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REVIEW ARTICLE

Current Trends in Microbial Bioengineering Approaches and their Applications

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Abstract

Microbial bioengineering is an advanced, highly potential, modern era tool in the field of research and development. Manipulation in microbial metabolic mechanisms and their beneficial exploitation in sustainable future applications is the key to new and future microbial biotechnology research. The several benefits of microbial bioengineering include food, vaccine production, treatment of several genetic issues, in forensics, bioremediation of water and soil, biofuels and waste water treatment. Technological microbiology has evolved into a field of study that is primarily used in the production of products for the food, chemical, agricultural, and pharmaceutical industries. As the selection of enhanced microbial strains became more common over time and as other microorganisms were modified to produce goods to meet natural demands, technological microbiology also became more widely applied. With advantages many cons are also attached with it, in terms of increased crop diseases, expense etc. So, we have to use it judiciously for the betterment of the society.

Keywords: Environment, Microbial bioengineering, Sustainable future.

Introduction

Microorganisms are so tiny that they cannot be seen with the human eye and constitute for around 60% of all living things on earth (Caron *et al.*, 2009). Numerous diverse life forms of various sizes, shapes, and traits are referred to as "microbes." Bacteria, fungi, protists, viruses, and archaea are a few of these microorganisms. Both beneficial and harmful microbes can exist in nature. While some microorganisms can cause serious diseases and illnesses, degrade food, and have other negative impacts, others are crucial to preserve the balance of the ecosystem.

Since bioengineering led to interesting discoveries such as beer and wine, which can be made by a process called fermentation using yeast, a unicellular fungi belonging to phylum Ascomycota and family Saccharomycetaceae and other highly genetically manipulated strains. Robert Koch and Louis Pasteur proposed that a single pathogenic bacterium is capable of causing a particular disease, and Louis Pasteur created pure monoculture in a sterile environment. The discovery of these two scientists led to the building up of the understanding of Microbiology. Louis Pasteur also discovered that beer, wine, and alcohol can be made using certain microorganisms, and this invention has high profitable applicability even today (Buchholz and Collins, 2013). Later, Buchner gave the idea that all of a live cell's functions were governed by both pure chemical rules and a metaphysical "*vis vitalis.*" He claimed that the chemical basis for bioconversions was provided by enzymes (Buchhloz and Collins, 2014).

By understanding the configuration of products in microbial fermentation, various chemical products which were required in wartime, like acetone and butanol were manufactured. During Second World War the demands for penicillin, an antibiotic that is naturally obtained from penicillin mold, led to the increase in the manufacture of penicillin synthetically and of other antibiotics like streptomycin, etc. After the antibiotics, the secondary metabolites such as steroids came into recognition as they could be used as high value-added products (Bernardini *et al.*, 2018)

Research on the composition of microbial fermented products were a response to the production of citric acid, and other products like butanol and acetone, as well as chemical components required for hazardous substances, notably during times of war. At the time of World War 2 there was a high demand for penicillin which was artificially synthesised, and it was followed by an era of other antibiotic availability like streptomycin. A novel category of products with high value like steroids which are largely secondary

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metabolites were introduced by bio-transformation process. In the middle of the 20th century, biotechnology was becoming a recognised field of study. Government organisations in Japan, Germany, USA, UK and other countries began to pay attention to biotechnology in the 1970s and 1980s as an area with inventive potential for development and prosperous growth, propelling the field's spread. The genetic study and their relationships all through the course of evolution have greatly expanded the research in the field. This has been made possible by fundamental research in molecular biology, genetics and biochemistry. The range of accessible goods along with services has significantly increased during last few years. Gainful contributions boosted up Research and Development (R & D) by promoting and funding the creation of novel approaches, tools and businesses (Demain *et al.*, 2016).

The field of "New Biotechnology" have become one of the main subjects in research related to medicine and agriculture. Even though biotechnology continues to have an impact on a variety of artificial industries, such as the production of food, feed, and other products, as well as polymers, bio-fuels, and energy, it also has an impact on the process of developing and producing many of the most potent medicines. Considering life at the molecular level paves the path for the development of novel goods and successful, environmentally friendly production methods (Buchholz and Collins, 2013).

