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DEVELOPMENT OF MICROBIAL CONSORTIUM FOR BIOAUGMENTATION AND ELECTRICITY GENERATION FROM DAIRY EFFLUENT

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Abstract: Analysis of effluent from ten selected dairies from Nashik city (Maharshtra, India) with respect to the parameters recommended in Environment protection act 1986 and two additional parameters, showed pH, TSS, TS, BOD, COD and oil and fats much above the permissible limits. In the current study, a microbial consortium containing microaerophilic indigenous bacteria and yeasts and two exogenous bacteria named *E.coli* ATCC 20715 and *B.amyloliquifaciens* ATCC 15841 was prepared and used for bioaugmentation as well as generation of electricity from dairy effluent by constructing a Microbial Fuel Cell (MFC). For preparation of microbial consortium,30 indigenous yeasts and 25 indigenous bacteria were isolated from 10 selected dairy effluents. Two best indigenous bacteria and yeasts were selected and identified using 'VITEK-2'automated system and 18S-rRNA gene sequencing technology respectively. The bacteria were *Enterococcus casseliflavus* and *Enterococcus faecium* and yeasts were *Kluyveromyces lactis* strain 1 and 2. The exogenous bacteria selected were with known activity of extracellular protease and lipase. Three microbial consortia were prepared with different combinations of indigenous and exogenous organisms. In self degradation experiment of dairy effluents, maximum reduction in BOD was 20.01% after 6 days and pH became more acidic in the range 5.2 to 6.2. The best consortium gave maximum reduction of 64.47% in BOD and 65.43% in COD after 6 days when individual effluent was used. 10 % inoculum of the best consortium was found to be the most optimum for bioaugmentation. Reduction in parameters of individual dairy effluents with the treatment using 10% of the best consortium after 10 days was 87%, 86%, 64.37%, 67%, and 49.23% in TSS, TS, BOD, COD and oils and fats respectively. *E.coli* was found to be sensitive to common antibiotics like Amikacin, Chloramphenicol *etc*.The immobilized consortium showed 10 to 24 % more reduction after 6 days in all above mentioned parameters. Using the same consortium, electricity was generated in MFC. Maximum voltage obtained was 363 mV and % BOD reduction in MFC was 55.27% on 10^{th} day.

Keywords: Dairy effluent, Biological Oxygen Demand (BOD), Bioaugmentation, Microbial Consortium, Microbial Fuel Cell (MFC), Bioelectricity

INTRODUCTION

White Revolution is a renowned program in India implemented in 1970 under the

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guidance of National Dairy Development Board (NDDB) to increase the production of milk. Dr. Verghese Kuerin is honoured as the father of the Indian White Revolution [1]. Since 1998, owing to the efforts under the white revolution, India ranks first in the world in production and consumption of milk and milk products with the dairy industry contributing largely to the nation's economy. In 2019, the value of Indian dairy industry was worth INR 10,527 Billion. India has the largest bovine population in the world with dairy being an important source of income in rural India [2]; [1].

Among food industries, dairy industry is the most polluting, because it consumes large amount of water and generates a large volume of effluent, which is highly polluting and a cause for eutrophication in water bodies [3]. In dairies the process of washing and cleaning consists of rinsing milk cans, tanks and pipes for the purpose of removing milk residues and other impurities and of washing floors, and can generate 50 to 95% of the total effluent volume [4]. A typical untreated dairy effluent is characterized by high organic loads, with high levels of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) [5].

Discharge of untreated dairy waste in rivers causes massive pollution. Hence, after proper treatment only, industrial effluents should be discharged in water bodies. All major constituents of dairy effluent are bio-degradable. As a result, biological oxidation of organic constituents by natural micro flora carried out in activated sludge system or trickling filters is a common method used for its treatment [6]. However, this method needs vigorous aeration for which a large amount of electricity is consumed making it quite expensive.

Researchers have used different treatments for dairy effluent. Reverse osmosis of dairy wastewaters was used with good nutrient removal efficiency [3]. Some had prepared microbial consortia for aerobic treatment using *Bacillus, Serratia, Lactococcus, Enterococcus, Stenotrophomonas, Klebsiella and Escherichia* [6]. The best consortium was selected using fabricated dairy effluent and COD removal efficiency of the consortia was tested. Ozonization was used as a new treatment by using solidification reaction of milk fats and proteins [7]. Dairy effluent was subjected to lipid estimation and treated using Microalgae [8]. It was observed that high strength waste waters with COD more than 4000 mg/lit required a sequential anaerobic-aerobic treatment for removal of COD [9].

Enhancement of degradation using more efficient micro-organisms is described as bioaugmentation [10];[11]. Many researchers have used bioaugmentation successfully for treatment of various industrial wastes [12]. There are numerous reviews published on use of bioaugmentation for the treatment of industrial wastes [13];[14];[15]. For dairy effluents,

aerobic treatment with bioaugmentation has been tested by some researchers [6];[16]. Bioaugmentation without aeration may prove to be a cheaper alternative as there is no consumption of electricity for aeration.

Many studies reported that using a mixture of several microorganisms (Microbial consortium) is more effective than using a monoculture. [17]; [18]. It was mentioned in 2020 that single microorganism is not efficient in degradation of organic compounds [19]. A consortium with mixed organisms has better degradation capacities. Microbial consortium has higher potential of biodegradation as compared to a single organism [20]. In a consortium, co-existence of micro-organisms may produce a synergistic effect. Various metabolic pathways present in different organisms are helpful in increasing degradation rate of organic substrates to a larger extent. Microbial consortium is more robust than a single organism and can withstand adverse environmental conditions [21]. Mixed cultures assist to overcome feedback regulation and catabolite repression by allowing the use of one organism's product as substrate by the other [22].

Whole cell immobilization of microbes has received increasing interest in the field of waste treatment [23]. Advantage of this technique is, it helps retain high cell concentrations within the bioreactor and protect organisms from a hostile environment. Moreover, immobilized microorganisms can be used several times without significant loss of activity, proving to be cost effective [24];[25]. Therefore, immobilized microorganisms technology has been explored as promising technology for wastewater treatment in the past few decades [26].

A review was published on Microbial Fuel Cells (MFCs) which detailed early models of MFCs, latest modifications in the model, limitations of MFC technology and potential applications of MFC [27]. The mechanism of energy generation in MFC has been described in detail [28].

