

TABLE OF CONTENTS

Declaration by student	i
Certificate by the supervisor	ii
Certificate by the co-supervisor	iii
Certificate for plagiarism	iv
Anti-plagiarism software (as suggested by UGC/Turnitin) report of Ph.D. thesis (certified by Dean Research)	v
Certificate of successful completion of viva-voce of Ph.D.	xii
Undertaking for submission of Ph.D. thesis	xiii
Acknowledgements	xiv
Table of Contents	xviii
List of Figures	xxii
List of Tables	xxiii
Abbreviations	xxiv
 1. Introduction	 1-11
1.1 Background	1
1.2 Proposal	4
1.3 Research Hypothesis	9
1.4 Aims and Objectives	10
1.4.1 Objectives	11
2. Review of Literature	12-59
2.1 Dairy effluent	13
2.1.1 Types of effluent or processing water	13

2.2 Components of dairy effluent	15
2.3 Dairy effluent treatment approaches	16
2.3.1 Physico-chemical treatment	16
2.3.2 Biological treatment	18
2.4 Microalgae	19
2.4.1 Dual role of microalgae	21
2.4.1.1 As a bioremediator and producer of valuable lipids	21
2.5 Lipid producing microalgal strains	24
2.6 Biosynthesis of lipids in microalgae	25
2.7 Major factors affecting microalgal growth	27
2.7.1 pH	27
2.7.2 Salinity	30
2.7.3 Temperature	33
2.7.4 Light intensity	36
2.7.5 Macronutrient's concentration (carbon, nitrogen, phosphorus	41
2.7.6 Micronutrient's concentration	46
2.7.7 Mixing	47
2.7.8 Screening and selection of microalgae species	48
2.8 A source of value added lipid by-products	50
2.9 Applications of microalgal lipids	51
2.9.1 In food and nutrition field	52
2.9.2 In cosmetic industry	53
2.9.3 In Pharmaceuticals	54
2.9.4 As Biofuel	55
2.9.4.1 Biodiesel	56
2.9.4.2 Other fuel applications (Biohydrogen)	57
3. Materials and Methodology	61-84
3.1 Dairy waste water collection and preservation	61
3.2 Physicochemical analysis of dairy waste water	61
3.2.1 Colour and pH	62
3.2.2 TSS (Total suspended solids)	62

3.2.3 TD (Total dissolved solids)	62
3.2.4 TS (Total solids)	63
3.2.5 DO (Dissolved oxygen)	63
3.2.6 BOD (Biological oxygen demand)	64
3.2.7 COD (Chemical oxygen demand)	65
3.2.8 Metals analysis	66
3.2.9 Nitrate analysis	67
3.2.10 Phosphate analysis	68
3.3.1 Isolation of microalgae from dairy wastewatere	70
3.3.2. Growth media preparation	70
3.3.3 Culture conditions	71
3.3.4 Experimental design	72
3.4 Nutrient removal efficiency	72
3.5 Estimation of microalgal biomass	73
3.6 Extraction and identification of microalgal lipids	75
3.7 Molecular identification of isolated microalgae	76
3.7.1 18SrRNA sequencing	76
3.7.2 Amplification of DNA	78
3.7.3 BLAST (Basic local assignment search tool)	80
3.8 Phylogenetic analysis	83
4. Results	85-119
4.1 Physico-chemical analysis of dairy effluent	85
4.2 Screening for the isolation of potential microalgae in DWW	86
4.3 Microalgal growth and nutrient reduction rate	91
4.4 Nutrient removal in optimized conditions	96
4.5 Biomass and lipid productivity of selected microalgae	100
4.6 Identification of autochthonous microalgae strains from DWW	105
4.6.1 Primer details of DWW06 microalgae strain	109
4.6.2 Primer details od DWW08 microalgae strain	110
4.7 Lipid profiling through GCMS	114
5. Discussion	120-137

5.1 Physico-chemical analysis of DWW	120
5.2 Growth of potential microalgae isolates in DWW	123
5.3 Phycoremediation of dairy effluent	126
5.5 Lipid productivity of selected microalgae isolates	132
5.5 Identification of isolated microalgae strains from DWW	135
5.6 Lipid profiling through GCMS	135
6. Conclusion	138-141
7. Summary	142-147
7.1 Performance evaluation of isolated (DWW06)	
<i>Botryococcus</i> sp. (accession no. KF46941.1.)	145
7.2 Performance evaluation of isolated (DWW08) <i>Poterioochromonas</i>	
<i>melhamensis</i> (UPMCA0073)	146
References	148-168
Appendix	169-183

