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Research Article

UTILIZATION OF DAIRY WASTEWATER FOR POLLUTANTS REMOVAL AND HIGH LIPID BIOMASS PRODUCTION BY A NEWLY ISOLATED MICROALGAL STRAINS *CHLOROMONAS PLAYFAIRII* AND *DESMODESMUS OPOLIENSIS*

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Abstract

Two new unicellular microalgal species from milk processing unit situated at Anand, India were screened and tested for their growth rate and nutrient removal capability under dairy wastewater at outdoor conditions in a simple column aeration photobioreactor. Results of 18s rRNA sequence analysis indicated that these strains have a close relationship with *Chloromonas playfairii* and *Desmodesmus opoliensis* named as SBC 2 and SBC 3, respectively. Both the strains removed more than 92% of ammonical nitrogen and 99% of total phosphorus, whereas COD and BOD reductions were found to be more than 95%. Maximum biomass production was 1.7 and 1.2 g L^{-1} and lipid content was 15 and 12%, respectively from SBC 2 and SBC 3 after 15 d cultivation. This study demonstrates the potential of algae based biofuel production by coupling wastewater treatment with microalgae cultivation for high lipid containing biomass production using *Chloromonas playfairii* and *Desmodesmus opoliensis*.

Keywords: Microalgae; column aeration photobioreator; wastewater treatment; pollutant removal; biomass production; lipid content.

Introduction

Algae have recently received growing attention given its prospects as a source of renewable energy and its potential for CO$_2$ capture. A large quantity of water is consumed during microalgae cultivation, which occupies 10-20% of the total cost of algae production (Subhadra *et al*., 2011). The production of biofuels based on microalgae as feedstock is also associated with a high demand of nutrients, mostly nitrogen and phosphorus. Therefore, the major cost contributory factors for microalgal cultivation are nutrients and water supply. Indeed, due to the high cost and energy associated with chemical fertilizers as a source of nutrients, and given the relative unavailability of fresh water, inexpensive and easily accessible alternatives are necessary for sustainable microalgae based biofuels (Lam *et al*., 2012).

Wastewaters have been proposed to as an alternative to more expensive organic carbon sources (Abreu *et al*., 2012). The common inorganic compounds in wastewater are chloride, hydrogen, irons, nitrogen, phosphorus, sulfur and trace amounts of heavy metals (Muttamara *et al*., 1996). Many studies have focused on nutrient removal by micro algae from municipal wastewater, agricultural wastewater, industrial wastewater, brewery wastewater and dairy wastewater and dairy effluent (Roberts *et al*., 2013). Dairy industry is one of the major industries having economic

importance in agricultural sector. India is sharing about 13.1% of the total milk produced in the world (Sukumaran *et al*., 2014). There are 281 large and small scale dairy industries in India responsible for discharging of large quantities of solid and liquid wastes. Specifically, dairy industry is noted as one of the significant contributor to water pollution. Dairy industry generates 0.2-10 L of effluent per litre of milk processed (Sukumaran *et al*., 2014). Dairy wastewater is characterized by strong color, offensive odor, high BOD (40-48,000 mgL^{-1}), high COD (80-95,000 mg L^{-1}) Kushwaha *et al*., (2011). It also contains sufficient amount of nitrogen and phosphorus for microalgal growth. Generally, the dairy wastewater is treated in anaerobic digesters to remove the high organic loads that are a potential source of organic carbons. These organic carbons are largely used by microalgae for their metabolic requirement (Wang *et a*l., 2010).

In recent years, the use of microalgae in treatment and recycling of wastewater has attracted great interest due to their role of carbon oxide fixation and bioremediation. Microalgae can perform the dual role of wastewater bioremediation and high biomass and lipid production (Farooq *et al*., 2013).Thus, it is essential to have microalgae which can perform the dual role of wastewater treatment and high lipid containing biomass production which will

eventually leads to a promising route towards developing an economical production of biofuel.

Although closed algae cultivation systems and controlled conditions can provide suitable environment in terms of light, nutrients and temperature and they have the potential higher rates of biomass production and improved culture quality but they are expensive to operate at large scale. The design of a simple system utilizing natural sunlight efficiently under uncontrolled conditions is a real challenge. In the present study dairy wastewater from a milk processing industry was evaluated for pollutants removal and high lipid containing biomass production at outdoor conditions by a newly isolated microalgal strains using simple column aeration photobioreactor.

