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BIO-REMEDIATION OF DAIRY INDUSTRIAL SLUDGE THROUGH CULTIVATION OF MUSHROOM

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ABSTRACT:

Globally, the dairy sector is one of the most important sectors of the world. The milk processing industry is emerging during the last two decades due to enormous increase in the milk production. The waste water discharge form industries are major source of pollution and it affect the ecosystem. The degradation of environment results in adverse effect on living organism and agriculture. The overarching goal of the study is to reduce nutrients and pathogens from diary waste in Jaipur through a novel approach that utilizes remediation properties of mushrooms (higher macro fungi). Ames Assay and Respirometric Inhibition Assay are performed on extracts of mushrooms grown. Nutritional Analysis of grown Mushrooms is checked by various biochemical tests. It can be concluded from this study that DaS (Dairy Industrial Sludge) is suitable in enhancing the *Pleurotus* mushroom productivity. Cultivated mushrooms are found to have high biological yield. On the basis of higher yields, improved biological efficiency and richer protein content, Dairy Industrial Sludge after ETP treatment (when used at 50% concentrations), appear as a suitable option for substrate amendment of wheat straw for *Pleurotus citrinopileatus* cultivation.

Keywords: *Dairy, Industrial Sludge, Pleurotus, Biological efficiency, Protein, Bioremediation*

INTRODUCTION:

India has emerged as the largest milk producing country in the world with present level of annual milk production estimated as 94.5 million tonnes. It was assumed by Ministry of food processing Industries: MOFPI, that production level of milk would increase to 135 million tonnes by the year 2015. It has played a major role in the economy of Rajasthan. The dairy industry in India on an average has been reported to generate 6-10 litres of waste water per litre of the milk processed. The waste water of diary contain large quantities of milk constituents such as casein, lactose, fat, inorganic salt, besides detergents and sanitizers used for washing [18].

Detergents (Sodium hydroxide, Ethylene-diamine-tetra-acetic acid) represent the biggest portion of chemicals used in dairies; it may be alkaline or acidic. Many Dairy industries like Jaipur Dairy have waste treatment facilities such as Effluent Treatment Plant (ETP). Outcome of Effluent Treatment Plant is sludge and scum, a huge problem occurs in disposal of such waste discharged from these industries.

1.1 Waste Characteristics:

Among all industrial sectors, food processing units (Dairy Industry, edible and oil confectionary) are major contributor for waste water generation. The wastewater from food-processing industries is very rich in organic contents and may be a potential source for production of methane gas. There are over 18,550 food processing industries in India, emanating large quantities of wastes [6]. Dairy effluents contain dissolved sugars and proteins, fats, and possibly residues of additives.

A dairy often generates odors and, in some cases, dust, which need to be controlled. Most of the solid wastes can be processed into other products and by products. The key parameters are biochemical oxygen demand (BOD), with an average ranging from 28.55 to 29.15 milligrams per liter (mg $^{l-1}$) of Dairy sludge in the treated effluent; chemical oxygen demand (COD), is 6263.33 ± 805 ; total suspended solids, at 1.1–1.77 Ppt. [24]. Dairy Industrial waste is slightly alkaline which good sign for cultivation of any Oyster Mushrooms. Mushroom cultivation is of recent origin in India. It is mainly cultivated on the hills as it requires low temperature for its growth; however with the advent of modern cultivation technology it is now possible to cultivate this mushroom seasonally under uncontrolled conditions and throughout the year by employing environmentally controlled conditions. Mushroom cultivation slowly spread to North western plains of India (seasonal crop during winter). In Rajasthan, production of mushroom started in 1980 [25].

Among these *Agaricus* and *Pleurotus* contribute maximum to total world production of cultivated edible mushroom. The oyster mushrooms are botanically species of *Pleurotus* called as 'Dhingri' or 'Abalone'. They grow naturally in temperate or tropical forest on dead and decaying wooden logs or sometimes on outer bark of living trees. The fruit bodies of this mushroom are distinctly shelly or oyster shaped with different shades of white, cream, grey, yellow, pink or light brown depending upon the species. The oyster mushroom confers many advantages over other mushroom in terms of its ease for cultivation, role in biodegradation and bioremediation, extracellular enzymes production and nutriceuticals production [15,11]

Nutritional attributes of the oyster mushroom is being increasingly realized in recent times. Low in calories and high in protein as compared to rice, wheat, cabbage and milk, they are good sources of several vitamins including thiamine, riboflavin, niacin, biotin and ascorbic acids. The oyster mushrooms are good source of minerals and rich in carbohydrate and fibres. *Pleurotus* species represents the third largest group of cultivated edible mushrooms in the world, grown on a variety of plant residues, and they have been found to be nutritionally and gastronomically important. They may be cultivated on a large number of substrates, according to local availability in different regions of the world [11]. Out of 2000 species of recorded edible mushrooms, 100 are widely picked, 15-30 species are commonly eaten, 80 species are experimentally cultivated and 5-6 are produced on large scale [9].

