

Sustainable Biodiesel Production via *Chlorella vulgaris* and *Tetraselmis chuii* in food-based brewery Industrial Wastewater

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Sustainable Biodiesel Production via *Chlorella vulgaris* and *Tetraselmis chuii* in Food-based brewery Industrial Wastewater

Abstract

The microalgae *Chlorella vulgaris* and *Tetraselmis chuii* offer several attractive attributes as an aquaculture feed. Biomass production of *C. vulgaris* and *T. chuii* is relatively expensive because it requires high operation and substrate cost. Use of food industry waste is one of the promising solutions to reduce the substrate cost. In this research the impact of diverse concentration was filtered and centrifuged, food wastewater (FWW) was scrutinized as an alternative intermediate for microalgal bioremediation, lipid yield and cell growth. *C. vulgaris* had superior lipid accumulation and cell growth at 75 % level and *T. chuii* had enhanced lipid accumulation and cell growth at 75 % with the most explicit growth rate (0.27 d^{-1}) and maximum biomass formation rate ($0.1 - 0.18\text{ gL}^{-1}\text{ d}^{-1}$) and lipid content ($33.80 \pm 0.88\%$ and $35.04 \pm 2.05\%$) respectively. The microalgae cells not only improved with FWW feeding and the level of COD ($80.97 - 66.17\%$), BOD ($81.13 - 66.81\%$), TN ($55.88 - 22.06\%$) and certain heavy metals like zinc ($99.72 - 99.52\%$) manganese ($72.09 - 65.17\%$) nickel ($50.68 - 34.41\%$), NO_3N ($50.68 - 42.59\%$) ferric ($57.14 - 65.71\%$) cadmium ($83.51 - 86.64\%$) lead ($48.83 - 97.21\%$) and arsenic ($55.13 - 55.00\%$) were efficiently removed from FWW at different dilution concentrations. C18:0/C18:1 was the major fatty acids in lipid which show a vast potential of ADW007 for biodiesel fabrication and instantaneous bioremediation procedure using FWW.

Keywords: Food wastewater, *Chlorella vulgaris*, *Tetraselmis chuii*, Bioremediation, Lipid content.

Introduction

In country economy, food and brewery industries play a vital role. During the production processes it consumes large volumes of water and afterward liberates about 70% of it as wastewater [1]. Among diverse operations contributing to wastewater, the brewery, food dispensation and manufacturing operations generates a huge amount of wastewater and waste, beside with additional industrial effluents. Excessive amount of nitrogen and phosphorus is there in food industry waste and in streams it is a common issue [2]. Release of unprocessed and partially processed brewery wastewater in water bodies raises many environmental issues. The

main environmental issues by the process of breweries contain wastewater, water consumption, solid waste, by-product generation, energy exploit and its release to the air [3]. This observable fact shows environmental problems including, unnecessary growth of unwanted microbes that can cause damage to aquatic life forms, water scarcity as well as health-related issues in communities that are near the release region [4]. Green methodology for wastewater treatment, resource recovery and pollution remediation during biological means are progressively more popular in the world. To address these issues the use of microalgae is very effective. Microalgae is very environmentally friendly as well as cost effective water treatment technique is a best way to address these problems [5]. In present times, research about the progress of integrated processes for microalgae cultivation as well as simultaneous treatment of industrial sewage is a main concern [6]. The most important targeted industries contain food and breweries, textile industries, municipal sewage and dairy industries. Brewery wastewater is frequently enormous with high moisture content.

Since an organic compound is present (sugars, soluble starch, ethanol, volatile fatty acids), this effluent often has more chemical and biochemical oxygen demand (COD, BOD). Brewery effluent normally has a temperature range of 25 to 38 °C. Its pH values are variable and reliant on the quantity and kind of chemicals (such as soda caustic, phosphoric acid, nitric acid etc.) used in cleaning also sanitizing. There are also levels of phosphorus (P) and nitrogen (N) [7]. Microalgae beside with associated eukaryotic photosynthetic organisms as well as Fungi have the ability to produce peptides that can bind with heavy metals, resulting in the formation of organometallic complexes (heavy metals + peptides = organometallic complexes). This synthesis process is primarily localized within vacuoles, allowing for the proper regulation of heavy metal ion concentrations in the cytoplasm. By doing so, fungi prevent or neutralize the potential harmful effects of heavy metals. In contrast, eukaryotic and prokaryotic cells utilize different methods such as ATP-dependent efflux of heavy metals or enzymatic modification of speciation to achieve detoxification [8].

The primary objective of this research was to assess the potential or evaluate the capability of *T. chuii* and *C. vulgaris* to utilization of food-based brewery industrial (Murree brewery) wastewater inside cultivation systems, immediate nutrient elimination along with towering biomass along with lipid productivity for biofuels production. Murree brewery utilizes food

products like lemon, peach, apple, lychee, pineapple watermelon, pomegranate and wheat to making different kinds of drinks. The wastewater generated by this industry is a good resource of micro and macro nutrients and which is also a good source of food for microalgae. The main aim of research is to investigate the use of sieved as well as centrifuged FWW at diverse configuration in distils H₂O used for culturing *C. vulgaris* and *T. chuii*. The lipid content and biomass production were evaluated collectively among reduction in nutrient level COD, diverse heavy metals and BOD from FWW.

