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Production of Algal Biomass Using Different Dilutions of Textile Effluent Wastewater

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Abstract
The ability of micro-algae to grow on wastewater for biofuel production has been extensively studied as the wastewater from different industries could serve as a nutrient source for its growth. However, there is scanty information regarding the utilization of textile effluent wastewater as a nutrient source for algal biomass production. So, a laboratory study was conducted to elucidate the potential of textile effluent wastewater as an economical nutrient medium for improved algal biomass production and to compare it with conventionally used modified marine algae medium. Four algal strains belonging to family *Volvocaceae* (well surface, well depth, fresh water and well side) in five growth media using different dilutions of textile waste water (5, 10, 15 and 20%; v/v) and modified MA medium as control were investigated in triplicate. Algal biomass was harvested after each 24 hours i.e. 0, 24, 48, 72, 96, 120 and 144 hours. The results showed that algal biomass was increased with increasing dilution percentage of textile wastewater up to 15%, while a further increase in the dilution percentage (20%) resulted in reduced biomass in most of algal strains. The maximum increase in algal biomass production of well surface (177%) was noted after 144 h incubation followed by well side (55%) and fresh water (7%) strains compared to control. Well depth strain produced maximum biomass in modified MA medium and showed decreased growth under different dilutions of textile wastewater. In conclusion, the dilution percentage of 15% textile effluent wastewater could be used for maximum algal biomass production.
Keywords Algae biomass, different dilutions, textile wastewater, modified MA medium.

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Introduction

During the 21st century, access to economical and eco-friendly sustainable fuels and energy sources has been one of the greatest challenges around the world. The availability of fossil fuels would inevitably reduce due to continuous increase in demand and more cost required to extract new supplies; ultimately will result in increasing energy prices [1, 2]. The elevated prices of energy are directly linked with the food prices, which are also increasing due to the alarming increase in world population. Moreover, research efforts into alternatives in bio-based processes have been increased due to concerns about greenhouse gas emission caused by the use of conventional fuels derived from fossils. The focus of research has been directed towards the use of non-food competing biomasses like that of algae [3]. Moreover, change in land use to produce biofuels from food crops has increased the prices of food commodities [4]. This has become more intense problem for developing countries where more than 800 million individuals undergo starvation and hunger [5]. Recently, interest has been developed in algal based biofuels as one of the most economical alternative sources of renewable energy [6-9]. As an alternate renewable feedstock for making bioethanol, algal fuels have attained more consideration and are recognized as third generation biofuels [10]. Algal biofuels are favored due to many reasons e.g. Algae doesn’t compete for arable land, have faster growth, can grow under harsh conditions like under high salt and wastewater and produce more oil per unit land compared to conventional crop plants [5, 11-13]. Algae has been utilized for the production of bio-diesel, biogas, bio-ethanol and bio-hydrogen [9, 14]. Moreover, these could also be used for the production of bio-products like industrial, therapeutic, and nutraceutical co-products [2].

Algal biofuels hold a significant importance to meet the challenge of energy crisis, but these are not economically good at the current level of production and developments are required to improve its production and to make it more economical [15-16]. Nutrient requirements of algae are simple, i.e. can be grown on waste effluents from different industries [17]. To improve algal biomass through an economical source of nutrients could be a possible strategy in this regard. Recently, there is an increasing interest with regard to the feasibility of using municipal and industrial wastewater as a growth medium for an economical production of algal biomass [18-19]. Most of the studies have focused on the use of primary and secondary treated municipal wastewater, mainly in activated sludge plants as well as in municipal centrate, obtained from the sludge centrifuge [20]. Moreover, raw or untreated wastewater has also been investigated as growth media [21]. However, up to our knowledge, no study has been conducted in finding out the feasibility of using raw textile effluent wastewater. Industrial wastewater generally contains organic mass like proteins, carbohydrates, lipids, volatile acids and inorganic content containing sodium, calcium, potassium, magnesium, chlorine, sulfur, phosphate, bicarbonate and ammonium salts [22]. In addition, these effluents are also rich in cellulose, hemicelluloses, starch and other organic and inorganic compounds [23] and hence could act as a sustainable growth medium for algal feedstock [24]. The textile industry is one of the main industries in Pakistan. It is highly important in relations to its effects on the environment as it uses a large amount of water for its different processes like washing fibers, bleaching, dyeing and printing and yields highly contaminated wastewater with azodyes [22].

