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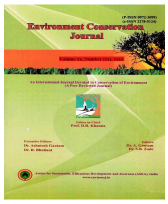

Analysis of the growth profile, biochemical composition and nutrient removal efficacy of *Spirulina* sp. NCIM 5143

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ABSTRACT

In the present manuscript, the growth profile of the microalgae *Spirulina* sp. NCIM 5143 was studied. Screening was performed on four commercial media, i.e., blue–green-11 (BG-11), Bold's basal medium (BBM), algal culture medium (ACM), Zarrouk's medium (ZM), and different concentrations (20%, 40%, 60%, 80%, and 100%) of unsterilized dairy effluent (UDE). Characterization of biomass was performed to assess its biochemical composition through various assays. Elemental composition and bioactive compound analysis were accomplished by inductively coupled plasma-atomic emission spectroscopy (ICP–AES) and gas chromatography–mass spectrometry (GC–MS), respectively. The results revealed that maximum values of most of the parameters, i.e., optical density (0.21), chlorophyll (2.00 mg/l), proteins (119.17 mg/l), and wet (4.06 g/l) and dry biomass weight (0.28 g/l), were found on ZM. For UDE, maximum growth parameters and the highest nutrient removal efficiency were obtained at 100% concentration. Biochemical analysis revealed that total Kjeldahl nitrogen (7.14±0.49%), crude protein (48.23± 3.34%), total antioxidant activity (3.07±0.03 mg AAE/g), and total phenols (8.88±1.93 mg GAE/g) were present in the biomass. Elemental and GC–MS analysis detected essential micronutrients and many bioactive compounds, respectively. Hence, this study proved that *Spirulina* sp. NCIM 5143 has the potential for the management of waste dairy effluent. This study also showed its cost-effectiveness, as the dairy effluent analyzed is used without any kind of sterilization. In addition, its biomass is rich in several essential elements, antioxidants, and bioactive compounds of therapeutic and nutraceutical importance.

Introduction

The dairy industry is the food industry of prime importance and produces an enormous volume of waste causing environmental contamination. Dairy industry effluent has a characteristic unpleasant odor that is offensive in nature, has high BOD (40–48,000 mg/l) and COD (80–95,000 mg/l) contents (Kushwaha *et al.,* 2011) with varying pH ranges (Kothari *et al.,* 2012), and contains ample amounts of nitrogen and phosphates, i.e., 14–830 mg/l and 9–280 mg/l, respectively (Gavala *et al.,* 1999). Dairy effluent is primarily alkaline; however, it turns acidic due to the fermentation of sugar present in milk in the form of lactose to lactic acid. Putrefaction of milk protein casein generates heavy and blackish sludge. Untreated dairy effluents are generally released into nearby water bodies, causing environmental pollution. Dairy effluent decomposes rapidly and reduces the dissolved oxygen levels of the receiving water bodies, causing anaerobic conditions and producing a foul smell. These water bodies further become breeding grounds for mosquitoes, flies, and other vectors harboring malaria, dengue fever, yellow fever, and chikungunya. Physical and chemical techniques are

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being employed for the management of these effluents. However, these traditional methods are not only expensive but also produce a great amount of sludge and are not as efficient (Yuan *et al.,* 2011). These constraints limit their use and prompted scientists to search for an alternate approach that is economical, efficient, and eco-friendly.

In recent years, microalgae have received much attention in the treatment and recycling of waste effluents. Microalgae can perform photosynthesis and can adapt to various environments. They have the astounding capability to thrive in nutrient-rich environments and consume them for sustaining and bioabsorbing heavy metals from effluents in their biomass. Mixotrophic cultivation of microalgae on dairy effluent has been reported to be successful, as this waste contains copious amounts of sugars and organic carbon (Girard *et al.,* 2014). All these characteristics make them remarkable means for sustainable and inexpensive waste effluent treatment. A microalgal sp. with enormous bioremediation potential is *Spirulina*. It is a blue–green microalga with a photoautotrophic mode of nutrition and requires mainly nutrients such as nitrate, urea, and ammonium salts for sustaining growth (Ariede *et al.,* 2017). *Spirulina* sp. employed for the treatment of dairy waste removed 80%, 72%, 61%, 56%, 71%, 56%, 77%, 54%, and 59% of nitrates, phosphorus, sulfate, total hardness, alkalinity, chloride, COD, TDS, calcium and magnesium hardness, respectively (Ahmed, 2014). Moreover, biomass harvested after effluent treatment is rich in many value-added products. *Spirulina* contains 70% proteins, 15 to 30% carbohydrates, and 3-9% lipids in its biomass along with other essential β-carotene, vitamins, and phycocyanin pigments (Andrade *et al*., 2019).

Several synthetic media have been used for microalgae cultivation, but the constraint associated with the use of these commercial media is that costs exceed the final products (Li *et al*., 2007). Therefore, it is imperative to search for alternative lower-cost substrates for microalgae cultivation. Dairy waste contains a sufficient amount of nitrogen and phosphates, which makes it an ideal medium for algal cultivation. The growth and biomass composition of *Spirulina* sp. depends upon several factors, including pH, salinity, temperature, and bicarbonate ions.

Although there are some studies on the characterization of microalgae, they are still fewer than the vast number of prevailing species of microalgae. Therefore, the current study was executed to analyze the growth profile, biochemical composition, and nutrient removal efficacy of *Spirulina* sp. NCIM 5143. To meet this end, the growth profile of *Spirulina* sp. NCIM 5143 was studied on four commercial media, viz. BG-11, ZM, BBM, and ACM and different concentrations of UDE. The biochemical composition of microalgal biomass was determined through various assays, and micronutrient composition and bioactive compounds were detected through inductively coupled plasma-atomic emission spectroscopy (ICP–AES) and gas chromatography–mass spectrometry (GC–MS) analysis.

Material and Methods

Collection of mother culture

The standard microalgal strain *Spirulina* sp. NCIM 5143 was procured from the National Collection of Industrial Microorganisms (NCIM) Laboratory, Pune, India.

Collection of dairy effluent

The dairy effluent sample was collected from a local dairy near gate no. 3, PAU, Ludhiana in plastic cans, sealed aseptically, and stored at -20°C until further analysis.

Morphology of *Spirulina* sp. NCIM 5143

The morphology of microalgal cells was observed and photographed at 40X under an Olympus 528293 microscope (Magnus Icon Freedom Model) using a Debro 5.1 Megapixel digital camera and Toup view software program.

Cultivation of microalgae in commercial media

Triplicate 250 ml Erlenmeyer flasks containing 100 ml of four different commercial media, i.e., BG-11 (Moghazy *et al*., 2019), BBM (Sorokina *et al*., 2020), ACM (Dar and Phutela, 2020), and ZM (Rajasekaran *et al*., 2016), were sterilized by autoclaving at 121 °C at 15 psi for 15 minutes. Inoculum was added at 10% inoculum. Flasks were maintained at 28±2 °C under light conditions of 54 μmol photons m^{-2} s^{-1} using compact fluorescent lamps maintaining a photoperiod cycle of 16:8 for 30 days. Growth was evaluated mainly in terms of change in optical density (at 750 nm) on every 3rd day, pigment concentration, i.e., chlorophyll (mg/l) on every 5th day, and dry biomass weight (g/l), carbohydrates, lipids, and protein content at the end of the 30-day growth period. Commercial media in which the highest values of all growth parameters were obtained, called respective growth media, were selected for further experimental analysis with UDE.

Cultivation of microalgae in dairy effluent

Triplicate Erlenmeyer flasks (250 ml) containing different concentrations of UDE, i.e., 20%, 40%, 60%, 80%, and 100%, were supplemented with the respective growth media to make a final volume of 100 ml. Inoculum was added at 10% inoculum. Flasks were maintained at 28±2 °C under light conditions of 54 μmol photons m^{-2} s^{-1} using compact fluorescent lamps maintaining a photoperiod cycle of 16:8 for 30 days. After completion of the incubation period, flasks were tested for various growth parameters of microalgae and reduction in various physicochemical parameters of unsterilized dairy effluent (UDE). A control consisting of only UDE without inoculum was run simultaneously.

Growth kinetic study

Microalgal growth kinetics were studied on commercial media as well as the concentration of UDE, whereby the highest rate of all the growth parameters was obtained using a modified nonlinear logistic equation (Dar, 2017) as given below:

$$\mathbf{Y} = \frac{\mathbf{A}}{\left[\mathbf{1} + \mathbf{exp}\left\{\mathbf{4}\left(\frac{\mu}{\mathbf{A}}\right)(\lambda - \mathbf{t}) + \mathbf{2}\right\}\right]}$$

where A is defined as the asymptote value (biomass g/l), μ is the growth rate (day^{-1}) and λ is the lag time (days). The fitting of the data in the model was done by using the MS Solver of Excel 2007.

Analytical methods

Physicochemical parameters

Standard protocols of APHA (2005) were followed for analyzing physicochemical parameters, i.e., dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS), before and after treatment with microalgae.

The nutrient removal efficacy of microalgae was calculated by the following formula (Ramsundar *et al*., 2017):

$$\textbf{Percent removal } (\%) = (\mathbf{IC} - \mathbf{FC})/\mathbf{IC} \times \mathbf{100}$$

where IC = initial concentration and FC = final concentration

Determination of total nitrogen and crude protein

The standard total Kjeldahl method (TKN) was used to determine the nitrogen content in a semiautomatic N-analyzer (Pelican Kelplus-KES06LR, Classic DX). Crude protein was calculated based on the expression: N × 6.25 (AOAC, 1990).

Determination of phosphorus

The method of Jackson (1967) was followed for the estimation of phosphorus content.