Researchers had isolated several organisms from tea garden soil, and screened for their efficiency of bioelectricity generation from household waste. They had constructed a two chambered microbial fuel cell. The potential isolate was identified as *Bacillus megaterium*. It was used in microbial fuel cell for generation of bio electricity [29].

Nashik is a city in Maharashtra state located in Western India. Its area is 264.2 Km^2 and in 2020 its population was 2.66 crores. There are plenty of small scale dairies in Nashik city. Most of the treatments employed at large scale dairies are highly expensive, not affordable to small scale dairies.

Survey of Nashik city indicated that the treatment given to effluent at most of the small scale dairies is not adequate and sufficient due to their unaffordability. Hence, in the current study, a cost effective bioaugmentation without aeration using an efficient microbial consortium is suggested. The consortium was then subjected to whole cell immobilization using gel entrapment with calcium alginate and studied for efficiency of biodegradation. Due to energy crisis, alternative sources of energy are in demand. Construction of Microbial Fuel Cell and generation of bioelectricity from the effluent is helpful in waste management without causing pollution. Hence generation of bioelectricity using the consortium developed by constructing a Microbial Fuel Cell (MFC) has been tried which is wealth from waste.

In present study, for developing microbial consortium, indigenous organisms were isolated from the effluent itself. Majority of these organisms are either facultatively anaerobic or microaerophilic. Hence, they do not require aeration for growth and biodegradation. Combination of indigenous organisms and few already tried exogenous organisms was used in the consortium.

2. MATERIAL AND METHOD

2.1 Sampling

1 liter untreated effluent was collected from each of 10 selected dairies located in central and peripheral parts of Nashik city (Maharashtra, India) in a sterile bottle for isolation of organisms and 5 lit in clean plastic can for analysis and treatment. Among the selected dairies, one was large scale, two were medium scale and remaining seven were small scale dairies. The main product of these dairies is milk. At some dairies curd, ghee and buttermilk are also produced. The sample collected was an effluent generated during washing of cans, filling milk into plastic bags or glass bottles and making other products like curd and butter milk. The effluents were thoroughly mixed and within 2 hours were subjected to analysis. In case of delay, the samples were refrigerated.

2.2 Analysis of dairy effluent

The dairy effluents were analyzed with respect to the parameters recommended for the analysis of dairy effluent by Environment Protection Act 1986.These include pH, Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), oil and fats. Additional parameters selected for analysis were Chemical Oxygen Demand (COD) and Total Solids (TS).

Protocols for analysis of dairy effluent used were as per ‘APHA manual’ (American Public Health Analysis) [30] or IS manual (Indian Standards). All tests were carried out in triplicates.

2.3 Checking degradation of organic matter by indigenous micro flora of the effluent.

500 ml sample from 10 selected dairies was separately filled in conical flasks, plugged and kept at room temperature for 6 days. % reduction in BOD_3 due to self degradation was checked after 6 days.

2.4 Isolation of indigenous bacteria and yeasts

Indigenous bacteria belonging to Lactic acid bacteria group and yeasts were isolated from dairy effluents using sterile Deman, Rogosa and Sharpe medium (DMRS), (gm/lit) peptone:10, yeast extract:5, Glucose:20, Tween80:1, Ammonium citrate:2, Sodium acetate:5, Magnesium sulphate:0.1, Dipotassium hydrogen phosphate:2, pH:6.5, Agar:2) and Sabouraud's agar (gm/lit) Dextrose: 40, Peptone: 10, pH: 5.6, Agar:20 respectively. Pure slant cultures have been prepared and preserved. Cultural and morphological characterization of these isolates has been carried out by conventional methods.

2.5 Selection of the best indigenous cultures

For selection of the best indigenous bacterial and yeast cultures, a modified nutrient broth was used with the composition (gm/lit) yeast extract:3, NaCl:5 and incorporated with 2% mixed effluent from 10 selected dairies. In this medium peptone from nutrient broth has been replaced by dairy effluent. The role of peptone is carbon and nitrogen source which is played by dairy effluent in the modified medium.

Isolates showing maximum growth, measured in terms of optical density (OD) at 540 nm using visible spectrophotometer (Systronics model) were selected as the best isolates.

2.6 Identification of the organisms

Strains of *E.coli* ATCC 20715 and *B.amyloliquifaciens* ATCC 15841 used as exogenous bacteria were confirmed by using VITEK 2 automated identification system.

Indigenous bacteria also have been identified using VITEK 2 automated system. VITEK2 is a fully automated system that performs bacterial identification

based on results of suitable biochemical tests and can give up to 99% identification of bacteria. For *E.coli*, 55 tests, for *Bacillus amyloliquifaciens*, 46 tests, for indigenous bacterium1, total 43 tests and for indigenous bacterium 2, total 43 tests have been carried out in VITEK 2. Indigenous yeasts were identified by 18S-rRNA sequencing technology at National Centre for Microbial Research (NCMR), Pune, India.

2.7 Confirmation of protease and lipase activity of exogenous bacteria

For protease test, sterile plates with a medium having the following composition were

prepared (gm/lit) Casein: 10, Gelatin:10, skimmed milk powder:10, Agar: 20. Wells were made with alcohol sterilized cork borer in the plate and filled with cell free supernatant of 24 hours old nutrient broth culture of *E.coli* ATCC 20715 and incubated at 37^0C for 24 hours. Due to extracellular degradation of proteins a clear zone is developed around a well which indicates a positive test [31].

For Lipase test sterile plates with a medium having following composition were prepared. Olive oil, 0.1ml, Phenol red, 0.01gm, Calcium chloride, 0.1gm, Agar, 2gm; Distilled water, 100 ml, pH, 7.4. The plates were inoculated with 24 hours old nutrient broth culture of *B.amyloliquifaciens* ATCC 15841 and incubated at 20^0C for 24 hours. Degradation of fats and lipids by extracellular lipase and production of fatty acids makes the medium acidic and phenol red changes its colour to yellow which is a positive test. The control plate shows original red colour [32].

