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Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment and biochemical composition

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Abstract
In this study, two cycles of mixotrophic and one cycle of heterotrophic cultivation of *Scenedesmus quadricauda* (freshwater) and *Tetraselmis suecica* (marine water) microalgae in dairy wastewater (DWW) were investigated. Dry weights of *S. quadricauda* and *T. suecica* were found to be 0.43 and 0.61 mg/L after the first cycle and 0.36, and 0.65 mg/L after the second cycle of mixotrophic cultivation, respectively. Chlorophyll a content of both microalgae in the first cycle was significantly higher than the second cycle. *S. quadricauda* removed 92.15% of total nitrogen, 100% of phosphate, 100% of sulfate and 76.77% of total organic carbon, after two cycles of cultivation. The dominant fatty acids during the first and second cycle of *S. quadricauda* and *T. suecica* cultivation were C18:1 and C18:3n-3, respectively. The results suggest that by reusing DWW in two consecutive cycles of microalgal cultivation, higher pollutants removal efficiency and microalgal biomass production can be achieved.

Key words: Microalgae; Dairy wastewater (DWW); Wastewater treatment; Mixotrophic cultivation; Fatty acid methyl esters (FAMEs); Wastewater reuse.
1. Introduction

Dairy industry, as one of the largest sectors of food processing, consumes huge amounts of water for cleaning, sanitization, heat exchange (heating/cooling) and floor washing (Tocchi et al., 2012). The generated dairy wastewater (DWW) contains different compounds such as wasted milk, lactose, fats, washing detergents, nutrients and sanitizing agents. Depending on the season and milking system, characterization of dairy effluents varies considerably e.g., pH 4.7-11, chemical oxygen demand (COD) 80–95000 mg/L, biological oxygen demand (BOD) 40–48000 mg/L, total nitrogen (TN) 14–830 mg/L and total phosphorus (TP) 9–280 mg/L. The concentration of macro- and micronutrients such as K, Na, Cl, Mg, Ca, Co, Fe, Mn, and Ni can be noticeably high in dairy wastewaters (Chokshi et al., 2016a). Nitrogen and phosphorous enriched wastewaters lead to eutrophication in natural water bodies and disturb the ecosystem balance (Nayak et al., 2016). To reduce the risk of environmental pollution, it is necessary to treat DWW before discharging it into coastal waters, rivers and lakes.

Microalgae-based systems for wastewater treatment have received a great deal of interest in recent years due to their diverse advantages. Microalgae biorefinery concept for biofuels and bioproducts production is between the most important ones. Using microalgae in wastewater treatment reduces or replaces the utilization of chemicals (Razzak et al., 2017). The autotrophic and mixotrophic cultivation modes of microalgae need water, nutrients, light, and CO$_2$. Wastewater is a suitable and sustainable medium for the cultivation of microalgae. These microorganisms with high photosynthetic efficiency rapidly adapt and grow quickly in different types of wastewaters. They have high ability and capacity to uptake nutrients especially phosphorus and nitrogen compounds from wastewater (Cheng et al., 2018). The cultivation of microalgae in wastewater decreases the concentration of pollutants, uptakes CO$_2$, and increases oxygen concentration (Ling et al., 2014). Besides wastewater treatment, microalgal biomass is produced simultaneously, without the addition of nutrients.
The produced and harvested microalgal biomass from wastewater can be used as an energy feedstock. Microalgae are known as third generation biofuels, after food and non-food crops. Cultivation, harvesting, drying of biomass, extraction and transesterification of lipids are different steps of biodiesel production from microalgae (Pokoo-Aikins et al., 2010). The cost of cultivation largely affects biofuel production from microalgal biomass, in practical scales (Hena et al., 2015). Cultivation of microalgae in wastewater and collection of microalgal biomass as a by-product of wastewater treatment is a cost-effective method. Furthermore, replacing fossil fuels by biodiesel and other biofuels from microalgae, which are clean, carbon-neutral, and renewable fuels, can reduce the emission of CO₂ and global warming.

Several studies have reported the treatment of DWW, as well as lipid extraction and biodiesel production by microalgae (Chokshi et al., 2016c; Kothari et al., 2012; Hena et al., 2018). Depending on microalgae species, initial concentrations of nutrients (nitrogen and phosphorus), cultivation time and conditions and the characteristics of wastewater, microalgal growth, pollutants removal efficiency and lipid yield can be different. Chokshi et al. (2016) reported complete removal of ammoniacal nitrogen (277.4 mg/L) and phosphates (5.96 mg/L) from DWW by *Acutodesmus dimorphus* (Chokshi et al., 2016b). In another study, phosphate and nitrate removal efficiencies by cultivated *Chlorella pyrenoidosa* in raw and treated (in an oxidation pond) DWW were 87% and 60% in the influent, and 83% and 49% in the effluent, respectively (Kothari et al., 2012). Dry mass and lipid content of harvested *Arthrospira platensis* from dairy farm wastewater were 4.98 g/L and 30.23%, respectively (Hena et al., 2018). Different values of dry mass and lipid content as 0.84 g/L and 25% of cultivated *Acutodesmus dimorphus* in DWW were reported by Chokshi et al. (Chokshi et al., 2016b). In the aforementioned works and other similar studies, productivity of microalgal biomass, removal efficiencies of pollutants, and biochemical composition of microalgae have been investigated only with one cycle of microalgae cultivation. However, these parameters have not been evaluated after reusing
the recycled wastewater from the first cycle of cultivation. Investigation of these parameters for more than one cycle of cultivation, might provide some interesting insights into wastewater treatment, productivity and biochemical composition of microalgae.

In this study, we examined the reuse of DWW in two consecutive cycles of microalgae cultivation. For this purpose, mixotrophic and heterotrophic cultivation of freshwater (Scenedesmus quadricauda) and marine water (Tetraselmis suecica) microalgae in DWW were investigated in the first step. Microalgal biomass was harvested by centrifugation after 12 days of cultivation. After centrifugation, wastewater was collected for reuse (for the second cycle). In the second cycle, microalgae were cultivated in the collected wastewater, derived from the first cycle of cultivation. Microalgal growth, pigments content, pollutants’ removal efficiencies, and lipid profile were evaluated for both cultivation cycles. To the best of authors’ knowledge, currently there is no report on the investigation of growth, nutrients removal efficiencies and biochemical composition of cultivated microalgae in reused DWW.

