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Characterization Of Molasses Spentwash Collected From United Spirits Ltd., Aleppey, India: A Preliminary Report

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ABSTRACT

Industries happen to be the major source of pollutants to the ecosystem. Techniques such as distillation and fermentation were known to human civilization since ages. Distilling industries are those concerned with the production of ethanol and distilled spirits such as rum, whisky, brandy, gin, cordials and liquors. Apart from other industries distillery is one of the industries which produces wastes characterized by high organic matter, disagreeable colour and odour. In fact alcohol distilleries are listed at the top in the "Red Category" industries as per the Ministry of Environment and Forests (MoEF) due to their high polluting potential. There are more than 319 distilleries producing 3.25 x 10^9 litres of alcohol and generating 40.4 x 10^{10} litres of wastewater annually in India alone. For the present study, digested molasses spent wash (DMSW) was collected from United Spirits Ltd. Aleppey, India after the biomethanation step in an anaerobic digester plant. Physical, chemical and biological characterization of the effluent was performed as per standard methods APHA, 1995. Effluent had a dark black brown colour, highly acidic with a very low pH (3.5-4.0),with electrical conductivity 24,500 units, total solids 13,600 mg/l, dissolved oxygen nil, Chloride 4,165mg/l, Nitrate 10mg/l, BOD 65,000 and COD 1, 45,000 mg/l.

Key Words: Characterization, Molasses Spent wash, Industrial Pollution, United Spirits LTD., Distillery, Biological Oxygen Demand and Chemical Oxygen Demand.

INTRODUCTION

Today industries are the major source of pollutants to the ecosystem. Distilling industries are those concerned with the production of ethanol and distilled spirits such as rum, whisky, brandy, gin, cordials and liquors. Now a day's alcohol distilleries have emerged as a prominent sector worldwide due to the large-scale industrial applications of alcohol in pharmaceuticals, food, perfumery etc. Moreover alcohol is used as an alternate fuel. There are more than 319 distilleries producing 3.25 x 10^9 l of alcohol and generating 40.4 x 10^{10} l of wastewater annually in India alone (Pant and Adholeya, 2007).Apart from other industries distillery is one of the industries which produces wastes characterized by high organic matter, disagreeable colour and odour. In fact alcohol distilleries are listed at the top in the "Red Category" industries as per the Ministry of Environment and Forests (MoEF) due to their high polluting potential (Tewari *et al.*, 2007).

The residue of the fermented mash which comes out as liquid waste is generally known by the term spent wash (Pathade 2003; Chandraraj and Gunasekharan 2004; Singh *et al.,* 2004). Spent wash is a very complex, caramelized and cumbersome recalcitrant agro-industrial waste with a temperature range of 70-80^{0}C at discharge point, deep black brown colour, low pH, high concentration of organic materials and solids. However the distillery effluent pollution load largely depends on the quality of molasses, unit operations for processing the molasses and process recovery of alcohols respectively (Pandey *et al.*,2003). The distillery effluent is characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), phenol compounds, sulphate and heavy metals (Pant and Adholeya, 2007).

For the present study, digested molasses spent wash (DMSW) was collected from United Spirits Ltd. Aleppey, India after the biomethanation step in an anaerobic digester plant. Physical, chemical and biological characterization of the effluent was performed as per standard methods (APHA, 1995). Parameters analyzed were pH, electrical conductivity, TDS, D O, chlorides, nitrates, Biological Oxygen Demand (BOD 3 days), Chemical Oxygen Demand (COD) and reducing sugars.

MATERIALS AND METHODS

Characterization of molasses spent wash

pH

pH of the spent wash was determined by pH meter.

Electrical Conductivity

Electrical conductivity was measured by conductivity cell potentiometer method.

Total solids

Total dissolved solids were estimated by Gravimetric after filtration. A known volume of spent wash was transferred to a pre-weighed evaporating dish, evaporated to dryness in an oven at 104 ± 1^{0}C, till a constant weight was achieved. The dried sample was cooled in desiccators and weighed again.

Dissolved oxygen

The dissolved oxygen content was estimated by following the Winkler's Azide modification titrimetric method. To about 300 ml of sample, one ml $MnSO_4$ solution followed by one ml alkali-iodide reagent were added, stoppered carefully to exclude air bubbles and mixed thoroughly. When the precipitate was settled sufficiently to leave a clear supernatant above the manganese hydroxide floc, one ml of concentrated H_2SO_4 was added. It was restoppered and mixed by inverting several times until dissolution was complete. This was titrated with 0.025 M $Na_2S_2O_3$ solution to a pale straw color. Few drops of starch solution were added and titration was continued till the first disappearance of blue colour. Volume of $Na_2S_2O_3$ used was noted for D.O determination.

