THE SHADES OF ENVIRONMENT

Pioneering Environmental Research: Faculty Perspectives from DRIEMS University

MR. PARTHA SARATHI SATAPATHY

The Shades of Environment

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" Dedicated To DRIEMS University, With Gratitude For Fostering Knowledge And Inspiring Excellence."

Preface

In the intricate web of life that blankets our planet, the environment stands as both the nurturer and the challenged. As we navigate the complexities of modern development, the need to understand and preserve the delicate balance of nature has never been more pressing. It is within this context that *The Shades of Environment* finds its genesis, emerging as a testament to the dedication and intellectual rigor of the faculty at DRIEMS University. This compendium is a collective endeavor, bringing together the diverse research efforts of our esteemed faculty members. Each chapter reflects a unique perspective and a specialized study, unified by a common goal: to enhance our understanding of the environment and to contribute to its preservation and sustainable management. The topics explored in this volume span a broad spectrum, from the intricate dynamics of ecosystems and biodiversity conservation to the sustainable utilization of natural resources and the impact of human activities on our planet. At DRIEMS University, we believe that research is the cornerstone of academic excellence and societal progress. Our commitment to fostering a research-driven environment is embodied in the pages of this book. The studies presented here not only highlight the innovative methodologies and scientific rigor employed by our researchers but also underscore the relevance of their work in addressing some of the most pressing environmental challenges of our time.

The Shades of Environment is more than just a collection of research papers; it is a reflection of our university's mission to inspire, educate, and lead in the realm of environmental science. It is our hope that this volume will serve as a valuable resource for students, researchers, policymakers, and anyone with a vested interest in the environment. Through the collective wisdom and expertise of our faculty, we aim to spark dialogue, inspire further research, and contribute to the global effort of preserving our natural world for future generations. We extend our heartfelt gratitude to the faculty members who have contributed their knowledge and time to this publication. Their unwavering dedication and passion for environmental research are the true essence of this book. We also acknowledge the support of DRIEMS University in making this publication possible, recognizing that institutional backing is vital for the continued advancement of research.

As you delve into the pages of *The Shades of Environment*, we invite you to explore the myriad ways in which our natural world can be understood, cherished, and sustained. May this book inspire you as it has inspired us, and may it serve as a beacon of knowledge and hope in our collective journey towards a sustainable future.

Acknowledgement

We would like to express our sincere gratitude to the esteemed leaders and visionaries of DRIEMS University for their unwavering support and dedication towards the publication of the book entitled "The Shades of Environment." This compilation of research papers by our distinguished faculty members across various departments of DRIEMS University reflects our commitment to advancing knowledge and understanding of environmental issues. Our heartfelt thanks to:

Dr. Pramod Chandra Rath, (Founder Chairman, DRIEMS University)

Er. Durga Prasad Rath, (Vice-Chairman, DRIEMS University),

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Prof. (Dr.) Susant Kumar Das,(Advisor, DRIEMS University)

Your visionary leadership, guidance, and unwavering support have been instrumental in making this publication a reality. We are immensely grateful for your encouragement and the platform you have provided for our faculty to share their invaluable research on environmental topics. This book stands as a testament to the collaborative spirit and academic excellence that DRIEMS University embodies.

Thank you for your continued commitment to fostering a culture of research and innovation at DRIEMS University.

Content

Chapter-1

Exploring Enriched Environments: A Novel Path in Treating Developmental Disorders Dr. Durga Prasad Mishra

Dean, Soop, Driems University

Background

The beneficial effects of enriched environments have been well-documented through extensive research, particularly in animal models where larger cages, sensory stimulating objects, and opportunities for social interaction and physical exercise have consistentlyshown to reduce emotional reactivity, mitigate abnormal behaviors, and enhance cognitive functioning. Recently, this research has been extended to humans, particularly focusing on children with neurodevelopmental disorders (NDDs). However, while environmental enrichment shows promise as a developmental intervention for children with NDDs, several methodological considerations need to be addressed for a comprehensive evaluation of its efficacy. These include the need for operational definitions and standardization of enriched environment treatments across studies, the incorporation of control groups and better control over potential confounding variables, and the development of a comprehensive theoretical framework capable of predicting how and when environmental enrichment will impact the trajectory of NDDs. Addressing these methodological factors is essential for advancing our understanding of the potential benefits of enriched environments in the context of developmental disorders and optimizing their implementation as therapeutic interventions.

Keywords: Neuroplasticity, animal model, Neurodevelopmental disorders,, Sensory stimulation, Cognition

Introduction

Enriched environments typically refer to environments that provide increased sensory, cognitive, and social stimulation compared to standard or deprived environments. These environments often include opportunities for physical activity, social interaction, exposure to novel stimuli, and cognitive challenges(van Praag et al., 2000). Enriched environments, characterized by sensory, cognitive, and social stimulation beyond the norm, offer a promising framework for intervention. In recent years, there has been growing interest in the potential of enriched environments as a therapeutic avenue for individuals with developmental disorders(Cooper and Zubek, 1958; Manosevitz, 1970). Enriched environments have been explored as a potential treatment or intervention for various developmental disorders, leveraging the notion that stimulating and nurturing

environments can positively impact cognitive, emotional, and social development(Stoddard and Wellman, 1940; Gruber, 1975). Let's delve into how these environments are being investigated as a means to foster positive outcomes in various developmental disorders.

Rise of Interest in Enriched Environments

The interest in enriched environments traces back to pioneering research conducted in the mid- 20th century, notably by Cooper and Zubek in 1958. Their seminal study laid the groundwork for understanding the effectsofenvironmentalcomplexityoncognitive function. In their experiment, they compared rats raised in standard laboratory conditions with those in enriched environments featuring various stimuli such as toys, tunnels, and social interactions. They found that rats reared in enriched environments displayed superior cognitive abilities and adaptive behaviors compared to their counterparts in standard cages.

Another significant contribution came from Manosevitz in 1970, who further explored the concept of environmental enrichment in rodents. His work corroborated earlier findings, demonstrating that enriched environments not only enhanced cognitive performance but also had profound effects on emotional resilience and stress management in animals.

These foundational studies inspired subsequent research, including investigations into the effects of enriched environments on human development and education. Stoddard and Wellman's work in 1940 and Gruber's research in 1975 explored the implications of environmental enrichment in educational settings, highlighting its potential to enhance learning outcomes and intellectual development in children.

Building upon this early ground work, more recent studies have elucidated then eurobiological mechanisms underlying the beneficial effects of enriched environments. For instance, van Praag et al. (2000) conducted research showing that environmental enrichment stimulates neurogenesis, synaptogenesis, and other processes associated with brain plasticity, thereby promoting cognitive function and resilience to neurological disorders.

Furthermore, Baroncelli et al. (2010) emphasized the role of multisensory stimulation in enriching environments, suggesting that exposure to diverse sensory experiences fosters exploratory behavior and neuronal plasticity, ultimately enhancing cognitive performance.

In essence, the interest in enriched environments originated from seminal studies in the fields of psychology, neuroscience, and education, which collectively demonstrated the profound impact of environmental complexity on cognitive function, emotional well-being, and neurodevelopment. Subsequent research has continued to refine our understanding of the mechanisms underlying these effects, paving the way for the

application of environmental enrichment in various contexts, including the treatment of neurodevelopmental disorders.

Using Animal Models of Enriched Environments

Several studies have investigated the effects of enriched environments on animal models of Neurodevelopmental Disorders (NDDs), offering insights into potential therapeutic benefits. Some key studies are:

1. Autism Spectrum Disorder(ASD):

Study: Baroncelli and colleagues (2010) delved into the effects of enriched environments on brain plasticity using a mouse model of ASD.

Findings: Their research illuminated the positive impact of enriched environments on neural plasticity. This suggests a potential avenue for mitigating ASD symptoms through environmental interventions.

2. Attention Deficit Hyperactivity Disorder(ADHD):

Study: The study by van Praag et al. (1999) is a pioneering investigation into the relationship between physical exercise, a key component of enriched environments, and neurogenesis in the mouse dentate gyrus.

Key Insight: Their findings suggest that voluntary exercise could enhance brain plasticity, offering promising implications for managing ADHD-like symptoms through environmental enrichment strategies.

3. Fragile X Syndrome(FXS):

Study: Restivo et al. (2005) examined the effects of environmental enrichment on behavioral and morphological abnormalities in a mouse model of FXS.

Significance: Their results highlight the potential therapeutic value ofenriched environments in promoting recovery from FXS-related deficits, underscoring the importance of environmental factors in managing neurodevelopmental disorders.

4. Down Syndrome:

Study: Sale et al. (2014) explored the impact ofenvironmentalenrichment on amblyopia recovery in adult mice, with implications for cognitive and sensory deficits observed in Down syndrome.

Implication: While not directly focused on Down syndrome, their findings suggest that enriched environments may modulate intracortical inhibition, offering potential benefits for addressing cognitive and sensory impairments associated with the condition.

These studies collectively underscore the potential therapeutic benefits of enriched environments for various NDDs in animal models, highlighting the importance of further research to elucidate underlying mechanisms and translate findings to human populations.

Unraveling the Impact: Research Insights

Research on enriched environments in children with Neurodevelopmental Disorders (NDDs) like Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), and Fragile X Syndrome (FXS) has garnered attention due to the need for evidence-based treatments and parental preferences for non-pharmacological interventions; while studies primarily focus on sensory integration therapy (SIT), exploring its potential benefits and limitations, recent investigations have extended to broader enriched environment approaches, with notable studies indicating symptom reduction and sustained benefits, yet emphasizingthe necessity for rigorous experimental designs to determine true efficacy (Halperin& Healey, 2011; Lang et al., 2012; Leong et al., 2015; Rusu et al., 2015; Woo & Leon, 2013; Woo et al., 2015; Aronoff et al., 2016; Dawson et al., 2010; Pajareya& Nopmaneejumruslers, 2011, 2012; Jernberg& Booth, 2009; Yochman et al., 2004; Salami et al., 2017; Oddi et al., 2015; Restivo et al., 2005).

Research on Enriched Environments and Developmental Disorders:

Researchers have been probing the effects of enriched environments across different developmental conditions. For instance:

a. Autism Spectrum Disorder(ASD):

Studies have shown that enriched environments can potentially ameliorate some symptoms associated with ASD. These environments may include structured activities, sensory integration therapy, and social skills training(Schneider and Przewłocki, 2005) .

b. Attention Deficit Hyperactivity Disorder(ADHD):

Enriched environments, with increased physical activity, cognitive challenges, and social interaction, have shown promise in improving attention and reducing hyperactivity in individuals with ADHD (Bucci and Rudebeck, 2016).

c. Down Syndrome:

Enriched environments, including early intervention programs that focuson cognitive stimulation and social interaction, have been associated with improved cognitive development and adaptive skills in individuals with Down syndrome (Fidler and Nadel, 2007) .

d. FXS:

Limited research exists, but animal models suggest potential benefits of social and sensory enrichment. Studies in humans are scarce, highlighting a need for further investigation (Oddi et al., 2015; Restivo et al., 2005; Dyer-Friedman et al., 2002; Glaser et al., 2003).

These studies collectively underscore the importance of enriched environments in improving outcomes for children with NDDs, while also emphasizing the necessity for rigorous experimental designs and longitudinal investigations to establish efficacy conclusively.

Deciphering the Mechanisms: How Do Enriched Environments Work?

Understanding the mechanisms underlying the therapeutic effects of enriched environments on neurodevelopmental disorders (NDDs) is crucial for optimizing their efficacy. While the exact mechanisms remain multifaceted and may vary depending on the disorder and individual characteristics, several key pathways have been proposed:

1. **Neuroplasticity:** Enriched environments promote neuroplasticity, facilitating the brain's ability to reorganize and adapt. Studies like Baroncelli et al. (2010) demonstrate that enriched environments positively influence neural plasticity, potentially mitigating symptoms associated with NDDs such as Autism Spectrum Disorder (ASD).
2. **Stress Reduction:** Environmental enrichment can mitigate stress responses, as evidenced by research like Griñán-Ferré et al. (2016), which shows that enriched environments prevent epigenetic changes associated with stress and inflammation. This stress reduction may alleviate symptoms in individuals with NDDs by reducing anxiety and improving emotional regulation.
3. **Sensory Stimulation:** Enriched environments provide diverse sensory experiences, which can enhance sensory integration and processing abilities. Aronoff et al. (2016) suggest that sensory enrichment may contribute to the therapeutic effects of enriched environments, addressing sensory sensitivities common in NDDs.
4. **Social Interaction:** Social engagement within enriched environments fosters social development and communication skills. Studies like Fountain et al. (2012) highlight the importance of social interaction in understanding developmental trajectories associated with NDDs such as ASD.
5. **Cognitive Engagement:** Enriched environments encourage cognitive engagement throughchallengingactivitiesandproblem-solving tasks. ResearchbyvanPraaget al. (1999) suggests that physical exercise, a component of enriched environments, may enhance cognitive function and alleviate symptoms of NDDs such as AttentionDeficit Hyperactivity Disorder (ADHD).
6. **Gene Expression and Epigenetic Modifications:** Environmental enrichment can influence gene expressionand epigenetic modifications, impacting neurodevelopment and synaptic plasticity. Griñán-Ferréet al.(2016)and Zhanget al.(2018)demonstrate that enriched environments prevent epigenetic changes associated with stress and inflammation, potentially offering long-lasting therapeutic benefits.

7. **Neurotransmitter Regulation**: Enriched environments may modulate neurotransmittersystems,affectingmoodregulation,attention,andlearning.Research by Restivo et al. (2005) suggests that environmental enrichment promotes behavioral and morphological recovery in a mouse model of Fragile X Syndrome (FXS),possibly through modulation of neurotransmitter systems.
8. **Neuroinflammation and Oxidative Stress:** Enriched environments have been shown to reduce neuroinflammation and oxidative stress, which are implicated in the pathogenesis of NDDs. Studies like Griñán-Ferré et al. (2016) and Sale et al. (2014) demonstrate that enriched environments prevent epigenetic changes associated with stress and inflammation, potentially mitigating neuronal damage and enhancing neuronal resilience.

By addressing these mechanisms, enriched environments offer a holistic approach to managing NDDs, potentially leading to improved outcomes and quality of life for affected individuals.

In summary, the efficacy of enriched environments is believedto stem from their influenceon neuroplasticity—the brain's ability to reorganize and adapt in response to experiences. Through mechanisms such as increased neurogenesis, enhanced synaptic connectivity, and modulation of neurotransmitter systems, enriched environments create an optimal milieu for promoting positive developmental outcomes(Sale et al., 2009).

Clinical Implications and Challenges

Navigating the landscape of enriched environments as a treatment modality fordevelopmental disorders presents both challenges and opportunities. Implementing and sustaining these interventions on a large scale pose logistical and resource-related hurdles, while the diverse responses among individuals underscore the necessity for personalized, tailored approaches to maximize efficacy. Despite these challenges, the potential benefits of enriched environments offer promising avenues for improving outcomes in individuals with developmental disorders(Akers and Hamilton, 2007) .

The adoption of enriched environments as a novel path in treating developmental disorders carries significant clinical implications and presents several challenges:

Clinical Implications:

1. **Holistic Approach:** Enriched environments offer a holistic approach to treating developmentaldisorders, addressingvariousaspectsofneurodevelopment.Thisaligns with research demonstrating the multifaceted benefits of enriched environments (Baroncelli et al., 2010).
2. **Complementary Therapy:** Enriched environments complement existing therapeutic interventions, potentially enhancing treatment outcomes (Restivo et al., 2005).

Combining enriched environments with behavioral therapies and medication can promote overall well-being.

3. **Early Intervention:** Early implementation of enriched environments during critical periodsofneuroplasticitymaymaximizetherapeuticbenefits(Inguaggiatoetal.,2017). Research suggests that early intervention can mitigate the long-term impact of developmental disorders (Bakermans-Kranenburg et al., 2008).
4. **Individualized Treatment:** Enriched environments can be tailored to individual needs, allowing for personalized interventions (Fountain et al., 2012). This individualized approachaligns withthe diverse natureofdevelopmentaldisorders and the importance of addressing unique characteristics and challenges.

Challenges:

1. **Standardization:** The lack of standardized protocols for implementing enriched environments leads to variability in intervention approaches (Baroncelli et al., 2010). Establishing standardized guidelines is crucial for ensuring consistency and reproducibility.
2. **Resource Intensity:** Enriched environments require substantial resources, raising concerns about equity and accessibility (van Praag et al., 1999). Access to enriched environments may be limited, particularly in underserved communities.
3. **Measurement and Evaluation:** Assessing the effectiveness of enriched environments poses challenges in defining outcome measures and evaluating treatment efficacy (Restivo et al., 2005). Clinically meaningful measures of progress need to be developed to accurately assess the impact of interventions.
4. **Sustainability:** Maintaining the effects of enriched environments over the long term presents challenges (Sale et al., 2014). Strategies for sustaining the benefits beyond the duration of the intervention need to be explored.
5. **Inter disciplinary Collaboration:** Effective implementation of enriched environments requires collaboration across multiple disciplines (Restivo et al., 2005). Interdisciplinary teamwork is essential for designing comprehensive interventions that address the complex needs of individuals with developmental disorders.

Addressing these challenges is crucial for realizing the full potential of enrichedenvironments as a novel path in treating developmental disorders (Baroncelli et al., 2010). Despite these challenges, the promising therapeutic benefits underscore the importance of continued research and innovation in this area.

In summary, enriched environments offer a holistic approach to addressing developmental disorders, capitalizing on the plasticity of the brain to promote positive outcomes incognitive, emotional, and social domains. However, further research and tailored interventions are needed to fully harness their potential in clinical settings.

Conclusion

In conclusion, the exploration of enriched environments as a novel therapeutic avenue for developmental disorders has its roots in pioneering research from the mid-20th century, highlighting the profound impact of environmental complexity on cognitive function and neurodevelopment. Over the years, interest in enriched environments has grown, fueled by a deeper understanding of the neurobiological mechanisms underlying their beneficial effects.

Studies utilizing animal models of neurodevelopmental disorders (NDDs) have provided valuable insights intothepotentialtherapeuticbenefitsofenrichedenvironments. Research in ASD, ADHD, Fragile X Syndrome, and Down Syndrome has demonstrated that enriched environments can positively influence neuroplasticity, cognitive function, emotional resilience, and behavioral outcomes.

However, translating findings from animal models to human populations poses challenges, including the lack of standardized protocols, resource intensity, and the need for interdisciplinary collaboration. Despite these challenges, enriched environments offer a holistic approach to addressing developmentaldisorders, complementing existing therapeutic interventions and promoting individualized treatment strategies.

Further research is needed to elucidate the mechanisms underlying the therapeutic effects of enrichedenvironmentsinhumans, considering factorssuchasgene-environment interactions, epigenetic modifications, and developmental trajectories of specific NDDs. Rigorous experimental designs and longitudinal investigations are essential for establishing the true efficacy of enriched environment interventions and optimizing their implementation in clinical settings.

By addressing these challenges and leveraging the potential benefits of enriched environments, we canpave the wayfor improved outcomes and qualityoflife for individuals with developmental disorders. Continued research and innovation in this area are crucial for realizing the fullpotentialofenriched environmentsasanovelpath intreating developmental disorders.

