Chapter -04

Results

4.1. Physico-Chemical Analysis of DWW:

The DWW which utilized in this present study, was analyised for its physico-chemical characteristic features which expressed in Table 4.1. The pH of DWW was found 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} respectively. The TP and TN values were 88.52 ± 1.98 and 95.60 ± 2.45 mg L^{-1}, respectively. Along these, it also consisted numerous minerals like; Na, Mg, Ca, K, Fe, Ni, Zn, and Mn, in minute quantity, which required for microalgae development and expansion.

Table no. 4.1 Physico-Chemical Analysis of Dairy Effluent

S.No.	Parameters	Dairy Effluent (Untreated)	Unit
1.	P^H	6.4 ± 0.02	-
2.	Colour	Milky White	-
3.	BOD	1278 ± 56.55	mg/L
4.	TS	1890 ± 34.41	mg/L
5.	TDS	1250.5 ± 24.81	mg/L
6.	TSS	639.5 ± 20.30	mg/L
7.	COD	2398 ± 102.52	mg/L
8.	Total Phosphate	88.52 ± 1.98	mg/L
9.	Total Nitrate	95.60 ± 2.45	mg/L
10.	Oil and Grease	13.6 ± 0.11	mg/L
Metals			
1.	Na	64.03 ± 6.88	mg/L
2.	K	17.7 ± 1.45	mg/L
3.	Mg	10.28 ± 0.12	mg/L
4.	Ca	33.231 ± 2.21	mg/L
5.	Fe	0.161 ± 0.10	mg/L
6.	Zn	0.055 ± 0.03	mg/L
7.	Mn	0.0084 ± 0.010±	mg/L
8.	Ni	0.0067 ± 0.00	mg/L
9.	Cr	ND	mg/L

*ND=not detected

4.2. *Screening for the Isolation of Potential Microalgae in DWW:*

Screening is an essential procedure of bio-process expansion to acquire a single species and consider the principle move in to the isolation of potential microalgae for the generation of valuable lipids and bioremediation of dairy effluent. However, autochthonous microalgae strains can be

efficiently utilized for pollutant removal from dairy effluent, CO_2 sequestration and production of valuable byproducts.

In this work, isolated microalgal species examined to determine their potency towards nutrient removal and biomass production using dairy wastewater as a cultivation media. A total of 10 autochthonous microalgae species were isolated from dairy effluent by applying standard microbiological techniques which quoted in the Section of material and methods 3.1. The steps involved in screening process, the isolation and selection of potential strains are depicted in the following images;

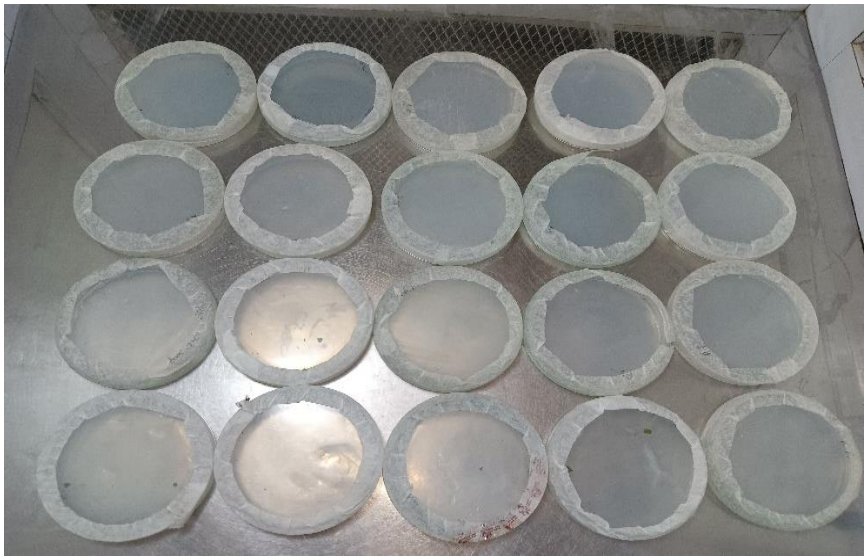


Figure no 4.1 Isolation of Microalgae from DWW

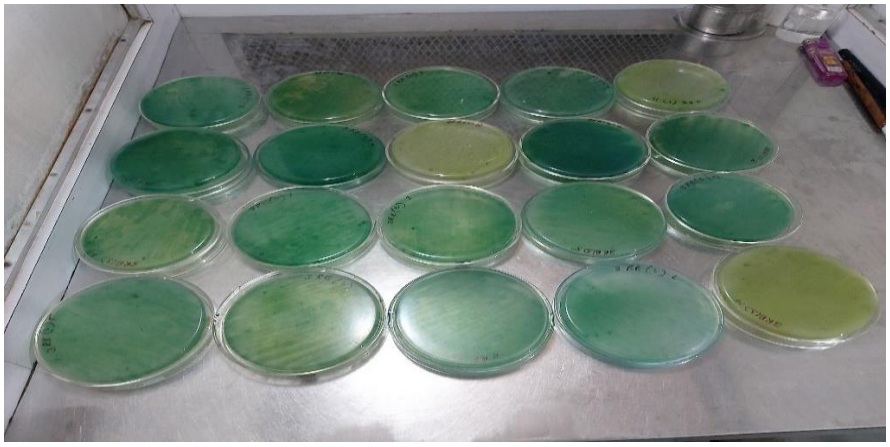


Figure no 4. 2 Growth of microalgae strains on DWW

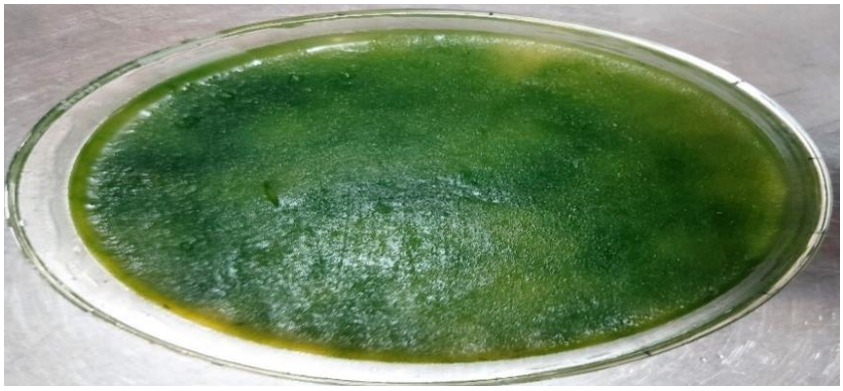


Figure 4. 3. (a)

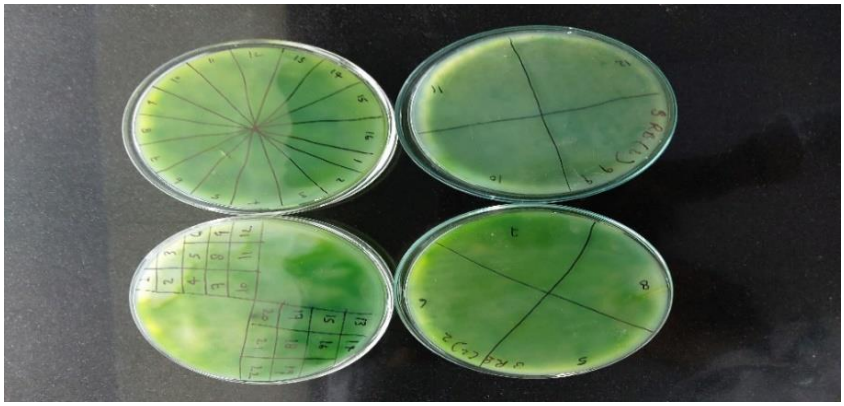


Figure 4. 3. (b)

Figure no 3. (a) Microalgae Consortium growth on Agar Plates Using DWW (b) The Sub culturing of Isolated Microalgae to Attain Pure Strains

Figure no 4. 4 Growth of Pure Microalgae Strains in Erlenmeyer Flasks

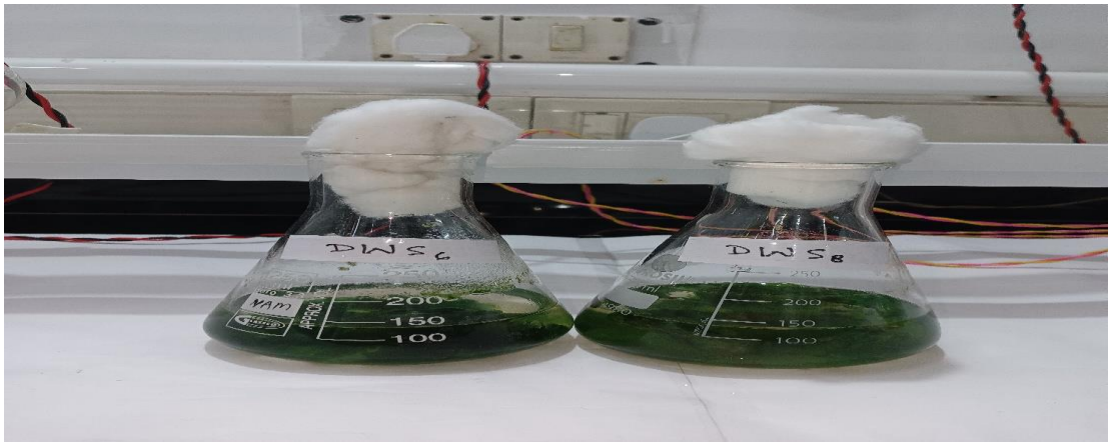


Figure no 4. 5 Selected Microalgae Strains (DWW06 and DWW08)

The growth of all isolated microalgal strains were measured at every alternate day, up to 15 days of batch cultivation in Erlenmeyer flasks which showed that they were growing well in dairy effluent medium. This observation revealed that dairy waste water can enhanced the microalgae growth by providing all the required nutrients and all isolates are comfortably adapted to it. The next

move, best performing 06 microalgal strains (DDW01, DDW04, DDW06, DDW08, DDW09 and DDW10) were selected (Fig. 4.1). These better acclimated microalgal strains were screened according to their biomass productivity. Data regarding the growth parameters was calculated as mentioned in the section no. 3 i.e, (Materials and Methods) segment. Table 4. 2. represents the biomass production in terms of DCW (g L^{-1}) of every acclimatized microalgal strain. The doubling time (T_d) and specific growth rate (μ) were estimated and are depicted in in the same i.e., Table 4. 2.

