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Mixotrophic Cultivation of *Coccomyxa subellipsoidea* Microalga on Industrial Dairy Wastewater as an Innovative Method for Biodiesel Lipids Production

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Abstract

Global demand for new energy resources is continuously raising as non-renewable fossil fuels cost and combustion rise over the years. Cultivation of *Coccomyxa subellipsoidea* HSSASE8 with accession No. KT277791.1 was cultivated on basal medium supplemented with different proportions (20, 40, 60, 80 or 100%) of sterile or unsterile wastewater in a bioreactor. The chemical component of dairy wastewater, biomass, lipid content, fatty acids profile, total nitrogen (TN) and total phosphorus removal (TP) were estimated. The maximum biomass production was at 60% dairy wastewater (DWW) dilutions (918.15±0.07 and 909.09±0.04 mg/L in unsterile and sterile conditions, respectively). The removal percentage of total N (310±132.9- 0.0 mg/L) and total P (279.5±56.2- mg/L) was at 100%. In addition, total organic carbon (TOC) ranged from 182.6-2.42 (94.58%) and 182.6-3.86 (83.47%) at 60% DWW dilution. The maximum lipid contents of the dry cell weight (DCW) were 75.16%±5.3 and 80.67±5.6 in sterile and unsterile conditions, respectively, while the fatty acids composition revealed that the highest yield of fatty acids (C16-C18) ranged between 68.54% and 72.54% (w/w) at unsterile condition compared with sterile condition (68.27- 71.34%).

Keywords: Microalgae; *C. subellipsoidea*, Mixotrophic, Photobioreactor; Biodiesel, Fatty acid profile

1. Introduction

Biodiesel is presently undergoing extensive attention owing to its excellent power as a bright, continuous and environmentally friendly energy source option compared to fossil fuels (Griffiths *et al*., 2012).The international concerns owing to exhausting petroleum reserves can be a reason for extending the sum of investigators on biodiesel production (Schenk *et al*., 1998). Successful algal biodiesel production mainly depends on picking the right species with vital properties, for instance, biomass and fatty acid productivity, respectively. Green microalgae in comparison to blue-green algae are found to be potential biodiesel feedstocks (Lei *et al*., 2012). The algae lipid content varies greatly according to different growth conditions which may vary to be within 1 to >50%. The eukaryotic algae have high levels of TAGs that is not common in cyanobacteria or other prokaryotes in general (Carolina *et al*., 2017).

The dairy industry is regarded as one of the dominant industries with strong commercial value in the horticultural district (Gavala *et al*., 1999). Meanwhile, dairy waste effluents represent one of the significant sources of water pollution.

That is the reason why there is a crucial need for the treatment of dairy wastewater (DWW) prior to consumption or disposal (Karadag *et al*., 2015; Rad and Lewis 2014). On the other hand, it has satisfactory minerals like N (14–830 mg/l), and P (9–280 mg/l) required for biological management, in addition to a high protein content, a high organic matter content, traces of heavy metals and easy biodegradability (Gavala *et al*., 1999 and Sarkar *et al*., 2006). In recent years, many studies proved that microalgae have to reach a vigorous measure of biomass viewed as third generation feedstock for biofuels and animal forage (Rad and Lewis 2014).

The coupling of microalgae cultivation with DWW treatment and recycling is represented in our study as an effective strategy for microalgae-based biofuel production (Gao *et al*., 2014 and Alvarez-Diaz *et al*., 2015). Therefore, the aim of this work was to cultivate *C. subellipsoidea* HSSASE8 microalga in DWW which has nutrients required for microalgae proliferation in order to achieve both benefits of mineral discharge and biomass production for biodiesel generation. In addition, the total fatty acid methyl esters (FAME) was measured by gas chromatography (instead of the determination of raw lipids using solvent extraction) as a signal for determining the efficiency of this alga in biodiesel production.

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2. Materials and Methods

2.1. Isolation and culture of microalgae

2.1.1. Samples collection

Coccomyxa subellipsoidea microalga was isolated from agricultural drainage water (Bahr hadus pump station No. 3 with Latitude/Longitude (31°02'07.0"N-31°44'35.1"E) in El-Daqhlia Governorate, Egypt) during summer of 2014 by the Department of Botany, Faculty of Science, Cairo University, Egypt and was registered in GenBank under the name of *C. subellipsoidea* HSSASE8 with Accession Number: (KT277791.1).

