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Review article

MICROBIAL BIOREMEDIATION OF SELECTED WASTEWATERS – A REVIEW

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ABSTRACT

When untreated wastewater effluent is discharged, especially into water, it makes the environment unhealthy, and thereby affecting entire life of humans, plants and other aquatic animals. This ultimately affects the quality of water and limits its utilization. The microbial bioremediation of wastewaters relating to the application of variety of microorganisms has established effective degradability of organic wastes in wastewaters which has attracted attention in modern time. Several studies have shown the bioremediation ability of indigenous microorganisms isolated from different types of wastewaters. The bioremediation potentials of these microorganisms is measured in terms of reduction in physicochemical parameters of the wastewaters e.g. the Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), total suspended solid (TSS), and significant inorganic parameters e.g. color, acidity, alkalinity, phosphorus, nitrate over a period of treatment time. The *Bacillus* genera, *Pseudomonas* species, *Alcaligens feacalis*, *Aspergillus* species, *Micrococcus* species, *Actinobacillus* species, *Burkholderia cepacia,* fungus like *Aspergillus niger*, *Aspergillus fumigatus*, *Geotrichum* sp. and some other isolated species of microorganisms have been found to be effective in bioremediation process of wastewaters. This paper examines the bioremediation abilities of microorganisms in abattoir wastewater, textile wastewater and effluent from palm oil mill. Significant decrease in organic and inorganic parameters of selected wastewater effluent indicates reduction in pollution. Synergism among microorganisms when used in consortium in the bioremediation also aids cleanup process. Microbial bioremediation of different wastewaters is discussed in this paper in brief.

Keywords: Abattoir, Bioremediation, Chemical Oxygen Demand, Environment, Pollution.

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INTRODUCTION

Bioremediation.

The term bioremediation is derived from two words; "bios" which refers to living organisms and "to remediate" which means to solve a problem. "Bioremediate" is a word which is used when referring to solving an environmental problem such as contaminated soil or groundwater using biological organisms. Bioremediation is the application of microorganisms to breakdown environmental contaminants into less toxic forms (1). Bioremediation is brought about by stimulating microorganisms to swiftly breakdown hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and groundwater. The stimulation of these microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptors/donors or some combinations thereby leading to an increase in organic pollutant degradation and bio-transformation. Energy and carbon are obtained through the metabolism of organic compounds by the microbes involved in bioremediation process (2). This process exploits the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (iv) oxide and water, or by conversion into microbial biomass. This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated site that are able to perform desired activities.

Need for bioremediation.

The major reason for the control of water and soil pollution and the consideration of bioremediation is firstly because of public health concerns, secondly, environmental conservation and finally the cost of environmental decontamination. Clean water is essential to agriculture and industry, and for most countries, water is a finite resource which has to be kept free from pollutants. Maintenance of safe potable water supplies is a major health concern around the world. The argument for uncontaminated soil is similar; even countries with very large areas of land tend to have their development concentrated in certain places. Having land so contaminated that it cannot be developed in those areas is a real financial and developmental burden. Also, there are known cases in which contamination has brought about public health problems. Land that is contaminated can also become a contaminated water problem if the contaminants migrate to groundwater. For many countries, ground water is a major source of drinking water. When the pollution traverses national boundaries, the issue becomes political. All of this speaks to the critical need for cost effective technologies, such as bioremediation, to help remove environmental contaminants

Microbial bioremediation.

Over the last 150 years, the number of organic chemicals released into the environment has increased vividly leaving an unprecedented chemical footprint on earth. Microorganisms are known to be the principal agents, which can clean up and change these complex hydrophilic organic molecules, once considered recalcitrant, to simple water soluble products. They first attack these organic chemicals by the enzymatic apparatus acquired during the course of enrichment, when they are exposed to these specific or structurally related compounds (2). Microbial bioremediation make use of naturally occurring bacteria and fungi which maybe either indigenous to the contaminated site or maybe isolated from somewhere else to breakdown or detoxify substances that are harmful to human health and/ the environment (3). Microbial bioremediation explores gene diversity and metabolic adaptability of microorganisms (2).

