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APPLICATION OF GENETICALLY MODIFIED ORGANISMS IN WASTE MANAGEMENT – A REVIEW

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Mankind has suffered many deleterious consequences from improper waste management following the advent of industrialization and xenobiotic wastes. Although there are several methods for treating waste such as physical methods which includes; reuse, recycling and landfills of waste, but the use of biological agents is preferred. Genetically Modified Organisms (GMOs) are microbes employed for enhanced degradation strategy which has greater prospects over the wild microbes. These bacteria can persist and transfer the genes to other microbes resulting in unintended effects in microbial community. The development of bioluminescent suicidal GMOs could be a way out. This review advocates for more research into this important area in order to tackle the perennial problems of waste management.

Key words: Composting, Anaerobic Digestion, Activated Sludge, Biosorption, Wastes

INTRODUCTION

Human activities such as oil and gas production, fossil fuel combustion, and activities from the industries and domestic environment generate a lot of waste. Basically, waste is seen as anything that has no value and unwanted by the owner or producer. According to Barik (1), waste can be defined as organic or inorganic waste materials produced out of household, commercial activities, and industries that have lost their value in the eyes of the first owner, but which may be of great value to somebody else. Wastes are materials that people would want to dispose of even when payments are required for their disposal (2).

In the prehistoric times, disposal of waste was not a problem because the number of population was small and there were vast lands available to the population at the time (3). The environment could easily absorb the volume of wastes produced without any form of degradation (4). However as population increased more wastes were generated which caused strain on the environment.

Also the industrial revolution of 16th century particularly brought people to live together in communities or cities. The high population in cities gives rise to high output of wastes resulting in poor management by indiscriminate littering and open dumping which served as breeding sites for rats and other vermin, posing significant risks to the public (3). This led to epidemics with high death tolls (4, 5, and 6).

This ugly development necessitates proper waste management methods to ensure a safe environment. According to (7) waste management is the process by which wastes are gathered, transported and processed before disposal of any remaining residues. Poor management of wastes results in the pollution of the soil, air and surface and underground water causing a major impact on public health (8). According to Amasuomo and Baird (3) waste management is the process where pollutants are detoxified to ensure a safe environment.

Common waste management methods employed globally include re-use, recycling or composting, incineration and land filling (8). According to Troschinetz and Mihelcic, (9) some methods are better than others. Apart from being costly, physicochemical method is ecologically unfriendly (10). Respiratory failure and sicknesses are associated with emissions from incinerators (8). Specific cancers like primary liver cancer, laryngeal cancer, soft tissue sarcoma and lung cancer, enlargement of bladder, leukemia and stomach cancer are also reported amongst people living close to incinerators (8). Direct land fill causes an impact on the environment due to the emission of greenhouse gases and unpleasant odour (11). Recycling of materials also has its own merits and demerits. For example, it is cheaper to collect metal scraps than extract them from raw materials. On the other hand, the recycling of broken glass is more costly than obtaining from raw materials; it is also more energy consuming to use glass chippings to replace stones in the road or street construction (11).

On the other hand, the use of biological agents (microorganisms) in waste management has been found to be considerably efficient (12), less costly and eco-friendly. Hence, the use of microorganisms in waste treatment is alternative solution to land fill which emits greenhouse gases and unpleasant odour (11).

Use of Microorganisms in Waste Treatment

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Wastes are made up of many components, some organic and others inorganic (1). Organic materials are used by microorganisms as a source of carbon under favourable conditions (13). Microorganisms have been utilized extensively in treating organic wastes due to their high efficiency in eliminating pathogens, and accelerating the degradation process (14). Microorganisms not only remove pollutants from the environment, but also eco-friendly and more effective (15). Their activities help to restore and maintain ecology through composting and anaerobic digesting (14, 15).

Among the most common methods of microbial treatment of solid organic wastes are composting and anaerobic digestions. For wastewater (sewage) treatments (16) identified some of the methods as activated sludge and oxidation ditch membrane bioreactor, convectional activated sludge, integrated anaerobic-aerobic fixed film reactor and waste water stabilization ponds.