Table no 4. 2. Biomass (DCW) of Microalgae in Different Dilutions of DWW

Time (Days)	DWW01	DWW04	DWW06	DWW08	DWW09	DWW10
0	0.062	0.074	0.064	0.072	0.082	0.092
3	0.123	0.125	0.133	0.243	0.122	0.247
5	0.430	0.459	0.523	0.534	0.511	0.398
7	0.654	0.764	0.813	0.865	0.658	0.687
9	0.866	0.954	1.009	1.008	0.786	0.986
11	0.987	1.034	1.125	1.134	0.985	1.003
13	1.023	1.002	1.243	1.256	1.024	1.067
15	1.103	1.132	1.385	1.478	1.056	1.223
μ (d^{-1})	0.191	0.181	0.204	0.201	0.170	1.72
T_d(day)	2.34	2.40	2.28	2.29	2.46	2.45

The values of DCW were the mean of triplicate values (SD≤3%)

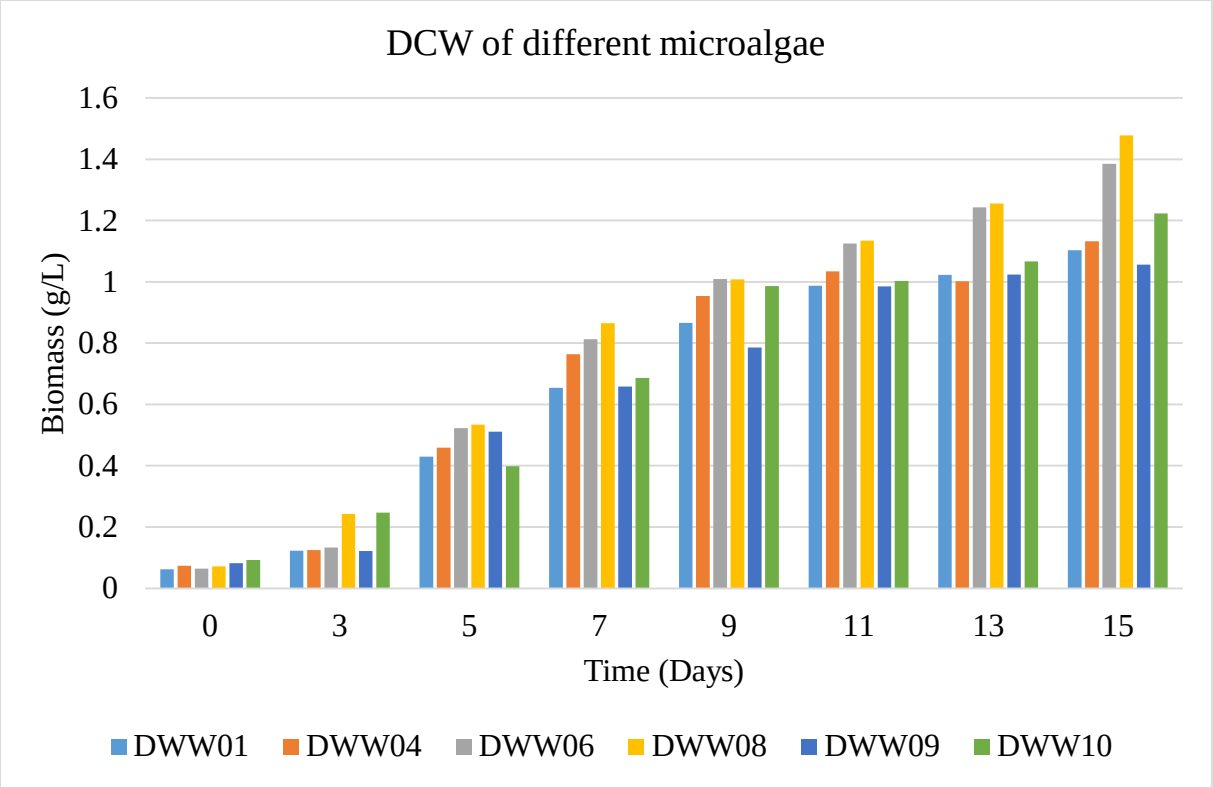


Figure 4. 6. Biomass of all isolated microalgae in dairy waste water

4.3 Microalgal Growth and Nutrient Reduction Rate:

Microalgae expansion and capacity of nutrient reduction, directly proportional to the metabolism of microalgae and the obtainability of nutrients in dairy effluent or dairy waste water. Further, two potential microalgae isolate namely DWW06 and DWW08 were selected based on their acclimatization and growth (dry cell weight) in dairy waste water The growth of microalgae species, examined by taken OD , at 650 nm by utilizing different dilutions [25% concentration (25 ml DWW +75 ml Bold's Basal media), 50% concentration (50 ml DWW +50 ml Bold's Basal media), 75% concentration (75 ml DWW + 25 ml Bold's Basal media), 100% concentration (100 ml DWW (dairy waste water)) and Control (100 ml BBM)] for promoting the growth and nutrients reduction from dairy waste water.

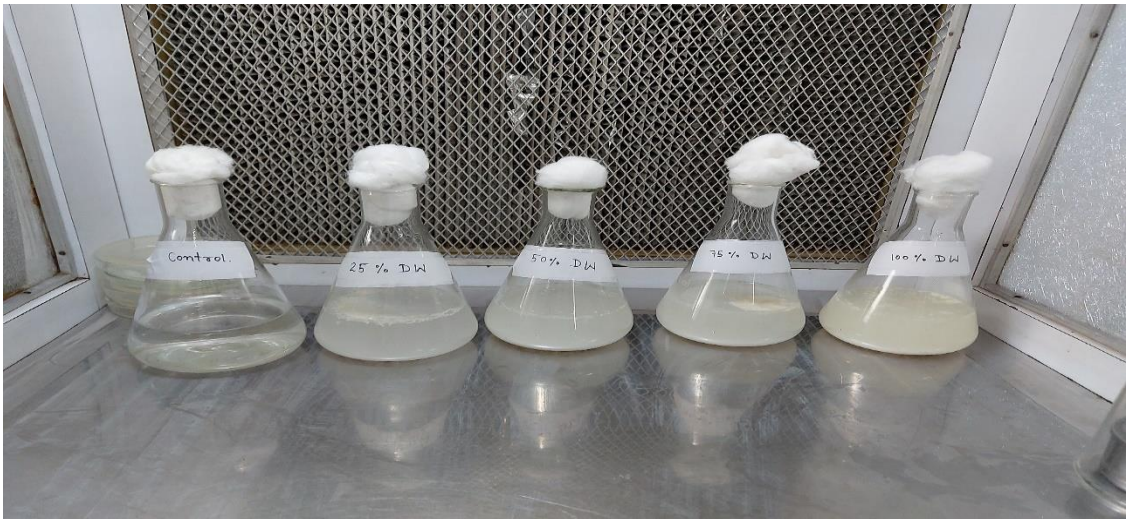


Figure 4. 7. Different Dilutions of Dairy Wastewater with BBM Media

Table no 4. 3 and Figure 4. 8 represents the microalgae growth, in terms of biomass of DWW06 strain. And Table no 4. 4 and Figure 4. 9 Shows the biomass of DWW08 strain in various dilutions of DWW (dairy waste water). The maximum biomass DCW yield was 1.478 ± 0.003g L^{-1}, after 15 day's batch culture by using an inoculum size of 0.072 g L^{-1} in case of DWW08. While DWW06 was able to attain 1.385 ± 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) Microalgae biomass was observed to declined moderately in dairy waste water, after fifteen days of batch culture. It was supposed that biomass yield based on the utilization of nutrients (TP and TN), in DWW. If the nutrients are utilized swiftly, then biomass enhanced gradually. It was observed that 75% conc. (75 ml DW+25 ml Bold's Basal media), presented the excel broth for growth and development of microalgae than any other dilutions of dairy waste water.

Table no 4.3. Biomass (DCW) of DWW06 in Different dilutions of DWW

Time (Days)	**Control (Bold's Basal media)**	**3:1 (25%DWW)**	**1:1 (50%DWW)**	**1:3 (75%DWW)**	**1:0 100%DWW**

0	0.064	0.064	0.064	0.064	0.064
3	0.123	0.126	0.134	0.243	0.122
5	0.430	0.459	0.523	0.534	0.511
7	0.655	0.764	0.813	0.865	0.658
9	0.866	0.954	0.989	1.008	0.786
11	0.987	1.034	1.002	1.134	0.985
13	1.025	1.002	1.034	1.256	1.024
15	1.122	1.221	1.288	1.385	1.250
μ (d^{-1})	0.196	0.196	0.200	0.204	0.198
T_d(day)	2.32	2.32	2.30	2.28	2.31

The DCW values = the mean values of triplicates (SD≤3)

Table no 4. 4 Biomass (DCW) of DWW08 Microalgae in Different Dilutions of DWW

Time (Days)	Control BBM Media)	3:1 (25%DWW)	1:1 (50%DWW)	1:3 (75%DWW)	1:0 100%DWW
0	0.072	0.072	0.072	0.072	0.072
3	0.123	0.126	0.134	0.243	0.122
5	0.430	0.459	0.523	0.534	0.511
7	0.655	0.764	0.813	0.865	0.658
9	0.866	0.954	0.989	1.008	0.786
11	0.987	1.034	1.002	1.134	0.985
13	1.025	1.002	1.034	1.256	1.024
15	1.128	1.145	1.265	1.478	1.056
μ (d^{-1})	0.183	0.184	0.191	0.201	0.179
T_d(day)	3.78	3.76	3.62	3.44	3.87

Dry cell weight (DCW) = The mean of triplicate values, (SD≤3%)