Rationale of using microorganisms for environmental bioremediation.

Research has revealed that the process of bioremediation is an efficient solution to tackle wastewater owing to the ability of microorganisms to survive, adapt and thrive within many environments, wastewater included (4). Some microorganisms produce broad range of extracellular enzymes including proteases, cellulases, amylases, and ureases which can degrade organic high molecular weight substances in soil. As opposed to cleaners with added enzymes, microorganisms can further metabolize some of these degradation products. The microorganisms used in the cleaning products are also claimed to outcompete unwanted microorganisms in colonizing surfaces by using up nutrients provided in the soil and from polluted surfaces. Producers claim a long term-effect because microorganisms will stay on the treated surface (as spores) and hinder recolonization by unwanted microbes. In this direction, a new technology named 'microbial cleaners' is coming into play. Microbial cleaners are the specially designed groups of bacteria and fungi that are capable of cleaning the polluted sites (5). Microbial cleaners are environmentally sound as most contain much lower levels of acids and surfactants.

Bioremediation strategies

Different techniques are employed depending on the degree of saturation and aeration of the site:

In-situ techniques

In situ techniques
In -situ technique is that which is applied to soil and water at the site with least disturbance. This technique is generally the most desirable options due to lower cost and fewer disturbances, since it provide the treatment in place avoiding excavation and transport of contaminants (6).

Ex-situ techniques

Ex- situ technique is applied to soil and water which has been removed from the site through excavation (soil) or pumping (water). This technique involves the excavation or removal of contaminated site (6).

Characteristic of organisms involved in bioremediation.

1. The organisms must possess the effective enzymes important in bioremediation.

2. The organism shall be able to survive and demonstrate its bioactivity under conditions of pollution.

3. The organism must be able to get access to the contaminant that may not be soluble in aqueous environments or severely absorbed to solid surfaces.

4. The substrate site of the contaminant must be accessible for the active site of the enzyme of role in bioremediation.

5. Contaminant and enzymatic system must come in close contact somewhere in or out of the cell.

6. Appropriately favorable environmental conditions must exist or be provided to arise the population of the potential bioremediant.

Principle of microbial bioremediation.

Microorganisms are suited to the task of contaminant destruction because they possess enzymes that allow them to use environmental contaminant as a food (7). All metabolic reactions are mediated by enzymes for example; the oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases. Many enzymes have a remarkably wide degradation capacity due to their non-specific substrate affinity. For bioremediation to be effective, microorganisms must enzymatically attack the pollutant and convert them to harmless products. Bioremediation technology is primarily based on biodegradation. It refers to complete removal of organic toxic pollutant into harmless compounds like carbon dioxide, inorganic compounds, and water. These compounds are generally safe for humans, animals, plants and aquatic organisms (8).

Wastewater

Wastewater simply means water whose properties (physical, chemical or biological) have been altered as a result of introduction of certain substances which render it unsafe for some purposes, like drinking. The daily activities of man are water dependent and therefore discharge waste into water. Some of these substances include body wastes (feces and urine), food scraps, toilet papers, fabric conditioners, household cleaners, chemicals, detergents and microorganisms which can make people ill and also cause harm to the environment (9). Much of water supplied end up as wastewater which makes its treatment vital. The treatment of wastewater is the process and technology that is used to remove most of the contaminants that are found in it to ensure a sound environment and good public health. The management of wastewater, therefore, means handling wastewater to protect the environment to ensure public health, economic, social and political security (9). According to its source, wastewater can be classified into either domestic or industrial wastewater. Domestic wastewater originates from household activities like washing from the kitchen, bathing, laundry, and water from flushing toilets while industrial wastewater originates from industrial activities like textile industries, paper and pulp mill industries, oil palm producing industries, food processing industries, and chemical producing industries. These industrial wastewaters are important habitat for various microbes (10). Figure 1 below shows the various types of wastewaters and sources.

