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BIOLOGICAL TREATMENT OF DISTILLERY WASTE WATER - AN OVERVIEW

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ABSTRACT

In whole world, cane molasses base distilleries are included under one of the polluting industries in concern to water pollution. After fermentation remains waste from bottom of distillation columns, termed stillage. This highly aqueous residue containing organic soluble is considered a troublesome and potentially polluting waste due to its extremely high BOD and COD values. The typical odour emanating from distilleries is a major nuisance. The color of the spent wash interferes with its oxygenation and self purification. The treatment of distillery wastes is a priority area for environmental sustenance and its quality. Due to the large volumes of effluents and presence of certain recalcitrant compounds the treatment of this stream is rather challenging by conventional methods. Therefore to supplement the existing treatments, a number of studies encompassing physic-chemical and biological treatments have been conducted. This review presents an account of the problem, biological treatment methods and role of enzymes in decolorizing waste water.

KEYWORDS: *Spent Wash, Decolourization, BOD, COD, Enzymes*

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INTRODUCTION

The waste water (effluent) generated from distillery is of two types viz. process waste water and non-process waste water. The non-process waste water is comparatively pure and as such can be recycled. The process waste waters of distillery consist of fermenter sludge, spent less and spent wash. Spent less is usually recycled. Fermenter sludge has higher biochemical oxygen demand (BOD) and lower volume as compared to spent wash. It is advisable to dewater fermenter sludge and dispose it off without mixing it with spent wash as it will increase the BOD of receiving stream. In India there are a number of large-scale distilleries integrated with sugar mills. The waste products from sugar mill comprise bagasse (residue from the sugarcane crushing), pressmud (mud and dirt residue from juice clarification) and molasses (final residue from sugar crystallization section), Bagasse is used in paper manufacturing and as fuel in boilers; molasses as raw material in distillery for alcohol production while pressmud have no direct industrial application [1]. The Indian distillery industry produces alcohol from molasses, a by- product of sugar manufacture. During 1990-91, India produced about 1,025 million

liters of alcohol; India produced per liter of alcohol, about 15,375 million liters of distillery effluent during 1990-91.The effluent foul smells, dark brown colored and has a very high BOD.

Characteristics of Distillery Waste Water

Generally the effluents from molasses based distilleries contain large amounts of dark brown coloured molasses spent wash (MSW). Because of its acidic pH, dark brown colour, High ash content, high percentage of dissolved organic and inorganic matter, MSW is one of the most difficult waste products to dispose. The biochemical Oxygen demand (BOD) and chemical oxygen demand (COD) of MSW range between 35,000– 50,000 and 100,000–150,000 mg L-1, respectively. Various studies conducted in South Africa, the COD of wine distillery wastewater ranged from 20 g/L to 30 g/L (18), while most of the studies on distillery wastewater reported concentration of COD range between 22 and 48 g/L. Distillery wastewaters contain phenolics compounds, mainly gallic acid, *p*-coumaric acid and gentisic acid, which impart high Antibacterial activity. Organic acids such as lactic acid (29% v/v), tartaric acid (27% v/v), succinic acid (26% v/v), acetic acid (10% v/v) and malic acid (8% v/v) also documented in distillery waste water. Apart from this distillery waste water also contains soluble proteins.

Table 1: Chemical Characteristics of Waste Water

Parameter	Distillery Waste Water	Vinasse	Raw Spent Wash	Molasses Water
pH	3.0-4.1	4.4-4.2	5.2	3.8
Alkalinity (meq/l)	---	-	2	6000
EC ($S.cm^{-1}$)				
Phenol (mg/l) -	346	-	2530	--
VFAs (g/l)	1.6	477	-	450
CODT (g/l)	100-120	-	37.5	80.5
CODS (g/l)	-	97.5	----	-
BOD5 (g/l)	30	42.23	--	-
TOC(mg/l)	-	36.28	--	-
VS (g/l)	50	-	-	79
VSS (g/l)	2.8	-	-	2.5
TS (g/l)	51.5-100	3.9	2.82	109
TSS (g/l)	-	-	-	-
MS (g/l)	-	-	-	30
MSS (mg/l)	-	100	-	1100
TN (g/l)	-	-	2.02	1.8
NH4+ (mg/l)	-	-	125400	-
NO3- (mg/l)	4900	-	-	-
TP (g/l)	-	-	0.24	-
$PO4^{--3}$(mg/l)	-	-	139	-

