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Study on the treatment of dairy effluent using *chlorella vulgaris* and production of biofuel (Algal treatment of dairy effluent)

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ABSTRACT

Effluents from dairy industries are the major concerns currently across the globe. Utilizing the liquid and gaseous wastes as the feedstock to grow the microalgae in a sterile photo bioreactor to produce value added products like omega-3 fatty acid, bio ethanol and biodiesel will not only minimize the ill effects of these pollutants but also help to increase the revenue of the respective company and also for the respective countries. Since dairy industrial effluents have different composition of nutrients, selecting appropriate microalgae is very important in this process. Hence an initial screening study is important before scaling up the process to a pilot scale and then to the industrial scale. Products can be extracted and tested for purity in a series of standardized purification methods (extraction, distillation, HPLC etc.). Moreover, the extent of water pollution and air pollution because of the hazardous effluents and flue gases generated by these industries will also be minimized. In addition, value added end products like omega-3 fatty acid, bio ethanol and biodiesel could be produced. Omega-3 fatty acid, thus produced will save the precious marine life which is otherwise killed for the extraction of oil. Moreover, the byproducts are excellent sustainable alternatives for fossil fuels.

KEYWORDS

Dairy effluent;
Microalgae;
Biodiesel.

INTRODUCTION

Dairy industry is an example of the industrial sector, in which the cleaning silos, tanks, heat exchangers, homogenizers, pipe sand and other equipments engenders a large amount of effluents with a high organic load. The effluents contain dissolved sugars, proteins, fats and possibly residues of additives. The key parameters are Biochemical Oxygen Demand (BOD), with an average ranging from 0.8 to 2.5 kilograms per metric ton (kg/t) of milk in the untreated effluent; Chemical Oxygen Demand (COD), which is normally about 1.5 times the BOD level; total suspended solids, at 100–1,000 milligrams per litre (mg/l); total dissolved solids: phosphorus (10–100 mg/l), and nitrogen (about 6% of the BOD level)[9]. Cream, butter, cheese, and whey production are major sources of BOD in wastewater. The waste load equivalents of specific milk constituents are: 1 kg of milk fat = 3 kg COD; 1 kg of lactose = 1.13 kg COD; and 1 kg of protein = 1.36 kg COD[9]. The wastewater may contain pathogens from contaminated materials or production processes.

Micro algae have attracted a great deal of appeal as they have a potential of producing biofuel which depends on the species and the cultivation conditions. They can also spontaneously convert CO_2 and nutrients into biomass in the presence of light at much higher rates than conventional oil producing crops[7]. The combination of algal carbon dioxide fixation, waste water treatment and biofuel production has been investigated by a number of researchers today. There are two main methods that are used for cultivating photoautotrophic microalgae namely raceway ponds and photo bioreactors[5]. Using algae for wastewater treatment offers some interesting advantages over conventional wastewater treatment. Advantages of algae wastewater treatment are a) Cost effective b) Low energy requirement c) Reductions in sludge formation d) GHG emission reduction e) Production of useful algal biomass[6,11].

The objective of this study is to design the cost effective and low energy requirement for waste water treatment with by-product formation by utilizing the Microalgae technology which provides low-cost effluent treatment and an effective approach to remove excess nutrients and other contaminants in tertiary wastewater treatment, while providing potentially valuable biomass.

MATERIALS & METHODS

The dairy effluent is collected from various sources for this study. The physiochemical characteristic of dairy effluent is studied in laboratory. The nutrient content of effluent varies to site. TABLE 1 shows the composition of effluent from the industry and farm.

This interesting study about the algal cultures which were used was provided by Phycospectrum Environmental Research Centre (PERC), Chennai-India. Among the eukaryotic, green microalgae of class *chlorophycea* and *cynophycea* produces the CO_2 tolerant species from genera include *chlorococcum*, *chlorella*, *chorococcum.* The species used in this current study is *chlorella vulgaris.*

The growth patterns of these species were observed in their respective synthetic media. The fresh water species *chlorella vulgaris* was cultured in

TABLE 1: Analysis of dairy effluent

S. No	Parameters	Dairy Industry Effluent	Dairy-farm Effluent	Unit
1.	pH	6.57	6.8	-
2.	Total Kjeladahl Nitrogen as N	77.03	1520	mg/l
3.	BOD, 5 days @ 20 ^oC	480	7800	mg/l
4.	Total Solids	3000	68000	mg/l
5.	Organic Nitrogen	44.61	880	mg/l
6.	Total Phosphorus as P	3.76	150	mg/l
7.	Total Organic Carbon	410	4200	mg/l
8.	Bulk Density	1	1.01	-
9.	Ammonium Nitrogen	31.44	650	mg/l

TABLE 2 : Cell count and pH of dairy effluent through days

Day	pH	Cell count (No of cells/ml)		
		Chlorella vul vulgaris	*Scendusmus quadricauda*	*Chroococcus turgidus*
Day 1	6.6	316	240	320
Day 3	6.8	960	288	496
Day 5	6.8	1760	558	728
Day 7	7.2	2240	1056	1104

