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Review

Biotechnology Contributions in Sustainable Environmental Development

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As wastewater is heterogeneous compound contains a lot of different organic and inorganic components especially in case of mixing industrial with municipal waste; biological treatment will face the problem of variations in quality of the plant bio-mass. Bacteria and protozoa in both municipal and industrial biological systems may be negatively impacted by temperature, toxic chemicals, high substrate loadings, or variable waste streams. A well designed and operated biological wastewater treatment plant provides an optimum environment for a microbial population to become acclimated to the incoming wastewater. As long as the basic requirements (dissolved oxygen, nutrients and micronutrients, pH and temperature) are met, along with a controlled nutrient source, the treatment objectives are usually be met. Problems arise when the plant become overloaded with a particular waste component to which the indigenous population of microorganisms cannot acclimate. This often results in erratic performance or a plant upset. Application of biotechnology and genetic engineering to find the proper strain(s) is the tool for maximization of pollutants removal. It is often stated that bioremediation is the most economically effective treatment technology available, costing at least one-third less than conventional incineration or land-fill methods. The idea of establish a microbial bank in order to provide the ideal strain(s) of microorganisms for management the removal of specific pollutant(s) beside production of materials with economic value from wastes will be discussed.

Keywords: Environmental pollution, Waste treatment, Bioremediation, Microbial additives and applications, Microbial Bank establishing and environment sustainability.

INTRODUCTION

The fresh water resources that attainable for humans consumption are poorly distributed across the globe (El-Dessouky and Ettouney, 2002), has been roughly calculated at 12,500–15,000 km^3/year (Postel *et al*., 1996) and it represents only 0.5% of the world's water (Shiklomanov, 2000; Seckler *et al.*,2003). Agricultural irrigation, industrial and domestic uses consume about 4000 km^3/year (Gleick, 1993), and is expected to increase to reach 4300–5000 km^3/ year in 2025. Fresh water supply increase is limited and expected not able to cover the high demands due to the population explosion, changes in community life style, industrial development, new lands irrigation beside the expected impacts of climate changes (Vörösmarty *et al*., 2000; Rockström, 2003; Seckler *et al*., 2003; Shannon *et al*., 2008; Godfray *et al*., 2010, Mulder *et al*., 2010). Water pollution by domestic, industrial and agricultural wastes are causes of water quality deterioration and reduction in safe water resources availability (Kemper, 2004; Foley *et al*., 2005; Coetser *et al*., 2007; Ritter *et al*., 2002; Fawell and Nieuwenhuijsen, 2003; Rodriguez-Mozaz *et al*., 2004; Falconer and Humpage, 2005). As a result of civilization, urbanization and industrialization, different and new wastes are generated and may be dumped into the environment harming the living creatures and may enter the food chain. Ecologically, all contaminants leading to imbalance in nature received global concern, but unfortunately with little/no reactions from those responsible to environmental and health protection. The natural result is continuation use of the contaminated resources without caring the adverse consequences.

Emerging/micro-pollutants e.g., endocrine disruptors, pharmaceuticals and personal care products do not respond to the traditional treatment processes and required the application of more advanced, costly and sophisticated treatment technologies. Decreasing the load of metals by dilution downstream (Bowman *et al*., 2002; Verstraeten *et al*., 2003), is not the solution, because the conventional drinking water treatment is not effectively remove the harmful residuals.

Solid wastes can result in negative impacts on health and the environment (Gupta and Mohapatra, 2003; Strong and Burgess, 2008) so, effective and efficient management program is urgently required.

Soil contamination with wastes led to losses in biodiversity. Pesticides and herbicides used by farmers represent additional sources of environmental pollution (Kumari *et al.*, 2013).