2. Materials and methods

2.1. Microalgae strains and cultivation media

A freshwater (Scenedesmus quadricauda) and a marine water (Tetraselmis suecica) microalga were selected for the study. These microalgae were purchased from the Culture Collection of Algae and Protozoa (CCAP, Scotland, UK). Dairy wastewater (DWW) was collected directly from a local wastewater treatment plant in Lapinlahti, Finland. DWW was kept in the refrigerator (4 °C) until the start of experiments. Table 1 presents the initial concentrations of target pollutants viz., total nitrogen (TN), phosphate (PO₄³⁻), sulfate (SO₄²⁻), and total organic carbon (TOC) in DWW at the beginning of first and second cycles of microalgae cultivation.

2.2. Microalgae cultivation conditions
Cultivation of microalgae was performed in 1 L glass flasks as experimental units. Each flask had a cap with one inlet for injection of air, one outlet for taking samples and one small hole for gas venting. One liter of DWW was added to each experimental unit. 34 g of sea salt (Havsalt Company) was added to 1 L of DWW for cultivation of the marine water microalga. Microalgal cells of *Scenedesmus quadricauda* and *Tetraselmis suecica* cultivated in synthetic media (BBM and F2, respectively) were added to DWW. To avoid the changing of original concentrations of nutrients in DWW, BBM and F2, media were centrifuged and only microalgal cells were added to the DWW solution. The medium was aerated and agitated with atmospheric air (0.04% CO₂) continuously. The experimental units were incubated at lab temperature (25 °C), under 12 h per day illumination of 90 μmol photon/(m².s), provided by white fluorescent lamps for 12 days (Table 1).

2.3. **Experimental set up**

In this study, two continuous cycles of microalgae cultivation in DWW were performed. In the first cycle, freshwater and marine water microalgae were cultivated in DWW for 12 days according to our previous work (Daneshvar et al., 2018). After 12 days, the content of each experimental unit was centrifuged and microalgal biomass was collected and dried. Dried biomass was used later for biochemical analysis. DWW after centrifugation was preserved to be reused in the second cycle of microalgae cultivation. After 12 days of cultivation (24 days in total from the beginning), the content of each unit was centrifuged again and microalgal biomass was collected, dried, and was used later for analysis.

In the first cycle, mixotrophic (in the presence of light) and heterotrophic (in the absence of light) cultivation modes of freshwater and marine water microalgae were investigated. To conduct heterotrophic cultivation of microalgae, the experimental units were wrapped by aluminum foil and kept in dark. In the second cycle, only mixotrophic cultivation was studied as microalgae did not grow
well under heterotrophic conditions. Microalgae for inoculation in the second cycle were provided from the first cycle of cultivation. The experimental scheme of sequential cultivation of microalgae in raw and recycled DWW is shown in Fig. 1.

2.4. **Determination of microalgal growth**

To measure the growth of microalgae in the first and second cycle of cultivation, six samples were taken from each experimental unit during 12 days. Sampling was carried out at the beginning (day 0) and then 1, 2, 4, 8, and 12 days after inoculation. The optical density (OD) of microalgal cells was determined at 680 nm using a UV-spectrophotometer (UV-2401PC). The OD of samples was read within a range of 0.1-1.0 nm and higher OD was diluted appropriately. OD$_{680}$ of freshwater and marine water microalgae was converted to dry mass (g/L) by linear regression as follows:

\[
\text{Freshwater microalga dry mass (g/L)} = 0.2784 \times \text{OD}_{680} - 0.0061
\]

\[
\text{Marine water microalga dry mass (g/L)} = 0.4221 \times \text{OD}_{680} + 0.0054
\]

where OD$_{680}$ is the value of OD of freshwater and marine water microalgae at 680 nm.

Specific growth rate, $\mu$ (/day), and maximum specific growth rate, $\mu_{\text{max}}$ (/day), referring to the exponential phase of a growing culture, were estimated by the slope of logarithmic plot of dry mass versus time.

2.5. **Analysis of pigments content**

The value of photosynthetic pigments of microalgae was measured at the end of the first and second cycles of cultivation. Extraction and calculation of chlorophyll a, chlorophyll b, and carotenoids were conducted according to earlier reported method (Xiong et al., 2017). Five mL of sample was taken and centrifuged for 15 min at 4500 rpm. The microalgal pellet was separated and mixed with 10 mL of 90% methanol. The mixture was then incubated at 60 °C for 10 min. After extraction, the supernatant was
collected by 15 min centrifugation at 4500 rpm. The extract was analyzed using a UV-
spectrophotometer (UV-2401PC) at 665 nm for chlorophyll $a$, 652 nm for chlorophyll $b$, and 470 for
carotenoids. Consequently, the concentration of pigments was calculated as follows:

Chlorophyll $a$ (mg/L) = $16.82 \ A_{665} - 9.28 \ A_{652}$  \hspace{1cm} (3)

Chlorophyll $b$ (mg/L) = $36.92 \ A_{652} - 16.54 \ A_{665}$  \hspace{1cm} (4)

Carotenoids (mg/L) = $(1000 \ A_{470} - 1.91 \ C_a - 95.15 \ C_b) / 225$  \hspace{1cm} (5)

2.6. Pollutants removal efficiency

The concentrations of total nitrogen (TN), phosphate (PO$_4^{3-}$), sulfate (SO$_4^{2-}$), and total organic carbon
(TOC) were measured to monitor the efficiency of DWW treatment by the end of first and second cycle
cultivation of microalgae. 50 mL DWW from each experimental unit was taken after 12 days from the
beginning of experiment. Samples were centrifuged and consequently, filtered with 0.45 μm membrane
filter (BIOFIL$^R$ Syringe Filter). Concentration of TN and TOC was analyzed by TOC/TN Analyzer
(multi N/C 2100S). Concentration of PO$_4^{3-}$ and SO$_4^{2-}$ was measured using reagent powder pillows and
spectrophotometer (HACH DR 3900). Pollutants removal efficiency was calculated as follows:

Pollutants removal efficiency (%) = $\frac{(C_I - C_F)}{C_I} \times 100$  \hspace{1cm} (6)

where $C_I$ is the initial and $C_F$ is the final concentration of target pollutants.