2.8 Development of microbial consortium

Exogenous bacteria *E.coli* ATCC 20715 *and Bacillus amyloliquifaciens* ATCC 15841 were grown in 100 ml sterile nutrient broth for 48 hours at 37^0C and 20^0C respectively. Fastest growing indigenous yeasts, isolated from dairy effluent were grown in 100 ml of Sabouraud's broth for 48 hours at 20^0C and two fastest growing indigenous bacteria isolated from dairy effluent were grown in 100 ml MRS broth separately for 48 hours at 20^0C. After incubation, all cultures were centrifuged at 3000rpm, pellets were washed with sterile saline. These steps repeated twice to avoid incorporation of nutrients from the medium. Pellets were re-suspended in volume of sterile saline equal to broth centrifuged. Each suspension was diluted to 0.1 optical density (O.D.) at 540 nm using spectrophotometer. Equal volumes of diluted cultures were mixed to prepare three different consortia as follows.

Consortium 1: The best indigenous bacteria 1 and 2+ The best indigenous yeasts 1and 2

Consortium 2: The best indigenous bacteria 1 and 2+ The best indigenous yeasts 1 and 2+ Exogenous bacterium 1

Consortium 3: The best indigenous bacteria 1 and 2+ The best indigenous yeasts 1and 2+ Exogenous bacterium 1+ Exogenous bacterium 2

2.9 Checking compatibility of organisms in the consortium

5ml of the all 3 consortia was individually inoculated in 100 ml sterile nutrient broth and incubated for 48 hours at room temperature. The broth was serially diluted up to 10^{-7} and growth of each type of organism was determined on specific media.

2.10 Selection of the best consortium

All 3 microbial consortia were studied with reference to reduction of BOD and COD of effluents from individual 10 selected dairies after 6 days at room temperature. Each effluent was inoculated with 5% consortium of above types and incubated at room temperature for 6 days. Initial and Final BOD and COD were estimated.

2.11 effect of inoculum size on BOD and COD reduction of mixed effluent

Based on results of BOD and COD reduction after 6 days, consortium 3 was selected as the best consortium. It was then used to study the effect of inoculum size for maximum BOD and COD reduction.

500 ml of mixed effluent was inoculated with 1%, 2.5%, 5%, 7.5%, 10% and 15% inoculum and incubated for 6 days at room temperature. Initial and final BOD and COD were estimated and % BOD and COD reduction after 6 days was calculated.

2.12 Bioaugmentation of individual dairy effluents with the best consortium

1 lit effluent from 10 selected dairies was inoculated individually with 10% (0.1 O.D. at 540 nm) of the best consortium and tested for pH, TSS, TS, BOD, COD, and oils and fats before and after 6 and 10 days incubation at room temperature.

2.13 Antibiotic sensitivity testing

Since *E.coli* is an opportunistic pathogen, its antibiotic sensitivity test was carried out by spreading 0.1 ml of 24 hours old culture on sterile Diagnostic Sensitivity Test agar (Hi Media), with alcohol sterilized forceps, antibiotic multi disc ring (Dynamicro Labs. Pvt. Ltd.) was placed on the plate. It was incubated at 37^0C for 24 hours. Size of zones of inhibition of growth of the organism around the antibiotic discs were measured and compared with standard chart for interpretation of results.

2.14 Whole cell immobilization of the consortium and application

100 ml of the consortium 3 (O.D. 0.1 at 540nm) was mixed with 200 ml of sterile 4% sodium alginate and with a sterile syringe added drop by drop in 1lit of ice chilled solution of 0.2 M sterile $CaCl_2$ to form beads. They were allowed to harden in the same amount of fresh ice cold, 0.2 M $CaCl_2$ solution for 24 hours in refrigerator, then washed with sterile distilled water, transferred to 1 lit mixed effluent [33]. Analysis with respect to the same parameters was carried out after 6 days and 10 days. To check the stability of beads, some were transferred to sterile nutrient broth, incubated at room temperature and increase in turbidity of the broth was recorded on spectrophotometer at 540nm.

2.15 Construction of microbial fuel cell (MFC) and generation of bioelectricity

A Two chambered MFC was constructed for checking bioelectricity generation. One was anodic chamber and the other was cathodic chamber. In both the chambers flat graphite electrodes (15cm x 3cm) were used. Electrodes were soaked in distilled water overnight before use. Wiped, dried and wrapped with copper wires. Ends of Copper wires were drawn out from the lids of both the chambers. Both the chambers were wiped with alcohol and dried. Anodic chamber was filled with 500 ml mixed dairy effluent (effluent from ten dairies was mixed in equal volumes). The anodic chamber was properly sealed to make it anaerobic. Effluent in the anodic chamber was inoculated with 10% of the best consortium O.D. of which was adjusted to 0.1 at 540 nm. Cathodic chamber was filled with phosphate buffer, pH 7. The chambers were connected to each other by a salt agar bridge (2.5% NaCl and 2.5% agar). Experiment was carried out for 10 consecutive days. On each day, 10 readings of voltage generated were recorded in mV at the same time using a multi-meter (DM3540A model). Initial Biological oxygen demand (BOD) of the mixed effluent and BOD after 10 days of treatment in MFC was determined.

3. RESULTS AND DISCUSSION

3.1 Sampling

Untreated dairy effluents collected from 10 selected dairies were white/yellowish in colour and significantly turbid. All samples had pungent smell. Effluents from ten dairies mixed in equal volume, appeared moderately turbid and also had pungent smell.

3.2 Analysis of dairy effluents

Results of initial pH, Total Suspended Solids (TSS), Total Solids (TS), BOD, COD, and oil and fats of individual effluents from ten selected dairies showed large variation. BOD of dairy 4 was 1817 mg/lit which was highest amongst them. COD estimated by open reflux method of dairy 1 was 985.66 mg/lit which was least amongst them. Total solids were in the range of 677 mg/lit to 2647mg/lit. Maximum TSS were 905.7 mg/lit. Oil and fats in all samples were found to be in the range of 200 to 733.33 mg/lit. pH of all samples was in the range of 6.3 to 7.6 (**Table 1**)

Table 1: Results of analysis for selected parameters of untreated dairy effluents

DAIRY NO	pH	TS mg/lit	TSS mg/lit	BOD mg/lit	COD mg/lit	Oil and fats mg/lit
1	7.1±0.1	946±10.39	92±1.73	320.33±19.39	985.66±16.25	200±10

