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RESEARCH ARTICLE

ANALYSIS ON BIODEGRADATION AND COLOUR REDUCTION OF DISTILLERY EFFLUENT SPENT WASH

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The disposal of large quantities of biodegradable waste without adequate treatment results in significant environmental pollution. The distillery effluent which is also known as spent wash is discharged as a waste from distilleries. This is the major source of aquatic and soil pollution because intense brown colour which results in the blockage of photosynthesis, eutrophication and low pH acidifies soil affecting agricultural crops. It is one of the most complex and cumbersome waste having very high BOD and COD content, highly acidic pH, with other organic and inorganic toxic constituents. Anaerobic digestion of biodegradable waste results in both energy generation and reduction of greenhouse gas emissions. The main objective of this project was to treat the spent wash by anaerobic digestion using methanogenic bacteria and reduction of colour, sulphate by the bacterium. Anaerobic digestion results in 69.09 % reduction in COD and the pH changes to acceptable level. In the present study, efficient degradation of sulphate and colour reduction of raw effluent spent wash was achieved using *Pseudomonas aeruginosa* (MTCC 2474). The organism exhibited 72.03 % of sulphate reduction, 47.6 % colour reduction and 76.62 % of COD reduction. Further the study revealed that sulphate is converted into hydrogen sulphide during microbial treatment of distillery effluent and the presence of excess sulphate was found inhibitory for degradation of distillery effluent and bio methanogenesis.

Key words: MCP, MP, *Pseudomonas aeruginosa, Trichoderma viridae, Vigna mungo.*

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INTRODUCTION

Distillery Industry and Environment

Distillery effluent also known as spent wash, is discharged as waste water from distillery factories based on fermentation of alcohol from molasses. The discharge of distillery stillage in India is in order of 12-15 times the amount of alcohol produced.

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Distillery effluent is one of the most complex, caramelized and cumbersome waste having very high BOD (35,000-50,000 ppm) and COD (85,000-130,000 ppm) content which is highly toxic to environment with other organic and inorganic constituents (Mirsa *et al.,* 1993). In India there are approximately 246 distilleries, which release annually 4 x 10^{12} kilolitre of spent wash.

Methods for distillery effluent treatment Physical treatment methods

Among the physical methods applied for distillery effluents are coarse and fine screens,

sedimentation, removal of oil and grease, dilution with water so as to reduce the pollution load, sun drying and incineration etc. These are simple methods for the treatment of effluents but are able to partly remove BOD load and come under primary treatment techniques. These methods cannot remove major BOD load which is present in dissolved form in sugar/distillery industry effluent and require large amount of water for dilution resulting in inflated volumes of effluent which require very large areas for drying. Therefore, these methods are not regarded as complete methods for the treatment of effluent.

Chemical Treatment Methods

These processes include those methods in which the change in effluent quality is brought about by means of chemical reactions. Removal of organic matter by chemical coagulation using lime and alum, chemical oxidation by chlorine or potassium permanganate or dichromate are very expensive and hence are not economical for treatment of effluent from distillery.

Biological Treatment Methods

Biological treatment methods are nothing but mere duplication of nature's own self purification process under controlled conditions. The biological devices can be divided into three categories.

1. Aerobic process
2. Semi aerobic process
3. Anaerobic process

Out of these, microbial anaerobic process with biogas recovery is most common successful.

Anaerobic Digestion

Anaerobic digestion is a process in which microorganisms break down biodegradable material in the absence of oxygen. The process is widely used to treat wastewater sludges and organic wastes because it provides volume and mass reduction of the input material. As part of an integrated waste management system, anaerobic digestion reduces the emission of landfill gas into the atmosphere. Anaerobic digestion is a renewable energy source because the process produces a methane and carbon dioxide rich biogas suitable for energy production helping replace fossil fuels. Also, the nutrient-rich solids left after digestion can be used as fertilizer (Ghosh *et al.*, 2002).

The digestion process begins with bacterial hydrolysis of the input materials in order to break down insoluble organic polymers such as carbohydrates and make them available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. Acetogenic bacteria then convert these resulting organic acids into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. Methanogenic bacteria finally are able to convert these products to methane and carbon dioxide.