Keeping in view the above literature, textile effluent wastewater could be utilized as an economical source of nutrients i.e. growth medium for algal biomass production. Based on this hypothesis, a laboratory study was conducted to elucidate the potential of textile effluent wastewater to serve as an economical nutrient medium for improved algal biomass compared to conventionally used modified MA medium. The objectives of the present study were as follows: 1) to elucidate the potential of textile effluent wastewater to serve as a growth medium for optimum growth of algae, 2) to find out the optimum dilution of textile effluent wastewater for the improved algal biomass production and 3) to find out the comparative efficacy of optimum dilution for enhanced production of algal biomass compared to commonly used growth medium.

Materials and Methods

The proposed research was carried out in the Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. Four different algal strain samples, i.e. well surface, well depth, fresh water and well side belonging to family *Volvocaceae* (Kingdom: *Plantae*, Phylum: *Chlorophyta*, Class: *Chlorophyceae*, Order: *Volvocales*; based on physical and microscopic analysis) were obtained from Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Their names were coded on the basis of their collection site.

Modified marine algae (MA) medium was used with the following composition (mg/l) for algal biomass production of different algal strains: 50 $Ca(NO_3)_2.4H_2O$; 100 KNO_3; 5.0 $CoCl_2.6H_2O$; 50 $NaNO_3$; 20 H_3BO_3; 0.5 $FeCl_3.6H_2O$; 5.0 $Na_2EDTA.2H_2O$; 40 Na_2SO_4; 50 $MgCl_2.6H_2O$; 100 βNa2glycerophosphate.$5H_2O$; 5.0 $MnCl_2.4H_2O$; 0.5 $ZnCl_2$; 0.8 $Na_2MoO_4.2H_2O$ and 500 Bicine [25]. The pH was adjusted to around 7.5-8.0 before autoclaving the medium at 121˚C.

Different dilutions of textile effluent wastewater collected from the direct outlet of the Dawood textile mill, Faisalabad were used and each treatment had five replicates. Four different dilutions (5%, 10%, 15% and 20%) were prepared with distilled water in 1L volumetric flask. For that, 50, 100, 150 and 200 ml of textile effluent wastewater was added in each 1L volumetric flask and made the volume up to the mark using distilled water. The pH was adjusted around 7.5–8.0 before autoclaving the medium at 121˚C. Algal strains were grown in separate plastic tubs (2.5 L capacity) placed in a controlled room with incandescent light and temperature control. The media volume in each case was maintained by marking a line in plastic tub and maintaining the volume up to the mark. Algal growth in terms of biomass (g) was observed on a daily basis.

The algal mass was harvested after each 24 hours, i.e. 0, 24, 48, 72, 96, 120 and after 144 hours. The treatments were arranged in a completely randomized design (CRD) in triplicate. Overall 60 plastic tubs were used in the whole experiment. For harvesting algal biomass, simple settling down technique was used. Alga containing culture was allowed to settle for twenty four hours and then water was removed without disturbing the settled algal culture. Algae with larger size were easily harvested by hand picking from growth medium and weighed using a digital balance.

The wastewater was analyzed for various physiochemical characteristics following standard methods (Table 1). Electrical conductivity and pH of the collected samples were recorded by using Jenway Model 4510 conductivity meter. The EC was converted into total soluble salts (meq L^{-1}) by following the method described by U.S. Salinity Laboratory Staff [26]. Nitrogen (N) contents were determined by using Kjeldahl distillation apparatus [27]. For phosphorus (P), 1.0 g dried and ground sample was digested by adding 25 ml of conc. HNO_3 followed by 20 ml of 60% $HClO_4$. After digestion, vanadomolybdo-phosphoric yellow color complex in nitric acid medium was added to the samples and placed for 10 minutes until the color was developed [28]. Then the total P content was determined by taking the reading of absorbance at 410 nm using a spectrophotometer (Beckman photometer 1211, London). The digested sample was used to determine potassium (K) contents by using flame photometer (Jenway PFP- 7). A standard curve was drawn with the help of standard series ranging from 2-10 ppm with KCl. The K contents of the sample were calculated by comparing the values with standard curve. Titrimetric method was used to measure the chemical oxygen demand (COD) of the wastewater sample [29].

