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Research Article

Qualitative and combined analysis of bio recycled dairy effluent waste water from Thiruvannamalai district in Tamilnadu, India.

***Anandaraj. K[1°], Elaiyaraja. R[1°], A. Jayakumar[2°] and K. Sathiyaseelan[2°].**

[°] Assistant Professor in PG Department of Microbiology, Shanmuga Industries Arts and Science College, Thiruvannamalai, Thiruvalluvar University, Tamilnadu.
*Corresponding author

Abstract

The present study was aimed to Bioremediation, Biorecycling and Biodegrade the dairy effluent via *Spirulina platensis* for particular period. The composition of the dairy effluent was analyzed before the inoculation of biomass of *Spirulina platensis* as well as after. Several factors determined especially biological oxygen demand (BOD), chemical oxygen demand (COD), Calcium and Nitrogen in the effluent. The algal biomass has been utilized the dairy pollutants and well grow. Bioremediation studies was studied for one month period, the samples was collected and analyzed on 2nd, 3rd, 4th, 6th, 8th and 10th days. From this study was noticed recovery and utilization of dairy wastes.

Keywords: BOD, COD, Nitrogen, Calcium, *Spirulina platensis,*

1.Introduction

India is the 2nd largest in the emerging economies in the world, with a GDP growth rate of 6.5%, 3rd largest economy in the world (based on PPP) with a GDP of US $ 4.4Trillion, and according to BRIC report published by Goldman Sachs, India will be the 2nd largest economy after China by the year 2035.
In our country was world's largest milk producer, accounting for more than 16% of world's total milk production, is the world's largest consumer of dairy products. The total amount of milk produced has tripled from 23 million tonnes back in 1973 to 95 million tonnes in 2008 and expected a production level of 135 million tonnes by 2015 but the projected demand for milk by 2021-22 estimated at 180 million tonnes which implies that milk production would have to be doubled.

Milk production is growing at 3.3% while consumption is growing at 5% leaving a gap between demand and supply. In order to meet the rapid growing demand and to increase the milk production, Union Govt has started a central scheme National Dairy Plan – Phase 1, for a period of 2011-12 to 2016 – 17.This scheme will be implemented with a total investment of about 2242 corers. This scheme main objective is to help provide rural milk producers with greater access to the organised milk –processing sector and thus to bridge the gap between the demand and supply of milk in the country.

The share of the total milk processing capacity by private sector is 44%of total installed capacity of 73 MLPD (Million Litres per Day) in the country. Therefore, the total share of the organized sector, both cooperatives as well as the private sector is barely 12%. What is, therefore, disquieting is that as much as 88% share of the total milk production is commanded by the unorganized sector. In order to attract promote Dairy Industry and attract more investment in this sector, govt has also reduced the excise duty of 16% to Zero on Dairy processing Machineries.

Dairy industry produce waste water rich in organic matter and thus leading to reaction leads to odorous, BOD and high COD containing water. Aerobic lagoons are widely used in warmer regions to store wastewater produced from food industries livestock's etc as it reduces the nuisances created due to odor. These lagoons are aerated by natural wind so they need large surface area compared to anaerobic lagoons hence anaerobic lagoons are widely used. But these anaerobic lagoons lead to odor problem and release of noxious gases. So to reduce the problems created by anaerobic lagoons works are been carried out on aerobic treatment to reduce over loading of organic matter in lagoons. Aerobic treatment of liquid waste produced from food industries and animals is evolving as one of pre-treatment option to reduce chemical oxygen demand, biological oxygen demand and odor problems. Most of the studies are on finding the optimum dosage of aeration for treatment of livestock's are carried on swine and piggery wastewater.

In the present study an effort has been made to evaluate the Effluent Treated Plant (ETP) provided for the treatment of wastewater generated by dairy industry. The study was limited to the performance evaluation of the ETP Plant of dairy industry and waste water treated to biorecycling (*Spirulina* cultivation). Characterization of wastewater from different units of processing plant and management strategies are not studied.

Mosulishvili *et al*.,*(*2002) and Eugenia *et al*., (2003) also reported to explained that the great potential of using blue–green algae *Spirulina platensis* as a matrix for the production of selenium- and iodine-containing pharmaceuticals is shown experimentally. The background levels of 31 major, minor and trace elements in *S. platensis* biomass were determined by means of epithermal neutron activation analysis. Algae growth rate controls directly and indirectly the nitrogen and phosphorus removal efficiency.

The maximum algae productivity is required for effective nutrient removal and must be considered as a key area of research. Likewise, low harvesting costs are also required for a cost-effective nutrient removal system. The use of filamentous microalgae with a high autoflocculation capacity and the use of immobilized cells have been investigated in this respect. Finally, to combine most of the achievements from key areas and to design integrated recycling systems (IRS) should be an ultimate and rewarding goal.