Fresh water protection against pollution through wastewater reuse requires the development of up-to-date treatment technologies to be used effectively, safely and economically. Usually, the traditional treatment systems are ineffective for removing a wide range of micro-pollutants and hence, remain soluble in the effluent (Ozaki, 2004; Servos *et. al.*, 2005; Urase and Kikuta, 2005). On the other hand, the application of membranes treatment technique for water/wastewater showed high efficiency for pollutants removal (Yoon *et al*., 2004; Yoon *et al.*, 2006), but high energy and costly membranes needed may represent a barrier against widening its application as alternative treatment.

2. Bioremediation for wastewater treatment

Bioremediation is one of the possible, cost-effective and safe technologies for overcoming the problems of environmental contamination. Through this technology, it is possible to degrade and/ or detoxify some pollutants to safe or less hazardous compounds using environmental friendly microorganisms (.Singh and Tripathi, 2007; Talley, 2005; Wasi *et al*., 2008). When contaminated material treatment is carried out at the same site, it is called in-situ bioremediation. The contaminated soil or groundwater is treated via infiltration process using oxygen or any other electron acceptor and solution of nutrients necessary for microbial activity to deal with the pollutants (Vidali, 2011). Chemotaxis is important to the study of in-situ bioremediat-ion because microorganism with this sensory phenomenon can move into the contaminated area according to the chemical concentration. The more microbial cells' chemotactic abilities, the more bioremediation process effectiveness and safety in contaminants treatment.

The bioremediation process byproducts could be assimilated by the same micro-organism used for treatment or by the indigenous microflora producing biomass or energy with production of CO_2 and H_2O.

Generally, the bioremediation process should be:

* Eco-friendly,

* Cost effective,

* Not resulted in addition of new microbial population to the indigenous flora especially in case of using genetically engineered strains affecting the biodiversity.

It is often stated that bioremediation is the most economical and effective treatment technology available, costing at least one-third less than conventional incineration or landfill methods (Zechendorf, 1999).

This review outlines the different process of bioremediation and how far it could be multi-beneficial, the fate of microbial additives in the environment, the production of bioremediation microbes, keeping the vitality and effectiveness of microbial additives, and finally a discussion of the idea of establishing a Microbial Bank (MB) to serve industrial, agricultural and home sectors will be covered.

3. Microbial additives

As wastewater usually contains both human wastes (mainly organics), mixed with industrial wastes (mainly inorganic compounds), it is possible to be classify as a heterogeneous compound. Therefore, the traditional biological treatment process may face the problem of variation in quality of the plant biomass depending on the influent composition and may be negatively impacted by temperature, presence of toxic chemicals, shock loadings, or variable waste streams, then the indigenous population can't acclimate and the plant may enter a case of upset. In order to have an adapted microbial population for the incoming waste to achieve the treatment objectives, providing a well designed and operated biological treatment processes beside the optimum environmental conditions for the microbial activities are essential.

Adaptation, selection and application of genetic engineering biotechnology are followed to collect a group of microorganisms that have the superiority in capability to solve certain pollution problem(s). This represent the main objective of establishing the MB in order to provide the proper and strong strain(s) in order to solve specific problem of treatment failure or maximization the pollutants removal from the waste before discharging on the environment. The application of these microbial additives in wastewater treatment plants besides improving the effluent quality it may decrease treatment process detention time and consequently increase the treatment plant capacity.

4. Regulatory approach towards application of microbial additives

The application of genetically engineered microorganisms (GEMs) for bioremediation is controversial. The U.S Environmental Protection Agency's (USEPAs) risk-based regulatory approach not encourages the application for engineered products in bioremediation (Miller, 1997). The entry of new genetically engineered microorganisms to the environment may affect the natural balance between the natural microorganisms. The USEPA Office of Pollution Prevention and Toxics regulates on a broad basis the production and application of GEMs through its Toxic Substances Control Act (TSCA) (Drobnik, 1999). It is supposed that introduction of foreign genetic materials in GEMs will increase the energy requirements and decrease the level of competence, considering the vast arrange of both biotic as well as abiotic factors (Giddings, 1998) which discourage deriving a competent modeling scheme for GEMs. To an extent that appears true, but the problem is further confounded by other issues over the perceived need for engineered organisms in bioremediation and cost competitiveness with other technical solutions. These problems are true not only for engineered microorganisms but also for application of natural organisms for use in areas such as trichloroethylene bioremediation (Kato, Davis, 1996). Anyway, genetic systems are numerous and through them it is possible to serve the development of bioremediation technology (Timmis and Pieper, 1999). For example, through control/change of metabolic pathway in microorganisms genetically it is possible to increase degradation rates, or orient the process to a new metabolic pathway. In addition, genetically engineered microorganisms could be environmentally used for monitoring, control toxicity and stress response (Menn et al., 2008).

