

Degradation and detoxification of distillery wastewater pollutants for environmental safety through bacteria

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Abstract - India, like many other developing nations, is concerned about the environmental degradation brought on by fast industrialisation, urbanisation, and population growth. Increased research is needed to successfully mitigate and solve the issues of environmental (soil, water, and air) pollution caused by numerous harmful chemicals emitted from many natural and human activities and its detrimental impacts on living organisms. When it comes to eradicating organic and inorganic contaminants from polluted sources, contemporary remediation techniques have shown to be better to their more traditional counterparts. Phenolic compounds, non-phenolic compounds, endocrine-disrupting chemicals, and heavy metals are only some of the most dangerous types of environmental contaminants. All throughout the world, governments are strongly supporting programmes that help reduce pollution. To ensure our civilization can continue to thrive while having a negligible effect on the environment, pollution must be contained or eliminated as soon as possible.

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INTRODUCTION

Every litre of alcohol produced by a distillery creates an average of 10 to 15 litres of effluent, making them one of the dirtiest companies in existence. Due to the increased demand for non-renewable energy sources, the manufacture of ethanol from agricultural products for use as an alternative fuel has drawn attention on a global scale. Ethanol is produced in distilleries that use cane sugar molasses, a major commodity in South America and Asia. From cane molasses, more than 13,000,000 m³ of alcohol is generated annually across the globe. India is the second-largest producer of ethanol in Asia, with an estimated annual output of 2300 million litres in 2006–07. In 1999, India was home to 285 distilleries, each of which produced 4,010 litres of effluent and 2,710,000 litres of alcohol. This number has risen to 319 as a result of yearly alcohol consumption of 3.25 x 10⁹ L and wastewater discharge of 40.4 10¹⁰ L. Waste wash that is dumped into already-existing water bodies depletes oxygen and poses a hazard to aquatic life. Tossing used wash onto the ground has a significant potential for environmental damage. Similar to the effects of land disposal, the soil's alkalinity diminishes, seedlings are unable to germinate, and plants perish. According to BOD estimates, India's 6.2 billion people contribute nearly seven times as much chemical pollution as distillery effluent. Effective processing and disposal of the produced sewage is a huge challenge for Indian distilleries due to the discharge criteria imposed by

the CPCB, the national organisation responsible for environmental compliance. Because phenolic compounds are toxic to methanogen bacteria, their presence in distillery waste may reduce the efficiency of this anaerobic process. Spent wash has a higher emission risk after anaerobic digestion because of the comparatively large residual COD and BOD of the treatments (BOD: 8,000-10,000 mg/L and COD: 36,000-40,000 mg/L). Therefore, before being discharged into the environment, ANDDW (anaerobically digested distillery wastewater) must be controlled using a practical and economical technology. Ligninolytic enzymes like laccase are found in bacteria, fungi, higher plants, lichen, and insects and may aid in the bioremediation of agricultural waste. To preserve the environment, aerobic procedures are utilised for the degradation/decolorization of hazardous and resistant substances present in a wide range of industrial wastes by microorganisms such as bacteria, fungus, actinomycetes, and others. [1, 2]

Treatment of distillery spent wash

Wastewater is often treated using either physical-chemical processes or biological methods. Sedimentation, flotation, screening, adsorption, coagulation, oxidation, ozone, electrolysis, reverse osmosis, ultra-filtration, and Nano filtration are only some of the methods that have been employed to remove harmful substances from water. The physical-chemical process includes drawbacks

including high costs and the need to re-treat the products, which increases treatment expenses. As a result, rather of entirely eliminating contaminants, these procedures just alter their condition. In contrast, biological solutions include the decomposition of contaminants, which permanently resolves the issue. After treatment, the biological approach yields a comparatively little quantity of product by converting a significant proportion of organism components into stabilised carbon dioxide or by eliminating organic matter from wastewater by producing methane gas. The biological treatment technique uses mostly microorganisms to resolve, detoxify, and segregate contaminants in wastewater. The biological approaches have been the ones that have been employed the most commonly across the globe because of their comparatively inexpensive cost and the variety of job development. [3]

i. Decolourization of effluent by fungi

Recently, the decolorization of distillery wastewaters has benefited from the employment of several basidiomycetes and ascomycetes species of fungus. Compared to yeast, filamentous fungi have a lower nucleic acid content in their biomass and are more resistant to environmental stresses including those posed by changes in temperature, pH, nutrients, and air flow. *Coriolus* sp. no. 20 is the first strain of the basidiomycete genus to use the melanoidin-extracting abilities of Mollasse wastewater. *Aspergillus fumigatus* G-2-6 and *Geotrichum candidum* are only two of the many fungi that have been employed in scientific papers. Subfamily *Trametes* *Aspergillus niger* *Flavodon flavus* Decolorizing distillery mill effluent using *Phanerochaete chrysosporium*.