The data collected were subjected to one way analysis of variance (ANOVA) using Statistix v. 8.1 software package [30]. The treatment means were compared by least significant difference (LSD) test at $P = 0.05$ [31].

Table 1 Physicochemical characteristics of textile waste water used for algal growth.

Characteristics	Units	Values
pH	-	6.99
EC	dS m^{-1}	3.5
Total soluble solids	me L^{-1}	35
Available phosphorus	mg kg^{-1}	7.25
Extractable potassium	mg kg^{-1}	194
Total nitrogen	%	1.76
COD	mg L^{-1}	925

Results

Growth in terms of biomass of four algal strains was observed using different dilutions of textile effluent wastewater and modified MA medium as a control. The detailed results observed in each growth medium are explained as follows: In case of modified MA medium, all the four strains of algae showed a significant increase in biomass with time (Fig. 1). The maximum biomass produced after 144 h treatment was noted in case of algal strain collected from fresh water (96.16 g) while that of the minimum after 144 h was recorded in case of algal strain collected from well surface (89.24 g). Regarding each strain, the increase in biomass after 144 h was 8.96, 20.18, 18.82 and 13.99 g in case well surface, well depth, fresh water and well side algal strain, respectively. So, the maximum increase after 144 h was noted in case of well depth strain while that of the minimum was noted in case of well surface. In this way, well depth algal strain showed a 125% increase in biomass compared to well surface algal strain.

All the four strains of algae showed a significant increase in biomass with time in case of textile effluent wastewater at 5% dilution (Fig. 2). The maximum biomass produced after 144 h treatment was noted in case of algal strain collected from well depth (93.52 g) while that of the minimum after 144 h was recorded in case of algal strain collected from fresh water (86.14 g). Regarding each strain, the increase in biomass after 144 h was 13.60, 18.16, 14.16 and 8.32 g in case well surface, well depth, fresh water and well side algal strain, respectively. So, the maximum increase after 144 h was noted in case of well depth strain while that of the minimum was noted in case of well side. In this way, well depth algal strain showed a 34% increase in biomass compared to well side algal strain at 5% dilution.

In textile effluent wastewater at 10% dilution, all the four strains of algae showed a significant increase in biomass with time (Fig. 3). The maximum biomass produced after 144 h treatment was noted in case of algal strain collected from well side (98.20 g) while that of the minimum after 144 h was recorded in case of algal strain collected from well surface (85.36 g). Regarding each strain, the increase in biomass after 144 h was 11.6, 16.04, 14.88 and 21.73 g in case well surface, well depth, fresh water and well side algal strain, respectively. So, the maximum increase in algal biomass after 144 h was noted in case of well side algal strain while that of the minimum was noted in case of well surface. In this way, well side algal strain showed a 87% increase in biomass compared to well surface algal strain at 10% dilution.

Similarly, all the four strains of algae showed a significant increase in biomass with time in textile effluent wastewater at 15% dilution (Fig. 4). However, the maximum biomass produced after 144 h was noted in case of algal strain collected from fresh water (98.33 g) while that of the minimum was recorded in case of algal strain collected from well side (89.56 g). Regarding each strain, the increase in biomass after 144 h was 22.11, 16.28, 20.21 and 19.30 g in case well surface, well depth, fresh water and well side algal strain, respectively. So, the

