# REVIEW ON THE APPLICATION OF GENETIC ENGINEERING/MOLECULAR BIOTECHNOLOGY IN ENVIRONMENTAL SECTORS

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## ABSTRACT

*Genetic engineering, the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms. The term* genetic engineering *initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction.*

*Creation of genetically modified organisms requires recombinant DNA. Genome of an organism. Creation of genetically modified organisms requires recombinant DNA. Recombinant DNA is a combination of DNA from different organisms or different locations in a given genome that would not normally be found in nature. Genetic engineering has different uses for Environmental sectors. There for the objective of this paper was to review on the Application of Genetic Engineering/Molecular biotechnology in Environmental sectors.*

**KEYWORDS:** *Recombinant DNA; Molecular biotechnology; Environmental sector; Genetically modified organism (GMO)*

# 1. INTRODUCTION

Genetic engineering, the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms. The term genetic engineering initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction. As such, the term embraced both artificial selection and all the interventions of biomedical techniques, among them artificial insemination, in vitro fertilization (e.g., "test-tube" babies), cloning, and gene manipulation.

The possibility for recombinant DNA technology emerged with the discovery of restriction enzymes in 1968 by Swiss microbiologist Werner Arber [13]. The following year American microbiologist Hamilton O. Smith purified so-called type II restriction enzymes, which were found to be essential to genetic engineering for their ability to cleave a specific site within the DNA (as opposed to type I restriction enzymes, which cleave DNA at random sites) [4]. Drawing on Smith's work, American molecular biologist Daniel Nathans helped advance the technique of DNA recombination in 1970–71 and demonstrated that type II enzymes could be useful in genetic studies [2]. Genetic engineering based on recombination was pioneered in 1973 by American biochemists Stanley N. Cohen and Herbert W. Boyer, who were among the first to cut DNA into fragments, rejoin different fragments, and insert the new genes into *E.* coli bacteria, which then reproduced Genetic engineering is the process by which scientists modify the genome of an organism. Creation of genetically modified organisms requires recombinant DNA [5]. Recombinant DNA is a combination of DNA from different organisms or different locations in a given genome that would not normally be found in nature. In most cases, use of recombinant DNA means that you have added an extra gene to an organism to alter a trait or add a new trait. Some uses of genetic engineering include improving the nutritional quality of food, creating pest-resistant crops, and creating infection-resistant livestock Organisms GMO. We're struggling with the fact that some diabetic patients are experiencing an allergic reaction in response to insulin isolated from cows and pigs. New DNA is obtained by either isolating or copying the genetic material of interest using recombinant DNA methods or by artificially synthesizing the DNA. A construct is usually created and used to insert this DNA into the host organism. The first recombinant DNA molecule was made by Paul Berg in 1972 by combining DNA from the monkey virus SV40 with the lambda virus [7]. As well as inserting genes, the process can be used to remove, or "knock out", genes. The new DNA can be inserted randomly, or targeted to a specific part of the genome.

An organism that is generated through genetic engineering is considered to be genetically modified (GM) and the resulting entity is a genetically modified organism (GMO). The first GMO was a bacterium generated by Herbert Boyer and Stanley Cohen in 1973[6]. Rudolf Jaenisch created the first GM animal when he inserted foreign DNA into a mouse in 1974[9]. The first company to focus on genetic engineering, Genentech, was founded in 1976 and started the production of human proteins [3]. Genetically engineered human insulin was produced in 1978 and insulin-producing bacteria were commercialised in 1982. Genetically modified food has been sold since 1994, with the release of the Flavr Savr tomato. The Flavr Savr was engineered to have a longer shelf life, but most current GM crops are modified to increase resistance to insects and herbicides. GloFish, the first GMO designed as a pet, was sold in the United States in December 2003. In 2016 salmon modified with a growth hormone were sold [11].
Therefore, the objective of this paper is to review on Application of Genetic Engineering/Molecular biotechnology in Environmental sectors

# 2. LITERATURE REVIEW

### 2.1 Concept of Genetic Engineering

By definition, genetic engineering is the direct alteration of an organism's genome which is achieved through manipulation of the DNA. This is achieved by using "recombinant DNA technology" which involves different techniques to insert, alter, or cut out pieces of DNA that contain one or more genes of interest. This is also known as genetic modification, gene transfer or transgenesis. Main focus of genetic engineering is:

- Gene isolation,
- Gene modification so that they can be transferred into and function within a new organism of a different species (transgenics) or the same species (cisgenics),
- Gene removal, and
- Evaluating the success of resultant gene combinations.

