# Chapter -05

# Discussion

## 5.1. Physico-chemical Analysis of Dairy Effluent:

Due to over increasing demands of milk products, dairy industries will continue to develop to fulfill the enhanced demand and hence, discharge huge volumes of Waste water. In dairy sector, approx. 0.2 -10 litre of effluent produces, in the preparation of 01 litre of milk or milk-products.[41] Dairy effluent having extremely high values of oxygen demanding waste, milk solids, pathogenic microorganisms, carbohydrates (lactose), oil and grease, phosphorus, nitrogen, sanitizers, cleaning agents and emulsifiers. Depending on the processing of different milk products, components of dairy wastewater vary accordingly. pH ranges between 4.0 –11.0, biochemical oxygen demand and COD ranges between 50–45,000 mg/L, and 90–95,000 mg/L, respectively.[17, 163] The amount of total phosphorus and nitrogen concentrations ranges between 9–280 mg/L and 14–830 mg/L respectively. The amount of inorganic salts (minerals) and heavy metals like; Ca, K, Mg, Na, Cl, Co, Fe, Mn, Zn, Ni and Mo can be considerably elevated in dairy effluents.[7] Generally, dairy effluents containing high concentration of lipids such as triacylgycerides, phospholipids, unsaturated and saturated fatty acids, carbohydrates like; lactose, glucose and galactose etc. Its high biological oxygen demands deplete the dissolved oxygen content and create anaerobic conditions very rapidly under the aquatic system.[4] Dairy effluent contains various inorganic and organic compounds of N and P, available in higher concentration and responsible for the alkalinity of the dairy waste and other than it also contains trace amount of some mono-valent and bi-valent cations like Na, Mg, Ca, Fe, K, Ni, Mn etc. Use of detergents and chlorine in the dairy industry further deteriorates the quality of effluent.

Developing countries such as India have immense potential for producing huge number of industrial effluents. According to the report, annually Indian dairy sector generate approx. 72.65 billion gallons of waste water.[164] Hence, these effluents are biologically active because of the presence of rich organic load the proper treatment before the disposal of dairy waste effluent has become the major problem for the vitality of the dairy industry and environment. Hence, the integrated sustainable proposal could be originated for the generation of microalgal biomass and waste remediation. Dairy effluent contains all the required nutrient for microalgae cultivation such as protein, fats, lactose, inorganic salts and metals. Apart from these, DE also having various types of N (Organic-N, nitrite, nitrates, and ammonium) and P as ortho, meta and para phosphate), which makes them most appropriate for microalgae culture.[9, 164, 165] The concentration of dairy effluent probably varied according the procedure and production of various milk products. The COD (chemical oxygen demand) is an indirect estimation of all types of organic and inorganic number of pollutants available in dairy effluent. The organic carbon (OC) from DWW enhanced the heterotrophic mode of cultivation in microalgae culture. Many researchers suggested that heterotrophic cultivation enhanced the microalgae biomass, lipid productivity and nutrients removal capacity.[166]