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Chapter-2

Biomedical Waste Managementin Hospitals -An Emerging Issue of India

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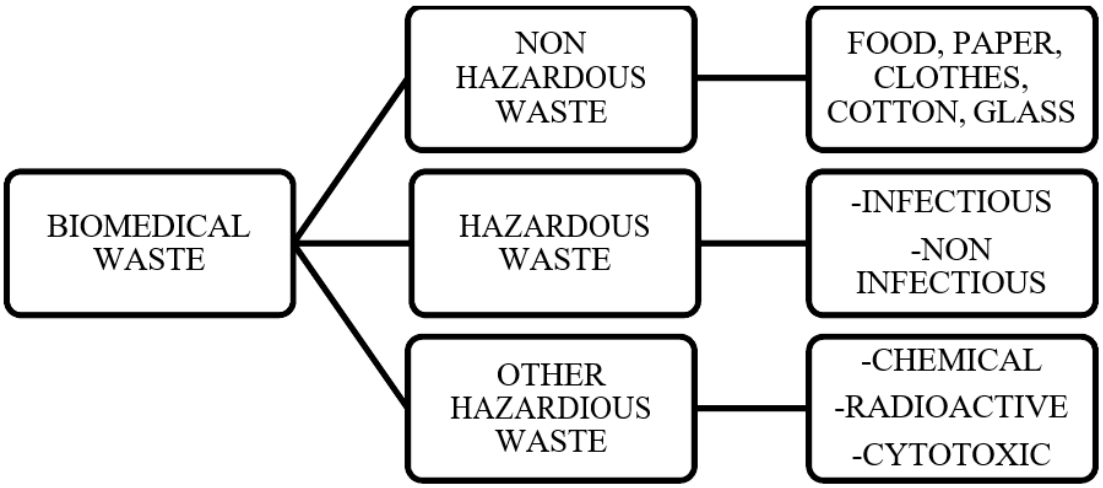
Introduction

"Hospitals have long been used to treat sick people, but we are unaware of the harmful effects their waste and squalor have on the environment and human health." It is now common knowledge that hospital waste poses a risk to the health of the general public, healthcare staff, and local flora and animals. Hospital trash in India was not previously separated before being disposed of in a landfill or incinerator. In India, recycling has historically been done from massive rubbish dumps where independent contractors or rag pickers manually sort the waste for recyclables. These workers then get in touch with the necessary businesses, who buy the waste from them.The majority of these rag pickers are women and children from the lowest socioeconomic classes; hence there is a general lack of awareness of health dangers. In turn, many of them become carriers of serious health risks to the general population by contracting infections through syringes, needles, and other biomedical waste. It is appropriate to call for additional legislation to ensure education, awareness, and health care facilities for their special status, in light of the health risks in the recycling industry, given their historical rejection from mainstream society (as "untouchables") and recent reemergence as a political front.

In this regard.The Bio Medical Waste (Management and Handling) Rules were notified in July 1998 after the act was passed by the Ministry of Environment and Forests in 1986. According to these regulations, it is the responsibility of every "occupier," that is, a person who has control over the institution or its premises, to make every effort to guarantee that waste created is managed in a manner that doesn't harm the environment or human health.

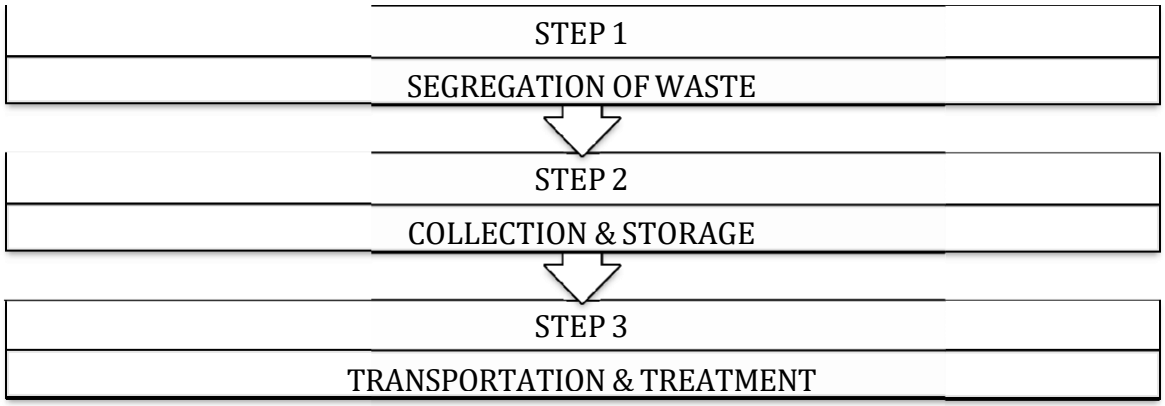
Classification of Biomedical Waste

The source of biomedical waste is the place or the location atwhich biomedical waste has been generated.The source of biomedical waste is classified into following types basedon the quantity of waste generated.

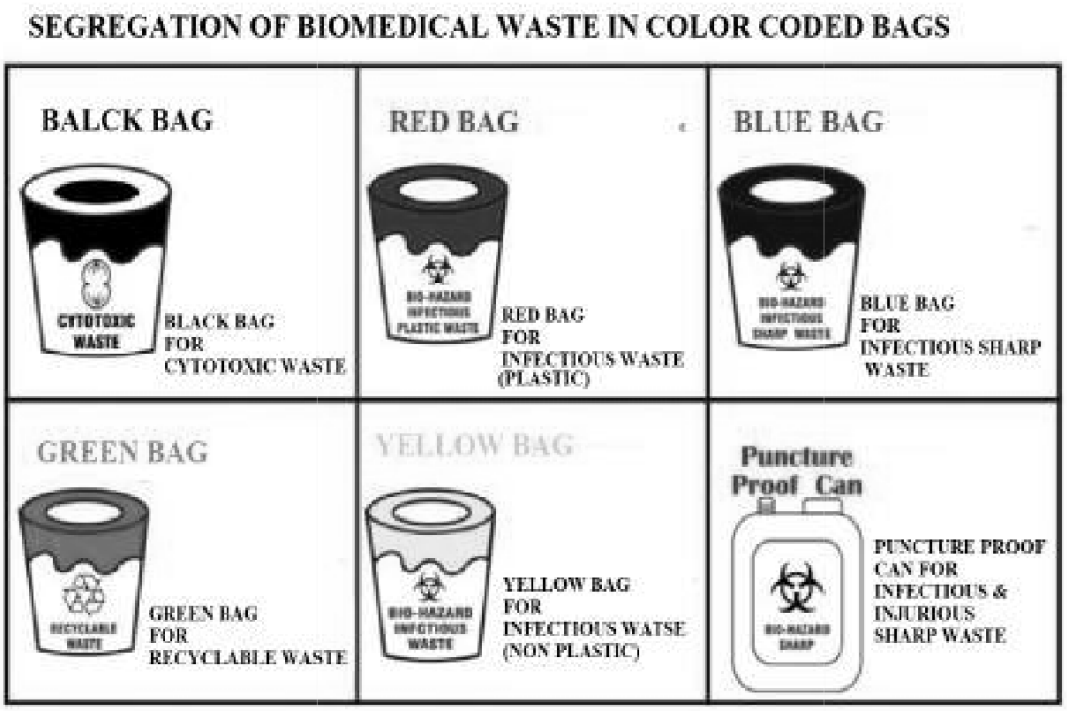


Steps For Waste Management

There is a big network of Health CareInstitutions in India. The hospital waste like bodyparts, organs, tissues, blood and body fluids alongwith soiled linen, cotton, bandage and plaster castsfrom infected and contaminated areas are veryessential to be properly collected, segregated,stored, transported, treated and disposed of in safemanner to prevent nosocomial or hospital acquiredinfection. The following steps are involved in management of waste in hospitals.



Segregation refers to the basic separation of different categories of waste generated at source and thereby reducing the risks as well as cost of handling and disposal. Segregation is the most crucial step in bio-medical waste management. Effective segregation alone can ensure effective biomedical waste management. The following color coded bags used for segregation of biomedical wastes.



The collected wastes are transported in trolleys or in enclosedwheelbarrow for treatment. The operator should ensure to avoidmanual loading. The bags / Container containing biomedical wastes must be tied/lidded before hauling for treatment. Vehicles used for transportingshould be special to avoid contact to, and direct contact with theoperator, scavengers and the public.

Categories of Bio-Medical Waste, Its Treatment And Disposal

Waste Category	Treatment & Disposal
Microbiology & Biotechnology Waste (wastes from laboratory)	Localautoclaving/micro-waving/incineration
Animal Waste (generated by veterinary hospitals colleges)	Incineration/deepburial
Human Anatomical Waste (human tissues, organs,body parts)	Incineration/deepburial
Waste sharps (needles, syringes, scalpels, blades)	Disinfection (chemicaltreatment/autoclaving g/microwaving andmutilation/shredding
Soiled Waste (Items contaminated with blood, and body fluids including cotton,dressings, soiled plaster casts	Incineration/autoclaving/microwaving
Discarded Medicines and Cytotoxic drugs(wastes comprising of outdated,contaminated and discarded medicines)	Incineration/destructionand drugs disposal in securedlandfills

Liquid Waste (waste generated from laboratory and washing, cleaning, house- keeping and disinfecting activities)	Disinfection by chemical treatment and discharge intodrains
Incineration Ash (ash from incineration of any bio-medical waste)	Disposal in municipal landfill
Chemical Waste (chemicals used in production of biological)	Chemical discharge into drainsfor liquids and secured landfillfor solids

Conclusion

The management of biomedical wastes poses a great challenge to the policyplanners, city administrators, medical personnel and workers in the recycling industry. Thereis a need for adopting a cost-effective system for providing better medical waste treatmentfacilities and reduce the amount of waste generation by awareness and education of allconcerned.

Chapter-3

Sustainable Development of Environment through Green Chemistry in Pharmaceutical Industries.

(Prof).Dr Chandra Sekhar Barik (Asst.Prof) Mr. Harish Mishra
School Of Pharmacy, Driems University

Green chemistry[1] in pharmaceutical industries plays a crucial role in promoting sustainable development by focusing on minimizing environmental impact throughout the entire lifecycle of pharmaceutical products. Here are some key ways it contributes[2-3]:

1. Reduced Hazardous Substances:

Green chemistry aims to design and use chemicals that are less hazardous to human health and the environment. This includes reducing or eliminating the use of toxic solvents, reagents, and by-products in pharmaceutical processes[4].

2. Improved Efficiency:

By optimizing synthetic routes and processes, green chemistry reduces resource consumption such as energy and water, leading to lower operational costs and environmental footprint.[4]

3. Biodegradability and Eco-Toxicity:

Green chemistry emphasizes the development of pharmaceutical products that are readily biodegradable and have minimal eco-toxicity, reducing their impact on ecosystems after disposal.[5]

4. Lifecycle Assessment:

It considers the environmental impact from the initial stages of drug discovery through manufacturing, distribution, use, and disposal. This holistic approach helps identify opportunities for improvement and innovation.[6]

5. Renewable Feed stocks:

Green chemistry encourages the use of renewable raw materials and feed stocks, reducing dependence on fossil fuels and contributing to a more sustainable resource base.[7]

6. Regulatory Compliance and Public Perception:

With increasing regulatory scrutiny and consumer awareness regarding environmental issues, adopting green chemistry practices enhances regulatory compliance and improves public perception of pharmaceutical companies.[8]

7. Innovation and Collaboration:

Green chemistry fosters innovation in pharmaceutical research and development by encouraging interdisciplinary collaboration and the adoption of new technologies that are both economically viable and environmentally friendly.[9-10]

Overall, integrating green chemistry principles into pharmaceutical industries not only supports sustainable development but also enhances competitiveness and promotes responsible stewardship of natural resources.

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Chapter-4

Curcumin: Can it Slow Cancer Growth in Human Body?

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At this time, there is not sufficient evidence to propose curcumin for stopping or treating most cancers, but studies are ongoing. Curcumin is a substance observed in the spice turmeric. Curcumin has lengthy been utilized in Asian medicinal drug to treat a selection of illnesses. Curcumin, a polyphenol extracted from Curcuma longa in 1815, has gained interest from scientists global for its biological activities (e.g., antioxidant, anti-inflammatory, antimicrobial, and antiviral), among which it's anticancer capacity has been the most defined and still stays beneath research. Laboratory and animal studies indicates that curcumin may additionally save you cancer, gradual the spread of most cancers, make chemotherapy greater powerful and shield healthy cells from damage by using radiation therapy. Studies of curcumin in people are nevertheless inside the early stages. Clinical trials are underway to investigate curcumin as a manner to prevent most cancers in human beings with precancerous conditions, as a most cancers remedy, and as a treatment for symptoms and signs and symptoms as a result of cancer treatments.

Clinical use of curcumin is still under investigation, both as a monotherapy and in combination with other drugs. In a phase I clinical trial, curcumin was used alone in 15 colorectal cancer patients as an oral formulation.It was reported the absence of toxicity, the development of significant diarrhea in two patients, and two patients showed stable disease after two months of curcumin treatment. An additional monotherapy clinical trial (phase II) of curcumin as an oral formulation was performed in 25 advanced pancreatic cancer patients. Despite the low levels of curcumin present in plasma two patients showed clinical biological activity. Indeed, in one patient a stable disease for >18 months was observed.The assessment of curcumin mixed with other drugs as chemotherapeutic or adjuvant to the standard treatments in cancer disease has been reported. The therapeutic effect of a combination of curcumin with imatinib (tyrosine kinase inhibitor) has been evaluated in 50 chronic myeloid leukemia patients. The mixed treatment was more effective than imatinib alone, although additional studies are needed to confirm the efficacy of the experimental combination. Furthermore, a combination of curcumin with anti-EGFR monoclonal antibodies in pretreated cSCC (cutaneous squamous cell carcinoma) patients has been described as a highly effective strategy in disease control.

Research is ongoing, and there isn't enough evidence to recommend curcumin at this time. As always, talk with your doctor before using any herbal supplement, including curcumin. It's known to interfere with certain medications, including some chemotherapy drugs.

Chapter-5

Harnessing Green Hydrogen For Environmental Sustainability

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Abstract:

For every scientific innovation nature stands behind us from which we derive ideas and mimic computational algorithm from its inhabitants because of their unique characteristic and try to model them for real life practical utility. We are hard pressed to preserve it. At the cost of inscicent urbanisation, industrialisation nature is at stake. The man made devastation is more pronounced. No doubt sometimes due to natures furry the environment is at bay. She cannot preserve its flours and fauna at all. Time has come for its survivability. Therefore , preservation of environment becomes a prime concern for all globally.

There are certain things like air pollution, emission of toxic gases, affluent coming out of industrial wastes, incessent cutting of woods. In such a situation green hydrogen taken as an alternatives. Hydrogen technologies are much more suited for mass deployment.

There is vast and growing application spectrum . There is huge demand for hydrogen technologies according to their potential to accelarate the transition to more sustainable form of energy. It still supports current energy models although with regional variations. Hydrogen is zero emission source of fuels for trains, buses and cars. It can be used as a feedstock gas for industries such as chemicals, refining and steel. In addition to this it is a source of heat and power for buildings. It can buffer energy generated from renewable sources.

Slowly we are moving towards a greener energy economy. Hydrogen offers many benefits. The first and foremost it supports gradual transition towards lower carbon sources of energy as it can be generated from natural gas and other from non-renewable by products. In addition to this , it can be used as an energy carrier. Looking into the future, it can be generated at the scale with zero carbon footprint using renewable energy unlike solar or wind power to split water by means of electrolysis. Hydrogen can be produced from a range of feedstock and natural resources. By using process like stream forming,H2can be generated from natural gas, LPG and nephtha. Naphtha regarded as a gray hydrogen. We also come across blue hydrogen, In addition to this , our technologies enable H2 to be generated from renewable energy sources that is green hydrogen.

Introduction:

Depending upon the target application, hydrogen needs further processing. The typical impurities are removed, separation of carbon dioxide, compression and cryogenic liquification. After processing, it needs to be transported to the point of use. With the help of equipment and techniques for efficient transport gaseous and liquid hydrogen to its destination or to store it until needed. Hydrogen is essential across wide range of industrial process. Based on our end-to-end expertise , we can offer an enriched portfolio of application technologies for this light weight gas ensuring best possible use in diversified industries unlike chemicals, refining, metal working and glass. It is a source of most sustainable future.

Explanation

India stresses the importance of integrating green hydrogen into any the any country's energy mix and changing the perception that is highly pressured and dangerous fuel. The energy ministry is trying to bring green hydrogen as fuel. Green hydrogen -generated energy must be accessible to every nook and corner of India to mitigate the ever increasing energy crisis. The impact of covid- 19 pandemic reinforced the necessity for young people to sustain their livelihood independently. The energy ministry in India, in particular should emphasise in their energy mission for the youth to improve their livelihood by supporting entrepreneurs and fostering ventures with strong socio-economic impacts. Green hydrogen sector's diverse opportunities beyond traditional roles, encompassing project development and other ancillary services necessary for industry's growth.

Efforts towards renewable hydrogen production centre around water electrolysis, where water gets splitted into hydrogen and oxygen using electricity. In general water electrolyzers consists of two electrodes an anode and a cathode . When it is dipped in water and separated by semi permeable separator. By means of an external electric circuit when connevcted to the electrodes to a power source. Water enters the electrolyzer and it is subjected to electrical current causing it to split into hydrogen and oxygen. A reduction occurs at the cathode to produce H2 and oxidation occurs at the anode to produce O2.These two reactions are respectively referred to as oxygen evolution reaction respectively. Electro catalysists usually of platinum group metals are required to reduce the over potential of the electro chemical reactions by adsorbing reactants on their surface to produce intermediates which promote transfer of charge in electrolyzer. These chemical principles can be applied in various electrolyzer configurations to produce H2 from water. These are the primary technologies used for industrial applications are alkanine electrolyzers, proton exchange membrane and splid oxide electrolyzers respectively. In India, NTPC Green Energy Limited ventures into with oil refiners and marketer HPCL and copper and aluminium marker HINDALCO industries to supply these companies with green hydrogen.

With an eye toward enabling a hydrogen economy a concerted efforts have been given with congregation of many technologies . Within the research domain of hydrogen production, storage and utilization in conventional fuel cells , various class of materials have been developed that ameleorates higher efficiencies and best possible utility. Specific attention has been given to catolyst materials that enable the green production of hydrogen from water, chemical and physical storage systems. The materils used in technical capacities within fuel cells.

Despite decades of research toward alternatives, fossil fuel accounts for more than 80%of the global energy consumption today. Facing twindling natural resources and burgeoning echological consequences , we are challenged with charting a sustainable course for modern life using renewable energy sources. This will require safe and reliable method s of converting ,storing and using energy that can compete with hydrocarbon fuels extracted from the earth. While optimal solutions may vary depending on the geographycal locations and availability of alternative energy enabling materials . The one proposed avenue is the use of hydro carbon as an energy carrier hydrogen fuel cells being a primary method of converting energy into electricity. The integrated system of hydrogen production ,storage and afterward utilization on a sociatal scale is referred to as hydrogen economy. Hydrogen being the potential candidate to act as a superior energy carrier compared to its fossil fuel counterparts.

Iberdola leads the global development of green hydrogen with over fifty major projects in eight countries . It includes green ammonia and green methanol . These are predominately used in countries like Spain, UK, Australia, Brazil and USA. It aims at responding to electrification and decarbonization. The primary sector heavily use it like industry and heavy transport. Anew green hydogen business echo system is established . It addresses various technological challenges of producing and supplying green hydrogen from clean energy sources. Green hydrogen becomes a key to our energy transition stategy in different sectors like transportation,aviation etc.Emergence og green hydrogen plant for the production of zero emission fertilizers.The Puertollano plant,the global leader, consists of hundred megawatt photovoltaic solar olant. It is basicaal a lithium -ion battery system with a storage capacity of 20 Mwh. It is one of the largest electrolytic hydrogen production systems of the order of 20MW. All of them harnessed from various network sources. It avoids emission of 48,ooot CO2per year.. Green hydrogen plant are for industrial use. Green hydrogen is an alternative to reduce emission are cares for our plant servivability. Decarbonization the planet is one of the goal s that countries all over the world have set a target to be achieved by the year 2050.

The ongoing war in Ukraine and waging of war between Israel and Hammas caused serious energy crisis due to lack of fossil fuels. It leads to an unprecedented rise in the price of gas and coal, causing Europe to import much more liquified natural gas than usual with surmounting problem of worsening climate change However, decarbonization

the plants suggests a different world by 2050: one that is more accessible, efficient and sustainable driven by clean energies such as green hydrogen. The technology is based on generation of hydrogen. It is a universal, light and highly reactive fuel obtained through chemical process known to be electrolysis.

This method uses an electrical current to separate hydrogen from oxygen in water. By this electricity is produced from renewable sources then we will produce energy without emitting carbon dioxide into atmosphere. As per international energy association statistics, this method of obtaining green hydrogen would definitely save nearly 830 tonnes of CO2 that are emitted annually when this gas is produced using conventional fossil fuels. By replacing all gray hydrogen globally would require 3000TWh/year from renewables. But the prevalent question arises about the viability of green hydrogen because of its high production cost ; the reasonable doubt that will disappear as the decarbonization of earth progresses and consequently the generation of renewable energy becomes cheaper. As hydrogen is most abundant chemical element in nature. As per statistics by IEA, the global demand for use as fuel has tripled ever since 1975 and reached to 70 million tonnes a year in 2018. In addition , green energy is a clean energy source that only emits water vapour and leaves no residue in the air unlike coal and oil. Hydrogen has long standing symbiotic relationship with industry . This gas has been used to fuel vehicles, airships and space ships since the beginning of 19^{th} century . The decarbonization of the global echonomy , a process that can not be underrated ,will give hydrogen more prominence . In addition , if its production cost falls by 50% by the end of 2030 as predicted by world hydrogen council, will undoubtedly be looking at one of the fuels of the future.

