Pure & Appl. Chem., Vol. 60, No. 6, pp. 837–840, 1988.
Printed in Great Britain.

Potential applications of biotechnology in the energy and the environment sectors

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Abstract - Biotechnological approaches to solving some of the problems of environmental pollution or developing better or alternate sources of energy are considered realistic and technically feasible within the near future. Some of the potential applications of biotechnology of interest to chemists in the energy and the environment sectors are considered briefly in this article.

Much of the applications of genetic engineering and biotechnology has primarily been directed in the areas of agriculture and human and animal health. This is evident from the reports of Drs. Chaleff and Demain in this volume. Considerable work is also being done in the area of chemical technology as reported by Dr. Kölbl. In contrast, the present day work going on in the energy sector or with regard to applications in the areas of environmental pollution problems is few and fragmentary, and no large scale applications are in sight in the near future. Yet, this technology holds considerable promise for potential applications in these areas; I would like to touch upon some of these potential applications in this short article.

With regard to the energy sector, one area of considerable importance is the enhanced production of ethanol, either as industrial alcohol or in the brewing and food or beverage industries. The consumption of ethanol in the United States is expected to rise sharply because of the curtailment in the use of lead in gasoline, since ethanol can increase the octane rating by several points as a 10% blend with gasoline. The use of such gasohol has been encouraged for several years, and may assume some importance in the future, depending upon the price of crude oil and its availability. About 80% of the fuel-grade alcohol in the U.S. is obtained by fermentation with yeasts, and considerable enhancement of such fermentation can be accomplished by genetic engineering. For example, yeasts are poor starch digesters, and in the fermentative production of ethanol from starch, enzymes such as α-amylase and glucoamylase are routinely added as pretreatment of the starch to convert it to linear oligomers and ultimately to glucose units, that are then fermented by the yeasts. Such fermentative production of ethanol can be considerably enhanced at a cheaper rate if the genes for such enzymes can be cloned in yeast so that the enzymatic pretreatment steps can be avoided. Toward this end, yeast strains containing *Aspergillus* glucoamylase genes have been constructed where the enzyme is not only glycosylated but is also excreted into the medium (Ref.1). Further improvements of this strain may allow economical fermentation of ethanol directly from starch by genetically engineered yeasts.

Another avenue for enhanced production of ethanol by yeasts would be to use cheaper substances such as whey, a by-product of cheese making, or agricultural biomass such as cellulose or hemicellulose. Considerable progress in the enhanced degradation of pentose sugars from hemicellulose has been reported (Ref.2); while the cellulase genes have been cloned from a number of sources, the development of an efficient cellulolytic yeast is still several years away because of the involvement of a number of enzymes (both endo and exo-glucanases and β-glucosidase) in cellulose degradation. On the other hand, lactose degrading yeast *Saccharomyces cerevisiae* has recently been constructed (Ref.3) by cloning into non-lactose fermenting *S. cerevisiae* strains the structional gene for β-galactosidase as well as the lactose permease gene. Thus future possibilities for providing ethanol from bulk waste materials look promising.

In addition to the quantity of fuel, the quality of fuels can also be improved by genetic engineering and biotechnology. For example, high sulfur coal and oil, when burned for power generation, often lead to large scale environmental pollution in the form of acid rain.

Biotechnological approaches to reducing the concentrations of organic and inorganic sulfur from high sulfur coal and oil have received some attention recently (Ref.4), although large scale processes for the desulfurization of coal or oil remain a long-term goal. Microbial enhancement of oil recovery, similarly, has been a subject of much discussion (Ref.5), although formidable problems remain to make a microbial process for oil recovery a reality (Ref.6).

A major cause of environmental pollution now-a-days is the release of highly toxic chemical compounds in the form of herbicides and pesticides, solvents, refrigerants and industrially useful compounds such as PCBs (polychlorinated biphenyls) in the environment. Many of these compounds are highly chlorinated, and are extremely persistent in nature. The persistence of highly chlorinated, synthetic compounds is believed to be due to a lack of utilization of such compounds by natural microorganisms, where the primary route of metabolism is bacterial co-oxidation, a slow non-energy yielding process (Ref.7). Recently, however, considerable efforts have been directed towards developing microbial strains either by genetic selection or by gene cloning that allows degradation of a number of highly chlorinated compounds (Ref. 8). Genetic studies with microorganisms capable of degrading simple chlorinated compounds have demonstrated that the degradative genes are often located on plasmids. Cloning of the degradative genes for a synthetic compound such as chlorocatechol and the nucleotide sequence homology of the degradative genes with those involved in the degradation of natural analogous compounds such as catechol have demonstrated considerable sequence homology among these genes (Fig.1), suggesting that new degradative functions against synthetic chlorinated compounds may have evolved by recruitment of genes encoding degradation of analogous compounds and then introducing mutational divergence to alter the substrate specificity of the gene products (Refs. 9-10).