Microbial anaerobic treatment of distillery effluent is accomplished into three stages, i.e. hydrolysis, acidogenesis and methanogenesis. In the biphasic treatment of spent waste the hydrolysis and acidogenesis is combined together while methanogenesis remains separate. Sometimes, all the three stages re combined in a single step with anaerobic digestion. In an anaerobic composition microorganisms are able to use molecules other than oxygen as terminal electron acceptor. This anaerobic decomposition ultimately results in the production of biogas consisting of methane (50-70%) carbon dioxide (25-45%) and small amount of hydrogen, nitrogen and hydrogen sulphide. The overall chemical reaction is often simplified to:

Spent wash → Hydrolysis, Acidogenesis,

Methanogenesis → $CH_4 + CO_2 + H_2 + N_2 + H_2S$

- Utilizing anaerobic digestion technologies can help to reduce the emission of greenhouse gases in a number of key ways:
- Replacement of fossil fuels
- Reducing methane emission from landfills
- Displacing industrially-produced chemical fertilizers
- Reducing vehicle movements
- Reducing electrical grid transportation losses.

There is no method appreciated worldwide to reduce sulphate which concentration exceeds the limit poses threat to environment and

the reduction of colour of distillery effluent (Livernoche *et al.,* 1983). Effort has been made to find the solution for these problems through this work.

MATERIALS AND METHODS

Anaerobic digestion

The Spent wash sample was collected in a lab scale digester of 1 litre capacity. The temperature of the spent wash was reduced from 80^0C to 37 ^{0}C. Then the sample was inoculated with methanogenic bacteria (digester sample) and mixed well for uniform mixing. The sample was collected at regular intervals and its physico-chemical parameters like pH, Total Solids, TSS, TDS, VA, Alkalinity, COD and BOD were analyzed (Nandy *et al.,* 2002).

Sulphate Reduction

The Spent wash was collected and stored at 4º C until usage to prevent the microbial growth and its action. 100 ml of sample was taken in a conical flask and inoculated with 10 ml of inoculum (Containing overnight culture of *Pseudomonas aeruginosa* (MTCC 2474). Then the sample was agitated at 170 – 200 rpm for uniform mixing of micro organism with spent wash sample (Uzal *et al.,* 2003). Initial NTU values were noted after inoculation. At regular intervals the sample was withdrawn and their NTU values were measured.

Effect of pH on Sulphate Reduction

The effect of pH on Sulphate reduction was determined by incubating 100 ml of sample in 250 ml conical flask at different pH by using 0.1 N HCl and 0.1 N NaOH. Then they are inoculated with 10 ml of culture medium containing *Pseudomonas aeruginosa* (MTCC 2474). The flask containing sample was shaken well for 5 minutes at 170 rpm in orbital shaker for uniform mixing of inoculum with spent wash sample.

Then the flask containing sample was incubated at room temperature. Their initial NTU readings are noted. At regular intervals the 10 ml of sample is withdrawn and to collected sample about 2ml of buffer solution and 0.1 g of Barium chloride was added and mixed well. After 5 minutes NTU readings are measured and tabulated. The percentage of reduction is calculated using standard formula.

Colour Reduction

About 100 ml of sample was taken in 5 conical flasks (250 ml). The sample was supplemented with 0.5% glucose, 0.1% KH_2PO_4, 0.05% KCl and 0.05% $MgSO_4.7H_2O$. These provide micro nutrients for the growth of micro organism. Then the flask containing sample was inoculated with 10 ml of 24 hours culture of *Pseudomonas aeruginosa* (MTCC 2474), *Bacillus subtilis* (MTCC 2414) and Mixed Culture (each 5 ml of *Pseudomonas aeruginosa* (MTCC 2474), *Bacillus subtilis* (MTCC 2414)). Sample was shaken well for 5 minutes at 170 rpm in orbital shaker for uniform mixing of inoculum with spent wash sample (Manisankar *et al.,* 2004). At regular intervals the sample was collected and the suspended solids were removed by centrifuging at 7000 rpm for 15 minutes followed by filtration (Dahiya *et al.,* 2001). The initial absorbance value was measured at 475 nm using spectrophotometer. Then the sample was incubated at room temperature. At regular intervals about 5 ml of sample is withdrawn and absorbance is measured at 475 nm was measured.

Calculation

% Reduction = (Initial OD – Final OD)/ InitialOD.

RESULTS AND DISCUSSION

In this work, we have explored the possibility of using microorganism for the digestion and degradation of spent wash. Anaerobic digestion is particularly suited to wet organic material and is commonly used for effluent and sewage treatment. Anaerobic digestion is a simple process that can greatly reduce the amount of organic matter which might otherwise be destined to be land filled or burnt in an incinerator.