In the present study, Edible Mushroom (*Pleurotus citrinopileatus*) is used as a bioremediating tool. It converts wastes biologically to harmless compounds.

Toxicity and edibility of cultivated mushrooms was evaluated. This is a sign of developing cultivation methods, modernization of the traditional methods and integration of mushroom cultivation with waste disposal and remediation. It can be both meet demands as nutritious food and a great bioremediating tool.

2. MATERIALS AND METHODS :

2.1 Study area:

The initial handling capacity of the dairy plant was 1.5 Lakh Lt. per day with a power plant of 10 MT per day capacity, which was commissioned in the year 1981 under Operation Flood Program 1 by National Dairy Development Board. Over the years, significant growth has been made in all fields i.e. procurement, processing and production of various milk and milk products. There marketing is done under the brand name of SARAS.

Jaipur Dairy Industry has its own ETP treatment plant. Outcome of Effluent Treatment Plant is sludge and scum, a huge problem occurs in disposal of such waste discharged from these industries. This sludge after ETP treatment is collected in Sludge drying beds, where they are dried. After which this sludge is transported in trucks in areas where land filling is required. In the present study the sludge just after final ETP treatment is collected and used in Mycoremediation.

2.2 Collection of effluent:

Semi solid sludge is collected from Sludge drying beds of Jaipur dairy. This sample is the last waste generated from Jaipur Dairy after ETP treatment. Samples were collected in a clean plastic container, were transferred to laboratory and stored at 4^{o}C until use for analysis.

2.3 Physico-chemical analysis of effluent:

Effluent samples were analyzed for physicochemical parameters such as pH, temperature, salinity, conductivity, BOD (Biological Oxygen Demand) and total dissolved solids (TDS) by water analyzer kit. Chemical Oxygen demand (COD) is done by the method as given in American Public Health Association (APHA) manual (1995). All these parameters were analyzed within 24 hrs. in each of the three replicates. (Table 1)

PARAMETERS	TREATED DAIRY EFFLUENT
TEMP. (^{O}C)	27.8 ± 3
TURBIDITY (NTU)	23.25 ± 2.7
TDS(ppt)	1.28 ± 0.48
SALINITY(ppt)	1.57 ± 0.36
CONDUCTIVITY(mS)	3.16 ± 0.27
pH	6.94 ± 0.31
BOD (mg/lit.)	29 ± 2.11
COD (mg/lit.)	6303.33 ± 133.9
COLOUR	Colourless

Table - 1: Analysis of different Physico-chemical parameters of treated dairy. Effluents from Dairy Industry: (mean ± standard deviation) 1ppt = 1000mg/l

2.4 Bioassays used for Genotoxicity evaluation of Dairy Sample and prepared Mushroom extracts:

2.4.1 Mutagenicity assay/ Ames assay-The tester strains of *Salmonella typhimurium* viz. TA98 and TA100 were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTEC), Chandigarh, India. The assay was carried out using the plate incorporation method described by Ames et al. (1975) and revised by Maron and Ames (1983). The samples were analysed with and without hepatic S9 fraction. Introduction of mammalian liver enzymes into prokaryotic system incorporates the aspect of mammalian metabolism into the *in vitro* test. Uninduced swiss albino Mice was used to prepare standard S9 mixture. Sodium azide was used as positive control and sterile distilled water was used as negative control. Five dose levels of individual samples (2, 5, 10, 50 and 100 µL) were tested and all the plates were run in duplicates. Each set of experiment was repeated twice.

This bioassay is used to analyze dairy effluent samples and further grown mushrooms on this effluent, were checked for mutagenicity. This test is called as Ames assay or *Salmonella* microsomal mutagenicity assay. The samples were analyzed with and without the hepatic S9 fraction, which incorporates an important aspect of mammalian metabolism into the in vitro test. Samples were tested on duplicate plates. Five dose levels (2 µl, 5 µl, 10 µl, 50 µl and 100 µl) of individual samples were tested. Positive control used for TA 98 and TA 100 is Sodium azide (1 µg/ plate: 594 revertants) respectively. On adding metabolic activation system i.e., S9 mix (prepared from uninduced liver of mouse as used by (Prival *et al.* 1982*)* revertants per plate in positive control increased as TA 98 (1 µg/plate: 481) and TA 100 (1 µg/plate: 897). Sterile distilled water was used as negative control. All readings were taken in duplicates. (Table 2)

