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Eco-Friendly Approach for Treating Dairy Effluent and Lipid Estimation Using Microalgae

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Authors' contributions

This work was carried out in collaboration between all authors. Author ASR has contributed for the concept development, author HRVNGR has designed the study, authors ABK and MS conducted the experimental work, drafted the manuscript and involved in literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: In this study, an attempt has been made to examine the utility of these species in treating dairy wastewater. Bioreactor was studied using immobilized *Chlorella vulgaris* and *Anabaena ambigua* to treat dairy effluent.
Study Design: The entire study including the treatment and filtration was conducted in Centre for biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, between November 2014 to February 2015.
Methodology: The treatment of dairy effluent consists of two stages; the first stage includes dairy effluent treatment using immobilised *Chlorella vulgaris* and *Anabeana ambigua*, while the second stage involves sand bed and coal bed filtration.
Results: *Chlorella vulgaris* reported a high lipid content of 12% when compared with *Anabaena ambigua* 5% after the cultivation period. Whereas the protein content of *Anabaena ambigua* (40%) was higher than *Chlorella vulgaris* (28%) when compared with after harvesting. Whilst ammonium nitrate was completely removed by bead treatment it was 96% reduction when treated with *Chlorella vulgaris*. A 98% removal of phosphates was achieved on an average after algal bead

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treatment for both species in both modes of operation. Moreover, a significant decrease in BOD and COD was achieved by this method.

Conclusion: It can be concluded that, the cultivation of algae in dairy effluent results the combined advantages of treating the dairy effluent and also producing algal biomass, which can further use of food additives for aquatic culture, animal and human feed, energies such as biogas and fuels and bio-fertiliser.

Keywords: Chlorella vulgaris; Anabaena ambigua; lipid; dairy effluent.

1. INTRODUCTION

In India milk is distributed to the consumers directly by the farmers. On the other hand, the remaining part is processed in dairy industry to manufacture various milk products like butter, cheese, yogurt, condensed milk, dried milk (milk powder), and ice cream etc with typical by-products such as buttermilk, whey and their derivatives. In India dairy industry is the major industry which causing water pollution [1]. Dairy industries require huge volumes of water for washing the equipments and floor which is causing polluted water from manufacturing process, utilities and service sections. Whilst most of the waste generation is from a dairy industry it may be spoiled and skimmed milk, their disposal remains a problematic issue. The dairy effluent contains nutrients, high COD and BOD which causes the serious threat to the water quality and damage to the aquatic life [2,3]. Moreover, its high biological oxygen demand (BOD) depletes the dissolved oxygen content of the aquatic system and creates anaerobic conditions as a function of time and resulting in serious threat.

Industries like the dairy and brewery produce effluents with high organic compounds. For achieving this, conventional physical and chemical methods, for instance, primary treatment methods involve screening, skimming, coagulation and sedimentation of unwanted solids which is cost intensive. In contrast, biological treatment methods for the effluents are eco-friendly [1,4]. While the secondary treatment involves aerobic and anaerobic methods, novel methods need to be developed for the treatment of dairy effluents as low-cost waste treatment methods are the prime need for many countries.

Microalgae can be useful as treating agents for dairy effluents and wastewater. The cultivation of algae in dairy effluent results the combined advantages of treating the dairy effluent and also producing algal biomass, which can further use of food additives for aquatic culture, animal and human feed, energies such as biogas and fuels and bio-fertiliser [5]. The microalgae were used to remove different kinds of inorganic and related substances which have been studied by several workers [6,7,8]. Algae serve as indicators of water pollution since they respond typically to many ions and toxicants. *Chlorella vulgaris* is the most common and effective species for the immobilization and nutrient removal purposes [9,10].

Therefore, the present study aims at the analysis of physico-chemical properties of dairy wastewater treated with *Chlorella vulgaris and Anabaena ambigua,* which are potential agents for treating wastewaters. Moreover, the biomass composition of these algae was also studied which has a great potential to contribute for alternate energy source. Recent studies suggest the role of *Chlorella vulgaris and Anabaena ambigua* in high yield of lipids.