Materials and Methods

2.1. Collection of diary effulent

The dairy effluent was collected from Heritage Foods India Limited, Somasipadi Pudur Village, Thiruvannamalai Dt., Tamil Nadu, India. The fresh dairy effluent was collected and stored in a sterile plastic utensil and stored at refrigerator until further use.

2.2. Collection of *Spirulina*

The cyanobacteria *Spirulina platensis* was collected from Blue Green Agrotech, Kariyamangalam, Thiruvannamalai, Tamil Nadu. The treatment of dairy effluent was studied by using *Spirulina platensis*. The stock culture was maintained in Zarrouk's medium, the inoculated flasks were kept at 20^0C in room with aeration and agitation.

2.3. Estimation of Biochemical Oxygen Demand [BOD] (Winkler, 1978)

The initial dissolved oxygen (DO) was determined by adding each 2 ml of manganese sulphate and alkali-iodide solution. The bottle was shaken for few minutes and the precipitate was dissolved using 2 ml of concentrated sulphuric acid. From this 100 ml of the sample was taken and titrated against 0.025 N standard sodium thiosulphate solution using starch as an indicator. The end point was the colour change from blue to colourless; the same procedure was repeated for the 5 days incubated sample.

Calculation

Biochemical oxygen demand was calculated using the formula.

BOD, mg/l = (DO of sample before incubation)-(DO of the sample after incubation) (5 days at 20.C)

Where,

$$D0, mg/l = \frac{CD \times N \times E \times 1000 \times 0.698 \times Vt}{\text{Volume of the sample}}$$

Where,

DO - Dissolved Oxygen,

CD - Correction factor,

N - Normality of sodium thiosulfate,
E - Equivalent of oxygen,
Vt - volume of titrant,
0.698 - convert parts per million of Oxygen.

2.5. Determination of Chemical Oxygen Demand (APHA, 1998)

20 ml of the diluted sample was taken in a (Chemical Oxygen Demand) COD flask. To this, 10 ml of 0.025 N potassium dichromate solutions was added. A pinch of sliver sulphate and mercuric sulphate were added, followed by the addition of 20 ml concentrated sulphuric acid. The contents were refluxed for about an hour. The flasks were removed, cooled and distilled water was added to a final volume of 100 ml. 2 to 3 drops of ferrous indicator was added, mixed thoroughly and titrated against 0.1 N ferrous ammonium sulphate. The end point was a sharp colour change from blue green to reddish brown.

Calculation

$$\text{COD, mg/l} = \frac{(A-B) \times \text{Normality of FAS} \times 1000 \times 8}{\text{Volume of Sample}}$$

Where

A = ml of titrate with blank.
B = ml of titrate with sample.

2.6. Estimation of Calcium (By EDTA Titrimetric Method)

Exactly 10 ml of the diluted sample was taken in a clean conical flask. To this, 2 ml of 1N sodium hydroxide was added to produce the pH of 12-12 and mixed well. A pinch of murexide indicator was added and titrated against the standard EDTA solution. The end point was the colour change from pink to purple.

Calculation

$$\text{Total hardness, mg/l (as } CaCo_3) = \frac{1\text{ml EDTA titrate} \times \text{Normality of EDTA} \times 1000}{1\text{ml sample taken for titration}}$$

2.7. Estimation of Total Nitrogen By Kjeldahl Method (Jessen-Hensen, 1932)

2.7.1. Sample digestion

40 ml of sample was taken in a 100 ml Kjeldahl flask. To this sample, 4 ml of concentrated sulphuric acid, 100 drops of 10% copper sulphate 6 g of potassium sulphate and 1 ml of 10% sodium chloride solutions were added. The flask was heated on a hot plate to avoid loss through foaming. After the water has boiled off, the sample was turned black due to decomposition of organic matter by sulphuric acid. As the digestion proceeds the colour of the sample was turned pale green continued heating for additional 30 min. the flask was cooled and the volume was made up to 100 ml with distilled water.

2.7.2. Distillation

About 25 ml of the digested sample was taken and the solution was made alkaline with sodium hydroxide using phenolphthalane indicator. The distillation was started after immersing the tip of the condenser in 50 ml of boric acid solution in a conical flask 100 ml of the distillate was collected.

2.7.3. Titration

0.5ml of the mixed indicator solution was added to the distillate and titrated against 0.02N sulphuric acid. Endpoint was the colour change from pale green to lavender. A blank was also conducted starting from the digestion step to final titration.