Table 2: Mutagenicity Ratio of *Salmonella* TA98 and TA 100 in Ames test on Dairy Industrial waste effluent sample and Mushroom extracts :

Sample	Dose (µl)	Mutagenicity Ratio TA98		Mutagenicity Ratio TA100	
		-S9	+S9	-S9	+S9
Mushroom extract (100% DaS)	2	–	–	–	–
	5	–	–	–	–
	10	–	–	–	–
	50	–	–	–	–
	100	–	–	–	–
Mushroom extract (75% DaS)	2	–	–	–	–
	5	–	–	–	–
	10	–	–	–	–

	50	–	–	–	–
	100	–	–	–	–
	2	–	–	–	–
Mushroom	5	–	–	–	–
extract	10	–	–	–	–
(50% DaS)	50	–	–	–	–
	100	–	–	–	–
	2	–	–	–	–
Mushroom	5	–	–	–	–
extract	10	–	–	–	–
(100% WS)	50	–	–	–	–
	100	–	–	–	–

+, Ratio greater than 2.0 indicating possible mutagenicity; – ,Ratio less than 2.0 indicating non – mutagenicity

2.4.2 *Saccharomyces cerevisiae* respiration inhibition assay:

This is other test to analyze cytotoxicity of samples. A commercial brand of dried baker's yeast (*Saccharomyces cerevisiae*) was used for this assay. Baker's yeast assay (1984) was carried out by preparing a 1% (v/v) suspension of yeast in sterilized saline solution (0.85% NaC1) as the suspending fluid. The yeast suspension was stirred for 15 min to break up yeast floc. Test sample (0.2 mL) was added to 0.8 mL of yeast suspension and incubated for 30 min at 30°C with shaking. Different doses of samples (2, 5, 10, 50 and 100µL) were analysed.

To this, 0.1 mL of 0.2% INT solution and 0.1 mL of 10% solution of yeast extract were added and the mixture incubated in the dark at 30°C for 1 hr with shaking. The reaction was stopped with 0.1 mL of 37% formaldehyde. The proportion of respiring cells was determined as follows: one or two loopfuls of the yeast suspension was spread on a glass slide, air dried, counterstained with 0.025% malachite green and blotted after 1 min; 500 cells were examined with bright field microscopy (100x) and the number of respiring cells (green cells with red INT-formazan crystals) and non respiring cells (green cells) were determined. (Table - 3).

Mushroom Extracts	Inhibition Percentage at max. 200 µl (%)
Mushroom extract (100% DaS)	22.3
Mushroom extract (75% DaS)	22.2
Mushroom extract (50% DaS)	23.4
Mushroom extract (100% WS)	22.9

Table – 3 Inhibition percentage of Mushroom extracts by Baker's Yeast bioassay.

All reagents used were of analytical grade, supplied by Himedia Laboratories Limited (India) and Sigma Aldrich (India).

3. Collection of Mushroom strains:

The experiment is conducted in Environmental Molecular Microbiology Laboratory, University of Rajasthan, Jaipur. Pure culture of spawn of *Pleurotus citrinopileatus* is obtained from Agricultural Institute, Durgapura, Jaipur.

3.1 Spawn preparation:

Pure cultures of spawns are obtained from Agricultural Institute, Durgapura, Jaipur where they are prepared on large scale through following process:

The spawn was prepared in a polystyrene bag. Wheat grains boiled in a water bath for 20 min and mixed with 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$. The grains were inoculated with actively growing mycelium of the *Pleurotus citrinopileatus* from malt extract slant and incubated for mycelia growth for 14 days until the mycelium fully covered the grains in a room temperature maintained at 30°C. The method of [22] for spawn preparation was adopted for this study. Completely covering the grains with mycelium rapidly colonizes the bulk growing substrate [23].

3.2 Cultivation:

Collected spawns of *Pleurotus citrinopileatus* are stored until experiment at 20°C - 25°C temperature in pre sterilized area. For cultivation of oyster mushrooms last sludge of Dairy Industry is used. This type of waste is said to be rich in protein content. The effluent (watery substance obtained after ETP treatment) was mixed with semi - solid sludge obtained from Dairy Industry. Due to mixing, thick slurry of composite waste was obtained; 1.25 mg^{l-1} of 40% formalin solution (v/v) and 0.75 g/l (w/v) of anti-fungal substance bavistin were added to the slurry (Kulreshtha *et al.* 2010). After 18 hrs, slurry was filtered through a clean cloth and collected sludge is spread on the surface cleaned previously by formalin, to remove excessive water and maintain 80% humidity. Waste was then sterilized by steam for 1 hr.

