toxic metals, making their treatment costlier (Gasperi *et al.*, 2008).

In addition, these effluents are also rich in cellulose, hemicelluloses, starch, carbohydrates and other organic and inorganic compounds (Lara *et al.*, 2002) and hence can act as sustainable growth medium for the algal feedstock. In such ambience, microalgae can grow effectively accumulating nutrients and metals, making them sustainable and suitable for low cost wastewater treatment. (De-Bashan and Bashan, 2010).

Moreover, species like *Chlorella vulgaris* has shown promising results with the highest lipid content (42%) when grown in wastewaters (Feng,*et al.*, 2011). This suggests a setup of wastewater high rate algal ponds (HRAPs) near the industrial areas to trap sustainable and renewable source of energy. Wastewater treatments HRAPs are presently the only economic and eco-friendly systems to produce biofuels (Park, Craggs and Shilton, 2011).

This excellent capability of microalgae gives it a huge potential to be used in wastewater treatment plants. The dairy effluents consist of milk, milk products and enormous quantity of water. The pH of the effluent is alkaline and the organic content is considerably high. The effluent affects the aesthetic value of the receiving water its alkaline pH causes damage to aquatic life (Jin Hur *et al.*, 2010).

3. Methodology

3.1 Sample collection and screening of microalgae

Water and soil samples were collected by random sampling method from dairy site, Anand, Gujarat. The algae were isolated and purified through serial dilution followed by cultivation in streak plates or liquid media methods respectively and regular observation under microscope (Figure 1).

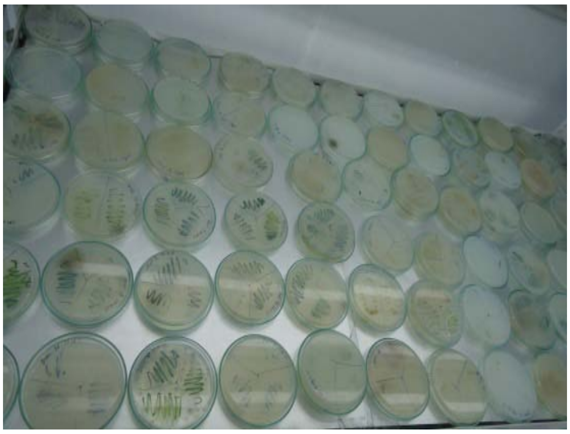

Figure 1: Isolation of microalgae from dairy wastewater habitat

3.2 Media composition

Samples were enriched in different modified media, Bold's Basal medium (Starr and Zeikus, 1993). The mineral salt medium contained (g/l): $NaNO_3$,1.5; K_2HPO_4,0.04; $MgSO_4.7H_2O$,0.075; $CaCl_2.2H_2O$,0.036; Citric Acid,0.006; Ammonium Ferric Citrate,0.006; EDTA Na_2; 0.001; Na_2CO_3,0.02;H_3BO_3,0.028;$MnCl_2.4H_2O$,0.0018;$ZnSO_4.7H_2O$, 0.0002; $Na_2MoO_4.2H_2O$,0.00039; $CuSO_4.5H_2O$,0.00008; Co $(NO_3)_2.6H_2O$,0.00005 at pH 6.6. BG11 medium (Rippka *et al.*, 1979) contained (g/l): $NaNO_3$,0.25; K_2HPO_4,0.075; $MgSO_4.7H_2O$,0.075; $CaCl_2.2H_2O$,0.025; KH_2PO_4,0.175;NaCl,0.025,EDTA,0.05;KOH,0.03;$FeSO_4$,0.0049;H_3BO_3,0.028;$MnCl_2.4H_2O$,0.0018;$ZnSO_4.7H_2O$,0.0002;$Na_2MoO_4.2H_2O$,0.00039;$CuSO_4.5H_2O$,0.00008;$Co(NO_3)_2.6H_2O$, 0.00004 at pH 7.5. The flasks were sterilized by autoclaving at 121 °C for 15 min at 15 Ibs pressure. These medium were used for further experiments.

3.3 Growth conditions of selected microalgae

Growth condition of microalgae were tested under laboratory conditions as follows: continues shaking of the strain at 150 rpm incubated under standard temperature condition of about 25±2 ˚C under a photoperiod of 12:12 h light dark cycle at light intensity of 35 µmol photon m^{-2} s^{-1}. To evaluate the suitably of dairy wastewater as enrichment medium for growing the selected SBC 39 and SBC 212, experiments were set up under control laboratory conditions with different proportions of wastewater (1%, 5%, 10%, 20%, 40%, 50% and 80%) by replacing BBM and BG11 medium (Figure 2).