Determination of total phenols

Total phenols were estimated as per Taga *et al.* (1984).

Determination of total antioxidant activity

For estimation of total antioxidant activity, the procedure of Prieto *et al.* (1999) was followed.

Determination of DPPH radical scavenging activity

The standard protocol was used to estimate the DPPH radical scavenging activity. The percent scavenging effect (%) was calculated using the equation:

$$\textbf{Scavenging effect (\%)} = (1-(A_{sample}-A_{blank})/A_{blank}) \times 100$$

Determination of pigment content

Chlorophyll was analyzed by the protocol developed by El-Baky *et al.* (2008). The equations given by Lichtenthaler (1987) were used to calculate chlorophyll pigment.

$$\mathbf{Chl\ a + b = 7.05 * A661.6 + 18.09 * A644.8}$$
$$\mathbf{Chl\ a = 11.24 * A661.6 - 2.04 * A644.8}$$
$$\mathbf{Chl\ b = 20.13 * A644.8 - 4.19 * A661.6}$$

Determination of lipids
The sulfo-phospho-vanillin (SPV) assay is used to determine the lipid content in microalgal biomass (Mishra *et al*., 2014).

Determination of carbohydrates
The protocol devised by Dubios *et al.* (1956) was adopted for the estimation of carbohydrate content in microalgal samples.

Determination of proteins
The procedure given by Lowry *et al.* (1951) was employed for estimating total soluble proteins.

Elemental analysis by ICP–AES
The elemental composition of *Spirulina* sp. NCIM 5143 was determined through inductively coupled plasma-atomic emission spectroscopy (ICP–AES) (iCAP 6000 SERIES, ICP Spectrometer, Icap 6300 Duo, Thermo Electron Corporation, UK). The wet digestion method was used to digest the sample for further analysis (Hseu, 2004).

GC–MS analysis
The processing of samples for GC–MS analysis was performed according to the procedure of Krishnakumar *et al*. (2013). Lyophilized microalgal biomass was extracted with 50 mL of 80% methanol for 2 hours and filtered, and then the residue was again digested for 2 hours with methanol. The same extraction process was repeated 3 times. Then, sample extracts were vacuum evaporated at 45 °C, filtered, dissolved in 80% methanol, and stored in amber glass bottles for analysis by GC–MS (Thermo Trace 1300 GC coupled with Thermo TSQ 800 Triple Quadrupole MS) equipped with a splitless injector. The column was a BP 5MS with dimensions of 30 m×0.25×0.25 µm employing helium as a carrier gas with a flow rate of 1.0 ml/min. Initially, the oven temperature was programmed at 50 °C for 4.0 min, reprogrammed to 250 °C at a rate of 5 °C/min for 1.0 min, then again programmed to 280 °C at a rate of 15 °C/min and held for 18 min at 280 °C, with an injector temperature of 260 °C. Mass spectra (range=m/z 40–650) of the injected sample (1 µl) were collected. A comparative search of different mass spectra was carried out in the National Institute of Standards and Technology (NIST) library (2.0) for the identification of compounds detected in the analysis.

Statistical analysis
Experiments were completed in triplicate. Values are depicted as the mean±standard deviation. Values superscripted by different letters in tables represent the significant difference between the values based on Tukey's HSD Multiple Range test using IBM SPSS Statistics 22.

Results and Discussion

Morphology of *Spirulina* sp. NCIM 5143
Spirulina sp. NCIM 5143 cells were viewed under a microscope to determine their cellular morphology. Under 40 X, cells were large and filamentous. They were long unicellular nonheterocystous filamentous cells that grew in the form of a tightly coiled right- or left-handed helix (Plate I).

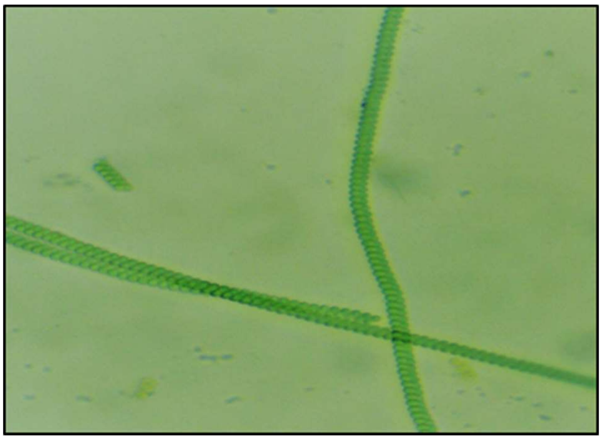

Plate I: *Spirulina* sp. NCIM 5143 cells under the Olympus 528293 microscope

Growth profile of *Spirulina* sp. NCIM 5143 on commercial media
Four commercial growth media were screened to find the best media for *Spirulina* sp. NCIM 5143, favoring its maximal growth. The growth and productivity of microalgal biomass are largely determined by the nutritional composition of the culture media (Madkour *et al*., 2012). The highest optical density (0.21) was recorded on ZM, followed by BBM, BG-11, and ACM, which showed absorbances of 0.20, 0.19, and 0.18, respectively. The lag phase of a very short duration was observed in all four commercial growth media and had a long exponential phase and pursued this

phase until the completion of the experimental period, i.e., 30 days. No stationary or death phase was detected (Fig. 1). This may be because a longer time is required for the decay phase to commence (Kodihalli *et al.,* 2018), but in this study, the cultivation time was only 30 days. The concentration of microalgal biomass can be determined by its optical density. The studied wavelength of 750 nm was selected for absorbance measurements because this wavelength measures in accordance with the light scattered and no light will absorb by the pigments present in the given sample (Yap *et al.,* 2018). Chlorophyll content followed a similar trend as optical density (Fig. 2). The highest chlorophyll content (2.00 mg/l) was recorded on ZM, followed by BG-11 (1.66 mg/l), BBM (1.54 mg/l), and ACM (1.21 mg/l). The chlorophyll content increased in the exponential phase and continued until the end of the experimental period. Hence, the most favorable commercial media for *Spirulina* sp. NCIM 5143 according to the current study is ZM. Similarly, Pandey *et al.* (2010) also reported that the most favorable media for *Spirulina maxima* is ZM. Other studies reported that the best growth medium for *Spirulina* (*Arthrospira fusiformis*) was LCMA medium compared to ZM. Madkour *et al*. (2012) found chlorophyll contents of 0.0701 ± 0.0089 µg/l and 0.0685 ± 0.0024 µg/l in *Spirulina platensis* grown on ZM and reduced cost media, respectively. Hence, the screening experiment revealed ZM as the most suitable medium for *Spirulina* sp. NCIM 5143.

Growth profile of *Spirulina* sp. NCIM 5143 on UDE

Cultivation of *Spirulina* sp. NCIM 5143 was carried out on different concentrations of UDE supplemented with ZM (Plate II). The results showed that the optical density and chlorophyll content increased from 20 to 100%. Absorbance was higher at all concentrations of UDE than in the control (0.23). The maximum optical density (1.77) was observed at 100% UDE, followed by 80% UDE (1.68), 60% UDE (1.61), 40% UDE (1.51), and 20% UDE (1.40). The microalgal strain showed a small or no prominent lag phase. However, the exponential phase lasted up to the 30th day (Fig 1). Maximum chlorophyll (4.59) was observed at 100% UDE, followed by 80% UDE (4.51), 60% UDE (4.11), 40% UDE (4.02), and 20% UDE (3.54), which were significantly higher than the control (2.34) (Fig. 2). The rationale behind the increase in microalgal growth with increasing concentrations of UDE might be because of the availability of essential nutrients required for sustaining microalgal growth because at lower concentrations, nutrient levels are not sufficient to support growth. Kothari *et al.* (2012) observed that the algal strain *Chlorella pyrenoidosa* showed the highest growth at a 75% concentration of dairy wastewater. The employment of UDE as algal culture media serves a dual purpose. First, it provides an inexpensive and readily available culture medium for microalgal cultivation. Second, dairy effluent is a waste product of the dairy industry that has no use and is generally discarded without any treatment. Hence, it prevents environmental pollution.

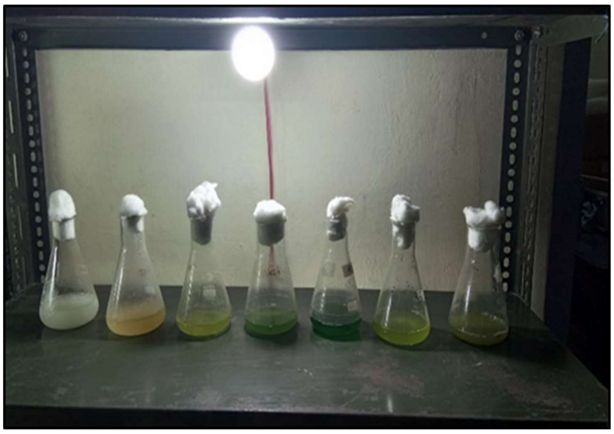

Plate II: Microalgae cultivation on dairy effluent

Biochemical constitution of *Spirulina* sp. NCIM 5143 on ZM

Proteins, lipids, carbohydrates, dry and wet biomass of *Spirulina* sp. NCIM 5143 growing on four culture media and different concentrations of UDE was done to predict the effect of nutrients present in UDE to support the biochemical composition of microalgae (Fig 1 & 2). The highest wet biomass (4.06 g/l) and dry biomass (0.28 g/l) were observed in ZM, while the highest protein (119.17 mg/l), carbohydrate (74.90 mg/l), and lipid contents (29.87 mg/l) were found in ZM, BBM, and BG-11 (Table 1). These observations again support the fact that ZM supports the maximum growth of *Spirulina* sp. NCIM 5143. As ZM (2.50 g/l) is rich in nitrogen, the highest proteins were found in algal biomass growing in ZM. Bajwa *et al*. (2017) also observed a similar trend in different parameters, viz. biomass yield, chlorophyll content, total carbohydrate, protein, and lipid production for four microalgae strains (*Chlorococcum aquaticum, Scenedesmus obliquus, Nannochloropsis oculata, Chlorella pyrenoidosa*) grown on five media (BG-11, BBM, Modified HS CHU#10, Modified Hoagland Medium, Half strength CH#10 medium). The microalgal strains in the current study showed different growth rates and different values of various biochemical parameters in different media. This is because in their natural cultural conditions and habitats, different species of microalgae show varied physiological needs (Falkowski, 1984). Our results are in agreement with those of Michael *et al*. (2019), where the highest protein (65.00 ± 0.26%) and highest lipid content (6.84 ± 0.05%) were observed in ZM, while the carbohydrate content (15.29 ± 0.41%) was higher in LCMA medium than in ZM media. Carbohydrates present in *Spirulina* are cellulose and sugar-free, which confers them the property of easy digestibility and is ideal for diabetic and obese patients (Braga *et al*., 2018). The lipids in *Spirulina* are enriched in PUFAs such as DHA, EPA, and ALA and are free from cholesterol, which is favorable in diseases such as atherosclerosis, obesity, and blood pressure (Anvara and Nowruzib, 2014).