2.1.2. Media preparation

Sterilized and raw dairy wastewater was used for the preparation of modified Basal Bold medium containing(g/L) 0.25; $NaNO_3$, 1.25; $MgSO_4 \cdot 7H_2O$, 1.0; KH_2PO_4, 1.25; EDTA, 0.5; boric acid, 0.1142; $CaCl_2$, 0.111; $FeSO_4 \cdot 7H_2O$, 0.0498; $ZnSO_4 \cdot 7H_2O$, 0.0382; $CuSO_4 \cdot 5H_2O$, 0.0157; $MnCl_2 \cdot 4H_2O$, 0.0144; $Na_2MoO_4 \cdot 2H_2O$, 0.01192 and $CoNO_3 \cdot 6H_2O$, 0.0049 in 1 L distilled water and the pH of the medium was adjusted to 6.5 prior to autoclaving.

2.1.3. Characteristics of wastewaters

The DWW used in this research was collected from a local dairy transformation plant in Golden Pack Company, 6 October City, Egypt. It was immediately divided into two groups. The first one was the unsterile dairy wastewater which is primary effluent wastewater refined with a clean cloth to exclude large fragments and kept for a week to sit down any visible solid molecules. After that, the wastewater was centrifuged at 11,000 (g) for 15 min to eliminate microscopic particles. The second group was the sterile dairy wastewater which was refined using 0.2 µm nylon microfilters to get rid of the suspended solid particles and microbes, followed with autoclaving the supernatant.

2.1.4. Microalga growth

The microalgal seed culture was inoculated at 10% concentration (V/V) per100 mL of Bold Basal Medium (BBM) in a 250 mL Erlenmeyer flask (The American Public Health Association, 2012), and further incubated in a shaker under continuous illumination with white fluorescent light of 2000 lux intensity at 150 rpm and 28 °C for 14 days. The sterile algal culture was developed by streptomycin and penicillin antibiotics treatment (Sigma-Aldrich, USA) to remove commensal bacteria in the cultures and to verify the axenic status of microalgae (Droop, 1967). The microalgae suspension in BBM was adjusted using a spectrophotometer to 0.6± 0.05 optical density at 540 nm for further experiments.

2.2. Optimization of biomass and lipid accumulation

To evaluate the suitability of DWW as an enriching medium for microalgae growing under mixotrophic conditions, five different sets of experiments were conducted. Partial substitution of the BBM medium with sterile/non-sterile DWW was tested by supplementing 1L flasks with 500 mL working volumes by five various proportions 20% (A), 40% (B), 60% (C), 80% (D)or 100 (E) of (sterile/non-sterile DWW(v/v). Inoculate 10 % (20mL) of the *C. subellipsoidea* HSSASE8 microalga suspension (V inoculum/V BBM). The experiments were carried out at 25°C, pH (7.5), and 150 rpm with cool-white fluorescent light illumination at 2000 lux intensity The experiments were carried out in three replicates (n=3). Culture growth was estimated by measuring dry cell weight (DCW) and lipid productivity after 20 days.

For large scale production under controllable mixotrophic conditions, 100 ml of *C. subellipsoidea* HSSASE8 growing suspension in 7L bioreactor (BioFlo 110, New Brunswick Scientific, USA) containing 2L running volume of the experiments sets (A-E) of sterile/non-sterile dairy wastewater DWW (v/v)) as optimum concentrations showed high growth biomass and lipid content. The main structure consists of a vessel, a fundamental control unit for beginning and performing process criteria, and a function unit to regulate temperature (25°C), turbulence, and pumps for continuous sterile aeration (150 mL/min) for 20 days under illumination intensity (2000 lux). The experiments were conducted at Bioprocess Development dept., GEBRI, SRTA-City in three replicate. DCW and fatty acids were measured at the end of the culture period at the Central lap of SRTA-City.

2.3. Analytical methods

2.3.1. Microalgae growth

As mention before, the algal growth and nutrients consumed in the growing batches followed daily during the experimental period by taking a sample volume of 10mL from growth suspension. Centrifuged at 11,000 (g) for 15 min, the supernatants were diluted and investigated for BOD, COD, TN, and TP to measure the mineral consumption. Development of algal cells in BBM supplemented with various concentrations of DWW was checked by determining the optical density at 680 nm using UV/visible spectrophotometer (Optizen, Korea).

2.3.2. Biomass production and lipid content

The algal biomass was harvested after growth of algal for twenty days batch culture and the dry weight (g/L) of culture suspension sample was determined as described by Droop (1967), weighing a dried sample of the culture suspensions. About 10 mL growth cultures were filtered through pre-weighed filter papers (Whatman GF/C 1.2mm, 90mm in width). Then, they were dried overnight at 110 ℃ and evaluated again at cabinet temperature to determine dry biomass accumulation per liter of culture broth (g/L). The OD of the microalgal suspension was measured at 680nm using a spectrophotometer. When needed, the sample was diluted to present an absorbance between 0.1–1.0. The OD_{680} was then switched to a dry cell weight (DCW) applying a linear relationship between OD_{680} and DCW (g/L), (The American Public Health Association., 1998).