Pros And Cons of The Energy Source:

* **100% Sustainability:**

It does not emit polluting gas during either combustion or in production .

* **Storability:**

It is easy to store by which it can be used subsequently for other purposes and at times other than It is highly volatile and flammable element. Extensive safety measures are required to prevent leakage and explosion.

* **Impact of Green Hydrogen:**

Hydrogen as a fuel is a reality in contries like United States, Russia, China, France and Germany. Others like Japan are going even further and aspire to become a hydrogen economy. Electricity and drinking water generator. These two elements are by reacting hydrogen and oxygen together in a fuel cell. This process is very useful during space mission.

* **Energy Storage:**

The compressed hydrogen tanks are capable of storing energy for long period of time and are reliably easier to handle than lithium-ion batteries because they are of lighter weight.

Transport Mobility:

The use of hydrogen storage tank and transportation dynamics. There is use of super capacitors. This has been engineered to address the ever growing demand for high capacity, fast charging energy storage and power solutions. It offers distinctive material combinations for delivering high pulse power and capacitance density.

As per our national green hydrogen mission , India set its sight on becoming energy independent by the end o 2047 and achieving net zero by the year 2070.In order to achieve this target, increasing renewable energy use across all economic sectors is the focal point to India's energy transition.

On the other hand hydrogen can be utilized for prolonged duration storage of renewable energy by replacing conventional fossil fuels in industry , clean transportation and potentially decarbonized power generation , aviation and marine transport . The national green hydrogen mission was approved in the year 2022.The intented objectives are: A global leader in green hydrogen production. Opportunity creation for green hydrogen and its derivatives. Reduction in dependence on fossil fuels.

* **Energy Security :**

By producing green hydrogen from local renewable energy sources , the countries can lessen their reliance on imported fossil fuels . It improves energy security and reducing geopolitical risks. This diversification of energy sources can contribute to a more resilient and robust energy infrastructure.

* **Economic growth and creation of job opportunities :**

The development and deployment of green hydrogen technologies can spur innovation, economic growth and more job creation. As mot of the countries invest in infrastructure need to produce, store and transport green hydrogen. New industries and employment opportunities will definitely emerge. It will definitely support a more sustainable and inclusive global economy. The importance of green hydrogen lies in its novelities and new insights have been evolved. Green hydrogen is widely considered a much more promising solution for decarbonizing various sectors unlike transportation and industrial processes. However, its adoptation faces several challenges and barriers. Strategies and policies to be implemented The strategies and policies for green hydrogen deployment aims at facilitating the development and adaptation green hydrogen as a sustainable and clean energy carrier. It encompasses a wide range of measures which includes ambitious targets by providing financial incentives.

*** Impact on climate change :**

Green hydrogen is produced by means of electrolysis of water using renewable energy sources unlike solar, wind and hydropower. This process results in zero green house emission and making green hydrogen a clean and sustainable alternative to fossil fuels. By incorporating green hydrogen into the global energy mix up can lessen our dependence on carbon intensive energy sources and can significantly decrease the emission that are responsible for climate change.

*** Storage of energy and it's flexibility :**

Green hydrogen can be stored and transported easily. It makes an ideal solution for energy storage and grid balancing. This is particularly important as the world increasingly relies on intermittent renewable energy sources. It requires effective storage solutions to maintain grid stability. Green hydrogen can be converted back into electricity using fuel cells. It can combust to generate heat by providing a more flexible and reliable source of energy for multi-faceted applications.

*** Decarbonization :**

There are certain industries for heave transport , aviation and even steel manufacturing are difficult to electrify or decarbonizing using conventional renewable energy sources . Green hydrogen can serve as a low carbon fuel or feedstock in these sectors by providing a pathway to reduce emissions in areas in which other solutions may be less feasible. More and more investment in research and development and promoting infrastructure development and promoting infrastructure development

The key element of strategies and policies for green hydrogen deployment.

*** Green hydrogen as energy vector:**

It mainly highlights current state of hydrogen value chain from generation of end use. Hydrogen generation and distribution also it includes transmission. Hydrogen is more technically viable and being energy vector for various applications ranging from small scale power supply in off-grid modes to large scale chemical energy exports. However, as hydrogen is naturally unavailable in its purest form so traditionally reliant industries such as oil refining and fertilizers have sourced it through emission intensive gasification and reforming of fossil fuels. The deployment of hydrogen as an alternative energy vector has been broadly discussed.

Conclusion:

New direction of hydrogen production Water electrolysis as an energy intensive process that benefits from the use of catalysts . Because of the canonical hydrogen evolution catalysts is Pt and the oxygen evolution catalysts is RuO2 an important research focus for green hydrogen production has been development of catalysts that rival scarce metals in terms of performance but with reduce metal loading. The recent material research landscape in this area can be visualized in many ways. . We begin with presenting the

most commonly co-occuring concepts found to be is important in each respective study in a broad clustered network. From high level conceptual analysis we see that hydrogen evolution reaction and water splitting concepts are indexed with similar frequencies . While oxygen evolution reaction commonly occurs as well. This finding underscores the studies of overall water splitting must consider both oxygen evolution reaction and hydrogen evolution reaction respectively. In addition to this photo catalysts are becoming a common research then since 2021 and it significantly overlap with several nanomaterial related concept. The reason behind it photo catalysts co-occur as approximately the same rate as electo chemical reaction catalysts manifests how important it becomes. Finally the inclusion of surface oriented concepts unlike surface structure, surface area and pore size shows the relevance of surface phenomena in catalysts design. In between 2011 through 2021 , there is fivefold increase in green hygrogen production as per statistics. This is driven by concomitant increase in both journal article and patents. By experiencing rapid growth within this decade the publishing volume appears to be levelling off. The relative prevalance of most common nanomaterils inthis research are normalized. For given hydrogen production the nano particle concept is most common. It is followed by nanosheets and nano composites. The popularity of nanoparticle is well known with Pt nanoparticles being considered among top performing HER electrocatalysts.

Concerning the most commonly and equally popular nano sheets, the material chemistry has been significantly enamored with The evolution of two dimensional material for last fifteen years . As a result diverse set of products can be promptly be formed into atomically thin dimension to give rise to novel and useful phenomena for catalysis. When it is combined with nanocomposites materials high surface area catalysts can be formed which take advantage of nanoscale effects such as quantum confinement , surface plasmon resonance as well as internal interfacial effects.It includes aforementioned semiconductor heterojunction and schottky junction . In addition to trantition metals dichalconides C3N4. Nanosheets are used for green hydrogen production have thus far included layered hydroxides ,graphine, Mxenes, Bismuth oxyhalides , halde pervoskites and 2D MOFS, and covalent organic frameworks.

There is vast selection of electronic materials available in the toolbox of synthetic method impart control over particle size , shape, doping and defects ,crystallimity material interfaces. Thus it motivates a large number of observed studies on nanoscale morphology in case of green hydrogen production. The substance information that are found within any research publication provides additional insights. Analysis of relevant substance classes within this progression over time reveals several research treands.. Increase in compounds of the classes alloy and elements shows the exploration chemical space for alternative electrolysts to Pt. And as components in composite materials as electro and photocatalysts. The coordination compounds on the otherhand ,show an increase in material diversity. During the period of time metal-organic framework based on metal organic derived materials saw increased interest in heterogeneous catalysts. The

application of semiconductor engineering to photo catalysis saw compounds of the classes of general inorganic and oxide are increasesingly being applied to green hydrogen production throughout the decade along with their uses as catalysts supports. Finally polymers began to be studied at large as components in heterojunction catalysts as tunable and stand-alone porous catalysts and as precursors to be engineered carboneous catalysts materials.

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Chapter-6

Medication Error (ME) and the Ways to Prevent it by Nursing Interns

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Introduction: Medication errors (MEs) pose a serious concern in healthcare as they compromise patient safety and can lead to adverse outcomes. These errors may occur during prescribing, dispensing, and administration stages. Nursing interns, who are often at the forefront of patient care, play a pivotal role in preventing MEs. This article investigates the causes of medication errors and discusses the strategies that nursing interns can employ to reduce these errors.

Understanding Medication Errors

Definition and Types of Medication Errors

Medication errors are mistakes that can happen when using medication. These errors can cause harm to the patient and can be prevented. There are different types of medication errors.

1. **Prescribing Errors:** Improper selection of medication, incorrect dosage, or inaccurate route of administration.
2. **Omission Errors:** Noncompliance with medication administration.
3. **Wrong Time Errors:** Medication is administered outside of the prescribed time span.
4. **Unauthorized Drug Errors:** Administration of a drug not authorised by a legitimate prescriber.
5. **Dosage Errors:** Incorrect dosage delivery, either too much or too little..
6. **Administration Errors:** The administration method is incorrect.
7. **Monitoring Errors:** Failure to monitor and adjust therapy as required can have serious consequences and impact your progress.

Causes of Medication Errors

Several factors contribute to medication errors, including:

- **Communication Failures:** The occurrence of miscommunication among healthcare providers, patients, and caregivers is a matter that needs attention.

- **Human Factors:** Fatigue, stress, and inexperience.
- **System Failures:** Flaws in healthcare processes and systems.
- **Environmental Factors:** Distracting or chaotic work environments.
- **Knowledge Deficits:** Lack of knowledge about medications and their proper administration.

Strategies To Prevent Medication Errors

1. Education and Training

Nursing interns must complete thorough education and training programs that prioritize the criticality of medication safety. This encompasses:

- **Pharmacology Education:** Understanding the pharmacokinetics and pharmacodynamics of medications.
- **Simulation Training:** Participating in simulated scenarios to practice medication administration and error prevention.
- **Ongoing Education:** Attending workshops and conferences to stay updated on best practices and new developments in medication safety.

2. Effective Communication

To prevent medication errors, clear and effective communication is absolutely critical. Nursing interns must adhere to the following guidelines:

- **Use SBAR (Situation, Background, Assessment, Recommendation):** A standardized communication tool to ensure clear and concise information exchange.
- **Verify Orders:** Double-check medication orders with prescribers and pharmacists, especially if there is any ambiguity.
- **Engage Patients:** Encourage patients to be active participants in their care by asking questions and confirming their medications.

3. Adherence to Protocols`

Strict adherence to established protocols and guidelines can significantly reduce the risk of medication errors. Nursing interns should:

- **Follow the "Five Rights":** Ensure the right patient, right drug, right dose, right route, and right time.
- **Utilize Checklists:** Implement checklists to verify each step of the medication administration process.
- **Double-Check High-Risk Medications:** Collaborate with colleagues to double-check medications that are prone to errors, such as anticoagulants and insulin.

4. Use of Technology

Utilizing advanced technology can greatly improve the safety of medication administration. It's essential for nursing interns to have a strong command of the following:

- **Electronic Health Records (EHRs):** To access and verify accurate patient information and medication orders.
- **Barcode Medication Administration (BCMA):** To ensure the correct medication is administered to the right patient.
- **Automated Dispensing Cabinets (ADCs):** To reduce the risk of dispensing errors.

5. Creating a Culture of Safety

Fostering a culture of safety within healthcare settings is essential. Nursing interns should:

- **Report Errors and Near Misses:** Encourage a non-punitive environment where errors and near misses are reported and analyzed to prevent recurrence.
- **Participate in Safety Audits:** Engage in regular audits and reviews of medication administration practices.
- **Advocate for Safe Staffing Levels:** Ensure adequate staffing to reduce workload and fatigue, which can contribute to errors.

Conclusion

Medication errors pose a significant threat to patient safety, but nursing interns can play a vital role in preventing these errors through education, effective communication, adherence to protocols, the use of technology, and fostering a culture of safety. By adopting these strategies, nursing interns can contribute to safer healthcare environments and improve patient outcomes. It is imperative that healthcare institutions support and empower nursing interns with the necessary resources and training to mitigate the risk of medication errors.

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Chapter-7

Advanced Treatment Methods In Medical Sciences By Using Cationic Polymers

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Abstract :

Cationic polymers can be defined as polymers containing positive groups in their structures and these types of polymers can be synthesized by using positively charged particles. These types of polymers parade idiosyncratic chemical and physical utilization which permits these polymers for moreover changes offered for the utilization in advanced treatment methods in medical sciences. Due to developments in science and current research, we have witnessed considerable uses for these polymers. Cationic polymers can be used in various processes like drug delivery, tissue engineering, gene delivery, hydrogels, micelles, nanoparticles, antibacterial agents, and biofilm inhibitors due to their biodegradable properties and less toxic.

Keywords: Cationic polymers, chitosan, cyclodextrin, gelatin, cellulose

Introduction:

Cationic polymers can be defined as polymers containing positive groups in their structures and these types of polymers can be synthesized by using positively charged particles. These types of polymers parade idiosyncratic chemical and physical utilization which permits these polymers for moreover changes offered for the utilization in advanced treatment methods in medical sciences. Due to developments in science and current research, we have witnessed considerable uses for these polymers. Numerous health-giving applications are successfully investigated by using both cationic and anionic polymers. One of them can be described as combinatorial therapy, by combining basic short peptides, and proteins with cationic polymers. Ionic compounds can be formed by mixing anionic and cationic polymers[1]. As compared to negatively charged polymers, Cationic polymers are largely analyzed and form ionic bonds with various proteins, DNA, and RNA. Which can be used as the future treatment method for different healing processes. It can be used in gene delivery by the condensation process of nucleic acid with a cationic polymer and it cannot be destroyed by the action of enzymes. Apart from this, it can be used in antibiotic modifications, eradication of biofilms, phage

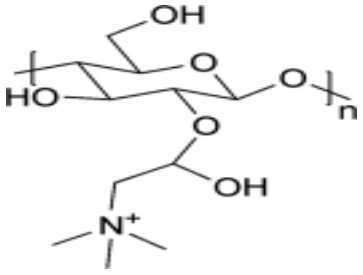
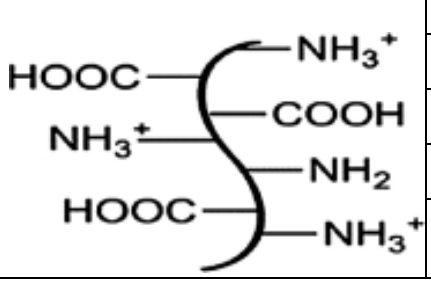
therapy, transdermal drug delivery, chronic wound healing, and other various treatment methods. Common examples of cationic polymers include poly(ethyleneimine) (PEI), poly-L- (lysine) (PLL), poly[2-(N, N-dimethylamine)ethyl methacrylate] (PDMAEMA) and chitosan. These polymers can be synthesized in the lab by using chemical groups dextrans and cyclodextrin. Maximum numbers of Cationic polymers having amine groups that are acidified. Every Cationic polymer has different numbers of hydrogen groups[2]. They can be seen in linear, branched, hyperbranched, and dendrimer- like. An example of a linear cationic polymer is PLL. Another one is PEI which is not only a branched but also a linear compound. Some of these contain positive groups in their side chain and others in their primary structure. Current research focuses on the formation of block copolymers containing polycationic backbone and the water-hating group as a side chain. An example is PEG-PLL. Which is informed in this review[3].

Cationic Polymers:

Different kinds of health issues can be treated with cationic polymer because it can be used as biomaterials. Major factors that are responsible for its activity are polymeric chain flexibility, H-bond formation, hydrophobic interactions, electrostatic forces or charge transfer potential, amine group and its neighboring functionalities, pKa, and nucleophilic character[4].

Types of Cationic Polymer:

Cationic polymers can be divided into two types natural and synthetic. Natural cationic polymer can be derived from zero-toxic natural products which are obtained from renewable resources. These are biocompatible, biodegradable, and possess low immunogenicity [5].

Cationic Polymer	Nature	Structure	uses
Cationic	Polysaccharide		Tissue Engineering
cellulose	Drug delivery		Gene Delivery
	Linear glucose		Drug delivery
	units linked by		
	b-1,4-D-linkage		
Cationic	Protein		Drug Delivery
gelatin	18 Non-		Tissue engineering
	uniformly		Gene Therapy
	distributed		
	amino acids		

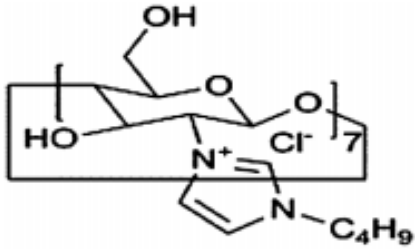
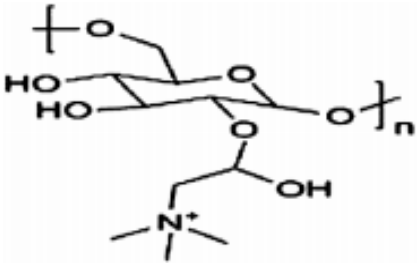
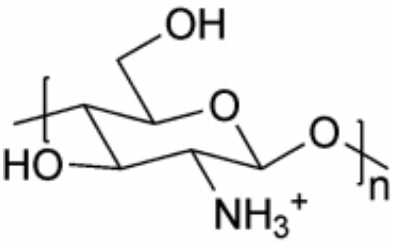
Cationic	Polysaccharide		Drug Delivery
cyclodextrin	Drug delivery		Tissue engineering
	Cyclic glucose		Gene Therapy
	units linked by		
	a-1,4-linkage		
Cationic	Polysaccharide		Drug Delivery
dextran	Drug delivery		Tissue engineering
	43, 86–88		Gene Therapy
	Glucose units		
	linked by a-1,6-		
	linkages		
Cationic	Polysaccharide		Drug Delivery
chitosan	Drug delivery		Tissue engineering
	27, 67, 68		Gene Therapy
	N-acetyl		
	glucosamine and		
	D-glucosamine		

Table 01: Natural Cationic Polymers and Uses

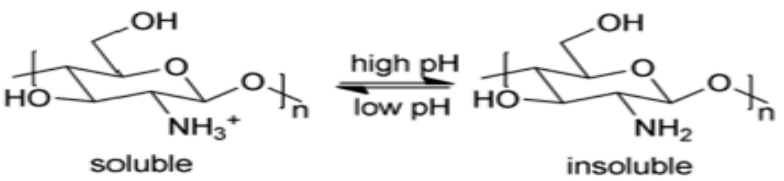
Cationic Cyclodextrin :

Cyclodextrins are cationic polymers obtained from carbohydrates (Starch), which are synthesized by bacteria. It is torus in shape and has cyclic oligomers of glucose-containing six to eight glucose units linked by a-1,4-bonds. The internal structure of it contains hydrocarbon and oxygen connecting the glucose groups[6]. Outside of this cationic polymer has water-compatible exterior parts. Internal structure of polymer containing macrocyclic ring. B-cyclodextrin is primarily considered as cyclodextrin rather than six (alpha, a), seven (beta, b), or eight (gamma, g) glucopyranose units. It is used in medical science due to its monodisperse saccharide compound, easily engineered with chelating ligands favorable toxicology. Another advantage of this polymer is less immunogenicity for which it can be used in cell targeting groups[7]. These polymers have the property to adhere with adenine, guanine, cytosine, uracil, and increased rate of gene therapy in biotechnology. In addition, CDs have already been mixed with polycationic polymers and dendritic vectors. Due to these reasons, it is used in various medical treatments. Yang et al. reported that the formation of various cyclodextrin

combined with oligo ethylenimine (OEI) bearing various chain lengths connected to the internal structure of this cationic polymer. A cationic star polymer is synthesized by mixing OH groups of 6 C6H12O6 and combined with OEI chains. This chemical reaction is formulated by combining 1,10 - carbonyldiimidazole (CDI), followed by a reaction with a large excess of OEI resulting in a-CD-OEI star polymers. Which is shown figure below. the molar ratio of CDI or OEI to a-CD was maintained at above 100, which proved that it does not contain any intra- or intermolecular crosslinking[8]. The group of Qian et al. prepared a range of novel cationic polymers and considered them as potential drug carriers. They reported that cationic b-CD of more MW and less cationic charge density showed good drug inclusion and dissolution properties. The formation of cationic b-CD was created via one-step condensation using epichlorohydrin choline chloride. The incorporation of the drug was also combined via b-CD–PEI conjugates[9]. Lu et al. proved that a b-CD–PEI cationic polymer containing 5-fluoro-20 -deoxyuridine showed its efficiency as a gene delivery system for glioma cancer treatment. CDI was again used in this study for the formation of 5-fluoro-20 - deoxyuridine to be combined with it to form b-CD–PEI. Davis et al reported that the utilization of CD-containing polymers for gene delivery. They have evaluated the potential to incorporate cyclodextrin into cationic polymers. Davis et al. reported that the utilization of cationic polymer-bearing CD is used in gene therapy. Along with this, they reported how to enhance the cationic properties of modified cyclodextrin[10]. This reaction is completed by combining cationic bifunctionalized co-monomers and bifunctionalized CD monomers. Bis(hydrogen carbonate) salt of 6A,6D-dideoxy-6A,6D-di(2- amino ethane thio)-b-CD hexahydrate with dimethyl suberimidate (DMS) combine with cyclodextrin to combine with Nucleic acid. Modified polymers are further mixed with pDNA (B5 kbp) which is negatively charged and has a size of 100nm -150 nm after this it can be used in vitro cell transfection efficiency comparable to that toxicity[11].