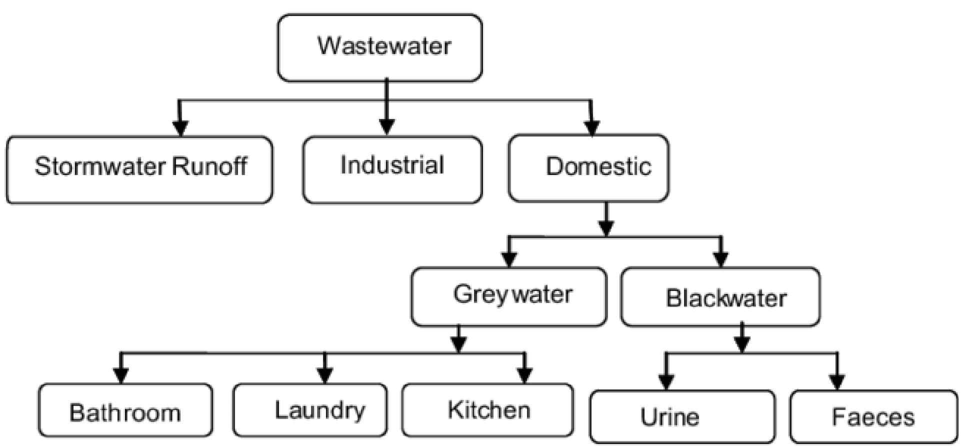

Figure1.Types of wastewaters

Microbial bioremediation of abattoir wastewater.

Abattoirs, also known as slaughterhouses are industries that are concerned with the commercial slaughtering of animals such as cattle among others, and processing of meat for human consumption (11). Abattoirs are approved, hygienic, registered and authorized ground that is exclusively meant for slaughtering, processing and preservation of meat products for human use (12). Wastewater is generated and released during the slaughter of animals for meat in slaughter houses (13). As the demand for meat increases, the release of wastewater from slaughter houses also increases (14, 15). Abattoirs are often sited near water bodies where different untreated waste products of the industry are discharged and this is of immense concern to public health authorities (16). The main components of abattoir wastewater include condemned organs, bones, carcasses, animal feces, blood, fat, hides, urine, and carcass trimming (16,). Studies revealed that wastewater from slaughter houses is typically composed of 99% liquid and 1% solid particles. The water produced by slaughterhouses is large and contains organic matter, suspended solids, nitrogen and phosphorus compounds in high concentration (17). The highest toxic waste of all component of abattoir wastewater water is blood, followed by fat (13). Blood is rich in nutrients, phosphorus and nitrate, it is one of the major dissolved component of abattoir wastewater and has the highest chemical oxygen demand of any wastewater from abattoir operations (18). It has biochemical oxygen demand of about 450,000 mg/L and chemical oxygen demand of 375,000mg/L (19). Many studies have shown that the physicochemical parameters of abattoir wastewater are higher than the permissible standard set by regulatory bodies (20). Abattoir activities have also been found to be associated with some diseases such as diarrhea, typhoid fever, pneumonia, asthmas, wool sorter diseases, respiratory and chest diseases. Four bacteria isolated from wastewater effluent from an abattoir are *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa* (21). After screening for bioremediation potentiality; *B. subtilis* and *P. aeruginosa* were selected and used singly and in consortium for the bioremediation of the wastewater. The results indicated reduction in the value of some of the physico-chemical parameters of the wastewater after a period of 28 days as against its values before bioremediation. From the results obtained, there was steady reduction in the physicochemical properties of the sterile abattoir wastewater treated with bacterial isolates indicating a reduction in the pollutants of the wastewater. *Bacillus subtilis* RN40 for example reduced the COD from 173.08 mg/L to 64.59 mg/L, BOD of the effluent from 259.00 mg/L to 75.12mg/L. *P. aeruginosa* DM1 reduced COD and BOD form 173.08 mg/L and 259.00 mg/L to 71.79 mg/L and 97.12 mg/L respectively (21). Treatment by consortium showed more reduction when compared to the individual isolate; this result agrees with the work carried out by other researcher (22, 23), where they observed that *B. subtilis* and *P. aeruginosa* reduced the BOD of Yamuna water and lipid rich wastewater (24). The consortium indicated the maximum reduction of COD followed by *B. subtilis* and *P. aeruginosa* (21). Nitrate concentration of the abattoir wastewater before treatment is often higher than the permissible limits set by standard bodies, the reduction of nitrates in wastewater by either the consortium or single species of isolated microorganism show that the process of denitrification took place during treatment (25). The high concentration of phosphate in the wastewater before treatment can be attributed to the high fecal content, blood, stomach and intestine content present in the wastewater (15). Wastewater samples must have less than 50mg/L of nitrates and 0.5mg/L of phosphate before it is released into aquatic environment.