μ = Higher specific growth rate

Td=Doubling time

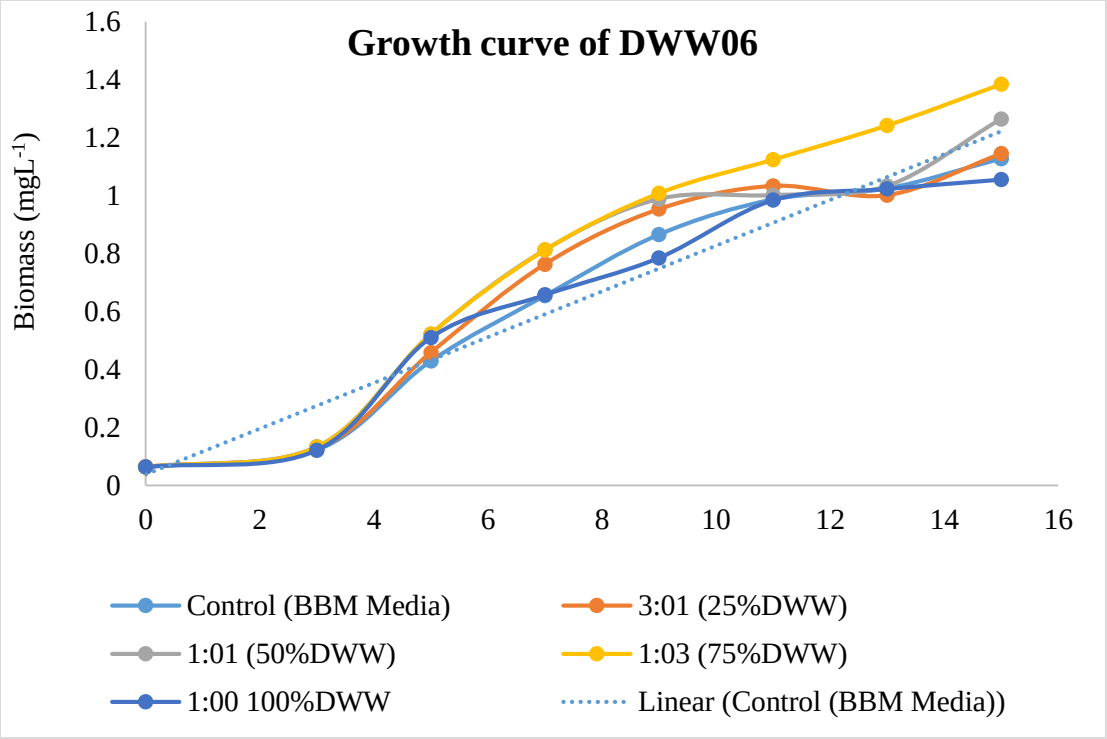


Figure no 4. 8 Growth curve of DWW06 in different dilutions of dairy waste water

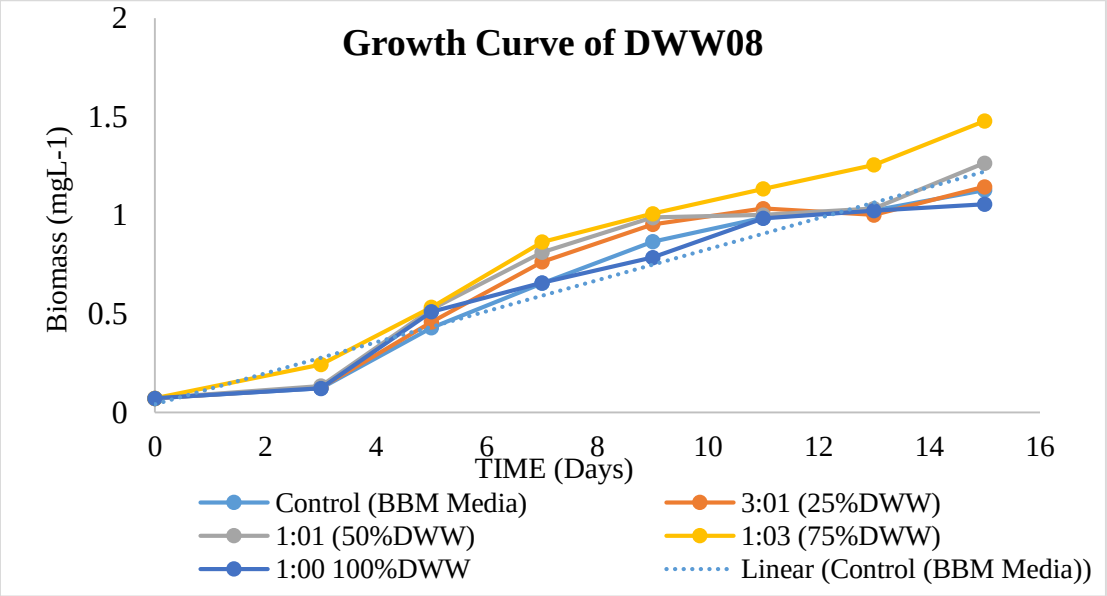


Figure no 4.9 Growth curve of DDW08 in different concentrations of dairy waste water

4.4. Nutrient Removal in Optimized Conditions:

Chemical oxygen demand is the indirect amount of all types of inorganic and organic contents available in dairy effluent. Figure no 4.10 and figure no 4.11. presents the change COD levels as well as the reducing capacity of two potential microalgae strains in different concentrations of DWW + BBM respectively. The isolates DWW06 and DWW08 were able to eradicate more than 88% of COD with > 142 mgL-1 d^{-1} of reduction rate from DWW, after 15 days of batch culture, in 75% DWW+25 % BBM. Both the potential isolated microalgae strains DWW06 and DWW08 provided almost similar COD removal i.e., 86.45% to 88.84% respectively. 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.

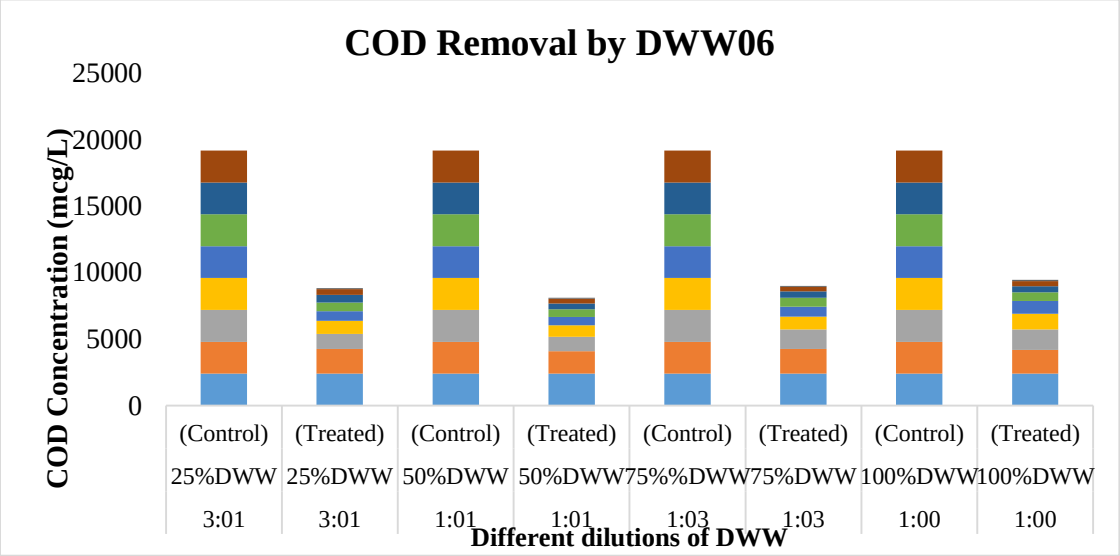


Figure 4. 10. COD Removal Rate by DWW06 Microalgae Isolate Over 15 Days

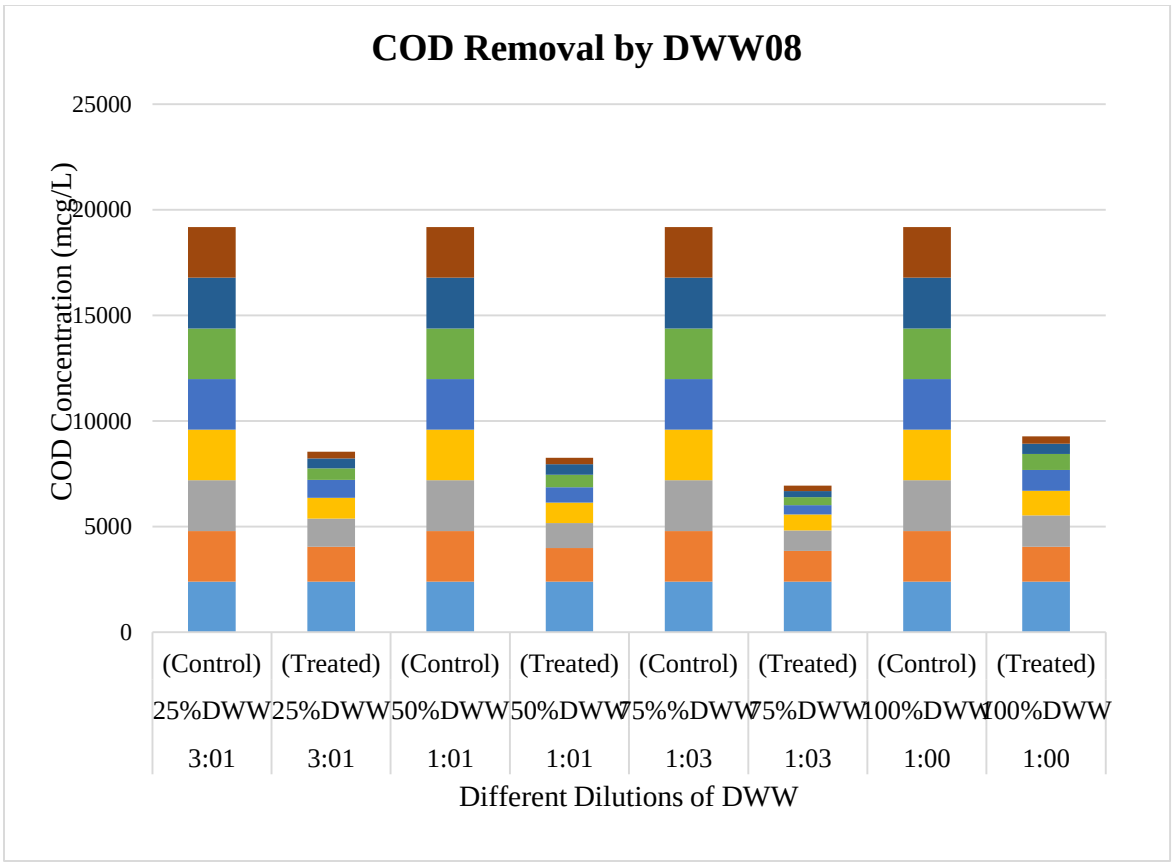


Figure 4. 11 COD Removal Rate by DWW08 Microalgae Isolate Over 15 Days

Both the isolated microalgae (DWW06 and DWW08) successfully removed 87.45% 85.90 % of nitrate from DWW respectively. Nitrate removal by DWW06 was represented by Figure 4.12 And nitrate removal by DWW08 was depicted in Figure 4.13. While phosphate removal 88.96% and 86.45% respectively by both strains DWW06 and DWW08. Phosphate removal by DWW06 was depicted by Figure 4.14 and phosphate removal in different dilutions of dairy effluent by DWW08 was represented by Figure 4.15. The NO_3^- values, the mean of triplicates (SD≤ 4%)