Dairy effluent, utilized in this study, was analyzed for its physico-chemical characteristic features, these were as followed; the pH was slightly acidic i.e. (6.4 ± 0.2), in nature. While, the BOD and COD values were 1278 ± 56.55mg $L^{-1}$ and 2398 ± 102.52 mg $L^{-1}$ individually. The values of TP and TN was 88.52 ± 1.98 and 95.60 ± 2.45 mg $L^{-1}$, individually. Along with, it also had different metals like; Na, Mg, Ca, K, Mn, Fe, Ni, and Zn, in minor quantities, which required for microalgae proper development and expansion. Many other previous studies were also found the similar properties of dairy waste water. Panday et al. 2019, estimated higher Chemical oxygen demand, it was being (3800 ± 400 mg $L^{-1}$), while the values of nitrate, TP and ortho-Phosphate were (55.5 ± 0.11 mg $L^{-1}$), (46.1 ± 0.27 mg $L^{-1}$), and (25.63 ± 2.89 mg $L^{-1}$) individually, in RDE (raw dairy effluent). While in SDE (simulated dairy effluent) COD was 4000± 112 mg $L^{-1}$, nitrate 108.49±2.36 mg $L^{-1}$, total phosphorus 82.88±1.89 mg $L^{-1}$, and ortho-phosphate was 56.28±1.17 mg $L^{-1}$.[9] In another experimental work, the level of COD was evaluated very high 19,607 mg $L^{-1}$. Whereas, phosphorus was found 108 mg $L^{-1}$ mg $L^{-1}$. TN and protein nitrogen concentrations were 593 mg $L^{-1}$, and 3785 mg $L^{-1}$ individually in D1 cycle (first reuse of DWW).[3] In another research Khemka and Saraf, 2017 also found the similar physicochemical parameters in dairy waste water. they observed COD level was 2470 mg $L^{-1}$, which was almost similar to the present study. All forms of nitrogen concentration, including (total nitrogen, $NO_3^-$ N, $NO_2^-$ N) were found (160 mg $L^{-1}$, - 94.3 mg $L^{-1}$, - 48.3 mg $L^{-1}$respectively). Whereas, the concentration of phosphorus and total phosphate were 120.6 mg $L^{-1}$, and 183.4 mg $L^{-1}$. [165] Chokshi et al. 2016, observed in an experimental study, the levels of COD, nitrite, ammonical nitrogen, which were 2493.33 mg $L^{-1}$, 0.95 mg $L^{-1}$, and 277.40 mg $L^{-1}$ respectively. While the phosphorus level was 5.96 mg $L^{-1}$, in dairy industry waste water.[164] Lu et al. 2015 also evaluated the physico chemical properties of dairy industry discharge. They documented indistinguishable results like the previous ones. The COD, TKN (total kjeldahl nitrogen), ammonical nitrogen concentrations were 2593.00 mg $L^{-1}$, 283.00 mg $L^{-1}$, 181.50 mg $L^{-1}$, individually. While, the amount of total phosphorus was 115.90 mg $L^{-1}$.[166] Similarly, Shivsharan et al. 2013, estimated higher BOD and COD values i.e. 1448.00 mg $L^{-1}$and 672.5 mg $L^{-1}$respectively in dairy industry wastewater.[167] In another research, Amini et al. 2013, reported higher BOD and COD levels were 13054 $gL^{-1}$ and 11000 $gL^{-1}$in dairy industrial waste water used in this work. While the observed value of nitrogen in terms of TKN and $NO_3^+$ were 69.32 $gL^{-1}$ and 6.62 $gL^{-1}$ respectively. The phosphorus concentration was measured 13.12 $gL^{-1}$ .[168]

## 5.2. Growth of Potential Microalgae Isolates in DWW:

Screening is an essential procedure of bio-process expansion to acquire a single species. Isolation of microalgae consider the first move in the selection of potential microalgae for the generation of valuable lipids and phyco-remediation of dairy effluent. However, autochthonous microalgae can be efficiently utilized for nutrient removal from dairy effluent, and production of valuable lipids. Typically, dairy effluent needs some pre-treatment process; such as pH adjustment, addition of metals, different dilutions, and supply the external carbon source etc. before the inoculation of microalgae.[166] Many researchers have been conducted several experiments to estimate the microalgal biomass and lipid yield using dairy effluent as nutrient source. Daneshvar et al. 2019, concluded that *scenedesmus quadricauda* achieved 0.43 $gL^{-1}$ microalgae biomass when cultivated in dairy effluent for 12 days.[7] In another study, it was estimated that maximum microalgae biomass was 1.22 $gL^{-1}$ obtained by a native isolated microalgae strain (*Scenedesmus sp.*) after 12 days of batch culture in raw dairy waste water.[9] Similarly, Kumar et al. 2019, documented that *Ascloris sp.* was able to attain 2.23 $gL^{-1}$ biomass, when cultivated in dairy effluent up to 11 days.[169] Chokshi et al. 2016, observed, increased biomass and cell number in an experimental study. They found that biomass yield of *Acutodesmus dimorphous* was 0.84 $gL^{-1}$ while, utilizing RDW after $4^{th}$ day of cultivation, which was 5-6 times more, when compared to BG-11 cultivation media (0.149 $gL^{-1}$). Likewise, the microalgal cell count of *Acutodesmus dimorphous* (from $0.47x10^7$ to $3.48x10^7$ cells $mL^{-1}$) was also estimated which was approximately 7 times increase, after 4 days of culture in raw dairy waste.[164] In another research it was observed that microalgae culture was easily adapted in raw dairy wastewater and produced optimum biomass production (0.86 $gL^{-1}$) in 20 % dairy waste water, in 8 days, under laboratory conditions.[170] Lu et al. 2015, observed that *Chlorella* sp. was flourished in unsterilized dairy effluent in open pond culture and attained biomass yield of 110.0 $mgL^{-1}d^{-1}$. When *Chlorella* sp. shifted in indoor lab conditions to raw DWW, optimum biomass yield was 2.25 $gL^{1}$ and 3.05 $gL^{-1}$ by using 0.17 $gL^{-1}$ and 0.34 $gL^{-1}$ initial concentrations respectively, after eight days of cultivation. Additionally, reported that initial inoculum amount has also influenced the microalgae biomass.[166] Qin et al. 2014, conducted an experiment on *C. vulgaris* in raw and UV treated dairy effluent. They observed the biomass yield ranged from 0.861-1.870 $gL^{-1}$.[171] Kothari et al. 2012, demonstrated that *Chlorella pyrenoidosa* NCIM 2738 had shown greater biomass in 75 % dilution of the dairy effluent, when compare with other concentrations.[172] The microalgae cell no. of *Chlorella zofingiensis* was also enhanced (from $3.0 \times 10^6$ to $11.0 \times 10^6$ cells $mL^{-1}$) at the end of five days' batch culture, which was four-fold increments in raw dairy waste water.[173]