Biological Methods in Integrated Waste Management

Composting

It is a controlled biological exothermic oxidation of organic matter aerobically followed by a maturing phase, carried out by a dynamic and rapid succession of microbial populations (17). Composting is the process where organic waste materials are decomposed by microorganisms to produce humus. It involves mesophilic and thermophilic bacteria, fungi and actinonycetes to convert and stabilize the organic wastes to humus (18). These microbes degrade complex materials such as lignin, protein, chitin, and cellulose (12).

Cellulolytic microorganisms of the genera *Pseudomonas, Bacillus, Acinetobacter, Micrococcus, Citrobacter, Penicillium, Chaetomium, Aspergillus, Fusarium, Geotrichum* and *Bipolaris* play a vital role in the degradation of cellulose materials (14, 12) observed that the use of thermophilic cellulolytic microorganisms in composting agricultural wastes could reduce dependency on industrial cellulase, thermal and chemical pretreatments. Similarly, Zhou *et al* encouraged co-composting of food wastes and Chinese medicine herbal residues as it inhibits activities of *Alternaria solani* and *Fusarium oxysporium* which cause plant diseases.

Composting is beneficial to land (soil) in several ways including; as a soil conditioner, a fertilizer is source of humic acids and act as a natural pesticide for soil. It is also useful for erosion control; land reclamation and land fill cover (10). Compared to the other methods of waste management, composting is cheap and ecofriendly (20, 21).

Anaerobic Digestion

The organic materials used in composting can be applied in anaerobic digestion to produce biofuel or biogas (12). A mixture or consortium of microorganisms is used to degrade organic matter in the absence of oxygen to produce methane gas. It comprises of four steps: hydrolysis, acidogenesis, acetogenesis and methanogens, out of which the hydrolysis of lignocellulose materials is the rate determining step (22). It is therefore paramount to utilize a consortium of microorganisms with high lignocellulosic degradation capacity to ensure success of the biogas production, in a cost effective way (12).

White rot fungi such as *Phanerochaete drysosporium, Pleurotusostreatus, Coriolusversicolor, Cyathusstercoreus, Ceriporiopsissubvermispora*, produce lignolytic extracellular oxidative enzymes that degrade lignin, leaving the decayed wood whitish in water and fibrous in texture (23).

The white-rot fungi are the common class of fungi used for biofuel production (24). However, the white-rot fungi while in association with other microbes that degrade the hemicellulose and cellulose parts of the organic matter leading to the production of methane gas. The effluents can be used for fertilizer production.

The management of organic wastes by biogas production provides the two fold advantages of greenhouse gases minimization and renewable energy generation (11).

Membrane Aerated Biofilm Reactor

The Membrane Aerated Biofilm Reactor (MABR) process is a promising biofilm technology for treating surface water (25). Surface water becomes polluted through the discharge of sewage and municipal waste water, industrial wastewater, and over usage of fertilizers and pesticides (26). This usually leads to eutrophication, posing treat and danger to human and animal health.

In this technology, permeable hollow fiber membranes are used for attachment of biofilm and also for the supply of oxygen with high efficiency at appropriate pressure (27). As the oxygen flows through the hollow of the membrane, it diffuses along the thickness of the biofilm towards the wastewater and is utilized by microorganisms. Meanwhile, the pollutants of the waste water penetrate into the depth of the biofilm, creating a counter diffusion mechanism, enhancing the removal of the pollutants (28). MABR is applied in treatment of municipal wastewater, industrial wastewater, pharmaceutical wastewater, dyes and so on to remove carbon and nitrogen (28).

Activated Sludge

Activated Sludge is composed of bacteria, fungi and protozoa (29). It is a process whereby natural microorganisms are employed to remove organic carbon, nitrogen and phosphorus from wastewater. The organisms are cultivated in aeration tanks and they utilize the dissolved and sometimes suspended organic particles in the wastewater to grow. As a suspended growth biological treatment process, activated sludge utilizes a dense microbial culture in suspension to biodegrade organic material under aerobic conditions and forms a biological floc for solid separation in the settling unit. Typical retention times are 5 – 14 hours in conventional units. Once the dissolved organic solids are removed, the microorganisms are separated from the water through gravity settling, and the water is discharged into rivers (29, 30).

Wastewater Stabilization Pond

Conventional Biological/Biochemical Method of sewage treatment processes are used in resources limited countries (31). Wastewater is discharged into a pond where natural factors act to reduce the pollutants and pathogens. Some of these factors include; time, temperature increase, high PH (>9), ultraviolet rays and high concentration of dissolved oxygen (32). Helminthes in the wastewater also die off. When significant levels of pollutants and pathogens have been reduced, the wastewater is used for irrigation (33, 34).