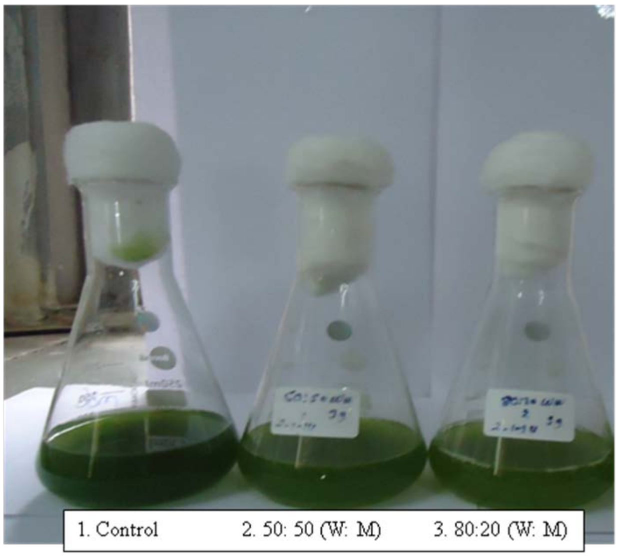

Figure 2: Growth of microalgae strains with different concentration of dairy wastewater with medium

3.4 Analytical method

Collected samples were observed under light microscope used for observing and images were captured with RADICAL RxLR-3 microscope fitted with camera and photomicrographic system. The identification was done using keys in standard monographs of Desikachary, (1959); Phillipose, (1967); Iyengar and Desikachary (1981).

The biomass of the entire cultures was measured by weighing dried sample of the culture suspensions. Filter papers (Whatman GF/C 0.7µm, 47mm in diameter) stored in a constant room temperature (23±2°C) were pre-weighed. Then, samples of 5ml of each alga culture were filtered through the filter papers. They were dried over night at 110°C and then weighed again at room temperature. The biomass content was calculated.

3.5. Determination of Lipids

Nile red (9-(Diethylamino)-5H benzo [∞] phenoxazin-5-one) has been shown to be quite useful in detecting neutral lipids in many different microalgae. The use of the Synergy™ H4 Multi-Mode Microplate Reader to monitor lipid levels in algal strains using fluorescence. Another method was used as solvents - lipid extraction method. Freeze-dried algal mass was extracted with methanol containing 10% DMSO according to Chiara *et al.*, (2002) method. The solvent with the biomass was heated at 45°C and stirred for 45 minutes and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant removed and the pellet was re-extracted with a mixture of diethyl ether and hexane (1:1 v/v). Added equal volume of water to the solvent mixture and supernatants so as to form a ratio of 1:1(v/v). The mixture was centrifuged again and the upper phase was collected. The water phase was re-extracted and the organic phases that contain total lipid were combined and evaporated to dryness under nitrogen protection. Thereafter, the total lipids were measured gravimetrically after freeze drying for 24 h.

4. Result and Discussion

Samples from diary wastewater were collected from dairy site Anand, Gujarat. Microscopic observations revealed that dairy wastewater supported an indigenous population of microalgae belonging to Cyanophycean, Chlorophycean and Bacillariophycean groups with filamentous forms being predominant. Species of *Oscillatoria*, *Phormidium*, *Spirulina*, *Leptolyngbya*, *Planktolyngbya*, *Geitlernema* sp. *Chroococcus*, *Chlorella*, *Scenedesmus* sp *Pithophora*, *Stigeloclonium*, *Navicula* and *Nitzschia* were isolated and maintained at SPRERI (Table 1 and 2).

Table 1: Physico-chemical parameters of different treatment tanks of dairy wastewater plant, Anand.

Parameters	*Site 1*	*Site 2*	*Site 3*	*Site 4*	*Site 5*	*Site 6*
pH	6.21	8.2	7.46	8.20	8.09	8.26
Temperature (°C)	30.1	29.7	29.8	29.9	29.9	29.9
Conductivity (µS/cm)	2264	1754	1821	1772	1830	1776
TDS (mg/l)	1600	1240	1290	1250	1300	1260
Salinity (mg/l)	1300	1010	1040	1020	1040	1020

Site: 1 Storage tank 1, Site: 2 Flotation tank, Site: 3 Storage tank 2, Site: 4 Clarification tank Site: 5 Pasteurization, Site: 6 Homogenization tank.

Microalgal distribution, richness and its nature depends on the quality of the wastewater. Therefore, the physico-chemical properties of all the sites were analyzed and documented (Table 1). The wastewater was varied from acidic to alkaline, pH between 6.2 - 8.26. The highest pH was observed in site number 6 and the lowest in storage tank 1. Conductivity and the concentrations of major ions were found varying widely from 1754 – 2264 in different sites. The salinity varied from 1010-1300 (mg/l) whereas the temperature ranged from 29.7-30.1 ˚C.