In the present work, microalgae isolates were examined to determine their potency towards nutrient remediation and biomass production using dairy wastewater as a culture medium. From 10 autochthonous microalgae isolates, best performing 02 microalgal strains namely (DDW06 and DDW8) were selected. These both were efficiently acclimatized in dairy effluent. Isolate microalgae were produced higher biomass and lipid yield in 75% concentration of dairy waste water, in 15 days of batch culture. The DWW08 microalgae isolate was achieved the optimum biomass or DCW (dry cell weight) $1.478 \pm 0.003$g $L^{-1}$ after 15 days of batch culture by using an inoculum size of 0.072 g $L^{-1}$, While DWW06 was able to attain $1.385 \pm 0.002$ g $L^{-1}$ from the inoculum 0.064 g $L^{-1}$ in 75% conc. (75 ml DW+25 ml Bold's Basal media). These findings were quite similar with the previously reported biomass yield of microalgae grown in dairy waste water. Hence, autochthonous microalgae culture efficiently utilized nutrients present in effluent, and generated expanded biomass.[9, 60]

## 5. 3. Phycoremediation of Dairy Effluent:

The nutrient reduction efficiency directly depends on the metabolism of microalgae isolates, the nutrient's availability and abiotic and biotic environmental factors in DWW. It was supposed that microalgae biomass production directly proportional to the assimilation of nutrients, available in the DWW. If the nutrients are assimilated quickly, biomass enhanced consecutively. Chemical oxygen demand is the indirect estimation of all types of inorganic and organic pollutants available in effluents. Microalgae are popularly known to consuming various forms of organic and inorganic pollutants from dairy waste water. Daneshvar et al. 2019 reported that *scenedesmus quadricauda* removed 76.77 % COD level, when cultured in dairy effluent for 12 days.[7] Similarly, Kumar et al. 2019, documented that *Ascloris sp.* was able to reduce COD concentration up to 95.10 %, when cultivated in dairy effluent after 11 days of batch culture.[169] Panday et al. 2019, observed the maximum COD reduction range varied between 84.92 % to 92.92 % (from 4000.00 $mgL^{-1}$ to 323.88 $mgL^{-}$1) with isolated six microalgae strains by using dairy effluent as culture media.[9] Chokshi et al. 2016, observed that *Acutodemsus dimorphus* cultured in raw dairy waste water and removed 91.71 % of chemical oxygen demand from 2593.33 to 215.00 $mgL^{-1}$ in four days of batch cultivation with reduction rate, 579.5 $mgL^{-1}d^{-1}$. At the end of 4 days, no further change in COD concentration.[164] Tsolcha et al. 2016 documented 92.3±2.0 % of chemical oxygen demand removal after 7 days' culture of green microalgae *Choricystis* sp. in dairy effluent.[174] In another study, it was observed that the percentage of chemical oxygen demand reduction (54.82 %) and removal rate of 41.31 $mgL^{-1}d^{-1}$ with *Chlorella sp*. cultivated in 25% unsterilized dairy effluent in open pond conditions.

