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Biodegradation of dairy effluent by using microbial isolates obtained from activated sludge

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ABSTRACT

Water resources are of significant importance to human beings. The present investigation was carried out for biodegradation of dairy effluent by using selected aerobic microbial isolates and a model having layers of sawdust and activated charcoal as filtering media. Yeast isolates (DSI_1) and two bacterial isolates (DSI_2 and DSI_3) were obtained from the dairy sludge. A mixed culture (DSI_4) was prepared by taking 1:1, DSI_1 and DSI_3 to treat the effluent and check its efficiency. After aeration period of 48 h, mixed culture of dairy sludge isolates proved to be most efficient in treatment of effluent. DSI_2 showed least reduction in chemical oxygen demand. After aeration, the reduction efficiency of DSI_4 was highest by 47.52% in biological oxygen demand in comparison with other isolates. DSI_3 was second most effective in reduction of water parameters mainly electrical conductivity, totals solids, chemical oxygen demand and biological oxygen demand.

1. Introduction

Rapid growth of industries has not only enhanced the productivity but also resulted in release of toxic substances into the environment, creating health hazards. It has seriously affected normal operations of ecosystems, flora and fauna. In recent years, considerable attention has been paid to the

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industrial wastes, which are usually discharged on land or into different water bodies. This is likely to result in the degradation of environment [1]. Various physicochemical techniques have been studied for their applicability in treatment of wastewaters [2]. These mainly include sedimentation, screening, aeration, filtration, flotation, degassification, chlorination, ozonation, neutralization, coagulation, sorption, ion exchange, etc. Several limitations of physicochemical methods including partial treatment, higher cost, generation of secondary pollutants, higher quantity solids and use of chemicals agents make the biological methods a favorable alternative for the removal of pollutants. Waste materials associated with the food industry including the wastes generated by the dairy industry namely sludge, heavy organic matter, fats, oil & grease, fatty acids, nitrogenous compounds are notables [3]. Of all industrial activities, the food sector has one of the highest consumptions of water and is one of the biggest producers of effluents per unit of production; in addition they generate a large volume of sludge in biological treatment [4]. In aerobic systems, the sludge production is about 0.5 kg per kg of removed chemical oxygen demand (COD) and in anaerobic systems about 0.1 kg per kg of removed COD [70]. Due to high pollution load of dairy wastewater, the milk-processing industries discharging untreated/partially treated wastewater cause serious environmental problems [5]. Nutrients present in dairy effluent such as nitrogen and so forth lead to eutrophication of receiving waters [6]. Dairy wastewater deserves special attention since its levels of potential contaminants typically exceed those levels considered hazardous for domestic wastewater [62]. Numerous attempts have been made to solve this problem by the activated sludge process where wastewater containing organic matter is aerated with microorganisms to metabolize the suspended and soluble organic matter. Nutrients mainly nitrogen and phosphorous from wastewaters could be reused for nutrient balance in such treatment processes. Dairy industry is found all over the world, but their manufacturing process varies tremendously [9]. This sector generates huge volume of wastewater and its pollution is primarily organic [6,10]. Dairy industry is of crucial importance to India. The country has enacted Water (prevention and control of pollution) Act, 1974 and amendments in order to treat the effluents generated by the industries and maintain wholesomeness of the natural water resources. Milk production in India has developed significantly in the past few decades from a low volume of [7,8] million tons in 1951 to 110 million tons in 2009. India's dairy sector has great potential to influence the world dairy market in long run if adequate technological progresses along with structural changes are introduced [8].

Water is a major utility in dairy industry, which results in significant effluent volumes being generated; hence the challenge of its disposal cannot be ignored. The dairy industry on an average has been reported to generate 6–10 L of wastewater per liter of the milk processed [11]. It is estimated that about 2% of the total milk processed is wasted into drains [12]. Dairy raw wastewater is characterized by high concentrations and fluctuations of organic matter and nutrient loads [61]. The composition varies depending on the operations and products [13]. The wastewater of dairy contain large quantities of milk constituents such as casein, lactose, inorganic salt, besides detergents and sanitizers used for washing [14]. The recycling of nutrients through land application of dairy waste effluent requires usage of crops capable of utilizing these nutrients [15]. Industrial effluents rich in organic matter and plant nutrients for agriculture are considered as cheaper way of disposal [16]. Dairy effluents contain dissolved sugars and proteins, fats and possibly residues of additives and are the main contributors to the organic load of these wastewaters [17]. Due to the presence of high organic load, dairy effluents degrade rapidly and deplete the DO (dissolve oxygen) level of the receiving streams and become the propagation place for mosquitoes and flies carrying malaria and other perilous disease such as dengue fever, yellow fever and chicken guinea [18]. The wastes are also characterized by strong butyric acid odor and heavy black flocculated sludge masses [19]. The dairy industries produce effluents rich in fats, oils and greases (FOGs) and can have negative impacts on wastewater treatment systems [20] as often cause foul odors, blockage of pipes and sewer lines. Volatile fatty acids (VFA) are among the most abundant volatile organic compounds in dairy manure and are associated with odor nuisance [65]. Raw milk contains of ammonia nitrogen and presence of 50 mg/L of nitrogen in wastewater stream is due to 1% loss of milk [21]. Presence of nitrate can cause methemoglobinemia if converted to nitrite [6] and contaminate groundwater. Presence of nitrogen in dairy effluent is another major problem that once converted may contaminate ground water with nitrate [22].