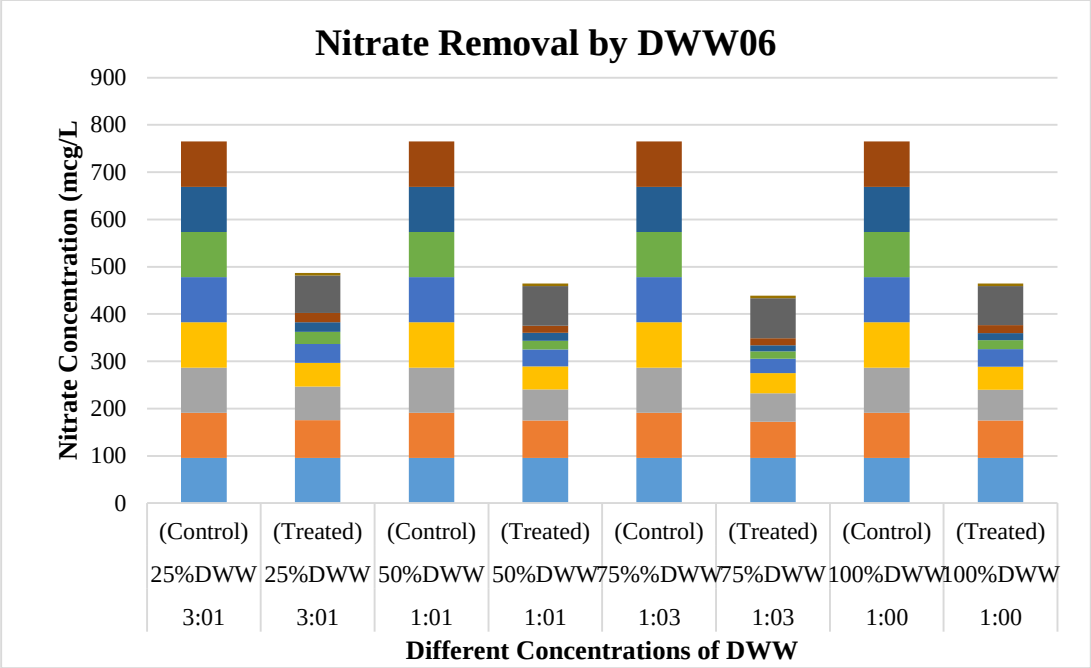


Figure 4. 12. Nitrate removal by DWW06 Microalgae Isolate

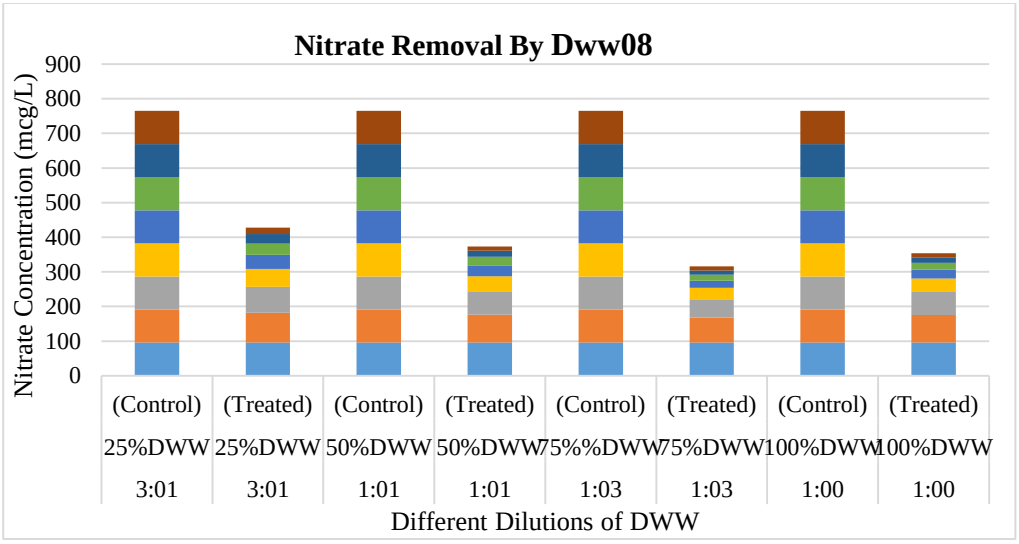


Figure 4. 13 Nitrate removal by DWW08 Microalgae Isolate

The PO_4^{-3} values, the mean of triplicates (SD≤ 3%)

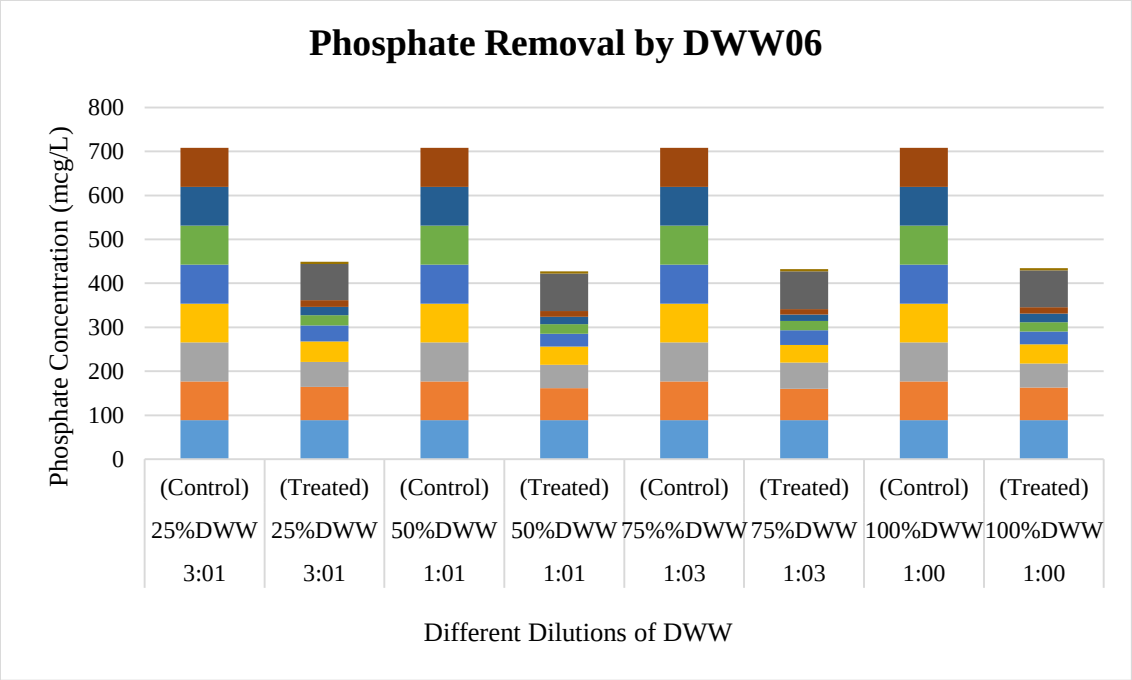


Figure 4. 14 Phosphate Removal by DWW06 Microalgae Isolate

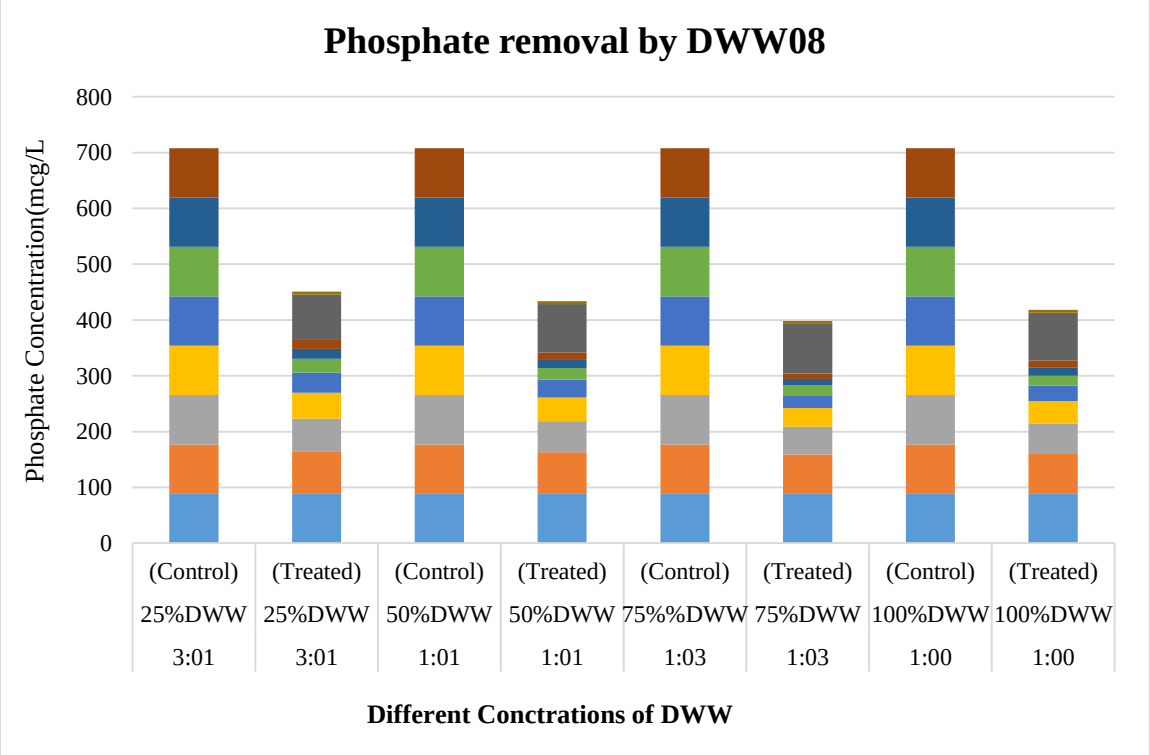


Figure 4. 15 Phosphate Removal by DWW08 Microalgae Isolate

4. 5 Biomass and Lipid Productivity of Selected Microalgae:

The microalgae growth and nutrient reduction, concurrently occurring in DWW. Microalgae biomass and lipid productivity were considerably depending on the reduction of nutrients in the DWW. Lipid productivity in microalgal biomass is the decisive variable to examine their potency. Table 4. 5. and Table 4. 6. represents the microalgae biomass attribute in terms of BP, LP and LC% of the autochthonous microalgae in various concentrations of dairy waste water. DWW08 strain, presented 93.73 mg L^{-1} d^{-1} BP. While, DWW06 strain showed 88.00 mg L^{-1} d^{-1} BP in 75 % conc. of dairy waste water, which were the higher BP among these DWW conc. DWW08 microalgae strain, was also capable to store an elevated content of lipids (31.60 % w/w) in 75% conc. of dairy waste water followed by 100% dairy waste water (28.46% w/w), 50% conc. of dairy waste water (26.50% w/w), control (Bold's Basal media) (23.45% w/w), and 25% conc. of dairy waste water (22.60% w/w). While DWW06 accumulated 29.80% w/w lipid content (LC %) in 75% conc. of dairy waste water followed by 50% conc. of dairy waste water (27.70% w/w), 100% conc. of dairy waste water (26.88% w/w), 25% DWW (24.34% w/w) and control (Bold's Basal Media) (21.45% w/w).