Biochemical constitution of *Spirulina* sp. NCIM 5143 on UDE

In UDE, the highest wet biomass (2.80 g/l), dry biomass (0.15 g/l), protein (162.33 mg/l), carbohydrate (49.72 mg/l), and lipid content (99.80 mg/l) were observed at the 100% DE concentration (Table 1).

Table 1: Biochemical profile of *Spirulina* sp. NCIM 5143 on commercial media and dairy effluent (DE)

Growth Media	Wet biomass (g/l)	Dry biomass (g/l)	Protein (mg/l)	Carbohydrate (mg/l)	Lipid (mg/l)
BG-11	1.23^{h}	0.11^{bcd}	90.63^{h}	34.89^{e}	29.87^{f}
ACM	3.77^{b}	0.12^{bc}	87.42^{i}	45.54^{c}	17.20^{h}
BBM	2.40^{d}	0.10^{cde}	98.30^{g}	74.90^{a}	11.52^{i}
ZM	4.06^{a}	0.28^{a}	119.17^{d}	22.33^{h}	19.69^{g}
0% UDE	4.03	0.31	118.96	22.19	18.73
20% UDE	1.82^{g}	0.02^{g}	98.68^{f}	16.70^{i}	48.24^{e}
40% UDE	1.92^{f}	0.04^{fg}	115.99^{e}	33.24^{g}	50.73^{d}
60% UDE	2.69^{c}	0.06^{efg}	145.42^{c}	34.58^{f}	63.16^{c}
80% UDE	2.70^{c}	0.07^{def}	153.99^{b}	36.33^{d}	82.70^{b}
100% UDE	2.80^{c}	0.15^{b}	162.33^{a}	49.72^{b}	99.80^{a}

DE= Dairy effluent. Values superscripted by different letters in the column differ significantly (P≤0.05) from each other

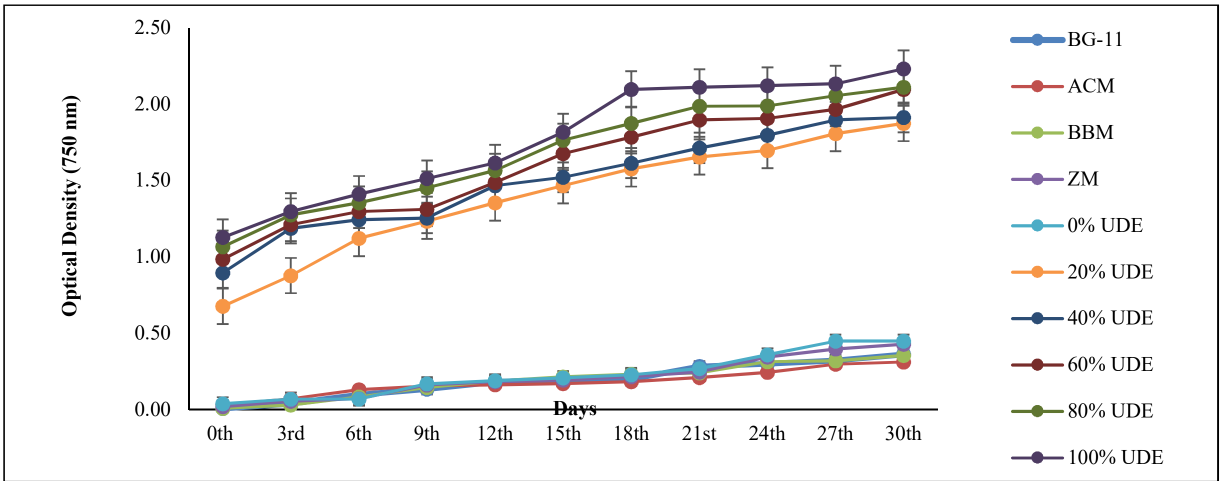

Figure 1: Growth profile of *Spirulina* sp. NCIM 5143

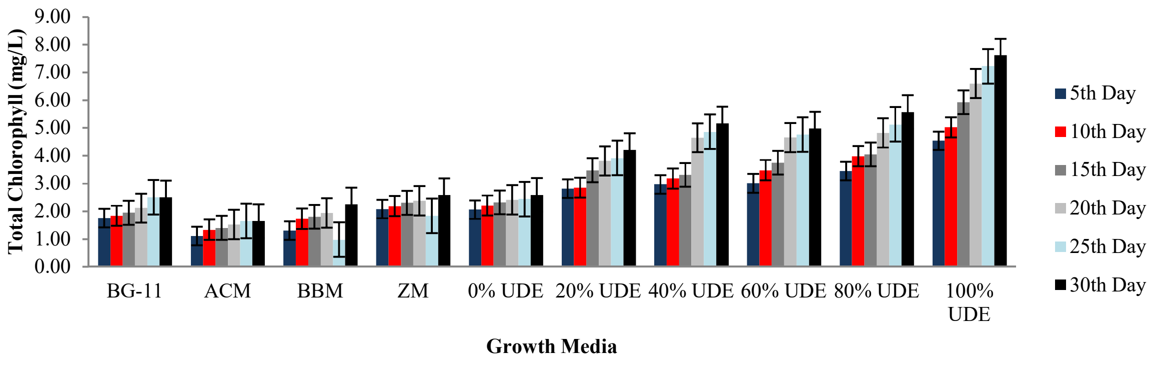

Figure 2: Total chlorophyll of *Spirulina* sp. NCIM 5143

It was observed in this study that all the parameters, i.e., optical density, chlorophyll, carbohydrates, proteins, and lipids, were higher in UDE than in commercial media. Salla *et al.* (2016) found that mixotrophic growth of *Spirulina platensis* grown on ZM supplemented with whey residues and lactose showed increased production of biomass and carbohydrates. In another study, Girard *et al.* (2014) observed that *Scenedesmus obliquus* under mixotrophic culture (in standard media supplemented with 40% whey) showed higher specific growth and biomass compared to standard media only. Similar results are obtained in our study, whereby all the growth parameters are higher in the mixotrophic culture of *Spirulina* sp. NCIM 5143 on dairy effluent compared to any of the four commercial media. This may be because compared to heterotrophic and autotrophic cultures, mixotrophic cultures are less affected by photoinhibition and have the additional advantage of assimilating both substrates and performing photosynthesis because both carbon sources maintain fixation and the organic carbon source maintains the acetyl-CoA pool (Mohan *et al.*, 2015).

Growth kinetic study

A study of microalgal growth kinetics by a nonlinear logistic model showed that the value of asymptote A, which determines the biomass production potential, was 0.65 at a growth rate per day (μ (day^{-1})) of 0.02 with a lag phase of 4.26 days in ZM media. In the case of UDE, the value of asymptote A was 2.02 with a growth rate per day (μ (day^{-1}) of 0.25 with no lag phase at 100% DE. The absence of a lag phase in 100% UDE indicated the microalga's potential to adapt to the new environment immediately and hence increase its nutrient removal ability (Daneshavar *et al.*, 2018). Similarly, Cardoso *et al.* (2020) also observed that *Spirulina* sp. LEB 18 showed no lag phase when grown in wastewater from aquaculture containing 25% and 50% ZM.

