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RESEARCH ARTICLE

REDUCTION IN PHYSICO CHEMICAL PARAMETERS BY DAIRY EFFLUENT ADAPTED BACTERIA

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Abstract

Global environmental pollution is one of the major issues, griping earth day by day. The dairy industry effluent was the second most important single source of pollution in streams. Dairy is one of the major agriculture industries and dairy wastewater problem is larger in developing countries because all milk is processed industrially. Dairy is having particular characteristics of effluents and hence has the different effluent related problems. These problems can be revealed only after the factual study of various physico-chemical characteristics. The present study would be aimed at physico-chemical characterization of untreated and treated effluent from Jaipur dairy and an attempt would be made to characterize bacterial strain from untreated effluent samples and study its effect on treated effluent in terms of COD reduction with different intervals and different inoculations. The bacteria used in the present study is a gram negative bacillus the confirmatory tests of which are under progress in our laboratory . The strain was inoculated in nutrient Broth(NB) and its $O.D._{660}$ was monitored at regular basis. The parameters taken into consideration for physicochemical characteristics were colour, temperature, pH, DO, BOD, COD, acidity , alkalinity, TDS, chloride, nitrate, phosphate, hardness, oil & greese. The values are expressed as mg/l except colour temperature and pH. The values of different parameters were expressed as Mean ± standard deviation. The study revealed that the dairy effluent is slightly alkaline in nature, and other values of treated effluent for pollution indicators like DO, COD, chloride were in compliance with ISI guidelines, so it could be concluded that the functioning of effluent treatment plant is average and it needs to be monitored at regular basis.It was observed that effective COD reduction was found in 1 % inoculum at 20 days.

Keywords: pollution, physico-chemical characterstics, DO, COD, chloride.

Introduction

Water pollution is one of the major problem in a todays scenario. Dairy industry is among the most polluting of the food industries in regard to its large water consumption. Dairy is one of the major industries causing water pollution. Waste water generated in a dairy contains highly putrescible organic constituents. This necessitates prompt and adequate treatment of the waste water before its disposal to the environment. Almost all the organic to biological treatment-either aerobic or anaerobic. Rapid growth of industries has not only enhanced the productivity but also resulted in the production and release of toxic substances into the environment, creating health hazards and affected normal operations, flora and fauna. These wastes are potential pollutants when they produce harmful effects on the environment and generally released in the form of solids, liquid effluents and slurries containing a spectrum of inorganic &organic

compounds. Natural attenuation is viewed as the best solution for cleaning up many waste sites and will save billions of dollars in cleanup costs. The biotreatment, bioremediation and Natural Attenuation area have both basic research and field application foci for the environment biotechnology (Singh et.al., 2012). In order to have proper processes in the ETP, Characterization of waste water, treatability studies and planning of proper units and processes for effluent treatment is very much necessary. (Chaiudhari and Dhoble ,2010) Treatment using natural coagulant *Moringa oleifera* was carried out for the treated effluent from the plant.(Harush et.al.2011) Chemical oxygen demand is an indicator of oxidizable matter present in waste water. In concern to dairy industry this is a important parameter to check the organic load in effluent of treatment plant. The present study would be aimed at physico- chemical characterization of untreated and treated effluent from Jaipur dairy and an attempt would be made to characterize bacterial strain from untreated effluent samples and study its effect on treated effluent in terms of COD reduction with different intervals and different inoculations. (Shivsharan. et.al., 2013, Kumar. et al 2012)

Materials and methods

Chemicals

All the chemicals used in the study were of analytical grade. The laboratory glass wares used were washed with detergents and rinsed with distilled water then oven baked at 200°C overnight, prior to use.

Study area

The study was carried out at SARAS DAIRY JAIPUR. Saras Dairy (Federal unit of RCDF Dairy development was initiated by the state government in the early seventies under the auspices of Rajasthan State Dairy Development Corporation (RSDDC) registered in 1975.

Sampling Site

Sampling site of above study was effluent treatment plant (ETP) of saras dairy Jaipur.