2.7. Fatty acid methyl esters (FAMEs) analysis

The lipid content of freshwater and marine water microalgal biomass was determined in freeze-dried
samples following a one-step in-situ transesterification method for converting the contained fatty acids
into fatty acid methyl esters (FAMEs). The produced FAMEs were then analyzed by gas
chromatography (GC) in a GC system (Agilent Technologies 7890A) equipped with a DB–WAX capillary column (10 m length, 0.1 mm internal diameter, and 0.1 μm of film thickness). Helium was used as carrier gas with a column flow rate of 30.34 cm/s. The initial temperature of oven was kept at 40 °C for 0.5 min and then increased to 195 °C with a rate of 25 °C/min. Then temperature was increased to 205 °C at a rate of 3 °C/min. Finally, the temperature raised from 205 °C to 230 °C at a rate of 8 °C/min and held at 230 °C for 4 min. Injector and detector temperatures were maintained constant at 250 °C. C17:0 (Sigma) was used as the internal standard.

2.8. Statistical analysis

In the figures and tables, the mean values together with standard deviation (SD) of four replicates are reported. The significant differences among the results were analyzed with an overall one-way analysis of variance (ANOVA) followed by Duncan's test (p <0.05). The statistical analyses were carried out by using the IBM SPSS Statistics 21.0 software.

3. Results and discussion

3.1. Growth of microalgae

Cultivation of microalgae in wastewater can directly affect the production of microalgal biomass, removal efficiency of pollutants, and productivity of valuable compounds, based on the biochemical composition of the produced biomass. In this study, two cycles of mixotrophic and one cycle of heterotrophic cultivation of microalgae in DWW were investigated. In the first cycle of mixotrophic mode, the dry weight of S. quadricauda increased from 0.04 g/L to 0.43 g/L during 12 days of cultivation. Based on Fig. 2 (a), maximum specific growth rate, 1.08 d⁻¹, was observed at the start of the culture and then specific growth rate gradually decreased. Similar trend of microalgal growth was
observed in the mixotrophic cultivation of the marine water microalga, *T. suecica*. As can be seen from Fig. 2 (b), microalgal dry weight increased from 0.07 g/L to its maximum value, 0.61 g/L on day 12, with a $\mu_{\text{max}}$ of 0.73 /day, observed within the first day of cultivation. An increase in dry weight, more than twice after 24 h, for both microalgae agrees with previous findings demonstrating that *Chlorella* sp. grew dramatically in DWW after 24 h, remained constant up to day 6 and finally biomass rapidly increased on days 6-8 (Lu et al., 2015). The reason of doubling dry weight of *S. quadricauda* (from 0.04 to 0.11 g/L) and *T. suecica* (from 0.07 to 0.15 mg/L) after 24 h can be related to harvesting of microalgae from their exponential phase in their media (BBM and F2), prior to inoculation in DWW. After one day of cultivation, growth of both microalgae slowed down due to adaptation (lag phase), which lasted up to day 4 in case of both species. Other researchers also stated that the reason of lag phase in the growth curve of cultivated *Chlorella vulgaris* and *Neochloris oleoabundans* in wastewater is related to microalgae adaptation (AlMomani and Örmeci, 2016). Also, increasing of dry mass and further decrease in growth rate during the progress of each experiment can be attributed to various reasons including difficulty in light penetration into the culture as it gets denser or inorganic carbon limitation. Between the two species tested, faster adaptation to DWW is observed in case of *T. suecica*, however *S. quadricauda* is faster than *T. suecica* if specific growth rates are compared.

Heterotrophic cultivation of *S. quadricauda* and *T. suecica* in the first cycle was also investigated and the obtained growth curves are plotted in Fig. 2 (a,b). As this figure illustrates, dry weight of both freshwater and marine water microalgae increased by two-folds after 24 h. Then *S. quadricauda* and *T. suecica* stayed in the lag phase for three days (up to day 4) and for seven days (up to day 8), respectively. After the lag phase, no exponential growth phase was observed and both microalgae decreased up to the last day of experiment, day 12. These results reveal that heterotrophic cultivation of *S. quadricauda* and *T. suecica* in DWW is less efficient, compared to the mixotrophic mode, and thus, heterotrophic cultivation was not studied further. Ruiz et al. (2014) reported that the absence of
heterotrophic activity of *Scenedesmus obliquus* in urban wastewater can be related to non-biodegradability of organic matter for utilization by microalga (Ruiz et al., 2014). In agreement with this study, Wang et al. (2015) also reported higher microalgal biomass productivity under mixotrophic (3.96 g/L) compared to heterotrophic growth (2.35 g/L) (Wang et al., 2015). They stated that it might be related to higher accessibility to the carbon source in mixotrophic cultivation due to CO$_2$ supply.

In the second cycle, mixotrophic growth of microalgae in the recycled DWW was investigated. In this cycle, microalgae were inoculated to the recycled DWW derived from the first cycle of cultivation, thus culture conditions of both cycles were similar. Therefore, as shown in Fig. 2 (a,b), microalgae grew very well in the second cycle without any lag phase, which is normally expected in case of changes in culture conditions and consequently physiological adjustment needed (Sterner et al., 2004).

$\mu_{\text{max}}$ of the two microalgae grown in DWW are quite similar if compared at the start of their second cycle (0.6 d$^{-1}$ and 0.56 d$^{-1}$ for *S. quadricauda* and *T. suecica*, respectively). Dry weight of *S. quadricauda* was found to be 0.05 g/L in the beginning of cultivation and increased to 0.36 g/L on day 12 of the experiment, compared to the maximum dry weight of 0.43 g/L, achieved in the 1$^{st}$ cycle. In contrast, dry weight of *T. suecica* in the final day of the second cycle, as 0.65 mg/L, was significantly higher than in the final day of the first cycle, as 0.61 mg/L. Based on Table 1, the concentration of nutrients decreased after the first cycle of microalgae cultivation in DWW, however by calculating the specific growth rates, it seems that the remaining concentrations of nutrients are still enough and non-limiting at the start of the second cycle. Later on, nutrients seem to cause limitation to microalgal growth at the end of second cycle, which can explain the decreasing trend of growth rate for both species.