Nutrient removal efficacy of *Spirulina* sp. NCIM 5143

The high-level nutrient profile of UDE made it an ideal medium for algal growth. Dairy effluent does not contain any pathogens or toxic components but has vast amounts of oils, oxygen-demanding waste, and total suspended matter contributing to its polluting nature. Whey constitutes nutrients (55%) present in milk and is a useful waste generated by the milk industry with a considerable amount of organic content capable of supporting the mixotrophic and heterotrophic growth of microalgae (Sales *et al.*, 2017). In this study, the physicochemical parameters of UDE were analyzed to determine the nutrient removal efficiency of *Spirulina* sp. NCIM 5143. The results showed that *Spirulina* sp. NCIM 5143 cultivated on different concentrations of UDE at lab scale conditions reduced these undesirable characteristics to a greater extent. UDE was procured in the morning immediately after milk processing operations. Microalgae require organic carbon, nitrates, and phosphates for growth. The organic carbon and energy needs of microalgae are fulfilled by consuming the dissolved oxygen in wastewater. This organic carbon is used in the form of BOD and COD (Sarfraz *et al.*, 2021). Changes in various physicochemical parameters of UDE after cultivation of *Spirulina* sp. NCIM 5143 are described below (Table 2).UDE had an off-white color with a temperature of 42 °C, pH of 6.5, and an offensive odor. The decomposition of lactose present in milk into lactic acid under aerobic conditions contributed to the pH in the acidic range (Joseph, 1995). *Spirulina* sp. NCIM 5143 showed a maximum percent reduction in various physicochemical parameters at 100% UDE (Table 2). pH gives the measure of H^+ or OH^- ion activity of the solution by determining the acidity, alkalinity, or neutrality. All the concentrations of UDE showed significantly higher pH values than the control (6.50). The highest percent increase in the pH of the UDE after treatment with *Spirulina* sp. NCIM 5143 was 55.30% (10.11) in 100% DE, which was found to be significantly higher than the percent increase in pH at 80% UDE (51.77%) (9.88), 60% UDE (36.71%) (8.90), 40% UDE (21.97%) (7.94) and 20% UDE (10.25%) (7.21). Enhanced carbonates due to the photosynthetic activity of the microalgae may be the reason for increased pH. During the light period, microalgae use inorganic carbon sources autotrophically for the photosynthesis process, increasing the pH content (Kumar *et al.*, 2014).BOD is the measure of the quantity of oxygen required by the microbial population to oxidize the organic matter in a waste (Bhutiani and Ahamad 2108; Bhutiani *et al.*, 2021). For BOD, the provision of a standard nutrient supply and pH conditions is mandatory. Low oxygen solubility and strong-strength wastes are diluted to assure that demand does not exceed available oxygen (Verma and Singh, 2017). No dissolved oxygen was detected in the control, while the BOD was 11,000 mg/l. The results of statistical analysis showed that the highest percent reduction in BOD was 45.45% (6,000 mg/l) at 100% UDE, which was on par with the percent reduction at 80% UDE (45.45%) (6,000 mg/l). This was followed by 60% UDE (27.27%) (8,000 mg/l), and a minimum percent reduction was obtained at 40% and 20% UDE (9.09%) (6,000 mg/l) (Table 2). The ability of microorganisms present in wastewater to oxidize organic matter into CO_2 and water is defined as the BOD. The major objective of wastewater treatment is BOD removal. The reduction in BOD was due to a decrease in dissolved organic compounds and their derivatives (Kotteswari *et al.,* 2012). Fats, nutrients, lactose, detergents, protein, and inorganic salts in UDE may be responsible for high BOD and COD (Porwal *et al*., 2015).BOD alone does not give precise information about the organic matter content of wastewater because various toxins present in wastewater affect the validity of BOD tests (Hendricsk and David, 2007; Ahamad et al., 2023). Hence, a better estimate of organic matter is confirmed by COD. The COD content in the control experiment was 11,500 mg/l. The high amount of organic content in UDE is another reason for the higher COD value because COD accounts for the amount of nonbiodegradable organic matter present in effluents (Malaviya and Rathore, 2001). The reduction in COD was also maximum at 100% UDE (56.52%), followed by 80% UDE (47.82%) and 60% UDE (47.82%) (Table 2).

Table 2: Percent reduction in physicochemical parameters of dairy effluent after treatment with *Spirulina* sp. NCIM 5143

Growth media	pH	Biological Oxygen Demand (mg/l)	Chemical Oxygen Demand (mg/l)	Total Solids (mg/l)	Total Dissolved Solids (mg/l)	Total Suspended Solids (mg/l)	Total Phosphorus mg/l)	Total Kjeldahl Nitrogen (mg/l)	Crude Protein content (mg/l)
Control	6.50	11,000.00	11,500.00	24,190.00	23,600.00	1,000.00	10.88	195.55	1319.96
20% DE	7.21 (10.75) [e]	10,000.00 (9.09) [c]	10,000.00 (13.04) [c]	0.14 (99.99) [a]	17.94 (99.92) [b]	0.35 (99.96) [a]	6.40 (32.82) [d]	131.34 (32.83) [d]	0.08 (32.83) [d]
40% DE	7.94 (21.97) [d]	10,000.00 (9.09) [c]	10,000.00 (13.04) [c]	0.10 (99.99) [a]	2.34 (99.99) [a]	0.20 (99.98) [a]	6.60 (41.77) [c]	113.83 (41.79) [c]	0.07 (41.78) [c]
60% DE	8.90 (36.71) [c]	8,000.00 (27.27) [b]	6,000.00 (47.82) [b]	0.06 (99.99) [a]	2.06 (99.99) [a]	0.20 (99.98) [a]	6.70 (50.77) [b]	96.25 (50.78) [b]	0.06 (50.77) [b]
80% DE	9.88 (51.77) [b]	6,000.00 (45.45) [a]	6,000.00 (47.82) [b]	0.66 (99.99) [a]	0.04 (99.99) [a]	0.22 (99.98) [a]	7.40 (59.69) [a]	78.80 (59.70) [a]	0.05 (59.70) [a]
100% DE	10.11 (55.30) [a]	6,000.00 (45.45) [a]	5,000.00 (56.52) [a]	0.14 (99.99) [a]	0.78 (99.99) [a]	0.17 (99.98) [a]	7.90 (59.69) [a]	78.80 (59.70) [a]	0.05 (59.70) [a]

DE=Dairy effluent. Values in parentheses show percent reduction. Values superscripted by different letters in the column differ significantly (P≤0.05) from each other

In addition to CO_2, the inherent potential of microalgae to metabolize organic compounds as a source of energy might be responsible for the reduction in COD, which is an indirect means of organic components in the effluent (Hu *et al.,* 2012).The TS content is due to the prevalence of compounds such as carbonates, bicarbonates, chlorides, sulfate, phosphate, nitrate, calcium, magnesium, sodium, potassium, manganese, and organic matter (Sahana and Shirnalli, 2018; Bhutiani et al., 2018; Bojago et al., 2023). TS in the control was 24,190.00 mg/l. The maximum percent reduction of total solids (99.99%) was at par at 20% (0.14 mg/l), 40% (0.10 mg/l), 60% (0.06 mg/l) and 80% UDE (0.66 mg/l), and 100% UDE (0.14 mg/l) (Table 2).TSS quality checks the wastewater after treatment in the plant and is defined as the dry weight of particles trapped by a filter (Chikwe and Onojake, 2016). UDE is characterized by higher levels of TDS, which is contributed by a higher concentration of biodegradable organic matter (Kotteswari *et al.,* 2012). The concentration of TSS was 1,000.00 mg/l in the control. The maximum reduction in TSS was at 100% UDE (99.98%) (0.17 mg/l) (Table 2). The reason might be the transformation of the TSS present in UDE into dissolved materials that are assimilated by the algae (Rao *et al.,* 2011).The total dissolved solid content of UDE (control) was 23,600.00 mg/l. The percent reduction in TDS (99.98%) was highest and similar at all concentrations, i.e., 100% (0.78 mg/l), 80% (0.04 mg/l), 60% (2.06 mg/l) and 40% UDE (2.34 mg/l), except for 20% UDE (99.92%) (17.94 mg/l), which was significantly lower than the others (Table 2). The presence of sodium (Na^+) and chloride (Cl^-) ions is attributed to the employment of a great number of cleaners (alkaline) in the dairy plant (Demirel *et al.,* 2005). *Spirulina* sp. growth in mixotrophic culture is supported by the availability of glucose, acetate, or glycerol, which are sources of organic carbon (Cardoso *et al*., 2020). The high TDS percent reduction in the present study reflects that the utilization of specific salts by *Spirulina* sp. NCIM 5143 is required to meet its metabolic needs. Phosphorus deficiency greatly affects the ability to grow, chlorophyll synthesis, and cellular metabolism because the Calvin cycle and many phosphorylation syntheses are dependent on it (Liang *et al.,* 2013). On the other hand, excess phosphorus causes less growth and leaf necrosis and reduces zinc availability (Loneragan and Webb, 1993). The maximum percent reduction in total phosphorus was 59.69% (7.90 mg/l) at 100% UDE and 80% UDE (59.69%) (7.40 mg/l) compared to the control (10.88 mg/l) (Table 2). Algae use phosphorus for sustaining their growth and development, synthesizing their biomass and phospholipids, adenosine triphosphate (ATP) molecules, intracellular polyphosphate compounds, and nucleic acids, which are assimilated as inorganic orthophosphate, in the form of $H_2PO_4^-$ or HPO_4^{2-} (Ding *et al.,* 2015), which might be the most appropriate reason for phosphorus reduction. Removal of phosphorus from industrial effluent is a complex process. Orthophosphates are the preferred form for assimilation and for growth and synthesis of nucleic acids and several value-added compounds, such as astaxanthin and PUFAs.The preferred forms of nitrogen used by plants are ammonium (NH_4^+) and nitrate (NO_3^-). Nitrogen deficiency affects the productivity and growth of plants, whereas surplus nitrogen causes groundwater pollution and is harmful to humans (Akao *et al*., 2021). The total Kjeldahl nitrogen (TKN) and crude protein content were 195.55 and 1319.96 mg/l, respectively, in the control experiment. Total Kjeldahl nitrogen and crude protein content showed the highest percent reduction at 100 and 80% UDE (59.70%) and the lowest at 20% UDE (32.83%) (Table 2). Amino acid and protein synthesis requires nitrogen (Sialve *et al*., 2009). High rates of nitrogen removal were observed in the study because of the presence of the enzymes nitrate and nitrite reductase, which reduce nitrate ions (NO^-_3) to nitrite ions (NO^-_2) and then to ammonium ions (NH_4^+) (Salama *et al*., 2017). Cardoso *et al*. (2020) observed 72.11% nitrate and nitrite (79.28%) nitrate and 72.72% TDS removal rates by *Spirulina* sp. LEB 18. Therefore, both nitrogen and phosphorus elements in water and soil should be within permissible limits because both the excess and deficiency of these elements negatively affect living organisms. Sahana and Shirnalli (2018) reported significant reduction efficiency in various physicochemical parameters (pH, total solids, chemical oxygen demand, nitrate, phosphate) in 40% of untreated effluent and 100% of treated effluent after treatment with *Chlorella*

MA-6 microalgae. Previously, Choi *et al.* (2016) reported that *Chlorella vulgaris* after 10 days of treatment reduced the biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), total nitrogen (TN), and total phosphorus (TP) contents in dairy wastewater by 85.61%, 80.62%, 29.10%, 85.47%, and 65.96%, respectively.