At the end of experiments (20 days), lipid content analyzed gravimetrically according to solvent-based extraction method (Bligh and Dyer, 1959; Araujo *et al.,* 2013). Briefly, 35 mL of microalgal culture were centrifuged at 3900 (g) and 4 °C for 20 min. The supernatant was thrown and the algal cells were resuspended in 2.5 mL of distilled water. 1.25 mL of chloroform and 2.5 mL of methanol were added. The mixture was applied to a sonication procedure for 30 min with overnight shaking. Then, 1.25 mL of chloroform were added and the mixture was sonicated for 30 min with shaking for 2 h. 1.25 mL distilled water was added with

shaking for 1 h. the mixture was then centrifuged at 3900 (g) and 4 °C for 5 min. The lipid-chloroform layer (in the bottom) was genteelly pipetted and transferred in a new tube, resuspended in 5 mL of deionized water and vortexed for 30 s at room temperature. 2.5 mL of chloroform were added with shaking for 1 h. The mixture was centrifuged at 3900 (g) and 4 °C for 5 min. The lipid-chloroform layer was gently pipetted and transferred in a new tube, washed with 5 mL of NaCl solution (5 %) and vortexed. The mixture was centrifuged at 3900 (g) and 4 °C for 5 min. The chloroform layer was pipetted and transferred in a pre-weighed tube. Chloroform was dry in the oven at 105°C for 1h and total lipid weight was calculated.

2.3.3. Transesterification of lipid into Fatty acid methyl esters(FAME)

The algal lipid was transesterified into fatty acid methyl esters (FAME) where most extraction methods were based on rout of Bligh and Dryer in 1959 as reported by (Lewis *et al*, 2000). Each 10 mg of lipid was dissolved in 2 mL of hexane and 0.2 mL of freshly prepared methanolic KOH (2M) as a catalyst. The mixture was Vortexed for 2 to 5 min followed by brief centrifugation. The upper hexane layer was gathered for FAME analysis.

2.3.4. Fatty acids profiles

The algal cells utilized for fatty acid profiles analysis were collected by centrifugation at 5000 rpm and 4°C for 30 min, and the algal pellets were lyophilized by a freeze dryer. Lipid and fatty acid composition analysis was performed as described by Jean-Michel Girard (2014) with slight modification. Briefly, 15 to 20mg of freeze-dried algal biomass was dissolved in 5 mL of sulfuric acid/methanol (2:98, v/v). The mixture was warmed to reflux using Dien-Stark apparatus. After cooling, the reaction mixture was rinsed twice with saturated sodium hydrogen carbonate aqueous solution and evaporated over anhydrous sodium sulfate. The fatty acid methyl esters (FAMEs) gained as mentioned by Ichihara *et al.*, (1996) dissolving analysis by 5 column (30 m × 0.25 mm, film thickness 0.25 um) with helium as carrier gas at 1.33 mL/ min. The injection port held at 210°C, the detector temperature adapted to 230°C. The segregation ratio kept 1:10 and ionization voltage maintained at 70 eV. One µL specimen injected. The oven computed as follows: at 40 °C for 2 min and afterward raised to 210°C at 5°C/min at which the column retained for 5 min. The fatty acids were classified by matching the retention times with those of standard (Fatty acids -Sigma-Aldrich) and assessed by relating their peak area with that of the internal standard.

2.4. Statistical analysis

All the data obtained from the experiments replica was assessed by calculating the standard deviation of the means, and the Least Significant Difference (LSD) between the mean values was calculated at the 0.05 levels using SPSS V.16

3. Result

3.1. Characteristics of dairy wastewater

The components of the main effluent dairy wastewater used in this experiment were illustrated in Table 1. Mineral concentrations, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Phosphorus (TP) and Total Nitrogen (TN) were calculated corresponding to the standard method as specified by The American Public Health Association (APHA, 2012). The diverse components including Na, K, Ca, Fe and Mn were tested by inductively coupled plasma-atomic emission spectrometry (ICP-MS, Agilent Technologies, Japan).

Table 1. Physico-chemical properties of the primary effluent dairy wastewater.

Parameters	Units	Dairy wastewater
Chemical oxygen demand	mg O_2/L	2453.6±361.2
Biochemical oxygen demand	mg O_2/L	1645.2±156.26
Total organic carbon	mg/L	182.65±13.2
Total suspended solids	mg/L	674±83.7
Total phosphate	mg/L	279.5±56.2
Total nitrogen	mg/L	310±132.9
pH		7.5±0.3
Na	mg/L	517±46.2
K	mg/L	5420±487.7
Ca	mg/L	1273±940.1
Fe	mg/L	31±6.4
Mn	mg/L	16.7±3.8

Data were shown by mean values ± standard deviation of three individual repetitions

3.2. Growth biomass evaluation

Significant lipid composition is one of the major benchmarks for the choice of microalgae strains as a sustainable source for the production of biofuel. The freshwater algae confirmed in the existing work were chosen not solely on the principle of a great lipid and fatty acid composition but further on excessive growth rates and significant cell density with little cost. Upon cultivation of microalga, the water samples were analyzed for physicochemical properties (Table 1).