The presence of phosphate in water bodies causes environmental problem like eutrophication (21). From (21), it reveals that the consortium effectively biodegraded the sterile abattoir wastewater, this shows that the consortium collaborated in the degradation of a wide range of substrate under a short period of time (26). The decreased level of the physicochemical properties confirms the presence of synergy interactions in the consortium. The efficacy in the reduction of the various physicochemical parameters as demonstrated by the bacterial isolates indicates their ability to adapt and survive naturally in the presence of abattoir wastewater and the microorganisms possess degradative enzymes for the degradation of the wastewater (21).

Microbial bioremediation of Textile effluent.

Textile industries are concerned with processes such as pretreatment of clothing materials, dyeing, printing and finishing operations. These production processes consume large amounts of energy and water hence they also produce a substantial amount of waste products (27). As demands for textile products increased, the textile industry and its waste waters have increased proportionally making it one of the main sources of severe pollution problems worldwide. The effluent generated from this industry affects people and the environment as it contaminates the ground water which alters the natural hydrologic cycle. Effluent from textile industries contains numerous types of chemicals for example; dispersants, leveling agents, acids, alkalis and various dyes. This alters the pH, increases the BOD, COD, and gives the receiving water bodies intense colorations limiting the use of these water resources and the ecosystem is also affected (27). Dye in wastewater from textile and dyestuff industries must be treated due to their impact on water bodies and growing public concern over their toxic nature and carcinogenicity. Thus, colour elimination in wastewater is today the principal problem in relation to the textile industries, since it is the first contaminant recognized in textile wastewater and has to be removed before discharging into receiving water body (28). Because of the high BOD, the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources (28). Therefore the effluents with high COD level are toxic to biological life. Dyeing causes an easy recognition of pollution via colour. The most widely dyes used in most textile industries include Procion Blue HERD (RB-160), Remazol Violet 5R, (RV-5R) Vinylsul phonyl, monaazo and Remozol Brown GR (RBR-18), Azo dye. Although, there is limited understanding of the microbial degradation and decolorisation of a dye, results indicate that maximum dyes adopt reductive process of degradation. *Bacillus* spp, *Alcaligenes* spp, *Acinetobacter* spp are essential bacteria useful in bioremediation of halogenated aromatic compounds and textile effluents. Decolorisation and degradation can also detoxify the effluent effectively without leaving any residues. In recent years, considerable interest has been generated in studying microbial azo dye degradation (28). Several studies noted that textile effluent are highly colored and saline, it contain non-biodegradable compounds, and are high in BOD, COD. A combination of *P. aeruginosa*, *Alcaligenes faecalis* and *Pseudomonas putida* C15 was found capable of degrading all the dyes most efficiently compared to the other consortia. A temperature of about 29°C - 30°C and pH of 7.2 was reported for optimal degradation and decolorisation of azo dyes (28). Previous studies reported that pH of 7.00 was found to be most suitable for maximum decolorisation of dye effluent. In a research, (29) isolated 15 bacterial isolates in a study of textile effluent; five belong to *Pseudomonas* genera while six belongs to *Bacillus* genera. Others were *Alcaligenes faecalis, Aeromonas. hydrophila*, *Actinobacillus* species and *Burkholderia cepacia*. Isolation, characterization of these bacterial isolates and biodegradation / decolorisation potential was carried out for 10 days in the Laboratory. Nine bacterial isolates exhibited high growth potential when inoculated on minimum basal medium at different concentrations with rapid degradation of textile effluents in 48 hour, and they were presumptively selected for bioremediation / decolorisation studies (29). *P. aeruginosa*, *P. putida*, *B. megaterium* and *Aeromonas hydrophila* caused a reduction in organic parameters of textile effluents. These isolates probably have acquired natural adaptation to survive in the presence of the textile effluents, and had the degradative enzymes for degradation of effluents. This support the findings of other researchers. An increase in DO level in the effluents after bioremediation with *P. aeruginosa*, *B. Subtilis* might be due to oxygenic nature of bacterial (29). The overall analyses of increase in DO and the removal of TSS, TDS, COD, BOD, and EC observed agrees with the observations from other researchers (29). Some literature reported that *Citrobacter* sp. decolorizes several recalcitrant triphenylmethane and azo dyes by adsorption mechanism. *Enterococcus feacalis* effectively degraded reactive orange 16. Two bacterial strains *Bacillus cereus* (KEB-7) and *B. pumulis* (KEB-10) decolorized indigo dye from textile effluents. Also the decolorisation of textile azo dyes by the strain of *Staphylococcus arlettae* VN-11 by aerobic process was effective in azo decolorisation. Fungus *Geotricum* sp. CCMI 1019 was found capable of decolorizing large amount of dyes chiefly reactive black 5, reactive red 158 and reactive yellow 27. Other fungus like *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* sp. were reported to decolorize both reactive and direct dye solution (30).