Biochemical analysis of *Spirulina* sp. biomass

Total Kjeldahl Nitrogen (TKN) and crude protein (CP)

The results of the biochemical characterization of *Spirulina* sp. NCIM 5143 by various assays showed that TKN and CP were 7.14±0.49% and 48.23±3.34%, respectively. The protein content in *Spirulina,* as reported by Sharoba (2014), was 62.84%, which was higher than that obtained in this study. The protein content of microalgae varies widely based on the species, environmental conditions, and protocols used for measurement (Maehre *et al*., 2018). Exceptionally high levels of protein content in *Spirulina sp.* are not common even in microbes except bacteria such as *Cellulomonas,* which is reported to have 80% dry-weight protein content (Kameshwari *et al*., 2020). However, it also has high levels of nucleic acid content, which can cause many detrimental health conditions, such as gout. In *Spirulina,* even after the presence of high levels of proteins, the nucleic acid content is less than 5% of the dry weight (Anvara and Nowruzib, 2014). As seen from the above results, the protein content determined by the total Kjeldahl method (48.23±3.34%) was much higher than that obtained from Lowry's method (119.17 mg/l). A possible explanation for this could be that the Kjeldahl method determines the proteins in the sample based on the amount of nitrogen detected in the sample by simply multiplying the nitrogen content by a conversion factor (6.25), and all nitrogen in food samples is protein bound. Varied relative nitrogen content is observed between amino acids, and accordingly, different food proteins have a variable composition of amino acids. Moreover, nitrogen is present in several compounds, such as nitrate, ammonia, urea, nucleic acids, free amino acids, chlorophylls, and alkaloids, called nonprotein nitrogen, and variable relative contents contain nitrogen. Many workers have also reported that this conversion factor (6.25) overestimates the total protein count (Maehre *et al*., 2018**)**.

Total phenols

Total phenols in *Spirulina* sp. NCIM 5143 was 8.88 ±1.93 mg GAE/g. Different researchers reported varied values of phenolic compounds in *Spirulina,* e.g., El-Baky *et al.* (2009) reported that total phenols in *Spirulina maxima* were 12.94 ± 0.93 mg GAE/g, while phenolic compounds reported by Sehghiri *et al*. (2019) in *Arthrospira platensis* were 4.19 ± 0.21 mg GAE/g. According to Rechner *et al.* (2001), phenylalanine amino acid is a precursor for the enzyme ammonia lyase, which converts it into trans-cinnamic acid, then to cumaric acid, and caffeic acid, and ultimately several chemical reactions finally convert these into phenols and flavonoids in green algae. Phenolic compounds from algae have been reported to ward off oxidative damage to DNA, proteins, and lipids, which have a crucial role in cancer and brain dysfunctions such as diseases (Droge, 2002). Li *et al*. (2007) reported phenolic components of 23 microalgae strains to differ from 2.12 to 39.87 mg GAE g^{-1}, 0.01 to 9.80 mg GAE g^{-1}, and 0.95 to 10.68 mg GAE g^{-1} in hexane, ethyl acetate, and water fractions, respectively.

Total antioxidant activity and DPPH radical scavenging activity

The total antioxidant activity of *Spirulina* sp. NCIM 5143 was 3.07±0.03 mg AAE/g. In this study, good DPPH radical scavenging activity was recorded in *Spirulina* sp. NCIM 5143. The DPPH radical scavenging activity showed maximum % inhibition (75.07±0.09%) at the highest concentration (1000 µg/ml), followed by 500 µg/ml (41.20±0.30%) and 250 µg/ml (20.95±0.11%). This was lower than the antioxidant activity of the standard (ascorbic acid) at three different concentrations, i.e., 250, 500, and 1000 µg/ml (80.00±0.12, 90.00±0.45 and 109.88±0.11, respectively). Abd El-Baky *et al*. (2007) found that antioxidants inhibit the lipid peroxidation reaction by free radical scavenging activity, and their effect on DPPH activity was due to their ability to donate a hydrogen atom. Antioxidants inhibiting lipid peroxidation are generally validated by the free radical scavenging mechanism. In this study, methanolic extracts of *Spirulina sp.* NCIM 5143 containing phenols halted the extension of the chain reaction in the lipid oxidation reaction via the donation of a hydrogen atom to free radicals.

Phytochemical analysis

Phytochemical detection assays showed that quinones, tannins, saponins, terpenoids, and steroids were present in *Spirulina* sp. NCIM 5143. The results of the present study for phytochemical screening are in agreement with a study reported by Mane and Chakraborty (2018) that phytochemicals such as alkaloids, terpenoids, steroids, saponins, phenols, flavonoids, tannins, coumarins, and quinines are present in *Spirulina platensis.* Seghiri *et al*. (2019) studied the characterization of *Arthrospira platensis* and showed that the protein, carbohydrate, mineral, crude fiber, lipid, ash, flavonoid, and phenolic contents were 76.65 ± 0.15%, 6.46 ± 0.32%, 20.91 ± 0.88%, 4.07 ± 1.42%, 2.45 ± 0.82%, 14.56 ± 0.74, 15.60 ± 2.74 mg RE/g dw, and 4.19 ± 0.21 mg GAE/g dw, respectively. Moreover, they further reported that higher antioxidant activity (23 mg TE/g dw) was observed in methanolic extracts of algae, and these algae are safe for consumption as human food.

ICP–AES

Micronutrient analysis of *Spirulina* sp. NCIM 5143 showed that essential elements such as calcium (21.33 mg/kg), magnesium (28.96 mg/kg), iron (7.34 mg/kg), phosphorus (86.30 mg/kg), boron (1.16 mg/kg), copper (0.16 mg/kg), manganese (0.74 mg/kg), and zinc (0.64 mg/kg) were present. These elements have a variety of functions in various important metabolic activities. Micronutrients such as magnesium, calcium, and iron, which are metallic in nature and found in *Spirulina* sp. NCIM 5143, are required for the regulation of protein and chlorophyll synthesis, osmotic regulation, and nitrogen assimilation in microalgae (Beltrán-Rocha *et al*., 2017). Wuang *et al.* (2016) previously reported that *Spirulina platensis* growing on wastewater contained nitrogen (7.8%), phosphorus (0.8%), potassium (1.6%), and calcium (0.4%) in its biomass. Likewise, our results agree with those of Liestianty *et al*. (2019), who reported that *Spirulina* sp. contains essential micronutrients such as potassium (K), phosphorus (P), calcium (Ca), zinc (Zn), sodium (Na), manganese (Mn), magnesium (Mg) and iron (Fe), as analyzed by ICP–OES. These elements are of utmost importance for human nutrition. Maintenance of tissues, formation of bone and teeth, role as cofactors and coenzymes, body function regulation, and several other biochemical and physiological activities of the body are some of the important functions of micronutrients. For the maintenance of health throughout a lifetime, these micronutrients are essential (Gernand *et al*., 2016). Calcium, sodium, magnesium, phosphorus, and potassium are the five prime minerals in the human body, while all other remaining inorganic elements are called trace elements, including iodine (I), molybdenum (Mo), copper (Cu), chlorine (Cl), zinc (Zn), manganese (Mn), selenium (Se), sulfur (S), iron (Fe) and cobalt (Co). Bones and teeth contain approximately 99% calcium and constitute up to 920 g to 1200 g of the total body weight (Berdanier*et al*., 2013). Sodium, chlorine, sulfur, magnesium, and potassium are the major nutrients that constitute 0.85% of the total body weight (Awuchi and Godswill, 2020).

Heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni) were completely absent in *Spirulina* sp. NCIM 5143. The WHO has imposed certain specific limits for heavy metal concentrations in plants (Table 3). The maximum permissible limits for cadmium, chromium, nickel, and lead in plants are 0.005, 0.01, 0.05, and 0.05 ppm, respectively. The concentrations of heavy metals in *Spirulina* sp. NCIM 5143 were well below the permissible limits of the WHO, which ensures its safety for consumption purposes. Rebolloso-Fuentes *et al*. (2001) observed that calcium, potassium, sodium, magnesium, zinc, iron, manganese, copper, nickel and cobalt were present at concentrations of 972, 533, 659, 316, 103, 136, 3.4, 35, 0.22 and <0.1 mg, respectively, while heavy metals such as cadmium and lead were absent per 100 g dry biomass weight of marine microalgae *Nannochloropsis* spp.

