Research Article

Physico chemical and Bacteriological Characterization of Cheese Processing Effluent and their Effect on *Vigna mungo* Growth

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ABSTRACT

The present study was aimed with Cheese processing effluent sample were collected from Sri Ganapathy Dairy farms, RS Puram, Coimbatore District. These samples were subjected to analyze the physico-chemical parameters include pH, Salinity, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Solids (TS) before and after treatment. Estimation of Chloride, Phosphate and Calcium content were assessed by titrimetric and turbidity method respectively. The cheese processing effluent was serially diluted and transferred (10^{-4} to 10^{-6} dilution) to the nutrient agar medium and incubated. After incubation two bacterial species namely *Lactobacillus bulgaricus* and *Lactobacillus fermentum* were isolated and identified using morphological and biochemical characters. Among these, *Lactobacillus bulgaricus* were effectively degrade the effluent sample compared with *Lactobacillus fermentum.* The effect of treated effluent (*Lactobacillus bulgaricus* and *Lactobacillus fermentum)* on growth of *Vigna mungo* was determined by pot culture experiment. After 28th day of seedlings, the better response observed in morphological and phytochemical characteristics of *Vigna mungo* were recorded in T_2 than other treatments.

Keywords: Biochemical test, Cheese Processing Effluent, Gram's staining, Lactic acid bacteria, *Vigna mungo*.

INTRODUCTION

The dairy industry produces different products such as milk, butter, yoghurt, ice-cream and various types of desserts and cheese, the characteristics of these effluents also vary widely both in quantity and quality, depending on the type of system and the methods of operation used. Dairy wastewater contains milk solids, detergents, sanitizers, milk wastes and cleaning water. It is characterized by high concentrations of nutrient, organic and inorganic contents.[1]

Dairy industry is a large scale food production industry and plays an important role in causing water pollution. Waste water coming out from dairy industry is categorized as raw waste and activated sludge which have to be treated by taking various parameters under consideration. This is achieved by assessment of several physico-chemical parameters of dairy waste water with raw waste which includes determination of pH, temperature, acidity, alkalinity, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), out of which COD is the most important parameter.

Dairy wastes are plentiful in dairy industries. The wastes contain high organic matters and the disposal of the effluents may cause severe environmental pollution. The dairy industry generates residues from which whey is the most important wastewater produced, with an extremely high organic load. Whey is a by-product of cheese and casein manufactures; mainly consists of carbohydrates, lactose, salts, lactic acid, proteins and fat respectively. It has a high Biological Oxygen Demand which is caused by its protein and carbohydrate content and it creates a problem when disposed as wastewater. The worldwide production of fluid whey by the cheese and casein industries runs into millions of tons and yet effective utilization of this material is not well developed.

A steady rise in the demand for milk and milk products in many countries has led to advancements in Veterinary Science, which has subsequently led to steady growth in the production of milk per head of cattle. This has caused enormous growth of dairy industries in most countries of the world. Consequently, the amount of wastewater generated and discharged from these industries has also increased.

The dairy industry wastewaters are primarily generated from the cleaning and washing operations in the milk processing plants. It is estimated that about 2% of the total milk processed is wasted into drains. The wastewater generated from milk processing can be separated into two groups - the first group concerns wastewater having high flow rates and the second concerns the effluents produced in small milk transformation units (cheese production for instance). Dairy wastewater is characterized by high Biological-Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) concentrations and generally contains fats, nutrients, lactose, as well as detergents and sanitizing agents. Nutrients lead to eutrophication of receiving waters, and detergents affect the aquatic life. Due to the high pollution load of dairy wastewater, the milk-processing industries discharging untreated/partially treated wastewater cause serious environmental problems. Moreover, the Indian government has imposed very strict rules and regulations for the effluent discharge to protect the environment. Thus, appropriate treatment

methods are required so as to meet the effluent discharge standards.[2]

The objective of the present study was to determine physico-chemical parameters of raw effluent and treated effluent. The efficiency of selected isolates in degradation of cheese processing effluent and to study the effect of treated effluent (*Lactobacillus bulgaricus* and *Lactobacillus fermentum)* on *Vigna mungo* growth by pot culture experiment.

MATERIALS AND METHODS

Sample collection and Physico chemical characterization of wastes[3]

The raw effluent samples were collected from Sri Ganapathy Dairy farms, RS Puram, Coimbatore District, Tamil Nadu, South India. The collected samples were carried out into laboratory using sterile bottle. The physico- chemical characteristics of samples such as pH, Salinity, Temperature, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Solids, Total Dissolved Solids, Total Suspended Solids, were analyzed. Chloride, Phosphate and Calcium content were assessed by titrimetric and turbidity method respectively.