The concept that genes encoding the degradation of synthetic chlorinated compounds evolve by recruitment and mutational divergence of genes encoding degradation of their natural structurally-analogous counterparts has allowed us to develop a strain of Pseudomonas cepacia under strong selection in presence of plasmid gene pools in a continuous culture, that can utilize a persistent compound such as 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) as its sole source of carbon and energy at a rapid rate. This strain can not only completely dechlorinate 2,4,5-T and its metabolic intermediate 2,4,5-trichlorophenol, but a number of other chlorophenols including pentachlorophenol (Ref.8).

The strain is also very effective in removing large quantities of 2,4,5-T from contaminated soil within a few weeks, suggesting that such strains may be of practical value in removing the toxic chemicals from contaminated sites (Ref.8)

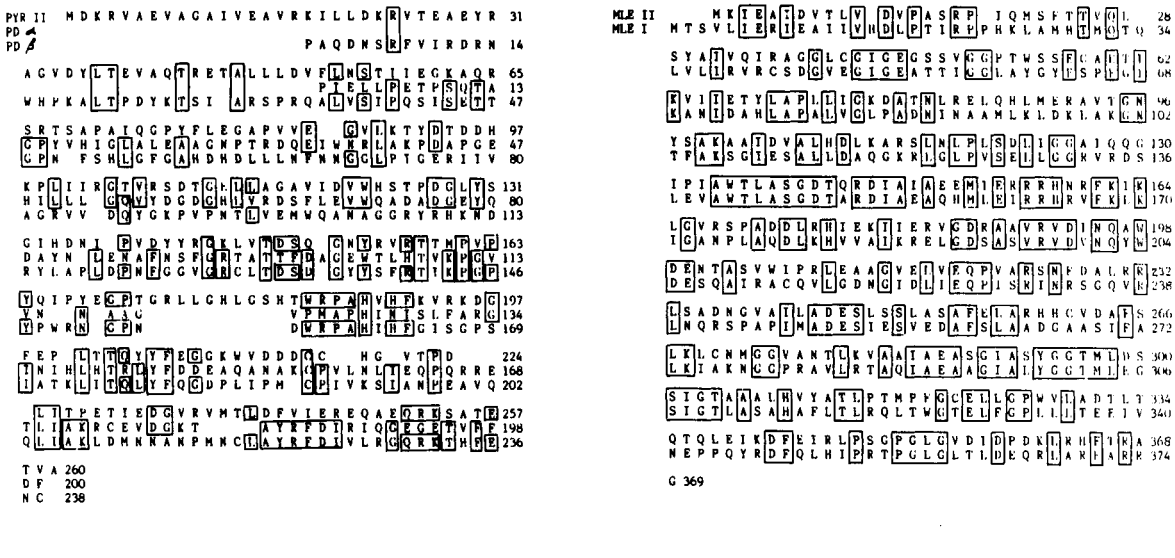

Fig. 1 Amino acid sequence homology between pyrocatechase II (PYRII) and protocatechuate dioxygenase subunits α and β (PDα, PDβ), cycloisomerase II (MLE II) and cycloisomerase I (MLE I), and dienelactone hydrolase (DLH) and enol-lactone hydrolase (ELH). The amino acid sequence for PYRII, MLEII, MLEI and DLH has been determined from the DNA nucleotide sequences while that for PDα, PDβ and ELH has been determined from the purified proteins (see Refs. 9 and 10).

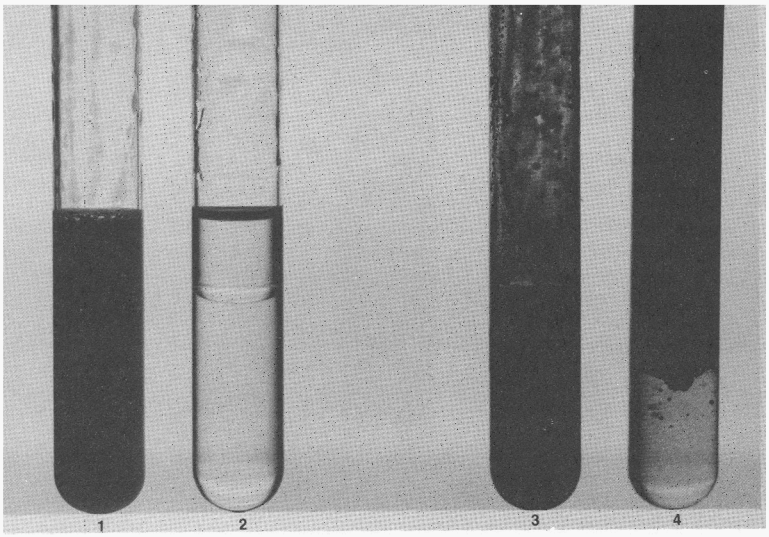

Fig. 2 Emulsification of liquid alkanes and crude oil by the P. aeruginosa emulsifier EM. Left tubes: (1) growth medium containing hexadecane and EM shaken together; (2) growth medium and hexadecane shaken together before EM production. Right tubes: (3) crude oil and EM in water shaken together; (4) crude oil and water without EM shaken together.

