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Dairy Effluent to Irrigation Water: A Holistic Bioremediation Process Using Microalgal Treatment

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Abstract

Improper discharge of dairy wastewater in rivers and lakes without proper treatment causes environment pollution, contaminates water and has negative impact on human health. The high cost of nutrients and decreasing availability of fresh water are the major bottlenecks that currently hinder commercialization of microalgae. Dairy industrial waste water is one such source which when utilized for microalgae cultivation leads to bioremediation of waste water along with algal biomass. The development of rapid and cost effective cultivation methods of potential microalgae using industrial wastewater is still in nascent stage and must compete with the existing biological systems. A potential microalgal strain was isolated from dairy industrial effluent contaminated water and genetically identified as a close relative of Ascochloris sp (ADW007). Indoor microalgal cultivation studies were conducted in controlled conditions of light and temperature while outdoor pilot-scale experiments were performed in errant conditions using semicylindrical open barrels. The rate of biomass productivity of ADW007 was improved with raw dairy wastewater (RDW) as growth nutrient in indoor bench-scale (102.74±13.78 mg/L/d) and outdoor pilot-scale cultivations (206.19±4.59 mg/L/d) when compared with the algal growth in synthetic BG 11 medium (86±4.25 mg/L/d) and TAP medium (98.82±2.03mg/L/d), respectively. Similarly, in outdoor conditions the lipid content reached maximum to 35.1±2.65 % with volumetric and areal lipid productivities of 72.23±6.88 mg/L/d and 9.63±0.91 g/m2/d, respectively. With this the estimated annual algal oil production is nearly 31.4±2.99 KL/ha/yr or 20495±1953 gallons/acre/yr, if cultivated throughout the year. Post-harvesting process includes hollow fiber filtration followed by activated carbon treatment and resulted in 95±1%, 80±2 % and 99±0.3% reduction of Chemical Oxygen Demand (COD), nitrate and total phosphate (TP), respectively. The microalgal treated water was suitable for irrigation and meet the standards of CPCB.

Keywords

Dairy effluent; Microalgae; Bioremediation; Irrigation water

1. Introduction

The high cost of nutrients and decreasing availability of fresh water are the major bottlenecks that currently hinder and pose a great challenge for commercialization of microalgae. Dairy industrial waste water is one such source of nutrients and water which when utilized for microalgae cultivation leads to bioremediation of waste water in addition to algae biomass. Recently, use of microalgae for treatment of dairy industrial waste water for simultaneous removal of high concentration of nitrogen and phosphorus is well studied [1, 2, 3]. Though waste water treatment usually involves additional cost but if the treatment of waste water itself produces valuable raw materials, cleans water there by reducing water pollution and complies with international standards, it takes the industry in the direction of profitability and sustainability [1].

Wastewater effluents from dairy industries are rich in ammoniacal nitrogen content, organic and inorganic phosphates and other valuable nutrients which support microalgae cultivation and growth. Moreover, the integration of microalgal cultivation and bioremediation processes reduces the overall capital expenditure on post-treatment processes of wastewater for safe discharge.

Evaluation of rate of algal biomass and lipid productivity are two important parameters that need to be considered during microalgal cultivation in order to test the feasibility of employing dairy wastewater in culture medium.

The study conducted here evaluates the potential of an isolated microalga *Ascochloris* sp. ADW007 for

utilization of raw dairy wastewater in indoor as well as outdoor cultivation systems, simultaneous nutrient removal along with high biomass and lipid productivity. No studies have been reported on bioremediation of dairy waste water using *Ascochloris* sp. and its growth, biomass and lipid productivity. Here we have attempted to evaluate the outdoor cultivation of microalga / Ascochloris without external supplementation of CO_2, no sterilization of the dairy wastewater or addition of bacterial inhibitors, pH adjustments or addition of other carbon sources or nutrients. A simple cost-effective batch-type outdoor cultivation system was developed that could be easily scaled up for mass production of microalgal biomass. Besides evaluation of lipid productivities, this study also involves generation of utilizable water after treatment of highly turbid, high pollutant dairy wastewater which meets the Indian Central Pollution Control Board water quality standards designated for Type E water. Overall the process developed here is simple, integrated, low-cost producing zero waste generating main products in terms of high lipid containing (oleaginous) microalgal biomass for biodiesel production and utilizable water for irrigation.