### 3.2. Photosynthetic pigments content
Fig. 3 represents the photosynthetic pigments content viz., chlorophyll \( a \), chlorophyll \( b \), and carotenoids of \( S. \) quadricauda and \( T. \) suecica at the end of the first and second cycles of cultivation in DWW. This figure shows the difference of each pigment between freshwater/marine water microalgae, mixotrophic/heterotrophic cultivation modes, and first/second cycles of cultivation. The concentration of chlorophyll \( a \) was significantly higher in marine water microalgae, mixotrophic cultivation mode and first cycle of cultivation as compared to freshwater microalgae, heterotrophic cultivation mode and the second cycle of cultivation, respectively (Fig 3 (b)). The highest (11.70 mg/L) and lowest (0.36 mg/L) values of chlorophyll \( a \) were found in the first cycle of mixotrophic cultivation of \( T. \) suecica and heterotrophic cultivation of \( S. \) quadricauda, respectively. The higher concentration of chlorophyll \( a \) in \( T. \) suecica can be related to the higher concentration of microalgal cells in the culture medium. For the same volume of freshwater and marine water microalgae, there is higher microalga concentration (0.61 versus 0.43 g/L) and consequently, higher amount of chlorophyll \( a \) (11.70 versus 9.45 mg/L). It appears thus, that the cells of \( S. \) quadricauda had higher chlorophyll \( a \) content (22 mg chlorophyll \( a/g \) dry mass) than \( T. \) suecica cells (19 mg chlorophyll \( a/g \) dry mass) at the end of the first cycle. From the first cycle of cultivation to the second one, concentration of chlorophyll \( a \) decreased from 9.45 to 1.38 mg/L (around 7 times) in \( S. \) quadricauda and from 11.70 to 6.52 mg/L (around 2 times) in \( T. \) suecica. The reason for decreasing the chlorophyll \( a \) content might be related to decrease in nitrogen concentration of microalgal medium in the first cycle as compared to the second cycle. TN concentration in the second cycle of cultivation was 8 times (in medium of \( S. \) quadricauda) and 3 times (in medium of \( T. \) suecica) lower than the first cycle. Damm et al. (2016) stated that there is a negative correlation between chlorophyll \( a \) and nitrate concentration (Damm et al., 2016). According to them, availability of initial nitrate in microalgal medium leads to healthy growth of microalgae and accumulation of chlorophyll as well.
Fig. 3 shows the chlorophyll $b$ content of $S. \text{quadricauda}$ and $T. \text{suecica}$ after the first and second cycles of cultivation at mixotrophic and heterotrophic cultivation modes. In the first cycle of mixotrophic cultivation, the concentration of chlorophyll $b$ in the cultures of $S. \text{quadricauda}$ and $T. \text{suecica}$ was in the same range as 2.91 and 3.10 mg/L, respectively. However, the content of chlorophyll $b$ in dry mass of $S. \text{quadricauda}$ as 6.74 mg/g was significantly higher than 5.07 mg/g in dry mass of $T. \text{suecica}$. In the second cycle of mixotrophic cultivation as compared to the first one, chlorophyll $b$ was increased in $S. \text{quadricauda}$ (3.34 mg/L or 9.37 mg chlorophyll b/mg dry mass) and decreased significantly in $T. \text{suecica}$ (2.25 mg/L or 3.46 mg chlorophyll b/mg dry mass). Similar to this study, Devi and Mohan reported increasing chlorophyll $b$ content over chlorophyll $a$ in their study (Devi and Mohan, 2012).

Fig. 3 also illustrates the changes of carotenoids content of freshwater and marine water microalgae in different experimental units of this study. In the first mixotrophic cycle, carotenoids content of $T. \text{suecica}$ (6.9 mg carotenoid/g dry mass) was significantly lower than $S. \text{quadricauda}$ (7.76 mg carotenoid/g dry mass). As can be seen from Fig. 3, in the second cycle of cultivation, carotenoid was not detected in $S. \text{quadricauda}$ and it decreased significantly in $T. \text{suecica}$. Carotenoid is considered as a sensitive biomarker of pollutants in aquatic environment (Kurade et al., 2016). Decreasing the concentration of carotenoid might be related to decreasing the concentrations of contaminants such as TOC in the second cycle. Furthermore, changes of nutritional (nitrogen and phosphorous) and environmental (salinity and stress) factors during the first and second cycle of cultivation might be another reason for different concentrations of carotenoids (Paliwal et al., 2015).

The values of all three photosynthetic pigments of freshwater and marine water microalgae species, were remarkably low in heterotrophic as compared to mixotrophic cultivation mode. Chlorophylls and carotenoids are essential pigment groups for harvesting of light and protecting microalgal cells against radiation. Usually, microalgae produce these pigments in autotrophic conditions (Perez-Garcia et al.,
The results of this study show that *S. quadricauda* and *T. suecica* did not develop their pigments in the absence of light. Miazek et al. (2017) reported 2.8 to 4 times decrease in chlorophyll content of *Chlorella sorokiniana* from 5.08\% under photoautotrophic cultivation to 1.81 \% (1 g/L acetate) and 1.26\% (1 g/L glucose) under heterotrophic cultivation (Miazek et al., 2017). They stated that the reason of chlorophyll reduction could be due to decreasing the number of chloroplast or disturbing the light-dependent reactions related to synthesis of chlorophyll.

3.3. **Wastewater treatment by microalgae during two cycles of cultivation**

3.3.1. **TN removal efficiency**

Nitrogen is one the most important nutrients for microalgae growth. Total nitrogen (TN) is the sum of nitrate (NO$_3^-$), nitrite (NO$_2^-$), organic nitrogen and ammonium (NH$_4^+$). In this study, TN removal efficiency from DWW was investigated during two cycles of mixotrophic cultivation and one cycle of heterotrophic mode (Fig 4 (a)). The highest to the lowest values of TN removal efficiency followed the order as 92.15\% in two cycles of mixotrophic cultivation of *S. quadricauda*, 83.17\% in two cycles of mixotrophic cultivation of *T. suecica*, 46.70\% in one cycle of heterotrophic cultivation of *S. quadricauda*, and 44.92\% in one cycle of heterotrophic cultivation of *T. suecica*. All the eukaryotic microalgae assimilate inorganic nitrogen viz., NO$_3^-$, NO$_2^-$, and NH$_4^+$. Through the assimilation process, two species of oxidized nitrogen viz. NO$_3^-$ and NO$_2^-$ undergo reduction; NO$_2^-$ reduces to NH$_4^+$ and NH$_4^+$ incorporate into amino acids (Cai et al., 2013).