Wheat straw (WS) was soaked in water containing formalin and bavistin, for 18 hrs and sterilized before using it as a substrate. Wheat straw is used

as a positive control. Wheat straw (WS) and Dairy Industrial Sludge (DaS) is mixed in relative proportions as mentioned.

100% WS (wheat straw) – Positive control
50% DaS + WS (dairy sludge + wheat straw) – 1:1
75% DaS + WS (dairy sludge + wheat straw) - 3:4
100% DaS (dairy waste)

After cooling, 500 gram of each of mixed proportions is transferred into polythene bags. Two bags of each combination of substrates (equiv. 500 g of substrate) spawned with *Pleurotus citrinopileatus* were filled and hanged in humid chamber [11]

Small holes at 3cm distance are made on polythene bags for air exchange. After inoculation, polythene bags were incubated at 22°C±1°C in humid chamber. When *Pleurotus* mycelia fully colonized the substrate, polythene bags were removed and substrate bundles were hanged at 16°C± 1°C in any slightly lighted chamber.

Humidity and Temperature is regularly checked at regular intervals by hygrometer. To maintain humidity bundles were sprayed with water twice a day. Two bags of each combination of substrate were prepared. Each substrate bundle was observed daily and time was recorded for mature fruiting bodies formation. After formation of mature fruiting body, mushrooms were harvested. Harvesting was done in minimum three flushes.

The fruiting bodies of *P. citrinopileatus* grow in clusters. They are of white colour with slightly brown caps. Caps range from 20–65 mm (6 cm) in diameter. The flesh is thin and white and without a strong smell. Mushroom stalk are cylindrical, white in colour, often curved or bent about 20–50 mm (5 cm) long. The gills are white, closely spaced, and run down the stem.

Table 6-i Time periods (mean± standard deviation) of different phases of *P. citrinopileatus* mushroom cultivation in days:

Waste substrate for cultivation of *Pleurotus*	Spawn running time (in days)	Pin head formation (in days)	Complete fruiting body formation (in days)
100 % WS	18 ± 1	24 ± 4	29 ± 3.6
50% DaS + WS	20 ± 2	24 ± 2	29 ± 2
75% DaS + WS	22± 2.65	25 ± 3.61	31.33± 3.06
100% DaS	22 ± 2.6	24 ± 4.3	31 ± 1.7

Table 6-ii Measurement of Mushroom: *Pleurotus citrinopileatus* (mean ± standard deviation)

Substrate (used for cultivation)	Stalk length (cm)	Pileus Diameter (cm)
100 % WS	4.5 ± 0.5	5.5 ± 0.5
50% DaS + WS	4.57 ± 0.93	5.3 ± 0.5
75% DaS + WS	3.70 ± 0.26	4.47 ± 0.06
100% DaS	2.67 ± 0.29	3.33 ± 0.42

4. Chemical toxicity test of mushroom:

A drop of juice was pressed out of the fresh body of mushroom on piece of newspaper. After the spot had dried hydrochloric acid was dropped on it. A blue spot indicates presence of toxins [10].

5. Extraction of grown Mushroom:

Fruiting bodies are shade dried and powdered. 100 gm of powdered mushroom is use to prepare extract. Extracts is prepared through soxhlet extraction using 95% methanol/ethanol as solvent. The filtered extract was kept in 10-20 ml glass vials and kept in refrigerator or deep freezer at 1-4°C for further use.

Soxhlet extraction is one of the oldest techniques for isolating metabolites from natural material. The technique is used for the isolation and enrichment of analytes of medium and low volatility and thermal stability [14].

6. Genotoxicity Analysis of Mushroom extracts:

Prepared extract is used for genotoxicity analysis through Ames assay (*Salmonella* mutagenicity Assay) and Respirometric Inhibition assay as discussed above using respective doses (2, 5, 10, 50 and 100µL) of prepared mushroom extracts.

7. Nutritional analysis of Mushroom :

7.1 Protein content:

The protein content of extracts of mushroom grown on 100% DaS, 50% DaS, 75% DaS and 100% WS was carried out by the method described by Lowry *et al*. (1951) using Bovine Serum Albumin (BSA) as standard (Table - 4).The optical density of each specimen was read at 650 nm using spectrophotometer [20].

7.2 Total Carbohydrate:

Total carbohydrates in the mushroom samples were estimated by Anthrone method, 100 mg of sample was hydrolysed in a boiling water bath for 3 hrs with 5 ml of 2.5 N HCl and after boiling, neutralized with sodium carbonate until the effervescence ceased, 0.5 ml of aliquot was taken and cooled rapidly. Absorbance was measured at 630 nm(Table - 4). Standard graph was plotted by using glucose [5].