Isolation and Identification[4]

The nutrient agar medium was sterilized and poured into separate petridishes and allowed to solidify. Then the serially diluted sediment soil and raw effluent samples were inoculated by spread plate method and incubated at 28^{o}C for 24 hours. After incubation, the plates were observed for isolation and identification of bacteria by Gram staining, Motility test and Biochemical test.

Treatment of Cheese Processing Effluent[5]

The laboratory experiment was conducted to evaluate the effect of bacterial *sp* on cheese processing effluent with three replicates and control. 300 ml of cheese processing effluent was taken in 500 ml of conical flask at four numbers in two set, one is control in each set. In first set, three flasks were inoculated with *Lactobacillus bulgaricus*. And another set of flasks were inoculated with *Lactobacillus fermentum*. Then the flasks were incubated in laboratory shaker at 37^{o}C for 15 days for degradation of cheese processing effluent. The effect of bacterial *sp* was assessed by changes in the pH, Salinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Dissolved Oxygen (DO), Total Solids (TS), Total Suspended Solids (TSS), Phosphate, Chloride, Calcium after the degradation of cheese processing effluent in the bacterial *sp* treated wastewater samples.

Pot Culture Experiment[6]

After seedlings of *Vigna mungo* were transplanted in four pots of equal size 20 cm in height and 6 cm in dm. Garden soil was used as the culture medium. The pots were provided with water facilities. There were 4 treatments resulting from combination of Raw effluent, *Lactobacillus bulgaricus* treated effluent, *Lactobacillus fermentum* treated effluent and Control. The pots were maintained in the open shade at the temperature of 27^{o}C – 30^{o}C. After 7th, 14th, 21st and 28th days of growth, 4 plants per pot were removed from all samples and studied for the following morphological parameters. They were, Height of the plant (in cm), Number of leaves (per plant), Number of roots (per plant), Shoot length (in cm), Root length (in cm) and Root nodules (per plant).

Statistical Analysis[7]

Statistical analysis was performed by calculating Mean ± standard deviation.

RESULTS AND DISCUSSION

The present study was carried out with the raw effluent samples were collected from Sri Ganapathy Dairy farms, RS Puram, Coimbatore district in Tamil Nadu, South India. From this sample, bacterial organisms were isolated and identified. The bacterial isolates were selected to assess the efficiency of treatment in effluent. The physico-chemical characteristics of samples such as pH, Salinity, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Solids (TS), Estimation of Chloride, Phosphate and Calcium before and after treatment were analysed.[8] (Table 2)

The bacterial species were isolated from raw effluent sample by serial dilution technique. Different bacterial colonies were observed in Nutrient Agar medium. The bacterial colonies were identified by Gram staining and biochemical test. Based on the morphological and biochemical characteristics, the isolates were confirmed as *Lactobacillus bulgaricus* and *Lactobacillus fermentum.*[9] (Table 1)

Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates

Morphological and Biochemical Characters	*Lactobacillus bulgaricus*	*Lactobacillus fermentum*
Gram Staining	Gram positive	Gram positive
Shape	Rods	Rods
Motility	Non Motile	Non Motile
Indole	-	-
Methyl red	+	+
Voges Proskauer	-	-
Catalase	-	+
Oxidase	-	-
Citrate	-	-
Urease	-	-

"+": Indicates Positive, "-": Indicates Negative

Analysis of Physico chemical Characteristics of Cheese Processing Raw Effluent and Treated Effluent

The physico chemical characteristics of cheese processing effluent were analyzed before and after treatment. The raw effluent sample was acidic in nature (pH 5). After the

treatment, the pH level was slightly increased and reached neutral pH. The physico-chemical parameters of the raw effluent samples were calculated as follows, pH (5), Salinity (13 mg/l), Total Solid (800 mg/l), Total Dissolved Solids (600 mg/l), Total Suspended Solids (200 mg/l), Biological Oxygen Demand (360 mg/l), Chemical Oxygen Demand (544 mg/l), Phosphate (320 mg/l), Chloride (60 mg/l), and Calcium (48 mg/l).The physico-chemical parameters of treated effluent sample compared with raw effluent. Parameters of *Lactobacillus bulgaricus* treated effluent was calculated as follows, pH (7.5), Salinity (16 mg/l), Total Solids (260 mg/l), Total Dissolved Solids (220 mg/l), Total Suspended Solids (40 mg/l), Biological Oxygen Demand (300 mg/l), Chemical Oxygen Demand (42 mg/l), Phosphate (280 mg/l), Chloride (40 mg/l) and Calcium (38 mg/l). *Lactobacillus fermentum* treated effluent was calculated as follows, pH (7), Salinity (12 mg/l), Total Solids (200 mg/l), Total Dissolved Solids (170 mg/l), Total Suspended Solids (30 mg/l), Biological Oxygen Demand (330 mg/l), Chemical Oxygen Demand (48 mg/l), Phosphate (200 mg/l), Chloride (36 mg/l) and Calcium (28 mg/l).

