



Research Article

BIOREMEDIATION OF SUGAR WASH USING NATURAL SCAVENGERS

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ABSTRACT

Effluent emanating from distillery industries is dark colored, acidic, with high biological oxygen demand, chemical oxygen demand, and liquid consisting of biodegradable organic and inorganic constituents, which cannot be disposed directly into water bodies. Hence, the effluent released from a distillery industry was collected and analyzed for various physico-chemical characters. Due to the higher strength of the spent wash, it was diluted with the sugar effluent at 1:1 ratio (spent wash 50 ml + sugar effluent 50 ml). Thus the obtained sugar wash was biologically treated using the indigenous microorganisms. The isolated microorganisms were identified as *Enterobacter*(T1)sp., *Corynebacterium*(T2)sp., *Micrococcus*(T3)sp., *Corynebacterium*(T4)sp. Upon treatment with the isolated microorganisms the level of TDS, COD was found to be lowered along with the color removal. As a level of reduction 12.76% of TDS was found to be reduced with *Corynebacterium*(T2)sp., and 16% of COD and 9% of color removal with *Corynebacterium*(T4)sp., with a 96 hours of treatment. Consortia microbial treatment has shown 28% of color removal, 32% TDS and 35% of COD reduction at the same detention time. The results revealed that the possibility of using indigenous microorganisms especially consortia for the treatment of sugar wash.

Keywords: Physico-chemical, indigenous, sugar wash, spent wash, effluent.

INTRODUCTION

Manufacturing pollution is one of the evils at present facing in India and several efforts are being vigorously pursued to control it in various industries spanning length and breadth of the country. Untreated effluents are highly toxic to the plants, fishes or other aquatic organisms, at higher pH and the sulphide in the effluents are of environmental concern (WHO, 2000). Industries happen to be the major source of pollutants, which contaminate the ecosystem to a maximum. Wastewater from industries includes employees' sanitary waste, process wastes from industrial, waters and relatively uncontaminated water from heating and cooling operations (Glyn et al 1996). With the rapid growth of industries (sugar, paper, tannery, textile, sago, dye industries) in the country, pollution of natural water by industrial waste water has increased tremendously. Among the various industries distillery is one of the industries that

consumes huge quantities of water and consequently generate large volume of spent wash. India is the largest producer of sugar in the world. Among the effluent discharging industries, sugar cum distillery mills plays a major role in polluting the water bodies. Diverse sugar industry effluents disposed of in soil and water cause major pollution problems. The sugar industry plays an important role in the economic development of India, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial Ecosystems (Ayyasamy et al., 2008). Use of these industrial effluent and sewage sludge on agricultural land has become a common practice in India as a result of which these toxic metals can be transferred and get accumulated into plant tissues from soil. These metals have damaging effects on plants themselves and may become a health problem to man and animals. The distillery sector is one of the seventeen categories of major polluting

industries in India. These units generate large volume of dark brown coloured wastewater, which is known as "spent wash". (MoEF). Liquid wastes from breweries and distilleries possess a characteristically high pollution load and have continued to pose a critical problem of environmental pollution in many countries. The distillery effluents, when drained into a water source, make it susceptible for the propagation of harmful microbes. A typical cane molasses based distillery generates 15 L of spent wash effluent per liter of ethanol produced. Around 212 distillery units in India generate more than 30 billion liters of spent wash annually (Rajor et al., 2003). Alcohol is produced from sugarcane molasses. The molasses is fermented with yeast and alcohol is distilled from fermented wash, leaving behind a large volume of foul-smelling colored waste water, generally known as spent wash or distillery effluent. Spent wash is a strongly acidic, dark brown colored, hydrophilic viscous liquid waste with strong objectionable odor (Jain et al., 2002) dark brown color of spent wash is mainly because of the presence of brown polymeric melanoidin pigments, which are highly recalcitrant. Melanoidin pigments are formed by the non-enzymatic amino carbonyl reaction i.e. Maillard reaction (Raghukumar et al., 2001). Sugar factory effluent that has not been treated properly has an unpleasant odor when released into the environment (Radosevich et al 1997). About 10-15L of spent wash is generated for every liter of alcohol. This spent wash is characterized by disagreeable color, odor and higher BOD, COD and TDS. Some places of Indogangetic plains and Tamil Nadu, especially Trichy, was contaminated severely with brown spent wash (Vasanthi et al 2006). Due to the presence of solids especially organic matter spent wash reduce the dissolved oxygen in the receiving water bodies. Spent wash was reported to result in thermal pollution because of high temperature, which could disturb the ecological balance of flora and fauna. The high dissolved solids content of the waste makes it unsuitable for irrigation or for being discharged into the water course. The caramel color is also objectionable (Trivedy 2003). Distillery wastewater therefore needs treatment prior to disposal either on land or into water bodies.

