ISBN No. 978-81-957386-3-2

# RECENT ADVANCES IN THE FIELD OF LIFE SCIENCES-VOL 1

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PUBLISHED BY
**SAI COLLEGE**
Street-69, Sector-6, Bhilai (C.G.)

**PREFACE**

We are delighted to publish our book entitled “Recent Advances in the Field of Life Sciences - Vol 1". This book is the compilation of esteemed chapter of acknowledged experts in the fields of Life Science. This book is published in the hopes of sharing the excitement found in the study of Life Science. We developed this digital book with the goal of helping people achieve that feeling of accomplishment. The chapters in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

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Sai Publication Sai College

Sector-6, Bhilai, Dist Durg (Chhattisgarh)



ISBN: 978-81-957386-3-2

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# CHAPTER – 1
# NUCLEAR POLYHEDROSIS VIRUSES IN INSECT PESTS MANAGEMENT

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**Abstract**

Insect pests are responsible for causing economic losses ranging from 10 to 18 percent in farm produce, primarily due to shifts in global climate patterns. The improper use and excessive reliance on pesticides have resulted in the development of resistance in insect pests, leading to increased input costs. To address this issue, utilizing nuclear polyhedrosis viruses as a pest control method has emerged as an eco-friendly alternative. The application of nuclear polyhedrosis virus (NPV) offers a sustainable and targeted approach to managing agricultural and forestry pests. It is essential to comprehend the operational mechanisms of NPV, its application techniques, and the potential impacts on non-target species and human health in order to maximize its efficacy while minimizing associated risks.

**Key Words:** NPC, Virus, Pest Management, Insect and IPM

## Introduction:

Crop production worldwide is declining annually due to various factors such as weeds, insect pests, and pathogens including viruses, bacteria, and fungi. These challenges are particularly prevalent in tropical and subtropical regions where monoculture farming is common. Over the past few decades, synthetic chemical pesticides have been extensively utilized for pest control. However, these synthetic insecticides not only harm beneficial fauna like entomopathogens but also have negative impacts on humans, birds, animals, and the environment. The unavailability, high cost, persistence in the environment, and development of insect resistance have led farmers to refrain from using synthetic chemical control methods. Instead, integrated pest management (IPM) has been embraced by the public, which combines cultural, physiological, chemical, and biological controls to mitigate insecticide resistance and environmental risks (Chandler *et al*., 2011).

## 1. Overview of Nuclear Polyhedrosis Viruses:

Nuclear polyhedrosis virus (NPV) which are part of the baculoviruses (BV) family, exhibit specificity towards a range of insect pests that affect economically important crops and forests pests as indicated by (Tang *et al*. 2011). The BV family comprises around 600 viruses, with two main genera being NPV and Granuloviruses (GV). Studies by (Tang *et al*. 2011) and (Zhang *et al*. 2015) have highlighted the significant potential of NPVs in managing various lepidopterous pests.

### 1.1. History of NPV:

In 1892, Germany pioneered the introduction of NPV to combat *Lymantriamonacha* in pine forests. By 1913, the United States conducted the first field application of NPV against gypsy moths, with a

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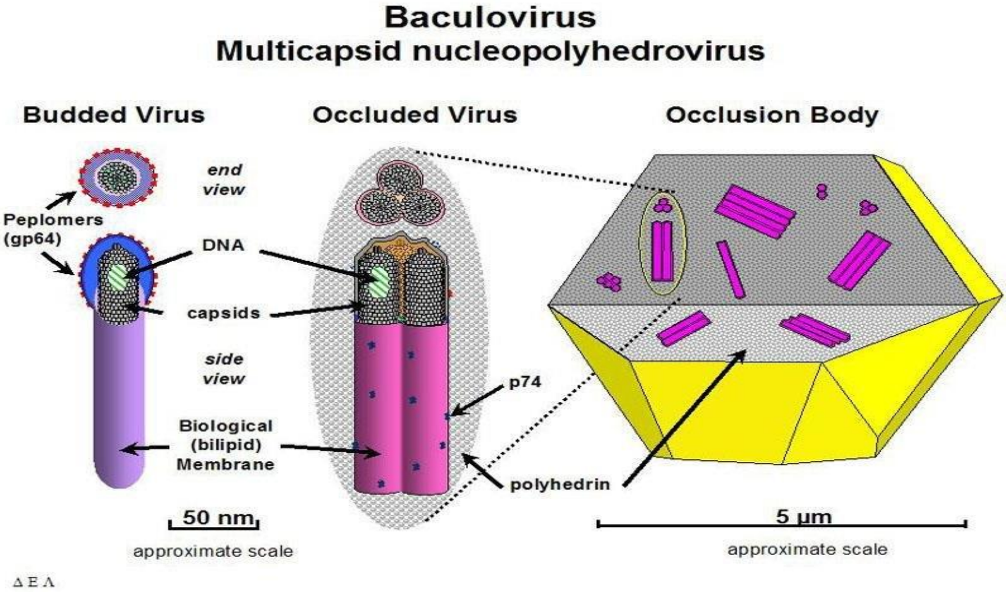
Californian farmer utilizing NPV-killed larvae of *C.eurytheme* as an inoculum for alfalfa crops. The year 1975 marked the registration of the first viral insecticide (Elcar) in the U.S.A, specifically targeting cotton bollworm (*HelicoverpaZea*). Baculoviruses offer a viable alternative to chemical insecticides for pest control due to their lack of residue effects and minimal impact on beneficial predators and parasites. Helicoverpa and Heliothis are known to infest over 65 different crops, including maize, wheat, sorghum, legumes, solanaceous crops, and malvaceous crops. Several significant species of Helicoverpa, Spodoptera and Heliothis, such as *Helicoverpaarmigera*, *Helicoverpaassulta*, *Heliothisvirescens*, and *Helicoverpazea*, *Spodopteralitura*have shown sensitivity to NPV.

**1.2. The Structure of NPV:**

The Nuclear polyhedrosis virus is a pathogen that is obligatory in nature. It possesses a rod-shaped nucleocapsid, measuring approximately 250-400 nanometers in length and 40-70 nanometers in width. This nucleocapsid contains double-stranded circular DNA (Maeda *et al*1993:Tinsley 1985). Enclosed within an envelope known as virions, the nucleocapsids are embedded within a proteinaceous polyhedral occlusion body (Beniya 1996: Grula 1981). Upon exposure to alkaline gut juice, the viruses are released and proceed to attack various cellular components such as the nuclei of cell tissue, fat bodies, hemocytes, tracheal matrix, ganglia, and the brain [Harrap1970].




**Structure and key features of an NPV (Lynn, D. USDA, 2006)**

**1.3. Factors That Influence Effectiveness of NPV:**

Temperature has a major impact on the effective control of insect pests in crop fields and such parameters were as follows.

- Daytime temperatures of 25–35°C are ideal for NPV activity.
- NPV activity is slower under cooler conditions (do not apply below 18°C).
- Larvae may stop feeding below 15°C, and take a while to recover. Delay applying NPV until temperatures have warmed to above 20°C following a cold night.
- Larvae death may take longer in cooler weather, but this will not influence overall damage levels.

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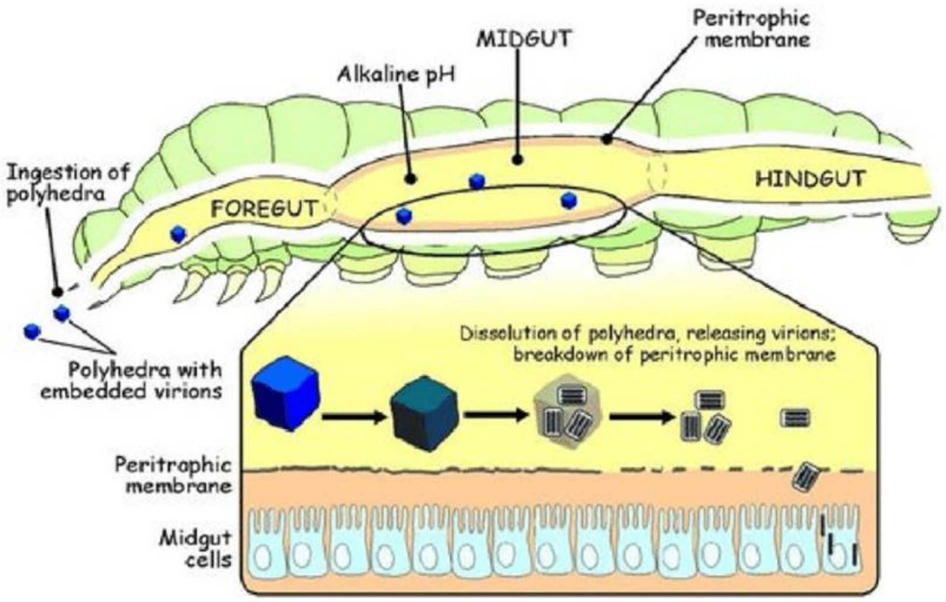
**1.4. Infection Process:**

The typical mode of infection occurs through the ingestion of polyhedra or, in certain instances, virions. The larvae are susceptible to infection, and when polyhedra are consumed along with their food, the occlusion protinacious bodies begin to dissolve under alkaline conditions with a pH greater than 9. The virions then proceed to infect the epithelial cells of the midgut. Within the nucleus of these infected cells, new virions are generated and subsequently infect cells in the hemocoel and other tissues, such as the fat body (Rahman and Gopinathan 2004). Within these tissues, the virions become occluded within polyhedra, and this process continues until cell lysis occurs. Approximately 24 to 72 hours after infection, the presence of polyhedra can be observed within the nuclei of infected cells. Following a few days, the larvae perish and are scattered across the foliage and soil. Predators and parasites then consume or disperse the infected larvae to other insects or locations

(Entwistle 1983). Infected insects exhibit a dull coloration, reduced activity, and the larvae display a reddish-pink hue on their ventral side. As the infection progresses, the larvae become limp, their skin becomes exceedingly fragile, and eventually ruptures [Deb 2015] Infected larvae often hang upside down on plants, a phenomenon referred to as "tree top disease" or "wipfelkrankheit." The diseases caused by NPV in silkworms are known as Grasserie (Babu et al 2009)




**1.5. Baculovirus (NPV) Transmission Routes:**

After larvae consume OBs while feeding on contaminated foliage, a portion of the infected individuals undergoes lethal disease and releases OBs onto the host plant, facilitating transmission to a susceptible host (red arrow). Rainfall washes OBs from foliage into the soil, allowing their transportation back to plants through biotic and abiotic factors (black arrows). On the other hand, insects that consume OBs and survive may continue their development, eventually pupating and emerging as covertly infected adults (blue arrows). These adults have the ability to disperse before laying eggs, thereby passing the infection to their offspring. Vertical transmission can persist over multiple generations until the activation of an elicitor or stress factor (orange arrow) triggers the covert infection to transform into lethal disease, thus restarting the horizontal transmission cycle (red arrows).

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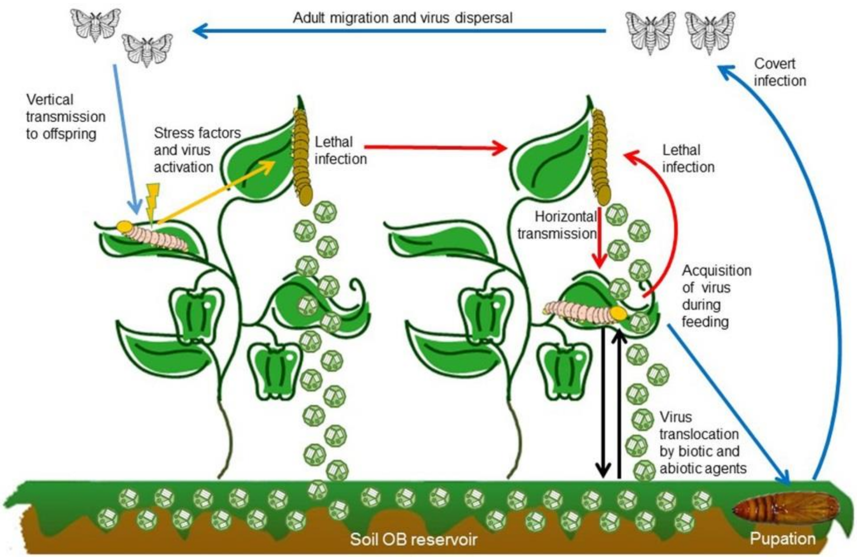
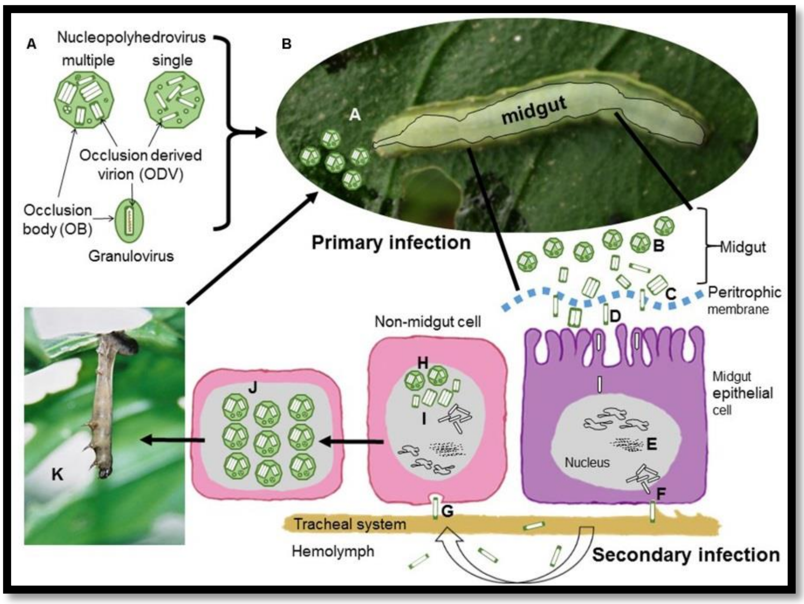
### 1.6. The Infection Symptoms of NPV:




NPV-infected larvae may exhibit initial symptoms of either turning white and granular or becoming very dark. In some cases, these larvae may ascend to the uppermost part of the crop canopy, cease feeding, and display a limp posture, ultimately hanging from the upper leaves or stems. Consequently, this condition is commonly referred to as "caterpillar wilt" or "tree top" disease. Conversely, larvae affected by a granulosis virus may undergo a transformation where they assume a milky white appearance and cease their feeding activities.

## 2. Insect Pests Management by NPV:

### 2.1. Application of NPV




To enhance the effectiveness of NPV, it is recommended to spray it late in the day when peak sunshine or evening escape sun's rays, in order to protect the microorganism particles. Previous studies have shown that adding an ultraviolet light absorbent, such as robin blue, to the spray solution can improve the effectiveness of NPV. Additionally, the inclusion of an adhesive like teepoland a phago-stimulant like jiggery has been suggested to enhance the efficacy of NPV. The dosage of NPV application varies between 250-500 LE/ha, depending on the density of the foliage.

### 2.2. Application Systems:

**High-volume**: For the application of nuclear polyhedrosis viruses in crops, various spraying systems can be utilized. High-volume applications can be carried out using an azo-propen hand sprayer equipped with a birchmeier helicon sapphire nozzle100, with a pressure of 4 bar. It is recommended to position the sprayer nozzle 50 cm above the crop during the spraying process.



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**Low-volume**: Low-volume spinning disc applications can be performed using micron-ulva eight spinning discs. The sprayer nozzle should be positioned 75 cm above the crop during spraying, and the droplet angle should be set at 50 degrees. It is advised to apply 3 ml of virus suspension in water with 20% mineral oil per square meter.

**Ultra-low**:Ultra low volume electrodynamic sprayer In the case of an ultra-low volume electrodynamic sprayer, an electrod provided by ICI is utilized. The sprayer nozzle should be positioned 50 cm above the crop during application.

### 2.3. Major Insect Pests and Their Management by NPV:

#### 2.3.1. Management of *Spodopteralitura* by NPV:

*Spodoptera litura*, a member of the Lepidoptera family Noctuidae, is a highly destructive pest that feeds on various crops. Its global significance arises from the extensive damage it causes and its resistance to insecticides. In the control of *Spodoptera litura* on cabbage, the use of SlNPV (*Spodoptera litura* nucleopolyhedrovirus) has shown promise as an alternative method. Research suggests that SlNPV can effectively combat this pest when applied at a rate of 500 liters per hectare. The application involves two sprays, with the first spray administered 45 days after transplanting cabbage in the field, followed by a second spray at the 60th day after transplanting.

**Table 1 Worldwide infestation by larvaeof *Spodoptera*spp. on various plant species**

| | | | | |
|---|---|---|---|---|
| strawberry | strawberry | strawberry | strawberry | strawberry |
| Lettuce | Lettuce | Lettuce | Lettuce | Lettuce |
| sage | sage | sage | sage | sage |
| Citrus | Citrus | Citrus | Citrus | Citrus |
| coffee | coffee | coffee | coffee | coffee |
| sugar beat | sugar beat | sugar beat | sugar beat | sugar beat |
| Maize | Maize | Maize | Maize | Maize |
| Potato | Potato | Potato | Potato | Potato |

#### 2.3.2. Management of *Helicoverpaarmigera* by NPV:

*Helicoverpa armigera*, a member of the Lepidoptera family Noctuidae, holds great significance as a lepidopteran pest. It is considered the most important pest of its kind in the region. This pest poses a serious threat to various economically important agricultural crops, including citrus. During spring, the moths of *Helicoverpa armigera* lay their eggs on or near the blossoms, further exacerbating the damage caused by this pest. A concentration of 1.15x107 OBs/ml of HaNPV is sprayed using a knapsack resulted in a100% reduction in *H. armigera* larval infestation within 7 days on tomato plants in ahot house environment. A 10-fold lower concentration, 1.15x106 OBs/mL, resulted in a 100%reduction within 16 days.

**Table 2: Insect pests of plants and their management by NPV**

| Scientific name of Host insect | Common name in India | Associated crops |
|---|---|---|
| *Helicoverpaarmigera* | cotton bollworm | Cotton, Tomato, Wheat |


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| | | |
|---|---|---|
| *Helicoverpazea* | Corn earworm | Maize |
| *Heliothisvirescens* | Tobacco Budworm | cotton |
| *Hyphantriacunea* | Fall webworm | Mulberry |
| *Buzurasuppressaria* | Tea looper | Tea & Tung oil tree |
| *Autographacalifornica* | Alfalfa looper | Cabbage, cotton & Ornamentals |
| *Anticarsiagemmatalis* | Bean caterpillar | Soybean |
| *Spodopteralitura* | Tobacco cutworm | Vegetables, rice and peanuts |
| *Spodopteraexigua* | Army worm | Cabbage, Cotton, Amaranthus and Sunflower |
| *Spodopterafrugiperda* | Fall armyworm | Maize |
| *Spodopteralittoralis* | cotton leaf worm | Cotton |
| *Lymentiradispar* | Gypsy moth | Forest |
| *Mamestrabrassicae* | Cabbage moth | Cabbage |

**Conclusion:**

In summary, this chapter paper establishes that organic foods are a secure choice for human consumption. NPV serves as an excellent alternative to chemical pesticides, ensuring minimal harm to the environment. Although the initial cost may be slightly higher, the implementation of in vivo techniques allows for the long-term replacement of chemical pesticides. To produce NPV effectively, it is essential to train and equip farmers with the necessary skills.

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ISBN: 978-81-957386-3-2

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ISBN: 978-81-957386-3-2

# CHAPTER- 2

# PRESSMUD WASTE: A POTENTIAL SOURCE FOR CELLULOLYTIC MICROORGANISMS

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## Abstract

Cellulose is an abundant natural biopolymer on earth and most dominating Agriculture waste. This cellulosic biomass is a renewable resource with great potential for bioconversion to value added byproduct. Cellulases are the enzymes which hydrolysis cellulosic biomass and are being produced by the microorganism utilizing cellulosic matter for their growth through extracellular release of bacteria. However second attempts have been reported for cellulosic activity of bacterial isolates and optimization studies to scale up the results. The present review deals with a detailed study about cellulase activity by various microorganisms and their possible application. Cellulase enzyme can be degraded by cellulolytic bacteria and fungi. This enzyme has various unique industrial applications and it has been considered as major group of industrial enzyme. Three main byproducts of the sugarcane industry are Pressmud, Molasses, and Bagasses. Microorganism such as bacteria and fungi are good producer of cellulolytic enzymes. Pressmud is waste created by the compressed sugar industry waste from cane juice filteration. Pressmud contains a high percentage of nutrients hence can be used as fertilizer. Its application also increases the bacterial, fungal and actinomycetes population. These microorganisms play an important role in the decomposition of organic materials to release beneficial nutrients for plant growth.

**Keywords-** Microorganism, Cellulase, Pressmud, Optimization, Cellulolytic microorganism.

## Introduction:

Sugarcane is one of the important crops in India and plays pivotal role in both agriculture and industrial economy of the country. In India Approximately 5.2 Million tones of sugarcane pressmud are generate as agro waste from sugarcane industries. An agriculture waste is a cheap source of cellulose for the production of different useful products all over the world. The most abundant renewable organic compounds in the biosphere is cellulose, Three major structural polymer combined to make up lignocellulose are called cellulose (a homopolymer of β D glucosyl unit Hemicellulase and lignin) (Saccharum and Officinarum,2018).

Different by product have been obtained from the sugar industry and these also play a key role in promoting a number of subsidiary industries. Sugarcane is a multipurpose crop used as a fundamental raw material for the production of paper boards, electricity sugar ethanol and auxiliary products the feeding of sugarcane is an important source of energy Molasses can be used for the production of ethanol on large scale by a distillery. Sugarcane process Bagasses has became a usual option for the

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sugar induatry in the generation of electricity and ethanol. In the paper industry Bagasses in a substitute for wood pulp and paper industry for economic and environmental sustainability.

Cellulase is a class of enzyme that catalyzes the hydrolysis of cellulose. Cellulase is a multiple enzymes system consisting of endo 1,4 β-D glucanase and Exo-1,4-β-D glucanase along with the cellobiose. Cellulases are expressed by a wide spectrum of microorganism in nature screening and isolation of cellulase –producing microbes from nature is one of the important ways to get novel cellulases. These newly screened microbes are source of new cellulase genes with diverse property to have cellulolytic ability.(Kuhand*et.al.*,2011).

Enzyme produced by these microorganism is commercially available for agriculture and industrial uses improvement at microbial stain for the over production of industrial product has been the hallmark of all commercial fermentation processes. Numerous microorganisms that are able to degrade cellulose have been isolated, screened and identified. However many studies have put more emphasis on cellulose degrading bacteria from agriculture, industrial and municipal waste because the cellulase that they produced are easily to extract, and some of the bacterial cellulases have been used as commercial cellulase .The study aimed to isolate and screen the cellulase producing-bacteria from sugar industry waste and purify and characterize the cellulase produced by the isolated bacteria strain.

Several studies on isolation and characterization of cellulose-degrading bacteria from industrial waste indicated that only a small number of bacteria can produces large amount of bioactive compounds that are capable of complete hydrolysis of crystalline cellulose in vitro. Among different industrial waste, sugar industry waste such as press mud are mainly cellulosic in nature and the microorganism present there have the capacity to degrade cellulose into glucose units for their normal growth and development. The study was conducted to screening, optimization, purification and characterization of cellulase from cellulase producing bacteria present in press mud.( Shanmugapriya,*et,al.*,2020)

Cellulases are inducible enzymes which are synthesized by microorganism during their growth on cellulosic material several microorganism can produce cellulase enzyme including fungi, bacteria, and actinomycetes. The present study was to isolate a potential organic solvent thermostable alkalophilic cellulase producing bacteria from natural ecosystem the cellulase enzyme was applied for purification and characterization of different parameter and purification was applied for the ethanol production and many industrial uses.

**What is Cellulase:**

Cellulases are the enzymes which hydrolysis cellulosic biomass and are being produced by the microorganism grown over cellulosic matters. Cellulases is an important enzyme which can be obtained from cheap agro wastes ,as well as cellulose protein can be degraded by using different types of fermentation. Cellulose protein can be degraded by cellulase enzyme produced by cellulosic bacteria and fungi. This enzyme has various unique industrial applications and it has been considered as major group of industrial enzyme.

Cellulose degradation is carried out by the enzymes called “cellulases”, responsible for the hydrolysis of β-1, 4linkages present in cellulose. Cellulases are extremely important enzymes both industrially

 ISBN: 978-81-957386-3-2

and in the natural world, because they play a major role in the global carbon cycle by degrading insoluble cellulose to soluble sugars. Cellulases are the most diverse class of enzymes that catalyze the hydrolysis of a single substrate, because there are three types of cellulases, endocellulases, exocellulases, and andendoglucanases.

**Endoglucanase:-**Endoglucanase often called CMCasehydrolyzescarboxymethyl cellulose or acid swollen amorphous cellulose, soluble derivatives of cellulose such as carboxymethyl cellulose (CMC) cello-oligosaccharide due to which there is a rapid decreases in chain length along with a slow increases in reducing groups Endoglucanase also acts on cellodextrins ,the intermediate products of cellulose hydrolysis ,and converts them to cellobioase and glucose (Sharada*et.al.*,2014).

**Exoglucanase:-**Exoglucanase and cellohydrobiolases degrades cellulose by splitting of the cellobioase units from the nonreducing ends of cellulose polysaccharides chains librating ether glucose or cellobiolose as major product.Cellobiohydrolase does not degrade cotton promptly, but can affect considerable saccharification of microcrystalline substrate such as Avicel, amorphous cellulose, and cellooligosaccharide. However, they are inactive against cellobioase or substituted soluble cellulose such as CMC (Sadhu and Maitri, 2013)

**B-Glucosidase/Cellobiose:-**β-Glycosidase hydrolase cellobioase or cello-oligosaccharides to glucose and are also involved in transglycosylation reaction of β-glucosidic linkages of glucose conjugates.

Beta-gylcosidase, endo-1, 4 D- glucanase (endoglucanase), and exo-1, 4-D-glucanase are the three enzymes that makeup cellulase. These three enzymes work together to hydrolyze Cellulose in a synergetic manner for complete and effective cellulose hydrolysis.

**What is Cellulolytic Properties:**

Most of the enzymes are imported at huge costs and there is a need for its commercial industrial applications. High cost and low activity are the major impediments to the commercial uses of cellulases. Cellulases are cellulose degrading enzymes with a great potential to convert cellulosic material into its subunit-glucose cellulases have been commercially available for more then 30 years, and these enzymes have represented a target for both academics as well as industrial research. The promising cellulolytic microorganism can be employed for the production of cellulolytic enzymes such as CMCase, FPase and β-glucosidase by using different agro-residues as the carbon source during submerged fermentation (Mishra*et, al.*, 2018).

The properties of the cellulases are playing a vital role in different type of industries. Cellulases are the most diverse class of enzymes that catalyze the hydrolysis of a single substrate. Cellulose is the main source of carbon is decomposed by the cellulase enzymes which hydrolyse β-1,4 glycosidic bonds. The ability to synthesis cellulase is shown by both bacteria among others *Bacillus, Cellulomonas, Pseudomonas,* and fungi *Aspergillus, Trichoderma, Penicillium* (Maki *et.al.*,2017).

**What are Cellulolytic Microorganism:**

Cellulolytic microbes are primarily carbohydrate degrades and are generally unable to use protein or lipids as energy source for growth. Cellulolytic microbes notably the bacteria Cellulomonas and most fungi can utilize a variety of others carbohydrate in addition to cellulose. The ability to secrete large amount of extracellular proteins is characteristics of certain fungi and bacteria are most suited for

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production of higher levels of extracellular cellulases. One of the most extensively studied fungi is *Trichoderma reesei,* which converts native as well as derived cellulose to glucose. Most commonly studied cellulolytic organism include:-
Fungal species- *Trichoderma, Humicola, Penicilium, Aspergillus.*

Bacterial species- *Baccillus, Pseudomonas, Cellulomonsa, Actinomycetes, Streptomyces, Acetobacter, Actinomycetes and Streptomyces.* (Watanabe and Tokuda,2011).

Microorganism such as bacteria and fungi are good producer of cellulolytic enzymes,althrough fungi were more suitable for cellulase producer due to their extracellular properties. On a commercial scale, several fungal and bacterial strain have been utilized to produce cellulases commercially, substrate such as cellulose or CMC have been used to produce cellulase. Both fungi and bacteria have been exploited for their abilities to produce a wide variety of cellulases and hemicelluloses. Most emphasis has been placed on the use of fungi because of their capability to produce copious amounts of cellulolytic enzymes and often less complex then bacterial cellulase and easy for extraction and purification. Microorganism produced cellulases but it is now clear that some insects, mollusks, nematodes and protozoa also produce cellulases. When termites, ruminant or shipworm utilize cellulose as an energy source microorganism usually are involved in its degradation (Weimer *et.al.*, 2016).

**Substrate used as cellulase source and types:**
Sugar can be produced from sugarcane by converting into raw juices and after heating and addition of lime, at the end of evaporation steps, the matter changed into raw sugar. Molasses is obtained during evaporation. Usually by products known is as pressmud also obtained during clarification. Pressmud is mostly used for fish food and as a fertilizer for crops. During the processing of sugar, various wastes such as Bagasses, Pressmud, Molasses and waste water also produced.
Sugarcane is one of the most popular ingredients in the manufacturing of sugar and ethanol. Sugar and alcohol generate complex for major type of waste products. Pressmud is the waste created by the compressed sugar industry waste from the cane juice filteration. It is a good fertilizer source around 12 millions tons of pressmud are produced by sugar mill in India as waste from double sulphicationprocesses. The pressmud can be used for energy production as it contains a higher fuel. It is also a strong biogas source because it contains approximately 5-15% sugar content .Pressmud is focused on the utility of fertilizer because it is rich in many micronutrients (Zechel and Withers, 2011).

Sugarcane waste residues are grouped into three kinds of wastes, molasses, bagasse and pressmud. A huge amount of sugarcane baggase is produced and destroyed or burnt in developing countries insufficient causing environmental pollution. Both these wastes are the same in properties but they are used as different bio-resource. In the sugar manufacturing industry, sugarcane stalks are chopped into small pieces and cane juices is obtained. Residual material that will be cleaft behind from this operation, consisting of fibrous remain of the cane sugar is known as bagasses. The precipitation in the from of sludge after filteration is called filter cake or pressmud (Sharada, *et.al*., 2014).

Molasses are by product generated during the sugar crystallization process. The composition of molasses depends upon the type of sugarcane, the agro-climate condition of the crop growing region,


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ISBN: 978-81-957386-3-2

sugar production process, storage and handling. Molasses is mostly used in alcohol distilleries because of containing formative sugars that are the best source of carbon for the metabolism of microorganism. Sugarcane molasses based distilleries produced 7 to 15l alcohol that is categorized by high chemical oxygen demand and low pH and brown colour (Vimal *et.al.* 2013).

**Other Studies and Application of Cellulase Enzyme:**

Agriculture and industrial wastes are among the main causes of environmental pollution. Their conversion into useful products may reduce the intensity of the problems caused by them. Sugarcane industry waste include pressmud,baggases ,molasses a large quantity is left form lands to be decomposed by microorganism such as bacteria and fungi (Okafor *et.al.*,2019). The most industrial important material other then food stuff affected by microorganism are cellulose and wood products, proper utilization of these wastes in the environments will eliminate pollution and convert them into useful byproducts ( Milata*et.al.*,2018). Enzyme production is the first crucial step in enzyme technology which needs to be paid much attention since it determines the economic feasibility of the process. Considerable number of cellulase has been produced from fungal species such as *Aspergillus* and *penicilium*. Several fermentation condition play fundamental roles on cellulaseproduction,among which fermentation method,carbon source, nitrogen source,pH ,temperature, salt/metal ion effect ,incubation time, aeration.(Saini *et.al.*,2017).

Cellulolytic enzyme play an important role in natural biodegradatation processes in which plant lignocellulosic materials are efficiently degrade by cellulolytic fungi,bacteria,actinomycetes and protozoa. Industry, these enzymes have found novel applications in the production of fermentable sugar and ethanol organic acids, detergent and other chemical cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization. CMC acts as good indicator of cellulytic ability, isolated fungal and bacterial strains were primarily screened by activity zone technique with Congo-red dye. Congo red dye is a met achromatic dye that can react with cellulose. The CMC is stained red to deep pink. After the treatment of CMC with cellulase enzyme, excess dye was made to note the substrate utilization zone around the colony. The parameter like temperature, pH, and substrate concentration and incubation time was optimized for selected bacterial strain. For the optimization of temperature and fermentation process.

Cellulase enzyme used after purification, the purification of crude cellulase in different types of purification methods like-Ammonium sulphate precipitation, Ion exchange chromatography, and SDS-PAGE chromatography of cellulase enzyme purity. After purification cellulase enzyme used in different types of industries like-food industry, improvement of animal feeds, fermentation technology, textile industry, detergent and cosmetics industry, paper and pulp industry, brewing and wine industry. Cellulases have been a target for academic and industrial research and are currently being applied in many industries (Bhat, 2016, Kuhadet.al. 2018). Many researches have been conducted to find out industry beneficiary strain to increase the yield of cellulase production.

**Industrial application of cellulase (Premalatha*et.al.*, 2015) (Singh *et.al.*,2017)**

| Industry | Process |
|---|---|
| Pulp and paper | • Recycling of pulp. |

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| | |
|---|---|
| industry | • Removal of ink and polluted particles :Bioremediation of waste<br>• Improvement of sheet strength and brightness; Cleans the surface of fibers ;Improve the strength properties ;Brightness;Cleaniliness;Fairness ;Deinking; Reduce the chlorine requirement. |
| Bio-fuel production | • Production of biofuel and ethanol<br>• Production of second and third generation biofuel |
| Textile industry | • Wet processing<br>• Bio stoning of jeans,Biopolishing,Removal of dyes:Improvementsin softness<br>• De-fibrillation of Lycocell;Bio-carbonization and wool Scouring |
| Laundary and detergent industry | • Used in detergents for the removal of dirt and for enhancing the glossier appearance, brightness and smoothness of the fabrics. |
| Food and feed industry | • Improves the methods of stabilization, extraction of fruit and vegetable juices; found productive by improving the colour and texture.<br>• Decreases viscosity, improve flavor properties; reduces the bittemess of citrus fruits<br>• Improve the animal feed by providing extra supplementary support for better digestion and also cause gain in the weight |
| Agricultural industry | • Degrade the cell wall of the plant pathogens and keep plant safe from dangerous diseases; improve roots, crops yield ,germination of seed ,plant growth<br>• Improves soil quality thus it limited the use of mineral fertilizer |
| Treatment of diseases | • Can be used for the diagnosis and treatment of many disesases like Phytobenzoars,Blinding keratitis is and Granulomatous Amoebic Encephalitis |
| Waste management | • By utilizing cellulosic waste, it convert them<br>• Into valuable products; Cellulosic waste can also be used as a carbon source in fermentation process |
| Flavonoids extraction | • Used in the isolation of carotenoids and other Flavonoids |
| Research purposes | • Useful for studying single cell protein; In plants studies such as metabolic and genomics ,Virology; for isolation of protoplast |
| Paper and pulp industry | • In papermaking ,adding cellulase in the paper pulp can increase the yield of micro fibers and water retentively |

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**Discussion:**

Pressmud is a sugar mill waste component which is a very useful source of fertilizers as well as industrial used. The major use that has recently been developed in India is in biocomposting,where it is treated with the spent wash from the distillery. The concept of biological degradation of organic waste by anaerobic digestion for the generation of methane has been used by waste management industries for many years. Pressmud is an industrial waste available from the sugar mills.(Bhat,2017). Cellulases are produced by the majority of the systematic groups of organism:microorganism, protista, plants; however, the industry uses mostly enzymes produced by microorganisms. The first commercial cellulases were derived from fungi, Trichoderma *and Aspergillus*. Recently, the importance of bacterial cellulases has been rising. Cellulolytic enzymes are produced by aerobic and anaerobic bacteria eg.-*Bacillus, Butyrivibrio, Cellulomonas, Clostridium, Paenibacillus and Ruminococcus.* Large number of enzymes produced by resistance to harsh environmental conditions, spore-forming bacteria of the genus *Bacillus* and related genera seem to be most interesting. Despite that, only a small number of spore-forming bacteria can synthesize a large amount of cellulase capable of cystalline cellulose degradation in vitro (Agyei, 2010).

The sugar industry generates many toxic substances in the environment. The main by-products generate from the sugar industry pressmud, Bagasse, fly ash, molasses. These by-products are converted to obtain valuable products will decreases environmental pollution to large extent. The sugar industry should be considered as an economic resource and its by-products can be changed into useful products. Optimization studies and exploitation of cellulose degradation potential of the isolation in various industries such as cellulases based detergent ,paper and pulp,textile,food,fermentation,andlignocellulosic bioconversion (Hadikana*et.al.*,2018).

**Conclusion:**

Cellulase production from microorganism is gaining attention worldwide due to its increasing demand. Therefore, extensive research has been done in different agro waste material produces of cellulases and already conquered the world enzyme global market to great extent. Cellulases can be produced from microbial strains through solid state and submerged fermentation processes at industrial scale by optimizing temperature, pH and nutritional requirements of growth media. Microbial strains can be improved through mutagenic treatments for enhancing the production of cellulases. The stability and recyclability of cellulases can be enhanced through immobilization on solid supports. Cellulases have potential application in various industries such as food and feed, laundary and detergent, paper and pulp, textiles, biofuels and agriculture industries. It is also employed for research purposes and in the treatment of diseases. Further advancement in biotechnology and microbiology are still needed to unlock the potential capabilities of cellulases.

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 ISBN: 978-81-957386-3-2

# CHAPTER -3

# THE POTENTIAL OF PSEUDOMONAS TO CLEAN UP THE ENVIRONMENT POLLUTION

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**Abstract**

Bioremediation harnesses microorganisms to detoxify stubborn substances from the environment, reducing pollution levels. This eco-friendly process, requiring less energy than conventional methods, utilizes bacteria to remove pollutants from air, water, and soil. Screening for degrading microorganisms is crucial in this process, with bacteria being excellent decomposers of complex pollutants. Pseudomonas, common soil bacteria, exhibit metabolic diversity, enabling the breakdown of various substances like pesticides, aromatics, and oils. Genetically engineered Pseudomonas strains have shown success in bioremediation, with engineered genes enhancing natural degradation processes. With their unique metabolic pathways, genetically engineered microorganisms offer promising potential for pollution cleanup, making *Pseudomonas* a valuable candidate for environmental restoration.

Keywords: Bioremediation, Pollutants, Hydrocarbon

## Introduction:

Environmental pollution, stemming from various human activities like urbanization, industrialization, and agriculture, poses grave threats to ecosystems and human health. Key contributors include waste generation, airborne dust, industrial emissions, and agricultural chemical usage. This pollution is comprised of a complex mix of hazardous substances known as xenobiotics, including azodyes, phenolics, PAHs, and pesticides, among others. Xenobiotics persist in the environment, posing long-term risks due to slow degradation, disrupting vital processes and climate patterns, and exhibiting harmful effects on biota and human health. Addressing these challenges necessitates coordinated efforts, sustainable urban planning, responsible waste management, eco-friendly agricultural practices, and public awareness alongside policy measures to mitigate their impact and ensure environmental sustainability.[1]

The bioremediation of xenobiotic compounds by microorganisms is a vital process through which these substances can be detoxified or eliminated from the environment, mitigating pollution. Bioremediation, utilizing microorganisms like bacteria, fungi, and yeast, is an effective method for treating contaminated environments. Bacteria are particularly efficient at degrading pollutants like pesticides, hydrocarbons, and detoxifying metals.[2][3]

## Pseudomonas involve in environment cleanup:

Pseudomonas bacteria, commonly found in water and soil, are essential in science and technology due to their versatile metabolisms and capability to metabolise various organic compounds. Pseudomonas species encompass various strains with diverse roles, ranging from human pathogens like P.

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aeruginosa to plant pathogens such as *P. syringae*. Additionally, several species serve as plant growth-promoting rhizobacteria (PGPR), notably *P. fluorescens, P.brassicacearum, P. protegens, and P. chlororaphis*.[4].Certain strains of these species are utilised in bioremediation due to their capacity to produce bioactive metabolites, making them valuable in breaking down aromatic hydrocarbons, oil, petroleum products, and pesticides. [5,6]. Their diverse metabolic pathways enable them to metabolize a wide range of low-molecular-weight compounds, including chlorinated aliphatic hydrocarbons like phenoxyalkanoic acid herbicides. Overall, Pseudomonas bacteria are highly adaptable and suitable for bioremediation due to their broad enzymatic specificity and widespread environmental adaptability [7]. P. aeruginosa shows significant promise in treating environmentally damaging substances, especially heavy oil, diesel, and kerosene in contaminated water bodies [8, 9, 10]. Top of Form *Pseudomonas fluorescens*, a nonpathogenic soil bacterium, produces a greenish fluorescent pigment and shows potential in detoxifying diverse water pollutants and mitigating heavy metal pollution, making them valuable in environmental remediation. *Pseudomonas putida* plays a crucial role in treating petroleum-contaminated land[11].Additionally, *P. putida* has the ability to degrade pollutants in extreme environmental conditions such as high temperatures, extreme pH levels, and exposure to toxins or inhibitory solvents[12,13]

**Hydrocarbon degradation:**

Petroleum-based hydrocarbons represent the most commonly encountered hydrocarbon contaminants. Petroleum is a complex mixture of alkenes, saturated and branched alkanes, homo- and heterocyclic naphthenes, as well as aromatics. Polyaromatic hydrocarbons, or PAHs, are multicycle hydrocarbons generated through the incomplete combustion of hydrocarbons[14].Despite their toxic nature, hydrocarbons can serve as substrates for living organisms. Numerous studies highlight Pseudomonas potential for bioremediation in degrading petroleum hydrocarbons. [15,16]. Top of Form Pseudomonas have a wide range of gene-encoding enzymes such as alkane monooxygenase, ring-hydroxylating dioxygenase, naphthalene dioxygenase, catechol 2,3-dioxygenase, and aliphatic alcohol dehydrogenase for degradation and transformation of hydrocarbon [17,18]. *Pseudomonas aeruginosa strain DQ8* oxidises n-alkanes through a terminal oxidation pathway and PAHs via two pathways: monooxygenation at C-9 and dioxygenation at C-3 and C-418. Pseudomonas putida ATCC-27853; *Pseudomonas aeruginosa (ATCC-27853); and Pseudomonas putida G7* demonstrated effective degradation of naphthalene [19, 20]. An endophytic strain, *Pseudomonas aeruginosa L10*, has genes linked to the breakdown of petroleum hydrocarbons, encoding aldehyde dehydrogenase, alcohol dehydrogenase, monooxygenase, and dioxygenase[21]. Naphthalene dioxygenase from Pseudomonas sp. strain NCIB 9816-4 and toluene dioxygenase from *Pseudomonas putida F1* are the two enzyme systems that start the breakdown of aromatic hydrocarbons[22].Halophilic *Pseudomonas sp. Strain BZ-3*, isolated from crude oil-contaminated soil, efficiently degrades PAHs, specifically phenanthrene (PHE), across various salinities and pH levels[23].*Pseudomonas*, in particular, is noteworthy for its ability to produce biosurfactants, enhancing the degradation of hydrophobic hydrocarbon pollutants. Due to their simple chemical structure, compatibility with diverse environments, low toxicity, and remarkable selectivity facilitated by specific functional groups, biosurfactants offer numerous advantages compared to conventional surfactants. Biosurfactants, such as rhamnolipid produced by *Pseudomonas sp.*, play a crucial role in hydrocarbon absorption, influenced by factors like molecular weight, concentration, and salinity. (24) Many researchers

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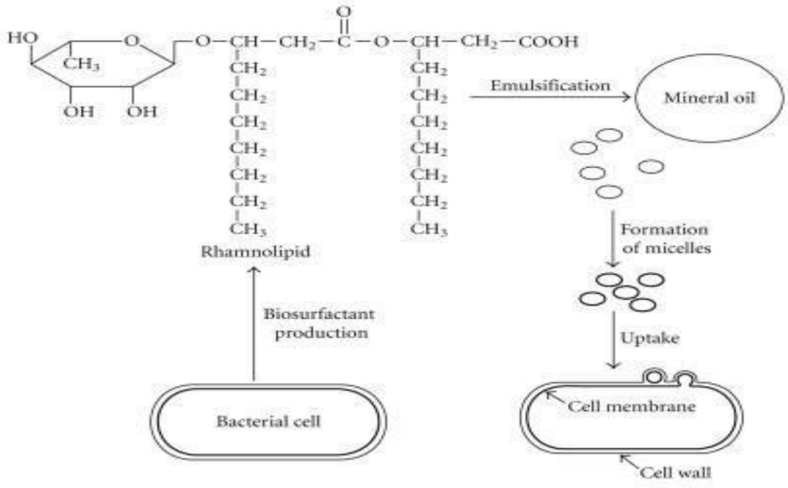
reported that *P. aeruginosa PG1, Pseudomonas aeruginosa MR0, P. aeruginosa S5, and Pseudomonas putida AM-b1* have produced biosurfactants that efficiently degrade hydrocarbons. [25,26,27,28]




Biosurfactant produced by Pseudomonas sp in the uptake of hydrocarbons Source[29]

**Removal of pesticides:**

Pesticides play a crucial role in enhancing food production by protecting plants against pests. However, their widespread use poses risks to ecosystems and human health when they contaminate natural resources. Pesticides encompass various groups, including insecticides, fungicides, herbicides, and disinfectants, each with distinct chemical properties. Exposure to pesticides, both through occupational and environmental routes, has been linked to numerous health issues such as immune suppression, hormone disruption, reduced intelligence, reproductive abnormalities, infertility, endocrine disorders, and neurological and behavioural issues, particularly in children and cancer[30, 31]. Microbial degradation has gained prominence in recent years as a method to break down pesticides into harmless or less toxic compounds, such as CO2 and H2O. The pesticide degradation by *Pseudomonas* occurs through various degrative enzymes,completely degraded or broken down it into smaller, non-toxic molecules. *Pseudomonas* exhibits strong pesticide degradation capabilities, facilitated by a plasmid carrying the degrading gene. This plasmid can transfer to other bacterial strains in the environment, endowing them with pesticide degradative abilities and potentially offering a selective advantage in specific environmental conditions.[32]

| Pesticides | Organism | Reference |
|---|---|---|
| Chlorpyrifos | *P. stutzeri B-CP5* | 33 |
| Isoproturon | *Pseudomonas aeruginosa* strain JS-11 | 34 |
| Propiconazole | *Pseudomonas aeruginosa* strain (PS-4) | 35 |
| Beta-cypermethrin | *Pseudomonas aeruginosa PAO1* | 36 |
| Imidacloprid | *Pseudomonas plecoglossicida* MBSB-12 | 37 |
| Chlorpyrifos | *Pseudomonas fluorescens* strain CD5 | 38 |
| Atrazine | *Pseudomonas fluorescens strain c50* | 39 |

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| | | |
|---|---|---|
| DDT | *Pseudomonas putida T5* | 40 |
| Carbofuran | *Pseudomonas* | 41 |
| Endosulfan,Chlorpyrifos, Malathion | *Pseudomonas frederiksbergensis* | 42 |
| Trifluralin | *Pseudomonas fluorescens* | 43 |

**Heavy metal detoxification:**

Heavy metals are indeed ubiquitous in the environment, and while they occur naturally, human activities significantly contribute to their release and accumulation in various ecosystems. Industrial processes, mining, smelting, and agricultural practices are primary sources of heavy metal pollution. Many researchers have noted the role of Pseudomonas in accumulating metals. These bacteria possess adaptive mechanisms to survive and grow in metal-contaminated environments [44]. Various resistance strategies against heavy metals include membrane transport systems, which facilitate the cellular accumulation of specific metals. In *P. aeruginosa*, P-type ATPase HmtA (heavy metal transporter A) leads to the accumulation of zinc and copper to the cytoplasm [45]. Metal sequestration, in which bacteria utilize specific binding components to sequester metals. Siderophores potentially facilitate bacterial attachment to metals. *Pseudomonas aeruginosa* produces pyoverdine, a siderophore that can bind covalently to TiO2 and iron(III) oxide [46]. Active efflux systems actively transport heavy metals out of the cell. In *P. putida KT2440* Czc and Cad systems that are responsible for cadmium efflux [47], enzymatic conversion, in which bacteria possess enzymes capable of converting toxic metal ions into less harmful forms, *Pseudomonas sp. MERCC_1942T*, having a mer operon that includes mer A-encoded mercuric reductase, transforms Hg2+ (reactive mercury) into Hg0 (volatile mercury) and confers a narrow spectrum resistance on the bacterium[48].

**Use of immobilized strain :**

To enhance the survival and persistence of bacterial cells in contaminated sites, it's essential to immobilize them. There are various support materials and immobilization techniques available, depending on the specific application. Immobilized P. fluorescens G7 efficiently removes Cd and Zn from contaminated soil [49].*Pseudomonas*strain NGK 1 cells immobilized in polyacrylamide, agar, and alginate degrade naphthalene. [50] immobilized Pseudomonas fluorescens SM1 strain in calcium alginate is used for the removal of hydrocarbons and heavy metals in water bodies [51]. Studies have extensively explored the degradation efficiencies of s-triazine herbicides by immobilized strains of *Pseudomonas sp. ADP* was immobilized in zeolite to degrade atrazine, specifically in sterile laboratory soil, with field applications yet to be explored. Immobilization techniques have been found to increase the rate of biodegradation, particularly in adverse environments[51].

**Genetically engineered microorganism [GEMs]**

The rise in environmental pollution has resulted in the development of genetically engineered microbes (GEMs) for bioremediation. These engineered microorganisms have stronger degradative capacity and rapid adaptation to diverse contaminants. A change in the catabolic gene of *Pseudomonas* putida enhances its ability to utilise substrates. [52] The TOL plasmid pathway of Pseudomonas putida has undergone extensive manipulation to broaden its catabolic capacity for

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various branched compounds. TOL metabolic pathway is modified to utilize 4-ethylbenzoate as a substrate by altering the pathway regulator XylS or by adjusting the substrate specificity of enzymes like catechol-2,3-dioxygenase.[53] *Pseudomonas putida PpY101* that had been genetically altered and carried the recombinant plasmid pSR134, which confers resistance to mercury [54], By inserting the methyl parathion (MP)-degrading gene and the enhanced green fluorescent protein (EGFP) gene into *P. putida X4* , a multifunctional strain of *Pseudomonas putida X3* was developed successfully.[55] Most research on genetically engineered microbial bioremediation relies on laboratory-based experiments. There are few examples of using GEM in natural ecosystems. But the introduction of these strains into natural environments generates ecological issues, including the stability of the plasmids integrated into these strains and the ultimate fate of these strains. in the surroundings.[56]

**Conclusion**

Bioremediation presents an alternative approach to detoxifying contaminants, harnessing the natural abilities of Pseudomonas. These organisms utilize contaminants as nutrients during their normal life cycles. Through their metabolic processes, they can convert chemical contaminants into harmless or less toxic substances, effectively neutralizing them. This research aims to offer an improved solution for bioremediating spilled petroleum hydrocarbons in soil and water ecosystems. It will shed light on the distribution of microorganisms in the environment with the capability to degrade hydrocarbons, as well as investigate how microorganisms respond to various types of petroleum oils**.**

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ISBN: 978-81-957386-3-2

# CHAPTER- 4

# BIOPRINTING: APPLICATION

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## Abstract

Bioprinting stands at the forefront of tissue engineering and regenerative medicine, revolutionizing the fabrication of complex three-dimensional (3D) structures that mimic native tissues and organs. This chapter provides an in-depth exploration of bioprinting, encompassing its principles, technological advancements, applications, challenges, and future directions. Fundamentally, bioprinting involves the precise deposition of living cells, biomaterials, and bioactive factors layer by layer, drawing from materials science, biology, and engineering. Various bioprinting methods, including laser-assisted, extrusion-based, stereolithography, and inkjet bioprinting, offer distinct capabilities and challenges, shaping the landscape of bioprinting research.Central to bioprinting are living cells, selected based on intended tissue functions, viability, and functionality. Biomaterials, serving as supportive frameworks, provide mechanical stability and biochemical cues necessary for tissue development. Bioprinting processes vary, with inkjet bioprinting, extrusion-based bioprinting, laser-assisted bioprinting, and stereolithography each offering unique capabilities.The applications of bioprinting span across tissue engineering, regenerative medicine, organ transplantation, disease modeling, drug discovery, and personalized medicine. Bioprinted constructs have been utilized for skin regeneration, cartilage and bone repair, vascularized tissues, organ transplantation, disease modeling, and personalized treatments, offering solutions to complex healthcare challenges.Despite significant advancements, bioprinting faces challenges such as scalability, vascularization, and regulatory approval. Interdisciplinary collaboration, technological innovation, and robust regulatory frameworks are essential to overcome these challenges. Looking ahead, the future of bioprinting holds immense potential for revolutionizing healthcare and advancing regenerative medicine.Bioprinting represents a paradigm shift in tissue engineering, offering unprecedented opportunities for fabricating functional biological structures with living cells. By harnessing the power of bioprinting, researchers and clinicians are poised to address complex healthcare challenges and improve patient outcomes, ushering in a new era of regenerative medicine and personalized therapeutics.

**Keywords:** Bioprinting, applications, challenges, future directions, disease modeling, drug discovery, personalized medicine.

ISBN: 978-81-957386-3-2

**Introduction:**

Bioprinting, an innovative field at the intersection of tissue engineering and regenerative medicine that enables the precise deposition of living cells, biomaterials, and bioactive factors to create complex three-dimensional (3D) structures that mimic the native architecture of tissues and organs (Murphy & Atala, 2014). This chapter delves into the intricacies of bioprinting, exploring the fundamental principles, technological advancements, applications, challenges, and future prospects of this transformative field.

**Principle of Bioprinting:**

Fundamentally, bioprinting is the process of creating biological tissues and organs layer by layer by combining concepts from materials science, biology, and engineering (Mandrycky, Wang, Kim, & Kim, 2016) There are now several bioprinting methods available, including as laser-assisted bioprinting, extrusion-based bioprinting, stereolithography, and inkjet bioprinting(Ozbolat & Hospodiuk, 2016). Each technology offers unique advantages and challenges, shaping the landscape of bioprinting research and applications.

**Building Blocks: Living Cells in Bioprinting:**

Central to bioprinting is the utilization of living cells as building blocks. These cells, which include stem cells, primary cells, and cell lines, can be obtained from a range of tissues and organs (Bishop et al., 2017). The intended use and required tissue functions influence the cell type selection (Murphy & Atala, 2014). Ensuring cell viability, proliferation, and functionality throughout the bioprinting process is paramount for the successful fabrication of functional tissues.