2. MATERIALS AND METHODS

Anabaena ambigua species (NCIM NO: 2785) was collected from National Centre for Industrial Microorganisms (NCIM), Pune, India and *Chlorella vulgaris species* was isolated from local pond and characterized. Stock culture of *Chlorella vulgaris and Anabaena ambigua* was grown photoautotrophically in media containing BG11 and maintained at 26ºC under continuous light in two 100 mL flasks separately. The BG11 media contained K_2HPO_4-0.04 g, $NaNO_3$-1.5 g, $CaCl_2 \cdot 2H_2O$-0.036 g, $MgSO_4 \cdot 7H_2O$-0.075 g, $NaCO_3$-0.02 g, Citricacid-0.006 g, H_3BO_3-0.00286 g, $MnCl_2 \cdot 4H_2O$-0.00181 g, $Na_2MoO_4 \cdot 2H_2O$- 0.00039 g, $CuSO_4 \cdot 5H_2O$-0.00008 g, $ZnSO_4 \cdot 7H_2O$-0.00022 g, $Co(NO_3)_2 \cdot 6H_2O$-0.00005 g, Na_2EDTA-0.00001 g, $(NH_4)_6Mo_7O_{24}.4H_2O$-0.003 g. From the stock culture the algal cells was inoculated in a two 1000 mL flasks containing 700 mL of fresh BG11 media separately at 26ºC for five days under continuous illumination of 35 µmol m^{-2} s^{-1} on an orbital shaker at 100 rpm. Then after 5 days the culture was used and inoculated 10% of volume

for the preparation of immobilized microalgal beads. The algal beads are done using 0.1 M Calcium chloride and 2% sodium alginate and the average diameter of the algal beads was 0.665 m. The dairy effluent is collected from A.P dairy industry, Hyderabad, Telangana, India.

2.1 Effluent Treatment

Stage1: Photo-bioreactor for dairy effluent treatment, 100gms of *Chlorella vulgaris and Anabaena ambigua* immobilized beads were weighed approximately and inoculated in five1-litre polycarbonate (PCB) bottles set on a wooden rack as shown in Fig. 1. A light intensity of 60 µmol m^{-2} s^{-1} was maintained at 26ºC for five days. Air supply is provided artificially through air pumps at 1 L min^{-1} for all 10 bottles continuously to ensure the suspension of immobilized beads in dairy effluent and thorough contact of immobilized beads with the nutrients present in dairy effluent. The filtered dairy effluent is analyzed for its biochemical composition.

Stage2: A two level sand bed and coal bed was prepared for the filtration of algal treated dairy water. The first level contains 3 layers of large, medium, small stones and pebbles each 5 cm and each layer is covered by fine sand of about 3 cm in height. In the second level of filtration a charcoal bed is spread up to 7 cm to 10 cm as shown in the Fig. 2. The algal treated water from the first level slowly drips into the second level and the treated water will be collected in a bottle after passing through coal bed. The treated dairy effluent after sand bed filtration, coal bed was again analyzed and compared with untreated dairy effluent (control).

2.2 Fish Collection

The specimens of *Danio rerio* (5–7 cm long; weight 1.50±0.25 g) were used for toxicity tests. These were obtained from a local aquarium shop, Hyderabad. The fishes were transported from the shopping polythene bags which are oxygenated to the laboratory and immediately transferred into 50 L capacity containing well – aerated un-chlorinated water. The fishes were allowed to acclimatize for 15 days before the experiments were conducted. The fishes were fed with fish food containing wheat flour, soya meal during the acclimation period. Fishes which are healthy and active were used for experiments. The dissolved oxygen, temperature and pH used for acclimatization was 5.0-6.5 mg/L, 30ºC, 7.0±0.2 respectively.

2.3 Carbohydrate Estimation

The carbohydrate content was analyzed based on the procedure by Anthrone method. 1.0 g of dried algal pellet was acidified by adding 200 mL 2.5 M HCl. The above acidified solution was then hydrolyzed at 100ºC for 30 min. Then the solution was neutralized to pH 7. Total volume was adjusted to 1000 mL. The filtered sample was subjected to Anthrone assay by Loewus [11].

2.4 Lipid Estimation

Extraction of lipid was done following the protocol of Bligh and Dyer [12]. The cells were harvested by centrifugation at 10,000 rpm for 10 min at 4ºC. The cells were washed once with distilled water and re-centrifuged. The pellet was, then, subjected to wet weight estimation and then dried in oven for 2 hours at 80ºC. For 1 g of algal biomass, 2 mL of methanol and 1 mL of chloroform was added and kept for 18 hours at 25ºC. The mixture was agitated in vortex for 2 min. 1 mL of chloroform was again added and the mixture was shaken vigorously for 1 min. After that, 1 mL of distilled water was added and the mixture was vortex again for 2min. The layers were separated by centrifugation for 10 min at 2000 rpm. The lower layer was separated and the procedure was again repeated with the pellet. The two supernatants collected were allowed to stand for 2 h. Lower organic layer with the lipids was transferred to a clean pre-weighed vial (W1). Evaporation was carried out in hot air oven at 80ºC for 50 min. The weight of the vial was again recorded (W2). Lipid content was calculated by subtracting W1from W2 and was expressed as % dry cell weight.