Water management in dairy industry is well documented, but effluent production and disposal remain a problematic issue. To enable dairy industry to contribute in water conservation, an efficient and cost-effective treatment technology has to be developed. Dairy wastewaters are generally treated using biological methods such as activated sludge process, aerated lagoons, trickling filters, sequencing batch reactor (SBR), anaerobic sludge blanket (UASB) reactor and anaerobic filters [23]. Many dairy factories employ continuous biological treatment systems in order to treat wastewater. In some cases, anaerobic/aerobic/anoxic series processes have been studied and applied [24]. The process of seeding inoculation of microorganisms for degrading waste materials on streams, rivers and treatment tanks has been rapidly increasing practice in many countries because it is economical and the application is uncomplicated [25]. Bioremediation is any process that uses living microorganisms or their enzymes, to return a polluted environment to its original condition. As such, it uses relatively low-cost, low-technology techniques, such as using environment-friendly microorganisms which generally have a high public acceptance and can be used on the site [26]. It constitutes the use of natural biota and their processes for pollution reduction and the end products are non-hazardous [27]. The process of biodegradation is a well-established and powerful technique for treating domestic and industrial effluents [28]. The performance of a biological process is often enhanced through bioaugmentation of one or more species of specialized microorganisms [55,56]. Microbial populations have an amazing and extensive capacity to degrade variety of organic compounds [29]. Naturally occurring microorganisms thrive on many of the complex compounds contained in wastewater. Small size, high surface area-to-volume ratio and large contact interfaces with their surrounding environment, are some of the ideal features of microorganisms as bioindicators of chemical pollutants [54]. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. To get an efficient biological wastewater treatment it is very important to know the wastewater microbiota composition and biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and physical-chemical conditions [30]. *Pseudomonas sps.* can be used as a bioremediation tool for the treatment of effluent from leather and other industries [59]. Usually there is a large amount of heterotrophic microorganisms which belong to the following species: *Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Enterobacter, Streptococcus faecalis, Escherichia coli* etc. The yeasts belonging to genus *Saccharomyces, Candida, Cryptococcus,* are frequently found in dairy wastewaters [31]. Pepper et al. [32] stated that the bioavailability of compounds in a given system is very important factor determining biodegradability of the system. Schneider and Topalova [57] reported that municipal wastewater treatment inoculums were as efficient as commercial inoculums in removing the COD and phenols from dairy wastewater. Hesnawi et al. [58] suggested the need for highly specialized strains for efficient treatment of wastewater.

To combat the excess environmental burdens, efficient and environmentally safe organic waste treatment technologies are needed [63,64]. The bioengineered technologies adapted for each type of organic and toxic wastes are required to achieve high treatment efficiencies. The present investigation was carried out to see the bioremediation of dairy effluent rich in organic nutrients and to test the ability of some selected aerobic microbial cultures to degrade organic matter from dairy effluent with the help of model associated with filtration medium. The study was conducted in two phases (1) Isolation of microorganisms from dairy sludge and (2) Bioremediation of the dairy effluent. We hypothesized that the microorganisms already present in the dairy effluent can be used as a means for bioremediation.

2. Materials and methods

2.1. Sample collection

Fresh dairy effluent sample was obtained from a dairy effluent treatment plant located in Pune of Maharashtra. The sample was collected in a 5 L plastic container. The container used for sample collection was pre-treated by washing with alcohol and later rinsed for three times with distilled water. It was dried in an oven for 1 h at 30 ± 5 °C and allowed to cool to room temperature. At the collection point, container was rinsed with the sample thrice and then filled, corked tightly and taken

to the laboratory of Department of Environmental Science, Fergusson College, Pune for further analysis. The sample was stored at a temperature below 4 °C to avoid any physico-chemical changes in the effluent. The sludge sample was collected from the V-Notch chamber of the effluent treatment plant. The sample was collected in sterile glass bottle. The bottle used for sample collection was autoclaved and dried in an oven before collecting the sample.

2.2. Analysis of the sludge sample

The wet mount is a preparation of a sample to observe structure of microorganisms. A sterile inoculating loop was used to place a loopful of sample on a slide. It was then covered immediately with a cover slip. Before the preparation dried, it was observed under microscope. Monochrome staining was also carried out to study the sludge. Smear was prepared and heat fixed. Then smear was treated with 5–7 drops of crystal violet stain. It was allowed to react for 60 to 120 s. The staining solution was poured off and the slide was washed by gentle flow of tap water. The slide was dried in air and finally examined under oil emulsion lens.

2.3. Isolation of microorganisms

One gram sludge sample was inoculated in Erlenmeyer flasks containing 100 ml of enrichment cultural media namely sterile Nutrient Broth (N.B.) and Sabouraud's Glucose Broth (S.B.) respectively. The flasks were kept on rotary shaker at 100 rpm at room temperature for 24–96 h. One loopful enriched sample from N.B. flask was streaked on N.A. (Nutrient Agar) plates and one loopful enriched sample from S.B. was streaked on Sabouraud's Agar (S.A.) plates. N.A. plates were incubated for 24 h at room temperature, while the S.A. plates were incubated for 3 days at room temperature. Plating was done in triplicate for each medium. Isolated colonies were further studied for Gram's staining and identified on the basis of Gram nature. Colonies on S. A. (yeast isolate) were separated on the basis of monochrome staining.