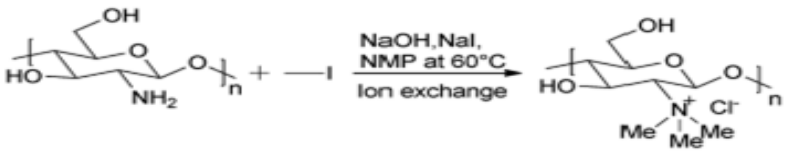
Chitosan:

Chitosan can be made up of obtained with PEI and Lipofectamine while preserving a reduced distributed N-acetyl glucosamine and D-glucosamine arranged in different ways. Chitosan is basic in nature having Ph 6-6.5. Due to the changing nature of Ph., it is suitable for various treatment methods[12]. The image of chitosan is shown below[13].

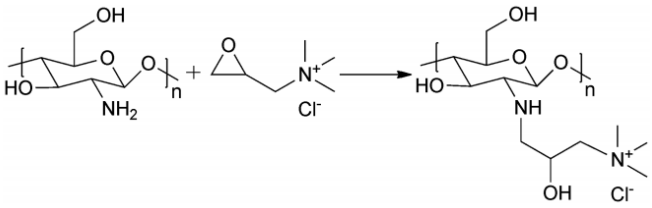


When the Pka value is more than the Ph of chitosan it is responsible for the formation of polyelectrolyte. At normal Ph, it helps in biomolecule interactions. It shows some properties like the removal of the acetyl group which helps in cell signaling. Communication between cells occurs due to the formation of polyelectrolytes[14]. It can

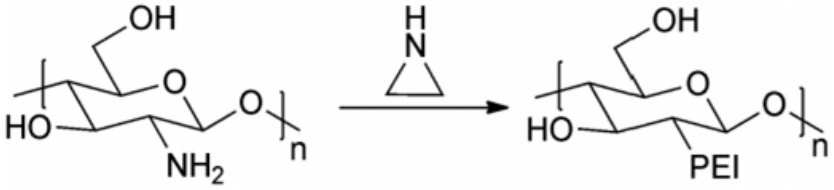
be used in chronic wound healing due to its antibacterial properties. The disadvantage of naturally obtained chitosan isits low solubility. Apart from this synthesized chitosan shows various properties towards therapeutics application. It contains major three reactive sides for the changes like amine groups like primary, secondary, and tertiary groups along with hydroxyl moiety and glucosidic group. The major step in the alteration of chitosan is successfully done by quaternization of the NH2 group and the addition of small chemical compounds to its primary structure. After the alteration it does not impact its original properties but it can enhance its antibacterial properties for which it can be successfully used for biofilm removal[14,15,16].Synthetic chitosan shows perfect bonding with biomolecules like DNA, and RNA, and enhances its property to form stable complexes. Chitosan is easily mixed with water which can be used in variable Ph. The mechanism for the quaternization reaction of chitosan involves a combination of CH3I with it. This process increases its mucoadhesive activity which is directly proportional to the degree of quaternization. For which it can be used in the delivery of genes. Formation of Tetramethyl chitosan is successfully done by combining CH3I with NaOH in NMP Temp 60 degrees. 2nd method involves the addition of Cl ion. Reaction is mentioned below[17].



Jia et al. researched that chitosan showed antimicrobial properties against Multidrug resistance bacteria (MDR) by combining it with N,N, Ntrimethyl, N-propyl-N, N-dimethyl, N-furfurylN, N- dimethyl and, N-diethylmethylamino. Apart from this oligomers of chitosan enhanced the antimicrobial activity of antibiotics. It can be used in the formation of engineered antibiotics[18].Yang et al. researched that combining folic acid with chitosan acts as an anti-cancer agent. It is used successfully for drug delivery of 5-aminolevulinic acid. During the delivery process of chitosan, it is involved in the biochemical mechanism of folic acid synthesis. 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) is mixed with chitosan to form complexes with folic acid. The molarity of the solution is 1: 0.02 to 1: 0.2 for the amine groups of chitosan to folic acid. An engineered chitosan is formed by combining transactivating transcriptional activator peptide (TATp) with folic acid to form alteration in derivative octadecyl-quaternized lysine-modified chitosan (OQLCS).Combining folate- PEG with N-((2-Hydroxy-3-trimethylammonium)propyl)chitosan chloride (HTCC), enhances the drug delivery property of paclitaxel, which is easily soluble in water. Glycidyl-trimethyl-ammonium chloride mixed with to form complexes[19].

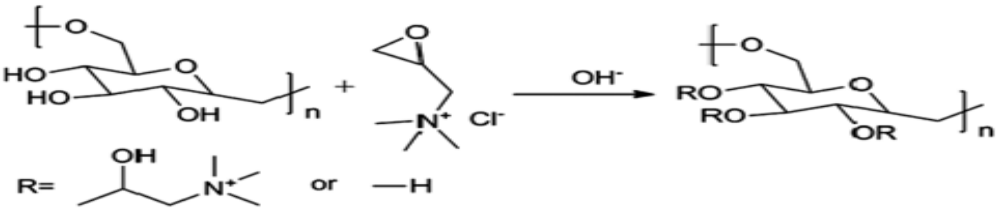


Another chemical reaction involves a combination of chitosan with PLL. Modified chitosan reduced cell toxicity and enhanced its binding capacity towards DNA[20].



Cationic Dextran:

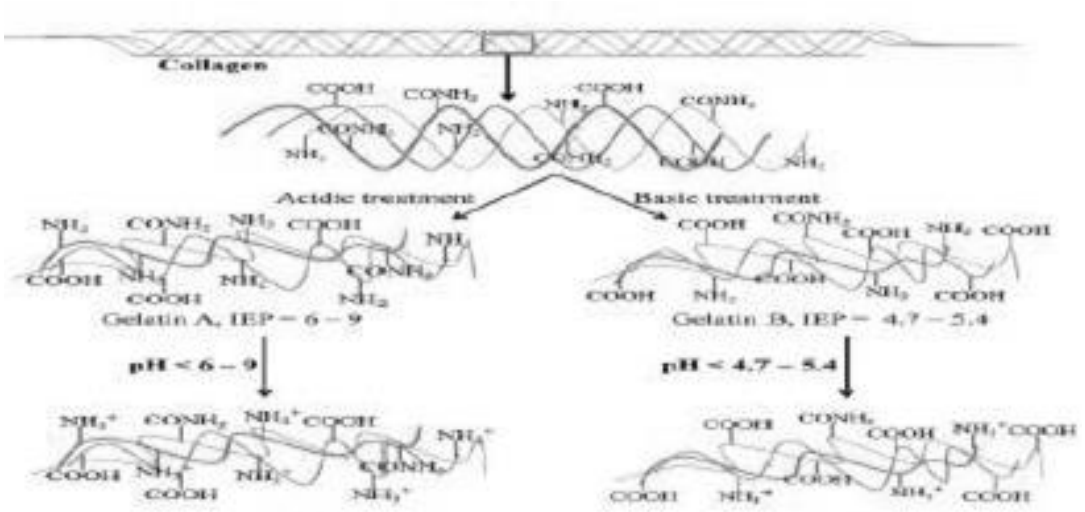
It is composed of glucose molecules connected through 1,6- linkages polysaccharides. It dissolved in water and was independent of ionic concentration. Dextran can be used in gene therapy, and drug delivery due to its biodegradability[21,22]. In the process of polymerization, it can be easily modified and enhance its antibacterial properties towards superbugs. For example, diethyl aminoethyl–dextran and dextran–spermine are used in the delivery of DNA and RNA. Kaminski et al. researched the replacement of the hydroxyl group with glycidyl trimethylammonium chloride for anticoagulant treatment[23].



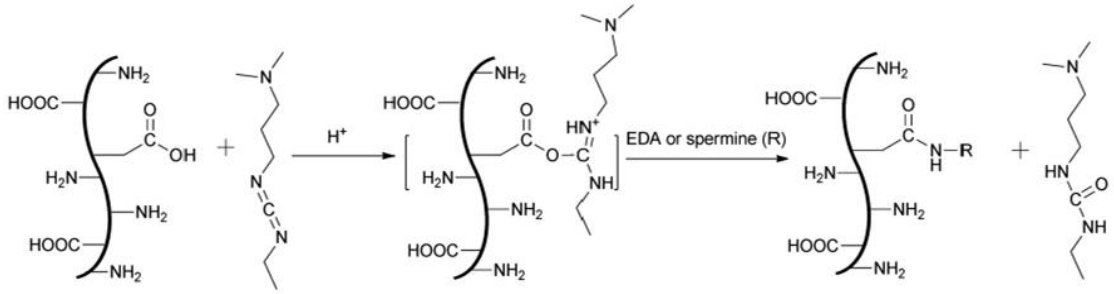
During this substitution reaction degree of polymerization ranged between 0.50 to 0.65 GTMAC groups per glucose unit[24]. This modified cationic polymer combined with heparin to increase its binding capacity towards biomolecules. A combination of spermine and dextran showed an increase in transfection properties in DNA and grafted spermine groups. Along with this, these cationicpolymersare used in the transfection of extra circular DNA in bacteria. It is used in the delivery of si-RNAby combining acetyl dextran with spermine. This modified cationic polymer is used in the delivery of proteins[25].

Gelatin:

Gelatin is a gel-like polymer obtained from collagen protein by the process of breaking of short peptide chain[26]. It is used in various pharmacy research companies and medical science. It is eco- friendly in nature and made up of amino acids such as lysine and arginine. These amino acids are responsible for its cationic properties. It is divided into two types Gelatin A & Gelatin B having PI 4 of 6–9 and 4.7–5.4 respectively[27,28]. The process of formation of gelatin from collagen is shown below.



Here COOH groups are formed from amino acids such as glutamine and asparagine, It is responsible for its acidic properties and it is negatively charged. Cationic strength is more in gelatin with acidic properties. It is widely used in nanomaterials and approved by the US Food and Drug Administration (FDA)[28,29]. By utilization of carbodiimide chemistry, adds hydrogen ions to gelatin. Morimoto et al. have researched that the addition of ethylenediamine (EDA) or spermine through an EDC-mediated reaction to gelatin, enhanced the cationic property. Details of reactions are shown below[30].



Modified gelatin is not dependent on Ph and it is easily combined with negatively charged biomolecules[31]. Xu et al. researched in vitro by applying insulin-like growth factor (IGF)-1 to gelatin for drug delivery. From this study, it is understood that engineered gelatin showed stable bonds with IGF-1 in culture. Amino acid combined with gelatin combined with protein-enhanced its antibacterial properties. For which it is suitable for tissue engineering, drug delivery, and eradication of biofilm[32].

Cationic Cellulose:

Cellulose is a polysaccharide, composed of b-1,4-D-glucan[33]. It is a major component cell wall along with this it shows various properties such as including hydrophilicity, biodegradability, and antibacterial properties. Modification of cellulose with the help of glycidyl ammonium salts in the presence of NaOH. Song et al. had researched the quaternization of cellulose in an aqueous solution[34].During this process, cellulose was mixed with sodium hydroxide and urea. After that 3- chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTA) is combined with it in a basic medium, and used in the etherification process. During this reaction, quaternized cellulose and epoxide were formed as end products[35]. Diols are synthesized as side products during the formation of this cationic end product. This modified cellulose can be used in drug delivery and it is one of the future medical methods to be used treatment of nosocomial infections. We can also synthesizecationic-engineered cellulose by homogeneous method, these are not mixed with H2O and other organic chemical compounds due to their high resonating power of hydrogen bonds[36]. Song et al. reported on the formation of engineered quaternized cellulose. It is prepared by two-step methods. In the primary step, nitrogen is mixed with water-soluble quaternized cellulose in the process of Sodium Hydroxide -urea solution. Cellulose contains hydroxyl functional groups mixed with hexadecyl bromide, Which enhances the water-hating property of the synthesized cationic polymer. It can be used as Micelles for the delivery of rarely water-mixed drugs. Other examples of modified cellulose are, hydroxypropyl cellulose (HPC) and hydroxyethyl cellulose (HEC), which are used in the composite film, the formation of antibiotics[37]. Another derivative of this cationic polymer was reported by Xu et al. which is made up of HPC backbones and cationic PDMAEMA side chains and used in studies as novel non-viral gene vectors. It appears as comb comb-shaped cationic polymer. By using trans radical polymerization the long HPC backbone (HPD) is mixed with short PDMAEMA chains during this process bromo-iso-butyryl terminated HPC (HPC-Br) is the macroinitiator. At the end of the reaction, we can get quaternary ammonium HPD (quaternized HPD)[38]. Fayazpour et al reported the application of this polymer in gene delivery in HEC/plasmid DNA (pDNA) nanoparticles[39].

Artificial Cationic Polymers:

Not only natural cationic polymers but also artificial cationic polymers are synthesized in the lab and used as novel approaches toward advanced research tools for future medical sciences. Limitations of natural polymers can be overcome by using synthetic cationic polymers. We can easily modify the artificial cationic polymer as compared to natural cationic polymers. The bioactivefunctional groups can be easily incorporated into the synthetic polymeric system to form specific MWs and block structures with degradable connectors if necessary. These unique qualities of these polymers are enhanced in the field of the therapeutic potential and their eco-friendly nature towards green chemistry

due to their less toxic byproducts. Examples of synthetic cationic polymers along with their structures and applications are mentioned in the picture below[40,41,42,43].

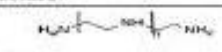
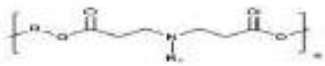
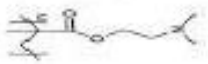
Cationic polymer	Nature	Structure	Application
PEI	LPEIs contain all secondary amines RPEIs contain primary, secondary and tertiary amines		Drug delivery Tissue engineering Gene delivery
PLL	Homopolymer of the amino acid L-lysine		Drug delivery Tissue engineering Gene delivery
PAA	Synthetic cationic polymer containing tertiary amino groups		Drug delivery Tissue engineering Gene delivery
PAE	Amine containing polyesters		Drug delivery Tissue engineering Gene delivery
PDMAEMA	Synthetic cationic polymer containing tertiary amino groups		Drug delivery Tissue engineering Gene delivery

Table: 02 Synthetic polymer and their uses

Properties of Cationic Polymer:

Synthesis and formation of cationic polymer depend upon some factors. It is essential to alter some acquired properties of these polymers to achieve their perfect function in Research. It is based on 5 factors: which are (I) stimuli-responsive, (II) antimicrobial, (III) antioxidant, (IV) antitumor, and (V) anti-inflammatory properties.

1. Stimuli-Responsive Cationic Polymers:

Based on developments in science and research methodology, medical science requires highly brainy techniques to overcome dangerous diseases with fewer side effects. Mainly it involves the separation of DNA, RNA, nucleotides, managed gene delivery, etc. The responsive property of cationic polymer is very impressive in the field of targeted gene therapy. It is impacted by a signaling process to the affected region by the disease. Examples of outside stimuli are temperature, pH, ionic concentration,

light, magnetic field, electric field, and chemicals. Which depends upon the structural and functional group of cationic polymer[44,45,46,47].

2. Antimicrobial Properties of Cationic Polymers

Antimicrobial resistance is a major problem faced by us due to biofilm and indirectly it impacts our day-to-day life. Multi-drug-resistant bacteria – the superbugs – are responsible for various diseases in us. It's time to discover new engineered antibiotics in combination with a cationic polymer to enhance the life span of classical antibiotics[48,49,50,51].

3. Anti-Oxidant Nature :

Cellular reactants which require oxidation reaction for their metabolism, are inhibited antioxidants. Apart from this, cationic polymers act as reactive oxygen species (ROS). Mitochondria produces superoxide radicals, hydrogen peroxide, and hydroxyl radicals

which are mixed cationic polymers to enhance their antioxidant properties. These properties indirectly deal with cell signaling and, the immune system[56,57].

4. Anticancer properties:

Cationic in combination with anti-tumor agents helps reduce the growth of cancer.DMAEMA was incorporated with N-isopropyl acrylamide (poly(NIPAM-co-DMAEMA)) and evaluated as nanoparticle c for the controlled release of a water-hating anticancer agent, 7-ethyl-10-hydroxy- camptothecin (SN-38).243 The thermo-sensitive poly(NIPAM-co-DMAEMA) nanoparticles were synthesized by free radical polymerization[58,59,60].

Future Prospectives & Conclusion:

The primary aim of this review was to draw attentionto some of the recent advances in the field of cationic polymer-mediated treatment processes in medical science. Cationic polymers can be used in various processes like drug delivery, tissue engineering, gene delivery, hydrogels, micelles, nanoparticles, antibacterial agents, and biofilm inhibitors due to their biodegradable properties and less toxic. Nowadays modern problem requires novel techniques to be solved such as cationic polymer. It can be used as an emerging new approach towards medical science by alteration in their structure. This hasty review regarding cationic polymer will help researchers for further modification and utilization in health science.

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Chapter-8

Analyzing PM2.5 levels across diverse zones in Cuttack, Odisha

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Abstract

Public health is severely threatened by severe PM2.5 exposure. This study presents a comprehensive assessment of PM 2.5concentration in seven different places(Lunahar, Balisahi, SCB Medical college, CMC Dumpyard, Netaji Bus terminal, Jagatpur Industrial Estate and Tangi)of Cuttack,Odisha starting from the month November 2023 to March 2024. Digital air quality monitoring equipment was utilized for data collection purposes .The above study also focuses on establishing a relationship between the pollutant concentration, temperature fluctuation and humidity. Data analysis revels significance disparities in pollutant concentration among locations and months, with industrial areas exhibiting consistently higher level compared to residential or commercial areas.From November to March, air quality data revealed fluctuating PM2.5 levels across locations. In November, SCB Medical College recorded the highest morning PM2.5 levels, peaking at 203.00 μg/m³. January showed significant increases at Lunahar, alongside consistently high PM2.5 levels at SCB Medical College and CMC Dumpyard. By February and March, PM2.5 levels decreased, yet SCB Medical College and Jagatpur Industrial Estate still exhibited elevated readings.

Key words: Air quality monitoring, PM2.5 exposure, Temperature fluctuation, Humidity, Industrial areas, Residential areas, Pollutant concentration.

1. Introduction

Air plays a significant role in regulating Earth's climate. However, human activities have led to an increase in greenhouse gas concentrations, contributing to global warming and climate change. The quality of the air we breathe directly impacts our health and well-

being. Pollutants such as particulate matter, ozone, nitrogen dioxide, and sulphur dioxide can have harmful effects on respiratory and cardiovascular systems, leading to health problems like asthma, bronchitis, and cardiovascular diseases. Maintaining good air quality is essential for public health. Particulate matter (PM), especially fine particles like PM2.5, can penetrate deep into the respiratory system, causing health issues such as respiratory infections, asthma, bronchitis, cardiovascular diseases, and even premature death. These particles can evade the body's natural defences, reaching deeper into the lungs and bloodstream. PM also impairs visibility by scattering and absorbing sunlight, affecting aviation, transportation, and scenic views, and contributing to haze and smog in urban areas, creating unpleasant living conditions.PM originates from natural sources like wildfires, volcanic eruptions, and dust storms, as well as anthropogenic sources such as vehicle emissions, industrial processes, construction activities, and agricultural practices. Combustion of fossil fuels, biomass burning, and diesel-powered vehicles are significant contributors to urban air pollution.The permissible annual average concentration of PM2.5 according to Central Pollution Control Board (CPCB) in India, the National Ambient Air Quality Standards (NAAQS) is 40 micrograms per cubic meter (μg/m³), and the 24-hour average should not exceed 60 μg/m³.