GC–MS analysis

GC–MS is a high-value and authentic technique for the identification of bioactive compounds in a given sample. GC–MS analysis of methanolic extracts of *Spirulina* sp. NCIM 5143 detected several bioactive compounds (Fig. 3) (Table 4). A total of twelve compounds of therapeutic and nutraceutical value were present in the biomass. The major compounds were as follows: phytol (26.15%), hexadecanoic acid, methyl ester (18.82%), trans-13-octadecenoic acid, methyl ester (22.31%), and 9,12-octadecadienoic acid, methyl ester (20.75%). Phytol was present as a major compound (26.15%) at a retention time (RT) of 38.87. Phytol is a component of various pharmaceuticals used for prophylaxis, prevention, and treatment of hypercholesterolemia, for maintaining normal levels of cholesterol in serum, obesity, insulin resistance, diabetes, atherosclerosis, and related cardiovascular diseases (Olofsson, 2011). Many plants used in Chinese medicines traditionally contain phytol, which has low toxicity, is

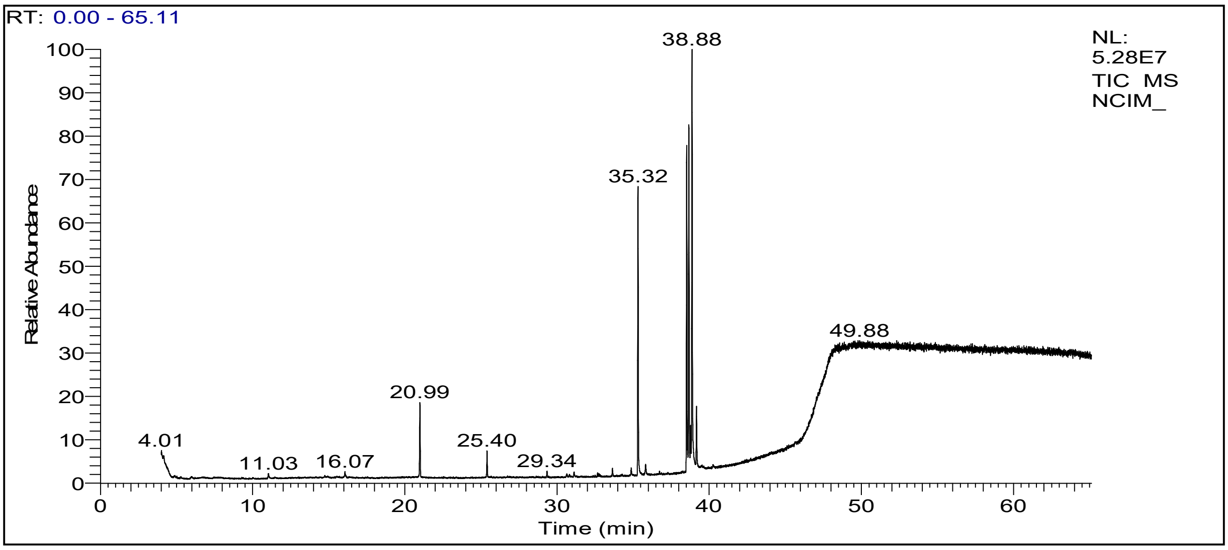

Figure 3: Peaks showing various bioactive compounds in Spirulina sp. NCIM 5143 as revealed in the GC–MS profile

Table 3: Maximum permissible limits for heavy metals in plants and soil

Element	Symbol	WHO Limits (ppm)
Cadmium	Cd	0.005
Chromium	Cr	0.01
Copper	Cu	1.00
Iron	Fe	0.30
Manganese	Mn	0.01
Nickel	Ni	0.05
Lead	Pb	0.05
Zinc	Zn	5.00

Source: Musa *et al.*, 2016.

inexpensive, and is used in pharmaceuticals (Yu *et al.*, 2009). The fatty acid compound 9,12-octadecadienoic acid, methyl ester (20.75%), found at RT 38.52, has been reported to have an anticancer effect (Yu *et al.,* 2005). Trans-13-octadecenoic acid, methyl ester (22.31%) at RT 38.66 has anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene-D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, and flavor properties (Krishnamoorthy and Subramaniam, 2014). Similarly, at an RT of 35.32, hexadecanoic acid, methyl ester (18.82%) was detected, which was reported to have antibacterial and antifungal properties (Chandrasekaran *et al.,* 2011), anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicidal, insectifuge, antihistaminic, antieczemic, antiacne, alpha-reductase inhibitor, antiandrogenic, anti arthritic, and anticoronary properties (Krishna moorthy and Subramaniam, 2014). Organosil oxane compounds such as Cyclohexasiloxane dodeca methyl,Cycloheptasiloxanetetradecamethyl, Cyclo detected in methanolic extracts of *Spirulina* sp. NCIM 5143. Cyclohexasiloxanedodecamethyl has antimicrobial, antifouling immunomodulatory, and antitumor activities, and cyclo heptasil oxane tetradecamethyl has skin-conditioning agent, fragrance, and antimicrobial properties (Chaudhary and Tripathy, 2015). Jubie and Dhanaba (2012) discovered eight compounds in the GC–MS octasil oxane, and hexadecamethyl were also analysis of *Spirulina platensis*. Among them, stearic acid, gamma-linolenic acid, linoleic acid,heptadecanoic acid, and oleic acid were the five major compounds. Soltani *et al.* (2005) reported many fatty acids and volatile components, such as phytol, fucosterol, neophytadiene, or palmitic, palmitoleic, and oleic acids, from liquid extracts from cyanobacteria by GC–MS and HPLC-DAD.

Table 4: Bioactive compound analysis by GC–MS

SN	Compound name	Molecular Formula	Area (%)	Retention time (RT)
1.	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	4.87	20.99
2.	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$	1.33	25.41
3.	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	0.03	29.34
4.	Z-3-Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	0.13	33.64
5.	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$	0.07	34.87
6.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.82	35.32
7.	Dibutyl phthalate	$C_{16}H_{22}O_4$	0.24	35.82
8.	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	20.75	38.52
9.	Trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	22.31	38.66
10.	6-Octadecenoic acid, methyl ester, (Z)	$C_{19}H_{36}O_2$	1.85	38.78
11.	Phytol	$C_{20}H_{40}O$	26.15	38.87
12.	Methyl stearate	$C_{19}H_{38}O_2$	3.46	39.17

The main mandate of performing GC–MS analysis of *Spirulina* sp. NCIM 5143 is to explore its biochemical constitution, which further has application as a functional food. GC–MS analysis of *Spirulina* sp. NCIM 5143 revealed the presence of phytol (26.15%), hexadecanoic acid, methyl ester (18.82%), trans-13-octadecenoic acid, methyl ester (22.31%), and 9,12-octadecadienoic acid, methyl ester (20.75%) as major bioactive compounds. Each of these compounds has an important biological function. A functional food contains compounds that have biological and physiological importance, and it also confers several health advantages. The compounds present in such foods that provide health benefits have been generally termed bioactive compounds. Bioactive compounds are of great significance because they are endowed with antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, and antitumor activities. Therefore, they protect humans from damaging free radicals and reactive oxygen species (ROS) (Banwo *et al*., 2021). Microalgal biomass is also enriched in these bioactive compounds, as previously reported by many workers. The compounds produced by microalgae possess myofibroblast differentiation-inducing, anti-proliferative, angiotensin I-converting enzyme inhibitory, antioxidant, antimicrobial, anti-chymotrypsin, anti-parasitic, anti-trypsin, anti-elastase, and hepatic fibrosis inhibitory activities (Saha and Murray, 2018).

Conclusion

Microalgae are endowed with the remarkable property of treating waste effluent, thus recycling water sources along with biomass enriched with many valuable compounds, such as phenols, antioxidants, proteins, lipids, carbohydrates, and phytocompounds. The present study evaluates the nutrient removal efficiency of *Spirulina* sp. NCIM 5143 along with its biochemical characterization. The results showed that *Spirulina* sp. NCIM 5143 was able to use pollutants present in dairy effluent to meet its growth requirements. This showed that wastewater treatment by microalgae can be employed either as a secondary or tertiary treatment step. In addition, biomass harvested after treatment is enriched in many valuable compounds for human health. The absence of heavy metals in microalgal biomass ensures their safety for human consumption purposes.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abd El-Baky, H.H., El Baz, F.K., & El-Baroty, G.S. (2007). Production of carotenoids from marine microalgae and its evaluation as safe food colorant and lowering cholesterol agents. *American-Eurasian Journal of Agricultural and Environmental Science,* 2(6), 792-800.

Ahamad, F., Sharma, A. K., & Tyagi, S. K. (2023). A Study on Comparative Assessment of Water Quality of Dal and Nigeen Lakes of Jammu and Kashmir, India. *Agro Environmental Sustainability*, *1*(1), 48-56.

Akao, P.K., Singh, B., Kaur, P., Sor, A., Avni, A., Dhir, A., Verma, S., Kapoor, S., Phutela, U.G., Satpute, S., Sharma, S., Avisar, D., Sandha, K.S. & Mamane, H. (2021). Coupled microalgal–bacterial biofilm for enhanced wastewater treatment without energy investment. *Journal of Water Process Engineering*. Vol. 41:102029 https://doi.org/10.1016/j.jwpe.2021.102029

Andrade, B.B., Cardoso, L.G., Assis, D.J., Costa, J.A.V., Druzian, J.I., & Lima, S.T.C. (2019). Production and characterization of Spirulina sp. LEB 18 cultured in reused Zarrouk's medium in a raceway-type bioreactor. *Bioresource Technology,* 284, 340–348. https://doi.org/10.1016/j. biotech.2019.03.144.

Anvara, A.A., & Nowruzib, B. (2014). Nutritional value of spirulina and its use in the preparation of some complementary baby food formulas. *Journal of Agroalimentary Processes and Technologies*, 20, 330-350.

AOAC, (1990). Association of official analytical chemists, official methods of analysis. 15th ed. Washington DC.