2. Materials and Methods

2.1 Medium and Chemicals

Synthetic media (BG 11 and TAP) were used for preliminary enrichment of microalgal biomass [4, 5]. Analytical grade chemicals were used for microalgal growth in synthetic media, lipid extraction studies and other biochemical experiments. Raw dairy wastewater (RDW) was obtained from Amul dairy, one of the largest dairy industry in India, located in Anand district, Gujarat, India. In this study, RDW represents undiluted 100% raw dairy wastewater, unless specified separately. Physico-chemical characterization of the RDW includes pH, conductivity, total nitrogen (TN), total phosphorus (TP), organic and inorganic phosphates, total hardness, ammonia, nitrates, Total Dissolved Solids (TDS), COD, sulphate, chloride and Sodium Adsorption Ratio (SAR) measurements [6].

2.2 Isolation and identification of microalgae

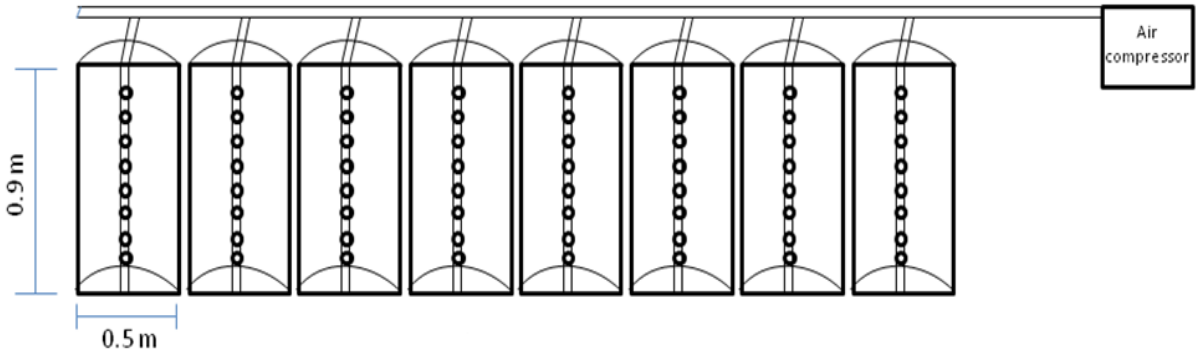
Microalgae samples were collected and isolated from nearby water bodies contaminated with dairy processing effluent treatment plants located in Anand district, Gujarat, India. Water samples were collected in sterile 50 ml sealed falcon tubes and were stored at 4 ^oC until isolation. Physico-chemical analysis of water samples were performed following the standard methods described in [7]. Primary screening was done by suitable dilution of samples in sterile distilled water and spread plating on a sterile agar media supplemented with BG11 medium. The petriplates were then incubated for a period of 7-15 days in a temperature regulated growth room maintained at 25± 1^oC in the presence of light (3978W/m^2) by providing light/dark cycle of 8h: 16h. The pure microalgal cells were isolated and then cultured and grown in BG11 medium for biomass enrichment under similar conditions as described above. 28S rDNA was isolated from 7 day BG11 grown microalga culture using plant DNA isolation kit (Qiagen). Consensus sequence of 574 bp 28S rDNA was generated using aligner software. BLAST analysis was performed using NCBI genbank database, multiple sequence alignment using Clustal X, distance matrix using RDP database and phylogenetic tree was constructed using MEGA5.