Table-4 Showing Nutritional content and moisture of sample and grown mushrooms using different substrates :

Substrate	Protein (µg/ml)	Total Carbohydrate (µg/ml)
Sample(before sun dried)	791.02	894.26
100% DaS	748.33	845.57
75% DaS + WS	733.78	830.21
50% DaS + WS	731.02	824.64
100% WS	680.77	823.20

8. Yield and Biological efficiency:

Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom (Table - 5).The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula [1].

$$\textbf{Biological Efficiency} = \frac{\text{Fresh weight of mushrooms per bag (x)}}{\text{Dry weight of substrate per bag (y)}} \times 100$$

Table 5: Showing Biological Efficiency and Total Yield:

9. CALCULATION:

9.1 Data analysis for Ames test:

The most common method of evaluation of data from the mutagenicity assay is the "two fold rule" according to which doubling of spontaneous reversion rate at one or two test chemical concentrations constitutes a positive response (Mortelmans *et al*., 2000). This rule specifies that if a test compound doubles or more than doubles mean spontaneous mutation frequency obtained on the day of testing, then the compound is considered significantly mutagenic. Using this procedure the following criteria were used to interpret results:

Positive -A compound is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *S. typhimurium*. A compound is considered a weak mutagen if it produces a reproducible dose- related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies.

Negative - A compound is considered to be a non-mutagen if no dose-related increase in the number of revertant colonies is observed in at least two independent experiments.

Inconclusive -If a compound cannot be identified clearly as a mutagen or a non-mutagen, the results are classified as inconclusive (e.g. if there is one elevated count).

For this analysis the dose-related increases in the number of revertant colonies were observed for the test compounds and mutagenicity ratios were calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants)to average spontaneous revertants on negative con+trol plates (spontaneous revertants) [21].

Substrates	**100% WS**		**50% Das+ WS**		**75% DaS + WS**		**100% DaS**	
	Fresh wt.(gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt.(gm)	Fresh wt.(gm)	Dry wt.(gm)
I Flush	214.43±0.40	31.27±0.46	215.47±0.45	27.27 ±0.46	180.23±0.32	20.20±0.35	185.20±0.26	26.17±0.29
II Flush	140.53±0.46	18.20±0.35	170.27 ±0.46	18.27 ± 0.46	65.30 ± 0.52	15.33±0.29	80.03±0.06	18.19±0.27
III Flush	18 ±0.50	8.47±0.45	28.2± 0.35	5.03 ± 0.05	15.27 ± 0.46	3.27±0.46	29.20±0.20	5.02±0.03
Total Yield	372.97±0.58	57.93±0.86	413.94±0.94	50.57 ± 0.49	260.80±0.92	38.80±0.98	294.43±.45	49.37±0.59
Biological Efficiency	74.59%		82.79%		52.16%		58.89%	

Figure 2 Mushroom extracts : TA98

Figure 2-i : Showing Number of induced revertants with S9 on different samples

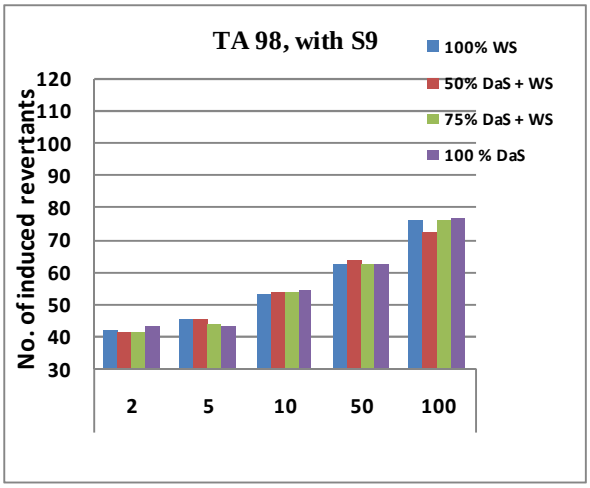
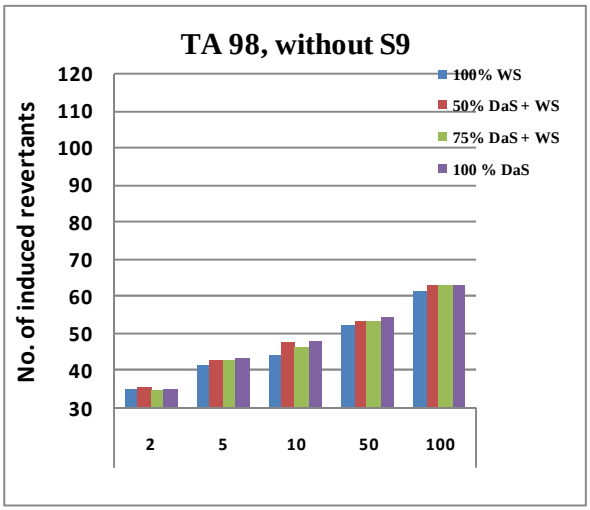