Materials and Methods

Organism and Growth Conditions

Microalgal strains used in the present study were isolated from water samples from dairy industry site, Anand, Gujarat. The samples were collected aseptically from sites that appeared to contain algal growth and were enriched in sterilized Blue-Green (BG 11) medium as described by Rippka et al., 1979. Initial screening was done based on morphological identification at Sardar Patel Renewable Energy Research Institute (SPRERI), later the algal strains were sent for 18s ribosomal RNA gene sequencing YAAZH XENOMICS, Chennai. The purified microalgal strains were maintained in 1.5% agar BG 11 medium.

Seed Cultivation

The microalgae seed were cultivated in 2000 mL autoclaved BG 11 medium in 3000 mL round bottom flask. Individual purified colonies were inoculated into the medium and the flasks were incubated in a growth chamber at $25 \pm 2°C$ under a photoperiod of 12:12 h light dark cycle at light intensity of 35 µmol photon $m^{-2} s^{-1}$.

Dairy Wastewater Collection

Dairy wastewater was collected from a local milk processing industry situated in Anand, Gujarat, India. The sample was filtered using ordinary cloth filter to remove suspended solids prior to microalgal cultivation and stored at 4°C until further used.

Determination of Physico-Chemical Characteristics of Dairy Wastewater

Physicochemical characteristics of dairy wastewater analyzed every 5th day starting from 1st day of inoculation upto 15 days according to the standard method given in APHA 1998. Nutrient consumption was evaluated by measuring their variations by collecting the samples at regular intervals till the last day of the experiment. Each sample was centrifuged at 5500 rpm for 30 min and supernatant was collected for analysis of other parameters such as nitrate, ammonia, phosphate, chloride, fluoride, sulphate, iron, total hardness, magnesium hardness, calcium

hardness according to standard methods. Total phosphorus content was determined colorimetrically following the UV/Vis spectrophotometric method (Wei et al., 20012). Reduction in organic pollution load was calculated by measuring the chemical oxygen demand (COD) and biological oxygen demand (BOD) before and after micro algal growth. Change in pH during algal growth was measured by using a Eutech Cyberscan pH510 (Eutech instruments, The Netherlands). Lipid content was measured by the method described by Chiara et al., 2002. Microalgae dry weight gram per litre (g L^{-1}) was measured according to the method previously reported by APHA 1998. Microalgal cells were harvested by centrifugation and washed twice with deionized water. Microalgal pellets were dried overnight at 105°C for dry weight measurement. All parameters were estimated in triplicates and data are expressed as mean ± SD.

Indoor Experiments

Suitability of dairy wastewater as an enrichment medium for growing microalgae i.e. SBC 2 (Chloromonas playfairii) and SBC 3 (Desmodesmus opoliensis), preliminary experiments were set-up in the laboratory at different proportions of dairy wastewater and BG 11 medium. The tested ratios of dairy wastewater to BG 11 were 10:90, 20:80, 40:60, 50:50 and 80:20, respectively. BG11 medium was used as a control. The experimental flasks were inoculated with 10% (v/v) inoculum. The other supportive conditions were as described in section 2.2.

Outdoor Experiments

To confirm the laboratory findings, two bench scale column aeration photobioreactor were designed, fabricated and tested with 80% dairy wastewater and 20% BG11 medium combination (Fig.1). Each reactor was fabricated with 5 mm thick acrylic material. The height of the reactor was 1220 mm and diameter was 300 mm. There was a capillary tube inserted in the centre of the reactor with a dimension of 1500 mm height and 12 mm diameter. The total volume of the reactor was 86 L and the effective volume of the reactor was 60 L. Total area of the reactor was 1.23 m^2. The reactors were operated at ambient conditions for 15 days. Samples were collected at regular intervals till to estimate the nutrient removal and biomass and lipid production.