Areas closer to industrial zones exhibit moderate air pollution levels, ranging from 51 to 75 on the AQI scale. The region surrounding steel industries shows signs of transitioning from moderate to heavy pollution in the near future(Bhuyan P K, et al (2010)). The study conducted in Rohtak city, Haryana, monitored ambient air quality using High Volume Sampler. NO2 exceeded standards at four sites in winter. Ozone levels were below standards but higher in summer. SPM concentrations surpassed safety limits across all sites and seasons.(Shukla V, et al 2010). Transport emissions and increasing construction activities contribute to pollution, exacerbated by rising vehicle volume and traffic patterns. Results revealed that criteria pollutants SPM, CO, SO2, and NO2 either exceeded or approached limits, highlighting the need for continuous monitoring and control mechanisms. (Balashanmugam P, et al (2012)). Prolonged exposure to PM2.5 increases the likelihood of developing type 2 diabetes mellitus, although further research is needed to definitively establish the link between PM2.5 and GDM (gestational diabetes mellitus). Implementing effective measures to reduce PM2.5 exposure in vulnerable populations, particularly pregnant women, would be prudent (D He, et al. (2017)).The various survey shows that there is positive relationship between air pollution levels and sick days, suggesting that reducing pollution may decrease illness (Nayak Tapaswini and Chowdhury Indrani Roy (2018)). In Sambalpur, Odisha, air pollutant monitoring was conducted at four stations for a year, following CPCB guidelines. Gaseous pollutants remained within standards, but particulate matters exceeded limits. Meteorology and anthropogenic activities influenced pollutant dispersion. Continuous monitoring and source reduction for particulate matters are crucial for improving air quality(Sahu C & Sahu S.K (2019)). Meteorological analysis showed a significant negative correlation

between relative humidity and pollutants (Sharma Rajat & Kumar Ashutosh (2023)).The study conducted , focusing on Odisha's remote opencast coal mining region, confirms that proximity to mining increases respiratory illness (RI) likelihood, potentially influenced by underreporting bias. Regression analyses highlight the significance of variables like distance from mine, treatment, and per capita income, indicating higher RI likelihood in closer proximity to mines and in treatment villages (Chowdhury Indrani Roy, et al. (2024)).

This study presents a comprehensive summary of the seasonal variation of PM2.5 concentration in seven different places(Lunahar , Balisahi ,SCB Medical college ,CMC Dumpyard, Netaji Bus terminal ,Jagatpur Industrial Estate and Tangi) of Cuttack, Odisha starting from the month November 2023 to March 2024., based on ground observations taken by using digital air quality monitor. This analysis reveals the observation-based patterns of human activity and local temporal characteristics of emissions in each place, and hence provides valuable input for comparison studies. The results of this study are valuable for the designation and implementation of mitigation policies on a city level aimed at improving air quality to meet the Indian NAAQS standards.

2. Instrument Used And Methodology

2.1 Study Area

Cuttack is situated at latitude 20°30' north and longitude 85°50' east, located on a fertile delta at the confluence of the Mahanadi and Kathajodi rivers. The city is approximately 35 kilometres from the Bay of Bengal and experiences significant tidal influence from the rivers.

With a population of around 1.5 million, Cuttack serves as a major urban and industrial centre in Odisha. Given its proximity to industrial areas and the heavy vehicle traffic in the city, measuring the ambient concentration of PM2.5 is critical. Regular monitoring helps assess exposure levels and identify pollution sources, facilitating effective management of air quality and safeguarding the health of residents.

2.2 GPS Positions of Sampling Locations

Table 1: GPS Locations of Study Area

Category	Location	Longitude	Latitude
Residential	Balisahi	86.110002	20.483637
Residential	Lunahar	86.108904	20.482
Constructionsite/ Health care	SCBmedical college	85.892237	20.477769
Wastedisposalsite	CMC Dumpyard	85.849514	20.474956

Trafficcrossing/bus stand	NetajiBusterminal	85.903008	20.447488
Industrial	JagatpurIndustrial Estate	85.920215	20.495293
Trafficcrossing	Tangi	85.997602	20.555828

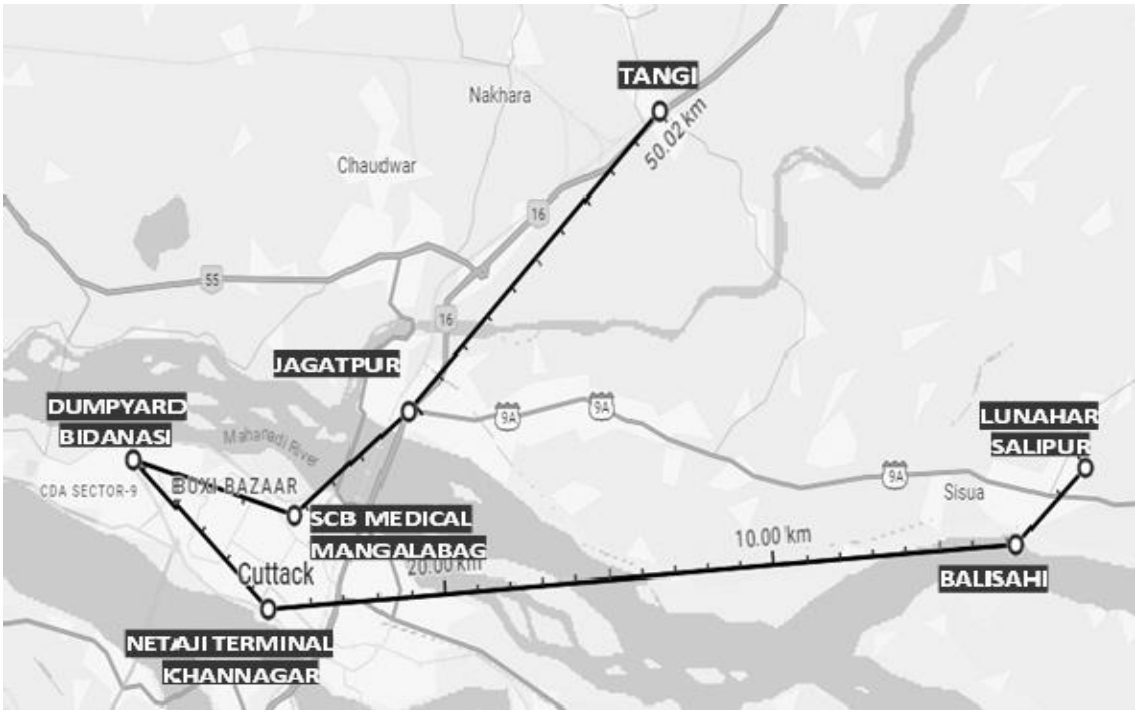

Fig.1. Study area showing sampling locations

Instrument Specifications

Fig.2: Digital Air Quality Monitor

This scientific air quality detection device integrates multiple air sensors and includes a built-in fan, enabling real-time monitoring of formaldehyde (HCHO), total volatile organic compounds (TVOC), PM2.5/10, AQI, temperature, and humidity, displayed on its digital LCD screen.

Data Collection And Analysis Technique

The entire research endeavor spanned from November 2023 to March 2024, encompassing duration of five months. Data collection occurred at seven designated locations on the 14th day of each month, encompassing three time slots: morning (6:00 am), mid-day (1:00 pm), and evening (7:00 pm). Digital air quality monitoring equipment was utilized for data collection purposes. Subsequently, the Air Quality Index (AQI) values for PM2.5 were computed utilizing the formula stipulated by the Central Pollution Control Board (CPCB). The computation process was entirely conducted using formulas within the Excel application. Following the calculation of AQI values for pm 2.5 for each month, comprehensive analysis ensued. The principal objective of the analysis was to ascertain compliance with permissible limits for pollutant concentrations and to establish correlations between meteorological parameters (temperature and humidity) and pollutant (PM2.5) concentrations.

Fig.3. Field images

Fig.4. Field images

3.1 Formula Used For AQI Calculation

1. Formula used:

$$Ip=\left(\frac{IHi-ILow}{BPHi-BpLow}\right)(Cp-BpLow)+ILow$$

Ip : The air quality index

Cp : The pollutant concentration

BpLo : The concentration break point that is ≤ Bp

BpHi : The concentration break point that is ≥ Bp

ILow :The index break point corresponding to Bplow

IHigh :The index breakpoint corresponding to Bphigh

Table 2: Standard AQI Values for Different Pollutants (as per CPCB standards)

AQI Category (Range)	PM_{10} 24-hr	$PM_{2.5}$ 24-hr	NO_2 24-hr	O_3 8-hr	CO 8-hr (mg/m³)	SO_2 24-hr	NH_3 24-hr	Pb 24-hr
Good (0-50)	0-50	0-30	0-40	0-50	0-1.0	0-40	0-200	0-0.5
Satisfactory (51-100)	51-100	31-60	41-80	51-100	1.1-2.0	41-80	201-400	0.6 –1.0
Moderate (101-200)	101-250	61-90	81-180	101-168	2.1- 10	81-380	401-800	1.1-2.0
Poor (201-300)	251-350	91-120	181-280	169-208	10.1-17	381-800	801-1200	2.1-3.0
Very Poor (301-400)	351-430	121-250	281-400	209-748*	17.1-34	801-1600	1201-1800	3.1-3.5
Severe (401-500)	430 +	250+	400+	748+*	34+	1600+	1800+	3.5+

*One hourly monitoring (for mathematical calculation only)

Image credit: National Air Quality Index Report by Central Pollution Control Board

Results:

4.1 AQI Values For PM2.5

Table 3: AQI values of Study Area during different time period

Slno.	**Month: November Timing**			
	Place	Morning	Mid-day	Evening
1.	Lunahar	561.86	104.41	53.21
2.	Balisahi	145.38	114.66	83.93
3.	SCBMedicalCollege	121.48	112.24	22.48
4.	CMC Dumpyard	22.48	112.00	295.59
5.	NetajiBusTerminal	87.34	80.83	115.66
6.	JagatpurIndustrial Estate	200.00	159.03	60.03
7.	Tangi	60.03	112.24	165.86
	December			
1.	Lunahar	330.16	261.86	244.98
2.	Balisahi	352.42	269.53	254.19
3.	SCBMedicalCollege	304.84	254.19	312.51
4.	CMC Dumpyard	296.40	269.53	363.16
5.	NetajiBusTerminal	309.44	250.35	297.16
6.	JagatpurIndustrialEstate	317.88	268	286.42
7.	Tangi	265.70	261.09	268.77
	January			
1.	Lunahar	286.34	166.86	825.72
2.	Balisahi	221.48	91.76	777.93
3.	SCBMedicalCollege	979.34	276.10	207.83
4.	CMC Dumpyard	1027.14	265.86	344.38
5.	NetajiBusTerminal	610.66	163.45	388.76
6.	JagatpurIndustrial Estate	125.90	433.14	170.28
7.	Tangi	78.10	474.10	170.28
	February			
1.	Lunahar	327.09	284.12	296.40

2.	Balisahi	330.93	254.19	281.81
3.	SCBMedicalCollege	289.49	226.56	234.23
4.	CMC Dumpyard	244.98	228.09	223.49
5.	NetajiBusTerminal	223.49	229.63	225.02
6.	JagatpurIndustrial Estate	250.35	286.42	282.58
7.	Tangi	259.56	317.88	301.00
	March			
1.	Lunahar	108.45	89.86	138.86
2.	Balisahi	100.00	110.14	130.41
3.	SCBMedicalCollege	52.69	34.10	32.41
4.	CMC Dumpyard	51.00	35.79	29.03
5.	NetajiBusTerminal	54.38	29.03	23.97
6.	JagatpurIndustrial Estate	115.21	267.28	91.55
7.	Tangi	91.55	240.24	203.07

Conclusion And Discussion

From the above study and analysis it was found out those Industrial areas like Jagatpur Industrial Estate show higher pollutant levels compared to residential or commercial areas like Netaji Bus Terminal. There are fluctuations in pollutant concentrations across months. For example, PM2.5 levels at SCB Medical College seem to decrease from November to January but increase again in February. As the temperature and humidity increases, the pollutant concentration is also getting increased. The analysis also aimed to ascertain compliance with permissible limits for pollutant concentrations. By comparing the measured concentrations with established standards, it was possible to identify areas of concern and prioritize mitigation efforts. Continued monitoring of air quality parameters beyond the study period will provide valuable insights into seasonal trends, long-term variations, and the effectiveness of mitigation measures implemented over time. Further research focusing on the health impacts of air pollution in Cuttack, particularly among vulnerable populations, can inform public health policies and interventions aimed at reducing health risks associated with poor air quality. Integrating meteorological data with air quality measurements will enhance our understanding of the factors influencing pollutant dispersion and accumulation, enabling more accurate air quality forecasting and management.

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Chapter-9

Innovative Strategies for Enhancing Food Security: Integrating Biotechnology and Agroecology

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Abstract

Food security at the global scale is one of the major problems that the world confronts, with a rapid growth in the population and wide spread of adverse environmental factors. This work evaluates the possibility of applying biotechnological and agroecological technologies as new Sustainable ways to boost world food safety. Biotechnology enables GM crops with additional features, including pest resistance, drought tolerance and high nutrient content. On the other side agroecology recommends and preserves cultivation approaches like rotation farming and diverse intercropping. Trials conducted all around the broad agro ecological areas indicate that mixing these techniques provides more crop yields than the conventional methods. This integrated approach, which promotes productivity as well as soil health, biodiversity, and ecosystem resilience, stands for an ecologically sound agricultural system that can sustain human life and build a more resilient nature. Bending the rules of bureaucracy and creating joint work of biotechnologists with agro ecologists are determinative in the world-wide popularization of these methods among farmers. Policy and institutional capacities licensing conjunctive research and participatory methods will be crucial. To sum up, the combination of biotechnology and agroecology increases food security freeing it from shocks or reversals and the fair and sustainable farmer and consumerwelfare.

Keywords: Access to food, genetics, agriculture parallel systems, agricultural productivity andclimate change.

Introduction

Secure food supply, namely the fact that the required food is readily available and it is nutritious and safe for active and healthy living, is one of the most important challenges on a global scale. Hungeris still a fact of life for nearly half a billion people around the world with the FAO projections pointingto an even more inexorable growth of the number of undernourished and suffering people since 2019 (FAO et al., 2020). The world population is projected to surge by 9.7 billion by 2050 and hencethe strategies to secure food for all is the need of the hour (UN, 2019). Biotechnology and agroecology are two ways that have so far failed to tap into their potentials and may be seen as beingmutually

non- interdependent. Synergizing these strategies by means of interdisciplinary innovationcould be a way to bring about a sustainable and efficient food security to the population.

Biotechnology taken in general is a set of technologies that brings about the modification of living organisms to achieve certain valuable products or to improve plants, animals, or microorganisms. In "agriculture," biotechnologies such as gene engineering, marker-assisted breeding, and tissue culture are genetically modified to produce crops that yield higher crops, better nutrition, and resistance to diseases, pests, and stresses from the environment (Prado et al., 2014). Genetically modified crops like the insect-resistant and herbicide-tolerant have been a factor for the improvement of the global economy and environment (Klümper & Qaim, 2014). Besides genome modification, and other biotechnologies that have been proved to give hope for sustainablyenhancing global food security (Abdallah et al., 2015).

Agroecology makes use of natural concepts and rules to ensure equilibrium between plants and animals, people and environment with a consideration of socio-economic factors (Dalgaard et al., 2003). Agroecological practices, namely, crop rotation, intercropping, usage of natural soil conditioners, and IPM are the key agro practices. The results show clearly that agroecology is sustainable crop diversification for increasing yields and food security especially for small farmers (FAO, 2018). Agroecology does not only develop the sustainability factors like to include the growth of biodiversity, to boost the resilience of our climate as well as farmers' empowerment (HLPE, 2019).

Despite attracting attention because of their much anticipated benefits, traditional approaches having biotechnology and agroecology in isolation involve some weakness. Although most of the preexisting genetic engineering crops serve the purpose of facilitating weed and pest control for large-scale farmers they do not, directly, help in improving the life of small holders in the underdeveloped nations (Stone & Glover, 2016). The lack of biotechnology projects in crops such as cowpeas, fava beans and millets which are the nutritional source for the poor farmers is evident since only a small number of projects have focused on the plants. On the other hand, agroecological intensification tolls an avenue that may perhaps not increase global food production at a rate that is sufficient to match with future demand (Garnett et al., 2013).

Taking advantage of the coordination between biotechnology and agroecology would be a great way to powerfully unleash their joint potential of delivering sustainable food security at different scales of agriculture, i.e., smallholder farms and commercial plots. Smallholder farmers would be able to participate in research undertaking project through community-based participatory research projects (Ceccarelli et al., 2009) designing and improving locally grown crops with various genetically engineered traits that would qualify for low-input agriculture in diverse agro-ecosystems. Genome editing has attractive prospects for boosting nutrition and the propagation of orphan crops that are

necessary in subsistence farming in a short time. (Abdallah et al., 2015) In case of big farms, the combination of ecological principles like crop rotation or intercropping with genetically engineered crops will increase the sustainability, as shown in the research by Davis et al. (2012). Policy and institutional changes where biotechnologists and agroecologists interact and work together will be critical to the discovery and application of the new possibilities that stem from the mix of distinct social-ecological contexts (Foley et al., 2011).

To conclude, a fruitful and accomplishable process would be through modern, holistic and flexible methods of food production that would be unique to specific regions. Effectively, the combination of the potential of biotechnology and agroecology can be the right and the most powerful way of modification. This can be used from small subsistence farms all the way up to the backbone of the commercial operations. Even though there are institutional barriers now that stop cooperation between these two communities, both sides must find ways of overcoming and breaking these barriers. The future seems to lie in participatory research. It would involve a concerted effort to integrate biotechnology into agroecology with an aim of designing innovations that are both equitable and novel. This will help in sustainably enhancing food security on a global scale.

Material and Methods

1. Study Design

Researchers sought to discover how technology and agroecology could be used together better in order to improve food stability. We employed a mixed-method strategy consisting of articles reviews, field experiments and expert interviews that allow us to get a 360-degree view of the specific topic under study.

2. Field Experiments Experimental Site Selection

Experimentation sites were chosen to represent different agroecological zones across the continent which includes factors such as climate variability, types of soils and cropping patterns. Sites selection was carried out together with agricultural authorities in accordance with local environmental requirements to meet expectations of future real-world situations. According to the given sentence,the pollution level in each city is dependent on the specific pollution emissions from the city's economic activities and the meteorological conditions of that location.

3. Crop Selection

Our target was on the staple food crops, which contributed strongly to economic status and nutrition, in the specific geographic regions. The choice of major crops, like maize, rice, wheat, and soybeans, as the main crops was made on the basis of their considerable cultivation on a global scale as well as their wide consumption.

4. Experimental Design

Treatment was allocated by randomized complete block design, and the experimental sites were organized as the blocks to avoid biasing the results. The three replications of each approach were performed to ensure that the effect of variability was as small as possible and also each approach had enough statistical stability.

5. Biotechnology Interventions

Biotech procedures consist of genetically engineered crop plants with resistant pests, drought tolerance and extra nutrients. These alternative crop varieties are used as a comparative group between conventional and organic crops to study the performance in alternate agro ecologies.

6. Agroecological Practices

The agroecological model consisted of all kinds of sustainable farming methods amongst all of them were intercropping, crop rotation, agroforestry, and IPM practices. They were conducted with the intention of increasing soil fertility, variety of plant species, and resistance to the possible impacts of climate change as well as reducing dependency on imported substances.

7. Data Collection

Data harvesting involved a system of continuing crops' growth tracking, pest presence, soil properties, and production parameters, thus, being recorded permanently all the way through the crop cycles. Before data collection, there were standard protocols which were followed closely at all sites in order to do comparisons.

8. Expert Interviews

Farmer communities, agricultural policy experts, researchers, farmer unions and non-government organization representatives took part in expert interviews with the purpose to enrich the discussion with qualitative aspects from socio-economic and political sides concerning integration of biotechnology and agroecology for food security. Through the use of semi-structured interviews, the author had the chance to explore the interview data in as much depth as possible, which enabled them to get into details.

9. Data Analysis

Quantitative data were analyzed by the means of the most relevant statistical techniques, such as analysis of variance (ANOVA) and linear regression, to see how biotechnology manipulation, and agroecological practices impact crop yields. Qualitative data and experts' opinions were analyzed after combining to unveil the major patterns and general understanding.

10. Ethical Considerations

This study was in compliance with the ethical standards for use of genetically modified organisms (GMOs) and participation in individual interviews was one of its procedures

that required written informed consent from those involved. Likewise, we applied transparency and integrity in reporting, regarding research findings that will, in turn, promote trust and accountability.

