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Bioaugmentation for Dairy Wastewater

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Abstract: ***Five fungal sp. were isolated from dairy effluent collected from Verka Milk Plant Industrial Area-1, Chandigarh, India in winter and summer season in the year 2014 and identified as Alternaria sp. (D_1W & D_4S), Fusarium sp. (D_3S), Aspergillus sp. (D_2W & D_5S) by lactophenol cotton blue staining. Aerobic and anaerobic degradation was carried out by inoculating the dairy effluent with the fungal isolates and maintaining the volume at 500mL followed with the addition of D-glucose (4g/L) after every 24 hours for the period of 5 days. During aerobic degradation aeration rate of 4L/min and stirring rate of 10 rpm was maintained. Anaerobic degradation was carried out at stirring rate of 5 rpm without aeration. The various physico-chemical parameters like COD, TDS, EC, salinity and pH were checked after every 24 hours for 5 consecutive days. Under aerobic conditions D_1W effectively degraded the effluent by reducing COD upto 90.59%. Under anaerobic conditions D_4S had shown maximum reduction in TDS (83.78%), COD (83.07%), EC (80.86%) and salinity (76.19%). The present study concluded that Alternaria sp. isolated from different seasons proved to be promising strain for biodegradation of organic compounds present in dairy effluent and can further be exploited for industrial scale applications.***

Keywords: Dairy effluent, COD, bioaugmentation, *Alternaria sp,* fungal isolates

1. Introduction

The dairy industry is generally considered the biggest source of food processing wastewater and producers of large amount of effluent (0.2 to 10 litres of effluent per litre of processed milk) [1]. Dairy effluents have an alkaline pH value (6.6–12.2) and are rich in organic matter, 4000 mg/L COD and also contains biodegradable carbohydrates [2], [3]. This type of industrial wastewater contains milk and, therefore, ammonium (from the amino acids) and phosphate (from the caseins) [4]. Typically, dairy effluents are much more concentrated than domestic sewage and the main contributors of organic load to these effluents are lactose, fats and proteins originated from milk [5]. Due to their very high concentration level of organic matter, these effluents can create serious problems by putting an organic burden on local municipal sewage treatment systems [6].

There is an increasing cost of excess slugde treatment and disposal owing to strictly enforced environmental and legislative requirements on discharge of excess sludge [7]. A viable alternative to organic pollution removal is bioaugmentation strategy which is defined as addition of bacteria, nutrients and growth factors to enhance biodiversity and efficacy of wastewater or other pollution degrading systems. The vast majority of organic compounds produced in nature or through human manufacture is degraded aerobically with molecular O_2 as terminal electron acceptor. These processes involve the use of free or dissolved oxygen by microorganisms (aerobes) in the conversion of organic wastes to biomass and CO_2 [8].

Anaerobic degradation can be defined as a biological conversion process without external electron acceptor such as oxygen as in aerobic processes or nitrate/sulphate as in anoxic processes and involves conversion of organic carbon to its most oxidized state (CO_2), and its most reduced state (CH_4) by subsequent oxidation and reduction reactions [9]. Both European community and U.S. consider the anaerobic treatment as the most promising approach for future in sustainable development [10]. The lower cost of anaerobic treatment equipment makes this an attractive alternative for the dairy industry [11]. Several factors make anaerobic treatment a good choice for the treatment of dairy wastewater, high organic concentrations, the low energy requirements for anaerobic processes, reduce sludge production, and the generation of methane gas [12].

Research has been conducted to understand bioremediation for environmental pollutants such as aromatic compounds that are among the most prevalent and persistent environmental pollutants [13]. Currently a wide range of microorganisms (bacteria, yeast, fungi, algae) are being studied for use in bioremediation processes. Several workers have also described the application of bioremediation of dairy effluent by using fungi where fungi appeared to show higher degradation rates of organic matter. In addition to extracellular enzyme production, fungal cell walls and their components play a major role in biosorption of toxic compounds during waste water treatment [14]. Keeping in view the importance of fungi in removal of pollutants the present study was aimed to find efficiency of fungal isolates of two different seasons to treat dairy wastewater under aerobic and anaerobic conditions.