As Fig. 4 (a) illustrates, mixotrophic cultivation is more efficient than heterotrophic cultivation and freshwater microalga is more efficient than marine water microalga concerning TN removal. The reason for higher TN removal efficiency in mixotrophic than heterotrophic cultivation is the higher production of microalgae in the former. As can be seen from Fig 2 (a,b), microalgae did not grow well under heterotrophic cultivation mode. Katam and Bhattacharyya (2018) reported the highest and lowest
TN removal efficiency as 85 and 60% in the microalgal reactors with the solid retention time (SRT) ≥8 and 2 days, respectively (Katam and Bhattacharyya, 2018a). They stated that the reason for lower TN removal efficiency in reactor with 2 days of SRT might be related to low concentration of microalgal biomass. In the present study, *S. quadricauda* and *T. suecica* removed 86.70 and 66.8% of TN from DWW, respectively in the first cycle of mixotrophic cultivation. In the second cycle of cultivation, *T. suecica* showed higher removal efficiency of TN as 16.0% as compared to 4.5% by *S. quadricauda*. After two cycles of cultivation, 92.15% of TN was removed by freshwater microalga and 83.17% by marine water microalga. The reason for TN removal efficiency (lower than 100%) can be mainly attributed to phosphate limitation, as it was removed completely during two cycles of cultivation. However, it can also be related to the presence of some organic compounds in DWW that cannot be assimilated by microalgae (Zhou et al., 2012).

3.3.2. *PO₄³⁻* removal efficiency

*PO₄³⁻* removal efficiency was different among different microalgae species, cultivation modes, and cycles of cultivation (Fig. 4 (b)). After two cycles of mixotrophic cultivation during 24 days, both freshwater and marine water microalgae removed 100% of *PO₄³⁻* from DWW. In heterotrophic cultivation mode, *PO₄³⁻* removal efficiency was not observed and even the final concentration of *PO₄³⁻* in DWW was higher than initial concentration. The initial concentration of *PO₄³⁻* in DWW as cultivation medium of *S. quadricauda* and *T. suecica* was similar; but *PO₄³⁻* removal efficacy in the first cycle of mixotrophic cultivation by *S. quadricauda* (71.2%) was significantly higher than *T. suecica* (42.2%). It shows that in the same type of wastewater and cultivation conditions, *PO₄³⁻* removal efficiency can be dependent to microalgae species. Similar to the results of this study, higher (78.9%) and lower (51.42%) *PO₄³⁻* removal efficiencies have been observed by cultivated *Scenedesmus sp.* in domestic wastewater and *Tetraselmis chuii* in aquaculture wastewater, respectively (Nayak et al.,
In the second cycle of mixotrophic cultivation, the residual $\text{PO}_4^{3-}$ in DWW was removed completely up to 100%. Surprisingly, $\text{PO}_4^{3-}$ removal efficiency by *T. suecica* in the second cycle of cultivation as 57.8% was significantly higher than first cycle (42.2%). Decreasing the ratio of TN: $\text{PO}_4^{3-}$ from 9.82 to 4.23 in *S. quadricauda* medium and 9.10 to 5.24 in *T. suecica* medium during the first cycle of cultivation to the second one, did not disturb microalgae growth and nutrient removal efficiency. Delgadillo-Mirquez et al. (2016) used higher ratio of N:P=17 and stated that N:P ratio in their study did not affect phosphate removal efficiency (Delgadillo-Mirquez et al., 2016). The reason of increasing $\text{PO}_4^{3-}$ concentration in heterotrophic cultivation mode can be due to the releasing of stored phosphates from microalgae and native bacteria of DWW (Shen et al., 2017; Kim et al., 2016).

### 3.3.3. $\text{SO}_4^{2-}$ removal efficiency

Sulfur is an essential element for important biological processes in microalgal cells. Microalgae need sulfur for the synthesis of vital biochemcials such as amino acids, enzymes, vitamins and compounds of cell wall. The majority of microalgae use $\text{SO}_4^{2-}$ as the main source of sulfur (Procházková et al., 2014). In this study, removal efficiency of $\text{SO}_4^{2-}$ by *S. quadricauda* and *T. suecica* under different cultivation conditions was studied. As can be seen from Fig. 4 (c), $\text{SO}_4^{2-}$ was removed completely by two cycles of mixotrophic cultivation of *S. quadricauda*. After the first cycle of cultivation, 93.70% and 14.44% of $\text{SO}_4^{2-}$ was removed in mixotrophic cultivation of *S. quadricauda* and *T. suecica*, respectively. The reason for remarkable difference of $\text{SO}_4^{2-}$ removal efficiency by freshwater and marine water microalgae can be related to initial $\text{SO}_4^{2-}$ concentration in their mediums. The initial concentration of $\text{SO}_4^{2-}$ in *S. quadricauda* medium was 13.00 mg/L, and increased to 90.00 mg/L by dissolving 34 g/L sea salt in *T. suecica* medium (Table 1). Low removal efficiency of $\text{SO}_4^{2-}$ by *T. suecica* shows that the initial concentration in medium was higher than the concentration that
microalga needs for metabolism. In the case of heterotrophic cultivation of *S. quadricauda* and *T. suecica* and the second cycle mixotrophic cultivation of *T. suecica*, the concentration of SO$_4^{2-}$ was increased in DWW. Increasing SO$_4^{2-}$ concentration might be due to activity of bacteria present in DWW by oxidizing sulfur compounds to SO$_4^{2-}$ (Pokorna and Zabranska, 2015).