Calculation

The amount of total nitrogen present in the sample was calculated using the formula,

$$\text{Total nitrogen, mg/l} = \frac{(S-B) \times \text{Normality of Sulphuric acid} \times 1000 \times 14}{\text{Volume of sample}}$$

Where,

S = volume of sulphuric acid consumed for sample.

B = Volume of sulphuric acid consumed for blank.

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3.Results

The effect of physico-chemical parameters on the treatment of dairy effluent by using the cyanobacterium *Spirulina platensis* was analysed. The effluent colour, pH, total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were analysed.

Raw dairy effluent physiochemical properties were recorded in (Table-1).

Table1. Characteristics of Raw Dairy Effluent

S.NO	CHARACTERISTICS	RESULT
1.	Colour	White
2.	Odour	Pudrid milk odour
3.	pH	8.3
4.	Total suspended solids mg/ml	312
5	Dissolved oxygen mg/ml	Nil
6.	Biochemical oxygen demand mg/ml	800
7.	Chemical oxygen demand mg/ml	960
8.	Total Kjeldahl Nitrogen (as N) Mg/ml	64.4
9.	Sulphide (as S) mg/ml	0.1
10	Calcium (as CaCo3)	20

3.1. Physiochemical properties of dairy effluent with treatment of S*pirulina platensis*

3.1.1. Biochemical oxygen demand

The BOD content of the raw dairy effluents was 921 mg/ml and the BOD was found to be lowered after treatment with *Spirulina.* The treatment was carried out for about 10 days. BOD level was lowered from 921 mg/ml to 158 mg/ml when the raw effluent was treated with *Spirulin*a. During treatment, the BOD of the effluent was reduced by 83%.The average percentage reduction of BOD at various stages of treatment with *Spirulina* is given in table-2.

Table-2.Estimation Of BOD (Biochemical Oxygen Demand)

S. NO	LABELLING (DAYS)	BOD (mg/l)
1	Sample(II)	865.00
2	Sample(IV)	753.84
3	Sample(VI)	582.32
4	Sample(VIII)	251.28
5	Sample(X)	157.92

3.1.2. Chemical Oxygen Demand

The COD content of the dairy effluent was found to be 980 mg/ml. *S. plantensis* was inoculated in the raw dairy effluent and the reduction in COD was estimated for a period of 10 days. The results showed that there was a reduction in COD level of the effluent when it was treated with *Spirulina.* During treatment COD level was reduced to about 92%, which was showed in table-3.

Table-3.Estimation of COD (Chemical Oxygen Demand)

S. NO	LABELLING (DAYS)	COD (mg/l)	AVERAGE % OF REDUCTION
1	Sample(II)	960	2.08
2	Sample(IV)	720	26.53
3	Sample(VI)	560	42.85
4	Sample (VIII)	320	67.35
5	Sample(X)	80	91.84

3.1.3. Total Calcium

The initial level of Calcium in the dairy effluent was 20 mg/ml. The raw effluent was inoculated with *Spirulna platensis* and the treatment was continued for about 10 days. The Calcium level was reduced in the course of treatment by 80 %.(Table-4).

Table-4.Estimation of Calcium.

S. NO	LABELLING (DAYS)	AMOUNT OF CALCIUM (mg/l)	AVERAGE % OF REDUCTION
1	Sample(II)	18	10
2	Sample(IV)	14	30
3	Sample(VI)	10	50
4	Sample(VIII)	8	60
5	Sample(X)	4	10

3.1.4. Total Nitrogen

The initial level in the raw dairy effluent was 77 mg/ml. After treatment with *Spirulina* for a period of 10 days, the Nitrogen level was reduced from 77 mg/ml to 14 mg/ml. About 82% of Nitrogen reduced in the dairy effluent. The average percentage reduction of total kjeldhal Nitrogen in the dairy effluent is given in the Table -5.

Table-5.Estimation Of Total nitrogen

S. NO	LABELLING (DAYS)	AMOUNT OF NITROGEN (mg/l)	AVERAGE % OF REDUCTION
1	Sample(II)	64.4	16.4
2	Sample(IV)	35.0	54.54
3	Sample(VI)	29.4	61.81
4	Sample(VIII)	25.9	66.36
5	Sample(X)	14	81.81

4.Discussion

Productivity, nutrient content and nitrogen and phosphorous recovery values from algal biomass grown in anaerobically digested dairy manure shows increasing level productivity unto (1 g of DW m-2d-1), and nutrient content (upto 6.5% N and 1.1%P) (Kebede-wethead *et al*., 2003), as like this *Spirulina platensis* in dairy effluent reduces the BOD, COD, nitrogen and calcium in considerable amount. This is revealed in *Spirulina platensis* which was grown in the dairy effluent like increase in the carbohydrate, protein and its biomass higher than in Zarrouk's medium.