2.3 Microalgae growth

Growth studies were performed in both indoor bench-scale and outdoor pilot-scale conditions. Rate of algal growth, lipid production and nutrient removal from raw dairy wastewater were evaluated. Indoor bench-scale cultivations were performed in BG11, TAP and RDW as culture media in 25 L capacity photobioreactor. The growth containers were incubated for 5 to 17 days in a temperature regulated growth room maintained at 25± 1^oC and light/dark cycle of 8 h: 16 h. Outdoor cultivation studies were performed in small semi-cylindrical polypropylene troughs (0.9 m x 0.5 m). Maximum working culture volume of 60 L was achieved in each trough at 0.20 m depth. Scale-up cultivation was achieved at a total cultivation volume of 480 L in one batch using eight troughs placed in series adjacent to each other. Schematic representation of outdoor pilot-scale microalgae cultivation system is shown in Fig 1. 1 mm pore size perforated PVC tubes of 0.75 cm i.d. (inner diameter) were placed at the bottom of each trough and interconnected with valve junctions and regulators. At one terminal end of the tubes, an air compressor (capacity 0.35 hp) was connected for aeration in troughs. 10% (*v/v*) freshly grown algal culture was used as inoculum. Culture samples were withdrawn at regular intervals and evaluated for algal growth, biomass and lipid analysis and physico-

chemical properties of RDW were studied pre-microalgal treatment and post-microalgal treatment.

(A)



(B)

Figure 1. Outdoor cultivation of microalgae. (A) Schematic representation of outdoor pilot-scale microalgae cultivation. (B) Pictorial view of the cultivation system.

2.4 Post-harvesting process

Tangential flow hollow fiber filtration (TFF) was performed with a 0.2 µm Microza hollow fiber microfiltration cartridge (Pall filtration systems, USA) for enriching microalgal biomass concentration. The retentate *i.e.* concentrated biomass obtained from TFF treatment was collected and then passed through 200 GSM non-woven geotextile membrane and then the wet algal biomass was dewatered using horizontal batch-type solar tunnel dryer. The algal free treated water was passed through activated carbon filter (SUPRAcapTM^{50}, Pall filtration systems, USA). Physico-chemical properties of the treated water were studied at each step of post-processing.

2.5 Lipid analysis

Algal lipid was extracted following standard chloroform/methanol (1:1) extraction method [8]. Further to enhance the total lipid extract the dried biomass was subjected to microwave pretreatment (2400 hz) for 5 min with 30s pulse interval prior to chemical treatment [9]. The lipid fraction was separated and solvent was evaporated under vacuum.

2.6 Biomass measurement, growth rate and biomass productivity calculation

Microalgal culture samples grown in BG11, TAP and RDW were collected at regular growth intervals (24h) and were subjected to centrifugation at 10000 rpm for 15 min, and the pellet was dried at 60^{o}C to determine the microalgal biomass dry weight (g/L) [10]. Different equations used for calculating growth rate, areal and volumetric biomass productivity, lipid content, volumetric lipid productivity and estimated bio-oil production are shown in Figure 2.

2.7 Analytical methods

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Temperature, pH, electrical conductivity and TDS were monitored at regular intervals during microalgal growth using electrode probe based equipment (Eutech instruments Cyberscan series 600 Portable Meter). Light intensity for indoor and outdoor cultivation was measured using digital lux meter (TES-1332A, TES Electrical Electronic Corp. Taiwan). All the experiments related to microalgal growth, biomass and lipid contents, bioremediation and harvesting processes were performed for three times and the aggregated mean values are presented. To present the significance of the data in the current study, values of all samples are presented as mean ± SD, minimum and maximum by applying Minitab (Version 13.2, State College, PA., USA). An integrated process for microalgal cultivation using dairy waste water with simultaneous waste water bioremediation was demonstrated in the current investigation. The entire process flow with individual process steps are shown in Figure 3.

3. Results and Discussion

A potential microalgal strain designated as ADW007 was isolated from dairy effluent contaminated water bodies. Nucleotide blast analysis of ADW007 showed 81% sequence identity (Expect value 3e-92) with *Ascochloris multinucleata* UTEX 2013 26s and 28s ribosomal RNA gene sequences (NCBI GenBank ID: AF395492.1 and AF277652.1) (Fig.3) The gene sequence was submitted to NCBI with GenBank ID: KU725690.1

$$\ln \frac{x}{xo} = \mu t$$

$$\text{Areal Biomass Productivity} = \frac{(\text{Final culture density} - \text{Initial culture density}) \times \text{Working volume}}{\text{Batch run time} \times \text{Carpet area}}$$