The *C. subellipsoidea* HSSASE8 microalgae were grown in BBM media supplemented with five different dilutions (20% (A), 40% (B), 60% (C), 80% (D) and 100 (E)) of sterile or unsterile DWW under a mixotrophic condition at a small scale in shake flask experiment (Figure 1A) and at large scale in bioreactor batch experiments up to 10 days (Figure 1B).

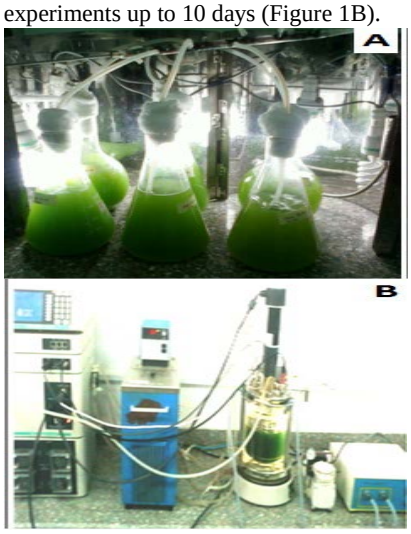

Figure 1. Growth optimization of C. subellipsoidea microalgae in BBM media supplemented with 20%, 40%, 60%, 80% and 100 of sterile/unsterile Dairy using shake flak methods(A) and bioreactor for large scale biomass production & lipid productivity (B).

All biomasses of sterile and unsterile batches showed a continual increase in biomasses with time (Figure 2). This increase in biomass reached the maximum after 8 days in

A, B, C and E treatments, while it reached the maximum after 7 days in D treatment and then slightly decreased in the next cultivation time (48h). The growth of *C. subellipsoidea* HSSASE8 microalga was greatly promoted by DWW treatment, and the most abundant biomass was achieved at 60% (918.15 mg/L and 776.09 mg/L for unsterile and sterile DWW dilutions, respectively). This was followed by biomasses in unsterile condition at 80% (573.01mg/L) and 100% (563.11mg/L) of DWW. On the other hand, the minimum biomasses were obtained at 20% (9.72mg/L) in sterile DWW dilutions.

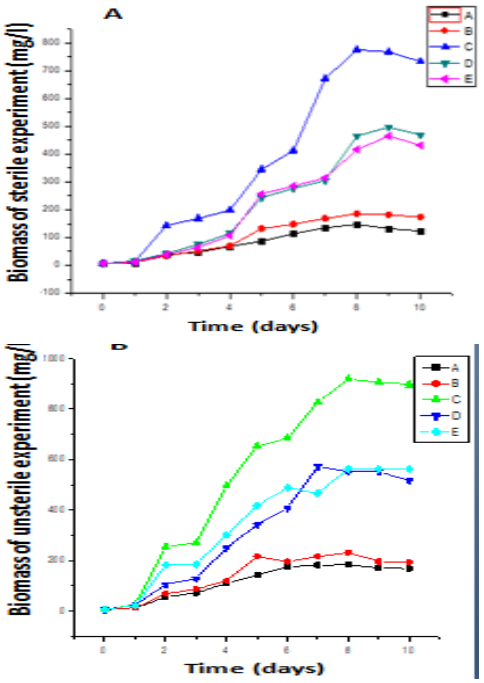

Figure 2. Growth biomass curve (DW) of *C. subellipsoidea* microalga in basal medium supplemented with different dilutions; 20% (A), 40% (B), 60% (C), 80% (D and 100 (E) of sterile DWW (A) and unsterile DWW (B) within 10 days.