Table no 4.5. Growth Characteristics of DDW06 Isolate

Growth characteristics	Control (BBM Media)	3:1 (25%DWW)	1:1 (50%DWW)	1:3 (75%DWW)	1:0 (100%DWW)
DCW (g/l)	1.122 ± 0.004	1.221 ± 0.002	1.288 ± 0.004	1.385 ± 0.005	1.250 ± 0.004
LC % (W/W)	21.45 ± 0.002	24.35 ± 0.002	27.70 ± 0.003	29.80 ± 0.002	26.88 ± 0.003
BP (mg/l/d)	70.5 ± 0.005	77.1 ± 0.001	81.6 ± 0.003	88.0 ± 0.003	79.0 ± 0.002
LP (mg/l/d)	15.12 ± 0.002	18.77 ± 0.002	22.60 ± 0.003	26.22 ± 0.004	21.23 ± 0.006

Table no 4. 6. Growth Characteristics of DDW08 Isolate

Growth characteristics	Control (BBM Media)	3:1 (25%DWW)	1:1 (50%DWW)	1:3 (75%DWW)	1:0 100%DWW
DCW (g/l)	1.128 ± 0.005	1.145± 0.002	1.265± 0.003	1.478 ±0.003	1.056±0.004
LC% (W/W)	23.45 ± 0.004	22.60± 0.004	26.50 ±0.002	31.60± 0.003	28.46±0.003
BP (mg/l/d)	70.40 ± 0.004	71.53± 0.003	79.53 ±0.002	93.73± 0.006	65.6± 0.004
LP (mg/l/d)	16.50 ± 0.003	16.16± 0.005	21.07± 0.006	29.61± 0.003	18.66± .002

From the observations of these experiments, it has been concluded that 75% dilution proves the suitable dilution for maximum biomass and lipid yield. After that microalgae were grown in large tank having the capacity of 10 litter for achieving huge volume which required for the lipid extraction process. Microalgae growth was visualized by the following figure 4.16 and extracted lipid presented by figure no 4. 1

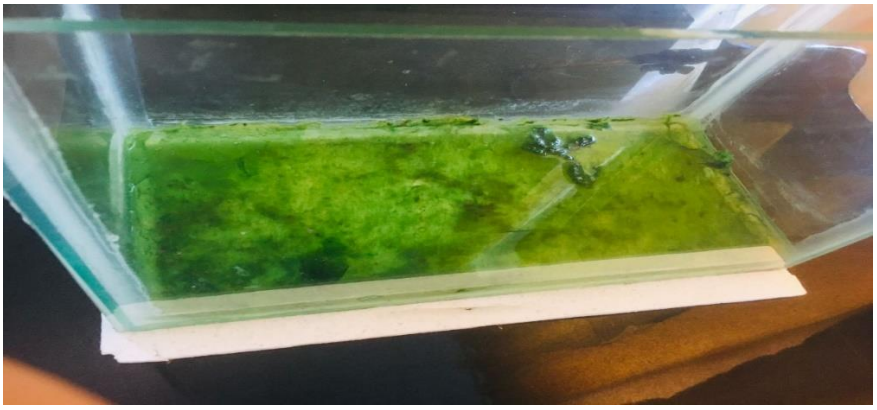
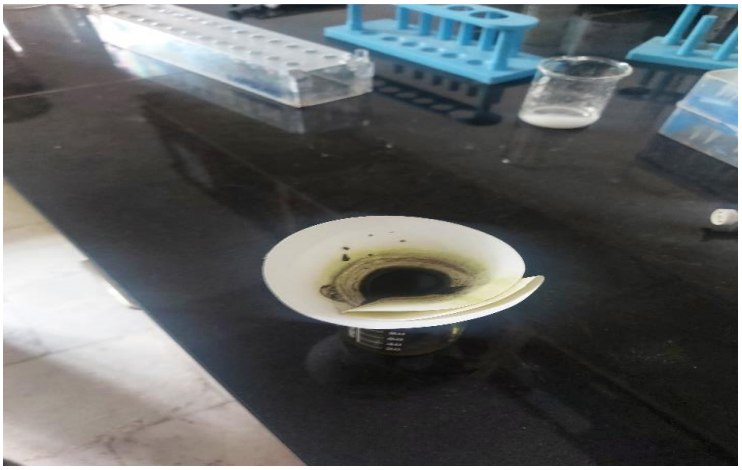


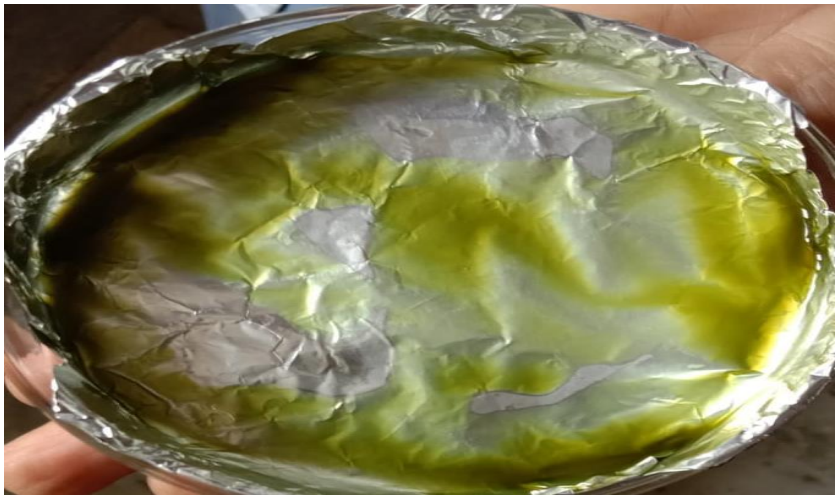
Figure 4. 16. Microalgae Cultivation in Large Tank

(a)

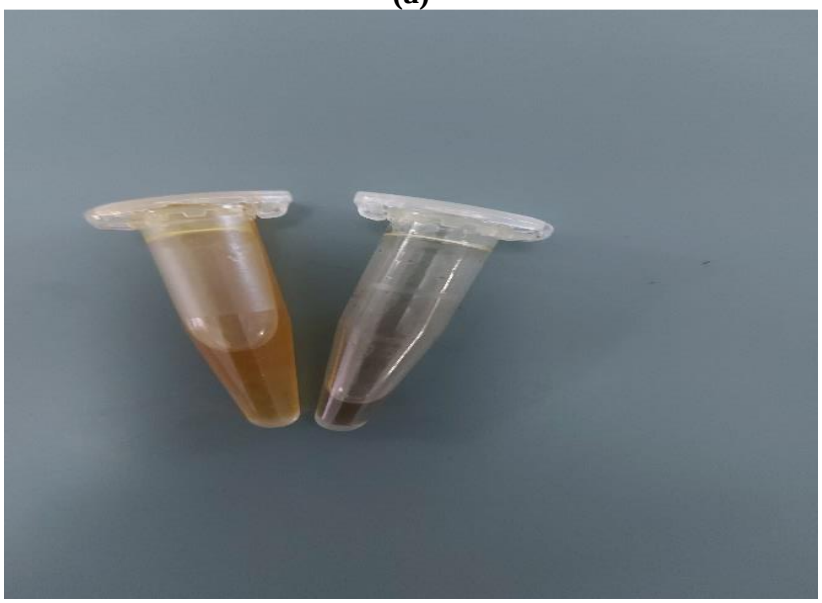


(b)

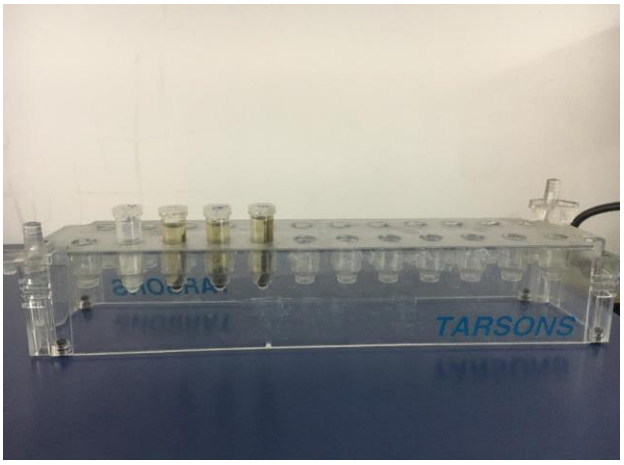
Figure no 4. 17. (a) Harvested Microalgal Biomass and (b) Dried Microalgal Biomass



(a)



(b)



(c)

Figure no 4. 18. (a) Extracted Lipids from Microalgae; (b) DWW06 and (c) DWW08

4.6 Identification of Autochthonous Microalgae Strains from Dairy Waste Water:

The microscopic investigation disclosed the individuality and morphology of isolated microalgae strains.[153] The figure no 4. 19 shown the microscopic view of both selected potential microalgae isolates DWW06 and DWW08 representing the morphology and purity. According to the primary morphological characterization, the DWW06, microalgae strain was identified as *Botryococcus sp.* as the next closest homologue was found to be *Botryococcus sp.* PK (a chlorophyte). After that, for amplifying the 18S rRNA gene segment from the autochthonous microalgae sp., universal primers were utilized. These amplified sequences were deposited to the NCBI database. These BLAST hits sequences represented of 96.36% identity with *Botryococcus sp*. having accession no. of KF746941.1. (Figure 4. 20. Phylogenetic tree of DWW06, was concluded by utilizing 18S rRNA and ITS1-5.8S-ITS2.