2	6.8±0.1	1689±27.22	543.7±4.16	1274±16.82	3459±46.04	300±10
3	6.3±0.1	2463.33±33.72	437±6.24	1362±60.09	3702±25.05	550±10
4	6.8±0.1	2254±24.85	634.3±29.83	1817±37.85	3164±141.05	633.33±52.9
5	7±0.1	2647±46.50	905.7±9.07	994.3±14.01	2772±10.78	466.66±57.73
6	6.8±0.1	749.66±43.93	312.7±7.21	758.7±44.24	2364.33±57.32	466.66±57.73
7	7.6±0.1	2184±55.74	545±12.05	761±6.02	2325±15	300±10
8	6.6±0.1	677±19.97	349±4.50	406±10.69	1269±60.34	300±10
9	6.5±0.1	2403.66±50.50	677±5.13	2613±29.71	5452±42.33	203.33±10
10	6.4±0.1	1866±60.89	393.33±4.16	830±14	2620±53.37	733.33±57.73
E.P.L	**6.5 to 8.5**	**500**	**150**	**30**	**250**	**10**

E.P.L.-Environmental Permissible limits

Researchers had carried out analysis of untreated dairy effluent from one dairy and got BOD 1268 mg/lit, COD 2398 mg/lit, TS 2342mg/lit and TSS 626.5mg/lit [34]. Others carried out physicochemical analysis of dairy effluent and reported 644mg/lit TSS, 1166mg/lit BOD and 2248mg/lit COD [35]. Results of analysis of dairy effluents obtained in the current study are comparable with the results obtained by earlier researchers.

3.3 Self degradation of dairy effluents by indigenous micro flora

Dairy effluent has its own microbial flora which mainly consists of bacteria and yeasts. When effluent from ten dairies was kept to undergo self- degradation, efficiency of the self micro flora of the effluents was seen to be low indicated by less reduction in BOD (maximum 20.01%), after 6 days. pH was seen to become more acidic after 6 days in the range of 5.2 to 5.7.

3.4 Isolation of indigenous organisms

DMRS agar was used for isolation of indigenous bacteria which mainly consist of a group of lactic acid bacteria. DMRS agar is a nutrient rich medium recommended for the isolation of lactic acid bacteria. Sabouraud's agar was used for isolation of indigenous yeasts. Due to acidic pH and high sugar content of this medium, it favours growth of yeasts and suppresses growth of bacteria.

30 bacteria and 35 yeasts were isolated from ten dairy effluents. The bacterial isolates were numbered as B1, B2, B3---B25 and yeasts were numbered as Y1, Y2, Y3---Y30.

Majority of the bacterial isolates were Gram positive cocci and few were Gram positive rods. Yeasts exhibited large oval Gram positive cells.

Researchers had used only exogenous bacteria for the preparation of microbial consortium [6]. However, mixed consortium of indigenous and exogenous organisms would have better efficiency. Indigenous organisms are already adapted to the environment of dairy effluent and have greater efficiency of degradation of organic matter. Hence in the current study a consortium was prepared using both indigenous and exogenous organisms.

3.5 Selection of the best indigenous organisms

Some researchers mentioned that measurement of optical density can be used for measurement of growth of bacteria [36]. In current study, in order to select the best indigenous bacteria and yeasts a modified medium was designed which contained 2% mixed dairy effluent as the carbon and nitrogen source. Highest turbidity produced in the modified medium indicated maximum growth of the organism. When OD of 48 hours old cultures of yeasts and bacteria in the modified medium was recorded using visible spectrophotometer (Systronics, model166) at 540nm. Y17 and Y18 of yeast cultures and B15 and B13 of bacterial cultures showed highest OD. Hence, they were selected as the best indigenous bacteria and yeasts respectively, for incorporation into the consortium.

3.6 Identification of indigenous bacteria, yeasts and confirmation of exogenous

Bacteria

Indigenous bacteria were identified by VITEK-2 as *Enterococcus casseliflavus* and *Enterococcus faecium* and both the yeasts have been identified by 18 S-rRNA gene sequencing at NCMR, Pune, India as *Kluyveromyces lactis*. Both bacteria belong to lactic acid group of bacteria. Identity of both exogenous bacteria was confirmed by VITEK-2automated system.

Researchers had isolated protease producing bacteria from food processing industries. They identified those isolates using VITEK-2 automated system. They got 85.5% probability of accurate identification. They have mentioned several advantages of this system over conventional methods used for identification of bacteria [37]. Researchers in 1997 had mentioned that traditional methods for identification of yeasts based on biochemical, physiological and morphological criteria are time-consuming, laborious and very similar species may be difficult to distinguish hence, they had used 18S rRNA gene sequencing technology for the identification of yeasts isolated from yoghurt [38].

3.7 Confirmation of protease and lipase activity of exogenous bacteria

Two exogenous bacteria, *E.coli* and *Bacillus amyloliquifaciens* procured earlier from ATCC culture collection center were selected for use in consortium. They were known to produce extracellular protease and lipase respectively. However their enzymatic activities were confirmed by protease test using a medium incorporated with various proteins. **Figure 1** shows clear zone around the well filled with cell free broth of *E.coli* due to degradation of proteins by extracellular protease, indicating positive test. No clear zone is seen around the control well filled with sterile distilled water. Lipase test was carried out using a medium incorporated with various sources of fats. **Figure 2** shows development of yellow colour in the 'test'plate streaked with culture of *Bacillus amyloliquifaciens* due to degradation of fats, production of fatty acids changing colour of phenol red to yellow which is a positive test. The 'control' plate is red in colour indicating no degradation of fats.

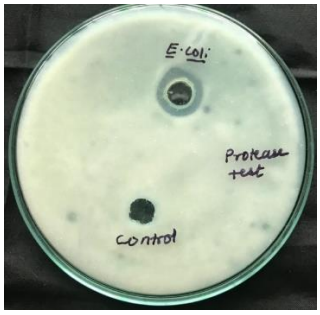

Figure 1: Protease activity of *E.coli*

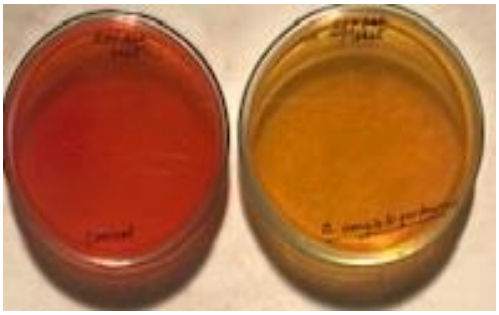

Figure 2: Lipase activity of *B.amyloliquifaciens*

3.8 Development of microbial consortia

Three different consortia were developed with different combinations of indigenous bacteria and yeasts and two exogenous bacteria. Indigenous bacteria and yeasts are already adapted to the parameters of dairy effluent. Hence, a consortium with only indigenous bacteria and yeasts (consortium 1) was prepared and tested. When indigenous organisms were combined with exogenous bacteria with known protease and lipase activities in consortia 2

and 3 greater degradation efficiency was expected. The best consortium (Consortium 3) consisted of *Enterococcus casseliflavus, Enterococcus faecium, Kluyveromyces lactis* (*both strains*), *E.coli* ATCC 20715 and *Bacillus amyloliquifaciens* ATCC15841.