Figure 2 - ii: Showing Number of induced revertants without S9 on different samples



TA 100

Figure 2 - iii: Showing Number of induced revertants with S9 on different samples

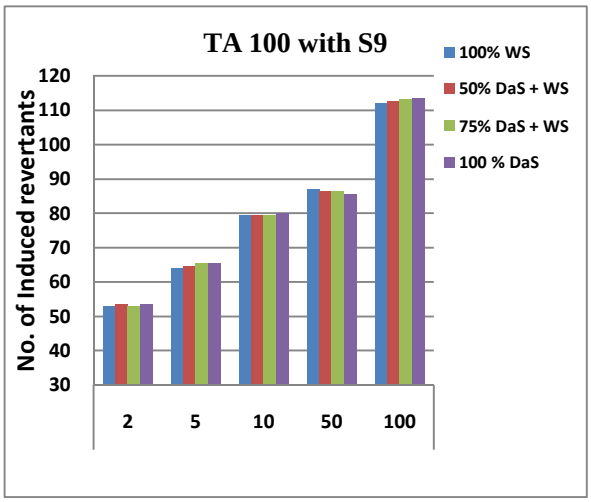
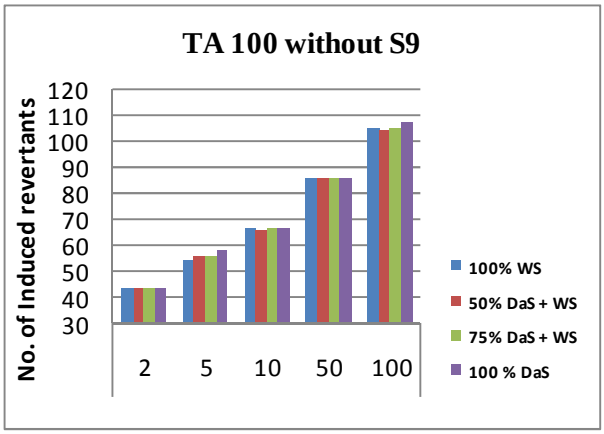


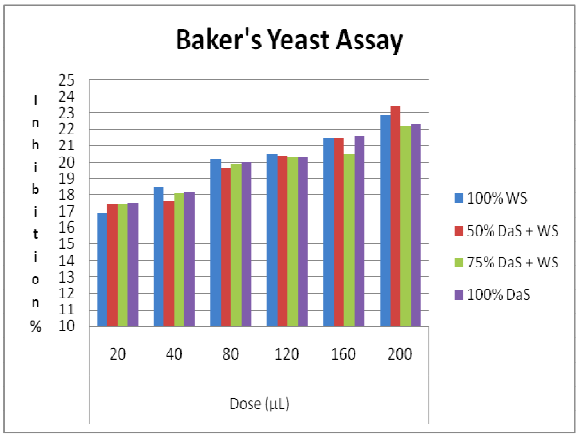
Figure 2 – iv: Showing Number of induced revertants without S9 on different samples



9.2 Interpretation of test results for *S. cerevisiae* Respiration Inhibition Assay:

Results of Baker‘s yeast assay were expressed in terms of percent inhibition of respiring cells compared with negative controls. Total 500 cells were examined in which number of respiring cells and number of non – respiring were counted. Interpretation of the test was done by calculation of Inhibition percentage.

Figure 3 : Showing Inhibition Percentages at different doses of Mushroom Extracts



10. RESULTS:

10.1 Growth Characteristics:

(Table 6-i) shows 22± 2.6 days is taken by mushrooms on 100% DaS for spawn running, 24±4.3 for pin head formation and average 31± 1.7 days for complete fruiting body formation. The shortest number of days for complete fruiting body formation is taken by mushrooms on wheat straw i.e., average 29 days.

Longest period was average 31 days days on 100% DaS (Table 6(i)).

10.2 Mushroom measurement:

10.2.1 Stalk length: Length of the stalk of *P. citrinopileatus* differed on different substrates. Highest stalk length was recorded on 50% DaS (Average 4.5cm). The lowest stalk length was recorded on 100% DaS, which was average 2.67cm (Table 6-ii). Stalk length of mushrooms on 50% DaS and that of on 100%WS (4.5cm).