### 2.2 Process and Techniques of Genetic Engineering

Most recombinant DNA technology involves the insertion of foreign genes into the plasmids of common laboratory strains of bacteria. Plasmids are small rings of DNA; they are not part of the bacterium's chromosome (the main repository of the organism's genetic information). A subsequent generation of genetic engineering techniques that emerged in the early 21st century centred on gene editing. Gene editing, based on a technology known as CRISPR-Cas9, allows researchers to customize a living organism's genetic sequence by making very specific changes to its DNA. Gene editing has a wide array of applications, being used for the genetic modification of crop plants and livestock and of laboratory model organisms (e.g., mice).

The correction of genetic errors associated with disease in animals suggests that gene editing has potential applications in gene therapy for humans. Creating a GMO is a multi-step process. Genetic engineers must first choose what gene they wish to insert into the organism. This is driven by what the aim is for the resultant organism and is built on earlier research. Genetic screens can be carried out to determine potential genes and further tests then usedtoidentify the best candidates. The development of microarrays, transcriptomics and genome sequencing has made it much easier to find suitable genes. Luck also plays its part; the round-up ready gene was discovered after scientists noticed a bacterium thriving in the presence of the herbicide. GE is the important tool and various techniques are used for transferring genes from one organism to another. These techniques include: Agrobacterium mediated, microinjection, microinjection, electroporation mediated, PEG/liposome mediated.

**2.2.1 Gene isolation and cloning**

The next step is to isolate the candidate gene. The cell containing the gene is opened and the DNA is purified. The gene is separated by using restriction enzymes to cut the DNA into fragments or polymerase chain reaction (PCR) to amplify up the gene segment. These segments can then be extracted through gel electrophoresis. If the chosen gene or the donor organism's genome has been well studied it may already be accessible from a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can also be artificially synthesized. Once isolated the gene is ligated into a plasmid that is then inserted into a bacterium.

The plasmid is replicated when the bacteria divide; ensuring unlimited copies of the gene are available. Before the gene is inserted into the target organism it must be combined with other genetic elements. These include a promoter and terminator region, A selectable marker gene is added, which in most cases confers antibiotic resistance, so researchers can easily determine which cells have been successfully transformed. A selectable marker gene is added, which in most cases confers antibiotic resistance, so researchers can easily determine which cells have been successfully transformed. The gene can also be modified at this stage for better expression or effectiveness. These manipulations are carried out using recombinant DNA techniques, such as restriction digests, ligations and molecular cloning.

**2.2.2 Inserting DNA into the host genome**

There are a number of techniques used to insert genetic material into the host genome. Some bacteria can naturally take up foreign DNA. This ability can be induced in other bacteria via stress (e.g. thermal or electric shock), which increases the cell membrane's permeability to DNA; up-taken DNA can either integrate with the genome or exist as extra chromosomal DNA. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cell's nuclear envelope directly into the nucleus, or through the use of viral vectors. In plants the DNA is often inserted using *Agrobacterium*-mediated recombination, taking advantage of the *Agrobacterium* T-DNA sequence that allows natural insertion of genetic material into plant cells. Other methods include biolistic, where particles of gold or tungsten are coated with DNA and then shot into young plant cells, and electroporation, which involves using an electric shock to make the cell membrane permeable to plasmid DNA.

Due to the damage caused to the cells and DNA the transformation efficiency of biolistic and electroporation is lower than agro bacterial transformation and microinjection. As only a single cell is transformed with genetic material, the organism must be regenerated from that single cell. In plants this is accomplished through the use of tissue culture. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. Selectable markers are used to easily differentiate transformed from untransformed cells. These markers are usually present in the transgenic organism, although a number of strategies have been developed that can remove the selectable marker from the mature transgenic plant. Further testing using PCR, Southern hybridization, and DNA sequencing is conducted to confirm that an organism contains the new gene. These tests can also confirm the chromosomal location and copy number of the inserted gene. The presence of the gene does not guarantee it will be expressed at appropriate levels in the target tissue so methods that look for and measure the gene products (RNA and protein) are also used. These include northern hybridization, quantitative RT-PCR, inserted randomly within the host genome or targeted to a specific location. The technique of gene targeting uses homologous recombination to make desired changes to a specific endogenous gene. This tends to occur at a relatively low frequency in plants and animals and generally requires the use of selectable markers. There are four families of engineered nucleases: mega nucleases, zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the Cas9-guideRNA system (adapted from CRISPR). TALEN and CRISPR are the two most commonly used and each have its own advantages. TALENs have greater target specificity, while CRISPR is easier to design and more efficient. In addition to enhancing gene targeting, engineered nucleases can be used to introduce mutations at endogenous genes that generate a gene knockout.