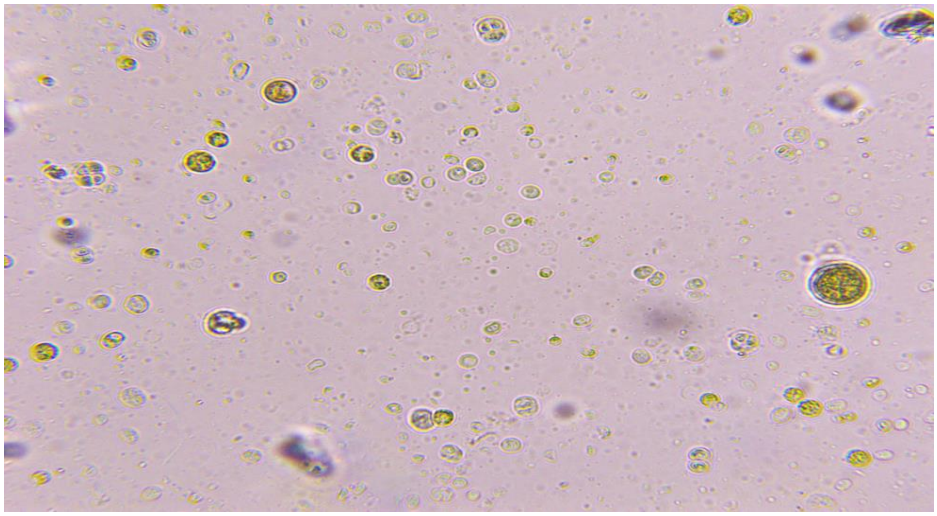


Figure 4. 19. (A)

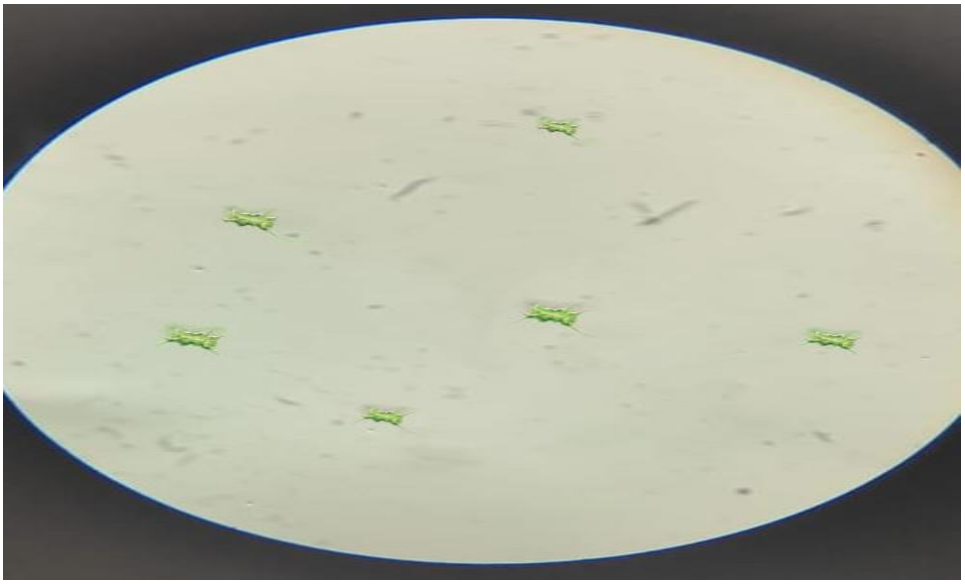


Figure 4. 19. (B)

Figure 4. 19. Microscopic view of potential microalgae isolates from dairy effluent

(A) ***Botryococcus sp.*** **PK (DWW06);**

(B) ***Poterioochromonas malhamensis*** **(DWW08)**

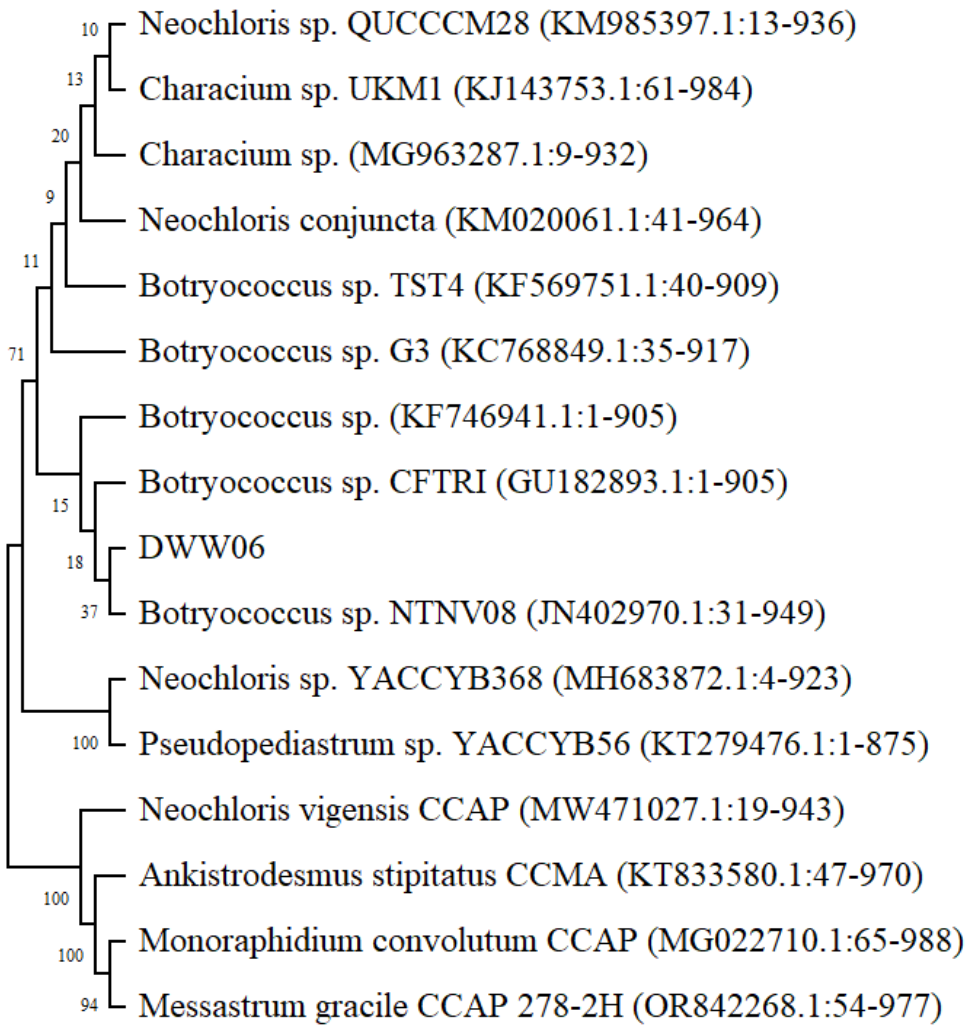


Fig. 4. 20 Phylogenetic trees of DWW06, was interpreted by utilizing 18S rRNA and ITS1–5.8S-ITS2

While, DWW08 strain was recognized as a chrysophyte or golden-brown microalgae which named *Poterioochromonas species.* After that, to amplify the 18S rRNA segment from DWW08 microalgae sp., universal primers were applied. These amplified fragments were deposited to the NCBI database. These BLAST hits designated (97.30%) homology with *Poterioochromonas malhamensis* species UPMC-A0073 Sequence id: MK834582. This strain represented the next nearest similarity with

Achnanthidium saprophilum Sequence id: MN592667.1. (Figure 4. 21 Evolutionary tree of DWW08, was interpreted by following 18S rRNA and ITS1-5.8S-ITS2.

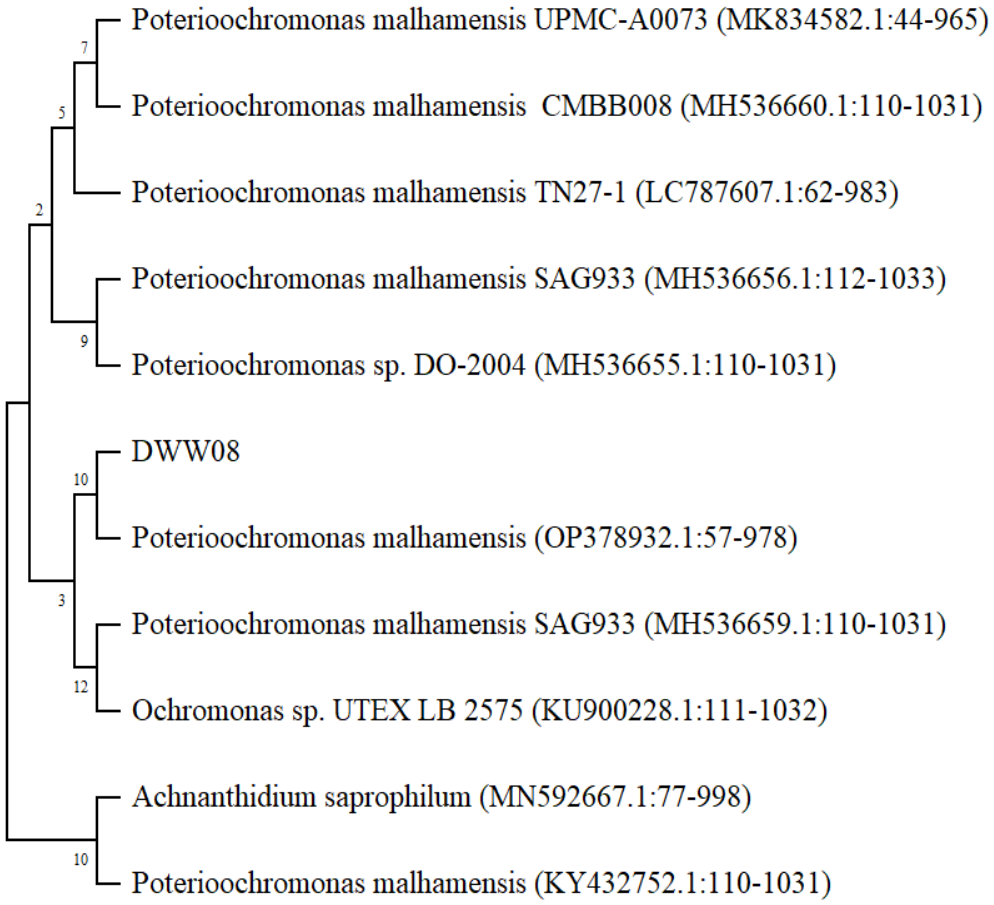


Figure no 4. 21. Phylogenetic tree of DWW08, was interpreted by utilizing 18S rRNA and ITS1–5.8S-ITS2

In case of DWW06 the 18S rDNA sequences showed the maximum 96.36% homology with *Botryococcus* species (accession no. of KF746941.1.) whereas DWW08 exhibited (97.30%) homology with *Poterioochromonas malhamensis* UPMC-A0073).) Evolutionary tree for both strains were prepared with a software aligner system. The evolutionary intervals were determined by applying Jukes and Cantor method. All these detailed examinations were concluded in MEGA6.