**Biomaterials and Scaffolds:**

Biomaterials serve as the supportive framework for bioprinted tissues, providing mechanical stability, structural integrity, and biochemical cues necessary for cell growth and tissue development. Hydrogels, engineered polymers, and decellularized extracellular frameworks are commonly utilized as framework materials(Groll et al., 2019).Bioinks, formulated from a combination of cells and biomaterials, facilitate the precise deposition of cellular components during the bioprinting process.

**Bioprinting Processes and Techniques:**

Bioprinting processes vary based on the chosen technology, each offering unique capabilities and limitations. Inkjet bioprinting utilizes bead discharge instruments to store cell-laden beads onto a substrate(Song et al., 2016).-Extrusion-based bioprinting employs pneumatic or mechanical forces to extrude bioink through a nozzle, enabling the deposition of continuous strands of biomaterials and cells. Laser-assisted bioprinting utilizes lasers to precisely position cells and biomaterials onto a substrate, while stereolithography employs light-sensitive polymers to create 3D structures through photopolymerization.

**Applications of Bioprinting:**

Bioprinting has developed as a flexible innovation with a wide run of applications over numerous areas, revolutionizing regions such as tissue building, regenerative medication, organ transplantation, illness modeling, medicate revelation, and personalized pharmaceutical(Murphy & Atala, 2014).This

ISBN: 978-81-957386-3-2

section explores in detail the diverse applications of bioprinting and their potential impact on healthcare and research.

**1. Tissue Engineering and Regenerative Medicine:**

Bioprinting offers phenomenal capabilities for creating complex, utilitarian tissues with exact control over the spatial course of action of cells, biomaterials, and bioactive components(Mandrycky et al., 2016)Tissue engineering aims to develop artificial substitutes that restore, maintain, or improve tissue function, making it particularly relevant for applications such as:

- Skin Bioprinting: Bioprinted skin substitutes have been developed for wound healing applications, providing a viable alternative to traditional skin grafts. These constructs mimic the native skin structure and promote wound closure, reducing the risk of infection and scarring(Mandrycky et al., 2016).
- Cartilage and Bone Regeneration: Bioprinting enables the fabrication of scaffolds seeded with chondrocytes or osteoblasts for the regeneration of cartilage and bone tissues. These constructs promote tissue integration, stimulate new tissue formation, and offer potential solutions for treating osteoarthritis, bone defects, and fractures.
- Vascularized Tissues: Bioprinting techniques are being developed to incorporate vascular networks within bioprinted tissues, enabling the perfusion of nutrients and oxygen to cells throughout the construct. Vascularized tissues hold promise for transplantable organs, such as kidneys and livers, as well as for repairing ischemic tissues and promoting angiogenesis.

**2. Organ Transplantation and Replacement:**

The shortage of donor organs for transplantation has spurred efforts to develop alternative approaches for organ replacement and regeneration. Bioprinting offers a promising solution by enabling the fabrication of patient-specific tissues and organs tailored to individual anatomical and immunological requirements(Murphy & Atala, 2014). Key applications include::

- Bioprinted Organs: Bioprinting offers a promising arrangement by empowering the creation of patient-specific tissues and organs custom fitted to person anatomical and immunological prerequisites. Key applications incorporate:
- Bioprinted Organs:

Analysts are working towards bioprinting complex organs, such as hearts, kidneys, and livers, employing a combination of cells, biomaterials, and bioactive variables. These bioprinted organs have the potential to overcome the confinements of benefactor organ accessibility, safe dismissal, and long-term join survival.

- Organ-on-a-Chip Models:

Bioprinting is utilized to make organ-on-a-chip models that imitate the structure and work of human organs on scaled down stages.

**3. Disease Modeling and Drug Discovery:**

Bioprinting enables the fabrication of 3D tissue models that recapitulate the complexity and functionality of native tissues, making it an invaluable tool for disease modeling and drug

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discovery(Bishop et al., 2017). Bioprinted tissue models offer a few points of interest over conventional 2D cell culture frameworks, counting:

- Patient-Specific Models: Bioprinting allows for the creation of patient-specific tissue models using cells derived from patient samples, such as induced pluripotent stem cells (iPSCs). These models capture the genetic diversity and disease phenotypes observed in patient populations, offering insights into disease mechanisms and personalized treatment strategies.
- High-Throughput Screening: Bioprinted tissue models can be used for high-throughput drug screening to evaluate the efficacy and safety of potential therapeutics. These models empower analysts to evaluate medicate reactions in physiologically important situations, driving to more exact forecasts of medicate viability and poisonous quality(Murphy & Atala, 2014)

**4. Personalized Medicine and Patient-Specific Treatments:**

Bioprinting holds promise for personalized medicine by providing customized solutions tailored to individual patient needs(Murphy &Atala, 2014). Personalized tissue constructs and organoids can be fabricated using patient-specific cells and biomaterials, offering the following benefits(Groll et al., 2016):

- Patient-Specific Implants: Bioprinting allows for the fabrication of patient-specific implants, such as bone grafts, cartilage scaffolds, and dental implants, customized to match the patient's anatomy and biomechanical properties. These implants promote tissue integration, reduce the risk of rejection, and enhance patient outcomes.
- Drug Response Prediction: Bioprinted tissue models derived from patient cells can be used to predict individual drug responses and optimize treatment regimens. These models enable clinicians to select the most effective therapies based on the patient's genetic profile, disease stage, and treatment history, leading to improved therapeutic outcomes and reduced adverse effects.

Overall, the applications of bioprinting are vast and hold immense potential for advancing healthcare, research, and personalized medicine. By harnessing the power of bioprinting, researchers and clinicians can develop innovative solutions for tissue repair, organ replacement, disease modeling, and drug discovery, ultimately improving patient outcomes and quality of life.

**Challenges and Future Directions:**

Despite significant advancements, bioprinting still faces challenges related to scalability, vascularization, innervation, biocompatibility, and regulatory approval(Groll et al., 2016). Overcoming these challenges requires interdisciplinary collaboration, technological innovation, and robust regulatory frameworks. Looking ahead, the future of bioprinting holds immense potential for revolutionizing healthcare and ushering in a new era of regenerative medicine and personalized therapeutics.

**Conclusion:**

Bioprinting represents a paradigm shift in tissue engineering and regenerative medicine, offering unprecedented opportunities for the fabrication of functional biological structures with living cells. By harnessing the power of bioprinting, researchers and clinicians are poised to address complex healthcare challenges, enhance patient outcomes, and improve quality of life. As the field continues to

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evolve, bioprinting holds the promise of transforming the landscape of medicine and advancing the frontiers of science

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ISBN: 978-81-957386-3-2

# CHAPTER- 5

# DIVERSITY AND PHOSPHATE-SOLUBILIZING ABILITY OF *BACILLUS* AND *PSEUDOMONAS* SPECIES ISOLATED FROM DIFFERENT AGRICULTURAL FIELDS

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**Abstract**

One essential nutrient for plants is phosphorus. It is involved in numerous physiological processes, including the division of cells, photosynthesis, the establishment of a strong root system, and the use of carbohydrates. In contrast to cash crops, the current study will concentrate on the diversity and activity of PSB in citrus fields. One hundred soil samples were taken from different localities in Katoltahsil (vidarbha), and these samples underwent PSB isolation and identification. Each of the 50 isolates used in this investigation came from both normal and citrus fields. Out of 50 isolates from different crop fields, the results indicated that four isolates (PSBV 14 and 22) had the maximum phosphate solubilizing capacity (28 mm).However, among the 50 strains found in the citrus field, two isolates (PSBC)

**Key words:** Phosphate solubilization, general crop fields, citrus crop field, diversity and PSB

## Introduction:

Plant development and productivity depend on phosphorus. It is crucial to numerous physiological processes in plants, including cell division, photosynthetic processes, the formation of strong root systems, and the use of carbohydrates. A lack of phosphorus causes the leaves to turn brown, along with undersized leaves, feeble steam, and sluggish growth. It also causes cation precipitate or poorly soluble organic compounds to stick to the particles. Iron, aluminium, and calcium phosphates are the primary inorganic phosphates found in soil. (Baby, 2001). Although phosphorus is a necessary mineral for plants, it is frequently unavailable because of its fixation in the soil. Insoluble phosphate was solubilized by Phosphate Solubilizing Bacteria (PSB) and made accessible to the plant (Bhattacharya and Jain, 2000). The average amount of phosphorus in Indian soil is 0.05%, which makes up 0.2% of plant material. In acidic soil, the main phosphate components are iron and aluminium phosphates. When it comes to nutrients needed for the growth of microorganisms and plants, phosphorus is second only to nitrogen in neutral to alkaline soil. However, most phosphorus is unavailable to plants. The portions of plants above ground receive only 1% to 2% of their total phosphorus intake.

In order to transform insoluble phosphate into a soluble form and supply plants with phosphorous, PSB secretes organic acids and enzymes. Furthermore, PSB generates vitamins, amino acids, and compounds that promote plant growth. According to studies, using PSB has improved crop growth and yield by 10–20% for a variety of crops, including oats, coffee, tea, bananas, mustard, maize, rice, sorghum, barley, chickpea, soybeans, groundnut, sugar beet, cabbage, and tomatoes (Ponmungan and Gopy, 2006). Phosphate solubilizing microorganisms (PSM) come in several forms in soil. These

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include Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Aspergillus, Penicillium, Fusarium, and others. These microbes can use a variety of phosphate sources as their only supply of phosphate in the media, including apatite, rock phosphate, tri-calcium phosphate, and FeSo. The creation of a cleared zone surrounding each colony is a sign that they are being used.

They secret organic acid such as acetic acid, lactic acid, succinic acid, propionic acid, formic acid etc. Consequently, bound form phosphates are solubilised and charge molecule of phosphorus is absorbed by the plant. Therefore, the PSM save 30-50 kg/ha of supper phosphate and increase crop yield up to 200-500 kg/ha. Phosphorus was probably discovered around 1669 by a German Alchemist, H. Brandt in Hamburg He derived the word phosphorus from its property Greek terminology "phos" meaning "light" and" phorus" meaning "bringing it exist in two allotropic forms yellow (white) and red (brown) troms (GoelandPathode, 2004).

**Review of Literature:**

In small quantity as aluminium and iron phosphate and largely in the form of rock phosphate, it is also found in soil as insoluble phosphates, soluble phosphates, organic phosphate and residual phosphates (GoelandPathode, 2004, Dardarwal, 1992). Assimilation of phosphate from organic compound by plants and microorganism take place through the enzyme "phosphate" which is present in wide variety of soil microorganism plant can absorb phosphate only in soluble from The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil. "A large fraction of soil microbes can dissolve insoluble Inorganic phosphate present in the soil and make them available to the plants (Bhattacharya and lain, 2000) (Richardson, 2001).

The medium used to estimate the population density of phosphate solubilizers show a clear zone around the colonies indicating phosphate solubilization. Phosphorus the "Master key element in crop production is next only to nitrogen as a major plant nutrient due to chemical fixation of phosphate it remains largely unavailable to growing plant. In alkaline soil, the predominal form of fixed phosphate is tri calcium phosphate carried out by majority of bacteria and fungi (Gaur ,1990).

Bromheld (1959) found dissolution of rock phosphate by fungi mainly by the secretion of organic acids. Yin (1988) observed the solubilization of hydroxyapatite and rock phosphate by a variety of Gram negative and positive bacteria, fungi and Actinomycetes under liquid medium conditions. Gaur (1974) found that TCP and hydroxyapatite were easily Solubilizers than RP by different groups of bacteria normally, TCP is solubilized with an equal case by fungi and bacteria but fungi solubilized RP in more amount than bacteria.

Vidharbha is referred to as the "California of India" due to the fact that citrus—specifically, lemon, orange, and sweet lemon—is the primary crop grown on the 100,000 hectares of land in Vidharbha, Maharashtra. 40% of the citrus produced in our nation is produced in Vidharbha, which has a sizable area under cultivation and contributes around 50% of the nation's productivity. Vidarbha's citrus fruits pique scholarly curiosity and inspire study
The evaluation of PSB diversity and its solubilizing activity in citrus fields in comparison to cash crops was the main goal of the current study. This study aims to evaluate the effectiveness of

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phosphate solubilization of isolates in citrus crops, which will improve phosphate intake and increase productivity.

**Materials and methods:**

Vidharbha is called as California of India for citrus as citrus is the main crop of this region of Maharashtra in Vidharbha around 100,000 hectare of land under cultivation of citrus that is lemon, orange, sweet lemon . In our country 40% of citrus is produce un Vidharbha such a large area under cultivation ton's share of around 50% in country productivity Citrus of Vidharbha attract academic interest and motivate to research.

**Isolation of Phosphate Solubilizing Bacteria (PSB):**

The purpose of collecting soil samples from various crop field in Katol tehsil was to isolate bacteria that dissolved phosphate (PSB). The samples were used to isolate and identify the organisms after being air dried in the shade. In all, 60 soil samples were gathered from different villages inside the Katol tehsil, and those samples underwent the same day's processing and analysis. A systematic protocol was followed for sample collection, which included properly mixing all of the cores together to guarantee a homogenous combination. Then, about half of the combined sample roughly one cup was placed into each plastic bag.

**Sterilization of Medium and Inoculation:**

Media. Petri dishes, were kept in an autoclave for sterilization at 121 °c for 20 min at 15 lb pressure after sterilization, plating of these media and inoculation the soil suspension by int inoculation and incubate at 37°c or 2 days. At the end of incubation, PSB colonies were identified by the formation of clear zone of phosphate Solubilisation around the bacterial colony (De, freitas et. al. 1997).

**Identification & Analysis of Phosphate Solubilizing Activity:**

The organisms were identified on the basis of standard procedure including gram staining and IMVIC test (Indole, MR. VP, Citrate test) for identification of Phosphate Solubilization bacteria selective medium were used.

The phosphate Solubilizing activities of isolated strains were observed by zone of phosphate solubilization on Pikovskaya's agar medium.

**Results and Discussion:**

In the current investigation, a total of sixty isolates were collected from areas used for citrus and general crops. The results showed that two isolates (PSBV 14 and 22) had the greatest capacity to bind phosphate, measuring 28 mm, out of the 30 isolates from different crop areas. However, out of the 30 strains found in the citrus field, PSBC 16 was the isolate with the largest solubilization zone, measuring up to 25 mm. Additionally, a 24 mm zone of phosphate solubilization was shown by strains PSBC 22, 24, and 25. Comparing the isolates to the citrus field, only 11 of the isolates were found to have a zone of phosphate solubilization larger than 20 mm, whereas 17 of the isolates carried out research on the microorganisms that solubilize phosphorus in subtropical soil. They stated that a critical characteristic of bacteria that encourage plant growth and raise plant yields is their capacity to change insoluble forms of phosphorus into an accessible form. It has been demonstrated that phosphate solubilizing bacteria can be used as inoculants to increase plant uptake of phosphorus. In

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this work, 36 strains of phosphate-solubilizing bacteria from central Taiwan were identified, tested, and described by the researchers showed this range. (Chen et al.)

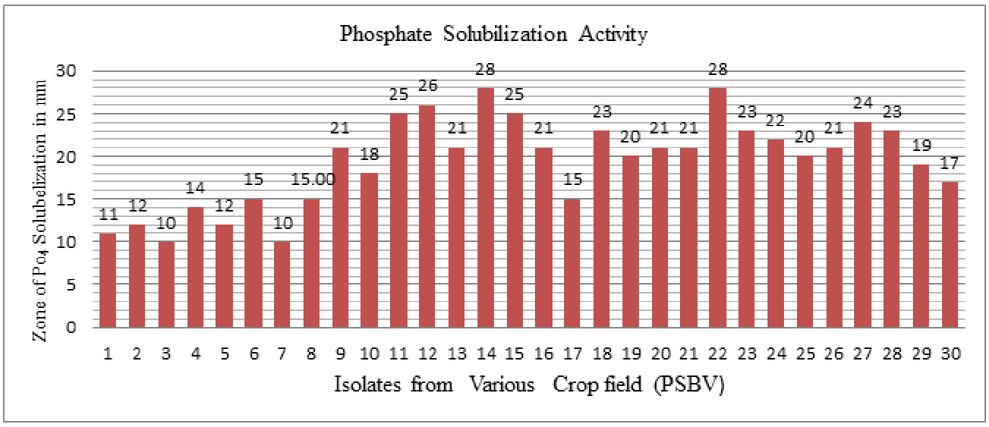


**Fig . 1: Phosphate Solubilization Activity of normal crops fields**

In various crop fields, 18 isolates (PSV) were showed highest phosphate Solubilization activity whereas 12 isolates solubilized the phosphate moderately (Fig. 1)

In 2006 Ponmurugan and Gopi studied the distribution pattern and screening of phosphate solubilising. After isolating and analysing bacteria from a variety of food and fodder crops, it was found that the groundnut rhizosphere had the highest PSB population levels, whereas the rhizospheres of ragi, sorghum, and maize had the lowest. According to this data, as one gets farther away from the plants, the distribution of PSB in the Rhizosphere soils decreases. In addition, the phosphate solubilization ability, growth regulator production, phosphatase activity, PII changes, and titrable acidity of the PSB isolates were assessed.

The Isolates Phosphate Solubilizing abilities varied greatly, according to the data .Furthermore, under in vitro settings, each isolate showed the capacity to secrete acid phosphatase and phytohormones like gibberellic acid (GA3) and indole acetic acid (IAA). Common phosphate solubilizers from both fields, such as Bacillus species, Pseudomonas, Enterobacter, and Erwinia species, were isolated for this investigation. The highest zone of solubilization was found in Bacillus species, whereas Pseudomonas, Enterobacter, and Erwinia displayed lower phosphate solubilization activity in both fields. It could be observed from the data that the distribution pattern of PSB in the Rhizosphere soils showed that the Population levels decreased with the distance of soil sampling from the plants A wide variation the capacity of solubilize phosphorous by the PSB isolates was observed, Further, all the isolates were able to secrete phytohormones like gibberelic acid (GA3) and Indole acetic acid (IAA) and acid phosphatase under in vitro condition.

In present study total of 30 isolate were isolated from citrus field. The results analysis were showed that isolates PSBC 16 isolate showed highest phosphate solubilization zone ie 25mm where isolate no. PSBC3. PSBCS, PSBC9 and PSBC 26, showed low phosphate solubilization zone (Fig. 2).

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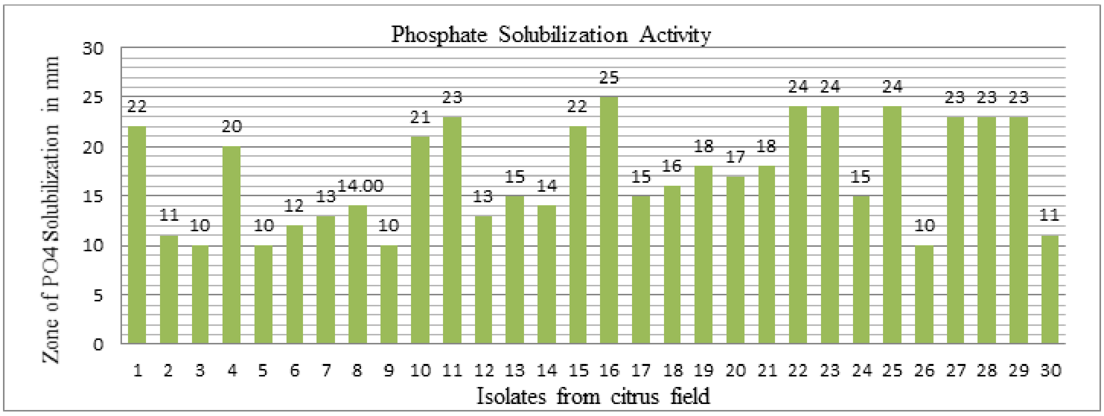


Fig . 2 Phosphate Solubilization Activity of citrus field

The study of phosphorus solubilization in isolates from both sane agricultural fields and citrus fields yielded interesting results. Specifically, 17 isolates had a phosphate solubilization degree of 20mm, while 28 isolates had a degree greater than 10mm. Furthermore, three isolates exhibited a higher level of phosphate solubilization, measuring at least 25mm. In the citrus field, 11 isolates had a phosphorus solubilization degree greater than 20mm, whereas 25 isolates had a degree of 10mm. Interestingly, only one isolate in the citrus field showed a higher level of phosphorus solubilization, measuring at least 25mm (Fig. 1 & 2). On days 3, 5, 7, and 10, at concentrations of 0.4 µg/ml, 0.5 µg/ml, 0.7 µg/ml, and 1.5 µg/ml, respectively, the solubilization of phosphate was seen in PSBC 11. Analogously, in PSBC 23, phosphate was solubilized on days 3, 5, 7, and 10, at doses of 0.2 µg/ml, 0.4 µg/ml, 0.8 µg/ml, and 1.5 µg/ml, in that order (Fig 3).

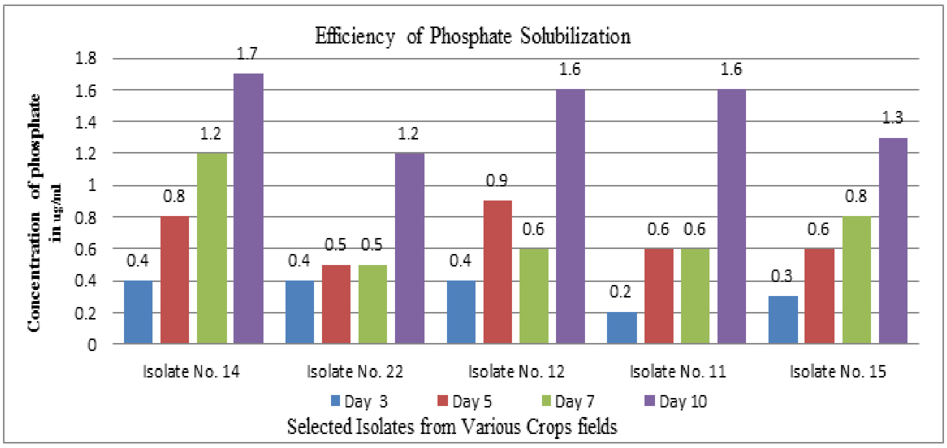


Fig No. 3 Phosphate Solubilizing Efficiency of Isolates from Citrus Field.

The efficiency of Phosphate solubilization was checked by using colorimetric method. The results and data obtained from various crop field (PSBV) and Citrus Crop filed (PSBC) were compared. Only five isolates (PSBV) out of the 30 isolates were chosen for evaluation of phosphate solubilization, according to the results. As seen in Figure 3, the effectiveness of phosphate solubilization was assessed at various intervals (3, 5, 7, and 10 days). By the end of the tenth day, PSBV 14, PSBV 22, PSBV 12, PSBV 11, and PSBV 15 showed the highest solubilization. On days

ISBN: 978-81-957386-3-2

3, 5, 7, and 10, PSBV 14 showed phosphate levels of 0.4µg/ml, 0.8µg/ml, 1.2µg/ml, and 1.7µg/ml, respectively. On days 3, 5, 7, and 10, PSBV 22 showed phosphate solubilization levels of 0.4µg/ml, 0.5µg/ml, 0.5µg/ml, and 1.2µg/ml, respectively. On days 3, 5, and 6, PSBV 12 showed phosphate solubilization levels of 0.4µg/ml, 0.9µg/ml, 0.6µg/ml, and 1.6µg/ml.

**Conclusion:**

In present study from both field the common phosphate solubilizers *Bacillus spp. Pseudomonas,Enterobacter, Erwinia spp.* were isolated. It was found that the Bacillus spp given highest zone of solubilization whereas *Pseudomonas, Enterobacter, Erwinia* show low phosphate solubilization.

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 ISBN: 978-81-957386-3-2

# CHAPTER - 6

# IMPROVED BIOREMEDIATION OF OIL SPILLS BY USING MICROORGANISM

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## Abstract

Mycoremediation is a form of bioremediation that employs fungus to biologically remove or dissolve poisons from the surfaces of earth and water. *Penicillium chrysogenum*, a fungus used in mycoremediation, has been found to use monooxygenases to break down aromatic hydrocarbons into trans-diols. Oil leaks, whether accidental or deliberate, have a significant negative impact on the ecosystem. Oil leaks can have catastrophic consequences for the ecosystem, economy, and welfare of both vegetation and creatures. Global pollution is growing due to the differences in natural and human actions leading to the contamination of various land and marine environments with heavy metals and chemical and organic compounds. Many recent studies shown that other fungi have also been found to be capable of breaking down hydrocarbons and other xenobiotic compounds, such as heavy metals, into harmless forms. Monocyclic aromatic compounds (benzene, toluene, ethylbenzene, and xylene) are discovered to be destroyed by *Penicillium chrysogenum* alone (BTEX). Penicillium species, particularly *Penicillium chrysogenum*, get little focus in studies of the bioremediation and biodegradation of hydrocarbons and other xenobiotics, despite the prevalence of fungus in the trash. The highlight of this paper is new fungus isolate *Penicillium chrysogenum* has potential for bioremediation of oil spills.

**Key words**: Bioremediation, Oil spills, Mycoremediation, Biodegradation.

## Introduction:

The technique of using biological processes inside microorganisms or plants to break down or store toxic hydrocarbons, heavy metals, and other combustible organic compounds contained in fossil fuels is known as bioremediation of petroleum-contaminated ecosystems. Every day oil leaks happen at different levels, just as they do in all parts of the petroleum supply chain, creating a complex web of problems for the environment and overall health. Bioremediation is less time-consuming, less expensive, and does not cause mechanical or chemical harm, whereas traditional clean-up approaches like chemical or physical confinement and elimination typically produce immediate results. To assist reactions, bioremediation operations must maintain optimum circumstances for the target organism or biological pathways, such as pH, RED-OX potential, temperature, moisture, oxygen abundance, nutrient availability, soil composition, and pollutant structure. The three principal bioremediation strategies utilised for petroleum leaks are microbial remediation, phytoremediation, and mycoremediation. Bioremediation has been utilised in several notable oil spills, notably the 1989 Exxon Valdez catastrophe, in which fertilizer supply to the damaged shoreline increased biodegradation rates [1].

 ISBN: 978-81-957386-3-2

Living things are either directly or tangentially subjected to petroleum in several ways. Some industrial by-products from gasoline refineries and factories that produce other goods can be extremely poisonous. These harmful substances are accidentally released into the environment daily, and if the impact of unintended crude oil spills around the world is linked to this effect, then these combined unregulated petroleum sources are the primary cause of environmental contamination. The toxicity and accessibility of hydrocarbon compounds for bacteria digestion are influenced by their molecular and physical properties. Depending on the sort of petroleum component, the method of exposure, and the duration of contact, petroleum is poisonous and can be fatal. Crude oil contains chemicals and dispersants that can have a variety of negative health impacts [2].

Depending on the degree of vulnerability and contact in both humans and animals. Crude oil includes highly poisonous chemicals that can harm any internal system in the human body, including the neurological, pulmonary, cardiovascular, immunological, reproductive, olfactory, and hormonal systems, as well as the liver and kidney. As a result, they can induce a variety of illnesses and ailments. The following people are more prone to the detrimental effects of petroleumoil:

- Babies, early children, and toddlers in development.
- Women who are pregnant.
- People who are currently suffering from serious health problems.
- People who exist in situations that jeopardize their well-being [4].

Untreated oil toxins can cause immediate or prolonged damage to internal systems. Furthermore, oil refineries generate massive quantities of sticky sludge and petroleum waste. The Mobil Corporation and the United States Environmental Protection Agency used microorganisms to restore Alaskan beaches polluted by the Valdez oil spill this method is known as bioremediation [3].

Cleaning up oil spills can be done in several methods. The sort of oil discharged, the position, how near it is too susceptible areas, and other natural factors all affect the methods used to clear up an oil leak. Techniques involving mechanics, pharmacology, and molecules are all available. Examples of motorized methods include booms, skimmers, and car vacuums. Chemical methods include dispersants, surface cleaners, and surface gatherers, for instance. Microbiological communities, enzyme additives, and nutrition additives are a few biological techniques used to hasten pollutant biodegradation. Oil zapper is an inoculants developed in India that uses a range of bacterium species to transform oil spillage and greasy debris. Oil zapper irrigation reduced greasy waste contamination in soil from 13.41% to 0.5% over the course of 360 days. It decreased to 11.35% in the region that wasn't polluted. The oil zapper performed well in comparably extensive field experiments [5].

**Oil Spills:**

When gasoline escapes from pipelines, ocean-going ships, or other sources, an oil mishap happens. It happens frequently and has serious consequences for the environment.

**1. Oil Spills in the ocean:**

All living things, including the earth's ecology, are negatively impacted by oil leaks in the water. On the other side, poisonous substances imperil aquatic life. Most of us are aware of oil platforms and how petroleum is extracted from the seabed. The same oil is used in a variety of medicinal business operations, transit, and building.

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Oil spills from ships that happen during loading, offloading, discharge, ballasting, tank repair, or when they are near offshore platforms, drilling sites, or wells are the major sources of ocean contamination. A gaseous petroleum substance being discharged into the environment as a result of human action is the essence of an oil spill.

Ocean water contamination is also influenced by the type of oil that was unintentionally or intentionally discharged into the ocean. Examples of oil include crude oil, hydrocarbon residues, purified petroleum products like gasoline or motor fuel, oil combined with garbage, or greasy waste. Light oil, like gasoline, does not linger for very long after being released into the atmosphere. It is extremely combustible and toxic, but it evaporates quickly [6].

**The worst oil spills in history:**

Kuwait purposefully released 400 and 450 million gallons of petroleum into the Persian Gulf during the Gulf War in 1991. It is regarded as the worst oil leak in history because it was the only one that was premeditated. In 2010, the Deepwater Horizon oil leak, also known as the BP oil disaster, released the most underwater oil ever, spilling roughly 4.9 million gallons of petroleum into the Gulf of Mexico. 140 million barrels of petroleum oil were discharged after an oil well was drilled in Mexico's Bay of Campeche. Sparks caused unintentional detonation, and it took almost a year to halt the ongoing oil spill.

In 1991, an oil tanker exploded off the coast of Angola, releasing between 50 and 80 million barrels of crude into the ocean. Due to this, a large amount of sediment caught fire and blazed for three days before the ship fell. The ship M/T Haven was recommissioned in 1991 despite being in bad condition and having been struck by a projectile during the Iran-Iraq conflict. For more than ten years, the fuel from this ship's shipwreck in the Mediterranean Sea leaked [6].

**Bioremediation for oil spills:**

An approach to eliminating oil contamination from the environment and water is oil accident bioremediation. Most oil spills happen on ships, seriously harming the maritime ecosystem. Hazardous materials are dumped into the seas by oil seepage as a result of ship accidents, carelessness, and catastrophes. Contaminated water reacts with the soil to make it even dirtier. To remove dangerous substances from the land and oceans, a lot of work and money is required. Bioremediation is one such effective method for removing toxins from land and water and making them safe for marine and ground species [7].

**Bioremediation methods for oil spills:**

Ex situ or in situ methods are both used to remove harmful chemicals from the earth. The type and level of contamination determine the methods that are used. The decomposition of compounds is aided by the enzymes created by microorganisms. The compounds that are taken by the soil or water cause them to become translucent.

1. **Mechanical Methods:**

   Typically, mechanical techniques are used as confinement techniques to stop oil from dispersing. Booms, drifting obstacles constructed from a variety of materials, are useful for holding oil with varying viscosities and densities and for facilitating swift removal or retrieval. Unfortunately, using


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ISBN: 978-81-957386-3-2

platforms can be time-consuming and costly because precise handling is needed to help position the net below the surface. Booms come in a variety of shapes and sizes, but they all contain oil and need to be removed using a distinct technique. Booms provide confinement, which improves the efficiency of other mechanical techniques like dredging and sorbents. Booms that can withstand fire can be used to hold oil for in-situ combustion [8].
Skimmers and buoys are used to clear oil from the sea's surface and stop it from dispersing further. Weir skimmers, oleophilic skimmers, and vacuum skimmers are the three different kinds of skimmers. The most popular type of skimmer used in maritime oil leaks is the weir skimmer, which collects oil through gravity force. As an alternative, oleophilic skimmers use chains, disks, or belts made of oleophilic materials to pick out specific oil spills. Oil is basically "vacuumed" from the water's top by suction skimmers. Even though skimmers have been in use since the 1970s, technology has not significantly advanced since then. Skimmers are one of the fastest recovery techniques, but they are labour-intensive and prone to blocking, making them unsuitable for employees due to possible health risks connected with oil leak cleaning. Skimmers may be costly if human work is required, and blockage reduces productivity [2, 7].

Another frequently used mechanical healing technique is sorbents. To bond the liquid oil in a solid or semisolid structure, sorbents are either adsorbent or absorbent substances. Typically, sorbents are used to get rid of any remaining oil or oil that is closer to the coast or in wetlands. Cheap natural biological materials like grass, wheat, shavings, hair, feathers, cotton, and alfalfa are examples of sorbents. As an alternative, sorbents can be made from manufactured materials or natural inert materials like clay or sand. The sorbents with ingested oil must be swiftly taken from the accident location after placement. Sorbents may start to descend to the ocean bottom if they are not taken out as soon as possible. However, recent research has revealed novel types of oil-sorbent materials that can not only preferentially absorb oil but also hold onto it in adverse weather. Such "sponge-like" materials with specialized surface chemistry might make it possible to remove oil from the accident location quickly and efficiently. You can read more about the benefits, constraints, and guiding principles of these novel resources elsewhere [7-8].

Mechanical techniques enable for the actual retrieval of oil and have been used in every major oil leak since the 1969 Santa Barbara oil spill. These techniques have proven successful at confining oil and stopping dispersal, but there are significant restrictions. For one, confinement localizes oil, which could increase the effect on the local maritime ecosystem, although fast clearance can surmount this restriction. Weather factors can significantly influence these techniques. Calm waterways are required to avoid blockage of skimmers and enable sorbents to remain on the water surface. Additionally, a significant drawback of mechanical techniques is that further treatment of the oil is needed, including removing the gathered oil from detritus and water, bodily removal, or additionalchemicaltreatments. Alternatively, heat and chemical techniques enable simple elimination without any further preparation [9].

**2. Thermal Methods:**
In situ combustion needs only two pieces of equipment: fire-resistant barriers to confine the gasoline and an igniter. The oil confined by the booms is lit using surface-deployed lighters from a vessel, land,


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or aerially deployed ignitors from aircraft. Ignitors may include paraffin, propane, or butane flames, grass burners, or fuel-soaked sorbents. Surface combustion in situ is one of the most efficient and fast ways of oil elimination, but it is only useful for a limited time due to the natural emulsification of the oil. The fluid must be 3 mm thick and water concentration must be less than 25% for combustion. When used in placid waterways previous to emulsifying the film in water, elimination can be as high as 95%. In situ burning causes serious air pollution, sinkage of charred debris, and the danger of additional flames or blasts [10,18].

**3.Chemical Methods:**

Chemical techniques, or those that make use of chemical substances, have been used to stop the spread and lessen the effects of oil spills. Smaller particles of the slurry are created thanks to emulsifiers and dispersants. Alternatives include oil herders, which gather oil and "herd" it into thicker, insoluble-in-water slurry that can be lit for in-place combustion. These techniques are typically successful in most weather situations, including choppy or frigid seas, where conventional mechanical techniques are frequently ineffective.

By lowering the air-water surface tension, oil shepherds, who gather oil, constrict the oil into thicker slurry, allowing a prolonged in-situ combustion process. Chemical oil herders usually contain silicone, are nonbiodegradable, and linger in water. At this point, it is unclear how silicone-based caretakers will affect the ecosystem and biology [4, 18].

In more recent oil leaks, toxic chemical emulsifiers and detergents have been used. As a result, chemical techniques have become more contentious, as the active chemicals used in the Deepwater Horizon oil leak were shown to be harmful to aquatic life's health. There have been few studies on the long-term environmental effects of chemical lubricants, and thefindings are presently contradictory. One potential answer is to depend more heavily on bioremediation [11].

**4.Natural Methods:**

The natural process of oil breakdown by local microbes is known as bioremediation. Hundreds of organisms can break down oil in dirt or water, including fungi, algae, and bacteria. Bioremediation can be very effective, and it was a key technique for cleaning up after the Exxon Valdez spill. The solubility of the oil and minerals plays a major role in the efficiency of bacteria decomposition. Methods for improving bioremediation frequently aim to increase the nutrients that restrict development, but doing so can extend the cleaning period by as much as 2-4 weeks by lengthening the microbe latency period (the period before the cells start to reproduce). Mechanical and chemical cleaning techniques may impede bioremediation. Chemical methods may prevent the microbes from destroying the oil by raising its toxicity, while mechanical methods frequently involve the location of the oil, which can impede the development of many microorganisms due to a drop in resources like sunshine and air [12].

- **Using bacterial species:**

Pseudomonas species can degrade hydrocarbons found in petroleum and fuel, lessening the effect of oil leaks. *P. mendocina* and *P. putida* can decompose polycyclic aromatic compounds, and *P.*

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*mendocina* and *P. putida* can eliminate toluene. *P. veronii* can degrade a wide range of aromatic organic substances. These oil-based substances are consumed by microbes because they serve as metabolic fuels. Because they are abundant in water and sediment, these microorganisms can help clean up oil leaks. As the quantity of these microbes increases, the bioremediation process accelerates. Microbes such as Achromobacter, Flavobacterium, Acinetobacter, and others assist in bioremediation. [13].

- **Using fungal species:**

*Penicillium* species can be found in food, interior air, and soil. *Penicillium chrysogenum* has been discovered on desiccated cereals, salted meat, and a variety of other foods with low water activity. It is found not only in food, but also in internal air spaces, salty sediments, and water (marine water) [10, 14]. Many studies have demonstrated that Penicillium strains are excellent hydrocarbon-assimilating bacteria, with many studies demonstrating their ability to convert xenobiotic substances like phenol into less mutagenic products. Phenol is a common natural contaminant produced by many businesses.

*Penicillium chrysogenum* is capable of degrading phenol molecules, heavy metals (such as lead, copper, and iron), and monocyclic aromatic hydrocarbons (benzene, toluene, ethyl benzene, and xylene) [13-14]. Low molecular weight aromatics, the most soluble oil components, volatile aromatic compounds, the most toxic in raw and treated oils, and toxins are all examples of BTEX. Even though combustion can eliminate a large number of them (i.e., by natural method). Most hydrocarbon products contain 20-50% of them, with fuel oil containing 75% and gasoline and paraffin containing 100%. Because of their solvent effect on cell walls, mono-aromatic hydrocarbons are toxic to some microbes, but in tiny quantities, they are readily biodegradable under aerobic circumstances.

*Penicillium chrysogenum* and other fungi frequently use monooxygenases to oxidize aromatic compounds and create trans-diol. Low molecular weight aromatics are more soluble at higher temperatures, with soil having the greatest degradation rates, 20-300c in some freshwater environments, and 15-200c in the seas [14].

**Table: Showing examples of oil-degrading microorganisms.**

| Microorganism | Genera | Oil components |
|---|---|---|
| **Bacteria** | *Alcanivorax* | *n*-alkanes (C8–C32) |
| | *Actinomyces* | *n*-alkanes |
| | *Bacillus* | *n*-alkanes (C13–C30) and aromatic |
| | *Gordonia* | *n*-alkanes |
| | *Marinobacter* | *n*-alkanes |
| | *Pseudomonas* | *n*-alkanes (C14–C30) and resins |
| | *Cycloclasticus* | Aromatics |
| | *Rhodococcus* | *n*-alkanes (C13–C17) and aromatic |
| | *Staphylococcus* | Aromatics |
| **Cyanobacteria** | *Anabaena* | *n*-alkanes |
| | *Aphanocapsa* | *n*-alkanes and aromatics |
| | *Microcoleus* | Aromatics |

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| | | |
|---|---|---|
| | *Oscillatoria* | Aromatics |
| | *Nostoc* | Aromatics |
| | *Plectonema* | *n*-alkanes and aromatics |
| **Algae** | *Amphora* | Aromatics |
| | *Chlamydomonas* | Aromatics |
| | *Chlorella* | Aromatics |
| | *Dunaliella* | Aromatics |
| | *Prototheca* | Aromatics |
| | *Ulva* | Aromatics |
| **Fungi** | *Aspergillus* | *n*-alkanes and aromatics |
| | *Candida* | *n*-alkanes and aromatics |
| | *Fusarium* | *n*-alkanes and aromatics |
| | *Penicillium* | *n*-alkanes (C11–C25) and aromatic |
| | *Trichoderma* | *n*-alkanes and aromatics |

**Impacts of oil spills:**

Oil spills can have disastrous effects on marine and shoreline ecosystems. The information provided below explains the various detrimental effects of oil spills on various environments, the economy, and the well-being of creatures and flora [15].

1. **Environmental Impact:**

An oil spill, whether intentional or unintentional, has a major environmental effect. The thick liquid distributes on the sea surface during an oil discharge in water, creating dense oil sheen. Chemical substances that evaporate lose 20-40% of their bulk. A trace quantity may dissolve in water as well. What remains is oil residue, which creates thick foam on the water's surface over time. Some of the leftover oil may now solidify and drift in the water as tar particulates. Furthermore, some oil remnants disintegrate (photolysis) and deteriorate in saline water by Biodegradation process. When this oil hits the coasts, it reacts with the silt, stones, boulders, flora, and animals and plants in the land environment, contaminating the natural environment.
Toxic acid rain and unpredictable climate change are two of the most serious consequences of oil leaks, both of which are produced by oil vapours in the atmosphere [9,15].

2. **Economic Impact:**

In addition to the ecosystem, a country's economy suffers a serious loss. Oil is a natural and finite source of energy, so any loss of quantities has serious consequences. The impact on a country's economy. The tourist industry also experienced a downturn. This is so that many recreational activities, like scuba diving, sailing, snorkelling, and fishing, are prohibited in the immediate wake of the leak until the clean-up is complete. The most affected industry by these oil spills is the fisheries industry [16-17]. A health risk arises from the pollution of salmon and other marine items. The oil spill has also harmed numerous fishing boats and equipment, so fishing areas are still closed until it is

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cleared up. Because many of their commercial activities rely on saltwater, power plants, desalination plants, and nuclear power plants are also impacted (Narayani, 2010).

3. **Health Impact:**

Benzene, propylene glycol, polypropylene glycol butyl ether, and hydro-treated light hydrocarbon distillates are some of the dangerous chemicals present in unrefined oil. Health risks from contact with these chemicals may also exist. Working in unclean environments has been linked to severe headaches, back pain, dizziness, rashes, runny eyes, and sore throat. Animals' red blood cells are also harmed by the harmful compounds in the oil, which also leads to severe breathing problems. These compounds are even riskier because they are naturally poisonous. A lot of food poisoning cases near the coasts where such events have occurred are directly caused by tainted seafood, which is another problem. In some cases, an oil spill has also been linked to a decrease in the mental health of those residing in these areas [17]. Therefore, whether it is plant, animal, or human life, the negative effects of an oil discharge are fatal in nature. Aquatic life, vegetation close to beaches, and workers who remove detritus are the most vulnerable victims. Such man-made disasters that worsen environmental degradation can undoubtedly be avoided to avoid such disastrous consequences.[5,17]

4. **Impact on marine life:**

Depending on the sort of oil, a thick coating can develop on the bodies of fish and aquatic creatures. Here are some additional materials on the subject:

- Bird feathers are damaged, destroying their beneficial properties.
- Oil spills have an impact on fur seals because the oil adheres to their fur.
- Whales and dolphins are not affected by the oil because their slippery bodies prevent it from entering their bodies.
- If the oil sticks to the flipper, marine animals will drown.
- Ulcers and other internal problems in the body can lead to disease and death.
- Problems with appetite and weight loss in birds, wildlife, and marine life.
- Problems with the digestive system, resulting in a fragile body.
- A sense of alienation among community members as the smelling ability of seals and other marine animals is reduced, making them feel lost because they are unable to detect their surroundings. [18,25]

**5.Impact on birds:**

The structure, look, and protective qualities of aquatic creatures are altered by the oil as it penetrates the ground. They are affected by this and are consequently more vulnerable to environmental shifts [26]. having water-based levity. Furthermore, these birds are unable to fly, rendering them helpless in the company of predators. Additionally, they ingest the oil when they wash or shave, completely soaking their plumage. As a result, they suffer from impaired liver function, kidney damage, and irritation of their intestinal system. These issues lead to dehydration and aberrant biochemical processes, which are made worse by a constrained capacity for hunting. If no one intervenes, these creatures will die [7, 19, 27].

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**6.Impact on aquatic plants and animals:**

The smallest aquatic creatures that reside on the ocean floor are the ones most harmed by oil spills. Young fish, algae, seaweed, mussels, clams, dolphins, tortoises, plankton, and fish are all affected. The hydrocarbon layer on top makes it difficult for sunlight to travel through, which stops photosynthesis and causes marine vegetation to suffer as well. One of the most alarming facts is that this disrupts the entire water food chain while also reducing the number of plants and creatures. [20,28]

Sea Otters are unable to produce air gaps or bubbles when their fur gets greasy. These clouds serve as balance aids and barriers, helping people survive in arctic conditions. They die because they are unable to protect themselves from the shift or maintain the necessary air spaces when the oil builds up on their dense hair. For the therapy of grease, sickness, or fractured bones, these otters are periodically taken away and placed under monitoring [21].

Whales may consume the released oil directly or tangentially. Either way, it gets to their blowhole and helps them breathe. Consuming oil closes the blowhole, making it impossible for them to breathe and murder them. Whales regularly consume microscopic fish that have been through oil on their journey to becoming sustenance. The whale becomes sick and dies due to the oil entering its system [22].

**Advantages of bioremediation:**

Bioremediation has numerous benefits over conventional ways of cleaning up maritime oil spills, including the following:

- One of the main advantages of bioremediation is the cost savings and time saved by workers clearing up a polluted site. When used properly, bioremediation offers significant financial benefits over traditional clean-up techniques. Following the Exxon Valdez accident, the cost of bioremediating 120 kilometres of shoreline was less than the cost of manually washing the strand for one day. Another way bioremediation saves money is that, unlike traditional methods, it continuously cleans the contaminated area without the need for workers. This saves money that would otherwise be spent on work hours, and it also allows for more time to be spent on bioremediation research.
- Bioremediation is also beneficial because of its ecologically friendly strategy. No foreign or toxic substances are brought into the site, unlike chemical techniques.
- It is also ecologically beneficial because it does not disturb the native habitat, which is frequently caused by physical and chemical cleaning methods.
- Bioremediation allows natural organisms to degrade toxic hydrocarbons into basic compounds that are non-hazardous to the environment, removing the need to remove and relocate the toxic compounds. The removal of the need to transport the oil and contaminated soils lowers the risk of additional oil spills while also saving energy and money that would have been spent in the transportation process. Because of these environmental benefits, bioremediation is a common method among the general public. With today's restricted resources, this is a highly endorsed technology that delights the public, resulting in governmental support and funding for further research.

**Disadvantages of bioremediation:**

The slowness of bioremediation for marine time oil spills is one of its most important drawbacks:

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- Oil spills can jeopardize a variety of habitats, environments, and businesses, and based on the seriousness of the release, bioremediation may not always be the best choice.
- Numerous factors influence whether bioremediation is competent and feasible for cleaning up different oil spills. It may be challenging to provide adequate nutrient concentrations to oil-degrading microbes depending on where the discharge happens and the circumstances of the water.
- When an oil spill happens offshore, the energy and waves are usually higher, which can result in the rapid loss and dilution of nutrients given by biostimulation.
- Bioaugmentation has flaws, especially the rivalry that forms between native and foreign microbes, rendering it a failed bioremediation technique.
- Another drawback of bioremediation is the difficulty of testing it in the environment. This is because many variables and circumstances can only be controlled in laboratory experiments, not in the field.

**Conclusion:**

Hydrocarbons, the main constituents of crude oil, as well as other xenobiotics, such as heavy metals, which can be harmful to humans and other forms of life even in low quantities, can be remediated and degraded by microorganisms, particularly fungi and bacteria. In addition to its ability to save time and money, bioremediation has the benefit of being ecologically friendly. No alien or dangerous compounds are brought into the area, in opposition to pharmaceutical methods.

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Published by : Sai College, Bhilai

ISBN: 978-81-957386-3-2

# CHAPTER - 7
# DIETARY PREFERENCES OF BIRDS IN INDIA: METHODOLOGICAL APPROACHES FOR ASSESSMENT

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**Abstract**

This chapter explores the dietary preferences of birds in India and the methodologies used to assess them. With over 1,300 species, India hosts diverse avian communities influenced by habitat type, seasons, and human activities. Methodologies include field observations, dietary analysis, feeding studies, stable isotope analysis, remote sensing, and modeling. These approaches offer insights into bird foraging ecology and are crucial for conservation amidst urbanization, climate change, invasive species, and agricultural practices. Understanding avian dietary preferences is essential for preserving India's rich biodiversity.

**Keywords:** Avian dietary preferences, Bird ecology, Methodological approaches, Conservation, India's avifauna.

## 1. Introduction:

Birds, with their exquisite plumage, melodious songs, and diverse behaviors, have long captivated the imagination of humanity. In the vast subcontinent of India, with its rich tapestry of ecosystems ranging from lush rainforests to arid deserts, birds hold a particularly special place. The dietary preferences of birds in India, influenced by a myriad of factors including habitat type, seasonal variations, and evolutionary history, offer a fascinating within this region.

India's avian diversity is staggering, boasting over 1,300 species, representing roughly 13% of the world's avifauna. This wealth of birdlife is attributed to the country's diverse geographical features, including the towering Himalayas in the north, the expansive Thar Desert in the west, the dense forests of the Western Ghats, Gangetic plains. Each of these regions harbors its own unique avian communities, each with its own set of dietary preferences shaped by the local environment.Understanding the dietary habits of birds is crucial not only for unraveling the intricacies of their ecology but also for informing conservation efforts and managing ecosystems effectively. Birds play pivotal roles as predators, prey, and seed dispersers, shaping the dynamics of the ecosystems they inhabit. By studying their dietary preferences,shedding light on broader ecological processes.The dietary preferences of birds are highly diverse, reflecting adaptations to exploit a wide array of food resources. While some species are strict specialists, relying on a narrow range of food items, others are generalists, capable of feeding on a variety of foods. The factors influencing these dietary preferences are multifaceted, encompassing ecological, physiological, and behavioral considerations. For instance, the morphology of a bird's beak often correlates with its feeding habits, with long, slender bills adapted for probing nectar from flowers, while robust, hooked bills are suited for tearing flesh.

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In India, the dietary preferences of birds exhibit remarkable variability across different habitats. In the lush rainforests of the Western Ghats, for example, frugivorous species such as the Malabar Grey Hornbill (Ocycerosgriseus) and the Indian Pitta (Pitta brachyura) thrive on a bounty of ripe fruits and berries. Meanwhile, in the arid scrublands of Rajasthan, birds like the Indian Courser (Cursoriuscoromandelicus) and the Cream-colored Courser (Cursorius cursor) have evolved to feed on insects and small reptiles, adapting to the harsh conditions of their environment.Seasonal fluctuations further influence the dietary preferences of birds in India. During the monsoon season, when the landscape is transformed into a verdant paradise, insectivorous birds such as warblers, flycatchers, and drongos abound, taking advantage of the abundance of insects that emerge after the rains. In contrast, during the dry season, when water sources become scarce, waterbirds congregate around remaining water bodies, feeding on fish, crustaceans, and aquatic plants.Moreover, human activities and anthropogenic changes are increasingly impacting the dietary preferences of birds in India. Habitat destruction, pollution, and climate change are altering the availability of food resources, forcing birds to adapt or perish. For instance, the proliferation of monoculture agriculture has led to declines in insect populations, affecting the availability of food for insectivorous birds. Similarly, the pollution of water bodies with pesticides and industrial waste has detrimental effects on aquatic ecosystems, impacting the food sources of waterbirds.

## 2. Birds’ classification based on the food preferences:

### 2.1 Insectivores:

These birds primarily feed on insects and other small invertebrates. They play a crucial role in controlling insect populations. Examples include Asian Green Bee-eaters, which raid beehives for food, and warblers that hunt insects in trees and shrubs.Insectivory is the most common pattern of food consumption in birds. Birds exhibit diverse dietary preferences, with some specializing in specific insect types while others opportunistically consume a wide variety. Remarkably, nearly 80% of bird families incorporate insects into their diet. Avian’s parents, especially granivorous, frugivorous, nectarivorous, and herbivorous species, diligently feed their rapidly growing offspring a diet rich in insects, spiders, and other invertebrates. The nutritional composition of insects varies, contingent upon species and life cycle stage. Adult insects boast protein levels ranging from 50% to 75%, alongside 5% to 35% lipid content, with minimal nonchitin carbohydrates. Meanwhile, butterfly and moth larvae contain 40% to 70% protein and 10% to 40% lipid. Intriguingly, the amino acid profile of insects closely aligns with avian requirements, surpassing that of plant proteins and rivaling vertebrate prey. Insects serve as valuable sources of phosphorus, trace minerals, and vitamins for birds, albeit with low calcium content. However, their chitinous exoskeleton poses a challenge for digestion. Insectivorous birds possess enlarged proventriculi, equipped with pepsin and hydrochloric acid for efficient protein breakdown. Gizzard size varies: birds consuming soft-bodied insects have smaller gizzards, while those preying on hard-bodied insects exhibit larger, more muscular gizzards. Additionally, insectivores tend to have diminutive ceca.