Besides this, they also observed, COD removal percentage and COD reduction rate, in controlled indoor conditions and estimated that 83.33% and 1.25 $mgL^{-1}d^{-1}$ chemical oxygen demand removal percentage and reduction rate, respectively when *Chlorella* sp. cultured in 25% unsterilized dairy effluent. They concluded that open pond culture analogously less proficient than indoor culture.[166] One another research also resulted, about 90.0% removal of COD after eight days of batch cultivation in raw dairy effluent.[170] In another research, it was examined that 93 % reduction (from 984 to 60 $mgL^{-1}$) of chemical oxygen demand and 82 % removal (from 37 to 7 $mgL^{-1}$) of biological oxygen demand levels, at the end of twelfth and fifteenth days of batch culture, of *Chloroccum* sp. in dairy effluent, respectively. It also reported that 73 % of chemical oxygen demand removal within initial 3 days of batch culture.[41] The slow removal of chemical oxygen demand in later could be because of the availability of remaining organic carbon (slowest biodegradable components). In the present research, both the potential microalgae isolates, DWW06 and DWW08 were showed almost similar COD removal efficiency i.e. 88.84% and 86.45% of COD from (from 2388 $mgL^{-1}$ to 324.92 $mgL^{-1}$ and 267.61 $mgL^{-1}$) respectively by DWW06 and DWW08, after 15 days of cultivation, in 75% DWW+25 % BBM. Extreme reduction in the COD concentration suggested that both microalgal strains effectively metabolize and utilized the organic contents available in culture media for their growth and development.

Microalgae isolates were effectively eradicating all types of nitrogen; organic and inorganic (nitrate, nitrite, urea and ammonium) from dairy effluent.[175] In microalgae culture, absorption of $NO_3^-$ occurs through the following ways; as nitrate transported into the microalgal cell's cytoplasm, here an enzyme, nitrate reductase converting this nitrate to nitrite, which later entered into the chloroplast, where it reduces into ammonium by an enzyme, nitrite reductase.[176] Then, it is assimilated into carbon chain by synthesizing glutamate, via the glutamine synthase cycle.[18] Hence, for microalgae growth and development, ammonical nitrogen has been suggested the most preferring form of nitrogen available in wastewater, because of it's simple metabolism which needs less energy for its assimilation. Several previous studies represented the effective eradication of nitrogen from dairy waste water by using microalgae culture.