3.9 Compatibility test for all 3 consortia

Results of compatibility test for organisms used in all 3 consortia indicated that they were able to survive together. No organism was found to be completely inhibited due to presence of other organisms.

3.10 Selection of the best consortium

Consortium 1 showed more than 35% reduction in BOD in five dairy effluents. It gave above 40% reduction in two dairy effluents and in remaining three reduction was between 35% to 39%. Maximum reduction in BOD obtained with consortium 1 was 46.12%. Consortium 2 showed reduction in BOD more than 35 % in five dairy effluents of which four dairy effluents showed reduction in BOD between 42 to 47 % and only one dairy showed 35 % reduction in BOD. Maximum reduction obtained using consortium 2 was 47.14%. Consortium 3 showed reduction in BOD more than 40% in eight dairy effluents. In 4 dairy effluents reduction in BOD was near about 50%. In two dairy effluents reduction was between 41% to 43% and in remaining two, it was above 60%. Maximum reduction obtained with consortium 3 was 64.47%.

Consortium 1 showed more than 40% COD reduction in 4 dairy effluents among which three showed COD reduction in the range of 43 to 46% and one showed near 60%. Maximum reduction in COD obtained with consortium 1 was 60.34%. Consortium 2 showed more than 40% reduction in 5 dairy effluents of which four dairy effluents showed reduction between 42 to 49% and one dairy around 60%. Maximum reduction was found to be 61.34%. Consortium 3 showed more than 40% reduction in COD of 7 dairy effluents. Maximum reduction in COD obtained with consortium 3 was 65.43 %. Among seven dairies four dairy effluents gave COD reduction between 42 to 48 % and in two dairy effluents it was about 53%. From these results, consortium 3 was selected as the best consortium and used for bioaugmentation.

3.11 Effect of inoculum size on BOD and COD reduction of mixed effluent

When effect of inoculum size on BOD and COD reduction of mixed effluent after 6 days was studied, 10% and 15% v/v inoculum gave 58.18 and 58.84% reduction in BOD and 40.11 and 40.38% reduction in COD respectively. Since there was less difference in reduction of BOD and COD by 10% and 15 % inoculum sizes, 10% v/v inoculum size was selected for bioaugmentation study.

3.12 Bioaugmentation of individual dairy effluents with the best consortium

3.12.1 Reduction in pH

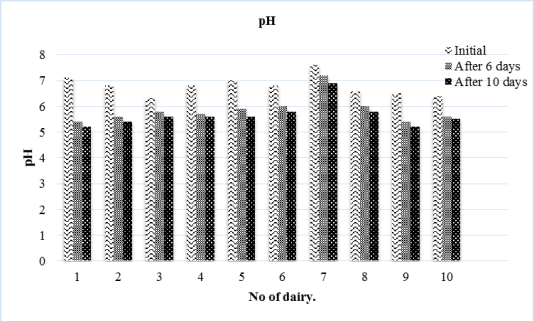

Figure 1: reduction in pH after 6 and 10 days

When individual dairy effluents from 10 selected dairies were treated with 10% v/v consortium 3 (O.D.0.1 at 540 nm), pH was seen to decrease from the range of 6.3-7.6 to 5.4 -7.2 after 6 days and to a range of 5.2-6.9 after 10 days (**Figure 1**) Researcher in 2014 had used microbial consortium for treatment of dairy effluent. They also had noted drop in pH from 7.19 to 7.03 after 72 hours [20]. Others in 2011 observed that pH of effluent gradually decreased from 7.2 to 7.5 to 5.0 after 12 days when an anaerobic treatment was given to dairy effluent [39]. Such decrease in pH may be attributed to fermentation of lactose present in dairy effluent by micro organisms and production of lactic acid.

3.12.2 Reduction in TSS

consortium 3 (O.D.0.1 at 540 nm). Among all dairy effluents, minimum reduction in TSS was observed to be 38.5% i.e. from 393.33 mg/lit (initial) to 241.66 mg/lit (final) and maximum reduction was 75.36% i.e. from 92 mg/lit (initial) to 22.66 mg/lit (final) after 6 days of treatment. Minimum reduction in TSS after 10 days of treatment was 39.83% i.e from 393.33 mg/lit (initial) to 236.66 mg/lit (final) and maximum reduction in TSS was 87.68% i.e. from 92 mg/lit (initial) to 11.33 mg/lit (final) (**Figure 2**).

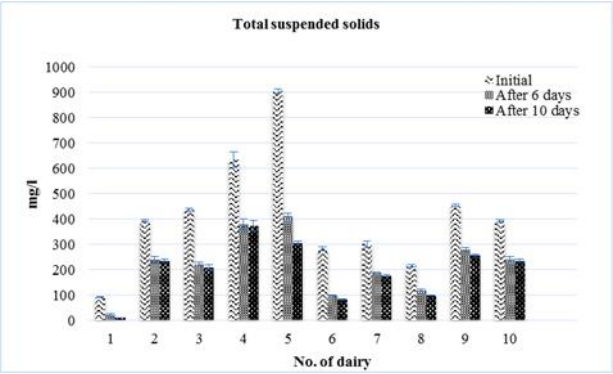

Figure 2: TSS reduction after 6 and 10 days

Researchers in 2013 tried vermicomposting for treatment of dairy effluent. They noticed reduction in TSS by 85-90% from dairy effluent due to ingestion and biodegradation of organic wastes caused by earthworms [40]. In current study, bioaugmentation is used which is a simpler technique. Reduction in TSS by using microbial consortium is comparable with the results obtained by other researchers.