Field release of GEM (Pseudomonas *fluorescens* strain designated HK 44) to be applied for bioremediation purposes has achieved firstly by University of Tennessee in collaboration with Oak Ridge National Laboratory ,USA (Ripp et al., 2000). The origin of the GE strain was gas plant facility heavily contaminated with polyaromatic hydrocarbons (PAHs). The plasmid responsible for catabolism of naphthalene (pUTK21) was introduced to form the GE strain (King et al., 1990). Additionally, strain HK44 contains a transposon-based bioluminescence-producing lux gene fused within a promoter for the naphthalene catabolic gene (Chatterjee; Meighen, 1995). Therefore, strain HK44 is capable of sensing to naphthalene and/or for intermediate metabolite salicylate as environmental contaminants and responding through detectable bioluminescence signaling.

5. Applications of biotechnology in the form of microbial additives for better life

5.1. Agriculture

The application of microbial additives was extended to cover a wide range of life aspects included the mechanistic basis for beneficial effects. Their effect may include degradation processes with or without intermediates production such as organic acids, amino acids, vitamins, phytohormones and growth regulators. Therefore, the beneficial effects in the agriculture field include soil fertility improvement and promote plant growth and crop yield (Iwahori and Nakagawara, 1996;Yamada and Xu, 2000), increase seeds germination, inhibit harmful pathogens, promotes decomposition of organic matter; plant materials that reducing the undesirable effect of undecomposed materials and raise cell activity; root cells division (Bhoyar *et al*; 2013).

5.2. Sewage treatment

Generally, the microbial additives are more than one microorganism, when works together they are able to lowering the pollutants load (Higa and Wididana, 1991). After their introduction into waste treatment process, a period of time is needed to be adapted and at the same time they may face competition by the indigenous organisms. The added organisms should be able to exponentially grow in order to perform their role perfectly. The expected results are improvement of effluent wastewater quality, beside increase the efficiency and effectiveness levels of the treatment processes.

5.3. Industrial wastes

Due to the high population growth rate, production of food, agricultural products and fiber were increased to cover their needs. Industrialization, new industries, and changes in populations standards of living, all demands increase in daily use of new chemicals and water which should be associated with increase in production of different wastes that may be release to the environment causing serious pollution problems. Most pollutants in industrial wastes, for their health impacts, standards are regulate their presence in the discharged effluents on water streams. Some of these contaminants are biodegradable while others are non degradable and toxic such as insecticides and heavy metals. The traditional techniques for the removal of metal ions from industrial wastes are incapable of reducing concentrations to the level recommended by the regulations or prohibitively expensive. Discharged non degradable materials into aquatic systems may be accumulated in water and/or sediments. The negative impacts of metals pollution on ecosystem function vary significantly and are of economic and public-health importance. Generally, environmental awareness is increased and pollutants release becoming increasingly strict, leading to urgently need for new and sophisticated advanced treatment technologies (Gadd, 1992). Biological activities may be responsible on undergo biotransformation and/or immobilization for metals resulting in stunning effects such as metal precipitation (Watterson, 1992). Biotechnological approaches to the recession of toxic metals pollution consist of selectively using and enhancing these natural processes to treat particular waste. The processes by which microorganisms interact with toxic metals are very diverse (Scott and Palmer, 1990).