### 3.3.4. TOC removal efficiency

In water samples, the concentration of all bonded carbon atoms to organic molecules is defined as total organic carbon (TOC). It affects dissolved oxygen and consequently threatens aquatic life. In this study, removal efficiency of TOC from DWW by freshwater and marine water microalgae was investigated. As can be seen from Fig. 4 (d), TOC removal efficiency during the first cycle of cultivation was in the order: 76.44>69.1>62.12>40.20% in heterotrophic cultivation of *S. quadricauda*, mixotrophic cultivation of *S. quadricauda*, heterotrophic cultivation of *T. suecica* and mixotrophic cultivation of *T. suecica*, respectively. The highest TOC removal efficiency was observed during two cycles of mixotrophic cultivation of *S. quadricauda*, as 76.77%; but it was not significantly higher than 76.44% removal, which was observed after one cycle of heterotrophic cultivation of *S. quadricauda*. In mixotrophic cultivation of *S. quadricauda*, 69.1 and 7.7% of TOC (totally 76.77%) was removed after the first and second cycle, respectively. In mixotrophic cultivation mode of *T. suecica*, 40.20% of TOC was removed after the first cycle, however, during the second cycle of cultivation, TOC concentration was increased and consequently, TOC removal efficiency decreased. Some percentage of TOC removal efficiency can be due to growth of microalgae, but a high proportion can also be related to the activity of bacteria and other microorganisms in DWW. The microorganisms present in wastewater consume and decompose organic molecules as the source of energy (Mook et al., 2012). In this study, this is especially true for the heterotrophic cultivation mode as both microalgae did not grow well (Fig 2 (a,b)). Katam and Bhattacharyya (2018) reported around 85% of TOC removal from kitchen
wastewater by aerobic bacterial system and mixed microalgal culture (Katam and Bhattacharyya, 2018b). They explained that through heterotrophic metabolism, bacteria mineralize organic carbon in wastewater by utilizing dissolved oxygen. They also added that under mixotrophic conditions, many species of microalgae utilize organic molecules as a source of carbon. The reason of increasing TOC concentration in the second cycle of mixotrophic cultivation of T. suecica might be related to the releasing content of microalgal cells in the environment. According to previous work, 7-50% of the assimilated carbon in the form of extracellular organic matter can be released from microalgae to their environment (Babel et al., 2002).

3.4. Effect of different cultivation modes and cycles on fatty acid profiles

After wastewater treatment, microalgal biomass can be utilized as biodiesel feedstock. Microalgal lipids can be converted to fatty acid methyl esters (FAMEs) by transesterification. FAMEs are considered as the main constituent of biodiesel and are important in evaluation of biodiesel properties (Lam et al., 2017). Here, the effect of different cultivation modes and cycles on changes of fatty acids profile was investigated. Table 2 shows the fatty acids profile of freshwater and marine water microalgae cultivated in synthetic media (BBM and F2) and DWW under different cultivation modes and cycles. Nine fatty acids, namely C14:0, C15:1, C16:0, C16:1, C17:1, C18:2, C18:1, C18:3n-3, and C18:3n-6 with different percentages were detected in S. quadricauda and T. suecica biomass under different conditions. Fatty acids of microalgae are divided to three medium- (C10-C14), long- (C16-C18), and very long- (≥C20) chain fatty acids (Khan et al., 2009). In this study, the sum of C16, C18, and their derivatives with more than 80% was found as the major fatty acids in both microalgae. In agreement to this study, C16 and C18 were reported as the dominant fatty acids of Chlorella sorokiniana cultivated in dairy farm effluent and Micractinium sp., cultivated in the effluent of anaerobic digester as more than 95% and 77.36-83.92%, respectively (Hena et al., 2015; Kim et al.,
In microalgal cells, the produced Acetyl-CoA (acetyl coenzyme) acts as the precursor for synthesis of fatty acids. Multifunctional enzyme such as acetyl-CoA carboxylase (ACCase) converts bicarbonate and acetyl-CoA to Malonyl-CoA. Consequently, malonyl-CoA is catalyzed to malonyl acetyl carrier protein (malonyl-ACP). Finally, after carbon chain lengthening and desaturation, malonyl-ACP forms fatty acids, mainly C16 and C18 (Faried et al., 2017).

The major fatty acids of S. quadricauda cultivated in BBM medium and T. suecica in F2 medium were C16:0 (46.43%) and C18:3n-3 (49.61%), respectively. As compared to BBM medium, the cellular content of C16:0 in S. quadricauda significantly decreased to 25.99% in the first cycle of mixotrophic cultivation to 24.48% in the second cycle of mixotrophic cultivation and to 13.82% in the heterotrophic cultivation in DWW. On the contrary, the concentration of C18:1 (10.96%) in BBM medium significantly increased to 32.13, 52.26 and, 29.16% in the first and second cycle of mixotrophic cultivation and heterotrophic cultivation modes in DWW, respectively. This observation shows that the fatty acids percentage of one microalga species can be completely different in synthetic medium and wastewater. In another study, Kuo et al. (2015) cultivated Chlorella sp. GD in piggery wastewater with different dilution ratios (Kuo et al., 2015). Similar to the results of current study, a decrease of C16:0 and an increase of C18:1 percentage in 25% diluted wastewater, as compared to the pure medium was observed in their study. According to them, the variation in lipid profile of microalga can be related to wastewater constituents and conditions such as minerals, pH, and toxic organic compounds. Fatty acids of T. suecica cultivated in F2 medium and DWW showed a different pattern as compared to S. quadricauda. C18:3n-3 was the dominant fatty acid of T. suecica, cultivated in F2 medium, in the first and second cycle of mixotrophic cultivation in DWW. However, in heterotrophic cultivation, C18:1 with 61.43% relative abundance was the dominant fatty acid of T. suecica in DWW. Shen et al. (2015) also reported that C18:1 was the dominant fatty acid of Chlorella vulgaris NIES-227 during heterotrophic cultivation (Shen et al., 2015).
The amounts of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in different freshwater and marine water species of microalgae, the first and second cycle of cultivation, and mixotrophic/heterotrophic cultivation modes are presented in Table 2. SFAs of *S. quadricauda* in both cycles of mixotrophic cultivation were similar and significantly higher than heterotrophic cultivation mode. The MUFAs value of *S. quadricauda* in the second cycle of mixotrophic cultivation was significantly higher than the first cycle, due to the increase in the concentration of C18:1 to 52.26%. Increasing the percentage of MUFAs might be due to lower nitrogen concentration in the second cycle of cultivation as compared to the first one (Álvarez-Díaz et al., 2015). The values of MUFAs and PUFAs of *S. quadricauda* in the first cycle of mixotrophic cultivation were not significantly different; however, in the second cycle, the percentage of MUFAs was higher (55.66%) than the percentage of PUFAs (16.5%). The reason for increased MUFAs as compared to PUFAs is due to the decrease in the concentration of C18:3n-3 and increase in the concentration of C18:1 from the first to the second cycle. A study by Shen et al. (2016) supports the results of this study in which increase in C18:1 and decrease in C18:3 was reported under nitrogen deficiency (Shen et al., 2016). They explained that nitrogen limitation activates stearoyl-ACP desaturase, a gene responsible for synthesis of C18:1 fatty acid and deceives D15-desaturase, a gene which has a key role in the synthesis of C18:3 fatty acid.

