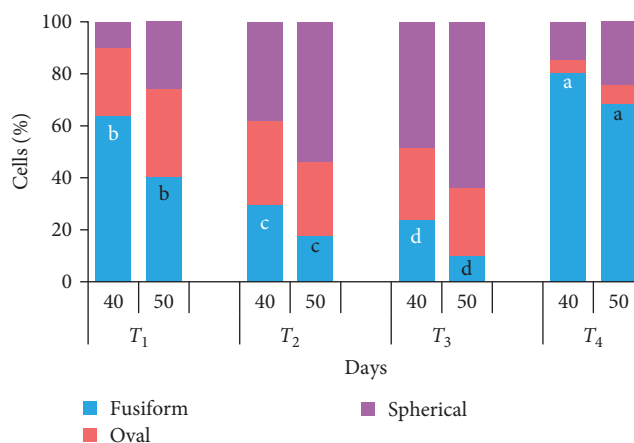


FIGURE 12: Morphology of *M. littorale* grown in different DRPS concentrations and BBM (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM) on 40th and 50th days. Means with different letters (bold white letters for 40th day and black letters for 50th day) are significantly different from one another (DMRT, $P < 0.05$).

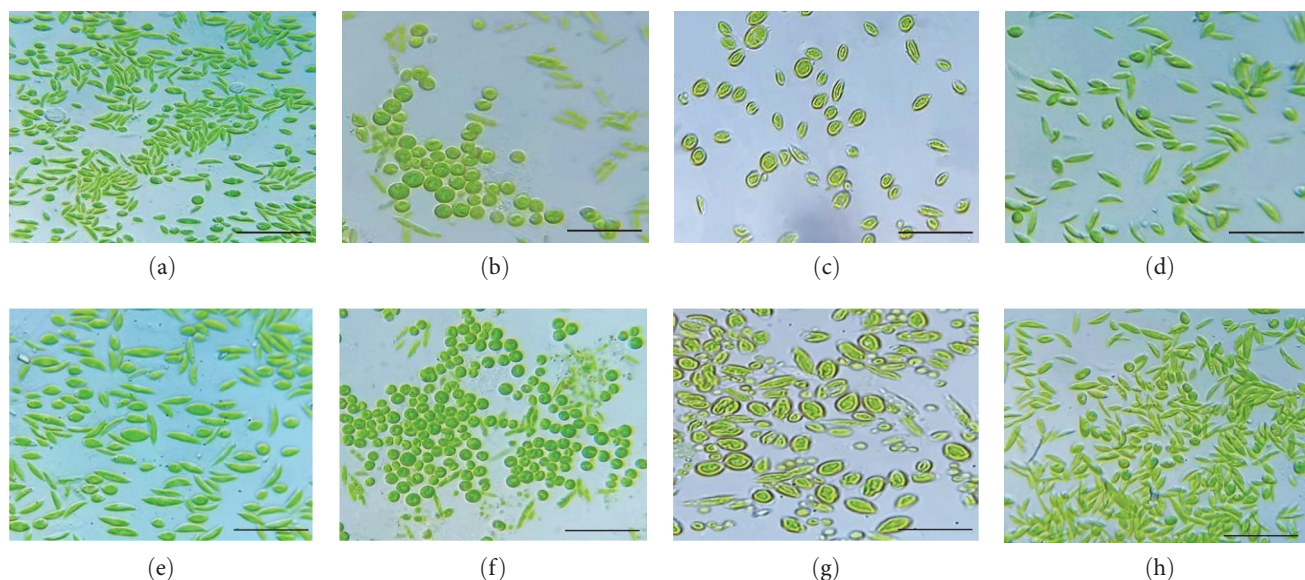


FIGURE 13: Morphological changes of *M. littorale* at the mid-stationary phase where (a–d) depict phenotypic changes of cells in the four treatments ($a = T_1$, 25% of DRPS; $b = T_2$, 50% of DRPS; $c = T_3$, 75% of DRPS; $d = T_4$, BBM) on 40th day and (e–h) show phenotypic changes of cells in the four treatments ($e = T_1$, 25% of DRPS; $f = T_2$, 50% of DRPS; $g = T_3$, 75% of DRPS; $h = T_4$, BBM) on 50th day of the culture period. (Each scale bar: 10 μm ; 40 \times magnification; B-510BT OPTIKA, Italy).