Results and Discussion

Isolation and Identification of Microalgae

A total of 15 algal cultures were isolated from a dairy industry site, Anand, Gujarat, India by serial dilution method and were purified by plating and regular observation under microscope. Out of 15 cultures, two microalgal strains (SBC 2 and SBC 3) were selected based on their nutrient removal capacity and biomass and lipid production in the laboratory (data not shown). Ribosomal RNA gene sequencing from the isolates was searched against GenBank using BLAST. The phylogeny analysis of

sequence was performed followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequences (Edgar 2004). The resulting aligned sequences were cured using the programme Gblocks 0.91b. This Gblocks eliminates poorly aligned positions and divergent regions (remove alignment noise) Talavera *et al.*, 2007). Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as substitution model. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering (Dereeper *et al.*, 2008). Based on sequence data the two

microalgal strains SBC 2 and SBC 3 were closely related to *Chloromonas playfairii* and *Desmodesmus opoliensis* based on 97 and 100% sequence similarities, respectively (Fig.1 & 2).The nucleotide sequence was submitted to NCBI database and the GenBank accession numbers for SBC 2 and SBC 3 are KT360945 and KT360946, respectively.

Characteristics of Dairy Wastewater
The physico-chemical characteristics of dairy wastewater are shown in Table 1. The samples were analyzed after micro algal growth and the data was shown in Table 2 and 3.

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ATGAAGCCTCTACCCAGTTGCGGCCCATATAACGGGTCCTTGGTTAGCCTCTGCACTTCAGCTGATCCAG
CCTATGATGTTACTCGTGCGCTGGAACAACCAGATCCACATCCGTATACCCACAGGGAGTATGGGGAGTG
ATATTAACCCGACACTGAGGCAGACATGCTCTCGGCCGAAGCCTCGAGCGCAATATGCGATTCAAAGATT
CGATGATTCACGGAATTATGCAATTCACACTACGTATCTCATTTCGCTGCGTTCTTCATCGTTGCGAGAG
CCAAGATATCCGTTGTTGAGAG
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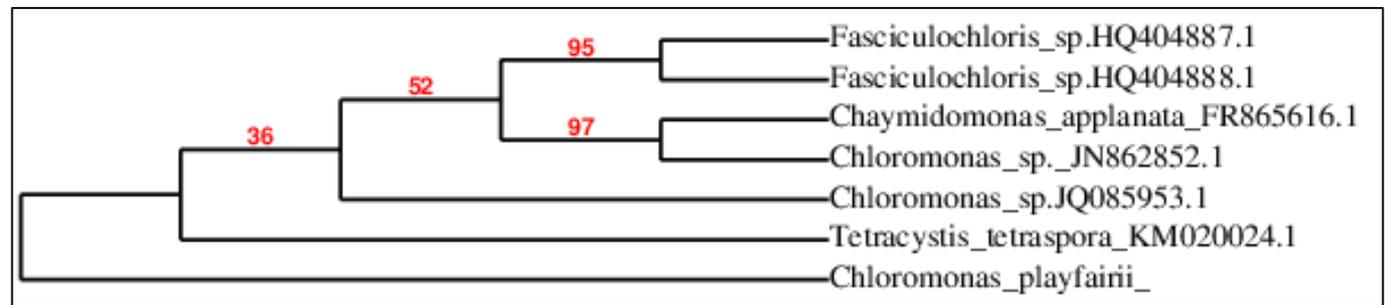