5.3.1. Heavy metals and biological treatment

The use and application of biotechnological approaches to the regression of toxic metals pollution on biological wastewater treatment (Cimino and Caristi, 1990) is today an attractive technique. In practice, there are three general categories of biological process for treating liquid wastes containing toxic metals namely; biosorptiion, extra-cellular precipitation, and uptake by biopolymers and other molecules originated from microbial cells.

Biosorption is used to include uptake by whole living or dead biomass via physico-chemical mechanisms such as adsorption or ion exchange. For living biomass, the process is dependent on metabolic uptake mechanisms, while under certain conditions it is mainly metabolism-independent process (Tobin *et al*., 1988). Usually, the main site of metal accumulation is the cell wall (Volesky, 1990). Exposure of the microorganisms to environment polluted with a toxic heavy metal, an initial reduction in microbial numbers and species diversity was observed (Thomas *et al*., 1980), this was followed by development of resistant population (Tyler, 1981). Therefore, the success of biosorption process is dependent on application with effluents containing sub-toxic concentrations of metal.

The other approach is to use adapted strains or genetically engineered strains to resist the high concentration of toxic metal that may exist in the waste. Such environments are subjected to a myriad of toxic xenobiotic compounds that challenge the integrity of the indigenous flora. Typically, the community responds giving

a removal efficiency of ≥90%. A fixed-bed double canister containing about 20 kg of biomass granules is used for small volumes (≤15 L/min), whereas a large, fluidized pulsed-bed system containing 80-90 kg of biomass is used for large flows (≥35 L/min). After loading, metals are deprived from the biomass using sulphuric acid, sodium hydroxide of complex agents, and recovered using chemical methods. The granules are regenerated by alkali treatment for reuse (Whitlock and Smith, 1989).

Immobilized particles, of diameter 0.7-1.3 mm, containing *Rhizopus arrhizus* biomass with 12-23% added polymer gave improved uranium removal at low polymer contents and lower particle diameter (Tsezos and Deutchmann, 1990, Volesky and Tsezos, 1981). Biomass from Cyano, yeast, algae and plants was used effectively as beads for Zn^{+2} adsorption. Hydrochloric or nitric acid was used as eluent, and the biosorpent can be reused for more than 120 cycles (Brierley, 1990). Such complex systems clearly utilize other mechanisms, such as precipitation and complex systems depend on precipitation, entrapment beside biosrption has been used to concentrate the metals forming sediment and reducing the environmental mobility.

Hydrogen sulphides (H_2S) from sulphate-reducing bacteria (SRBs) forms metal sulph-ides which precipitate (Brierley, 1990). Sludge-blanket reactor using SRBs with ethanol as the growth substrate is recommended in that respect. Methanogenic bacteria activities can remove acetate produced by SRBs, leaving an effluent with low BOD. Excess H_2S is possible to be removed from waste gases using a $ZnSO_4$ solution. Cells of *Citrobacter* sp. have

a surface-located acid-type phosphatase enzyme that release HPO_4 from a supplied substrate, such as, glycerol 2-phsphate, and precipitates divalent cations (M^{2+}) as $MHPO_4$ at cell surfaces (M = Ca, Ba, Pb, Sr or Sn). This process may have a potential application where phosphate-containing organic substrates are present in metal containing effluents (Macaskie, 1990).