Fig.1 Sampling site

Fig. 2 Sampling site

Sampling

The untreated wastewater was sampled after the screening of particulate matter. Treated wastewater was sampled from aeration tank. (ETP, Jaipurdairy) in accordance with standard procedures.

Effluent analysis

All the samples were analyzed for colour, pH and temperature DO, COD, BOD, alkalinity, acidity, free CO_2 TDS, hardness, chloride, and nitrogen as nitrate and phosphorus as phosphate , sulphate, oil & greece in accordance with standard procedures (APHA, 1998) Color, temperature , pH and DO of the effluents were recorded at the sampling point. Clean and pre sterilized plastic bottles were used for sample collection (Banupriya et.al., 2012).

These bottles were stored at 4°c.in laboratry. For microbiological analysis samples were collected in BOD bottles.

Fig 3.samples

The samples were designated as-
1. DIUT: Dairy influent (Untreated)
2. DET: Dairy effluent (Treated)
3 Control

Microbiological analysis

The aim of this study is to evaluate the dairy wastewater microbiota and its biochemical activities, in order to obtain pure cultures adapted for wastewater treatment.To get an efficient biological wastewater treatment it is very important to know the wastewater microbiota composition and the biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and the physical -chemical conditions .

Isolation identification and biochemical characterization

For microbiological studies sample was taken from untreated water .It was serially diluted to 10^{-10} dilution.after that they were plated on nutrient agar with the following composition (gm /l);

NUTRIENT AGAR: peptone 5, meat extract 1, yeast extract 2, Nacl 5, agar 15, pH 7

Following this procedure we obtained mixed culture plate and from this plate we isolated pre dominant strain and prepared pure culture of this strain(Shivsharan et.al.2013)

Fig.4 Mixed culture

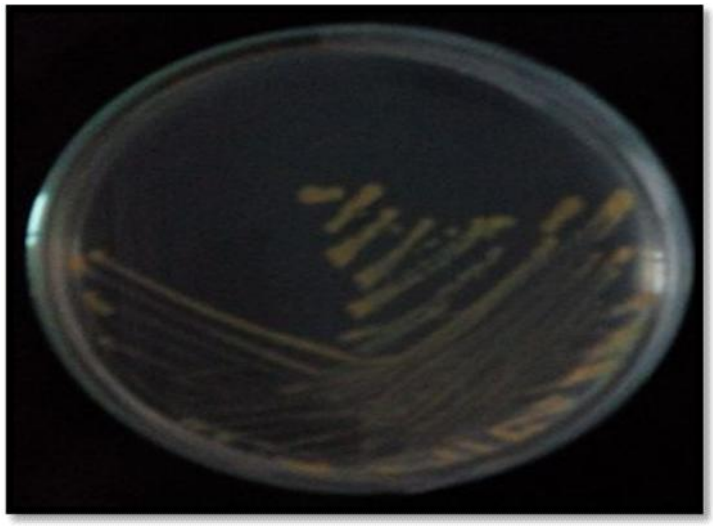
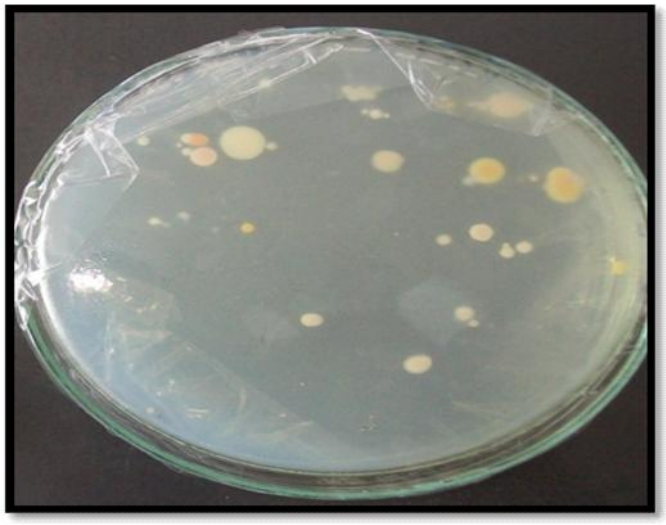


Fig.5 pure culture



COD Reduction by selected bacterial strain:

This strain was inoculated in nutrient Broth with following composition (gm l):

Nutrient broth:
Peptone 5, meat extracts 1, yeast extract 2, Nacl 5, pH 7

Through this procedure we obtained mixed culture plate .Its $O.D._{660}$ was monitored at regular basis and standarized at 0.6.(Harush et al 2011**)** At this stage this strain was inoculated with different concentration(0.1%, 0.5%, 1 %) in treated water of ETP plant. The bacterium used in the present study is a gram negative bacillus the confirmatory tests of which are under progress in our laboratory. COD Reduction was monitored at different intervals (0

day, 5th day, 10 th day, 15th day, 20th day). There were 4 groups in this experiment:

DIB 0.1%

DIB 0.5%

DIB 1%

CONTROL

Results and discussion

Colour

The colour of untreated water is milky white while colour of treated water is brown. Due to microbial activity sludge generation is high and it results in brown colour of water. Colour change in dairy effluent has been studied by (Sharma et al 2013).

Temperature

Most biological activity occurs when the water temperature is between 10°C-30°C.In the present study, the range of temperature for influent was 16°C and the temperature ranged 18^0 C in effluent. This slight increase in temperature may be attributed to vigorous biological activity primarily because of mesophils during the secondary treatment. Varition in influent and effluent water temperature has been studied in sugar mill. Weqar 2013

pH

pH gives an idea about the concentration of carbonate, bicarbonate and CO2 in water. pH of influent water was 6 whereas it was 9 in effluent water . This increase in pH may be attributed to alkalophilic micro-organisms which are a part of indigenous micro-flora. .(Sharma et al .2013)

DO

DO is an important parameter to assess the quality of water. Oxygen is removed from the water by respiration and decomposition of organic matter by indigenous micro flora present in effluent. DO of influent is 6mg/l and that of effluent was found to be 8 mg/l, which is in compliance with ISI guidelines. Presumably, increase in DO of effluent is due to degradation of organic matter by indigenous micro flora and the biofilms which is of phenomenal importance during secondary treatment. Kolhe &Pawar 2011observed the DO of Dairy industry is ranging between 0-3.5mg /l ..

BOD

BOD is the measure of amount O_2 required by the microorganism to oxidize the organic content in water. BOD in raw effluent was found to be 314 mg/L which is reduced to 280 mg/L after secondary treatment. BOD is reduced after secondary treatment .BOD value reduction was also observed Kavitha et al 2012 observed the reduction of BOD value after treatment in pharmaceutical industry.

COD

COD is a chemical oxidation process that bring out the true picture of water quality. In the present study, COD of influent was 739 mg/l and that of effluent was 430mg/l . COD is reduced after tretment.(Chaudhari. D.H. and. Dhoble R.M).

Chloride

High concentration of chloride is a clear cut index of pollution. Direct discharge of untreated industrial waste may also increase the salt content in water. In the presentstudy, Chloride content of influent was found be in the range 335 mg/l and that of effluent was 380 mg /l which was within the permissible limits of600 mg/ L according to the ISI standards .

TDS

The total solid concentration in waste effluent represents the colloidal form and dissolved species. The probable reason for the fluctuation of value of total solid and subsequent the value of dissolved solids due to content collision of these colloidal particles. The rate of collision of aggregated process is also influenced by PH of these effluents. (Manu et. al 2011) The value of TDS for effllluent was 900 mg /l.(The effect of high TDS (total dissolved solids) value of effluents of textile and dairy industry on the germination inhibition and subsequent seedling growth on wheat plant was also studied by Varma and Sharma 2012.