Parameters such as the pH, alkalinity, electrical conductivity (EC), total chemical oxygen demand (COD), soluble chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD), total organic carbon (TOC), phenol, volatile fatty acids (VFA), volatile solids (VS), volatile suspended solids (VSS), total solids (TS), total suspended solids (TSS), mixed solids (MS), mixed suspended solids (MSS), total nitrogen (TN), ammonia (NH4+), nitrates (NO3-), total phosphorus (TP) and phosphates (PO43-) are reported. In general, distillery wastewaters are acidic, have a brown colour and have a high content of organic substances that varies according to the raw material distilled [2, 4, 5, 6, 7]. The average [3] values for COD are 7 to 40 g/l and for BOD5 5.5 to 20 g/l [8, 9, 10]. In other examples, the concentration of organic substances is very high, ranging from 20 to 150 g/l COD [11, 12, 13.14,15,16]. In studies conducted in South Africa, the COD (whether soluble or total was not specified) of wine distillery wastewater ranged from 20 g/l to 30 g/l [17] while

Driessen *et al*. (1994) [18] reported COD between 22 and 48 g/l (Table.1).

Pollution and Toxicity of Distillery Effluent

Rapid growth of distilleries in India resulted into substantial increase in industrial pollutant load. There are 254 distilleries in India producing 1000 million liters of alcohol and 3.5x 10^8 kiloliters of effluent each year [19]. The industrial wastes generated by various distillery units are posing serious threat to the adjoining aquatic and terrestrial habitats due to practice of discharging them into nearby waste water courses and lands [20]. The distillery effluents have high BOD, COD, phenols & heavy metals [21]. The colour of the effluent persists even after the anaerobic treatment and poses a serious threat to environment. The water bodies receiving colour wastes got colored and affect the penetration of light in aquatic ecosystems, which in turn affect the aquatic life [22]. Therefore, it is essential to reduce the toxic level of various pollutants in the distillery effluent before discharging them into nearby watercourses or lands.

TREATMENT METHODS OF DISTILLERY WASTE WATER

A new strategy is required involving novel materials; methods and process integration options/technology for waste water treatment. There are several different methods for treatment of distillery effluent. They are as follows:

- Physico-Chemical Treatment Methods.
- Biological Treatment Methods.
  - Aerobic Treatment.
  - Anaerobic Treatment.
  - Enzymatic Treatment.

Physico-Chemical Treatment Methods

Coagulation: Reduction of repulsive forces through addition of coagulant.

Flocculation: Physical process by which particle contact and agglomeration occurs.

Ion Exchange: To separate ionized molecules (organic as well as inorganic) from aqueous solution as well as contaminants in organic streams.

Hydrodynamic Cavitation Technology: Cavitation is the formation, growth and collapse of cavities/bubbles, releasing large amount of energy & generating oxidizing agents in waste water.

Membrane Technology: The effluent collected from the distillery industry is highly acidic with P^H range of around 3. Hence, it is neutralized using sodium hydroxide. The neutralized solution has a lot of suspended solids, so the filtration is carried out to remove the suspended solids with fine-pore thin cloth or by using some membranes.

Therefore, environmental biotechnology today is dominated by attempts to find ways of dealing with growing industrialization and the problems it causes, such as production of toxic wastewaters. Amongst solutions being attempted, bioremediation is the most popular [23]. Bioremediation encompasses all processes that occur in order to transform the environment altered by contaminants back to its original state [23]. The exact processes that can be used to achieve the desired outcomes differ, but they all have the same principle: to use microorganisms and then enzymes they produce to remove contaminants. Therefore bioremediation and waste treatment technologies are gaining momentum [23].

