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BIOREMEDIATION AND ITS APPLICATION IN WASTE MANAGEMENT

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ABSTRACT

Pyrethroid, carbamate and organophosphate compounds were introduced to replace the recalcitrant and hazardous chlorinated pesticides. Although newly introduced pesticides were considered to be biodegradable, some of them are highly toxic and their residues are found in the environment. Moreover, some of these pesticides generates metabolites that are also toxic. A serious environmental concern is the contamination of ecosystems due to pesticide discharges from manufacturing plant, surface runoff, leaching, accidental spills and other resources. Safe and economical disposal of pesticides is a current problem of significant magnitude. Although microorganisms capable of degrading pesticides have been isolated. However, knowledge about bioremediation of these toxic compound in the soil and water environments is sparse. In this review, the role of microorganisms and the essential factors that enhanced the biodegradation process are described. This information may be helpful in designing on-site bioremediation systems (e.g. on-site bioreactor) for the degradation of toxic compounds like pesticides which are resistant otherwise to conventional treatment.

Key words: Recalcitrant, pesticides, ecosystem, degradation, microorganism, conventional treatment.

INTRODUCTION

The intensive nature of modern agricultural practices has led to the development and widespread use of synthetic pesticides in our environment. Pesticides fight against diseases and save crops from pests. However, their indiscriminate use has become a serious problem. They have been frequently detected in the water bodies in various regions of the world including Pakistan (Khurshid, 1990; Currie and Williamson, 1995; Schreibet *et al*., 1995; Elefsiniotis and Mangat, 1996; Kreuger *et al*., 1999; Liess *et al*., 1999).

According to an estimate Pakistan uses more than 45,000 tones of different types of pesticides. Since 1980, the pesticide consumption has risen many folds. Organophosphates and pyrethroids pesticides dominate the market, accounting for about 85 to 90 percent of total pesticide consumption. Over 70 percent pesticides are used on cotton crop production and the rest on rice, sugarcane, fruits and vegetables (Dhaliwal and Balwinder, 2000).

The advantage resulting from pesticide application is generally undisputed, but the residues of the applied pesticides stay in the environment (air, soil, ground and surface water) for variable period of time, which poses serious threats to environment and indeed can lead to acute and chronic effects on human life causing damage to health or even death (Boraiko, 1980). These compounds may be toxic, mutagenic or carcinogenic and may be bioaccumulated or biomagnified by the biota (Sharp, 1986; Axelson, 1987). According to World Health Organization (WHO) study, worldwide three million people suffered from pesticide poisonings with about 220,000 deaths per year. It is also reported that because of the misuse and mishandling of pesticides, the incidence of pesticide poisoning is 13 times higher in developing world although nearly 80 percent of the world pesticide production is consumed in industrialized countries (Forget, 1989).

In Pakistan besides pesticide contamination from agricultural field, the agricultural industries are also contributing relatively high quantities of toxic pesticides into the environment, as most of them have either no treatment facilities or have grossly inadequate arrangement. At present the Karachi coastal region has become the dumping ground of hazardous waste, receiving huge quantity of untreated domestic, industrial and agricultural wastes. Pesticides waste treatment technologies are therefore needed to prevent water pollution and to comply with increasing regulatory pressure. For sustainable development, country should implement control over waste disposal in order to avoid high cleanup cost in future.

Recently, the bioremediation (biological treatment system) has been proven to be a suitable method for the treatment of polluted aquifers containing hazardous waste that could be implemented either in situ or off-site in specially designed reactors or wastewater treatment plants. Moreover, in most cases, it has been found the most cost-effective and environmentally friendly treatment method. According to literature, bioremediation success depends upon the physical and chemical characteristics of the substrate, such as nutrient status and pH, and is influenced by environmental factors such as temperature (Comeau *et al*. 1993) and biotic factors such as inoculum density (Ramadan *et al*. 1990). Therefore, the present study described the use of potential microorganisms and the optimum conditions that support the growth and biodegradation of pesticides in an aquatic environment using biological treatment system. Such studies would be a valuable addition in the improvement in designing and operation of biomechanical treatment system used for the degradation of toxic compounds like pesticides which are resistant otherwise to conventional treatment.