2. Materials and Methods

2.1 Collection of Effluent Sample

For the present study the effluent was collected from Verka Milk Plant Industrial Area-1, Chandigarh, India in different seasons in the months of February, March, April and May'2014 in sterile plastic container and was stored at 4°C for future investigation.

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2.2 Isolation and Identification of Fungal Cells

The dairy effluent was serially diluted using sterile pipettes from 10^{-1} to 10^{-5} dilution. For enumeration of fungi PDA medium was used. The dairy effluent (0.1 mL) was spread on the solidified PDA plates and incubated at 25ºC for 48 hours. To obtain pure culture, the cultures were repeatedly streaked on PDA medium and incubated at 25°C for 72 hours. The isolated fungi were identified by colony morphology and microscopic observation. The isolated fungal cultures were identified using Lactophenol cotton blue staining method. Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) medium were used for the maintenance of isolated fungal cultures.

2.3 Preparation of Fungal Seed Cultures

100 mL of PDB was prepared by suspending 2.4 g in 100 mL of distilled water and sterilized by autoclaving. The broth was inoculated with the isolated fungal strains and incubated at 25ºC in rotary shaker at 120 rpm for 5 days.

2.4 Physico-Chemical Analysis of Dairy Effluent

Determination of Chemical Oxygen Demand of the various samples was done by using titrimetric method [15]. Electrical Conductivity (EC), Total Dissolved Solids (TDS) and salinity were determined using the Deluxe Water & Soil analysis kit from M. S. Electronics (India Pvt. Ltd.).

2.5 Bioaugmentation on a Lab Scale

The bioaugmentation experiment was carried out on the dairy effluent inoculated with the fungal isolates by addition of D-Glucose (4g/L) after every 24 hours under aerobic and anaerobic conditions [7]. The various physico-chemical parameters of the sample: COD, TDS, Salinity, E.C, pH etc were recorded for 5 consecutive days.

2.5.1 Experiment Setup

For degradation of dairy effluent 1L borosil beaker of 15 cm height, 10 cm diameter with working volume of 500 mL was used. The top of the beaker was closed by using cardboard provided with connections for inlet and outlet of effluent. In aerobic treatment the beaker was connected to the aquarium pump in order to provide the aeration rate of 4 L/min allowing the production of fine air bubbles to ensure an efficient oxygen transfer for 5 days of incubation. The sample was agitated to ensure homogeneity and appropriate dissolved oxygen and magnetic stirrer was used to provide the incubation temperature of 25ºC and stirring rate of 10 rpm while in anaerobic treatment stirring rate of 5 rpm was maintained. After every 24 hours 2g of D-Glucose was added and total volume of set up was maintained at 500 mL by addition of dairy effluent.

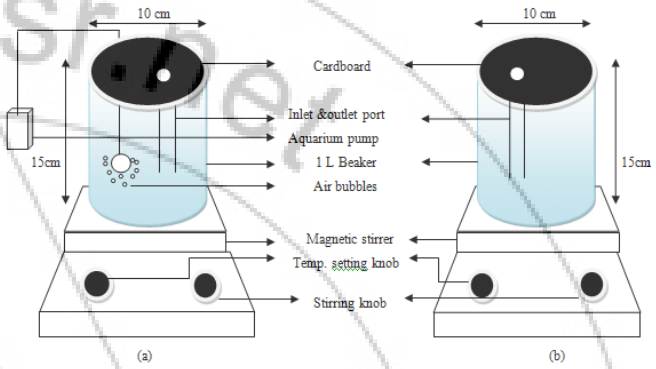

Figure 1: Diagram of the experimental set up of (a) aerobic treatment and (b) anaerobic treatment of dairy effluent for bioaugmentation on a lab scale.

3. Results and Discussion

3.1 Identification of effluent degrading strain from the dairy effluent

The five fungal strains were isolated from dairy effluent, D_1W & D_2W were isolated in winter season and identified as *Alternaria sp.* and *Aspergillus sp*. D_3S, D_4S & D_5S were isolated in summer season and identified as *Fusarium sp, Alternaria sp.* & *Aspergillus sp*. All the five strains were characterized morphologically and microscopically as shown in Table 1.