Sulphate

Sulphate in one of the major cation occurring in natural water. When water is over loaded with organic waste to point that oxygen is removed then

Table 1. physicochemical parameters of influent and effluent generated from Jaipur dairy

PARAMETERS	UNITS	UNTREATED	TREATED	REFERENCE VALUE *
COLOUR	-	WHITE	BROWN	-
TEMPERATURE	$0^{O}C$	16	19	-shall not exceed 5^{0}c above the receiving water temperature
PH		6	9	8.5
DO	mg/l	2.3	4.5	4-6
BOD	mg/l	314	280	50
COD	mg/l	739	430	250
ALKALINITY	mg/l	158	280	590
TDS	mg/l	1400	900	1500
CHLORIDE	mg/l	335	380	600
HARDNESS	mg/l	240	215	600
NITRATE	mg/l	24	12	10
SULPHATE	mg/l	240	160	350
PHOSPHATE	mg/l	3.4	2.7	2
OIL&GREECE	mg/l	5	3	10

Table 2. biochemical characterization :

Character	+ve/-ve
Gram stain reaction	- rod
Agar slant characteristic	Abundant,thin, white growth, with media turning green
Lactose fermentation	-
Dextrose Fermentation	-
Sucrose Fermentation	-
Indole production	+
Methyl red test	-
Voges-Prauskauer	-
Citrate utilisation	-
Catalase	+
Oxidase	+
Gelatin hydrolysis	+
Starch	-
Lipid hydrolysis	+
Nitrate	+

Table 2. COD Reduction by selected bacterial strain

Strain	Initial COD	0thday	5thday	10thday	15thday	20thday /FINAL COD
DIB 0.1%	610	518	416	371	273	210
DIB 0.5%	610	512	336	288	243	195
DIB 1 %	610	448	304	225	177	150
CONTROL	610	607	596.8	594	592	586

Fig.6. COD Reduction by selected bacterial strain (%)

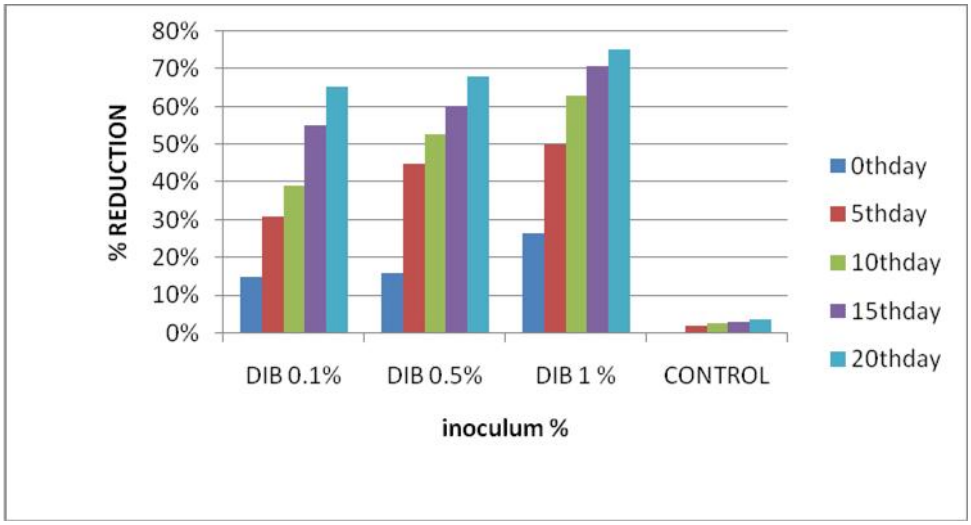


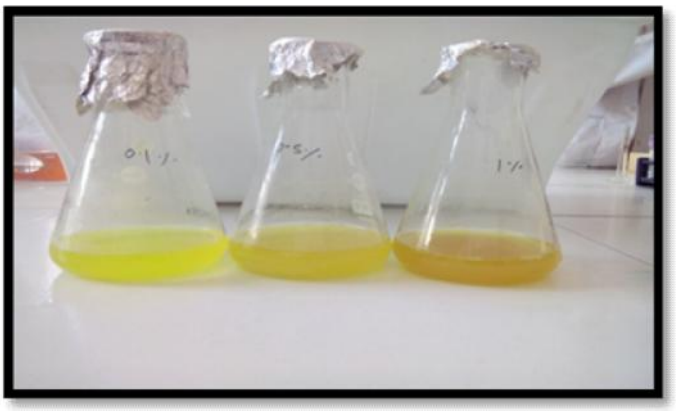
FIG. 7 COD O DAY

FIG.8 COD 5TH DAY

FIG. 9 COD 10th DAY

FIG. 10 COD 15th DAY

FIG. 11 COD 20th DAY



sulphate as an electron acceptor is often used for breakdown of organic matter to produce H2S and produce rotton egg smell. In the present study the values of sulphate for untreated effluent was240 mg/l and that of treated effluent was 160 mg/l. observed the sugar (Siddiqui and Waseem Muhammad 2012) mill effluent was having sulphate of untreated effluent is 760 mg/l and treated effluent showed 420mg/l.