Panday et al. 2019 reported, 100% $NO_3^{-1}$ removal (from 108.49 mgL$^{-}$1to 0.00 mgL$^{-}$1) by ASK14 strain (native isolate) by the end of day 12 cultivation in dairy effluent.[9] Daneshvar et al. 2019, reported that *scenedesmus quadricauda* removed 92.15 % nitrate reduction, when grown in dairy effluent for 12 days.[7] While in another research, Kumar et al. 2019 reported that *Ascloris sp.* was capable to reduce nitrate concentration 79.10 %, when cultivated in dairy effluent after 11 days of batch culture.[169] Khemka and Saraf, 2017 also observed that nitrogen reduction 66.25 % (from 160±2.06 to 54±1.4 mgL$^{-1}$) by *Desertifilumtharense* at the end of 15 days' cultivation in DWW. whereas the nitrogen reduction rate was observed that the highest (6.58 mgL$^{-1}$d$^{-1}$) in 25 % unsterilized dairy effluent and 3.21 and 2.51-times increased from 5 % and 10 % unstrelized dairy effluent, respectively.[165] Chokshi et al. 2016 concluded, *Acutodesmus dimorphus* was consumed $NH_4^+$-N (ammonical nitrate) (277.4 mgL$^{-1}$) and phosphate (5.96 mgL$^{-1}$) from simulated dairy waste.[164] In an experimental study, *Acutodesmus dimorphous* cultured in unsterilized DWW. This strain was successfully removed (100 %) nitrite (from 0.95 to 0.00 mg L$^{-1}$) while, $NH_4^+$-N (from 277.40 to 0.00 mg L$^{-1}$) in microalgae cultivation after 4 and 6 days respectively.[164] In another study represented complete eradication of total Kjeldahl nitrogen (TKN) by using integrated culture (bacteria and microalgae) in 35 % dairy effluent, after 7 days. The bacterial and microalgae consortium dominated by *Choricystis sp*.[174] In another research, it was reported that microalgae consortium was capable to reduce 83.20 % (from 73.8 to 12.4 mg L$^{-1}$) of ammonium nitrogen after eight days of cultivation, by using 20 % DWW.[170] In other experimental work, it was observed that total phyco-remediation of ammonical nitrogen after 6 day's culture of *Chlorella* species, in 75 % concentration of dairy effluent. While TN removal was reported varied from 83.38 to 85.17 % after eight days of batch cultivation of *Chlorella* strain, with different dilutions (5-25%) unsterilized dairy effluent.[166] An inclusive research demonstrated that the consumption of TN by *N. salina* at the end of 10 days' cultivation at 3 % and 6 % dairy effluent dilutions.[177] Yadavalli and Heggers 2013, also reported total reduction of $NH_3$-N from dairy effluent by employing immobilized *Chlorella pyrenoidosa*.[178] In another study, the nitrate eradication by cultivated *Chlorella pyrenoidosa* in untreated and pre-treated dairy effluent were 87% in raw dairy waste, whereas, 83% in the pre-treated dairy waste.[172] Both the isolated microalgae DWW06 and DWW08 successfully removed 87.45% 85.90 % of total nitrate from (from 95.60 mgL$^{-}$1 to 14.43 and 11.99 mgL$^{-}$1) respectively by the end of day 15 cultivation in 75% concentration of dairy effluent.

The phosphorus is an essential element for energy transfer (ATPs, NADPHs etc.), plasma membrane synthesis and nucleic acid (DNA, & RNA) formation. Phosphate ($PO_4^{-3}$) has been available in enormous amounts in raw dairy effluent. In another research also reported that 97.7% phosphate removal by using *Scenedesmus sp*.in dairy effluent after 11 days of cultivation.[38] Another group of researchers found 51.84% reduction in phosphate concentration by using *Chlorella vulgaris* in dairy effluent cultivation medium.[179] While the assimilation of $PO_4^{-3}$ has been observed to be moderate, when compared with $NO_3^{-1}$. Although, it has been 100 % removed (from 5.96 to 0.00 mgL$^{-1}$) by *Acutodesmus dimorphous* after eight days of batch cultivation, in raw dairy effluent.[163] Likewise, Daneshvar et al. 2019 also reported that *scenedesmus quadricauda* showed 100 % phosphate consumption, when grown in dairy effluent for 12 days.[7] Panday et al. 2019 reported, 95.81 % $PO_4^{-3}$ removal (from 82.88 mgL$^{-}$1to 3.47 mgL$^{-}$1) by ASK25 strain (native isolate) by the end of day 12 cultivation in dairy effluent.[9] While in another research, Kumar et al. 2019 reported that *Ascloris sp.* was capable to reduce phosphate concentration 98.10 %, when cultivated in dairy effluent after 11 days of batch culture.[169] Khemka and Saraf 2017, while working on *Desertifilum tharense* MSAK01 effectively reduced 56.5 % of total phosphate (from 183.4 ± 1.14 to 79.7 ± 0.3 $mgL^{-1}$) in 15 days of culture in dairy effluent.[165] In another study, it was reported that 88.20 to 100 % eradication of $PO_4^{-3}$ by microalgal consortium leaded by chlorophyte, *Choricystis* strain at the end of 7 day's batch culture with different concentrations of secondary cheese whey wastewater (SCWW).[174] In another experimental work it was documented that 99 % reduction of total phosphate at the end of 10-day's batch culture of *N. salina* in 3 % and 6 % dilutions of dairy waste water.[177] The optimum phosphorus eradication had based on nitrogen/phosphorus ratio, which perhaps, the capacity of *N. salina* to consume maximum phosphorous. Yadavalli and Heggers 2013 also observed 98 % of phosphate reduction within 96 h by immobilized cells of *Chlorella pyrenoidosa* employing dairy effluent as cultivation medium.[178] Similarly, Kothari et al. 2012 also observed that 87 % of $PO_4^{-3}$ reduction by *Chlorella pyrenoidosa* NCIM 2738 after 10 days of batch cultivation in dairy effluent.[172] In the present study the phosphorus consumption was observed 86.44% and 88.90% (from 88.52 $mgL^{-1}$ to11.99 and 9.77 $mgL^{-}$1) by both strains DWW06 and DWW08 respectively.