3.3. Nutrients removal

In the present study, a primary parameter for monitoring DWW effluent quality (Table 1) indicated that the initial nutrients loads TN (310mg/l), TP (279.5mg/l), TOC (182.65 mg/l) of DWW effluent were beyond the minimal discharge values (TN 15–20 mg/l, TP 0.5–1.0 mg/l, TOC 20–30 mg/l) established by State Environmental Protection Administration of China (SEPAC). An overview of the elimination of macro and microelement showed a particular decline in these elements in all concentrations of dairy wastewater. The usage of nutrient elements by *C. subellipsoidea* HSSASE8 microalga enhanced their production and decreased the nutrient accumulation in the dairy wastewater, aiding the purpose of the advanced wastewater management and biomass production. Therefore, the strength of *C. subellipsoidea* HSSASE8 to remove TN, TP and TOC was studied in bioreactor running under different dilutions of DWW up to 10 days in bioreactor batch culture, and the data are shown in Figures3. An efficient performance in reductions of TN, TP and TOC were signed by *C. subellipsoidea* HSSASE8 cells as a return to DWW substrate feeding. Specifically, the measured consumption figures of TN ranged from 301 to 0.0mg/w (100%) (Figure 3A and B) was achieved in 60% DWW batches in the first 7thand 8thcultivation days of unsterile and sterile conditions, respectively. Moreover, the consumption rates of TP ranged from 279.5-0.0 mg/w (100%) (Figure 3C and D) were in 60% DWW batches in the 9thand 10thcultivation days of unsterile and sterile conditions respectively whereas the reduction values of TOC ranged from 182.6-2.42 mg/w (94.58%), and 182.6-3.86 mg/w (83.47%) (Figure 3E and F) was measured in 60% DWW batches in the 10thcultivation day in unsterile and sterile conditions, respectively. It is obvious that a maximum TN, TP, and TOC reduction by *C. subellipsoidea* HSSASE8 was recorded in 60% unsterile DWW culture media, which is higher than that at 60% sterile DWW culture batches. It was remarkable that *C. subellipsoidea* HSSASE8 growth slowed down when TN, TP, and TOC nutrients were no longer detected in DWW culture batch media, suggesting that the exhaustion of these nutrients may limit the growth of microalga.

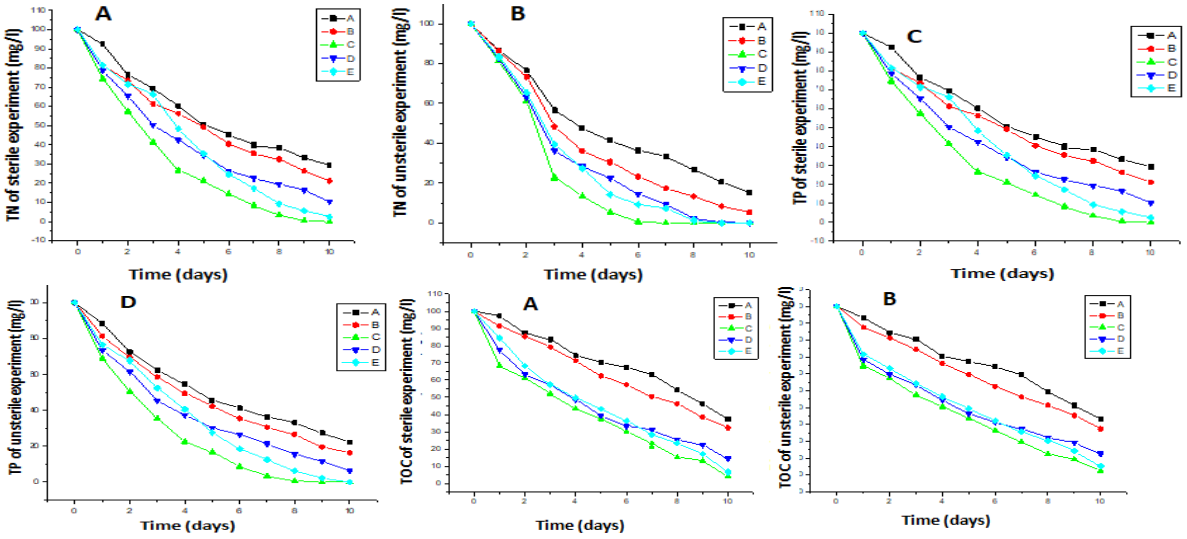

Figure 3. Nutrients removal of TN (A & B), TP (C & D) and TOC (E & F) versus time by C. subellipsoidea microalgae cultivated in basal medium supplemented with different dilutions; 20% (A), 40% (B), 60% (C), 80% (D and 100 (E) of sterile/unsterile Dairy WW.

C. subellipsoidea HSSASE8 fulfills the essential benchmarks for biodiesel production, essentially excessive lipid content and massive growth yield, adding another interest of usage of drain water and alleviate environmental deterioration. In a request to assess its cost-benefit an alternative competitive C-N source likes DWW of varied concentrations that was appeared as a good nutrient supplement and may be used for algal cultivation that agrees with that found by Ian Charles Woertz (2007).