Materials and Methods

Materials

Raw food-based brewery wastewater was collected from Murree brewery industry Islamabad Pakistan, which used different food products in making drinks. In order to evade biodegradation and contamination sample were store in plastic container at 4°C. Dust and sand particles were removed from FWW by filtration and autoclaved for water quality analysis and *C. vulgaris* and *T. chuii* cultivation substrate

Methods

Microalgal cultivation

C. vulgaris and *T. chuii* were imported from Fisheries Research Center Institute (FRI), Sungai Petani, Pulau Sayak and Kedah, Malaysia. The culture flasks were shaking periodically under ambient conditions of temperature and light (28±5°C, pH 7-8 and 24-hours illumination), lipid content and algal growth were recorded every alternate day. Algal samples were centrifuged for 10 mins at 4000 rpm after harvesting. The supernatant and pellet were analyzed for chemical properties and cell growth.

Food-based brewery wastewater collection

Raw food-based brewery wastewater was collected from Murree brewery industry Islamabad Pakistan, which used different food products in making drinks. In order to evade biodegradation and contamination sample were store in plastic container at 4°C. Dust and sand particles were removed from FWW by filtration, furthermore sample was subjected to centrifugation and the

resulting supernatant from waste material was utilized as culture media, while pellet was retained aside used for subsequent examination. Supernatant remained diluted up to 25, 50, 75 with 100% in distil water; the supernatant underwent sterilization by subjecting it to a temperature of 120°C for a duration of 40 minutes. Before use, the pH of the FWW medium was adjusted to fall within the range of 7-8. Additionally, the sample underwent an extra filtration step.

Chemical composition of FWW

According to the standard procedure offered by HACH, COD was measured using a spectrophotometer DR2800 and the 5000-Reactor 140 Digested Method. To 150°C, the DR5000-Reactor was preheated. Two milliliters of each standard's diluted FWW were introduced after diluting one milliliter of the sample at ratio of 1:50, 1:100 and 1:250 with FWW and distilled water; the suitable Vials containing a high-range COD digestion reagent were chosen for the experiment. To establish a blank, a quantity of 2cc of distilled water was added. Vials were taken out after two hours and let to cool for 20 minutes before being read. For a COD test, The COD reading in milligrams per liter (mg/L) was recorded after recalling the previously saved HACH program 435 HR COD (HACH, USA 1997).

BOD was measured using a BOD track in accordance with the HACH-provided Standard Method. A volume of 1 milliliter of the substance was diluted using FWW and distilled water at ratios of 1:100 and 1:250, respectively. Subsequently, 95 milliliters of the sample were carefully transferred into a dedicated 300 mL BOD track designed to ensure complete filling without any air gaps, and it was sealed tightly. Each sample bottle was equipped with a 3.8 cm magnetic stir bar, which had been utilized during the preparation of the four samples. At 20°C, the incubator was set up with the instrument. To conduct the BOD test, the preprogrammed HACH program for duration of 5.2 days was selected. The range of 156-701 mg/L was chosen for the expected BOD concentration. After 5 days, the reading was conducted, and for each sample bottle, the screen showed 157 BOD in mg L⁻¹ (HACH, USA 1997). As per Standard Method of APHA, TN was measured using a TN Analyzer (TOC-VCSH SHIMADZU). In the following ratios: 1:50, 1:250, and 1:100. Atomic absorption spectrometer was used for the analysis of heavy metals. Following equation is used for removal efficiency of BOD, COD, TN, as well as heavy metals:

$$\text{Removal efficiency (\%)} = A_i - A_f / A_i \times 100$$

Quantification of Cell Dry Weight (DW)

Microalgal biomass was analyzed on day 18 with the help of microfiber 0.47μ Whitman filter paper. To measure the biomass yield, the microalgal culture (10-50 ml) was filtered with the help of a filter paper which is previously dried and weighed. 10-50 ml of distilled water will be filtered by using the control filter paper and dried in the same way. Algal dry weight will be calculated by the formula:

$$\text{Algal dry weight (g L}^{-1}\text{): } \frac{\text{DW}_A - \text{DW}_C}{V} \times 100$$

DW_A = dry weight left on the algal filter

DW_C = dry weight left on control filter paper

V = the total volume of sample culture.

The biomass obtained was expressed in grams per liter (g L^{-1}) respectively t_1 and t_2 were the culture times at the initial and final measurement.

Lipid extraction and Fatty acid analysis

The modified Bligh and Dyer [9] method was used for extraction of a total lipid fraction from biological matrices which are based on a solvent system that may contain The mixture consisting of chloroform, methanol, and water in a volumetric ratio of 2:2:1:8 (v/v/v) was prepared. 30-50 ml microalgae culture was taken and centrifuged for 5 minutes at 5000x. The homogenized tissue sample was mixed with 4ml of chloroform and 2ml of methanol. After carefully vortexing, 2ml KCl (0.9%) was added. The mixture was centrifuged at 3500rpm 5 mins to take away the lower chloroform phase. It was dried by nitrogen evaporator, and weight was measured. Non-extractable residues were present in the mixture of chloroform/methanol/water. It was give a maximum product for non-polar lipids like triacylglycerol. Lipid analysis was performed by suspending the lipid in a tube with 1.5 ml hexane and then 4.5ml 2% H_2SO_4 was added in methanol. It was incubated for 2.5h at 85°C. After cooling, 2ml KCl was added and then shaking it for 5 mins. The supernatant was transferred into a 1.5ml tube. It was rotary evaporated for 15

mins and then centrifuged at 12000rpm for 5 mins after centrifugation the supernatant was transferred into a GC tube for fatty acid analysis.

Dried percentage of the lipids was considered as:

$$\text{Lipid Content (\%)} = \frac{W_2 - W_1}{\text{Fresh weight of pellet}} * 100$$

Where

W_1 = previously weighed test tube

W_2 = test tube with lipid content

W_d = algal dry weight.