$$\text{Volumetric biomass productivity} = \text{Areal biomass productivity} \times \frac{\text{Carpet area}}{\text{Volume of reactor}}$$

$$\text{Lipid content} = \frac{\text{Weight of lipid}}{\text{Weight of dry biomass}} \times 100$$

$$\text{Volumetric lipid productivity} = \text{Volumetric biomass productivity} \times \text{Lipid\%}$$

$$\text{Estimated annual biooil production} = \left(\frac{\text{Volumetric lipid productivity}}{\text{Lipid density}}\right) X \left(\frac{\text{No. of days of operation}}{\left(\frac{\text{area}}{\text{Volume of reactor}}\right)}\right)$$

Figure 2. Equations for calculating growth rate, areal and volumetric biomass productivity, lipid content, volumetric lipid productivity and estimated bio-oil production
Where x_o = initial biomass concentration, at time t_o; x = final biomass concentration and μ=specific growth rate of the culture

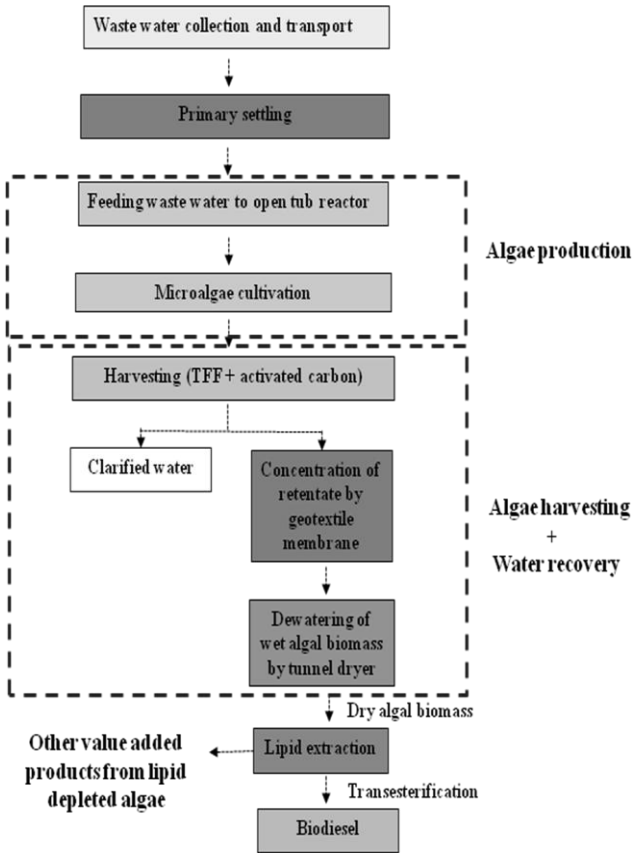

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Figure 3. Process flow diagram for different steps involved in the integrated approach for microalgae production using dairy waste water and post harvesting process