Biological Treatment Methods

Biological treatments have been recognized as effective methods of treatment for highly polluted industrial wastewaters. Both anaerobic and aerobic systems are commonly used to treat the waste waters from agro-industrial plants.

Anaerobic Treatment

Application of anaerobic digestion to distillery effluents is a preferable primary treatment option. Since aerobic processes have higher nutrient requirements and cause operational difficulties in treating high organic strength wastewaters, employing these methods in primary treatment of stillage would result in lower cost-efficiency. Most of the anaerobic technologies applied so far in the treatment of high organic strength wastewaters-municipal and originated from other industry branches-were employed for effluents from ethanol manufacture, achieving high levels of pollutants decay. R.Tomczak-Wandzel, et al., [24] reported that anaerobic treatment of brewery waste water in UASB system could be good method of organic, easily biodegradable wastewater utilization. The achieved efficiency of COD removal was satisfactory; it reached over 95%.During the experiment the properties of anaerobic granular sludge was also controlled. Goodwin and Stuart (1994) [25] studied two identical UASB reactors operated in parallel as duplicates for 327 days for the treatment of malt whisky pot ale and achieved COD reductions of up to 90% for influent concentrations of 3526-52129mg L^{-1}. When the OLR s of 15kg m^{-3} day and above were used, the COD removal efficiency dropped to less than 20% in one of the duplicate reactors. A mesophilic two-stage system consisting of an anaerobic filter (AF) and an UASB reactor was found suitable for anaerobic digestion of distillery waste, enabling better conditions for the methanogenic phase [26].

Aerobic Treatment: The post anaerobic treatment stage still has high organic loading and is high dark brown in colour, hence it is generally followed by a secondary, aerobic treatment. These are some aerobic treatment methods:

Aquaculture: The post methanated effluent has been used for pisciculture near Chennai city in southern India. The BOD is reduced to nearly zero and the initiative yields about 50 tons per hectare per year of fish. [27]

Constructed Wetlands: Billore et al., (2001**)** [27] have demonstrated that the post-anaerobic treated effluent had a BOD of about 2500 mg/l and a COD of nearly 14,000 mg/l. A pre-treatment chamber filled with gravel was used to capture the suspended solids. All the cells were filled with gravel up to varying heights and cells three and four supported the plants *Typha latipholia* and *Phragmites karka* respectively. The overall retention time was 14.4 d and the treatment resulted in 64% COD, 85% BOD, 42% total solids and 79% phosphorus content reduction. In another study, a laboratory scale CW employing T. latipholia was used to treat diluted distillery effluent [28]. A root zone of 1.5_0.3_0.3 m, filled with 75% sand and gravel and 25% soil was used and the diluted effluent was applied after 4 weeks of planting. The system resulted in 76% COD reduction in 7 d which increased marginally to 78% COD reduction in 10 d. The BOD reduction was 22% and 47% on days 7 and 10, respectively.

Biocomposting: Biocomposting is an aerobic, thermophilic process resulting in a product rich in humus which is thus used as a fertilizer. This is a popular option adopted by several Indian distilleries attached to sugar mills with adequate land availability. The spent wash, either directly, or after biomethanation is sprayed in a controlled manner on sugarcane pressmud. The latter is the filter cake obtained during juice clarification in the manufacture of sugar. Biocomposting is an aerobic, thermophilic process resulting in a product rich in humus which is thus used as a fertilizer. To enhance the efficiency of aerobic systems, several workers have focused on treatment by pure cultures. Further, aerobic treatment has also been examined as a precursor to anaerobic treatment. In studies on both beet spent wash and molasses, aerobic

pretreatment of beet spent wash with Penicillium decumbens resulted in about 74% reduction in phenolics content and 40% reduction in colour [29].