4.6. 1 Primer Details of DWW06 Microalgae strain:

The PCR product size ~1.2 kb

18s rDNA Microalgae universal primers:

18s-F Primer 5'CCTGGTTGATCCTGCCAG3'

18s-R Primer 5'TTGATCCTTCTGCAGGTTCA3'

(Yuan et al. (2011)

Aligned Sequence Data of Sample - (1213 bp)

AAGTTGCTCTTAAGATATATTGTTTATACTGTGAACTGCGAATGGCTCATTAAATCAGT

TATAGTTTATTTGATGGTACCTCCTACTCGGATAACCGTAGTAATTCTAGAGCTAATAC

GTGCGTAAAACCCGACTTCTGGAAGGGTCGTATATATTAGATAAAAGGCCGACCGGGC

TCTGCCCGACCCGCGGTGAATCATGATATCTTCACGAATCGCATAGCCTTGTGCTGGCG

ATGTTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATG

GTGGTAACGGGTGACGGAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG

GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGA

GGTAGTGACAATAAATAACATTTCCGGGCACATCGTGTCTGGAAAATGGAATGAGTAC

AATCTAAATCCCTTAACGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG

TAATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGA

TTTCGGGTGGGTTCTAGCGGTCCGCTTCGGTGAGTACTGCTATGGCCTTCCTTTCTACG

GGGGACGGGCTCCTGGGATTAACTTCTCGGGATCCGGATTCCGTGATGATACTTTGAGT

AAATTGGAGTGTTCAAAGCAAGCCTGCGCTCTGAACATTTTTAGCATGGAATATCACG

ATAGGACTCTGGGCCTATCTTGTTGGTCTGTAGGACCAGAGTAATGATTTAGAGGGAC

AGTCCGGGGGGTGATTTCGTATTTTCAATTGGTCAGAAGGTGAAATTTCTTGGGATTAT

ATGAAAAGAACGAAACTAACTGGCGAAAGCAATTTTGGCCCGAGGATTGTTTTTCTAT

TAATTCAAAGAACCGAAAAGGTTGGGGGGGGGCTTCGGAAAAACGAAATTAGAAATA

ACCGGCGGAAAGTCTCCAACCAATAAACGGAATTGCCGAACCTTAAGGGGAATTGAGC

AAGAACGCGCTCCTCCCAATTTTGGAAGGTGAGAATCTCAT

4.6.2 Primer Details of DWW08 Microalgae strain:

The PCR product size ~1.2 kb

18s rDNA Microalgae universal primers:

18s Forward primer : 5' GTAGTCATATGCTTGTCT 3'

18s Reverse Primer : 5’CTTCCGTCAATTCCTTTAAG 3’

Aligned Sequence Data of Sample - (927 bp)

TAGTTTATTGGATGGTCCATGGCTACTGGGATACCCGTAGTAATTTCTAGAGCTAATCC

ATGCATAAATCCCTGATTTCTGGAAAGGGTGTACTTATTAGATGGAACCCAATGCGGG

GCAACCCGGATCTTGGTGATTCATAATAATTTTCGGATCGATCGTAGGATCGAT

GCATCATTCAAGTTTCTGCCCTATCAGCTTTGGATGGTAGGGTATTGGCCTACCATGGC

ATTTACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCT

ACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACCCAATCCTGACACAG

GGAGGTAGTGACAATAAATAACAATGTCGGGCTTTCTTAAGTCTGACAATTGGAATGA

GAACAATTTAAATCCCTTATCGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCC

GCGGTAATTCCAGCTCCAATAGCGTATACTAAAGTTGTTGCAGTTAAAAAGCT

CGTAGTTGAATTTCTGGTCTCGAGACGCGGCCAGCCCCAAGGGGCCAGTGCTGTCGAT

TGGGACCATCCTCGAGAAGAACATATCTGTCATTGAGTTGATGGGTATGGGACTCTCGT
CATTTACTGTGAGCAAAATAGAGTGTTCAAAGCAGGCTTACGCCGTTGAAT
ACATTAGCATGGAATAATAAGATAGGACCTTGGTCTATTTTGTTGGTTAGTTCTCCAAG
GTAATGATTAATAGGGACAGTTGGGGGTATTCGTATTCAATTGTCAGAGGTGAAATTCT
TGGATTTATGGAAGACGAACTACTGCGAAAGCATTTACCAAGGATGTTTTCATTAATCA
AGAACGAAAGTTAGGGGATCGAAGATGATTAGATACCATCGTAGTCTTAACCATAAAC
TATGCCGACTAGGGA.

4.6. 3. Gel Images Description:

Figure no 4. 22. Gel Image for Genomic DNA

Lane Description:

2: DNA Marker;

3. Sample DWW06;

4. Sample DWW08

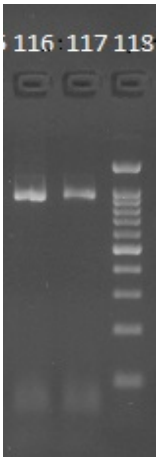
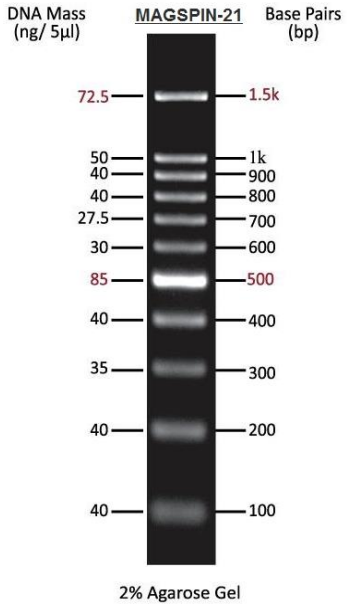


Figure no 4.23. Gel photo for PCR amplification with 18S primers

Lane Description:116. Sample DWW06; 117 Sample DWW08. 118. DNA Marker

Ladder Specification



4.7 Lipid Profiling Through GCMS:

The neutral lipids and hydrocarbons yield by both (DWW06 and DWW08) microalgae strains, in DWW were analyzed by utilizing GCMS (Gas Chromatography-Mass Spectrometry). Major fatty acids or lipids, produced by DWW06 were (C16:1, C16:0, C18:1, C18:0, C18:2, and C20:0) found along with (C4:0, C6:0, C8:0, C12:1, C14:1 and C14:0) and other lipids with a minor amount, which are showed in Table no. 13 and Figure 21. The value of saturated lipid content of DWW06 strain was 29.27 %, the unsaturated lipid content was 66.85%, and 3.88 % others. While, the major lipids, synthesized by DWW08 were (C16:0, C16:1, C18:1, and C18:0) observed (C14:0, C20:1) and other lipids with minor value, which showed by Table no 14 and Figure 22. The value of saturated lipid content of DWW08 strain was 26.83%, the unsaturated lipid content was 72.25%, and 0.99% other fatty acids. The lipids of C16 to C18, were the main lipids whereas, the lipids of C14 and C20 were available in lower value in both strains.

Table no 4. 7. Fatty acid percent (%) Composition of DWW06 Isolate Microalgae by GC-MS

S.no	Retention time (min)	Content %	Lipid name in abbreviated form	Name of compound
1.	09.21	1.09	C4:0	Butane (hydrocarbon)
2.	10.23	1.04	C6:0	Hexane (hydrocarbon)
3.	10.88	1.56	C8:0	Octane (hydrocarbon
4.	12.21	1.26	C12:1	Dodecane (cis-9)
5.	14.88	2.20	C14:1	Tetradecenoic acid (cis-9)

6.	15.56	2.17	C14:0	Tetradecanoic acid
7.	18.09	30.17	C16:1	Hexadecenoic acid (cis-9)
8.	18.81	10.46	C16:0	Hexadecanoic acid
9.	19.11	14.51	C18:1	9- octadecenoic acid
10.	19.59	12.04	C18:2	9,12- octadecedinoic acid
11.	21.86	2.71	C18:3	9,12,15- octadecetrinoic acid
12.	22.45	8.29	C18:0	Octadecanoic acid
13.	23.26	4.66	C20:0	Eicosanoic acid
14.	25.22	1.66	C20:1	Eicosenoic acid (cis-17)
15.	27.45	1.21	C22:1	Docosanoic acid (cis-9)
16.	27.97	1.09	C22:2	Docosanoic acid (cis-7,12)

Table no 4. 8. Fatty acid Percent (%) Composition of DWW08 Isolate Microalgae by GC-MS

S.no	**Retention time (min)**	**Content %**	**Lipid name in abbreviated form**	**Name of compound**
1.	10.21	1.09	C8:1	Octa 2-en (hydrocarbon)
2.	12.24	1.06	C12:1	Dodecane 9-en
3.	12.88	1.17	C12:0	Dodecane
4.	13.26	3.40	C14:1	Tetradecenoic acid (cis-9)

5.	16.26	5.17	C14:0	Tetradecanoic acid
6.	18.09	38.17	C16:1	Hexadecenoic acid (cis-9)
7.	18.81	6.46	C16:0	Hexadecanoic acid
8.	19.13	15.71	C18:1	9- octadecenoic acid
9.	19.68	6.04	C18:2	9,12- octadecedinoic acid
10.	20.82	2.78	C18:3	9,12,15- octadecetrinoic acid
11.	21.15	15.22	C18:0	Octadecanoic acid
12.	24.56	3.26	C20:1	Eicosenoic acid (cis-11)
13.	25.63	2.89	C20:5	Eicosapentaenoic acid (cis-5,8,11,1,17)