Table 1 Anaerobic Digestion – Estimation of pH, COD of Samples at regular intervals

S.No	Days	pH	COD (x 10^3 mg/l)	% COD Reduction
1.	0	4.14	85.225	-
2.	1	4.15	83.910	1.54
3.	3	4.46	79.146	7.13
4.	5	5.28	72.112	15.39
5	7	5.93	64.800	23.97
6.	9	6.56	51.162	39.97
7.	11	7.02	44.081	48.28
8.	13	7.43	35.423	58.44
9.	15	8.11	26.341	69.09

Pressure from environmentally-related legislation on solid waste disposal methods in developed countries has increased the application of anaerobic digestion as a process for reducing waste volumes and generating useful by-products. These facilities are called mechanical biological treatment plants. Methane and power produced in anaerobic digestion facilities can be utilized to replace energy derived from fossil fuels, and hence reduce emissions of greenhouse gases. This is due to the fact that the carbon in biodegradable material is part of a carbon cycle. If the putrescible waste processed in anaerobic digesters was disposed of in a landfill, it would break down naturally and often anaerobically. In this case the gas will eventually escape into the atmosphere. As methane is about twenty times more potent as a greenhouse gas as carbon dioxide this has significant negative environmental effects. Digestate liquor can be used as a fertilizer supplying vital nutrients to soils. The solid, fibrous component of digestate can be used as a soil conditioner.

Blonskaja *et al.,* 2003 reported that digestate typically contains elements such as lignin that cannot be broken down by the anaerobic microorganisms. Also the digestate may contain ammonia that is phytotoxic and will hamper the growth of plants if it is used as a soil improving material. For these two reasons maturation or composting stage may be employed after digestion. Lignin and other materials are available for degradation by aerobic microorganisms such as fungi helping reduce the overall volume of the material for transport.

During this maturation the ammonia will be broken down into nitrates, improving the fertility of the material and making it more suitable as a soil improver. Large composting stages are typically utilized by dry anaerobic digestion technologies. The final output from anaerobic digestion systems is water. This water originates both from the moisture content of the original waste that was treated but also includes water produced during the microbial reactions in the digestion systems.

Table 2 Anaerobic Digestion – Estimation of TS, TDS and TSS of Samples at regular intervals

S.No	Days	TS (x 10^3 mg/l)	TDS (x 10^3 mg/l)	TSS (x 10^3 mg/l)
1.	0	95.00	86.00	9.00
2.	1	93.50	84.00	9.50
3.	3	89.50	79.00	10.50
4.	5	84.00	73.00	11.00
5	7	80.00	67.00	13.00
6.	9	76.00	60.50	15.50
7.	11	72.25	56.00	16.25
8.	13	69.00	52.00	17.00
9.	15	66.75	48.00	18.75

Anaerobic Digestion

The disposal of large quantities of biodegradable waste without adequate treatment results in significant environmental pollution. The Physico-chemical characteristics of spent wash before anaerobic digestion was analyzed and the measurements are recorded (Table: 1). The pH of spent wash was found to be 4.27 and the temperature was found to be 82 °C. The COD and BOD of the spent wash were found to be 85225 and 42150 mg/l respectively. The Sulphate concentration in the spent wash was found to be 3200 mg/l.

The spent wash was digested anaerobically and the parameters like pH, COD, TS, TSS, and TDS were recorded at regular intervals (Table: 2). The methanogenic bacterium increases the pH from 4.14 to 8.11 and the COD of the sample was

reduced upto 69.09 % over a period of 15 days. The variation in pH and COD reduction of the sample was shown in Figure 1 and 2.

Table 3 Estimation of Sulphate Reduction in Raw Spent Wash treated with *Pseudomonas aeruginosa* (MTCC 2474)

S.No	Days	Ntu Readings	Sulphate concentration (mg/l)	% Reduction
1.	0	80.64	3200	-
2.	1	73.92	2933	8.34
3	2	60.73	2409	24.71
4.	3	52.42	2080	35.00
5.	4	40.82	1620	49.37
6.	5	31.96	1268	60.38
7.	6	26.74	1061	66.84
8.	7	22.55	895	72.03

Sulphate and Colour Reduction of Effluent Spent Wash

The sample was inoculated with *Pseudomonas aeruginosa* (MTCC 2474) for sulphate reduction in spent wash. Initially the sulphate degrading capacity of microorganism was tested using copper sulphate solution in which the sulphates of increasing concentration from 0.1 to 0.6 were prepared and inoculated with *Pseudomonas aeruginosa* (MTCC 2474) and incubated over a period of 7 days. It was found that the microorganism was capable to degrade upto 0.4 g of sulphate per 100 ml above which it was found the growth of microorganism was slow which implies the concentration of sulphate above 0.4 g was found toxic for the microbial growth.