3.12.3 Reduction in TS

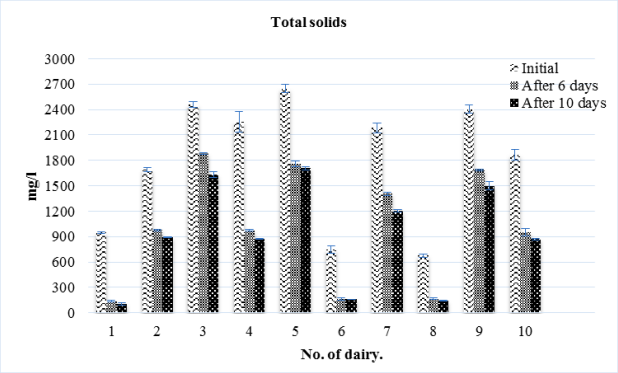

Figure3 : TS reduction after 6 and 10 days

Individual dairy effluents from 10 selected dairies were treated with 10% v/v consortium 3 (O.D.0.1 at 540 nm). Among all dairy effluents, minimum reduction is TS was observed to be 23.67i.e. from 2463.33 mg/lit (initial) to 1880.33 mg/lit (final) and maximum reduction was 85.80% i.e. from 946 mg/lit (initial) to 134.33 mg/lit (final) after 6 days. Minimum reduction in TS after 10 days of treatment was 33.82% i.e. from 2463.33 mg/lit (initial) to 1630 mg/lit (final) and maximum reduction in TS was 88.83% i.e. from 946 mg/lit (initial) to 105.66 mg/lit (final) (**Figure 3**).

Researchers in their paper published in 2015, reported TS in effluent collected from dairy effluent treatment plant located in Pune, Maharashtra as 2342mg/lit in the month of December 2010 and observed reduction in TS to 1354mg/lit by inoculating organisms from sewage sludge under aerobic treatment and reduction in TS from 1964mg/lit to 1276 mg/lit was reported in the month of January 2011 with the same treatment [34]. In current study the treatment employed is not aerobic still comparable reduction in TS has been observed.

3.12.4 Reduction in BOD

Individual dairy effluents from 10 selected dairies were treated with 10% v/v consortium 3 (O.D.0.1 at 540 nm). Among all dairy effluents, minimum reduction in BOD was observed to be 29% i.e. from 2613 mg/lit (initial) to 1855.33 mg/lit (final) and maximum reduction was 65.76% i.e. from 333 mg/lit (initial) to 114 mg/lit (final) after 6 days. Minimum reduction in BOD after 10 days of treatment was 34.51% i.e. from 1362 mg/lit (initial) to 892 mg/lit

(final) and maximum reduction in BOD was 69.17% i.e. from 333 mg/lit (initial) to 102.66 mg/lit (final) (**Figure 4**).

Researchers used artificial wetland for treating dairy waste where they observed 80-90% reduction in BOD [41]. Others tried vermicomposting for treatment of dairy effluent. They noticed reduction in BOD by over 85%. In current study reduction in BOD achieved by bioaugmentation using microbial consortium is comparatively less but the technique employed is simpler [40].

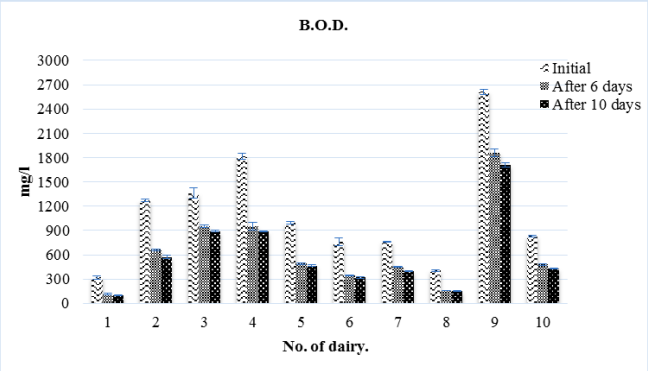

Figure 4: BOD reduction after 6 and 10 days

3.12.5 Reduction in COD

Individual dairy effluents from 10 selected dairies were treated with 10% v/v consortium 3 (O.D.0.1 at 540 nm). Among all dairy effluents, minimum reduction in COD was observed to be 26.42% i.e. from 3702 mg/lit (initial) to 2723.67 mg/lit (final) and maximum reduction was 66.65% i.e. from 985.66 mg/lit (initial) to 329.66 mg/lit (final) after 6 days. Minimum reduction in COD after 10 days of treatment was 30.01% i.e from 3702 mg/lit (initial) to 2591 mg/lit (final) and maximum reduction in COD was 67.47% i.e. from 985.66 mg/lit (initial) to 320.66 mg/lit (final) (**Figure 5**).

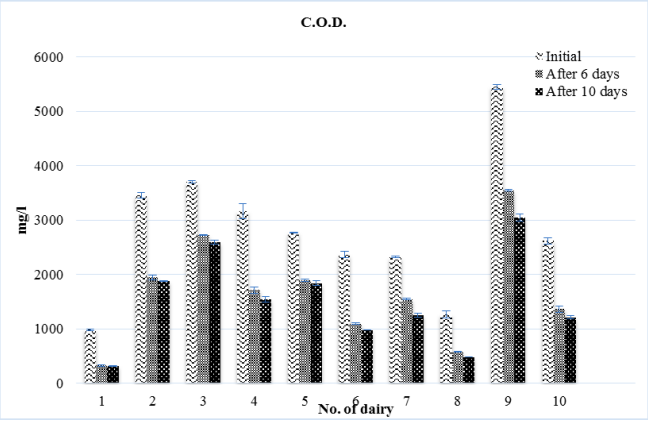

Figure 5: COD reduction after 6 and 10 days

Researchers had carried out phytoremediation of dairy waste collected from a well known dairy named ‘Gokul’ located at Kolhapur in Maharashtra using aquatic plants like water hyacinth, duckweed for phytoremediation. Water hyacinth was found to be most suitable for phytoremediation with reduction in COD from 810mg/lit to less than 200mg/lit being obtained in 5 days. However, it was unsuitable for effluents with COD more than 1000mg/lit [42]. Others in 2011 observed that 72% COD reduction an anaerobic treatment reactor packed with fire bricks after 6 days. The results obtained in current study are parallel with these results [40].

