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Microbial Diversity: A Key Driver of Environmental Biotechnology

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Abstract: *Microorganisms have been part of man's environment from the earliest times. In the past microorganisms were seen as enemies. However, a better understanding has improved our perception of their diversity and utility. Molecular biological techniques, particularly the study of small sub-units rRNAs and the respective genes, have provided new insight into microbial diversity confirming the inadequacy of our initial knowledge and estimates. New molecular techniques have enhanced our ability to investigate the dynamics of microbial communities and utilize them for the benefit of man and the environment.They are applied to wastewater treatment, remediation of contaminated environment, recovery of toxic heavy metalsfrom aqueous solution, and Microbial Enhanced Oil Recovery (MEOR).Their potential is tremendous and could be further enhanced by application of recombinant DNA techniques and advances in genomics. Microbial diversity has become friends in environmental management.*

Key words: *Bioremediation, Phytoremediation, MEOR,*

1. INTRODUCTION

Microorganisms are vital components of all ecosystems and have been playing very important role in the environment from time immemorial. Evolution has it that microorganisms made it possible for the early CO_2-dominated atmosphere to slowly change into one with a high proportion of oxygen, enabling the formation of conducive conditions for the evolution of all higher forms of life, particularly for the photosynthetic activity of plants [2] which formed the basis of the food chain on which other forms of life are sustained. However, our knowledge of microbial diversity was mainly limited to their small sized nature which hampered probe into their existence. The invention of microscope by Han and Zacharias Jansen, and the development of culture techniques by Robert Kock were major in our early understanding of microorganisms and microbial diversity. These discoveries supplied tools that were central to the development of bacterial taxonomy. The magnitude of microbial diversity discovered using these tools, as high as it may look, have been proved rudimentary when compared with the microbial diversity being observed using more modern techniques. The statement that approximately 4, 12, and 5% of all viruses, bacteria and fungi (Table 1) are already known can be nothing but a grouse underestimation of actual microbial diversity [1]. Our perception of microbial diversity has changed with the discovery of better methods of study of microbial diversity. Molecular biological techniques, and particularly the study of small sub-units rRNAs and the respective genes, have provided new insight into microbial diversity confirming the inadequacy of our initial knowledge and estimates. As illustration of this explosive discovery is the division, 250 rDNA sequences have been reported that define at least eight major subdivisions [6]. New bacteria or sludge capable of degrading chemicals previously known as non-biodegradable are also being detected every year[17].

A) Microbial Diversity

Because of their physiological diversity, microorganisms play major roles in the cycling of chemical elements within the biosphere [24]. These microbes break down and transform wide variety of inorganic and organic compounds to obtain energy for their growth. The wide range of ecology in which microbes are found has further increased the variety of substrates that could be utilized by them and today biotechnology has taken advantage of this attribute in environmental management. Among microorganisms are thermophils, mesophils, acidophils, chemophiles, etc [3]. In the past, microorganisms has been utilized in the treatment of waste (composting) and food processing, today however, biotechnology application of microorganisms to environmental management is vast and cover such areas as waste water treatment, bioremediation of contaminated sites, mineral extraction, environment monitoring, etc.

Table 1: Known numbers of microbial species and of some higher organisms (modified after Bull et al., 1992 in Avgustin G., 1998).

Group	**Known Species**	**Group**	**Known Species**
Viruses	5,000	Insects	800,000
Bacteria	4,760	Fish	19,000
Fungi	69,000	Birds	9,198
Algae	40,000	Mammals	41,70
Protozoa	30,800		