Limitations of Natural Microorganisms

As noted above, when human population was small, the environment readily absorbed their wastes without degradation. However around 16th century human population increased grossly, likewise the volume of wastes produced, straining the microbes that could degrade those wastes.

In addition, during the industrial revolution xenobiotic wastes were also introduced, comprising metals and glass (35), plastics and heavy metals and radioactive compounds. These wastes required a longer time for degradation compared to the then agricultural wastes and household wastes. The natural microbes began to fall short in cleaning up the wastes given the volume, frequency and nature of the pollutants. Thus, man began to have problem with wastes and sought various ways of waste management.

Various physiochemical methods like land filling, incineration, recycling, and so many others were tried but they had their shortcomings. Bioremediation was found to be the best option due to enhanced degradation, low cost, eco-friendliness, and self-sustainability. How organisms present in nature are mostly limited by low degradation capacity and require long time for degradation especially for xenobiotic waste wastes. Genetically modified organisms (GMOs) can be a solution to the limitations of physiochemical methods. GMOs are organisms whose genetic material has been artificially modified to change their characteristics in one way or another so that they can be applied for a specific purpose such as protect crops by providing resistance to a specific disease or insect and even ensuring greater food production (36). This is achieved through genetic engineering or recombinant DNA techniques. It is used to enhance the ability of an organism to metabolize a xenobiotic through the detection of genes associated with degradation, transforming it into appropriate bioremediation agents (37).

Application of GMOs in Waste Management

The application of GMOs in waste management is called bioremediation. This is well-organized approaches which is applied to breakdown or transform contaminants to a less toxic or non-toxic element and compound (38). Bioremediation involves bioaccumulation which uses living and actively metabolizing cells and biosorption which can utilize both dead and living cells. In biosorption, the metal ions adsorb to the external walls of the microbes through electrostatic forces of attraction (39). Microbial cell surfaces are negatively charged and they bind to the cationic heavy metals and enhance their removal through biosorption.

Transgenic plants and or bacteria are used in bioremediation of polluted soils. GMOs exhibit enhanced degradability of a wide range of xenobiotics and have potential for bioremediation of wastes from various environmental sources (40). These GMOs have higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions (41). GMOs have shown potential for bioremediation applications in soil, groundwater, activated sludge environments and wastewater treatment (38).

GMOs and Wastewater Treatment

Wastewater is a mixture of many pollutants; among the most serious are heavy metals and radioactive compounds. Sources of heavy metal contamination include mining and smelting of ores, effluent from storage batteries, automobile exhaust, fertilizers, pesticides and many others (37). The metals and metalloids that contaminate water are of ecological concern and include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold and nickel (37, 39). Heavy metal contamination is of environmental concern because of their bioaccumulation and non-biodegradability in nature (42).

Heavy metals have many deleterious effects on cell structures including destabilization of the structures and biomolecules (cell wall, enzymes, DNA, RNA) thus, inducing replication defects and hereditary genetic disorders and cancers (43). In the soil, they can accumulate and persist for a long time, negatively influencing geochemical cycle (44). The negative effects of heavy metals in the environment are not restricted to fauna and flora but also to human health. According to (45, 46), some health challenges implicated with heavy metal pollution are; it disrupt metabolic activities and genetic makeup while others affect embryonic or fetal development. There is also the implication of cancer developmental disorder, neurological and behavioral changes often found in children.

Bioremediation is used to transform toxic heavy metals into less toxic forms. Bioremediation strategy is based on the ability of microbes to attract metals from contaminated sites and store them in their cells. Microorganisms including algae, bacteria, fungi and yeast are used in bioremediation.

