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Research article

From waste to resource: Effects of digested rotten potato supernatant on the growth, total biomass and nutrient composition of *Chlorella vulgaris*

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ABSTRACT

A study was carried out to evaluate the growth performance of *Chlorella vulgaris*, a green microalga, in three different concentrations of digested rotten potato supernatant (DRPS) for 16 days. *C. vulgaris* was grown in 20 % (T_1), 40 % (T_2), and 60 % (T_3) of the DRPS and at the same time in Bold Basal Medium (BBM) as a control (T_4). A significantly highest cell density of *C. vulgaris* was found in T_1 ($192.83 \pm 1.75 \times 10^5$ cells mL^{-1}) in comparison to T_2 ($136.83 \pm 5.58 \times 10^5$ cells mL^{-1}), and T_3 ($99.11 \pm 5.38 \times 10^5$ cells mL^{-1}) ($p < 0.001$ for all comparisons) while the cell density at T_1 ($192.83 \pm 1.75 \times 10^5$ cells mL^{-1}) and T_4 ($180.907 \pm 4.58 \times 10^5$ cells mL^{-1}) did not differ significantly ($p = 0.227$). Moreover, the mean daily division rate of *C. vulgaris* was significantly higher in T_1 (0.340 ± 0.001 divisions day^{-1}) in comparison to other concentrations of DRPS ($p < 0.001$ for all comparisons). The maximum value of total biomass (1.07 ± 0.10 g L^{-1}) was found in T_1 which was statistically similar to those in T_4 and T_2. In addition, there was no significant difference between the mean maximum values of chlorophyll-*a* content and optical density of *C. vulgaris* in T_1 and T_4. The highest protein content of 42.67 ± 0.57 % was observed in T_4 which was significantly higher than T_1 (39.43 ± 1.67 %) ($p = 0.027$). It is also worth mentioning that there was no significant difference in the crude lipid content of the microalgae grown in T_1 (10.06 ± 0.17 %) and T_4 (9.88 ± 0.14 %) ($p = 0.616$). Hence, 20 % DRPS can be used as an alternative culture media of BBM for *C. vulgaris* with a broad aim to accelerate the sustainable advancement of microalgal production.

1. Introduction

The microalgal culture industry has been rapidly growing, particularly considering their demand for the production of nutraceuticals, and increased utilization in food and feed applications [1]. Microalgae are the biological starting point of energy flow through the aquatic food chain [2]. They are widely found in both marine and freshwater environments and play a significant role as a crucial food for zooplankton, shellfish, the larvae of crustaceans, and finfishes [3–5]. There are diverse species of astaxanthin-producing green microalgae that have great prospects in aquaculture [6] and are importantly utilized because they are

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rich in health-promoting biomolecules such as immunostimulants, growth enhancers, and anti-inflammatory agents [2,7]. Furthermore, microalgae are recognized as one of the promising sources of biofuel due to their higher ability to produce biomass within a short time and higher lipid content [8,9].

The freshwater microalga *C. vulgaris* is a green microalga that is rich in pigments, essential amino acids [7], and the beneficial phytonutrient chlorella growth factor (CGF) [10], which has great commercial importance due to its overall nutrition qualities such as high protein content, low fat, minerals, and antioxidants [11], anti-cancer properties [12], as well as high digestibility properties [13]. Its utilization in the aquaculture sector is regarded as a sustainable and environmentally beneficial source because of its outstanding nutritional properties [7,14]. In addition, *C. vulgaris* is also used to enhance body composition, immunological biomarker responses, growth performance, and feed utilization in prawns, resulting in disease resistance [15]. Also, it has carotenoids, which can improve the pigmentation of the skin colour of ornamental fish and the colour of the muscle in food fish [16]. Moreover, *C. vulgaris* is considered one of the most important microalgae in aquatic bioremediation techniques [17]. Furthermore, considering the reduction in phosphate and total nitrogen levels upon the integration of *C. vulgaris* in aquaculture, there is potential for the microalgae to serve as a means to mitigate pollution within aquaculture effluent [18,19]. *C. vulgaris* is also utilized as a natural antibiotic that may be a promising alternative to conventional synthetic drugs with a broader spectrum of activities against pathogenic infections [7,20], and exhibits other health-enhancing qualities such as anti-tumor, antiviral, and potent immunomodulation, indicating the potential for medical uses [21]. Consequently, *C. vulgaris* holds substantial commercial value and interest, necessitating large-scale production to fully unlock its myriad possibilities.

One of the main obstacles to the large-scale production of microalgae is the costly chemical medium [22]. To address this situation, the goal of this study was to use vegetable waste to prepare low-cost culture media. It was expected that *C. vulgaris* could be cultured using an inexpensive medium (DRPS). Therefore, from an economic standpoint, microalgae in digested rotten potato supernatant may have a lot of prospects.

According to the Bangladesh Bureau of Statistics (BBS), Bangladesh produced 9.887 million tons of potatoes in 2021, which was higher than the production (9.606 million tons) in the previous year, 2020 [23]. Despite the rising potato production in Bangladesh, cold storage facilities are facing challenges in maintaining quality and preserving substantial quantities of potatoes, resulting in a considerable amount being spoiled [24]. Instead of commercial media (eg., BBM), potato waste might be utilized as the raw material in the production of microalgal culture medium. In previous studies, the use of DRPS improved the growth of *Spirulina platensis* [25]. Moreover, Ritu et al. [8] explored that a lower concentration of DRPS (25 %) increased biomass production and the lipid content of *Monoraphidium littorale* compared to commercial Bold Basal Media. Therefore, the purpose of this study was to assess how DRPS affects the growth of *C. vulgaris*. This experiment hypothesized that DRPS will increase the growth, total biomass and nutrient composition of *C. vulgaris*. Therefore, the objective of the experiment was to investigate the feasibility of utilizing DRPS for the cultivation of *C. vulgaris*, while assessing its growth, total biomass production and nutrient composition.

2. Materials and methods

2.1. Isolation of microalgae and media preparation

C. vulgaris was collected from freshwater ponds beside the Faculty of Fisheries, Bangladesh Agricultural University, Bangladesh. Isolation of pure cells of *C. vulgaris* was accomplished by streaked agar plating and the serial dilution procedure. *C. vulgaris* stock cultures were grown in Bold Basal Medium (BBM) at temperature 26 ± 2 °C, light intensity 60 μmol m^{-2} s^{-1} and photoperiod 12:12 h, L:D.