2.4. Gram's staining (method of Hucker and Conn)

Smear was prepared and heat fixed. The smear was treated with Hucker's Crystal Violet for 1 min and was then removed by Gram's Iodine to react for 1 minute.The smear was then washed with water and treated with 95% ethyl alcohol for 10–15 s. Smear was washed with water and again treated with safranin for 30 s. The smear was then washed, dried and examined.

2.5. Maintenance of cultures

The yeast isolate was maintained on S. A. slant while bacterial isolates were maintained on N. A. slants and at 4 °C.

2.6. Inoculum preparation

Each microbial isolate with 0.1 ml suspension was inoculated in 100 ml inoculum medium. The flasks were kept on rotary shaker at 150 rpm for 24 h at 32 °C. Similarly, 0.1 ml of suspension of Yeast isolate was inoculated in 100 ml inoculum medium. The flask was kept on rotary shaker at 150 rpm for 3 days at 32 °C.

To study the biodegradation efficiency of the microbial isolates, biomass of actively growing cells was prepared. For the same, each bacterial isolate was grown on 50 ml nutrient broth and 50 ml Sabouraud's broth for yeast at 150 rpm on orbital shaker at 24 to 48 h for 32 °C. Activity growing culture of each isolate was washed with sterile deionized water thrice and centrifuged at 10,000 rpm for 10 min to get wet pellet of each isolate. The pellet was resuspended in sterile deionized water till turbidity reaches at or above that of McFarland 0.5 standard [69].

2.7. Experimental setup and working

A two stage model was set up for the experimental treatment of the effluent (Fig. 1). The design of the model was obtained from the model suggested by RamaKrishna and Ligy [33] and the model suggested by Arumugam and Sabarethinam [34] for treatment of dairy wastewater. The model was modified as per the requirements of present study. Two used plastic bottles of capacity 1.5 L each were reused for making two columns (considered as primary and secondary tanks) of the model. The bottles were cut from the bottom and inverted to make columns. They were rested on iron rings which were nailed onto a wooden plank. Holes were made at required positions in the bottles and transparent silicon pipes were connected using an adhesive, araldite. An aerator was used to provide continuous aeration and maintain desired level of dissolved oxygen above 5 mg/L. Activated charcoal powder and sawdust were used for filtration following the microbial treatment in secondary tank. The activated charcoal powder was heated up in an oven at 70 °C for 2 h before use. Sawdust was obtained from a plywood shop located in Timber Market of Pune. Thus the experimental model was constructed from reused plastic bottles and filtration materials (Fig. 1).

The two columns of the treatment cum filtration unit were washed with alcohol to make it sterile and then rinsed with sterile distilled water. The laboratory scale 1.5 L reactor (primary tank) was then fed with 1 L autoclaved untreated dairy wastewater. The autoclaved effluent was cooled to room temperature and then added to the reactor. Then 10 ml of microbial culture was added to the effluent. An aerator was inserted into the reactor and the open top portion of the reactor was covered with aluminum foil paper. The aerator was used to maintain desired level of dissolved oxygen above 5 mg/L in the effluent and to support the proper growth and survival of the aerobic microorganisms used for the study. The aeration was provided for a period of 48 h. The effluent was given a retention time of 48 h in the primary tank where the microorganisms were allowed to carry out degradation. After 48 h, the aeration was stopped and effluent was allowed to stand for 1 h to allow settling of the sludge formed. The treated effluent from primary tank was then allowed to flow into secondary tank through outlet pipe of the primary tank. The filtration was carried out in secondary tank containing one inch layer of activated charcoal powder at the bottom of bottle and one inch layer of sawdust above it.

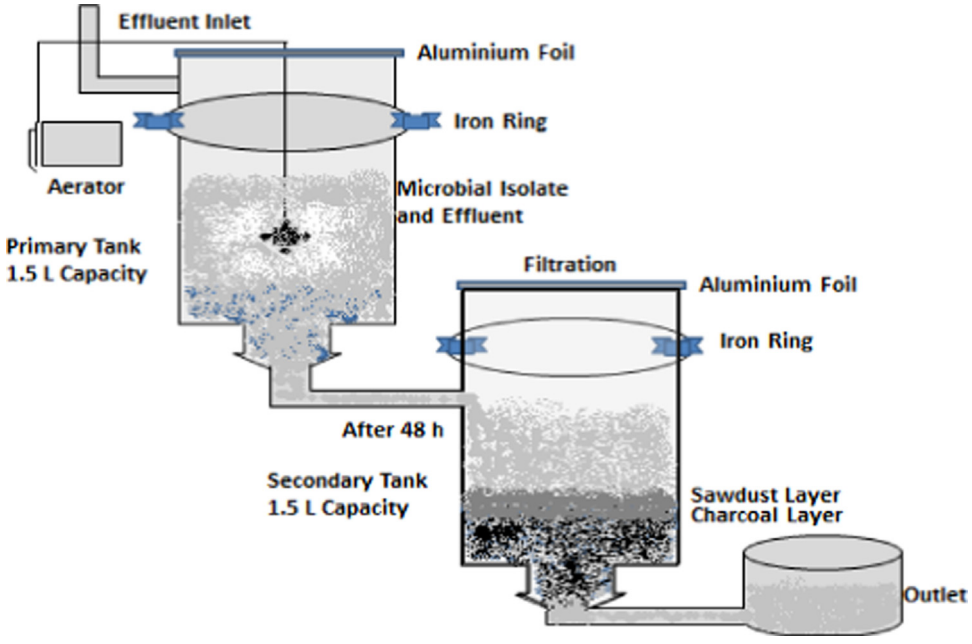

Fig. 1. Experimental set up for treatment of dairy efflunt by using microbial isoates.