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3.4. The lipid content and fatty acid profile

The lipid contents and fatty acid profiles represent a potential indicator of biodiesel yield. Lipids substances biosynthetically related to fatty acids and their derivatives (Chisti, 2007). In the present study, the total lipid content of the *C. subellipsoidea* HSSASE8 microalgae after 10 days was determined. Results prove that the process of partial replacement to synthetic media with dairy wastewater decrease the comprehensive cost of biofuel production. The total lipid content of *C. subellipsoidea* HSSASE8 cultivated in sterile or unsterile DWW supplemented media was improved and directly proportional to the algal biomass concentration, ranged from 34.46to 80.67% (DCW) response to the cultivation in growth media supplemented with different dilutions (A-E) of sterile or unsterile DWW substrate. It was obvious in Figure 4 that the total lipid content varied significantly across the different medium treatment strategies. It was clear that the increase in the lipid content in unsterile treatments was higher than that in sterile treatments. The highest lipid contents (39.66% and 47.62% for sterile and unsterile substrate, respectively) were achieved by incorporation of 60% of DWW. However, the minimal lipid contents (24.3 and 27.30% for sterile and unsterile substrate, respectively) were recorded at 20% of DWW.

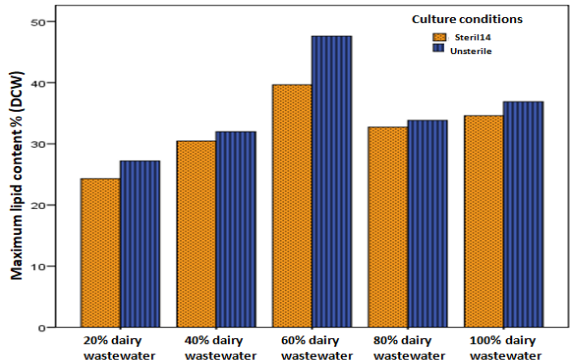

Figure 4. Maximum lipid content in dry biomass produced by *C. subellipsoidea* cultivated in basal medium supplemented with different concentration; 20% (A), 40% (B), 60% (C), and 80 % (D and 100 (E) of sterile/unsterile Dairy WW.

4. Discussion

It is noteworthy that the growth characteristics of *C. subellipsoidea* HSSASE8 as successful, promising commercial microalgae strain in DWW supplemented media under bioreactor patch condition that allows rapid biomass production with high lipid content. The algal growth stimulated and increased as the DWW dilutions (20%, 40%, 80% and 100%, v/v) increased either in sterile or unsterile experiments. Overall, the unsterile treatments exhibited a significant increase in biomass production as compared with sterilized DWW. The growth of *C. subellipsoidea* HSSASE8 microalga was greatly promoted by DWW. The increase of DWW concentration in cultivation media enhanced the lipid content accumulation (Chisti, 2007 and Ian Charles Woertz, 2009) with the tested microalgae grown in DWW and a possible feedstock for biodiesel where they reported that the total lipid content of the algae ranged from 8% to 29% of algal dry mass. A group (Kothari *et al.,* 2013) at California Polytechnic State University worked on algae grown on dairy and municipal wastewater for simultaneous nutrient discharge and lipid production for biofuel feedstock. The research pointed out that the lipid productivity reached 2.8 $g/m^2/d$ and lipid content varied from 4.9%-29% corresponding to 11,000 L/ha/yr (1,200 gallons/acre/year). The relative lipid content on the 10^{th} day (1.6 g) and 15^{th} day (1.2 g) of the batch experiment was noticed to be richer than that reached in BG-11 growth medium on the 10^{th} day (1.27 g) and 15^{th} day (1.0 g).

Results of a previous study (Mahendraperumal *et al*., 2014) indicated clearly a significant reduction in the content of phosphorus (44.36mg/l), ammonias nitrogen (20.73 mg/l) and total organic carbon (3701 mg/l) as compared to raw wastewater. Our results agree with the results showed by Jimenez-Perez *et al*. (2004) in that great TN uptake reached by *S. intermedius* and *Nannochloris sp.*

The increment in overall lipid content under nitrogen limitation can be accepted because the enzymes involved in lipid synthesis are less responsive to a reduction in cellular soluble protein matter than those involved in carbohydrate synthesis (Ian Charles Woertz *et al.,* 2009). They were well declared that, under N-deficient conditions, algal cells often have an excess of carbon metabolites and smaller NP uptake rates of *Chlorella kessleri* developed in artificial wastewater that showed efficiency removal of N and P under high illumination. Distinctly, Nitrogen diminished to 136.5 from 168.1 mg NO_3-N/l in 3 days, while the effectiveness of removed phosphate was hardly 8 - 20% of the original compositions (Lee and Lee, 2001). Nitrogen is an effective macro component of microalgae ranging from 1 to 10% of the growing biomass, and still a key element for improving the lipid content in algal cells (Miao and Wu, 2007). Phosphorus is essential for growth and alternative processes, dealing with energy delivery and biosynthesis of DNA (Ebrahimian *et al*., 2014). The chief process for TN and TP removal is assumed to be biomass uptake. This utilization of nutrients from wastewater demonstrates the potential cost savings when compared to the purchase of fertilizers. The massive uptake of minerals by biomass points out that the biomass junk left behind after oil extraction will have the power as a crop manure.