Statistical Analysis

Origin Pro 2018 was used to generate the PCA data analysis for the screening step and the heat map visualization was built into the origin software. For this research a complete randomized design was employed. The study consisted of four different treatments, each with three replicates. The data was subjected to statistical analysis using a one-way analysis of variance (ANOVA) method with aid of statistics 8.1 software, to measure the main difference between the treatments means with P-value below 0.05 was considered statistically significant in this study. Experiments were carried out in triplicate, and the data was presented as the mean $\pm$ with accompanying standard deviation.

Result and Discussion

Lipid Content and Cell Growth

Fig.1 and 2 shows the effect of dissimilar composition of FWW on cell growth of *C. vulgaris*, and *T. chuii*. Highest optical density 0.93 and 0.90 was obtained under 50% and 25% FWW for *C. vulgaris*, and *T. chuii* respectively. The maximum biomass formation rate was obtained from 0.16 to 0.18 g⁻¹L⁻¹d⁻¹, with the doubling time of 2.4 to 4.7 days (Table 1-2). The maximum specific growth rate ($\mu_{\max}$) was found to be between 0.14-0.27 d⁻¹. Additionally, the lipid content (33.80 $\pm$ 0.88% - 35.04 $\pm$ 2.05%) was obtained under 75 % FWW for *C. vulgaris*, and *T. chuii*.

At a 95% confidence level, a statistically significant difference was observed in the mean dry weight and lipid content among different levels of FWW dilution for both species ($P < 0.05$). Results of studied freshwater microalgal growth show differences with that of marine microalgae which reports cell density of 48.7×10^6 cells mL^{-1} and 66.2×10^6 cells mL^{-1} for *T. suecica* and *N. oculata* respectively [10].

Analyzing the chemical composition of FWW

Levels of BOD, COD, and TN

C. vulgaris achieved 81.13 % BOD, 80.97% COD, and 55.88 % TN at 100% of food wastewater (Table3). However, *T. chuii* show maximum removal as 66.81 % BOD, 68.00 % COD, at 50% dilution and 22.06% TN at 100% represented by table 4 respectively [11] *Scenedesmus obliquus* is utilized for both brewery wastewater treatment and biomass production, achieving a maximum reduction of 57.5% and 20.8% in COD (Chemical Oxygen Demand) and TN (Total Nitrogen), respectively.

Heavy Metal Removal

Microalgae have a considerable capability of fascinating and eliminating heavy metals from wastewater. The result showed in table (3-4) that the *C. vulgaris* and *T. chuii* removed heavy metals (Zn, Mn, Ni, Fe, Cr, Cd, Pb, Se, Al, Ba, As, Co and NO_3N) from food wastewater. The wastewater was diluted 25, 50, 75 and 100 times before applied to cultivate microalgae. *C. vulgaris* showed the maximum removal of different heavy metal at different dilutions for zinc it achieved 99.72 % at 25% dilution for manganese 72.09% removal achieved at 100% for nickel 50.68% removal observed at 100% ferric removal achieved up to 57.14% at 100% dilution chromium showed 64.13% removal at 100% dilution 83.51% removal observed for cadmium at 100% dilution 48.83% removal observed for lead at 50% selenium showed 35.21 at 75% dilution 35.37% removal efficiency was observed for aluminum at 50% and barium showed 75.24% removal at 100% dilution arsenic indicated 55.13% removal at 100% dilution NO_3N removal observed as 38.88% at 100% dilution and 29.09% removal efficiency was observed for cobalt at 25% dilution of food wastewater. *T. chuii* showed the maximum removal of different heavy metal at different dilutions for zinc it achieved 99.52 % at 75% dilution for manganese 65.17% removal achieved at 50% for nickel 34.41% removal observed at 100% ferric removal achieved

up to 65.71% at 100% dilution chromium showed 51.58% removal at 100% dilution 86.64% removal observed for cadmium at 50% dilution 97.21% removal observed for lead at 25% selenium showed 45.19 at 50% dilution 52.23% removal efficiency was observed for aluminum at 75% and barium showed 70.71% removal at 50% dilution arsenic indicated 54.69% removal at 75% dilution NO₃N removal observed as 42.59% at 100% dilution and 25.25% removal efficiency was observed for cobalt at 100% dilution of food wastewater. The phycoremediation process typically occurs over a period ranging from 3 to 26 days, during which heavy metals and nutrients are reduced to minimal levels [12]. [13] Reported the removal of heavy metals by microalgae *Scenedesmus species*. The results demonstrate that concentration number 1 (C1) is the concentration, that removes heavy metals from the treatment, as evidenced by the removal efficiency for iron was 88.22% and 69.63%, for copper it was 60% and 53.85%, and for zinc (C4 concentration) it was 75.61% and 76.63%, respectively [14]. The study reported the capability of *Scenedesmus* sp. to effectively treat wet market wastewater samples in Malaysia. The results demonstrated significant removal rates, including 73.01% for TN (Total Nitrogen) and 87.6% for TP (Total Phosphorus). Moreover, the removal percentages for heavy metals were found to range from 59.33% for Fe (Iron) to 100% for Cu (Copper) and 79.65% for Zn (Zinc).