On completion of filtration, treated effluent was tested for various physicochemical parameters in laboratory.

2.8. Analytical methods

The methods of analysis were in consistent with the standard methods mentioned in 'Handbook of Water Analysis' by S. K. Maiti [35].

The pH was determined by direct measurement with a pH meter while electrical conductivity (EC) and total dissolved solids (TDS) were determined by using a handy Elumech EC-TDS meter. The turbidity and sulfate contents of the sample were measured by Nephalometric method. Chloride content was determined by Argentometric titration, while total solids (TS) and Oil & grease (O&G) were estimated by gravimetric method. Total Suspended Solids (TSS) were determined by the equation, TSS = TS-TDS. Biological oxygen demand (BOD) was estimated by preparing required volume of dilution water with the addition of nutrients and incubation period of five days at 20 °C while chemical oxygen demand (COD) determination was based on rapid dichromate oxidation method.

3. Results and discussion

The results obtained of the initial physicochemical analysis of dairy raw and treated effluents are shown in Table 1. The effluents were tested in two successive months namely December 2010 and January 2011 in order to check the variations in physiochemical parameters. The inlet was untreated effluent coming from various sections of dairy processing units, also referred as raw effluent which is

Table 1
Characterization of Dairy Effluent.

Sr. No.	Parameters	Months			
		December 2010		January 2011	
		Inlet (untreated)	Outlet (treated)	Inlet (untreated)	Outlet (treated)
1	pH	6.03 (±0.21)	7.6 (±0.14)	6.06 (±0.29)	7.4 (±0.14)
2	Color	Milky	Clear	Milky	Clear
3	Turbidity	1144.2 (±12.31)	21.2 (±0.52)	1173.4 (±14.34)	20.6 (±0.41)
4	Electrical Conductivity	436.2 (±6.98)	139.6 (±5.64)	430.8 (±8.12)	147.7 (±4.27)
5	Total Suspended Solids	626.6 (±8.79)	96.4 (±2.87)	601.6 (±3.46)	96.3 (±5.11)
6	Total Dissolved Solids	1715.4 (±6.12)	1216.6 (±9.33)	1362.4 (±4.57)	1179.7 (±5.48)
7	Total Solids	2342 (±15.69)	1354 (±12.57)	1964 (±8.46)	1276 (±19.65)
8	Chemical Oxygen Demand	2398 (±16.98)	280 (±4.56)	2332 (±21.89)	260 (±4.35)
9	Biochemical Oxygen Demand	1268 (±12.65)	104 (±2.21)	1210 (±11.23)	106 (±2.64)
10	Oil and Grease	156.5 (±3.64)	12.3 (±0.34)	154.9 (±2.67)	13.3 (±1.24)
11	Chlorides	135.9 (±1.12)	105.42 (±1.54)	141.86 (±1.89)	102.7 (±1.36)
12	Sulfates	84.6 (±0.58)	49.46 (±0.89)	83.06 (±1.03)	53.2 (±0.67)

Each value is expressed as mg L^{-1} [Except pH, turbidity (NTU) and EC (μS)].
Values in parenthesis indicate standard deviation.
Each value is a mean of three determinations.

Table 2
Characteristics of the Isolated Microorganisms.

Characters	DSI_1 (Yeast Isolate)	DSI_2 (Bacterial Isolate)	DSI_3 (Bacterial Isolate)
Size	1 mm	2 mm	3 mm
Shape	Circular	Circular	Irregular
Color	White	White (greenish pigment)	White
Margin	Entire	Entire	Irregular
Elevation	Convex	Flat	Convex
Opacity	Translucent	Translucent	Opaque
Consistency	Sticky	Moist	Moist
Gram staining	–	Gram negative	Gram positive
Microscopic characteristics	Oval shape	Short rods	Long rods
Culture media	Sabouraud's agar	Nutrient agar	Nutrient agar

Table 3
Treatment of Dairy Effluent by Using Various Microbial Isolates after Aeration and Filtration.