Chlorides

Total chlorides were estimated by the Argentometric titration method. Spent wash sample 0.1ml (diluted 100 times) was mixed with 3ml aluminium hydroxide suspension and the solution was allowed to settle. To the filtrate, 1ml potassium chromate indicator solution was added and titrated with silver nitrate titrant to a pinkish yellow end point.

Nitrates

Total nitrogen was determined by following microkjeldahl method. The sample was digested with concentrated sulphuric acid and then distilled with excess of 40 per cent sodium hydroxide. Ammonia liberated was trapped in four per cent boric acid. Nitrogen content was determined by back titration against sulphuric acid using mixed indicator.

Biochemical Oxygen Demand (B.O.D)

Biological Oxygen Demand was estimated by Bottle Incubation Method: For 3-Days at 27°C. Spent wash sample 0.1ml (diluted 100 times) was filled to overflowing in an air tight B.O.D bottle of 300 ml and incubated at 27^0 C for three days in a B.O.D incubator. Dissolved oxygen was measured initially and after incubation, and the B.O.D was computed from the difference between initial and final D.O.[9] Controls were also included.

Chemical Oxygen Demand (C.O.D)

Chemical oxygen demand was estimated by following the "Open reflux method"**.** To the sample aliquots (0.1 to 1ml) suitably diluted to 50mL with distilled water in a 500mL refluxing flask, 1g $HgSO_4$, few glass beads, and 5mL sulphuric acid reagent were added, mixed and cooled. To this 25mL of 0.0417M $K_2Cr_2O_7$ solution was added and mixed. The flask was connected to the condenser for refluxing. Additional 70mL sulphuric acid reagent was added to this through the open end of condenser, with swirling and mixing.

The whole mixture was refluxed for 2 hours; cooled and washed down the condenser with distilled water to double the volume of contents and cooled again. After digestion, the remaining unreduced potassium dichromate was titrated with ferrous ammonium sulphate using ferroin indicator, until a colour change from bluish green to reddish brown was observed. Also refluxed and titrated a distilled water blank with reagents only.

Estimation of Reducing Sugars

Reducing sugars in spent wash was estimated by DNS method**.** The sample (0.1ml) was made up to 2ml with distilled water, to which 3ml Dinitro Salicylic acid reagent was added and boiled for 5 minutes in a boiling water bath, cooled and made up to 10ml with distilled water. Absorbance was read at 540 nm against blank. Reducing sugar (mg) present per ml of sample was calculated from the standard glucose graph.

RESULT AND DISCUSSION

Characterization of Molasses Spent Wash

The effluent was characterized as per the standard methods APHA, 1995. Effluent had a dark black brown colour, highly acidic with a very low pH (3.5-4.0) with electrical conductivity 24,500, total solids 13,600 mg/l, dissolved oxygen nil, Chloride 4,165mg/l, Nitrate 10mg/l, BOD 65,000 and COD 1, 45,000 mg/l. Table-1.

Table 1: Characterization of Molasses Spent Wash

No	Effluent Sample	Results
1	Colour and appearance	Black
2	pH	3.5
3	Conductivity	24,500 units
4	Total solids	13,600 mg/l
5	Dissolved oxygen	Nil
6	Chlorides	4165 mg/l
7	Nitrates	10 mg/l
8	Biological Oxygen Demand	65,000 mg/l
9	Chemical Oxygen Demand	1,45,000 mg/l

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MSW is one of the most difficult waste products to dispose because of its low pH, high temperature, dark brown colour, high ash content and high percentage of dissolved organic and inorganic matter. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD), the index of its polluting character, typically ranged between (35,000-50,000 mg/l) and (100,000-150,000 mg/l), respectively (Beltran et *al.*, 1999). Similarly Nandy *et al.*, (2002) reported that the spent wash was acidic (pH 3.94-4.30), dark brown liquid with high BOD (45000-100000 mg/l) and COD (90000-210000 mg/l)and emits obnoxious odour.

The studies conducted by Pandey *et al.*, (2003) reported that the anaerobically digested spent wash before treatment had a greenish dark brown color, burnt sugar like odour, pH 7.2, BOD (5000-6500 mg/l), COD (34,800 mg/l), total dissolved solids

(4500-4620 mg/l), sulphates (160 mg/l), potassium (850 mg/l), free chlorine (800 mg/l) etc.