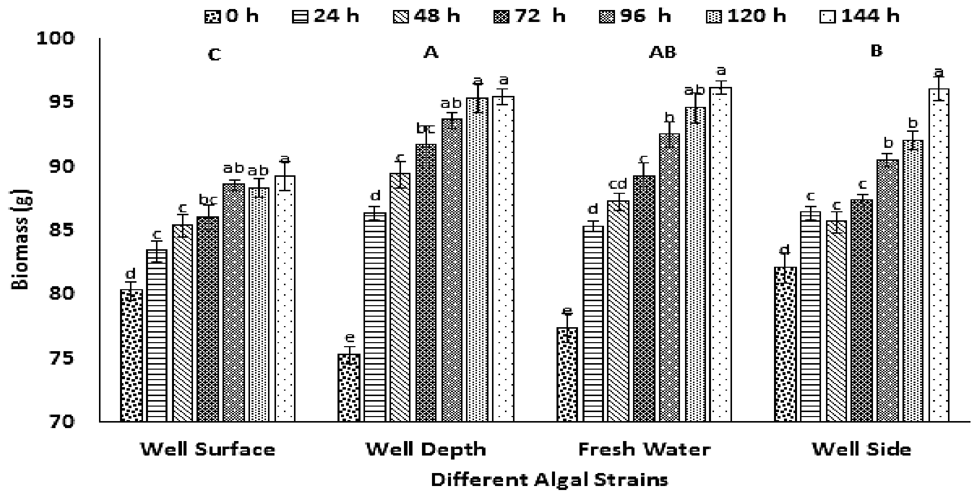

Fig. 1 Effect of modified MA medium on the biomass of different algal strains. Different letters describe significant differences at P = 0.05.

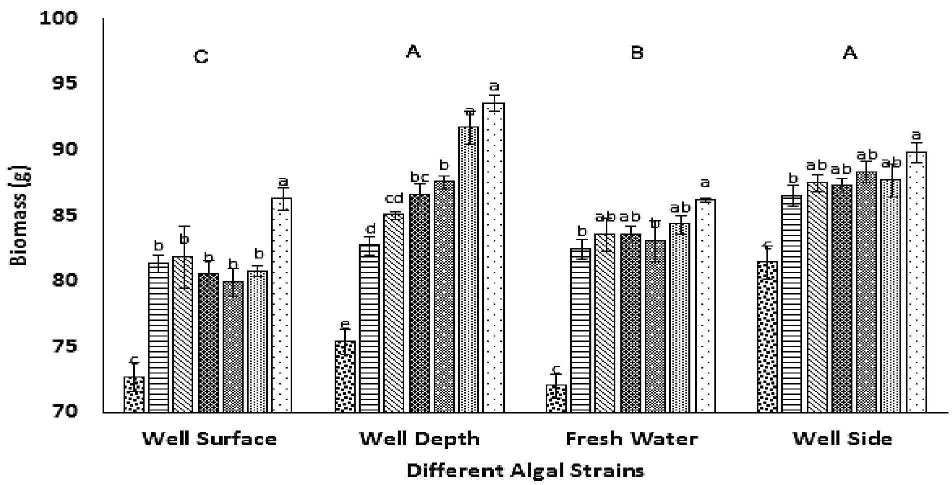

Fig. 2 Effect of 5% dilution of textile waste water on the biomass of different algal strains. Different letters describe significant differences at P = 0.05.

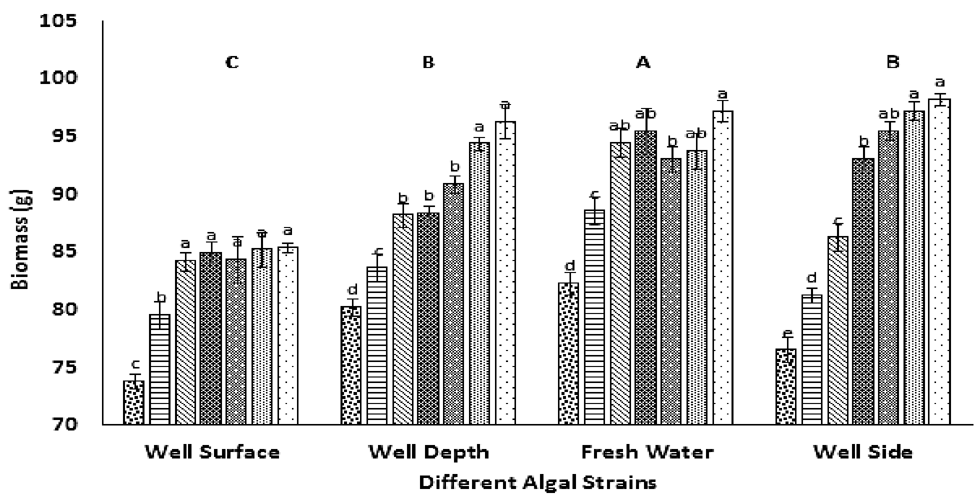

Fig. 3 Effect of 10% dilution of textile waste water on the biomass of different algal strains. Different letters describe significant differences at P = 0.05.