Table 1
Composition of the chemicals for preparation of stock solution required for BBM that was used for *Chlorella vulgaris* culture[a].

No.	Chemicals	g L^{-1}
1	$NaNO_3$	25.00
2	$MgSO_4$. $7H_2O$	7.50
3	NaCl	2.50
4	K_2HPO_4	7.50
5	KH_2PO_4	17.50
6	$CaCl_2$. $2H_2O$	2.50
7	Trace Elements	
	$ZnSO_4$. $7H_2O$	4.42
	$MnCl_2$. $4H_2O$	1.44
	MoO_3	0.71
	$CuSO_4$. $5H_2O$	1.57
	Co $(NO_3)_2$.$6H_2O$	0.49
8	H_3BO_3	11.40
9	EDTA-KOH solution:	
	EDTA Na_2	50.00
	KOH	31.00
10	$FeSO_4$.$7H_2O$ with 1.0 mL Concentrated H_2SO_4	4.98

[a] Z.H. Scientific and Chemicals Mart, Dhaka, Bangladesh is the supplier of the above-mentioned chemicals.

To prepare BBM, 10 mL of each of the first six mentioned ingredients of the stock solutions (Sl Nos. 1 to 6), and 1 mL of each of the remaining four stock solutions (Sl Nos. 7 to 10) listed in Table 1 were mixed in a 1 L volumetric flask and made up to 1 L by adding distilled water (DW). The pH of the BBM media was 8.43 ± 0.15. It was then sterilized at 120 °C for 15 min using an autoclave (Model SS-V35HD, WINCOM, China).

Partially or fully rotten potatoes (dark spots or bruises can form on the surface, indicating softness and slight discolouration) were collected from the commercial market, Bangladesh Agricultural University Campus, Mymensingh, Bangladesh. After that the rotten potatoes were mashed and digested at 50 g L^{-1} with continuous aeration in a 10 L glass jar for 22 days. When the colour of the solution turned light reddish, it indicated the completion of digestion. At this stage, all the rotten potatoes were thoroughly macerated and degraded into soluble compounds. The digestion period of rotten potatoes depends on the nature of the rotten potatoes used (chopped or blended paste). We didn't use any enzyme for the digestion process. After obtaining a light reddish-coloured supernatant, particulate materials were removed using a net with a mesh size of 30 μm. The physicochemical properties of the DRPS were analyzed. Then, 9.0 g L^{-1} sodium bicarbonate ($NaHCO_3$) was added to DRPS as an additional carbon source and 0.20 mL L^{-1} micronutrient solution (Table 2) as essential trace elements necessary for proper growth and photosynthesis of the microalgae. Following DRPS preparation, three DRPS levels [20 % (200 mL DRPS + 800 mL DW), 40 % (400 mL DRPS + 600 mL DW, and 60 % (600 mL DRPS + 400 mL DW)] were prepared with three replications of each to examine the development of *C. vulgaris*.

2.2. Analyses of proximate composition of the rotten potato and C. vulgaris

Horwitz's standard technique was used to investigate the proximate composition of the rotten potatoes, including moisture, crude protein, crude lipid, crude fiber, ash, and nitrogen-free extract (NFE) [26]. A similar technique was used for the determination of the crude protein and crude lipid content of *C. vulgaris*. The standard micro-Kjeldahl nitrogen method was used to estimate the crude protein content utilizing a Behrotest® InKjel M digesting device and Behr S 1 steam distillation apparatus (both Labor-Technik GmbH, Dusseldorf, Germany). Before 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:

Percent (%) crude protein = % Nitrogen × Conversion factor (5.85 for plant-based sample)

% Nitrogen = [Milliequivalent of N_2 × Strength of HCl × Titrant used (mL)/Weight of sample (g)] × 100

Where, Milliequivalent of N_2 = 0.014; Strength of HCl = 0.2 N.

For the analysis of the crude lipid content, samples were dried in the oven at 105 °C and then extracted the fat with acetone in a Soxhlet Extractor for near about 4 h. The crude lipid content of the sample was estimated by using the equation:

Percent (%) crude lipid content = {(D – B)/A} × 100

Where, D = Weight of crude lipid with the beaker; B = Empty beaker weight, and A = Weight of sample.

2.3. Growth conditions of C. vulgaris

C. vulgaris was cultivated using T_1 (20 %), T_2 (40 %), T_3 (60 % of DRPS), and T_4 (BBM, as a control) media (Fig. 1). The initial cell concentration in each treatment was 1.36 x 10^5 cells mL^{-1}, sourced from a culture in the logarithmic phase maintained in BBM. The experiment was carried out in 1000 mL Erlenmeyer flasks holding 700 mL of culture medium with three replications in each treatment under a light intensity of 60 μmol m^{-2} s^{-1} at a photoperiod of 12:12 h, L:D, with continuous aeration for 16 days. A Sedgwick-Rafter Chamber was used to count the cell density immediately after inoculation and on every other day for up to 16 days.

The number of cell divisions per day (K) for the 10-day growing period was determined using the following equation [27].

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

Where, C_t and C_0 are the cell concentrations at times t and 0 respectively.

Table 2
Composition of the micronutrient solution[a].

No.	Chemicals	g L^{-1}
1	H_3BO_4	2.86
2	$MnCl_2$. $4H_2O$	1.81
3	$ZnSO_4$. $7H_2O$	0.22
4	$CuSO_4$. $5H_2O$	0.08
5	MoO_3	0.01
6	$CoCl_2$. $6H_2O$	0.01

[a] Z.H. Scientific and Chemicals Mart, Dhaka, Bangladesh is the supplier of the above-mentioned chemicals.

