

Isolation and Characterization of Bacterial Strains In Terms Of COD Reduction from Dairy Waste Water

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Abstract: Dairy industry is a large scale food production industry and plays an important role in causing water pollution. 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. Bioremediation strategies are often more beneficial than traditional strategies because it can be implemented *in situ*. Dairy waste water is characterized by high biological oxygen demand (BOD) and Chemical oxygen demand (COD) Concentrations. The bacterial isolation was done from untreated effluent and then the raw waste was treated with the isolates to observe reduction in COD level. 05 bacterial strains were isolated from dairy waste water out of which consortia with 1 % inoculum at 20 days was effective in reducing the level of COD and thus helping in bioremediation.

Keywords - Dairy waste water, COD, Bioremediation, Microorganisms

INTRODUCTION

Dairy industry is a large scale food production industry and plays an important role in causing water pollution waste water coming out from Dairy industry is categorized as raw waste and activated sludge which have to be treated by taking various parameters of dairy waste water with raw waste which includes determination of pH, temperature, acidity, alkalinity, total dissolved solids, Chemical

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Oxygen Demand out of which COD is the most important parameter¹. Dairy wastewater are characterized by high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) concentration and generally contain fats, nutrients, lactose, detergent, sanitizing agents as well as milk constituents such as casein, lactose, fat, inorganic salts. 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 conditions². Microbial digestion of dairy food wastewater offers many advantages over other treatments in that a high level of by waste stabilization is achieved with much lower levels of sludge. As microbial digesters become increasingly used in dairy plants, more research should be directed toward selecting the best cultures that maximize environmental problem from dairy waste³.

The isolation of bacteria and the study of their identification have been hampered by the unreliability of conventional microbiological techniques⁴. This is largely due to their morphological variations and inconsistent characteristics and different biochemical Characteristics Dairy waste water are generally treated using biological methods such as activated sludge process, aerated lagoons, trickling filters, sequencing batch reactors, anaerobic sludge blanket reactor, anaerobic filters^{5, 6.} In the present study, instead of these biological methods the dairy effluent was treated with different concentrations of bacteria in order to get best results⁷.

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, in accordance with standard procedures.

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. The microbiological characterization was carried out by serial dilution followed by plaring on nutrient media. Based on their morphological and biochemical characteristics, the isolated cultures were grouped into various genera as outlined in the *Bergeys manual of determinative bacteriology*

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 strain⁶.

The biochemical analysis of isolated cultures was done by following Cappuccino and Sherman, 2002.

COD Reduction by Bacterial Strains: These strains were inoculated in nutrient Broth with following composition (gml):

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 standardized at 0.6.At this stage these strains were inoculated⁸ with different concentration (0.1%, 0.5%, 1%) in treated water of ETP plant. COD Reduction was monitored at different intervals (0 day, 5thday, 10thday, 15th day, 20th day). They were grouped as follows:

Dai A0.1%	Dai B0.1%	Dai C 0.1%	Dai D 0.1%	Dai E 0.1%
Dai A 0.5%	DaiB 0.5%	Dai C0.5%	Dai D0.5%	Dai E 0.5%
Dai A 1%	Dai B1%	DaiC1%	Dai D1 %	Dai E 1 %
CON.(Dai A)	CON.(DaiB)	CON.(Dai C)	CON.(Dai D)	CON.(Dai E)

RESULTS AND DISCUSSION

1. Biochemical Characterization: After obtaining pure culture we did biochemical characterization for the confirmation of strain. Following test was performed for the biochemical characterization:

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S.No.		Pseudomonas	Staphylo	Serratia	Bacillus	Micrococcus
		(Dai-A)	Coccus	(Dai -C)	(Dai -D)	(Dai -E)
			(Dai -B)			
1.	Gram Stain	Rod (-)	Cocci (+)	-	Rod (+)	cocci (+)
2	Agar Slant cultural	abundant, thin	Abundant,	Red,	Abundant,	soft, smooth,
	Characteristics	white growth.	opaque, golden	Mucoid	opaque.	vellow, growth
		with medium	growth	Colonies	white waxy	J , 8
		with meanin	growin	coronics	growth	
3	Lactose	-	А	-	-	-
4	Dextrose	-	А	+	А	-
5	Sucrose	-	А	+	А	-
6	H ₂ S. Production	-	-	+	-	-
7	NO ₃ Reduction	+	+	+	+	±
8	Indole Production	-	-	+	-	-
9	MR Reaction	-	+	+	-	-
10	VP Reaction	-	±	+	±	-
11	Citrate Use	+	-	+	-	-
12	Urease activity	-	-	-	-	+
13	Catalas activity	+	+	+	+	+
14	Oxidase	+	-	-	-	-
15	Gelatin	+ Rapid	+	+	+ (Rapid)	+ slow
16	Starch	-	-	+	+	-
17	Lipid	+	+	-	<u>+</u>	-

Table 1. Divencinical characterization of mulgenous Dacteria

2. Morphological Characterization:



Fig.3: Mixed Culture

3. Culture Characteristics:



(A)





(B)

(D)



(E)