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Ascochloris multinucleata 28S ribosomal RNA gene, partial sequence	1057	1057	100%	0.0	100%	KU725690.1
Ascochloris multinucleata strain UTEX 2013 26S ribosomal RNA gene, partial sequence	350	350	75%	3e-92	81%	AF395492.1
Ascochloris multinucleata strain UTEX 2013 28S ribosomal RNA gene, partial sequence	350	350	75%	3e-92	81%	AF277652.1
Uncultured soil fungus clone NCD_LSU_otu3790 28S ribosomal RNA gene, partial sequence	348	348	71%	1e-91	82%	KF568760.1
Dunaliella salina strain CCAP 19/3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, com	340	340	71%	2e-89	82%	EF473744.1
Dunaliella tertiolecta isolate PL1 28S ribosomal RNA gene, partial sequence	337	337	69%	2e-88	82%	KC415758.1
Dunaliella salina strain CCAP 19/18 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, co	337	337	69%	2e-88	82%	EF473746.1
Dunaliella tertiolecta strain Dtsi 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, comple	337	337	69%	2e-88	82%	EF473730.1
Stephanosphaera sp. UTEX-LB 2409 strain UTEX LB 2409 26S ribosomal RNA gene, partial sequence	329	329	70%	4e-86	81%	AF395512.1
Dunaliella parva strain UTEX LB 1983 28S ribosomal RNA gene, partial sequence	329	329	68%	4e-86	82%	AF183473.1
Dunaliella parva strain SAG 19-1 26S ribosomal RNA gene, partial sequence	329	329	68%	4e-86	82%	DQ015726.1
Gongrosira papuasica strain UTEX 1916 26S ribosomal RNA gene, partial sequence	326	326	70%	5e-85	81%	DQ015756.1
Dunaliella tertiolecta 28S ribosomal RNA gene, partial sequence	326	326	66%	5e-85	82%	AF036311.1
Chlorococcum diplobionticum strain UTEX 950 26S ribosomal RNA gene, partial sequence	324	324	64%	2e-84	83%	AF395505.1
Haematococcus zimbabwiensis strain UTEX LB 1758 28S ribosomal RNA gene, partial sequence	322	322	70%	7e-84	81%	AF183475.1
Dunaliella sp. CCMP1641 strain CCMP 1641 26S ribosomal RNA gene, partial sequence	320	320	65%	3e-83	82%	DQ015746.1
Chlorosarcinopsis bastropiensis strain UTEX 1698 26S ribosomal RNA gene, partial sequence	318	318	67%	9e-83	81%	DQ015736.1
Chlamydomonas applanata strain UTEX 225 26S ribosomal RNA gene, partial sequence	316	316	67%	3e-82	82%	AF395495.1
Hamakko caudatus gene for 26S rRNA, partial sequence, strain: KzCl-4-1	315	315	66%	1e-81	82%	LC066337.1

Figure 3. Nucleotide blast analysis of ADW007 for microalgal identification

3.1 Biomass growth and productivity

The growth rate of *Ascochloris* sp. ADW007 in bench-scale was highest in RDW followed by TAP and BG11 culture media (Table 1). The biomass yield (g/L) in bench scale studies for RDW, TAP and BG11 were 1.74±0.23, 1.68±0.03 and 1.46±0.07 respectively. Detailed biomass and lipid productivities are given in Table 1. All the calculations were based on dry weight basis (dwb) after measuring the moisture content of the wet biomass. The biomass production in BG11 in bench scale was reduced to 1.46±0.007g/L, which was nearly 19.1% lower compared to 100% RDW. However, when growth studies were performed in nutrient rich synthetic TAP medium, the biomass yield was elevated to 1.68±0.005 g/L on 7th day. Higher biomass productivities in RDW and TAP might be due to the presence of high amounts of ammoniacal nitrogen (0.1-0.3 g/L) in culture media. A series of individual troughs were placed side-by-side occupying a total area of 3.6 m^2 with maximum cultivation volume of 480 L (Fig 1 B). Even though there was undulation of illumination intensity and temperature in outdoor cultivation system, when compared to indoor cultivation the rate of biomass productivity in outdoor cultivation was significantly higher. Compared to the biomass productivity in synthetic BG 11 medium (86.82±4.25 mg/L/d) the growth rate of microalga was boosted significantly with RDW in indoor bench-scale (103.23± 13.78 mg/L/d) and outdoor pilot-scale cultivation (206±4.59 mg/L/d).

Table 1. Biomass and lipid productivities of *Ascochloris* sp. ADW007

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Cultivation Medium	Biomass (g/L)	Biomass productivity (g/L/d)	Areal biomass productivity ($g/m^2/d$)	Lipid (%)	Lipid productivity (g/L/d)	Areal lipid productivity ($g/m^2/d$)	Expected Bio oil yield (gallons/acre/year)
Indoor Bench Scale							
BG11	1.46±0.07	0.086±0.004	17.21±0.84	10.6±0.51	0.0091±0.002	1.83±0.03	2593.17±47.77
TAP	1.68±0.03	0.099±0.002	19.76±0.41	29.2±0.92	0.029±0.001	5.77±0.28	8190.27±398.93
RDW	1.74±0.23	0.103±0.013	20.55±2.76	31.7±1.35	0.032±0.005	3.69±2.69	9270.02±1568.44
Outdoor Pilot Scale							
RDW	1.44±0.03	0.206±0.0045	27.49±0.61	35.1±2.65	0.072±0.0068	9.63±0.91	20495.68±1957.79