ADVANTAGES OF BIOREMEDIATION

Biological means i.e. microbial metabolism provide an excellent alternative to various conventional physical/chemical methods such as volatilization, evaporation, photooxidation, absorption and hydrolysis etc. used for the decontamination of toxic wastes (Park *et al*.,1990). These methods are not efficient and very expensive to employ (Morgan and Watkinson, 1984). According to Nicholas and Giamporcaro (1989), cost of bioremediation can be as little as 1 percent of off-site incineration. Off-site biological treatment system provide an excellent alternative to this problem. This is the only means to completely mineralize many toxic compounds (Atlas and Pramer, 1990) and have the following advantages:

- They are ecologically sound, a natural process
- The target chemicals are completely destroyed or detoxified into harmless intermediates finally assimilating them forming carbon dioxide and water
- Biologically-based treatment is reported to be less costly as it employs growing the microorganisms at the expense of the toxic chemicals whereas conventional methods may use costly chemicals/manpower etc.
- Operating conditions are less extreme, elaborated equipment and controls are not needed.
- Bioremediation can often be accomplished where the problem is located, eliminating the need to transport large quantities of contaminated wastes off site.

REQUIREMENTS FOR BIOREMEDIATION

i) Microbes

If active microbes are not indigenous in the waste, they must be introduced by way of inocculation. The introduced microorganisms either may be naturally occurring types or genetically engineered to attack the hazardous waste. Seeding contaminated sites with competent microflora produced in fermenters and used to speed up bioremediation is the approach known as bioaugmentation. Thomas and Ward (1989) found that the treatment of chlorinated aliphatic compounds (e.g. carbon tetrachloride, chloroform, tetrachloroethylene) generally requires mixed cultures. However, Murthy *et al*., (1988) have shown that the correct consortium or organisms is central for optimizing the degrading activity.

Genetically engineered microorganisms (GEMs) in natural environment generally do not work well when placed in competition with other microbes in a practical setting. Consequently, they eventually disappear or function very slow and inefficiently. This resulted in a general abandonment of the use of genetically engineered microbes by most bioremediation companies (Eitan and Morris, 1996). Therefore, interest are developed towards the use of microbes that have been isolated from natural environment.

In the laboratory (and theoretically in the field) it is possible to acclimate naturally occurring soil microbes to specific chemicals and eventually select out strains that can utilize these chemicals as a carbon source. This process takes time during which increasing concentrations of the chemical are added slowly. At the end of the process an enriched culture of microbes results. These enriched cultures would be useful to help destroy chemicals in contaminated soil and groundwater. In practice, things are a bit more complicated. For one thing, these selected microbes may not be able to survive when fed low levels of the chemical in soil or water. They may not be able to compete with other soil microbes or they may need additional nutrients to thrive. The differences in soil composition at each site make it imperative to tailor the nutrients added to these enriched cultures to achieve successful chemical breakdown. If the indigenous microbial population is extremely small or insufficiently large, it may be brought to proper size by the introduction of a treatment regimen based on the enrichment approach.

According to literature review, Nicolas and Giamporcaro (1989) stated that an estimated 42 different pollutants can be biodegraded. These organisms have been isolated, identified, and studied. *Pseudomonas putida* was found to remediate PCB in the soil, another species of *Pseudomonas* able to detoxify chlorinated aliphatic solvents. Whereas, *Corynebacterium* sp. mineralizes p-nitrophenyl in lake water and *Arthobacter* sp. decontaminate the chlorinated aromatics.

Several soil bacteria with the ability to degrade pesticides have been isolated for bioremediation of pesticide wastes. These include a metamitron-degrading *Rhodococcus* sp. (Parekh *et al.*, 1994), a chlorpyrifos -degrading *Flavobacterium* sp. (Mallick *et al*., 1999), an atrazine degrading *Agrobacterium radiobacter* (Struthers *et al.*, 1998), and an iprodione-degrading *Arthrobacter* sp. (Mercadier *et al.,* 1996). A consortium of degradative microbial population consisting of six bacterial species has been isolated from soil and identified as *Sphingomonas paucimobilis*, *Acinetobacter baumannii*, *Chryseomonas luteola*, *P. aureofaciens*, *P. cepacia* and *P. fluorescens* which were able to utilize diclofop-methyl as the sole source of carbon and energy (Smith and Adkins, 1996). In further studies with the soil, two *P. putida* strains were isolated which were able to utilize diclofop-methyl as a source of carbon and energy (Karpouzas and Walker, 2000). These studies suggested that the cultures of bacteria with the ability to degrade specific compounds can be used for the bioremediation of pesticide polluted sites (Assaf and Turco 1994, Duquenne *et al*., 1996, Shelton *et al*., 1996).