While some wild types of microbes such as *Pseudomonas veronii, Bacillus cereus, Barkholderia* spp., *Penicillium canescens, Aspergillus fumigatus, Saccharomyces cerevisiae and Candida utilis* have been shown to remove heavy metals (37), their bioaccumulation capacity is little compared to the genetically engineered strain. For example, recent studies have demonstrated the ability of genetically modified fungi like *Aspergillus* and *Penicillium*, and yeasts like *Saccharomyces cerevisiae* to remove three times larger quantities of heavy metals (37). Bhakta *et al.* (47) also reported that species of *Escherichia coli, Bacillus subtilis, Saccharomyces boulardii*, *Enterococcus faecium* and *Staphylococcus aureus* have been engineered to remove heavy metals from water bodies with high efficiency compared to wild microbes. *Deinococcus geothermalis* have been engineered to remediate sites of radioactive wastes contamination. Coelho, Fujita and Hashimoto (37, 48) did a feasibility study for treatment of wastewater using engineered microorganisms *Escherichia coli* and *Pseudomonas putida* compared to wild strains. The result showed higher degradative activity in terms of the shortness of time used for the remediation and the reduction in the concentration of the pollutant at the end of the bioremediation process as compared to the wild strains under similar conditions (48).

The interesting thing is that, these modified organisms grow faster, withstand metal toxicity higher and other extreme environmental conditions like PH and temperature better than the wild types. These characteristics make them better candidate to remove higher quantities of metal pollutants.

GMOs have also been constructed for the bioremediation of hydrocarbon polluted sites. The first example of a genetically engineered microbe for bioremediation was *Pseudomonas fluorescence* HKAA which degrades naphthalene (49). Following this success, other strains, *P. putida* (NRRL B-5473) and *P. aeruginosa* (NRRL B-5472) were engineered for enhanced naphthalene, salicylase and camphor degradation (37).

Bioremediation involves bioaccumulation which uses living and actively metabolizing cells and biosorption which can utilize dead cells. In biosorption, the metal ions adsorb to the external wall of the microbes through electrostatic forces of attraction (39). Microbial cell surfaces are negatively charged and they can easily bind to the cationic heavy metals (50, 51 and 52).

Uses of GMOs in Remediation of Xenobiotic in Industrial Wastes

Industrialization is key to civilization and human development. However, industries produce wastes which were not known hitherto. Such xenobiotic wastes are usually recalcitrant and resistant to microbial degradation. One of such xenobiotic is 1, 2, 3 - Trichloropropane (TCP).

1, 2, 3 - Trichloropropane is a chlorinated hydrocarbon that is toxic, carcinogenic and recalcitrant (53). TCP is used in the paint industry as a cleaning agent and as intermediate for the production of polysulfone liquid polymers and hexafluoropropylene. Due to improper waste disposal, TCP often sips into underground water, posing a serious human health challenge (54).

Anaerobically, TCP like other chloropropanes can be reduced via dechlorination (55). However, no naturally occurring microbial culture has been described to degrade TCP under aerobic conditions (53). In a previous report, (54) genetically engineered a strain of *Pseudomonas putida* called MC4 - 5222 was used for TCP bioremediation under aerobic conditions. Laboratory experimentation showed 95 to 97% removal efficiency over a period of 48 days. According to the researchers, this organism is probably the best example of a genetically constructed bacterium that grows on a recalcitrant chemical.

Use of GMOs in Degradation of Plastics

Plastics are a range of synthetic or semi synthetic materials that can be molded into objects of different shapes in form of polyethylene, polypropylene, polystyrene etc. (56). Plastics are usually resistant to degradation and constitute environmental hazard especially to aquatic life and humans feeding on sea foods. They require hundreds to a thousand years to be degraded by natural microbes such as *Aspergillus glaucus, A. niger,* and species of *Streptococcus, Staphylococcus, Micrococcus* (Gram positive), *Moraxella* and *Pseudomonas* (Gram negative) (57), *Bacillus mycoides,* and *B. subtilis* (58).

Although many microbes have been identified as plastic degraders, plastics constitute a major problem of pollution in most urban centers in Africa (58) because of the long duration required for their degradation. Recently, there has been a glee of hope as researchers working in the University of Portsmouth engineered a double mutant of the enzyme Polyethylene terephthaletase, which degrades plastics in a matter of days (59). This followed a study on a newly discovered bacterium, *Ideonella sakaiensis* 201-F6, with unusual ability to use poly- (ethylene terephthalate) as its major carbon and energy source for growth reported by (60).

The researchers from the University of Portsmouth are hopeful that this engineered enzyme would be inserted into a thermophilic bacterium with capacity to degrade plastics at temperatures above 70°C.