Many previous studies provide the moreover similar evidence which support the present study in nutrient removal from dairy waste water. Table no 5.1. showing the comparative nutrient eradication from dairy waste water by microalgae culture.

**Table no 5.1. Cultivation of microalgae in DWW and nutrient reduction percentages (COD, $NO_3^-$ and $PO_4^{3-}$)**

| Microalgae | Biomass (g/l) | Time (days) | COD removal % | Nitrate removal % | Phosphate removal % | Reference |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. Protothecoides* | 3.30 | 10 | 65.00 | 43.00 | 77.00 | Gramegna et al, 2020 |
| *Scenedesmus sp.* | 1.75 | 11 | 89.30 | 88.41 | 97.07 | Mercedo et al, 2020 |
| *C.reinhardtii* | 1.70 | 10 | 76.00 | 65.00 | 87.00 | Gramegna et al, 2020 |
| *Chlorella vulgaris* | _ | _ | _ | 57.01 | 51.84 | Kalaji et al, 2020 |
| *Scenedesmus sp.* | 1.22 | 12 | 90.50 | 100 | 91.24 | Panday et al, 2019 |
| *Scenedesmus quadricauda* | 0.43 | 12 | 76.77 | 92.15 | 100 | Daneshvar et al, 2019 |
| *Ascocloris sp.* | 2.23 | 11 | 95.10 | 79.10 | 98.10 | Kumar et al, 2019 |
| *Desertifilum tharense* MSAK01 | 0.8-1.11 | | 94 | 98 | 56 | Khemka & Saraf, 2017 |
| *Acutodesmus dimorphus* | 0.840 | 04 | 90.1 | 100.0 | 100.0 | Choskshi et al. 2016 |
| *Chlorella vulgaris* | 1.23 | | 80.62 | 85.47 | 65.96 | Choi, 2016 |
| Microalgal consortium *(Chlorella sp./ C. zofingiensis/ Scenedesmus sp.)* | 4.72- 5.41 | | 57.01- 62.86 | 87.04- 91.02 | 91.16- 95.96 | Qin et al., 2016 |
| *Chlorella sp.* | 3.05 | 08 | 81.68±0.54 | 81.57± 2.29 | 99.73±0.38 | Lu et al. 2015 |
| *Microalga consortium* | | | 91 | 82 | 99.7 | Tsolcha et al. 2015 |
| *Chlorococcum sp. RAP 13* | 1.94 | 15 | 93 | - | - | Ummalyma& Sukumaran 2014 |
| *Clamydomonas polypyrenoideum* | | 10 | 50 | 90 | 70 | Kothari et el. 2013 |
| *Chlorella pyrenoidosa* | | 04 | 84.55 | 98.29 | 97.1 | Yadavalli & Heggers, 2013 |
| *Chlorella pyrenoidosa* | 6.8 | 11 | - | 60 | 87 | Kothari et el. 2012 |
| *Chlorella vulgaris* | 0.69 | 14 | 86 | 97 | 96 | Feng et al. 2011 |
| *Poterioochromonas Melhamensis* UPMC A0073 | 1.478 | 15 | 88.84 | 87.45 | 88.96 | In this study |

| | | | | | | |
|---|---|---|---|---|---|---|
| Botryococcus strain (Accession no. KF746941.1.) | 1.385 | 15 | 86.45 | 84.96 | 86.44 | In this study |