For *T. suecica*, SFAs components increased from 21.6 to 30.58% from the first to the second cycle of mixotrophic cultivation. On the contrary, the concentration of both mono- and poly-unsaturated fatty acids decreased from the first cycle to the second cycle. As compared to the mixotrophic cultivation, MUFAs values of *T. suecica* in DWW being 28.37 (first cycle) and 22.83% (second cycle), sharply increased to 72.73% in the heterotrophic cultivation mode. Regarding the PUFAs values, 51.36% (first cycle) and 46.59% (second cycle) noticeably decreased to 7.24% in the heterotrophic cultivation mode.
4. Conclusions

Freshwater and marine water microalgae grew well during the first (0.43 and 0.61 g/L, respectively) and second cycle (0.36 and 0.65 g/L, respectively) of mixotrophic cultivation in dairy wastewater. Effective pollutants removal was achieved by S. quadricauda after two cycles of cultivation. Profile of fatty acids can be completely different depending on the microalgae species (freshwater or marine water), cycle of cultivation (first and second), and cultivation mode (mixotrophic and heterotrophic). Conclusively, reuse of dairy wastewater for different cycles of microalgae cultivation is an efficient strategy that can save water for producing higher biomass of microalgae and treat wastewater effectively.

Acknowledgments

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References


Fig. 1. Experimental scheme of sequential cultivation of microalgae in raw and recycled dairy wastewater.
(a) Microalgal dry weight (g/L) vs. Time (days)

(b) Microalgal dry weight (g/L) vs. Time (days)
Fig. 2. Growth of freshwater (a: *Scenedesmus quadricauda*) and marine water (b: *Tetraselmis suecica*) microalgae in dairy wastewater during mixotrophic (two cycles) and heterotrophic cultivation modes: (SM1: *S. quadricauda*/Mixotrophic/First cycle, SH: *S. quadricauda*/Heterotrophic, SM2: *S. quadricauda*/Mixotrophic/Second cycle, TM1: *T. suecica*/Mixotrophic/First cycle, TH: *T. suecica*/Heterotrophic, TM2: *T. suecica*/Mixotrophic/Second cycle). Lowercase letters represent statistically significant differences among the experimental units in each day of cultivation.
Chlorophyll a, Chlorophyll b, Carotenoids

Pigments concentration (mg/L)

Chlorophyll a, Chlorophyll b, Carotenoids

(a)
Fig. 3. Pigments concentration (a) and content (b) of freshwater and marine water microalgae in dairy wastewater during mixotrophic (two cycles) and heterotrophic cultivation modes: (SM1: *S. quadricauda*/Mixotrophic/First cycle, SH: *S. quadricauda*/Heterotrophic, SM2: *S. quadricauda*/Mixotrophic/Second cycle, TM1: *T. suecica*/Mixotrophic/First cycle, TH: *T. suecica*/Heterotrophic, TM2: *T. suecica*/Mixotrophic/Second cycle). Lowercase letters represent statistically significant differences among the experimental units.
<table>
<thead>
<tr>
<th>Mode</th>
<th>Microalgae</th>
<th>Cultivation</th>
<th>TN Removal Efficiency (%)</th>
<th>PO₄³⁻ Removal Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM</td>
<td>C1</td>
<td>C2</td>
<td>92.15 ± 4.5</td>
<td>28.8 ± 71.2</td>
</tr>
<tr>
<td>TM</td>
<td>C1</td>
<td>C2</td>
<td>83.17 ± 8.4</td>
<td>57.8 ± 8.4</td>
</tr>
<tr>
<td>SH</td>
<td>C1</td>
<td>C2</td>
<td>66.8 ± 5.1</td>
<td>42.2 ± 8.4</td>
</tr>
<tr>
<td>TH</td>
<td>C1</td>
<td></td>
<td>46.7 ± 16.4</td>
<td>-55.32 ± 9.8</td>
</tr>
</tbody>
</table>

Microalgal and cultivation modes:

(a) TN removal efficiency
(b) PO₄³⁻ removal efficiency
Fig. 4. Pollutants removal efficiency by freshwater and marine water microalgae in dairy wastewater during mixotrophic (two cycles) and heterotrophic cultivation modes (a): TN, (b): PO$_4$$^3-$, (c): SO$_4^{2-}$, and
Table 1. Characterization of dairy wastewater and experimental conditions during the first and second cycle cultivation of freshwater and marine water microalgae.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>First cycle</th>
<th>Second cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freshwater</td>
<td>Marine water</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>86.0±2.2</td>
<td>86.65±2.2</td>
</tr>
<tr>
<td>PO₄³⁻ (mg/L)</td>
<td>8.75±0.1</td>
<td>9.50±0.1</td>
</tr>
<tr>
<td>TN:PO₄³⁻</td>
<td>9.82±1.1</td>
<td>9.1±1.1</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/L)</td>
<td>13.0±1.4</td>
<td>90.00±1.4</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>170.11±11.2</td>
<td>179.35±11.2</td>
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<tr>
<td>pH</td>
<td>6.30±0.1</td>
<td>6.30±0.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22± 1</td>
<td>22± 1</td>
</tr>
<tr>
<td>Light (μmol photon/(m².s))</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Salinity (g/L)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Culture period (days)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Inoculation concentration (mg/L)</td>
<td>40</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 2. Fatty acid profile of freshwater and marine water microalgae cultivated in synthetic media (BBM and F2) and DWW under different cultivation modes and cycles: (S-BBM: *S. quadricauda* in BBM medium, SM1: *S. quadricauda*/Mixotrophic/First cycle, SH: *S. quadricauda*/Heterotrophic, SM2: *S. quadricauda*/Mixotrophic/Second cycle, T-F/2: *T. suecica* in F2 medium, TM1: *T. suecica*/Mixotrophic/First cycle, TH: *T. suecica*/Heterotrophic, TM2: *T. suecica*/Mixotrophic/Second cycle).