Result And Discussion

Treatment	Crop Yield (kg/ha)
Control (Conventional)	5000
Biotechnology (GM)	6000
Agroecology	5500
Biotechnology + Agroecology	6500

Table 1: Crop Yield Performance under Different Experimental Treatments

This table compares crop yields in kilograms per hectare (kg/ha) across four different agricultural treatment methods: the Conventional/control, biotechnology as a Genetically Modified Organism (GMO) crops, agroecology, and a hybrid of biotechnology and agroecology. The comparative or the traditional method is the use of modern industrialized agriculture synthetic fertilizers and pesticides. Therefore 5,000 kg/ha at the table was my outcome. Biotechnology means modifying crops by expressing gene engineering and having their DNA altered to produce desired characters such as resistance to pests or diseases. Biotech yield is 6,000 kg/ha, and the table presents a 20 % percent higher yield value compared to the conventional crop. Agroecology is based on ecological knowledge and is developed through non-chemical pest control, plant association with other plants. Agroecology got the yield of 5,500 kg/ha, which is 10% more than sourced through conventional approaches. To sum up, taking biotechnological methods and agroecological practices together surmounted the yield of 6,500 kg/ha norms, which meant 30% more compared to conventional agriculture. The results imply that biotechnology and agroecology can be a source for boosted crop yields when they are in competition with conventional farming methods. Therefore, the generated results are consistent with literatures that transgenic pest-resistant crops increase yields by 22-29% (Klümper & Qaim, 2014) and agricultural systems that improve yields via enrichment of soils and pest controls through agroecological methods (Reganold & Wachter, 2016).

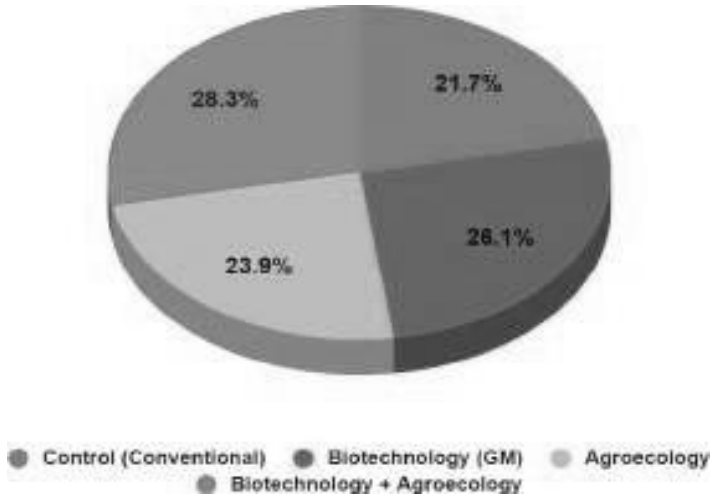

Figure 1: Crop Yield Performance under Different Experimental Treatments

Subsequently that indicates this table it is shown biotechnology and agroecology can have a multiple effect, the total yields over both of which separately therefore. More research is required on hybrid agricultural systems which are a vital part of the future of agriculture in order to foster sustainable food production. This table is provisional but shows that the integrated approach is stronger than the single-faceted strategy at promoting food security as it provides higher yield with a lower environmental footprint.

Trait	**Conventional**	**Biotechnology (GM)**	**Agroecology**
Pest Resistance	Low	High	Low
Drought Tolerance	Low	High	Medium
Nutrient Content	Standard	Enhanced	Standard

Table 2: Impact of Biotechnology Interventions on Crop Traits

This table compares three agricultural approaches—conventional, biotechnology using genetic modification (GM), and agroecological—across three key traits: pest resistance, water conservation, and improved nutrient profile. The vast majority of conventional crops have a low level of the inherent resistance against pests and drought, thus the production of these crops depends more on irrigating and using synthetic pesticides to yield enough crops (Altieri et. al., 2012). Biotechnology seeks to design a genetic code that is tolerant to the pressures these stresses put on the organismsso that the reliance on external inputs like fertilizers and pesticides is reduced. For instance, the Bacillus thuringiensis (Bt) genes have been transformed and integrated into crop plants, giving thema capacity to produce insecticidal proteins for pest control (Tabashnik et al., 2013). The biotech drought tolerance may comprise modifying stress-response pathways toward

the enhancing of yield even when under water shortage. The biotech additionally deals with more nutrient rich types of crops, like Golden Rice which is rich in Vitamin A (Bollinedi et al., 2017). Despite this, consumer acceptance and regulation of genetically modified (GM) crops act as the key barriers for the massive productivity of this (Funk & Kennedy, 2016).

Agroecological techniques are the mechanism which embraces ecological concepts to reinforce the agroecosystem within the farm (Altieri et al., 2012). Technics like intercropping, crop rotation and natural management of the soil on-farm elevate the biodiversity and natural pest control and at the same time no changes in the nutrient content in cultivars are observed. Water conservation is moderate—improved over conventional by methods like optimized water harvesting apart from the ability of biotech varieties. Increasing biofortification employing conventional breeding instead of genetically engineered food is the approach to achieve high nutrient content. The main point is thatin the agroecology point of view the resilience and sustainability is achieved without the use of the synthetic inputs (Reganold & Wachter, 2016).

Soil Parameter	**Before Treatment**	**After Treatment**
Organic Matter (%)	2.3	3.5
pH	6.0	6.5
Nitrogen (ppm)	50	70

Table 3: Soil Health Parameters under Agroecological Practices

The table depicts three basic soil characteristics - organic matter, pH, and nitrogen - both before and after some organic input and additives. The contents of organic matter percentage increase from 2.3% before treatment to 3.5% after it, which suggests that there was a content enrichment during treatment. The pH values that are measured before and after showed a small shift from acidic to more alkaline. The other tool applied to solve our problem is nitrogen addition. The level of nitrogen rose from 50 ppm before the treatment to 70 ppm after the treatment. Organic matter is a major indicator of soil health and vitality, and it means a lot (Horwath, 2015). The combined proportion with organics of 3.5% greater than double was that of 2.3% being 30% higher as indicated by the soil test (Magdoff & Weil, 2004). The higher soil organic matter, in general, provides the ground for many types of soil organisms and biological processes that are of greater significance for plants and their development. The extra may be the addition of compost, manure or other organic amendments which are food of a soil food web.

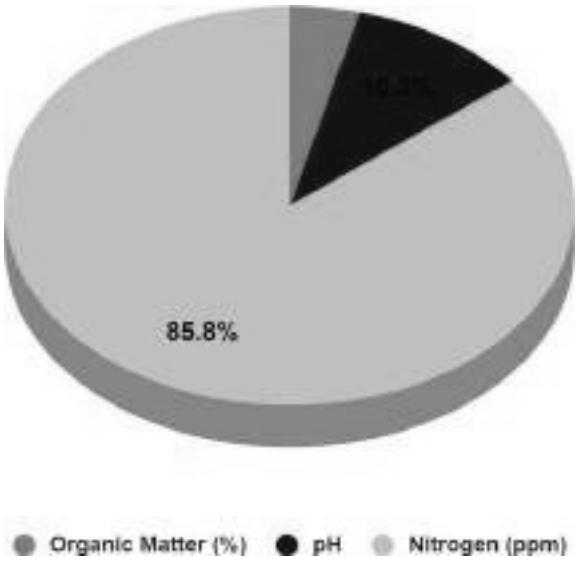

Figure 2: Soil Health Parameters under Agroecological Practices

The pH shift of the soil evidenced from 6.0 to 6.5 after the treatment demonstrates that the soil is not acidic. Most key nutrients of plants tend to move towards their ideal range of pH 6.5-7.0 (Bailey, 2009). Among the various options, liming agents containing calcium or/and magnesium are most often applied to acidic soils to make them more favorable to plants (Havlin et. al., 2014). The soil in this case received enough fertilizer to be able to operate near the ideal pH range.

Ultimately, the increasing level of nitrogen from pretreatment mixtures of 50 ppm and post- treatment for 70 ppm suggests higher availability of nitrogen for plants. Plants utilize nitrogen for growth very efficiently, but excessive amounts can create problems (Fageria, & Baligar, 2005). A 20 ppm increase may originate from fertilizers, manures, composts, and other sources that are rich in nitrogen-based compounds (Shaviv & Mikkelsen, 1993). This study would end with the fact whether the levels of nitrogen are now higher than normal or just right one. Generally, the soil particle distribution and nutrient status were altered from bad to good and the productivity was enhanced when these soil parameters were analyzed before and after treatment. The data points to applicationof valuable materials such organic matter, liming agents and nitrogen fertilizing, which should in turn promote plant growth.

Conclusion

Finally, incorporating biotechnology and agroecology are the choices that may be the best way to get sustainable food security. The reason why the integration of these two methods offers the synergy that takes into account the issues of growing food demand and preventing environmental deterioration lies in the duality of their approaches. Genetic engineering is one of biotechnology's many skills, helping crops to develop

characteristics of pest resistance, drought tolerance, and enriched nutrient content, thus, contributing to increased crop productivity. Nevertheless, there are issues to be borne in mind such as obstacles to regulatory issues and consumer acceptance for its wider use. Agroecology, in contrast, is a farming approach, which assumes a central role in promoting biodiversity at the farm level, rich soils and natural pest control. Agroecology incorporates crop rotation, inter-cropping and integrated pest management to overcome vulnerability and reduce the need for artificial inputs. The results of field tests confirm that the combination of biotechnology and agroecology is able to achieve even greater achievements than any of the methods applied individually. This combined method will not only increase production but also fosters soil health, biodiversity and ecological resilience. It also promotes smallholder farmers' participation in improvement and innovation via community-based projects. However, in order to get started, exploration and dismantling of institutional barriers, as well as development of collaboration between biotechnologists and agroecologists, will be needed. The amendment and the establishment of the institutions that support interdisciplinary work as well as research in the fields of blended agricultural systems are necessary. Alongside this we will support participatory researchand remain transparent and accountable in the reporting of results which will build confidence and trust within the corresponding agricultural community. The symbiosis of biotechnology and agroecology bring the systemic and sustainable solution to the issue of food security all over the world. By combining the unique benefits of both methods, we can be sure of a more stable, equitable, and environmental- friendly food production setup.

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Chapter-10

Plastic Waste Management During COVID-19: A Review

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Abstract

The COVID-19 pandemic has led to a substantial rise in plastic waste, primarily driven by the widespread use of personal protective equipment (PPE), single-use plastics, and packaging materials. This increase has significantly strained waste management systems unprepared for such a surge. The rise in PPE usage, necessary for health and safety, has resulted in massive quantities of single-use masks, gloves, and face shields. Concurrently, lockdowns and social distancing have increased reliance on takeout and delivery services, further escalating the volume of plastic packaging.This review explores the impact of the pandemic on plastic waste generation, highlighting the challenges faced by waste management systems, such as overwhelmed recycling programs and increased landfill use. The pandemic has disrupted traditional waste management practices, leading to more plastics being incinerated or improperly disposed of, thus exacerbating environmental pollution.To address these challenges, innovative solutions and sustainable practices are essential. Advanced recycling technologies, such as chemical recycling, can enhance plastic recovery and reuse. Promoting biodegradable and compostable materials can also reduce the environmental footprint of plastic waste. Policy measures, including extended producer responsibility (EPR) and single- use plastic bans, are crucial for driving systemic change. Additionally, public awareness and education about proper waste disposal and the environmental impacts of plastic waste are vital for fostering sustainable behaviors.

1. Introduction

The COVID-19 pandemic has substantially altered global consumption patterns, resulting in a significant increase in plastic waste. The heightened demand for personal protective equipment (PPE) such as masks, gloves, and face shields, along with the surge in single-use plastics for food packaging and medical supplies, has exacerbated this issue. The demand for PPE has skyrocketed as governments and health organizations have sought to protect frontline workers and the general public from the virus [1]. Masks, gloves, and other protective gear are predominantly made from plastics such as polypropylene, which are designed for single use to prevent cross-contamination. Consequently, disposing of billions of these items has led to a dramatic increase in medical waste. The inadequate

disposal of PPE has also become a significant environmental concern, as improperly discarded masks and gloves are increasingly found in natural environments, contributing to plastic pollution in oceans and waterways [2].

The food industry has similarly seen a rise in plastic use, driven by lockdowns and social distancing measures that have increased reliance on takeout and delivery services. Single-use plastic packaging has been favored for its perceived hygiene benefits, leading to higher volumes of waste [3]. The increase in home deliveries has also contributed to more packaging waste, with consumers receiving goods in plastic wrapping and containers. These changes in consumption patterns have overwhelmed existing waste management systems, which were not designed to handle such a sudden and massive influx of plastic waste. Disruptions in waste collection and recycling services have further compounded the challenges in plastic waste management [4]. Many recycling programs have been suspended or reduced due to health concerns for workers, leading to more plastics being sent to landfills or incinerated. The suspension of these programs has meant that a larger proportion of plastic waste is being diverted to landfills, where it can persist for hundreds of years, or to incineration, which releases harmful pollutants into the atmosphere. This has increased the environmental footprint of plastic waste and highlighted the limitations of current waste management systems [5].

Several strategies for sustainable management must be considered to address the environmental implications of COVID-19-related plastic waste. First, developing advanced recycling technologies, such as chemical recycling, can improve the recovery and reuse of plastic materials [6]. Chemical recycling breaks down plastics into their original monomers, creating new plastics without degrading quality. This process can handle mixed and contaminated plastics that are often unsuitable for mechanical recycling, thus significantly reducing the amount of plastic waste in landfills or incinerators.Promoting using biodegradable and compostable materials is another crucial strategy [7]. These alternatives, made from natural sources like cornstarch or sugarcane, can decompose more efficiently than conventional plastics, thus reducing their environmental impact. However, for these materials to be effective, appropriate composting infrastructure must be in place, and public awareness about proper disposal practices must be increased. Without the necessary facilities and education, biodegradable plastics may not break down as intended, leading to environmental issues similar to conventional plastics [8].

Policy interventions play a pivotal role in driving systemic change. Implementing stringent plastic production, use, and disposal regulations can enforce more sustainable practices across various sectors. Policies promoting extended producer responsibility (EPR) require manufacturers to take accountability for the entire lifecycle of their products, incentivizing the design of more sustainable and recyclable products [9]. Additionally, implementing bans or restrictions on certain single-use plastics, such as

plastic bags, straws, and cutlery, can significantly reduce the volume of plastic waste generated.Public awareness and education are essential components of effective plastic waste management. Educating the public about the environmental impacts of plastic waste and the importance of proper waste disposal can foster more sustainable behaviors [10]. Awareness campaigns can highlight the benefits of reducing, reusing, and recycling plastic products, encouraging individuals to make environmentally conscious choices. Promoting reusable items, such as cloth masks, shopping bags, and water bottles, can help decrease reliance on single-use plastics.

2. Impact of Covid-19 on Plastic Waste Generation

The COVID-19 pandemic has led to an unprecedented increase in plastic waste, presenting significant environmental challenges. One of the primary contributors to this surge is the healthcare sector. With the rise in COVID-19 cases, the demand for personal protective equipment (PPE) such as masks, gloves, face shields, and gowns has skyrocketed [11]. These items, predominantly made from various forms of plastic, are designed for single-use to prevent contamination and the spread of the virus. Consequently, the mass production and subsequent disposal of PPE have significantly increased plastic waste, overwhelming waste management systems already under strain.In addition to the healthcare sector, the food industry has contributed substantially to the rise in plastic waste [12]. Lockdowns and social distancing measures have forced many people to rely heavily on takeout and delivery services, as dining out became restricted and cooking at home was not always feasible for everyone. This shift has dramatically increased single-use plastic packaging, including containers, cutlery, and bags. Restaurants and food delivery services, aiming to ensure safety and hygiene, have favored disposable options, further adding to the plastic waste burden [13].

Consumer behavior during the pandemic has also played a critical role in exacerbating the plastic waste crisis. With many people confined to their homes due to lockdowns and remote working arrangements, online shopping and home deliveries have surged. This increase in e- commerce has led to higher volumes of packaging waste, much of which is plastic [14]. Products ordered online are often over-packaged to ensure they arrive intact, resulting in excessive use of plastic bubble wrap, air pillows, and shrink wrap. The convenience of online shopping and safety concerns related to the virus have made consumers more reliant on these services, thereby generating more plastic waste.The pandemic has also posed significant global challenges to waste management systems [15]. The increased volume of plastic waste from PPE, food packaging, and online shopping has overwhelmed waste management infrastructures. Many recycling programs have been suspended or reduced due to health and safety concerns for workers, leading to more plastics being sent to landfills or incinerated. This disruption has undermined efforts to recycle and manage waste sustainably, further contributing to environmental degradation [16]. Safely disposing contaminated medical waste, such as used PPE, has added another layer of complexity. This type of waste requires specialized handling and

disposal methods to prevent the spread of the virus, but the sheer volume has made it difficult to manage effectively. As a result, there has been an increase in environmental leakage, with improperly disposed PPE ending up in oceans and waterways, exacerbating the already critical issue of plastic pollution [17].

Given these challenges, it is essential to explore strategies for mitigating the environmental impacts of plastic waste during the COVID-19 pandemic. One promising approach is the development of enhanced recycling technologies [18]. Traditional mechanical recycling methods have limitations, particularly when dealing with contaminated or mixed plastic waste. Advanced recycling techniques, such as chemical recycling, offer the potential to break down plastics into their original monomers, creating new plastic products without degradation of quality. These technologies can improve the recovery and reuse of plastic materials, reducing the reliance on virgin plastics and decreasing the overall environmental footprint [19].Promoting the use of biodegradable and compostable materials is another crucial strategy. Biodegradable plastics, made from natural sources like cornstarch or sugarcane, can decompose more efficiently than conventional plastics, reducing their environmental impact. However, it is important to ensure that these materials are disposed of in appropriate composting facilities, as they may not break down effectively in traditional landfills [20]. Increasing investment in and access to composting infrastructure can support the widespread adoption of biodegradable alternatives, helping mitigate single-use plastics' environmental impact.

Policy interventions are vital in driving systemic change in plastic waste management. Governments can implement stringent plastic production and disposal regulations to encourage more sustainable practices. Policies promoting extended producer responsibility (EPR) require manufacturers to take responsibility for the entire lifecycle of their products, including end-of-life disposal. This approach incentivizes companies to design products with recyclability and sustainability in mind [21]. Additionally, implementing bans or restrictions on certain single-use plastics, such as plastic bags and straws, can reduce the overall volume of plastic waste.Public awareness and education are also critical components of effective plastic waste management. Educating the public about the environmental impacts of plastic waste and the importance of proper waste disposal can foster more sustainable behaviors [22]. Campaigns promoting reusable items, such as cloth masks and shopping bags, can help reduce reliance on single-use plastics. Encouraging consumers to make environmentally conscious choices, such as selecting products with minimal or recyclable packaging, can also reduce plastic waste [23].

Innovative waste management solutions can enhance local capacity to manage plastic waste. Decentralized waste management systems, such as community-based recycling initiatives, can provide localized solutions more adaptable to specific needs and

challenges [24]. These initiatives can engage communities in waste reduction efforts, fostering a sense of responsibility and ownership. Additionally, integrating digital technologies, such as smart bins and waste-tracking apps, can improve the efficiency and effectiveness of waste collection and recycling processes.Examining case studies and best practices from countries and organizations successfully managing plastic waste can provide valuable insights and inspiration [25]. For instance, countries with robust recycling infrastructures and strict plastic waste regulations have demonstrated effective strategies for reducing plastic pollution. Corporate initiatives prioritizing sustainable packaging and waste reduction commitments can also serve as models for other businesses. Highlighting grassroots initiatives that have successfully reduced plastic waste locally can showcase the power of community-driven efforts [26].

3. Challenges in Plastic Waste Management

The COVID-19 pandemic has created numerous challenges in plastic waste management, significantly impacting the environment. One of the most pressing issues is the increased volume of plastic waste. The sudden surge in the use of personal protective equipment (PPE) such as masks, gloves, face shields, and gowns, all of which are typically designed for single- use, has resulted in an overwhelming amount of waste [27]. The healthcare sector, in particular, has seen an exponential increase in the consumption and disposal of these items. Hospitals and healthcare facilities, in their efforts to curb the spread of the virus and protect both patients and staff, have generated unprecedented quantities of plastic waste. This surge has overwhelmed existing waste management systems, which were already under strain before the pandemic [28]. The sheer volume of discarded PPE, combined with the regular plastic waste from households and industries, has pushed waste management infrastructures to their limits, leading to significant operational challenges.