Microbial cultures can be designed to be operated as continuous culture, batch culture, or semi-batch cultures. Accordingly, treatment systems based on those cultures can be operated on a continuous basis, a batch basis, or a semi-batch basis. With continuous systems, activity and reproduction must be at the rate sufficient to compensate for the continuous discharge of microbes. A continuous culture has the advantage of being maintained such that its active microbial population consist principally of microbes at the most effective stage of their growth. Requirement of batch cultures are not as critical as those of continuous cultures. An advantage is that they can be operated either in a growth or in a non-growth mode. The non-growth mode is used in the production of enzymes or certain products, such that the products are less contaminated with other materials. The non-growth mode requires full-size populations. They are kept in a non-growing, but active condition by eliminating all nitrogen sources.

ii) Nutrients

Phosphorus and nitrogen are referred to as macronutrients because the synthesis of cellular tissue requires much more of these than other nutrients. Frequently, nitrogen and phosphorus are not available in sufficient amounts in hazardous waste and must be added, usually as ammonia and orthophosphate. Normally, no carbon source is required to be added to the culture in the presence of hazardous waste. However, some toxic molecules may be so resistant that their carbon is only difficulty available to the active organisms. Under such a circumstance, it may be necessary to add carbon to the extent that the active population can remain sufficiently large.

In addition to nitrogen and phosphorus, many other inorganic nutrients are needed at lower concentrations to ensure unhindered metabolism. These micronutrients include sulfur, potassium, calcium, magnesium, iron, nickel, copper, zinc, various vitamins, and others. The micronutrients should have a minimum concentration of 1 to 100 μg/L (US.EPA.,1989). In most cases, all micronutrients can be obtained by bacteria from environment and do not have to be added, particularly if the waste is a contaminated soil or one that has been in contact with soil (e.g. ground water). However, the biological treatment of an industrial process wastewater may require the addition of micronutrients.

iii. Environmental conditions

Temperature: For efficient biodegradation, the optimum temperature of active microbes are required to be maintained in the treatment system. Each species of microorganism has a distinct optimum growth temperature, and microorganisms have been isolated that are able to survive and grow across a wide range of temperatures.

Oxygen: Oxygen [O_2] availability is an important environmental factor, because most microbes capable of attacking toxic molecules are either obligate or facultative aerobes. Usually, facultative aerobes function more efficiently under aerobic conditions. Consequently most biological systems for treating toxic wastes are designed to be operational under aerobic conditions. However, within the past decade it has been demonstrated that the breakdown of certain wastes (e.g., halogenated hydrocarbons, some pesticides) can only be broken down under a combination of the two conditions, namely, an aerobic phase and an anaerobic phase in series.

The end product of aerobic processes usually are increased microbial mass, carbon dioxide, and various intermediates. Aerobic processes generally are assumed to be more efficient than anaerobic processes. At any rate, they are characterized by the foul odors that accompany anaerobic processes. Much higher temperatures also are attainable with aerobic processes. Certain anaerobic processes have the advantage of producing useful end products, e.g., methane, ethanol, lactic acid; whereas, the utility of aerobic processes usually is limited to effectiveness in treatment.

pH: The growth of bacteria depends on pH. Most bacteria grow best in a relatively narrow range around neutrality (i.e., in a range of pH of 6 to 8). Die-off typically occurs below a pH of 4 to 5 and above a pH of 9 to 9.5. In the treatment system, microbial activity can alter the pH of the surrounding environment. Examples include anaerobic fermentation, which converts organic compounds to organic acids, depressing pH. Nitrification also lowers pH, as does the carbon dioxide produced by aerobic degradation. The breakdown of organonitrogen compound can raise pH by releasing NH_4. If these pH changes are significant and not buffered, the altered pH can create a microbial growth environment that is inhibitory or toxic to the microbial populations present (Michael, *et al*., 2001).

TECHNOLOGY AND PROCESSES USED IN BIOREMEDIATION

Basically, the function of technology and processing (i.e., of treatment), is to bring the active microbes, their nutrients, and the materials to be detoxified (broken down) into close contact. This should be done in manner which maintain environment, nutritional, and operational conditions at an optimum level with respect to the needs of the active organisms responsible for breaking down the toxic component of the waste.

TYPES OF TREATMENT

Normally, hazardous wastes are processed before they are discharged into the external environment. In other words, they no longer are a threat to the quality of the environment.

i. Treatment of liquid wastes

The majority of the biological treatment systems are aerobic bioreactors that rely on aeration to support microbial growth. Several types of these aerobic suspended growth systems used for the removal of carbonaceous organic matter include:

- activated – sludge
- aerated lagoons
- trickling filter
- rotating biological contactor

Of these, the activated–sludge is one of the most commonly used method for the secondary treatment of domestic wastewater and several organics toxicants in wastewater (Grady, 1986; Hannah *et al*., 1988).