Microbial Engineering

Naturally microorganisms have been used therapeutically for over a century, but the arrival of new ways for inheritance manipulation has created unknown openings to develop new bioengineered microbes with high remedial productiveness. For instance, obligate anaerobic bacteria, such as *Clostridia* spores, were employed for cancer treatment. Because necrotic regions are a common and distinctive feature of solid tumours, these bacteria are specialised for them. They should therefore provide patients a high level of safety. Even though *Clostridia*'s germination was restricted to hypoxic areas, the toxicity of its exotoxins made this species of bacteria highly lethal. The α-toxin and other virulence factors had been eliminated from possible therapeutic strains of *Clostridia* in order to boost their safety (Felgner *et al.*, 2016). Even live bacterial cells are orally delivered as therapeutics in the form of probiotics for the treatment of acute infectious diarrhoea in infants and the prevention of antibiotic associated diarrhoea and nosocomial acquired diarrhoea. (Prakash and Bhathena, 2005). Tremendous variability among microorganisms as well as a vast potential for metabolic and inheritable variation have been produced by the phylogenetic processes that enable microbial growth and survival in a variety of environmental contexts. Microorganisms have an immense amount of potential and can be used as tools in farming, processing, and the environment.

Microbial bioengineering is the process of redesigning and modifying microbial systems for application in biotechnological processes. To advance the creation of microbial cell workshops, we create and use novel technologies. The utilisation of integrated genomics techniques for bioengineering target identification and genome streamlining to produce simpler, stable, and highly defined strains as microbial structures for novel biotechnological experiments are key components of this strategy.

Microbial engineering approaches (fig.1) are regarded as a crucial and significant field of study in applied science and engineering. A microbial architect manipulates microbes and creates new applications for them while working on the biological, chemical, and engineering dimensions of biotechnology (Timmis *et al.*, 2016).

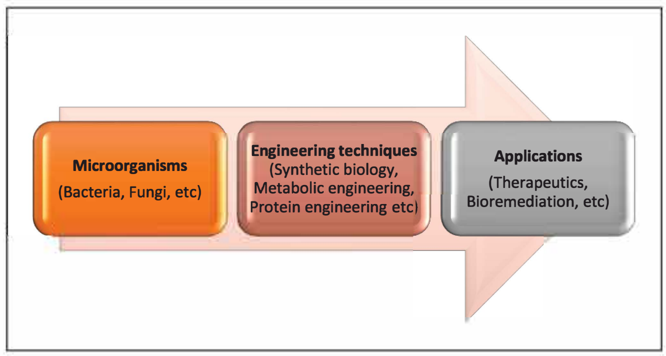

Fig.1. Overview of Microbial Bioengineering

Microorganisms as Tools

Microbiology at present is one of the fast-evolving disciplines in biological field. It includes studies on physiology, ecology, development, and clinical/ immunological aspects of microorganisms, including the benefits to society, which can be achieved by exploiting the microbial genetic and metabolic system (Maheshwari and Jayant, 2016). It is a huge employment initiator worldwide, as well as a promising area in biological sciences for exploration. Microorganisms are of great significance to both humans and the ecosystem. Hence, it should be high precedence to make use of the subject for smart work in R & D and also for the creation of employment. The present review enlightens the tremendous beneficial aspects of microbes and microbiology to mankind and its surroundings. The composition focuses generally on the trends in the microbiological investigation, processed work, extent, and future of microbiology on a global scale. In the 1970s era, *Escherichia coli* was genetically altered, enabling the artificial insulin production, the very first product made employing tools and techniques of recombinant DNA and in 1982 certified through the U.S. -Food and Drug Administration as reported by Johnson (1983).

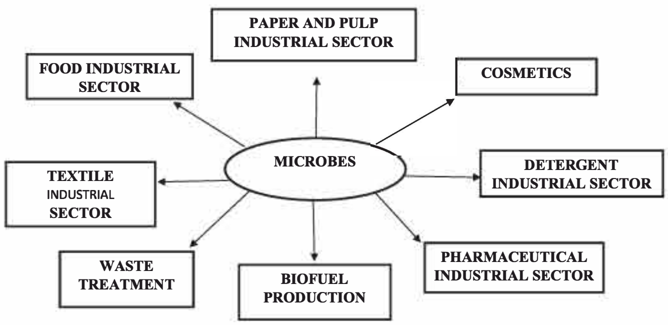

Fig.2. Applications of Microbial Bioengineering

Applications of Microbial Bioengineering

Applications of microbial bioengineering help us know prokaryotic and eukaryotic cell systems that are relevant for industry and that have been genetically altered and created. Agriculture, bioremediation, and industrial sectors all hold enormous potential for microbial community or individual bioengineering (fig.2).

