"Isolation of Microorganism from Dairy Effluent for Activated Sludge Treatment"

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Abstract

On current trends, it is estimated that, in the year 2030 most of our water resources would be exhausted or totally destroyed (UK Trade and Investment, 2002). Most water bodies have the ability to self-purify whereby they are pendent on environmental conditions, pollutant load and water retention times (Sanders and Yevjevich, 1996).But the natural contaminants are coupled with artificial pollutants, which are a result of human activities, the pollution load becomes too much for the water body to handle. The main pollutant derived from the industrial wastewaters are organic and inorganic substances,solved or in suspension, with different harmfulness degree (Banu, 1998). In the early 1960`s U.S. senate committee noted that dairy industry was second most important single source of pollution in streams (worners-1976).The dairy waste is basically organic and slightly alkaline in nature, when discharged in to streams without treatment, result in rapid depletion of dissolved oxygen(DO) and encourage the growth of algae i.e. eutrophication(Forsberg, 1998). Due to the overuse of surfactants in dairy, the waste can become unamenable to the biological treatment. The treatment of dairy wastewater, so that it is microbiologically and chemically acceptable for use in flush and irrigation applications, is of great importance (Ibekwe et al. 2003).Every Dairy is having particular characteristics of effluents and hence has the different effluent related problems. These problems can be revealed by effective treatment of the effluent a possible by the study of wastewater microbiota and to identify some new active strains adapted to the wastewater physical-chemical conditions, which metabolize organic compounds, similar to those which determine the pollution of wastewaters such as starch, casein, basic carbohydrates and lactic acid (Mihaela Palela et.al;2007).There were identified strains able to produce a fast biodegradation of the organic compounds. Normally to isolate microbial culture of endogenous origin for the development of microbial biomass which can be used effectively under Activated sludge treatment to stabilize the effluent. For that purpose bacteria isolated from effluent and study morphological, cultural and biochemical characteristics and identified with the help of Bergey's Mannuals of Systematic Bacteriology (1984)

Keyword: Dairy Effluent, Activated Sludge, Endogenous Culture, Isolation & Preliminary Identification , Microbiological And Biochemical Characterization.

1. Introduction:

Dairy wastewater disposal represents a major environmental problem. Numerous effective attempts have been made to resolve this problem by the activated sludge process is the mostly used biological treatment ever(Dashika Naidoo ;2005). This review discusses microorganisms associated with microbial digestion of dairy wastewater, biochemistry of the process, factors affecting microbial digestion, and efforts to develop defined cultures. 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 (Janczukowicz et al., 2007).Microbial digestion of dairy food wastewater offers many advantages over other treatments in that a high level of 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 waste. The microbes responsible for the organic and inorganic luxury uptake occurring in the treatment plant(G.W. Fuhs & M.Chen ;1975) The isolation of bacteria and the study of their identification have been hampered by the unreliability of conventional microbiological techniques. This is largely due to their morphological variations and inconsistent characteristics and different biochemical Characteristics. To fully understand their role in promoting activated sludge process, bacteria need to be characterized . The aim of this study was, therefore, the bacteria in pure culture isolated. fifteen different cultures were used for this study. The cultures were identified using Bergey's Mannuals of Systematic Bacteriology(1984).

2. Material And Methods:

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 (Janczukowicz *et al.*, 2007).

1.**Selection of source for isolation of micro organisms:**The dairy effluent samples were collected from "Shivamrut Dudh Utpadak Sahkari Sangh Maryadit, Vijaynagar (Vizori) Akluj". The sample was collected in a clean sterile plastic container and stored at 4oC until the analysis was carried out. (Trivedy and Goel ; 1984).Effluent samples were collected in duplicate from each station in pre sterilized bottles.

2.Primary screening: For primary screening the Dilutions of whey sample are prepared in distilled water. Selective dilutions are spread on nutrient agar, incubated and for 24hrs at room temperature.After incubation, isolated colonies are selected for further study.These selected colonies were restricted on nutrient agar for isolation by four quadrant method.These well isolated colonies were purified on nutrient agar and preserved on nutrient agar slant at 4^{o}C for long time for further studies.