The sulphate reduction in the spent wash by using *Pseudomonas aeruginosa* (MTCC 2474) was observed (Dewalle *et al.,* 1976). The percentage of reduction was 72.09 % over a period of 7 days. The results are shown in table 3. The reduction in sulphate concentration and percentage of sulphate reduction at regular intervals, by the microorganism was shown in figure 3 and 4. The effect of pH on sulphate reduction were determined and optimized (Fumi *et al.,* 1995) (Table: 4). From the graph the reduction was high at the pH values of 6 when compared to other values (Figure: 5). After sulphate reduction the COD was 19926 mg/l whose reduction percent was 76.62 (Table: 5).

Table 4 Estimation of pH effect on Sulphate Reduction in Raw Spent Wash treated with *Pseudomonas aeruginosa* (MTCC 2474)

S.No	pH	Sulphate Concentration (mg/l)		% Reduction
		0^{th} Day	7^{th} Day	
1.	4	3260	1155	64.57
2.	5	3241	1019	68.55
3	6	3223	844	73.81
4.	7	3202	876	72.64
5.	8	3173	958	68.81
6.	9	31s42	1055	66.42

The colour reduction of effluent spent wash was tested using the cultures of *Pseudomonas aeruginosa* (MTCC 2474), *Bacillus subtilis* (MTCC 2414) and mixed culture (*Pseudomonas aeruginosa* (MTCC 2474) and *Bacillus subtilis* (MTCC 2414)). The reduction in colour by these microorganisms was shown in table 6, 7 and 8. The percentage of reduction was 47.6 %, 37.85 % and 42.9 % respectively. Thus the bacterium *Pseudomonas aeruginosa* (MTCC 2474) was found to degrade more efficiently when compared to other 2 cultures, which were shown in figure 6.

CONCLUSION

Distillery industry contributes to one of the major industrial pollution with generation of large amount of effluent called as spent wash. Effluent originating from distilleries leads to extensive soil and water pollution. Elimination of pollutants and colour from distillery effluent is becoming increasingly important from environmental and aesthetic point of view. Due to the large volumes of effluent and presence of certain recalcitrant compounds, the treatment of this stream is rather challenging by conventional methods.

Anaerobic digestion of biodegradable waste results in both energy generation and reduction of greenhouse gas emissions thus reducing the land fill gas. It would not only replace the use of fossil fuels, in various applications but would also utilize

Table 5 Estimation of cod of sample at regular intervals treated with *Pseudomonas aeruginosa* (MTCC 2474)

S.No	Days	COD (x	% REDUCTION
1.	0	85.225	-
2.	1	79.464	6.76
3.	2	75.100	11.88
4.	3	61.344	28.02
5	4	48.791	42.75
6.	5	35.351	58.52
7.	6	26.164	69.30
8.	7	19.926	76.62

Table 6 Color Reduction of Spent Wash by using *Pseudomonas aeruginosa* (MTCC 2474)

S.NO	Days	OD at 475 nm	% Reduction
1.	0	1.000	-
2.	1	0.941	5.9
3.	2	0.875	12.5
4.	3	0.802	19.8
5	4	0.726	27.4
6.	5	0.638	36.2
7.	6	0.524	47.6

Table 7 Color Reduction of Spent Wash by using *Bacillus subtilis* (MTCC 2414)

S.No	Days	OD at 475 nm	% Reduction
1.	0	0.996	-
2.	1	0.966	3.01
3.	2	0.912	8.43
4.	3	0.847	14.96
5	4	0.765	23.19
6.	5	0.692	30.52
7.	6	0.619	37.85

Table 8 Color Reduction of Spent Wash by using Mixed Culture

S.No	Days	OD at 475 nm	% Reduction
1.	0	1.000	-
2.	1	0.962	3.8
3.	2	0.878	12.2
4.	3	0.804	19.6
5	4	0.745	25.5
6.	5	0.687	31.3
7.	6	0.571	42.9

Days Vs pH Variation

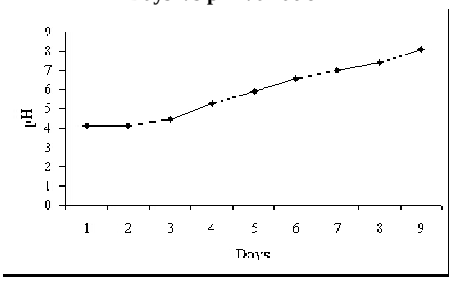


Figure 1: Graph showing variation in pH of the Sample during Anaerobic Digestion of Spent Wash