3.12.6 Reduction in oil and fats

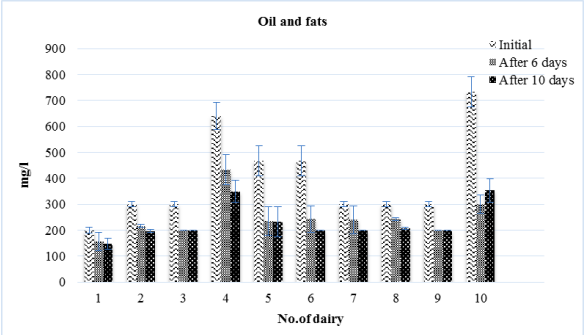

Figure 6: Oil and fats reduction after 6 and 10 days

Individual dairy effluents from 10 selected dairies were treated with 10% v/v consortium 3 (O.D.0.1 at 540 nm). Among all dairy effluents, minimum reduction in oil and fats was observed to be 4.91 % i.e. from 203.33 mg/lit (initial) to 193.33 mg/lit (final) and maximum reduction was 33.33% i.e. from 300 mg/lit (initial) to 200 mg/lit (final) after 6 days. Minimum reduction in oil and fats after 10 days of treatment was 4.91% i.e from 203.33 mg/lit (initial) to 193.33 mg/lit (final) and maximum reduction in oil and fats was 34.44% i.e. from 300 mg/lit (initial) to 196.66 mg/lit (final) (Figure 4.2f).

Researchers in 2015, reported oil and fats in effluent collected from dairy effluent treatment plant located in Pune, Maharashtra as156.5mg/lit in the month of December 2010 and observed reduction in oil and fats to12.3 mg/lit by inoculating organisms from sewage sludge under aerobic treatment and reduction in oil and fats from 154.9 mg/lit to 13.3 mg/lit was reported in the month of January 2011 with the same treatment [34]. In current study comparatively less reduction in oil and fats has been achieved. However treatment used is not aerobic hence it is more cost effective.

3.13 Antibiotic sensitivity test

Since *E.coli* is an opportunistic pathogen, it was subjected to antibiotic sensitivity test. When multi discs of antibiotics were placed on sterile diagnostic sensitivity test agar spread with 24 hours old broth culture of *E.coli*, large clear zones of inhibition of growth of the bacterium were observed around several antibiotics like Amikacin, Ciprofloxacin, Ampicillin Sulbactam, Gentamicin *etc*. The size of clear zones of inhibition was compared with the standard table. The results indicated that the strain of *E.coli* selected as exogenous bacterium was sensitive to all common antibiotics. **(Figure7**). These antibiotics can be used for treating infections caused by this organism. Hence, though, it is an opportunistic pathogen, it is safe to use it in consortium for bioaugmentation. Moreover, earlier researchers in 2014, had already used it for the same purpose [6].

Figure 7: Antibiotic sensitivity test for *E.coli* ATCC 20715

3.14 Whole cell immobilization of the consortium and application

Whole cell immobilization of consortium 3 using calcium alginate resulted into formation of firm beads (**Figure 8**) which were easy to handle as compared to free cell suspension.

Researchers have elaborated several advantages of whole cell immobilization in fermentation industries [43]. Others in 2013 had used the same technique of gel entrapment using calcium alginate, used in the current study, for whole cell immobilization of *Chlorella pyrenoidosa* and combined it with sand bed filtration. They could achieve reduction in BOD of dairy effluent from 1200mg/lit to 32mg/lit and in COD from 2900mg/lit to 106 mg/lit after 4 days with continuous air supply [44].

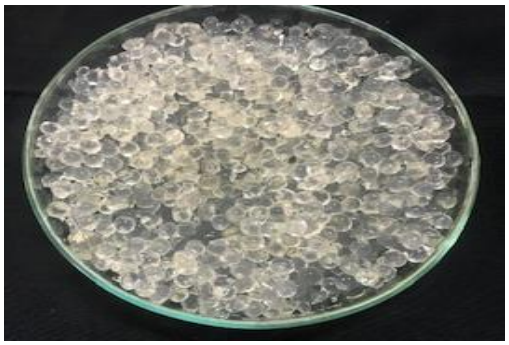

Figure 8: Alginate beads of Immobilized consortium 3

When stability of beads with immobilized consortium 3 was checked, cells were found to remain in beads up to 3 days. Then after, oozing of cells was observed which was indicated by increase in turbidity of nutrient broth in which beads were inoculated. (**Figure 9**).

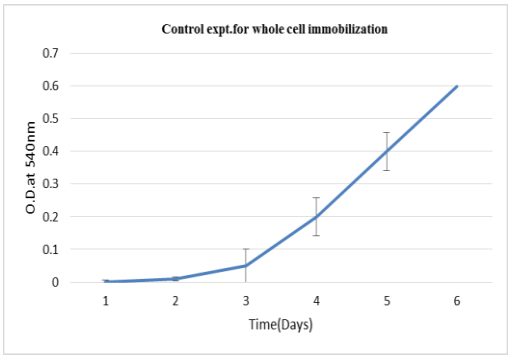

Figure 9: Oozing of organisms from alginate beads

Since, oozing of cells from the beads was observed after 3 days, sustainability study means possibility of re-use of beads was not carried out. Immobilized consortium can be said to have better field application because handling of beads is easier than handling free cells. Immobilized consortium in form of beads can be supplied to small scale dairy owners for the treatment of their effluent.

When bioaugentation of mixed dairy effluent was carried out using free cells and the immobilized consortium, difference of 10 to 24 % reduction after 6 days in TSS, TS, BOD, COD and oil and fats and 10 to 13 % after 10 days was observed (**Figures 10 to 15**). It indicated that Immobilized consortium was more efficient in degradation of organic matter present in dairy effluents.