Table 1: Morphological & Microscopic Characteristics of isolated fungal strains

Morphological & Microscopic Characteristics	*D_1W, D_4S (Alternaria sp.)*	*D_2W, D_5S (Aspergillus sp.)*	*D_3S (Fusarium sp.)*
Colony colour	Grayish-green with gray edges	White become black as culture matures	Wooly, white changing colour to pink
Colony shape	Circular	Circular	Circular
Conidia	Multicelled spores	Singlecelled spores	Multicelled spores
Conidia shape	Pear-shaped	Form of chains	Oval-shaped
Conidia colour	Brown	Dark brown to black and rough walled	Brown
Hyphae	Dark septate hyphae	Hyaline septate hyphae	Hyaline septate hyphae
Conidiophores	Short	Long with spherical vesicles at the apex	Short multi-branched

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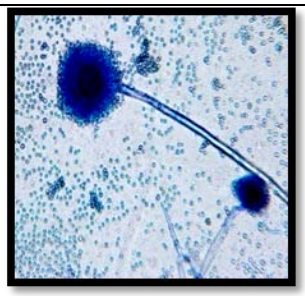
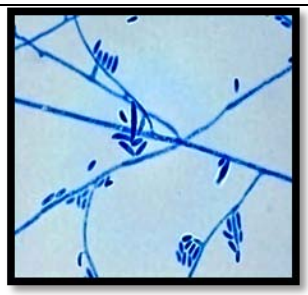
Microscopic image			

3.2 Bioaugmentation on a Lab Scale

Bioaugmentation was carried out under two experimental set ups i.e aerobic and anaerobic conditions. Various physico-chemical parameters were recorded and are presented in Table 2.

3.2.1 Colour

Colour is a qualitative characteristic that can be used to assess the general condition of wastewater. The removal of colour from wastewater is often more important than the removal of soluble colourless organics, which normally contribute to the major BOD load [16]. Colour affects the other parameters like temperature, DO, BOD etc [17]. In the present study the colour of untreated effluent was white in winter and black in summer whereas after aerobic and anaerobic treatment treated effluent appeared as blackish-brown for all fungal isolates except for D_3S (*Fusarium sp.*) which appeared to be cream in colour after aerobic treatment and orange after anaerobic treatment.

3.2.2 Salinity

Higher salinity values were observed during the treatment due to increase in solubility of solids, while the value lowered due to decrease in temperature which lowered the solubility of solids [18]. It was observed that no reduction in salinity was recorded for all the fungal isolates during aerobic treatment while D_4S (*Alternaria sp.*) had reduced salinity to 0.50ppt with 76.19% reduction on 4th day during anaerobic treatment.

3.2.3 Electrical Conductivity (E.C)

During aerobic treatment D_1W, D_2W, D_5S had shown higher values of E.C throughout the treatment whereas D_1W, D_2W and D_3S had shown maximum increase in E.C values during anaerobic treatment. No reduction in E.C was recorded for all the fungal isolates during aerobic treatment. D_4S (*Alternaria sp.*) had shown highest E.C percentage reduction of 80.86% on 4th day of anaerobic treatment.

3.2.4 Total Dissolved Solids (TDS)

The total solid concentration in waste effluent represents the colloidal form and dissolved species. The probable reason for the fluctuation of value of total solid may be due to content collision of these colloidal particles [19]. Increase in concentration of TDS was due to increased amount of dissolved solids in water [18]. Among all the fungal isolates only D_3S (*Fusarium sp.*) had shown reduction of 92.30% with TDS recorded as 0.12 ppt on 5th day of aerobic treatment. D_4S (*Alternaria sp.*) on 4th day (83.78%) of anaerobic treatment had shown significant reduction with TDS value recorded as 0.24ppt.