### 2.2 Carnivores

Carnivorous birds consume meat, including small vertebrates like frogs, lizards, and snakes. Birds of prey (raptors) like vultures and eagles fall into this category.Carnivores, a diverse group that includes

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falcons, hawks, kites, eagles, vultures, owls, and shrikes, exhibit specialized feeding behaviors. Similarly, piscivores, which encompass various falcons, hawks, eagles, owls, kingfishers, grebes, wading birds (such as herons and egrets), and mergansers, have evolved unique adaptations for capturing aquatic prey. The nutrient composition of vertebrate foods remains relatively consistent, with variations primarily in fat content and subsequent caloric density. While mammals tend to possess higher fat levels than birds or lizards, the overall fat content varies significantly across vertebrate species, influenced by factors such as age and individual variation. Remarkably, the protein quality in vertebrate foods consistently meets the essential amino acid requirements of birds. In addition to protein, vertebrate prey provides readily digestible vitamins and minerals. The water content of these foods' ranges from 50% to 80%, often sufficient to meet the hydration needs of birds in diverse environments, rendering an external water source unnecessary. Adaptations in beak morphology enhance prey capture efficiency. Carnivorous birds possess pointed beaks with sharp edges, while piscivores exhibit hooked tips for securing slippery fish. Some species even feature toothlike patterns or rough tongues to aid in tearing apart prey. Vultures, known for their scavenging habits, exhibit minimal feathering on their heads, reducing contamination from tissue debris. An expandable esophagus, enlarged at the junction with the proventriculus, facilitates efficient food passage. In certain species (e.g., falcons), a distinct crop assists in food storage. The proventriculus and gizzard, both enlarged, accommodate substantial meal sizes rich in protein. Interestingly, the gizzard lacks distinct pairs of thin and thick muscles, functioning more as a mixer than a grinder. The pancreas, relatively diminutive in carnivorous and piscivorous birds compared to granivores and herbivores, reflects their specialized dietary needs. The high digestibility of flesh renders amylase unnecessary, accounting for the pancreas' reduced size. Additionally, the small intestine, relative to other avian species, facilitates efficient nutrient absorption. Many carnivorous and piscivorous birds harbor vestigial ceca, remnants of their evolutionary history. When these birds consume whole vertebrate prey, they access a comprehensive nutritional package devoid of deficiencies. Remarkably, this prey includes essential nutrients such as water, which is crucial for avian survival. Indigestible components—bones and skin—are efficiently egested, allowing for optimal digestion. The metabolizable energy derived from vertebrate food hovers around 75%, sustaining these specialized birds. Hydrochloric acid and pepsin, secreted from the proventriculus, play pivotal roles in breaking down the prey. Notably, most carnivorous and piscivorous birds raise altricial young—precocial offspring requiring extensive parental care. This reproductive strategy likely stems from the physical prowess and skill necessary for locating and capturing vertebrate prey.

**2.3 Frugivores:**

Frugivorous birds, specialized fruit eaters, play a vital role in ecosystems by consuming fruits and facilitating seed dispersal. Their feeding habits contribute to forest regeneration and encourage biodiversity. Let's delve into the implications of this ecological interaction. In rapidly urbanizing areas like Delhi, the conversion of agricultural land to urban spaces has been substantial. Between 2000 and 2016, cultivated land decreased from 52,816 to 34,750 hectares. While this urban growth is impressive, it poses challenges for wildlife. Notably, several bird species rely on grains produced by agriculture. As farmland diminishes, their food supply dwindles, potentially impacting their long-term survival. Frugivorous birds, including the vibrant rose-ringed parakeets, thrive on fleshy fruits. These birds seek out ripe, nutritious offerings, avoiding unripe green fruit due to its lower energy content

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and higher toxin levels. By consuming fruits, they inadvertently aid plants in seed dispersal. As they fly across diverse habitats, frugivores carry seeds far from the parent plant, promoting forest regeneration. The interaction between frugivorous birds and medicinal fruiting trees exemplifies an essential ecological process. These birds not only disperse seeds but also connect living and non-living components within ecosystems. Biodiversity—the rich tapestry of different species—thrives when frugivoresfulfill their role as seed carriers. Beyond frugivores, avian diets span a spectrum. Insectivorous birds hunt insects, carnivorous birds' prey on other animals, and omnivorous birds consume a mix of plant and animal matter. The beak, a defining feature, adapts to each bird's food preferences and environment.

**2.4 Granivores:**

Granivorous birds primarily eat seeds. They play a role in seed dispersal and plant regeneration. Sparrows and doves are common granivores in India. If we take the example of Delhi, between 2000 and 2016, the area under cultivation decreased from 52,816 to 34,750 hectares, and the main reason behind this decline can be attributed to the fast pace of urbanisation. Even in the fringe areas of the city, the area under farmlands is decreasing rapidly. While on the one hand, this decline in cultivated land showcases the speed of urbanisation and growth of the city, on the other hand, it will surely impact several species that are dependent on the grains produced by agriculture. Wheat and rice crop from agriculture provide a large amount of feed to granivorous birds, and a decline in crop production will surely impact their numbers in the long term. Urbanization has led to significant changes in landscapes worldwide, impacting various ecological processes and species. Among the affected groups, granivorous birds—those that primarily feed on seeds—face particular challenges due to the decline in grasslands and agricultural areas within cities. In this article, we explore the implications of urban development on granivorous bird populations and discuss potential conservation strategies. The decline of grasslands and agricultural spaces in cities directly affects granivorous birds. These habitats provide essential resources such as food, shelter, and nesting sites. Unfortunately, rapid urban expansion and development have led to the conversion of these areas into concrete jungles, leaving little room for natural vegetation. While some urbanites engage in positive practices, such as placing bird feeders on balconies, these efforts only benefit a subset of bird species. Moreover, the impact is limited. Granivorous birds require more extensive and diverse habitats to thrive. Despite the challenges, there are opportunities to conserve granivorous birds within cities. Remnant forest patches and wetlands can serve as critical refuges. By preserving these small pockets of urban grasslands, we can provide both food resources and suitable habitats for granivorous species. The House Sparrow, once common in urban areas, faced a decline due to habitat loss and other factors. However, concerted efforts by concerned citizens have led to its resurgence. Similar awareness campaigns can help highlight lesser-known granivorous species and encourage their conservation.

**2.5 Omnivores:**

Omnivorous birds have a varied diet, including both plant and animal matter. House crows, for instance, consume everything from dried chapatis and bread to insects, larvae, and garbage waste. For example, the black drongo and myna were found to be very aggressive birds. They aggressively drive-away other species of birds for food resources. These birds fly very fast and forage on insects like dragonflies, grasshoppers, moths and butterflies and small invertebrates like ants, termites and

ISBN: 978-81-957386-3-2

small worms present in the soil. These are very opportunistic birds and often feed on the eggs and nestlings of other birds. Few instances have been observed where the black Drongo were seen feeding on the nectar of the bright-orange flowers of the Indian coral tree. These two birds have been found to be very compatible with each other, most probably due to their similar feeding habits. The Asian koel, cuckoo, show a marked sexual dimorphism. The male cuckoo is bluish-black in color and the female is dull greyish or brownish in color and has spotted plumage while their belly side shows stripes. The koels are "brood parasites". They lay their eggs in the nest of crows, ravens, black drongo and mynas. Their young ones are raised by their foster parents. These birds are generally omnivores but mostly feed on fruits and berries available in the wild. These birds are mostly seen in pairs on the higher branches of mango and banyan trees. They also feed on the poisonous fruits of yellow oleander flowers (Cascabelathevetia). Besides, they also feed on small invertebrates. The male species produces a sweet 'koo-ooo' sound repeatedly, mostly during breeding season.

**2.6 Piscivores:**

Piscivorous birds, adept at catching fish, are commonly observed near water bodies. Among them, species like egrets and herons fall into this category. Recent research highlights the substantial economic impact of piscivorous bird predation on pond fish in small-scale aquaculture systems. This study reveals that piscivorous birds pose a significant challenge for small-scale fish farmers. Their predation on pond fish results in economic losses that cannot be ignored. The implications extend beyond individual farms, affecting the overall sustainability of rural aquaculture. To address this issue, habitat management practices play a crucial role. Strategies should focus on deterring the aggregation of predatory bird species around ponds. Key measures include: Excessive vegetation around ponds attracts piscivorous birds. Managing vegetation can discourage their presence. Locating ponds closer to farm buildings minimizes bird access and reduces losses. Stocking ponds with diverse fish species, such as tilapia and catfish, may deter birds that exclusively prey on tilapia. only a few piscivorous bird species account for most of the pond-fish losses experienced by farmers. Focusing on these specific species is essential for effective mitigation. Expanding assessments beyond the current study—across a wider range of farms and incorporating non-avian predators—will enhance our understanding of pond fish losses. Detailed assessments of fish predation rates will provide a more accurate basis for policy decisions supporting profitable aquaculture ventures in rural areas.

**2.7 Nectarivores:**

Nectar-feeding birds, such as sunbirds and barbets, play a crucial role in pollination by visiting flowers for nectar.Birds, those free-living avian beings, wield remarkable influence over the reproductive success of plant species across the globe. Their role extends beyond mere survival; it shapes the very fabric of ecosystems. In this article, we delve into the intricate dance between birds and plants, emphasizing the critical importance of their interactions. Birds, like skilled choreographers, orchestrate the pollination process. As they flit from flower to flower, seeking energy-rich nectar, they inadvertently become carriers of life. Pollen grains from one flower adhere to their feathers, beaks, and bodies, only to be deposited onto the stigma of the next flower they visit. This transfer of genetic material ensures fertilization and seed production, perpetuating plant lineages. Flowers, too, play their part. They beckon birds with vibrant hues—reds, oranges, and yellows—like a grand opening act. Their nectar, hidden deep within tubular or cup-shaped blossoms, lures avian

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visitors. These floral structures accommodate birds, providing sturdy perches for landing and easy access to nectar. The absence of overpowering scents caters to the bird's limited sense of smell. Alas, our feathered allies face challenges. Habitat clearance, urban sprawl encroaching upon their homes, rampant tree felling, and the degradation of water bodies threaten their existence. As we witness these global changes, we must recognize the urgency of documenting these threats and formulating effective conservation strategies. The first step lies in identifying the plants that sustain bird populations. Food, nectar, and shelter plants—these form the backbone of avian survival. By cataloging these essential species, we pave the way for targeted conservation efforts. Each bird species plays a unique role in pollination. Their decline reverberates through ecosystems. In a recent survey across diverse Indian locations, approximately 20 bird species were documented alongside their associated nectar plants. This knowledge informs our conservation approach. Our strategy intertwines birds and plants. By safeguarding their habitats, promoting native flora, and raising awareness, we can mitigate the threats faced by both. It's a delicate balance—one that ensures thriving ecosystems and sustains our avian allies.

**2.8 Herbivores:**

Herbivorous birds, their beaks adapted for plant consumption, play a pivotal role in shaping ecosystems. Their diet includes leaves, buds, and fruits, making them essential agents in plant dynamics. Ducks and geese, too, fall into this herbivorous category, their feeding habits influencing local flora. To understand how urbanization impacts bird communities, researchers evaluated diversity patterns along a five-stage gradient in and around Amravati city, situated on the Deccan Plateau in Central India. The study spanned the months of January to April from 2010 to 2013. A remarkable 112,829 birds, representing 89 species, were meticulously identified across the region.

**3. Cause of different dietary preferences**

Birds, like any other living creatures, can adapt their dietary preferences based on various factors such as changes in environment, availability of food sources, competition with other species, and human activities. In India, there have been observed changes in the dietary preferences of birds due to several reasons:Species richness exhibited a clear trend: from the rural forest (with 73 species) to more urbanized areas (reaching a minimum of 29 species at the heart of Amravati city). Urbanization, it seems, curtails avian diversity. Along the urbanization gradient, bird assemblages favored smaller species. These adaptable birds thrive in human-altered landscapes. The composition of bird diets also shifted. Frugivorous and omnivorous species gained prominence, while insectivorous species declined. Urban environments seem to encourage fruit consumption and dietary flexibility. Local (alpha) diversity accounted for 50.1% of the total (gamma) diversity. This suggests that within a given area, species richness remains relatively stable. Urbanization stages contributed 36.2% to overall diversity. The transition from rural to urban habitats significantly shapes avian communities. Interestingly, within-stage local diversity played a minor role (2.7%). Urbanization tends to homogenize bird assemblages, creating fairly uniform communities.

**3.1 Urbanization and Habitat Loss:**

With the rapid urbanization and habitat loss occurring in many parts of India, birds may have to adapt to new food sources available in urban environments. For example, some bird species might start

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feeding on human-provided food like bread crumbs or leftovers from garbage dumps. Rapid urbanization, agricultural expansion, deforestation, and industrial development continue to encroach upon natural habitats critical for avian species. Fragmentation of habitats disrupts breeding, foraging, and migration patterns, leading to population declines. He primary reason for this decline is the loss of natural habitat. As human activities alter landscapes, birds struggle to find suitable places for nesting, feeding, and shelter. Invasive species disrupt ecosystems, further exacerbating the problem. A recent study conducted in India sheds light on the urgent conservation needs of bird species. The State of India's Birds 2023 report reveals crucial findings: 178 wild bird species in India, including teals, sarus cranes, and greater flamingos, require immediate attention. These species are imperiled by habitat loss or degradation and the impacts of climate change. While the populations of the Asian Koel and Indian peafowl have increased significantly, most bird species are in decline. The study analysed 942 bird species based on 30 million field observations submitted by birdwatchers to the online platform eBird. Among these, 348 species had sufficient data over 25 years, and 60% of them showed long-term decline.

**3.2 Climate Change:**

Climate change can lead to alterations in the distribution and availability of certain plant and insect species, which are essential food sources for many birds. Birds may shift their preferences to new plant species that are better adapted to changing climate conditions. Changing climatic patterns affect bird habitats, migration routes, and food availability. Rising temperatures, altered precipitation patterns, and extreme weather events can disrupt breeding cycles, shift distribution ranges, and lead to mismatches between the timing of breeding and food availability. A study titled "Projected Shifts in Bird Distribution in India under Climate Change" revealed that 66–73% of bird species in India are likely to shift either to higher elevations or northwards by 2070. Long-distance migratory birds are particularly vulnerable to these shifts, as their habitats are more affected by climate change. By 2070, nearly 75% of endemic bird species in India are likely to experience reduced climatically suitable areas. The impact of climate change on partially migratory and migratory species is higher (62.88% and 68.01%, respectively) compared to sedentary species (54.08%). Researchers modelled 42 species out of the 78 endemic species in India to understand these effects.

**3.3 Invasive Species:** The introduction of invasive plant and animal species can also affect the dietary preferences of birds. Invasive plants may replace native vegetation, altering the food available to birds. Similarly, invasive insect species can become significant food sources for birds, leading to changes in their diets. Among them are teals, sarus cranes, and greater flamingos. Several bird species in the Delhi-NCR region need urgent conservation efforts. These include the white-rumped vulture, Palla's fish eagle, Sarus crane, Indian skimmer, bristled grassbird, and common pochard.A recent study highlights that invasive plants pose a serious threat to natural and agricultural landscapes in India. Two prominent invasive species are Lantana camara and Chromolaenaodorata. These plants exhibit robust resilience to climate change, making their spread across landscapes more challenging. Control and eradication efforts are essential, but they come with economic losses as invasives overtake habitats. Experts recommend leveraging green bonds to manage invasive species effectively Approximately 66% of India's wilderness areas have been invaded by these aggressive plants.

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**3.4 Agricultural Practices:**

Shifts in cropping patterns, can impact the availability of insects and seeds that birds rely on for food. This can lead to changes in the dietary preferences of birds, with some species adapting to new agricultural landscapes while others may struggle to find suitable food sources.

**3.5 Human Feeding:**

Feeding birds in parks, gardens, and other urban areas can also influence their dietary preferences. Birds may become accustomed to human-provided food sources such as bird feeders, leading to changes in their natural foraging behaviors. Conflicts between humans and birds, particularly in agricultural areas, can arise due to crop damage caused by certain bird species, leading to retaliatory killings or habitat destruction. Human-wildlife conflict often leads to conflict-related killing. People may kill animals in self-defence or as pre-emptive or retaliatory measures. Unfortunately, this can drive species to the brink of extinction. Globally, more than 75% of wild cat species, as well as other terrestrial and marine carnivores (such as polar bears and Mediterranean monk seals) and large herbivores (like elephants), are affected by conflict-related killing.

**3.6 Migration Patterns:**

Changes in migration patterns, which can be influenced by factors like climate change and habitat loss, can also affect the dietary preferences of birds. Birds may need to adapt their diets to different food sources available along their migration routes.

**3.7 Biomagnification:**

It is an ecological phenomenon that poses a significant threat to various species, including birds. Biomagnification occurs when toxic chemicals, like DDT, persist in the environment and are indirectly consumed by organisms through their food.In aquatic ecosystems, smaller species like zooplankton ingest these toxins, which then pass up the food chain to larger fishes, humans, and birds.Birds, being higher up in the food chain, accumulate these toxins in their bodies. The concentration of these chemicals increases as they move through different trophic levels.DDT, being non-biodegradable, remains in soil or water for years, perpetuating this dangerous process DDT and Bird Eggshells: DichloroDiphenylTrichloroethane (DDT), a pesticide widely used in India for pest control, has serious implications for both human health and the environment. During the 1940s and 1950s, DDT was extensively employed to reduce mosquito populations, but this led to a rapid decline in bird populations. DDT affects Bioaccumulation refers to the gradual accumulation of toxins within an organism's body over time. Biomagnification, on the other hand, specifically involves the transfer of these toxins through the food chain, affecting every trophic level.

**4. Methodologies used for the Assessment of dietary preferences:**

Understanding the dietary preferences of birds in India requires a multifaceted approach that encompasses various methodologies. These methodological approaches provide valuable insights into the feeding ecology of avian species and help inform conservation efforts. Below, we delve into the key methodologies used for assessing the dietary preferences of birds in India:

**a) Field Observations:** Field observations are foundational to studying bird diets. Researchers spend extensive time observing bird behavior in their natural habitats, documenting feeding locations,


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 ISBN: 978-81-957386-3-2

food types consumed, and foraging techniques. Systematic surveys conducted across different habitats yield valuable data on the dietary habits of diverse bird species.

b) **Dietary Analysis:** Dietary analysis involves examining stomach contents or fecal samples of birds to identify consumed food items. This method provides quantitative data on the proportion of different food items in a bird's diet, offering insights into its feeding preferences and ecological niche. Techniques such as microscopic analysis and DNA barcoding enhance the accuracy of food item identification.

c) **Feeding Studies:** Feeding studies entail controlled experiments to investigate bird feeding preferences. Researchers offer birds various food items and observe their choices to determine dietary preferences and nutritional requirements. These experiments provide valuable insights into the factors influencing bird food selection and foraging behavior.

d) **Stable Isotope Analysis:** Stable isotope analysis is a powerful tool for studying long-term dietary patterns of birds. By analyzing stable isotopes in bird tissues like feathers, researchers can infer the types of food consumed and trophic relationships. This method provides insights into bird migration patterns, habitat use, and ecosystem dynamics over time.

e) **Remote Sensing:** Remote sensing techniques, including satellite imagery and GIS mapping, aid in assessing habitat quality and food availability for birds. Analyzing vegetation cover, land use patterns, and environmental variables helps predict suitable foraging habitats for different bird species. Remote sensing data complement field observations and support conservation planning efforts.

f) **Modeling Approaches**: Modeling approaches, such as niche modeling and resource selection functions, predict bird dietary preferences based on environmental variables. Integrating data on habitat characteristics, climate, and food availability enables researchers to identify critical foraging areas and inform conservation strategies effectively.

**Conclusion:**

In conclusion, the dietary preferences of birds in India reflect a complex interplay of ecological, environmental, and anthropogenic factors. From the lush rainforests of the Western Ghats to the arid scrublands of Rajasthan, avian species have evolved diverse feeding habits to exploit the rich tapestry of habitats across the subcontinent. Understanding these dietary preferences is essential for unraveling the intricacies of avian ecology, informing conservation strategies, and managing ecosystems effectively.Methodologically, a range of approaches, from field observations to stable isotope analysis and remote sensing, are employed to assess bird diets comprehensively. These methodologies provide valuable insights into the factors shaping bird food selection, foraging behavior, and ecosystem dynamics. By integrating these approaches, researchers can gain a holistic understanding of the dietary preferences of birds in India and their implications for ecosystem health and biodiversity conservation.

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ISBN: 978-81-957386-3-2

# CHAPTER - 8

# SYNBIOTICS COUPLED NANOPARTICLES – A MECHANISM OF EFFICACY ENHANCEMENT

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## Abstract

In the past ten years, research on probiotics and prebiotics combined as synbiotics to improve human and animal health has increased, including numerous clinical trials that have evaluated a wide variety of synbiotic formulations. These investigations and the available synbiotic commercial applications are outlined in this review. We evaluate the purported health benefits of synbiotic applications, as well as the ecological and therapeutic considerations that should be made when creating synbiotics, and we examine the significance of these ideas for future study in this area.Changes in the microbial makeup of the GI tract have been linked to a decline in the host's immunity, barrier, and other functioning processes. These changes might be brought on either directly by the gut microbiota or indirectly via a changed metabolite production. Although the microbiota composition in the human GI tract is surprisingly stable, certain synbiotic therapy can effectively change it. When chosen rationally, taking into account ecological and evolutionary factors, these treatments may provide significant benefits for human health.

**Keywords:** Synbiotics, clinical trials, therpy, Gastrointestinal tract, Microbiota

## Introduction:

Prebiotics and probiotics contain tiny particles known as nanoparticles, which typically have dimensions between 1 and 100 nm on the nanometer scale. Prebiotics are non-digestible food components that help the growth of good bacteria in the gut, whereas probiotics are living microorganisms that provide health advantages when taken in adequate quantities. This is the major distinction between prebiotics and probiotics. (1)

Nanoparticles are hypothesized to have contributed to the emergence of the first living things on Earth in the primordial environment. These particles could have provided a safe environment for chemical processes that resulted in the development of life and acted as a template for the construction of biomolecules like nucleic acids and proteins. (2)

Nanoparticles are particles that are utilized to transport probiotics to the gut in the setting of probiotics. This includes encapsulating probiotics in nanoparticles, which can shield them from the harsh environment of the stomach and improve their chance of survival in the intestine. This might be

 ISBN: 978-81-957386-3-2

accomplished by putting the probiotics inside a nanoparticle consisting of a protein, polysaccharide, or liposome. Additionally, probiotics can be shielded with nanoparticles against environmental stresses including heat, light, and pH fluctuations, preserving their stability and viability. (3)

In order to selectively provoke the growth and stimulate the metabolism of one or more health-promoting bacteria, the synbiotic agents are a combination of prebiotics and probiotics. They have positive effects on the host by enhancing the activity and survival of beneficial microorganisms in the gastrointestinal tract. The prebiotic and probiotic selection criteria and needs are the most crucial aspect of the design of synbiotics and should be clearly stated. (4)

**Modulation of Gut Microbiota:**

The administration of live microorganisms was one of the first methods to be suggested for modifying the gut microbiota. In fact, long before the name "probiotic" was properly defined, what we now refer to as probiotics have been produced and ingested for more than a century. Current definitions of probiotics state that they are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Numerous clinicians recommend probiotics to patients for a range of conditions, including antibiotic-associated diarrhoea, acute gastroenteritis, general GI disorders [like irritable bowel syndrome (IBS) and infant colic], mild ulcerative colitis (UC), and to improve lactose digestion. There are hundreds of probiotic strains and products on the market.(5)

Probiotics have been linked to a number of health benefits, including the treatment of antibiotic-associated diarrhoea, the prevention of necrotizing enterocolitis in preterm neonates, the maintenance and induction of IBD remission, the prevention and control of hyperglycemia and improvements in total cholesterol, high-density lipoproteins (HDLs), and tumor necrosis factor (TNF)-α in patients with nonalcoholic fatty liver disease (NAFLD) and reduction of glucose and insulin as well as a homeostatic model assessment of insulin resistance in diabetes patients (6-7)

The usefulness of probiotics in avoiding or lessening the severity of infectious diarrhoea and respiratory tract infections that are caused by antibiotic use is further discussed by Jonkers (2016). (8)

Prebiotics may offer health benefits that are not dependent on fermentation. Human milk oligosaccharides, for instance, may shield young children against infections by preventing bacteria from adhering to the epithelial cells lining the GI tract. Similar outcomes have reported for GOSs. Resistant starch has recently been shown to have microbiota-independent effects on increased insulin sensitivity and other metabolic advantages. (9-10)

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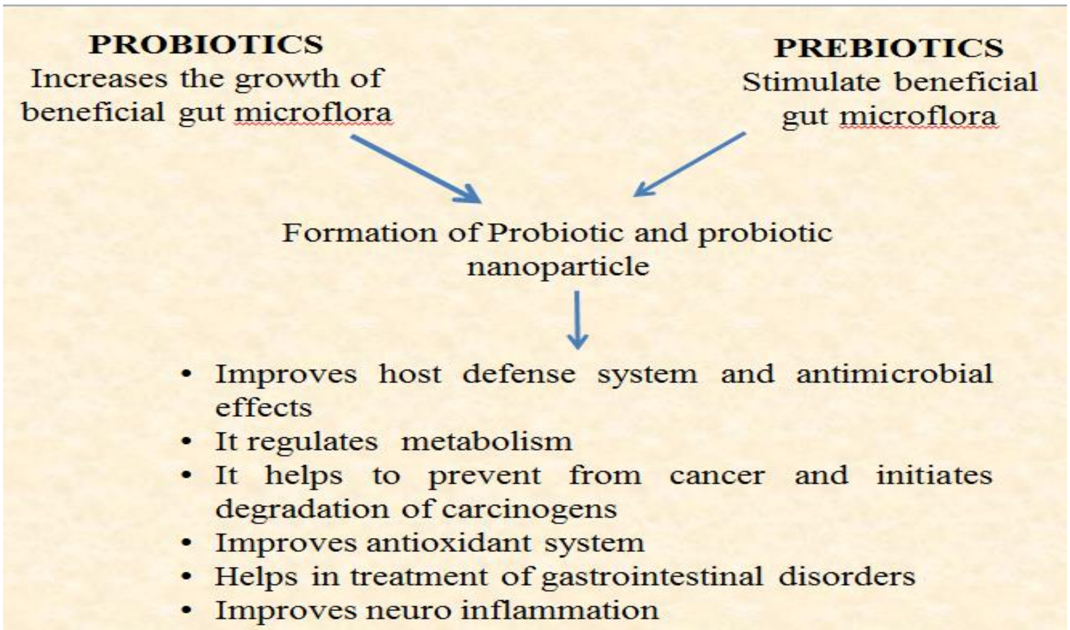


**SYNBIOTICS: Effects on human health in clinical studies**

| S.No. | Disease phenotype | Type of synbiotic | Outcome | Reference(s) |
|---|---|---|---|---|
| 1 | Ulcerative Colitis | Probiotic Bifidobacteriumlongum<br><br>Prebiotic Psyllium | improved quality of life as per IBDQ (inflammatory bowel disease questionnaire) score , Improvement of endoscopic grading compared to standard therapy group,<br><br>Inflammatory markers improved,<br><br>Synbiotic is safe and effective | (11-12) |
| 2 | Inflammatory markers in adult patients with overweight or obesity | Probiotic: Lactobacillus sporogenes, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacteriumlongum, fructooligosaccharide, inulin | Some synbiotics and prebiotics may have immunomodulatory effects | (13) |

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| | | | | |
|---|---|---|---|---|
| 3 | Perioperative effect for elective abdominal surgery | Abstract for oral presentation, thus none given | Postoperative infectious complications reduced by synbiotics; no effect on mortality or noninfectious complications | (14) |
| 4 | Improvement of high fasting blood glucose | Probiotic: Lactobacillus casei, Streptococcus thermophilus, BifidobacteriumbrevePrebiotic: fructooligosaccharide; | Subanalysis on synbiotics showed no significant improvement | (15) |
| 5 | Reduction of postoperative complications | fructooligosaccharide; | Synbiotics reduced surgical site infections, sepsis, length of hospital stay, and duration of antibiotic treatment better than pro- or prebiotics alone; no effect on mortality | (16) |
| 6 | Prevention of infections after GI surgery | Probiotic: Lactobacillus casei, Streptococcus thermophilus, Bifidobacteriumbreve, Lactobacillus plantarum, Pediococcuspentosaceus Prebiotic: fructooligosaccharide; galactooligosaccharide, oligofructose, β-glucan | Subgroup analysis of synbiotic trials showed no health benefits due to synbiotics | (17) |

**Synbiotics in human health:**

**1. Antibacterial properties:**

Due to their synergistic net health advantages, synbiotics, which are the combination of probiotics and prebiotics, have recently gained interest. Antibiotics have been replaced with probiotics in some cases. The probiotic *Lactobacillus plantarum* (LP) has demonstrated substantial antibacterial action against the significant cattle pathogen Escherichia coli K99. In this study, the antibacterial efficacy of LP treated with phthalylpullulan nanoparticles (PPN) was studied. It's interesting to note that when PPNs were given to LP, the PPNs were internalised into the LP by a method that required both energy and the galactose transporter. Additionally, PPN-treated LP released more plantaricin, a naturally



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 ISBN: 978-81-957386-3-2

occurring antibacterial peptide, compared to untreated or pullulan-treated LP.Additionally, PPN-treated LP shown greater antibacterial activity than untreated or pullulan-treated LP against Gram-positive Listeria monocytogenes and Gram-negative Escherichia coli K99. The PPN-treated LP's improved antibacterial abilities are assumed to be the result of intracellular activation. Overall, our study introduces a novel strategy for LP plantaricin synthesis via PPN internalization-induced intracellular activation. (18)

**2. Enhancement of oral bioavailability of natural compounds:**

By increasing the solubility of hydrophobic and hydrophilic compounds in aqueous medium, protecting them from acid and enzymatic degradation in the gastrointestinal tract, and promoting their permeability across mucosal membranes, encapsulation in polymer NPs. (NPs) is a well-recognized method for improving oral bioavailability. Prebiotics, probiotics, and natural antibacterial chemicals are also suggested to be encapsulated in nanostructures rather than bigger delivery methods since they allow for more mucous layer penetration as well as lower and longer dosages. Due to their potential to stick to the intestinal mucosa, NPs with mucoadhesive capacity are specifically created to lengthen the residence time at the intestinal absorption sites.The enterocytes, or intestine absorptive cells, should, however, also be accessible via the mucosa. The size and surface characteristics of NPs can be directed towards a certain mucus-interaction mechanism by utilising the right materials. Polymer NPs are frequently used in the pharmaceutical industry for oral administration applications because their surface characteristics may be easily adjusted to enhance mucoadhesive capacity and mucus penetrating ability. Biopolymer NPs have drawn attention recently as mucoadhesive delivery systems for possible food applications due to their biodegradability, biocompatibility, and non-toxicity.This review intends to draw attention to the use of food-grade biopolymer NPs to improve the delivery of prebiotics, probiotics, and antibiotics along the gastrointestinal tract and to boost the bioavailability of natural compounds based on their mucoadhesive potential. This also provides a very basic overview of the intricate mechanism underlying mucoadhesion as well as typical techniques for assessing mucoadhesiveness in nanoparticulate systems. (19)

**3. As antibacterial peptides:**

Probiotics and prebiotics combined to form synbiotics have synergistic effects on the wellbeing of the host. Antibiotics have been replaced with probiotics in some cases. Pediococcusacidilactici (PA), a probiotic, has enhanced animal output and demonstrated strong antibacterial efficacy against Salmonella Gallinarum (SG), a significant poultry disease. Prebiotics like inulin are frequently utilised to boost animal development and health. This study's primary goal is to determine how future in vivo applications of inulin nanoparticles (INs)-internalized PA encapsulated in alginate/chitosan/alginate (ACA) microcapsules (MCs) will be affected by their antibacterial activity. By using DLS and FE-SEM, the prepared phthalylINs (PINs) were identified.By measuring 1H-NMR, it was determined that the amount of phthal groups in phthalyl inulin was 25.1 mol.-%. The PINs' diameters, as determined by DLS, were roughly 203 nm. Using flow cytometry and confocal microscopy, internalisation into PA was verified. Co-culture antimicrobial tests on SG were used to measure the antibacterial activity of PIN-internalized probiotics that were encapsulated in ACA MCs.

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ACA MCs loaded with PA/inulin or PA demonstrated a lower antibacterial capacity than PIN-internalized probiotics. It's interesting to note that pediocin was created significantly higher in the culture medium when PINs were treated with PA and encapsulated into ACA MCs as a natural antimicrobial peptide, compared to other groups using inulin-loaded ACA MCs and PA-encapsulated into ACA MCs. (20)

**4. As nanonutraceuticals:**

Due to its capacity to increase the bioavailability of the loaded active components, nanotechnology has been used increasingly in nutraceuticals over the past few years, leading to better therapeutic/nutraceutical outcomes. This research focuses on nanoprebiotics and nanoprobiotics, which refer to the loading of a group of substances (such as prebiotics, probiotics, and synbiotics) in nanoparticles that operate as digestive tract absorption enhancers.The basic characteristics of prebiotics and probiotics are emphasised in this manuscript, along with a discussion of new nanotechnology applications in their preparation. Additionally, current research methodologies are presented, with a focus on the possible application of nanofibers for probiotic administration. Synbiotic-based nanoparticles are a cutting-edge development in this field. It is necessary to conduct research on this important area, encompassing effectiveness, bioavailability, and safety issues, as there are only a few experimental investigations on nanoprebiotics and nanoprobiotics available in the scientific literature. (21)

**5. As Bacteriocin:**

Probiotics have received a lot of interest recently as an antibiotic alternative due to the growing bacterial resistance brought on by antibiotic abuse. We created phthalyl dextran nanoparticles (PDNs) by conjugating phthalic anhydride with dextran to make a prebiotic and examined its effects on the cellular and antibacterial capabilities of the probiotics. Prebiotics are known to increase the activity of probiotics. First, we discovered that temperature, time, and glucose transporters all affected how well probiotics internalised PDNs. Internalisation of PDNs increased probiotics' production of antimicrobial peptides through a self-defense mechanism, and as a result, probiotics themselves had stronger antibacterial activity against Gram-positive and -negative microorganisms.Additionally, the pediocin that PDN-internalized probiotics produced has the power to suppress pathogenic gut infections and change the composition of the gut microbiome in living organisms. When Pediococcusacidilactidi is internalised in mice, its heightened antimicrobial properties may result in a reduction in pathogen species and an increase in beneficial bacteria. Additionally, the gut microbiome's composition changed, which prevented the microflora's variety from declining. Our findings provide a new route for probiotic modulation and its application in tackling the problem of bacterial resistance by showing that PDNs, a novel form of prebiotic, can control probiotic bacterial metabolism.(22)

**6. In nutraceuticals:**

It has long been established that including probiotics and prebiotics in your diet has positive benefits on your internal health. However, this may experience a loss of vitality along the

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digestive tract's absorption, resulting in a meagre intestinal delivery of probiotic active components. Nanotechnology has recently been fervently applied to increase the bioavailability of active substances. Versatile nanoparticle (NP) forms have been developed for usage with probiotics, prebiotics, synbiotics, or various combinations of these. The characteristic organic substances found in the NPs that are currently popular include sugars, proteins, fats, and others. Inorganic substances include oxides of silver, titanium, and magnesium, among others. This review critically explains the new connection between probiotics and prebiotics in nanotechnology for various uses in neutraceuticals. In this review, formulations of nanoprobiotics and nanoprebiotics—which function as efficient drug delivery systems—are thoroughly reviewed. These formulations also have qualities that are anti-cancerous, anti-microbial, anti-oxidant, and photo-protective. The limited amount of scientific study on probiotics and prebiotics that is now available suggests dynamic research investigations on the bioavailability of loaded active components and the efficient drug delivery system by taking environmental and food safety concerns into account. (23)

**7. Inflammatory bowel diseases (IBD):**

The two IBDs that affect the GI tract are Crohn's disease (CD) and ulcerative colitis(UC). Both diseases affect people who are genetically predisposed and are exposed to unidentified environmental risk factors. IBD's aetiology has been thoroughly researched, although the pathogenesis of the illness is not fully understood. While CD affects the whole GI tract, UC typically affects the distal small intestine and colon with transmural inflammation and is occasionally linked with granulomas, the inflammation's features are different in each condition. In contrast, the mucosa of the colon is typically the only area in which UC inflammation occurs. In contrast, the mucosa of the colon is typically the only area in which UC inflammation occurs. Both UC and CD have a relapsing and remitting nature that significantly lowers quality of life when the disease is present. (24-25)

In clinical research, a number of synbiotic formulations have been utilised to treat IBD. Patients with UC received a mixture of 6 g of inulin/oligofructose (Synergy 1R) and CFU of *B. longum*. The test strain was derived from a healthy human individual, and its adhesion to epithelial cells, bile salt resistance, aerotoxin tolerance, and acid tolerance were all evaluated. Also demonstrated in vitro was its capacity to draw energy from the prebiotic substrate.It was also demonstrated that the organism changed the expression of cytokines in an epithelial cell line called HT29 and decreased proinflammatory cytokine levels in culture. 18 patients were used in the clinical investigation, split into placebo and synbiotic groups, and given their corresponding medicines twice daily for 4 weeks. Comparatively to the placebo group, the synbiotic treatment reduced inflammation, promoted epithelial tissue regeneration, decreased mRNA levels of human -defensins, and decreased TNF- and IL-1 levels. The probiotic strain's survival was not assessed in a strain-specific manner, however in the synbiotic group, bifidobacteria-specific rRNA levels increased 42-fold compared to roughly fivefold in the placebo group. However, this study did not look into the probiotic's effects on their own. (26)

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**8. Diarrhoea:**

Infectious disorders that result in loose or liquid bowel movements with increased frequency, water content, and volume are frequently the cause of diarrheal diseases. According to Dinleyici et al. (2013), diarrhoea is the main cause of hospitalisations, morbidity, and mortality worldwide. Children with acute diarrhoea were examined using a multistrain mixture of FOSs (626 mg) and the probiotics Lactobacillus, Bifidobacterium, and Enterococcus. Additionally, intravenous treatment and oral rehydration salts (ORSs) were administered. The synbiotic decreased the length of diarrhoea (P 0.0001) and hospital stay (P = 0.002) as compared to a control group (getting only ORSs and/or intravenous therapy). These kids' gut microbiome wasn't examined. (27)

Children's susceptibility to vomiting, diarrhoea, and different diseases was examined in relation to a mixture of S. thermophilus, L. bulgaricus, Bifidobacteriumanimalis subsp. lactis, and inulin (1 g). Children who received synbiotic treatment had considerably fewer days with a fever but significantly more days with watery stools after 16 weeks of treatment (P 0.05). There was no microbiome analysis or evaluation of the individual component effects in this RCT. (28)

Patients with HIV and AIDS typically complain of GI symptoms like diarrhoea. Using a maltodextrin placebo, da Silveira et al. (2017) evaluated the effectiveness of FOSs (6 g) and a combination of L. paracasei, L. rhamnosus, L. acidophilus, and B. lactis. 64 patients made use oftherapies twice each day for six months together with a customised food schedule. The incidence of diarrhoea was greatly reduced in both the synbiotic and placebo groups, and there was no significant difference between them. There was no investigation of the gut microbiota, and the choice of this synbiotic combination was not explained in detail. (29)

**9. Irritable Bowel Syndrome:**

Abdominal pain, bloating, diarrhoea, constipation, or alternating periods of these symptoms are the hallmarks of IBS, an intestinal condition. Visceral hypersensitivity, genetics, the gut microbiota, ongoing low-grade inflammation, and environment are all risk factors for this condition although the exact reason is unknown. IBS may affect 11% of people worldwide, with females and younger people experiencing it more frequently. Current IBS treatments include physiological interventions, dietary modifications, pharmaceutical medications, and regulation of the gut microbiota. An RCT examined the impact of dietary fibre (80% inulin, 20% oligofructose) and L. acidophilus, B. animalis subsp. lactis, S. thermophilus, and fermented milk on the gut microbiota of 30 persons with IBS. Relative'afferent pupillary defect and real-time polymerase chain reaction tests of faecal samples revealed the presence of all three treatment bacteria in the synbiotic group, which decreased during the one-week follow-up. Within the synbiotic therapy group, there was no discernible difference in the number of the test bacteria; no strains were found in the control group. This study did not evaluate how the synbiotic therapy affected IBS symptoms.(30-32)

**10. Colon cancer:**

Third most prevalent type of cancer is colorectal cancer. Colon cancer is influenced by environmental variables in addition to hereditary ones, including radiation, chemical carcinogens, and nutrition. Current therapies have a low success rate and a high risk of consequences. According to research, up to one-third of colon cancers may be avoided by maintaining a healthy weight, diet, and level of

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physical exercise .Numerous investigations on probiotics, prebiotics, and synbiotics in rodent models imply that these treatments may be both preventive and therapeutic. (33)

The impact of the synbiotic combination of resistant starch (12.5 g) and B. lactis (5 109 CFU) on early colorectal carcinogenesis markers was examined in a four-week crossover trial in 20 healthy volunteers. Additionally included were prebiotic-only, probiotic-only, and placebo arms. Denaturing gradient gel electrophoresis and quantitative real-time polymerase chain reaction were used to do complete studies of the faecalmicrobiota in order to measure levels of B. lactis.Results demonstrated that compared to a placebo or pro- or prebiotic therapies alone, the synbiotic treatment dramatically altered the gut flora more. However, there was no discernible difference in B. lactis levels between the probiotic and synbiotic therapy groups.There were no differences between the therapy groups in terms of the SCFA profile, faecal ammonia or pH, serum inflammatory markers, or epithelial variables.(34)

11. **Kidney and liver disease**:

According to Jha et al. (2013), between 6 and 10% of individuals have chronic renal disease, and Charytoniuk et al. (2017) found that the prevalence of NAFLD varies from 6.3% to 33%.

According to Koppe et al. (2015), Lambert et al. (2015), and Vitetta&Gobe (2013), pro- and prebiotics have both been proposed as potential treatments for various disorders. There have been a number of synbiotic clinical trials for kidney and liver disorders recently. Patients with chronic kidney disease were given a synbiotic that contained a nine-strain cocktail (bifidobacteria, lactobacilli, and streptococci) and a FOS/GOS mixture.In this short study (n = 31), the majority of the evaluated biomarkers were unaffected by the treatment, although microbiota analysis showed an increase in bifidobacteria abundance and a decrease in Ruminococcaceae. For the treatment of liver illness, B. longum and FOSs were also combined . The microbiome was not examined, and individual components were not included as controls, despite improvements in immunological biomarkers and liver function being seen in both experiments. (35- 39)

FOSs (125 mg) and a seven-strain mixture with species of Lactobacillus, Streptococcus, and Bifidobacterium (2 108 CFU total) were tested on 50 NAFLD patients. According to this RCT, there was a larger reduction of fibrosis and steatosis in the synbiotic group. Similar to this, in an RCT with 80 NAFLD patients, the same synbiotic cocktail reduced the degree of steatosis but did not reduce the levels of alanine aminotransferase and aspartate transaminase when compared to a placebo. The combination of the same cocktail with vitamin E reduced hepatic transaminase levels and produced substantial variations in total cholesterol and LDL cholesterol when compared to the control groups. (40-41)

**Commercialization of synbiotics problems and future aspects:**

Probiotic sales are expected to reach $40 billion worldwide by 2018, while prebiotic sales may soon surpass $5 billion. Several technological and scientific obstacles still stand in the way of potential market opportunities, according to Van den Nieuwboer et al. (2016). The biggest obstacle, according to Van den Nieuwboer et al. (2016), was the "difficulty in demonstrating clinical efficacy." The amount of proof needed to substantiate a health claim differs from nation to nation, which complicates the matter. The FDA and EFSA have not yet approved any probiotic or synbiotic

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combination-related health claims. Additionally, such goods need to be identified as either food or pharmaceutical products. (42-45)

According to FDA regulations, any substance—including probiotics—that is consumed with the intention of treating, diagnosing, preventing, or mitigating disease is deemed to be a medicine and must go through the same regulatory procedures as any brand-new pharmaceutical. The European Union's regulations on nutrition and health claims aim to (a) ensure that they are "clear, accurate, and based on scientific evidence" and (b) outlaw items with "claims that could mislead consumers." In the end, any health claims made for a product must be supported by high-quality human-intervention studies (42).

Consumers frequently receive synbiotic products in the form of food, including a variety of cultured dairy and nondairy goods. Prebiotics can be used in the majority of food applications, however probiotics (and synbiotics) may not be as practicable in many non-refrigerated foods due to their environmental sensitivity. However, new microencapsulation technologies could result in a range of new synbiotic products, such as desserts, confections, juices, cheeses, or chocolate. These technologies shield the bacteria from potentially harmful processing treatments. (46-48)

It's interesting that many pre- and synbiotic products only have trace levels of the prebiotic component in each serving, which may not be enough to have any positive effects on health. Low doses are utilised, possibly partly for financial reasons, in part to prevent negative GI problems. Numerous conditions must be met in order for synbiotic combinations to be developed that are therapeutically successful. As said before, figuring out the minimal effective doses of each component is typically anticipated. It can be difficult to include suitable controls in synbiotic experiments. To confirm additive or synergistic effects, probiotic-only and prebiotic-only controls may also be required in addition to standard placebo controls. It is important to explain the rationale behind the probiotic and prebiotic choices. It's significant to note that proving causality necessitates precise test strain detection, quantification, and enrichment in the GI tract.(49)

To ascertain whether crossfeeding or other ecological occurrences, such as niche competition, niche partitioning, or niche exclusion with the resident microbiota, happened, changes introduced to the gut microbiota should also be evaluated.

**Conclusion:**

Changes in the microbial makeup of the GI tract have been linked to a decline in the host's immunity, barrier, and other functioning processes. These changes might be brought on either directly by the gut microbiota or indirectly via a changed metabolite production. Although the microbiota composition in the human GI tract is surprisingly stable, certain synbiotic therapy can effectively change it. When chosen rationally, taking into account ecological and evolutionary factors, these treatments may provide significant benefits for human health.Antibiotics have been replaced with probiotics in some cases. The probiotic Lactobacillus plantarum (LP) has demonstrated substantial antibacterial action against the significant cattle pathogen Escherichia coli K99. Nanoparticles are particles that are utilised to transport probiotics to the gut in the setting of probiotics. This includes encapsulating

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probiotics in nanoparticles, which can shield them from the harsh environment of the stomach and improve their chance of survival in the intestine. Antibiotics have been replaced with probiotics in some cases. Pediococcusacidilactici (PA), a probiotic, has enhanced animal output and demonstrated strong antibacterial efficacy against Salmonella Gallinarum (SG), a significant poultry disease.

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ISBN: 978-81-957386-3-2

# CHAPTER- 9
# PREVALENCE OF ANTIBIOTIC RESISTANT *E. COLI* ISOLATED FROM DRINKING WATER OF PUBLIC SCHOOLS IN RURAL AREAS

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**Abstract**

Public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic bacteria. *Escherichia coli (E. coli)* bacteria, found in the digestive tract of animals, can get into the environment, and if contacted by people, can cause health problems and sickness. Among the coli form bacteria, Escherichia coli is the most abundant and best indicator of water quality and presence of pathogens. It comprises 97%of fecal coliform bacteria in human faces and available indicator of fecal contamination. Global E. coli-related morbidities and mortalities are high. In present study the drinking water samples were collected from water taps of various schools and colleges of Katol and nearby cities. Katol is a city and municipal council in Nagpur District of Maharashtra.The study concludes that drinking water sources at schools in Katol and the surrounding area are frequently contaminated with E. Coli resistant to antibiotics. Gentamicin is the most effective antibiotic, with Ciprofloxacin and Cephalothin being the least effective. The *E. Coli* isolates showed different patterns in response to routinely used antibiotics. For the majority of the schools, well water was the main supply of water. Taking care to handle the water properly needs to be considered. In summary, it's evident that *E. Coli* seems to be the most reliable marker of the bacteriological quality of water. The isolates showed a range of antibiotic-use-common resistance and sensitivity patterns.

**Keywords:** Public and environmental health, Indicators of water quality, Fecal Contamination

## Introduction:

Our world is covered in water, which is the basis for the existence of organic life. It controls our weather, sculpts our mountains, and cuts our oceans. Water is a mixture of chemicals. Water's remarkable capacity to dissolve a wide range of compounds has led to its characterization as the "universal solvent". In addition to being vital for social welfare and economic growth, access to clean, fresh water is also necessary for food production, health, and the fight against poverty. Water aids in preserving the body's internal organs' moisture content.

A single cell's water content ranges from 45% to 95%, while microorganisms hold 80% of their body weight in water, and humans contain 70% of their body weight in water. The human body uses it as a heat regulator, and the average human body has 42 liters of water (WHO, 2003). A loss of 2.7 liters of bodily fluids can cause weakness, dehydration, and headaches. Water is vital and essential for both the environment and humans, and it is especially significant when it comes to drinking water. Wells, canals, and dams demonstrate the value of water and how human activity affects the water cycle.

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Water is a vital element in each of our lives. Clean water is important to one's good health. Water is a natural resource and is essential to sustain life. There are two main sources of water: surface water and groundwater. Surface Water is found in lakes, rivers, and reservoirs. Groundwater lies under the surface of the land, where it travels through and fills openings in the rocks (Frederick, 2011).

Safe drinking water must be free of harmful germs in order to protect the environment and public health. Animals' digestive tracts contain the bacteria Escherichia coli (*E. coli*), which can enter the environment and infect humans when they come into touch with it. (Dev et al., 1991) The traits, behaviors, and resistance of the diseases that might spread through tainted drinking water are varied. Low pressure conditions, low residual chlorine concentrations, and pipe breaks, which can allow pathogenic microorganisms to infiltrate, are some of the causes that can lead to microbial contamination of drinking water (Singh and McFeters, 1990).

Water-borne diseases are one of the major public health problems in developing countries. It is estimated that contaminated water has caused more than 20 million deaths of which more than 80% were among children under age five.

**Review of Literature:**

According to Mekonen Wolditsadik, Jida Leta et al. (2014), there were differences in the average indicator bacteria (E. coli) collected from each site. The shower sample had the highest average E. Coli levels (203.57 cfu/100 ml), followed by the cafeteria (138.57 cfu/100 ml), and the spring sample had the lowest counts (80.7 cfu/100 ml). The shower had the greatest mean *E. Coli* colony counts (203.57 CFU/100 ml) and the lowest mean counts (80.7 CFU/100 ml). There was an E. Coli colony in the spring sample. According to Frederick Adzitey et al. (2015), Escherichia coli isolates from 56 different water samples were tested against nine distinct antibiotics. There was significant resistance to Vancomycin (94.64%) and erythromycin (85.71%).

According to Adinortey et al. (2012), a total of 389 E. Col isolates were collected, of which 128 and 261 came from ambient and clinical samples, respectively. Imipenem sensitivity was 100% present in all E. Coli isolates. Clinical E. Coli isolates were reported to have relatively low percentage sensitivity to ampicillin (0-24.1%), tetracycline (16.0-28.4%), cotrimoxazole (16.8-22.0%), cefuroxime (27.6-43.2%), and nalidixic acid (22.1-47.8%). Carnot, Carneiro, et al. (2014) confirmed the presence of plasmids that generated bacterial resistance when isolating and identifying E. coli from natural water samples. From two distinct stations, a water treatment station and a water capitation station—they obtained 24 water samples.

According to Garba et al. (2009),they studied biochemical analysis of the samples, 41 (45.5%) of the 63 confirmed Escherichia coli isolates came from well water, 14 (23.3%) from tap water, and 8 (13.3%) from packaged water. The bulk of the isolates were found to have moderate susceptibility to Sceptrin and resistance to Amoxicillin, while they were highly susceptible to Chloramphenicol, Gentamycin, Pefloxacin, Triviid, Augmentin, Streptomycin, Sparfloxacin, and Ciprofloxacin, according to the susceptibility profile of the isolates to nine antimicrobial agents. A total of 448 water samples, including tap and mineral water, were examined by Hassan Momtaz et al. (2013). 34 (7.58%), 4 (0.89%), and 3 (0.66%) of the 448 water samples tested positive for Salmonella species, Escherichia coli, and Vibrio cholera, respectively, according to the culture method.

har et al. (2007) separated the total and fecal coliform bacteria from every drinking water sample taken from Khairpur City's surface reservoir, which serves as the city's main water supply. Both the

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total and fecal coliform (E. coli) levels (log10 3.0-3.94 CFU/100 mL and log10 1.46-2.47 CFU/100 mL) exceeded the maximum microbiological contaminant level (MMCL) set by the World Health Organization. Human and animal contamination was shown to be the cause of the contamination, according to Ibekwe et al.'s (2011) investigation of the antimicrobial resistance pattern of E. coli isolated from minor channels emerging from the middle Santa Ana River in Southern California. Of the 600 isolates, 24 percent showed resistance to multiple antimicrobial agents.

**Materials and Methods:**

The study was conducted at Nabira Mahavidyalaya, Katol in Nagpur district of Maharashtra State. It is the administrative headquarters of Katol taluka, one of the 14 talukas of this district. Water samples were collected from water taps of various schools and colleges of Katol and nearby cities. Katol is a city and municipal council in Nagpur District of Maharashtra. Katol is located at 21.27°N 78.58° E

A total of 50 samples were collected from various school's drinking water. Study was conducted between December 2019 to January 2020. Water samples are collected to estimate the presence and number or population of *E .coli.*. The water samples were collected from sources and collected in sterile bottles. The sampling bottles were labeled and sealed with paper tape. After collection of sample test tubes were tightly closed bottle to avoid any contamination and to protect from environmental pathogen contamination. Then transported to the laboratory for microbiological analysis.

**Bacteriological analysis:**

Bacteriological water analysis is a method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. Methods used in culturing of water.

**Antibiotics susceptibility testing:**

Antibiotic sensitivity profiles of *E. coli* isolates were studied against different antibiotics, bacteria were grown in nutrient broth. Mueller-Hinton Agar (Hi-media) was used as a medium to study the susceptibility to antibiotics. The Modified Kirby-Bauer disk diffusion method was used to evaluate the susceptibility or resistance of E. coli isolates to 11 selected antibiotics, i.e. erythromycin (15 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cephalothin (30 µg), penicillin-G (10 units), cotrimoxazole (25 µg), ceftriaxone (30µg), Vancomycin (30 µg), amoxicillin (10µg), ampicillin (10 µg). (W. Kirby, et.al., 1966).

**Results and Analysis:**

Out of 50 water samples, in 12 water samples *E.coli* were isolated. All positive tubes from presumptive test were streaked on Eosin methylene blue (EMB) agar for the detection of coliform colonies especially E. coli. Positive confirmed samples that showed typical coliform colonies i.e. metallic green sheen colonies of E. coli. All samples are then subjected for antibiotic susceptibility test. The average indicator bacteria (*E.coli*) obtained from all sites were different (Fig No. 1).