The microalga cellular content of *C. subellipsoidea* HSSASE8 was significantly increased when inoculated in growth medium with different proportions 20, 40, 60, 80, and 100% of sterile/unsterile DWW within 10 cultivation days. Our results clearly show the increase in total fatty acids in an unsterile treat as twice as in sterile treats. Moreover, our results show the increase in total polysaturated fatty acids in unsterile treatments as twice as in sterile conditions and all detected fatty acids including carbon chain length, branching of the chain, and degree of

unsaturation acids as revealed by GC analyses, The fatty acid composition summarized in the (Table 2 and Figure 5) showed that the highest yield of unsaturated fatty acids (C16-C18) ranged from (68.54-72.54% w/w) at unsterile substrates as compared with (68.27- 71.34%) in sterile conditions. Fatty acid profiles characterized with particular of the high-proportioned palmitic acid (C16:0), behenic acid (C22:0), linolenic acid (C18:3) and linoleic acid (C18:2) were the dominant fatty acids occurring in the accumulated lipids in *C. subellipsoidea* HSSASE8 during growth on sterile and unsterile DWW growth media. In addition in the unsterile conditions, linoleic acid and linolenic polyunsaturated fatty acid (PUFA) was significantly enhanced up to 1.5- 2 folds at the 60% DWW concentration as compared with 20% unsterile experiment. Some earlier review lumped all polyunsaturated fatty acids together as essential fatty acids. Essential fatty acids play an important role in the life and death of cardiac cells (Kaur *et al*., 2014).

The ratio of saturated FA was commonly two folds greater than that of unsaturated one during production on DWW growth medium. This is a suitable fatty acid profile for biodiesel production, since biodiesel gained from saturated oils have a further oxidative stability and fewer NOx emissions. Overall, that the relative fatty acids accumulated in the *C. subellipsoidea* HSSASE8 cells grown in unsterile DWW batches were significantly higher than those grown in sterile DWW treatments may be attributed to the indigenous wastewater bacteria that exhibit a potential role in degradation *of* organic compounds enhancing the growth and biomass productivity rate of *C. subellipsoidea* HSSASE8 cells as compared with sterile condition. Meanwhile, nitrogen limited growth often enhances algal lipid metabolism (Fields *et al*., 2014 and Griffits *et al*., 2012) and the existence of bacteria in algal cultures can improve NH_4-N removal from wastewaters. While algae efficiently pick up NH_4-N, bacteria take part in NH_4-N removal through nitrification (Wang, 2015). The fatty acids recommended as feedstock for high-quality biodiesel were characterized with C16–C18 long-chain groups, as well as a certain degree of unsaturation. As summarized in Table 2, *C. subellipsoidea* HSSASE8 cells grown in unsterile and sterile DWW produced a high proportion of C16-C18 fatty acids (68.54-72.54% w/w). Our results provide a foundation for improving the yield of lipid-based biodiesel production from cultivation of *C. subellipsoidea* HSSASE8 on DWW in the future, in addition to a nutrients removal result in wastewater treatment.

Table 1. Composition of fatty acids (%, w/w) in *C. subellipsoidea* grown on basal medium supplemented with different dilutions of sterile/unsterile DWW.

Fatty acid profile	Growth media condition	Dairy wastewater proportions				
		20% (A)	40% (B)	60% (C)	80% (D)	100%(E)
		Fatty acid composition (%, w/w)				
Lauric acid (C12:0)	Sterile	1.70±8.83	2.38±0.03	2.73±0.03	3.71±0.03	3.5±0.02
	Unsterile	1.77±0.09	3.41±0.02	3.14±1.15	3.82±0.03	3.63±0.04
Myristic acid (C14:0)	Sterile	1.1±0.02	1.27±0.03	2.83±0.04	1.58±0.03	1.55±0.04
	Unsterile	1.19±0.04	1.33±0.03	2.09±0.05	1.62±0.03	1.64±0.04
Palmitic acid (C16:0)	Sterile	5.4±0.35	8.77±0.05	16.87±0.08	9.53±0.12	12.42±0.08
	Unsterile	5.72±0.02	8.91±0.04	18.46±0.04	10.4±0.03	13.49±0.1
hexadecatrienoic acid (C16:3)	Sterile	0.73±0.02	1.23±0.15	2.2±0.05	1.79±0.03	1.66±0.01
	Unsterile	0.82±0.04	1.24±0.04	3.45±0.03	1.92±0.02	1.74±0.02
Stearic acid (C18:0)	Sterile	3.95±0.07	5.3±0.06	7.69±0.06	6.69±0.03	6.82±0.03
	Unsterile	4.33±0.04	5.60±0.03	9.15±0.06	6.92±0.03	7.22±0.04
Oleic acid (C18:1)	Sterile	2.28±0.06	2.86±0.06	4.94±0.09	3.2±0.07	3.44±0.07
	Unsterile	2.42±0.04	3.15±0.04	5.31±0.03	3.37±0.03	3.6±0.03
Linoleic acid (C18:2)	Sterile	5.28±0.05	5.92±0.08	8.57±0.15	6.27±0.14	6.14±0.06
	Unsterile	5.47±0.05	6.20±0.03	8.71±0.04	6.50±0.02	6.41±0.05
Linolenic acid (C18:3)	Sterile	5.96±0.08	8.43±0.04	12.99±0.11	8.63±0.07	9.67±0.03
	Unsterile	6.23±0.11	8.63±0.03	13.43±0.04	9.75±0.05	10.47±0.06
Arachidic acid (C20:0)	Sterile	0.42±0.03	0.71±0.03	2.1±0.04	1.58±0.02	1.52±0.03
	Unsterile	0.69±0.02	0.84±0.02	2.31±0.03	1.75±0.03	1.65±0.03
Behenic acid (C22:0)	Sterile	7.64±0.03	8.2±0.06	14.24±0.09	9.60±0.03	12.09±0.13
	Unsterile	7.82±0.04	9.44±0.02	14.61±0.07	10.2±0.03	12.49±0.02