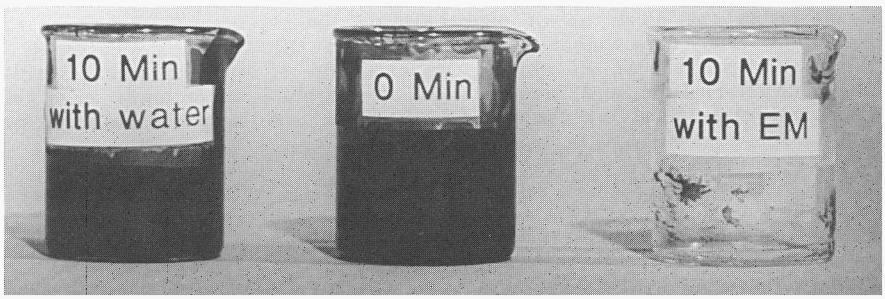

Fig. 3. Removal of adhering oil from solid surfaces with EM.

An interesting feature of the 2,4,5-T degrading strain is its ability to form surface active compound (s) that would emulsify 2,4,5-T (Ref.11). It is likely that during recruitment and evolution of the 2,4,5-T degradative genes, the genes for the formation of the emulsifier were also recruited to facilitate the uptake of this compound. Normally, highly chlorinated compounds such as PCBs, TCDD (2,3,7,8-tetrachloro-dibenzo-p-dioxin) etc. are very hydrophobic, and are unavailable to the bacterial cells because of their insolubility in water. The hydrophobic nature of most highly chlorinated compounds must have contributed to the persistence of the compounds, since microorganisms need not only to evolve the degradative genes for these compounds, but also genes for the synthesis of appropriate emulsifiers to facilitate their uptake in an aqueous environment. This situation is reminescent of the microbial degradation of hydrocarbons present in crude oil, most of which are also highly hydrophobic. How do microorganisms grow rapidly with normal alkanes then? We have shown previously that a strain of P. aeruginosa that can rapidly degrade both liquid (C10 to C18) and solid (C20 to C36) alkanes produces a surface active agent (called EM or emulsifier) that is absolutely essential for allowing the cells to utilize liquid alkanes (Ref.12). A mutant incapable of producing the EM is unable to utilize liquid alkanes but would grow readily with solid alkanes; this mutant will also grow rapidly with liquid alkanes if the EM is added exogenously. Thus it is clear that EM is absolutely essential for this strain of P. aeruginosa to grow with liquid alkanes. Much of the work concerning the purification and application of the EM in oil recovery and oil pollution control is being conducted by Petrogen, Inc. of Arlington Heights, Illinois. Figure 2 demonstrates the ability of the EM to emulsify both hexadecane as well as crude oil, while Fig. 3 demonstrates the ability of the EM to remove adhering oil from a solid surface (S. Banerjee, J.F. Karns and A.M. Chakrabarty, patent pending). The ability of the EM to extract heavy oil from tar sands, and the final recovery of the extracted oil by deemulsification with lime or $MgCl_2$ is shown in Fig. 4.

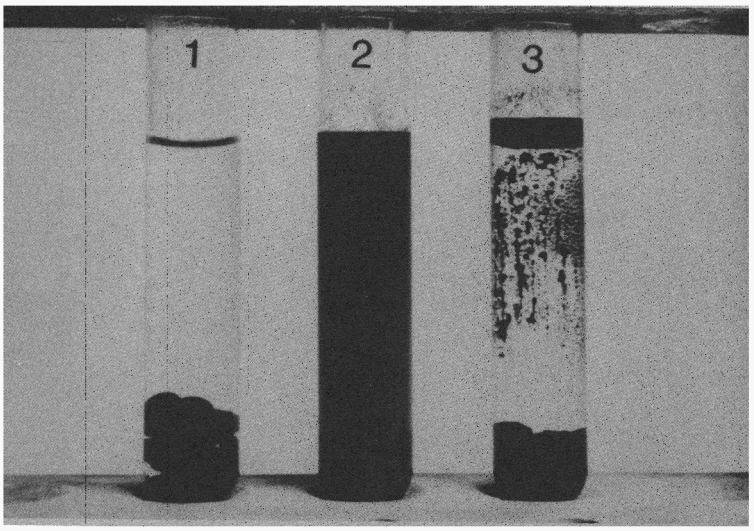

Fig. 4 Recovery of heavy oil from Canadian tar sands by EM. Tube 1: tar sands in water; tube 2: tar sands extracted with water containing EM; tube 3: tar sands extracted with water containing EM, and the emulsified oil deemulsified by addition of O.1M $MgCl_2$.

In conclusion, microbial surfactants appear to be useful in the energy sector not only in enhancing oil recovery but may also be highly useful in controlling environmental pollution from spilled oil or in the clean up of oil-containing drums, barrels and tanks (Ref. 12). In an actual field trial in collaboration with Kuwait Oil Company, Petrogen Inc. has recovered substantial amount of oil during the clean up of large oil storage tanks by the EM. Indeed, characterization and identification of microbial surfactants may well be one of the most viable routes to solving the oil as well as our toxic chemical pollution problems.

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