Transformation of metal and metalloid species by oxidation/reduction, methylation and dealkylation can be done by microorganisms (Gadd, 1992). Continuous cultures of mercuric reductase bacterial species can reduce Hg^{2+} to Hg^0, which is volatilized (Wilkinson *et al*., 1989). Also, organomercuricals may be detoxified by organo-mercurical lyase followed by mercuric reductase (Gadd, 1990). Many bacteria, algae, fungi, and yeasts can reduce Au (III) to elemental Au (0), and Ag^+ to elemental Ag^0 , which is deposited on culture vessels (Kierans *et al*., 1991). Fluidized beds of alginate-and polyacrylamide-immobilized algae; for example, *Chlorella vulgaris* and *Spirulina platensis* have been used to remove a variety of metals including Cu, Pb, Zn, and Au, from mixtures, and several schemes for selective recovery have been devised (Harris and Ramelow, 1990). Microbial transformations of arsenic and chromium species are also associated with detoxification process and may have a role in wastewater treatment (Williams and Silver, 1984). Treatment of arsenic contained waste with arsenite oxidase-producing bacteria, which stimulate the conversion of arsenite [As (III)] to arsenate [As (V)] can improve certain removal methods of arsenic from waste, since As (V) is precipitated more easily by Fe^{3+} than As (III). Chromate ($Cr\ O_4^{2-}$) reducing bacteria, for example, *Enterobacter cloacae*, are resistant to high chromate concentrations and can reduce anaerobically Cr

O_4^{2-} to precipitated Cr (III) (Fuji *et al*., 1990). A fed-batch process and dialysis cultures were both effective for CrO_4^{2-} detoxification (Komori *et al*., 1990). Microbial dealkylation of organometallic compounds such as organotins can result in the formation of ionic species which could possibly be removed using a biosorptive process (Volesky, 1990). Biomethylated metal derivatives, although toxic, are often volatilized and eliminated from a system (Trevors, 1986).

Practically all biological materials have a high affinity for toxic metals (Beveridge, 1989). Several mechanisms by which metals interact with microbial cell walls envelopes are well established (Lovely *et al*., 1991); however, some biomolecules function specifically to bind metals and are induced by their presence. For example, carboxyl groups of the peptidoglycan in Gram-negative organisms are the main metal-binding site (McLean and Beveridge, 1990). Some bacterial species such as *Klebsiella*, *Enterobacter aerogenes*, *Arthrobacter viscosus* and *Pseudomonas* form capsules or slime layers (exopolymers) which composed of polysaccharides, glyco-proteins and lipopolysaccharides (Scott and Palmer, 1990), has metal binding capacity through association with protein. Generally, a correlation exists between high anionic charge and metal complexing capacity. Additionally, there may be deposition the metal in a chemically altered form (Beveridge, 1989). The application of both genetic and protein engineering could lead to peptides or other biopolymers with enhanced metal specifically stability and other useful properties. Specific metal-binding proteins and peptides have been recorded in yeasts (Winge *et al*,., 1989).

Other molecules with significant metal-binding abilities, for example, fungal pigments melanin, may be over-produce as a result of exposure to sublethal concentrations of metal and may act as sites for metal binding (Bell and Wheeler, 1986, Gadd and DeRome, 1988, Volesky, 1990). Many fungi have high chitin content in their cell walls, and this polymer of N-acetyl glucosamine is an effective metal biosorpent (Tsezos and Volesky, 1981). Chitosan and other chitin derivatives are also having a significant biosorptive capability that can be enhanced by chemical treatment (Wales and Sagar, 1990). Metallothioneins are small cysteine-rich polypeptides that can bind essential metals, such as Cu and Zn as well as non-essential metals such as Cd. They mediate Cu resistance in *Sacchromyces cervisiae* (Fogel *et al*., 1988) and also bind other metals (Berka *et al*., 1988).

As summary, the increasing contamination of wastewater by metal ions is worrying environmental problems because they are non-biodegradable, highly toxic and have a probable carcinogenic effect. If directly discharged into the sewerage system they may seriously damage the biological treatment as well as make the activated sludge unsafe for application as fertilizer (Madoni *et al*., 1996). The removal of metal ions from the effluent via traditional techniques, such as lime precipitation, is unable to reduce metals concentration to the safe values recommended in the reuse guidelines.