Possible uses of the algal biomass includes its uses a feed supplement or slow release fertilizer. A recent assessment of the nutrient recovery potential and economic cost of an on - farm algal turf scrubbers (ATS) treatment systems to treat dairy manure effluent used "best case" values that were extrapolated from laboratory results (Piazzro *et al*., 2006).

Arthospira removes the nutrient from pig waste water about 84%-96% of NH_4, 72-87% of P (Olguin *et al*., 1997). The growth of *spirulina* in anaerobically digested waste water (ADW) was poor but can be enhanced by addition of $NaHCO_3$- upto 4% to 8% as a carbon source, which gives maximum production at 1.4 mg/l. it also showed the removal of COD upto 80% BOD upto 95% and cations such as K-98%, Calcium-95% and sodium-90%. Anions such as Chloride -81%, Nitrate-52%, P-76% and Sulphate

upto 6% were removed from effluent heavy metals eg. N was removed upto 57% and pb upto 75% in the growth period.(Jayant doke *et al*., 2004). In this study various components have been reduced like Nitrogen 77.13% and Calcium 80% from dairy effluent using Spirulina *platensis*.

The growth of *Spirulina* is higher in desalinated waste water about 91.5% than the Paoletti medium and Paoletti medium with 1 g/l of NaCl (Harriet Volkmann *et a*l., 2008). *Spirulina platensis* was successfully grown in diluted real human urine to achieve biomass production and Oxygen evolution. Thus it is possible to use *Spirulina platensis* to regenerate nutrient matter, and at the same time to assimilate Co_2 and release Oxygen with in the life support system (Feng Dao-lun *et a*l., 2005).

Mixed culture of cyanobacteria shows that removal efficiency of linden contaminated effluent about 91.6% and 100% indicates the potential of natural resources as efficient agents for pollution control (Ebstesam *et al*., 2008). After stabilization, sedimentation and bioremediation with wolfia for 15 days the dairy effluent showed decline toxicity about 73.5%. This is similar to that in current study reveals that paddy seeds are grown will in treated dairy effluent.

As like *Spirulina platensis* in the dairy effluent which was grown for 10 days, algae removed ammonia total nitrogen, total phosphorous, and COD by 100%, 75.7%-82.8%, 62.5%-74.7% and 27.4%-38.4% respectively in differently diluted dairy manure (Liang *et al*., 2009). Liu *et al*., (2001) studies showed that cultivating *Spirulina* in pretreated dairy effluent removes COD, PO_4, total P, total N, NH_4 -N are 54.83%, 100%, 76.06%, 76.42%, and 92.46%, respectively. *Spirulina platensis* well suited for removal of cadmium (Rangaswamy *et al*., 2001).

The pH limits for effluent water prescribed Tamil Nadu Pollution control Board (TNPCB) is in the range of 5.5 to 9.0. The pH of dairy effluent is 8.3 it increase the alkanity of the soil. The BOD content in industrial effluent for discharge into land water supply is 250ml/l. The TNPCB limit of BOD content in industrial effluent for discharge into the water bodies is 30mg/l. The BOD level of the *Spirulina platensis* treated dairy effluent was 157.92 mg/l. when this particular effluent is discharged into any bodies, they may get diluted. But the BOD level of the dairy effluent is 157.92 mg/l not within the limits prescribed by TNPCB.

The raise in hardness of the dairy effluent is due to the presence of Calcium, Mg, and some other divalent metallic ions. The increase in calcium and Mg content of the effluent is due to it is contact with fly ash. The calcium and Mg salts get dissolved in the effluent and hence shows increase in the concentration of the calcium and Mg in the effluent. The level of nitrogen in the effluent is very low. Dairy effluent has a nitrogen content of only 77 mg/l. Even the small amounts of calcium and nitrogen found in the effluent are degraded and reduced by the *Spirulina platensis*.

Thus in studies on *Spirulina platensis* in the dairy effluent had observed 90% of nitrogen removal and 99% of calcium (Chung et al., 1978). *Spirulina platensis* grows well in dairy effluent. As because of the level of the calcium and nitrogen in the treated dairy effluent are within the limits prescribed by TNPCB, even though the effluent is discharged into the water body, that may not lead to eutrophication.

Certain factor present in the dairy effluent supported the best growth of *Spirulina platensis* in the raw dairy effluent. This was indirectly confirmed by an increase in protein, carbohydrate and chlorophyll content, when compared with growth in the Zarrouk's medium. When the effluent is disposed without dilution for irrigation, lead to the accumulation of salts in the soil. The putrescible matter in the effluent may cause a soil to be dogged and thus that area cannot be used for other purpose.

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