Role of Enzymes in Decolorization of Wastewater

Paper and pulp mills, molasses based-alcohol distilleries, tanneries, dye-making units and textiles are some of the major industries that produce and discharge highly colored effluents. Each of these industrial effluents creates some specific problem besides producing aesthetically unacceptable intense coloring of soil and water bodies. They block the passage of light to the lower depths of the aquatic system resulting incessation of photosynthesis, leading to anaerobic conditions, which in turn result in the death of aquatic life causing foul smelling toxic waters. Besides these paper mill effluents are highly alkaline and alter the P^H of the soil and water bodies into which they are discharged.

Lignin peroxidases (LiP), manganese dependent peroxidases (MnP) and laccase are the three major lignin-degrading enzymes with great potential in industrial applications [30]. Recently, laccase lignin peroxidase, xylanase, endo-1, 4-β-*D*-glucanase production by *Aspergillus sp*. on agricultural waste of banana under solid state fermentation. Decolorization activity involved two types of intracellular enzymes, sugar-dependent and sugar-independent. One of these enzymes required no sugar and oxygen for appearance of the activity and could decolorize MWW upto20% darkness and 11-17% of synthetic melanoidins. It was demonstrated that decolourization was dependent on glucose oxidase levels in the culture medium. The activity of MnP and percentage decolourization of MSW by the isolate NIOCC #312 did not correlate but there was a direct co-relation between concentration of glucose oxidase and decolourization of MSW.Peroxidases like horse radish peroxidase (HRP) manganese peroxidases and lignin peroxidases are ferric ion containing heme proteins and require peroxidases like H_2O_2 for their functioning. The azo groups in the dyes are converted to amines by reductive splitting relatively easily under anaerobic conditions. The anaerobic reduction of certain azo dyes, however, yields aromatic amines that are potentially carcinogenic.

Potential Decolourizing Oxidative Enzymes

For living cells, the major decolorization mechanism in biodegradation is the production of lignin modifying enzymes (LME), laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) to mineralize synthetic lignin or dye. However, the relative contributions of LiP, MnP and laccase to the decolorization of dyes may be different for each organism. Lignin-modifying enzymes are essential for lignin degradation, however for lignin mineralization they often combine with other processes involving oxidative enzymes. An older concept of ligninolysis reemerges, enzymatic ‘‘combustion’’. By extension, this enzyme-assisted process is applicable to the degradation of many other recalcitrant molecules including dyes. The main LME are oxidoreductases, i.e., two types of peroxidases, LiP and MnP and a phenoloxidase, Laccase.

Table 2: Enzyme Mediated Decolorization of Some Dyes

Sl. No.	Substrate (s)	Enzyme
1.	3-(4 dimethyl amino-1 phenylazo) Benzene sulfonic acid	Laccase from *Trametes villosa*
2.	Acid Orange6, Acid Orange 7,Methyl orange and Methyl Red	Mixture of Bacterial Oxidoreductases from Slidge Methanogens
3.	Direct Yellow	HRP from *Armoracia rusticana*
4.	Acid Blue	Laccase from *cladosporium cladosporioides*
5.	Tartazine and Ponceau	Azoreductase from Green Algae
6.	Reactive yellow, reactive black,reactive red and direct blue	Azoreductase from staphylococcus arlettae

Despite of advantages of enzymatic waste water treatment, the major limitation in the use of enzymes is their prohibitive cost. Currently effluent treatment using enzymes on a large scale is not economically viable. However, if maximum reusability of enzymes is achieved through the use of standardized immobilization procedures, the running cost can be lowered considerably (Table.2).