Sr. No.	Parameters	Untreated Effluent	DSI_1 (Yeast Isolate)		DSI_2 (Bacterial Isolate)		DSI_3 (Bacterial Isolate)		DSI_4 (Mixed Culture)	
			After Aeration	After Filtration	After Aeration	After Filtration	After Aeration	After Filtration	After Aeration	After Filtration
1	pH	5.73 (±0.23)	6.3 (±0.15)	6.53 (±0.19)	6.9 (±0.21)	7.36 (±0.15)	5.7 (±0.0.9)	6.87 (±0.16)	5.9 (±0.18)	7.22 (±0.21)
2	Color	Milky White	Creamy white	Clear	Creamy white	Slightly Turbid	Light green	Almost Clear	Faint green	Clear
3	Turbidity	1049.4 (±12.33)	562.0 (±10.34)	9.0 (±0.34)	870.9 (±10.23)	16.6 (±1.02)	694.3 (±10.59)	6.2 (±0.11)	491 (±4.98)	2.1 (±0.18)
4	Electrical Conductivity	496.6 (±8.59)	284.1 (±7.95)	61.1 (±7.59)	359.3 (±5.64)	82.2 (±6.32)	274.3 (±8.94)	57.5 (±5.61)	182.5 (±5.48)	64.8 (±4.69)
5	Total Suspended Solids	629.3 (±7.56)	510.8 (±6.54)	5.2 (±0.14)	561.1 (±8.56)	8.2 (±0.19)	469.4 (±10.22)	3.1 (±0.18)	418.4 (±6.32)	2.9 (±0.32)
6	Total Dissolved Solids	1470.7 (±18.56)	1089.2 (±16.21)	470 (±8.95)	1248.9 (±16.32)	959.8 (±14.65)	1020.6 (±12.56)	479.9 (±10.84)	971.6 (±12.13)	449.1 (±8.16)
7	Total Solids	2100 (±19.74)	1600 (±14.24)	475 (±6.14)	1810.0 (±18.65)	968.0 (±8.94)	1490.0 (±19.54)	483.0 (±5.61)	1390.0 (±18.94)	452.0 (±8.32)
8	Chemical Oxygen Demand	2148 (±23.12)	1228.0 (±13.24)	570.0 (±10.12)	1470 (±13.87)	710 (±8.65)	1140.0 (±13.64)	320 (±8.61)	1060.0 (±19.46)	300.0 (±7.34)
9	Biochemical Oxygen Demand	1010 (±18.45)	790.0 (±9.75)	320.0 (±3.64)	920 (±5.89)	490.0 (±4.32)	610.0 (±7.23)	170.0 (±4.17)	530.0 (±12.34)	150.0 (±2.89)
10	Oil and Grease	135.8 (±2.91)	75.1 (±3.01)	3.5 (±0.21)	115.3 (±2.88)	7.5 (±0.45)	85.0 (±1.98)	4.2 (±0.12)	55.5 (±2.12)	2.5 (±0.11)
11	Chlorides	146.9 (±2.14)	139.1 (±1.95)	132.4 (±2.86)	145.3 (±2.10)	138.6 (±2.45)	129.2 (±1.78)	122.3 (±1.15)	131.8 (±2.96)	119.4 (±2.15)
12	Sulfates	86.8 (±1.12)	80.6 (±1.59)	74.3 (±2.11)	82.9 (±1.75)	79.1 (±2.05)	76.9 (±1.0)	69.8 (±1.65)	71.4 (±1.96)	67.7 (±1.23)

Each value is expressed as mg L^{-1} [Except pH, turbidity (NTU) and EC (μS/cm)].
Values in parenthesis indicate standard deviation.
Each value is a mean of three determinations.

taken in effluent treatment plant. The treated effluent collected at outlet of the treatment plant was also collected for characterization. The raw effluent entering in treatment plant was milky but at the outlet, after treatment it was clear. The changes in color and odor of the dairy effluent might be due to action of alga which decomposed the organic matter present in both untreated and treated effluent.

These findings were in accordance with the work of Verma and Madamwar [36]. The turbidity of the untreated effluent was 1144.2 mg/L in December while it was observed to be 1173.4 mg/L in January 2011. The electrical conductivity was observed to be 436.2 μS/cm (December) to 430.8 μS/cm (January). The COD values of the untreated effluent were variable by 2398 mg/L in December while 2332 mg/L in January. The BOD value of inlet was also with higher pollution potential and was observed to be higher in December by 1268 mg/L. The oil and grease contents were not much variable but considerable in terms of pollution potential with 156.5 mg/L in December. The values of chlorides and sulfates of the inlet were 141.86 mg/L and 84.6 mg/L in the months of January and December respectively.

Yeast (DSI_1) isolate and two bacterial isolates (DSI_2 & DSI_3) were obtained from dairy sludge. After Gram method and microscopic observation, characteristic specifications of dairy sludge isolates (DSI's) were carried out (Table 2). Three isolates were named as DSI_1, DSI_2 and DSI_3. These selected microorganisms were examined for their ability to reduce parameters from dairy effluent. The results showed that they were capable of reducing the COD, BOD and other parameters effectively from dairy effluent (Table 3). It was observed that the DSI_1 and DSI_3 showed better results than DSI_2, so, a mixed culture (DSI_4) was prepared by taking 1:1 DSI_1 and DSI_3 to treat the effluent and check its efficiency as compared to single cultures. The mixed culture was then named as DSI_4 and was used for further study.

3.1. pH

The pH of the untreated sample was acidic. It was variable even after treatment by using microbial isolates and observed to be slightly acidic or alkaline. Overall after filtration the values were nearer to neutral. The pH of dairy wastewaters depends on the nature of end product and can assortment from 6.6 to 12.2 [37,38]. The pH varies in the range of 4.7–11 [39]. The effluent indicating acidic conditions could have an adverse effect on soil and microflora [40].

3.2. Turbidity

The reduction in turbidity due to isolates DSI_1, DSI_2, DSI_3 and DSI_4 after aeration period of 48 h was 46.45, 17.01, 33.84 and 53.21% respectively (Table 4). It can be seen that DSI_4 caused highest reduction in turbidity among the four cultures. After filtration the reductions obtained were 99.14, 98.42, 99.41,

Table 4
Percent (%) Reduction in Various Physiochemical Parameters of Dairy Effluent after Aeration and Filtration (control values of respective parameters were considered as 100% of the untreated effluent).