## 5.4 Lipid Productivity of Selected Microalgae Isolates:

Nutrient removal and generation of biomass, concurrently occurring in DWW. Microalgae biomass or dry cell weight and lipid synthesis were highly enhanced with the reduction of pollutants in the dairy effluent. Lipid production in microalgae is the limiting factor to decide their potentiality. various researches have been conducted to evaluate biomass of microalgae for lipid generation utilizing dairy effluent as cultivation media. The previous reports suggested that microalgae flourished in different dilutions of dairy effluent have extremely greater lipid yield when differentiate with other cultivation medim. *Tetraselmis sp.* capable to accumulated 51.65 % (wt.) lipid content grown in dairy effluent at the end of 10 day's batch culture.[1] *Scenedesmus sp.* successfully accumulated 51.00 % (wt.) LC with 507 $mgL^{-1}d^{-1}$ LP, when cultured in dairy effluent after 11 days of batch culture.[39] *A. protothecoides* and *C. reinhardtii* both microalgae stored 18.50 and 12.00 % (wt.) lipid content with 592 and 204 $mgL^{-1}d^{-1}$ lipid productivity respectively, cultured in dairy effluent after 10 days' cultivation.[3] *Scenedesmus sp.* stored 30.70 % (wt.) LC with 31.16 $mgL^{-1}d^{-1}$ LP, cultured in raw dairy waste water after 12 days of batch culture.[9] *Ascocloris sp.* ADW007accumulated 34.98 % (wt.) LC with 207 $mgL^{-1}d^{-1}$ LP cultured in dairy waste water, after 11 days of batch cultivation.[169] *Chlorella sp.* stored 23.00 % (wt.) LC with 107.83 $mgL^{-1}d^{-1}$ LP, when using dairy effluent as growth medium after 12 days of cultivation.[20] *Arthrospira sp.* able to stored 30.45 % (wt.) lipid content grown in DWW after 11 days of batch culture.[180] *Acutodesmus dimorphous* stored 25.05 % (wt.) LC with 50 $mgL^{-1}d^{-1}$ LP, in dairy effluent and also reported that 1.2-fold greater when compared with BG11 growth medium (21.08 % wt.) at the end of 8 day's culture.[164] It was also documented that microalgal consortium accumulated 19 to 21 % (wt.) lipid at the end of 8 day's culture, grown in DE.[21] In other studies, reported that *Choricystis* sp. dominated microalgal-bacteria culture stored optimum lipid content 13.4±2.0 % wt. when cultured in 17 % SCWW (secondary cheese whey wastewater).[174] In another experimental work, *Chlorella sorokiniana* was accumulated 11-52 $mgL^{-1}d^{-1}$ LP, when cultivated in raw dairy farm effluent.[181] *Chloroccum* sp. stored 31 % lipid content when cultivated in raw DWW mixotrophically, while in heterotrophically, stored 39 % and 42 % lipid when cultivated with 4 % and 6 % of glycerol (exterior carbon resource) supply, respectively.[41] It was suggested that external carbon source supply to affect the microalgae growth positively in heterotrophic conditions of microalgal culture. It is the economic range of carbon resource mostly biofuel industry waste glycerol is utilized as a carbon source.[41] Cai et al. 2013 observed that *Nanochloropsis salina* accumulated 35 % (wt.) lipid content when cultivated in 3% effluent supplied amount and it was also reported that with enhanced supplied rate (from 3 to 24 %) resulted lowering the LP from 29.2 $mgL^{-1}d^{-1}$ to 14 $mgL^{-1}d^{-1}$.[177] Kothari et al. 2013a also observed that *Chlamydomonas polypyrenoideum* stored 42% (wt) lipid content when grown in dairy waste.[42] In the present work, the DWW06 showed lipid content 29.80 ± 0.002 % (w/w) and 26.22 ± 0.004 mg $L^{-1}$ $d^{-1}$ LP in 75 % dairy waste water. The lipid content in other conc. of DWW were followed by 50% dairy waste (27.70 ± 0.003% w/w), 100% DWW (26.88 ± 0.003 % w/w), 25% DWW (24.35 ± 0.002 % w/w) and control (Bold's basal media) (21.45 ± 0.002 % w/w). While, the DWW08 microalgae sp. was accumulated the higher amount of lipid content i.e., 31.60 ± 0.003 % (w/w) with 29.61 ± 0.003 mg $L^{-1}$ $d^{-1}$ LP in 75% concentration of dairy waste water. Which were higher lipid content among these DWW concentrations, followed by 100% dairy wastewater (28.46 ± 0.003 % w/w), 50% dairy waste water (26.50 ± 0.002 % w/w), control (BBM) (23.45 ± 0.004 % w/w), and 25% dairy waste water (22.60 ± 0.004 % w/w).