- Microbes used in wastewater treatment, which aims to cleanse wastewater and remove contaminants, are one of the most significant bioengineered communities that are extensively used.
- The capacity for the transfer of energy to certain system strategically is now made possible by electro microbiology. Due to the fact that an electron is accepted or donated by specific electrode at a precise redox potential which also makes it possible for others to be rejected, the utilization of these balanced electrodes as either electron donors or acceptors has made it possible to stimulate certain natural important pathways inside the community.
- Similar to an existing petroleum refinery, a variety of distinct marketable products are being created via the proposed bio-refinery which is specifically designed and totally utilising the input substrate(s).
- A prime example of early success in designed pure cultures is insulin generated in *E. coli*.
- Crop diseases caused by fungi are responsible for significant productivity losses worldwide. These fungal infections can be efficiently handled and crop productivity loss can be minimised by specifically implementing helpful microbial genes in plant systems through the transgenic technique.
- Biocatalysts with biotechnological value include microbial thermostable lipases. They carry out a variety of reactions, including as aminolysis, acidolysis, hydrolysis, esterification, interesterification, and transesterification. They are used in a wide variety of artificial applications, such as the creation of biological laundry detergents, flavouring food additives and reducing environmental pollution, resolving the pitch issue in the pulp and paper industry, creating biosensors for medical diagnosis, and serving as the active ingredient in pharmaceuticals.
- Despite being very small and invisible to the naked eye, microorganisms perform a far larger function than other species. The genes found inside the bacterial cell are the primary causes of this substantial and vital role. Bacterial cells have the flexibility to live in practically any circumstance, favourable or unfavourable, thanks to their genes and gene products. In a strict sense, bacterial cells interact with other species for their own survival, but the outcome has an impact on plants and people, either positively or negatively. With a biological process that produces around 175 million tonnes of nitrogen every year, nitrogen fixation is one of nature's most remarkable and important phenomena. The nif gene governs this large amount of nitrogen fixing (Li *et al.*, 2020).
- As consumer awareness of environmental issues and sustainability increases, the cosmetics industry is progressively concentrating on microorganisms in order to identify more stable and sustainable ways of manufacturing biologically deduced particles. Microorganisms use in biotechnology aren't only the most recent trend but also represent a vast resource for the longer term of the cosmetics industry in addition to multiple other sectors.
- Microorganisms have long been used to serve the demands and requirements of humans, e.g., to maintain food, such as vegetables, fruits and beverages, milk and products like cheese, fermented products, breads, as well as improving the quality of the product. The oldest best-known fermentation process for beer production is via sugar conversion to alcohol by the action of yeasts. In general, fermentation refers to an enzymatically controlled process that breaks down energy-rich compounds,

such as carbohydrates, into other, simpler substances, often carbon dioxide and alcohol, or organic acid (Heshof *et al.*, 2016).

- Better and engineered microorganisms for biocontrol of animal and plant pathogen, superior quality of vaccines, improved disease-diagnostic methods and tools pests, decreased virulence in animal and plant pathogens via modifications, recently developed industrial catalysts and fermentation organisms, and for bioremediation of land and aquatic environment polluted by toxic substances newly discovered microbial agents are just a few of the breakthroughs made possible by microbial bioengineering, using genome studies and tools (Sivasubramaniam and Franks, 2016).
- Research on microbial genomics and microbial bioengineering is essential for advances in biotechnology, value-added products, human nutrition and functional foods, plant and animal protection, and basic agricultural science research (Vitorino and Bessa, 2017).
- The terrorist attack that used anthrax in the fall of 2001 brought attention to the employment of microorganisms in criminal acts (Crupi *et al.*, 2003). In epidemiological investigations, microbiologists primarily look into the origin of microorganisms. The colloquium focused on the research and educational requirements to facilitate the use of microbial forensics in criminal investigations and, as a result, the prosecution of bio crimes, including acts of bioterrorism to overcome difficulties in microbiological forensics, such as identifying a microorganism exploited in a bioattack and the bioattack's perpetrators (Keim, 2003). The application of microbial forensics to assist in resolving bio crimes was discussed (fig. 3, 4).