Table 2: Physico-chemical parameters of dairy effluent after treatment with fungal isolates under aerobic and anaerobic conditions

Fungal isolates	*Incubation days*									
	1st day (control)		*2nd day*		*3rd day*		*4th day*		*5th day*	
	A	*A_N*	*A*	*A_N*	*A*	*A_N*	*A*	*A_N*	*A*	*A_N*
	Salinity (ppt)									
D_1W	2.40	4.3	3.50	7.4	10.9	7.7	12.20	10.9	20.2	20.1
D_2W	2.30	11.2	4.90	24.8	5.70	16.2	7.30	16.7	14.3	10
D_3S	2.60	12.1	7.90	31.6	12.3	17.3	9.10	17.4	9.50	19.2
D_4S	2.10	2.1	4.10	1.9	2.3	2.2	4.8	0.50	5.2	2.1
D_5S	1.80	1.7	8.10	1.5	18.0	1.6	13.3	1.7	14.2	1.9
	Electrical Conductivity (mS)									
D_1W	0.36	3.86	2.90	6.28	9.58	6.67	10.72	9.88	16.60	16.93
D_2W	2.08	11.32	4.30	18.64	5.26	15.76	6.12	16.05	13.81	10.28
D_3S	2.37	11.05	7.19	19.76	11.03	16.16	8.06	16.38	2.60	17.35
D_4S	2.30	2.30	4.39	2.11	2.86	2.35	6.44	0.44	4.89	2.28
D_5S	1.97	1.72	8.39	1.68	17.29	1.79	14.48	1.84	16.2	1.94
	Total Dissolved Solids (ppt)									
D_1W	1.24	2.53	2.00	4.14	6.24	4.36	6.97	6.45	11.96	12.3
D_2W	1.36	7.48	2.82	15.64	3.42	10.70	4.90	11.05	8.96	6.68
D_3S	1.56	7.14	4.45	16.99	7.12	11.30	5.25	11.55	0.12	13.42
D_4S	1.48	1.48	2.75	1.37	4.98	1.53	5.36	0.24	3.62	1.47
D_5S	1.31	1.12	5.15	1.08	12.87	1.16	9.47	1.20	10.53	1.24
	pH									
D_1W	7	14	8	9.8	13	8	11	7	7.80	10

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D_2W	7.9	8	7	6	7	7	6	8	6	6
D_3S	7	14	6.9	13	6	14	6.5	11	6.8	11
D_4S	8	8	8.3	6	10	7	7	7	7	7
D_5S	12	12	9	6	8	7	5	7	6	4
	Chemical Oxygen Demand (mg/L)									
D_1W	468	132	544	152	600	160	80	264	44	168
D_2W	372	176	164	212	96	148	260	360	248	132
D_3S	384	144	176	132	720	88	508	84	308	80
D_4S	520	520	320	156	276	88	240	120	175	145
D_5S	500	500	140	220	248	224	312	190	364	172

Note: *A*- aerobic treatment; A_N- anaerobic treatment

3.2.5 Hydrogen Ion Concentration (pH)

pH is one of the important biotic factors that serves as an index for pollution. It is therefore, necessary to evaluate effluent with respect to the pH value. pH value indicates acidic to alkaline nature of effluent. The wide variation in the pH value of effluent can affect the survival of various micro organisms [19]. For each of the experimental set up, fluctuation of pH was recorded during experimental period. For D_1W and D_3S treated wastewater pH was neutral on 1st day of aerobic treatment while it was slightly alkaline in nature for D_2W and D_4S while strongly alkaline in case of D_5S. The pH of the effluent was alkaline in nature on 1st day of anaerobic treatment for each of the experimental set up. On 5th day of aerobic and anaerobic treatment pH for D_4S treated effluent was neutral, D_2W was slightly acidic, D_1W was alkaline while D_3S was neutral in aerobic treatment and alkaline in anaerobic treatment whereas D_5S was slightly acidic in aerobic treatment and strongly acidic in anaerobic treatment. The pH values were lowered after aerobic and anaerobic treatment.

3.2.6 Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) determines the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. The COD is a test which is used to measure pollution of domestic and industrial waste. It is useful in pinpointing toxic condition and presence of biological resistant substances. During aerobic treatment D_1W (*Alternaria sp.*) was the most efficient strain with COD reduction of 90.59% on 5th day by reducing COD values to 175mg/L and 82.90% on 4th day with COD recorded as 240mg/L followed by D_2W (*Aspergillus sp.*) with 74.19% COD reduction on 3rd day and D_5S (*Aspergillus sp.*) with 72% reduction on 2nd day of aerobic treatment. Figure 2 explains the percentage COD reduction order among the various fungal isolates during aerobic treatment is as follows: D_1W (5th day) > D_2W (3rd day) > D_5S (2nd day) > D_4S (5th day) > D_3S (2nd day).