Other treatment technologies that should be followed such as ion exchange, activated carbon, and electrolytic treatments are costly. The use of microorganisms fo treating wastewater containing toxic metal ions is an attractive technique (Akthar *et al*., 1996). It is clear that some microbiological methods for treating metal containing wastes offer potentially efficient and cost-effective alternatives or supportive to existing treatment technologies (Kuyucak,1990). Research has proved that many microorganisms possess different detoxification mechanisms that make them capable to resist and even grow in the presence of toxic metals at high concentrations. Among the detoxification mechanisms there are chemical transformations to more volatile compounds or to ions with different valences, accumulation of dissolved and particulate metals via biosorption and/or bioprecipitation. The ability to accumulate metals is further attractive for the recovery of valuable or economically important metals from wastes (DeRome and Gadd, 1991). These different detoxification mechanisms may be exploited to remove toxic metals from effluents to reduce environmental deterioration. Whether these, or other, biotreatment realize their full potential depends on further investment and exploitation by receptive industries.

5.3.2. Organic pollutants

Two examples will be considered as organic pollutants that need more efficient and reliable biological treatment processes.

5.3.2.1. Coking plant industrial waste effluent (Phenolic compounds)

Phenolics may be present at concentrations as high as several grams/ liter in coking plant effluent, with phenol (70%) and *p*-cresol (25%) of the total phenols (Ganczarczyk, 1979). The interaction between waste constituents is important to choose the more efficient and reliable biological treatment processes. It is known that many *Pseudomonas* spp. has the ability to utilize different organics including many aromatic and phenolic compounds as growth substrate beside their wide activity that makes it the first choice for application in waste treatment. For example, Bettmann and Rehm (1985) investigated the use of gel-entrapped *Ps. putida* in a continuous-flow, fluidized-bed bioreactor for the treatment of phenol and mixed phenolics. At a sufficiently high aeration rate (5 VVM) and at 14.9 h as mean residence time, complete degradation of total phenolics was observed when feeding a mixture of phenol (1 g/l), *O*-, *m*-, and *p*-cresols (100 mg/L of each) and 4-chlorophenol (130 mg/L). Clarke and Ornston (1975) discussed the regulation of metabolic pathways in *Pseudomonas*. Two important characteristics of the *Pseudmonas* include firstly, convergence of catabolic pathways which allows the efficient utilization a wide growth substrates, and secondly the non-specificity enzyme induction that allows the simultaneous utilization of several similar substrates without an excess of reductant genetic coding for enzyme induction. Evidence for the non-specificity of enzyme induction comes from observing the lag time for growth after changing the growth substrate. Claus and Walker (1964) found that toluene-grown organisms were able to oxidize toluene, benzene, catechol, 3-methylcatechol, benzyl alcohol, *o*-cresol and *m*-cresol. Sala-Trepat *et al*. (1972) reported that enzyme production induced by phenol and cresols was non-specific. The dehydrogenase involved in the oxidation of phenol and *p*-cresol was found to be induced by *o*-and *m*-cresol but nonfunctional to metabolise them. The catabolic pathways for phenol and *p*-cresol have been illustrated and are essentially identical (Clarke and Ornston, 1975).

The biodegradation of different organic pollutants by different *Pseudomonas spp* can be expected to proceed with different rates controlled by many factors including, the pollutant composition, temperature, salinity, pH, beside the availability of inorganic nutrients and oxygen (Anonymous, 1967). In addition, substrate utilization rates cannot be assumed to be independent of one another, especially if there was a rate controlling enzymatic reaction at the convergence of the catabolic pathways. The types of organic materials in which a microbial community was previously living can play an important role in determining the response of the same community to new compounds. By another words, previous exposure to an organic compound will often increase the rate at which a microbial community can degrade it. This has been demonstrated with both pollutants (Haller, 1978) and natural substrates (Hollibaugh, 1979). However, very little research has been conducted which has characterized the effect of exposure to organic substrates other than the compound of interest on the rates of degradation of that compound, except in highly polluted environments. The organic exposure history of most aquatic microbial communities is dominated by naturally occurring substrates (amino acids, carbohydrates, and fatty acids) which are easily assimilated by aquatic microbial communities (Seki, 1982).