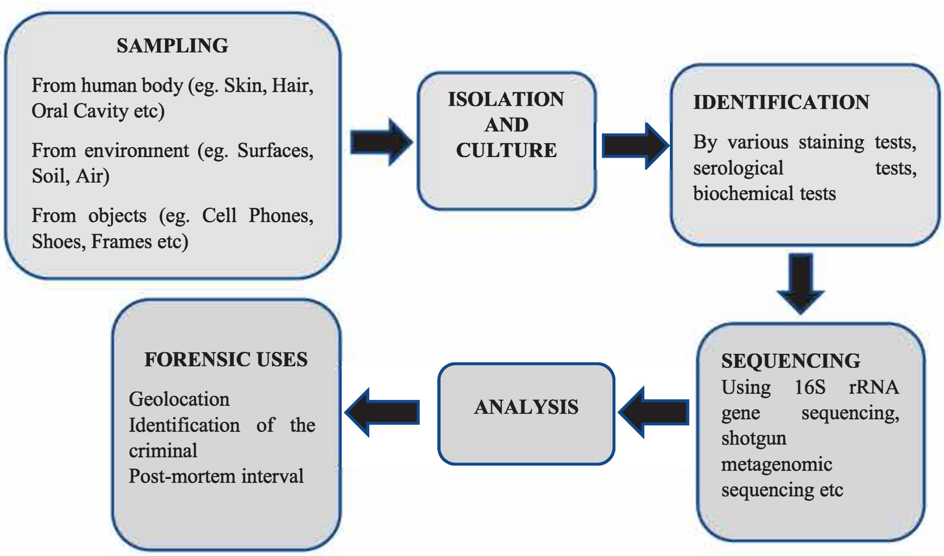

Fig.3. Microorganisms assisting in resolving biocrimes

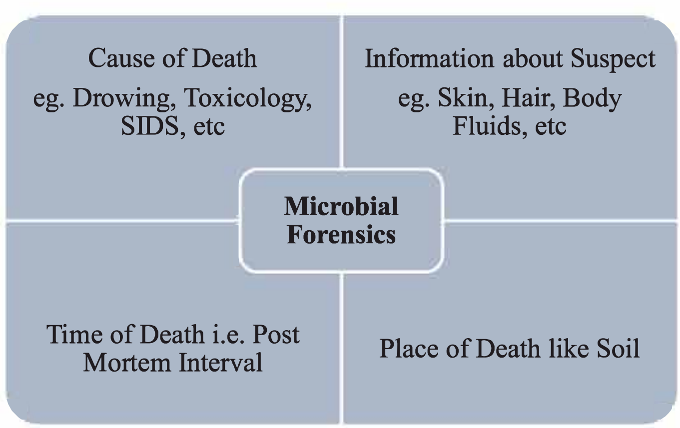

Fig.4. Applications of Microorganisms in Forensics

CRISPR-Enabled Tools for Engineering Microbial Genomes

Prokaryotic DNA segments called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) include short base sequence repetitions. In between these short repeats, a spacer DNA made from bacteriophage or plasmid DNA is inserted. Geneticists and medical researchers can edit a portion of the genome by deleting, inserting, or changing DNA sequences using the novel CRISPR-Cas 9 technology. It is now the more affordable, quick, adaptable, and straightforward technology for genetic manipulation and is creating a stir in the scientific community. Nucleotide repeats and spacers are two distinct features of this unique section of DNA. Repeated sequences of nucleotides, the DNA's building blocks, are dispersed across a CRISPR region. These repeating sequences contain DNA fragments called spacers. The spacers in bacteria come from earlier viruses that assaulted the organism. They work as a memory bank, allowing bacteria to identify viruses and defend themselves from further attacks. The *Streptococcus thermophilus* bacteria were utilised, which are typically present in yoghurt and other dairy cultures, as their model as published in the journal Science in 2007. It was noticed that additional spacers were added to the CRISPR area. Furthermore, the DNA sequence of these spacers closely resembled portions of the genome of the virus. Additionally, the spacers were changed by being removed or fixed in new viral DNA sequences. The bacteria's resistance was modified to be used as a specific virus attack in this way. Thus, the scientists established that CRISPRs are essential for controlling bacterial immunity. The prokaryotic defence mechanism uses CRISPR-Cas9 to stop the introduction of foreign DNA from bacteriophage and plasmid segments.