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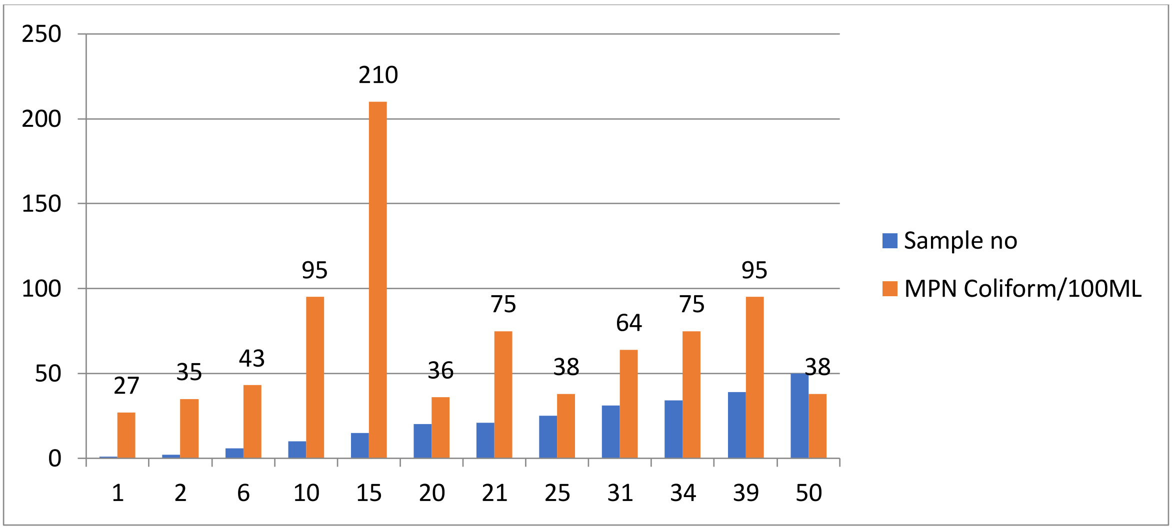


**Fig: - 1. No. of Coliforms/100 ml sample (< 9 coliforms /100 ml)**

The isolation of coliform especially *E. coli* from water sources is attributable to contamination by human and animal origin and this is of health significance as these organisms have generally been agent of gastroenteritis in humans. The tap water found positive might have been contaminated by water hoses connected to the tap, which was normally left on the ground after used and reused without cleaning.Following data provides descriptive information about bacterial results. Fig 1 showed that out of all 50 water samples 12 water samples were not fit for drinking purposes. Out of these sample no, PS 15 showed the maximum coliform count i.e. 210 coliform / 100 ml. As per observed physical condition and the storage procedure of the drinking water in public educational institute the water is contaminated with the coliforms (Fig 1).

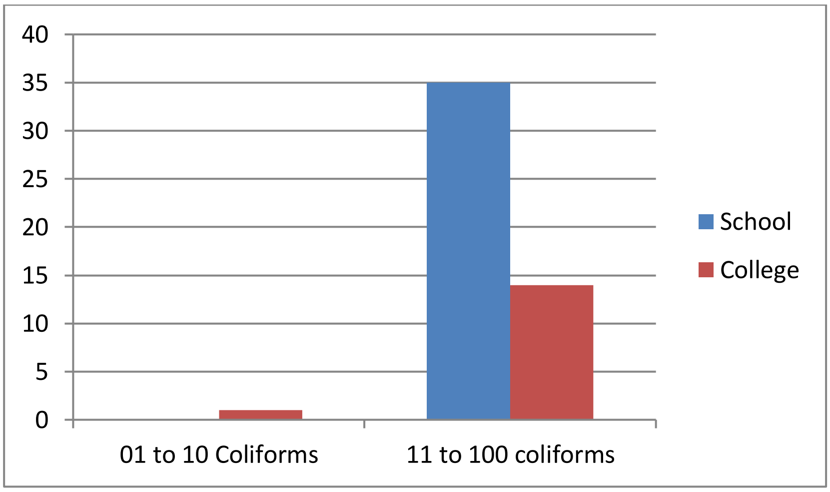


**Fig. 2:- Number of Non Potable water in educational institutes**

In present study when the data was analyzed it was found that the drinking water of public schools are not suitable for drinking purpose as compare to higher educational institutes. Coliform range between 11-100 was mostly found in the public schools drinking water. Poor hygienic condition and lack of health education in school going children's also leads the contamination in drinking water (Fig 2).

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**Table no 1-Antibiotic Susceptibility Test (mm)**

| Sample no | Erythromycin | Chloramphenicol | Gentamicin | Cefotaxime | Penicillin-G | Cotrimoxazole | Cephalothin | Vancomycin | Amoxicillin | Ampicillin | Ciprofloxacin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N1 | 00 | 15 | 15 | 18 | 0 | 22 | 0 | 11 | 10 | 24 | 10 |
| N2 | 10 | 27 | 25 | 17 | 0 | 21 | 18 | 00 | 00 | 28 | 36 |
| N6 | 15 | 38 | 28 | 22 | 12 | 24 | 00 | 12 | 15 | 00 | 00 |
| N10 | 20 | 00 | 30 | 22 | 14 | 0 | 0 | 22 | 12 | 00 | 00 |
| N15 | 00 | 00 | 10 | 00 | 0 | 25 | 15 | 0 | 13 | 14 | 00 |
| N20 | 00 | 12 | 36 | 00 | 15 | 10 | 11 | 27 | 00 | 36 | 23 |
| N21 | 00 | 27 | 0 | 00 | 10 | 15 | 14 | 00 | 20 | 00 | 00 |
| N25 | 24 | 00 | 20 | 35 | 25 | 30 | 11 | 13 | 15 | 24 | 03 |
| N31 | 30 | 25 | 25 | 00 | 30 | 15 | 00 | 00 | 10 | 15 | 10 |
| N34 | 14 | 17 | 15 | 25 | 14 | 00 | 20 | 11 | 18 | 22 | 15 |
| N39 | 00 | 4 | 35 | 00 | 10 | 10 | 15 | 13 | 15 | 10 | 00 |
| N50 | 26 | 00 | 12 | 10 | 13 | 00 | 22 | 15 | 15 | 20 | 12 |

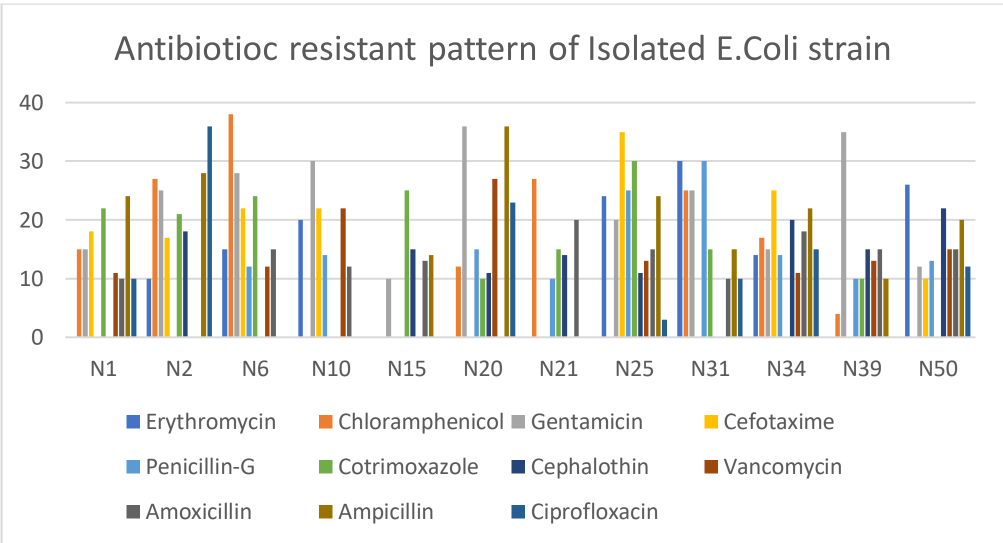



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**Fig: 2 – No of Coliforms /100 ml of collected water samples< 9 coliforms**

| Sample no | Erythromycin | Chloramphenicol | Gentamicin | Cefotaxime | Penicillin-G | Cotrimoxazole | Cephalothin | Vancomycin | Amoxicillin | Ampicillin | Ciprofloxacin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N1 | R | S | S | S | R | S | S | S | S | S | S |
| N2 | S | S | S | S | R | S | S | R | R | S | S |
| N6 | S | S | S | S | S | S | S | R | S | R | R |
| N10 | S | R | S | S | S | R | R | S | S | R | R |
| N15 | R | R | S | R | R | S | S | R | S | S | R |
| N20 | R | S | S | R | S | S | S | S | R | S | S |
| N21 | R | S | S | R | S | S | S | S | S | S | S |
| N25 | S | R | S | S | S | S | S | S | S | S | S |
| N31 | S | S | S | R | S | S | S | R | R | S | S |
| N34 | S | S | S | S | S | R | S | S | S | S | S |
| N39 | R | S | S | R | S | S | S | S | S | S | R |
| N50 | S | R | S | S | S | R | S | S | S | S | S |

**Table no 7- Antibiotic susceptibility pattern S= Sensitive R= Resistant**

In a present study isolate N1 is resistant to Erythromycin, penicillin-G and there is no zone found. Isolate N1 is sensitive to Chloramphenicol and Gentamicin measure 15 mm zone, Cefotaxime measure 18mm zone, Cotrimoxazole measure 22 mm zone, Vancomycin measure 11 mm zone, Amoxicillin measure 10 mm zone, Ampicillin measure 24 mm zone, Ciprofloxacin measure 10 mm zone.Isolate N21 is resistant to Erythromycin, Gentamicin, Cefotaxime, Vancomycin, Ampicillin, Ciprofloxacin and there is no zone found. Isolate N21 is sensitive to Chloramphenicol 27 measure mm zone, Penicillin-G measures 10 mm zone, Cotrimoxazole measure 15 mm zone, Cephalothin measure 14 mm zone and also sensitive to Amoxicillin measures 20 mm zone.

Isolate N31 is resistant to Cefotaxime, Cephalothin, Vancomycin and there is no zone found. Isolate N31 is sensitive to Erythromycin measure 30 mm zone, Chloramphenicol measures 25mm zone, Gentamicin measure 25 mm zone, Cotrimoxazole measure 15 mm zone, Amoxicillin measure 10 mm zone, Ampicillin measure 15 mm zone and also sensitive to Ciprofloxacin measure 10 mm zone.

Analysis of antibiotic sensitivity and resistance pattern showed that out of 12 isolates,Isolated E. coli was showed maximum resistance to Gentamicin followedby Cefotaxime, Cotrimoxazole, Chloramphenicol, Ampicillin, Erythromycin, Amoxicillin, Cefotaxime, Vancomycin, Penicillin-G, Cephalothin, Ciprofloxacin. Thus finding recommended that Gentamicin, chloramphenicol, Cotrimoxazole are the best choice of drugs against *E.coli* infection.

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**Photo Plate I**

Fig. 1: Sources of Drinking Water

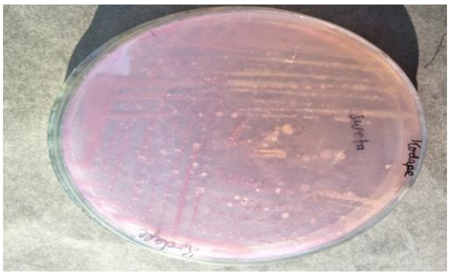

Fig:2 : Culture of *E.Coli* on Selective Media

Fig: 3 Biochemical Characterization of isolates

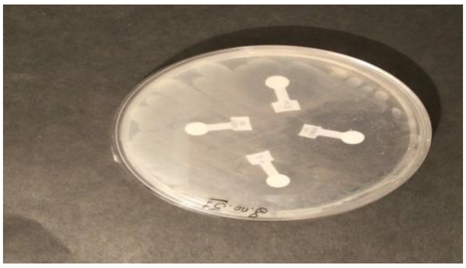

Fig:4 Antibiotic Susceptibility Test

**Conclusion:**

It can concluded from the study that antibiotic resistant *E.coli* is common in school's drinking water source in Katol and surrounding area. Ciprofloxacin is the least effective antibiotic followed by Cephalothin while Gentamicin is the most effective antibiotic. The E. coli isolates also varied patterns to commonly antibiotics used. Major source of water was well water for most of the schools. Attention should be given to proper handling of the water. On conclusion it is clear that E. coli appears to be the best indicator of bacteriological quality of water. So water authorities should have steps to control coliforms in drinking water to prevent from water borne disease. Water, especially water from a private water source like a well, should be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate E. coli.

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**Published by : Sai College, Bhilai**

 ISBN: 978-81-957386-3-2

# CHAPTER -10
# MICROBIAL BIOTECHNOLOGY: HARNESSING MICROBES FOR SUSTAINABLE RESOURCE UTILIZATION

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**Abstract**

Microbial biotechnology stands at the forefront of scientific innovation, offering transformative solutions to address pressing challenges in sustainability and resource utilization. This interdisciplinary field leverages the metabolic diversity and adaptability of microorganisms to develop sustainable processes for energy production, environmental remediation, agriculture, and healthcare. By harnessing the inherent capabilities of microbes, researchers can engineer novel biotechnological solutions to mitigate environmental pollution, enhance food security, and advance medical therapeutics. This abstract explores the multifaceted applications of microbial biotechnology, from biofuel production and bioremediation to the development of microbial-based products for agriculture and pharmaceuticals. Through a comprehensive examination of the principles, technologies, and real-world applications of microbial biotechnology, this abstract highlight its pivotal role in shaping a more sustainable future for humanity and the planet.

**Keywords:** Microorganisms,Sustainable Resource Utilization,Bioproduction,Bioremediation,Bio-based Materials,Sustainability

**Introduction:**

In the intricate tapestry of life on Earth, microorganisms occupy a fundamental niche, exerting profound influences on ecosystems, human health, and industrial processes. Over millennia, humanity has harnessed the power of microbes for various purposes, from fermenting food and producing antibiotics to wastewater treatment and biofuel generation. As the world grapples with pressing environmental challenges and seeks sustainable solutions for resource utilization, microbial biotechnology emerges as a pivotal frontier, offering innovative pathways to address these complex issues.

Microbial biotechnology encompasses a diverse array of techniques and applications aimed at leveraging the metabolic capabilities of microorganisms to enhance resource utilization sustainably. Through genetic engineering, metabolic engineering, and synthetic biology, scientists manipulate microbial pathways to optimize production processes, mitigate environmental pollutants, and develop novel bio-based materials. This interdisciplinary field draws upon insights from microbiology, genetics, biochemistry, and engineering to design tailored microbial systems that offer unprecedented versatility and efficiency.

 ISBN: 978-81-957386-3-2

At its core, microbial biotechnology embodies the ethos of sustainability, seeking to minimize environmental impact while maximizing resource efficiency. By harnessing the inherent metabolic diversity of microorganisms, researchers unlock innovative solutions to longstanding challenges, ranging from waste valorization and renewable energy production to bioremediation and pharmaceutical development. Moreover, the scalability and adaptability of microbial systems render them invaluable tools for addressing the urgent imperatives of a rapidly changing world.

In this chapter, we embark on a journey through the realms of microbial biotechnology, exploring the remarkable potential of microbes as agents of sustainable resource utilization. We delve into the principles underpinning microbial metabolism, examine cutting-edge technologies driving innovation in the field, and survey diverse applications spanning agriculture, industry, and environmental remediation. Through a multidisciplinary lens, we illuminate the transformative impact of microbial biotechnology on global sustainability efforts, underscoring its pivotal role in shaping a more resilient and harmonious relationship between humanity and the biosphere.

**Fundamentals of microbial metabolism:**

Microorganisms exhibit an extraordinary array of metabolic pathways, allowing them to thrive in diverse environments and fulfill critical ecological roles. This metabolic diversity encompasses a spectrum of biochemical processes that enable microorganisms to obtain energy, synthesize essential biomolecules, and interact with their surroundings. Understanding the metabolic diversity of microorganisms is fundamental to elucidating their ecological significance, harnessing their biotechnological potential, and addressing challenges in fields ranging from environmental science to medicine.

The metabolic diversity of microorganisms represents a vast reservoir of biochemical innovation with profound implications for both natural ecosystems and human endeavors. From driving global nutrient cycles to serving as sources of biotechnological innovation, microorganisms continue to inspire awe and fascination with their metabolic prowess. Continued exploration of microbial metabolism promises to unlock new frontiers in environmental sustainability, human health, and industrial innovation.

**Metabolic Diversity of Microorganism:**

Microorganisms exhibit remarkable metabolic diversity, allowing them to thrive in diverse environments and fulfill critical ecological roles. This diversity encompasses a spectrum of biochemical pathways that enable microorganisms to obtain energy, synthesize biomolecules, and interact with their surroundings. Understanding the metabolic diversity of microorganisms is crucial for elucidating their ecological significance, harnessing their biotechnological potential, and addressing challenges in fields such as environmental science and medicine.

**2.1.1 Overview of Microbial Metabolism:** Microbial metabolism involves a complex network of biochemical reactions that occur within cells to sustain life processes. This includes processes such as energy generation, carbon assimilation, and biosynthesis of essential


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ISBN: 978-81-957386-3-2

molecules. Key metabolic pathways include glycolysis, the Krebs cycle, and the electron transport chain, which are fundamental to cellular respiration and energy production (Madigan et al., 2014).

**2.1.2 Energy Generation Pathways:** Microorganisms utilize various energy sources to drive metabolic processes. Photosynthetic microorganisms harness light energy to produce organic molecules through photosynthesis, while chemotrophic microorganisms derive energy from chemical compounds through processes such as oxidative phosphorylation and fermentation (Nielsen &Keasling, 2016).

**2.1.3 Carbon Assimilation Strategies:** Microorganisms employ diverse strategies for carbon assimilation, depending on their nutritional requirements and environmental conditions. Autotrophic microorganisms fix carbon dioxide to synthesize organic compounds using energy from light or chemical reactions, whereas heterotrophic microorganisms utilize organic carbon sources for growth and metabolism (Woolston et al., 2018).

**2.1.4 Adaptations to Environmental Niches:** Microorganisms have evolved specialized metabolic pathways to thrive in diverse environmental niches, including extreme environments such as hot springs, acidic soils, and deep-sea hydrothermal vents. These adaptations allow microorganisms to exploit unique energy sources and withstand harsh conditions (Pandey et al., 2000).

**2.1.5 Metabolic Interactions and Community Dynamics:** Microorganisms often interact with each other within complex microbial communities, forming symbiotic relationships that influence metabolic processes and ecosystem dynamics. Cross-feeding, syntrophy, and quorum sensing are examples of metabolic interactions that regulate community structure and function (Hug et al., 2016).

**2.2. Metabolic pathways**:

Metabolic pathways are intricate networks of biochemical reactions within cells that are crucial for energy production, synthesis of biomolecules, and maintenance of cellular functions. Among the most fundamental metabolic pathways are glycolysis, the Krebs cycle (also known as the citric acid cycle or tricarboxylic acid cycle), and the electron transport chain. Together, these pathways play a central role in cellular respiration, the process by which cells extract energy from nutrients and convert it into a form usable for cellular activities.

***2.2.1*** **Glycolysis:** Glycolysis is the initial step in the breakdown of glucose, a common sugar molecule, to produce energy in the form of ATP (adenosine triphosphate) and NADH (nicotinamide adenine dinucleotide). This pathway occurs in the cytoplasm of cells and consists of a series of enzymatic reactions that convert glucose into pyruvate. Key steps in glycolysis include phosphorylation of glucose, cleavage into two molecules of glyceraldehyde-3-phosphate, oxidation and phosphorylation to form ATP and NADH, and ultimately, the production of pyruvate. Glycolysis is anaerobic, meaning it does not require oxygen, and serves as the primary energy-generating pathway in conditions of low oxygen availability.

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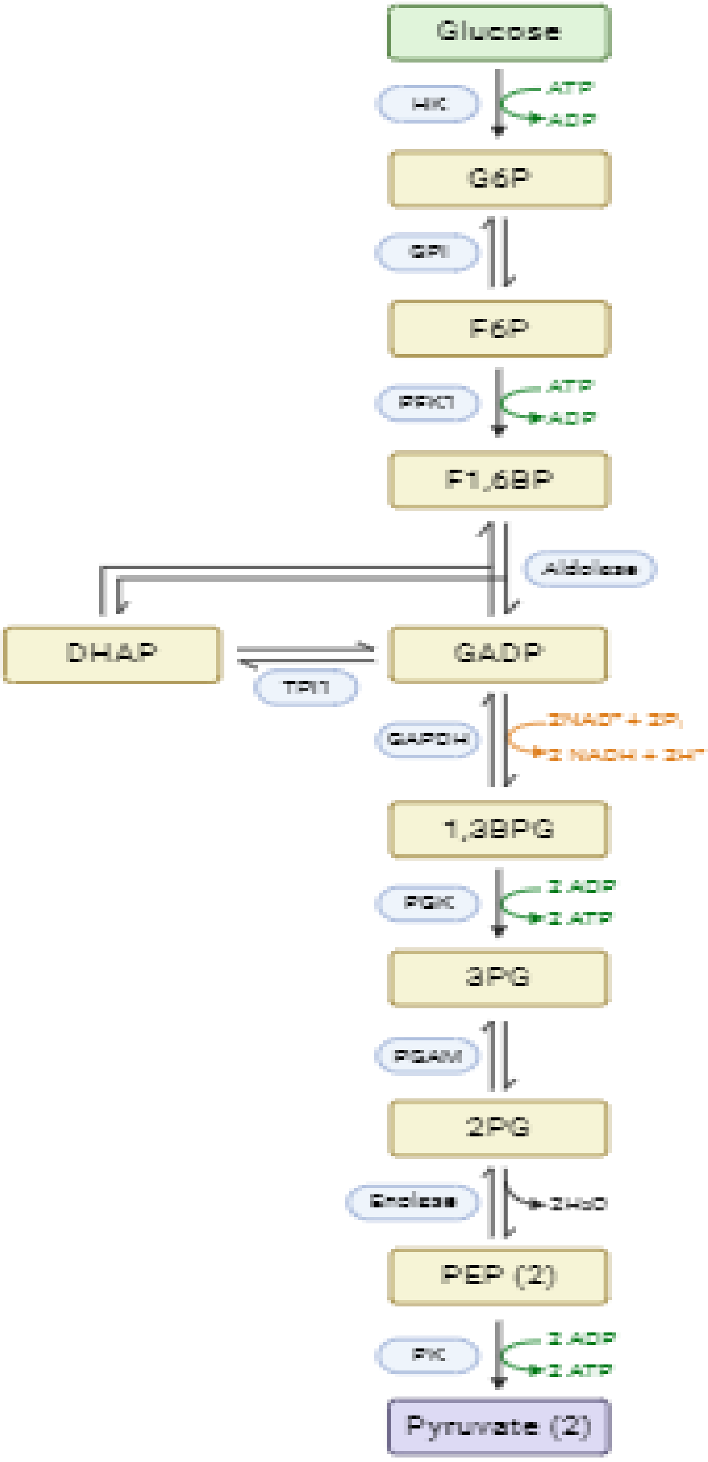


***2.2.2*** **Krebs Cycle:** The Krebs cycle is a central metabolic pathway that occurs in the mitochondria of eukaryotic cells and the cytoplasm of prokaryotic cells. It serves as the hub of cellular respiration, linking glycolysis and the electron transport chain. The cycle begins with the conversion of pyruvate, derived from glycolysis, into acetyl-CoA, which enters the cycle. Acetyl-CoA undergoes a series of enzymatic reactions, releasing carbon dioxide and generating high-energy electron carriers NADH and FADH2. These electron carriers carry

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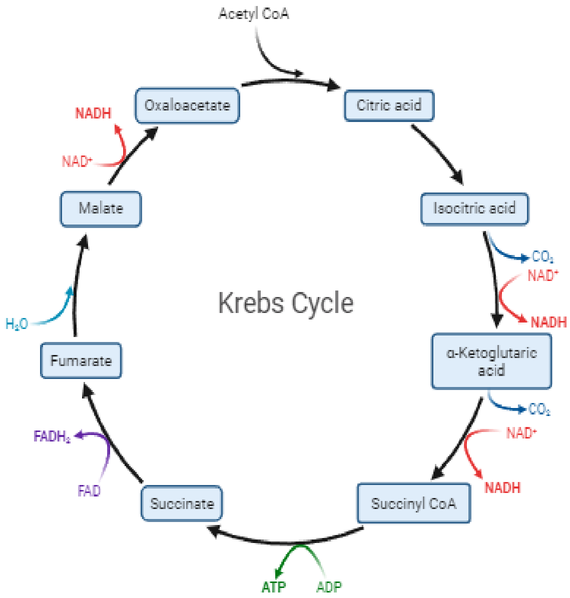
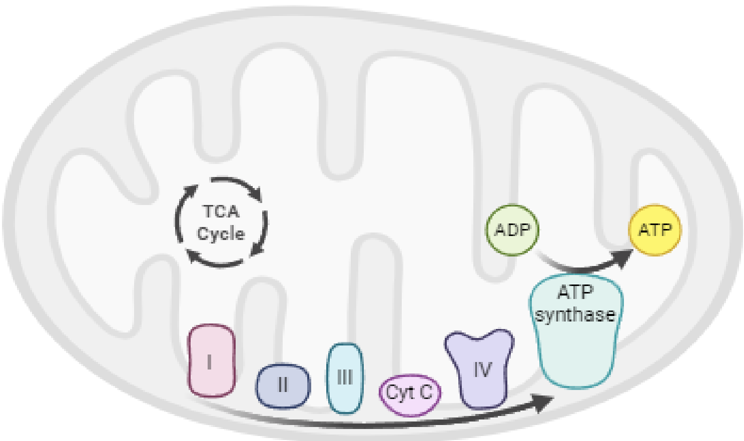
electrons to the electron transport chain for ATP production. The Krebs cycle also generates ATP directly through substrate-level phosphorylation. Overall, the Krebs cycle completes the oxidation of glucose, yielding carbon dioxide, ATP, and electron carriers for the electron transport chain.




***2.2.3*** **Electron Transport Chain:** The electron transport chain is a series of protein complexes located in the inner mitochondrial membrane in eukaryotic cells and the plasma membrane in prokaryotic cells. It is the final stage of cellular respiration and is responsible for the majority of ATP production through oxidative phosphorylation. The electron transport chain accepts electrons from NADH and FADH2, which were generated by glycolysis and the Krebs cycle, and transfers them through a series of redox reactions. As electrons pass through the protein complexes, protons are pumped across the membrane, creating an electrochemical gradient. This proton gradient drives ATP synthesis by ATP synthase, a process known aschemiosmosis.

In aerobic respiration, oxygen acts as the ultimate electron acceptor in the electron transport chain, where it binds with protons to generate water. This pivotal role underscores the indispensability of oxygen in this metabolic process.



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In summary, glycolysis, the Krebs cycle, and the electron transport chain are interconnected metabolic pathways that play critical roles in cellular energy production and metabolism. Together, they enable cells to efficiently extract energy from nutrients, regulate cellular processes, and maintain homeostasis. Understanding the intricacies of these pathways is essential for unraveling the complexities of cellular physiology and metabolism.

## 3 Microbial Biotechnology in Agriculture:

Microbial biotechnology has emerged as a promising approach to address challenges in modern agriculture, offering innovative solutions for enhancing crop productivity, soil fertility, and pest management. By harnessing the metabolic capabilities of microorganisms, researchers have developed novel strategies to promote sustainable agricultural practices while reducing reliance on synthetic inputs. This detailed note explores the diverse applications of microbial biotechnology in agriculture, highlighting its potential to revolutionize farming systems and contribute to global food security.

### 3.1. Plant-Microbe Interactions:

Microorganisms play essential roles in promoting plant growth and health through symbiotic interactions. Beneficial microbes, such as rhizobia, mycorrhizal fungi, and plant growth-promoting bacteria (PGPB), form associations with plant roots, facilitating nutrient uptake, disease resistance, and stress tolerance (Berendsen et al., 2012). Understanding the dynamics of these interactions is key to harnessing microbial biotechnology for agricultural benefits.

### 3.2. Biofertilizers and Bio stimulants:

Microbial inoculants, including biofertilizers and bio stimulants, are microbial-based products applied to enhance soil fertility and plant growth. Biofertilizers contain beneficial microorganisms capable of fixing atmospheric nitrogen, solubilizing phosphorus, or enhancing nutrient availability to plants (Bashan et al., 2014). Biostimulants, on the other hand, consist of microbial metabolites or microbial-derived compounds that stimulate plant growth and improve stress tolerance (Du Jardin, 2015). These microbial products offer sustainable alternatives to chemical fertilizers and pesticides, promoting soil health and reducing environmental impacts.

### 3.3. Biological Control of Plant Diseases:

Microbial biotechnology has revolutionized biological control strategies for managing plant diseases. Biocontrol agents, such as fungi, bacteria, and viruses, are used to suppress phytopathogens and protect crops from infections (Haidar et al., 2016). For example, certain strains of Bacillus and Pseudomonas species produce antimicrobial compounds that inhibit the growth of plant pathogens, while mycoparasitic fungi parasitize and kill fungal pathogens (Lorito et al., 2010). Harnessing the antagonistic activities of beneficial microbes offers sustainable and environmentally friendly alternatives to chemical pesticides.


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**3.4. Microbial Amendments for Soil Health:**

Microbial inoculants and organic amendments are utilized to improve soil health and fertility in agricultural systems. Soil probiotics, such as compost teas and microbial consortia, introduce beneficial microorganisms into the soil, enhancing nutrient cycling, organic matter decomposition, and soil structure (Bonanomi et al., 2018). Additionally, biochar, a carbon-rich material produced from organic waste, serves as a substrate for microbial colonization and promotes soil microbial diversity and activity (Lehmann et al., 2011). These microbial amendments contribute to soil sustainability, mitigating soil degradation and erosion while enhancing crop productivity.

## 4 Industrial Applications of Microbial Biotechnology:

Microbial biotechnology has revolutionized industrial processes, offering sustainable and cost-effective solutions for the production of various compounds, chemicals, and materials. By harnessing the metabolic diversity and biochemical capabilities of microorganisms, industrial biotechnology has transformed sectors such as pharmaceuticals, chemicals, energy, and environmental remediation. This detailed note explores the diverse applications of microbial biotechnology in industrial settings, highlighting its significance in advancing manufacturing processes, reducing environmental impact, and driving innovation.

**4.1. Bioproduction of Industrial Chemicals:** Microbial biotechnology is widely utilized in the production of industrial chemicals, including organic acids, amino acids, enzymes, and specialty chemicals. Microorganisms such as bacteria, yeast, and fungi are genetically engineered or selected for their ability to produce specific compounds through fermentation or enzymatic processes (Chen & Nielsen, 2013). Examples include the production of lactic acid, citric acid, and ethanol for use in food, pharmaceuticals, and biofuels.

**4.2. Bioremediation and Waste Treatment:** Microbial biotechnology plays a crucial role in environmental remediation and waste treatment processes. Microorganisms capable of degrading pollutants, such as hydrocarbons, pesticides, and heavy metals, are employed to clean up contaminated sites and wastewater (Hassanshahian et al., 2014). Bioremediation technologies leverage microbial metabolism to transform hazardous substances into non-toxic or less harmful byproducts, contributing to environmental sustainability.

**4.3. Biopharmaceutical Production:** The pharmaceutical industry relies on microbial biotechnology for the production of biopharmaceuticals, including vaccines, antibodies, hormones, and enzymes. Microbial hosts such as Escherichia coli, Bacillus subtilis, and yeast are engineered to express recombinant proteins with therapeutic properties (Walsh, 2018). Microbial fermentation processes enable large-scale production of biologics, offering advantages in cost-effectiveness, scalability, and product quality.

**4.4. Biofuel and Renewable Energy:** Microbial biotechnology holds promise for the development of biofuels and renewable energy sources as alternatives to fossil fuels. Microorganisms such as algae, bacteria, and yeast are engineered to produce biofuels such as ethanol, biodiesel, and hydrogen through fermentation or photosynthesis (Peralta-Yahya et al., 2012). These biofuels offer

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sustainable and carbon-neutral alternatives for transportation and energy production, contributing to efforts to mitigate climate change.

**4.5. Industrial Enzymes and Biocatalysis:** Microbial enzymes are widely used as biocatalysts in industrial processes for the production of chemicals, pharmaceuticals, food, and textiles. Enzymes such as proteases, amylases, and lipases are employed to catalyze specific chemical reactions, increasing process efficiency and reducing energy consumption (Adrio & Demain, 2014). Microbial biocatalysis offers advantages in terms of substrate specificity, reaction selectivity, and environmental compatibility.

## 5 Microbial Biotechnology in Healthcare and Pharmaceuticals:

Microbial biotechnology plays a pivotal role in healthcare and pharmaceutical industries, offering innovative solutions for drug discovery, production of biopharmaceuticals, and development of novel therapies. By harnessing the metabolic capabilities and genetic engineering techniques, microorganisms have become invaluable tools for addressing medical challenges and improving human health. This detailed note explores the diverse applications of microbial biotechnology in healthcare and pharmaceuticals, highlighting its significance in drug development, disease treatment, and biomanufacturing.

### 5.1. Antibiotic Discovery and Development:

Microbial biotechnology has been instrumental in the discovery and development of antibiotics, which are essential for treating bacterial infections. Microorganisms such as bacteria and fungi produce a wide array of bioactive compounds, including antibiotics, antimicrobial peptides, and secondary metabolites, which exhibit potent antimicrobial properties (Demain & Sanchez, 2009). Through screening of microbial natural products and genetic engineering of microbial hosts, novel antibiotics with improved efficacy and specificity can be developed to combat antibiotic-resistant pathogens.

### 5.2. Microbial Synthesis of Therapeutic Compounds:

Microorganisms are utilized for the production of various therapeutic compounds, including antibiotics, vaccines, hormones, and enzymes. Microbial fermentation processes enable large-scale production of recombinant proteins and small molecules with therapeutic applications (Demain & Fang, 2001). Microbial hosts such as Escherichia coli, yeast, and filamentous fungi are engineered to express recombinant proteins with pharmaceutical properties, offering cost-effective and scalable production platforms for biopharmaceuticals.

### 5.3. Probiotics and Microbiome-based Therapies:

Microbial biotechnology has led to the development of probiotics and microbiome-based therapies for maintaining gut health and treating gastrointestinal disorders. Probiotics are live microorganisms that confer health benefits when administered in adequate amounts (Hill et al., 2014). These beneficial bacteria, such as Lactobacillus and Bifidobacterium species, help restore microbial balance in the gut, enhance immune function, and alleviate symptoms of digestive disorders. Microbiome-based therapies involve manipulation of the gut microbiota composition to promote health and treat diseases such as inflammatory bowel disease and obesity.

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5.4. **Drug Delivery Systems:**

Microbial biotechnology is utilized in the development of drug delivery systems for targeted delivery of therapeutic agents to specific tissues or cells. Microbial vectors such as attenuated bacteria and viruses are engineered to deliver therapeutic genes or drug payloads to target sites within the body (Forbes, 2010). These microbial carriers offer advantages such as high specificity, low immunogenicity, and sustained release of therapeutic compounds, enhancing the efficacy and safety of drug delivery systems.

5.5. **Genome Editing and Gene Therapy:**

Advancements in microbial biotechnology have facilitated the development of genome editing tools for gene therapy and precision medicine. Microbial-based systems such as CRISPR-Cas9 enable precise modification of genes within microbial hosts or mammalian cells, offering potential treatments for genetic disorders, cancer, and infectious diseases (Doudna & Charpentier, 2014). Genome editing technologies hold promise for personalized medicine and targeted therapies, revolutionizing the treatment of various medical conditions.

## 6. Future Perspectives and Challenges

As microbial biotechnology continues to evolve, it holds immense promise for addressing global challenges related to sustainability and resource utilization. However, several future perspectives and challenges must be considered to fully realize the potential of microbial biotechnology in promoting sustainable practices and mitigating environmental impacts.

6.1. **Enhanced Metabolic Engineering:** Future advancements in metabolic engineering techniques will enable the development of more efficient microbial strains capable of producing valuable compounds with higher yields and purity. Engineering microbial pathways for improved substrate utilization, metabolic flux, and product specificity will be crucial for optimizing bioproduction processes and enhancing resource efficiency.

6.2. **Integration of Omics Technologies:** The integration of omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, will provide deeper insights into microbial metabolism and function. Systems biology approaches will facilitate the comprehensive characterization of microbial systems, enabling the design of tailored solutions for sustainable resource utilization and environmental remediation.

6.3. **Microbial Consortia Engineering:**

Harnessing the synergistic interactions between microorganisms within microbial consortia offers novel opportunities for enhancing bioproduction processes and ecosystem services. Engineering microbial consortia with complementary metabolic capabilities can improve substrate utilization efficiency, stability, and resilience to environmental fluctuations, enabling sustainable resource utilization in diverse contexts.

6.4. **Sustainable Bioprocessing:**

The development of sustainable bioprocessing technologies will be essential for minimizing environmental impact and resource consumption in microbial biotechnology applications. Integration of renewable feedstocks, green solvents, and energy-efficient processes will reduce


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the carbon footprint and enhance the sustainability of bioproduction processes across various industries.

6.5. **Addressing Regulatory and Ethical Considerations:**
As microbial biotechnology advances, regulatory frameworks and ethical considerations must evolve to ensure the safe and responsible deployment of biotechnological solutions. Comprehensive risk assessments, biosafety measures, and ethical guidelines will be essential for addressing potential environmental, health, and social implications of microbial biotechnology applications.

6.6. **Overcoming Technological and Economic Barriers:** Despite the tremendous potential of microbial biotechnology, technological and economic barriers remain significant challenges to widespread adoption and commercialization. Research efforts focused on reducing production costs, improving scalability, and developing robust bioprocessing platforms will be essential for realizing the economic viability of microbial biotechnology applications.

6.7. **Promoting Public Awareness and Engagement:** Public awareness and acceptance of microbial biotechnology will be critical for fostering trust and engagement among stakeholders, including policymakers, industry leaders, and the general public. Education, outreach, and transparent communication about the benefits, risks, and ethical considerations of microbial biotechnology will be essential for building societal support and facilitating responsible innovation.

## 7. Case Studies in Microbial Biotechnology:

Case studies in microbial biotechnology provide valuable insights into the diverse applications of microorganisms in various fields, including agriculture, healthcare, environmental remediation, and industrial bioprocessing. By examining real-world examples, researchers and practitioners can gain a deeper understanding of the challenges, opportunities, and impact of microbial biotechnology on society and the environment. This detailed note presents a selection of case studies that highlight the innovative use of microorganisms to address pressing challenges and advance scientific knowledge.

7.1. **Bioremediation of Oil Spills:** The Deepwater Horizon oil spill in the Gulf of Mexico in 2010 prompted a massive cleanup effort using microbial bioremediation techniques. Microorganisms, particularly hydrocarbon-degrading bacteria and archaea, played a crucial role in breaking down crude oil components and reducing environmental damage (Head et al., 2006). Case studies of microbial communities in oil-contaminated environments have provided insights into the mechanisms of hydrocarbon degradation and the factors influencing microbial activity, informing future strategies for oil spill response and environmental management.

7.2. **Production of Biofuels from Lignocellulosic Biomass:** The conversion of lignocellulosic biomass into biofuels such as ethanol represents a promising avenue for sustainable energy production. Microbial biotechnology plays a key role in this process, with microorganisms capable of fermenting sugars derived from biomass feedstocks. Case studies have demonstrated the feasibility of using engineered microorganisms, such as yeast and bacteria, to produce biofuels from lignocellulosic materials, highlighting the challenges of optimizing fermentation conditions and metabolic pathways (Himmel et al., 2007). These studies provide valuable insights into improving the efficiency and scalability of biofuel production processes.

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7.3. **Development of Novel Antibiotics:** The rise of antibiotic-resistant bacteria has spurred efforts to discover and develop novel antibiotics to combat infectious diseases. Microbial biotechnology offers promising avenues for antibiotic discovery through the screening of microbial natural products and the engineering of biosynthetic pathways. Case studies of antibiotic-producing microorganisms, such as Streptomyces species, have led to the identification of new bioactive compounds with therapeutic potential (Demain & Sanchez, 2009). These studies highlight the importance of biodiversity conservation and genetic manipulation in antibiotic discovery efforts.

7.4. **Bioproduction of Industrial Enzymes:** Industrial enzymes produced by microorganisms have diverse applications in biocatalysis, food processing, detergent manufacturing, and bioremediation. Case studies have demonstrated the use of microbial biotechnology to engineer enzyme-producing microorganisms for enhanced production of industrial enzymes. By optimizing fermentation conditions, metabolic pathways, and genetic expression systems, researchers have successfully scaled up the production of enzymes such as proteases, amylases, and lipases for industrial applications (Adrio & Demain, 2014). These studies showcase the potential of microbial biotechnology to drive innovation and efficiency in enzyme production processes.

7.5. **Microbial Synthesis of Pharmaceuticals:** Microorganisms are used to produce a wide range of pharmaceutical compounds, including antibiotics, anticancer agents, vaccines, and biologics. Case studies have highlighted the use of microbial biotechnology in the production of recombinant proteins and small molecules for pharmaceutical applications. By engineering microbial hosts and optimizing fermentation processes, researchers have achieved high yields and purity of pharmaceutical products, paving the way for cost-effective and scalable production methods (Walsh, 2018). These studies underscore the importance of microbial biotechnology in advancing drug discovery and biomanufacturing technologies.

## 8. Conclusion:

Microbial biotechnology holds immense potential to shape a sustainable future by addressing pressing environmental, social, and economic challenges. As we look ahead, the role of microbial biotechnology in promoting sustainability becomes increasingly critical, offering innovative solutions across various sectors. In conclusion, microbial biotechnology holds immense potential for sustainable resource utilization and environmental stewardship. However, addressing future perspectives and challenges will require interdisciplinary collaboration, technological innovation, and proactive engagement with stakeholders to harness the full transformative power of microbial biotechnology in shaping a more sustainable and resilient future.

Here are some areas where microbial biotechnology can contribute to a sustainable future:

8.1. **Sustainable Agriculture:** Microbial biotechnology can revolutionize agriculture by enhancing soil fertility, improving crop yields, and reducing reliance on chemical inputs. Microbial inoculants, biofertilizers, and biostimulants containing beneficial microorganisms can promote plant growth, nutrient uptake, and stress tolerance, contributing to sustainable agricultural practices (Berendsen et al., 2012). Additionally, microbial-based biocontrol agents

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offer eco-friendly alternatives for managing pests and diseases, minimizing environmental impacts and preserving ecosystem health.

8.2. **Environmental Remediation:** Microbial biotechnology plays a crucial role in environmental remediation efforts, offering cost-effective and sustainable solutions for cleaning up contaminated sites and mitigating pollution. Microorganisms capable of degrading pollutants, such as hydrocarbons, heavy metals, and pesticides, can be harnessed for bioremediation applications (Hassanshahian et al., 2014). Engineered microbial consortia and bioreactor systems can enhance the efficiency of pollutant degradation processes, facilitating the restoration of ecosystems and safeguarding human health.

8.3. **Renewable Energy Production:** Microbial biotechnology holds promise for advancing renewable energy production through biofuel generation, microbial fuel cells, and biogas production. Microorganisms such as algae, bacteria, and fungi can convert organic matter into biofuels such as ethanol, biodiesel, and hydrogen through fermentation or photosynthesis (Peralta-Yahya et al., 2012). Harnessing microbial metabolism for bioenergy production offers sustainable alternatives to fossil fuels, reducing greenhouse gas emissions and mitigating climate change.

8.4. **Circular Economy and Waste Valorization:** Microbial biotechnology can support the transition to a circular economy by valorizing waste streams and converting them into valuable products. Microbial fermentation processes can transform organic waste materials, agricultural residues, and wastewater into bio-based chemicals, biopolymers, and biofuels (Carpenter et al., 2018). By closing the loop on waste streams and promoting resource recovery, microbial biotechnology contributes to resource efficiency, waste reduction, and environmental sustainability.

8.5. **Healthcare and Biopharmaceuticals:** Microbial biotechnology plays a vital role in healthcare and biopharmaceutical industries, offering innovative therapies, vaccines, and diagnostics. Engineered microorganisms serve as production hosts for recombinant proteins, antibodies, and therapeutic compounds, enabling scalable and cost-effective manufacturing of biologics (Walsh, 2018). Microbial-based probiotics and microbiome-based therapies also hold promise for promoting human health, preventing diseases, and enhancing well-being.

Looking ahead, the continued advancement of microbial biotechnology will require interdisciplinary collaboration, technological innovation, and policy support to realize its full potential in promoting sustainability. By harnessing the power of microorganisms, we can create a more resilient, equitable, and sustainable future for generations to come.

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ISBN: 978-81-957386-3-2

# CHAPTER- 11

# EXPLORING HEAVY METAL RESILIENCE IN FRESHWATER CYANOBACTERIA: INSIGHTS FROM EXPERIMENTAL INVESTIGATIONS

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**Abstract**

Autotrophic microorganisms inhabiting water bodies demonstrate remarkable capability to proliferate under optimal conditions, yielding substantial biomass with minimal effort. These microorganisms are recognized for their significant role in absorbing heavy metals from contaminated water sources in which they thrive. While essential for the proper functioning of living organisms, metals such as Cu, As, Pb, and Hg can induce severe toxic effects when present in excess or deficiency, depending on the organism, metal type, concentration, and environmental factors. This study designed to examine the impact of heavy metals, including Cu, As, Pb, and Hg, on cyanobacterial species, considering their essential roles in the correct functioning of living organisms, but also acknowledging that excess or deficiency of these metals can lead to severe toxic effects. In this investigation, two Cyanobacterial isolates Synchocystis sp. and Oscillatoria sp. were cultivated using diverse growth media, both nitrogen plus and nitrogen free, and evaluated for their heavy metal tolerance under experimental conditions employing the dilution method across different concentrations (5, 10, 15ppm) and for different incubation periods. The outcomes were assessed in terms of OD550 and dry biomass, considering the variability in responses dependent on the organism, metal type, concentration, and environmental conditions. These findings suggest that both Synchocystis sp. and Oscillatoria sp. could potentially be utilized for environmental remediation purposes

**Key Words**: Heavy Metals, Toxicity, Tolerance, Cyanobacteria, Growth

## Introduction:

Cyanobacteria are the major group of photosynthetic prokaryotes. Their habitats vary from fresh and marine water to terrestrial environment. Heavy metals have been released to the environment over extensive periods of time by natural procedureand by anthropogenicactions generate their anomalous concentration. These metals are unswervingly or circuitouslycaught up inthe entire phases of microbial growth and enter in to higher levels of food chains. All these metals can be lethal to biotic components and in a fewcases the valuabletoenormously narrowranges.

Many cyanobacterial sp. are reported notable affinity for heavy metals (Gale and Wixson, 1979 andAudholia*et al.* 1993),these cells have developed natural methods of responding to metals such as copper, lead, cadmium, arsenic and mercury through passive accumulation in cells and through surface binding to a range of functional groups.They have also been found to eliminateharmful metals from the environment.

 ISBN: 978-81-957386-3-2

In the present study, two abundantly growing indigenous Cyanobacteria species were studied under experimental conditions to obtain large biomass and their comparative tolerance for some heavy metals.

**Material and methods:**

**Isolation and Selection of best Growth medium** – Two strainsfrom different water bodies of Durg and Rajnandgaon area (Chhattisgarh), viz. *Synchocystis sp*. and *Oscillatoriasp*was found to have higher ability to grow in mass quantity under culture conditions. Monocultures of *Synchocystis sp*. and *Oscillatoria sp*. were developed by isolationand identified by the monographs provided byDesikacharya (1959) and Presscott (1962).The isolates were grown on different growth medium such as Allen's and Arnon's broth, BG-11 broth, Chu 10 broth, Pringschim's broth (nitrogen plus and nitrogen free) axenically at 28±2°C temperature with continuous illumination of intensities of 2000 Lux. Growth were compared by $OD_{550}$ and recovery of dry biomass (Yoshida *et al*. 2005).

**Heavy metal tolerance study** – The monocultures of*Synchocystis sp*. and *Oscillatoria sp*. were screened for isolation of heavy metal tolerance under experimental conditions. Sterilized solution of different heavy metals Arsenic Sulphite (As), Cuprous chloride (Cu), Mercorous oxide (Hg), and Lead carbonate (Pb)at the rate of 5, 10, 15ppm individually, were aseptically supplemented with suitable solid culture medium. Tolerant colonies were selected and re-cultured for two more times and finally transferred to heavy metal containing broth. Developmentof both monocultures was evaluated by $OD_{550}$ and recovery of dry biomass and compared with control (Yoshida *et al*. 2005).

**Analysis of data** – Five replicates were maintained throughout the studies and data were analyzed by suitable statistical methods (Muthukumar*et al*. 2007).

**Result and discussion:**

The monocultures were grown on different growth medium individually and observedafter 30 days for growth in terms of $OD_{55O}$ and recovery of Dry biomass (Table -1 and 2).

**Table - 1 Selection of Best growth media**

| S.No. | Growth Media | ***Synchocystis sp.*** | | | | ***Oscillatoria sp.*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $Od_{500nm}$ | | Dry Weight (g) | | $OD_{500nm}$ | | Dry Weight (g) | |
| | | $+N_2$ | $-N_2$ | $+N_2$ | $-N_2$ | $+N_2$ | $-N_2$ | $+N_2$ | $-N_2$ |
| 1 | Allen's &Arnon's | 0.57 | 0.60 | 0.95 | 1.00 | 0.04 | 0.05 | 1.00 | 0.62 |
| 2 | BG-11 | 0.58 | 0.63 | 0.97 | 1.02 | 0.07 | 0.09 | 1.07 | 0.60 |
| 3 | Chu#10 | 0.00 | 0.18 | 0.00 | 1.05 | 0.09 | 0.10 | 0.64 | 0.80 |
| 4 | Pringhschim's | 0.00 | 0.64 | 0.00 | 1.01 | 0.06 | 0.12 | 0.59 | 0.85 |

BG 11(nitrogen free) media and Pringhschim's broth (nitrogen free) media were found aspreeminentmedia for *Synchocystis sp*. and *Oscillatoria sp*. respectively in terms of $OD_{55O}$ and Dry biomass.

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**Table -2 Growth studies of*Synchocystissp*. and *Oscillatoria sp.* in different incubation periods**

| S.No. | Incubation period (Days) | *Synchocystissp.* BG 11(-$N_2$) | | *Oscillatoriasp.* Pringhschimsbroth (-$N_2$) | |
|---|---|---|---|---|---|
| | | $OD_{550}$ | Dry weight* (g) | $OD_{550}$ | Dry weight* (g) |
| 1 | 5 | 0.60 | 1.02 | 0.11 | 0.85 |
| 2 | 10 | 0.62 | | 0.16 | |
| 3 | 15 | 0.63 | | *0.*19 | |

Note: *after 15 days of incubation

The effect of Arsenic Sulphide (Table - 3) was found more tolerable by*Oscillatoria sp*. as compared to *Synchocystis sp*. yet,both the organisms responded positive growth in terms of $OD_{550}$ andDry biomass up to 10 days of incubation and up to 15ppm concentration.

**Table - 3 Screening of *Synchocystissp*. and *Oscillatoria*sp for Arsenic tolerance.**

| S.No. | Concentra tion of Heavy Metal | *Synchocystissp.* BG 11(-$N_2$) + Arsenic sulphite | | | | *Oscillatoriasp.* Pringhschimsbroth (-$N_2$) +Arsenic sulphite | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $OD_{550}$ | | | Dry weight* (g) | $OD_{550}$ | | | Dry weight* (g) |
| | | I | II | III | | I | II | III | |
| 1 | 5 ppm | 0.967 | 0.810 | 0.372 | 1.06 | 1.137 | 0.979 | 0.391 | 1.10 |
| 2 | 10ppm | 1.081 | 0.796 | 0.298 | 1.02 | 1.161 | 0.887 | 0.406 | 1.05 |
| 3 | 15ppm | 1.017 | 0.791 | 0.243 | 1.00 | 1.155 | 0.819 | 0.360 | 1.01 |

Note: I, II, III – 5, 10, and 15 days of incubation
*after 15 days of incubation

The Mercurous Oxide also responded higher in terms of biomass (Table - 4) for both of the test cyanobacteria in 5ppm concentration. The higher concentration still, resulted in continuingdecline of growth up to 15ppm concentration and 15days of incubation respectively.

**Table – 4 Screening of *Synchocystissp*. and *Oscillatoria*sp for Mercurytolerance**

| S.No. | Concentra tion of Heavy Metal | *Synchocystissp.* BG 11(-$N_2$) + Mercorous oxide | | | | Oscillatoria sp. Pringhschims broth (-$N_2$) + Mercorous oxide | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $OD_{550}$ | | | Dry weight* (g) | $OD_{550}$ | | | Dry weight* (g) |
| | | I | II | III | | I | II | III | |
| 1 | 5 ppm | 1.060 | 0.959 | 0.569 | 1.26 | 0.783 | 0.796 | 1.00 | 1.14 |
| 2 | 10ppm | 0.987 | 0.689 | 0.345 | 0.93 | 0.658 | 0.599 | 0.321 | 1.11 |
| 3 | 15ppm | 0.991 | 0.639 | 0.301 | 1.03 | 0.688 | 0.639 | 0.317 | 1.08 |

 ISBN: 978-81-957386-3-2

Note: I, II, III – 5, 10, and 15 days of incubation
*after 15 days of incubation

Effect of Lead tolerance is depicted in Table - 5 reveals that Lead Carbonate affected well up to 10days of incubation in 5 and 10ppm concentration. There was a decline in growthin 10ppm concentration and 15days of incubation respectively.

**Table – 5 Screening of *Synchocystis sp.* and *Oscillatoria*sp for Leadtolerance**

| S.No. | Concentration of Heavy Metal | *Synchocystissp.* BG 11(-$N_2$) + Lead carbonate | | | | *Oscillatoria sp.* Pringhschims broth (-$N_2$) + Lead carbonate | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $OD_{550}$ | | | Dry weight* (g) | $OD_{550}$ | | | Dry weight* (g) |
| | | I | II | III | | I | II | III | |
| 1 | 5 ppm | 1.149 | 0.695 | 0.418 | 1.17 | 1.056 | 0.887 | 0.346 | 1.17 |
| 2 | 10ppm | 1.046 | 0.688 | 0.318 | 0.96 | 1.051 | 0.796 | 0.459 | 0.83 |
| 3 | 15ppm | 1.040 | 0.639 | 0.278 | 0.98 | 1.012 | 0.695 | 0.305 | 0.68 |

Note: I,II,III – 5,10, and 15 days of incubation
*after 15 days of incubation

Screening for copper tolerance (Table - 6) reveals that Cuprous Chloride is also exaggerated fine up to 10ppm for both of the test cyanobacterial sp. The higher concentration and larger incubation days gradually resulted in decrease in the growth as compared to control (Table - 2).
Amongst heavy metals Copper, Arsenic, Lead and Mercury are reported to necessitate for accurate performance of existing organisms although surplus or insufficiency of these metals can endorse rigorous toxic effects, which depends on the type of organism, the nature and concentration of the metal and environmental conditions. This study was focused on the effects of heavy metals on cyanobacterial species in terms of $OD_{550}$ and Dry biomass. Mann *et al.*, (2002)studied that the picometer concentrations Cu that occur in the oceans appear to be noxious to some cyanobacteria. Reported by Kowalewsla*et al.*, (1992)the favorable range of Cu concentration is extremely narrow, it's required as an dynamic centre in numerous proteins and enzymes, nevertheless it is still lethal to some cyanobacteria.Kupper and Kroneck also studied the heavy metal uptake by plants and cyanobacteria. In adsorption studies on heavy metals by Kumaran*et al.* the isolated cyanobacteria (*Nostoc sp.*) were accumulated more than 91% of the heavy metals from the estuarine water.