2.5 Protein Estimation

Protein is estimated using Lowry's method [13]. 0.1 g algal powder is taken and 2 mL of 5% TCA is added. The mixture is centrifuged at 2000 rpm for 10 min. Solution containing pellet is dissolved in 1.5 mL of 0.1 N NaOH. From the above prepared solution 0.2 mL is taken into 20 mL test tube and 5 mL of alkaline copper solution is added. After incubation at room temperature for 15 min, 0.5 mL of Folin - Ciocalten reagent is added. Again the mixture is incubated for 30 min and OD values were taken at 540 nm.

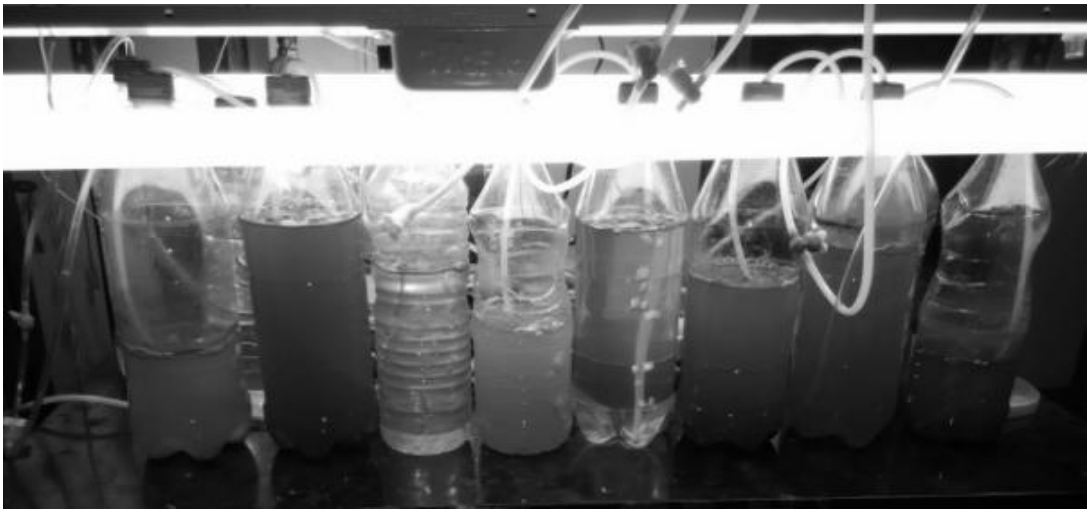

Fig. 1. Photobioreactor with immobilized algal beads

Fig. 2. Sand bead and coal bed apparatus

3. RESULTS AND DISCUSSION

3.1 Biochemical Analysis of Algal Biomass

After five days of the growth period the algal beads of both *Chlorella vulgaris* and *Anabaena ambigua* were separated from the dairy water using nylon filter cloth. Earlier studies mentioned that even at high concentrations (upto 900 mg/L) of phosphates, algal immobilized beads tend to be stable in acidic pH ranges up 4 to 15 days *Dainty* [13]. Hence the beads stability was maintained throughout the experiment. The fully bulged algal beads of diameter 1.078 m for *Chlorella vulgaris* and the diameter of *Anabaena ambigua* is 1.056 were analyzed for lipid, carbohydrate and protein content. The high protein content of algal biomass was supported by *Rodolfo* [15].

3.2 Dairy Effluent Treatment

3.2.1 Water analysis of dairy effluent with *Chlorella vulgaris* and *Anabaena ambigua*

Various chemical parameters were analyzed for algal treated water. Further, the treated water was passed through sand bed and charcoal. Table 1 shows the dairy effluent treatment using *Chlorella vulgaris and Anabaena ambigua*.

3.2.2 Nitrogen and phosphate removal

The phosphates are reduced gradually after algal bead treatment and sand bed filtration respectively as the days progressed. The ammonium nitrate was almost completely reduced after two stage filtration process for both the algal treatment. There was a significant decrease of N and P which may be because of algal uptake and adsorption on alginate gels were found to be major processes involved in the removal of N and phosphate in present study. The 5th day was showing the maximum reduction of nitrogen and phosphates. Previous studies [16,17,18,19] have reported that air-stripping of ammonia is a possible mechanism for removal in an intensively aerated microalgal system with

alkaline pH resulting from photosynthetic activity and aeration.

3.2.3 Effect on Ph

The untreated dairy effluent has an acidic pH value initially but after algal beads treatment and sand bed filtration the treated effluent is shifted to alkaline pH range. Ammonium nitrates could be lost via ammonia volatilization while phosphates was removed by chemical precipitation, because of which alkaline pH was recorded in the two stage treatment system.