According to R. Ravikumar *et al.*, (2011), raw distillery effluent had a reddish color, total Solids (4285±.005 mg/l), total dissolved solids (3980 mg/l), total suspended solids (255 mg /l), pH (6.4), total alkalinity (1437.5 mg/l), total hardness (565 mg/l),calcium hardness (455 mg/l), calcium (182.2 mg/l), chloride (860.87 mg/l), BOD (544.5 mg/l),COD (2433 mg/l), sulphates (160 mg/l), potassium (850 mg/l) and free chlorine (800 m/l).[12] Similarly according to Murthy *et al* (2009), the spent wash had a dark brown color,total suspended solids (25040 mg/l), total dissolved solids (38140 mg/l), pH (4.0), chloride (59981 mg/l)' BOD (41946 mg/l), COD (125,000 mg/l), sulphates (25920 mg/l), potassium (4360 mg/l).

Whereas Dikshit *et al.*, (2006), reported that the raw effluent had a very high organic content. BOD is generally of the order of (40, 000-50,000 ppm) and the COD is of the order of **(**80,000-1, 00,000 ppm).The wastewater of distillery had dark brown colour, mainly due to the presence of caramelized sugar, which was produced in the processes employed in extracting maximum quantity of sugar by the sugar mills.

According to Chavan *et al.*, (2006), the distillery effluent had a dark brown color, sweet odour, total sugars (13,4,000-85,000 mg/l), total dissolved solids (8900 mg/l), pH (2-4.0), free chlorine (58 40mg/l)' BOD (70,840 mg/l), COD (1,46,380 mg/l), sulphates (1100 mg/l), phosphorous (5100 mg/l), magnesium (2160 mg/l), Iron (140 mg/l) and Oil and grease (196.5 mg/l).

Studies done by Piyush Malaviya *et al.*, (2011), observed that the distillery waste water was light brown in colour with unpleasant odour. The average values of various physicochemical characteristics of the 100% effluent exhibited high chemical oxygen demand COD 2496 mgL^{-1}, chloride concentration (799.7 mg/l)' total dissolved solids (1408 mg/l) and osmotic pressure (Op), 0.79 atmospheres.Different concentrations of the effluent showed increasing trend of electrical conductivity (EC), Op and TDS with increasing concentration of the effluent. Minimum values of EC 0.8 mS cm-1, Op 0.29 atmosphere and TDS 512 mgL^{-1} were observed in 20% effluent concentration (E20), while maximum values were detected in 100% effluent concentration (E100) i.e. 2.2 mS cm-1 EC, 0.79 atmosphere Op and 1408 mg/l TDS. There was a decreasing trend of pH with increasing concentrations of the effluent with highest pH (6.93) in 20% effluent and lowest (5.53) in 100% effluent concentration.

According to S. Mohana *et al.*, (2007), the distillery effluent was characterized by very low pH (3.0-4.5), total solids (110,000-190,000 mg/l), total dissolved solids (90,000-150,000 mg/l), total volatile solids (80,000-120,000 mg/l),total suspended solids (13,000-15,000mg/l), Chlorides (8000-8500 mg/l), BOD (50,000-60,000 mg/l), COD (1,10,000-190,000 mg/l), sulphates (7500-9000 mg/l), phosphate (2500-2700 mg/l), total nitrogen (5000-7000mg/l),phenols (8000-10,000 mg/l).

With respect to the studies conducted by Chandralata Raghukumar *et al.*, (2001), the raw MSW was obtained from a local distillery unit manufacturing alcohol from sugarcane molasses. The raw MSW was dark brown in color and according to the data provided by the distillery, had a pH of 4.3 and contained B.O.D of 42,000 mg/l and C.O.D of 80,000 mg/l.

The untreated effluent was characterized by dark colour, high temperature, and low pH, high ash content and high percentage of dissolved organic and inorganic matter. The conventionally treated distillery effluent still contains chemical oxygen demand\ (COD) around (25-30,000 mg/l) and was dark brown in color (Deepak Pant & Alok Adholeya, 2010).

Distillery wastewater is the major source of soil and water pollution due to high BOD (23,000 mg/l), COD (47,400 mg/l), TDS (10,480 mg/l), phenolics (510 mg/l), sulfate (3,786 mg/l) and phosphate (739.0 mg/l) (Bharagava *et al.,* 2008). These values are much higher than the permissible limit 40, 120, 2,100, 0.5, 750, and 1.0 mgL^1 respectively (Ram Chandra and Singh S. K. 2010).

SUMMARY AND CONCLUSION

Wastewaters coming from distilleries contain high concentration of organic and inorganic substances causing significant polluting phenomena. In order to sustain our global water supply, many environmental operation programs have been established to address pollution issues. Numerous environmental directives, regulations and legislations have been issued in order to define quality standards for water. The high chemical oxygen demand, biological oxygen demand, suspended solids, conductivity, salinity and total dissolved solids still pose serious economic issues for the industries since these have been employed as major parameters for effluent discharge. In order to abide by the stringent government policies on pollution control, the industries have now been forced to look for more effective treatment technologies which would not only be environment friendly but also cost effective. Due to high cost and generation of secondary pollutants, treatment of waste waters by physical or chemical methods was found not feasible. Consequently, the wastewater needs to undergo extensive treatment in order to meet the stipulated environmental demands. But treating the effluent, up to the standards prescribed by the respective regulatory authorities, still remains a challenge today.