3. Characterization of the bacterial isolates : By following methods.
3.1 Cultural Characteristics: The various typical colony characteristics of the bacterial isolates were recorded after their appearance on nutrient agar after 24 hours of incubation at 28^{o}C as described in sale (1974) Benson (1990) and Frobisher (1968).

3.2 Staining and Morphology: The Gram Characteristics and morphology of the isolates were studied by Gram staining method as described in Desai and Desai (1980).

4. Biochemical characterization: following two methods were used.
4.1 Enzymatic activities and 4.2 Carbohydrates utilization
4.1 Enzymatic activities: The extra-cellular production of various enzymes and their activities were studied using suitable method and materials described below.

4.1.1 Catalase:A loop full of growth of each bacterial isolate from nutrient agar slant was stirred in 30.0 v/v hydrogen peroxide and observed for evolution of gas as described in Blazevic (1975).

4.1.2.Gelatinase: Spot inoculation of the Bacterial isolates was done on sterile gelatin agar containing 0.4 % gelatin and incubated at 28oC for 24 hours. Gelatin hydrolyzing ability of micro-organisms was detected as the appearance of the clear zone after the addition of frazieres solution on the medium as described in Blazevic (1975).
4.1.3.Nitrate Reductase: Reduction of nitrate to nitrite was tested by inoculating bacterial isolates in tubes containing sterile peptone nitrate broth and detected by the sulphanilic acid - naphthyl-amine reaction as described in (Blazevic-1975).

4.1.4. Urease: Slants of sterile Christensen's urea agar (1946) were inoculated with the bacterial isolates and incubated at 28^{o}C for 24 hours Hydrolysis of urea was detected by the appearance of the pink colour in the medium as described in Blazevic (1975).

4.1.5. Oxidase: Colonies of the bacterial isolates are transferred by a glass rod on the filter paper strip moistened with freshly prepared 1% tetramethyl-1-p-phenylene diamine (TAPA) solution and observed for the appearance of a deep blue colour on the strip to indicate a positive result.

4.1.6.Starch hydrolysis: Spot inoculation of bacterial isolates was done on sterile starch agar containing 1% starch and incubated at 28^{o}C for 24 hours and then Lugols iodine was poured on the plates to detect zones of starch hydrolysis around and beneath the colonies.

4.1.7.Hugh and Leifsons Test: The bacterial isolates were inoculated in to the two tubes of Hugh and Leifsons Medium (Aerobic and Anaerobic) in order to check oxidative and fermentative metabolism of sugar and incubated at 28^{o}C for 24 hours. After incubation, tubes were observed for acid and gas production.

4.1.8.Hydrogen Sulphide Production Test: The bacterial isolates were inoculated in to sterile standard thiosulphate iron agar stab medium. Formation of black ferrious Sulphide precipitate in the medium indicated thiosulphate reduction.

4.2 Carbohydrates Utilization: Using peptone water as a basal medium in 5ml aliquots of each, the utilization of different carbohydrates by the bacterial isolates were studied. As described in Cruickshank (1973)The carbohydrates tested were :Disacclarides – Lactose, Maltose, Sucrose.;Hexoses- Glucose;Sugar alcohols – Mannitol, Sorbitol.;Sugar Vitamin – Inositol;Peptose – Xylose.

3. Result And Discussions:

Bacterial diversity has not been studied in detail in dairy effluent. However a few reports are available on the bacterial flora of certain effluents. Ravichandra et al. (2007) two strains of Thiobacillus sp were isolated from aerobic sludge of distillery and dairy effluent treatment plant . Diary industry effluent in Chennai *Bacillus Subtilis* $-galactosidase has been used as a probiotic source organism by *1Jayashree Natarajan et.al (2012)*

3.1Isolation of Bacterial cultures from Whey :

3.2 Isolation of Bacteria:

Total fifteen bacterial isolates were isolated using sterile nutrient agar medium after incubation at room temperature for 24 hrs. from the"Shivamrut Dudh Utpadak Sahkari Sangh Maryadit, Vijaynagar (Vizori) Akluj" dairy plant effluent.Micro flora of the effluents from a dairy factory in Tehran (Pegah Dairy Processing Plant) were isolated and screened for their ability to reduce the organic matter content and COD of the effluents by Maghsoodi et.al(2007).R. Prakashveni and M. Jagadeesan(2008)study 19 bacterial genera isolated from the Thanjavur Co-operative Milk Products dairy effluent *Alcaligens faecalis, Lueconostoc lactis* survive in all seasons .