Heat map for removal efficiency of *C. vulgaris* and *T. chuii* from food-based brewery industrial wastewater

A heat map was generated to visualize the data for *C. vulgaris* and *T. chuii*, representing values using different colors. Specifically, a cluster heat map was utilized, where the rows and columns of a data matrix were ordered based on clustering results (Fig. 3-4). In this heat map, magnitudes were organized into a fixed cell-sized matrix, with rows and columns representing different categories and phenomena. The arrangement of rows and columns was intentionally chosen, aiming to identify clusters or highlight them through statistical analysis. The cell size was arbitrary but large enough to ensure clear visibility. In the heat map, the maximum removal efficiency values were depicted in red color, while the minimum values were represented in white color for *C. vulgaris*. Similarly, for *T. chuii*, the maximum removal efficiency values were shown in red, while the minimum values were displayed in black. The cluster heat map showed dendrogram of hierarchical clustering based on average linkage and the euclidean distance for comparison of removal efficiency of different metals.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was used to identify the patterns, decrease the complexity of the datasets and to make the data easier to interpret while minimizing the loss of information. It created uncorrelated variables that consecutively maximized the variance and identified attributes which are the most different between all samples. Fig (5-6) shows PCA for removal efficiency of *C. vulgaris* and *T. chuii*. PC1 versus PC2 plot indicates the large spread of data for *T. chuii* as compared to *C. vulgaris*.

Conducting analysis of FAME

The composition of fatty acids presents in the recovered lipid from *C. vulgaris* and *T. chuii* cultured in 25%, 50%, 75% and 100% compositions of food wastewater with distilled H₂O is revealed in Table 5. The saturated fatty acids (SFA) include important fatty acids such as palmitic acid (C16:0), lauric acid (C12:0), and stearic acid (C18:0). On the other hand, the monounsaturated fatty acids (MUFA) category encompasses significant fatty acids like oleic acid (C18:1) and palmitoleic acid (C16:1). The content of saturated fatty acids of the *C. vulgaris* was 11.7% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (SFA) was 10.91, 15.26, 15.67, and 26.46 respectively. The amount of monounsaturated fatty acids was 18.62% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (MUFA) was 18.91, 22.85, 6.69, and 36.17 respectively. The amount of polyunsaturated fatty acids was 28.51% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (PUFA) was 11.24, 20.27, 27, and 25.37 respectively. Similarly, for *T. chuii* the content of saturated fatty acids was 5.24% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (SFA) was 5.1, 10.8, 10.8, and 8.91 respectively. The amount of monounsaturated fatty acids was 52.16% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (MUFA) was 48.2, 53.06, 38.53, and 26.9% respectively. The amount of polyunsaturated fatty acids was 10.2% under control condition and at 25%, 50%, 75% and 100% dilution the total content of (PUFA) was 1.71, 1.52, 9.52, and 6.15 respectively. Short-chain fatty acids (C12–C18) are the most important components of biodiesel and most of the fatty acids are present in the *C. vulgaris* and *T. chuii*, the long-chain fatty acids (C20–C22) are present in a lesser amount. The superiority parameters

of the biodiesel were predisposed by the fatty acid preparation of the oil. Collate to rapeseed oil, the algal oil was not as rich in polyunsaturated fatty acids.

Biodiesel Properties

The results of the FAME analysis of the transesterified lipids revealed the fatty acid composition of *C. vulgaris* and *T. chuii* (Table 6). This data was further utilized to work out the average degree of unsaturation which also helped to calculate the biodiesel properties. The ASTM D6751- 20a (B100) value for each biodiesel property is also mentioned. The biodiesel properties like viscosity, specific gravity, and cetane number of the lipids extracted from both *C. vulgaris* and *T. chuii* fall within the specified ASTM standards. In terms of physical properties, the kinematic viscosity and specific gravity completely satisfied the ranges specified by the ASTM D6751 and EN 14214 standards. The value of cetane number corresponded to the ASTM D6751 standard, the high heating value measurement stayed within the specifications outlined in the EN 14214 standards. However, the iodine values were low which indicate that biofuels contains a higher proportion of saturated fatty acids and fewer unsaturated fatty acids. The iodine value is a measure of the degree of unsaturation in a substance, particularly in fats and oils. It is determined by the amount of iodine, in grams, that can be absorbed by 100 grams of a substance. a low iodine value in biofuels indicates that the fuel is likely to have better oxidative stability, cold flow properties, and potentially higher energy content due to a higher proportion of saturated fatty acids. However, the ideal composition of biofuels can vary depending on the intended application, and a balance between saturated and unsaturated fatty acids is often sought to achieve optimal performance and characteristics. According to the ASTM D6751 the kinematic viscosity of biofuels should range between 1.9-6.0 mm²/s where according to EN 14214 it should vary between 3.5 -5 mm²/s. The kinematic viscosity of *C. vulgaris* was 4.67 mm²/s and for *T. chuii* it was 4.86 mm²/s which indicates that these biofuels can be utilized as blended diesel/biodiesel. Similarly, the specific gravity of both species grown under their optimum conditions was 0.879 which perfectly coincides with the ASTM D65751 standard and falls with the range of 0.86-0.9 specified by the EN 14214 standard. The ASTM D6751 and EN 14214 standard have not set down any ranges for the cloud point but since it indicates the temperature at which the biofuel will start to gel or form crystals so, the lower it is the better it will perform at cold temperatures. A higher cloud point is attributed to a higher PUFAs percentage whereas

although SFA and MUFAs are inversely related to the cold flow properties they still contribute oxidative stability to the biofuels [15]. Growing the two species under food wastewater significantly increased the percentage of PUFA, MUFA and SFAs in the algal triglycerides of the species under study. With respect to the cetane number, although an increase in the unsaturated fatty acids due to food waste media had favored the cloud point of the biofuels derived from our species. In a nutshell, our biofuels have a relatively greater content of unsaturated fatty acids with respect to saturated fatty acids. For this reason, properties that are positively correlated with PUFAs such as cloud point, viscosity, cetane number and specific gravity were within the appreciable ranges but other properties like, iodine value can be improved. Scientists reported that higher temperatures increase the accumulation of saturated fatty acids whereas; lower temperatures increase the accumulation of unsaturated fatty acids [16]. It was reported that at 22 °C, *C. luteoviridis* accumulated 40.7% PUFAs whereas at 27 °C, the percentage of SFAs was increased to 52.9% [17].

