STATE OF THE ART APPLICATIONS OF BIOTECHNOLOGY IN WASTEWATER TREATMENT

by

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Abstract

Biological waste treatment processes such as the activated sludge and biomethanation use naturally occurring microorganisms. With environmental biotechnology, the performance of these processes can be improved. Special strains can be isolated to treat xenobiotics and other poorly degradable substances found in municipal sewage and industrial wastes.

Introduction

Even before the discovery of the existence of microorganisms, man has produced fermented food and feeds. The same degrading action of microorganisms has been used in naturally biodegradable human wastes. For large scale treatment, man had to design artificial processes emulating the degrading activity of naturally occurring microorganisms. Microbiologists, engineers and chemists worked hand in hand in accomplishing this effort. Now we know that for biodegradable materials other than human wastes, the process is also applicable. These biological waste treatment schemes belong to one of the largest field of applications within biotechnology.

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Usually, biotechnology aims to synthesize products by using specially isolated pure strains of microorganisms under optimal conditions. Defined compositions of nutrient media (carbon source, energy source, nitrogen, sulfur, phosphorous, trace minerals, and vitamins) are used. The growth and yields of biomass and products under proper cultural conditions are related to the properties of the pure strain. The production of antibiotic, a valuable product, is an example of this development in the field of traditional biotechnology. Waste treatment biotechnology differs substantially from the traditional processes. (1) No valuable product is produced (except pure water and a clean environment--which will be valuable very soon). (2) The waste materials are often mixed. (3) Pure culture cannot be used and one can only rely in naturally present mixed population or enrichment of the mixed culture if possible. Therefore, the economics are quite different.

Industrial microbiologists have recognized the usefulness of microorganisms in waste treatment since 1914, with the development of the activated-sludge process. This process depends on a mixed culture of naturally-occurring microorganisms. Each strain has the ability to degrade a component of the waste material and to coexist together. An advancement was to improve the sludge by inoculation with a desired mixture of microorganisms. At present, pure cultures that are able to degrade specific compounds in industrial wastes are available. With genetic engineering, particular microorganisms can be tailored to degrade particular types of wastes. However, releasing these genetically-engineered microorganisms might affect the ecosystem adversely. At present, naturally mutated strains of microorganisms are being used in waste treatment. The state-of-the-art process is the supplementing of the existing bacterial population with bacteria that are capable of higher rates of reduction or are capable of degrading compounds that have previously been considered non-biodegradable. This paper considers the poorly degradable substances found in industrial wastes that are amenable to microbial treatment.

Xenobiotics and Pollutants.

Xenobiotics and pollutants are discharged to the environment through point sources or in a dispersed manner mostly through consumers. Microbiological techniques for the elimination of hazardous chemicals from the environment are often more difficult and mostly directed only to the symptom of pollution. At point sources, the pollutant concentration is high, the physical and chemical parameters are controllable, the chemical complexity is low and the biodegradation rate is high. These conditions allow the application of special strains of microorganisms for the treatment of poorly degradable substances.

Isolation and enrichment of waste-degrading microorganisms.

Experiments with pure cultures in single substrates form the basis of collecting knowledge of the biosynthetic pathways of poorly degradable compounds in microorganisms. Once a pure culture is available, the development of the biotechnological processes is possible. To isolate microorganisms with biodegradative potential, microbiologists use the technique of enrichment culture. The procedure is to allow the microorganisms with potential to grow in a medium with the poorly degradable compound as the growth-limiting source of an essential nutrient. Only those microorganisms that can degrade that substance will grow. A series of subcultures allows one to evaluate the success of enrichment. Sewage, where many microorganisms come in contact with xenobiotics, is the usual source for the enrichment of bacteria with degradative capability. However, isolates from natural environments where the compound of interest is found, is usually successful. This includes samples from industrial production lines, pesticide-treated soils, waste dumps, or industrial waste treatment plants.

Cultivation and Processing

Fermentation procedures are usually conventional starting with the slant, inoculum preparation, and sequential seeding to fermenters as large as 10,000 liters. Although mixed cultures are cultivated, sterile conditions are maintained to guard against contamination with *Salmonella, Staphylococcus,* and *Streptococcus.* The cultural conditions maintained produce microorganisms that a depressed and conditioned to their final environment. Centrifugation or filtration are the methods used for cell concentration. Spore-formers and air dried while non-spore-formers are freeze-dried. The cultures are blended with additives before final packaging.

Organic Compounds with Pollution Potential

For significant advances in the microbial treatment of wastes, identification of organic chemicals which resists degradation in conventional waste treatment plants is necessary. Upon identification, their degradation in existing treatment plants can be improved or appropriate specialized technologies for their biodegradation can be developed. Among the organic chemicals in the EPA list of priority pollutants are pesticides and metabolites, halogenated aliphatics, aromatics, nitroaromatics, chloroaromatics, polychlorinated biphenyls, phthalate esters, polycyclic aromatic hydrocarbons, and nitrosamines. The EPA list is useful in defining research in improving industrial wastewater treatment.

Aerobic and Anaerobic Metabolism

Aerobic metabolism consists of two processes: (1) The electron transfer from organic substrates to oxygen -- as source of energy for the cell, and (2) the addition of oxygen to the organic substrate -- prepares the substrate for further metabolism. The degradation of xenobiotics and difficulty degradable compound is important for the second part. In aromatic compounds, the ring cleavage is dependent in oxygen. The availability of molecular oxygen for reaction depends on several enzymes.. Researches on the anaerobic degradation of organic compounds are limited but the process will be important and attractive in the future.