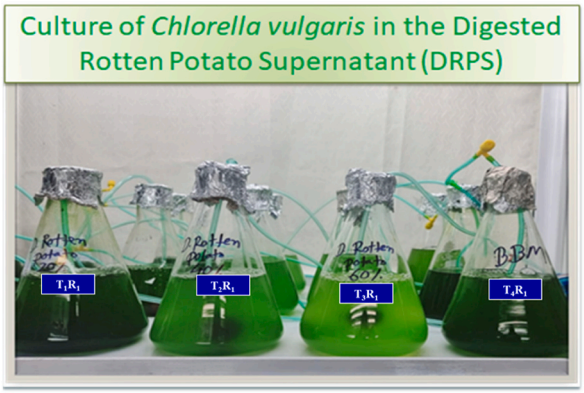

Fig. 1. Growth condition of *Chlorella vulgaris* in three concentrations of digested rotten potato supernatant ($T_1 = 20$ %, $T_2 = 40$ %, and $T_3 = 60$ %) and in bold basal media (T_4) on the 12th day of the growing period (exponential phase).

2.4. Determination of optical density, chlorophyll-a content and microalgal biomass produced

To estimate the optical density (OD) and the chlorophyll-*a* content, 15 mL of the *C. vulgaris* sample was filtered using Whatman GF/C glass microfiber filter paper (0.45 μm mesh size and 47 mm diameter) and kept in 20 mL tubes mixed with 10 mL of 100 % acetone, ground with a glass rod and kept in a refrigerator overnight (4 °C). The refrigerated samples were homogenized for 2 min before centrifugation at 4000 rpm for 10 min. To determine the chlorophyll-*a* concentration, the absorbance at 630 nm, 647 nm and 664 nm wavelengths and for the optical density determination, the absorbance at 620 nm wavelength was measured using a UV spectrophotometer (T60U-UV-Visible Spectrophotometer, 2 nm spectral bandwidth, Brand: PG Instruments, Model: MO00330001) against a blank at the room temperature of 21 ± 1 °C.

Chlorophyll-*a* content was calculated using the following formula [28]:

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

For measuring microalgal biomass concentration at the end of the experiment, 20 mL of the *Chlorella vulgaris* suspension sample was filtered through a Whatman GF/C glass microfiber filter paper (0.45 μm pore size and 47 mm diameter). Each loaded filter paper was dried in an oven at 90 °C until it reached a constant weight. The biomass was determined by subtracting the dried blank filter paper weight taken before use from the loaded filter paper weight after drying and calculated as g L^{-1} using the following equation.

$$\text{Microalgal Biomass } (\text{g/L}^{-1}) = \{(W_2 - W_1) / \text{Sample (mL)}\} \times 1000$$

Where, W_2 is the weight of the dried sample with the filter paper in g, and W_1 is the weight of the filter paper in g before use.

2.5. Cost analysis in preparation of BBM and DRPS media

A simple economic analysis was done to compare the preparation cost of the Bold Basal Media (BBM) and the Digested Rotten Potato Supernatant (DRPS) media used for the culture of the microalga *Chlorella vulgaris*. The production costs of the media were calculated according to the local price of the inputs (chemicals). The cost of the distilled water produced in the laboratory, glassware used during digestion of the rotten potato, electricity consumed etc. were not considered, and no cost was required for rotten potato.

2.6. Statistical analysis of the data

To normalize the percentage data, we performed an arcsin square root transformation on the proportion data of crude protein and crude lipid content (%) of *C. vulgaris*. All the data were normally distributed (Shapiro-Wilk test, $0.836 \leq W \leq 1.000$, $0.205 \leq p \leq 1.00$), had equal variances (Levene's Test, $\leq F_{3,8} \leq 0.578$ and $p = 0.68$) and were independent. We performed one-way ANOVA test to determine whether *C. vulgaris* grown under 3 different concentrations of the DRPS and BBM differed in their mean maximum cell density, total biomass, mean daily division rates, mean maximum chlorophyll-*a*, optical density, crude lipid and protein content (%) followed by pairwise post-hoc comparisons using a Tukey HSD correction. All statistical analyses were conducted using SPSS software (version 25.1, IBM SPSS Inc.). All tests were 2-tailed and the α was set at 0.05.

3. Results

3.1. Proximate composition of the rotten potato

The proximate composition of the utilized rotten potato was analyzed on a dry matter basis. The crude protein, crude lipid, ash,

crude fiber, and nitrogen-free extract (NFE) content in the rotten potatoes were 12.93 ± 0.04 %, 10.48 ± 0.31 %, 17.93 % ± 0.15, 20.92 ± 0.11 %, and 37.74 ± 0.09 %, respectively.

3.2. C. vulgaris isolation and identification

During the isolation period, after streaking agar plates several times, an unidentified green microalga was observed, which was then serially diluted in test tubes to obtain a pure culture. Moreover, using the microscope (B-510BT OPTIKA, Italy), we observed that the isolated microalga was morphologically similar under the Chlorellaceae family, closely matched *C. vulgaris*, and had ellipsoidal and spherical shapes without flagella or mucilage around the cell (Fig. 2).

3.3. Physico-chemical properties of the supernatant of the digested rotten potato

The physicochemical properties of the rotten potatoes before and after the digestion process are shown in Table 3. The physico-chemical properties of the digested rotten potato supernatant (DRPS) at different concentrations (20 %, 40 %, 60 %) and the BBM exhibited distinct trends (Table 4). The temperature observed in all concentrations of the DRPS and in the BBM were relatively close, ranging between 23.15 °C and 23.47 °C. DRPS showed a trend of increasing pH with higher concentrations, while the BBM displayed a decreasing trend in pH. A slight decrease in dissolved oxygen (DO) was observed in the DRPS with higher concentrations but all the values were in optimum range. The DRPS exhibited a substantial increase in electric conductivity (EC) and total dissolved solids (TDS) with rising concentrations, whereas the BBM consistently displayed lower values compared to DRPS. A clear increase in available nitrogen content was evident with higher concentrations of DRPS. Similar to nitrogen, available phosphorus content also demonstrated a substantial rise with increasing concentrations of DRPS, with BBM having notably higher values compared to DRPS.