Microbial engineering techniques have recently undergone a revolution due to CRISPR-Cas technologies. The productivity and scope of engineering efforts have increased thanks to the genome editing and non-editing operations of multiple CRISPR-Cas systems, which have also created new opportunities for modifying the genomes of non-model organisms. We must create better, more versatile methods to manipulate these systems as the variety of species utilised in biotechnological processes grows. The recent developments in genome-scale engineering in model organisms like *Escherichia coli* and *Saccharomyces cerevisiae*, as well as technologically advanced techniques for high-throughput, effective genome-scale engineering have been employed. The non-editing CRISPR-Cas applications are there for RNA editing, labelling, epigenetic remodelling, changing gene expression, and designing synthetic gene circuits. Finally, there is still a need of areas to be explored in order to broaden the potential applications and improve the effectiveness of these newest techniques (Tarasava *et al.*, 2018).

Bioremediation

The biological process used by microorganisms to break down contaminants is known as bioremediation. Today, numerous toxins have polluted the entire environment and the most effective solution for addressing this difficulty is microorganism's application. Because of their metabolic activity, microorganisms have potential to thrive in almost every harsh environment on the earth. Through the all-encompassing microbial activities, bioremediation is heavily involved in the eradication, immobilisation, detoxification or degradation of a variety of chemical toxins, pollutants or wastes and physical harmful items in the environment. The elementary idea is the enzymatic metabolization of various pollutants such pesticides, hydrocarbons, dyes, effluents, oil, heavy metals and other substances that are harmful to the environment. Abiotic and biotic environments, which impact the rate of deterioration, fall into two categories. Various techniques are currently used throughout the world; some popular ones are biostimulation, bioaugmentation, bioventilation, and attenuation. Due of their unique applications, each bioremediation strategy has its own benefits (Pal *et al.*, 2020)

Types of Bioremediation

Under the category of bioremediation procedures, there are numerous types of treatment technologies or methods. Biostimulation, attenuation, augmentation are the basic bioremediation techniques which are discussed below.

Biostimulation

This type of method involves adding certain nutrients to the site (soil or groundwater) to encourage the activity of local microorganisms which initially, provide trace nutrients, growth supplements, and fertilisers. The rate and pathway of their metabolism are increased in the second step by providing and maintaining additional ecological needs such as oxygen, temperature and pH. By activating the operons for the bioremediation enzymes, the presence of a small amount of pollutants can also act as a stimulant. Most frequently, this kind of planned course is followed by adding nutrients and oxygen to aid activity of native microorganisms in the habitat. Because all bacteria require molecules which are high in nitrogen, phosphorus, and carbon, these nutrients are the essential building blocks of life that enable microbes to develop their fundamental needs, such as cell biomass, energy generation, and enzymes to digest toxic substances and pollutants (De Vrieze *et al.*, 2017).

Bioattenuation

The elimination of toxic contaminant concentrations from the soil or water environment is known as bioattenuation or natural attenuation. It is carried out via different biological processes, such as anaerobic and aerobic biodegradation, uptake by plants and animals and by various physical phenomena, such as a dispersion, sorption/desorption, dilution, diffusion, volatilization, dispersion, and chemical reactions (complexation, ion exchange, abiotic transformation). The more inclusive concept of natural attenuation includes intrinsic remediation or biotransformation. Bioremediation process is accelerated by biostimulation or bioaugmentation if natural attenuation isn't quick or thorough enough.

Bioaugmentation

Bioaugmentation that is one of the major biodegradation pathways is the technique of introducing pollutant and contaminant degrading microbes which may be natural, synthetic or exotic to a contaminated area in order to increase the native microbial populations' ability for biodegradation. Microbes are taken from the cleanup site, individually cultivated, genetically altered, and then released back there. For evidence, soil and groundwater contaminated with chlorinated ethenes like tetrachloroethylene and trichloroethylene are home to the necessary bacteria. It is used to make sure that the *in-situ* microbes could totally eliminate and transform the so mentioned pollutants into harmless chloride and ethylene.