Hydrocarbons

In nature, numerous microorganisms use hydrocarbons for growth and energy source. The microbes oxidize the terminal methyl group in the aliphatic hydrocarbons. The hydrocarbon becomes a fatty acid. In general, each species can degrade limited kinds of hydrocarbons. For example , *Methanomonas methanooxidans* can attack only methane. *Nocardia paraffinicum* and some species of *Pseudomonas* can utilized several hydrocarbons, not all necessarily found in petroleum.

Since benzenoid structures are the most common organic compounds in nature, microorganisms attack them fairly well. However, aromatic polycyclics and those with uncommon substituents (e.g., polychlorinated biphenyls) are difficult to degrade. The degradation of aromatic compounds start with ring cleavage. Examples of microbe that carry this attack are *pseudomonas putida* and *P. ovalis*.

Halogenated Compounds

Halogenated compounds find uses as solvents, aerosol propellants, lead scavengers, nematocides, and fumigants, among others. *Pseudomonas* species and *Xantobacter autophicus* are able to degrade these compounds. Halogenated aromatic compounds used solvents, lubricants, intermediates in synthesis, insulators, plasticizers, etc. are degraded via halocatechal formation or dehalogenation before ring cleavage. Examples of these microorganisms are *Pseudomonas* and *Athrobacter* species.

Nitroaromatic Compounds

Nitroaromatic compounds used in the manufacture of dyes, drugs, pesticides, explosives, and industrial solvents are toxic. As the simpler compounds are completely biodegradable, more complex nitroaromatics such as 2, 4, 6-trinitrotoluene are not degraded. In aerobic conditions, polymerization may take place, while in anaerobic condition, transformation to amines may take place.

Polychlorinated Biphenyl

Polychlorinated biphenyls used in transformer oils, capacitor dielectrics, and heat transformer liquids are toxic to animals and man. *Acinetobacter* and *Alcaligenes* species are capable of transforming many PCB's. PCB's containing more than four chlorines are resistant to degradation.

Lignin and Lignosulfonates

Biomass consists of cellulose, hemicellulose, and lignin. Lignin acts as the cementing material in lignocellulosic materials and protects the structure from microbial degradation. The biodegradation of lignin is important because of its increasing number of industrial uses. Lignosulfonates, which are more resistant to biodegradation than lignin, are the waste products in the sulfite process for pulp and paper manufacture. White rot fungi can degrade lignosulfonates. Other fungi and mixed microbes promote precipitation via polymerization.

Surfactants

Commercial detergents contain 10 to 20% surfactants for cleaning purposes. Anionic surfactants is not biodegradable. A substituted linear alkyl benzene sulfonate is more biodegradable but accumulates in sewage systems. Cultures of adapted microorganisms degrade alkyl sulfates and alkyl sulfonates quite rapidly.

Synthetic Dyes

At present, 3500 dyes are in use (out of 40,000 dyes and pigments with 7,000 different chemical structures). Therefore we cannot make generalizations of their biodegradability. The textile and dyestuff industries are responsible for the entry of dyes into the environment, although in small amounts. A model for biodegradation is the action of a *Pseudomonas* species on azo dyes to produce biomass, carbon dioxide, water, and ammonia. In actual situations, we can use mixed cultures of adapted microorganisms.

Pesticides

Highly efficient and long-lasting, early synthetic organic pesticides were useful but accumulate in the ecosphere. More degradable or metabolizable types have been developed. Organochlorines such as DDT can be mineralized by *Pseudomonas aeroginosa* although very slowly. Some pure or mixed cultures can act on organophosphorus insecticides. Other cultures can use striazines as growth nutrient.

Synthetic Polymers

Because of their high molecular weights, plastics are extremely resistant to microbial attack. Some bacteria attack only polymers with low molecular weights. *Acinobacter* and *Monaxella* species attack polybutadiene with a degree of polymerization of 43, while *Pseudomonas* species degrade polystyrene with a degree of polymerization of 3. Plasticizers in plastics are more prone to microbial attack. These substances are degraded by microorganisms from soil and sewage.

Industrial Waste Treatment

Researches on biodegradation of xenobiotics and pollutants are accumulating. The results of the microbial, biological, and genetic studies will eventually improve the practical application of the treatment processes on the industrial scale. With higher degree of sophistication being attained, more specific microbial strains for the biodegradation of particular wastes will be utilized.

Ideally, waste treatment plants using microorganisms should emulate industrial fermenters, However, aseptic condition is not possible and the system is confronted with varying composition, temperature, and volume. With constantly changing toxic loads, the microorganisms may be harmed. Intermittent feeding might washout desirable strains. However, whatever problems are encountered, several microbial processes are now successful. Special mixed cultures of mutant bacteria for specific type of wastes are now available commercially. They are now more efficient in that they consume less energy than conventional schemes. Biological processes are now increasingly more attractive, efficient, and most of all, economical.

Conclusion

With genetic engineering, specific or multiple activities of microbial cultures is now a reality. This is leading to new approaches in waste treatment. New microbial species that have been genetically altered and which could not be found in nature can be patented. The accumulation of research and studies as well as the results of the current state of the art application of biotechnology in waste treatment will lead to more efficient waste-treatment systems.

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