3.4. Cell density, mean daily division rate and biomass production of Chlorella vulgaris

The cell densities of *C. vulgaris* in different treatments with culture days are shown in Fig. 3a. The highest cell density of *C. vulgaris* was recorded in T_1 (192.83 ± 1.75 × 10^5 cells mL^{-1}) followed by T_4 (180.91 ± 4.58 × 10^5 cells mL^{-1}), T_2 (136.83 ± 5.58 × 10^5 cells mL^{-1}) and in T_3 (99.11 ± 5.38 × 10^5 cells mL^{-1}). Notably, the peak cell density in T_1 and T_4 was found on the 10th day of culture, while in T_2 and T_3 it was on the 12th day of culture. The cell densities of *C. vulgaris* varied between three different concentrations of DRPS and BBM (One-way ANOVA, $F_{3,\ 8} = 273.63$, $p < 0.001$) (Fig. 3b). A significantly highest cell density of *C. vulgaris* was reported in T_1 among the treatment groups (T_1 vs T_2: $p < 0.001$, T_1 vs T_3: $p < 0.001$), while the cell density at T_1 and T_4 did not differ ($p = 0.227$).

Indicatively, the total biomass production of *C. vulgaris* also varied between treatment groups (One-way ANOVA, $F_{3,\ 8} = 7.305$, $p = 0.011$) (Fig. 3c). There was no significant difference in the total biomass of *C. vulgaris* between T_1 and T_2 as well as between T_1 and T_4 (T_1 vs T_2: $p = 0.118$, T_1 vs T_4: $p = 0.980$), while significantly lower density found in T_3 in comparison to T_1 ($p = 0.016$). Additionally, it was evident that the higher DRPS concentrations led to reduced total biomass production in T_2 and T_3.

In addition, the mean daily division rates plotted as a function of different concentrations of DRPS and BBM are shown in Fig. 4. The best growth rate of *C. vulgaris* was observed jointly in T_1 and T_4, both showed the same division rate of 0.340 ± 0.001 divisions day^{-1} ($p = 0.919$). This growth rate was significantly higher in comparison to T_2 (0.285 ± 0.005 divisions day^{-1}) and T_3 (0.239 ± 0.010 divisions day^{-1}) ($p < 0.001$ for all comparisons).

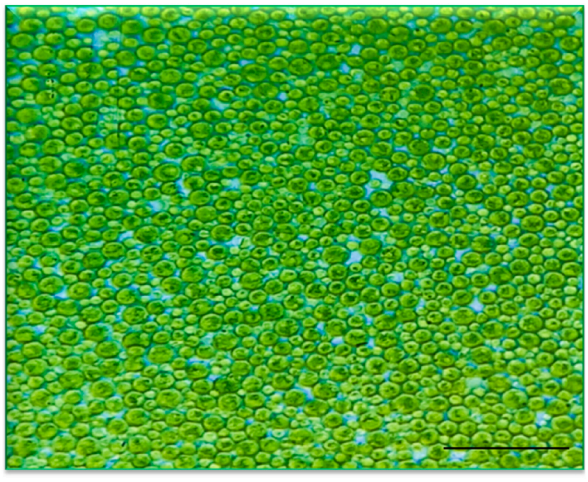

Fig. 2. Pure cells of the green microalga, *Chlorella vulgaris* (scale bar: 10 μm; 40 × magnification; B-510BT OPTIKA, Italy). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Observed physicochemical properties (mean ± SD) of the liquid rotten potato before digestion and the digested rotten potato supernatant (DRPS).

Sl. No.	Characteristics	Liquid rotten potato before digestion	Digested rotten potato supernatant (DRPS)
1	Colour	Reddish	Light Reddish
2	Temperature (°C)	24.30 ± 2.30	23.40 ± 1.30
3	pH	9.20 ± 0.12	10.45 ± 0.05
4	Total solids (mg L^{-1})	2212.00 ± 5.14	1734 ± 9.45
5	Available N (NO_3–N) (mg L^{-1})	18.56 ± 0.45	33.45 ± 0.19
6	Available P (PO_3–P) (mg L^{-1})	36.45 ± 0.06	58.22 ± 0.06

Table 4
Observed physico-chemical properties (mean ± SD) of the three different digested rotten potato supernatant (DRPS) media and the BBM during the culture of *Chlorella vulgaris*.

Parameters	Concentrations of DRPS and BBM			
	20 %	40 %	60 %	BBM
Temperature (°C)	23.41 ± 0.14	23.34 ± 0.15	23.15 ± 0.16	23.47 ± 0.11
pH	9.38 ± 0.13	10.11 ± 0.17	10.22 ± 0.13	8.43 ± 0.15
DO (mg L^{-1})	6.16 ± 0.15	5.51 ± 0.14	5.15 ± 0.05	6.31 ± 0.15
Electric conductivity (μS cm^{-1})	1034.74 ± 0.15	2108.78 ± 9.65	3106.19 ± 9.87	776.22 ± 2.33
Total Dissolved Solids (mg L^{-1})	672.31 ± 9.61	1043.33 ± 0.07	1723.93 ± 9.19	399.85 ± 7.82
Available N (NO_3–N) (mg L^{-1})	11.67 ± 0.07	19.67 ± 0.15	28.32 ± 0.07	41.67 ± 0.07
Available P (PO_4–P) (mg L^{-1})	20.23 ± 0.12	31.75 ± 0.06	42.00 ± 0.05	106.22 ± 0.04

3.5. Effects on the chlorophyll-a content and optical density

The values of chlorophyll-*a* and OD showed the same pattern as the growth of *C. vulgaris* in terms of cell density and growth rate (Fig. 5). There was a statistically significant effect of treatment groups on the mean maximum values of chlorophyll-*a* content (One-way ANOVA, $F_{3,\ 8} = 108.151$, $p < 0.001$) and optical density of *C. vulgaris* (One-way ANOVA, $F_{3,\ 8} = 488.500$, $p < 0.001$). Among the four treatments, T_1 appeared to support the significantly best production of chlorophyll-*a* with a value of 16.107 ± 1.285 mg in comparison to T_2 and T_3 (T_1 vs T_2: $p < 0.001$, T_1 vs T_3: $p < 0.001$), while there was no significant difference of chlorophyll-*a* content between T_1 and T_4 ($p = 0.727$) (Fig. 5b). In T_2 and T_3 the chlorophyll-*a* content was much lower due to significantly lower growth of *C. vulgaris* than in T_1 and T_4, and it was possibly for the reason of higher DRPS concentrations in T_2 and T_3.