5.3.2.2. Waste of the kraft pulping process

The largest industrial process for the delignification of lignocellulose is still the kraft pulping process. Despite vigorous research efforts in the past, the economic viability of biological hydrolysis of lignocellulose has yet to be explained (Ladisch and Tsao, 1986).

In the kraft pulping process, lignocellulose is broken down chemically to produce fiber with the resultant production of large amounts of monomeric carbohydrates.

Glucoisosaccharinic acid (GISA) is a major by-product of kraft pulping, it is a deoxyaldonic acid produced from alkaline degradation of glucose, glucomannan, and starch (Sjostrom, 1981, Reintjes and Cooper, 1984). Presently these compounds are burnt during the recycle of inorganics, but they may have greater economic value as substrates for bioconversion to useful chemicals using bacterial isolates that are capable of CISA utilization as the sole carbon source.

6. Microbial Bank (MB)

6.1. Technology

The application of biotechnology is the way to solve the problem that occupy the interest of all environmentalists that is the high negative impacts resulting from the discharge of untreated/ insufficiently treated wastes on the environment and all creations. In the natural environment, microorganisms and their enzymes produced playing significant role in biodegradation and metabolizing of different compounds producing energy and biomass. The enzymes facilitate the phase of metabolism of complex compounds into simple ones (catabolism). This in turn needs the process of food conversion into energy supplying the microorganism. Other group of microorganisms has the ability to uptake/transform the industrial toxic pollutants. Use of state-of-the art biotechnology to produce selectively adapted and/or genetically engineered strains with pollutants removal superiority as well as the beneficial microorganisms for the industrial sector and our daily life, needs the availability of the following characteristics in the strains produced:

*High efficient that consistently produce better results in biological treatment systems under wide range of variations in waste characteristics and environmental conditions.,

*can breakdown/remove a broader spectrum of pollutants at higher rates than the indigenous microorganisms and produce high quality effluent,

* can compete with the environmental indigenous organisms,

*have the ability to resist, transform, precipitate, adsorb or uptake heavy metals,

*have the specificity of enzyme induction systems and the convergence of catabolic pathways characters,

*have the ability to logarithmically reproduce,

*genetically stable and health, environmentally safe,

*easy and safe to handle,

*can serve industrial byproducts as a substrate for microbial growth and produce valuable products.

Genetic engineering or molecular breeding which has recently developed in biotechnology field, is an attractive and effective way for transfer and collection of the required effectively characters from the selected and acclimated strains in one or more strains (transfer and recombinant plasmids) in order to maximize their applications (Fujita *et al*., 1991). The stability of the recombinant plasmid in the host strains, which is one of the most important problems in application of GEMs should be investigated. Production and providing the market with different groups of effective microbial additive that can handle the problems of pollution and environmental protection should be performed through an accredited entity that is proposed to be the MB.

6.2 Main Objectives Highlights

The MB is suggested to be established in order to serve and assist in minimizing/ /eliminate the negative pollutants impacts on the environment and creations. That may be achieved through the application of science and biotechnology for providing the wastewater treatment investors with selected, high active microbial strains that can work on specific compound(s) in the presence of suppressive materials. Helping to find out the more specific organisms for a specific contaminant in order to maximize the waste treatment processes and reduce the pollutants load in the effluent would leads to widening the base of wastewater reuse besides increasing the capacity of wastewater treatment plants and reduction of waste treatment problems. Production of other beneficial microorganisms that could serve the industrial, agricultural as well as daily life sectors (section 6.3) are other aims for the MB project.

To reach these objectives successfully, **a** group of biologists, genetic engineers, engineers, economists as well as technologists should work together for designing the production line, testing the product efficiency under adverse environmental conditions and shelf life products stability and helping, providing the advice and products developments.