Conclusion

The present study is aimed to the impact of diverse concentration was filtered and centrifuged, food wastewater (FWW) was scrutinized as alternative intermediate for microalgal bioremediation, lipid yield and cell growth. *C. vulgaris* had superior lipid accumulation and cell growth at 75 % level and *T. chuii* had enhanced lipid accumulation and cell growth at 75 % level with maximum specific growth rate (0.27 d^{-1} - 0.27 d^{-1}) and maximum biomass formation rate (0.1 and $0.18 \text{ gL}^{-1} \text{ d}^{-1}$) with lipid content ($33.80 \pm 0.88 \%$ and $35.04 \pm 2.05 \%$) respectively. The microalgae cells revealed enhanced performance when fed with FWW, effectively reducing levels of COD, BOD, TN, nitrate ions as well as specific heavy metals such as zinc, manganese, nickel, ferric, cadmium, lead, and arsenic across varying dilution concentrations. The lipid composition of both the species was primarily comprised of C18:0/C18:1 fatty acids, indicating significant potential for biodiesel production and instant bioremediation of FWW.

Statements and Declarations

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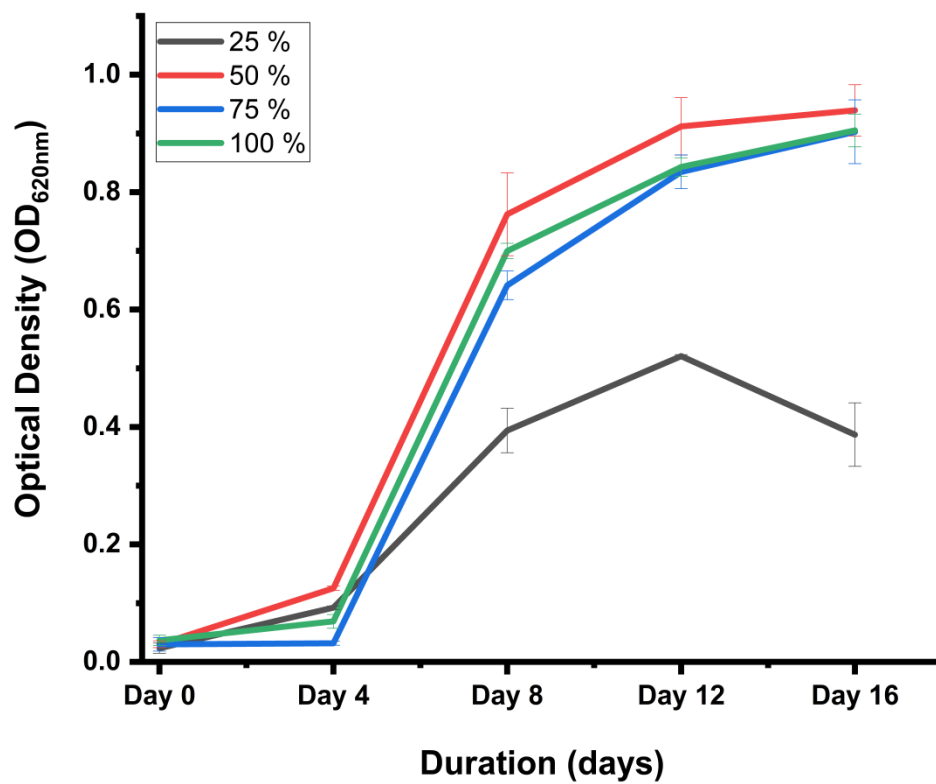


Fig. 1 Cell density of *C. vulgaris* in food-based wastewater.

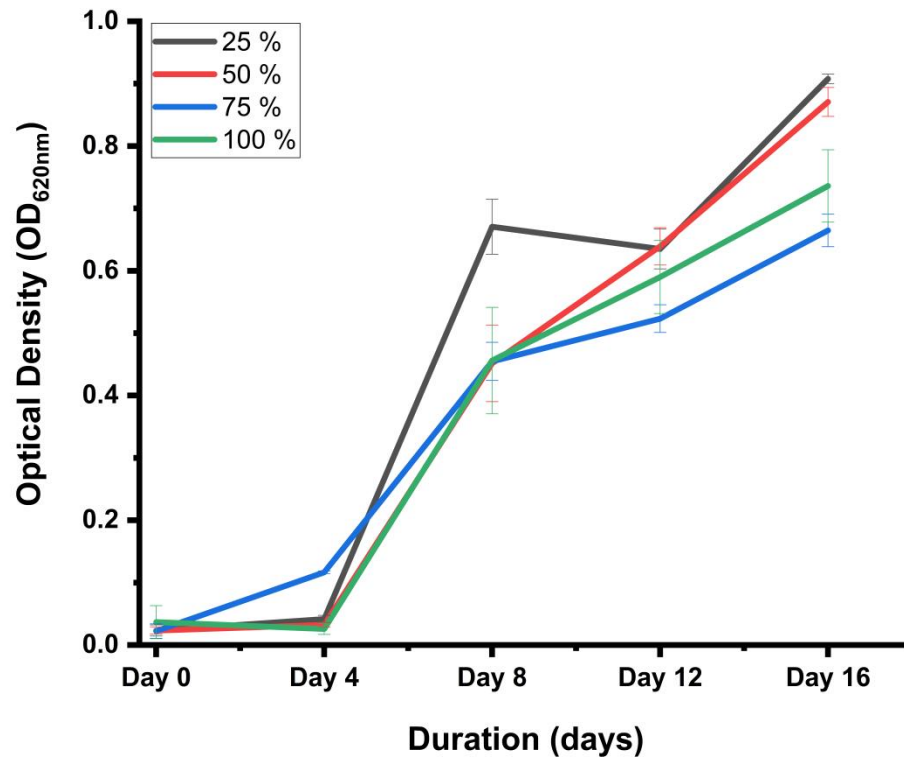


Fig. 2 Cell density of *T. chuui* in food-based wastewater.