The OD of *C. vulgaris* was found to be decreased with the increase of the DRPS concentration (Fig. 5c). In T_1 (1.536 ± 0.050) and T_4 (1.516 ± 0.030), the OD was significantly higher than in T_2 (0.863 ± 0.025) and T_3 (0.693 ± 0.015) ($p < 0.001$ for all comparisons) (Fig. 5d). At T_2 and T_3, low values of optical density were distinguished along with low growth of *C. vulgaris*.

3.6. Effects on the crude protein and crude lipid content of C. vulgaris

The crude protein and lipid content of *C. vulgaris* were found to be varied between the treatment groups (crude protein: One-way ANOVA, $F_{3,\ 8} = 54.643$, $p < 0.001$; crude lipid: One-way ANOVA, $F_{3,\ 8} = 206.645$, $p < 0.001$) (Fig. 6). The significantly highest crude protein content of 42.67 ± 0.57 % was observed in T_4 in comparison to other treatments (T_1 vs T_2: $p < 0.001$, T_1 vs T_3: $p = 0.005$, T_1 vs T_4: $p = 0.027$). Moreover, in T_2 and T_3, there was no significant difference in protein accumulation ($p = 0.051$), with corresponding crude protein contents of 32.14 ± 0.80 % and 34.98 ± 1.01 % respectively.

Among the tested samples, T_1 displayed significantly highest crude lipid content of 10.06 % in comparison to T_2 and T_3 ($p < 0.001$ for all comparisons). There was no statistically significant distinction between the lipid content of T_1 and T_4 ($p = 0.616$). In contrast, a difference was observed in the lipid levels between T_2 and T_3, indicating that T_2 had a significantly higher lipid content compared to T_3 ($p < 0.001$).

3.7. Analysis of the culture media (BBM and DRPS) preparation cost

The cost of chemicals required in preparation of BBM is shown in Table 5, and micronutrient solution in Table 6. In preparation of 1L BBM, it required BDT 9.99 (US$ 0.085; US$ 1 = BDT 117.45). In preparation of DRPS media, 9 g of $NaHCO_3$ (BDT 2.25; local price of $NaHCO_3$: BDT 250/kg) and 0.2 mL of micronutrient solution (BDT 0.01) was added per 1L of 100 % DRPS media. The cost required in preparation of 1L 100 % DRPS media was BDT 2.26 (BDT 2.25 for $NaHCO_3$ + BDT 0.01 for micronutrient solution) (US$ 0.019). In the preparation of 1L each of 20 %, 40 %, and 60 % DRPS media, it required 0.45, 0.90, and 1.36 BDT, respectively. The analysis showed that the cost of chemicals in preparation of BBM (BDT 9.99 L^{-1}; US$ 0.085 L^{-1}; US$ 1 = BDT 117.45) was about 22 times higher than in preparation of 20 % DRPS media (BDT 0.45 L^{-1}; US$ 0.0038 L^{-1}; US$ 1 = BDT 117.45).

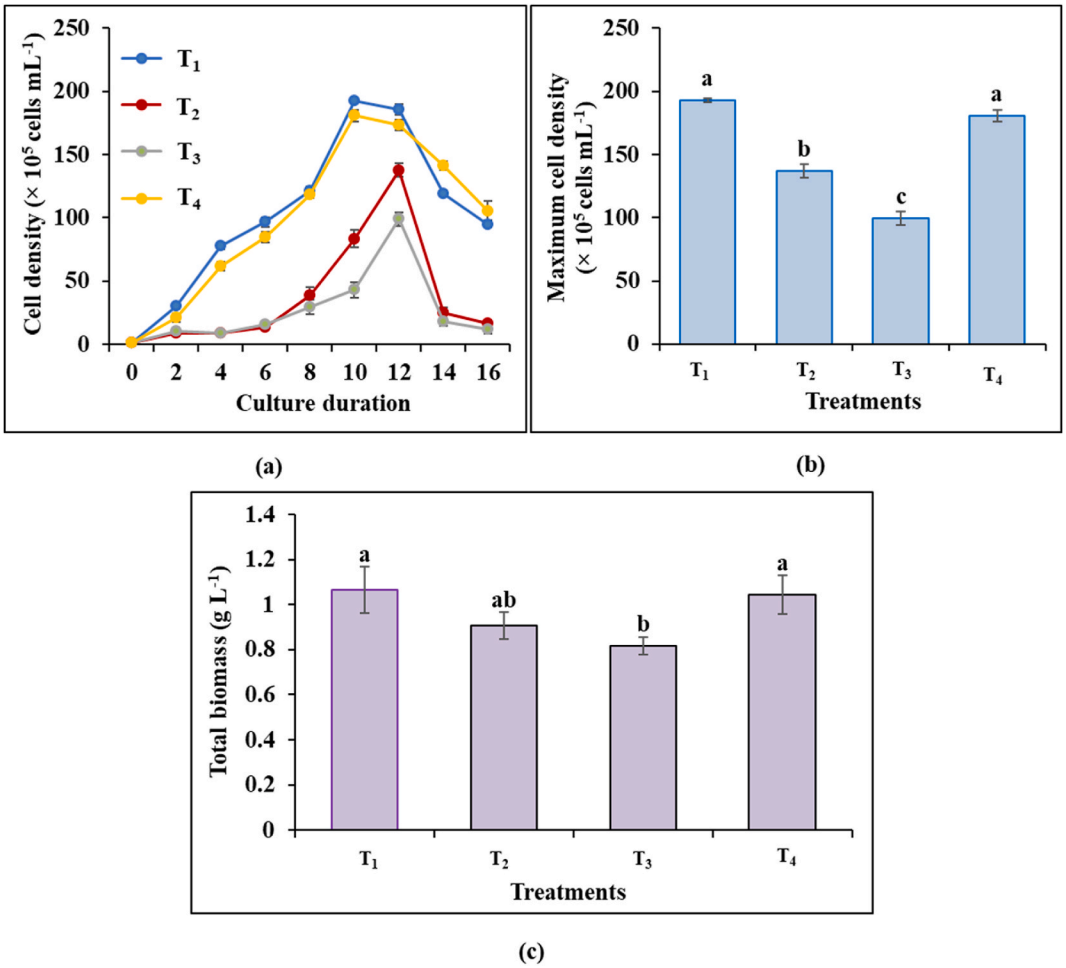

Fig. 3. (a) Mean (±SD) values of cell densities (× 10^5 cells mL^{-1}) during the culture period (measured on every alternate day from the initial day to 16th day), **(b)** mean (±SD) maximum values of cell density, and **(c)** mean (±SD) values of the total biomass (g L^{-1}) of *Chlorella vulgaris* grown in different concentrations of digested rotten potato supernatant and in bold basal media on the 16th day of culture period (n = 3/treatment) (T_1 = 20 % concentration of DRPS; T_2 = 40 % concentration of DRPS; T_3 = 60 % concentration of DRPS; and T_4 = BBM). Different small-case letters on bars represent statistically significant differences of means between treatments at α = 0.05.