Microbial bioremediation of Palm oil mill effluent (POME)

The Palm oil industry is one of the leading agricultural industries in the world. Malaysia for example, has an average palm oil production of more than 13 million tons per year (31). The production of palm oil results in the generation of a large quantity of polluted effluent called palm oil mill effluents (POME) (32). POME is the waste that is released by palm oil mills during the extraction of oil from palm fruit bunches. 1t of crude palm oil production requires about 5-7.5t of water (32). Over half of this water ends up as POME. Recently, so much attention has being drawn to environmental hazards caused by the direct discharge of this effluent without adequate treatment. POME is mainly generated from sterilization and clarification of palm oil, in which a large amount of steam and hot water are used. POME is a thick brownish liquid that is compiled with high concentrations of total solids, oil and grease, COD and BOD. Raw POME is warm, acidic with pH ranging between 4- 5 (10). Although, POME is non toxic, but it has unpleasant smell and its COD and BOD values are high and can cause great pollution and environmental problem to the receiving water bodies (31). POME has unfavorable environmental effects including land and aquatic ecosystem contamination, loss of biodiversity and increase in COD and BOD in environment (33). The challenge of converting POME into environmental friendly waste brought about a well-organized treatment and efficient removal method (10). Table 1 below gives an insight into the characteristics of raw POME

Table 1.Characteristics of raw palm oil mill effluent and discharge standard limits

Parameter	Raw POME	Discharge standard Malaysia
COD	61,200-75,900	-
BOD	30,500-34,393	100
Total suspended solid	12,800-14,467	400
Oil and grease	145-191	50
pH	4.37-4.74	5-9

Source: (32). (All parameters are in mg/L except pH)