3.3 Characterization of the bacterial isolates:

Morphological, cultured and biochemical characteristics of the isolates were studied. Micro flora of the effluents from a dairy factory in Tehran (Pegah Dairy Processing Plant) were isolated and screened for their ability to reduce the organic matter content and COD of the effluents by Maghsoodi et.al(2007)

3.4Cultural characteristics :

The data on the cultural characteristics of the bacterial isolates on nutrient agar is presented in table:1.The colonies of the isolates on nutrient agar were circular to irregular ranging in size from <1mm to 2mm. The colour of colonies was dirty white and yellowish mostly. The colonies of all the isolates have regular to irregular margins with mostly flat to convex elevation, moist consistency and opaque to translucent in nature. Jain *et. al* (2001) isolated three different bacterial species such as *Bacillusmegateriu, B. cereus and Xanthomonas fregariae* from distillery wastes effluent.

3.5 Staining and Morphology:

The bacterial isolates on nutrient agar were stained for their morphological characters and the results are presented in table 2.Out of the fifteen three were gram-negative short rod namely 3, 11, 13,Isolate 1,2,4,6 &12 are gram positive rods and isolate number 8,9, & 10 are gram positive cocci .Isolate number 2,4,& 6 were seen to produce endospore .Isolate 1 to 10 all are motile and 11 to 15 are non-motile.Similar finding recorded that Sree kumar et.al (2010) have successfully isolated a new strain of spore forming Bacilli that it capable fermenting lactose from dairy effluents.

3.6 Enzymatic activities:

Out of total fifteen isolates tested for enzymatic activity, eight isolates with number 2,5,6,7,8,9,14,15 show amylase activity and 1,2,3,10,11,12,13 show Urease activity, 1,2,3,5,6,7,8,10,11 show Gelatinase activity. Nitrate reductase activity is shown by isolates 2, 4, 5,6,7,8,9,10,15.Catalase activity is shown by isolates viz 2,3,4,5,6,8,9,10,13,14 while isolates viz 1, 3,4,5,7,8,10,11,12,13 shows Oxidase activity. Caseinase activity shown by all the isolates except No. 2, 5, 8, 11, 12, 15.While none of the bacterial isolate shows the hydrogen Sulphide activity. Isolate number 10 show all test positive except H_2S & amylase and Isolate No. 15 show all test negative except nitrate reduction H.L. test & amylase activity. On the basis of biochemical characteristics of microbes it can be concluded that the abovementioned chemoorganotrophs utilize energy from dairy effluent and chemolithotrophs accelerates the corrosion process by converting ferros ion to ferric and its oxides (Muthukumar *et al.*, 2003, Dawood *et al.*, 1998, Jayaraman *et al.*, 1998, Rajasekar *et al.*, 2005).

4. Utilization Of Carbohydrates :

Glucose is the sugar which is most favored by bacteria as all isolates utilize it. Lactose and sucrose are also the sugar favored by the bacterial isolates as twelve isolates utilized it. Maltose, Mannitol and insitol were utilized by seven isolates. Isolate number 2 utilized all the sugars while isolate number 14 utilized all sugars.Isolate number 4 also utilized all the carbohydrates while isolate number 15 utilized only glucose and Mannitol as shown in table 5

Table 1 Colony Characters of bacterial isolates .

Bacterial isolate	Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
1	1	Circular	Whitish	Regular	Flat	Opaque	Moist
2	<1	Round	Dirty white	Irregular	Slight Convex	Opaque	Moist
3	2	Circular	Pinkish	Regular	Flat	Opaque	Moist
4	2	Circular	Dirty white	Regular	Slight Convex	Opaque	Moist
5	<1	Irregular	White	Entire	Flat	Transparent	Moist
6	2	Circular	Faint red	Entire	Concave	Transparent	Moist
7	1	Irregular	Yellowish	Regular	Convex	Transparent	Moist
8	2	Regular	White	Irregular	Flat	Transparent	Moist
9	2	Irregular	Yellowish	Irregular	Flat	Transparent	Moist
10	<1	Circular	Yellowish	Regular	Convex	Transparent	Moist
11	<1	Circular	Colorless	Entire	Convex	Translucent	Moist
12	1	Circular	Yellowish	Entire	Convex	Opaque	Moist
13	2	Circular	Colorless	Entire	Convex	Opaque	Moist
14	1	Circular	Faint orange	Entire	Convex	Opaque	Moist
15	1	Circular	Whitish	Irregular	Raised	Opaque	Moist