Table 2: Microalgae from different treatment tanks of dairy wastewater plant, Anand

S.No	*Organism*	*Collection sites*					
		S-1	**S-2**	**S-3**	**S- 4**	**S-5**	**S- 6**
	Cyanobacteria						
1	*Synechococcus* sp. 1	+	+	-	-	-	-
2	*Spirulina* sp.1	+	+	-	-	+	-
3	*Geitlerinema* sp.	-	+	+	-	-	-
4	*Plantolyngbya* sp	-	+	+	-	+	-
5	*Leptolyngbya* sp.	-	+	-	-	-	-
6	*Oscillatoria* sp.	+	+	+	-	-	-
7	*Phormidium* sp. 1	-	+	-	-	-	-
7	*Phormidium* sp. 2	+	+	+	+	+	+
8	*Phormidium* sp. 3	+	-	-	+	-	
	Chlorophyta						
9	***Scenedesmus*** sp	-	-	+	+	+	-
10	***Chlorella* sp**	-	-	+	-	+	-
11	*Oocystis* sp.	-	-	-	-	-	+
12	*Pithophora* sp.	-	-	-	+	+	+
13	*Stigeoclonium* sp.	-	-	-	+	+	-
14	**Bacillariophyta**						
	Navicula sp.	-	+	-	-	+	-
15	*Nitzschia* sp.	-	+	-	-	-	+

(+ present; - absent)

The comparative distribution of algal forms recorded in all the samples revealed the predominance of Cyanophycean, Chlorophycean and Bacillariophyceae members. Two promising microalgae namely SBC 39 (*Scenedesmus sp*) and SBC 212 (*Chlorella* sp) were selected from site 3 and 4 of dairy industries.

The analysis of physico-chemical properties of pre-treatment dairy effluent sample and post treatment effluent samples of the wastewater was followed by the method described by (Eaton *et al*., 1995). Physico-chemical properties of raw dairy wastewater are show in table 3:

Table 3: Physico-chemical properties of raw dairy wastewater

S. No.	*Parameters*	*Raw cheese whey water*
1.	Chemical oxygen demand (mg/l)	19,497
2.	Bio chemical oxygen demand (mg/l)	8950
3.	Total suspended solids (mg/l)	602
4.	Phosphorous (mg/l)	279
5.	Ammonical nitrogen (mg/l)	295
6.	Total organic carbon (mg/l)	29,434

Dairy wastewater was tested by selected microalgae strains and proved to be efficient in degrading the pollutant at a very fast rate as well as tolerant to grow against its toxicity. These findings are important regarding the practical use of such strain in large-scale bioremediation of dairy industries. Growth parameters of selected microalgae SBC 39 and SBC 212 were tested under different photoautotrophic conditions. The cheese whey stimulates the growth of both strains (Table.4).

Table 4: Effect of dairy wastewater using selected strains for microalgae biomass and lipid production

Strains	Medium	Biomass	Days	Total Lipid (%)	Days	Waste and medium combinations
SBC 39 *Scenedesmus sp*	BBM	0.8 ±0.01	6	24 ±0.01	10	80:20
	BG11	1.2 ±0.01	6	28±0.1	10	80:20
SBC 212 *Chlorella sp*	BBM	0.8±0.01	6	21±0.1	10	80:20
	BG11	0.9±0.01	6	23±0.1	10	80:20

(Errors presented here were standard deviation of duplicate experiments)

The combination of 80% wastewater and 20% BG11 medium supported the growth of SBC 39 and SBC 212 followed by 50% wastewater and 50% BG11. Biomass production was highest for 1.2 g/l and 0.9 g/l on 6th day. The highest lipid content was 28% and 23% in SBC 39 and SBC 212, respectively on 10th day. After cultivation of microalgae, the water samples were analyzed for physico-chemical properties. The present results are clearly indicating that a significant reduction in COD (87.43%), BOD (86.82) and TSS (83.98%), respectively. The content of phosphorus (44.36mg/l), ammonical nitrogen (20.73 mg/l) and total organic carbon (3701 mg/l) were also found to be decreased as compared to raw wastewater.

Ian Charles Woertz (2007) was tested microalgae in dairy wastewater and reported that lipid productivity of algae grown on dairy wastewater a possible feedstock for biodiesel. The total lipid content of the algae ranged from 8% to 29% of algal dry mass.

Woertz *et al*., 2009. Group worked on algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock at California Polytechnic State University. The lipid content ranged from 4.9%-29% and lipid productivity reached 2.8 g/m^2/d. which would be equivalent to 11,000 L/ha/yr (1,200 gallons/acre/year).

Kothari (2013) reported that production of biodiesel from microalgae *Chlamydomonas polypyrenoideum* grown on dairy industry wastewater. The lipid content of algal biomass grown on dairy wastewater on 10th day (1.6 g) and 15th day (1.2 g) of batch experiment was found to be higher than the lipid content of algal biomass grown in BG-11 growth medium on 10th day (1.27 g) and 15th day (1.0 g). The results indicating that the dairy wastewater found to be a good nutrient supplement and may be used for algal cultivation.

5. Conclusion

Algae grown on dairy wastewater media are a potential source of low-cost lipids for production of liquid biofuels. Microalgal distribution, richness and its depends on the quality of the wastewater. Therefore, the physico-chemical properties of six sites were analyzed and documented. The comparative distribution of algal forms recorded in all the samples revealed the predominance of Cyanophycean, Chlorophycean and Bacillariophyceae members. The data shows that the use of dairy wastewater as a replacement to synthetic media may reduce the overall cost of biofuel production. In future microalgae with dairy wastewater will be tested in suitable cultivation systems (photobioreactor and race way ponds) at outdoor conditions. It is mainly on the integrated approach of algae culture for oil-based biofuel production and bioremediation.

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