MATERIALS AND METHODS

Physico – chemical characterization of spent wash and sugar effluent:

Spent wash and sugar effluent were collected from sugar – cum distillery industry. The Physico – chemical characteristics of spent wash, sugar effluent such as Colour, Odor, pH, Temperature, Total solids (TS), Total dissolved solids (TDS), Total suspended solids (TSS), Sodium, Potassium, Sulphate, Chloride, Nitrate, Phosphate, Dissolved oxygen (DO), Biological oxygen demand (BOD) and Chemical oxygen demand (COD) were determined using standard analytical methods (APHA, 2012).

Dilution of spent wash with sugar effluent

Most of the distilleries are located along with sugar industries. The color, BOD and COD of sugar effluent was not so high when compared to spent wash. Hence, distillery spent wash was diluted with sugar effluent to obtain 50% of dilution. The combination of 50ml sugar effluent and 50ml of spent wash was named as sugar wash.

Physico-chemical characterization of sugar wash

The various characteristics of sugar wash such as, Colour, Odour, Temperature, pH, TS, TDS, TSS, DO, BOD, COD, Nitrate, Chloride, Sodium, Potassium, Phosphate and Sulphate were estimated as per APHA (2012).

Effect of microbial treatment on sugar wash

Isolation and identification of bacterial strains from the sugar wash

Isolation and identification of microbial strains from the sugar wash were done using the pour plate technique (Atlas et al. 1995). The bacterial strains isolated from the effluent were screened for color, TDS and COD removal capability by growing them in nutrient broth medium supplemented with different concentration of sugar wash.

Effect of microbes on the color, TDS and COD removal from the sugar wash

Accurately 100ml of sugar wash was taken into five sterile flasks. To the each flask, 5ml of appropriate inoculums of selected different types of bacterial culture was added and to the fifth flask, 5ml of microbial consortia was added aseptically and were incubated at 32+ 2°C. Flasks were taken out at different time intervals and the solutions were

filtered through Whatman No. 1 filter paper. The percent removal of color, TDS and COD was estimated.

RESULTS AND DISCUSSION

Physico-chemical characterization of spent wash and sugar effluent

The color and odor of the spent wash were very dark brown and objectionable respectively. The pH of the sample was of acidic range and the temperature was about 80°C. The DO of the spent wash was nil. Hence, the BOD and COD of spent wash were found to be 74,909 mg/L and 1,32,367 mg/L respectively. The TDS of spent wash was 99,100 mg/L. The amounts of chloride, nitrate, phosphate, sulphate and potassium were 7541mg/L, 2950 mg/L, 256 mg/L, 8112 mg/L and 10,800 mg/L respectively.

Inthorn et al. (2001) have been reported that the color was due to the presence of melanoidin pigments in the spent wash and these melanoidin pigments have been reported as natural polymers, highly resistant to biodegradation. Most malodour of an inorganic nature has been reported to arise from the anaerobic decomposition of compounds containing nitrogen and sulphur, such as ammonia, Hydrogen sulphide, mercaptans, amines, aldehydes, ketones, indole and skatole (Hwang et al. 1994).