Sr. No.	Parameters	DSI_1 (Yeast Isolate)		DSI_2 (Bacterial Isolate)		DSI_3 (Bacterial Isolate)		DSI_4 (Mixed Culture)	
		After Aeration	After Filtration	After Aeration	After Filtration	After Aeration	After Filtration	After Aeration	After Filtration
1	Turbidity	46.45	99.14	17.01	98.42	33.84	99.41	53.21	99.79
2	Electrical Conductivity	42.79	87.69	27.65	83.45	44.76	88.42	63.25	86.95
3	Total Suspended Solids	18.83	99.17	10.84	98.71	25.41	99.51	33.51	99.54
4	Total Dissolved Solids	25.94	68.04	15.08	34.74	30.6	67.37	33.94	69.46
5	Total Solids	23.81	77.38	13.81	53.9	29.05	77	33.81	78.48
6	Chemical Oxygen Demand	42.83	73.46	31.56	66.95	46.93	85.1	50.65	86.03
7	Biochemical Oxygen Demand	21.78	68.32	8.91	51.49	39.6	83.17	47.52	85.15
8	Oil and Grease	44.7	97.42	15.1	94.48	37.41	96.91	59.13	98.16
9	Chlorides	5.31	9.87	1.09	5.65	12.05	16.75	10.28	18.72
10	Sulfates	7.14	14.4	4.49	8.87	11.41	19.58	17.74	22

and 99.79% respectively for four cultures. Actual values of turbidity after filtration were 9, 6.2 and 2.1 NTU for DSI_1, DSI_3 and DSI_4 respectively. It is clear that the turbidity of effluent was decreased with the use of cultures and might be due to consumption of organics and suspended particles by microorganisms for their further growth and survival. Similar decrease in turbidity of dairy wastewater (88.3%) was reported by Cosa and Okoh [68] with the consortium of two marine species belonging to *Ocenobacillus* and *Halobacillus*. The measurement of turbidity is a key test of water quality and an important parameter for evaluating suitability of the effluent for irrigation purpose [41]. Turbidity is an important consideration in public water supplies for three major reasons namely aesthetics, filterability and disinfection. The bioflocculants produced form naturally occurring biota could have good turbidity and COD removal efficiencies in dairy wastewater [68].

3.3. Electrical conductivity (EC)

It can be seen that after aeration, the reduction efficiency of DSI_4 was highest with 63.25% reduction in EC (Table 4). However, DSI_3 showed better reduction efficiency (44.7%) than DSI_1 and DSI_2 with 42.79 and 27.65% respectively. After filtration of effluent, the reductions obtained were 87.69%, 83.45%, 88.42% and 86.95% respectively for DSI_1, DSI_2, DSI_3 and DSI_4. Actual values of EC after filtration were 61.1, 57.5 and 64.8 NTU for DSI_1, DSI_3 and DSI_4 respectively. The reduction in EC after aeration might be due to use of ions by microorganisms for their growth and survival. Reduction after filtration was associated with use of combined filtering agents consisting of sawdust and activated charcoal. The important ions that impart conductivity in water are Cl^-, SO_4^-, CO_3^-, HCO_3^- and NO_3^- and Ca^{++}, Mg^{++}, Na^+ and K^+. EC is an important water parameter which can be used for detection of impurities in water, quantitative measurement of ionic constituents dissolved in water which are important for boiler feed water and cooling water.

3.4. Total suspended solids (TSS)

The percent reduction in total suspended solids of treated dairy effluent after aeration and filtration is recorded in Table 4. It is depicted in the table that after aeration period of 48 h, the percent reductions shown by DSI_1 was 18.83. Reduction efficiency of DSI_2, DSI_3 and DSI_4 was 10.84, 25.41 and 33.51% respectively. It is clear that DSI_2 caused least reduction whereas DSI_4 caused highest reduction in TSS content. After filtration the reductions obtained were 99.17, 98.71, 99.51 and 99.54% by DSI_1, DSI_2, DSI_3 and DSI_4 respectively. The actual lowest values of TSS after filtration were recorded with isolates DSI_3 and DSI_4 by 3.1 and 2.9 mg/L respectively. The reduction in TSS after aeration might be due to use of suspended organics by microorganisms for their growth and development. The overall reduction after filtration is directly associated with the use of sawdust and activated charcoal. Shruthi et al. [72] reported 75.7% reduction in TSS of rubber processing effluent by using *Pseudomonas Sp.* Gaikwad et al. [66] had also reported a maximum of 79.76% reduction in TSS by using microbial consortia of various bacterial species namely *Pseudomonas, Actinomycetes, Bacillus, Staphylococcus and Streptomyces.* The concentration of suspended solids in dairy effluent varies in the range of 0.024–4.5 g/L [39]. High concentrations of suspended solids can cause many problems for stream health and aquatic life. It is major water parameter used to evaluate the strength of domestic wastewater and to determine efficiency of the treatment unit. Suspended solids in the wastewater originate from gelatinous milk and the curd fines or flavorings [42]. TSS reduce light penetration and as a result plant production, in the receiving water body by increasing turbidity and can also clog fish gills [43,44].

3.5. Total dissolved solids (TDS)

The results shown in Table 4 indicate that DSI_4 was most efficient in TDS reduction, with reduction efficiency of 33.94% after aeration. DSI_2 was least efficient in removal of TDS, by causing reduction of just 15.08%. The reductions caused by DSI_1 and DSI_3 were 25.94% and 30.60% respectively. After filtration, the reductions shown by DSI_1, DSI_2, DSI_3 and DSI_4 were 68.04%, 34.74%, 67.37% and 69.46% respectively. The actual lowest values of TDS after filtration were recorded with isolates DSI_1 and DSI_4

by 470 and 449.1 mg/L respectively. Shruthi et al. [72] reported 68.8% reduction in TDS of rubber processing effluent by using *Pseudomonas Sp.* Gaikwad et al. [66] had also reported a maximum of 74.36% reduction in TDS by using microbial consortia of various bacterial species namely *Pseudomonas, Actinomycetes, Bacillus, Staphylococcus and Streptomyces.* The presence of high level of total suspended solids and total dissolved solids is due to organic and inorganic matter present in the effluent. A large number of solids are found dissolved in natural waters, the common ones are carbonate, bicarbonates, chlorides, sulfates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron, magnesium etc. A high content of TDS reduces the utility of water for drinking, irrigation and industrial purposes.