Table 2. Physico-chemical characterization of raw dairy waste water and microalgal treated water

Parameters (mg/L)	CPCB (Type E)	RDW	Day 1	Day 7	Post-harvested water (Day7)		% reduction (over RDW)
					TFF treated	AC treated	
pH	6.0-8.5	3.69	5.26	8.5	9.68	9.61	--
DO	--	1.24	1.38	3.02	3.26	3.28	--
Biomass density (O.D).	--	--	0.23	3.01	--	--	--
Protein	--	--	38.61	85.33	--	--	--
Algae dry biomass (g/L)	--	--	0.23	1.44	--	--	--
Chemical oxygen demand	≤ 600	7110	6892	351	347	345	95
Total dissolved solids	≤ 2100	3720	3358	2870	ND	ND	23
Electrical Conductivity (mOhms/cm)	≤ 2250	1823	1897	2200	--	--	--
Chloride	--	199	183	100	100	98	51
Fluoride	--	4.833	4.10	0.46	0.44	0.38	92
Sulphate	≤ 1000	0.521	0.511	0.389	0.316	0.23	57
Boron (mg/L)	≤ 5	ND	ND	ND	--	--	--
Ammonia	--	46.5	36.22	5.56	4.91	4.85	89
Nitrate	--	137	115.80	43.01	30.62	27.7	80
Orthophosphate	--	31.0	23.2	0.57	Low	Low	100
Total inorganic phosphate	--	74.1	51.6	0.81	0.79	0.75	99
Total phosphate	--	105.1	73.1	2.01	1.96	1.83	99
Iron	--	5.17	4.69	0.96	0.85	0.84	84
Total hardness as $CaCO_3$	--	733	586	110	93	82	88
Calcium hardness	--	564	513	21	8.10	7.84	98
Magnesium hardness	--	9.85	8.94	3.99	3.71	3.69	37
Sodium absorption ratio	≤ 26	42	38	21	--	--	50

3.2 Post harvesting processes

Although microalgal cells could be completely separated by centrifugation method; but it is an energy intensive process. Since TFF is a well known developed technology for microalgae harvesting, here we have selected it for microalgae harvesting. A 30x fold concentration of microalgal cells was achieved after 0.7 m^2 TFF at a flow rate of 67 L/h/m^2. The total suspended solids were increased from 1.2 % to 36.0%. No microalgal cells were detected in the flow through and all the cells were retained with 0.2 µm filter membrane. Within 4 h the initial feed volume of 30 L was reduced to 0.6 L. Further dewatering of the

concentrated retentate using a geotextile membrane resulted in thick wet biomass containing 90 % moisture content.

3.3 Lipid production

The lipid contents from 7th day grown culture of ADW007 in BG11, TAP and RDW under indoor cultivation conditions were 10.63±0.51 %, 29.2±0.91 % and 31.7±1.34 % (*v/v*), with a volumetric productivity of 9.1±0.16 mg/L/d, 28.8±1.4 mg/L/d and 32.6±5.5 mg/L/d, respectively (Table 1). The lipid productivities obtained from this study were the year. Moreover, as compared to side-by-side the areal productivities and estimated bio-oil yields from indoor cultivation were lower than the outdoor cultivation conditions as shown in the Table 1. These clearly indicate scale-up outdoor cultivation has significantly improved the total bio-oil production utilizing raw dairy wastewater as sole source of growth nutrient for liquid biofuels applications.