10.2.2 Pileus Diameter: Diameter of pileus differed on different substrates. In case of mushrooms grown on 100% WS was found highest (5.5 cm) and the lowest (3.33 cm) diameter was recorded of mushroom grown on 100% DaS. Table (6-ii) shows little difference between the stalk length of mushrooms grown on 100% WS (positive control – 5.5cm) and those on 50% DaS that is 5.3cm. The increase of pileus diameter increase the yield, so in case of pileus diameter 50% DaS as a substrates gave approximately similar results as that of control.

10.3 Nutritional Analysis:

Table 4

It was found from the results that protein content showed variation in all the tested mushrooms. However, the highest protein content was found in mushroom grown on 100% DaS (748.33µg/ml) followed by Mushrooms grown on 75% DaS (733.78µg/ml), 50% DaS (731.02 µg/ml), 100%WS (680.77 µg/ml) respectively (Table 4)

Dairy sludge contains high amount of protein hence, maximum amount of protein is absorbed by mushrooms grown on 100% DaS.

Carbohydrate content also showed variation and the highest carbohydrate content was observed in mushroom grown on 100% DaS (845.57µg/ml) followed by Mushrooms grown on 75% DaS (830.21 µg/ml), 50% DaS (824.64 µg/ml), 100%WS (823.20 µg/ml) respectively (Table 4)

10.4 Biological Efficiency and Total Yield:

Pleurotus citrinopileatus was cultivated on Dairy Industrial Sludge using its different concentration with wheat straw. Total yield and biological efficiency of the mushroom was found to be lower on 100% DaS (58.89 %) and lowest on 75% DaS (52.16%). The mushroom cultivated on DaS is mixed with lignocellulosic wheat straw WS in 1:1 proportions (50%) shows highest Biological efficiency that is 82.79% . It is followed by 100% WS which shows B.E. of 74.59%. (Table 5). Similar results are shown for total yield . Highest yield was noted in 50% DaS (413.94 ± 0.94) followed by 100% WS (372.97 ± 0.58)

10.5 Genotoxicity Analysis:

Extracts (100% WS, 50% DaS+WS, 75% DaS+WS, 100% DaS) shows negligible genotoxicity even at higher doses with both TA98 and TA100 strains. Even when these extracts were tested in the presence of S9 enzyme mix, there was weak mutagenicity observed (mutagenicity ratio being less than 2). Even when the dose is increased, the extracts did not show any mutagenicity. The strain TA98 showed 35 – 65 induced revertants per 100 µl of extracts , in the absence of the S9 hepatic fraction while strain TA100 showed 43 – 107 induced revertants per 100 µl of extract, in the absence of the S9 hepatic fraction. This indicates that the extracts are non-genotoxic at higher dose level.

The addition of S9 mix produced same results, with strainTA98 it was 42 – 78 induced revertants per 100 µl of extract, in the presence of the S9 hepatic fraction while with strain TA 100 it is 53 – 114 induced revertants per100 µl of extract., in the presence of the S9 hepatic fraction. (Figure 2 – i, ii, iii , iv). All extracts have mutagenicity ratio less than 2. Hence, it could be inferred that the extracts shows no mutagenicity even if they are cultivated using dairy sludge after ETP treatment as substrates.

When extracts were tested with *S. cerevisiae* respiration inhibition assay, it shows percentage of Inhibition within limits. As it is shown in Figure 3. At 20µl all extracts shows about 15-18 inhibition percentage. When Inhibition percentage (of all extracts) was calculated at higher doses that is 200 µl it comes out to be within 50%. In the test, number of dead cell is within 50 i.e., 25 % of Inhibition percentage which can be interpreted as non cytotoxic) Mushrooms. Mushroom is non cytotoxic as we have taken the highest volume of sample 200 microlitres (Table - 3)

11. DISCUSSION:

Analysis of Dairy effluent is done after ETP treatment. Dairy effluent has high organic loads as

milk is its basic constituent with high levels of chemical oxygen demand, biological oxygen demand, oil & grease, nitrogen, phosphorous and TDS content [13].

In the present research, temperature of treated sample is 27^{o}C. Slight change (S.D ±3) is due to seasonal variation. Temperature is an important factor and has its effect on most chemical and biological reactions taking place in water and in organisms inhabiting aquatic media and will depend upon seasons and time of sampling. No specific limit for temperature is prescribed by WHO or ISI for the water quality use for the domestic purpose. The pollution loads in most of the dairy industries are in large quantity due to the use of sanitizers, acidic and alkaline detergents in manufacturing unit. The wide variation in the pH value of effluent can affect the rate of biological reaction and survival of various microorganisms.