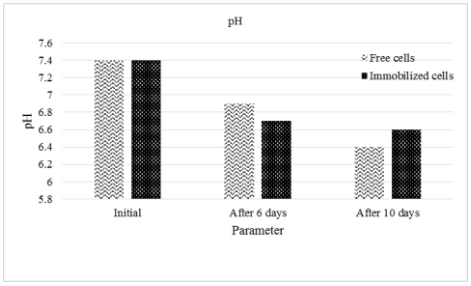

Figure 10: Change in pH by Free and immobilized cells after 6 and 10 days

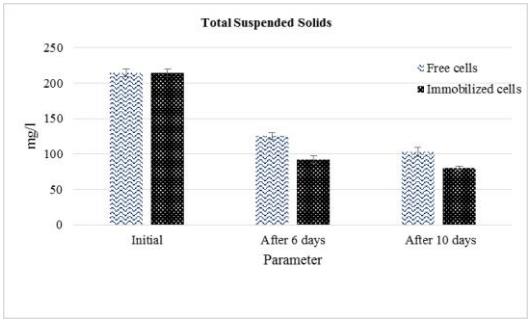

Figure: 11 Reduction in TSS by Free and immobilized cells after 6 and 10 days

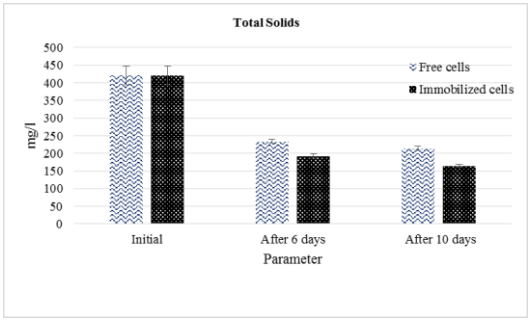

Figure: 12 Reduction in TS by Free and immobilized cells after 6 and 10 days

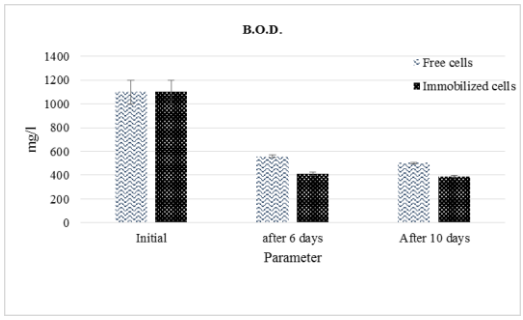

Figure: 13 Reduction in BOD by Free and immobilized cells after 6 and 10 days

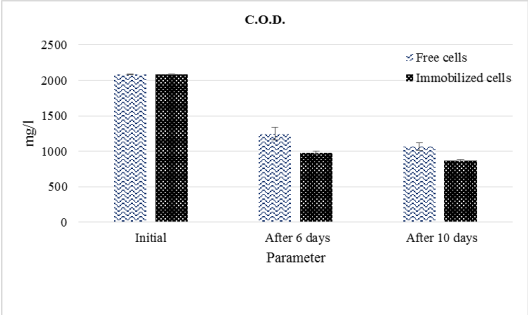

Figure: 14 Reduction in COD by Free and immobilized cells after 6 and 10 days

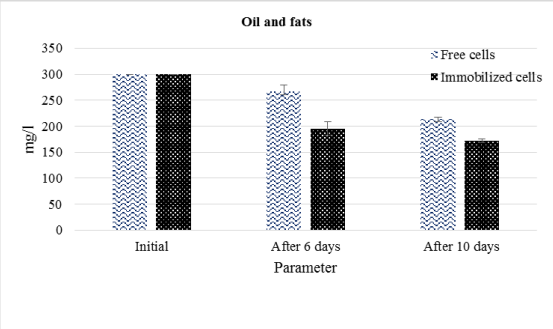

Figure: 15 Reduction in oil and fats by Free and immobilized cells after 6 and 10 days

3.15 Construction of microbial fuel cell (MFC) and generation of bioelectricity

Figure 16: Microbial Fuel Cell

Figure 16 is a two chambered laboratory model of microbial fuel cell. The multimeter connected is showing the reading of voltage generated 321.6 mV.

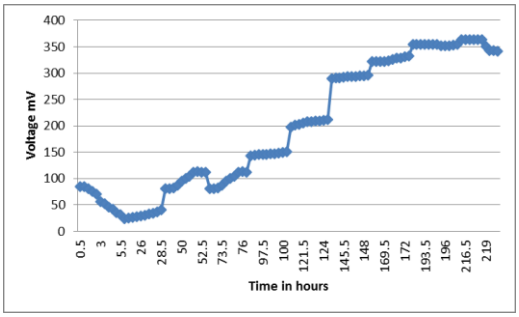

Figure 17: Generation of electricity in MFC

Figure 17 indicates that maximum 363 mV voltage could be generated in MFC from the dairy effluent on 8th day. Initial B.O.D. of the mixed effluent was 1100 mg/lit. After 10 days, B.O.D. was reduced to 492 mg/lit. There was 55.27% reduction in BOD of the effluent after 10 days.

Researchers had constructed a two chambered glass microbial fuel cell. The chambers were connected with 15 mm of salt bridge. They used artificial waste water and distillery waste water for generation of electricity. Maximum voltage generated using *Bacillus megaterium* was 491mV in 68 hours. However, they had used standardized artificial waste water rich in nutrients. They have mentioned that use of salt bridge instead of sophisticated membranes in MFC makes it cost effective [45].

Maximum voltage of 856mV was obtained from dairy effluent using sewage sludge as an inoculum. In their MFC model, sophisticated proton exchange membrane was used which is costlier and cathode was aerated making it still costlier [46].

In the current study, salt bridge has been used to make MFC cost effective. The microbial consortium worked efficiently in MFC and voltage obtained is comparable with others. Reduction in B.O.D. indicated simultaneous degradation of organic matter by the organisms in the consortium. The unit of MFC could have been kept for more days to achieve further decrease in BOD. Disposal of effluent after sufficient treatment in MFC unit would cause less pollution.

4. CONCLUSIONS

Analysis of effluent from ten dairies indicated that, there was a lot of variation in the parameters, indicating large variation in their composition. The degradation of organic matter in the effluent by its own flora was not sufficient. With microbial consortium there was much more degradation and reduction in BOD and all other parameters. Hence, it was concluded that the microbial consortium was working efficiently and was able to reduce all parameters significantly without aeration. All organisms in the consortium were able to survive and grow together. An opportunistic pathogen, *E.coli* was found to be sensitive to common antibiotics. Further reduction in the parameters like BOD and COD may be achieved using brief aerobic treatment followed by initial anaerobic treatment.

Whole cell immobilization using calcium alginate resulted into increase in degradation efficiency of microbial consortium. Though oozing of microorganisms from alginate beads was observed, the technique has better field application as handling of beads is more convenient than handling free cells.

Electricity has been successfully generated using a microbial fuel cell. Significant reduction in BOD of the effluent used in MFC was also noted. Generation of bioelectricity could have been continued further as it had not come down to very low level after 10 days and further reduction in BOD could have been obtained. Small scale dairy owners would like to adapt the technology because it is cheaper and the energy generated is a wealth from waste. For effective use of this treatment, small scale dairy owners should be trained to produce effluent with less organic matter by following proper techniques at each step. In future, the technology can be transferred to them.

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