3.4 Nutrient removal

Over 95±1 % of COD was reduced from the raw dairy wastewater after 7 day of microalgal cultivation both in indoor and outdoor cultivation. Maximum reduction of COD, total nitrogen and total phosphorus was 965±16, 19.7±1.2 and 15.1±0.9 mg/L/d in the treated water of 100 % RDW was observed (Table 2). The initial fluoride content in dairy wastewater was 4.83±0.8mg/L and nearly 92 % removal was seen in 7 days indicating ADW007 could effectively remove fluorine from fluoride contaminated waters. Besides, the physico-chemical characteristics of activated carbon treated water were within the limits of the Indian water quality standards of Central Pollution Control Board (CPCB) Type E water designated for irrigation usage (Table 2). Physico chemical characterization of raw dairy waste water and its comparison with microalgal treated water has been listed in Table 2.

4. Conclusion

The current study provided proof-of-concept for an integrated process comprising of a newly isolated microalgal strain belonging to *Ascochloris* sp. ADW007 in dairy waste water along with nutrient removal, high biomass and lipid productivity in a simple outdoor cultivation system. COD reduction and fluoride removal in dairy waste water reached comparatively higher than [11], where maximum of 29 % of lipid was produced using dairy wastewater in 6 d grown culture with a volumetric productivity of 17 mg/L/d. Besides, a 3-fold lower lipid was produced in BG11 medium compared to TAP and RDW. On the other hand, in outdoor conditions higher lipid yields were achieved in 7 days with a lipid concentration and volumetric and areal productivities of 35.1±2.65 %, 72.2±6.8 mg/L/d and 9.63±0.91 g/m^2/d respectively. This would be equivalent to 31.4±2.99 kL/ha/yr (20495±1953 gallons/acre/yr) of algal oil, if cultivated throughout

>90% after ADW007 treatment for period of 7 days. Pilot-scale cultivation studies were performed in outdoor conditions without external supplementation of additional nutrients and CO_2 in low-cost polypropylene semi- cylindrical barrels. With a biomass and lipid yields of 1.44±0.03 g/L and 35.1±2.65 g/L in 7 days using 100% RDW makes ADW007 as a potential 3rd generation biofuel feedstock for scale-up studies. Overall, this study gives a glimpse on an integrated approach on waste-to-biofuel and utilizable water that mainly has zero environmental concerns, provides a possible solution for availability of clean water and alternative renewable feedstocks for liquid fuels to meet the demand of developing countries such as India which is rapidly increasing its industrialization process.

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6. References

[i] Hena, S., Fatimah, S., Tabassum, S., (2015) Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resource Ind 10:1–14.

[ii] Lu, W., Wang, Z., Wang, X., Yuan, Z., (2015) Cultivation of *Chlorella* sp. using raw dairy

wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresour Technol 192:382-388.

[iii] Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., Xu, J., Yuan, Z., (2016) Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. Environ Sci Pollut Res Int 23:8379-8387.

[iv] Gorman, D.S., Levine, R.P., (1965) Cytochrome and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardi*. Proc Natl Acad Sci USA 54:1665-1669.

[v] Stanier, R.Y., Kunisawa, R., Mandel, M., CohenBazire, G., (1971) Purification and properties of unicellular bluegreen algae (Order Chroococcales). Bacteriol Rev 35:171-205.

[vi] Tchobanoglous, G., Burton, F. L., Stensel, H. D., (2003) In Metcalf and Eddy, Inc. Waste water Engineering, Treatment and Reuse. 4th edition. McGraw Hill Companies, Inc. USA.

[vii] American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1998. Standard Methods for the Examination of Water and Wastewater 20th Edition. United Book Press, Inc., Baltimore, Maryland.

[viii] Axelsson, M., Gentili, F., (2014) A single-step method for rapid extraction of total lipids from green microalgae. PLoS ONE 9: e89643.

[ix] Lee, J.Y., Yoo, C., Jun, S.Y., Ahn, C.Y., Oh, H.M., (2010) Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 101: S75–S77.

[x] Ding, J.F., Zhao, F.M., Cao, Y.F., Xing, L., Liu, W., Mei, S., Li, S.J., (2014) Cultivation of microalgae in dairy wastewater without sterilization. Int J Phytoremediation 17:222–227.

[xi] Woertz, I., Feffer, A., Lundquist, T., Nelson, Y., (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng 135:1115-1122.