APHA, (2005). Standard Methods for Examination of Water and Wastewater 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Ariede, M.B., Candido, T.M., Jacome, A.L.M., Velasco, M.V.R., de Carvalho, J.C.M., & Baby, A.R. (2017). Cosmetic attributes of algae—A review. *Algal Research,* 25, 483–487, doi:10.1016/j.algal.2017.05.019.

Awuchi, & Godswill, C. (2020). Health Benefits of Micronutrients (Vitamins and Minerals) and their Associated Deficiency Diseases: A Systematic Review. *International Journal of Food Sciences*, 3,1-32.

Bajwa, K., Bishnoi, N.R., Kirrolia, A., Sharma, J., & Gupta, S. (2017). Comparison of various growth media composition for physio-biochemical parameters of biodiesel producing microalgal species (*Chlorococcum aquaticum, Scenedesmus obliquus, Nannochloropsisoculata, Chlorella pyrenoidosa). European Journal of Biotechnology and Bioscience,*5, 27-31.

Banwo, K., Olojede, A.O., Adesulu-Dahunsi, A.T., Verma, D.K., Thakur, M., Tripathy, S., Singh, S., Patel, A.R., Gupta, A.K., Aguilar, C.N., Utama, G.L. (2021) Functional importance of bioactive compounds of foods with Potential Health Benefits: A review on recent trends. *Food Bioscience*. 43, 1-23.

Beltrán-Rocha, J.C., Guajardo-Barbosa, C., Barceló-Quinta, I.D. & López-Chuken, U.J. (2017). Biotratamiento de efluentes secundarios municipales utilizando microalgas: Efecto del pH, nutrientes (C, N y P) y enriquecimiento con CO_2. *Revista de Biologia Marina y Oceanografia*, 52, 417–427. https://doi.org/10.4067/s0718-19572017000300001

Berdanier, C.D., Dwyer, J.T., Johanna, T., & David, H. (2013). Handbook of Nutrition and Food (3rd ed.). CRC Press. p. 199. ISBN 978-1-4665-0572-8.

Bhutiani, R., Ahamad, F., & Ruhela, M. (2021). Effect of composition and depth of filter-bed on the efficiency of Sand-intermittent-filter treating the Industrial wastewater at Haridwar, India. *Journal of Applied and Natural Science*, *13*(1), 88-94.

Bhutiani, R., & Ahamad, F. (2018). Efficiency assessment of Sand Intermittent Filtration Technology for waste water Treatment. *International Journal of advance research in science and engineering (IJARSE)*, *7*(03), 503-512.

Bhutiani, R., Ahamad, F., Tyagi, V., & Ram, K. (2018). Evaluation of water quality of River Malin using water quality index (WQI) at Najibabad, Bijnor (UP) India. *Environment Conservation Journal*, *19*(1&2), 191-201.

Bojago, E., Tyagi, I., Ahamad, F., & Chandniha, S. K. (2023). GIS based spatial-temporal distribution of water quality parameters and heavy metals in drinking water: Ecological and health modelling. *Physics and Chemistry of the Earth, Parts A/B/C*, 103399.

Braga, V.S., Mastrantonio, D.J.S., Costa, J.A.V., & Morais, M.G. (2018). Cultivation strategy to stimulate high carbohydrate content in *Spirulina* biomass. *Bioresource Technology,* 269, 221–226. https://doi.org/10.1016/j.biortech.2018.08.105

Cardoso, L.G., Duarte, J.H., Costa, J.A.V., Assis, D. de J., Lemos, P.V.F., Druzian, J.I., Souza, C.O.de, Nunes, I.L., & Chinalia, F.A. (2020) *Spirulina* sp. as a Bioremediation Agent for Aquaculture Wastewater: Production of High Added Value Compounds and Estimation of Theoretical Biodiesel. *Bioenergy Research,* 14, 254–264

Chandrasekaran, M., Senthilkumar, A., & Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum L. European Review for Medical and Pharmacological Science,* 15, 775-80.

Chaudhary, R., & Tripathy, A. (2015). Isolation and Identification of Bioactive Compounds from Irpex Lacteus Wild Fleshy Fungi. *Journal of Pharmaceutical Sciences and Research,*7, 424-34.

Chikwe, T.N. & Onojake, M.C. (2016) An appraisal of Physicochemical Parameters and some Trace metals at the Disposal Points of Five Industrial Effluents in Trans-Amadi Industrial Area of Port Harcourt, Nigeria. *Journal of Applied Science and Environment Management*, 20, 31-37

Choi, H. J. (2016) Dairy wastewater treatment using microalgae for potential biodiesel application. *Environment Engineering Research,* 21, 393-400. https://doi.org/10.4491/eer.2015.151.

Daneshvar, E., Antikainen, L., Koutra, E., Kornaros, M., & Bhatnagar, A. (2018). Investigation on the feasibility of *Chlorella vulgaris* cultivation in a mixture of pulp and aquaculture effluents: treatment of wastewater and lipid extraction. *Bioresource Technology,* 255,104–110. https://doi.org/10.1016/j.biortech.2018.01.101.

Dar, R.A. (2017). Bioprospects of microalgal isolates from water logged area of Punjab for biogas production. Ph.D. thesis, Punjab Agricultural University, Ludhiana, Punjab.

Dar, R.A., & Phutela, U.G. (2020). Enzymatic and hydrothermal pretreatment of newly isolated *Spirulina subsalsa* BGLR6 biomass for enhanced biogas production. *Waste and Biomass Valorization,*11, 3639-3651.

Demirel, B., Yenigun, O., & Onay, T.T. (2005). Anaerobic treatment of dairy wastewaters: A review. *Process Biochemistry,* 40, 2583–95.

Ding, J., Zhao. F., Cao. Y., Xing, L., Liu, W., Mei, S., &Li, S. (2015). Cultivation of microalgae in dairy farm wastewater without sterilization. *International Journal of Phytoremediation,*17, 222-27.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry,* 28, 350–56.

El-Baky, H.H.A., El-Baz, F.K., & El-Baroty, G.S. (2008). Characterization of nutraceutical compounds in blue green alga *Spirulina maxima. Journal of Medicinal Plants Research,* 2, 292-300.

Falkowski, P.G. (1984). Physiological responses of phytoplankton to natural light regimes. *Journal of Plankton Research,* 6, 295-307.

Gernand, A.D., Schulze, K.J., Stewart, C.P., West, Jr. K.P., & Christian, P. (2016). Micronutrient deficiencies in pregnancy worldwide: Health effects and prevention. *Nature Reviews Endocrinology,* 12, 274–289.

Girard, J.M., Roy, M.L., Hafsa, M.B., Gagnon, J., Faucheux, N., Heitz, M., Tremblay, R., & Deschênes, J-S (2014). Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production. *Algae Research*, 5, 241–248.

Hendricks, I. & David, W. (2007). Water Treatment Unit Processes: Physical and Chemical. Boca Raton, FL: *CRC Press,* pp 44–62.

Hseu, Z. (2004). Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresource Technology,* 95, 53-59.

Hu, B., Min, M., Zhou, W., Li, Y., Mohr, M., Cheng, Y., Lei, H., Liu, Y., Lin, X., Chen, P., &Ruan, R. (2012). Influence of exogenous CO2 on biomass and lipid accumulation of microalgae *Auxenochlorella protothecoides* cultivated in concentrated municipal wastewater. *Applied Biochemistry and Biotechnology,* 166, 1661–73.

Jackson, M. L. (1967). Soil Chemical Analysis. Prentice -Hall, Inc., Englewood Cliffs, USA.

Joseph, K. (1995). Pollution control in dairy industry, paper presented at Tamil Nadu veterinary and animal sciences university at veterinary college on 10th Oct 1995, sponsored by the institute of food and dairy technology and Tamil Nadu pollution control board.

Jubie, S., & Dhanaba, S.P. (2012). Gas Chromatography- Mass Spectrometry Analysis and Antibacterial Activity of Fatty acid Mixture of *Spirulina platensis*. *Journal of Pharmaceutical Science and Research*, 4, 1836 - 1838

Kameshwari, V., Selvaraj, S., & Sundaramoorthy, S. (2020). Single Cell Protein Spirulina-A Nutrient Treasure. *Research Journal of Pharmacology and Pharmacodynamics,* 12(2), 49. https://doi.org/10.5958/2321-5836.2020.00010.5

Kodihalli, M.H., Sriappareddy, T., Haniyambadi, B.M.K., & Yelagiri, M.P. (2018). Screening of potential microalgal species from different natural environment for biodiesel production. *International Journal of Microbiology Research,* 10, 1052-57.

Kothari, R., Pathak, V.V., Kumar, V., & Singh, D.P. (2012). Experimental study for growth potential of unicellular alga *Chlorella pyrenoidosa* on dairy wastewater: An integrated approach for treatment and biofuel production. *Bioresource Technology,*116, 466–70.

Kothari, R., Prasad, R., Kumar, V., & Singh, D.P. (2013). Production of biodiesel from microalgae *Chlamydomonas polypyrenoideum* grown on dairy industry wastewater. *Bioresource Technology,*144, 499-503.

Kotteswari, M., Murugesan, S., & Kumar, R. (2012). Phycoremediation of Dairy Effluent by using the Microalgae *Nostoc* sp. *International Journal of Environmental Research and Development*, 2, 35-43.

Krishnakumar, S., Bai, V.D.M., & Rajan, R.A. (2013). Evaluation of bioactive metabolites from halophilic microalgae *Dunaliella salina* by GC–MS analysis. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5, 296-303.