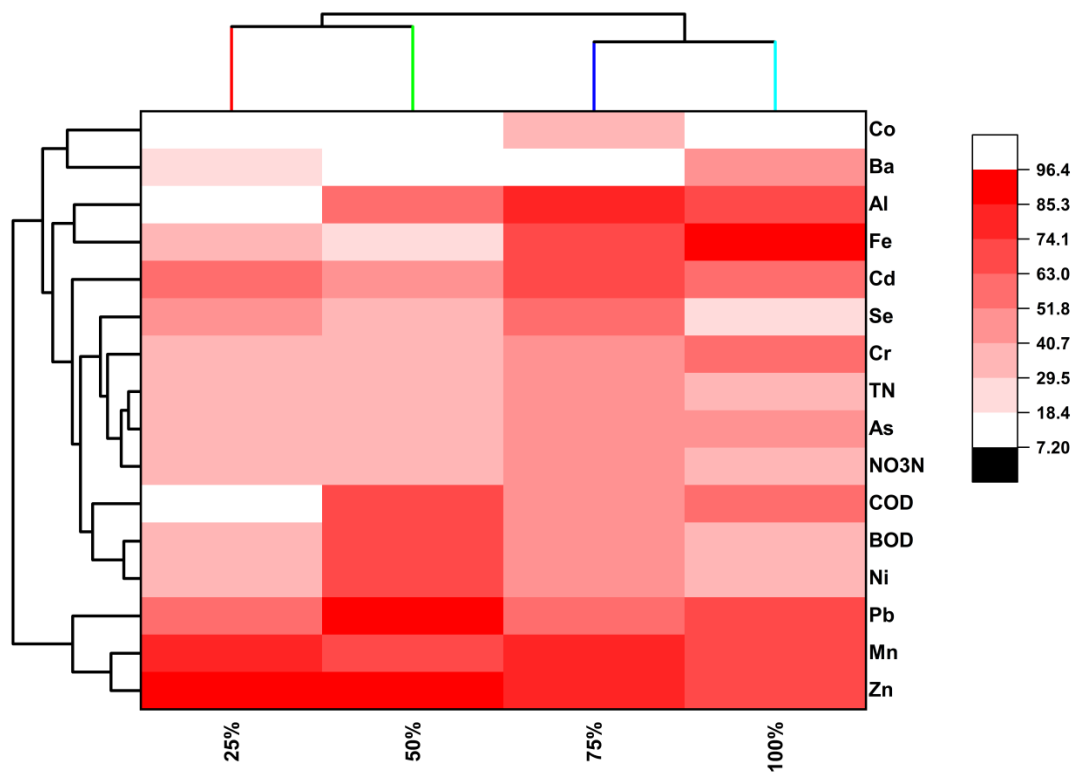


Fig. 3 Heat Map for *C. vulgaris* cultivated in food-based wastewater.

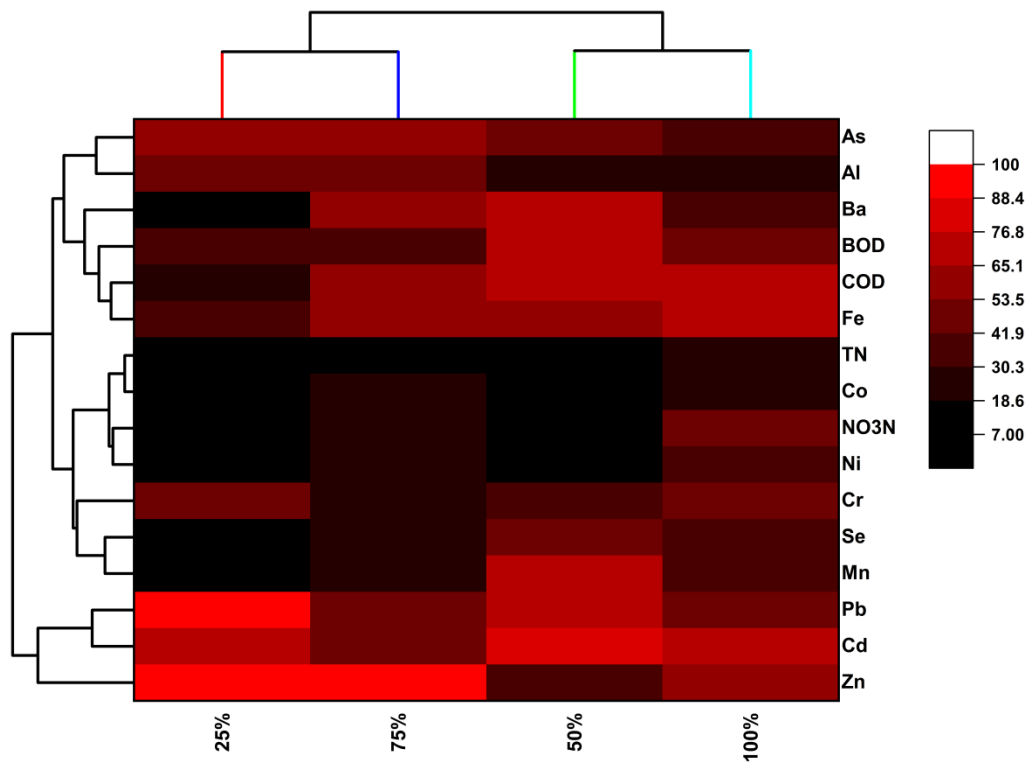


Fig. 4 Heat Map for *T. chuui* cultivated in food-based wastewater.