3.2.4 BOD and COD removal

Waste water of dairy industry contains large quantities of milk constituents such as casein, lactose, fat, inorganic salts, besides detergents and sanitizers used for washing. All the components contribute largely towards their high biochemical oxygen demand. High BOD and COD values lead to the deprival of oxygen for aquatic life of water. Initially in the dairy effluent contained BOD and COD as 1500 mg/L and 3300 mg/L respectively but it was completely decreased to 40 mg/L and 120 mg/L respectively for *Chlorella vulgaris* but in case of *Anabaena ambigua* it was 49 mg/L and 135 mg/L respectively. BOD and COD have decreased up to 86% and 81% respectively after algal bead treatment. The same were further reduced by 94% and 92% after sand bed filtration which was inacceptable levels prescribed by Andhra Pradesh pollution control board (APPCB), Andhra Pradesh, India. Dairy water after algal beads treatment and sand bed filtration each 1 liter were used for performing the mortality of studies on *Danio rerio* (zebra fish) and compared with control of pure dairy water effluent because of which the mortality of *Danio rerio* fishes drastically reduced from 100% to 0%. Previous work also showed that algal uptake had little effect on the removal of COD [20,21].

3.3 Biochemical Analysis

Immobilized *Chlorella vulgaris* and *Anabaena ambigua* were employed to treat effluent for 5 days respectively .Vital biochemical constituents like carbohydrates, lipids and proteins were analyzed during the cultivation of both species. Moreover, removal of organic contents like BOD and COD, nutrients like ammonical nitrogen and phosphates was also assessed showed in Table 2.

The toxicity of dairy effluent was evaluated using *Danio rerio* model showed in Table 3. Untreated dairy effluent was highly toxic and all the fish died immediately. However, when the fish were released into the effluent treated with immobilized beads of *Chlorella vulgaris* and *Anabaena ambigua* 20% mortality was observed which is however acceptable . This bead treated effluent when subjected to filter bed treatment, however showed no mortality and the treatment was found to be highly efficient.

Table 1. Shows dairy effluent treatment

Parameters	Untreated dairy effluent	*Chlorella vulgaris*		*Anabaena ambigua*		Acceptable limits as per APPCB
		Bead treated	Bed treated	Bead treated	Bed treated	
pH	6.82±0.02	8.42±0.04	8.26±0.02	8.51±0.05	8.35±0.02	5.5-9.0
BOD	1500mg/L	160 mg/L	40 mg/L	175mg/L	49 mg/L	<100 mg/L
COD	3300 mg/L	500 mg/L	120 mg/L	535 mg/L	135 mg/L	<250 mg/L
NH4+-N	40 mg/L	0.8 mg/L	0.4 mg/L	0.98 mg/L	0.45mg/L	<50 mg/L
Phosphates	35 mg/L	0.97 mg/L	0.41mg/L	0.99 mg/L	0.55mg/L	--

**All the above experiments were performed in triplicate and average of them was taken*

Table 2. In biochemical analysis of *Chlorella vulgaris* and *Anabaena ambigua*

Biochemical composition	*Chlorella vulgaris* (percentage)	*Anabaena ambigua* (percentage)
Carbohydrates	30	26
Lipids	12	5
Proteins	28	40

**All the above experiments were performed in triplicate and average of them was taken*

Table 3. Toxic levels of dairy effluent treated at various stages

S no	Sample	Survival rate (hr)								Toxic potential
		24 hr		48 hr		72 hr		96 hr		
		Cv	*Aa*	*Cv*	*Aa*	*Cv*	*Aa*	*Cv*	*Aa*	
1	Control (untreated dairy effluent)	0	0	0	0	0	0	0	0	Highly toxic
2	D.E after algal bead treatment	9	9	9	8	9	8	8	8	Acceptable
3	D.E after sand bed filtration	10	10	10	10	10	10	10	10	Non-toxic

Cv = Chlorella vulgaris; Aa = Anabeana ambigua

**All the above experiments were performed in triplicate and the mean values were tabulated*

4. CONCLUSION

The present study has shown that the dairy effluent was efficient for the growth of *Chlorella vulgaris* and *Anabaena ambigua*, with the significant lipid, carbohydrates, protein productivity. Both the cultures were optimized in BG11 media. Lipid and carbohydrate content was found high in *Chlorella vulgaris* when compared to *Anabaena ambigua*, which has high protein content. Ammonium nitrates and phosphates were almost completely removed by both algae. A significant decrease in both COD & BOD was achieved by the above mentioned method.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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