B)Microorganisms, Metal Solubilization and Environmental Management

Removal of metals from industrial waste water has, conventionally, been accomplished mainly by precipitation, ion exchange and electrolytic technologies [4]. The application of microbial biomass to the recovery of toxic heavy metals from an aqueous solution has been noted in recent decades [16] and more recently been used effectively for the removal of metal from industrial effluent ([30],[28],[25], [26])Several microorganisms such as yeast, fungi, algae and bacterial are able to take-up or accumulate heavy metal ions through the mechanism of metal-attraction of their cell wall or metabolism dependent intracellular metals uptake. Some important microorganisms that employ either of these techniques in the uptake of heavy metals and are being used in solubilisation process belong to the following genus, Acidithiobacilus sp. (*A.*ferrooxidans, *A. thiooxidans*, *A. Caldus*), Leptospirillum sp. (*L. ferrooxidans*, *L. thermoferrooxidans*, *L. ferriphilum*), Acidihpilum sp. (*A. acidiphilum*) [25], *Rhyzopusde[30], lemar,Aspergillusniger*[23], *sulfobolus,* Penicillin species (P. *chrysogonium* and *P. lilacinum*[22],and others. These microorganisms are able to oxidize metal sulphides to soluble metal sulphates which are washed out to extract the solubilized metals. Some organisms have conversion rates of organic acids reaching up to 90% of the carbon supplied[15]. Environmental biotechnology has taken advantage of the discovery of these organisms in the development of metal extraction process (mining) and also the remediation of metal contaminated sites (bioremediation). One of the leading applications of this technology is bioleaching. It is the process of using microorganisms to separate valuable metals from surrounding rocks and it is useful for recovery of valuable metals from low grade ore. In this process the microbes attach themselves to the sulphide crystals into soluble sulphates therefore dissolving the metal or play a catalytic role by accelerating the re-oxidation of ferrous iron to ferric iron which usually takes place very slowly in the absence of bacteria [2]. Some microorganisms could solubilize a range of metals for example *A. ferroxidans* and *Aspergillusniger* could solubilize copper, zinc, iron and nickel, while others can only effectively act on a single metal [27]. Some of these microorganisms are also not very effective at the predominant conditions within the processes there are applied. In microbial extraction of metals, two major processes are used; the irrigation process and the stirred tank process. In a stirred tank process, using *A. ferrooxidans*, *A.thiooxidans*, and *Leptospirillum* ina south African plant, it was found that *A.thio*oxidans and *Leptospirillum* were more effective than *A. ferrooxidans*[25]. Biotechnological tools are being applied to develop ‘supper bugs’ that are suitable for use in these processes taking advantage of microbial diversity. Using recombinant techniques, *A. ferrooxidans* could be engineered to cope with the conditions predominant in stirred tanks and would be more effective than *A.Thiooxidans* and *Leptospirillum*, since it already possess the ability to solubilize more metals. Also it has been found that metal solubilization process is more effective at higher temperature, however very few of the microbes with bioleaching potentials could strive well at such high temperatures as 70°C. Microbial diversity and environmental biology are expected to provide improvements in this direction through recombinant techniques.

Use of microorganisms in mineral extraction from ores, refractory gold or recovery of valuable metals from mining waste has gained acceptance and much popularity. The mere fact that this process has the potential to lower overall processing costs for current and future operations, increase the resource base by treating marginal deposits. Also, it lessons environmental concern by reducing emissions and impact on the landscape which is very contributory to the level of adaptation of the technology.Canada was in the forefront of early research in the application of microbial solubilization in mining in the 1970s and 1980s. however the first plants were built in south Africa, many others are found in Australia, Brazil Ghana and Peru with Ghana having the largest processing plant consisting of 24 tank units of 1,000,000 litres each [25]. The future will witness much more application of bio-mining, however with more shift to hotter processes, requiring thermophilic microorganisms as it has been recognized that biomining processes are more effective at high temperatures of 70°C and above.