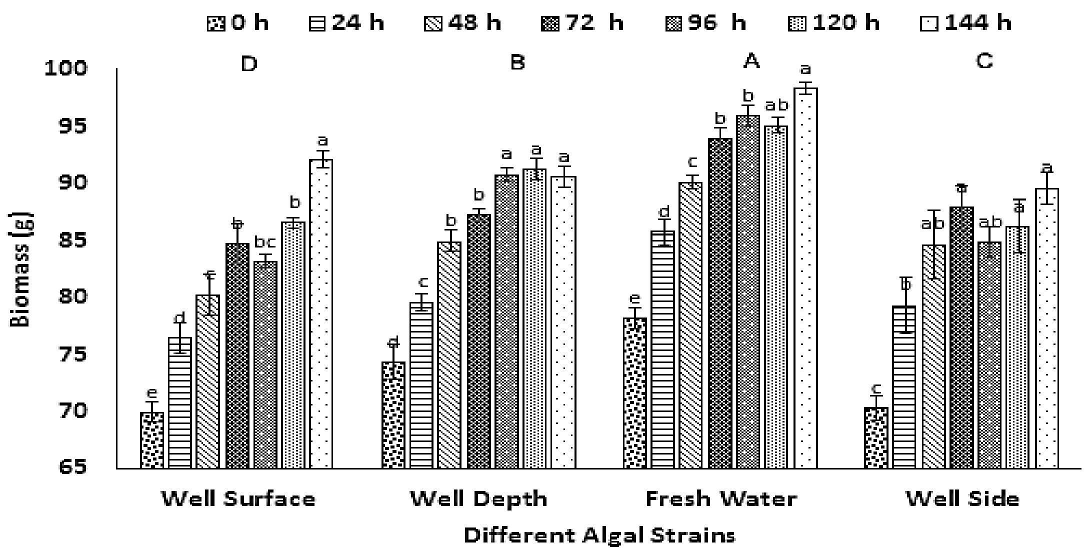

Fig. 4 Effect of 15% dilution of textile waste water on the biomass of different algal strains. Different letters describe significant differences at P = 0.05.

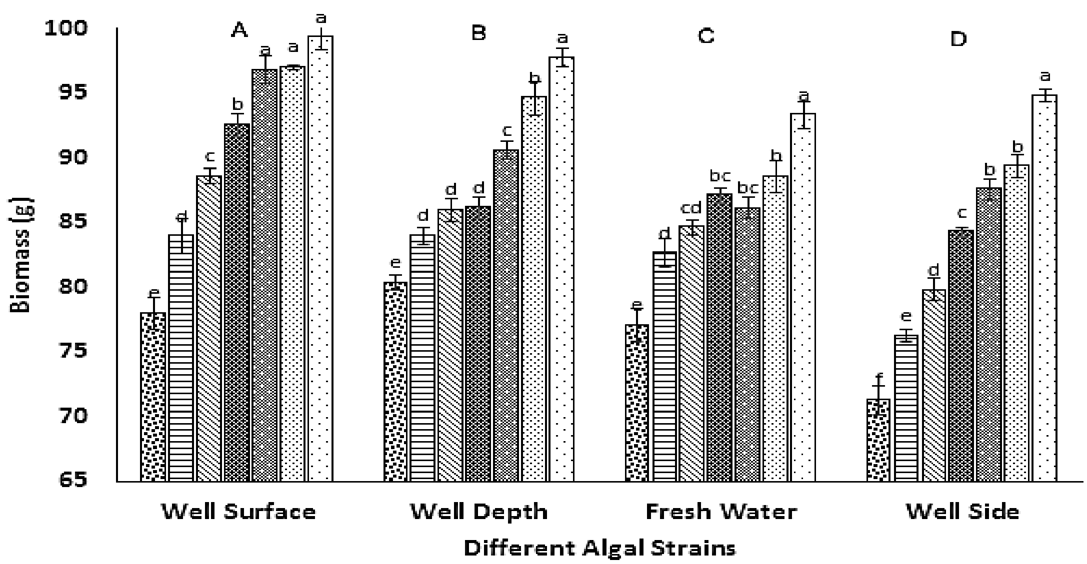

Fig. 5 Effect of 20% dilution of textile waste water on the biomass of different algal strains. Different letters describe significant differences at P = 0.05.

maximum increase after 144 h was noted in case of well surface strain while that of the minimum was noted in case of well depth. In this way, well surface algal strain showed a 36% increase in biomass compared to well depth algal strain at 15% dilution.