Fig.1: Sequence analysis of SBC 2 and phylogenetic tree

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TTTCGTTTGCATGCACACGCACTCTCTACTTGTGTACCGACGTTAGGTCAACACACGCAAGTGTGTGGCC
TACTAACCTACACACCATTGACCAACCACTGATCAAACCAAACTCTGAAGCTTTGACTGCCGTTAACCGG
CGGTTTTAACCAAGAACAACTCTCAACAACGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAA
TGCGATACGTAGTGTGAATTGCAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGCTCGACTC
CTCGGAGAAGAGCATGTCTGCCTCAGCGTCGGTTTACACCCTCACCCCTCCTCCCTACCAGGAGGCGCCT
ATCGTGCTTGCTCAAGCCGATAGCAGGGGTGGATCTGGCTCTCCCAATTGGATTCACCTCCGATTGGGTT
GGCTGAAGCACAGAGGCTTAAACTGGGACCCGATTCGGGCTCAACTGGATAGGTAGCAACACCCTCGGGT
GCCTACACGAAGTTGTGTCTGAGGACCTGGTTAGGAGCCAAGCAGGAAACGTGTTTCTAACACGTACTCT
GTATCGACCTGAGCTCAGTCAAGCAGCCCCGTCCTT
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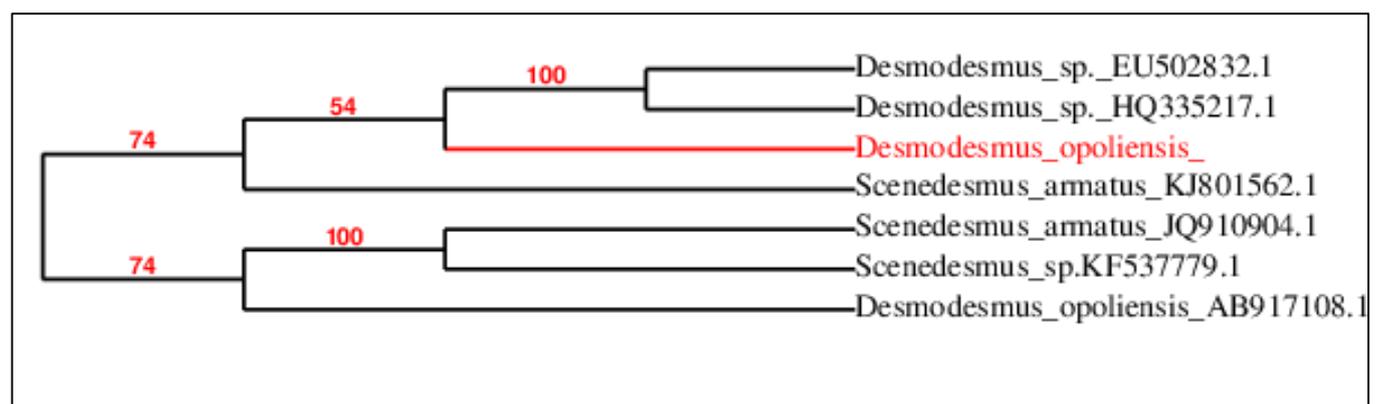

Fig. 2: Sequence analysis of SBC 3 and phylogenetic tree

Table 1: Physico-chemical characteristics of raw dairy wastewater

Parameters	Concentration
pH	6.24±0.3
Conductivity (ms)	12.17±0.5
COD (mg/L)	21690.00±3.5
BOD (mg/L)	8050.00±2.9
Total dissolved solids (mg/L)	7800.00±1.8
Chloride (mg/L)	1699.47±1.7
Fluoride (mg/L)	11.20±1.9
Sulphate (mg/L)	0.240±1.2
Nitrate (mg/L)	130.00±1.7
Ammonia (mg/L)	370.00±3.2
Total phosphate (mg/L)	400000.00±2.8
Iron (mg/L)	12.50±1.1
Total hardness as $CaCO_3$ mg/L	627.20±0.9
Calcium hardness as $CaCO_3$ mg/L	254.80±1.4
Magnesium hardness as $CaCO_3$ mg/L	372.40±0.8

Table 2: Pollutant reductions levels at different time points by SBC 2

Parameters	5th day	10th day	15th day
Chloride (mg/L)	886.00±1.1 (47.86)	836.77±1.0 (50.76)	864.69±0.9 (49.12)
Fluoride (mg/L)	1.50±0.9 (86.60)	1.46±0.5 (86.96)	0.00±0.6 (100.00)
Sulphate (mg/L)	0.11±1.0 (54.16)	0.10±1.0 (58.33)	0.06±1.2 (75.00)
Nitrate (mg/L)	37.00±1.6 (71.53)	30.00±1.5 (76.92)	34.04±1.2 (73.81)
Iron (mg/L)	2.60±1.4 (79.20)	2.40±1.5 (80.80)	2.23±1.0 (82.16)
Total hardness as $CaCO_3$ mg/L	276.00±0.5 (55.99)	234.14±0.8 (62.66)	181.26±0.4 (71.10)
Calcium hardness as $CaCO_3$ mg/L	64.18±1.3 (74.81)	44.21±1.1 (82.64)	28.46±1.0 (88.83)
Magnesium hardness as $CaCO_3$ mg/L	207.01±1.0 (44.41)	190.06±0.8 (48.96)	189.53±0.7 (49.10)

(Figures shown in brackets are % reduction)

Reduction of pollution load by SBC 2 and SBC 3 in dairy wastewater

As the laboratory results revealed that 80:20 combination reduced maximum amount of nutrients as well produced more biomass with high lipid content 1.9 and 1.4 g L^{-1} and lipid content of 17 and 11%, respectively from SBC 2 and SBC 3 microbial algal strains. Thus two bench scale column aeration photobioreactor (86 L) were operated at average ambient temperature for 15 days in 80:20 combinations (Fig.3 & 4). In view of the fact that microalgae could not grow well without air supply [15]. In the present study compressed air was supplied at a flow rate of 0.3 v/v/min (volume of air/volume of culture/min). Normally an aeration rate of 1 v/v/min is used in bench scale cultures (Sierra *et al*., 2008).