Bold Basal Medium (BBM) media. The mineral salt medium composition, per litre of distilled water used was 0.075 g K_2HPO_4, 0.014 g KH_2PO_4, 0.075 g $MgSO_4.7H_2O$, 0.09 g $NaNO_3$, 0.025 g $CaCl_2.2H_2O$, 0.025 g NaCl, 0.05 g EDTA-Na_4, 0.00498 g $FeSO_4.7H_2O$, 0.01142 g H_3BO_3, 0.232 mg $MnCl_2 \cdot 4H_2O$, 1.41 mg $ZnSO_4 \cdot 7H_2O$, 0.252 mg $CuSO_4 \cdot 5H_2O$, 0.192 mg $NaMoO_4 \cdot 5H_2O$, 0.080 mg $CoCl_2 \cdot 6H_2O$[10] .

Cell count of the micro algal culture was estimated by using Haemocytometer line method[10]. The no. of cells present in the matrix on the haemocytometer was observed under microscope with 40X resolution and the no. of cells present was noted[7].

No. of cells present / ml of the sample = No. of cells observed * 10^5

RESULTS & DISCUSSION

Species such as *chlorella vulagris, scenedesmus quadricauda, chrococcum humicola* are inoculated in dairy effluent to study the growth rate and other physiochemical changes as it would give result in large scale treatment[7]. The algae which gave the best reduction in the COD, BOD, phosphates and ammonical nitrogen was taken for further investigation (TABLE 2 and Figure 1 & 2).

Dairy effluent is rich in carbon source hence *C. vulgaris* shows a rapid growth. Algae are known to grow more abundantly in nutrient rich (eutrophic) waters leading frequently to algal blooms[4]. Algae are able to remove the nutrients from wastewater successfully and the lipid production is found to be similar to pure culture conditions[7].

Figure 1 and Figure 2 shows that there is a significant change in the effluent characteristics. *Chlorella vulgaris* species grows well in dairy effluent. The growth of *chlorella vulgaris* in effluent was primarily followed by counting algal cells under the microscope to analyse the contamination, tolerance to conditions like light, temperature, ionic strength, nutrient requirements, ease of harvesting and downstream processing impacts the success of large scale production.

After diluting the medium, the pH value was slightly decreased due to the addition of excess water. Gradually, the pH value increased until day nine of cultivation, probably due to consumption of bicarbonate ion in the medium as a sole source of carbon for algal growth and photosynthesis. This is in compliance with Sorensen et al. (1996), who maintained that, since algae use CO_2 (aq) from bicarbonate to compensate the lack of CO_2 from gas supply, this results in an increase in pH value.

It was observed in the current study that in the 1st week of experiment, the growth of *chlorella* under continuous illumination, was greater. This is due to the incidence of adequate light energy under continuous light in the 1st week of cultivation, during cell metabolism process. Therefore, *C. Vulgaris* is able to grow fast. When given aeration there was a rapid growth of *C. Vulgaris* in dairy effluent. This is because of photo oxidation reaction in the cells, owed to excess light that cannot be absorbed by the photosynthetic apparatus. The changes in pigments