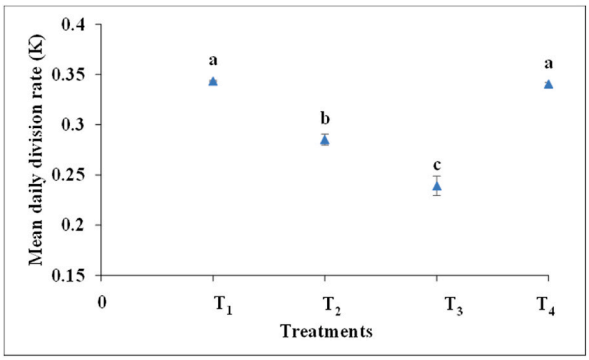

Fig. 4. Mean daily division rate of *Chlorella vulgaris* grown in different concentrations of digested rotten potato supernatant and in bold basal media (T_1 = 20 % concentration of DRPS; T_2 = 40 % concentration of DRPS; T_3 = 60 % concentration of DRPS; and T_4 = BBM). Each point and vertical line represent mean ± SD for three replicates. Different small-case letters represent statistically significant differences of means between treatments at α = 0.05.

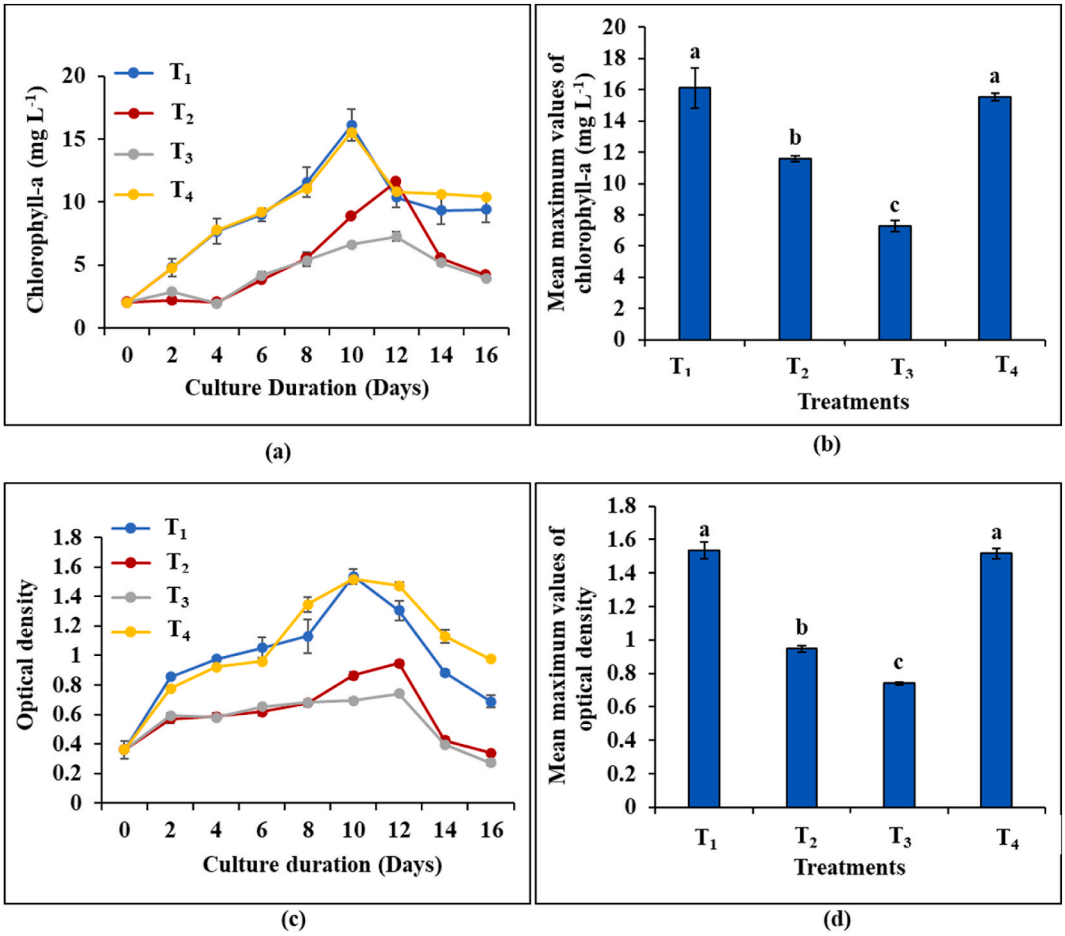

Fig. 5. (a) Growth curve of chlorophyll-*a* (mg L^{-1}), **(b)** mean maximum values of chlorophyll-*a* (mg L^{-1}), **(c)** growth curve of optical density, and **(d)** mean maximum values of optical density of *Chlorella vulgaris* grown in different concentrations of digested rotten potato supernatant and in bold basal media (T_1 = 20 % concentration of DRPS; T_2 = 40 % concentration of DRPS; T_3 = 60 % concentration of DRPS; and T_4 = BBM). Each point and vertical line represent mean ± SD for three replicates (n = 3/Treatment). Different letters on bars represent statistically significant differences of means between treatments at α = 0.05.