2) THE ROLE OF MICROORGANISMS IN REMEDIATION OF POLLUTED ENVIRONMENTS

Nature has an enormous potential to cycle materials and energy [17], using its abundant microbial diversity. There are innumerable microbes under the basic categories of bacteria, yeast or fungi which degrade or able to transform chemicals and compounds into simpler, more desirable, or less harmful or toxic substances. Some microorganisms and microbial communities have developed the ability to process recalcitrant, often xenobiotic compounds that do not form part of their central metabolism [7], into compounds which they can take up as nutrients for energy and growth. Species of *Acinectobacter* are known to be involved in biodegradation of a number of different pollutants such as biphenyl and chlorinated biphenyl, phenol, benzoate, crude oil, acetonitrite, and in the removal of phosphate or heavy metals [8]. Some other examples of important microbe with biodegradation ability include *Pseudomonas, Alcaligens, Sphingomonas, Rhodococcus, Mycobacterium* (degrading pesticides and hydrocarbons), *Phanaerobchaetechrysosporium*[31], *Acinectobacter sp*. [23]. This attribute of microbes forms the foundation of bioremediation. Bioremediation is a naturally occurring bioprocess that harnesses microbial and geochemical process to degrade or transform environmental contaminants.The problem of toxic wastes and environmental pollutants has become enormous due to industrialization. The conventional techniques used for remediation have been to dig up contaminated soil and remove to landfills, or to cap and contain the contaminated areas of the site [31]. This method did not solve the problem of detoxification of the contaminants and with time became more unacceptable to the society that has become more environmental safety conscious. Use of chemicals or high temperature incineration proved effective in the reducing the level of range of contaminants but involve complex technologies, are expensive and are fast loosing public acceptance, particularly incineration (ibid). Bioremediation offers great potential to destroy or at least render harmless various contaminants using naturally biological activity. The fact that this a low cost technique using naturally occurring organisms has made bioremediation more acceptable than other techniques. However, this process of cleansing could be slow and might not be able to completely remove the contaminants from the environment. The multitude of microbial diversity has provided an endless list of organism and potential organisms that could metabolize pollutants and could be applied in this process for environmental management. Apart from the fact that microorganisms adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in fresh water and hyper-saline water, in aerobic and anaerobic conditions, which has enabled them to survive and utilize substrates in unexpected environments such as hot springs, hyper-saline lake, and ocean floors, improved detection and isolation technique has improved the rate at which new useful microorganisms are being discovered for use in bioremediation. Combinatorial biotechnology is expected to further enhance the potential of microorganisms for use in bioremediation by creating microorganism with novel properties. This could be achieved through transfer by conjugation into a recipient strain plasmids that carry genes for different degradative pathways which will result in recombination forming a single larger “fusion” plasmid with combined function, if the two plasmids contain homologous regions of DNA [9]. Other genetic engineering techniques could also be applied as well. Genomics is expected to play a major role here through compilation of list of important genes in bioremediation, accurate identifications of gene functions, and forecast of possible combinations. The increasing number of microorganism with complete genome sequences particularly, those with biodegradation abilities will also be advantageous for utilization in further development of bioremediation.[7]was the first to create strain of bacteria with expanded degradative ability “super-bug” for bioremediation in 1970s. The super-bug contained octane-degrading/camphor-degrading plasmid, naphthalene-degrading plasmid and xylene-degrading plasmid and was patented in the USA.