6.3 Products

The products are multi-strained microorganisms similar to that found in the environment. They are usually harmless, in concentrated forms, perform tasks that preciously could only be done by harmful chemicals. However, unlike traditional chemicals, digestant microorganisms leave behind only harmless products or carbon dioxide and water.
The MB products can cover the following applications:

- **Odor control**. Bioactive products that digest the organic materials that causes of bad odor .in public restrooms, kitchens, carpets, wastewater treatment plants, and all porous surfaces.
- **Drain opener and maintainer**. It consists of high powered bacterial cultures may be combined with fast acting free enzymes. The product keep all drains free flowing by digesting the collected organic matter in drains.
- **Grease trap maintainer**. Greatly reduce odors and the need for frequent grease trap pump outs by using the bacterial additives that are able to digest grease, fats, and organic matter.
- **Septic tank maintainer**. Restore and maintain the biological activity of septic systems and cesspools by utilization the powerful waste digesting abilities of specifically selected microorganisms.
- **Toilet treatment.** Human waste and control odors treatment in portable toilets as well as airplanes, boats, trains, camps toilets has achieved with the powerful synergistic action of waste digesting microorganisms and fast acting free enzymes.
- **Animals waste control.** High potency digestants, designed for farm animal manure pits, lagoons, feedlots and pens would eliminate odors, reduce build-up of solids and increase capacity, will make pumping easier.
- **Wastewater treatment.** A bio-formulations will break down organic waste, control odors, reduce BOD, COD and TDS, increase the treatment capacity of units and produce better effluent quality.
- **Compost accelerator.** A blend of microorganisms and nutrients will breaks down lawn clippings and yard waste into beneficial compost, while minimize offensive odors..

6.4 Implementation

Microbial additives have been determined to be a feasible, low capital means of expanding both the treatment capacity and efficiency of the treatment facilities. These actions are required to bridge the gap between the "feasibility" and "implementation" stages of MB project to provide the investors with their needs.

The following steps should be followed in order to have successful applications results:

* Define the steps to implement a bioaugmentation program (system survey detailing the current design and operating conditions of the plant, the basic requirements for a biological plant, dosage recommendation).

*A study should be carried for the operational parameters in order to establish which type of bioreactor is the most efficient.

*The MB should produce microbial strains on economic basis and provide the client with clear instructions for successful application beside the necessary of precautions taken to have an effluent complying with the guidelines.

6.5 The Market

The products market is expected to be regionally firstly and will be expand to international level considering the issue of differences in environmental conditions from country to another that possible to have a negative impact on the microbial activities. Their application includes and covers many targets that aimed to eliminate the negative impacts of wastes on health and the environment. The MB will supply the rapidly growing sectors with the biological products that can solve their problems. Industrial sector would be involved as user for solving the industrial wastes problems in order to comply with the environmental laws.

6.6 Keys to the Success

 Developing groups of local and natural selected strains that are able to work together for the partial/complete degradation of different waste categories to less harmful compounds or CO_2 and H_2O or assimilation forming biomass beside that they should be effective for long time intervals and under different and harsh environmental conditions.

 Production the concentrated mixed strains in the form easy to be handled, need minimum preparation steps, and low production cost.

 Maximizing the shelf life of the selected strains by:

• Keeping the product in an airtight container,
• Keeping it stored out of direct sunlight,
• Storage at room temperature or in a cool place,
• Keeping it away from chemicals,
* Check the pH of the product, it should be within the range recorded on the label,
* Preparation should be carried according to the producer instructions. *Genetically stable as long as possible.

 Enhance the product reproduction rate by providing the essential nutrients needed to enter the logarithmic growth phase and to select the species that have low needs for specific nutrients.

 The selected microbial species should be safe (non pathogenic or opportunistic pathogens) and environment friendly.

 Have high competition ability with the indigenous organisms to maximize its persistence during the treatment process.

 Developing a scientific office and advanced laboratory for coverage the research needed, finding new strains and providing the advice and training for the consumers.

 Development the niche market allover the world and study their needs, environmental problems they face and wastes produced at present and in the future beside the environmental laws.

 Maintaining low overhead costs by monitoring and scheduling the product-ion.

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