Krishnamoorthy, K., & Subramaniam, P. (2014) Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi Using GC–MS. *International Scholarly Research Notices,* 2014,1-13

Kumar, K., Dasgupta, C.N., & Das, D. (2014). Cell growth kinetics of *Chlorella sorokiniana* and nutritional values of its biomass. *Bioresource Technology*, 167, 358–366. https://doi.org/10.1016/j.biortech.2014.05.118

Kushwaha, J.P., Srivastava, V.C., & Mall, I.D. (2011). An overview of various technologies for the treatment of dairy wastewaters. *Critical Reviews in Food Science and Nutrition,* 51, 442–52.

Li, X., Xu, H., & Wu, Q. (2007). Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnology and Bioengineering,* 98, 764-71.

Liang, K., Zhang, Q., Gu, M. & Cong, W. (2013). Effect of phosphorus on lipid accumulation in freshwater microalgae *Chlorella* sp. *Journal of Applied Phycology,* 25, 311-18.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology,* 148, 350-82.

Liestianty, D., Rodianawati, I., Arfah, R.A., Assa, A., Patimah, Sundari, &Muliadi. (2019). Nutritional analysis of *Spirulina* sp. to promote as superfood candidate. *IOP Conf. Series: Materials Science and Engineering*, 509,1-6.

Loneragan, J.F. & Webb M.J. (1993). Interactions between zinc and other nutrients affecting the growth of plants, Zinc Soils Plants, Springer Netherlands, Dordrecht, pp. 119–134.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with folin-phenol reagent. *Journal of Biological Chemistry,*193, 265-75.

Madkour, F.F., Kamil, A.E., & Nasr, H.S. (2012). Production and nutritive value of Spirulina latensis in reduced cost media. *Egyptian Journal of Aquatic Research*, 38, 51-7.

Maehre, H.K., Dalheim, L., Edvinsen, G.K., Elvevoll, E.O., & Jensen, I-J. (2018) Protein Determination-Method Matters. *Foods,*7, 1-11

Malaviya, P. & Rathore, V.S. (2001). A correlation study on some physicochemical quality parameters of pulp and paper mill effluents. *Pollution Research,* 20, 465-70.

Mane, R.S., & Chakraborty, B. (2018). Phytochemical screening of *Spirulina platensis* extracts from Rankala Lake Kolhapur, India. *Journal of Algal Biomass Utilization,* 9, 38-41.

Mishra, S.K., Suh, W.I., Farooq, W., Moon, M., Shrivastav, A., Park-Min, S., & Yang, J.W. (2014). Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. *Bioresource Technology,* 155, 330-33.

Moghazy, R.M. (2019). Activated biomass of the green microalga *Chlamydomonas variabilis* as an efficient biosorbent to remove methylene blue dye from aqueous solutions.*Water SA*, 45, 20-28.

Mohan, S.V., Rohit, M.V., Chiranjeevi, P., Chandra, R., & Navaneeth, B. (2015). Heterotrophic microalgae cultivation to synergize biodiesel production with waste remediation: Progress and perspectives. *Bioresource Technology*, 184, 169–178. https://doi.org/10.1016/j.biortech.2014.10.056

Musa D, Mu'azu A, Ujih U, Sabiu N, Abubakar M and Gebbe H (2016) Bioconcentration of Heavy Metals by Wild Plants Along Holomorphic Soils in Sule-Tankarkar Local Government Area, Jigawa State, Nigeria. *Journal of Natural Sciences Research*. 6:18-24.

Olofsson, P., Hultqvist, M., & Holmdahl, M. (2011). Phytol as a cholesterol lowering agent, Pub No.US2011/0015278A1.

Pandey, J.P., Tiwari, A., & Mishra, R.M. (2010). Evaluation of Biomass Production of *Spirulina maxima* on Different Reported Media. *Journal of Algal Biomass Utilization,* 1, 70 – 81.

Porwal, H.J., Mane, A.V., & Vehlhal, S.G. (2015). Biodegradation of dairy effluent by using microbial isolates obtained from activated sludge. *Water resource and industry,*9,1-15.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry,*269, 337-41.

Rajasekaran, C., Ajeesh, C.P.M., Balaji, S., Shalini, M., Siva, R., Das, R., Fulzele, D.P., & Kalaivani, T. (2016). Effect of Modified Zarrouk's Medium on Growth of Different *Spirulina* Strains. *Agriculture Technology and Biological Sciences,*13, 67-75.

Ramsundar, P., Abhishek, G., Singh, P., Pillay, K., & Bux, F. (2017). Evaluation of water activated sludge as a potential nutrient source for cultivation of *Chlorella sorokiniana*. *Microalgae Research,* 28, 108– 117. https://doi.org/10.1016/j.algal.2017.10.006

Rao, P.H., Ranjith, K.R., Raghavan, B.G., Subramanian, V.V., & Sivasubramanian, V. (2011). Application of phycoremediation technology in the treatment of wastewater from a leatherprocessing chemical manufacturing facility. *Water,* 37, N7-14.

Rebolloso-Fuentes, M. M., Navarro-Pérez, A., García-Camacho, F., Ramos-Miras, J. J., & Guil-Guerrero, J.L. (2001). Biomass Nutrient Profiles of the Microalga *Nannochloropsis. Journal of Agricultural and Food Chemistry*, 49*, 2966–2972.*

Rechner, A.R., Spencer, J.P.E., Kuhnle, G., Hahn, U., & Rice-Evans, C.A. (2001). Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. *Free Radical Biology and Medicine*, 30, 1213-1222.

Sahana, S.P., & Shirnalli, G.G. (2018). Effect of Microalgae on Physico-Chemical Properties of Different Dilutions of Untreated and Treated Dairy Industrial Effluent *International Journal of Current Microbiology and Applied Sciences,*7, 2979-93.

Salama, E., Jeon, B.H., Chang, S.W., Lee, S.H., Roh, H.S., Yang, S., Kurade, M.B., El-Dalatony, M.M., Kim, K.H., & Kim, S. (2017). Interactive effect of indole-3-acetic acid and diethyl aminoethyl hexanoate on the growth and fatty acid content of some microalgae for biodiesel production. *Journal of Cleaner Production,*168,1017–1024.

Sales, D.C., Rangel, A.H.N., Urbano, S.A., Freitas, A.R., Tonhati, H., Novaes, L.P., Pereira, M.I.B., & Borba, L.H.F. (2017). Relationship between mozzarella yield and milk composition, processing factors, and recovery of whey constituents. *Journal of Dairy Science,* 100, 4308–4321.

Salla, A.C.V., Margarites, A.C., Seibel, F.I., Holz, L.C., Brião, V.B., Bertolin, T.E., Colla, L.M., & Costa, J.A.V. (2016). Increase in the carbohydrate content of the microalgae Spirulina in culture by nutrient starvation and the addition of residues of whey protein concentrate. *Bioresource Technology*,209, 133–141

Sarfraz, R., Taneez, M., Sardar, S., Danish, L. & Hameed, A. (2021). Evaluation of *Desmodesmus subspicatus* for the treatment of wastewater. *International Journal of Environmental Analytical Chemistry*.

Seghiri, R., Kharbach, M., & Essamri, A. (2019). Functional Composition, Nutritional Properties, and Biological Activities of Moroccan *Spirulina* Microalga. *Journal of Food Quality*,2019,1-11.

Sharoba, A.M. (2014). Nutritional value of spirulina and its use in the preparation of some complementary baby food formulas. *Journal of Agroalimentary Processes and Technologies,* 20, 330-350.

Sialve, B., Bernet, N., & Bernard, O. (2009). Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*, 27, 409–416.

Soltani, N., Khavari-Nejad, R.A., Tabatabaei Yazdi, M., Shokravi, S.H. & Fernandez-Valiente, E. (2005). Screening of soil *cyano bacteria* for antibacterial antifungal activity. *Pharmaceutical Biology,* 43, 455-459.

Sorokina, K.N., Samoylova, Y.V., & Parmon, V.N. (2020). Comparative analysis of microalgae metabolism on BBM and municipal wastewater during salt induced lipid accumulation. *Bioresource Technology,* 11, 1-12.

Taga, M.S., Miller, E.E., & Pratt, D.E. (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of American Oil Chemists' Society*, 61, 928-31.

Verma, A. & Singh, A. (2017). Physico-Chemical Analysis of Dairy Industrial Effluent. *International Journal of Current Microbiology & Applied Sciences*. 6, 1769-1775.

World Health Organization (WHO) (1996). Permissible limits of heavy metals in soil and plants. Geneva, Switzerland.

Wuang, S.C., Khin, M.C., Chua, P.Q.D. & Luo, Y.D. (2016). Use of *Spirulina biomass* produced from treatment of aquaculture wastewater as agricultural fertilizers. Algal Research, 15,59–64.

Yap, P.Y., Jain, A., & Trau, D. (2018). Tip Biosystems Pte Ltd, Singapore.

Yen, G.C., & Chen, H.Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry,* 43, 27-32.

Yousaf, S., Khan, S., Sher, H, Afridi, I. & Ahmad, D. (2013). Canal water treatment with rapid sand filtration. *Soil Environment,* 32, 103-107.

Yu, F.R., Lian, X.Z., Guo, H.Y., McGuire, P.M., Li, R.D., Wang, R., & Yu, F.H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *Journal of Pharmacy and Pharmaceutical Sciences,* 8, 528-35.

Yu, L., Li, X., Liu, S., Xu, G., & Liang, Y. (2009). Comparative analysis of volatile constituents in Citrus Reticulata Blanco using GC–MS and alternative moving window factor analysis. *Journal of Separation Science,* 32, 3457-65.

Yuan, X., Kumar, A., Sahu, A.K., & Ergas, S.J. (2011). Impact of ammonia concentration on *Spirulina platensis* growth in an airlift photobioreactor. *Bioresource Technology,* 102, 3234–39.

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