REFERENCES

1. **APHA.** (1995**).** Standard methods for the examination of water and waste water. 19th ed. Washington, DC **(**8 pages).
2. .**Beltrán, P.,** Delgado, G., Navarro, A., Trujillo, F., Selander, R. K., Cravioto, A. (1999). Genetic diversity and population structure of *Vibrio Cholerae. Journal of Clinical Microbiology,* 37, 581-590.
3. **Bharagava, R.N.,** Chandra, R. (2010). Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites. *Biodegradation,* 21, 703-711.
4. **Chandralatha, Raghukumar., and Gauri, Rivonkar.** (2001). Decolorization of molasses spent wash by the white rot fungus *Flavadon flavus* isolated from a marine habitat. *Applied Microbiology and Biotechnology,* Volume 55, 510-514.

5. **Chandraraj, K.,** Gunasekaran, P. (2004). Bacterial alcoholic fermentation, in: Pandey A. (Ed.), Concise Encyclopedia of Bioresource Technology, Food Products Press, New York, USA. 327-333.
6. **Chavan, M.N.,** Kulkarani, M.V., Zope, V.P., Mahulikar, P.P. (2006). Microbial degradation of melanoidins in distillery spent wash by an indigenous isolate. *Indian J. Biotech,* 5, 416-421.
7. **Deepak, Pant.,** and Alok, Adholeya. (2010).Development of a novel fungal consortium for the treatment of molasses distillery waste water. *Environmentalist,* 30,178-182
8. **Dikshit, A.K.,** and Dhiman, Chakraborty. (2006). A techno-economic feasibility study on removal of persistent colour and COD from anaerobically digested distillery effluent: a case study from India. *Clean technologies and Environmental Policy,* volume 8, 273-285.
9. **Mohana, S.,** Desai, C., Madamwar, D. (2007). Biodegradation and decolourisation of anaerobically treated distillery spent wash by a novel bacterial consortium. *Biores. Technol,* 98, 333-399.
10. **Murthy, Z.V.P.,** Chaudhari, L.B. (2009).Treatment of distillery spent wash by combined UF and RO processes, *Global NEST J,* 11, 235-240.
11. **Nandy, T.,** Shastry, S., Kaul, S.N. (2002). Wastewater management in cane molasses distillery involving bioresource recovery. *Journal of Environmental Management,* 65 (1), 25-38.
12. **Pandey, R.A.,** Malhotra, A., Tankhiwale, S., Pande, S., Pathe, P.P., Kaul, S.N. (2003). Treatment of biologically treated distillery effluent—a case study. *Int. J. Environ.Study,* 60, 263-275.
13. **Pant, D.,** Adholeya. (2007). A Biological approaches for treatment of distillery wastewater: a review. *Bioresour Technol.* 98, 2321-2334. doi:10.1016/j.biortech.2006.09.027.
14. **Pathade, G.R.** (2003). A review of current technologies for distillery wastewater treatment, in: Goel P.K. (Ed.), Advances in Industrial Wastewater Treatment, ABD Publishers, Jaipur, India. 180-239.
15. **Piyush, Malaviya.,** and Anuradha, Sharma. (2011). Effect of distillery effluent on yield attributes of *Brassica napus* L. *Journal of Environ. Biol,* 32, 385-389.
16. **Ram Chandra.,** and Singh, S. K. (2010). Metabolic characterization by 1HNMR and mass spectrophotometric method from melanoidin degraded anaerobically treated distillery effluent by using isolated aerobic bacterial consortium. *Abstracts Presented in Association of Microbiologists of India Conference, Birla Institute of Scientific Research,* Jaipur, November 25-27, pp. 166-167.
17. **Ravikumar, R.,** Vasanthi, N.S., Saravanan, K. (2011). Single factorial experimental design for decolorizing anaerobically treated distillery spent wash using *cladosporium cladosporioides. Int. J. Environ. Sci. Technol,* 8, 97-106

18. **Singh, P.N.,** Robinson, T., Singh, D. (2004). Treatment of industrial effluents—distillery effluent, in: Pandey A. (Ed.), Concise Encyclopedia of Bioresource Technology, Food Products Press, New York, USA. 135-141.
19. **Tewari, P.K.,** Batra, V.S., Balakrishnan, M. (2007). Water management initiatives in sugarcane molasses based distilleries in India. *Res. Conserv. Recycl*, 52, 351-367.