**Table – 6 Screening of *Synchocystissp*. and *Oscillatoria*sp for Copper tolerance**

| S.No. | Concentration of Heavy Metal | *Synchocystissp.* BG 11(-$N_2$) + Cuprous chloride | | *Oscillatoria sp.* Pringhschims broth (-$N_2$) + Cuprous chloride | |
|---|---|---|---|---|---|
| | | $OD_{550}$ | Dry weight* | $OD_{550}$ | Dry weight* |


Published by : Sai College, Bhilai

 ISBN: 978-81-957386-3-2

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | (g) | | | | (g) |
| | | I | II | III | | I | II | III | |
| 1 | 5 ppm | 1.086 | 0.689 | 0.441 | 1.01 | 1.187 | 0.887 | 0.389 | 1.14 |
| 2 | 10ppm | 1.086 | 0.639 | 0.359 | 1.03 | 1.161 | 0.796 | 0.393 | 1.09 |
| 3 | 15ppm | 0.987 | 0.599 | 0.276 | 1.04 | 0.724 | 0.639 | 0.165 | 1.13 |

Note: I, II, III – 5, 10, and 15 days of incubation
*after 15 days of incubation

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ISBN: 978-81-957386-3-2

# CHAPTER- 12
# MUSCULAR DYSTROPHY: A RARE GENETIC DISORDER

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**Abstract**

Muscular dystrophies are a diverse set of hereditary illnesses with common clinical characteristics and dystrophic abnormalities on muscle biopsy. It is a hereditary myogenic condition marked by increasing muscle degeneration and weakening with varying distribution and severity. The term "muscular dystrophy" refers to a group of genetic illnesses that cause progressive, widespread muscle disease due to insufficient or absent glycoproteins in the muscle cell plasma membrane. They can be classified into numerous categories, including congenital dystrophy, based on the distribution of predominant muscular dystrophy: Duchenne and Becker, Emery-Dreifuss, distal, facioscapulo-humeral, oculopharyngeal, and limb-girdle. These dystrophies can cause substantial cardiac problems, even in the absence of clinically noticeable weakening. Knowledge of disease-specific consequences, the deployment of anticipatory treatment, and medical discoveries have altered the standard of care, resulting in an overall improvement in the clinical course, survival rate, and quality of life of afflicted persons. A better knowledge of the processes behind the molecular etiology of various ailments, along with the availability of preclinical models, is leading to the development of several new experimental techniques, some of which are currently in clinical trials. This information is critical for making an accurate diagnosis, as well as providing credible genetic counseling and prenatal diagnosis. This chapter discusses the genesis, clinical features, diagnosis, and therapy of muscular dystrophy, as well as the function of the word in treating people suffering from this ailment.

**Keywords**: muscular dystrophy, Duchenne and Becker, Emery-Dreifuss, congenital dystrophy, clinical feature

## Introduction:

A diverse collection of hereditary diseases known as muscular dystrophy are characterized by gradual degenerative muscle weakening and muscle tissue loss (which often begins in childhood). It affects muscles strength and action generalized or localized skeletal muscle and other organs may involve in it. It is a group of muscle disease that result in increasing weakening and breakdown of skeletal muscle over time. The disorder varies in terms of which muscles are affected most, the severity of weakness, the rate at which symptoms progress, and the onset of symptoms.Many will eventually lose their ability to walk, and some varieties are also linked to issues with other organs.A genetic condition called muscular dystrophy causes the body's muscles to progressively deteriorate. It results from inaccurate or absent genetic information, which stops the body from producing the necessary proteins for the development and upkeep of healthy muscles. It describes a collection of over thirty hereditary conditions that impair muscular function.Muscular dystrophy symptoms typically get worse with time. These conditions are a type of Myopathy, a disorder of skeletal muscles. Depending on the type, Muscular dystrophy can affect the ability to move, walk and perform daily activities. It

 ISBN: 978-81-957386-3-2

can also affect heart muscles and lungs function. Certain types of muscular dystrophy are inherited or emerge in childhood. other forms develop during adulthood (LaPelusa and Kentris, 2022).

**Types of muscular dystrophy:**

More than 30 types of muscular dystrophy are there. Some of the more common forms include (Fig: -1)

- Duchenne Muscular Dystrophy (DMD)
- Becker Muscular Dystrophy (BMD)
- Congenital Muscular Dystrophy (CMD)
- Limb- Girdle Muscular Dystrophy (LGMD)
- Facioscapulohumeral Muscular Dystrophy (FSHD)
- Emery-Dreifuss Muscular Dystrophy (EDMD)
- Distal Muscular Dystrophy
- Myotonic Muscular Dystrophy
- Oculopharyngeal Muscular Dystrophy (OPMD)

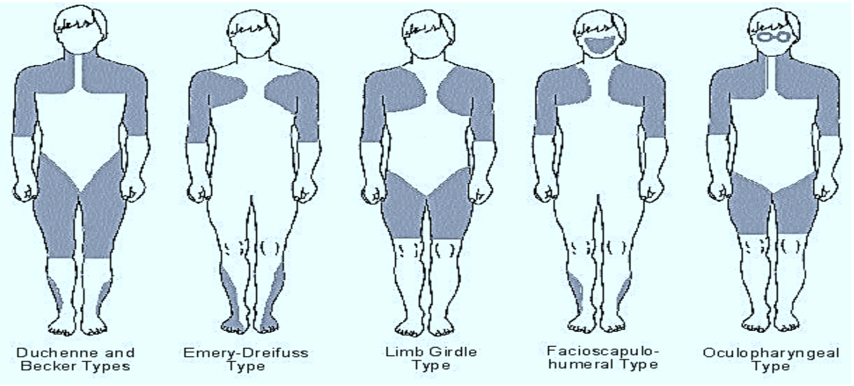


**Fig: -1 Main areas of muscle weakness in different types of dystrophy (de Greef *et al.,2018)***

**1. Duchenne Muscular Dystrophy (DMD):**

It is named after French neurologist Guillaume Benjamin Amand Duchenne.This type of muscular dystrophy is the most prevalent one. It will generally entrusted affects male children during birth (AMAB) but Sometime it also effects female children with mild symptoms. (Fig-2)As DMD progresses, it affects heart and lungs. (LaPelusa and Kentris, 2022).

**Etiology:** It is a X linked recessive inherited disorder It is common, mostly found in Male, Turner syndrome,1: 3500 live male birth,1/3 new Most mutation, 65% family history (Dooley *et al.*, 2010)



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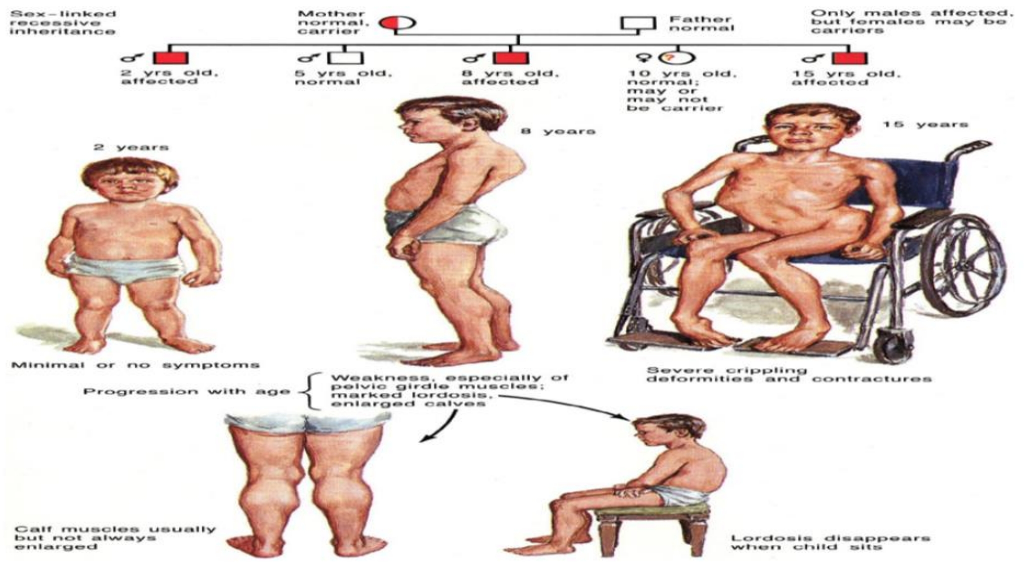


**Fig:2**

**Duchenne Muscular Dystrophy (Ryder *et al.*, 2017)**

**Clinical features of DMD: -**

- The typical age range for the clinical onset of muscle weakness is 2 to 3 years old.
- Myopathy symptoms may be seen from birth as laboratory and histologic evidence.
- It can be subdivided into 5 stages (Emery *et al.*, 2015)

**Stage-1-Presymptomatic**

- Creatine kinase usually elevated positive family history

**Stage-2- Early ambulatory**

- Clumsy & Waddling gait, manifesting in children aged 2-6years
- A gradual weakening of the proximal muscles, first affecting the lower limbs and then the arms, shoulders, and neck flexors.
- Possible toe- walking and Can climb stairs
- Gower's Sign- 'Climbing up legs' using the hand which rising from the floor

**Stage-3 – Late ambulatory**

- Greater challenges about the age of eight, the majority of patients start to have trouble climbing stairs, and their respiratory muscle power starts to gradually deteriorate.
- Cannot arise from the floor
- As the forced vital capacity steadily declines, nocturnal hypoxemia symptoms including fatigue and headaches in the early mornings appear.

**Stage-4 – Early non ambulatory**



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- Can self – propel for some time and able to maintain posture, Possible development of scoliosis

**Stage-5- Late non ambulatory**

- Scoliosis may worsen, particularly if a person becomes increasingly reliant on a wheelchair.
- Patients who are severely frail and confined to a wheelchair may experience terminal respiratory or cardiac failure by the time they are in their early 30s.
- Poor nutritional intake, Contracture may develop.

**Diagnosis:** Thomas test, Ober test, absent DTR, gait, and Meyeron sign-child evades truncal grip, Macroglossia, Immunoblotting: DNA mutation analysis, lack of dystrophin. (Guiraud *et al*.,2015)

**Treatment:**

- There's no known medical treatment for this illness. Treatment options for problems are numerous, and impacted children's quality of life can be greatly enhanced.
- Digoxin frequently has a positive initial effect on cardiac decompensation.
- Maintaining a healthy nutritional status is crucial. (Strober, 2006)

2. **Becker muscular dystrophy (BMD):**BMD, which is named after German physician Peter Emil Becker, is the second most prevalent kind of muscular dystrophy. It is milder version of DMD.The illness primarily affects AMAB individuals, while AFAB individuals may experience milder symptoms.Although BMD symptoms can start at any age between five and sixty, they usually start in adolescence. Individual differences exist in the severity of BMD. (fig-3). (Angelini *et al*.,1990)

   **Etiology:** Single gene defect, short arm X chromosome, Altered size and decreased amount of dystrophin.


**Fig: -3**

**Patients affected with BMD. Hypotrophy of quadriceps muscles (b, d, e, g, h), broad base posture (d, e), hypertrophy of the calves (a, e), and Gowers' maneuver (f). Some patients**

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**developed severe cardiomyopathy and required heart transplantation (g, h). Patient 1 (a), patient 2 (b), patient 3 (c, d), patient 4 (e), patient 5 (f), and patient 6 (g, h) (Angelini *et al*.,1990)**

**Clinical features, diagnosis and treatment:**

It is a less prevalent disorder, with a prevalence of 1 in 30000 live male births, similar to but less severe than DMD, onset at age greater than 7, pseudo hypertrophy of the calf muscles, a high rate of scoliosis in the Equinous and varas foot, and less frequent cardiac involvement. Diagnosis is same as DMD, increase CPK (< 200x) and decrease dystrophin. Similar to DMD, the forefoot Equinous receives plantar release and midfoot dorsal wedge osteotomy as treatment. (Thada *et al.*,2021)

**3. Congenital muscular dystrophy (CMD):**

Congenital, which meaning "present from birth," describes a class of muscular dystrophies known as CMD thatmanifest at or around birth. It is rear both in male and female.Overall muscle weakness and potential jointstiffness or looseness are symptoms of CMD. Depending on the kind, CMD might also include respiratory problems, cerebral or learning difficulties, seizures, visual problems, or spinal curvature (scoliosis). (Floriach *et al*., 2001)

**Etiology:** Autosomal recessive, Integrin, Fugutin defect (Yiu and Kornberg 2015)

**Clinical features, diagnosis and treatment:**

Stiffness of joint, congenital hip dislocation, subluxation, Achillis tendon contracture, talipes equinovarus,

Scoliosis. Diagnosis isMuscle Bx: Perimysial and endomysial fibrosis and treatment is Physical therapy,

Orthosis, Soft tissue release, Osteotomy (Balasubramanian *et al.*,2014)

**4. Limb- girdle muscular dystrophy (LGMD):**

The muscles in the upper arms, upper legs, shoulders, and hips are affected by LGMD.It impacts individuals of all ages.The prevalence of LGMD in the United States is about 2 per 100,000.It is linked to a weakening of the muscles of the upper arm, shoulder, and core hip. (Fig-4)Early onset and late middle age are both possible with LGMD, which typically advances extremely slowly.Muscle deterioration associated with LGMD can cause trouble reaching above the head and a Waddle-like gait. The heart and respiratory muscles may also be impacted in advanced stages of LGMD. It is common and more benign. (Nigro, 2003)

**Etiology: -**The chromosome 15q and 5q are autosomal recessive and dominant, respectively.

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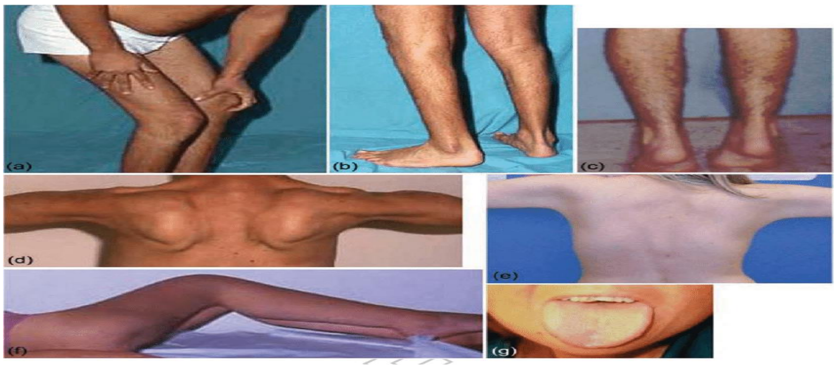


**Fig:-4 Clinical features frequently observed in patients with LGMD: Gower's manoeuvre in LGMD2A (a), atrophy of gastrocnemius muscle in distal Miyoshi myopathy (b), Achilles tendon retraction in LGMD2A (c), scapular winging and atrophy of scapular girdle muscles in LGMD2A (d), scoliosis in LGMD1F (e), severe hip and knee contractures in LGMD2C (f), tongue muscle hypertrophy or macroglossia in LGMD2E (g). (Politano *et al.*,2001)**

**Clinical features, diagnosis and treatment:**

Decade of onset: third, Initially: same distribution as DMD in the pelvic/shoulder m. (proximal to distal).

Diagnosis is Same clinical as DMD/BMD carriers, Moderately elevated CPK, Normal dystrophin Treatment is Similar to DMD, Scoliosis: mild, no Rx. (Magri *et al*., 2017)

**5. Facioscapulohumeral muscular dystrophy (FSHD):**

The face, shoulder, and upper arms are the main muscles groups affected by FSHD.(fig-5) (Sacconi et al., 2015) Symptoms tend to appear before age 20. About 4 out of 100,000 people in the U.S. have this form. Emerges in the early stages of adulthood or adolescence.They typically find it difficult to raise their arms,

whistle, and make certain facial gestures, such tightly shutting their eyelids. (Lewis *et al.*,2017)

**Etiology: -**Gene deficiency (FRG1), autosomal dominant, chromosome 4q35.


**Fig: -5 A 14-year old boy who first presented with symptoms of Facioscapulohumeral muscular dystrophy type I from age 11years. (a) asymmetrical facial weakness more on the left side and (b, c) marked winging of scapula. (Sacconi et al., 2015)**

**Clinical features, diagnosis and treatment:**

Age of onset: neither cardiac nor neurological; late childhood/early adulthood; winging scapula. Markedly decreasedshoulder flexion and abduction, Horizontal clavicles, Rare scoliosis. Diagnosis is PE, muscle biopsy, Normalserum CPK. Treatment is Posterior scpulocostal fusion/ stabilization (scapuloplexy) (Tawil *etal.*, 2015)



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**6. Emery-Dreifuss muscular dystrophy (EDMD):**

Children and young adults are the primary victims of EDMD. It tends to cause muscle weakness in shoulders,
upper arms and shins. (Fig-6) (Vytopil *et al*., 2003). EDMD also affects heart.It is a kind of muscular dystrophy that develops gradually and is marked by weakening in the heart and skeletal muscles. This type of musculardystrophy affects men more often than women. Male typical phenotype and female partial carrier.
(Bonne *et al.,* 1993)

**Etiology:** -X-linked recessive, Emerin protein (in nuclear membrane) etc.

**Fig:- 6 (A and B) Patients 1 and 3 exhibited shoulder, elbow and ankle contractures, standing on toes,
generalized muscle atrophy and limited neck mobility.(C) Patient 2, the daughter of Patient 1, exhibited a positive Gower's sign. (D) Patient 3 also exhibited Achilles tendon contracture. (Vytopil et al., 2003)**

**Clinical features, diagnosis and treatment:**

Muscle weakness, Contracture, Neck extension, elbow, Achillis tendon. Scoliosis: common, low incidence
of progressive. Abrupt death due to bradycardia and first- degree AV block. Diagnosis is Gower's sign,
Mildly/moderately elevated CPK, EMG: myopathic, Normal dystrophin. Treatment is Physical therapy:
Prevent contracture: neck, elbow, paraventral muscles, For slow progress elbow flexion contracture.
Soft tissue contracture: -Achillis lengthening, posterior ankle capsulotomy anterior transfer of tibialis posterior, Spinal stabilization, For curve > 40 degrees. Cardiologic intervention: - Cardiac pacemaker. (Emery and Emery, 1995)

**7. Distal muscular dystrophy:**

This kind affects the lower arms, lower legs, hands, and feet muscles. (Fig-7) (Olive *et al.*,2013) It tends to affect people in their 40s and 60s.Autosomal dominant trait. It is rear. Initially involvement: intrinsic hands, claves, tibialis posterior. Spread proximally, normal sensation present. (Murakami *et al.*,2005)


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**Fig-7 Distal limb weakness affecting both upper and lower extremities, with conspicuous muscle atrophy were seen (a: gastrocnemius and anterior tibial muscles, b: interosseus muscles of the hand) (Olivé *et al.*,2013)**

**8. Myotonic muscular dystrophy:**

This is the kind of muscular dystrophy that adult diagnoses for the most often. It affects adults AFAB and adults AMAB equally. Based on clinical and molecular presentation, two main types are identified:Steiner disease is the term for myotonic dystrophy type I (DM1), while proximal myotonic myopathy, a milder form of DMI, is the term for myotonic dystrophy type II (DM2). (Fig-8). (Jiménez et al.,2018) People with myotonic dystrophy have difficulty relaxing their muscles after using them.It can also lead to endocrine problems including diabetes and thyroid illness, as well as problems with the heart and lungs. (Mateos *et al.*, 2015)

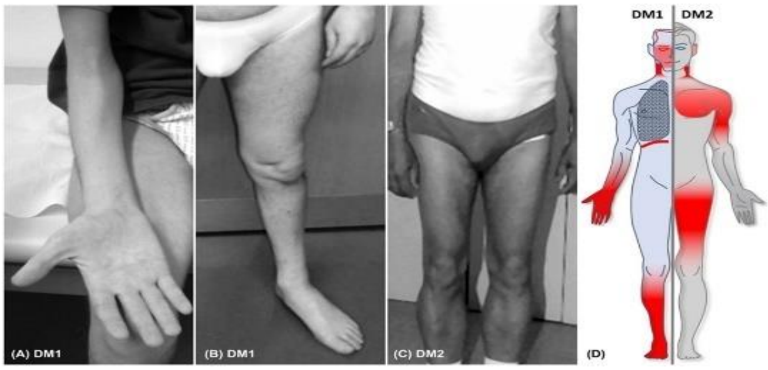


**Fig: -8 (A, B) Typical distal muscular atrophy in patients with DM1. (C) Atrophy of proximal muscles in a patient with DM2. (D) Figure illustrating the core phenotypes of DM1 (left) and DM2 (right). Regions of muscular involvement (weakness and atrophy) are highlighted in red. (Jiménez *et al*.,2018)**

**9. Oculopharyngeal muscular dystrophy (OPMD):**

OPMD affects the muscles of the throat and eyes. Symptoms include drooping eyelids (ptosis) and trouble swallowing (dysphagia) typically start to show up in the 40s or 50s. (Fig-9) (Semmler *et al*.,2007) About 1 in 100,000 people have OPMD. (Brais *et al.*, 1998)

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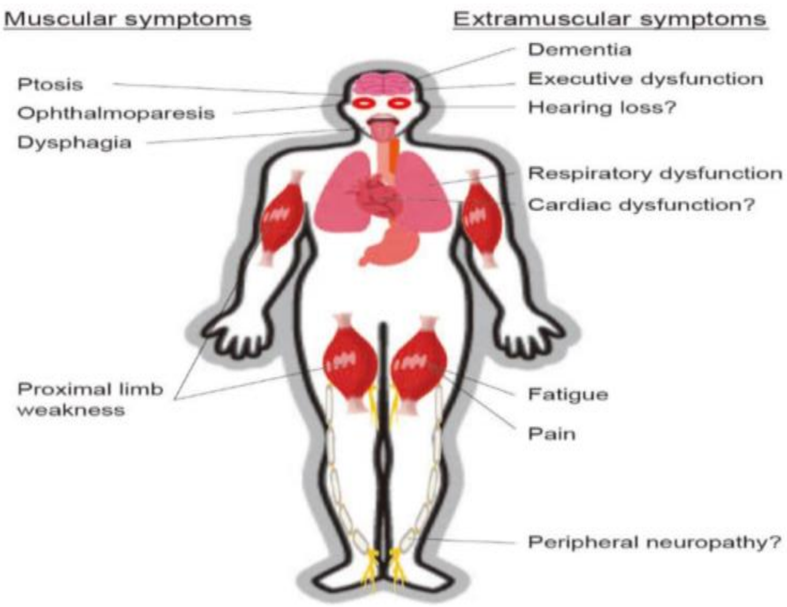


**Fig: - 9 Various muscular and extra muscular symptoms of oculopharyngeal muscular dystrophy (OPMD).A variety of extra muscular and muscular symptoms might be present in patients with OPMD. (Semmler *et al*.,2007)**

**Diagnosis, Treatment & Prevention of Muscular Dystrophy:**

Creatine kinase blood test, Genetic tests, Muscle biopsy, Electromyography (EMG). Treatment is Physical & Occupational Therapies, Corticosteroids, Mobility aids, Surgery, Heart care, Respiratory care, Speech therapy. Prevention is Healthy diet, prevent malnutrition, Drink lots of water to avoid dehydration, Exercise as much as possible according to your healthcare team’s recommendations, stay up to date on vaccines, quit smoking to protect your lungs and heart. (LaPelusa and Kentris, 2022)

**Conclusion:**

It is not possible to treat muscular dystrophy. Muscular dystrophies have devasting consequences. It can affect

anyone and surely will make someone’s life difficult. But it cannot be controlled.Although more recent medications are now accessible, their effectiveness has not been shown, thus further research is necessary to properly manage these medications and treatment alternatives.A variety of therapies are typically included, such as physical therapy to maintain strong, flexible muscles.

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ISBN: 978-81-957386-3-2

# CHAPTER- 13

# ANTIMICROBIAL POTENTIAL OF INDIAN SPICES AGAINST MULTIPLE DRUG RESISTANT BACTERIA

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**Abstract**

The excessive use of antibiotics in humans, animals and plants are some major causes of development of antibiotic resistance among bacteria.This increasing incidence of resistance to a wide range of antibiotic agents by a variety of organisms is a great challenge for modern medicine. The prevalence of antibiotic resistance in bacteria limits the effectiveness of medications even for common infections.So there is a crucial need of identifying safe and novel antimicrobial agents. Spices have been traditionally used in India since ancient times, as a flavoring agent as well as for preserving food products. In addition to enhancing flavors, they were used as folk medicines aimed to maintain proper sanitation, health, and hygiene and to increase longevity of life. Several spices such as carrom seeds, clove, ginger, black pepper, cumin, and asafoetida are commonly used in the Indian diet. Spices enhance the shelf life of foods through their antioxidant activity or their bacteriostatic and bactericidal activity. Therefore, spices have a great potential to be developed as new and safe therapeutic agents against Multi Drug Resistant (MDR) Bacteria. This review summarizes scientific studies on the antibacterial activity of several spices and their derivatives.

**Key words:**Antimicrobials, MDR, Indian spices.

## Overview:

The extensive use of antibiotics in human, animals and plants resulted in the frequent exposure of different concentrations of drugs by the commensal bacteria which is the major cause of evolutionof the antibiotic resistance in bacteria. Due to the lack of public awareness the occurrence of MDR in bacteria increases day by day. The major mechanisms involved inthe antibiotic resistance among bacteria are, through efflux pump bacteria canminimize the entry of antibiotics, inactivation of antibiotics by modifying or hydrolyzing it, and by modifying target site through mutation[1]. In recent years resistance against antibiotics amongst the pathogenic organisms is increasing at an alarming rate, therefore the treatment of infectious diseases is becoming a serious concern because antibiotics and other antimicrobial medicines become ineffective leading to the risk of disease spread and severe illness. It is predicted that by 2050, the drug-resistant infections will be the predominant cause of deaths exceeding cancer fatalities[2]. To check this situation there is a crucial need of identifying the novel and effective agents of antimicrobials with minimum side effects to the host cell.

India is referred to as the "Land of Spices" and the "Botanical Garden of the World" since it is the global leader in the production of traditional medicinal plants [3] In recent years there is a rising


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ISBN: 978-81-957386-3-2

interest about food safety and the search ofnatural antimicrobials to control food borne pathogens. Spices are some of the most commonly used natural antimicrobials in food [4].Spices are aromatic, pungent, seasoning agents generally used in small quantities in food preparations .Indian spices have history of more than 7000 years, centuries before Greece and Rome had been discovered. Spices are long been used to enhance flavor and aroma of food and become integral part of human diet. In addition they are also known for food preservation as well asmedicinal values [5]. In recent years there is a drastic increase in occurrences of multidrug- and disinfectant resistant bacteria in different food commodities resulting in the increase in morbidity and mortality [6]. Even though the scientific reasons of diseases were not known accurately, spices and condiments were traditionally utilized as curative and preventive agents.

**Spices as a natural antimicrobials:**

In addition to adding flavor and improving the taste of our food, herbs and spices provide us with a variety of dietary preventative ingredients. The majority of spices have various amounts of sugar, fatty oils, protein, starch, minerals, trace elements, alkaloids, and essential oils which are essential for good health. While certain alkaloids provide a stimulating effect on the neurological system, others boost appetite and may help the body to store protein. Food cannot be adequately digested without the activation of the lower digestive glands by spices. Essential oils promote the formation of mucus and bile in addition to increasing blood circulation to the skin and promoting the creation of white blood cells. They have antiseptic and antibacterial qualities, encourage sweating, and reduce inflammation. Tannin is naturally antibacterial and has an astringent impact on mucosal membranes.

A number of studies reported the antimicrobial potential of various Indian spices that reducemicrobial growth and can be used for preservation of foods .Preservative properties of spices are mainly through the inhibition of lipid oxidation and microbial growth [7,8].A number of research articles emphasizing the spices such as curcumin, clove, cinnamon, fenugreek seed, asafoetida,gooseberry, bay leaf, oregano, thyme, star anise, cumin, black pepper, garlic, coriander, and many more have antimicrobial properties, and they are used to treat antibacterial and antifungal infections [9,10,11].

Sulieman *et. al.*, (2023) summarizes a lot of works related to the antimicrobial potential of many spices [12]. The antibacterial activities of piperinefound in black pepper were reportedagainst various Gram-positive bacteria and Gram-negative bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella enterica*, *Klebsiella pneumonia*, and *Escherichia coli* [13]. Behbahani et. al., (2019) analyzed the effect of clove oil on bacterial cells and the SEM analysis revealed the cytoplasmic leakage leading to bacterial cell death [14]. Studies have shown that*Cuminum cyminum* L,*Crocus sativus* L. (saffron) and*Cinnamomum verum* J. display significant antibacterial activity fungicidal and antioxidant effects [15,16 17].The essential oils from *Coriandrum sativum* L. seeds showed potential antimicrobial activity against specific foodborne pathogens, including *Streptococcus pyogenes*, *Listeria monocytogenes*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella*etc[18].Essential oils extracted from ginger exhibited noteworthy antifungal efficacy against a wide spectrum of fungal strains[19].

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**Spices against MDR:**

Many researchers demonstrated the potential of spices against MDR bacteria all over the world. Synergistic effect of antibiotic and Cameroonian spices against MDR phenotypes was observed in a study [20]. Spices have been found to be effective against MDR urinary tract infecting bacteria [21,22]. Antibacterial and anti-biofilm activity of spices against MDR *Pseudomonas aeruginosa* has also been illustrated [23]. In a study conducted using Pakistani spices, effective antibacterial property against *Salmonella* and other multi-drug resistant bacteria was seen [24]. Similar study in Bangladesh Showed the antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* [25]. Research has shown interaction of South Asian Spices with Conventional Antibiotics against MDR *Mycobacterium*Abscesses and Cystic Fibrosis [26]. Spices have also been found to be effective against beta lactamase producing bacteria and food-borne pathogens, *Staphylococcus aureus* and *Salmonella enteritidis* [27,28].

**Bioactive components of spices and herbs:**

Each spice has a unique aroma and flavour, which is due to the phytochemicals or secondary metabolitespresent in it. These are known as bioactive compounds, such as phenolic acids, flavonoids, saponin, thiosulfinates, glucosinolates, alcohols, aldehydes, ketones, ethers and hydrocarbons and terpenes may present in different parts of the plants such as – flowers, buds, leaves, twigs, bark, fruits, seeds, roots etc. [29]. The antimicrobial and antioxidant properties are due to these components [8]. The following Table shows the different bioactive components and their possible role in antimicrobial and antioxidant activities. The most effective antibacterial component of spices are mostly aromatic phenolic compounds.The active ingredients in spices have the potential to exhibit antimicrobial properties through multiple mechanisms, such as disruption of cellular walls, disruption of cell membranes, leakage of cellular constituents, damage to proteins, involvement in intracellular enzymatic activities, interference with DNA and RNA synthesis, disruption of electron transport and nutrient uptake, leakage of cellular constituents, loss of intracellular energy production, and alteration of fatty acid and phospholipid components [30].

| Common name | Biological name | Bioactive compounds | Medicinal properties | Reference |
|---|---|---|---|---|
| Bay leaves | *Laurus nobilis* | 1,8-cineole, cinnamtannin | Antioxidant and antimicrobial | [31] |
| Black Cumin | *Nigella sativa L* | Thymoquinone, thymol, and α hederin | Antioxidant, antibacterial, Anticarcinogenic, anti-hypertensionanti-inflammatory, anti-diabetic | [32] |
| Black pepper | *Piper nigrum* | Piperine, piperidine Camphene, limonene, pinene, terpenes, isoquercetin, | Anti-microbial, Antioxidant, anti-inflammatory | [33] |
| Cinnamon | *Cinnamomum verum* | Cinnamaldehyde and transcinnamaldehyde | Antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and | [34] |

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| | | | | |
|---|---|---|---|---|
| | | | cardiovascular-disease-lowering | |
| Cloves | *Syzygim aromaticum* | Eugenol, gallic acid, flavonoids, phenolic acids | Antioxidant and antimicrobial | [35] |
| Coriander | *Coriandrum sativum* | Linalool, α-pinene, γ terpenene, camphor and limonene | Antimicrobial, antioxidant, antidiabetic, antimutagenic and antidepressant | [36] |
| Cumin | *Cuminum cyminum* | Cuminaldehyde | Antimicrobial, antioxidant, | [37] |
| Cardamum | *Amomum subulatum* | Limonene, 1,8-cineole, terpinolene, myrcene, caffeic acid, quercetin, kaempferol, luteolin, | analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, cardio-adaptogenic and hypolipidaemic activities | [38] |
| Fenugreek | *Trigonella foenum-graecum* | Sesquiterpenes, aromatic aldehydes, terpenes | Anticarcinogenic, antidiabetic, antioxidant, hypocholesterolemic, antilithogenic antimicrobial | [39] |
| Thyme | *Thymus vulgaris* | carvacrol, thymol, and pcymene, α-pinene, 1,8- cineole, camphor, linalool, and borneol | Antioxidant, antimicrobial | [40] |
| Turmeric | *Curcuma longa* | Curcumin, demethoxycurcumin and bisdemetoxicurcumin | antioxidant, anti-inflammatory, antibacterial, antifungal, antidiabetic | [41] |
| Ginger | *Zingiber officinale* | Gingerol, shogaols, paradols, quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione, terpene compounds | Antioxidant, anti-inflammatory, antimicrobial, cardiovascular protection, antiobesity activity, antidiabetic | [42] |
| Saffron | *Crocus sativus L* | Crocin, crocetin, safranal, and picrocrocin | Antioxidant, antiinflammatory, anticonvulsant, satiating, antihistamine | 43 |

**Conclusion:**

The spices have long been used for taste enhancement, colour and flavor in food,in addition to preserving food and treatment for various ailments. It is evident from several studies that many spices and their essential oils are efficient in destroying pathogenic microorganisms as well as the MDR strains. There is a remarkable increase in MDR strains continuously which is a big challenge for medical science. In this situation the drug formulation extracted from spices may be a promising,

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safe and eco-friendly alternative for therapeutic as well as prophylactics purpose. Although thorough knowledge and understanding of the precise mechanisms underlying the action of phytochemicals is essential for developing novel therapeutic agents.

**Future prospect:**

Researchers may explore innovative applications for bioactive substances obtained from spices, along with conventional food preservation methods. It is important to look deep into the mechanisms behind the antibacterial action of chemical substances derived from spices. Advanced methods, like molecular modelling may shed light on the specific interactions these substances have with microbial targets. Future research is also expected to focus on the safety issues from spices derived metabolites such as assessment of the toxicity, possible allergic responses, and any unexpected effects of prolonged exposure to these substances. These natural antimicrobials may play a crucial role in development of new therapeutic agents while reducing the risk of microbial resistance development**.**

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Published by : Sai College, Bhilai

ISBN: 978-81-957386-3-2

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 ISBN: 978-81-957386-3-2

# CHAPTER- 14
# A STUDY ON THE IMPACTOFSOLIDPARTICULATEMATTERDEPOSITSON VEGETATION

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## Abstract
In the current age of civilisation, air pollution is one of the major issues the world is currently facing. Particulate matter pollution, in particular, poses a risk both to the environment and public health. The biochemical, morphological, and biological status of green plants, as well as their responses, have been significantly influenced by the altered ambient environment caused by the particulate matter pollutant in urban areas. It turns out to be a useful indicator of the overall impact of particulate matter pollution as well as the detrimental effects of this pollution on vegetation when considering the context of the plants (wide distribution, increases the contact area, etc.). The chapter discusses how particulate matter pollution affects morphological characteristics like stomata structure, leaf area, flowering, growth, leaf number, as well as reproduction, along with biochemical parameters like pigment content, protein, enzymes, and sugar as physiological aspects like pH as well as relative water content. A brief overview of the effects of this matter on species diversity as well as climate change is also provided in the chapter. Additionally, the review highlights the genotoxic effects of particulate matter on plants.

**Key words:** particulate matter, ambient environment biochemical, morphological etc

## Introduction:
The effects of solid particle matter have been studied since the eighteenth century. In 1785, Sir Percival Pott published an epidemiological study about cancer among chimney sweepers (Goldberg 1985). Studies on the effects of dust particles on human health have been carried out for the past 30 years (Repace and Lowrey 1980; Spengler et al. 1980). However, phytotoxic chemicals like NO2, O3, and SO2 have received the majority of attention in studies on air pollution and its effects on plants (Farmer 1991a). Due in large part to growing traffic and human activity in urban areas, the general public's knowledge of CO2 issues and fine particle matter (PM, fine dust) has increased recently (Cwiklak et al. 2007 Because of this, research on how PM affects animals—including humans and the environment—has been growing in Germany and around the globe in recent years. The European Union (EU) increased the threshold-safety values for particulate matter in the air in European cities during this time. The EU guideline 1999/30/EG, which governs the maximum permissible concentration of fine dust particles less than or equal to 10 µm (PM10), came into force in January 2005. According to numerous estimates, the growing urbanization and pollution in Germany and other EU nations may make it impossible to maintain acceptable standards in many areas (Umweltbundesamt 2005).Particles in the air can have sizes ranging from 1 nm (0,001 µm) to 100 µm (Graedel and Crutzen 1994). Human health may suffer from fine particulate matter in the air (Lahl and Steven 2004). According to Hartog et al. (2003), the impact of fine dust on human health might

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range from brief impairments to a rise in mortality from acute respiratory infections and heart abnormalities... According to Donaldsona et al. (1998), ultrafine particles may irritate skin and trigger an allergic reaction that alters the inflammatory process. The reduced size of the particles makes it easier for them to enter the respiratory system. It has been shown that particles larger than 10 µm have the ability to enter the larynx (Sarangapani and Wexler 2000). But only few of them make it to the lung's alveoli and smaller airways (Pekkanen et al. 1997). Particles smaller than 10 µm can easily enter these organs and tissues, causing lifelong impairment. Particles smaller than 0.1 µm have the ability to pass through alveoli and enter the bloodstream, where they disperse throughout the body (Borm and Kreyling, 2004). Correlation studies examining the biological impact of dust particles of varying sizes remain incomplete. This may be partially explained by the various composition of naturally occurring dusts as well as the varied and heterogeneous health effects demonstrated by comparable dust particles in numerous biological model systems.

**Particulate matter:**

Particulate matter in the atmosphere is a mixture of many substances. Particulate matter deposition on vegetated surfaces is influenced by the chemistry and, to a lesser degree, the size distribution of these particles. Particulate matter may have an impact on vegetation by lowering the amount of light needed for photosynthesis and raising leaf temperature as a result of altered surface optical characteristics. The diffusion of gases into and out of leaves is controlled by particulate matter load, color, and particle size; changes in energy exchange are more significant. While some materials may be absorbed through the cuticle, alkaline particulate matter has the potential to damage the leaf surface. Urban areas' air quality is improved when vegetation removes particulate matter fromthe atmosphere enhances air quality in urban areas (Beckett *et al*, 1998, 2000; Freer-Smith *et al*., 2005) and near roadways (Smith, 1971; Freer-Smith *et al*., 1997). Particulate matter deposition on plants is contingent upon the dispersion path's leaf elements' dimensions and density, as well as the distribution of particle sizes. The impacts on the vigor, competitive viability, and reproductive fitness of individual plants mediate the effects of size-segregated particulate matter, as opposed to chemically hypothesized particulate matter, on ecosystem function. Large-leaved trees may offer efficient PM barriers near PM sources (such as highways or quarries), but they are less effective at blocking finer particles that move farther. Human health is greatly endangered by the small particles released, particularly from diesel automobiles, which have particulate size diameters of less than 10 µm (sometimes referred to as the PM10 percent). Older cars, overloading, and poor-quality diesel are the primary causes of this pollution.

In order to lower the amount of pollutants in the air, plants offer a vast leaf area that can be used for impingement, absorption, and storage of pollutants, with varying degrees of coverage depending on the species (Liu and Ding, 2008). Since plants are the first to absorb air pollutants, the use of plants as air pollution monitors has long been established. According to Joshi and Swami (2007), they serve as scavengers for a large number of airborne particles in the environment. A recent study by the Centre for Science and Environment (CSE) has revealed that air pollution has been killing nearly 52,000 people in 36 Indian cities every year pre-maturely while hospitalizing about 26 millions (Pattnaik and Pattnaik, 2000). Matysiak (2001) studied the contents of carotenoid in the needles of Pinussylvestris growing in polluted areas. Plants having more APTI values are more tolerant to air

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pollution than those having lower index value (Kulshrestha *et al*., 2003). Pandit and Prajapati (2003) studied accumulation of some trace elements in different species of Acacia like *A. nilotica*, *A. leucophloea* and *A. senegal* in reserved forests near Bhavnagar, Gujarat state and described air pollution tolerance among roadside plants exposed to varying degrees of traffic pollution. Wali *et al.* (2004) studied the effect of air pollution on plant growth, stomatal response and photosynthesis of *Althea officinalis*. Lone *et al*. (2005) studied dust pollution caused by vehicular traffic in Aligarh city Utter Pradesh state. Arya (2009) reviewed effect of air pollution on plants. According to Seyyednejad et al. (2009), air pollution in South Iran's petrochemical zones has a substantial impact on Callistemon citrinus's morphological and physiological responses. Agbaire (2009) and Agbaire and Esiefarienrhe (2009) found that several plants near oil exploration and gas plant sites in Delta State, Nigeria, have air pollution tolerance indices. The 2009 study by Honour et al. examined how herbaceous plants reacted to air pollution in metropolitan areas. A few common plants showed altered leaf surface structure as a result of particulate pollution, according to Rai et al. (2010). On Prosopisjuliflora, however, Koochak and SeyyedNejad (2010) noted the same result. Researchers Kapoor et al. (2009a, b), Govindaraju et al. (2010), and Bamniya et al. (2011a, b) examined the detrimental impacts of air pollution on a select few tree species' physiological activities.It has been generally accepted that dust originating from unpaved roads can aggravate respiratory ailments, create driving hazards and cause considerable discomfort to those living alongside these roads. However, it has only been recently that studies have been undertaken to establish the nature and extent of the road-dust problem.

The average density of dust present on leaf surfaces, on any dry day, was then calculatedThe average amount of dust present per unit of ground area per day was calculated using a range of high, medium, and low expected numbers of days per month on which road dust could occur, along with the average number of days of dry weather following a rainfall event and a road drying time difference for winter and summer. To determine the total average amount of dust present on leaf surfaces per unit of ground area per day, additional consideration was given to the quantity of leaf area per unit of ground area as well as the type of leaf surface (pubescent versus glabrous).

During the spring and summer, non-paved roads are treated with dust suppression and road stabilization chemicals based on magnesium chloride (MgCl2). By stabilizing soil and absorbing moisture from the environment to keep road surfaces damp, chloride-based dust suppressants are used to limit erosion and fugitive dust and save maintenance costs on unpaved roads (Addo et al., 2004; Piechota et al., 2004). Unpaved road dust can be a major source of atmospheric particulate matter, which has a variety of negative consequences on the environment and human health (Sanders et al., 1997; ECHC, 2001; Singh et al., 2003). The U.S. Environmental Protection Agency has established air quality standards for fine particulate matter (PM-10). Municipal road and bridge departments in arid climates can suppress PM-10 emissions on nonpaved roads by applied chemical dust suppression products (Singh *et al*., 2003).

Airborne particulate matter appears to be a major concern in Indian cities (Agarwal et al., 1999). There are several routes for airborne particulate matter to enter an organism or plant. It is a complex mixture of inorganic and organic elements of varied sizes. The majority of the acidity (hydrogen ion) and mutagenic activity of the particulate matter is found in the fine fraction (02.5 mm) (Van Houdt,

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1990; Beckett et al., 1998). Many plants are extremely vulnerable to air pollution, which can harm their leaves, impede the growth of the plant, and reduce primary productivity (Ulrich, 1984).

Air pollution can harm leaves on a variety of plants, hinder plant growth, and reduce primary productivity (Ulrich, 1984). The leaves have the most noticeable damage. Certain plants may experience difficulties with growth and reproduction, and sensitive species may see a decline in population, while tolerant species may flourish and take over the vegetation. Chlorosis, necrosis, and epinasty are the main harms that air pollution do to plants (Prasad and Choudhury, 1992; Katiyar and Dubey, 2000). Additionally, plants can lower the quality of the air, especially when they produce spores and pollens that become airborne (Burge et al., 1982; Owen et al., 1992).

The majority of the dust particle's effects on plants involve the possibility of stomata blockage and damage, which would impair respiration and photosynthesis. Additional consequences include cuticle and leaf surface wear and shadowing, which can lower photosynthetic capability (Iqbal and Shafig, 2001).

Since plants frequently react to atmospheric contamination in the same way that they do to drought and other environmental stressors, air pollution has been referred to as an extra stress on plants. The impact of air pollution on plants, whether through direct toxicity or by altering the host's physiology to make it more prone to infection. When pollution was severe, the plants would either entirely vanish or show signs of foliar necrosis. Previous research on the effects of air pollution on plants has also been conducted by a number of researchers using a variety of sensitive plants to examine foliar morphological and biochemical changes (Samal and Santra, 2002).

Generally speaking, it is thought that tolerant plants in metal-contaminated settings serve as trustworthy markers of pollution and disturbance. In settings where metals are present, tolerant plants are typically accumulators and excluders. Numerous researchers anticipate that robust roadside plants will contain hyper accumulator species (Okunola et al., 2007; Bakirdere and Yaman, 2008). It is acknowledged that phytosociological study of natural vegetation is a suitable and effective way to choose beneficial plant species from natural communities (Katsuno, 1977). A thorough review of the literature turned up a lot of articles on chemical and physical effects. The character and dispersal of motorway run-off (Bellinger et al., 1982), toxicity of sediments contaminated with road run-off (Boxall and Maltby, 1995), sand and 14 salt (Oberts, 1986), road dust (Keller and Lamprecht, 1995), salinity of motorway soils (Thompson et al., 1986), and landslides, erosion, and sediments (Haigh, et al., 1993) are among the subjects of research. Research on pollution detention ponds has been done (Yousef et al., 1986).

The chemical and physical impacts of road dust on the environment appear to have been the subject of relatively few research, some of which are particular to locations or biomes such the tundra and taiga (Forbes, 1995; Walker and Everett, 1987). Blockage of stomata and cell death are examples of physical impacts. Elements including Al, Cr, Fe, and Ni that are deposited in airborne road dust and impact biota through soil enrichment can have chemical consequences (Santelmann and Gorham, 1988). The impacts of different forms of dust on crops, grasslands, heathlands, trees, arctic bryophyte,

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and lichen communities were discussed in a recent literature review conducted by Farmer (1993). Dust can have an impact on transpiration, respiration, photosynthesis, and the effects of gaseous pollutants. Farmer found that Epiphytic lichens, Sphagnum and other mosses were the most sensitive of those studied.

**Summary:**

Plants are impacted by airborne particulate matter in different ways. In addition to the PM's composition, other factors that matter include the sort and location of the deposition, the climate, the soil, plant surface quality, and the environment's composition. Damage can take several forms, from a straightforward decrease in the target plant's photosynthetic efficiency to stomatal closure resulting in alterations to cells and tissues, necrosis in the leaves, chlorosis, etc. When PM is deposited on vegetation, the initial physiological response occurs on the leaf with lower net absorption efficiency. These responses occur instantly and intensify to a noteworthy degree in a matter of minutes following PM contact with the leaf surface. Depositions that last a long time alter the photochemistry, resulting in thicker branches and slower growth for the stem and leaves. Large-scale losses in the assimilate balance result from prolonged deposition of sediments across the plant surface. These impacts are particularly noticeable in the vicinity of the day mining industry, which in turn affects the neighboring vegetation's vigor and, ultimately, the plant productivity There haven't been many studies done to date on the potential abrasive properties of PM (like silicates) on vegetation in fields or greenhouses. This is mostly due to the fact that friction energy calculations, which are crucial for understanding these effects at the micro level, are highly challenging in the natural world. In the natural world, particularly during a sandstorm, wind turbulences can cause mechanical damage to plant surfaces, leading to a variety of physiological implications. Not enough research has been done on secondary impacts, such as an increase in illnesses and pests after PMs physically remove the protective leaf cuticle. A species' selective advantage over others may also change the effects of PM on natural plant communities. Eventually, the balance between various species within a plant community is altered as a result of alterations in soil chemistry brought on by PM deposition in the rhizosphere. Only a small number of studies have been conducted at the community level to yet, despite the need for immediate attention. It's clear that there are a lot of unanswered questions about how PM affects plants. We should anticipate further research in this field because densely populated places are increasingly using plants to filter particulate matter from the air.

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# CHAPTER- 15
# SEAWEEDS AS BIOPLASTIC

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**ABSTRACT**

Nowadays the prime focus is to search an alternative of petroleum-based plastic. Bioactive compounds present in seaweed have the capacity toform sustainable polymer. Polysaccharides such as agar, carrageenan and alginate are extracted through certain high-pressure techniques and then incorporated with other polymers, nanoparticles and solvents to increase the plasticity resulting in the formation of bioplastics. Seaweeds are found in a wide variety of ranges, depending on their pigment, habitat, nutrition, etc. Their habitat varies from species to species; most commonly, they inhabit the littoral zone, and some are even found in the benthic area, such as red seaweeds. It is now imperative to employ seaweed-derived biological polymers sustainably, as they are eco-friendly and abundant in nature. Since ancient times, macroalgae has been cultivated for consumption as food, and it still serves great nutritional value if included in a diet. Seaweeds would be a promising alternative for the production of "good" plastic due to their biodegradability, renewability, non-toxicity, and edibility. The chapter aims to highlight the potential of seaweeds to be used as a better option for developing-biological grade plastic.