digested food waste and brine than control (Johnson's medium). Furthermore, Habib et al. [34] recorded that *S. platensis* produced the highest amount of biomass $1135.65 \pm 9.56 \text{ mg L}^{-1}$ while cultured in 50% digested rotten tomato supernatant which is far lower than our study. Thus, the supernatant of digested rotten potato will be a novel medium for getting huge biomass from *M. littorale*. Pursuant to the literature, the microalgae *Monoraphidium* spp. mostly spectacularize a wide level of total lipids, and proteins in amounts between 19%–35% and 28%–45% consecutively [50]. Yee [18] also reported that the lipid content of *Monoraphidium* may vary from 19.9% to 43.5% on a dry weight basis and the lipid content can reach up to 56% relying on the

culture ambiance and mode of cultivation [51]. In the present study, both the maximum value of lipid content and protein content was recorded in T_1 , although the lipid and protein contents in T_2 and T_3 were statistically identical. Both the lipid content and protein content of T_1 recorded in the present study were close to the value reported in the literature by Díaz et al. [50] which was higher than the reported lipid content of *Monoraphidium* sp. QLY-1 (22.4%) [19], *Monoraphidium neglectum* SAG48.87 (17.8%) [52], and *Monoraphidium* sp. Dek19 (26.4%) [53] but lower than *Monoraphidium* sp. (28.92%) [54]. While, in contrast, decreased lipid accumulation was evident in T_4 which was 2.1-fold lower than T_1 but higher than the recorded value of

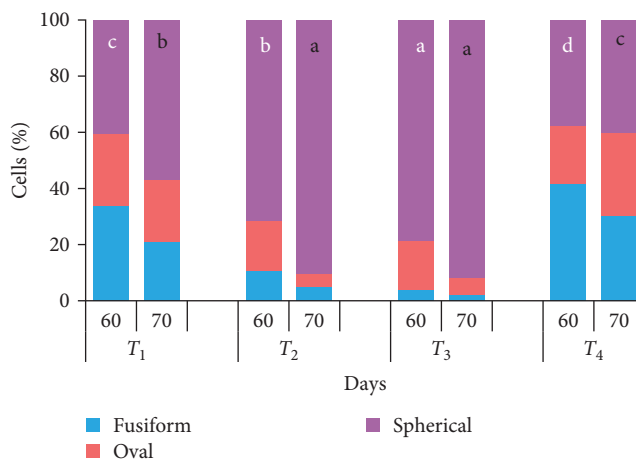


FIGURE 14: Morphology of *M. littorale* grown in different DRPS concentrations and BBM (T_1 = 25% concentration of DRPS; T_2 = 50% concentration of DRPS; T_3 = 75% concentration of DRPS; T_4 = BBM) on 60th and 70th days. Means with different letters (bold white letters for 60th day and black letters for 70th day) are significantly different from one another (DMRT, $P < 0.05$).

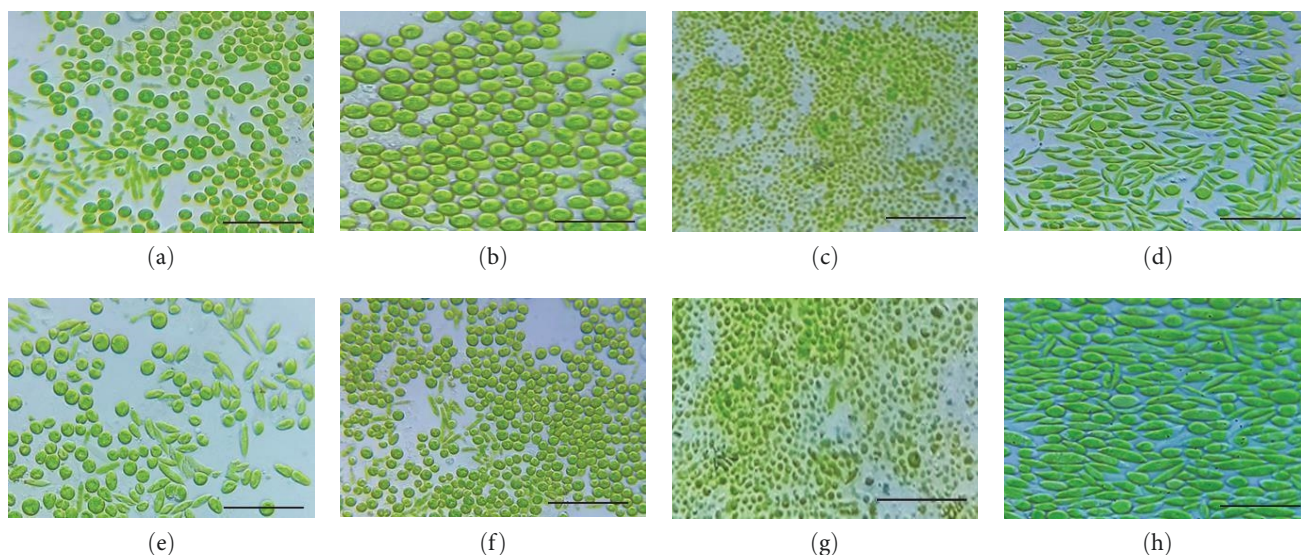


FIGURE 15: Morphological changes of *M. littorale* at the late-stationary phase where (a–d) depict phenotypic changes of cells in the four treatments ($a = T_1$, 25% of DRPS; $b = T_2$, 50% of DRPS; $c = T_3$, 75% of DRPS; $d = T_4$, BBM) on 60th day, and (e–h) show phenotypic changes of cells in the four treatments ($e = T_1$, 25% of DRPS; $f = T_2$, 50% of DRPS; $g = T_3$, 75% of DRPS; $h = T_4$, BBM) on 70th day of the culture period. (Each scale bar: 10 μm ; 40 \times magnification; B-510BT OPTIKA, Italy).