ii. Decolourization of effluent by bacteria

Different bacterial cultures that can decolorize distillery waste wash and perform bioremediation have been identified. Numerous bacterial strains that have adapted to larger quantities of distillery mill effluent have been isolated, according to different studies. These include *Pseudomonas putida*, *Lactobacillus hilgardii*, *Bacillus* sp., *Bacillus thuringiensis*, and *Pseudomonas aeruginosa*. Some studies used immobilised entire cells to do melanoidin decolorization. These strains were successful in significantly lowering BOD and COD levels. After treatment, the main byproducts were carbon dioxide, volatile acids, and biomass. Aside from fungi and bacteria, yeast and algae have also been extensively used for a long time to biodegrade complex, poisonous, and resistant substances found in distillery waste wash.

iii. Decolourization of effluent by algae

Cyanobacteria are thought to be the best choice for treating distillery effluent because, in addition to breaking down the polymers, they also oxygenate

the water, which lowers the BOD and COD levels. It studied how a marine cyanobacterium may be used to decolorize distillery waste wash and how it could use melanoidins as a source of carbon and nitrogen. This decolorization was caused by the marine filamentous, non-heterocystous form of *Oscillatoria boryana* BDU 92181 using the resistant biopolymer melanoidin as a nitrogen and carbon source. Scientists believe that the cyanobacterium's production of hydrogen peroxide, hydroxyl anions, and molecular oxygen during photosynthesis is responsible for the bleaching effect. [4]

iv. Role of bioreactors in treatment

a. Anaerobic reactors

A highly promising re-emerging technology, the anaerobic process for waste water treatment generates valuable biogas while requiring less energy and producing very little sludge. Due to their susceptibility to organic shock loadings, low pH, and the slow growth rate of anaerobic microbes, hydraulic retention times are lengthened (HRT). As a result, conventional mixed-reactor efficiency is typically compromised. Biomethanation using a biphasic system is the most effective treatment method for high strength wastewater due to its many benefits, such as the ability to maintain ideal conditions for buffering imbalances between organic acid production and consumption, stable performance, and higher methane concentration in the biogas produced.

b. Aerobic Reactors

The discarded wash still from a distillery that has undergone anaerobic treatment has too many organic pollutants for safe disposal. Aerobic treatment of anaerobically treated distillery waste wash has been attempted to further decrease the COD and BOD and to decolorize the major colourant, melanoidin. Over the last several decades, many different types of bacteria (pure and mixed culture), cyanobacteria, yeast, fungi, etc. have been identified as potential agents for degrading melanoidin and, therefore, decolorizing wastewater. [5]

v. Alternative technologies for decolourization

Interest in using more extensive methods of biological treatment has been sparked by new regulations for the disposal of sludge and industrial effluents. The technique known as sequencing batch reactor (SBR) has lately emerged as an appealing substitute choice for the elimination of numerous xenobiotic chemicals from wastewaters. According to preliminary research, wastewater from agro-industrial enterprises, including distilleries, is often treated using both aerobic and anaerobic systems. [6]

OPTIMIZATION METHODS FOR PROCESS PARAMETERS

The process optimization through statistical design is a common practice in biotechnology. In conventional methods numerous experiments have to be carried out to optimize all the parameters (factors) and to establish best possible culture condition by interrelating all the parameters. In recent years, use of statistical approach has gained lot of impetus for medium optimization and for understanding the interactions among various physico-chemical parameters. Statistical experimental design techniques are very useful tools for this purpose, as they can provide statistical models that assist in understanding the interaction of different variables and predict the maximized product formation. The use of statistically designed experiments can allow the rapid and economical determination of the optimal culture conditions with fewer experiments and minimal resources. Commonly used statistical methods are response surface methodology, Plankett-Burman design and Taguchi approach. [7]

i. Response surface methodology

A set of statistical tools and strategies for building and investigating an approximative functional connection between a response variable and a group of design factors may be summed up as the approach. The process calls for identifying the elements that significantly predict the response variable (the desired quality features). The first trial establishes the different levels to be applied for each component thought to be significant in predicting the response variable. Utilizing multiple regression analysis, data are evaluated. This results in an estimation of the model's unobserved parameters and a projected response function. Tests are run to make sure the model is adequate and to see if any words can be dropped altogether. RSM's fundamental approach consists of four steps: methods for entering the optimal zone, response behaviour there, assessment of the optimum circumstances, and verification. Typically, a first order model is used to start the experiment. Model lack of fit is found once the model parameters are evaluated. The fitted model is used to identify the region where more desired response values may be obtained if the first order model is sufficient.