3.6. Total solids (TS)

The total solids content of the treated dairy effluent with isolates and filtration media is recorded in Tables 3 and 4. From the results, it is evident that total solid content of the effluent decreased considerably in both columns of the treatment unit. DSI_4 brought highest degradation in TS content (33.81%) whereas, DSI_2 caused the least reduction by 13.81%. The percent reductions caused by DSI_1 and DSI_3 were 23.81% and 29.05% respectively after 48 h of aeration. The reduction efficiencies of DSI_1, DSI_2, and DSI_4 after filtration were 77.38%, 53.90%, 77% and 78.48% respectively. The actual lowest values of TS after filtration were recorded with isolates DSI_1 and DSI_4 by 475 and 452 mg/L respectively. Shruthi et al. [72] reported 73% reduction in TS of rubber processing effluent by using *Pseudomonas Sp.* The organic contents along with other mineral ions of the dairy effluent could have been used by microorganisms to cause overall reduction in total solids which also proves the efficiency in removal of nutrients. After filtration DSI_1 showed better results than DSI_3.

3.7. Chemical oxygen demand (COD)

It is evident from the results (Table 4) that the COD content of effluent after aeration was significantly reduced by DSI_1, DSI_2, DSI_3 and DSI_4 by 42.83, 31.56, 46.93 and 50.65% respectively. DSI_4 showed best reduction efficiency of the effluent, whereas; DSI_2 showed least reduction in COD. The results obtained after filtration indicate that DSI_4 showed 86.03% reduction in COD. Similarly after filtration the reductions shown by DSI_1, DSI_2 and DSI_3 were 73.46, 66.95 and 85.10% respectively. The lowest value of COD was recorded with isolate DSI_4 where it was 1060 mg/L after aeration while 300 mg/L after filtration. The reduction in COD values might be due to more amounts of nutrients present in the form of dissolved and organic nature which cultures could have used for growth. Our results are in accordance with the reduction in COD seen by Guillen-Jimenez [45] where maximum COD reduction was found up to 65–70%. Similar decrease in COD of the dairy wastewater (99.9%) was reported by Cosa and Okoh [68] with the consortium of two marine species belonging to *Ocenobacillus* and *Halobacillus*. Chatterjee and Pugaht [67] had also reported 67.1% and 48.3% reduction in COD of diary wastewater with use of two bacterial strains namely *Neisseria sp.* and *Citrobacter sp.* Vida et al. [71] had also reported COD reduction by 70.7% and 69.5% by using two bacterial isolates BP3 and BP4. The COD of dairy wastewater is mainly influenced by milk, cream or whey [46]. Among the major industries in India, dairy is one of the industries producing odorous and high COD containing wastewater [47]. Microflora of the effluents from a dairy factory in Tehran (Pegah Dairy Processing Plant) were isolated and studied to check reduction in COD of the effluents by Maghsoodi et al. [48].

3.8. Biochemical oxygen demand (BOD)

It can be seen that after aeration (Table 4), the reduction efficiency of DSI_4 was highest with 47.52% in BOD. The reductions caused by DSI_1 and DSI_3 after 48 h of aeration were 21.78 and 39.60% respectively. It was observed that DSI_2 showed very poor reduction efficiency by just 8.91%. Filtration caused further reductions in BOD values. The reductions obtained by DSI_1, DSI_2, DSI_3 and DSI_4 were 68.32, 51.49, 83.17 and 85.15% respectively. The lowest value of BOD was recorded with isolate DSI_4 where it was 530 mg/L after aeration while 150 mg/L after filtration. The significant decrease in BOD

values could be associated with consumption of organic material by microbes as a food source. Biochemical oxygen demand is widely used as an indication of water quality. Considerable reduction in COD, BOD has also been reported by Das and Santra [60], Gaikwad et al. [66] from wastewaters by using bacterial isolates. Like most other agro-industries the dairy industry generates strong wastewaters characterized by COD and high BOD absorptions representing their elevated organic content [49]. Dairy wastewaters are characterized by high BOD and COD values due to fats, nutrients, lactose, detergents, sanitizing agents, casein and inorganic salts. It is estimated that about 2% of total milk processed is wasted into drains [12,14]. The reduction of BOD can result in simultaneous reduction of coliform populations [50]. Yathavamoorthi et al. [51] measured a positive correlation of fecal coliform with BOD than with suspended solids and suggested that adsorption of fecal coliforms may be more important than sedimentation. Though high growth of microbes had consumed the oxygen present in the treatment column, continuous and excess of aeration had proved to be an important reason for the reduction of BOD in the first treatment column. The removal of organic matter and nutrients from the wastewater is an important aspect of biological treatment. Varied types of bacteria and microorganisms require oxygen to consume the organic matter which results in multiplication and overgrowth in the form of sludge, also referred as activated sludge. The activated sludge contains living and dead biomass along with organics and minerals. The excess activated sludge must be well removable and also requires re-pumping of the activated sludge at regular intervals in order to avoid contamination and efficient working of treatment plants. Effective reduction in sludge generation and good settling properties has been reported in pre-coagulated cheese whey wastewater by aerobic biodegradation [76].