In case of textile effluent wastewater at 20% dilution, all the four strains of algae showed a significant increase in biomass with time (Fig. 5). The maximum biomass produced after 144 h was noted in case of algal strain collected from well surface (99.32 g) while that of the minimum after 144 h was recorded in case of algal strain collected from fresh water (93.33 g). Regarding each strain, the increase in biomass after 144 h was 21.37, 17.41, 16.26 and 23.53 g in case well surface, well depth, fresh water and well side algal strain, respectively. So, the maximum increase after 144 h was noted in case of well side strain while that of the minimum was noted in case of fresh water algal strain. In this way, well depth algal strain showed a 45% increase in biomass compared to well surface algal strain.

Discussion

The results showed that the modified MA medium and different dilutions of textile effluent wastewater (5-20%) have significant effect on biomass production by different algal strains. Moreover, different algal strains produced significantly different biomass in each media showing that each strain was adapted to specific dilution of textile effluent wastewater. The textile industry is one of the main causes of environmental pollution and produces most of wastewater during dyeing and finishing processes. It produces about 35 billion tons of water during these processes annually. In general, textile effluent wastewater has high pH, EC and TSS. Moreover, it also contains large amounts of biodegradable and hardly biodegradable substances which include salts sizing agents, dyes, surfactants, volatile organic compounds, disodium terephthalate, ethylene glycol, toxic heavy metals, etc. [32-34]. Previously, the application of industrial wastewater resulted in reduced algal growth due to the presence of heavy metal pollutants and organic chemical toxins rather than N and P [18, 35].

In general, the algal biomass production was dependent on dilution percentage of textile wastewater and biomass increase was noted up to 5-15% dilution, while 20% dilution showed decrease in algal biomass in most of algal strains. There has been a well-known interaction between wastewater treatment and algae [36]. The responsible mechanisms behind this interaction are the provision of available forms of nutrients like organic carbon and inorganic N and P [18]. However, it has been found true in case of waste produced through chemical processing or through the generation of activated sludge. Although algae could be grown in modified MA medium, but it is a costly algal growth medium in terms of price. Waste water bears large amounts of N and P and also a rich source of organic and inorganic compounds like cellulose, starch and many others. However, there are also reports about the presence of toxic chemical like cadmium, chromium, zinc, etc. and organic chemical toxins like hydrocarbons, biocides, and surfactants [35, 37-38]. In order to reduce its toxicity, dilution strategy can serve better as it will help in reducing the toxic effect of toxic chemical in the textile effluent wastewater, ultimately would serve as a sustainable growth medium for the algae.

In this study, dilution strategy was tested to find out the optimum dilution for enhanced biomass production of algal biomass. We also found a toxic effect of wastewater when it was used at higher concentrations. Previously, it has been found that wastewater could serve as a source of micro- as well as macro-nutrients for algal growth or algae can take various nutrients, especially nitrogen and phosphorus and results in reducing the amount of nutrients in wastewater [22, 39-43]. Moreover, greater algal biomass may also be due to its ability to utilize azodyes present in the wastewater as a nitrogen source. Recently, it has been found under laboratory conditions that the algae has the potential to decolorize azodye contaminated wastewater through the process of phyto-remediation [44]. Other researchers have also reported the potential of algae in phyto-remediation of other wastes of leather industry, carpet mills effluent, pulp and paper industry, dairy manure effluent and municipal effluent [40, 45-48].

In conclusion, different dilutions of textile effluent wastewater can serve as a growth medium for optimum growth of algae in comparison to modified MA medium. Different algal strains produce maximum biomass under different dilutions depending on their sensitivity towards the toxicity of wastewater. Overall, the dilution percentage of 15% textile effluent wastewater could be used for algal biomass production without any hazardous effects on the studied algal strains.

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