All the physicochemical characteristic of treated effluent sample was significantly decreased when compared to raw effluent sample. Among the two bacterial species, *Lactobacillus bulgaricus* was effectively degrade the cheese processing effluent compared to *Lactobacillus fermentum.*

Table 2: Physico-Chemical Parameters of Cheese Processing Raw Effluent and Treated Effluent

Parameters	Raw effluent	*Lactobacillus bulgaricus* treated effluent	*Lactobacillus fermentum* treated effluent
pH	5	7.5	7
Salinity (mg/l)	13	16	12
Total Solids (mg/l)	800	260	200
Total Dissolved Solids (mg/l)	600	220	170
Total Suspended Solids (mg/l)	200	40	30
Biological Oxygen Demand (mg/l) Chemical Oxygen Demand (mg/l)	360 544	300 544	330 48
Phosphate (mg/l)	320	280	200
Chloride(mg/l)	60	40	36
Calcium(mg/l)	40	38	28

Table 3: Effect of Treated and Untreated Cheese Processing Effluent on Growth of *Vigna mungo*

Morphological Parameters	7th day				14th day				21st day				28th day			
	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C	T_1	T_2	T_3	C
Height of the plant (in cm)	1.2 ±1.3	2.0± 2.1	1.1± 1.2	1.1± 1.2	2.4± 2.3	2.6 ± 2.7	1.8 ± 1.9	1.5 ± 1.6	2.0 ± 2.1	2.8 ± 2.7	1.8 ± 2.2	1.8 ± 1.7	3.2 ± 3.3	3.6 ± 2.5	2.6 ± 2.5	1.5 ± 1.6
Number of leaves (per plant)	4 ± 5	6 ± 8	3 ± 4	2 ± 3	9 ± 8	10 ± 11	5 ± 6	3 ± 4	7 ± 6	9 ± 8	5 ± 6	4 ± 3	15 ± 14	18 ± 19	13± 12	12 ± 11
Number of roots (per Plant)	3± 2	4 ± 3	2 ± 3	1 ± 2	4 ± 5	7 ± 8	3 ± 4	2 ± 3	6± 7	8± 9	5 ± 4	4± 3	7 ± 8	8 ± 9	6± 5	4 ± 3
Shoot length (in cm)	0.3 ± 0.2	0.4± 0.3	0.2± 0.3	0.1± 0.2	0.5± 0.6	0.6± 0.7	0.3± 0.2	0.2± 0.1	0.7± 0.8	0.8± 0.9	0.6± 0.7	0.5± 0.6	0.7± 0.6	0.9± 0.8	0.5± 0.4	0.3± 0.2
Root length (in cm)	0.3 ± 0.4	0.6± 0.7	0.2± 0.3	0.1± 0.2	0.5± 0.6	0.7± 0.6	0.4± 0.5	0.3± 0.2	0.5± 0.4	0.6± 0.5	0.3± 0.2	0.1± 0.2	0.4± 0.3	0.5± 0.6	0.3± 0.2	0.1± 0.2
Root nodules (per Plant)	4 ± 5	5 ± 6	3± 4	2 ± 3	6 ± 7	8 ± 9	5 ± 6	4 ± 5	5 ± 6	6 ± 7	4 ± 5	3 ± 4	8 ± 7	9 ± 10	6 ± 5	5 ± 4

Table 4: Effect of *Lactobacillus bulgaricus* and *Lactobacillus fermentum* treated effluent on Chlorophyll Content of *Vigna mungo* (7th, 14th, 21st and 28th Days)

Treatment	Chlorophyll (mg/g)											
	7th day			14th day			21st day			28th day		
	A	B	Total	A	B	Total	A	B	Total	A	B	Total
T1	0.102	0.067	0.167	0.144	0.123	0.267	0.188	0.167	0.355	0.178	0.154	0.332
T2	0.107	0.070	0.177	0.158	0.135	0.293	0.190	0.172	0.362	0.181	0.168	0.349
T3	0.098	0.066	0.164	0.131	0.116	0.247	0.182	0.160	0.342	0.173	0.135	0.308
Control	0.073	0.053	0.126	0.112	0.098	0.210	0.180	0.143	0.323	0.162	0.128	0.290

A - Chlorophyll a; B – Chlorophyll b

Table 5: Effect of *Lactobacillus bulgaricus* and Lactobacillus *fermentum* treated effluent on Total Carbohydrate, Flavonoid and Phenol in the leaves of *Vigna mungo* (28th day)

Treatment	Carbohydrate (mg)	Phenol (µg/g)	Flavonoid (mg/g)
T1	16.0	62.2	97.5
T2	18.0	74.2	85.7
T3	15.8	74.7	72.1
Control	9.0	43.2	62.4

Note: T_1 – Raw effluent, T_2 - *Lactobacillus bulgaricus* treated effluent, T_3 - *Lactobacillus fermentum* treated effluent.