The data indicated that there was a gradual reduction in various parameters of the effluent treated with SBC 2 and 3, respectively (Table 2 & 3). During the experimentation changes in organic pollution load were monitored as COD, BOD and pH to evaluate the effect of algal growth on effluent treatment process. Initial COD and BOD of dairy wastewater were 21690 mg L^{-1} and 8050 mg L^{-1},

respectively. The COD of the dairy wastewater reduced from 29,000 mg L^{-1} to 2100 and 1400 mg L^{-1}, from SBC 2 and SBC 3, respectively after 15 days of algal cultivation. During the initial period the COD reductions from SBC 2 and SBC 3 microalgal strains were high as 72% and 71% (5th day) and latter (15th day) 96% and 90%, respectively was achieved (Fig. 5a & 5b). The slow reduction in COD reduction could be due to the presence of remaining carbon as some colloidal form or slowly biodegradable material (Sukumaran et al., 2014). Initial BOD value of 8050 mg L^{-1} was reduced to 70 and 110 mg L^{-1} from SBC 2 and SBC 3, respectively. In the present study higher COD and BOD removal efficiencies were observed compare to other researchers where the COD and BOD removal efficiencies were 88 and 89% [24] and 93 and 82%, respectively (Sukumaran et al., 2014).

Table 3: Pollutant reductions levels at different time points by SBC 3

| Parameters	5th day	10th day	15th day
Chloride (mg/L)	902.00±0.8 (46.92)	899.70±1.1 (47.05)	851.94±1.9 (49.87)
Fluoride (mg/L)	1.46±1.6 (86.96)	1.41±1.2 (87.41)	0.00±1.3 (100.00)
Sulphate (mg/L)	0.14±1.0 (41.66)	0.14±1.8 (41.66)	0.13±0.9 (45.83)
Nitrate (mg/L)	39.00±1.3 (70.00)	35.00±1.0 (73.07)	31.00±1.1 (76.15)
Iron (mg/L)	2.60±1.7 (79.20)	2.60±1.1 (79.20)	2.60±1.0 (79.20)
Total hardness as CaCO$_3$ mg/L	294.00±1.8 (53.12)	278.32±1.1 (55.62)	278.32±0.9 (55.62)
Calcium hardness as CaCO$_3$ mg/L	79.32±1.3 (68.86)	69.06±1.1 (72.89)	62.37±1.0 (75.52)
Magnesium hardness as CaCO$_3$ mg/L	211.68±1.5 (43.15)	203.84±1.8 (45.26)	199.92±1.0 (46.31)

(Figures shown in brackets are % reduction)
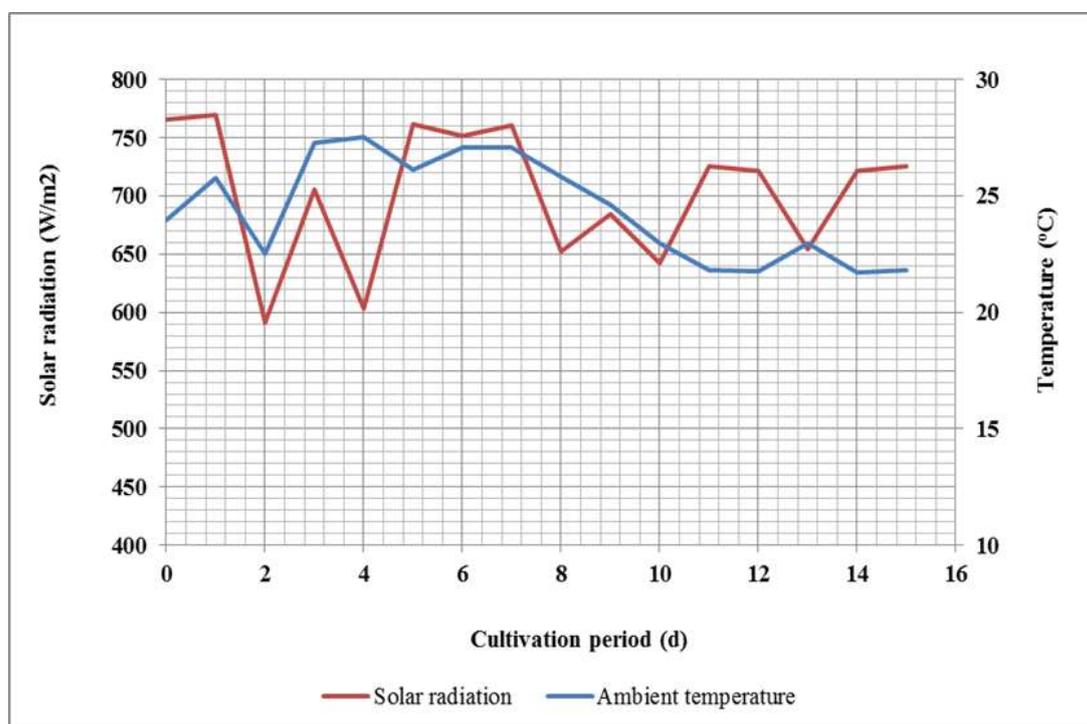
Fig. 3: The variation of irradiance and temperature of each day (10:00 am to 4:00 pm) under outdoor cultivation