M. griffithii NS16 (9.24%) [55] and lower than the *Monoraphidium* sp. KMN5 (35%) [47] grown in BBM. In another study, Dong et al. [31] recorded the highest lipid content (49.54%) of *Monoraphidium* sp. QLZ-3 in walnut shell extract media which was quite higher than in our study. Likewise, *Schizochytrium mangrovei* and *Chlorella pyrenoidosa* also showed elevated biomass production with a higher accumulation of lipids and proteins in food waste hydrolysate as a growth medium than in a conventional medium [56]. In another study, *C. vulgaris* (CPC 90) grown in vegetable waste media exhibited higher content of lipids [57]. Indicatively, the elevated protein content of 37.8% and lipid content of 26.4% were also found in *C. vulgaris* when cultivated under municipal food waste [58]. Wang et al. [59] also reported that

Chlorella sp. grew very well in food waste hydrolysate along with producing higher lipid content of 2.5 g L^{-1} . Considering the above-mentioned results, it is an absolute necessity to explore the latent applicability of nutrient-rich bio-wastes including agricultural wastes, vegetable wastes, food wastes, and fruit wastes for their timely and efficient utilization in triggering microalgal cultivation that can pave the way for feasible improved production with proven efficient nutrient recovery [60, 61].

Morphological changes are crucial for algae to survive in harsh environmental conditions [62]. This transformation can be triggered by media content, temperature, salinity, and pH variation in culture conditions [63]. In the present study, it was worth noting that the morphology of *M. littorale*

was found to be affected rapidly in T_3 and then T_2 during the experimental period. In contrast, T_1 and T_4 showed very slower morphological changes within the 70 days of the culture period than T_3 and T_2 . In these cultures, fusiform-like cells of *M. littorale* were reduced from 98% to 20.93% in T_1 and 100% to 30.3% in T_4 from day 20 to 70, respectively. In T_2 and T_3 , oval and spherical cells were evident in the early stationary phase, whereas late stationary phase cultures consisted of almost 90% of spherical cells. This is similar to the findings of Wang et al. [26] who demonstrated that fusiform cells of *Monoraphidium* were found markedly in the exponential phase, whereas spherical cells were observed predominantly in the late stationary phase. Moreover, during the late stationary phase, spherical cells of *M. littorale* were observed to be aggregated which may be occurred due to the secretion of a gelatinous substance from spherical cells [26]. It is worth noting that the morphology of *M. littorale* was found to be affected markedly by a high concentration of DRPS. This result also suggests that medium content is the major factor influencing morphotype alternation of *M. littorale*.

Therefore, the isolated green alga, *M. littorale*, grew under rotten potatoes may be a particularly promising source of algal feed for enhancing the growth of zooplankton and freshwater fish larvae in the hatchery by dint of their higher adaptability to wide environmental conditions as well as superior nutritional composition.

5. Conclusion

In conclusion, the result of the study showed that DRPS had a significant effect on the growth of *M. littorale* by producing higher biomass. The study noted that a lower concentration of DRPS (25%) supported the best growth of *M. littorale* in terms of cell density, growth rate, OD, and total biomass. Furthermore, *M. littorale*, grown in 25% DRPS concentration, showed higher content of crude lipid and crude protein that makes this species more potential for industrialization for the development of a bio-based economy. Moreover, the morphology of the cells of *M. littorale* was also found to be significantly affected by the elevating concentration of DRPS and very rapid alternation of the morphology of *M. littorale* was evident at 75% and 50% DRPS. Hence, a concentration of 25% DRPS is best for the growth of *M. littorale*. Due to the cost effectiveness of DRPS, marginal farmers can also easily culture *M. littorale* and utilize it as the first feed for rearing fish larvae directly or indirectly in aquaculture. Furthermore, the potential of this algal species can also be extended along with its successive utilization in aquaculture for rearing zooplankton and fish larvae. Hence, mass culture development of *M. littorale* with DRPS will decrease the total cost of microalgal production along with improving the growth and nutritional content of microalgae.

Data Availability

The data sets in this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jinnath Rehana Ritu took care of methodology, software, formal analysis, writing of original draft, and writing of review and editing. Saleha Khan took care of conceptualization, methodology, supervision, resources, investigation, validation, writing of review and editing, project administration, and funding acquisition. Md. Sakhawat Hossain and Md. Mahfuzul Haque took care of writing of review and editing.

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