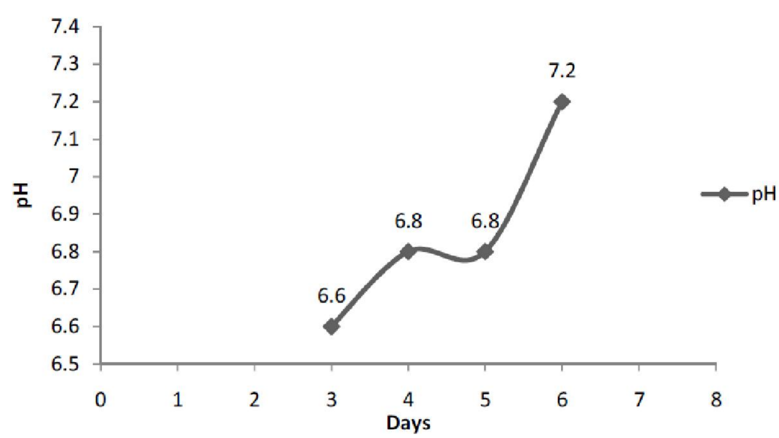

Figure 1 : Change of pH in dairy effluent through days

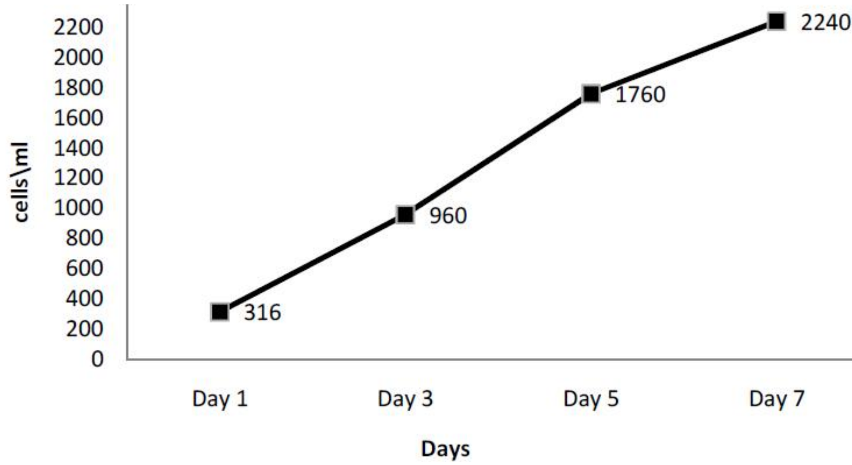

Figure 2 : Growth of *C.vulgaris* (cells/ml)

TABLE 3 : Presence of fatty acid in algal biomass

Component name	Carbon	Retention time (min)	Area
Palmitic acid	C 16	30.6	607.8
Stearic acid	C 18	33	214.5
Oleic acid	$C18_1$	35.5	145.6
Linolenic acid	$C\ 18_2$	37.2	709.6
Alpha linolinic acid	$C\ 18_3$	40.2	456.7
Moroctic acid	$C18_4$	44.6	387.8

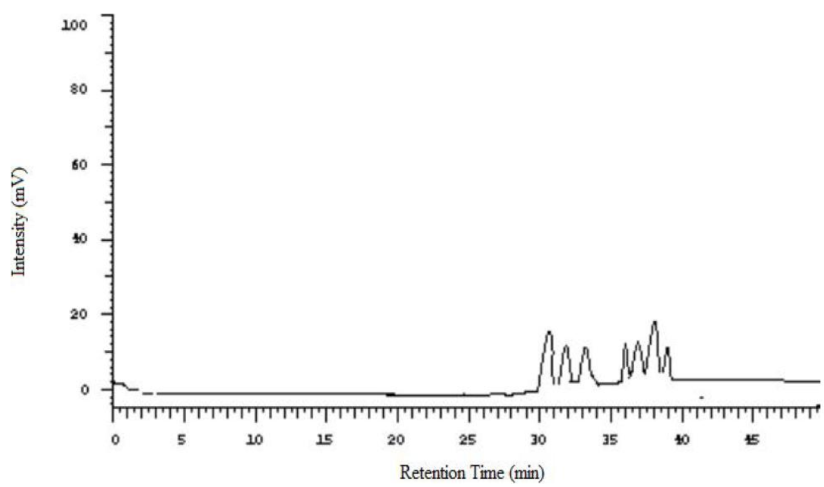

Figure 3 : Lipid present in algae biomass

are related to an adaptation mechanism, chlorophyll was reduced due to photo oxidation and carotenoids were increased to protect photo oxidative damage of the cell. In addition to that, in higher light energy the algae synthesized smaller photosynthetic units, most probably to prevent photo damage, however, in low light larger photosynthetic units are found probably to aid light harvesting.

Fatty acid content of algal biomass

Chlorella vulgaris were identified as species with the highest productivity of fatty acids relevant to trans -esterification reactions. The components were mainly linoleic acid, palmitic acid and oleic acid. To increase productivity of highly saturated fatty acids cultivation parameters, light intensity and temperature were varied.

A Total of 1.234% wet weight of lipids were present in the algae biomass grown in the dairy effluent. From TABLE 3 & 4 and Figure 3 it was found

TABLE 4 : Lipid content of algal biomass

Component name	Carbon	Weight present in biomass %
Palmitic acid	C 16	0.224
Stearic acid	C 18	0.129
Oleic acid	$C18_1$	0.1934
Linolenic acid	$C\ 18_2$	0.229
Alpha linolinic acid	$C\ 18_3$	0.3045
Moroctic acid	$C18_4$	0.1125

that moroctic acid which is one of the omega 3 fatty acid is highly present in the algal biomass. The lipid content of biomass may increase when algae is grown in photo bioreactor evidencing the possibility to switch the anabolic activity from the protein and DNA synthesis to the lipid accumulation[9]. The results indicate that there was no control over harvesting flow. Harvesting flow coverage had to be limited to only a few portions on the membranes where good harvesting could be observed. Controlled harvesting operation and maximum harvesting flow coverage is necessary for continuous algal growth.

CONCLUSION

Chlorella vulgaris using photo bioreactor by continuous process may be economically viable for bioactive based speciality product[5-2, 8]. On the other hand, due to its high capital and operating costs, fermentation of *chlorella* may be economically viable only for high-value particularity products (γ-linolenic acid, pigments, and protein) but not for large-volume commodity products like bio fuels[1]. The chemical composition of *chlorella vulgaris* by continuous process has a great potential to benefit the high-value constituents from algal biomass based on desired application.

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