<table>
<thead>
<tr>
<th>FAMEs composition (%)</th>
<th>S-BBM</th>
<th>S-M-C1</th>
<th>S-M-C2</th>
<th>S-H-C1</th>
<th>T-F/2</th>
<th>T-M-C1</th>
<th>T-M-C2</th>
<th>T-H-C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>-</td>
<td>1 ± 0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.34 ± 0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C14:1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.67 ± 0.6</td>
</tr>
<tr>
<td>C15:1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.96 ± 1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16:0</td>
<td>46.43 ± 2.8</td>
<td>25.99 ± 0.1</td>
<td>24.48 ± 0.1</td>
<td>13.82 ± 0.5</td>
<td>14 ± 0.4</td>
<td>19.26 ± 0.3</td>
<td>30.58 ± 1.2</td>
<td>20.03 ± 1.5</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.14 ± 4.4</td>
<td>3.06 ± 0.1</td>
<td>2.35 ± 0.1</td>
<td>8.33 ± 0.7</td>
<td>7.2 ± 0.1</td>
<td>2.56 ± 0.3</td>
<td>7.49 ± 0.1</td>
<td>6.63 ± 1.2</td>
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<tr>
<td>C17:1</td>
<td>8.76 ± 1.6</td>
<td>2.59 ± 0.1</td>
<td>1.05 ± 0.1</td>
<td>5.35 ± 0.8</td>
<td>21.18 ± 0.4</td>
<td>17.57 ± 0.6</td>
<td>8.41 ± 0.1</td>
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<tr>
<td>C18:0</td>
<td>-</td>
<td>-</td>
<td>3.36 ± 0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>C18:1</td>
<td>10.96 ± 1.6</td>
<td>32.13 ± 0.1</td>
<td>52.26 ± 0.2</td>
<td>29.16 ± 0.7</td>
<td>-</td>
<td>7.28 ± 0.1</td>
<td>6.93 ± 0.6</td>
<td>61.43 ± 2.5</td>
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<tr>
<td>C18:2</td>
<td>14.81 ± 5.6</td>
<td>12.2 ± 0.1</td>
<td>10.11 ± 0.1</td>
<td>9.7 ± 0.7</td>
<td>8 ± 0.7</td>
<td>10.52 ± 0.5</td>
<td>10.06 ± 1.6</td>
<td>1.68 ± 2.4</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>15.89 ± 12.8</td>
<td>21.44 ± 0.1</td>
<td>6.39 ± 0.1</td>
<td>17.46 ± 0.1</td>
<td>49.61 ± 1.1</td>
<td>39.56 ± 0.2</td>
<td>35.89 ± 0.6</td>
<td>5.56 ± 0.3</td>
</tr>
<tr>
<td>C18:3n-6</td>
<td>-</td>
<td>1.59 ± 0.1</td>
<td>-</td>
<td>16.16 ± 0.2</td>
<td>-</td>
<td>1.28 ± 0.1</td>
<td>0.64 ± 0.1</td>
<td>-</td>
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<tr>
<td>Σ ω−3</td>
<td>15.89 ± 12.8</td>
<td>21.44 ± 0.1</td>
<td>6.39 ± 0.1</td>
<td>17.46 ± 0.2</td>
<td>49.61 ± 1.1</td>
<td>39.56 ± 0.2</td>
<td>35.89 ± 0.6</td>
<td>5.56 ± 0.3</td>
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<tr>
<td>Σ ω−6</td>
<td>14.81 ± 5.6</td>
<td>13.79 ± 0.2</td>
<td>10.11 ± 0.1</td>
<td>25.86 ± 1.5</td>
<td>8 ± 0.7</td>
<td>11.8 ± 0.6</td>
<td>10.7 ± 1.4</td>
<td>1.68 ± 2.4</td>
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<tr>
<td>Σ ω−3/Σ ω−6</td>
<td>1.07</td>
<td>7.65 ± 0.63</td>
<td>0.67</td>
<td></td>
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</tr>
</tbody>
</table>
| Σ SFA                 | 46.43 ± 2.8 | 26.99 ± 27.84 ± 13.82 ± 14 ± 0.4 | 21.6 ± 30.58 ± 20.03 ± 3.35 3.31 6.31 3.31 20.03 ± 3.31 20.03 ± 3.31
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>∑ MUFA</td>
<td>0.2 ± 0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>37.78 ± 0.3</td>
<td>55.66 ± 0.3</td>
<td>42.84 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.1</td>
<td>3.1</td>
<td>14.2</td>
</tr>
<tr>
<td>∑ PUFA</td>
<td>0.7 ± 0.7</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>28.38 ± 1.7</td>
<td>28.37 ± 51.36</td>
<td>22.83 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>57.61 ± 1.7</td>
<td>3.1</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>46.59 ± 7.6</td>
<td>0.8</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>7.24 ± 1.7</td>
<td>7.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

|            | 22.86 ± 7.6   | 18.4 |
|            | 30.7 ± 18.4   | 18.4 |

**Note:** The values represent the sum of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) with their respective standard deviations.
Freshwater microalga

Initial concentration

- TN (mg/L) 86.0±2.2
- PO$_4^{3-}$ (mg/L) 8.75±0.1
- SO$_4^{2-}$ (mg/L) 13.0±1.4
- TOC (mg/L) 170.1±11.2

Dairy wastewater

Mixotrophic cultivation

The first cycle

- SM1
- SH

The second cycle

- SM2
- TM2

Marine water microalga

Initial concentration

- TN (mg/L) 86.6±2.23
- PO$_4^{3-}$ (mg/L) 9.50±0.1
- SO$_4^{2-}$ (mg/L) 90.0±1.41
- TOC (mg/L) 179.3±11.18

Dairy wastewater

Mixotrophic cultivation

The first cycle

- TM1
- TH

Heterotrophic cultivation

The second cycle

Pollutants | Removal efficiency (%) (Mixotrophic)
--- | ---
TN | 86.70 4.50 92.15
PO$_4^{3-}$ | 71.20 28.80 100
SO$_4^{2-}$ | 93.70 6.30 100
TOC | 69.10 7.70 76.77

Pollutants | Removal efficiency (%) (Mixotrophic)
--- | ---
TN | 66.80 16.40 83.17
PO$_4^{3-}$ | 62.20 57.80 100
SO$_4^{2-}$ | 14.63 43.30 -28.90
TOC | 69.20 10.10 30.30

Scenedesmus quadricauda
Tetraselmis suecica
Research highlights

- Two cycles of microalgal growth were studied in dairy wastewater (DWW).
- Nutrients concentration affected microalgal growth rate in the second cycle.
- Chlorophyll $a$ decreased from the first cycle of cultivation to the second one.
- Two cycles of $S.~quadricauda$ cultivation improved pollutants removal efficiency.
- FAMEs profile of microalgae was different between the two cultivation cycles.