LIST OF TABLES

Table 2.1	Types of microalgae: Based on their pigment and reserve food	20
Table 2.2	Some lipid containing micro-algal strains	24-25
Table 3.1	Stock solutions of components utilized for preparation of Bold's Basal medium	70-71
Table 4.1	Physico-chemical analysis of dairy effluent	86
Table 4.2	Biomass (DCW) of microalgae in different dilutions of DWW	90
Table 4.3	Biomass (DCW) of DWW06 microalgae in different dilutions of DWW	93
Table 4.4	Biomass (DCW) of DWW08 microalgae in different dilutions of DWW	94
Table 4.5	Growth characteristics of DWW06 isolate	101
Table 4.6	Growth characteristics of DWW08 isolate	101
Table 4.7	Fatty acid percent (%) composition of DWW06 isolate microalgae by GCMS	114
Table 4.8	Fatty acid percent (%) composition of DWW08 isolate microalgae by GC- MS	115
Table 5.1	Microalgae cultivation in DWW and nutrient reduction percentages (COD, NO ₃ ⁻ , PO ₄ ⁻³)	131-132
Table 5.2	Comparison of fatty acids lipid content of various microalgal sp. grown in DWW	136-137

LIST OF FIGURES

Figure 2.1	Schematic diagram of the biosynthesis of lipids in microalgae	26
Figure 2.2	Schematic diagram of applications of micro algal lipids	51
Figure 3.1	Steps of BLAST N analysis	81
Figure 4.1	Isolation of microalgae from DWW	87
Figure 4.2	Growth of microalgae strains on DWW	88
Figure 4.3	(a) Microalgae consortium growth on agar plates using DWW (b) The sub culturing of isolated microalgae to attain pure strains	88
Figure 4.4	Growth of pure microalgae strains in Erlenmeyer flasks	89
Figure 4.5	Selected microalgae strains (DWW06 and DWW08)	89
Figure 4.6	Biomass of all isolated microalgae in dairy waste water	91
Figure 4.7	Different dilutions of dairy wastewater with BBM media	92
Figure 4.8	Growth curve of DWW06 in different dilutions of DWW	95
Figure 4.9	Growth curve of DWW08 in different dilutions of DWW	95
Figure 4.10	COD removal rate by DWW06 microalgae isolate over 15 days	96
Figure 4.11	COD removal rate by DWW08 microalgae isolate over 15 days	97
Figure 4.12	Nitrate removal by DWW06 microalgae isolate	98
Figure 4.13	Nitrate removal by DWW08 microalgae isolate	98
Figure 4.14	Phosphate removal by DWW06 microalgae isolate	99
Figure 4.15	Phosphate removal by DWW08 microalgae isolate	99
Figure 4.16	Microalgae cultivation in large tank	101
Figure 4.17	(a) and (b) Harvested microalgal biomass	102
Figure 4.18	(a) Extracted lipids from microalgae (b) DWW06 and (c) DWW08	103- 104
Figure 4.19	Microscopic view of potential microalgae isolates from dairy effluent <i>Botryococcus sp.</i> PK (DWW06) (B) <i>Poterioochromonas melhamensis</i> (DWW08)	105
Figure 4.20	Phylogenetic tree of DWW06, was interpreted by utilizing 18S RNA and ITS1-5.8S-ITS2	106

Figure 4.21	Phylogenetic tree of DWW08, was interpreted by utilizing 18S RNA and ITS1-5.8S-ITS2	107
Figure 4.22	Gel image for genomic DNA	111
Figure 4.23	Gel photo for PCR amplification with 18S primers	111
Figure 4.24	Fatty acid % composition of DWW06 by GC-MS	115
Figure 4.25	Fatty acid % composition of DWW08 by GC-MS	115
Figure 4.26	GC-MS Chromatogram of fatty acids in lipid sample of DWW06 isolate	116
Figure 4.27	GC-MS Chromatogram of fatty acids in lipid sample of DWW08 isolate	117