The distillery spent wash is hot and highly acidic due to the presence of organic acids too. Binkly and Wolfrom (1953) have reported that 10% of acids were produced during the fermentation of molasses. This may be the reason for the acidic pH. And the total solids, total dissolved solids of the spent wash were so high, which may be the reason for higher BOD and COD. The colloidal nature of caramels and melanoidin also increase the amount of total solids, BOD and COD (Saxena and Rai 2000). Similar results have been reported by , Kasturibai and Ganga (1996), Srivastava and Pathak (1998). The higher values of TDS, BOD, COD and chloride proves the toxicity of the effluent and this effluent cannot be discharged into inland surface water, public sewers or as such for irrigational purpose because the values are not within the tolerance limit. Hence, the spent wash must be subjected to treatment prior to disposal.

The characterization of the sugar effluent was made (Table-2). The color and odor of the sugar effluent were pale yellow and sweet smell respectively. The pH of the sugar effluent was 6.5. The total solids, total dissolved solids, DO,

BOD and COD of sugar effluent were 3,500 mg/L, 3,100 mg/L, 0.7 mg/L, 5,040 mg/L and 6,200mg/L respectively.

Dilution of spent wash with sugar effluent

Many distilleries are located along with sugar industries. The color, higher BOD and COD of spent wash render them very difficult to be treated. Hence, the dilution of spent wash is utmost necessary. The physico – chemical characteristics such as colour, TDS, BOD and COD of sugar effluent was not so high when compared with spent wash. Reduction of rainfall and ground water resources has resulted in water crisis problem. Hence, sugar effluent was thought to be suitable diluents for the dilution of spent wash.

Effect of microbes on the colour, TDS and COD removal from the sugar wash

The treatment efficiency with micro-organisms was found to increase with time. The selected individual indigenous organisms were *Enterobacter* sp (T1), *Corynebacterium* sp(T2), *Micrococcus* sp(T3), and *Corynebacterium* sp (T4).

Table 4: Identification of heterotrophic bacteria

Similarly Indhurani (2005) has reported that 0%,50%,73% of color, TDS and COD removal were noticed with 5% diluted spent wash using the consortia which comprises sp of *Proteus* and *Enterobacter*. Normally the removal of toxicants such as heavy metals by micro –organisms has been attributed to various mechanisms such as binding to the cell surface and Intra cellular uptake (Stand berg et al. 1981). Kapdan et al ., (2000) have stated the removing dyes in aerobic conditions was mainly achieved by adsorbing the dyes on bacteria. During the degradation activated oxygen was produced that could carry out the initial attack on the stable structure of the dye (Hu, 2001).

Certain microorganisms have been reported to detoxify the xenobiotics through a process called co – metabolism in which the organic pollutant is detoxified without utilizing as a nutrient or energy sources. Whereas certain organisms are capable of removing the unwanted substances through mineralization process due to their genetic stability, enzyme activity, growth in the unfavorable environment and competitiveness (Gunasekaran, 1990).In the present investigation the efficiency of the consortia is higher than that of individual organisms. It may be due to their synergistic effects. Similar result has been recorded by Indhurani (2005). Also Asthana et al.(2001) have reported that color

Table 1: Physico-chemical characterization of spent wash

S. No	Parameters	Spent Wash
1	Colour	Dark brown
2	Odor	Objectionable
3	Temperature	80°C
4	pH	4.4
5	TS	1,05,510
6	TDS	99,100
7	TSS	6,410
8	DO	-
9	BOD	74,909
10	COD	1,32,367
11	Chloride	7,541
12	Nitrate	2,950
13	Phosphate	256
14	Potassium	10,800
15	Sodium	280
16	Sulphate	8,112

Table2: Physico-chemical characterization of sugar effluent

S. No	Parameters	Sugar Effluent
1	Colour	Pale Yellow
2	Odor	Sweet yellow
3	Temperature	45°C
4	pH	6.1
5	TS	3500
6	TDS	3100
7	TSS	400
8	DO	0.7
9	BOD	5,040
10	COD	6,200
11	Chloride	109
12	Nitrate	115
13	Phosphate	184
14	Potassium	695
15	Sodium	472
16	Sulphate	185

Table3: Physico-chemical characterization of sugar Wash

S.No	Parameters	Sugar Wash (50%)
1	Colour	Dark brown
2	Odour	Objectionable
3	pH	5.2
4	TS	58,020
5	TDS	57,160
6	TSS	860
7	DO	-
8	BOD	38,040
9	COD	69,558
10	Chloride	3,823
11	Nitrate	1,566
12	Phosphate	230
13	Potassium	6,134
14	Sodium	375
15	Sulphate	4100