Fig. 4: Cultivation of SBC 2 at outdoor conditions for the removal of pollutants from dairy wastewater
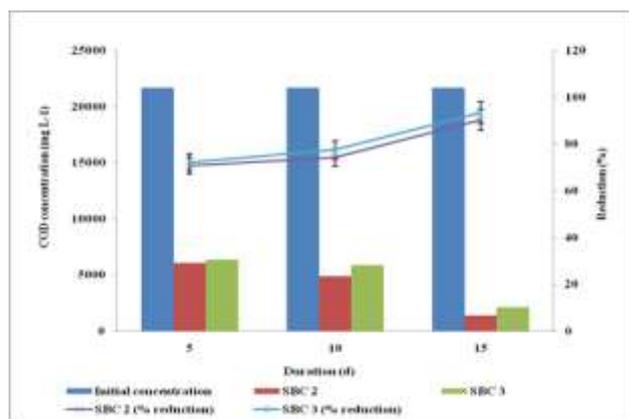

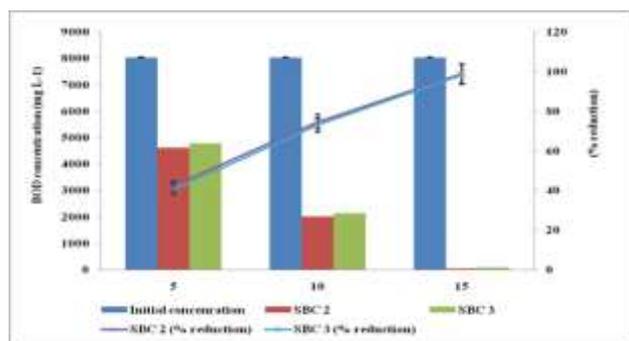**Fig.5a**: COD removal efficiency of SBC 2 and SBC 3

Fig. 5b: BOD removal efficiency of SBC 2 and SBC 3

One of the controlling factors to cultivate microalgae is the pH of the culture medium. Most algae are tolerant of fairy large changes in pH. Initial pH of the dairy wastewater was 6.4, which was gradually reached to 7.8-8.9 after growth of micro algae during effluent treatment process. pH should be in the range of 5.5-9.0 according to pollution control board standards and environmental protection. Sukumaran *et al*., 2014 also reported that pH was in the range of 7.5-8.8 when the dairy effluent was treated with *Chlorococcum* sp. RAP-13.