Oil and Grease

The oil and grease content of domestic and certain industrial waste waster and of sludge's is an important in handling and treatment of these material for ultimate disposal. Oil and grease may influence waste-water system. If present in excessive amount. They may interfere with an aerobic and anaerobic biological process and lead t decreased waste water treatment efficiency. A knowledge of quantity of oil and grease present in effluent is helpful in proper design and operation of waste water. Industrial waste contain high quantity of oil and grease which may cause a serous problem if discharged into water body without treatment. In the present study oil and grease of untreated effluent was 5 mg/l and treated effluent was 2.0 mg/ (Kolhe and Pawar, 2011**)**

Alkalinity

It is an important parameter which indicates the ability of water to neutralize acids from wastewater. The higher values of alkalinity are associated with increase in the presence of bicarbonates and carbonates from effluents. In the present study, alkalinity of influent was found be in the range 158mg /l and that of effluent was 280mg/l may be attributed to the higher pH value obtained (7.6-8.2).this value was under permissible limit of ISI (Sharma et.al. 2013)

Hardness

Hardness of water mainly depends upon the amount of calcium and magnesium salts or both. In the present study, value of hardness for influent was 240 mg /l and the values for effluent were 215 mg /l.

Phosphate

Phosphate is the soluble form of phosphorous mainly contributed through detergents and soaps widely used for cleaning purposes in milk Processing unit. In the present study, values for phosphate was 3.4 mg/l for influent and 2.7 mg/l for effluent. This lowering of phosphate level of effluent may be attributed to presence of microalgae which are efficient phosphate solubilisers and scavenge phosphates. (Sharma et.al., 2013)

Nitrate

In the present study, nitrate value for influent was in the range was 24 mg/l and that of effluent 12 mg/l and this reduction in nitrate content of treated effluent may be attributed to the heterotrophic nitrifying bacteria which forms a part of indigenous microflora.

Biochemical characterization

After obtaining pure culture we did biochemical characterization for the confirmation of strain. At first stage after gram staining it was negative rod shaped bacilli and after confirmative test it was concluded that this strain was *Pseudomonas* sp.

COD Reduction by selected bacterial strain

Broth cultures of Isolated bacterial strain were maintained in laboratory conditions**.** Its $O.D._{660}$ was monitored at regular basis and standarized at 0.6. At this stage this strain was inoculated with different concentration(0.1%, 0.5%, 1 %) in treated water of ETP plant. COD was measured at different intervals. It was on 0 day, 5^{th} day 10^{th} day, 15^{th} day , 20^{th} day .The results of COD reduction was as follows :

The reduction of COD was studied with different concentrations.. The results revealed that maximum COD reduction could be achieved in the flask incubated at 35°C as with 1 % inoculum. The selected Strain was found to have good potential to biodegrade the effluent generated from dairy industry. It was able to reduce COD load up to 76 %. It has been reported by Kumar, v. Et.al. 2011 in Agro-Based Pulp Mill Effluent.

Conclusion

Waste water quality can be maintained within safe limits better handling of plant. For treatment plant all the necessary units are there. The treat water is used for gardening purpose As far as the treatment plant is concerned, it is also efficient for handling any kind of variations in water. It is concluded that the treatment plant is working with satisfactory efficiency. As far as COD Reduction is concern the results showed that isolated bacteria strain from the effluent of dairy industry have the ability to, reduce the COD value up to 76 %.Treatment using natural coagulant *Moringa oleifera* was also studied for the treated effluent from the dairy plant. (Harush et.al., 2011). Reduction of COD level by using biosorption technique was also studied by Murali., (2013).

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