## 5.5 Identification of Isolated Microalgae Strains from Dairy waste water:

The DWW06, microalga was identified as *Botryococcus* sp. as the next closest homologue was found to be *Botryococcus sp.* PK (a chlorophyte). After that, amplification of 18S rDNA segment from the isolated microalgae sp., had done with universal primers. These amplified sequence were deposited to NCBI database. BLAST hits sequences indicated of 96.36% identity with *Botryococcus sp*. having accession no. of KF746941.1. While, DWW08, autochthonous microalge sp. was identified as *Poterioochromonas* strain, (golden brown microalgae). After, the amplification with universal primers, the amplicons of *Poterioochromonas sp.*, were sequenced and deposited to NCBI database. Afterwards, BLAST hits sequences designated (97.30%) homology with *Poterioochromonas malhamensis* species (UPMC-A0073), Sequence id: MK834582. Whereas, this strain represented the next nearest similarity with *Achnanthidium saprophilum* with Sequence id: MN592667.1

## 5.6. Lipid profiling through GCMS:

The neutral lipids and hydrocarbons produced by both (DWW06 and DWW08) the autochthonous microalgae strains, were analyzed by GCMS technique. Major fatty acids, produced by DWW06 were (C16:1, C16:0, C18:1, C18:2, C18:0 and C20:0) found along with (C4:0, C6:0, C8:0, C12:1, C14:1 and C14:0) and other lipids with a lesser amount. The saturated lipid content of DWW06 was 29.27 %, while, the unsaturated lipid content was 66.85%, and 3.88 % other form of hydrocarbons. Whereas, the major lipids, produced by DWW08 were (C16:0, C16:1, C18:1, and C18:0) together with (C14:0, C20:1) and other lipids in lesser amount. The saturated lipid content was 26.83%, the unsaturated lipid content was 72.25%, and 0.99% other hydrocabons. The lipids of

C16 to C18, were the main lipids whereas, the lipids of C14 and C20 were obtained with fewer quantity in both strains.

**Table no 5. 2.Comparison of fatty acids lipid content of various microalgal sp. grown in DWW:**

| ***Microalgal sp.*** | **Lipid %** | **Lipid production(mg/l/d)** | **Fatty acid composition%** | **Reference** |
|---|---|---|---|---|
| *Scenedesmus sp.* | 51.00 | 507.00 | C16:0 18.79<br>C18:2 20.05<br>C18:3 23.79 | Mercedo, et al, (2020) |
| *Tetraselmis sp.* | 51.65 | _ | C16:0 20.55<br>C16:1 10.45<br>C18:0 7.72<br>C18:1 13.95 | Swain et al, et al, (2020) |
| *A. protothecoides*<br>*C.reinhardtii* | 18.50<br>12.00 | 592.00<br>204.30 | | Gramegna et al, (2020) |
| *Ascocloris sp.* ADW007 | 34.98 | 207 | C16:0 6.0<br>C18:0 11.5<br>C18:2 5.30<br>C18:3 9.80<br>C20: 1 3.10<br>C22:6 6.60 | Kumar et al, (2019) |
| *Scenedesmus sp.* | 30.70 | | C16:0 29.23<br>C18:0 13.05<br>C18:1 46.20<br>C18:3 9.23 | Panday et al, (2019) |
| *Arthrospira platensis* | 30.45 | | | Hena et al,<br>(2018) |

| | | | | |
|---|---|---|---|---|
| *Chlorella sp.* | 23.00 | 107.83 | C16:0 8.44<br>C18:0 39.49<br>C18:1 27.54<br>C20:0 10.93 | Choi et al, (2018) |
| *Poterioochromonas Melhamensis* UPMC A0073 | 31.60 ± 0.003 | 29.61± 0.003 | C16:1 38.17<br>C16:0 6.46<br>C18:0 15.22<br>C18:1 15.71<br>C18:2 6.04 | In this study (Dhillon et al, (2023) |
| *Botryococcus sp*. accession no. KF746941.1. | 29.80 ± 0.002 | 26.22 ± 0.004 | C16:1 30.17<br>C16:0 10.46<br>C18:0 8.29<br>C18:1 14.51<br>C18:2 12.04<br>C 20:0 4.66 | In this study |