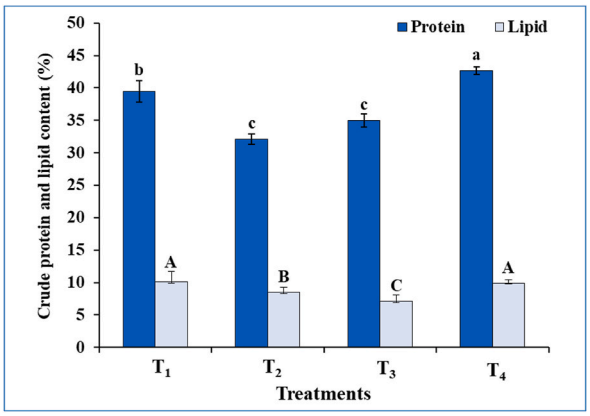

Fig. 6. Crude protein and lipid content (% on a dry weight basis) obtained from *C. vulgaris* cultured in different concentrations of digested rotten potato supernatant and in bold basal media (T_1 = 20 %, T_2 = 40 %, T_3 = 60 % concentration of DRPS, T_4 = BBM). Each bar and vertical line represent mean ± SD for three replicates. Different letters on bars represent statistically significant differences of means between treatments at α = 0.05.

Table 5
Price of the chemicals used in the preparation of BBM.

No.	Chemicals	Unit price BDT/kg	g L^{-1} used for stock solution	Stock solution used for 1L media (ml)	Price of 1 L Media (BDT)
1	$NaNO_3$	13000	25.00	10	3.25
2	$MgSO_4$. $7H_2O$	6000	7.50	10	0.45
3	NaCl	5500	2.50	10	0.14
4	K_2HPO_4	16500	7.50	10	1.24
5	KH_2PO_4	19000	17.50	10	3.33
6	$CaCl_2$. $2H_2O$	13500	2.50	10	0.34
7[a]	Trace Elements			1	0.08
	$ZnSO_4$. $7H_2O$	8000	4.42		
	$MnCl_2$. $4H_2O$	10500	1.44		
	MoO_3	19500	0.71		
	$CuSO_4$. $5H_2O$	5500	1.57		
	Co $(NO_3)_2.6H_2O$	19000	0.49		
8	H_3BO_3	11000	11.40	1	0.13
9[b]	EDTA-KOH solution:			1	0.98
	EDTA Na_2	15000	50.00		
	KOH	7500	31.00		
10	$FeSO_4.7H_2O$ with 1.0 mL Concentrated H_2SO_4	9500	4.98	1	0.05
Total cost in preparation of 1L BBM BDT 9.99 (US$ 0.085)					

BDT = Bangladeshi Taka.
[a] Price of $ZnSO_4$. $7H_2O$ + $MnCl_2$. $4H_2O$ + MoO_3 + $CuSO_4$. $5H_2O$ + Co $(NO_3)_2.6H_2O$
[b] Price of EDTA Na_2 + KOH

Table 6
Price of the chemicals used in the preparation of micronutrient solution that was used in DRPS media.

No.	Chemicals	Unit price BDT/kg	g L^{-1} used for 1L preparation of micronutrient	Price of 1L micronutrient solution in BDT
1	H_3BO_4	11000	2.86	31.46
2	$MnCl_2$ $4H_2O$	10500	1.81	19.01
3	$ZnSO_4$ $7H_2O$	8000	0.22	1.76
4	$CuSO_4$ $5H_2O$	5500	0.08	0.44
5	MoO_3	19500	0.01	0.20
6	$CoCl_2$ $6H_2O$	10500	0.01	0.11
Total cost in preparation of 1L micronutrient solution BDT 52.98				

BDT = Bangladeshi Taka.

4. Discussion

Utilizing accessible vegetable waste material as a valuable resource can serve as highly nutritious media for growing various microalgae, offering an efficient pathway for their substantial growth in an eco-friendly manner. Kadir et al. [29] explained that the cultivation of microalgae in wastewater offers cost-cutting benefits, and for example, several species demonstrated successful growth under such conditions including *Scenedesmus* sp. [30], *Acutodesmus dimorphus* [31], *C. vulgaris* [32,33] etc. Furthermore, assessment of the boost in microalgal growth supplementing vegetable waste emerges as a cost-efficient culture approach. The green microalgae such as *Asterarcys* sp. SPC, *Scenedesmus* sp. KT-U, *Scenedesmus* sp. KTWL-A, *Coelastrum* sp. T-E, and *Chlorella* sp. TWL-B showed the best growth rates in vegetable waste extracts [34]. Hence, it becomes crucial to explore the effect of rotten potato supernatant on the growth of *C. vulgaris* for the global upliftment of aquaculture. Consequently, this study focused on examining the cultivation of *C. vulgaris* under various DRPS treatments and BBM, revealing increased biomass growth at lower concentrations of DRPS.

Our study reveals that aerobic digestion of rotten potatoes significantly increases the concentrations of both nitrate and phosphate in the DRPS. During the aerobic digestion process, the organic nitrogen is converted to nitrate through the nitrification process, while organic phosphorus is mineralized to inorganic phosphate. The resultant digested supernatant, rich in nitrate and phosphate, provides a balanced nutrient profile that microalgae can readily assimilate. The highest cell density of *C. vulgaris* was found in T_1 which is statistically identical to the cell density of T_4. This observation substantiates that the T_1 serves as the most favourable growth medium for the green microalga *C. vulgaris* which is quite higher than the cell density of *C. vulgaris* (13×10^6 cells mL^{-1}) grown in wastewater [35]. Uddin et al. [36] recorded the highest cell density of *C. ellipsoidea* (200×10^5 cells mL^{-1}) in the supernatant of digested rotten potato powder which is quite similar to our findings. Furthermore, the highest growth of another green alga, *Monoraphidium littorale*, was similarly observed at the lowest concentration of DRPS (25 %) [8], further strengthening our findings. Furthermore, throughout the experimental period, T_1 showed significantly higher chlorophyll-*a* content, OD, and total biomass, which also indicated that a lower concentration of DRPS did not hinder the photosynthetic yield of *C. vulgaris*. Considering the mentioned facts, it seems that

culture media derived from rotten potatoes, particularly at a lower concentration of DRPS (T_1), can greatly influence the growth of *C. vulgaris* at the marginal stage.