POME contains a high concentration of organic matter: COD of 65,000mg/l, BOD of 48,000 mg/l and oil and grease greater than 2000mg/l. Today, the penetration of palm oil has been considered due to the entry of its effluents into the waterways and ecosystems, making it a particular concern towards the food chain interference and water consumption. This can cause considerable environmental problems such as land pollution and effectively suffocating aquatic life if discharged without effective treatment. Of late, various physical and chemical treatment processes have been designed to treat POME, the problem of TSS and chemical residues which is persistent after the treatment process remains to be resolved (34). Microbial bioremediation in recent studies gives an alternative solution to reduce the TSS and organic load content of the effluent. The biological treatment of POME depends enormously on consortium of microbial activities, which operate on the organic substrates present in the POME as supplements and eventually degrade these organic matters into simple by product such as methane, carbon dioxide hydrogen and water. Due to the high oil content of POME, it serves as a habitat for numerous groups of lipase producing microorganisms and hydrocarbon degraders. A variety of microorganisms have been investigated to be capable of biodegrading oil wastewater with high profits, however, amongst, the bacteria isolates screened for palm oil mill effluent biodegradability as reported, *Pseudomonas* spp. showed the highest BOD (53.33%) and COD (47.37%) reduction potential while *Fusarium* spp. and *Mucor* spp. showed the highest Oil and grease (64% each) reduction potential and *Mucor* spp. had the highest TSS reduction potential (23.20%). The employment of non-indigenous microorganisms like *Trichoderma viride* spores, *Saccharomyces cerevisiae*, T. *viride* mycelium for POME treatment was not effective (35). A study reported that the strain of *Bacillus cereus* 103PB isolated from POME is the best candidate and the most effective bacteria to use for the biological treatment of POME (32). The study used known bacteria isolated from POME as compared to anaerobic digestion techniques used by other investigators. The biological treatment depends to a great extent on active microorganisms, which utilizes the organic substances present in the POME as nutrients and eventually degrades these organic matters into simple by-products such as methane, carbon dioxide, hydrogen sulphide and water (35). The ability of bacteria and fungi isolates to biodegrade palm oil mill effluent was demonstrated in terms of reduction in BOD (mg/L), COD (mg/L), TSS (mg/L), oil and grease (mg/L). The reduction in value of these significant organic parameters is a product of microbial oil degradation due to hydrolysis of oil by lipase enzyme secreted by these microorganisms (31). A research on the reduction of organic load and biodegradation of palm oil mill effluent by aerobic indigenous mixed microbial consortium isolated from POME, treatment of POME by selected mixed microbial consortium was carried out (14). The first consortium was introduced as all bacteria-fungi combination, these organisms are *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigates* 107PF and *Aspergillus niger* 109PF. The second consortium was inoculated first with bacterial isolates (*Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB) and later with fungal isolates (*Aspergillus fumigates* 107PF and *Aspergillus niger* 109PF) and monitored for 50 days. The all bacterial fungi combination showed highest BOD reduction. For example 90% reduction was recorded after 40 days (14). While the bacterial -fungi stepwise achieved 80% BOD reduction after 35 days. Similarly for COD reduction, the all bacterial fungi combination showed 90% reduction by 40 days. Bacteria- fungi stepwise was able to achieve 82.28% reduction by 35 days of experiment (14). Reduction in BOD and COD by consortium indicates reduction in organic pollutant in the effluent. All bacteria-fungi combination showed significantly higher reduction of BOD, COD, TSS when compared to bacteria-fungi stepwise suggesting that these indigenous isolates are promising organism for application in industrial scale (14). It is therefore suggested that mixed microbial consortium will be effective and environmental friendly technology for the reduction of organic material from POME.

CONCLUSION

Wastewater is an important environmental pollutant which causes pollution of receiving water bodies if discharged untreated and also polluted the soil. The process of bioremediation is an important tool which has been employed for the treatment of wastewaters in modern times. Microbial bioremediation is a promising and the most ideal alternative technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. This environment friendly technology contains a range of organisms used for bioconversion, to clean up pollution and to degrade environmental pollutants. Employing microorganisms for the process of bioremediation is effective in the treatments of various wastewaters generated from industries before discharge into receiving water bodies. These microorganisms convert pollutants into non hazardous compounds like water and carbon dioxide and they help in environmental cleanup. Microbial bioremediation process is cheap, easily implemented and eco-friendly.

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