A) Microorganisms and Enhanced Oil Production

The concept of microbial enhanced oil recovery (MEOR) was proposed by Beckmen in 1926, but never received major attention till few decades ago due to skepticism of its potentials. In the past microorganisms were considered detrimental to the petroleum industry, however, it is now know that they could be beneficial in terms of oil recovery [5]. The extraction of crude oil from the oil reservoirs involves two stages-the primary, and the secondary stage. In the primary stage, the oil is forced out by the pressure within the reservoir. As more of the oil is extracted the pressure drops and so does the oil yield too. To continue the oil extraction process, the pressure of the reservoir has to be maintained and this is achieved by pumping steam to maintain the pressure of the reservoir is the secondary stage. After crude oil extraction by these methods; large quantities of “inextractable’ residual oil still remains in the reservoirs. This valuable but inextractable oil calls for more efficient extraction technique. The residual crude oil in reservoirs make up about 67% of the total petroleum reserves, indicating the relative inefficiency of primary and secondary production [5]. Microbial assistance could be called into play to improve efficiency. Microbial enhanced oil recovery aims at increasing the efficiency of oil extraction and reducing the percentage residual oil left within the reservoir at the end of the extraction process, by the use of microorganisms. Microorganisms produce several compounds that have the potential for enhanced oil recovery including bio-surfactants, biopolymers, carbon dioxide, acids and alcohol. They reduce the interfacial tension between oil and water, and oil and rock interface.Carbon dioxide may increase reservoir pressure and decrease the viscosity and gravity of the crude oil, allowing it to move more freely to the producing wells, while the acids dissolve carbonate rock, increasing its permeability and porosity. Several microorganisms are useful in MEOR processes. Some of these include *Desulfovibrio, ClostridiumArthrobacter, Mycobacterium, peptococcus, Xanthomonas sp.*([10], [13]). [18], reported the isolation of 22 microorganisms that produce biopolymers and emulsifiers with one strain able to grow in 10% salt concentration , over a pH range of 4.6 to 9.0, at temperature up to 50°C in the presence of crude oil. The number of microorganisms that have the potential for use cannot easily be quantified, considering microbial diversity and the tools presently available for their investigation. The potential use of microorganisms in EOR is confirmed and is already being applied in many oil fields. Harvey achieved 20 to 30% recovery of residual oil using yeast and bacteria and patented the process in USA [11]. A field test of MEOR by PetrogenInc. using 24 wells during 1983-1982, showed that four wells doubled production for six months, while twelve of the wells increased yield by 50% in three months [12]. Other reports follow similar trends [19]. The potentials of MEOR is tremendous and could be further enhanced by application of recombinant DNA techniques and advances in genomics.

B) Microorganisms and Waste Water Treatment

Microorganisms play very important role in removing organic matter from wastewater and prevention of eutrophication of the receiving waters. Wastewater treatment is a multi-step process involving the mechanical removal of large suspended particles (primary treatment), biological removal of organic compounds (secondary treatment), and the removal of inorganic compounds by chemical treatments (tertiary treatment). In the biological treatment at the secondary stage, the trickling system of the activated slug is the method used to expose the wastewater to microbial degradation[20]. In the trickling system, wastewater is sprayed over a column of pebbles inoculated with bacteria which carries out the mineralization of the organic matter in the water. Here the pebbles form the substrates on which the microbes attach and extract the organic compounds [14]. The activated slug process uses slime forming organisms e.g. *Zoogloearamigera* which grows to form flocs serving as a substratum for the bacteria that mineralize the organic matter in the wastewater [20]. As mineralization proceeds in the holding tank, by the growth of the bacteria, the flocs becomes heavy and drops to the bottom of the tank. It is harvested and sent to sludge digester. The application of microorganisms in wastewater treatment takes advantage of the fact that microbes, like all other living organisms, need nutrients, carbon and energy for survival and growth. The increasing discovery of new microorganisms could help in the discovery of microbes with better potentials for use in wastewater treatment.

3) CONCLUSIONS

It is very agreeable that microorganisms have great potentials for use in environmental management. In areas such as MEOR, a better understanding of the potential application of microorganisms has been enhanced tremendously by modern techniques used in the investigation of microorganisms and advances in combination DNA technology, genomics and bioinformatics. These tools have, not only, made it possible for easy quantification of microbial diversity, but have also opened a door to unlimited application of microbial diversity to environmental management and other fields. By integrating proper utilization of natural microbial capabilities with appropriate engineering designs to provide suitable growth environments, field implementation of bioprocesses and bioremediation methods can be more successful. However, successful application of bioprocesses and bioremediation techniques must address both the heterogeneous nature of many bioprocess, environments and contaminated waste sites and the complexity of using living organisms. This will throw more light on the potential environmental effects of introduction of microorganisms and 'super microorganisms' into new environments.

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