**Keywords:** Seaweeds, Bioplastic, Bioactive compounds, Biodegradability, Sustainability

## 1. Introduction:

Seaweeds, a macroalgal multicellular photosynthetic species found specifically in aquatic ecosystems, are a great source of oxygen and many other beneficiaries.Depending on the species, they contain a wide range of bioactive compounds such as polysaccharides, polyphenols, sterols, terpenes, carotenoides, vitamins, minerals and many more. Numerous of them hold a variety of medicinal properties including anti-tumoral, anti-bacterial, anti-viral effects. Around 168,971 species of seaweed have been discovered so far. Their pigments have led to theirclassification into distinct categories such as Rhodophyta (red seaweed), Phaeophyta(brown seaweed) and Chlorophyta (green seaweed) which includes red, brown and green pigments respectively.Seaweed is a common dietary source for many human communities. It provides several goods such as biofertilizer, biofuels, drugs and far more. It also contributes in the development of edible and biodegradable environment friendly polymers. [1-4]

Nowadays, the usage of plastic has increased enormously, especially for the purpose ofpackaging. The worldwide production of plastics exceeds 359 million tons.As the global population grows, it will rise even more. Approximately 8 million tons of plastic make their way into the ocean annually.Since these plastics are non-biodegradable, they do not break down naturally and end up piling up in landfills or polluting the environment. While burning or breaking them down through heat treatments can get rid of plastic waste, it also poses environmental risks. The increasing use of petroleum-based

ISBN: 978-81-957386-3-2

plastic could pose a threat to the environment. Switching to the material that poses the characteristics of plastic and also are biodegradable in nature and harmless to the human health is the prerequisite. [5, 6, 7]

"Bioplastics" are plastics that are originated from renewable biological resources and are easily degradable naturally. They do not cause any harmful effects to the environment, additionally; they are easily recyclable, need less energy consumption to produce, are non-toxic as compare to petro-based plastic and are eco-friendly. Major sources of bioplastic could be plants, bacteria and algae. Starch obtained from the plants and enzymes secreted by bacteria are used as sources for the production of bioplastic There are certain drawbacks as well,such as that if plants were used for large-scale production, it could affect the food chain and also take a long time for production. For bacteria, it would not be possible to secrete a huge amount of enzyme at a very fast rate as it is pH and temperature dependent making the process of accumulation of enzyme for the production of bioplastic a complex process. Algae, especiallymacroalgae or seaweeds, could prove be a great source for the production of bioplastic as it is easy to farm in natural conditions, harvesting can occur throughout the year, it is cost-effective and mass production is possible. Seaweeds are found deep in the oceans, which makes them survivable with little or no effort. The secondary metabolites found provide a defense mechanism against pathogens, make it resistant to microbes, and save it from predators. Some major anti-microbial compounds isolated from seaweeds includePhlorotannins, Bromophenols, and other halogenated compounds. [8, 9]

## 2. Properties suitable for plastic-like applications:

Plastics have brought convenience to our lives as they are being used for many purposes, such as packaging, making containers, and so on. But its non-degradability becomes a very big drawback for its use, and it also accumulates in the environment, causing harm to it. So bioplastics have become a solution to this, as they are easily decomposable. Seaweeds being used for the production of bioplastic make the process easier due to their widespread accessibility. Their cultivationbegan around the 1940s, and by the 1950s, they had reached large-scale production globally.Earlier, they were used as a dietary source only; later, the bioactives present made them suitable for use in many other purposes, such as pharmaceuticals, cosmoceuticals, nutraceuticals, biofuels, biofertilizers, and the production of polymers. This leads to an increase in demand for macroalgaeand its exploitation, and thus production. Seaweeds are rich in polysaccharides, which are the core material for bioplastic production. Polysaccharides contain polymer-positive compounds such as Carrageenan, Agar and Alginates. [10- 12]

### 2.1.Carrageenan:

Carrageenan is a sulfated polysaccharide derived from red seaweed (rhodophyta) and is water-soluble. It is a copolymer of α-(1-3)-D-galactose and β-(1-4)-D-galactose consisting of alternate glycosidic linkages (Figure 1). It can be classified into seven distinct types on the basis of the position of the sulfate group attached to the galactose unit: κ-carrageenan, ι-carrageenan, λ-carrageenan, γ-carrageenan, ν-carrageenan, ξ-carrageenan, and μ-carrageenan. Depending on the bonds they make, the various forms of carrageenan are present in mixed forms rather than pure forms. [13, 14]

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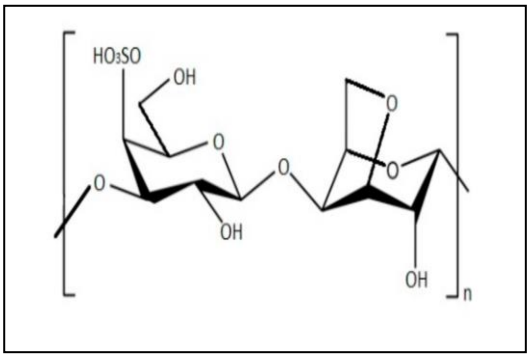


**FIGURE 1: κ-CARRAGEENAN- CHEMICAL STRUCTURE [3]**

The three major types of carrageenan used commercially include kappa (κ), iota (ι) and lambda (λ) forms. κ-carrageenanis obtained from *Kappaphycusalvarezii* species, and it forms strong and rigid gels. ι-carrageenanis obtained from *Eucheumadenticulatum*, and it forms gels that are weak and soft. Different species of the genera *Gigartina* and *Chondrus* provide λ-carrageenan. Carrageenan, due to its gelling, stabilizing, and thickening effects found its place in the food industry. [15]
The κ-, ι-, and λ-carrageenans have been authorized as food additives by the European Food Safety Agency (EFSA).Carrageenan has proven to be a strong polysaccharide for the synthesis of bioplastic, but due to its hydrophilicity, it tends to dissolve easily, which becomes its limitation; however, mixing it with other polymers, using plasticizers, and bonding with hydrophobic compounds could enhance its properties and increase its strength. [16 - 17]

**2.2. Agar:**

Agar is extracted from the cell wall of red seaweeds (called agarophytes), mostly from the genera *Gelidium* and *Gracilaria.* It can form viscous solutions and strong gels. Agar is primarily made up of D-galactose and its anhydride 3,6-anhydro-L-galactose (Figure 2). It is composed of agarose and agaropection in mixed proportions. Agar dissolves in warm water and solidifies while cooling down. [18,19]

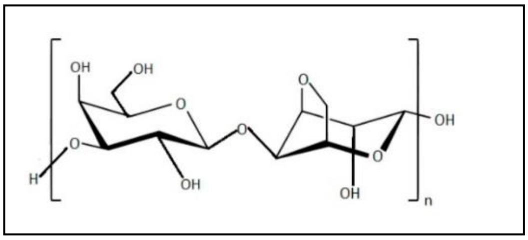


**FIGURE 2: AGAR- CHEMICAL STRUTURE [3]**

Agar gum has emerged as a source for biodegradable and edible films. Because of its widespread use and favorable outcomes, agar gum has been recognized as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration (FDA) since 1972.Due to its gelling and stabilizing properties it is widely used in the food industry, such as in the making of jams and jellies. The low hygroscopic property of agar is an advantage for food packaging; it also dissolves well with other bioactive substances and plasticizers to synthesize gels. [20, 21, 22]

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**2.3. Alginate:**

Alginates are extracted from brown seaweeds like *Laminaria japonica* and *Laminariadigitata.* It is also called alginic acid and is present in the cell wall and intercellular matrix of brown seaweed. They are linear polymers made up of two monomeric uronic acids, β-D-mannuronic acid (M) and α-L-guluronic acid (G) (Figure 3). Mannuronic acid provides linear and flexible orientation, and guluronic acid creates steric hindrance, both of which maintain the stiffness of the polymer. The varying ratios of both acids present in alginate form either brittle or elastic gel. [23]

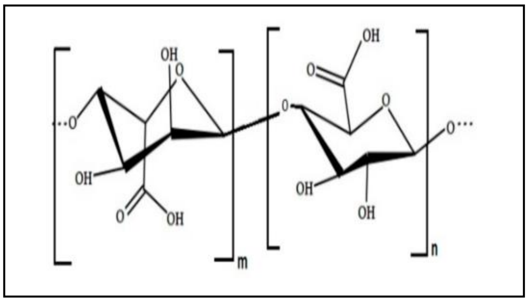

**FIGURE 3: ALGINATE- CHEMICAL STRUCTURE [3]**

For its insoluble film production, low toxicity and biocompatibility, alginate has been considered an interesting film matrix. Alginate films enhance food taste and appearance, act as effective oxygen barriers, and prohibit lipid oxidation. They are good stabilizers and thickeners and are used in the food, healthcare, and pharmaceutical industries. Alginate, being hydrophilic in nature, is required to combine with other matrix to make it water-resistant. Calcium could be added to alginate to make it more stable and resistant to the membrane, which can be a great initiative for harmless packaging. [23,24]

**3. Common seaweed species and their characteristics:**

Seaweeds are crucial renewable marine organisms that are grown in benthic water. They are of huge ecological value and great economic importance as well. There are varieties of macroalgae that can be found in the depths of the ocean. They have been divided into three groups based on the pigment. [25]

**3.1.Chlorophyta:**

Phylum Chlorophyta includes all green colored seaweeds found in marine ecosystems. They are good sources of certain bioactive compounds, such as polysaccharides, phenolic compounds, etc. They are primarily found in coastal areas.*Ulva*, *Enteromorpha*, *Chaetomorpha*,*Codium*,and*Caulerpa* are some common genera of green seaweeds. [1]

The nutritional elements found in green seaweeds vary from species to species and their growing season. They are considered to be a rich source of protein; they also includelectins, glycoproteins, and phycobiliproteins. Lectins bind with carbohydrates, which can interact with particular glycan structures in bacteria, fungi, viruses, and parasites. Lipids are crucial metabolites for sustaining human health. Seaweeds havelow lipid content- not more than 5% of their dry biomass. But they

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contain a high amount of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). These macroalgae are also rich in dietary fiber, vitamins, and trace minerals. [1, 26]

Xylan and Sulfated galactans are present primarily in green seaweeds as polysaccharides. Polyphenols such as phenolic acids, lignans, and flavonoids are largely present. Sterols and terpenes, due to their cytotoxic properties, have anti-tumoral and anti-bacterial characteristics. Chlorophyll a and chlorophyll b are major pigments found in green macroalgae, which impart a green color to them. These pigments also provide numerous health benefits. [26]

**3.2. Rhodophyta:**

Red-colored seaweed is included in the phylum Rhodophyta, which is the largest phylum of all macroalgae in terms of number and diversity. They are photosynthetic in nature and contain chlorophyll a and d and some other accessory pigments such as carotenoids (carotene, xanthophylls andfucoxanthin) and phycobiliproteins (phycoerythrin, phyocyanin). Compared to other macroalgae, red macroalgae can survive in more depth in marine water due to the presence of their accessory pigments. [27]

The cell wall of red seaweed consists of a fibrillar wall, an amorphous matrix, and a glycoprotein domain. Carrageenans and agar are polysaccharides present, which are calledphycocolloids due to their gel-forming ability. They also exhibit anti-thrombotic, anti-diabetic, and anti-inflammatory properties. Polysaccharides present are usable as prebiotics and dietary fibers for nutrition purposes. Red seaweeds containhigher amount of protein level than green and brown algae. Of the total proteins present, around 50% are phycobiliproteins. Modulation of the immune response can be observed due to the presence of bioactive compounds. Red seaweeds are also said to be functional foods with good potential. [28, 29]

**3.3. Phaeophyta:**

Brown seaweeds are being included in the phylum phaeophyta, which bears the pigment chlorophyll a and c and carotenoids (e.g.,xanthophylls). Fucoxanthin, a xanthophyll, is predominantly present abundantly in brown seaweeds, which impart a brown color to them. Brown seaweeds are reported to contain the highest amount of phytochemicals as compared to red and green macroalgae. [30]

Fucoxanthin accounts for over 10% of the total estimated production of carotenoids in nature. The presence of an allenic bond makes it the first allenic carotenoid, which also makes it highly antioxidant. Fucoxanthin has become a primary biological compound of brown seaweeds as it poses varieties of good properties such as anti-obesity, anti-inflammatory, anti-malarial, and anti-angiogenic, and it also holds protective effects on the liver, brain (blood vessels), and eyes. [31, 32]

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**TABLE- 1: TYPES OF SEAWEEDS**

| S.No. | Types of Seaweeds | Pigments | Examples | Reference |
|---|---|---|---|---|
| 1. | Chlorophyta | Chlorophyll a and b, | *Cladophoraprolifera, Ulvalacuca* | [1] |
| 2. | Rhodophyta | Chlorophyll a and d, phycoerythrin, phycocyanin | *Chondruscrispus, Gigartinapistillata* | [27] |
| 3. | Phaeophyta | Chlorophyll a and c, carotenoids, xanthophylls | *Laminaria japonica, Sargassumheterophyllum* | [31] |

**4. Processing methods for bioplastics:**

The production of bioplastic begins with the extraction of phycocolloids from the seaweeds, but first it needs to be clean to remove contaminants, sand, and other impurities. The extraction of agar and carrageenan starts with alkali pre-treament, which improves gelling properties. Hot water extraction is the next step, followed by alkali extraction to obtain the desired properties. Neutralization is done by removing excess solvents and chemicals through filtering and precipitation. The unwanted portion is eliminated, the pure sample is obtained, and lastly, it is dried out.The alginate extraction process is quite different from that of agar and carrageenan. It also begins with the cleaning of macroalgae, followed by pre-treatment with formaldehyde, which removes the pigments present. Hydrochloric acid (HCl) pre-treatment is done for the removal of formaldehyde residues and phenolic compounds; it also helps to convert insoluble organic salts into soluble ones. A pure sample is obtained after neutralization, and precipitation/filtration which is later dried out. Now the samples obtained could be used for commercial purposes. [3, 33, 34]

Some other methods useful in the extraction process include high-pressure techniques, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and enzyme-assisted extraction (EAE).If phycocolloid extracted is found suitable for production, then it is further treated or added with other solvents, polymers, plasticizers, or other materials so that the desired biopolymer could be developed. If the polysaccharide content of a particular species is high, then it gives a good yield of phycocolloids,favoring the production of plastic biofilms. [33]

*Eucheumaspinosum*, a red macroalgae, has been utilized for the synthesis of bioplastic by mixing the extracted polysaccharide with filler of sorghum stalk and glycerol as plasticizer. The addition of filler and plasticizer enhances the physical and mechanical strength of the polymer. The filler improves durability and hydrophilicity, and the plasticizer increases the flexibility of the polymer. [35]

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The production of bioplastic from seaweeds comes with lots of benefits, which mainly include biodegradability; some of them are edible as well due to their core material. There are certain drawbacks as well in bioplastics production,such as the durability of biopolymers and the large-scale production of macroalgae. Further research is needed for the development of techniques that may enhance the quality of the biodegradable polymer and its production. [36]

## 5. Future aspects and conclusion:

Seaweed is bio-sustainable; its growth rate is much faster than that of any other traditional crop. Seaweeds' bioactive compounds hold great potential for the future of bioplastic production, and the phycocolloids found have good plasticizing properties. The CEO of Sway, Julia Marsh, claims that replacing 25% of our plastic usage would only require 32% of the seaweed currently produced. While the data mentioned is quite significant, that it could eradicate a large portion of the plastic being used. Durability of the polymer becomes a real concern during the synthesis of bioplastic, which can be overcome by the addition of some other stabilizing components to the base material.

The metabolites obtained from seaweeds also possess many properties, such as, being anti-oxidant, anti-microbial, anti-fungal, anti-diabetic, anti-inflammatory, anti-tumoral, anti-malarial, and so on. It is also being used in the development of many pharmaceutical products and health supplements. If we incorporate it into our diet, it might become a superfood.

Seaweed production could be a matter of great interest due to its potential for synthesizing bio-based plastic, which could overcome the use of traditional petro-based plastic and ultimately help reduce plastic pollution.

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Published by : Sai College, Bhilai

ISBN: 978-81-957386-3-2

# CHAPTER - 16
# PROBIOTICS: BIOTECHNOLOGICAL APPLICATION

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**Abstract**

Probiotics, live microorganisms offering health benefits when consumed in adequate amounts, have garnered immense attention for their potential in various domains. This paper provides an overview of probiotics, delving into their origins, mechanisms of action, and applications across medical and industrial sectors.In the medical realm, probiotics play pivotal roles in supporting digestive health, bolstering immune function, and addressing conditions like irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Moreover, they hold promise in women's health, oral health, skin health, mental health, and overall well-being. Probiotics also mitigate antibiotic-associated side effects and maintain gastrointestinal health, underscoring their multifaceted therapeutic potential.Beyond healthcare, probiotics find application in diverse industrial sectors. They enhance agricultural productivity by promoting animal health and aiding wastewater treatment. Probiotics also facilitate bioremediation efforts, contribute to industrial fermentation processes, serve as biofertilizers, and are harnessed in pharmaceutical and biomedical applications.Probiotics offer a versatile solution for enhancing health and well-being, spanning from individual wellness to agricultural sustainability and environmental remediation. However, further research is warranted to elucidate their mechanisms and optimize their applications, ensuring their efficacy and safety across diverse contexts.

**Keywords**: Probiotics, live microorganisms, health benefits, application,.

**Introduction:**

Probiotics have gained significant attention in recent years for their potential to promote health and well-being(Hill et al., 2014). But what exactly are probiotics? Simply put, probiotics are live microorganisms, typically bacteria or yeast, that when consumed in adequate amounts, offer health benefits to the host(Sanders & Gibson, 2014).

These beneficial microorganisms are naturally found in our bodies, particularly in the digestive system, where they play a crucial role in maintaining a healthy balance of gut flora(Guarner&Malagelada, 2003). However, factors such as poor diet, stress, illness, or the use of antibiotics can disrupt this balance, leading to digestive issues and compromising overall health(Hill et al., 2014).

The concept of probiotics traces back thousands of years, with fermented foods like yogurt, kefir, and sauerkraut being traditional sources of these beneficial microbes(Quigley, 2017). It wasn't until the early 20th century that scientists began to study and understand the potential health benefits of probiotics more systematically (Hill et al., 2014).

Today, probiotics are widely researched and available in various forms, including supplements, foods, and beverages(Sanders & Gibson, 2014). They are commonly used to support digestive health,

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improve immune function, and even alleviate symptoms of certain medical conditions such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) (Hill et al., 2014).

The mechanisms through which probiotics exert their beneficial effects are diverse and multifaceted(Guarner&Malagelada, 2003; Hill et al., 2014; Quigley, 2017). They may help by:

- **Restoring the balance of gut flora:** Probiotics can replenish beneficial bacteria in the gut, helping to crowd out harmful pathogens and maintain a healthy microbial community.
- **Enhancing immune function:** Probiotics interact with the immune system, promoting the production of immune cells and antibodies that defend against infections and diseases.
- **Improving digestion:** Probiotics aid in the breakdown and absorption of nutrients, while also helping to regulate bowel movements and reduce symptoms of gastrointestinal discomfort.
- **Producing beneficial compounds:** Some probiotic strains produce substances like short-chain fatty acids, vitamins, and enzymes that contribute to overall health and well-being.

  It's important to note that not all probiotics are created equal. Different strains may have varying effects, and the benefits of specific strains can vary depending on the individual's health status and needs.

**Definition:**

Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014), have garnered significant attention for their potential to promote digestive health, boost immune function, and improve overall well-being. These beneficial microorganisms, predominantly bacteria but also including yeast and other microbes, play essential roles in maintaining the delicate balance of the gut microbiota and supporting various physiological functions in the human body.

**Examples of probiotics:**

1. Lactobacillus acidophilus: This probiotic is commonly found in yogurt and other fermented dairy products. It's known for its ability to support digestive health and boost the immune system.
2. Bifidobacteriumbifidum: Another probiotic strain often found in dairy products like yogurt, as well as in some fermented foods. It helps maintain a healthy balance of bacteria in the gut and may support digestion.
3. Lactobacillus rhamnosus: This probiotic strain is known for its ability to support gastrointestinal health and boost the immune system. It's often used in supplements and some dairy products.
4. Saccharomyces boulardii: Unlike most probiotics, this is a yeast rather than a bacterium. It's used to support digestive health, especially during and after antibiotic treatment, and to help prevent diarrhea.
5. Bifidobacteriumlongum: Found in various dairy products and supplements, this probiotic strain is associated with promoting a healthy balance of gut bacteria and supporting digestive health.

These are just a few examples, and there are many other probiotic strains with various health benefits

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**Application of Probiotics:**

**Medical Application:**

Probiotics have a wide range of applications across various aspects of health and wellness. Here are some common applications:

- **Digestive Health:** Probiotics are often used to support digestive health by helping to maintain a healthy balance of bacteria in the gut. They can aid in the digestion and absorption of nutrients, alleviate symptoms of digestive disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), and reduce the occurrence of diarrhea, including antibiotic-associated diarrhea(Hill et al., 2014).
- **Immune Support:** Certain probiotic strains have been shown to modulate the immune system and support immune function. They can help reduce the risk of infections, such as respiratory infections and urinary tract infections, and may also alleviate symptoms of allergies and autoimmune conditions(Plaza-Díaz et al., 2019).
- **Women's Health:** Probiotics are commonly used to support women's health, particularly in preventing and treating vaginal infections such as yeast infections and bacterial vaginosis(McFarland, 2015). Certain probiotic strains can help restore the balance of vaginal flora and reduce the risk of recurrent infections.
- **Oral Health:** Probiotics may play a role in promoting oral health by inhibiting the growth of harmful bacteria in the mouth, reducing plaque formation, and preventing conditions such as dental caries (cavities) and gum disease(Reid et al., 2010).
- **Skin Health:** Some research suggests that probiotics can benefit skin health by reducing inflammation, promoting wound healing, and alleviating symptoms of skin conditions such as acne, eczema, and rosacea. Probiotic skincare products are increasingly popular for their potential to support a healthy skin microbiome.
- **Mental Health:** The gut-brain axis refers to the bidirectional communication between the gut and the brain, and emerging research suggests that probiotics may influence mood and mental health(Gibson &Roberfroid, 1995). Certain probiotic strains have been studied for their potential to alleviate symptoms of depression, anxiety, and stress.
- **Overall Wellbeing:** While more research is needed, some people take probiotics as part of their daily wellness routine to support overall health and wellbeing. Probiotics are available in various forms, including capsules, tablets, powders, and fermented foods like yogurt, kefir, and kimchi.
- **Antibiotic-associated Side Effects:** Probiotics are commonly used to mitigate the side effects of antibiotic treatment, such as diarrhea and gastrointestinal disturbances. By replenishing beneficial bacteria that may be depleted by antibiotics, probiotics can help maintain gut health and reduce the risk of antibiotic-associated complications.
- **Gastrointestinal Health:** Probiotics are perhaps most well-known for their benefits in maintaining gastrointestinal health. They can help restore and maintain a healthy balance of gut bacteria, which is crucial for proper digestion and absorption of nutrients. Probiotics are used to alleviate symptoms of digestive disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), diarrhea (including antibiotic-associated diarrhea), constipation, and gastroenteritis(Hill et al., 2014).

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- **Prevention and Treatment of Vaginal Infections:** Certain probiotic strains, particularly Lactobacilli, are beneficial for women's health, especially in preventing and treating vaginal infections such as yeast infections (candidiasis) and bacterial vaginosis(McFarland, 2015). Probiotics help restore the natural balance of vaginal flora, creating an environment less conducive to the growth of harmful microorganisms.

These medical applications highlight the diverse potential benefits of probiotics in promoting overall health and well-being, although it's important to note that more research is needed to fully understand their mechanisms of action and therapeutic effects. As with any medical intervention, it's advisable to consult with a healthcare professional before using probiotics, especially if you have specific health concerns or medical conditions.or are pregnant or breastfeeding(Hill et al., 2014).

**Industrial application:**

Probiotics have a range of industrial applications beyond their use in dietary supplements and functional foods. Here are some notable industrial applications of probiotics:

- **Agriculture and Animal Feed:** Probiotics are commonly used in agriculture and animal husbandry to promote the health and growth of livestock, poultry, and aquaculture species. Probiotics added to animal feed can help improve digestion, nutrient absorption, and overall gut health, leading to better growth rates, feed efficiency, and disease resistance. They can also reduce the need for antibiotics in animal production, addressing concerns about antibiotic resistance.
- **Wastewater Treatment:** Certain strains of probiotic bacteria have been investigated for their potential use in wastewater treatment processes. These bacteria can help degrade organic matter, reduce odors, and remove pollutants from wastewater, contributing to more efficient and environmentally friendly treatment methods(Wang & Yu, 2020).
- **Bioremediation:** Probiotics have been explored for their ability to remediate environmental contaminants in soil and water. Certain probiotic microorganisms have the capability to degrade pollutants such as heavy metals, pesticides, and hydrocarbons, thereby helping to clean up contaminated sites and restore environmental health (Wang & Yu, 2020).
- **Biotechnology and Industrial Fermentation:** Probiotic bacteria are used in various biotechnological processes and industrial fermentations. They play roles in the production of fermented foods and beverages such as yogurt, cheese, sauerkraut, kimchi, and kombucha. Probiotics contribute to the fermentation process by producing desirable flavors, textures, and preservation effects, as well as enhancing nutritional value and shelf life(Gu et al., 2020).
- **Biofertilizers:** Probiotic microorganisms, particularly certain strains of nitrogen-fixing bacteria and phosphate-solubilizing bacteria, are used as biofertilizers to enhance soil fertility and plant growth. These beneficial microbes can improve nutrient availability to plants, enhance root development, and confer resistance to soil-borne pathogens, reducing the need for chemical fertilizers and pesticides in agriculture (Saha et al., 2020).
- **Pharmaceuticals and Biomedical:** Probiotics are utilized in the pharmaceutical industry for the development of novel therapeutics and biomedical products. They are studied for their potential in drug delivery systems, oral vaccines, and targeted treatments for conditions such as inflammatory bowel disease (IBD), colorectal cancer, and infections caused by antibiotic-resistant bacteria.

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These industrial applications highlight the versatility and potential of probiotics beyond their traditional use in human health and nutrition. Research and innovation continue to expand the scope of probiotic applications across diverse sectors, contributing to sustainable agriculture, environmental remediation, and advances in biotechnology and healthcare.

**Conclusion:**

The conclusion regarding probiotics is multifaceted and evolving as research continues. Generally, probiotics have shown promise in promoting gut health, aiding digestion, and potentially supporting immune function. However, the effectiveness of specific probiotic strains can vary, and more research is needed to fully understand their mechanisms of action and potential benefits.

Some studies suggest that certain probiotics may help alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Additionally, there is growing interest in the potential role of probiotics in managing conditions like allergies, eczema, and even mental health issues like anxiety and depression, although more evidence is needed in these areas.

It's important to note that probiotics are not a one-size-fits-all solution, and their efficacy can depend on various factors including the specific strain used, dosage, duration of use, and individual differences in gut microbiota composition.

In conclusion, while probiotics hold promise for promoting health and well-being, more research is needed to fully understand their potential benefits, optimal usage, and long-term effects. Individuals interested in using probiotics should consult with healthcare professionals to determine the most appropriate strains and dosages for their specific needs.

In conclusion, probiotics offer a promising avenue for promoting health and preventing disease by harnessing the power of beneficial microorganisms. As research in this field continues to evolve, we can expect to uncover even more insights into the diverse roles and potential applications of probiotics in improving human health.

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ISBN: 978-81-957386-3-2

# CHAPTER-17

# SPAGYRICS: CHALLENGES AND OPPORTUNITIES IN THE MORDEN WORLD

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**Abstract**

Spagyrics, an ancient alchemical practice, has seen a resurgence of interest in recent times due to its holistic approach to healing and transformation. This paper explores the challenges and opportunities presented by the integration of Spagyrics into the modern world. We examine the spiritualcontext of Spagyrics and its philosophical underpinnings, discussing its relevance in contemporary society. Furthermore, we analyse the practical challenges faced in adopting Spagyrics within the framework of modern healthcare and wellness practices. From regulatory hurdles to scientific validation, we address the obstacles that hinder the widespread acceptance of Spagyrics. However, amidst these challenges, we uncover promising opportunities for Spagyrics to contribute to personalized medicine, sustainable agriculture, and ecological restoration. By leveraging interdisciplinary approaches and embracing traditional wisdom alongside modern scientific methodologies, Spagyrics stands poised to offer unique solutions to pressing global issues. This chapter discuses spagyrics for further research and collaboration to unlock the full potential of Spagyrics in addressing the complex challenges of the modern world.

Keywords: spagyrics, research and development, challenges, opportunities.

**Introduction:**

Spagyrics is a term derived from two Greek words: "spao" meaning to separate or divide, and "ageiro" meaning to combine or unite. It refers to a holistic approach to alchemy that involves the separation, purification, and recombination of plant substances to create remedies or elixirs intended for healing and spiritual growth.

The practice of spagyrics dates back to ancient times, with roots in various cultures including Egyptian, Greek, and Arabic. Paracelsus, a Swiss alchemist and physician from the Renaissance period, is often credited with popularizing spagyric principles in the Western world. He believed that by isolating the essential components of plants and recombining them in precise proportions, their therapeutic properties could be maximized. (Thompson, 2019).

Spagyrics is often associated with herbal medicine and natural healing practices, as its focus on the holistic integration of body, mind, and spirit aligns with principles of holistic health and wellness. Practitioners of spagyrics believe that these remedies not only address physical ailments but also support spiritual and emotional well-being, promoting harmony and balance within the individual.

In recent years, there has been a resurgence of interest in spagyrics as people seek alternative and complementary approaches to healthcare. However, it's essential to note that spagyric preparations should be undertaken with care and expertise, as they involve working with potent plant substances and alchemical processes. (Garcia,2020).

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Spagyrics is a term derived from ancient Greek alchemy, which combines the words "spao" (to separate) and "ageiro" (to combine). It refers to a holistic approach to alchemical processes involving the separation, purification, and recombination of materials derived from plants, minerals, and metals to create medicinal and spiritual remedies.

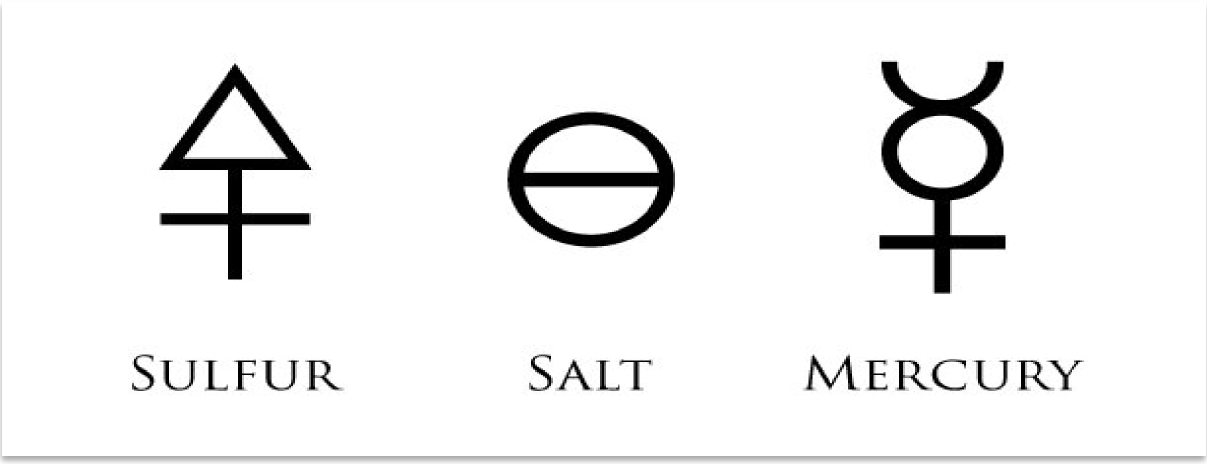
In spagyrics, the goal is not just to isolate particular chemical compounds for medicinal use, but to work with the entire plant or substance, including its physical, energetic, and spiritual aspects. This approach often involves various processes such as fermentation, distillation, extraction, and calcination to extract the essential components and purify them before recombining them in specific ratios. (Albert, 2018).

The process of spagyrics typically involves three main steps:

**Separation:** This step involves breaking down the plant material and separating its various components, such as essential oils, minerals, and other active compounds.

**Purification:** The separated components are then purified through various methods, such as distillation, filtration, or fermentation, to remove impurities and enhance their potency.

**Reunification:** Finally, the purified components are recombined in specific proportions to create a powerful and balanced medicinal remedy.




Spagyric preparations can include tinctures, essences, elixirs, and other herbal remedies that are believed to have enhanced potency and therapeutic effects due to the alchemical processes used in their creation. These preparations are often used in traditional and alternative medicine practices, as well as in spiritual and esoteric traditions for healing and personal transformation.

Spagyric remedies are believed to work on multiple levels, addressing not only physical ailments but also mental, emotional, and spiritual imbalances. Proponents of spagyrics often emphasize the importance of treating the whole person rather than just the symptoms of a specific illness. (Hughes, 2017).

Today, spagyrics continues to be practiced by herbalists, holistic healers, and alchemists around the world. While it is not as widely known or practiced as conventional medicine, it remains a fascinating and influential approach to natural healing and alchemical philosophy.

This process is freeing the body (salts) and allowing them to be further purified and eventually recombined with the soul (sulfur) and spirit (mercury).

The three primal alchemical properties and their correspondence in spagyric remedy are:

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• **Mercury**=water elements, representing the life essence of the plant, the very alcohol extract of the plant is the carrier of the life essence.

• **Salt**=earth element, representing the vegetable salts extracted from calcined ashes of plant body.

• **Sulphur**=fire element, virtue of plant, representing the volatile oil essence of the plant.

**Spagyrics and Spiritual Herbalism:**

It's an alchemical practice that involves the extraction, purification, and recombination of the essential components of plants, particularly focusing on medicinal and spiritual properties. Spagyrists utilize a combination of techniques including fermentation, distillation, and extraction to isolate the plant's essential oils, minerals, and other active constituents.

In the context of spiritual herbalism, spagyrics takes on a deeper significance. It's not just about creating herbal medicines, but also about tapping into the spiritual essence of plants. Practitioners believe that plants have inherent energetic qualities that can affect not just the physical body but also the mind and spirit. By working with plants in a conscious and intentional way, spiritual herbalists seek to connect with the plant's energy and use it for healing and spiritual growth. (Adams,2020).

In spiritual herbalism, plants are often seen as allies and teachers, guiding individuals on their spiritual journey. Herbal preparations such as tinctures, essences, and elixirs are used not only for physical healing but also for ritual, meditation, and ceremony. Each plant is believed to have its own unique energetic signature and healing properties, which can be used to address specific spiritual concerns or imbalances. Overall, spagyrics and spiritual herbalism represent holistic approaches to healing that integrate the physical, mental, and spiritual aspects of health and wellness. They draw on ancient wisdom and alchemical principles to unlock the full potential of plants for both healing and spiritual transformation. (Foster, 2021).

**3.How Do Spagyrics Work?**

Spagyrics is an ancient alchemical practice that involves the extraction, purification, and recombination of plant materials to create medicinal substances. The word "spagyric" comes from Greek roots meaning "to separate" and "to reunite." Spagyrics (which means extraction of divine gifts) is a technique considered to be a sister of Alchemy, with which one can extract the essence of materials from a plan t-based on the three kingdoms of nature; fire, water, and earth and then recombine them to create a single 'essence' which will contain superior. Spagyrics works to purify the blood. In fact, spagyrics cures the metabolic system in the body because metabolism is the first condition to make a blood. When food like protein, vitamins, minerals start to become fully metabolized then hundred percent will start to become poor blood. It starts to load in to the blood and creates oxygenated blood, thus metabolism begins to develop then the immune system becomes active and foreign particles remove from the body. The resulting spagyrics can contain some of the same active ingredients of the plant but in a concentrated form, allowing it to have a much more powerful effect. They work on the spiritual, energetic, and physical levels when consumed. (Bartlett, 2013).

Basic overview of how spagyrics work:( Pacholczyk,2016)

**1.Extraction:** Spagyric preparation typically begins with the extraction of plant material using a suitable solvent, such as alcohol or water. This step aims to draw out the plant's active constituents, including essential oils, alkaloids, and other bioactive compounds.

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**2.Purification:** After extraction, the plant material is subjected to purification processes to remove impurities and unwanted components. This may involve filtration, distillation, or other purification techniques to isolate the desired medicinal compounds.

**3.Calcination**: The purified plant material is then incinerated (calcined) at high temperatures to reduce it to ash. This step serves to release the mineral components of the plant and transform them into a more potent and bioavailable form.

**4.Extraction of Salts**: The ash obtained from calcination is dissolved in water, and the soluble mineral salts are extracted through various processes, such as filtration or precipitation. These mineral salts are believed to contain the essential life force or "soul" of the plant.

**5.Recombination**: Finally, the extracted mineral salts are reunited with the purified plant extracts, resulting in a potent and holistic medicinal preparation. This recombination process aims to restore the balance and synergy of the plant's active constituents, enhancing their therapeutic effects.

**4.HowSpagyrics is better in other system?**

Spagyrics offers several unique advantages compared to other herbal medicine systems:

1. **Holistic Approach**: Spagyrics considers both the physical and energetic aspects of plants, aiming to capture their full spectrum of healing properties. By integrating alchemical principles, it seeks to address not only the symptoms but also the underlying imbalances within the body. Garcia, R., & Patel, S. (2019).
2. **Potency and Synergy**: Through processes like purification, calcination, and recombination, spagyrics enhances the potency and synergy of plant compounds. This results in a more concentrated and balanced medicinal preparation compared to simple herbal extracts, potentially leading to more effective therapeutic outcomes.
3. **Bioavailability:** The alchemical processes in spagyrics are believed to increase the bioavailability of active compounds by transforming them into more readily absorbable forms. This may improve the body's ability to utilize the medicinal properties of plants, enhancing their therapeutic effects.
4. **Energetic Healing:** Spagyrics incorporates the concept of capturing the vital energy or "soul" of plants, which is believed to contribute to their healing properties. By working on both physical and energetic levels, spagyric remedies aim to promote holistic healing and overall well-being. (Adams and Moore,2018).
5. **Spiritual Connection:** Spagyrics has deep roots in alchemical and esoteric traditions, which emphasize the spiritual aspects of healing. Practitioners often view the preparation of spagyric remedies as a sacred and transformative process, fostering a deeper connection between the individual, the plant, and the natural world.

While spagyrics may offer these unique advantages, it's essential to recognize that its effectiveness may vary depending on individual beliefs, practices, and personal experiences. Additionally, scientific validation of spagyric remedies according to modern standards may be limited, as they are often based on traditional and philosophical principles rather than empirical evidence.

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**Bridging the Gap Between Food and Herbs in spagyrics:**

In spagyrics, there is indeed a fascinating bridge between food and herbs, as both are sources of nourishment and healing. Here's how spagyrics can bridge this gap:

1. **Nutritional Alchemy:** Spagyrics sees plants not only as sources of medicinal compounds but also as repositories of vital nutrients. By extracting and purifying these nutrients using alchemical processes, spagyric preparations can enhance their bioavailability and therapeutic potential. This approach can be particularly beneficial when working with herbs that are commonly used as food, such as culinary herbs and spices.
2. **Synergy of Food and Herbs:** Many herbs used in spagyrics are also culinary ingredients, rich in flavour and aroma. By combining these herbs with food or food-based preparations, such as tinctures or elixirs, spagyric remedies can be integrated into daily dietary practices. This allows individuals to incorporate medicinal herbs seamlessly into their diet, promoting both health and enjoyment. (Martinez & Johnson, 2019).
3. **Balancing Body and Spirit:** Food is not only about nourishing the body but also about nurturing the spirit. Similarly, spagyric preparations aim to balance physical healing with spiritual well-being. By infusing food with spagyric remedies, individuals can imbue their meals with not only nutritional benefits but also energetic and healing qualities, creating a harmonious blend of nourishment for both body and spirit.
4. **Culinary Alchemy:** Just as alchemy transforms base metals into gold, culinary alchemy transforms ordinary ingredients into extraordinary dishes. By incorporating spagyric preparations into culinary creations, chefs and home cooks can elevate the Flavours and health benefits of their meals. This culinary alchemy celebrates the synergistic relationship between food and herbs, transforming everyday eating into a sacred and healing experience.

Overall, bridging the gap between food and herbs in spagyrics offers a holistic approach to health and wellness, integrating the nutritional, medicinal, and spiritual aspects of plants into daily life. Whether enjoyed as part of a meal or taken as a medicinal remedy, spagyric preparations can enrich both the body and the soul, fostering a deeper connection to the natural world and the healing power of plants.

**Area Covered by Spagyrics Products:**

Spagyrics products can cover a wide range of areas within health and wellness, including:

1. **Physical Health:** Spagyric remedies are often used to address various physical health concerns, such as digestive issues, respiratory conditions, immune support, hormonal imbalances, and musculoskeletal problems. These products may include herbal tinctures, elixirs, balms, and salves formulated to target specific health issues and promote overall well-being.
2. **Mental and Emotional Wellness:** Spagyrics can also support mental and emotional health by addressing stress, anxiety, depression, insomnia, and cognitive function. Herbal formulations designed to promote relaxation, mood stabilization, mental clarity, and emotional balance are commonly used in spagyric practice. (Brown & Taylor, R. 2020).
3. **Spiritual and Energetic Healing:** Spagyrics products are believed to work on energetic levels, promoting spiritual growth, and supporting energetic balance. These remedies may include plant-based elixirs, essences, and vibrational remedies designed to facilitate personal transformation, spiritual awakening, and energetic healing.

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4. **Nutritional Support:** Some spagyrics products focus on providing nutritional support and enhancing overall vitality. These may include herbal tonics, dietary supplements, and superfood blends formulated to nourish the body, boost energy levels, and support optimal health and vitality.
5. **Beauty and Skincare:** Spagyrics can also be incorporated into beauty and skincare products to promote radiant skin, hair, and nails. Herbal-infused oils, serums, creams, and masks formulated with spagyric extracts are used to hydrate, rejuvenate, and protect the skin, as well as address specific skincare concerns.
6. **Environmental and Energetic Cleansing:** Spagyrics products are sometimes utilized for environmental and energetic cleansing purposes. These may include smudging sprays, room mists, and energetic clearing remedies designed to purify spaces, dispel negative energy, and create a harmonious environment for healing and growth.

Overall, the area covered by spagyrics products is extensive and encompasses various aspects of health, wellness, beauty, and spirituality. These products are often tailored to individual needs and preferences, offering a holistic approach to promoting health and well-being.

**Bridging the Gap Between Food and Herbs in spagyrics:**

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Overall, the area covered by spagyrics products is extensive and encompasses various aspects of health, wellness, beauty, and spirituality. These products are often tailored to individual needs and preferences, offering a holistic approach to promoting health and well-being.

**A Wide Range of Diseases Are Used in Spagyrics Products:**

Spagyrics products are primarily used in holistic and alternative medicine practices, where they are believed to address a wide range of physical, emotional, and spiritual imbalances. While spagyrics is not typically used to treat specific diseases in the conventional medical sense, its remedies are often employed to support overall health and well-being. Some of the conditions and areas where spagyrics products may be utilized include:

1. **Digestive Disorders:** Spagyric tinctures and remedies made from herbs such as ginger, peppermint, or chamomile may be used to support digestive health, alleviate indigestion, and promote gastrointestinal comfort.
2. **Immune Support: Certain** spagyric preparations, such as those made from echinacea, elderberry, or astragalus, are believed to boost the immune system and help the body defend against infections and illnesses. (Miller & Brown,2022).

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3. **Stress and Anxiety**: Spagyrics remedies derived from apoptogenic herbs like ashwagandha, rhodiola, or holy basil may be used to help manage stress, reduce anxiety, and promote emotional balance.
4. **Sleep Disorders**: Spagyric formulations containing herbs like valerian, passionflower, or hops are often used to support restful sleep and address insomnia or sleep disturbances.
5. **Pain Management:** Spagyric extracts from herbs such as arnica, devil's claw, or turmeric may be used topically or internally to help alleviate pain and inflammation associated with conditions like arthritis or muscular strains.
6. **Detoxification:** Spagyrics products may be utilized in detox protocols to support the body's natural detoxification processes and eliminate toxins. Herbs like dandelion, milk thistle, and burdock root are commonly used in spagyric detox blends.
7. **Energy Balance:** Some spagyrics remedies are believed to help balance the body's energy centres or chakras, promoting overall vitality and well-being. These formulations may include herbs with apoptogenic or tonifying properties. Khan, A., & Patel, M. (2023).
8. **Skin Conditions:** Spagyric preparations containing herbs like calendula, comfrey, or plantain may be used topically to soothe and heal various skin conditions such as eczema, psoriasis, or minor wounds. (Brown & Taylor,2020).

It's important to note that while many people find spagyrics remedies beneficial for supporting general health and addressing specific concerns, these products are not a substitute for professional medical advice or treatment. Individuals should consult with a qualified healthcare practitioner before using spagyrics or any other alternative remedies, especially if they have underlying health conditions or are taking medications.

**Research and Development of spagyrics:**

Research and development (R&D) in the field of spagyrics involves exploring the scientific basis, efficacy, safety, and applications of spagyric remedies. While spagyrics has its roots in ancient alchemical traditions, modern R&D efforts aim to bridge the gap between traditional knowledge and contemporary scientific understanding. Here are some key aspects of research and development in spagyrics:

1. **Botanical Studies:** R&D efforts in spagyrics often begin with botanical studies to identify and characterize medicinal plants. This involves studying the chemical composition, pharmacological properties, and potential therapeutic effects of plant extracts used in spagyric preparations.
2. **Pharmacological Research:** Researchers may conduct pharmacological studies to investigate the biological activities and mechanisms of action of spagyric remedies. This includes evaluating their antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and other pharmacological effects using in vitro and in vivo models. Albertus, Frater
3. **Bioavailability Studies:** Bioavailability studies are crucial for understanding how spagyric preparations are absorbed, distributed, metabolized, and eliminated in the body. Researchers may investigate the bioavailability of active compounds in spagyric remedies and compare them to conventional herbal extracts to determine their efficacy and therapeutic potential.
4. **Clinical Trials:** Clinical trials are essential for assessing the safety and efficacy of spagyric remedies in humans. Researchers may conduct randomized controlled trials (RCTs) to


Published by : Sai College, Bhilai

 ISBN: 978-81-957386-3-2

evaluate the effects of spagyric preparations on specific health conditions, such as digestive disorders, respiratory ailments, mental health disorders, and chronic diseases.

5. **Quality Control and Standardization:** Quality control measures are necessary to ensure the consistency, purity, and safety of spagyric products. Researchers may develop standardized methods for the extraction, purification, and analysis of spagyric preparations, as well as establish criteria for quality control and authentication of raw materials.
6. **Safety Assessment:** Safety assessment studies are conducted to evaluate the potential toxicity, adverse effects, and contraindications of spagyric remedies. Researchers may perform acute and chronic toxicity studies, as well as assess the genotoxicity, mutagenicity, and carcinogenicity of spagyric preparations to ensure their safety for human consumption.
7. **Formulation Development**: Formulation development involves optimizing the composition, dosage form, and delivery system of spagyric remedies to enhance their stability, bioavailability, and therapeutic efficacy. Researchers may explore innovative approaches, such as nanoencapsulation, to improve the delivery of active compounds in spagyric preparations.

Overall, research and development in spagyrics aim to validate its traditional practices, expand its therapeutic applications, and integrate it into modern healthcare systems. By combining scientific rigor with ancient wisdom, researchers strive to unlock the full potential of spagyric remedies for promoting health and well-being.

**Spagyrics Revolution:**

The term "spagyrics" refers to a branch of alchemy that involves the extraction, purification, and recombination of plant substances for medicinal and spiritual purposes. It originated in ancient Greece and was further developed during the Islamic Golden Age and the European Renaissance. However, in recent years, there has been a renewed interest and a kind of "spagyrics revolution" in the realm of alternative medicine and holistic healing.

Several factors contribute to this resurgence:

1. **Alternative Medicine Trends:** There has been a growing interest in alternative and complementary medicine approaches, as people seek more natural and holistic methods of healing. Spagyrics, with its focus on plant-based remedies and spiritual well-being, aligns well with this trend.
2. **Rediscovery of Traditional Knowledge:** Many traditional healing practices, including spagyrics, were sidelined with the advent of modern medicine but are now being rediscovered and reevaluated. This is partly due to dissatisfaction with the side effects and limitations of pharmaceutical drugs.3
3. **Holistic Health Movement:** The holistic health movement emphasizes the interconnectedness of the body, mind, and spirit. Spagyrics offers a holistic approach to healing, addressing not only physical ailments but also emotional and spiritual imbalances.
4. **Advancements in Herbal Medicine Research:** Scientific research on the medicinal properties of plants has advanced significantly in recent years. This has provided a scientific basis for the efficacy of many traditional herbal remedies, including those used in spagyrics.
5. **Cultural Shift Towards Sustainability:** There's a growing awareness of environmental issues and a desire to live more sustainably. Spagyrics promotes a deep respect for nature and

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the responsible use of plant resources, making it appealing to those who are environmentally conscious. (Paracelsus ,1988).

Overall, the "spagyrics revolution" represents a shift towards reclaiming ancient wisdom and integrating it with modern knowledge and practices to promote health and well-being in a holistic manner.

**Future Challenges and Opportunities of Spagyrics:**

Looking ahead, the field of spagyrics may encounter both challenges and opportunities as it continues to evolve and gain recognition. Here are some potential future challenges and opportunities for spagyrics:( Patel, 2023)

**Challenges:**

1. **Regulatory Hurdles**: Spagyrics involves the preparation and use of herbal extracts, which may face regulatory challenges in some jurisdictions. Ensuring compliance with regulations regarding herbal supplements and alternative medicines could be a hurdle for spagyric practitioners and businesses.
2. **Scientific Validation:** While there is growing anecdotal evidence and historical usage of spagyrics, scientific validation through rigorous clinical trials and research studies may be lacking. Obtaining funding and conducting such research to establish the efficacy and safety of spagyric remedies could be a challenge.
3. **Education and Training:** Spagyrics requires specialized knowledge and skills in alchemy, herbalism, and holistic healing practices. Providing comprehensive education and training programs for spagyric practitioners may be challenging, especially given the diverse backgrounds and interests of potential learners.
4. **Market Competition:** As interest in alternative medicine grows, the market for herbal remedies and holistic healing approaches becomes increasingly competitive. Spagyric practitioners and businesses may face challenges in differentiating themselves and establishing their unique value proposition in the market.

**Opportunities:**

1. **Integration with Modern Medicine:** There is growing interest in integrating complementary and alternative medicine (CAM) approaches with conventional medical practices. Spagyrics, with its holistic approach to healing, has the potential to complement mainstream medical treatments and contribute to integrative healthcare models.
2. **Personalized Medicine:** Spagyrics emphasizes the individualized approach to healing, taking into account a person's unique constitution and needs. As personalized medicine continues to gain traction, spagyric remedies tailored to individual patients could offer significant opportunities for customization and effectiveness.
3. **Research and Innovation:** Continued scientific research and innovation in the field of herbal medicine and alchemy could lead to new discoveries and advancements in spagyrics. Exploring the synergistic effects of plant extracts and refining spagyric extraction techniques could unlock new therapeutic potentials.

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4. **Consumer Demand for Natural Remedies:** With growing concerns about synthetic chemicals and pharmaceutical side effects, there is a rising demand for natural and plant-based remedies. Spagyrics, with its emphasis on organic and sustainably sourced ingredients, is well-positioned to capitalize on this trend.
5. **Global Outreach and Collaboration:** Spagyrics has roots in various cultures and traditions around the world. Building bridges between different healing traditions and fostering cross-cultural collaboration could enrich the practice of spagyrics and expand its reach to new audiences globally.

In navigating these challenges and seizing opportunities, stakeholders in the spagyrics community will need to collaborate, innovate, and advocate for the recognition and integration of spagyrics into mainstream healthcare systems

**Conclusion:**

The conclusion of a chapter on the Spagyrics system could summarize the key points discussed throughout the chapter and offer some final insights or reflections. In conclusion, the Spagyrics system presents a holistic approach to herbal medicine and alchemy that integrates principles from various philosophical and scientific traditions. Throughout this chapter, we have explored the foundational concepts of Spagyrics, including the principles of separation, purification, and recombination.

One of the defining features of Spagyrics is its emphasis on the unity of body, mind, and spirit. By treating the whole person rather than just the symptoms of illness, Spagyric remedies aim to restore balance and promote wellness on all levels of being. This holistic approach resonates with ancient healing traditions from cultures around the world, as well as with modern integrative medicine practices.

Moreover, the alchemical aspect of Spagyrics adds a spiritual dimension to the healing process, emphasizing the transformation of the individual on a deeper level. By working with the alchemical principles of sulphur, mercury, and salt, Spagyric practitioners seek to transmute not only physical ailments but also emotional, mental, and spiritual imbalances.

While Spagyrics may seem esoteric to some, its principles and practices offer valuable insights into the interconnectedness of all aspects of life. By incorporating the wisdom of nature and the wisdom of the alchemical tradition, Spagyrics provides a framework for understanding and promoting health and vitality in both individuals and communities.

As we continue to explore the potential of Spagyrics in modern times, it is essential to approach this ancient system with respect and humility, recognizing that our understanding is always evolving. By embracing the principles of Spagyrics with an open mind and a compassionate heart, we can tap into the transformative power of nature and unlock the full potential of healing for ourselves and for future generations.

ISBN: 978-81-957386-3-2

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ISBN: 978-81-957386-3-2

# CHAPTER- 18

# BIODEGRADATION STUDIES ON LOW DENSITY POLYETHYLENE BY MICROORGANISMS ISOLATED FROM SOIL

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**Abstract**

The plastics are synthetic material made from polymers, which are long chains of molecules. They have become an integral part of our everyday lives due to their durability, versatility and low cost of production.However, the improper disposal and excessive use of plastics have led to significant environmental issues.This research aims to biochemically identify and characterize polythene degrading bacteria from the various dumping site. The isolated microorganisms are screened and used for degradation of low density polyethylene. Through isolation and cultivation techniques the study focuses on determining the metabolic pathways involved in polyethylene degradation.The Biodegradation of low density polyethylene is done by using weight loss measurement and pH change. Enzymatic assays and molecular techniques are employed to identify key enzymes responsible for breaking down polythene.One potential isolate was identified through 16S rRNA sequence as *Enterobacter cloacae* .The comprehensive characterization of these bacteria provides insights into their potential applications for eco-friendly waste management and contributes to our understanding of microbial plastic degradation mechanisms.

**Key words:** Plastic, Polythene, Biodegradation, environmental issues,enzymatic assays.

## Introduction:

The name plastic is emanate from the Greek word “Plastikos” which means “capable of being shaped or molded”.This term accurately describes the property of plastic materials,which can be easily molded into different forms and used for various purpose.The plastic pollution has become a significant environmental concern worldwide (Divyalaxmi *et al.*,2016).The plastics having the property of light weight,strong and durability.Due to this property it is used in food clothing ,shelter,transportation,recreation and medical industry (Hussein *et.al*.2015).

The plastic is categorized into various kind such as Polyethylene (Low density polyethylene and High density polyethylene), Poly ethylene terepthalate, Nylon , Polypropylene, polystyrene,Polyvinyl chloride,Polyurethane.The low density Polyethylene(LDPE) is the most commonly used plastic.It is the polymer of ethylene monomer.(Alshehrei 2017 and Thomas *et.al*.,2015).The degradation of LDPE naturally in the environment is a long process.Before the bio-degradation of the polyethylene numerous environmental factors like temperature, air humidity,moisture content, pH, solar energy and polymer properties changes the surface of polyethylene.(Gupta *et.al*,2019).

Biodegradation is a natural process by which microorganisms break down the organic substance into simpler compounds.The microorganisms uses the simpler compounds as source of energy and nutrients for their growth and survival.Through metabolic processes microorganism converted these compounds into simpler form such as carbon dioxide or water.(Borghei *et al*.2010).

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The aim of the present study is to isolate and identify the low density polyethylene degrading bacteria and used for the degradation of polyethylene.

**Materials and methods:**

**1)Sample collection:**

The soil sample were collected from the various dumping site such as Borsi , Durg , Risali , Maroda , Kolihapuri of Durg region of Chhattisgarh.

The low density polyethylene films (0.910-0.940g/$cm^{3)}$ were collected from the local market of Durg ( Gajendiran *et al.*,2016 and Priyanka *et al.*,2011).

**1.Isolation of bacteria:**

The low density polyethylene degrading bacteria was isolated from the various soil sample by using minimal synthetic medium containing $NH_4NO_3$ (1.0g), $MgSO_4.7H_2O$ (0.2g), $K_2HPO_4$ (1.0g), $Cacl_2.2H_2O$ (0.1g), KCl (0.15g),Yeast extract (0.1g), $FeSO_4.6H_20$ (1.0mg),$ZnSO_4.7H_2O$ (1.0 mg),$MnSO_4$(1.0 mg),Tween 60 or 80 (0.01-0.50%(v/v)),Agar by serial dilution and spread plate method and incubated at $37^0$ C for 24 hrs.(Gilan *et.al.*,2004)

**2. Screening of polyethylene degrading bacteria:**

The screening of low density polyethylene degrading bacteria was done by zone of clearance methods by using the polyethylene glycol in minimal media.The zone of clearance around the colonies were observed by staining with commassie blue. (Singh *et al*.,2016).

**3. Biochemical Characterization:**

The bacterial isolates were initially identified by morphological and biochemical characteristics. Morphological characterization of the isolates was done by the Gram staining Biochemical characterization was done through IMViC ,Carbohydrate fermentation, Gelatine hydrolysis, Starch hydrolysis, Catalase, Urease production tests (Singh *et. al.*,2016 and Ariba Begam *et al.*,2015).

**4. Biodegradation Studies:**

**I. Weight loss measurement:**

The measurement of weight loss was done by inoculating the isolated bacteria into the minimal medium. The polyethylene film of 2×2 was inserted into the minimal medium and incubated in rotatory flask shaker at 120 rpm for 30 Days at $37^0$C.After incubation polyethylene films were washed with 2 % (v/v) sodium dodecyl sulfate solution and distilled water then dried overnight at $50^0$c and weight loss was calculated by using the formula (Abraham *et. al.*,2016 and Dede *et.al* 2013).

Weight loss %=initial weight−final weight initial weight×100

**II.pH Change:**

The polyethylene degradation is also observed by the pH change which results due to the metabolic activity.The microorganisms presents in the medium degrading the polyethylene film and use the carbon as source of energy and nutrients (Duddu *et.al.*, 2015).

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**III. Molecular identification:**

The bacterial strain which shows high potentiality in weight loss measurement and pH change was sequenced by16S rRNA.

**IV.Fourier transform infrared spectroscopy(FTIR):**

The surface analysis of polyethylene film was done by Fourier transform infrared spectroscopy.It is used for the analysis of changes in the structure by the breakdown of some bonds and the presence of functional group (Abraham *et.al*.,2016).