Additionally, the pandemic has caused considerable disruptions in recycling programs. Many recycling operations have been suspended or scaled back due to health and safety concerns for workers. Social distancing measures and fears of virus transmission have necessitated changes in the way recycling facilities operate, often reducing their capacity or halting activities altogether [29]. This reduction in recycling capabilities has meant that a larger proportion of plastic waste is being diverted to landfills or incinerated, rather than recycled. Landfills are becoming increasingly burdened with plastic waste, and the incineration of plastics, while reducing the volume of waste, releases harmful pollutants into the atmosphere. Therefore, the suspension of recycling programs has not only led to more plastics being sent to landfills but also contributed to environmental pollution and the loss of valuable recyclable materials [30].Managing healthcare waste, particularly contaminated PPE and medical waste, has posed significant challenges during the pandemic. The safe disposal of these materials is crucial to prevent the spread of COVID-19, but the scale of the waste generated has made this task daunting. Contaminated PPE, which must be handled as hazardous waste, requires specialized disposal methods to

ensure that it does not pose a risk to public health or the environment [31]. The increase in medical waste has placed additional pressure on waste management systems, which must now deal with both the increased volume and the hazardous nature of the waste. The proper segregation, collection, transportation, and disposal of medical waste have become critical operations, requiring enhanced protocols and infrastructure. However, many regions have struggled to keep up with the surge in waste, leading to instances of improper disposal and environmental contamination [32].

Improper disposal of PPE has also contributed to environmental leakage, significantly increasing plastic pollution in oceans and waterways. The improper handling and disposal of single-use PPE items have led to their widespread presence in natural environments. Masks, gloves, and other PPE items are often littered on streets, beaches, and other public spaces, eventually reaching rivers, oceans, and other water bodies [33]. This environmental leakage is unsightly and poses serious threats to marine life and ecosystems. Aquatic animals can mistake these plastic items for food, leading to ingestion and potentially fatal consequences. Additionally, the breakdown of plastics in the environment leads to the formation of microplastics, which further contaminate water sources and enter the food chain, affecting a wide range of organisms, including humans.The increased volume of plastic waste has highlighted the limitations of current waste management systems, which were not designed to handle such a sudden and massive influx of material [34]. Traditional waste management practices have proven inadequate in the face of the pandemic, necessitating urgent innovations and adaptations. Advanced recycling technologies, such as chemical recycling, offer potential solutions by enabling the breakdown of plastics into their original monomers, which can then be reused to create new plastic products. These technologies can help reduce the reliance on virgin plastics and improve the overall efficiency of recycling processes. However, the development and implementation of such technologies require significant investment and collaboration between governments, industries, and research institutions [35].

To address the issue of plastic pollution effectively, it is also essential to promote the use of biodegradable and compostable materials. These alternatives, made from natural sources such as cornstarch or sugarcane, can decompose more easily than conventional plastics, thereby reducing their environmental impact. However, the successful adoption of biodegradable materials depends on the availability of appropriate composting infrastructure [36]. Without proper facilities for the disposal and processing of biodegradable plastics, these materials may not break down as intended, particularly in conventional landfills. Investment in composting infrastructure and public education on the proper disposal of biodegradable materials are crucial for maximizing their environmental benefits.Policy interventions are pivotal in driving systemic change in plastic waste management. Governments can implement stringent plastic production, use, and disposal regulations to encourage more sustainable practices [37]. Policies promoting extended producer responsibility (EPR) hold manufacturers accountable for the entire

lifecycle of their products, incentivizing them to design products with recyclability and sustainability in mind. Additionally, implementing bans or restrictions on certain single-use plastics, such as plastic bags, straws, and utensils, can reduce the overall volume of plastic waste. Policymakers must also consider integrating economic instruments, such as taxes and subsidies, to promote the adoption of sustainable alternatives and support waste management initiatives [38].

Public awareness and education are critical components of effective plastic waste management. Educating the public about the environmental impacts of plastic waste and the importance of proper waste disposal can foster more sustainable behaviors. Awareness campaigns promoting reusable items, such as cloth masks, shopping bags, and water bottles, can help reduce reliance on single-use plastics [39]. Encouraging consumers to make environmentally conscious choices, such as selecting products with minimal or recyclable packaging, can also reduce plastic waste. Community engagement and participation in waste reduction initiatives are essential for building a culture of sustainability and environmental responsibility.Innovative waste management solutions can enhance local capacity to manage plastic waste more effectively [40]. Decentralized waste management systems, such as community-based recycling initiatives, can provide localized solutions adaptable to specific needs and challenges. These initiatives can engage communities in waste reduction efforts, fostering a sense of responsibility and ownership [41]. Additionally, integrating digital technologies, such as smart bins, waste tracking apps, and data analytics, can improve the efficiency and effectiveness of waste collection and recycling processes. Leveraging technology to optimize waste management operations can help mitigate the impact of increased plastic waste during the pandemic [42].

Examining case studies and best practices from countries and organizations successfully managing plastic waste can provide valuable insights and inspiration. For instance, countries with robust recycling infrastructures and strict plastic waste regulations have demonstrated effective strategies for reducing plastic pollution [43]. Corporate initiatives prioritizing sustainable packaging and waste reduction commitments can also serve as models for other businesses. Highlighting grassroots initiatives that have successfully reduced plastic waste locally can showcase the power of community-driven efforts. These examples can guide the development of comprehensive and integrated approaches to plastic waste management [44].

4. Strategies For Mitigating Environmental Impacts

Developing advanced recycling techniques is crucial in the fight against the escalating plastic waste crisis. Enhanced recycling technologies, such as chemical recycling, offer a promising solution by allowing for the recovery and reuse of plastic materials more effectively than traditional mechanical recycling [45]. Chemical recycling breaks down plastics into their original monomers, enabling the creation of new plastics without the

degradation of quality. This process can handle mixed and contaminated plastics that are often unsuitable for mechanical recycling. By transforming waste plastics into valuable raw materials, chemical recycling can significantly reduce the demand for virgin plastic production and lower the overall environmental footprint [46]. However, the widespread adoption of these technologies requires substantial investment in research and infrastructure and collaboration between governments, industries, and research institutions to overcome technical and economic challenges.

In addition to recycling advancements, promoting the use of biodegradable and compostable materials is another vital strategy for reducing the environmental impact of single-use plastics. Biodegradable plastics, made from natural sources like cornstarch or sugarcane, can decompose more readily than conventional plastics, thus mitigating their persistence in the environment [47]. Compostable materials further enhance this by breaking down into non- toxic components that can enrich the soil. However, for these materials to achieve their full potential, they must be disposed of in appropriate facilities that facilitate their decomposition. Traditional landfills and improper disposal can prevent biodegradable plastics from breaking down as intended, leading to environmental issues similar to those of conventional plastics [48]. Therefore, increasing investment in composting infrastructure and educating the public on proper disposal practices are essential to maximizing the benefits of biodegradable alternatives.

Policy interventions are critical in driving systemic change in plastic waste management. Implementing stringent plastic production, use, and disposal regulations can enforce more sustainable practices across various sectors [49]. Policies that promote extended producer responsibility (EPR) require manufacturers to take accountability for the entire lifecycle of their products, incentivizing the design of more sustainable and recyclable products. This approach shifts the burden of waste management from consumers and municipalities to producers, encouraging product design and waste reduction innovation. Additionally, implementing bans or restrictions on certain single-use plastics, such as plastic bags, straws, and cutlery, can significantly reduce the volume of plastic waste generated [50]. Policymakers can also introduce economic instruments, such as taxes on plastic production or subsidies for recycling initiatives, to promote sustainable practices and support waste management efforts. By creating a regulatory environment that prioritizes sustainability, governments can drive the transition towards a circular economy where materials are continually reused and recycled [51].

Public awareness and education are essential components of effective plastic waste management. Educating the public about the environmental impacts of plastic waste and the importance of proper waste disposal can foster more sustainable behaviors. Awareness campaigns can highlight the benefits of reducing, reusing, and recycling plastic products, encouraging individuals to make environmentally conscious choices [52]. For instance, promoting reusable items, such as cloth masks, shopping bags, and

water bottles, can help decrease reliance on single-use plastics. Additionally, educating consumers on the proper disposal of biodegradable and recyclable materials can enhance the efficiency of waste management systems. Community engagement is crucial in building a culture of sustainability and environmental responsibility [53]. Grassroots initiatives and local organizations can play a pivotal role in spreading awareness and driving behavior change at the community level. By empowering individuals with knowledge and practical solutions, society can collectively work towards reducing plastic waste and its environmental impact [54].

Innovative waste management solutions can enhance local capacity to manage plastic waste more effectively. Decentralized waste management systems, such as community-based recycling initiatives, provide localized solutions tailored to specific needs and challenges. These initiatives engage communities in waste reduction efforts, fostering a sense of responsibility and ownership over local waste management [55]. For example, neighborhood recycling programs can facilitate collecting and sorting recyclables, making it easier for residents to participate in recycling efforts. Additionally, integrating digital technologies, such as smart bins, waste tracking apps, and data analytics, can improve the efficiency and effectiveness of waste collection and recycling processes. Smart bins with sensors can monitor waste levels in real-time, optimizing collection routes and reducing operational costs. Waste tracking apps can provide residents with information on recycling schedules, proper disposal methods, and the environmental impact of their waste [56]. Data analytics can help waste management authorities identify patterns and areas for improvement, enabling more targeted and efficient interventions. By leveraging technology and community engagement, innovative waste management solutions can significantly enhance the capacity to manage plastic waste locally [57].

Examining case studies and best practices from countries and organizations successfully managing plastic waste can provide valuable insights and inspiration. For instance, countries with robust recycling infrastructures and strict plastic waste regulations have demonstrated effective strategies for reducing plastic pollution. These countries often have well-established EPR programs, advanced recycling technologies, and comprehensive public awareness campaigns [58]. Corporate initiatives prioritizing sustainable packaging and waste reduction commitments can also serve as models for other businesses. Companies that invest in sustainable packaging solutions, such as biodegradable materials or minimalistic designs, reduce their environmental footprint and appeal to environmentally conscious consumers. Highlighting grassroots initiatives that have successfully reduced plastic waste locally can showcase the power of community-driven efforts [59]. For example, community-led beach cleanups, recycling drives, and educational workshops can raise awareness and mobilize collective action against plastic pollution. These examples can guide the development of comprehensive and integrated approaches to plastic waste management, demonstrating the effectiveness of combined efforts from governments, businesses, and communities [60].

5. Conclusion

The COVID-19 pandemic has underscored the critical need for effective plastic waste management systems. The surge in personal protective equipment (PPE) usage and single-use plastics has strained waste management infrastructures, amplifying environmental concerns. Addressing this issue requires a comprehensive approach that integrates innovative technologies, policy interventions, and heightened public awareness.Innovative recycling technologies, such as chemical recycling, can significantly enhance plastic recovery and reuse, reducing the burden on landfills and incinerators. The promotion of biodegradable and compostable materials can further mitigate the environmental footprint of plastic waste.Policy interventions play a crucial role in driving change. Implementing measures like extended producer responsibility (EPR) and banning certain single-use plastics can enforce more sustainable practices across industries. These policies incentivize manufacturers to design products with recyclability in mind, fostering a circular economy.Public awareness and education are vital for promoting sustainable behaviors. Informing the public about

proper waste disposal and the environmental impacts of plastic waste can encourage more responsible consumption patterns.A multi-faceted approach involving stakeholders from various sectors—governments, businesses, and communities—is essential for achieving sustainable plastic waste management during and beyond the pandemic. By working collaboratively, we can mitigate the environmental impact of plastic waste and move towards a more sustainable future.

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Chapter-11

Isolation and Characterization of Cellulase Producing Bacteria from Soil

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Abstract

The present investigation was undertaken to isolate and Screen the Cellulase Producing Bacteria from Soil. Bacterial cultures were isolated from the soil sample collected from Botanical Garden, Karnatak University Campus, Karnataka, India. Four different substrates like Acacia arabica pod, Bauhinia forficata pod, Cassia surattensis pod and Peltophorum pterocarpum pods (as cellulose substrate) were used in the submerged production medium. A Total of 57 bacterial cultures were isolated based on Morphology and Biochemical characterization. Among all isolated strains, the three cellulolytic bacterial strains, maximum enzyme activity were showed in Bacillus cereus (0.440 IU/ml/min and 0.410 IU/ml/min), followed by Bacillus subtilius (0.357 IU/ml/min) and Bacillus thuringiensis (0.334 IU/ml/min) to the Acacia arabica pod. Acacia arabica pod showed maximum enzyme activity comparatively otherpods.

Keywords: Bacillus Species, CMC-Agar, Submerged fermentation,Substrates.

1. Introduction

Enzymes are delicate protein molecules necessary for life. Cellulose is the most abundant biomass on the earth (Venkata *et al.,* 2013) Plant biomass contains cellulose as the major component. Cellulose accounts for 50% of the dry weight of plant biomass and approximately 50% of the dry weight of secondary sources of biomasssuch as agricultural wastes (Haruta *et al.,* 2003). Presently huge amount of agricultural and industrial cellulosic wastes has been accumulating in environment. Cellulose has attracted worldwide attention as a renewable resource that can be converted into bio- based products and bioenergy (Xing-hua *et al.,* 2009). Celluloses are observed as the most important renewable resource for bioconversion. It has been become the economic interestto develop an effective method to hydrolyze the cellulosic biomass (Saraswati *et al.,* 2012).

Cellulose is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms, commonly by bacteria and fungi (Immanuel *et al.*, 2006). Cellulase is an important and essential kind of enzyme for carrying out the

depolymerization of 59 celluloses into fermentable sugar (Xing- hua *et al.,* 2009). Cellulases are the inducible bioactive compounds produced by microorganisms during their growth on Cellulosic matters (Lee and Koo, 2001). Cellulose degrading microorganisms can convert cellulose into soluble sugars either by acid and enzymatic hydrolysis. Thus, microbial cellulose utilization is responsible for one of the largest material flows in the biosphere (Lynd *et al.,* 2002). Increasing knowledge of mode of action of Cellulase; they were used in enzymatic hydrolysis of cellulosic substances (Kubicek *et al.,* 1993). Despite a worldwide and enormous utilization of natural cellulosic sources, there are still abundant quantities of cellulosic sources, cellulose containing raw materials and waste products that are not exploited or which could be used more efficiently (Sonia *et al.,* 2013). Cellulases are used in the textile industry for cotton softening and denim finishing, in laundry detergents for colour care, cleaning, in the food industry for mashing,in the pulp and paper industries for drainage improvement and fibre modification, and they are even used for pharmaceutical applications. Over all the cellulose enzymes will be commonly used in many industrial applications and the demands for more stable, highly active and specific enzymes will also grow rapidly (Cherry *et al.,* 2003) Cellulases form bacteria are also more effective catalysts. They may also be less inhibited by the presence of material that has already been hydrolyzed. The greatest potential importance is the ease with which bacteria can be genetically engineered (Arifin *et al.,* 2006). Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Some bacterial species viz., Cellulomonas species, *Pseudomonas* species, *Bacillus* species and *Micrococcus* have cellulolytic property (Nakamura and Kappamura, 1982). A large number of microorganisms are capable of degrading cellulose, only a few of them produces significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose *in vitro*. Numerous investigations have reported the degradation of cellulosic materials, but few studies have examined which microorganisms had met the industrial requirement (Lee and Koo, 2001). Bacteria which have high growth rate as compared to fungi have good potential to be used in cellulase production (Sonia *et al.,* 2013). Among bacteria, Bacillus species produce a number of extracellular enzymes including amylases, proteinases, and polysaccharide hydrolases (Mawadza *et al.,* 2000). For understanding the mechanism of cellulose degradation by cellulase, it is necessary to isolate, purify and characterize this enzyme. Therefore, the present investigation was designed to isolate and Screen the Cellulase Producing Bacteria fromSoil.

2. Materials And MethodsIsolation of Bacteria

Bacteria were isolated from the soil sample collected from Botanical Garden, Karnatak University Campus, Karnataka, India. Traditional serial dilution agar plating method was used for the isolation of cellulolytic bacteria. The medium used for cellulolytic bacteria contains 1.0 % peptone, 1.0 % carboxymethylcellulose (CMC), 0.2 % K2HPO4, 1 %

agar, 0.03 % MgSO4.7H2O, 0.25 % (NH4)2SO4 and 0.2 % gelatin at pH 7. The Plates were incubated for 48 hours at 30C.

Screening of Bacteria

The incubated CMC agar plates were flooded with 1 % Congo red and allowed to stand for 15 min at room temperature. 1M NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having clear zone were selected for identification and cellulase production. Further bacterial strains were purified by repeated streaking. The purified colonies were preserved at 4^{o}C.

Screening for cellulase enzymeDevelopment of Inoculum

The selected bacterial cultures were individually maintained on CMC agar slants at 4C. The selected bacterialcultures were inoculated in broth medium containing 0.03 % MgSO4, 0.2 % K2HPO4, 1 % glucose, 0.25 % (NH4)2SO4 and 1 % peptone at pH 7 for 24 Hrs of incubation period. After the incubation period these bacterialcells were used as inoculum.

Cellulase enzyme production by Submerged Fermentation Process

The isolated Bacterial strains were screened for cellulase enzyme production in submerged fermentation process. Fermentation medium was prepared by using powders of 1% *Acacia arabica* pod, *Bauhinia forficata*pod, Cassia *surattensis* pod and *Peltophorum pterocarpum* pod (as cellulose substrate), 0.2 % K2HPO4, 0.03 % MgSO4, 1 % peptone, 0.25 % (NH4)2SO4 and autoclaved at 121^{o}C for 15min. After autoclave, the medium was inoculated with 1 ml of bacterial isolates and incubated in a rotary shaker at 35C for 24 hrs of fermentation period with agitation speed of 140 rpm. After fermentation the broth was centrifuged at 14000 × g for 10 min at 4C. The supernatant obtained after centrifugation served as crude enzyme source.

Estimation of Cellulase enzyme

Estimation of Cellulase enzyme activity was assayed using Dinitrosalisic acid (DNS) reagent (Miller, 1959) by estimation of reducing sugars released from CMC. Crude enzyme was added to 0.5 ml of 1 % CMC in 0.05 M phosphate buffer and incubated at 50C for 30 min. After incubation, the reaction was stopped by the additionof 1.5ml of DNS reagent and boiled at 100C in water bath for 10 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using glucose calibration curve (Shoham *et al.,* 1999). One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 mol of glucose per minute under standard assay conditions (Muhammad *et al.,* 2012)

Morphological and biochemical characterization

The bacterial strains which produce cellulose enzyme were further subjected to morphological and MR VP test, Citrate utilization test, Starch hydrolysis test, Gelatin

hydrolysis test, Nitrate reduction test, Catalase test, Oxidase test, Glucose fermentation test, Lactose fermentation test, Indole test, Urea hydrolysis test, H2S production test. Molecular identification of cellulolytic bacteria

The strain which shows maximum cellulase activity was further subjected to molecular identification byanalysing 16S r RNA sequence.

Isolation of Genomic DNA

2 ml of overnight grown Nutrient broth culture was centrifuged at 10,000 rpm at 4^0C for 10 minutes. The pellet was re suspended in 10 min 10mM Tris, 100 mM Sodium chloride solution and centrifuged at 10,000 rpm 4^0C for 10 minutes. After discarding the supernatant, the pellet was re suspended in 100 µl of T50E20 buffer containing 20µl of lysozyme (50mg/ml) and incubated at 37^0C for 20 min, in that solution 1µl of RNase (10 mg/ml) was added and incubated at room temperature for 20 minutes. To this mixture 100µl of SDS (2% in T50E20) was added and incubated at 50^0C for 45 min with proper mixing. 2µl of Proteinase K (20mg/ml) was added and incubated at 55^0C for 30 min. The sample was extracted in same volume phenol, Chloroform and Iso-amyl alcohol (25:24:1) and DNA was precipitated with one volume of isopropanol and 0.1 volume of 3M of Sodium acetate. The pellet was washed with 70% Ethanol, dried and dissolved in 100 µl of T10E1 buffer and stored at -20^0C for further use. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation). The DNA was stored at 20ºC for further use (Modified method of Sadashiv and Kaliwal, 2013)

Identification of bacteria by sequencing of the 16s rRNA

PCR amplification was performed using Applied Biosystem verti thermal cycler. The primers for PCR amplification were obtained from Sigma-Aldrich.

Universal Primer (Lane, 1991)

27 forward 5 AGAGTTTCCTGGCTCAG 3 1492 reverse 5 ACGGCTACCTTGTTACGATT 3

The PCR was performed in 20µl reaction mixture containing 2µl of 10X assay buffer, 1µl dNTP mix of 2.5 mM, 0.5µl of mgcl2, 1µl each of forward and reverse primer (5pmol), 0.5µl of Taq polymerase, 1µl of template DNA and 13.5µl of HPLC grade water with the following amplification for 16s rRNA initial denaturation at 95ºC for 4 min followed by 38 cycles of denaturation, annealing and extension (94ºC for 1 min, 59.9ºC for 2 min and 72ºC for 2 min) and final extension at 72ºC for 20 min followed by hold for infinity at 4ºC. The presence of PCR products was determined by 2.5% agarose gel electrophoresis and to analyse the size of amplified PCR product DNA markers of 100bp was used which was provided by the Puregene. The amplifiedproduct was sent for sequencing to SciGenom Labs Pvt Ltd, Cochin, Kerala.