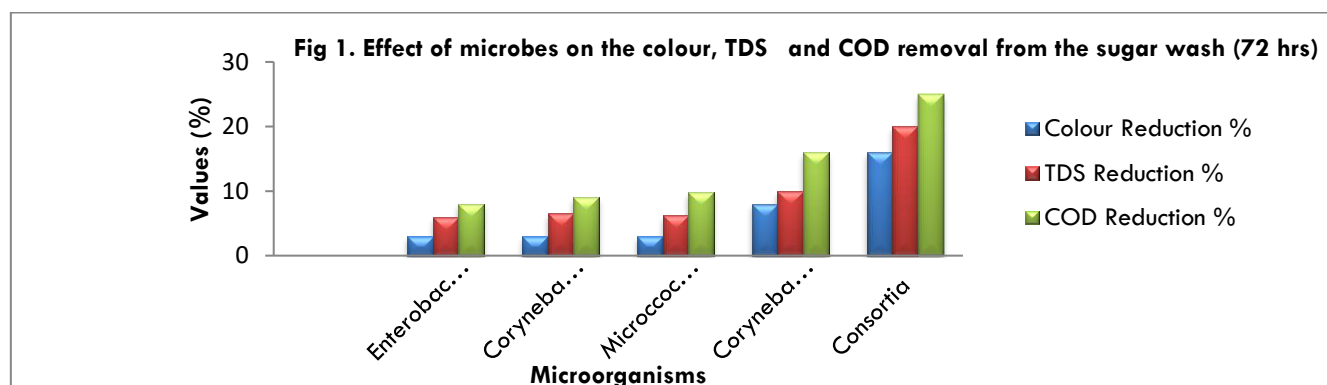


Table 4: Identification of heterotrophic bacteria

Characteristics	Name of the Genus			
	<i>Enterobacter</i> (T1) sp.	<i>Corynebacterium</i> (T2) sp.	<i>Micrococcus</i> (T3) sp.	<i>Corynebacterium</i> (T4) sp.
Shape	Rod	Rod	Cocci	Rod
Gram Staining	-	+	+	+
Motility	+	-	-	-
Spore	-	-	-	-
Oxidise	-	+	+	+
Urease	-	+	+	+
Indole	-	-	-	-
Citrate	+	+	+	+
Methyl Red	-	+	+	+
H ₂ S	-	-	-	-
Voges Proskaur	+	-	-	-

Table 5: Effect of microbes on the colour, TDS and COD Removal from the sugar washes (96 hrs)

S.No	Microorganism	Colour Removal	TDS	COD
		%	Reduction %	Reduction %
1	<i>Enterobacter</i> (T1) sp.	6	9	13
2	<i>Corynebacterium</i> (T2) sp.	8	12.76	14
3.	<i>Micrococcus</i> (T3) sp.	6	9.2	13
4.	<i>Corynebacterium</i> (T4) sp.	9	12	16
5.	Consortia	28	32	35

Table 6: Effect of microbes on the colour, TDS and COD removal from the sugar wash (72 hrs)

S.No	Microorganism	Colour Removal	TDS	COD
		%	Reduction %	Reduction %
1	<i>Enterobacter</i> (T1) sp.	6	9	13
2	<i>Corynebacterium</i> (T2) sp.	8	12.76	14
3.	<i>Micrococcus</i> (T3) sp.	6	9.2	13
4.	<i>Corynebacterium</i> (T4) sp.	9	12	16
5.	Consortia	28	32	35

removal efficiencies were 66.67% 63.9% and 75% for TA2, TA4 and mixed consortia respectively after a detention time of 72hr. The BOD removal efficiencies of the same sps were found to be 70%, 61% and 80% respectively after the retention time period of 48 hrs of incubation at 37°C temperature from the anaerobically treated spent wash.

From the investigation, it is clear that, the dilution of spent wash with sugar effluent can reduce the pollution strength and there is possibility for the treatment of raw sugar wash using low cost, eco-friendly, bioremediation techniques.

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