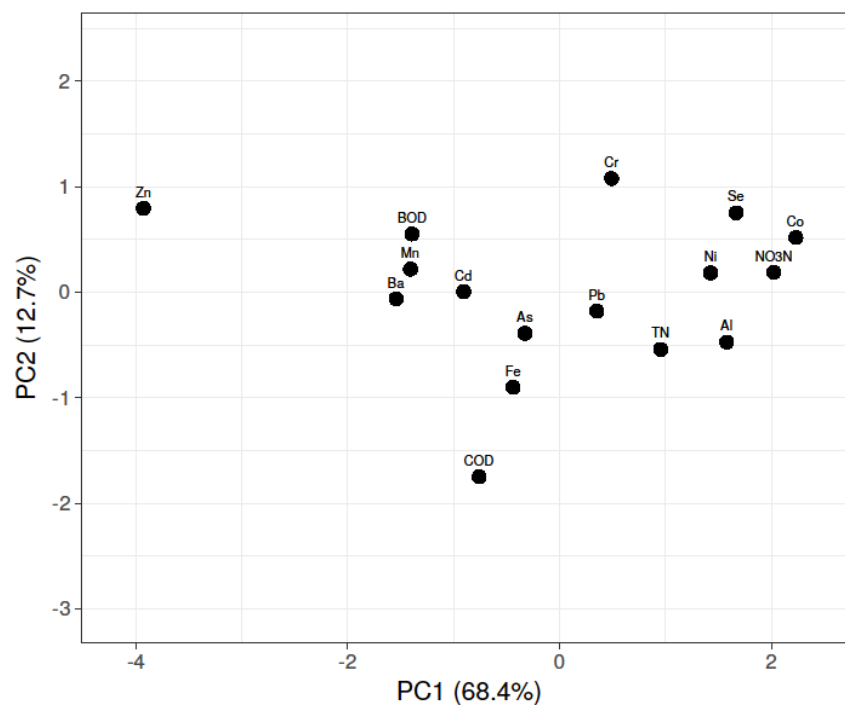


Fig. 5 PCA for *C. vulgaris* cultivated in food-based wastewater.

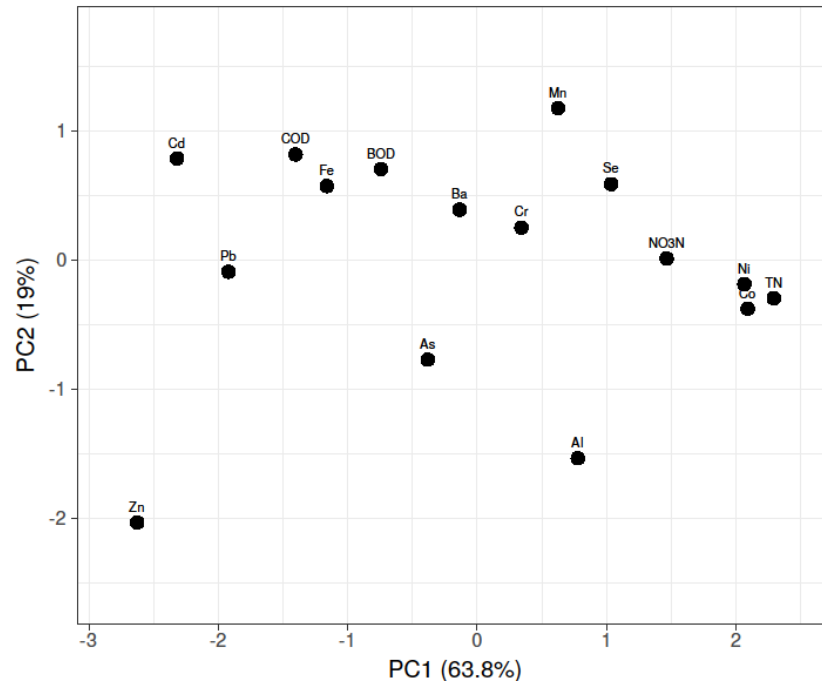


Fig. 6 PCA for *T. chuui* cultivated in food-based wastewater.