Optical density (OD) and chlorophyll-*a* content are often used as indicators of the growth and physiological status of microalgae like *C. vulgaris*. As microalgae grow and their population increases, the OD typically increases. Moreover, the higher chlorophyll-*a* content generally indicates healthier and more actively photosynthesizing cells in the culture media. In our investigation, the highest optical density was recorded as 1.54 ± 0.05 in T_1, followed by 1.52 ± 0.03 in T_4. These values notably exceeded the value (1.228 ± 0.039) reported by Haris et al. [37] for *C. vulgaris*. Additionally, Rasheedy et al. [38] documented OD values of 1.450 ± 0.002 and 1.054 ± 0.004 for *C. vulgaris* and *Oscillatoria* sp., respectively, which were lower than our present study. In addition, *C. vulgaris* demonstrated more pronounced growth in T_1, exhibiting the highest chlorophyll-*a* content of 16.10 ± 1.28 mg L^{-1}, surpassing the content (6.48 ± 0.67 mg L^{-1}) observed by Feng et al. [39] in membrane-treated distillery wastewater (MTDW). On the other hand, Chen et al. [40] examined *Auxenochlorella protothecoides*, AS-1, in bacterial culture media and found that when cell density increased, the OD of their cells also accelerated, which is much more similar to our present study. According to Diana et al. [41], a higher abundance of phytoplankton indicates higher chlorophyll-*a* content, as cell density is directly correlated with chlorophyll-*a*, matching the results of our current study.

In our current investigation, the biomass content decreased when the DRPS concentration increased. Probably, the decline in growth was caused by a reduction in chloroplasts and significant damage to the photosynthetic machinery [42]. The biomass content found in T_1 of newly isolated microalga, *C. vulgaris* was higher than the strains of *Chlorella sorokiniana* IG-W-96 (0.64 ± 0.14 g L^{-1}), *C. vulgaris* IG-R-96 (0.74 ± 0.07 g L^{-1}) and lower than the strain of *Chlorella* sp. IG-B-96 (1.21 ± 0.00 g L^{-1}) [43]. Furthermore, Zhang et al. [44] reported that *Chlorella sorokiniana* SDEC-18 and *Scenedesmus* SDEC-8 showed optimal biomass production of 0.42 ± 0.04 g L^{-1} and 0.55 ± 0.03 g L^{-1}, respectively, in the anaerobically digested kitchen waste effluent. In contrast, Tan et al. [45] unveiled that when grown in media made with organic fruit waste, *C. vulgaris* and *Haematococcus pluvialis* produced significant biomass concentrations. Moreover, Giwa et al. [46] described that *C. vulgaris* produced the highest biomass in digested food waste and brine compared to the control (Johnson's medium). Habib et al. [25] found the maximum biomass in kosaric medium (703.50 ± 9.50 mg L^{-1}) then in 50 % (678.71 ± 9.32 mg L^{-1}), 75 % (493.79 ± 8.33 mg L^{-1}) and 25 % (470.34 ± 8.15 mg L^{-1}) DRPS respectively where we got a maximum biomass in 20 % DRPS. This variation in growth may be due to species differences, methods of preparing DRPS and due to variation in NO_3–N and PO_4–P concentrations in the DRPS media. The NO_3–N and PO_4–P concentrations were quite higher in our culture media and this higher nutrient availability in our medium could have provided optimal conditions for microalgal growth, resulting in higher biomass yields. Therefore, using the supernatant of the digested rotten potato as a culture media can be an effective method to obtain a large biomass of *C. vulgaris*.

The highest protein content was found in T_4 whereas the highest crude lipid content was found in T_1. *C. vulgaris* produced higher biomass rich in lipids when grown in vegetable waste media [47]. Indicatively, *C. vulgaris* displayed a high protein content of 37.8 % and lipid content of 26.4 % when grown in municipal food waste [48]. Likewise, *Chlorella pyrenoidosa* and *Schizochytrium mangrovei* exhibited increased biomass production and higher accumulation of lipids and proteins when grown in food waste hydrolysate compared to a conventional medium [49].

A variety of environmental factors, including nutrients, light availability, temperature and salinity, collectively regulate the abundance of microalgae in natural ecosystems [50]. Surprisingly, despite lower nitrate and phosphate concentrations in different concentrations of the DRPS compared to BBM, *C. vulgaris* exhibited higher growth at lower DRPS concentrations (20 % DRPS). However, the higher growth was not sustained at higher DRPS concentrations (T_2 and T_3), where elevated pH, EC, and TDS were observed. The increased pH may lead to nutrient precipitation, while higher EC and TDS indicate a greater concentration of ions, potentially inducing osmotic stress. This complex interplay of nutrient availability, pH, and ion concentrations may affect algal growth at higher DRPS concentrations. This suggests a unique adaptability of *C. vulgaris* to lower levels of nitrogen (N) and phosphorus (P). Hence, a lower concentration of DRPS (T_1) is the best medium for increasing the growth, biomass and nutrient composition of *C. vulgaris*. Drawing from these findings, exploring the potential utilization of nutrient-rich bio-wastes such as agricultural, vegetable, food, and fruit wastes become very important. This exploration could significantly contribute to advancing microalgal cultivation by enhancing production and effectively recovering nutrients.

5. Conclusion

The study demonstrated that digested rotted potato supernatant (DRPS) is a suitable media for the culture of *C. vulgaris*. Through assessments of cell density, growth rate, optical density, chlorophyll-*a* content, and total biomass, it was found that *C. vulgaris* grow better in media having lower concentration (20 %) of DRPS. Furthermore, *C. vulgaris* grown in 20 % DRPS led to highest crude lipid and reasonably good protein content in the cells of the microalgae, showcasing its potential for fostering a bio-based economy. Additionally, this algal species holds promise for supporting aquaculture by aiding in the rearing of zooplankton and fish larvae. Consequently, the cultivation of *C. vulgaris* in DRPS not only reduces the overall cost of microalgal production but also enhances biomass production with enriched nutritive value.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Md Abu Sayim Shakil: Writing – original draft, Formal analysis. **Jinnath Rehana Ritu:** Writing – review & editing, Formal analysis. **Amina Akter:** Methodology, Investigation, Formal analysis, Data curation. **Naushin Fatima:** Formal analysis, Data curation. **Md Mahfuzul Haque:** Writing – review & editing. **Saleha Khan:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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