ii. Plankett-Burman Design and Uniform Design

For a systematic analysis of the target variables, Plankett-Burman design (PBD) and uniform design (UD) are efficient and successful methodologies. PBD is a successful screening method that significantly reduces the number of tests while providing as much data as feasible for the assessment of the target variables. Only the variables with the highest positive significance may be chosen out for further optimization; in subsequent experiments, elements with lower significance or

large negative effects on response value may be excluded. Within the intended scope, UD may be used to improve target parameters. The number of trials required by the UD technique is much lower when compared to other statistical designs of experiments, such as response surface design, since it is equal to the maximum number of planned levels of the target parameters. PDB and UD together provide a potent tool for process optimization. [8]

iii. Taguchi approach

The ideal amounts for each ingredient are established in order to obtain the appropriate levels of output while treating wastewater or producing enzymes in a batch reactor. Noise is the collective term for the variables that are difficult to manage and cause output volatility. The goal of optimization is to produce a resilient design that can still provide the intended results in the face of noise. This is accomplished by determining the role that various elements play, establishing the link between variables and the operational circumstances, and then establishing performance at the optimal levels as determined by a small number of well-defined experimental sets. The Taguchi technique makes it easier to determine the effects of certain factors and to draw connections between variables and operational circumstances. ANOVA (analysis of variance) and factors impact analysis of the experimental data produces results that are statistically significant and significantly reduce the number of trials.

iv. Enzymatic processes for decolourization

Several types of enzymes, such as peroxidases, oxidoreductases, cellulolytic enzymes, proteases, amylases, etc., have been shown to play important roles in different wastewater treatment processes. Paper and pulp mills, textile and dyeing firms, alcohol distilleries, and the leather industry are just few of the sectors that release effluents of varying vivid hues. The ligninolytic system is made up of two primary classes of enzymes, peroxidases (including lignin peroxidases and manganese peroxidases) and laccases. Although there is some evidence linking the presence and activity of fungal ligninolytic processes to the enzymatic system involved in the decolorization of melanoidin-containing wastewater, the precise nature of this relationship is still unclear. The multicopper blue oxidase laccase may be able to oxidise ortho- and para-diphenols and aromatic amines by removing an electron and a proton from a hydroxyl group to form a free radical. [9]

ADSORPTION ASSISTED DECOLOURIZATION

Several strategies for dealing with coloured wastewaters have been proposed in the literature. Biodegradation, chemical oxidation, and physicochemical processes all fall within this

category. It has been shown that, among the several physico-chemical treatment processes, adsorption is particularly attractive for the removal of the vast majority of organic molecules in wastewaters, especially at lower concentrations. Activated carbon is the most common kind of adsorbent used today. However, problems occur with the use of activated carbon because of the high prices associated with activation, regeneration, and disposal of the cleaning cycle concentrate. As a result, many scientists have joined the quest for low-cost, effective new adsorbents. Several low-cost adsorbents have been used for the treatment of various wastewaters, such as wood, coir pith, coal fly ash, bagasse fly ash (BFA), and bottom ash from coal-fired boilers. Biosorption is a physiochemical technique for removing impurities from water using either living or nonliving biological material.[10]

TREATMENT METHODS

For the purpose of lowering the pollutant load caused by distillery effluent, many approaches have been investigated. As a key method of lowering the contamination potential of distillery effluent, anaerobic treatment is frequently used. The alternatives presently used for treating molasses spentwash are shown in Figure 1. The following sections go through the key characteristics of the available alternatives.[11]