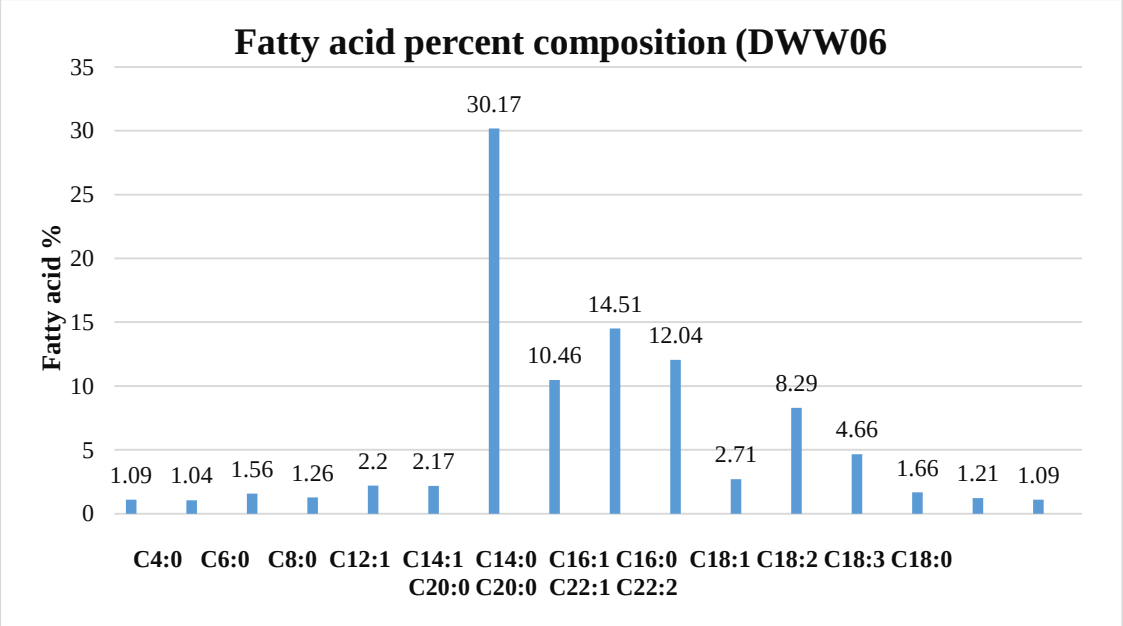


Figure 4. 24. Fatty acid % Composition of DWW06 by GC-MS

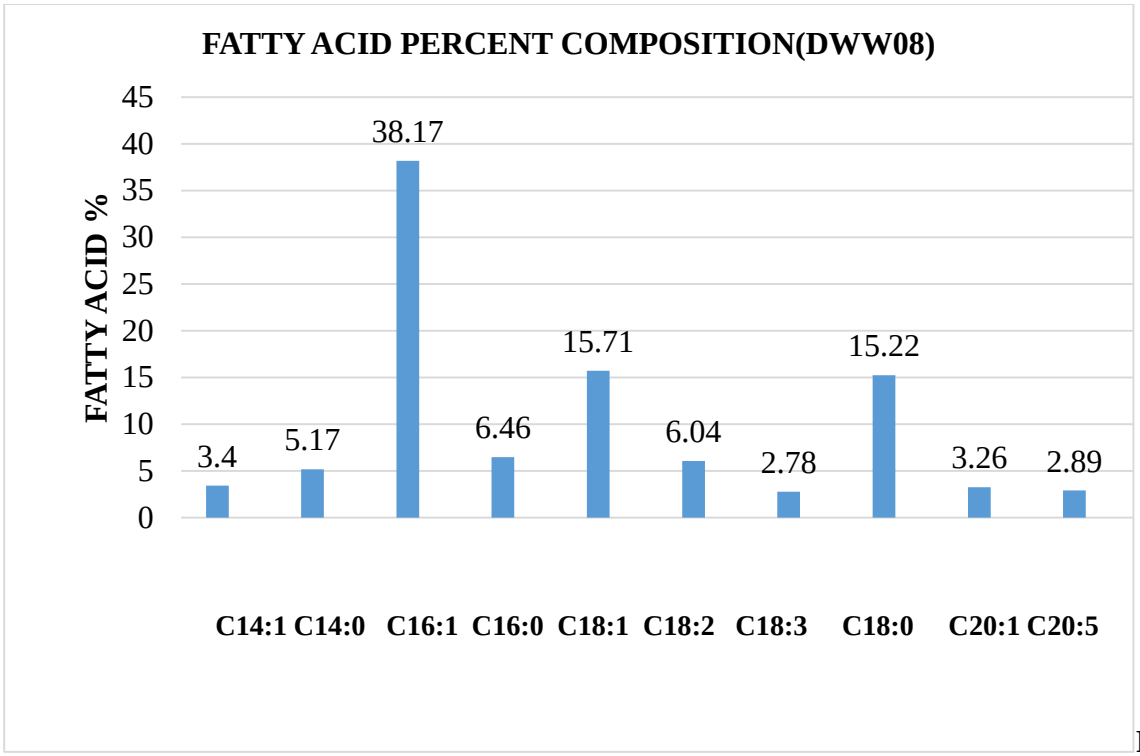


Figure 4. 25. Fatty acid Percent (%) Composition of DWW08 by GC-MS

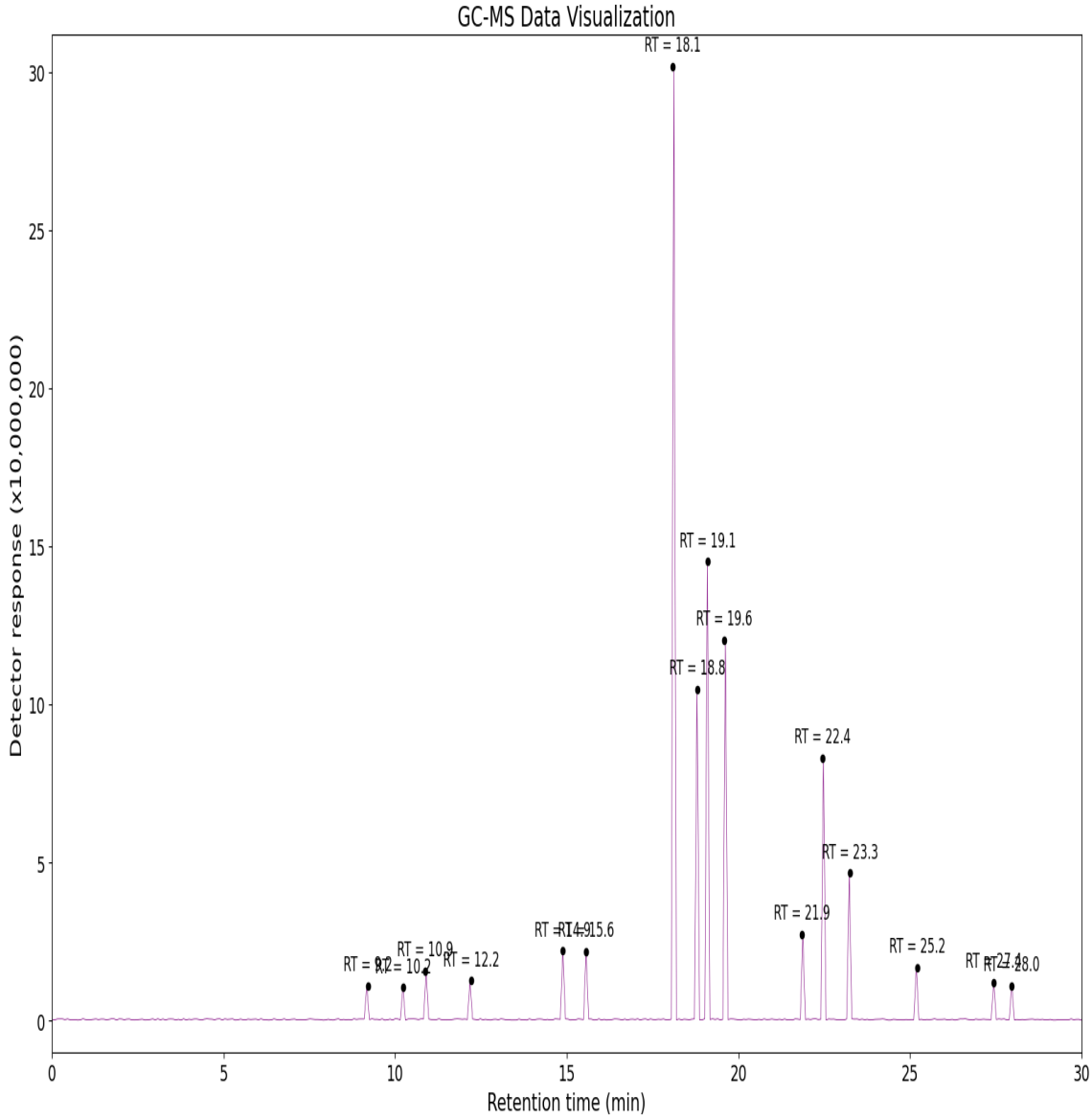


Figure 4. 26. GC-MS Chromatogram of fatty acids in lipid sample of DWW06 isolate

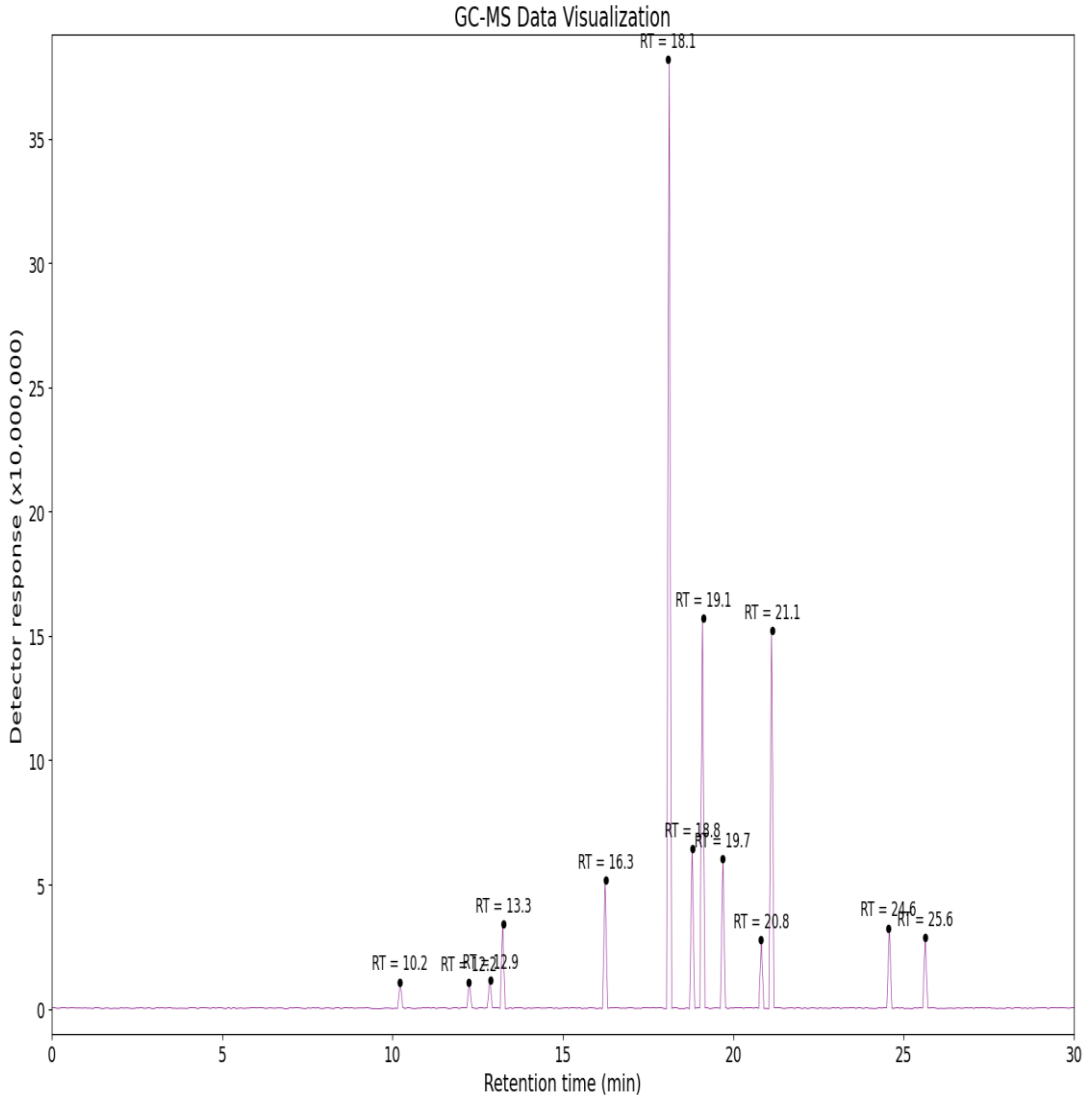


Figure 4. 27. GC-MS Chromatogram of fatty acids in lipid sample of DWW08 isolate