Role of Bacteria in Decolourization of Distillery Effluent

Microbial treatments employing pure bacterial culture have been reported frequently in past and recent years (Table 3). Bacterial strains were isolated from sewage and acclimatized on increasing concentrations of distillery waste were able to reduce COD by 80% in 4–5 days without any aeration .The major products left after treatment were biomass, carbon dioxide and volatile acids. An air bubble column reactor with activated sludge carrying self adapted microbial population in both free and immobilized on polyurethane particles was used for treating aerobic winery wastewater. The highest COD removal rate was with free activated sludge in the bubble column reactor. The most prominent bacterial species isolated from the reactor was *Pseudomonas* while *Bacillus* was isolated mostly from colonized carriers. *Pseudomonas fluorescens* decolourizes melanoidin wastewater (MWW) up to 76% under non-sterile conditions and up to 90% in sterile samples. The difference in decolourization might be due to the fact that melanoidin stability varies with pH and temperature and at higher temperature during sterilization melanoidin-pigments decompose to low molecular weight compounds. Under low oxygen condition, *Lactobacillus hilgardii* immobilized on Ca-alginate gel decolourized melanoidin solution very effectively. Acetogenic bacteria are capable of oxidative decomposition of melanoidins. Biodegradation of potato slops (distillation residue) by a mixed population of bacteria under thermophilic conditions up to 60 ^{o}C was achieved [31]. A COD removal of 77% was achieved under non-optimal conditions. Marine cyan bacteria such as *Oscillatoria boryna* have also been reported to degrade melanoidin due to production of H_2O_2, hydroxyl, perhydroxyl and active oxygen radicals, resulting in the decolourization of the effluent 96%, 81% and 26% decolorization of distillery effluent through bioflocculation by *Oscillatoria* sp., *Lyngbya* sp. and *Synechocystis* sp. respectively was also reported. Distillery spent wash, despite carrying high organic load contains little readily available carbon.

Isolation of bacterial strains capable of degrading recalcitrant compounds of anaerobically digested spent wash from soil of effluent discharge site. These were *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, *Aeromonas*, *Acinetobacter* and *Klebsiella* all of which could carry out degradation of some component of spent wash. Maximum 44% COD reduction was achieved using these bacterial strains either singly or collectively. An acetogenic bacterium was used to obtain a decolourization yield of 76.4% under optimal nutrient conditions. However, this value was only 7.3%, by using anaerobic pond. Also, it required sugar, especially glucose and fructose for decolourization of MWWs. The decolourization activity might be due to a sugar oxidase.

Table 3: Bacteria Employed for the Decolourization of Distillery Effluent

S. No	Name	Comments	Colour Removal (%)
1	*Acetobacter acetii*	The organism required sugar especially, glucose and fructose for decolourization of MWWs	76.4
2	*Bacillus smithii*	Decolourization occurred at 55 ^{o}C in 20 days under anaerobic conditions in presence of peptone or yeast extract as supplemental nutrient. Strain could not use MWW as sole carbon source	35.5

Table 3: Contd.,			
3	*Bacillus thuringiensis*	Addition of 1% of glucose as a supplementary carbon source was necessary	22
4	*Lactobacills hilgardii*	Immobilized cells of the heterofermentative lactic acid bacterium decolorized 40% of the melanoidins solution within 4 days aerobically	40
5	*Pseudomos aeruginosa*	The three strains were part of a consortium which decolorized the anaerobically digested spent wash in presence of basal salts and glucose	67
6	*Pseudomos fluorescens*	This decolourization was obtained by cellulose carrier coated with collagen. Reuse of decolorized cells reduced the decolourization efficiency	94
7	*Pseudomos putida*	The organism needed glucose as a carbon source, to produce hydrogen peroxide which reduced the colour	60
8	*Xanthomoas fragariae*	All the three strains needed glucose as carbon source and NH_4Cl as nitrogen source. The decolourization efficiency of free cells was better than immobilized cells	76

CONCLUSIONS

Industries using large quantities of water as distilleries, it is essential to treat and reuse their waste water. It has been observed that physic-chemical methods are capable of both organic and colour reduction. Whereas, biological treatment, especially with pure cultures, appears promising and cost-effective for colour removal. Generally microbial decolorization is an environment-eco friendly and cost competitive alternative to chemical decomposition process. While using microorganism use of media supplement pose extra burden on overall effluent treatment process. Further the emerging new treatment methods like enzymatic treatment have technological advantages and yet are in its infancy, requiring economical considerations in order to apply it on the plant scale.

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