Nitrogen is an important nutrient during the process of microalgae growth. Nitrogen is also an important macro element contributing to biomass production and also a critical factor in regulating algal lipid content (Min-Kyu *et al*., 2013).It was also reported in the literature that ammonium is among the most common chemical forms of nitrogen that can be readily absorbed by microalgae (Shaikh *et al*., 2013). Since nitrogen can be utilized as nitrate, nitrite or ammonium the production of microalge biomass response varied in the present study. In the same manner phosphorus is also essential for algal growth and involved in many cellular process, especially orthophosphate which is the preferred form supplied to microalgae and also in the biosynthesis of nucleic acid in algal cells (Min-Kyu *et al*., 2013).
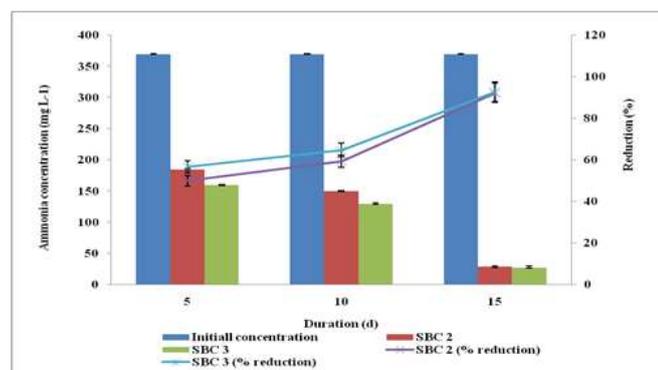
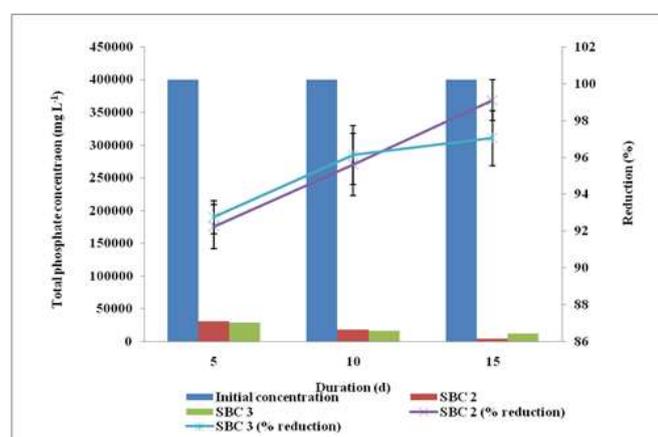**Fig. 6a:** Total ammonical nitrogen removal rates of SBC 2 and SBC 3

Fig. 6b: Total phosphate removal rates of SBC 2 and SBC 3

Table.2 and 3 show the removal of nitrogen, phosphorus and other pollutants from dairy wastewater by SBC 2 and SBC 3. It was observed that the concentrations of ammonical nitrogen and total phosphate were decreased rapidly due to their fast assimilation by SBC 2 and SBC 3 in the 5 days of cultivation and the ammonical nitrogen (> 92%) and total phosphate (>97%) were removed within 15 days (Fig. 6a & 6b). The concentration of nitrate (130 to 34 and 130 to 39 mg L^{-1}) and total phosphate (4,00,000 to 3560 and 4,00,000 to 11, 800 mg L^{-1}) were reduced by many folds in the effluent after treatment. Phosphate concentration decreased significantly, with more than 99% with SBC 2 and 97% with SBC 3, respectively (Fig.6b). The present results were in accordance with Franchino et al., 2013 who reported more than 94% removal. The nitrogen and phosphorus removal rates of Chlorella sp. have been reported to be 10.5 mg TN L^{-1}d^{-1} and 2.0 mg TP L^{-1}d^{-1} Aslan et al., 2006). Cho et al., 2011 reported that the concentrations of TN and TP in municipal wastewater were significantly decreased from 19.1 to 15 mg L^{-1} and from 3 to 0.2 mg L^{-1}, respectively after 9 days of Chlorella sp. cultivation. The present result demonstrates that removal nitrogen and phosphorus from dairy wastewater by microalgae in a controlled manner not only benefits rather than deteriorates the environment. On the other hand percent reduction in the level of fluoride and chloride were

almost similar from SBC 2 and SBC 3 (100 and 49%), respectively on 15th day of algal growth. The iron (2.23 and 2.60 mg L^{-1}) and fluoride (0.00 mg L^{-1}) concentrations were found in traces which are below the permissible limit. The permissible limit as per Indian standard specification for discharge of environmental pollutant for iron and fluoride were 3.0 mg L^{-1} and 2.0 mg L^{-1}, respectively.