ABBREVIATIONS

Accase	Acetyl CO-A Carboxylase
ALR	Air Lift Reactor
APHA	American Public Health Association
EPA	Eicosapentaenoic Acid
BBM	Bold's Basal Medium
BLASTN	Basic Local Alignment Search Tool
BOD	Biological Oxygen Demand
BP	Biomass Productivity
CN	Cetane Number
COD	Chemical Oxygen Demand
CPCB	Central Pollution Control Board
CV	Calorific Value
DCW	Dry Cell Weight
DE	Dairy Effluent
DETP	Dairy Effluent Treatment Plant
DFTW	Dairy Farm Treated Wastewater
DFW	Dairy Farm Wastewater
DGAT	Di acylglycerol Acyltransferase
DI	Dairy Industry
DNA	Deoxyribose Nucleic Acid
DNS	3,5- Di nitri salicylic Acid
DWW	Dairy Wastewater
EDTA	Ethylene diamine tetra acetic Acid
FAME	Fatty Acid Methyl Esters
FTIR	Fourier-Transform Infrared Spectroscopy
GC-MS	Gas Chromatography –Mass Spectrophotometry
GHG	Green House Gases
GPAT	Glycerol-3 Po4 Acyl Transferase
HC	Hydrocarbons
HV	Heating Value
IV	Iodine Value
LC	Lipid Content
LEMB	Lipid Extracted Microalgal Biomass
LHV	Light Heating Value
LP	Lipid Productivity
LY	Lipid Yield
MB	Microalgal Biomass
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NDDB	National Dairy Development Board
FA	Fatty Acid

PUFA	Polyunsaturated Fatty Acid
SFA	Saturated Fatty Acid
OD	Optical Density
OP	Open Pond
PBR	Photobioreactor
PCR	Polymerase Chain Reaction
PEMC	Phosphoenol Pyruvate
RDE	Raw Dairy Effluent
SDE	Simulated Dairy Effluent
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
TAG	Triacyl Glycerol
TDS	Total Dissolve Solids
TKN	Total Kjeldhal Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
WW	Waste Water
CaCl ₂ .2H ₂ O	Calcium chloride dehydrate
Co (NO ₃).2.6H ₂ O	Cobalt nitrate hexahydrate
CoCl ₂ .6H ₂ O	Cobalt dichloride hexahydrate
KH ₂ PO ₄	Potassium phosphate
FeSO ₄ .7H ₂ O	Iron sulfate heptahydrate
H ₃ BO ₃	Boric acid
MgSO ₄ . 7H ₂ O	Magnesium sulfate heptahydrate
MnCl ₂ .4 H ₂ O	Manganese chloride tetrahydrate
NaH ₂ PO ₄ .2H ₂ O	Sodium dihydrogen phosphate
Na ₂ MoO ₄ .2H ₂ O	Sodium molybdate dehydrate
NaNO ₃	Sodium nitrate
Na ₂ SiO ₃ .5H ₂ O	Sodium metasilicate pentahydrate
NH ₄ Cl	Ammonium Chloride
NH ₂ CONH ₂	Urea
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	Ammonium molybdate tetrahydrate
ZnSO ₄ .7 H ₂ O	Zinc sulfate heptahydrate
GC/MS	Gas chromatography/ Mass spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
AWTS	Algae based Wastewater Treatment System

NOTATION

\$	US dollar
%	Percent
°C	Degree centigrade
$\mu_{\max}$	Maximum Specific Growth Rate
Abs	Absorbance
Ca	Calcium
Cf	Final COD, Nitrate and Phosphate concentration (mgL^{-1})
Ci	Initial COD, Nitrate and Phosphate concentration (mgL^{-1})
Cu	Copper
Fe	Iron
$\text{gL}^{-1}\text{d}^{-1}$	Gram per litre per day
K	Potassium
$\text{mgL}^{-1}\text{d}^{-1}$	Milligram per litre per day
Mn	Manganese
N	Nitrogen
Na	Sodium
ng	Nanogram
Ni	Nickle
nm	Nano meter
N-NO ₃ -1	Nitrogen as Nitrate
t	Time (min.)
td	Doubling time (day^{-1})
Zn	Zinc
vvm	Volume of Air per Volume Medium