Days Vs % COD Reduction

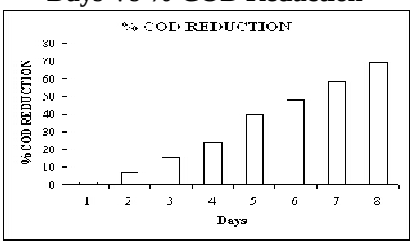


Figure 2: Graph showing percentage reduction of COD during Anaerobic Digestion of Raw Effluent Spent Wash

Days Vs Sulphate Concentration

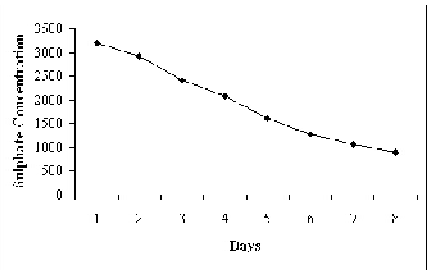


Figure 3: Graph showing Sulphate Concentration in Raw Spent Wash treated with *Pseudomonas aeruginosa* (MTCC 2474) at regular interval

Days Vs % Sulphate Reduction

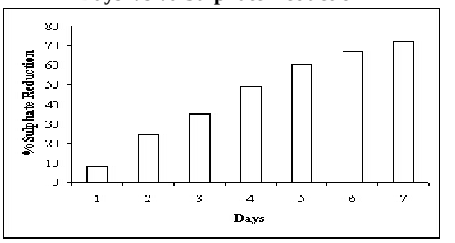


Figure 4: Graph showing Sulphate Reduction in Raw Spent Wash treated with *Pseudomonas aeruginosa* (MTCC 2474)

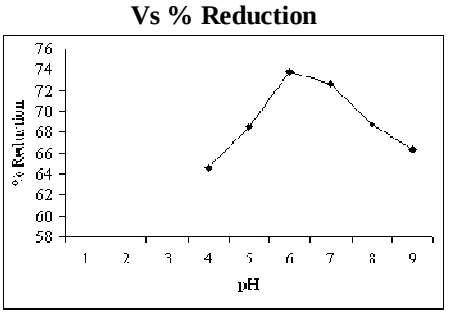
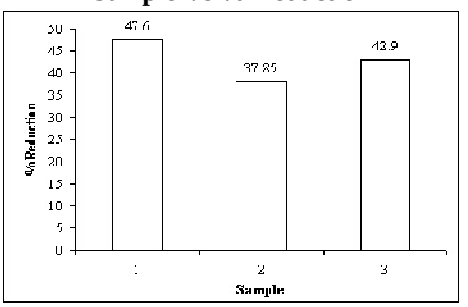


Figure 5: Graph Showing effect of pH on Sulphate Reduction of Raw Effluent Spent Wash



SAMPLE 1 - *Pseudomonas aeruginosa* (MTCC 2474).
SAMPLE 2 - *Bacillus subtilis* (MTCC 2414).
SAMPLE 3 - Mixed Culture of *Pseudomonas aeruginosa* (MTCC 2474) and *Bacillus subtilis* (MTCC 2414).

Figure 6: Graph showing percentage Color Reduction of Spent Wash treated with Microorganisms

methane generated from the waste. Anaerobic digestion of spent wash using methanogenic bacterium results in 69.09 % reduction in COD thus reducing the pollution load. Further 72.09 % of sulphate reduced in the effluent spent wash using *Pseudomonas aeruginosa* (MTCC 2474), during which the COD also reduced to 76.62 %. Thus sulphate reduction followed by anaerobic digestion results in increase in bio methanogenesis and reduction in hydrogen sulphide gas during the biogas production. The colour of the spent wash was reduced using the cultures of *Pseudomonas aeruginosa* (MTCC 2474), *Bacillus subtilis* (MTCC 2414) and mixed culture (*Pseudomonas aeruginosa* (MTCC 2474) and *Bacillus subtilis* (MTCC 2414). Of this *Pseudomonas aeruginosa* (MTCC 2474) was found to degrade more effectively turning dark blackish solution to light brownish colour with reduction percentage of 47.60 within a period of 7 days thus reducing the pollution load of soil and aquatic life.

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