Biomass and Lipid Productivity of SBC 2 and SBC 3 in Dairy Wastewater

The two microalgal species were tested for lipid production by evaluating biomass productivity and lipid content. The algal biomass was harvested on 15th d and was measured in terms of dry weight. SBC 2 showed the highest biomass production of 1.7 g L^{-1} while SBC 3 had the lowest biomass production of 1.2 g L^{-1}, respectively. The lipid content of SBC 2 was 15% of its dry weight, which was slightly higher than that of SBC 3 (12%), respectively. The important factor in the increase of the biomass and lipid content was the supply of organic carbon sources. The carbons available in wastewater are a source of alternative organic carbons. The results revealed that the presence of organic carbon indeed had a positive effect on the biomass and lipid productivity. Lipid composition and productivity depend on the growth conditions in the growth phase. The total lipid content varies between species ranging from very low (94.5%) to very high (60%). Biomass yield of 0.8 g L^{-1} was obtained when Chlorococcum sp. RAP.13 was cultivated under dairy effluent at mixotrophic conditions, while it increased to 1.478 and 1.94 g L^{-1} in heterotrophic conditions when supplemented with 4 and 6% biodiesel industry waste glycerol. The results also revealed that SBC 2 and SBC 3 at indoor conditions produced slightly higher biomass and lipid compared to the outdoor conditions. The higher biomass and lipid production in indoor level can be attributed to the controlled environment provided during the cultivation period. Weidong et al., 2015 also reported that higher biomass and lipid production were achieved in the indoor conditions compared to outdoor conditions may be due to fluctuation in the solar radiation and temperature when dairy wastewater was treated with Chlorella sp.

Growing algae in wastewaters for effective removal of nutrients as well as algal biomass accumulation can contribute a lot to the management of freshwater ecosystem by providing an environmentally sustainable approach. It was reported that the microalgae have been used as an alternative way to remove the pollution load from waste waters particularly organic carbon, nitrogen and phosphorus, which are unsuitable for water bodies. Thus the present investigation demonstrates that the tested microalgal strains were able to achieve similar or high biomass productivity and higher removal efficiencies in less residence time than reported earlier (Table 4). However, further exploration of practical yields of biodiesel from the

selected consortium is needed to turn the hypothesis into reality.

Table: 4 Bioremediation potential of dairy wastewater by various microalgal strains

Type of wastewater	Micro algal strain	Concentration of pollutants (mg L⁻¹)	Pollutants removal efficiency (%)	Biomass productivity (g L⁻¹ d⁻¹)	Cultivation period (d)	Reference
Dairy wastewater (Influent) (Indoor)		Total ammonical nitrogen: 282; Total dissolved phosphate: 1.51	Total ammonical nitrogen:60; Total phosphate: 87	0.45	15	[10]
Dairy wastewater (Effluent) (Indoor)	*Chlorella pyrenoidosa*	Total ammonical nitrogen: 282; Total phosphate: 21	Total ammonical nitrogen:43; Total phosphate: 83	0.32	15	[10]
	Chlorococcum sp. RAP-13 (Mixotrophic cultivation)			0.06	12	
Dairy effluent (Indoor)	*Chlorococcum* sp. RAP-13 (heterotrophic cultivation with addition of biodiesel waste glycerol)	COD: 984; BOD: 37	COD: 93; BOD: 82	0.12	15	[11]
Dairy wastewater (outdoor)	*Chlorella* sp.	COD: 2593; Total nitrogen: 283; total phosphorus: 115	COD: 54.82; total nitrogen: 83.83; total phosphorus: 65.33	0.11	8	[30]
	Chloromonas playfairii- SBC 2	COD: 21690; BOD:8050; Total ammonical nitrogen: 370; Total phosphate: 400000	COD: 92; BOD: 99; Total ammonical nitrogen: 92; Total phosphate:99	0.11	15	Present study
Dairy wastewater (outdoor)	*Desmodesmus opoliensis*- SBC 3	COD: 21690; BOD:8050; Total ammonical nitrogen: 370; Total phosphate: 400000	COD: 92; BOD: 99; Total ammonical nitrogen: 92; Total phosphate:99	0.08	15	Present study

Conclusion

The result in the present study showed that it is feasible to use dairy wastewater to cultivate microalgae for pollutants removal as well high biomass with high lipid production. Most importantly, the study also demonstrates that the in-house microalgal strains were found able to grow when grown under outdoor conditions (uncontrolled environment- i.e. fluctuation in the temperature and irradiance levels inherent in outdoor cultivation) thus can cut down the energy input and algal biomass production cost. Further experiments are in progress in large scale microalgae cultivation systems by using 100% replacement of synthetic medium.

Acknowledgements

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