Risks Related to the Use of GMOs

Many literatures express hope for the prospects of GMOs in several areas of human endeavors. Indeed, GMOs will be beneficial in waste management beyond what we have considered. However, the world is still skeptical regarding the field application of GMOs in waste management mainly because of two reasons: Horizontal transfer of recombinant genes and displacement of indigenous organisms (61, 62, and 63).

Horizontal Gene transfer is the acquisition of foreign genes through transformation, transduction and conjugation by organisms giving them access to new genes they did not inherit (62). This is a serious matter as unintended organisms pick up genes not expected of them. This brings about unexpected changes in their structure and function. It not only introduces a new gene to the recipient organisms, it also disrupts an endogenous gene, resulting to unpredictable and unintended effects (63). The effects could be short time and / or long time effects. Some could only be noticed as the recipient organisms become the dominant population and it could take a thousand generations (64). The effects could be diverse, from health to ecological impacts.

Apart from the risk of contaminating other organisms, the GMOs could outgrow the indigenous microbial population, leading to ecological imbalance. Nielsen and Townsend (65) frame GMOs as invasive species which will cause more harm than good. He justified his claim with scenarios from across the world.

Paull (66) reported the field trial of genetically engineered strain of pseudomonas designated HK44 for radiation purpose by the University of Tennessess and Oak Ridge National Laboratory. The *Pseudomonas fluorescens* was able to survive in harsh environmental conditions, contrary to some reports that GMOs will be unable to compete in real world conditions.

In addition, the monitoring and control of GMOs once released in the field, together with the organisms that will be transformed via horizontal gene transfer is also problematic. These and many more are some of the issues surrounding the public acceptance and field application of GMOs for waste management.

Possible Solutions and Areas of Further Research

The chief issues surrounding the field application of GMOs for waste management are their uncontrollable persistence and longevity, as well as horizontal gene transfer. To address the first issue, scientists are researching on exploring the antisense RNA- regulated plasmid addiction, proteic plasmid addiction, and inducible degradative operons of bacteria (40). This novel strategy will lead to the incorporation of suicidal genes in GMOs based on the knowledge of killer-anti-killer genes that automatically lyse the cell after the degradation of the xenobiotic. This will help to remove the GMO by autolysis thereby reducing the risk to human beings and the environment.

In addition, the experiment of (67) reported above, the *P. fluorescens* HK44 GMO released for studies was engineered to bioluminescent when physiologically active during biodegradation process. As the strain degraded the waste, it at the same time produced a bioluminescent signal that could be detected easily. This served as an effective means to monitor and control the GMO during the field trial.

As for gene transfer, one way of solving the problem is through composting (68). The elevated temperatures of composting (80-90°C), low PH (due to organic acid production), and production of toxic metabolites adversely affect the survival of the GMOs. The modified DNA released upon cell lysis can then be degraded by the extreme heat. The destruction of GMOs through composting has been widely reported in literature.

It is still pertinent to critically scrutinize the GMOs, all their possible effects (short and long term), and how to control them upon release. This needs to be done comprehensively before employing them in the field for remediation purposes. At present, their use is confined to closed and controlled systems like bioreactors and laboratories. So, environmental biotechnology has the objective of tackling and solving these problems so as to permit the use of microorganisms in bioremediation technologies. For this reason, it is necessary to support the activities of the indigenous microorganisms in polluted biotopes and to enhance their degradative abilities by bioaugmentation or biostimulation. Genetic engineering is also used to improve the biodegradation capabilities of microorganisms. Nevertheless, there are many risks associated to the use of GEM in the field. Whether or not such approaches are ultimately successful in bioremediation of pollutants may make a difference in our ability to reduce wastes, eliminate industrial pollution, and enjoy a more sustainable future. The earth is our only planet and we should not risk ruining it.

CONCLUSION

Civilization has brought with it the unwelcomed problem of wastes, particularly xenobiotic wastes. Over time, man has tried several methods to manage wastes but the most favourable method remains the use of biological agents due to their cheapness and environmental friendliness. Genetically modified organisms are attempted by human being to improve the remediation ability of the natural organisms in cleaning up environmental wastes. They have good prospects as they are faster in remediation and cheaper compared to other methods. However, their environmental friendliness has not been ascertained over the past few decades. This review therefore recommends that more research should be carried out to ascertain the most eco-friendly organism to be used in the bioremediation of wastes and to also ascertain that GMOs will not turn foes once released into our environment.

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