The method of adding genetically engineered microorganisms that function as bio-remediators to a system with objective to swiftly as well completely remove complicated contaminants is known as bioaugmentation. The ability of genetically altered microbes to bioremediate soil, groundwater, and activated sludge has been demonstrated. These organisms showed improved degrading capacities for a variety of chemical and physical contaminants (Abatenh *et al.*, 2017).

Microbial Genetics for Vaccine Development

The vaccine development is an example of advanced application of microbial cells (fig.5). For this application; from pathogens, genes are taken that can produce surface antigens competent enough of producing neutralising antibodies in the host which is attacked or invaded by the pathogen (Plitnick, 2013). A successful application of this process led to the creation of the now-commonly used hepatitis B virus (HBV) vaccine. Hepatitis B core antigen (HBcAg) genes have been found, and HBV DNA has been cloned in bacteria. *Escherichia coli* can synthesise small amounts of antigen, but to increase this, scientists have created plasmids that can express the antigen genes under the effective tryptophan (trp) operon regulatory region. Similar methods have been used to develop vaccines against various other animal and human diseases, and some of these preparations are currently being tested. The quite significant contribution of bioengineering is the development of fundamental research techniques on issues like antigenic variation, virulence determination, immunological response to viral antigens, virus receptors and many more. The core antigen of HBV may serve as a model for genetically engineered products that are useful diagnostic tools and have significant applications in immunological research with direct relevance to the creation of vaccines (Murray *et al.*, 1989).

Recombinant bio proteins produced by bioengineering methods, that are advantageous in biopharmaceutical applications. Bioengineering tools include a variety of methods for isolating and identifying the essential genes from a wide variety of microorganisms in the host plant genetic material in order to make organisms that are intrinsically transformed or altered due to novel features. For the manufacturing of medicinal goods, such as vaccines, monoclonal antibodies, cytokines, enzymes, and growth factors, bioengineering of microbes was extensively exploited (Sarsaiya *et al.*, 2019).

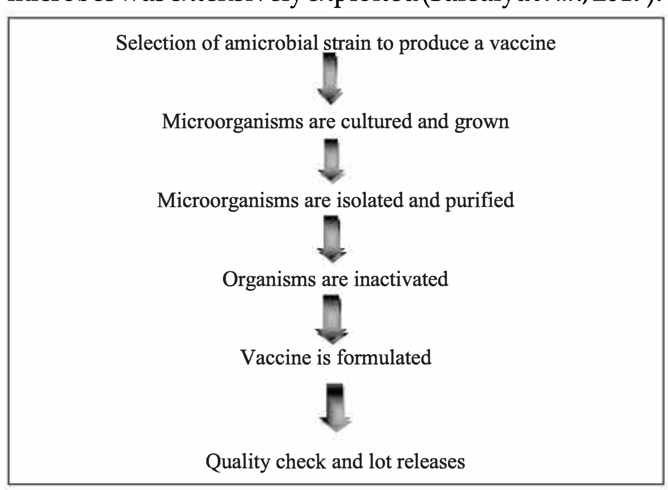

Fig.5. Vaccine Development from Microorganisms

Microbes for Making Biofuels

Many different scientific domains have a specific interest in microorganisms. They are present in the majority of the planet's surroundings, and their use as a fuelling agent is an important technological revolution. In the production of ethanol for biofuels from lignocellulose, a compound made of cellulose, hemicellulose, and lignin that makes up the plant cell wall, microorganisms are used (Antoni *et al.*, 2007). Cellulase is the enzyme that decomposes cellulose. The termite guts and soil found near volcanoes are two examples of these unusual ecosystems. An archaea bacterial species called *Sulfulobus solfatarticus* is discovered in volcanic waters close to Mount Vesuvius, Italy. Experiments with genetic modification have been conducted by researchers to improve this microorganism's capacity to produce the necessary enzymes (Prasetyo, 2002).

Trichoderma reesei is a fungus that is widely distributed in soil. It eats by releasing a considerable amount of cellulase. This fungus, degraded the tent and cloth cellulose of American soldiers, first identified during World War II, was accountable for 'jungle rot'. The fungus has been genetically engineered by businessmen from Canada to produce cellulase in copious amounts to break down straw into glucose, which was in next step converted into ethanol. They were thus successful in changing seventy five percent of straw into glucose (Sloothaak *et al.*, 2015).