**Result:**

**Morphological and Biochemical characteristics:**

Morphological and biochemical characteristics were performed for the identification of bacteria.Two different samples were identified by gram staining and biochemical tests(Table 1).The identified strains were *Klebsiella sp.and Enterobacter sp.*respectively.

**Table 1:Morphological and Biochemical characteristics**

| Bacterial isolates | Gram staining | Indol | MR | VP | Citrate | Starch Hydrolysis | Catalase | Urease | Gelatine | Fermentation of carbohydrates | Probably identified organism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BS1** | Gram –ve, Rod shaped | -ve | +ve | -ve | +ve | +ve | +ve | +ve | -ve | +ve | ***Klebsiella sp.*** |
| **BS6** | Gram –ve Rod shaped | -ve | -ve | +ve | +ve | +ve | -ve | -ve | -ve | -ve | ***Enterobact-er sp.*** |

**Weight loss measurement:**

The measurement of weight loss was done in the interval of 10 days by using mineral salt medium.The sample BS6 (Table 2) showed the high degradation rate.

**Table 2: Weight loss measurements**

| Sample code | Strain | Weight loss in 30 days | Weight loss in 60 Days |
|---|---|---|---|
| **BS1** | *Klebsiella sp.* | 20 % | 36.37 % |
| **BS6** | *Enterobacter sp.* | 30% | 40 % |


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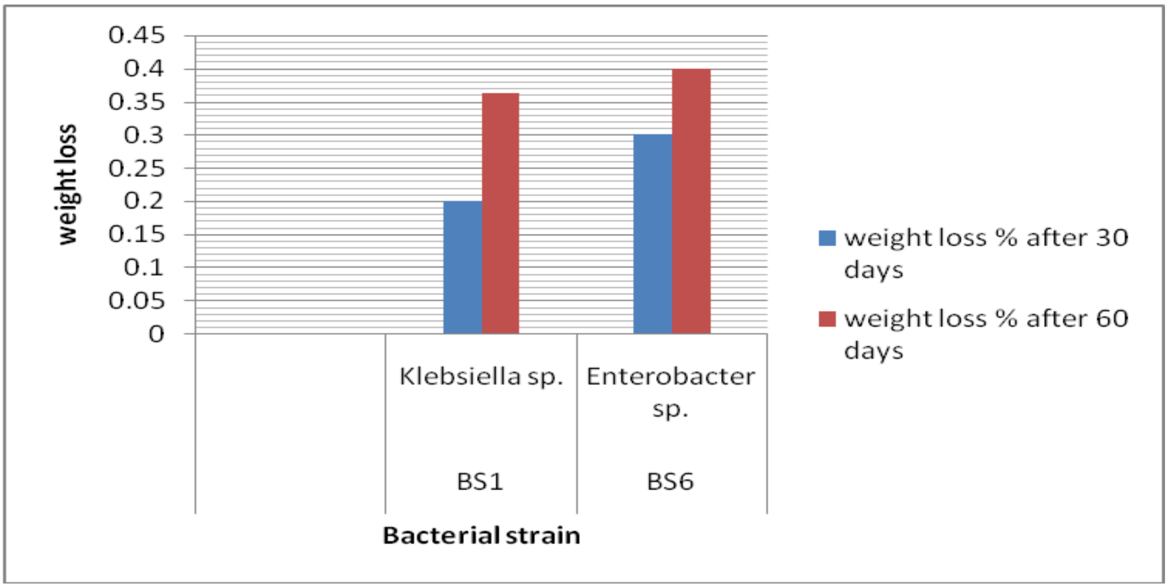


**Fig.1.Weight loss of lowdensity polyethylene at the interval of 30 days.**

**pH Change:**

The polyethylene degradation is also measured by the pH change at the interval of 10 days.The strain BS6 (Table 3) shows the confirmation pH change by the production of acid.

**Table 3:Measurement of pH change**

| Sample code | Strain | Initial pH | pH Change in 10 days | pH Change in 20 days | pH Change in 30 Days |
|---|---|---|---|---|---|
| **BS1** | ***Klebsiella sp.*** | **7.0** | **6.76** | **6.33** | **6.00** |
| **BS6** | ***Enterobacter sp.*** | **7.0** | **6.70** | **6.42** | **5.90** |

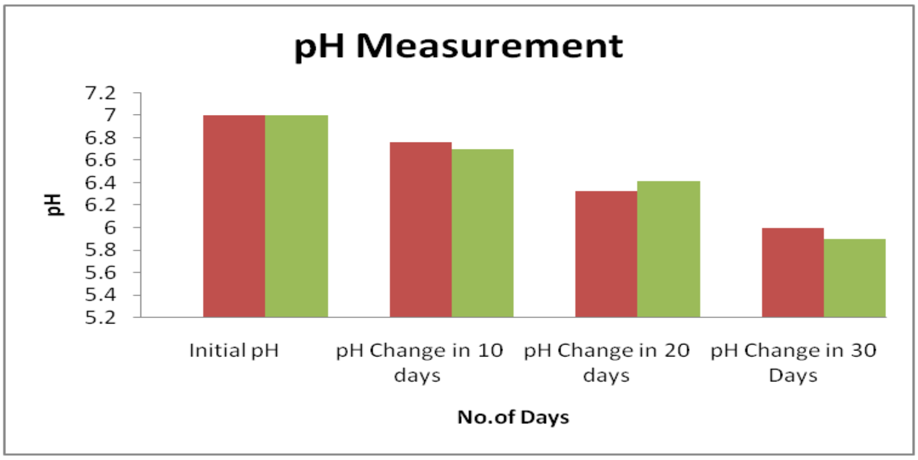


**Fig.2.pH Change at the interval of 10 days**

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**Molecular identification:**

The bacterial strain which shows high potentiality in weight loss measurement and pH change was sequenced by16S rRNA.The Microbe was identified as ***Enterobacter cloacae***as it showed highest similarity of 92.19 % with ***Enterobacter cloacae*** strain CTRTIPSB2-1 16S ribosomal RNA gene.


**Fig.3Phylogenetic analysis of *Enterobacter cloacae*strain CTRTIPSB2-1 16S ribosomal RNA gene.**

**Fourier transform infrared spectroscopy(FTIR):**

The surface analysis of polyethylene film was done by Fourier transform infrared spectroscopy.It is used for the analysis of changes in the structure and the presence of functional group.(Das *et.al.*,2015)

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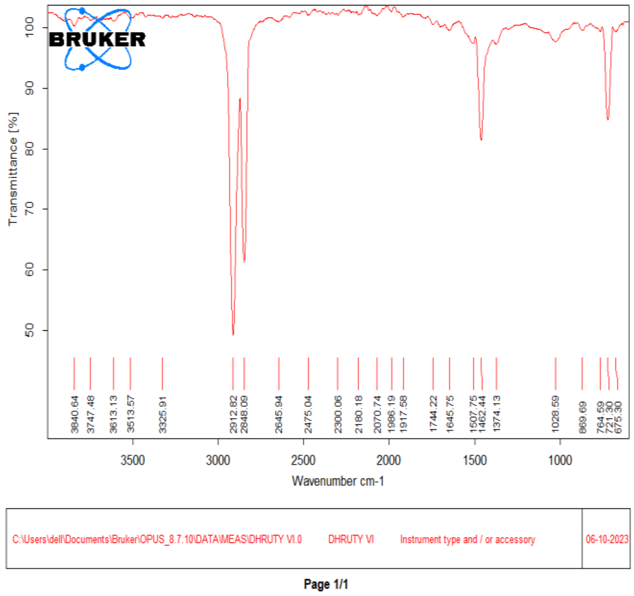


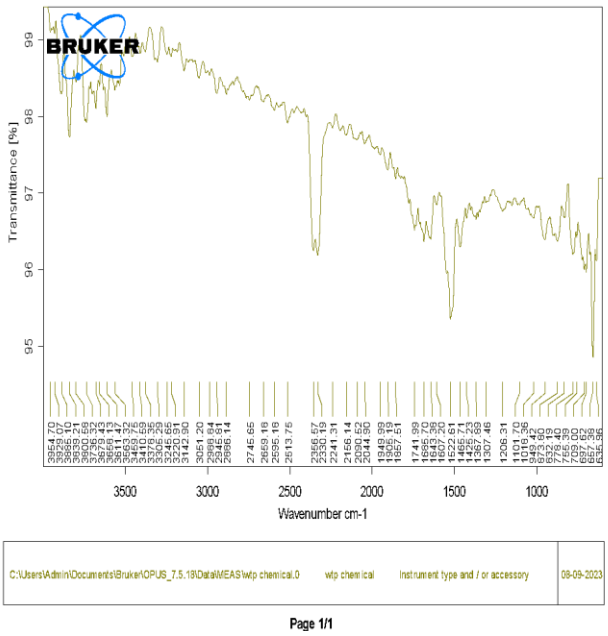


**Fig.4. FTIR spectrum of bio degradation of low density polyethylene film after 60 days of incubation (a)FTIR spectrum of strain BS6 (b) FTIR spectrum of control**

**Conclusion and discussion:**

Bacteria play a significant role in the degradation of polyethylene. Through a process called Biodegradation,certain strains of bacteria have been found to possess the necessary enzymes to break down polyethylene molecules into smaller compounds.This degradation process occurs in the presence of oxygen ,with bacteria utilizing polyethylene as a carbon and energy source. Research has shown that the activity of these polyethylene degrading bacteria can lead to a reduction in the amount of plastic waste in the environment.The ability of bacteria to break down polyethylene holds immense potential for developing sustainable solutions to plastic pollution.The Biodegradation process was confirmed by the weight loss,pH change studies at the time interval. The FTIR studies shows the chemical change in the pollutant leading formation of simple compounds.The molecular characterization of the potential isolates is ***Enterobacter cloacae.***

ISBN: 978-81-957386-3-2

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ISBN: 978-81-957386-3-2

# CHAPTER- 19
# CRISPR-CAS GENOME EDITING

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## Abstract

CRISPR-Cas technology has emerged as a powerful tool for precise genome editing, providing unprecedented control of genetic variation in many organisms. This review provides an overview of recent advances in CRISPR-Cas technology and its various applications in basic research, therapeutic interventions, and agricultural improvement. The versatility and efficiency of CRISPR-Cas systems have allowed scientists to achieve targeted changes with unprecedented precision, revolutionizing our ability to manipulate the genetic code. In basic research, CRISPR-Cas has facilitated the study of gene function, regulation, and disease mechanisms, leading to deep insights into biological processes. In addition, CRISPR-based therapies show promise in the treatment of genetic disorders, infectious diseases and cancer, offering potential therapeutic options where conventional therapies fall short. In agriculture, CRISPR-Cas technology has the potential to revolutionize crop improvement by enabling precise changes to improve yield, disease resistance and nutritional value. Despite its enormous potential, challenges such as off-site effects and ethical considerations remain, highlighting the need for continued research and ethical oversight. Overall, CRISPR-Cas genome editing represents a paradigm shift in biotechnology and offers unprecedented opportunities to address fundamental questions in biology and address pressing challenges in human health and food safety. .

**Keywords:** Crispr, enormous potential, therapeutic interventions, revolutionizing, genome editing.

**Introduction to CRISPR CAS technology:**

**Cas proteins**: Cas proteins are enzymes that play a crucial role in the CRISPR-Cas system. They act as molecular scissors capable of cutting DNA at specific points. Different Cas proteins have different functions, but they generally work together with a guide RNA to locate and cleave target DNA.**Guide RNA** (gRNA): guide RNA is a synthetic RNA molecule that directs Cas proteins to a desired position in the DNA sequence. It consists of a framework sequence required for Cas protein binding and a variable sequence that defines the target DNA site. The gRNA is designed to complement the DNA sequence of the target gene, directing the Cas protein to the exact site for editing. **Target DNA**: This refers to the specific DNA sequence in the genome that the CRISPR-Cas system is programmed to modify. Cas protein binds to target DNA under the guidance of gRNA and induces a double-strand break (DSB) at or near the desired site.

**Mechanism of action:**

1. Recognition: The gRNA is designed to be complementary to a specific sequence in the target DNA. After synthesis, the gRNA forms a complex with the Cas protein.
2. Binding: The Cas-gRNA complex scans the genome for a DNA sequence that matches the gRNA target sequence. When the Cas protein finds a complementary sequence, it binds to the target DNA.

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3. Cleavage: Upon binding to the target DNA, the Cas protein induces a double-strand break (DSB) in the DNA molecule. This break can be repaired by cellular DNA repair mechanisms, which can lead to gene deletion, insertion or replacement depending on the editing strategy used.

**Comparison with other genome editing technologies:**

CRISPR-Cas technology offers several advantages over other genome editing technologies such as TALEN (Transcription Activator-Like Effector Nucleases) and zinc finger nucleases:-

1. Ease of design: CRISPR-Cas systems are relatively easy to design and implement compared to TALENs and zinc finger nucleases, which require more complex protein engineering.
2. Versatility: CRISPR-Cas systems can be easily reprogrammed to target different DNA sequences simply by changing the sequence of the guide RNA. This versatility makes the CRISPR-Cas technology more adaptable to different editing applications.
3. Efficiency: CRISPR-Cas systems generally have higher editing efficiency compared to TALEN systems and zinc finger nucleases, resulting in more reliable and consistent results.
4. Cost: CRISPR-Cas technology is generally more cost-effective due to its simplicity and versatility, making it available to more researchers and laboratories.

**Key and Components tools:**

1. **Cas proteins:-** • Cas9: Cas9 from Streptococcus pyogenes is the best known and most used protein related to CRISPR. It acts as a molecular scissors and cuts DNA at specific target sequences guided by single-stranded RNA (sgRNA).
    - Cas12a (formerly Cpf1): Cas12a is an alternative to Cas9 and has some distinct advantages, such as stepwise DNA production. cuts and identification of different target sequences (T-rich PAM sites). It can be used in applications similar to Cas9, including genome editing and gene regulation.
    - Cas13: Unlike Cas9 and Cas12a, which target DNA, Cas13 targets RNA. It can be used for RNA editing, RNA knockdown and diagnostic applications. Cas13 is notable for its ability to target and cleave specific RNA sequences .
2. **Guide RNA (gRNA) selection and optimization:-•** Design principles: Several factors must be considered when designing gRNAs, including target specificity, off-target effects, binding efficiency and target-site accessibility. Tools such as CRISPR design software help select optimal gRNA sequences based on these criteria.
    - Optimization: gRNA sequences can be optimized to improve efficiency and specificity. This may include editing the sequence to improve target detection or minimize off-target effects .
3. **Delivery methods:-** • Viral vectors: Viral vectors such as lentiviruses and adeno-associated viruses (AAV) are commonly used to deliver CRISPR components into target cells. They ensure efficient delivery and stable integration of CRISPR machinery into the host genome.
    - **Lipid nanoparticles**: Lipid-based nanoparticles are non-viral delivery systems used to encapsulate and protect CRISPR components, facilitating their delivery into cells. They are particularly useful for in vivo applications.


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• **Electroporation:** Electroporation uses short electrical pulses to create temporary pores in the cell membrane, allowing CRISPR components to enter the cell. It is a versatile method suitable for multiple cell types and can be used for both in vitro and ex vivo applications.

• **Other methods:** Other delivery methods include microinjection, cell penetrating peptides and physical methods such as ultrasound delivery. they have their advantages and limitations depending on the specific test requirements.

**Applications in basic research:**

CRISPR-Cas technology is used in basic research to determine gene function and biological processes:-

1. Gene function studies:- • Knockout experiments: CRISPR-Cas9 is widely used to create gene knockouts by inducing double strand breaks (DSBs) at specific genomic loci. By disrupting a target gene, researchers can study its function and the phenotypic consequences of its loss.

   • Example: Using CRISPR-Cas9 to delete the BRCA1 gene in human cell lines to study its role in DNA repair and breast cancer susceptibility.

   • Knock-in experiments: CRISPR-Cas technology allows genetic sequences to be precisely aligned to specific genomic locations. This allows researchers to introduce mutations, reporter genes or regulatory elements to study their effects on gene function.

   • Example: inserting a fluorescent reporter gene into the genome of a model organism to monitor the expression pattern of a target gene during development.

Gene regulation experiments: CRISPR-based tools such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) allow targeted modulation of gene expression without changing the underlying DNA sequence.

Example: Using CRISPR to simultaneously silence the expression of multiple genes to study their shared role in a biological pathway.

**Elucidation of biological processes and disease mechanisms:**

Functional genomics: CRISPR-based screening methods such as CRISPR deletion screens and CRISPR activation/inhibition screens enable large-scale interrogation of gene function to identify key players in biological processes or diseases. traces. Example: performing a genome-wide CRISPR deletion screen to identify genes important for cancer cell viability or drug resistance.

Modeling human disease: CRISPR-Cas technology can be used to create accurate disease models in a variety of organisms, including cell lines. . . and animal models. These models can be used to study diseases, identify therapeutic targets, and test potential therapies. Example: Generation of patient-specific induced pluripotent stem cells (iPSC) carrying disease-associated mutations and using CRISPR-Cas9 to correct or introduce mutations to study the pathogenesis of genetic disorders.

Functional annotation of genomes: CRISPR-based tools are used to annotate non-coding regions of the genome, such as regulatory elements and non-coding RNAs, to understand their role in gene regulation and disease. Example: CRISPR-based functional annotation .commentary examining genes involved in the regulation of gene expression to identify enhancers during development or disease progression.

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**Therapeutic interventions:**

CRISPR-based Approaches to Treat Genetic Disorders: Gene Repair: CRISPR-Cas technology can be used to correct disease-causing mutations by precisely changing the DNA sequence of a target locus. This approach is particularly promising for monogenic disorders caused by single nucleotide mutations or small insertions/deletions.

Gene disruption: In cases where the disease phenotype is due to the expression of a faulty gene, CRISPR-Cas can be used to disrupt the gene knockout , its inhibitory expression and improvement of the disease phenotype.

Gene regulation: CRISPR-based tools such as CRISPRi and CRISPRa can change gene expression levels without changing the underlying DNA sequence. This approach can be used to up- or down-regulate the expression of specific genes involved in disease pathways.

Clinical trials using CRISPR-Cas technology:- • Although CRISPR-based therapies are still in the early stages of clinical development, several clinical trials are underway to evaluate the safety and efficacy of these approaches in the treatment of various genetic disorders.

Examples Ongoing clinical trials include blood disorders (eg sickle cell anemia and beta-thalassemia) genetic studies for treatment, as well as studies targeting genetic forms of blindness, muscular dystrophy and certain types of cancer.

These clinical studies mainly involves ex vivo editing of a patient's cells (eg, hematopoietic stem cells), followed by transplantation back to the patient or local delivery of CRISPR components to specific tissues.

3. **Challenges and Considerations in Translation of CRISPR-based Therapeutics:**

Off-Target Effects: One of the main concerns of CRISPR-based therapeutics is the potential for off-target editing when unintended changes are made to genomic sites (eg, the target. sequence) .Strategies to minimize off-target effects include optimizing gRNA design, using high-quality Cas enzymes, and using sensitive detection methods to detect off-target events.

• Immune responses: The immune system can recognize CRISPR components as Cas proteins or viruses. . delivery vectors that cause immune reactions that may limit the effectiveness of therapy or pose safety risks to the patient. Strategies to mitigate immune responses include the use of non-immunogenic delivery vehicles and the development of Cas proteins to reduce immunogenicity.

• Delivery challenges: Effective delivery of CRISPR components to target tissues or cells remains a challenge, especially for in vivo applications. Improvements in delivery methods, such as the development of tissue-specific targeting strategies and new delivery vehicles, are essential to the success of CRISPR-based therapies.

**Agricultural Applications:**

CRISPR-Cas technology has enormous potential in agricultural applications, revolutionizing crop improvement efforts. Here's an overview of its use, successful case studies, and regulatory and mainstream adoption considerations.

1. Improving Crop Traits: • Yield Enhancement: CRISPR-Cas technology enables precise modification of genes associated with yield-related traits, such as plant architecture, flowering time,


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and photosynthetic efficiency. By optimizing these traits, researchers aim to increase crop productivity and yield.

• Nutritional Enhancement: CRISPR-Cas can be used to enhance the nutritional content of crops by modifying genes involved in nutrient metabolism and accumulation. For example, researchers are working on increasing the levels of essential vitamins, minerals, and antioxidants in staple crops to address nutritional deficiencies.

• Biotic and Abiotic Stress Resistance: CRISPR-Cas offers a powerful tool for developing crops with enhanced resistance to pests, diseases, and environmental stresses such as drought, salinity, and extreme temperatures. By editing genes involved in stress response pathways, researchers can engineer crops with improved resilience under challenging growing conditions.

2. Case Studies of Successful Applications:

• Wheat Rust Resistance: Researchers used CRISPR-Cas9 to edit genes associated with susceptibility to wheat rust, a devastating fungal disease. By introducing mutations in these genes, they conferred permanent resistance to several strains of pathogens and offered a sustainable solution to protect wheat crops

. • High-yield rice: CRISPR-Cas technology was used to modify genes involved in regulating grain size and yield in rice By precisely modifying these genes, researchers have developed rice varieties with greatly increased grain yield without compromising other agronomic traits.

• Nutrient-enriched corn: Researchers have used CRISPR-Cas9 to improve levels of the previtamin beta-carotene in corn kernels. By modifying genes involved in the carotenoid biosynthetic pathway, they developed corn varieties with better nutritional value that improved vitamin A deficiency in areas that depend on corn as a staple food.

3. Regulatory Considerations and Public Acceptance:

• Regulatory Landscape: Legislation on CRISPR-Cas modified crops varies by country and region. Genome-modified crops are considered non-genetically modified organisms (GMOs) by some regulatory agencies, while others are subject to regulations similar to traditional GMOs.

• Public acceptance: public perception of CRISPR-Cas genetically modified crops can vary depending of factors. such as transparency. , safety and perceived benefits. Efforts to engage stakeholders, provide clear communication about the technology and its applications, and address safety concerns are essential for fostering public acceptance.

**Biotechnological and industrial uses**:

CRISPR-based technologies have increasingly found applications in industrial biotechnology, providing precise and efficient tools for strain engineering, enzyme optimization and bioprocessing. Here is an overview of CRISPR-based bioengineering applications and examples of successful applications:-

1. CRISPR-based applications in bioprocessing:- • Stem technology: CRISPR-Cas systems enable targeted modification of microbial genomes to improve their efficiency in bioprocessing applications. This includes improving properties such as substrate utilization, product yield and environmental tolerance.

   • Enzyme optimization: CRISPR-based approaches can be used to create enzymes to improve catalytic activity, substrate specificity and stability. By precisely editing the genes encoding

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enzymes, scientists can tailor their properties to optimize bioprocesses such as pharmaceutical synthesis and biofuel production.

• Pathway engineering: CRISPR-Cas technology facilitates the creation and optimization of metabolic pathways for the biosynthesis of valuable compounds, including .drugs, biochemicals and biofuels. This involves assembling synthetic pathways in microbial hosts and optimizing pathway flux through targeted genetic modifications.

2. Examples of successful applications:- • Production of biofuels: scientists used CRISPR-Cas technology to engineer microbial strains to improve the production of biofuels such as ethanol, butanol and biodiesel. This includes increasing the ethanol yield of yeast strains by altering genes involved in ethanol fermentation pathways and improving the tolerance of microbial hosts to toxic fermentation byproducts.

Pharmaceutical synthesis:- CRISPR-Cas has been used to optimize production in microbial hosts. pharmaceutical compounds, including antibiotics, antivirals and anticancer drugs. By designing microbial strains with better productivity and performance, researchers aim to reduce production costs and increase the availability of therapeutic compounds.

Enzyme engineering:- CRISPR-based approaches have been used to optimize the properties of enzymes, including enzymes used in industrial biocatalysis stability, substrate specificity and catalytic efficiency. This includes the design of enzymes for applications such as biopolymer synthesis, food processing and detergent formulation.

**Future Directions and Challenges:**

Future directions and challenges:- • Efficient screening: Advances in CRISPR-based technologies, such as multiplexed genome editing and high-throughput screening methods, allow rapid characterization and optimization of microbial strains and enzymes for industrial bioprocessing applications.

• Regulatory considerations: CRISPR-edited organisms and the regulatory environment surrounding the use of products in industrial settings is evolving. Regulatory agencies are evaluating the safety and environmental impact of CRISPR-modified organisms and developing guidelines for their commercial use.

• Scale-up and commercialization: As CRISPR-based biotechnologies advance, scaling up bioprocesses for large-scale production remains a challenge. commercialization This includes optimization of fermentation conditions, further processing and purification of products to meet the requirements of industrial production.

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ISBN: 978-81-957386-3-2

# CHAPTER- 20
# BIOFUEL: A GREEN ALTERNATIVE

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## Introduction:

Biofuel refers to renewable fuels derived from organic materials, such as plants, algae, or waste. These fuels are considered an alternative to conventional fossil fuels because they can be produced from renewable resources, potentially reducing greenhouse gas emissions and dependence on non-renewable energy sources.

Common types include ethanol from crops like corn or sugarcane, biodiesel from vegetable oils or animal fats, and biogas produced from organic waste.

Microorganisms encompass microscopic living organisms like bacteria, fungi, protozoa, and algae, existing ubiquitously on Earth and playing pivotal roles in diverse ecosystems. These minute organisms participate in nutrient recycling, decomposition and symbiotic relationships. In biofuel production, selected microorganisms are utilized for their capacity to convert organic matter into usable energy sources. This demonstrates their multifaceted and impactful roles across various fields, from medicine to environmental sustainability. Biofuels produced by microorganisms offer a promising renewable energy source. (Khan *et al.*, 2021)

## Production of biofuels:

Utilizing the biological processes of microorganisms, biofuels are created from organic materials. Microbes like bacteria, yeast, and algae play key roles, employing their metabolic activities to ferment or break down biomass into fuels like ethanol, biodiesel, or biogas. This method offers an environmentally sustainable alternative to traditional fossil fuels (Ganesan *et al.* 2020). (Fig. 1)

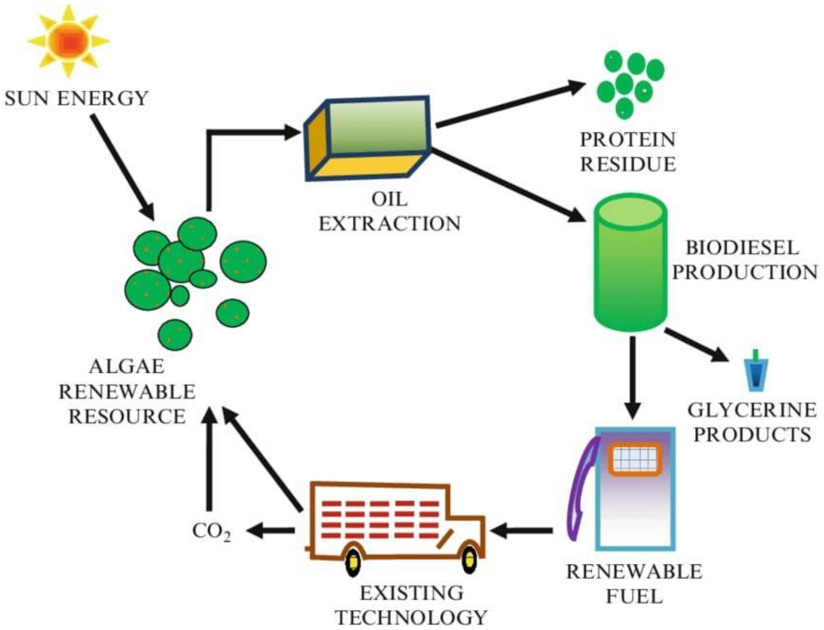


**Fig. 1: Production of biofuel by algae**

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**The process involved in biofuel production by microorganisms:**

This process can vary based on the specific microorganisms used, the feedstock employed, and the type of biofuel being produced. Additionally, research and advancements continue to refine and optimize each stage of biofuel production by microorganisms for increased efficiency and sustainability. Biofuel production by microorganisms involves several key steps:

- Feedstock Selection: Diverse organic materials, ranging from agricultural residues e.g., corn stover, sugarcane bagasse to energy crops such as switchgrass and algae and organic waste food scraps, animal fats, serve as the foundation for biofuel production.
- Preparation: Selected feedstock undergoes preparatory treatments, including grinding, chopping, or other processes to enhance accessibility and breakdown by microorganisms.
- Microorganism Selection: Tailored microorganisms, meticulously chosen for their efficiency in converting the designated feedstock into biofuels, encompass bacteria e.g., Escherichia coli, yeast e.g., Saccharomyces cerevisiae, or specific algae strains.
- Fermentation/Breakdown: Employing fermentation or enzymatic mechanisms, microorganisms intricately break down the prepared feedstock. For instance, bacteria and yeast adeptly transform sugars into ethanol via fermentation, while algae exhibit lipid synthesis suitable for biodiesel production.
- Harvesting/Extraction: Extraction of the resultant biofuel from the microbial culture medium occurs, utilizing methodologies such as filtration, centrifugation, or distillation, depending on the specific biofuel type.
- Refinement/Processing: Ensuring biofuel purity and meeting stringent quality standards necessitates subsequent refining processes. Distillation, filtration, or tailored chemical treatments are implemented to eliminate impurities and enhance biofuel quality.
- Utilization: The refined biofuel product stands prepared for multifaceted applications. Direct usage of ethanol as a fuel or its blending with gasoline, alongside biodiesel utilization in diesel engines with minimal modifications, exemplify the broad spectrum of potential applications. (Fig. 2; Fig. 3)


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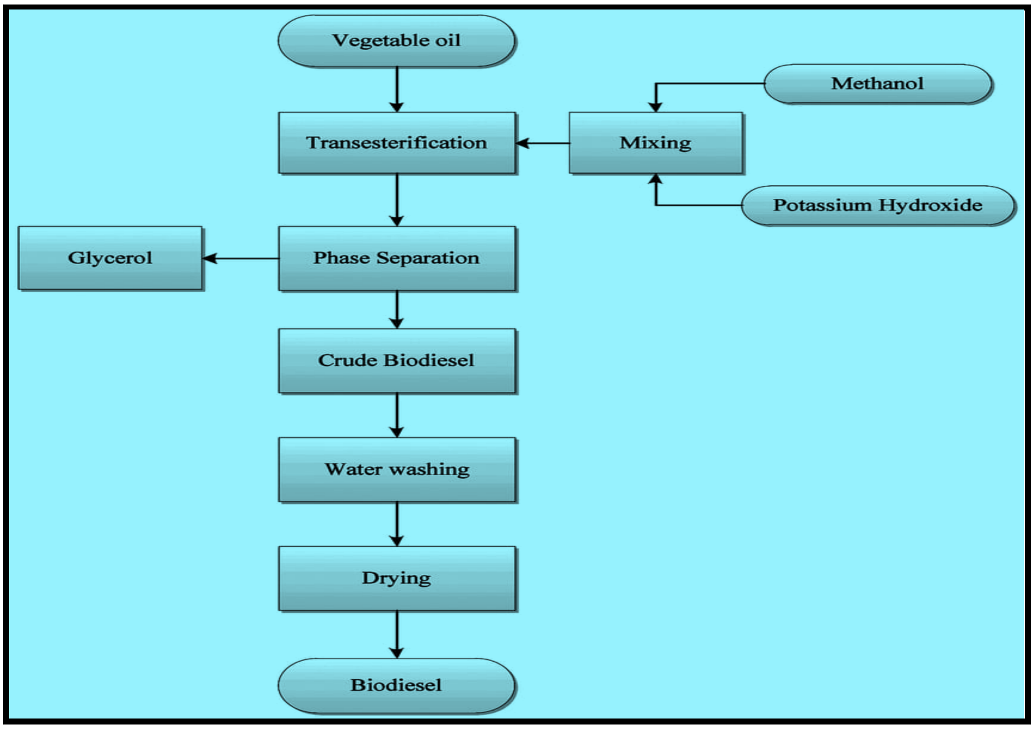


**Fig. 2: Production of biofuel**

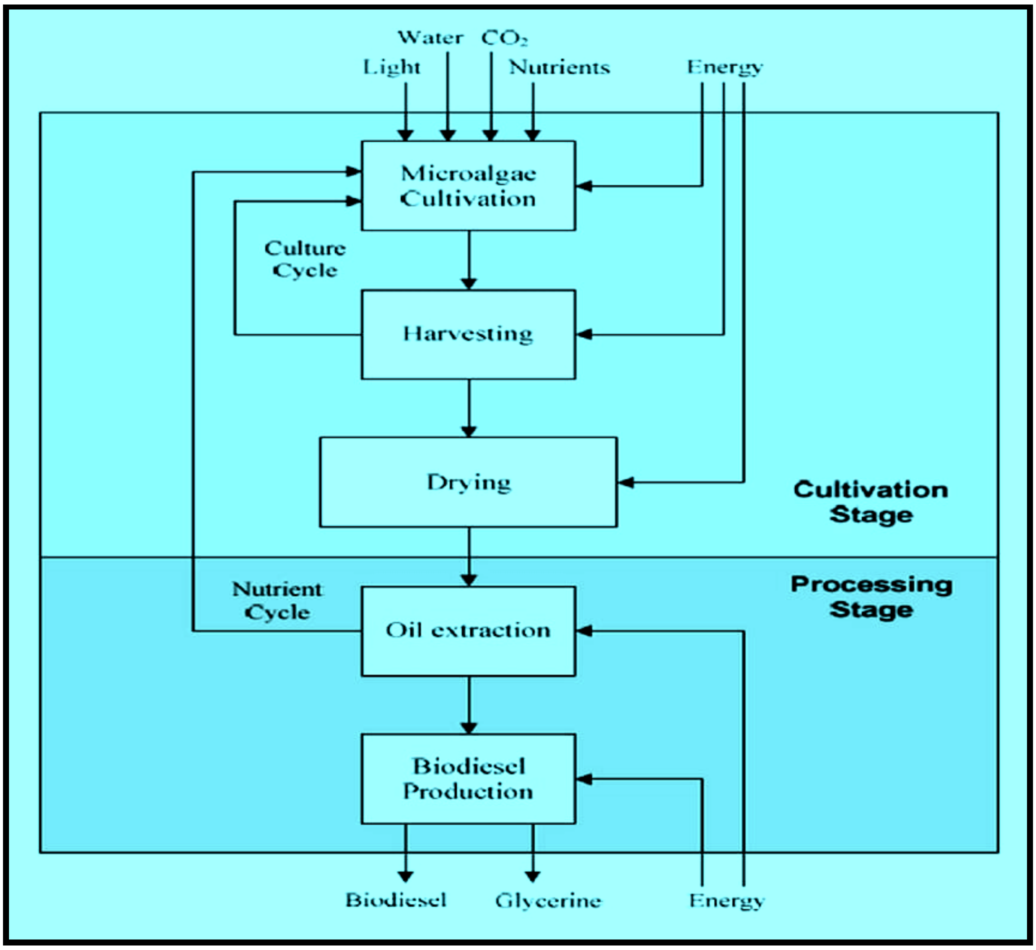


**Fig. 3: Production of biofuel by microorganism**


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**Scope of biofuels:**

The scope of biofuels is vast and spans various sectors and applications. Here are some key areas:

- Transportation: Biofuels can be used as a substitute for gasoline and diesel in vehicles, airplanes, and ships. Ethanol and biodiesel are commonly used in transportation.
- Energy Generation: Biofuels can be utilized in power plants to generate electricity. Biogas produced from organic waste is an example commonly used for this purpose.
- Industrial Applications: Industries can use biofuels as an energy source in manufacturing processes, reducing reliance on fossil fuels.
- Environmental Impact: Biofuels have the potential to reduce greenhouse gas emissions compared to conventional fuels, contributing to efforts in combating climate change.
- Agriculture and Waste Management: Biofuels encourage the use of agricultural waste and organic materials, promoting sustainable practices and reducing waste.
- Economic Opportunities: The biofuel industry offers opportunities for innovation, research, and investment in sustainable energy sources.

**The scope for biofuels in Chhattisgarh:**

In Chhattisgarh, the scope for biofuels is significant due to the state's rich agricultural resources and focus on sustainable development. Here are some aspects highlighting the potential scope:

- Agricultural Resources: Chhattisgarh has abundant agricultural land and produces a variety of crops like rice, maize, sugarcane, and oilseeds. These resources can be used for biofuel production, especially ethanol from sugarcane and biodiesel from oilseeds.
- Government Initiatives: The state government might have initiatives or policies promoting biofuel production, encouraging farmers to engage in crops suitable for biofuel production and supporting related industries. (Sori *et al.* 2015)
- Employment Opportunities: Biofuel production could create employment opportunities, especially in rural areas, through farming, processing units, and associated industries.
- Environmental Sustainability: Embracing biofuels aligns with environmental sustainability goals by reducing carbon emissions and promoting cleaner energy sources.
- Research and Development: There might be opportunities for research and development in improving biofuel production techniques and optimizing crop yields for biofuel purposes.
- Energy Security: Using biofuels can contribute to the state's energy security by reducing dependency on imported fossil fuels. (Rodionova *et al.* 2017)

**Need for biofuel production:**

Harnessing the capabilities of microorganisms for biofuel production aligns with the pursuit of sustainable, environmentally friendly and economically viable energy solutions, making it a crucial area of research and development in the renewable energy sector and development in the quest for a more sustainable energy future.

The need for biofuel production by microorganisms arises due to several critical reasons:

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- Environmental Concerns: Fossil fuel consumption contributes to climate change and environmental degradation. Biofuels produced by microorganisms offer a more sustainable and eco-friendly alternative, potentially reducing greenhouse gas emissions.
- Finite Nature of Fossil Fuels: Fossil fuels are limited and non-renewable. Biofuels derived from microorganisms utilize renewable resources like organic waste, algae, and crops, reducing dependency on finite fossil fuel reserves.
- Energy Security: Biofuels diversify the energy mix, contributing to energy security by decreasing reliance on imported fossil fuels and offering a more resilient energy system.
- Economic Benefits: Biofuel production can create economic opportunities by stimulating investment, innovation, and job creation in the renewable energy sector.
- Waste Management: Utilizing microorganisms to produce biofuels allows for the conversion of organic waste into valuable energy sources, addressing waste management issues.
- Global Sustainability Goals: Producing biofuels aligns with global sustainability targets by promoting cleaner energy sources and reducing the carbon footprint associated with traditional fossil fuels.

**Importance of biofuel production by microorganisms:**

The importance of biofuel production by microorganisms lies in its capacity to offer renewable energy sources, significantly reduce greenhouse gas emissions, and promote sustainable practices. Leveraging microorganisms for biofuel production enables the utilization of renewable resources such as agricultural waste, algae, and organic matter, thereby diminishing reliance on finite fossil fuel reserves. Moreover, it fosters economic growth by creating opportunities for innovation, research, and employment in the renewable energy sector.

Addressing environmental concerns related to fossil fuel consumption, microorganism-produced biofuels offer a more eco-friendly alternative, contributing to mitigating climate change and supporting global sustainability goals. The transformative potential of biofuels derived from microorganisms is evident in their diverse feedstock options and their ability to convert various organic materials, including waste products, into valuable energy sources. This not only addresses waste management issues but also contributes to global efforts in promoting cleaner energy sources and reducing the carbon footprint associated with traditional fossil fuels. (Mahapatra *et al.* 2021)

**Conclusion:**

The imperative need for biofuel production by microorganisms arises from a confluence of environmental, economic, and global sustainability factors. This necessitates continued investment in research and development, recognizing the pivotal role of biofuels derived from microorganisms in shaping a more sustainable energy future.

Biofuels emerge as a viable prospect for ameliorating environmental apprehensions linked to conventional fuels. Despite their promise, the widescale integration of biofuels necessitates the resolution of challenges such as land use conflicts and the refinement of associated technologies. Persistent research endeavors are imperative to optimize their efficacy and ascertain a sustainable trajectory for the future of energy.

In conclusion, biofuels offer a promising alternative to conventional fossil fuels, contributing to reduced greenhouse gas emissions and increased energy sustainability. However, challenges such as


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technological advancements, and the overall environmental impact need careful consideration for the widespread adoption of biofuels. Continued research and development are essential to address these challenges and maximize the potential benefits of biofuel production.

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 ISBN: 978-81-957386-3-2

# CHAPTER- 21
# GUT MICROBIOTA AND AUTISM SPECTRUM DISORDER

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## Abstract

In recent years, many studies have shown bidirectional flow of information between gut and brain. The microbiota residing in the gut is shown to affect various aspects of human health mainly cardiological, neurological & auto-immune disorders. Although there are evidences suggesting the existence of this Brain-Gut axis, further research is needed to unravel the detailed mechanism. The utilization of this aspect for therapeutic purposes in various disorders requires further knowledge and intervention. This review focusses on one of the neurodevelopmental disorders that is Autism Spectrum disorder(ASD). There has been significant rise worldwide in the number of cases of ASD in recent years. ASD is a neurodevelopmental disorder that affects social communication, language and verbal skills of children to variable extents. This behavioural impairment interferes with the social life of the individual. Many studies have indicated a correlation between n gut microbiota and their metabolites on symptoms of autism. The role of diet, prebiotics and probiotics as a measure of mitigation of ASD is also emphasized in the present study.

**Keywords**- ASD, Gut microbiota, neurological disorders.

**Introduction:**

Researchers all over the world have been trying from past two decades to delve deep into the relationship of gut microbiome and autism spectrum disorder (ASD). Recent breakthrough researches have proved that there is a definite association between changes in gut microbiota and symptoms of individuals affected with ASD. Studies that confirm an intricate relationship between gut microbiome and ASD can prove to be beneficial for treatment and therapeutic purposes.

Autism spectrum disorder (ASD) is a complex neurodevelopmental issue specified by a wide range of cognitive, behavioural, and communication disabilities. It includes Asperger's syndrome (AS), and pervasive developmental disorder not otherwise specified (PDD-NOS) [1]. The prevalence of ASD in children and adolescents is 0.36% in Asia [2] and 1.85% in Western countries [3]. It is a spectrum disorder which means that the symptoms and the severity differs widely among affected individuals. Around 1 in 100 children are being diagnosed with this disorder [4]. Sometimes the characteristics are identifiable in very early stages but many a times it is diagnosed much later. Apart from challenges in social interaction, communication and verbal abilities, individuals sometimesdisplay atypical behaviour like repetitive activities, difficulty in transition from one task to another and sensory issues like heightened response to light, sound and touch. Individuals also vary in their dealing with these issues like some evolve over time and are able to live independently while others need lifetime assistance and support.Both genetic and environmental factors are known to contribute to this disorder. Genetic factors are found responsible in 10-20% of ASD cases [5].

This raises a question on etiology of rest of the occurrences. Environmental factors that leads to predisposition for ASD include air pollution, exposure to pesticides, occurrence of infections,

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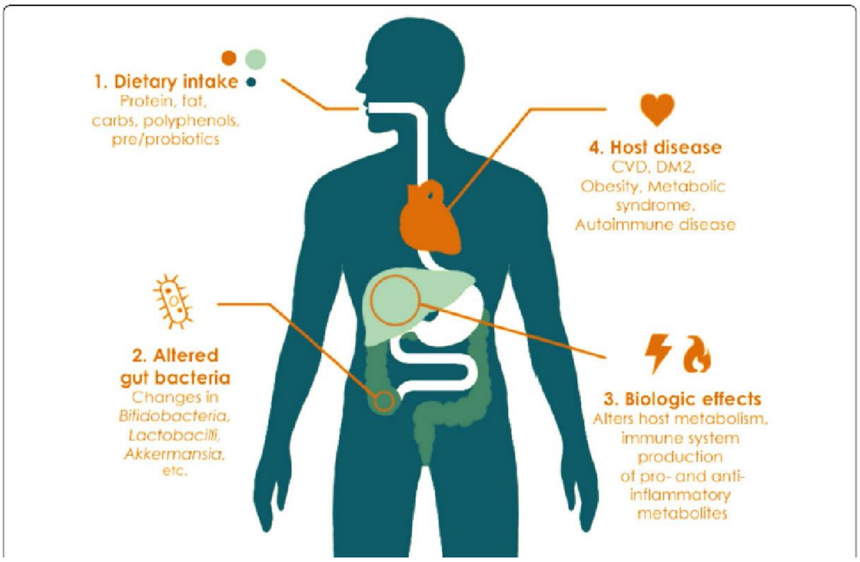
inflammation & usage of antibiotics by mother during pregnancy [6]. Apart from this, impairment of immune system and gastrointestinal disturbances have also been observed which amounts to severity of symptoms of ASD [7]. Several recent studies have shown the association of gut dysbiosis with behavioral patterns and intensity of ASD.
Gut microbiota can be defined as the collection of microbes present inside the human gut while gut microbiome is the term used to define their collective genome [8]. Its a non-genetic but heritable [9],contributor to behaviors& impacts development and function of the immune, metabolic, and nervous systems [10]. It is generally believed that the initial colonization starts at birth when maternal microbiota is acquired during vaginal delivery; however, recent studies suggests that this acquisition can have happened during gestation too .

The constituents of early-life microbiota depends on the delivery methods, hygiene, and feeding practices . Initially in an healthy infant gut there is predominance of *Bifidobacterium* and *Lactobacillus* however it varies due to change in diet and finally reaches a somewhat adult like composition around age 3 [11]. The microbiota of healthy adult gut comprises of *Firmicutes* and *Bacteroidetes*, along with lesser numbers of *Proteobacteria, Verrucomicrobia and Actinobacteria* [12]. In recent years the information regarding microbiota and its importance in maintaining health has increased along with the recognition that the loss and changes in gut microbiota can lead to immunological, cardiovascular, neurological and various other chronic disorders [13]. Alterations in gut microbiota can be attributed to antibiotic treatment, diet, stress and immune challenges which disturbs the balance between possible pathogenic microbes and beneficial commensals in the gut. This gut dysbiosis then cause many health issues including ASD [14]. Diet and other factors may alter the microbiome which may be only in the host or can be inherited by the offspring. Combined with the genetic factor, this may lead to alteration of the disease symptoms and its severity [15].
Its observed that even a ‘‘pathogenic’’ microbiome itself may lead to Initiation of disease in otherwise non-prone people [16]. For example, in a study wild type mice displayed specific behavioral patterns when transplanted with microbiota from patients with neurodegenerative issues[17].




Impact of Gut microbiota on human health. [18]

**Relation between Gut microbiota& autism:**
In a 2019 study by Sharon et al, it was seen that mice transplanted with microbiome associated with human ASD displayed ASD like behaviours compared to those transplanted with microbiome of

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typically developing humans. Extensive alternative splicing of risk genes was seen in brains of mice harbouring ASD microbiota [19]. Several other studies have shown the difference in gut bacterial communities between individuals with ASD and other typically developing ones. [20,21] and in corresponding mouse models of ASD as well [22]. Most diversified fecalmicrobiome profiles are seen in ASD subjects that have GI dysfunction, a common feature associated with autism [23]. Furthermore, specific interventions involving microorganisms like fecal transplantation, use of antibiotics, and probiotics have proven to be effective in few human trials [24]. In some studies, involving animal models of ASD, specific gut bacteria have shown to have therapeutic potential too [25]. Also, alterations in microbiome consequently changes the metabolic profiles, leading to changes in availability and variety of nutrients and microbial metabolites [26]. Differences in metabolomic profiles of ASD subjects and typically developed individuals have been observed in metabolomic profiles obtained by analyses of serum, feces and urine samples. Such studies uncovered the presence of many dysregulated compounds of microbial origin as well [27]. Particularly the differences found in amino acid transport and degradation among the two classes of individuals is worth attention as amino acids serve as precursors of many neuroactive compounds like neurotransmitters [28].

**Dysbiosis in Autism Spectrum Disorder:**

Multiple factors are known to cause dysbiosis in ASD individuals. Like high fat content in the diet of mother during pregnancy changes the gut microbiota in infants which may be associated with ASD [29]. Likewise, infants breast-fed for 6 months showed lesser risk of ASD and representation of *Clostridium difficile* in the Gut as compared to formula –fed infants [30]. Another factor affecting gut dysbiosis is usage of antibiotics in both humans and animal models, which shows long lasting impacts even if taken for a short span of time [31]. Research has shown that administration of antibiotics during the first 3 years of life to children leads to differences in compositions of gut microbiota [32]. Also, children treated with macrolide antibiotics displayed long lasting changes in their gut microbiota which may be linked to asthma and obesity [31]. Apart from ASD, several other conditions like as immunological defects, Crohn's disease, obesity, inflammatory bowel disease (IBD) etc are linked with gut dysbiosis [33].

Role of antibiotics was proven in another study where female mice were administered antiepileptic drug valproic acid (VPA) during pregnancy, which resulted in autism-like social behaviors and alterations in *Firmicutes* to *Bacteroidetes* ratio in the offspring [34]. There is a generalized increase in microflora and reduction in microbial diversity in ASD gut, which possibly leads to overgrowth of harmful bacteria which further leads to severity of autistic symptoms [35]. It is found in pyrosequencing of the gut microbiome from faecal samples that there is higher prevalence of *Bacteroidetes* and lower level of *Firmicutes* . Within the Firmicutes phylum, the increased representation of the genus *Clostridium* in autistic children has been observed in several researches [36]. Like in a study by Parracho et al. higher incidence of Clostridium histolyticum in autistic children than in the control group of healthy children was reported. Similarly several other studies confirmed the abundance of Clostridium perfringens in the stool samples of autistic children was [37]. These *Bacteroidetes* and other short chain fatty acid (SFA) producing bacteria modulate the gut-brain axis by producing their metabolites like propionic acid, which may influence the central nervous system and lead to autistic behavior [36]. De Angelis and colleagues also got similar results in faecal samples of autistic children where lesser number of Firmicutes compared to controls were seen [38].

Apart from these phyla ,reduced levels of *Prevotella, Coprococcus and Veillonellaceae*, which are involved in carbohydrate digestion and fermentation & lower levels of the genus Bifidobacterium, which has a possible protective role in autism due to its anti-inflammatory properties, have also been reported[36]. Furthermore there is increased prevalence of *Lactobacillus, Clostridium, Desulfovibrio,*



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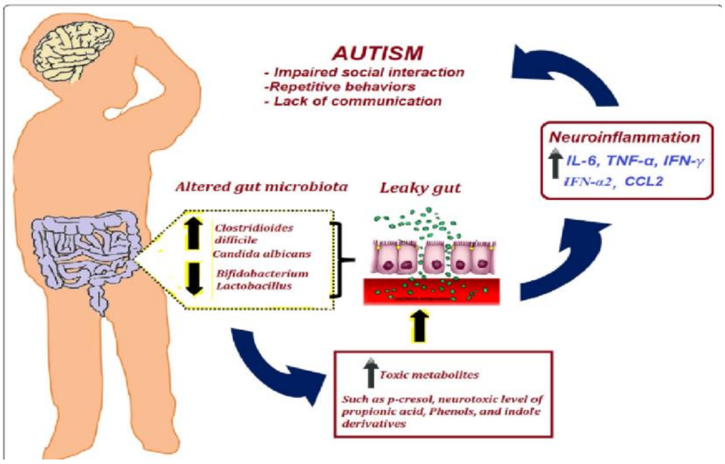
*Caloramator, Alistipes, Sarcina, Akkermansia,Sutterellaceae* and *Enterobacteriaceae* in children with ASD compared to healthy children [38]. Similarly an anaerobic bacillus, *Desulfovibrio,* that is resistant to common antibiotics such as cephalosporins, is also more frequently found in individuals with ASD [36]. Increased numbers of the genus *Sutterella,* which is linked to mucosal metabolism, were also observed in Duodenal biopsies of ASD affected individuals [39].

**Autism & GI disturbances:**

A wide range of GI disturbances like constipation, feeding issues, gastric reflux, abdominal pain and diarrhoea, have been found in ASD individuals. This led to the hypothesis of a possible connection between gut dysbiosis and autism [40]. Recent researches have shown that around 40% of ASD patients have complaints regarding GI disturbances [41]. Scientists have come up with the thought that altered microbiota associated with GI issues in a child that has genetic predisposition for ASD tends to express an autistic phenotype or may show severity of neurological & behavioural symptoms [42].Consequently an autistic child with GI disturbances may exhibit severe anxiety social withdrawal and irritability compared to the ones without GI disturbances. [43]. This is a vice versa effect where the severity of the neurobehavioral symptoms seem to increase the risk of having GI problems . Evidences have shown that these GI disturbances in autistic children may be associated to gut dysbiosis due to the inflammatory state [44], and the correlation between the two has been proved by the occurrence of abnormal faecal microbiota in both patients with inflammatory bowel syndrome and in Autistic children with GI disturbances. Researchers have shown that the transcriptional profile of ileal and colonic tissues of autistic children and individuals with inflammatory bowel disease were similar [45]. A recent research has shown that higher levels of proinflammatory cytokines are found in astrocytes derived from ASD people as compared to control group which may be the reason of changes in neuronal development and synapses in children with ASD [46].The whole idea behind this "inflammation hypothesis" is that distortion in the gut epithelial barrier which controls the movement of molecules from GI tract leads to altered gut permeability [44]. Gut microbiota and their metabolites play a crucial role in maintaining the integrity of this epithelial barrier and hence gut dysbiosis in ASD patients leads to the phenomenon called "leaky gut" [47].

This condition may permit the movement of bacteria and toxins such as LPS and metabolites that stimulate the immune system and produces an inflammatory state into the bloodstream [38]. Further this activated immune system then leads to the release of inflammatory molecules like cytokines and chemokines which impact the Central nervous system. As a consequence the early stages of brain development are modulated and leads to the onset of autism [48].




Association of disrupted gut microbiota and leaky gut in the pathogenesis of autism.Role of toxic metabolites & inflammation leading to onset of typical patterns of ASD [49].

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**Theureupetic approaches- prebiotics & probiotics:**

Presently there are no clear- cut or efficient therapies for ASD. The approved and suggested Treatment strategy for ASD essentially include occupational , behavioral& educational therapies along with psychological and pharmacological methods [50].Due to evidences indicating association of gut dysbiosis in ASD, researchers are working on the reclamation of healthy gut microbiota through administration of oral prebiotics & probiotics. Prebiotics can be defined as non-digestible compounds like oligosaccharides and inulin that are broken down in gastro intestinal tract and promote the colonization & proliferation of favourable gut bacteria such as *Lactobacilli* and *Bifidobacteria* [51]. A study showed the bifidogenic effect of galacto oligosaccharides in autistic as well as non-autistic individuals [52]. It has shown potential of suppressing the neuroendocrine stress response and improving attention span in healthy subjects [53].