Waste waters of dairy industry contain large quantities of milk constituents such as casein, lactose, fat, inorganic salts. Many detergents and sanitizers are used for washing. All these components contribute largely towards their high biochemical oxygen demand.

Trivedi *et al.* [4] observed the effluents of textile industry reported BOD value of mixed effluent ranged between 320 mg $^{l-1}$ to 720 mg $^{l-1}$ and final effluent 80 mg $^{l-1}$ to 640 mg $^{l-1}$. The maximum concentration of total dissolved solids is in summer, which increased in rainy seasons. While the minimum value, was found in winter probably because of stagnation. In summer most vegetation is decaying, so rise in the amount of dissolved solids was neutral as the products of decaying matter, which were settled in the water. 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 and subsequent the value of dissolved solids due to content collision of these colloidal particles [24].

The rate of collision of aggregated process is also influenced by pH of these effluents. In the rainy season less concentration of total dissolved solids are obtained. [7], studied textile industrial effluent and recorded total dissolved solid value, which ranges from 8500 mg $^{l-1}$ to 10,000 mg $^{l-1}$. In the present study, the total dissolved solid of treated effluent is reported as 1.28±0.48.

11.1 Growth characteristics:

Oyster mushrooms are known to produce extracellular enzymes, such as manganese peroxidise, laccase, lignin peroxidase, and aryl alcohol oxidase, which are capable of degrading lignocelluloses biomass [19].

Mushroom has utilized all substrates and their combinations for their mycelial growth and fruit body development although their magnitude varies among themselves. The mycelial growth of mushroom on diverse range of waste indicates that the substrate materials meet all the nutritional requirements desired for fungal growth. Material with high quality of lignin and cellulose contents take a longer time to initiate the pinning compared to the substrate with low content of lignin and cellulose. [19].

Here in both cases of stalk length and Pileus diameter, 50% DaS showed similar results when compared to positive control. Dairy sludge fulfils protein requirement and wheat straw provides lignin and cellulose to growing mushrooms.

Dhanam *et al.* [17] irrigated Paddy seeds in petri plates with various concentrations of dairy effluent (0 (control), 5, 140, 25, 50, 75 and 100%). Germination percentage and seedling growth was inhibited at 100% concentration may be due to osmotic pressure caused due to high dose. Osmotic pressure of the effluent at higher concentration of total salts making imbibitions was more difficult and retarded germination [12].

The results of *Salmonella* mutagenicity assay for mushroom samples are summarized in (Table 2) as the mutagenicity ratio of average induced reversions to spontaneous reversions. The extracts of grown mushrooms were prepared by Soxhelet using Alcohol as solvent. These prepared extracts were tested for their mutagenicity by using *Salmonella* strains. All extracts have mutagenicity ratio less than 2. Hence, it could be inferred that the extracts shows no mutagenicity even if they are cultivated using dairy sludge after ETP treatment as substrates. It is recommended that the analysis of properties of dairies wastewater on soil should be carried out periodically to ascertain

the extent of which dairy water can be use as soil amendment and its best usage as fertilizer. Dairy Effluent can be used to raise maize, rice, cassava, yam, vegetables and any other crops [16].

From the present study, we find that DaS (Dairy Industrial Sludge) could be suitably used for enhancing the *Pleurotus* mushroom productivity. While *P. citrinopileatus* performed better on wheat straw amended with DaS in 1:1 ratio. Oyster mushroom when grown on DaS + WS at 50% DaS concentration show maximum biological efficiency even greater than positive control (100% WS). Mushroom cultivation still proved to be one of the highly simple, beneficial and an economic method for disposing off the agricultural residues, such as wheat straw along with effective utilisation of the industrial effluents which are generated in abundance annually. Other methods for bioremediation of the industrial effluents could be further explored.

For a particular mushroom species, the degradation of hemicellulose and lignin was in accordance with the substrate dry-matter loss and so were the yield and biological efficiency. Greater losses in the dry-matter lead to higher yields, and hence, improved biological efficiency. In addition, the mushroom fruit bodies obtained with the use of effluent amendments had a higher protein content compared to that of the controls. Even when grown mushrooms tested for genotoxic potential they are found to be non- genotoxic and cytotoxic. The study is important from the point of view of resource recovery. Thus, on the basis of higher yields, improved biological efficiency and richer protein content, Dairy Industrial Sludge after ETP treatment (when used at 50% concentrations) appear as a suitable option for substrate amendment of wheat straw for *Pleurotus* species (*P. citrinopileatus*) cultivation.

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