Another important option is algae which turn carbon dioxide into sugar through photosynthesis that they employ to produce lipids. Though on a small-scale, scientists worldwide are using lipids to manufacture biodiesel and algal carbohydrates in laboratory bioreactors for the production of bioethanol (Matsuda, 2013). The concern of waste disposal is another one matter to be dealt. The waste is minimized given that the plant material like straw used is unpalatable. For that reason, a microbe-based system to manufacture biofuel is further economical, more proper and affordable. Additionally, it significantly lowers greenhouse emission and gas use.

Microbes as macro factories for production of pharmaceuticals and biochemicals

Secondary metabolites, commonly referred to as natural products, are produced by a variety of microorganisms, including bacteria, fungus, and plants. Many biochemicals which are different chemically and biologically and having medicinal properties, such as immunosuppressive, anti-inflammatory, antimicrobial and anticancer potential, have their origin in natural products. Many of these agents have been established as treatments or have the potential to be used therapeutically for human diseases (Firáková *et al.*, 2007). A wide range of biopharmaceutical products, including recombinant proteins, have been developed as a result of the most recent advancements in recombinant DNA technology in addition to natural products, offering significant improvements in the treatment of a variety of medical illnesses and conditions. Enzymatic synthesis or microbial fermentation is used to create fine compounds that have physiological activity, for instance additives, medications, cosmetics, flavouring agents and nutritional supplements for food, feed, and fertiliser. The required fine chemical can be produced by identifying the enzymes that catalyse the target process. The appropriate microbial hosts are then given the genes encoding these enzymes and are cultivated with cheap, naturally plentiful carbon sources and other nutrients. Through metabolic engineering, microbial cells are transformed into effective factories that produce compounds with increased yields. Researchers are summarizing current studies on the manufacture of fine biochemicals derived from biological sources and evaluate the possibility of synthetic bioengineering to further increase their productivity (Rasool, 2021).

Contemporary Microbial Bioengineering: Vista and Prospects

The use of microbial biotechnology in the ancient era was limited to meals and beverages. Microbial biotechnology advanced together with the advancement of physical sciences and gave rise to breakthroughs in bioscience, medical science, agricultural sustainability and food science. The advancement of biotechnological methods enables the rapid identification of novel compounds, precise nomenclature of microorganisms, or strain enhancement of well-known species via genetic engineering methods. Several helpful microorganisms are currently being used for a variety of purposes, although research has focused mostly on how microorganisms in agriculture might boost plant growth and act as a biocontrol agent for phytopathogens.

The main applications of microbial bioengineering in the modern era are the discovery and manipulation of various microbes for industrial applications and value addition in food products, exploring and producing secondary metabolites of extensive utilization, drug discovery and its production by microbes, and microbe-based biosensors. Additionally, microbes are used as bio factories to create a variety of biochemicals, fuel molecules and industrial polymers, and genetically engineered strains that are beneficial to the environment because of their ability to decompose or absorb substances. As a result, the use of microbial bioengineering has been beneficial for achieving environmental sustainability in the modern period for societal benefits (Maurya *et al.*, 2021).

Pros of bioengineering in the current scenario are summarized below:

- Bioengineering enhances medical interventions.
- It leads to resource conservation.
- Bioengineering can aid in pollution management.
- It reduces the prevalence of diseases.
- It enhances crop nutritional value.
- Bioengineering can be used in improving human health.
- Medical advancement opportunities are immense.
- It can improve crop yields.
- Pesticide usage can be minimized.
- Extending lifespan of foods is observed.
- It has the potential to detect whole microbial population.

Cons of bioengineering are plenty but benefits are far more as compared to negative aspects. Some of the disadvantages are as follows:

- Numerous species' survival may be in danger as a result of bioengineering.
- There is an increase in the number of plant pathogens that are spreading.
- It affects soil fertility adversely.
- It can be applied for destruction.
- It is very expensive.
- Data analysis is difficult.

Conclusion

The benefits and cons of biotechnology are diverse. Before using a particular technology, it is vital to assess its advantages and disadvantages. Even though technology has certain problems, many nations have embraced it because of its tremendous advantages. For example, it can aid in the treatment of several disorders with genetic roots. It can be used to develop higher yielding crop types. Over time, bioengineering has advanced and helped produce high yields. Before making a decision, it is crucial to consider the benefits and drawbacks of biotechnology then we will be in a better position to choose wisely after reviewing the list of advantages and disadvantages above.

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