Compared to prebiotics, most of the researches focus on the administration of probiotics. Probiotics are living harmless microorganisms that can prove beneficial for health in Various conditions, such as Inflammatory Bowel Disease(IBD) , obesity, colorectal cancer, and neurological diseases [48].Various pre-clinical trials have shown the potential benefits of probiotics in treatment of neurological disorders [46], which are further confirmed by human clinical trials [54]. The efficiency of probiotics in reducing gut inflammation and improvement of GI symptoms in children with IBD [55], has led to the suggestion that these may decrease inflammation and improve Social Behavior of children with ASD as well [40]. Hsiao and colleagues observed that The oral administration of Bacteroides fragilis (1 x $10^9$ CFU) in a pre-clinical model of maternal immune-activated offspring displayed improved gut permeability, recolonization of normal intestinal microbiota and ameliorated autistic behavioral patterns [47].

This indicated microbial intervention as an efficient therapeutic approach for ASD. In later studies it was seen that administration of specific bacteria and not many other species could alleviate symtoms associated with ASD. For instance it was observed that *Lactobacillusreuteri*, but not other species of probiotics, could lead to reversal of social behavioural abnormalities only and not the repetitive behaviours and anxiety. Also , Lactobacillus reuteri successfully improved the mesolimbic dopamine reward system by enhancing the oxytocin levels, which is thought to be disrupted in ASD [56].Several probiotic vs placebo trials are done using strains like *L. plantarum*, *L. acidophilus*, *L. rhamnosus* ,*Bifidobacteria* ,*Streptococci* etc. with ASD patients .Compared to the group which was given placebo ,the probiotic administered group displayed improvement and behavior and emotions. Inspite of all this there are certain limitations in the clinical trials done using probiotics, like non homogenous group, small sample size and variation in the assessment strategy of the ASD symptoms before and after treatment.

**Conclusion & Future prospects:**

Many studies done on animal models and humans have demonstrated a mutual correlation changes in gut microbiota ad Autism spectrum . Many researchers have proved that there are significant differences between gut microbial composition of ASD individuals and their normal counterparts. In last few years research emphasis has been given to understanding the etiology& treatment of ASD in association with gut microbiota. However, the existing data do not give a definite idea of characteristics and unique profile of ASD. Sometimes conflicting results are seen probably due to the heterogeneity of the subjects enrolled. The challenge in front of scientists right now is that the currently available data from clinical trials does not give a unique characteristic profile of ASD and many a times conflicting findings arise probably due to lack of homogeneity in samples. For instance GI disturbances are prevalent in ASD cases and the severity of symptoms may be attributed to it too. But still there are many ASD cases devoid of GI issues. Thus it can be concluded that there is a range

ISBN: 978-81-957386-3-2

of inflammation found in these patients which can be the reason of non-uniform findings of studies correlating ASD and Gut dysbiosis. . Further clinical studies need to be designed for standardization of ASD treatment by manipulation of GUT microbiota. While planning such researches multiple factors influencing the gut microbiota need to carefully considered.

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 ISBN: 978-81-957386-3-2

# CHAPTER-22
# FUNGAL WARRIORS: UNVEILING THE POTENTIAL OF ENTOMOPATHOGENIC FUNGI FOR SUSTAINABLE PEST MANAGEMENT

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**Abstract**

Entomopathogenic fungi have garnered increasing attention as biocontrol agents for managing insect pests in diverse agricultural and horticultural systems. This interest stems from their potential as natural regulators of insect populations, offering an eco-friendly alternative to chemical pesticides. Their unique mode of action involves penetrating the insect's cuticle and producing toxins, leading to effective pest control while minimizing environmental and human health concerns. Despite challenges such as development costs, entomopathogenic fungi have demonstrated promising results in reducing pest populations and managing plant diseases. Their integration into integrated pest management (IPM) strategies aligns with principles of sustainable agriculture, offering targeted pest control with reduced reliance on synthetic chemicals. Noteworthy fungal species such as *Beauveriabassiana, Metarhiziumanisopliae, Paecilomycesfumosoroseus, and Verticilliumlecanii* have shown efficacy against a wide range of pests, highlighting their versatility and potential for practical application. Ongoing research focuses on optimizing formulations, understanding mode of action, enhancing environmental compatibility, and developing resistance management strategies, further enhancing the prospects of entomopathogenic fungi as key components of sustainable pest management solutions.

**Keywords-**Sustainable Pest Management, Integrated Pest Management, Eco Friendly, Entomopathogenic Fungi.

## Introduction:

In recent years, there has been growing interest in the use of entomopathogenic fungi as biocontrol agents for the management of insect pests in various agricultural and horticultural systems (Ambethgar et al. 2018). These fungi have shown great potential as an alternative to chemical pesticides, as they are natural regulators of insect populations and can effectively control pest species without causing harm to the environment or human health (Arau´jojpm and hughesdp 2016). Furthermore, entomopathogenic fungi have a unique mode of action, infecting their hosts by penetrating through the cuticle and producing toxins that kill the insects. Additionally, they can also induce systemic resistance in plants against pathogens, making them a versatile and effective tool for pest management. The development and registration of entomopathogenic fungi as biocontrol agents, however, can be costly, which limits their availability and makes them relatively expensive compared to chemical pesticides. (Ambethgar, 2018)

Despite the cost limitations, entomopathogenic fungi have shown promising results in reducing insect pest populations and managing plant diseases. Moreover, their use as biocontrol agents aligns with the principles of sustainable agriculture, as they are less toxic and more flexible than chemical

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pesticides (Boopathi et al. 2015). Furthermore, their specificity in targeting pest species and their ability to adapt to different environmental conditions make them a valuable asset for integrated pest management strategies. Overall, entomopathogenic fungi offer a promising approach for the control of insect pests in agriculture and horticulture. In conclusion, entomopathogenic fungi have shown great potential as biocontrol agents for the management of insect pests in agricultural and horticultural systems. Their unique mode of action, ability to induce systemic resistance in plants, and compatibility with principles of sustainable agriculture make them a valuable tool for integrated pest management strategies and the reduction of reliance on chemical pesticides (Butt et al. 2016). The development and registration of entomopathogenic fungi as biocontrol agents can be costly, leading to limited availability and higher costs compared to chemical pesticides. However, their effectiveness and environmental benefits make them a worthwhile investment for farmers and agricultural systems seeking sustainable pest management solutions. In conclusion, entomopathogenic fungi have shown great potential as biocontrol agents for the management of insect pests in agricultural and horticultural systems. Their unique mode of action, ability to induce systemic resistance in plants, and compatibility with principles of sustainable agriculture make them a valuable tool for integrated pest management strategies and the reduction of reliance on chemical pesticides (Arau´jojpm&hughesdp 2016).

## **Entomopathogenic Fungi**:

Fungi-based insecticides, also known as fungal biopesticides or entomopathogenic fungi, are a type of biocontrol agent used in pest management. They are derived from naturally occurring fungi that infect and kill insect pests (Boopathi et al. 2015). There are several species of fungi used as biopesticides. Some common ones include:

***Beauveriabassiana*:**Research on *Beauveria sp*. as biopesticides has been extensive due to their effectiveness against a wide range of insect pests and their eco-friendly nature. Here are some key areas of research and findings related to *Beauveriasp.* as biopesticides: (Haraprasad et al. 2001, Keswani et al. 2013,Posadas et al. 2012, Ramakuwela et al.2020)

**Efficacy Against Target Pests:**

Numerous studies have demonstrated the efficacy of *Beauveriabassiana* and other *Beauveria* species against various insect pests such as aphids, whiteflies, beetles, caterpillars, thrips, and mites.

Field trials and laboratory experiments have shown significant mortality rates among target pest populations after exposure to Beauveria-based formulations.

**Mode of Action and Pathogenicity:**

Research has focused on understanding the mode of action of *Beauveria* species against insects. These fungi infect pests through contact and ingestion, colonizing the insect's body and causing mortality through fungal growth and toxin production.

Studies have also investigated the pathogenicity factors of *Beauveria*, including enzymes and secondary metabolites involved in host invasion and insect mortality.

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**Formulation Development:**
Researchers have worked on developing effective formulations of *Beauveria*-based biopesticides, including formulations for spray applications, granules, dusts, and seed coatings.

Formulation studies aim to enhance the stability, shelf life, and efficacy of Beauveria products under different environmental conditions.

**Impact on Non-Target Organisms:**
Evaluations of Beauveriabiopesticides have included assessments of their impact on non-target organisms such as beneficial insects, birds, mammals, and aquatic species.

Research efforts focus on ensuring the safety and minimal ecological impact of Beauveria-based products on non-target organisms.

**Field Performance and Integration in IPM Programs:**
**F**ield trials have demonstrated the effectiveness of Beauveriabiopesticides in real-world agricultural and horticultural settings.

Integration of Beauveria products in integrated pest management (IPM) programs has shown synergistic effects when combined with other control methods such as cultural practices and biological agents.

**Resistance Management and Sustainability:**
Studies on resistance management strategies aim to prevent the development of resistance in target pest populations against Beauveria-based biopesticides.

Research also focuses on the sustainable use of Beauveria products, including optimal application timing, dosage rates, and rotation strategies to maximize efficacy and minimize environmental impact.

Overall, research on Beauveria species as biopesticides highlights their potential as effective and sustainable alternatives to chemical insecticides in pest management programs.

**Metarhiziumanisopliae:**Research on *M. anisopliae* as a bioinsecticide has yielded valuable insights into its efficacy, mode of action, formulation, and applications in pest management. Here are some key aspects of research on *M. anisopliae* as a bioinsecticide: (Castro et al. 2016, Hu et al. 2002, Pattemore et al. 2014, Yosriet al.2018)

**Efficacy Against Target Pests:**
*M. anisopliae* is known for its broad-spectrum activity against a wide range of insect pests, including beetles, termites, grasshoppers, locusts, and certain fly species.

Field trials and laboratory studies have demonstrated the efficacy of *Metarhizium*-based formulations in controlling target pest populations.


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**Mode of Action and Pathogenicity:**
Research has elucidated the mode of action of *M. anisopliae*, which involves attachment to the insect's cuticle, penetration, and colonization of the host's body.

The fungus then grows inside the insect, producing enzymes and toxins that lead to mortality, often within a few days after infection.

**Formulation Development:**
Studies have focused on developing efficient formulations of *Metarhizium*-based bioinsecticides for different application methods such as sprays, granules, and dusts.

Formulation research aims to improve shelf life, stability under varying environmental conditions, and adherence to target surfaces for optimal pest control.

**Environmental Impact and Safety:**
Evaluations of *Metarhizium*-based bioinsecticides have included assessments of their impact on non-target organisms, soil health, and ecological balance.

Research efforts emphasize the safety and minimal environmental impact of *Metarhizium*products, particularly in comparison to chemical insecticides.

**Integration in Integrated Pest Management (IPM) Programs:**
*M. anisopliae* is often integrated into integrated pest management (IPM) strategies, where it complements other control methods such as cultural practices, biological agents, and selective pesticides.

Field studies have shown the synergistic effects of combining *Metarhizium*-based bioinsecticides with other IPM measures to enhance pest suppression and reduce reliance on synthetic chemicals.

**Resistance Management and Sustainability:**
Research on resistance management strategies aims to prevent the development of resistance in target pest populations against *Metarhizium*-based bioinsecticides.

Sustainable use practices, including application timing, dosage optimization, and rotation strategies, are studied to maximize efficacy and prolong the effectiveness of *Metarhizium* products.

Overall, research on *M. anisopliae* as a bioinsecticide underscores its potential as an effective, environmentally friendly, and sustainable tool for pest management across various agricultural, forestry, and public health applications.

**Paecilomycesfumosoroseus:** Research on *P. fumosoroseus* as a bioinsecticide has focused on its efficacy against specific pest species, mode of action, formulation development, environmental impact, and integration into pest management strategies (Zimmermann G,2008). Here are some key

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points regarding *P. fumosoroseus* as a bioinsecticide based on research findings: (Raiet al.2014, Wakil et al. 2017, Zimmermann 2008)

**Efficacy Against Target Pests:**
*P. fumosoroseus* shows effectiveness against a range of insect pests, including whiteflies, aphids, thrips, and certain mites.

Field trials and laboratory studies have demonstrated its ability to reduce pest populations and provide control under varying environmental conditions.

**Mode of Action and Pathogenicity:**
Research has investigated the mode of action of *P. fumosoroseus*, which involves attachment to the insect's cuticle, penetration, and colonization of the host's body.

The fungus then proliferates inside the pest, leading to mortality through the production of enzymes, toxins, and physical disruption of the insect's tissues.

**Formulation Development:**
Studies have explored different formulations of Paecilomycesfumosoroseus for application as bioinsecticides, including liquid sprays, granules, and dusts.

Formulation research aims to improve the stability, shelf life, and efficacy of *Paecilomyces*-based products for practical use in pest management programs.

**Environmental Impact and Safety:**
Evaluations of P.*fumosoroseus*bioinsecticides have considered their impact on non-target organisms, soil ecology, and overall environmental safety.

Studies focus on ensuring that *Paecilomyces*-based products have minimal adverse effects on beneficial insects, birds, mammals, and aquatic life.

**Integration in Integrated Pest Management (IPM) Programs:**
P. fumosoroseus is often integrated into integrated pest management (IPM) strategies, where it complements other control methods such as cultural practices, biological agents, and selective pesticides.

Research demonstrates the synergistic effects of combining *Paecilomyces*-based bioinsecticides with other IPM measures to enhance pest suppression and reduce reliance on synthetic chemicals.

**Resistance Management and Sustainable Use Practices:**
Research on resistance management strategies aims to prevent the development of resistance in target pest populations against *Paecilomyces*-based bioinsecticides.

 ISBN: 978-81-957386-3-2

Sustainable use practices, including application timing, dosage optimization, and rotation strategies, are studied to maximize efficacy and prolong the effectiveness of *P. fumosoroseus* products.

Overall, research indicates that *P. fumosoroseus* holds promise as an effective and environmentally friendly bioinsecticide for managing various insect pests in agriculture, horticulture, and other sectors.

**Verticilliumlecanii (Lecanicilliumlecanii):***Verticilliumlecanii*, also known as *Lecanicilliumlecanii*, is a fungal species commonly used as a bioinsecticide due to its efficacy against several insect pests. Here are key aspects of research and findings related to *V. lecanii* as a bioinsecticide: (Royet al.2010)

**Target Pest Control:**

V. lecanii is effective against a range of insect pests including aphids, whiteflies, mealybugs, thrips, and certain scale insects.

Field trials and laboratory experiments have demonstrated its ability to reduce pest populations and provide effective control in various crops and ornamental plants.

**Mode of Action and Pathogenicity:**

Research has investigated the mode of action of V. lecanii, which involves attachment to the insect's cuticle, penetration, and colonization of the host's body.

The fungus proliferates inside the insect, leading to mortality through the production of enzymes, toxins, and physical disruption of the insect's tissues.

**Formulation Development:**

Studies have focused on developing formulations of V. lecanii for practical application as a bioinsecticide, including liquid sprays, granules, and dusts.

Formulation research aims to enhance the stability, shelf life, and efficacy of *Verticillium*-based products under different environmental conditions.

**Environmental Impact and Safety:**

Evaluations of*V.lecanii*bioinsecticides have considered their impact on non-target organisms, soil ecology, and overall environmental safety.

Research aims to ensure that *Verticillium*-based products have minimal adverse effects on beneficial insects, birds, mammals, and aquatic life.

**Integration in Integrated Pest Management (IPM) Programs:**

*V. lecanii* is often integrated into integrated pest management (IPM) strategies, where it complements other control methods such as cultural practices, biological agents, and selective pesticides.

Studies demonstrate the synergistic effects of combining *Verticillium*-based bioinsecticides with other IPM measures to enhance pest suppression and reduce reliance on synthetic chemicals.

 ISBN: 978-81-957386-3-2

**Resistance Management and Sustainable Use Practices:**

Research on resistance management strategies aims to prevent the development of resistance in target pest populations against *Verticillium*-based bioinsecticides.

Sustainable use practices, including application timing, dosage optimization, and rotation strategies, are studied to maximize efficacy and prolong the effectiveness of *V. lecanii* products.

Overall, research indicates that *V. lecanii* is a promising bioinsecticide for managing various insect pests in agriculture, horticulture, and other sectors.

These fungi-based insecticides have shown varying degrees of effectiveness depending on factors such as the target pest species, environmental conditions, and application methods. Research and field trials continue to assess their efficacy and optimize their use in pest management programs. These fungi infect insects through contact, ingestion, or both. Once inside the insect, they grow and produce toxins that eventually kill the pest. This mode of action makes them environmentally friendly and less harmful to non-target organisms. Fungi-based insecticides can be highly effective under the right conditions (Prasad et al. 2017). Factors such as humidity, temperature, and the susceptibility of the target pest influence their efficacy. They are often used in integrated pest management (IPM) strategies alongside other control methods. Compared to synthetic chemical insecticides, fungi-based insecticides have lower environmental impact. They are biodegradable and pose less risk to beneficial insects, birds, and mammals (Gulet al.2014). Fungi-based insecticides are applied in various forms such as sprays, dusts, granules, and baits. They are used in agriculture, forestry, horticulture, and public health programs to control insect pests. Ongoing research focuses on improving the efficacy, formulation, and application methods of fungal biopesticides. Scientists are also exploring new fungal species with potential insecticidal properties (Prasad et al. 2017).

The market for fungi-based insecticides, also known as fungal biopesticides, has been experiencing significant growth and interest in recent years. With growing concerns about environmental sustainability and the impact of chemical pesticides, there is a rising demand for bio-based alternatives such as fungal biopesticides. Agriculture, horticulture, and forestry sectors are actively seeking sustainable pest management solutions, driving the adoption of biocontrol agents like fungi-based insecticides. (Pattemoreet al. 2014)

Government regulations and consumer preferences Favor IPM approaches, contributing to the market growth of biopesticides including fungal products. Ongoing research and development efforts focus on improving the efficacy, formulation, and application methods of fungal biopesticides. Scientists are exploring new fungal strains with enhanced insecticidal properties and studying their interactions with target pests for better control outcomes. The market for fungal biopesticides is not limited to agriculture but also extends to public health programs, forestry, turf management, and urban pest control (Keswaniet al.2019). Applications in urban areas for mosquito control and management of household pests are gaining traction, contributing to market diversification. Increased investments from both public and private sectors in biopesticide research and production facilities are driving market growth. Collaborations between research institutions, biotechnology companies, and agricultural organizations are fostering innovation and market expansion. Regulatory agencies are providing support for biopesticide registration and certification, facilitating market access for fungal

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biocontrol products. Certifications such as organic and eco-friendly labels further enhance the market prospects for fungi-based insecticides among environmentally conscious consumers. Overall, the market trends indicate a positive outlook for fungi-based insecticides, driven by the demand for sustainable pest management solutions, advancements in research, expanding applications, and regulatory support. (Posadas, 2012, Prasad et al. 2017,Wang et al. 2014, Yosriet al.2018)

## Recent research:

Recent research on fungi-based biopesticides has focused on several key areas to enhance their efficacy, sustainability, and practical application. Here are some notable trends and findings from recent studies:

1. **Strain Selection and Screening:** (Kozłowskaet al. 2019)
    - Researchers are exploring new fungal strains with enhanced insecticidal properties and tolerance to environmental conditions. Screening processes aim to identify strains that are effective against specific target pests.
    - Studies have focused on isolating and characterizing indigenous fungal species with potential biopesticidal properties, contributing to the development of region-specific biocontrol agents.
2. **Mode of Action Studies: (**Roy et al. 2010)
    - Recent research has delved deeper into understanding the mode of action of fungi-based biopesticides against insect pests. This includes elucidating the biochemical and molecular mechanisms involved in fungal infection, colonization, and insect mortality (Butt et al. 2016).
    - Advances in omics technologies such as genomics, transcriptomics, and proteomics have provided insights into the interactions between fungi and their target pests at the molecular level.
3. **Formulation Innovations:** (Wang et al. 2014)
    - Formulation research has focused on improving the stability, shelf life, and application efficiency of fungi-based biopesticides. Novel formulations such as microencapsulation, nanoemulsions, and biofilms are being explored to enhance product efficacy and reduce environmental impact.
    - Efforts are underway to develop biopesticide formulations compatible with integrated pest management (IPM) approaches and sustainable agriculture practices.
4. **Biological Compatibility and Safety:** (Butt et al. 2016, Das et al. 2019)
    Recent studies have assessed the compatibility of fungi-based biopesticides with beneficial insects, non-target organisms, and the environment. Research aims to ensure minimal ecological disruption and maximum safety in biopesticide use.

    - Bioassays and field trials evaluate the impact of biopesticides on non-target species, soil microbiota, and ecosystem services to determine their overall environmental safety.

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5. **Resistance Management Strategies:** (Humber et al. 2016, Keswaniet al. 2019)
    - Research on resistance management strategies for fungi-based biopesticides is ongoing. Studies focus on understanding the mechanisms of resistance development in target pest populations and implementing strategies to mitigate resistance.
    - Rotation of biopesticides, combination with other control methods, and deployment of synergistic formulations are explored to delay resistance and prolong biopesticide effectiveness.
6. **Field Trials and Commercialization:** (Rajmohanet al. 2020)
    - Field trials in diverse agroecosystems validate the efficacy and practicality of fungi-based biopesticides under real-world conditions. Research collaborations between academia, industry, and agricultural stakeholders facilitate the transition of promising biopesticides from lab to field.
    - Market analysis and economic assessments accompany research efforts to evaluate the feasibility, scalability, and market potential of fungi-based biopesticides for commercialization.

Overall, recent research on fungi-based biopesticides reflects a multidisciplinary approach encompassing biotechnology, ecology, agronomy, and pest management. The aim is to develop sustainable, effective, and environmentally friendly solutions for pest control in agriculture and related sectors.

In conclusion, the use of entomopathogenic fungi as biocontrol agents holds great promise for reducing insect pests and managing plant diseases in agriculture. Their ability to effectively target and control insect populations, their compatibility with sustainable agriculture practices, and their potential for integrated pest management make them a valuable asset in the quest for sustainable pest management solutions in agriculture and horticulture. Their unique mode of action, ability to induce systemic resistance in plants, and compatibility with principles of sustainable agriculture make them a valuable tool for integrated pest management strategies and the reduction of reliance on chemical pesticides.

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**Published by : Sai College, Bhilai**

 ISBN: 978-81-957386-3-2

# CHAPTER- 23
# BIOFUEL PRODUCTION BY *SACCHAROMYCES CEREVISIAE*

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**Abstract**

Biofuel is a fuel which is produced from a short time span biomass and by the very slow natural processes through the involvement in the formation of fossil fuels, such as oil. Biofuel can be produced from plants, agricultural product, domestic waste and industrial biowaste. One popular biofuel made from plants is ethanol. Mainly ethanol produced from plants residues such as corn, sugar beet and sugar cane. Plant produces large amount of sugar which can be easily degraded and fermented by the microorganisms like yeast cell *i.e. Saccharomyces cerevisiae*.Several types of microorganisms such as bacteria, fungi, algae and yeast can be used for the production of biofuels. Numerous microorganisms have been reported for production of biofuels such as biogas, bioethanol/biobutanol, bioelectricity and biohydrogen.Microbes produces biofuels in both aerobic and anaerobic fermentation conditions. Microorganisms consume the organic substrate and further utilize through metabolic process which generates the useful products. These products produce energy which can be used as a fuel.

**Key words:** Biofuel, *Saccharomyces cerevisiae*, Bio waste, Ethanol, Fermentation.

## Introduction:

In the Ancient time fossil fuels used as a main energy resource which is causes worldwide problems such as global warming and environmental pollutions (Hoekman, 2009; Kiran*et al*., 2014). Biofuels finds to be ecofriendly, sustainable and renewable energy by the Government, energy sector and industries (Shafiee and Tpal, 2009; Demirbas, 2009). Total 40% of renewable energies consume in the world in the form of liquid biofuels (Tan *et al*., 2008). Liquid biofuels reduces the greenhouse gases emissions and contributes to regional development, creation of job opportunities and supply security (Demirbas and Balat, 2006). In 1984, France and Germany started to use the bioethanol as a fuel forICEs (Internal Combustion Engines). Bioethanol is mostly used as biofuel in transportation sector (Demirbas and Karslioglu, 2007).Brazil was utilize the bioethanol in initiated since 1925, in1980s many countries considered as an alternative fuel, United States and Europe used until the early 1900s.In the year 2015 United States produces largest ethanol production of nearly 15 billion gallons. Brazil and United State contributes 85% world’s ethanol production (Demirbas, 2009).

## Properties of Bioethanol:

Bioethanol is also known as ethyl alcohol andtheir chemical formula is $C_2H_5OH$ or EtOH. It can be directly used as pure ethanol (blended) and with gasoline improver (octane enhancer)toproduce “gasohol” (Staniszewski, 2007). Bioethanol is less toxic, produces lesser air-borne pollutants and readily biodegradable (John *et al*., 2011).

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**Yeast (*Saccharomyces cerevisiae:***

*Saccharomyces cerevisiae* is a eukaryotes organism. It is widely used in industrial processes such as ethanol production (Parapouli*et al*., 2020). Currently, over 100 billion liters of alcohol is produced annuallyin worldwide.

*Saccharomyces cerevisiae* belongs to the group Ascomycetes, phylum: Ascomycota; subphylum: Saccharomycotina; class: Saccharomycetes; order: Saccharomycetales (Suh*et al*., 2006).Guilliermond (1912) firstly published yeast taxonomy, in which taxonomy *Saccharomyces* had 46 species in 6 groups according to their fermentative potential with sugars.Since that time, many work in*Saccharomyces*and make important changes in the group*Saccharomyces sensustricto*,in 41 species within the genus. According to Vaughan-Martin *et al*. (1971), the species included in the genus *Saccharomyces*are *Saccharomyces bayanus*(*Saccharomyces bayanusvar.uvarum*and *Saccharomyces bayanusvar.bayanus*), *Saccharomycesarboricolus*, *Saccharomycescariocanus*, *Saccharomyceskudriavzevii*, *Saccharomyces cerevisiae*, *Saccharomycesmikatae*, *Saccharomyces pastorianus* and *Saccharomycesparadoxus*(Figure 1).Thisspecies isa model eukaryotic organism for commercial production of ethanol studied for many years (Madigan *et al*., 2016).

*Saccharomyces cerevisiae* being most potable organism of production of alcohol in industries (Walker and Walker, 2018).Ithas many desirable properties for production of ethanol in industries such as rapid growth, high ethanol productivity, great yield, efficient glucose anaerobic metabolism and high tolerance to different types of environmental stress factors (low pH, high ethanol concentration and low oxygen level) (Dmytruk*et al*., 2017).

A typical *Saccharomyces cerevisiae* has ahaploid cell their genomic DNA of generally 12,000 kb (divided into 16 linear chromosomes), chromosome size ranging from around 200 to 2200 kb (Bergman, 2001; Kellis*et al*., 2004). *Saccharomyces cerevisiae* also have most important characteristics such as easily growth, nonpathogenicity and susceptible to isolated mutants and transformation techniques. According to *Saccharomyces* Genome Project it is the first eukaryotic organism with a sequenced genome, in these project observed that the presence of more than 6,608 open reading frames or open reading matrix (ORFs) which 5,797 encoded polypeptides. *Saccharomyces cerevisiae* has 4,666 proteins in the *Saccharomyces* Genome Database (Goffeau*et al*., 1996; Wiederhold*et al*., 2010). Phylogenetic analyses point their evolutionary development that mark the adaptation and favoring the growth at lower or higher temperatures. Lip *et al*. (2020) phenotypically screened 12 industrial yeast strains and laboratory strain CEN.PK113-7D was significant differences in maximum growth rates and temperature tolerance (between 12°C and 40°C)

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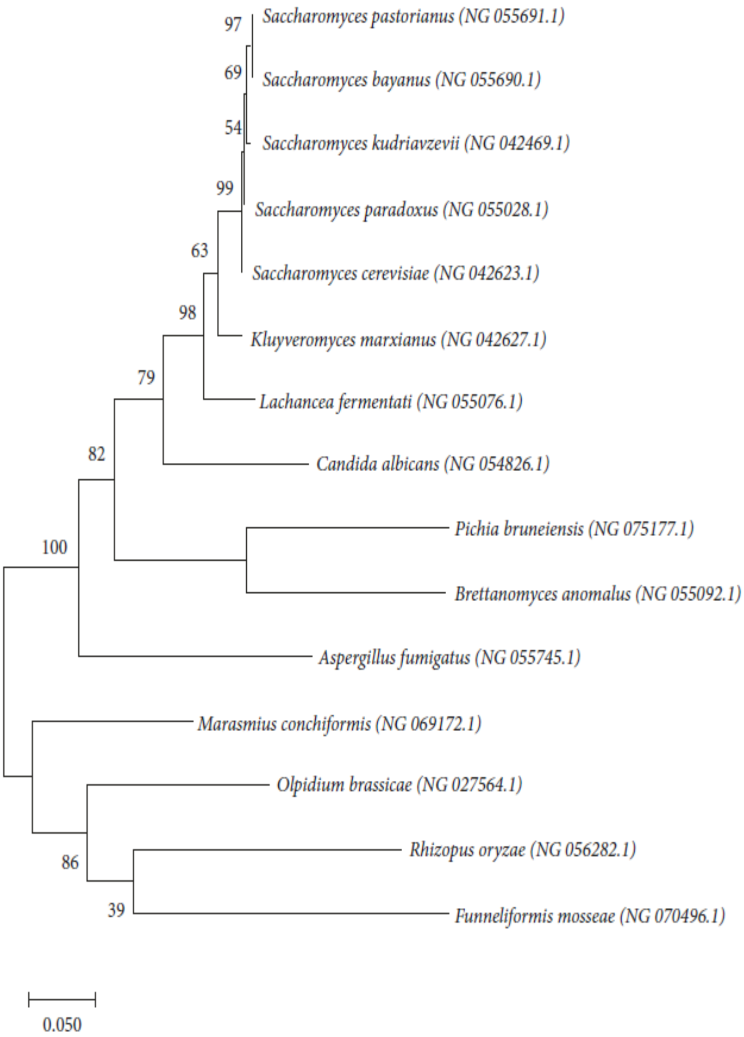


**Figure 1:**Phylogenetic tree according the 28s rDNA sequences labeled with their database accession numbers (Fernandes*et al.*, 2022).

**Isolation of *Saccharomyces cerevisiae* from Environment:**

The discovery of yeast being increasing year on year.Currently only 1 % of yeast species has known, which is represents approximately 1500 species. Totally 150000 yeast species is present in the Earth (Barriga*et al.*, 2011). Many natural yeast strains exhibit superior complex traits, such as temperature tolerance and inhibitor, which can be very beneficial for ethanol production industries. Thermotolerant and Ethanoltolerant strains that can resist the stresses and can be isolated from the natural resources such as water, soil, plants, and animals (Dujon*et al.*, 2004; Gallone*et al.*, 2016). Some studied shows *Saccharomyces cerevisiae* isolated from different sources such as mangoes, grape berries, tree bark (*Tapiriraguianensis*(*Tapirira*), *Quercusrubra*), orange peel, pineapples and fermented musts (Filho*et al.*, 2005; Suranska*et al.*, 2016; Beato*et al.*, 2016; Nasir *et al.*, 2017; Gobira*et al.*, 2017; Pandey *et al.*, 2019;Lee *et al.*, 2019;Tra Bi *et al.*, 2019). Environmentally isolated yeast strains can also lead to advances in the ethanol production of first and second-generation (Favaro*et al.*, 2019).


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**Production of Bioethanol:**

Production of biofuels from the renewable feedstocks has been capture extensive scientific attention. Biofules used to supply alternative fuels and energy. Bioethanol is most important and interesting biofuels due to their positive impact on the environment. Mostly bioethanol is produced from sugar and starch containing raw materials. Mainly bioethanol produced from hydrolysis, saccharification, and fermentation and distillation process from sugar, starch and cellulose (Figure 2). A variety of feedstocks involves from the first generation rich in starch (wheat, rice, corn, sweet potato, potato, cassava and barley) and sucrose (sugar cane, sweet sorghum, sugar beet and fruits), second generation comes from lignocellulosic biomass such as grasses, wood and straw and third generation from algal biomass (microalgae and macroalgae) used in bioethanol production (Nigam and Singh, 2011).

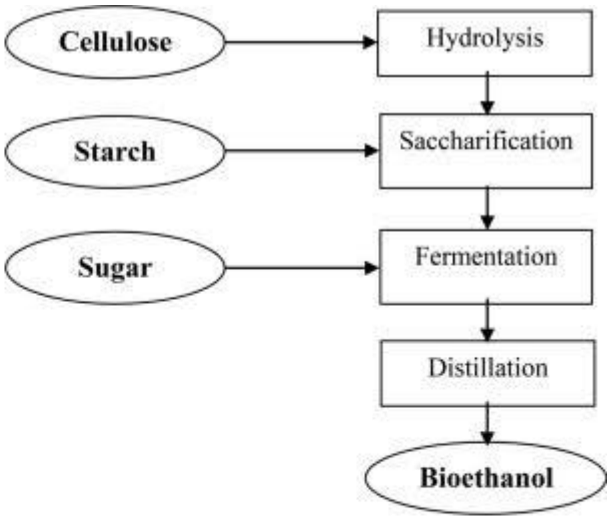


**Figure 2:** Bioethanol production from sugar, starch and cellulose (Chisti and Karimi, 2022).

In the first-generation ethanol production process some improvements are involved like long-lasting lifespans during the harvest, selection of yeastwith high fermentation speeds, elevated sugar-to-ethanol conversion rates, dominance, low output of glycerol, good fermentation capacity,genetic stability, low foam levels, fast growth, high ethanol productivity, efficient anaerobic glucose metabolism, high yield, and high tolerance (high ethanol levels, low oxygen and lower pH), tolerance to high concentrations of substrate and ethanol, good fermentation efficiency, resistance to acidity and high temperaturesflocculence, substrate consumption speeds, elevated cell growth speeds, high productivity and elevated ethanol output (Vasconcelos, 2015; MohdAzhar*et al*., 2017). Ethanol produced from corn, firstly starch must be solubilized and after two enzymatic steps fermentable sugars should be obtained(Moraes*et al*., 1999; ToksoyOner, 2006).

Use of microbial sources produces enzyme like $\alpha$-amylase has been prevalent for many decades in starch-based industries; however, some selected bacteria and fungi meet the criteria for commercial production of ethanol (Mobini-Dehkordi and AfzalJavan, 2012). New strain of yeast can produceethanol directly from starch without the separatesaccharification process, which cansupports the stressorsduring fermentation.

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In the second-generation biofuel produced from lignocellulose biomass (cellulose-hemicellulose-lignin composed feedstock) based on IEF (International Energy Agency) (Figure 3) (Bacovsky, 2010). As compare to starch-based first-generation ethanol (1GE) production lignocellulosic biomass include agricultural wastes (wheat, corn stover, or rice straw), grass, sugarcane bagasse, domestic waste, and dedicated energy crops (Switchgrass and Chinese silver grass) based second-generation ethanol (2GE) production is preferable over to sugar because of the absence of competition in the process (Sanchez and Cardona, 2008; Nigam and Singh, 2011; Kumar *et al*., 2016).

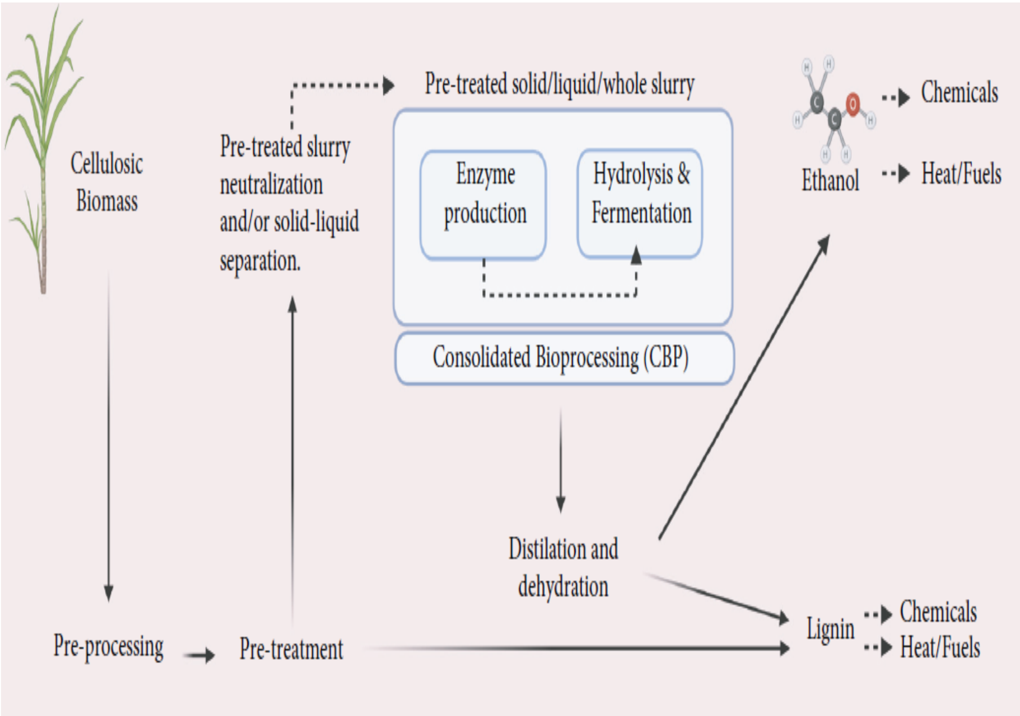


**Figure 3:** Bioethanol production in second-generation (Fernandes*et al*., 2022).

For improving second generation production includes additional glucose, pentose sugar (abundant in lignocellulose hydrolysates), L-arabinose and xylose utilize by the microorganisms and simultaneously ferment different hydrolyzed sugars and resistant to inhibitors (Dmytruk*et al*., 2017). *Saccharomyces cerevisiae* has been widely used and engineered for lignocellulosic valorization in second generation ethanol production (Cunha *et al*., 2020). For high yield of ethanol some fermentation conditions were applied, *Saccharomyces cerevisiae* growth will increase 10%, mesophilic condition (growth from 25°C to 30°C) (Dorta*et al*., 2006).

**Conclusion:**

*Saccharomyces cerevisiae* is the highest used organism in first and secondgeneration ethanol production, but improvements are needed, first generation ethanol production requires yeast directly produce ethanol from starch without separate saccharification process and yeast can produces high ethanol in high temperature during fermentation. Second-generation ethanol produces from lignocellulose will require ethanol produces from at least presence of glucose and xylose and that tolerance to inhibitors such as furans, phenolic compounds and weak acidsand exhibit thermotolerance.For highest production of ethanol significant advances have already achieved by

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hybridization, adaptation and genetic engineering (target specific). Several studies define the diversity of *Saccharomyces* species with superior characteristics that could be beneficial for industrial alcohol production. For high yield of ethanol production we can directly change *Saccharomyces* metabolic activity through functional genomics process. Science must cooperate in improving the existing industrial strains of *Saccharomyces* species and in developing new phenotypes.

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ISBN: 978-81-957386-3-2

# CHAPTER- 24

# APPLICATIONS OF MICROORGANISMS IN INDUSTRIAL WASTE TREATMENT

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**Abstract**

The increasing population has resulted in the growing demands and needs of people. With the continuous improvement of human socialization and the increasing frequency of social and economic activities, the problem of environmental pollution is becoming more and more serious, and the pollution of water body is especially prominent. The elevation of industrialization and industrial activities has generated plenty of solid and liquid wastes. Water contamination is mostly caused by the discharge of household and industrial sewage. The biological treatment of sewage strengthens the water's ability to purify itself. The microorganisms in the sewage must first be isolated from the water oncethe pollutants have been removed. Microbes are environmentally, economically, and socially important. Enzymes, probiotics, biofuels like bioethanol, hydrogen gas, and many more from centuries. There are many bacterium uses the free oxygen within the water to degrade the pollutants in the wastewater and then converts it into energy that it can use to grow and reproduce. Waste management, wastewater treatment, and bioremediation all involve the use of microorganisms and their enzymes. The broad category of sanitation includes the treatment of wastewater. The primary agents in the breakdown of organic compounds in wastewater are microorganisms and their enzymes. They play a crucial part and serve as the primary engineers in controlling all ecological processes. Currently, on an industrial scale, these microbes are playing important roles to clean up the industrial waste.

**Keywords:** Microbes, Pollution, Waste, Wastewater, Biological Treatment

## Introduction:

Population is increasing along with the growing demands of people which simultaneouslyis increasing the amount of wastes generated that gets discharged into the waterbodies. The increased urbanization has resulted in development of industrialization at a greater pace. As the number of industries are growing so is the growth of wastes coming out from them. The effluents from pharmaceutical, electrical, chemical, and agro-food industries are continuously getting mixed into the water and is worsening the quality of water intensively. There is a large difference between domestic and industrial wastewater. The wastewater emerging from industries have a high concentration of incorporated salts of metals in them (Ng, 2006).

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*Fig1: The waste thrown outside in open areas polluting the surroundings.*
*(https://static.toiimg.com/thumb/msid-67985046,imgsize-284614,width-400,resizemode-4/67985046.jpg*

*Fig 2: Solid wastes from different industries and households that is being mixed into the water bodies causing them to pollute.*
*( https://static.toiimg.com/thumb/msid-56850280,imgsize-1179610,width-400,resizemode-4/56850280.jpg)*

Water gets contaminated by generation and mixing of wastes from industries, households, agricultural lands etc. into the waterbodies (Crini & Lichtfouse, 2018). Different heavy metals such as copper, lead, mercury, cadmium, iron, etc. are released inthe industrial effluents. These metals are hazardous to human life and should be isolatedfrom the wastewater before releasing them into the water bodies. This water can be treated and used for further use, and it will aid in generation of treated water along with pure metals that will be of great benefit both to the resources and the environment (Duttaet al., 2021). Generally, the term ‘wastewater treatment’ is intended to eliminate the suspended as well as the incorporated solid wasters in the water by means of various methods in the treatment plants (Rao, 2018). For treating the wastewater from industries, anaerobic methods are highly implemented along with the aerobic processes.

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The microorganisms in anaerobic condition acts upon the pollutants and transform them into nutrient rich products (Vítězová et al., 2020). Different microbes such as bacteria, fungi etc. play a vital role in the treatment of industrial wastewater by means of biological applications. These microbes help in transforming the complex metal compounds into smaller and simpler configuration so as to separate them effortlessly (Dutta et al., 2021). Microbes such as bacteria aids in aerobic as well as anaerobic treatment of wastewater. They are capable of metabolizing most of the organic substances. Similarly, some fungi help to stabilize the organic compounds present in industrial wastewater. In activated sludge the microorganism's breakdown the biodegradable wastewater controlled conditions (Adebayo & Obiekezie, 2018). These microorganisms produce various enzymes that act on the pollutants and ferment and convert them into less harmful and enriched products. Such microbial enzymes have either oxidizing, reducing, hydrolyzing or metabolic character and act as catalysts aiding in different applications (Pandey et al., 2017).

**Industrial Wastewater:**

The tremendous shoot up in the number of populations is resulting in the growth of industrial sectors which is directly or indirectly increasing the requirement of water. In addition to that there is no control over water pollution due to the waste released from such industries that gets dispersed into the waterbodies and we are left with very less amount of freshwater on our planet. Therefore, measures should be taken to treat this wastewater before releasing it into the waterbodies. Among various types of wastewaters, the most prominent and hazardous is the industrial wastewater (Ahmed etal., 2021).

Fig3: The wastes from the industries getting discharged into the waterbodies.
( https://ozoniq.tech/wp-content/uploads/2020/12/Ozoniq_appl_industrial.jpg )

Many heavy metals that get dissolved in the waterbodies are toxic in nature and are hazardous to different life forms. Some of them are Lead (Pb), Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), etc. that cause deadly diseases in humans. Therefore, the industrial wastewater containing these harmful elements should be first treated well before disseminating it into the waterbodies (Barakat, 2011).

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Table1: Characteristics of Industrial Wastewater along with its compositions (Munter, 2003).

| | | |
|---|---|---|
| **Physical Features of Industrial Wastewater** | | |
| 1 | Solid Content | Suspended solids (40-65%)<br>Settleable solids (~60%) |
| 2 | Colour | Light brown (upto 6 hours old)<br>Grey (partially decomposed)<br>Dark grey / Black (excess bacterial decomposed in anaerobic environment) |
| 3 | Temperature | ~7-18°C (cold climate)<br>~13-24°C(warm climate) |
| **Chemical Features of Industrial Wastewater** | | |
| 4 | Inorganic Compounds | *Ammonia, chlorides, nitrates, Hydrogen sulphide* |
| 5 | Heavy Metals | *Iron, Zinc, Cobalt, Lead, Mercury, Cadmium, Chromium, Copper* |
| 6 | Organic Compounds | *Pesticides, polycyclic aromatic hydrocarbons, phenols, PCBs* |

**Wastewater Treatment Types**

Wastewater treatment requires time, energy, and cost. Any method that can optimize the wastewater treatment process is very important because one of the goals of sustainable development is to reduce energy consumption and reduce strategic processes across the globe. Wastewater treatment could be done in physical, chemical, and biological methods.

**i) Physical Treatment of Wastewater**

Physical wastewater treatment refers to the removal of pollutants without altering the biochemical properties of the contaminants, as such treatment processes ignore the influence of any chemical or biological agents. The first phase in every wastewater treatment system is filtering. This procedure involves the removal of nonbiodegradable pollutants from a wastewater treatment plant. (Ahmed et al.,2021).

**i) Chemical Treatment of Wastewater**

This method employs chemicals and comprises the separation or transformation of pollutants by different chemical reactions. This approach is quite expensive. Chemical precipitation is a popular method for removing heavy metals from inorganic wastewater. The dissolved metal ions are transformed to the insoluble solid phase by a chemical interaction with a precipitant agent such as lime after the pH is adjusted to basic conditions (Kurniawan et al., 2006).

**ii) Biological Treatment of Wastewater**

The biological method of treating the wastewater is classified into aerobic and anaerobic

 ISBN: 978-81-957386-3-2

methods with the employment of microbes. It is very common to use the microbes in wastewater treatment. These methods are used to remove biodegradable, soluble, organic and nutrient substances from wastewater. Some bacteria, colloidal and suspended substances, and other components are combined together to generate a flocculent particle with high adsorption and breakdown of organic matter and superior settling performance (Dong et al., 2018). The industrial wastewater is mostly treated by the anaerobic microbes (Bacteria). This process initiates from the hydrolysis of organic compounds by different enzymes which are then fermented by acidogens. Then after methanogenesis is carried out by methanogens. To achieve these methods various reactors are employed (Hussain et al.,2022).

**Industrial Wastewater Treatment**

To avoid the harmful consequences, the wastewater treatment plants are assembled for the treatment of effluents emerging from these industries Before being discharged into the water bodies. Search plants work on a level-to-level processes thus removing harmful and toxic substances from the water (Caicedo et al., 2019). The processes include primary treatment in which sedimentation is achieved then secondary treatment and finally the tertiary treatment is carried out. The biological treatment of sludge is carried out by help of different microorganisms to remove the organic matter from them and it also aids in nitrification (Muoio et al., 2019).


*Fig 4: An industrial wastewater treatment plant.*
(https://www.netsolwater.com/netsol-water/assets/img/product-images/Working_of_industrial_waste_water_treatment_plant.jpg)

Some techniques involving the treatment of industrial wastewater are-adsorption, coagulation, ozonation, membrane filtration, chemical oxidation, ion exchange as well as biological treatments. In adsorption many adsorbents are used such as silica, biochar, activated carbon etc. for the elimination of heavy metals as well as the organic chemicals that are incorporated into the wastewater (Rathi & Puranik, 2002). Biochar, an adsorbent manifested higher adsorption capacity for dyes in the industrial wastewater (Ambaye et al., 2021). The oxidation process is equally important in removal of the several dyes and reducing the BOD and COD values of wastewaters (Mandal et al., 2010). The biological method of treating the wastewater

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coming from industries is of great importance. The microorganisms aids in this process by acting upon the pollutants by the enzymes

released by them in aerobic or anaerobic condition (Pandey et al., 2017).

*Table 2: Types of Industries and effluents released by them* (Meena et al., 2022; Awaleh &Soubaneh, 2014; Jaseem et al., 2017).

| | **Industries** | **Effluents released** |
|---|---|---|
| 1 | Textile industries | Organic compounds i.e., less biodegradable<br>Hazardous heavy metals<br>Toxic chemical dyes.<br>Carcinogenic substances |
| 2 | Pulp and Paper Industries | Organic as well as inorganic complex pollutants (phenol, benzoic acid, heavy metals, and EDCs) |
| 3 | Food Industry | Mostly organic substances<br>Increased BOD and COD containing dairy wastewaters |
| 4 | Pharmaceutical Industry | Toxic chemicals, reactive and corrosive wastes |
| 5 | Chemical Industries | Carcinogenic elements<br>Non-biodegradable matter |

**Biological Method of Industrial Wastewater Treatment by Microorganisms:** Microbes are crucial when it comes to treatment of wastewater in our environment. Their capacity to decompose and degenerate the organic pollutants and transform it into nutrient enriched substances i.e., valuable to mankind. Pollutants such as heavy metals, dyes, complex chemicals, and other organic matter present in the industrial wastewater are degraded by enzymatic activities of the microbes by their superior metabolic activities (Szymanski & Patterson, 2003). Nitrogen is removed from the wastewater by bacteria such as *Nitrosococcus mobilis* (for oxidation of ammonia) and *Nitrospira* (for oxidation of nitrite). Phosphorus is removed by Acinetobacter spp. (polyphosphate accumulating organisms) and some other glycogen accumulating organisms (Daims et al., 2006). These microbes aid in lowering the level of COD of various wastes in the effluents generated from industries. The chemolithotropic bacteria such as *Thiobaccilus ferrooxidants* uses inorganic compounds for their reactions and furthermore, they aid in bringing down the toxicity level of industrial effluents and permits the growth of microorganisms in it as a result intensifying the biodegradability (Mandal et al., 2010). Some algae such as species of Chlamydomonas and Euglena play a vital role in treatment of wastewater with their potentiality to amass the toxic and heavy metals, organic solutes and inorganic contaminants from the wastewater (Mondal & Palit, 2019). The biological processes employed for the treatment of wastewater aids in degeneration of toxins, abolition of nutrients and maintenance of biomass (Yang et al., 2020).

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**Bioreactors in Wastewater treatment:**

Since there is increase in contaminants in the water bodies due to the release of effluents into them by different industries. Bioreactors play a vital role in treatment of this wastewater. There are several bioreactors that are put into use such as packed bed reactors, rotating biological contractors, and different types of membrane bioreactors which makes use of various microorganisms for treating the industrial wastewater (Meena et al., 2022). The membrane bioreactors (abbreviated as MBR) have contributed a lot in the treatment of wastewater emerging from different industries. It makes use of combined process of membrane filtration and the biological treatment of activated sludge. It consists of a membrane i.e. semi permeable and some suspended growth bioreactors that aids in the biochemical reactions. Further ultra-filtration and micro filtration is carried out (Fazal et al., 2015). These filtration membranes are employed to discrete the biomass or the activated sludge from the water that is treated. The membranes ensure the total removal of the small solid particles that remain suspended in water along with the microorganisms in it. The pore size of these membranes are approx. 0.1-0.5 micrometres (µm). For treatment of industrial waste, the membranes are laid down either in the external filtration, in the variation tank or in the tubular side streams type of layout (Ornel & Krause, 2006).

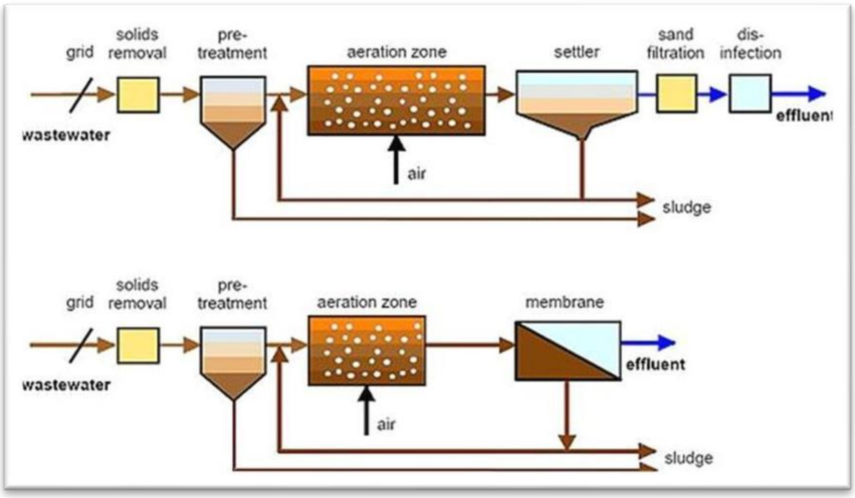


Fig 5: Diagram showing the process of conventional treatment of activated sludge (at top) vs the membrane bioreactor treatment (at bottom).
( https://upload.wikimedia.org/wikipedia/en/c/c0/MBRvsASP_Schematic.jpg )

Similarly, the anaerobic membrane bioreactors have demonstrated to be a methodical process for treatment of wastewater emerging from industries. It contains of two consecutive processes such as methanogenesis as well as acidogenesis along with the employment of microorganisms. Soluble microbial products are generated during these two processes where they get degraded by the acidogens whilst acidogenesis and also while microbial decay (Charfi et al., 2017).

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## Applications of microorganisms in industrial waste treatment

Microorganisms are prevalent in nature and perform a variety of functions that impact human life. They play a dominant role in the conservation of numerous natural as well as man-made complexes in the environment. Many microorganisms such as bacteria, fungus, protozoa, and metazoans have been utilized in waste management by breaking down waste metabolites and transforming them into organic fertilizers or beneficial compost which can be further used in organic farming. Certain noxious or toxi contaminants may be digested and transformed into harmless products by various typesof microorganisms by the process known as bioremediation (Hussain et al., 2022).

## Conclusion

Since due to the increasing industrialisation, the requirement of water is increasing and along with it the pollution of this water is also increasing. The wastewater from these industries directly gets discharged into the large water bodies resulting in a threat to human as well as animal life. Therefore, to reduce this risk, the effluents from industries must first be treated then should be released into the water source. many technologies and treatment processes have been implemented to serve this means such as employing the use of different microbes in treatment plants that helps in removal of harmful and toxic materials from the untreated wastewater. Microorganisms are indispensable to mankind when it comes to overcome the obstacles that deals with the survival of living beings. The utilization of microorganisms along with the microbiological approach has added an advantageous prospect in wastewater treatment and management. These microbes with their complex metabolic activities release enzymes that helps in degradation of harmful chemical and organic pollutants that are hazardous to the surroundings.

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Published and Printed by : (First Edition : May 2024)

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ISBN : 978-81-957386-3-2