3.9. Oil and grease (O&G)

From the results recorded in Table 4, it is clear that DSI_4 has the best O&G degrading ability among the four selected isolates after aeration. The reductions caused by DSI_1, DSI_2, DSI_3 and DSI_4 were 44.70, 15.10, 37.41 and 59.13% respectively. The least reduction was shown by DSI_2. After filtration, reductions in O&G content were 97.42, 94.48, 96.91 and 98.16% as caused by DSI_1, DSI_2, DSI_3 and DSI_4 respectively. The values of O&G with DSI_4 were 55.5 and 2.5 mg/L after aeration and filtration respectively. Microbial isolates might have used content of O & G as a nutrient for their growth and survival. Also the yeast showed sticky colonies, which might indicate that yeast had O & G retaining capacity. The yeast must have encapsulated the O & G. Oil and grease determination on raw and settled wastewater gives a measure of effectiveness of primary settling tanks. It may cause surface films and shoreline deposits leading to environmental degradation. The variations in the O & G degradation of the various cultures could depend on lipase system and physical properties of substrate [52]. Oil and grease is composed primarily of a fatty matter from animal and vegetable sources, hydrocarbons of petroleum origin, certain organic dyes and chlorophyll [53].

3.10. Chlorides

The highest reduction in concentration of chlorides was observed by DSI_3 by 12.05% after aeration (Table 4). Reductions caused by DSI_1, DSI_2 and DSI_4 were 5.31, 1.09 and 10.28% respectively. It is clear from the results that DSI_2 showed least reduction in chloride concentration. Results showed that after filtration DSI_4 showed highest reduction of 18.72% followed by DSI_3, DSI_1 and DSI_2 showing 16.75, 9.87 and 5.65% reductions respectively. The values of chlorides with DSI_4 were 131.8 and 119.4 mg/L after aeration and filtration respectively. It is observed that the isolated organisms were not very efficient in reducing the chloride concentration of the effluent. The reductions obtained after filtration might be due to combination of the filtering media as absorbed/adsorbed by sawdust and activated carbon. Reduction in chloride content of sugar mill effluent had been studied by Saranraj et al. [73] by using *Bacillus subtilis, Serratia marcescens, Enterobacter asburiae, Pseudomonas fluorescens, Bacillus weihenstephanensis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Brevibacterium halotolerance and Proteus mirabilis.* Chloride is one of the major anions found in water and are generally combined with calcium, magnesium, or sodium. Small amounts of chlorides are required for normal cell functions in plant and animal life.

3.11. Sulfates

It can be clearly seen from the results recorded in Table 4, that after aeration period of 48 h, DSI_1 and DSI_2 showed poor reductions in sulfates i.e. 7.14 and 4.49% respectively. The reductions caused by DSI_3 and DSI_4 were 11.41 and 17.74% respectively. Results obtained after filtration indicate that reductions caused by DSI_1, DSI_2, DSI_3 and DSI_4 were 14.40, 8.87, 19.58 and 22.0% respectively. The lowest values of sulfates with DSI_4 were 71.4 and 67.7 mg/L after aeration and filtration respectively. From the results obtained, it is clear that the overall sulfate reduction efficiencies of the four isolates were quite low and maximum sulfate reduction had occurred due filtration media present in second column of the treatment unit. Reduction in sulfate content of sugar mill effluent had been reported by Saranraj et al. [73] by using various bacterial cultures. High concentration of sulfate in water can have laxative effect [74] when combined with calcium and magnesium, the two most common constituents of hardness. Water with appreciable amounts of sulfates form hard scales in boilers and heat exchangers [75]. Sulfates also cause odor and corrosion of sewer in anaerobic condition because it gets converted to hydrogen sulfide.

4. Conclusion

The present investigation indicated that after aeration period of 48 h, DSI_4 (mixed culture) was proved to be the most effective in reducing selected physiochemical parameters of water. DSI_3 (bacterial isolate) was second most effective in reduction of EC, TSS, TDS, TS, COD, BOD, chlorides and sulfates. However, DSI_1 (yeast isolate) found to be more effective to reduce turbidity and O&G as compared with DSI_2 and DSI_3. DSI_2 (bacterial isolate) showed least reduction in all selected water parameters as compared to other three cultures. The results of present study also showed best treatment after filtration as there were significant reductions in all the selected water parameters. The possible reason could be associated with high adsorption ability of activated charcoal and absorption by saw dust. Findings reveal that DSI_3 (bacterial isolate) would be the best option among three selected isolates for the treatment of dairy effluent. However, the mixed culture would prove to be more effective and beneficial than a single culture. We suggest that the addition of any one of these microbial cultures to the activated sludge process will increase overall efficiency of the treatment system. It can also reduce bulking problems of the activated sludge by preventing load of organic matter from becoming too high. We also urge for further studies to determine exact mechanism of bioremediation, isolation, identification of microbes from dairy effluents and reduction of water quality parameters. This is necessary to improve the efficiency of wastewater treatment systems. Activated charcoal powder and sawdust have ability to remove variety of compounds from contaminated waters. Such filtering agents should be included in treatment processes wherever applicable. Though activated charcoal is expensive, it could be used in combination with other filtering agents such as sawdust as used in the present investigation.

Conflict of interest

The authors declare no conflict of interest.

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