Activated sludge

In the activated sludge, the organic material in the influent wastewater is utilized by the microorganism (activated sludge biomass) for the synthesis of new cells in the aerator. Oxygen supply is required for respiration as well as to maintain microorganism activity. The effluent from aerator is purified by settling of the microbial flocs in the final settler. A portion of the concentrated settled sludge is recycled to the aerator to maintain enough microorganism amount in the system. Part of the excess sludge is regularly removed. As a result, purification occurs progressively with shorter aeration periods and the sludge become more active. Finally, a type of sludge is obtained containing more actively growing microbial cells, which can treat wastewater in a shorter period of time (Bitton, 1998). Barker and Dold (1995) observed good COD and nitrogen balances on different types of laboratory scale activated sludge system. Eliosov and Argaman (1995) reported that in an activated sludge system the degradation rate of settleable particulate matter in raw domestic wastewater was slower than that of non-settleable particulate matter, which was due to the physical characteristics of the two types of particles. Muller *et al*., (1980) observed the removal of volatile non-biodegradable organic compounds from wastewater by the activated sludge system.

Generally, the principal components of an activated sludge treatment systems are 1) Physical treatment unit for screening, grit removal, and primary settling; 2) Diffuser or mechanical aerator; 3) Aeration basin 4) Sludge separation tank 5) Sludge return system; and 6) Excess sludge disposal system.

Although, there are several modification of the conventional activated sludge process (Nathanson, 1986; U.S.EPA, 1977), but fundamentally they are all similar. Activated sludge systems have found wide spread applicability in situation where there is high BOD, TOC, or COD concentrations in the wastewater (U.S. EPA., 1989). Among the disadvantages are the need to take elaborated measures to control the gaseous emissions generated by the intense aeration characteristics of the activated sludge system. In addition, the activated sludge process is sensitive to rapid changes in environmental and operational factors.

ii. Treatment of solid hazardous wastes

Composting and land treatment (land farming) are the two most commonly used methods for treatment of solid hazardous wastes.

Land Treatment

Land treatment define as a waste treatment and disposal process whereby a waste is mixed with or incorporated into the surface soil and is degraded, transformed, or immobilized through proper management. Principal factors involved in land treatment are the characteristics of waste; presence of microbial populations capable of attacking the hazardous wastes; and the pH, moisture content, temperature, and nutrient content of soil.

Among the advantages of landfarming are: 1) Because the site is continuously monitored, the potential of wastes to migrate is sharply reduced or even eliminated. 2) Waste treated site can be used for recreational purpose. Disadvantages include the following six: 1) Waste storage may be required 2) Land farming is land intensive as well as management intensive. 3) Air and odor emissions, may become nuisances sand hazardous to the health. 4) An improperly designed and operated facility can lead to the development of adverse environmental impacts. 5) Site selection and permitting may become time consuming. 6) As is true with most treatment systems, landfarming is suitable only for selected wastes.

Composting

The principles and technology characteristics of composting in general are applicable to the composting of hazardous wastes. For composting hazardous wastes and sludges characterized by a high moisture content or an amorphous consistency would be mixed with a bulking agent. Similarly a bulking agent would be also added to dilute a bactericidal waste to a level tolerated by needed microbes. If the C/N is too high, nitrogen is added. The same applies to all other nutrients.

Compost Technology

Crop residue contaminated with pesticides or other toxic organic can be satisfactorily and safely treated by windrow composting. However, safety demand that all incoming material as well as the working environment be carefully monitored and controlled. For certain waste (toxic or resistant wastes), a well designed, constructed and operated in-vessel systems would be indicated, because they permit good control for input of solid, liquid, gaseous emissions.

In comparison with incineration, composting has following advantages: 1) Lower capital and operational costs. 2) Its impacts are less adverse. Regarding landfarming, it is safer in terms of impact upon the water, land, and air resources. 3) Retention times are shorter. 4) Land requirements are correspondingly less. An additional advantage is the destructive effect exerted on less persistent pesticides (e.g., Malathion, carbaryls) by the combination of physical and chemical factors generated in the compost process.

Composting has some definite disadvantages that sharply constrain its utilization in hazardous waste treatment and disposal. For example, costs and the work involved in properly preparing a particular hazardous waste material for composting may be significantly high. Another consideration is the cost of necessary precautionary measures (enclosure, etc.).

A consideration of the future prospects of composting hazardous wastes must take into account the advantages and disadvantages inherently characteristics of composting as compared with those inherent in physical, chemical, and thermal methods of waste treatment. It was found that the sizeable volumes of the agricultural residues involved in treatment account for the economic advantage of composting over incineration. Therefore the difficulties and expenses of incinerating hazardous waste as compared to composting them are responsible for compost option.

CONCLUSIONS

To sum up, the following points may be concluded:

- The study may be helpful in understanding the role of microorganisms involved in the biodegradation of pesticide waste.
- Study may provide knowledge of basic principles involved in the operation of in-situ or on-site pesticide bioremediation process and therefore would be helpful in designing a more efficient treatment system.
- The study will be valuable to the scientist and engineers who try to develop methods for the use of engineered and indigenous organisms in the cleanup of contaminated soils and water.

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