Isolate No.	Gram Character	Sporulation	Shape of cell	Arrangement Of cell	Motility
1	Positive	-	Short rod	Pairs	Motile
2	Positive	+	Long rod	Long Chain	Motile
3	Negative	-	Short rod	Pairs	Motile
4	Positive	+	Long rod	Long Chain	Motile
5	Negative	-	Rods	Single	Motile
6	Positive	+	Rods	Single	Motile
7	Negative	-	Rods	Pairs	Motile
8	Positive	-	Cocci	Chains	Motile
9	Positive	-	Cocci	Bunches	Motile
10	Positive	-	Cocci	Bunches	Motile
11	Negative	-	Short rod	Chains	Non motile
12	Positive	-	Rods	Chains	Non motile
13	Negative	-	Short rod	Chains	Non motile
14	Negative	-	Rods	Chains	Non motile
15	Negative	-	Rods	Chains	Non motile

Table: 2 Staining and Morphology of the bacterial isolates.

Test No.	Catalase	Oxidase	H_2S	Nitrate Reduction	H.L. Test	Gelatinase	Urease	Amylase	Caseinase
1	-	+	-	-	-	+	+	-	+
2	+	-	-	+	+	+	+	+	-
3	+	+	-	-	+	+	+	-	+
4	+	+	-	+	-	-	-	-	+
5	+	+	-	+	+	+	-	+	-
6	+	-	-	+	+	+	-	+	+
7	-	+	-	+	-	+	-	+	+
8	+	+	-	+	-	+	-	+	-
9	+	-	-	+	+	-	-	+	+
10	+	+	-	+	+	+	+	-	+
11	-	+	-	-	-	+	+	-	-
12	-	+	-	-	+	-	+	-	-
13	+	+	-	-	+	-	+	-	+
14	+	-	-	-	+	-	-	+	+
15	-	-	-	+	+	-	-	+	-

Table 3 Biochemical Characteristics of the bacterial isolates.

Where " +" : Positive test
" -" : Negative test

Table 4 Utilization of carbohydrates by the bacterial isolates

Isolate No.	Lactose	Sucrose	Glucose	Maltose	Mannitol	Inositol	Xylose
1	+	+	+	+	+	-	-
2	+	+	+	+	+	+	+
3	-	-	+	-	+	+	-
4	+	+	+	+	+	+	+
5	+	-	+	+	-	-	-
6	+	+	+	-	-	+	+
7	-	+	+	-	-	+	-
8	+	+	+	+	-	-	-
9	+	+	+	+	-	-	-
10	+	+	+	+	+	-	-
11	+	+	+	+	-	+	+
12	-	+	+	+	-	+	+
13	+	-	+	+	+	-	-
14	+	+	+	+	+	+	+
15	-	-	+	-	+	-	-

Where , "+" -positive test
"-" -negative test

Table 5 Tentative identification of bacterial isolate from Bergey's Mannuals of Systematic Bacteriology

Sr. No.	Isolate Number	Tentative identification
1	1	*Lactobacillus*
2	2	*Bacillus*
3	3	*Pseudomonas*
4	4	*Bacillus*
5	5	*Azotobacter*
6	6	*Arthrobacter*
7	7	*Zoogloea*
8	8	*Micro bacterium*
9	9	*Staphyllococcus*
10	10	*Micrococcus*
11	11	*Cardiobacterium*
12	12	*Bifidobacterium*
13	13	*Pasterurella*
14	14	*Escherichia*
15	15	*Eikenella*

5. Conclusions :

According to the results, bacterial isolate are the most effective organisms for the activated sludge treatment to reduce the organic matter of high BOD- COD concentration of dairy effluent. It also decreased the carbohydrate ,nitrate to nitrite, fat, gelatin, urea, starch and protein content of the waste. We suggest that the addition of bacterium to the microbial mixture of the activated sludge will increase the overall efficiency of the treatment system. It can 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 effluents of Dairy.

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