Data were shown by mean values ± standard deviation of three individual repetitions.

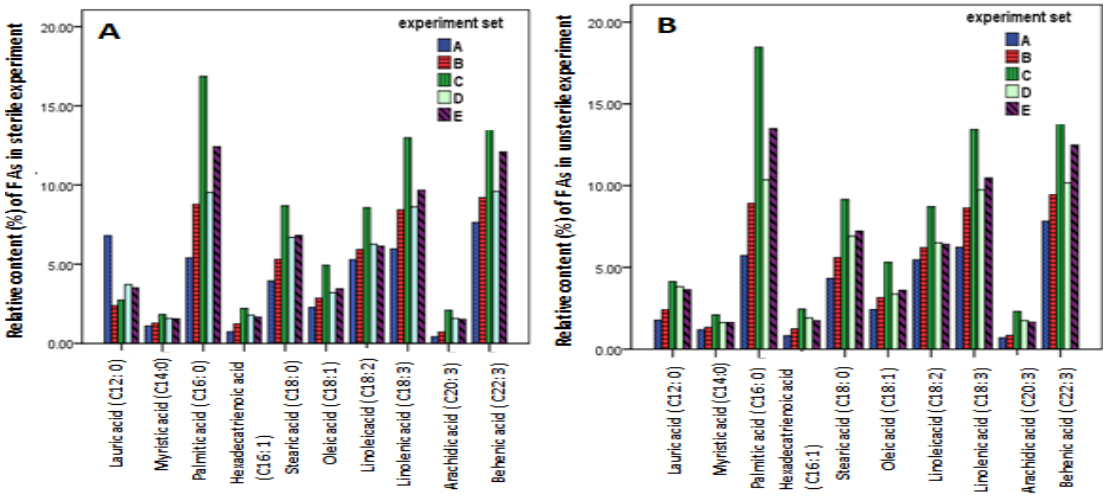

Figure 5. Fatty acid profile of lipids produced by C. subellipsoidea cultivated in basal medium supplemented with different concentration; 20% (A), 40% (B), 60% (C), 80% (D and 100 (E) of sterile/unsterile Dairy WW.

5. Conclusion

The study suggests that *Coccomyxa subellipsoidea* HSSASE8 cells, in particular, may show adequate growth in components of dairy wastewater, such as cheese whey, as the limiting nitrogen source, resulting in cost-effectual and feasible alternative commercial mediums for biomass production, without requiring expensive carbon sources in cultivation medium. In addition, the close elimination of TN, TP, and (94.58%) of TOC by C. subellipsoidea HSSASE8 with the support of DWW operation offers a reasonable solution of waste control. The gas chromatography evaluation proved that the FA analysis is advantageous when concluding the fitness of algal lipids for applications as biodiesel or nutritional demands. *Coccomyxa subellipsoidea* HSSASE8 oils have a high ratio of 16 -18 carbon chained fatty acids as reasonable biodiesel candidates (Lin and Lin, 2012). Moreover, in this study, a significant build- up in neutral lipid composition and conversion of fatty acid distribution with a powerful improvement in the magnitude of essential polyunsaturated fatty acids were obtained. Future research should concentrate on bioengineering of *Coccomyxa subellipsoidea* HSSASE8 for high lipid accumulation because it would be one of the highly promising ways to meet the energy demand using microalgae as a feedstock for third generation biofuel.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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