Fig.4: Dai-A, Dai-B, Dai-C, Dai-D, Dai-E

COD Reduction by Selected Bacterial Strain: Broth cultures of isolated bacterial strains were maintained in laboratory conditions. Its $O.D_{.660}$ was monitored at regular basis and standardized at 0.6. At this stage these strains were 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, 5th day 10th day, 15 th day, 20 th day The reduction of COD was studied with different concentrations.. The results

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revealed that maximum COD reduction could be achieved in the flask incubated at 35°C as with 1 % inoculum. The selected Strains were 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*etal*.⁹. 2011 in Agro-Based Pulp Mill Effluent. The results of COD reduction were as follows:

Table 1: COD Reduction by Selected Bacterial Strains:

Strain 1 – Dai A

Dai A	Initial COD	Othday	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

Microcosm analysis of COD reduction by pseudomonas sp.

Table 2: CODReduction by Selected Bacterial Strains:

Strain 2- Dai B

Dai B	Initial COD	Othday	5thday	10thday	15thday	20thday
						/FINAL
						COD
DIB 0.1%	610	608	595	580	540	515
DIB 0.5%	610	604	590	570	525	510
DIB 1 %	610	602	584	545	491	450
CONTROL	610	607	596.8	594	592	586

Microcosm analysis of COD reduction by seratia sp.

Table 3: COD Reduction by Selected Bacterial Strains

Strain 3- Dai C

Dai C	Initial COD	Othday	5thday	10thday	15thday	20thday
						/FINAL
						COD
DIB 0.1%	610	520	407	376	325	275
DIB 0.5%	610	510	485	316	275	208
DIB 1 %	610	502	490	380	235	194
CONTROL	610	607	596.8	594	592	586

Microcosm analysis of CODs reduction by staphylococcus sp.

Table -4: CODReduction by Selected Bacterial Strains

Strain 4- Dai D

Dai D	Initial COD	Othday	5thday	10thday	15thday	20thday
						/FINAL
						COD
DIB 0.1%	610	590	470	365	345	280
DIB 0.5%	610	570	490	378	330	274
DIB 1 %	610	535	480	370	273	220
CONTROL	610	607	596.8	594	592	586

Microcosm analysis of COD reduction by bacillus sp.

Table 5: CODReduction by Selected Bacterial Strain

Strain 5- Dai E

Dai E	Initial COD	Othday	5thday	10thday	15thday	20thday
						/FINAL
						COD
DIB 0.1%	610	518	416	371	273	210
DIB 0.5%	610	515	435	388	260	202
DIB 1 %	610	510	490	320	240	198
CONTROL	610	607	596.8	594	592	586

Microcosm analysis of COD reduction by micrococcus sp.

Table 6: COD Reduction by Selected Bacterial Strains

Strain 6- ConsD

ConsDs	Initial COD	Othday	5thday	10thday	15thday	20thday
						/FINAL
						COD
DIB 0.1%	610	578	498	388	267	210
DIB 0.5%	610	565	490	370	230	206
DIB 1 %	610	508	475	315	198	145
CONTROL	610	607	596.8	594	592	586

Microcosm analysis of COD reduction by consortia



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Fig.5:% COD reduction by *Pseudomonas sp.*



Fig.6:% COD reduction by *Seratia sp.*



Fig.7:% COD reduction by Staphylococcus sp.

Fig.8:% COD reduction by Bacillus sp





Fig.10:% COD reduction by consortia



Fig.11: COD reduction by all isolated strain with 1 %inoculation

The graphical representation shows that the COD value of the effluent is continuously decreasing and it indicates that the strains used for treating the effluent are helpful in COD reduction. The COD reduction percentage was calculated by using following formula –

%COD Reduction =COD Value Initial-COD Value final / Initial COD value x100

The results revealed that maximum COD reduction could be achieved in the flask incubated at 35° C as with 1 % inoculums with consortia which reduce the COD up to 76 %.. It has been reported by Kumar*et al.*⁹ inAgro-Based Pulp Mill Effluent.It was also reported by Chatterjee&Pugaht¹⁰.

CONCLUSION

In the present study, the bacterial isolates from untreated waste water were used for treatment of dairy waste water. The partial identification of all these isolates were done according to *Bergys Manualof Determinative Bacteriology* and they are shown to be *Pseudomonas sp.,Seratia sp.,Staphylococcus sp., Bacilus sp., micrococcus sp.,* As far as COD Reduction is concern the results showed that isolated bacteria strain from the influent of dairy industry have the ability to, reduce the COD value up to 76 % Best results were obtained in consortia which reduce the COD up to 76 %...

Treatment using natural coagulant Moringaoleifera was also studied for the treated effluent from the dairy plant⁸.Reduction of COD level by technique was also studied by Murali¹¹. We suggest that the addition of Consortia with this appropriate percentage to the microbial mixture of the activated sludge will increase the overall efficiency of the treatment system. It can Ibekue*etal.*¹² also reduce the bulking problems of the activated sludge by preventing the load of the organic matter from becoming too high. Thus industrial effluents from different industries may have been playing an important role in our social economy and creating serious problems solved by these isolates for the treatment of the effluent of dairy.

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