401 **Table 1** Kinetics and Lipid studies of *C. vulgaris* in food wastewater

<i>C. vulgaris</i>	Maximum biomass formation rate, X_{max} (gL ⁻¹ d ⁻¹)	Maximum specific growth rate, μ_{max} (d ⁻¹)	Doubling time, t_d (d ⁻¹)	Lipid Content (%)
25% Food Waste	0.13	0.11	5.8	20.33 ± 1.52
50% Food Waste	0.17	0.16	4.1	25.66 ± 0.57
75% Food Waste	0.16	0.27	2.4	33.80 ± 0.88
100% Food Waste	0.14	0.21	3.2	29.08 ± 0.87

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Table 2 Kinetics and Lipid studies of *T. chuii* in food wastewater

<i>T. chuii</i>	Maximum biomass formation rate, $X_{\max}$ (gL ⁻¹ d ⁻¹)	Maximum specific growth rate, $\mu_{\max}$ (d ⁻¹)	Doubling time, t_d (d ⁻¹)	Lipid Content (%)
25% Food Waste	0.11	0.25	2.69	22.33 ± 1.52
50% Food Waste	0.10	0.27	2.52	29.05 ± 1.00
75% Food Waste	0.18	0.14	4.78	35.04 ± 2.05
100% Food Waste	0.10	0.27	2.47	31.43 ± 1.54

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Table 3 Removal efficiency (%) of *C. vulgaris* for food wastewater

Heavy Metals	25% Food Waste	50% Food Waste	75% Food Waste	100% Food Waste
Zn	92.94	64.84	99.72	87.85
Mn	55.08	47.08	60.68	72.09
Ni	15.74	18.37	27.50	50.68
NO ₃ N	6.06	14.28	26.08	38.88
Fe	25.80	55.76	48.14	57.14
Cr	55.19	16.22	24.21	64.13
Cd	30.84	36.31	61.47	83.51
Pb	44.71	48.83	31.61	31.02
Se	26.55	18.77	35.21	21.03
Al	20.14	35.37	13.68	33.78
Ba	73.92	56.70	37.10	75.24
As	45.63	51.01	34.58	55.13
Co	29.09	20.55	14.42	13.70
BOD	74.94	42.57	40.75	81.13
COD	23.39	65.04	31.15	80.97
TN	15.38	33.87	25	55.88

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Table 4 Removal Efficiency (%) of *T. chuii* for food wastewater

Heavy Metals	25% Food Waste	50% Food Waste	75% Food Waste	100% Food Waste
Zn	93.48	41.22	99.52	57.03
Mn	17.09	65.17	20.65	39.77
Ni	7.49	8.00	21.42	34.41
NO ₃ N	18.18	14.29	21.74	42.59
Fe	35.48	55.77	53.70	65.71
Cr	44.90	31.42	21.78	51.85
Cd	73.98	86.64	49.51	65.16
Pb	97.21	75.83	44.49	51.25
Se	15.67	45.19	23.97	37.63
Al	48.32	24.41	52.23	21.83
Ba	15.33	70.71	55.87	36.70
As	55.00	42.29	54.69	40.29
Co	12.21	16.16	23.77	25.25
BOD	41.48	66.81	40.75	52.92
COD	27.36	68.00	60.49	65.55
TN	12.31	17.74	18.33	22.06

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Table 5 Fatty acid profile of *C. vulgaris* and *T. chuii* at different concentrations of food wastewater

Experimental		Total fatty acids composition (%)										
Conditions		C12:0 Lauric Acid	C16:0 Palmitic Acid	C16:1 Palmitoleic Acid	C18:0 Stearic Acid	C18:1 Oleic Acid	C18:2 n6 Linoleic Acid	C18:3 n6 Gamma- Linoleic Acid	C20:4 Eicosa- tetraenoic acid	C20:5 Eicosa- pentaenoic Acid	C22:5 Docosa- hexaenoic Acid	C22:6 Docosa- hexaenoic Acid
<i>C. vulgaris</i>	Control	4.26	2.13	12.275	5.315	6.355	7.84	1.08	2.81	2.25	9.55	4.98
	25%	4.53	2.32	14.47	4.06	4.44	2.61	0.64	1.03	1.9	3.2	1.86
	50%	6.11	4.64	20.21	4.51	2.64	3.52	0.88	0.46	3.2	8.55	3.66
	75%	6.235	4.28	2.14	5.165	4.555	4.52	3.37	4.43	5.65	5.375	3.66
	100%	9.84	7.16	24.51	9.46	11.66	3.225	0.71	1.85	5.3	8.05	6.24
<i>T. chuii</i>	Control	1.55	1.04	14.06	2.65	38.2	3.33	2.07	1.04	0.73	0.91	2.12
	25%	1.24	1.62	20.46	2.24	27.74	1.71	ND	ND	ND	ND	ND
	50%	2.63	4.71	24.67	3.46	28.39	1.52	ND	ND	ND	ND	ND
	75%	3.80	1.58	17.12	5.42	21.41	2.1025	2.185	1.092	2.01	1.12	1.02
	100%	3.06	1.58	10.09	4.27	16.82	1.19	1.13	0.565	1.65	0.94	0.68

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Table 6 Biodiesel properties of *C. vulgaris* and *T. chuii* at different concentrations of food wastewater

Experimental Conditions		Kinematic Viscosity (mm ² /sec) Limit: 1.9-6.0	Specific Gravity Limit: 0.86-0.90	Cloud Point (°C) Limit: Variable	Cetane Number (min) Limit: 47	Iodine Value Limit: 96.5	Higher Heating Value (MJ/Kg) Limit: 40-41 (for oil seed crops)
<i>C. vulgaris</i>	Control	4.50	0.879	5.052	46.26	95.91	40.50
	25%	4.83	0.876	11.927	46.12	57.63	39.60
	50%	4.69	0.877	9.074	46.18	73.51	39.97
	75%	4.56	0.878	6.355	46.23	88.66	40.33
	100%	4.55	0.878	6.203	46.24	89.50	40.35
<i>T. chuii</i>	Control	4.501	0.879	5.081	46.266	95.751	40.499
	25%	4.805	0.876	11.505	46.131	59.981	39.653
	50%	4.746	0.877	10.247	46.158	66.987	39.819
	75%	4.553	0.878	6.185	46.242	89.608	40.354
	100%	4.763	0.876	10.623	46.150	64.890	39.769

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