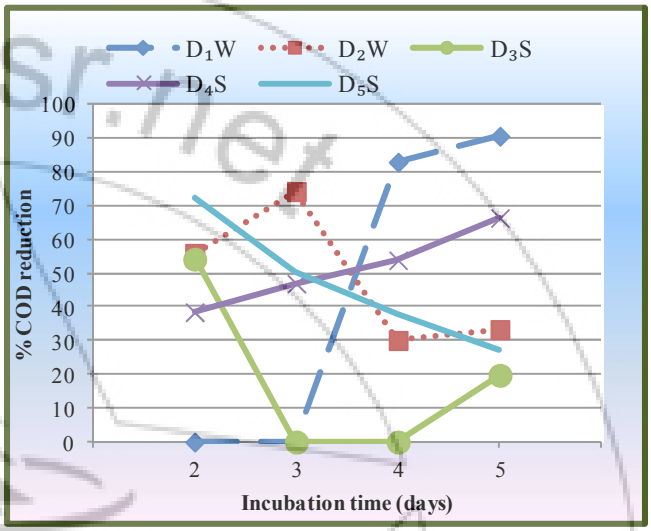

Figure 2: Variation in percentage COD reduction of dairy effluent by various fungal isolates under aerobic conditions.

As evident from the Figure 3, percentage COD reduction varied from 8.33% to 83.07% in the various setups of anaerobic treatment. Among these, the lowest percentage reduction was found in the set up using D_3S (8.33%) on 2nd day and the highest in the set up using D_4S (83.07%) on 3rd day of treatment which then decreased to 76.92% on 4th day and 72.11% on 5th day while D_1W had not shown any COD reduction throughout the treatment. The percentage COD reduction during anaerobic treatment followed the order: D_4S (3rd day) > D_5S (5th day) > D_3S (5th day) > D_2W (5th day).

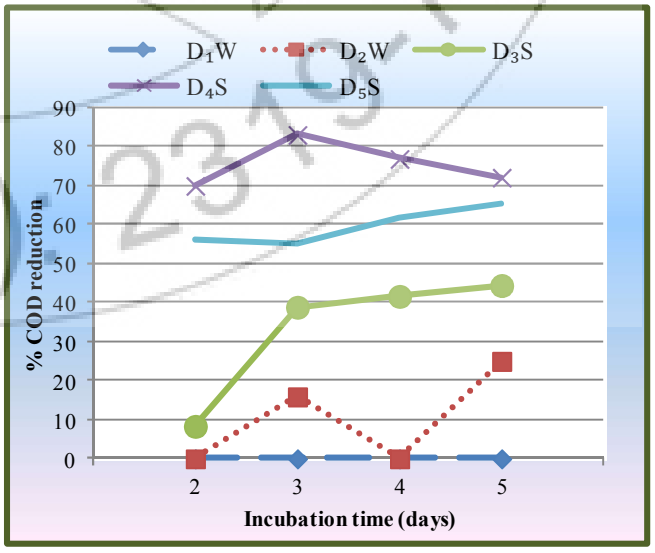

Figure 3: Variation in percentage COD reduction of dairy

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effluent by various fungal isolates under anaerobic conditions.

4. Conclusion

In the present study different fungal strains showed variable results in percentage reduction of various physico-chemical parameters. Selected fungal isolates had not shown any reduction in E.C, TDS and salinity under aerobic conditions except for *Fusarium sp.* (D_3S) in TDS. *Alternaria sp.* (D_1W) of winter season under aerobic conditions effectively degraded the effluent by reducing COD values while *Alternaria sp.* (D_4S) proved to be the best as it had reduced organic load by reducing all physico-chemical parameters under anaerobic conditions. Results concluded that *Alternaria sp.* is promising strain and can further be exploited for industrial scale applications.

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