Pot culture Experiment

The effect of treated effluent (*Lactobacillus bulgaricus* and *Lactobacillus fermentum*) on the growth of *Vigna mungo* was studied and compared with control. After seed inoculation, 7th, 14th, 21st and 28th day to observe the morphological characteristics and phytochemical analysis. After 28th day of seedling, the better response observed in Height (3.6 ± 2.5), Number of leaves (18 ± 19), Shoot length (0.9 ± 0.8), Root length (8 ± 9) and Root nodules (9 ± 10) were recorded in T_2 than other treatments include T_1, T_3 and Control (Table 3).

Phytochemical Analysis

Quantitative determination test for Carbohydrate, Chlorophyll, flavonoids and phenols were performed (Table 4 and 5).

Physico-chemical characterization of five whey samples collected during May 2007. Mean values of physical characteristics such as pH, Total Dissolved Solids (9.97 mg/l), Total Suspended Solids (1120 mg/l), Total Solids (253.6 mg/l) were recorded respectively. The results indicated that pollution parameter levels of wastewater samples of dairy industry tested in this study was found high the same results obtained by School of Veterinary Medicine, Department of Food Hygiene and Technology.[10]

The physico- chemical properties of dairy effluent was white in colour and unpleasant. pH value indicates acidic to alkaline nature of effluent. The wide variation in the pH value of effluent can affect the survival of various microorganisms. Total Dissolved Solids (2100 mg/l) is higher when compared to permission value. The Total solid concentration in waste effluent represents the colloidal form and dissolved species. The probable reason for the fluctuation of value of total solids may be due to content collision of these colloidal particles. The rate of collision of aggregated process is also influenced by pH of the effluents. BOD value of the effluent is 30 mg/l, compared to the standard value of 50% mg/l. Low value of BOD may be attributed to the quantity of Total Solids, Total Suspended Solids in effluents as well as to the quantitative number of microbial population.[11]

CONCLUSION

In the present study, the bacterial isolates from effluent were used for treatment of dairy waste water. It has been determined as the conclusion that the BOD, COD, TSS, TDS, TS in the wastewater of the milk industry prior to treatment are high. It has been observed on the other hand that the values determined in the wastewater samples obtained the treatment have been reduced to comply with the legal limits. Based on these findings, we have come to the conclusion that the treatment of the wastewater of the milk industry is inevitable for the prevention of the increase of the loads, of which the source is the milk industry, for the protection of the environmental health and the preservation of the ecological balance. Thus this treatment technology can be considered as a potential plant for industrial wastewater treatment.

REFERENCES

1. Rico JL, Garcia Encina PA, Anaerobic treatment of cheese-production wastewater using a UASB reactor, Bioresource Technol., 37, 1991, 271-276.
2. Srikar Sai, Biological Treatment of Dairy Industry Waste Water and its comparison with other treatment Techniques, International Journal of Sciences Biotechnology and Pharma Research, 3(5), 2000, 19-23.
3. Qiang Yu, Hengyi Lei, Zhong Li, HuaLiang Li, Physical and chemical properties of waste activated sludge after microwave treatment, Water Research, 44, 2010, 2841-2489.
4. Aneja KP, Determination of Temperature, World Journal of Microbiology and Biotechnology, 19, 2003, 653-657.
5. Sandeep Pandey J, Physico- chemical analysis and effect of distillery effluent on seed germination of wheat, pea, and lady's finger, Journal of Agricultural and Biological Sciences, 2, 2007, 6-8.
6. Parvathi K, Venkateshwarlu K, Raw AS, Effect of pesticides on development of *Glomus mosseae* in groundnut *(Arachis hypogeo),* Pans Br. Mycol. Soc., 42, 1985, 421-438.
7. Gupta SP, Statistical methods, Measures of central tendency and Measure of dispersion, Sultan Chan and sons Educational publishers, New Delhi, 3(4), 2004, 180 -181.
8. Vijayakumar S, Microbial Diversity in Rubber Industry Effluent, International Journal of Pharmacy and Biological Sciences, 3(2), 2012, 123-131.
9. Krieg NR, Holt JG, Bergey's Manual of Systematic Bacteriology, Vol. 1, eds: Williams and Wilkins, Baltimore, 1984, 189-194.
10. Trivedy RK, Goel PK, Chemical and Biological Methods for Water Pollution Studies, Environmental Publication, India, 2(3), 1986, 180-183.
11. Kolhe AS, VP Pawar, Physico- chemical analysis of effluent Dairy industry, Recent Research in science and technology, 3(5), 2011, 29-32.

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