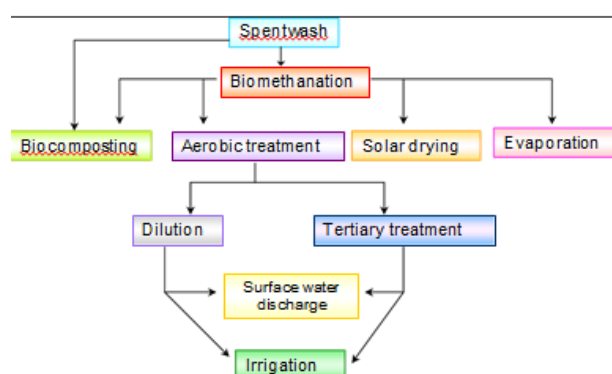


Figure 1 Options for spentwash treatment

BIOLOGICAL TREATMENT METHODS

Anaerobic Treatment

Anaerobic digestion is becoming more popular because it produces biogas, which can be used to meet some of the energy need. To bioremediate distillery spentwash, anaerobic digestion is often used as the primary treatment because of its positive reputation as a cost-effective, ecologically benign, and socially acceptable method. Anaerobic digestion works quite well with wastewater from vineyards and distilleries. Anaerobic treatment of distillery effluent is widespread practise, and many types of high-rate anaerobic reactors have been tried in both pilot and full-scale operations. Aerobic treatment on its own is

impractical because of the high energy costs associated with aeration, cooling, and other operations. More than half of the COD in wastewater may be converted into biogas by anaerobic treatment which can be utilised to create steam in the boilers and meet the unit's energy demands.[12]

BACTERIAL STRAINS IN DISTILLERY WASTEWATER TREATMENT

There has been a lot of talk about how using microbial treatments with pure bacterial culture may speed up aerobic breakdown. Under aerobic circumstances, *Pseudomonas putida* and *Aeromonas* species were used in a two-stage bioreactor to treat distillery effluent. With an influent COD of 5,000 mg/L, the COD removal rate was 66% after 24 hours thanks to the ability of *Aeromonas* species to use the refractory compounds of distillery waste wash as sole source of carbon. The addition of Peptide also led to a drop in colour by 60% and a loss of 44% COD. Sewage bacterial strains were identified by Kumar and Viswanathan (1991) and adapted to increasing concentrations of distillery waste. It only took these strains four to five days to significantly lower the COD levels without the use of aeration.[13]

Algal strains in distillery wastewater treatment

Melanoidin degradation in distillery effluent has also been attributed to marine cyanobacteria, namely *Oscillatoria boryana*. As a result of photosynthesis, the cyanobacterium generated hydrogen peroxide, hydroxyl anions, and molecular oxygen, all of which contributed to a 75% decolorization of the melanoidin pigment (0.1% w/v). Decolorization of distillery effluent using bioflocculation of *Oscillatoria* sp., *Lyngbya* sp., and *Synechocystis* sp. was reported at 96%, 81%, and 26% respectively. During the stationary phase, marine cyanobacteria excrete a wide variety of colloidal substances, including lipopolysaccharides, proteins, polyhydroxy butyrate, polyhydroxy alkonate, etc., that contain reactive groups such as OO- or half ester sulphate (OSO₃-), which then form electrostatic complexes with the active cationic site of organic matter, causing flocculation. Decolorization relies heavily on this bioflocculation process.

Fungal strains in distillery wastewater treatment

In recent years, researchers have looked at the ability of several fungal strains to decolorize melanoidins and molasses spentwash. White rot fungi may be able to degrade recalcitrant lignolytic substances, melanoidins, and polyaromatic compounds with the help of their highly developed extracellular and non-specific enzymatic systems. According to (Benito et al. To perform this very

broad task, the fungus requires a lignolytic enzyme system consisting of lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, and an enzyme that generates hydrogen peroxide (H₂O₂). They are able to oxidise both phenolic and non-phenolic compounds in the presence of oxygen.[14]

CONCLUSION

The findings of the present research suggest that distillery wastes may include a variety of bacterial species (wastewater and sludge). Following the application of first nutritional enrichment techniques, nine (9) bacterial strains were discovered (DS, DS1-DS8). The ability of eight distinct bacterial strains to thrive on various distillery wastewater dosages and produce manganese peroxidase activity on GPYM agar plates that had distillery wastewater added was examined. The screening was successful for four of these strains (DS1, DS3, DS4, and DS5). It was possible to recognise and distinguish between the various bacterial strains even at the microscopic and morphological levels. Gram-positive, coccus-like bacteria were found in strains DS3 and DS4, while gram-negative, rod-shaped bacteria were found in strain DS5.

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