**2.3 Applications of Genetic Engineering in Environmental sectors**

Genetic engineering has advanced the understanding of many theoretical and practical aspects of gene function and organization. Through recombinant DNA techniques, bacteria have been created that are capable of synthesizing human insulin, human growth hormone, alpha interferon, a hepatitis B vaccine, and other medically useful substances. Plants may be genetically adjusted to enable them to fix nitrogen, and genetic diseases can possibly be corrected by replacing dysfunctional genes with normally functioning genes. Nevertheless, special concern has been focused on such achievements for fear that they might result in the introduction of unfavorable and possibly dangerous traits into

microorganisms that were previously free of them—e.g., resistance to antibiotics, production of toxins, or a tendency to cause disease. Likewise, the application of gene editing in humans has raised ethical concerns, particularly regarding its potential use to alter traits such as intelligence and beauty.

ENVIRONMENTAL REMEDIATION BY
BIOTREATMENT/ BIOREMEDIATION

Environmental hazards and risks that occur as a result of accumulated toxic chemicals or other waste and pollutants could be reduced or eliminated through the application of biotechnology in the form of (bio)treatment/(bio)remediating historic pollution as well as addressing pollution result.

**2.3.1 Environmental Monitoring Inspecting**

Surrounding our living environment or in the municipal health, there are many places having pathogen or virus that need environmental monitor and inspection. For instance, we have to monitor and inspect or measure the microbial growth and decline, drinking water, and the sewer microorganism in water treatment plant. There are two main groups which can help us to monitor and inspect the species of the pathogen or virus:

❖ **PCR, RT-PCR, Quantitative Competitive-PCR**

The polymerase chain reaction (PCR) is a technique widely used in molecular biology. It derives its name from one of its components, a DNA polymerase used to amplify (or replicate) & a piece of DNA (the target) by in vitro enzymatic replication. By recombining a lot of this species, we can get the sequence data base, and make the phylogenetic tree. Then, we can use the phylogenetic tree to provide the microbial characteristic of the environmental samples or pure culture. Because the virus is not only DNA, but also RNA which needs to do the reverse process called reverse transcription- polymerase chain reaction (RT-PCR). The Quantitative competitive PCR (QC-PCR) is the fragment data of PCR adding into the sample which has the target model that we want (Kim et al, 2002) [6]. Through the previous process, the high concentration target model is very similar to our sample.

❖ **FISH**

FISH (Fluorescent in situ hybridization) is a cytogenetic technique which can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes binding parts of the chromosome to show a high degree of sequence similarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to the chromosome. FISH is often used for finding specific features in DNA. These features can be used in genetic counseling, medicine, and species identification. (Wilderer et al, 2002) [14]. For the environmental application, we can use FISH not only to detect the bacteria and virus easily, but also to get the accurate location in our sample. Therefore, this powerful technique will let the environmental monitoring and inspecting become easier.

**2.3.2 Environmental Management and Risk Evaluation**

The new molecular biotechnology can be used on the management, risk evaluation and pollution prevention of the environmental jobs. Microarray genomics, DNA chips, antibody or protein chips, all can be used for inspection and examination, such as toxicity pollutants, chemical substances, and bacteria and microorganisms. Gene chip is one kind of new technology of DNA microarray. It can analyze the gene expression in a whole bunch of genes. This technology does not like the traditional technology, which are very expensive and spend a lot of time to inspect or examine a lot of microorganisms to confirm the pollutants. On the contrary, it can reduce not only the inspecting time but also the number of biological experiments. Recently, most of environmental toxicity inspections usually used this technology to shorten the period of biological tests. Also, if we need to use amount of animals' experiments and just focus on one type of pollution, the new bioassay technology, such as DNA microarray, can detect the cadmium chloride ($CdCl_2$) or trichloroethylene in the liver of our examination quickly. Furthermore, the other technology, like DNA-chip, PCR and genotoxic Effects, are both expected nice tools for environmental detection. Therefore, after those technologies, are widely applied to solve a great number of environmental management problems and decrease the risk of the environmental evaluation.

**2.3.3 Soil and Ground Water Treatment**

There are a lot of pollutants throughout the soil and ground water; Trichloroethylene (TCE) is one type of pollutants in which we mostly can find. It can decompose at aerobic and anaerobic condition and need to process during co-metabolism and at aerobic condition. The research indicates enzyme includes soluble and particulate methane monooxygenase (sMMO & pMMO); it usually reduces and form dichloroethylene (DCE) and vinyl chloride (VC) at anaerobic condition, and it will become completely mineralization after totally oxidation. In aerobic or anaerobic bioreactor (CANOXIS or UASB), after TCE decomposes microorganisms by using PCR to understand its' composition, we can design the appropriate primers to research functional genomics. (Tresse, 2005) [12]. Furthermore, in order to decompose those pollutants, we need appropriate enzymes in microorganisms' bodies to be one kind of proteins. Composing those proteins, we need DNA to transfer the message to mRNA. During the transferring process, we also need RNA polymeras. The position of DNA and RAN polymeras is at the special space on the promoter; therefore,

increasing the numbers of mRNA and enhancing the activity of the promoter can directly or indirectly multiply enzymes we need. As the result, we can increase the decomposition of microorganisms' ability. There are several similar techniques in Peter C.K Lau and Victor De Lorenzo's article (Peter et al, 1999): 1. Using starvation signals to stimulate the activity of promoter, it can make few polymerases do its' function; then, the DNA also can transfer to mRNA easier. 2. Using the other genies' recombination to produce hybrid, it will make original bacterium have widely substrate specificity to induce other pollutants degrading. 3. By inducing the genie mutation, it can increase the survival rate of. microorganism [8]. By the way of these techniques, it can increase the decomposition ability of microorganism and reduce the time spending of soil pollution treatment

**2.3.4 Molecular Nanotechnology**

While conventional chemistry uses inexact processes driven toward some balance to obtain inexact results, and biology exploits inexact processes to obtain definitive results, molecular nanotechnology would employ original definitive processes to obtain definitive results. The desire in molecular nanotechnology would balance molecular reactions in positional-controlled locations and orientations to obtain ideal chemical reactions and to build systems by further assembling the products of these reactions. Therefore, acid rain, global warming, ozone depletion have become the common language in everyone's daily life. Today, environmental sustainability relies on technology is pessimistic. However, emerging molecular nanotechnology takes the lead of all industry development, such as nanoelectronics, nanobiotechnology, nanomaterial, nanoenergy, and etc. It is the first one which offers radical tools for human beings to be on the upper hand in the struggle toward to sustainable and economic growth. Furthermore, it provides human civilization not only to remediate environmental liabilities since industrial revolution in the 18th century, but also to produce unlimited material and energy within ultra-green processes. For the following, I will briefly talk about the molecular nanotechnology application on the acid rain and smog, global warming, toxic wastes and water and soil contaminations.

**2.3.6 Monitoring the community structure of wastewater treatment plants:**

In today's world with its increasing human population and the concomitant pollution problems there is an increasing awareness of the necessity for environmental protection. Our ability to control the pollution caused by human activities is, in the long run, crucial for the further development of mankind. Wastewater treatment is one of the basic processes in this regard. In former times, the natural ecosystems were, by the so-called self-purification, able to deal with the much lower levels of pollution. Microorganisms mineralized the waste and made it available again for primary production. Interestingly enough, most modern wastewater treatment processes still rely on the action of complex microbial communities. And even today these communities are not deliberately assembled from individual species with known functions, but they remain the result of natural selection. The art of building an efficient wastewater treatment facility is still to make the best use of the natural processes, by condensing them in space and time, or as Curds and Hawkes wrote in 1975 "Used water treatment by biological oxidation plants may be regarded as the environmental control of the activity of the appropriate organisms" [1].

The group of organisms most directly involved in wastewater treatment is the bacteria. They dominate, both in numbers and biomass, all other groups and dominate the processes of mineralization and elimination of organic and inorganic nutrients. They are favored, in traditional high load plants that operate with short sludge retention times, by their low generation times. Modern low load systems have high retention times and also allow for the presence of more slowly growing bacteria and of organisms with a more complex organization such as flagellates, amoebae, ciliates or even worms and insect larvae. The protozoa and metazoa are able to feed on particulates, such as those coming in with the sewage or bacterial flocs. It is generally assumed that their primary role in the wastewater treatment is the clarification of the effluent.

## CONCLUSION

The term genetic engineering initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction.

As such, the term embraced both artificial selection and all the interventions of biomedical techniques, among them artificialinsemination,invitrofertilization(e.g."testtubeabies), cloning ,and gene manipulation. We can understand that the benefits from molecular biotechnology application, such as environmental monitoring and inspecting, pollutant removal, soil and groundwater treatment, molecular nanotechnology, green energy and so on, really make the great changes in our environment and living life. Through recombinant DNA techniques, bacteria have been created that are capable of synthesizing human insulin, human growth hormone, alpha interferon, a hepatitis B vaccine, and other medically useful substances. Plants may be genetically adjusted to enable them to fix nitrogen, and genetic diseases can possibly be corrected by replacing dysfunctional genes with normally functioning genes.

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