Construction of Phylogenetic Tree

By using the sequence, the bacteria were identified and constructed phylogenetic tree by using NCBI (http:// blast. ncbi. nlm. nih. Gov / Blast. cgi? PROGRAM = blastn & BLAST_ PROGRAMS=mega Blast & PAGE_TYPE=Blast Search & SHOW _ DEFAU LTS = on & LINK _ LOC = blasthome) and MEGA 5 Software)

3. Results And Discussion

A Total of 57 bacterial cultures were isolated based on Morphology and Biochemical characterization. The strains were subjected to Cellulase enzyme production by Submerged Fermentation Process by providing different powders of *Acacia arabica* pod, *Bauhinia forficata* pod, *Cassia surattensis* pod and *Peltophorum pterocarpum* pods (as cellulose substrate). Among all 57 tested bacterial strains B7 (0.440 IU/ml/min) showedmaximum enzyme activity, followed by B20 (0. 0.357 IU/ml/min), B37 (0.410 IU/ml/min) and B49 (0.334IU/ml/min) to the *Acacia arabica* pod comparatively other pods (Table 1) All the 57 strains (B1 to B57) were Gram +ve and showed positive for Methyl red test, Voges Proskauer test, Citrate utilization test, Starch hydrolysis test, Gelatin hydrolysis test, Nitrate reduction test, Catalase test, Oxidase test, Glucose fermentationtest, Lactose fermentation test and Negative to Indole test, Urea hydrolysis test, H2 S production test. The highest cellulatic enzyme production strains (B7, B20, B37 and B49) were further subjected to 16S rRNA. Thepartial amplification of 16S rRNA confirmed on the agarose gel electrophoresis. (Fig.1). By using NCBI and neighbour joining method in MEGA5 the strains were identified as *Bacillus cereus* (B7, B37) (Fig. 2), *Bacillus subtilis* (B20) (Fig. 3) and *Bacillus thuringiensis* (B49) (Fig. 4).

Cellulose is converted into fermentable sugars by the enzyme cellulase, and cellulase based bio- refinery technologies are versatile and flexible because they utilize cheaper substrates for enzyme synthesis (Mane *et al.,* 2007). The ability to degrade cellulose is a character distributed among a wide variety of aerobic, facultativeaerobic, anaerobic bacteria. Efforts are going on throughout the world to enhance the production and purity of bacterial cellulases (Sreeja *et al.,* 2013). Studying on cellulolytic activity has isolated various bacteria from different environmental sources. (Hatami *et al.,* 2008).

Different Substrates are used in the present study as a carbon source to produce good yield of cellulase enzyme. *Acacia arabica* pod shows maximum enzyme activity comparatively other pods. Similar attempts have been done by many researchers. Ashish Vyas *et al.,* (2005) used groundnut shell, Shuchi Singh *et al.,* (2013) used Rhinoceros Dung, Atchara Sudto *et al.,* (2008) used Agricultural waste for the production of cellulase enzyme. It has been reported that, physico chemical factors influence the growth of the organisms and also the Cellulase agro - residues by microorganisms depend on many factors, chemical Composition of the agro- residues (cellulose, hemicellulose, lignin, nitrogen, and minerals), presence of an activator or an inhibitor in the agro-residues, diffusion of the catabolite, and type of organisms for fermentation (Chinn *et al.,* 2006).

Several microorganisms have been discovered fordecades which have capacity to convert cellulose into simple sugars (Perez *et al.,* 2002).

Many efforts were taken to generate microorganisms with high ability to produce cellulase that can degrade native cellulose (Aristidou and Penttila, 2000). From the present study among all isolated strains, the three cellulolytic bacterial strains the maximum enzyme activity were showed in *Bacillus cereus* (0.440 IU/ml and 0.410 IU/ml), followed by *Bacillus subtilius* (0.357 IU/ml), and *Bacillus thuringiensis* (0.334 IU/ml) to the *Acacia arabica* pod. Similarly, Afza *et al.,* (2012) reported 45.42 U/mg cellulase production, Mukesh Kumar *et al.,* (2012) reported cellulase activity 66 U/ml from *Bacillus* cereus which showed more activity when compared to our study and in both studies the strain was confirmed by 16s rDNA method. Venkata *et al.,* (2013) also concluded the *Bacillus cereus* is the promising bacteria to produce cellulase. Bacillus cereus was found to produce the endoglucanase type cellulase (Afza *et al.,* (2012) and most of the isolated *B. cereus* / *B. thuringiensis* strains were found to produce extracellular enzymes (Celenk *et al.,* 2009).

In the present study *Bacillus subtilis* also has been isolated and showed cellulase activity. Similarly, Yu- Kyoung Kim, *et al.,* (2012), Ramalingam and Ramasamy, 2013 also reported the cellulase activity of 0.9 unit/mL and 0.140 U/ml respectively, which have high growth rate as compared to fungi, good potential to be used in cellulose production. However, the application of bacteria in producing cellulase is not widely used. (Sonia *et al.,* 2013).

Molecular methods being highly sensitive and selective currently used to identify microorganisms. Environmental conditions may have intense impact on morphological and physiological characteristics, hence the accurate identification of isolates turned out to be more difficult (Bakri *et al.,* 2010). The molecular techniques are more significant for the characterization of the new isolates, allowing grouping the strains. Furthermore, complex studies (microbiological, biochemical and molecular) are essential, when the identification of new isolate is the purpose of the investigation (Rahna *et al.,* 2013). Species-specific DNA sequences can be used for the identification of bacterial species. The 16s-23s rRNA has proven useful for identification of strains and species (Gurtler & Stanisich, 1996). In the present study the selected three different cellulolytic bacteria such as *Bacillus cereus*, *Bacillus subtilis* and *Bacillus thuringiensis* have been identified based on biochemical and 16s rRNA sequencing. The 16s rRNA sequencing makes it possible to identify anddistinguish closely related bacterial species. 16s rRNA method was also used by Shuchi *et al.,* (2013) Where they isolated cellulatic bacteria *Bacillus amyloliquefaciens* from Rhinoceros Dung. Rahna *et al.,* (2013) isolated *Bacillus subtilis* using cellulosic waste as carbon source. Therefore, present molecular identification work suggests that, the 16s rRNA sequencing is more accurate for the species identification.

Enzyme production is closely controlled in microorganisms and for improving its productivity, these controls can be improved. Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size, pH value, temperature, presence of inducers, medium additives, aeration, growth time, and so forth (Immanuel *et al.,* 2006). In enzyme fermentation process, the crude extracts contain different mixtures of proteins and undesirable products as organic acids and other metabolites. So that purification of the required favourable product must be taking place by different purification methods. (Mukesh Kumar *et al.,* 2012). Optimization of different physicochemical parameter of the production medium is required to get the maximum yield of the enzyme. Further studies were in progress to get high yield production, purification and applicationof cellulase.

Sl. No	Strain No	Enzyme activity (IU/ml/minute)			
		Acacia arabica	*Bauhinia forficate*	*Cassia surattensis*	*Peltophorum pterocarpum*
1	B7 (*Bacillus cereus*)	**0.440**	0.213	0.187	0.190
2	B20 (*Bacillus subtilis*)	**0.357**	0.201	0.189	0.178
3	B37 (*Bacillus cereus*)	**0.410**	0.217	0.203	0.187
4	B49 (*Bacillus thuringiensis*)	**0.334**	0.219	0.203	0.193

Table.1: Enzyme activity by different strains to different substrates

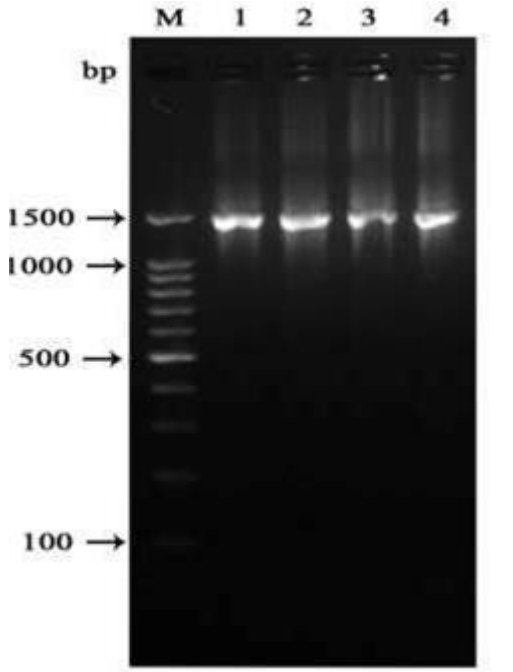


Fig.1: Agarose gel electrophoresis to PCR amplified DNA.

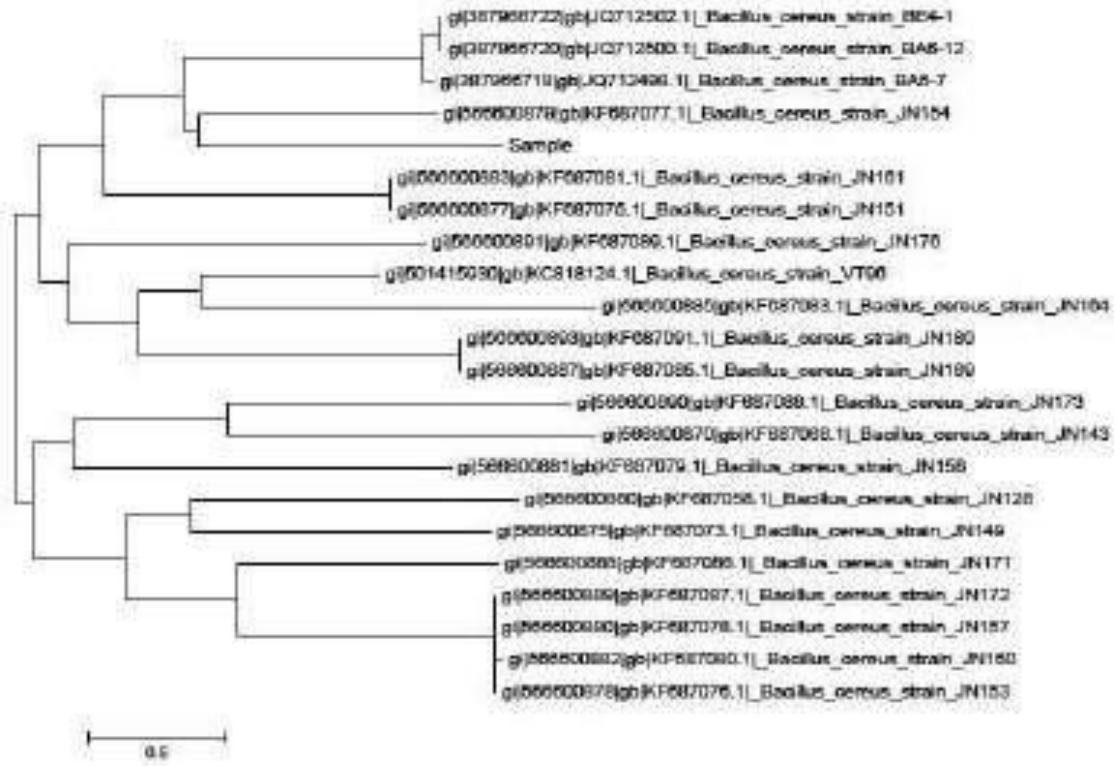


Fig.2 Phylogenetic tree of Bacillus cereus

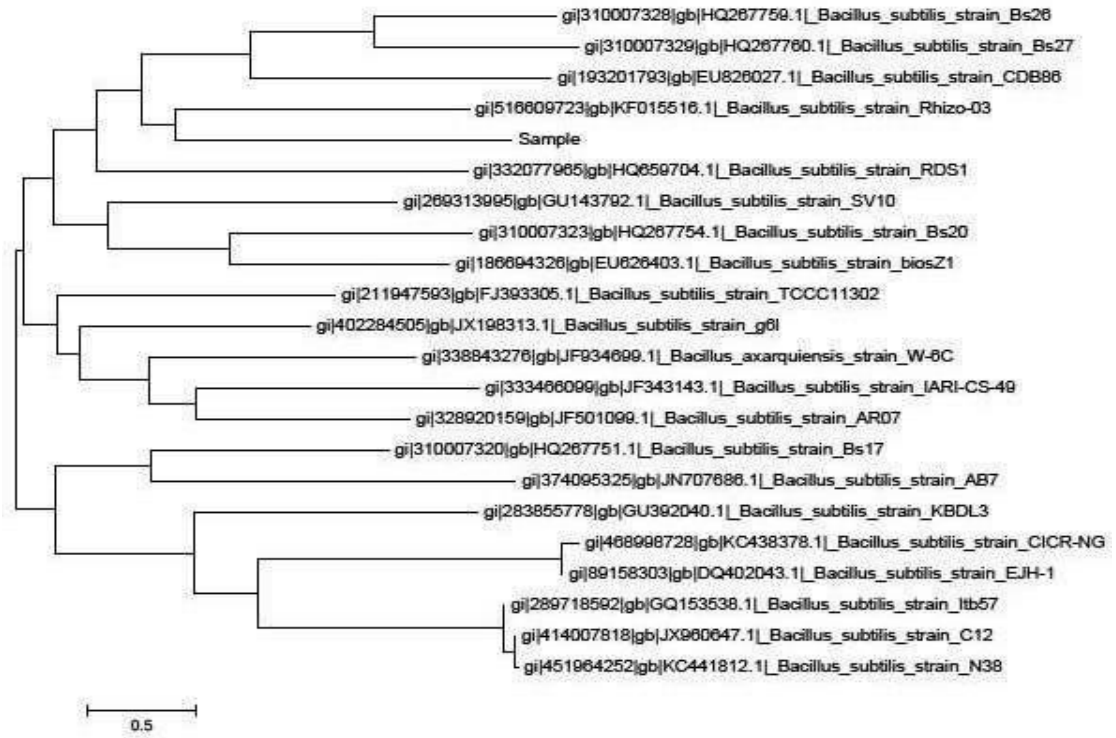


Fig.3 Phylogenetic tree of Bacillus subtilis

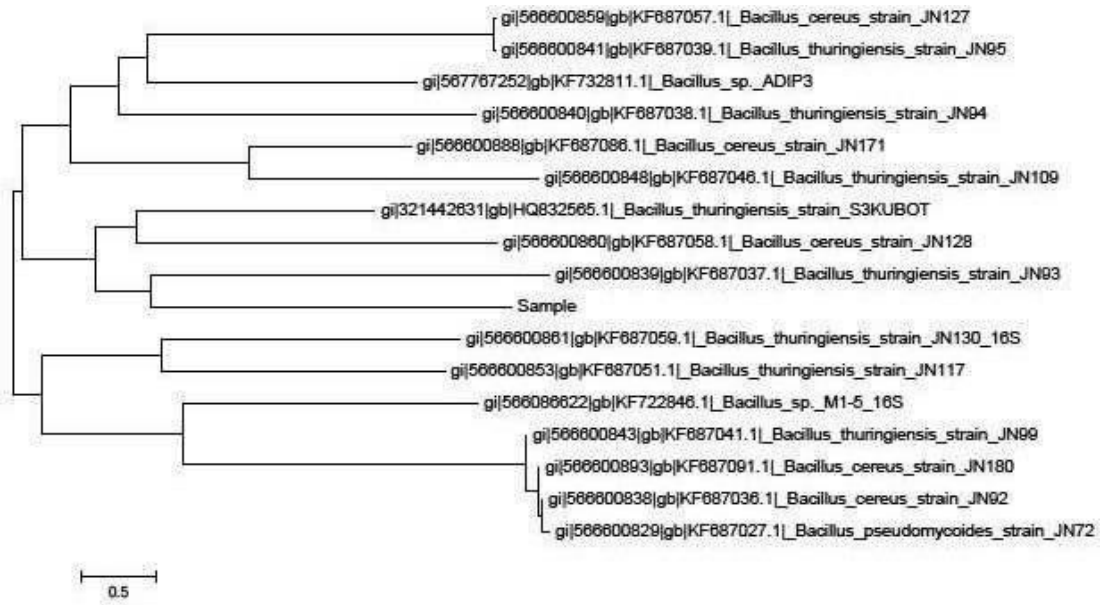


Fig.4 Phylogenetic tree of Bacillus thuringiensis

The purified cellulase can be used for various purposes in detergent industries, food industries, and pharmaceutical industries.

Test Report

Test name: Isolation and Screening of Cellulase producing Media, Pure Culture of Positive Isolates, Morphology, Biochemical Test: Catalase Test, Citrate utilization test, Gelatin Hydrolysis Test, Starch Hydrolysis Test, Carbohydrate Utilization, Methyl Red and Voges Proskauer Test

Test Sample: CL002 and CL005

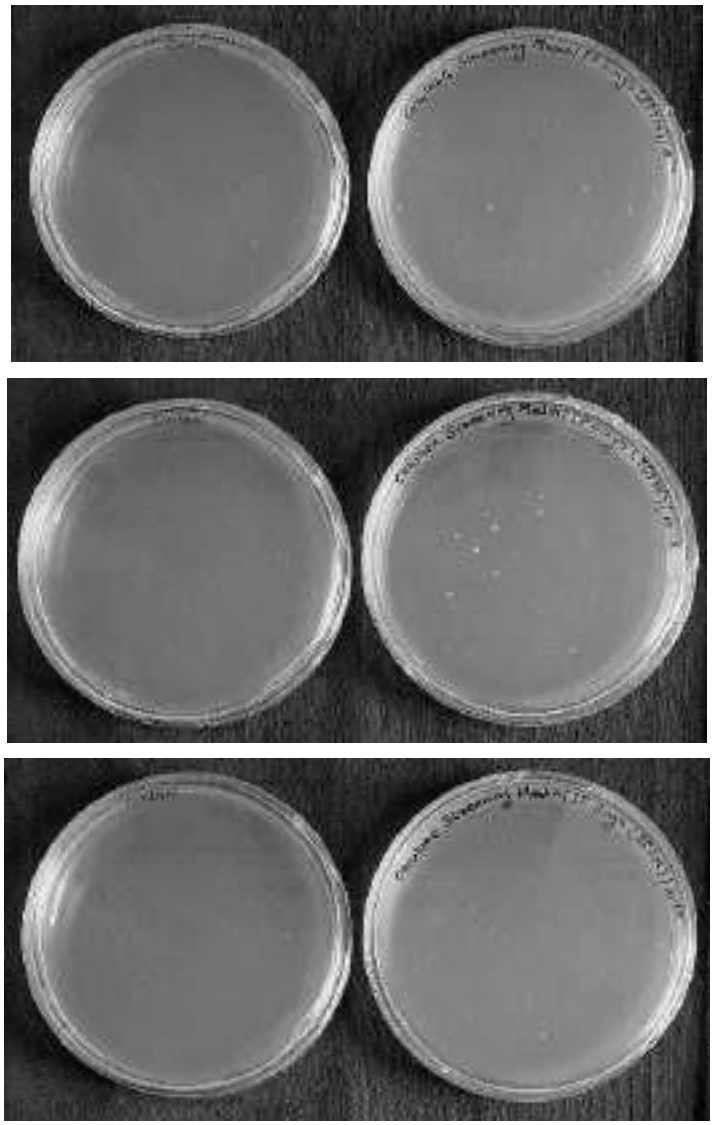
Test Method

Isolation And Screening of Cellulase Producing

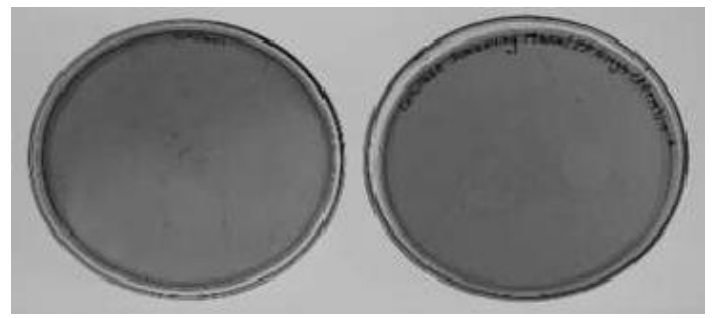
Bacteria Procedure:

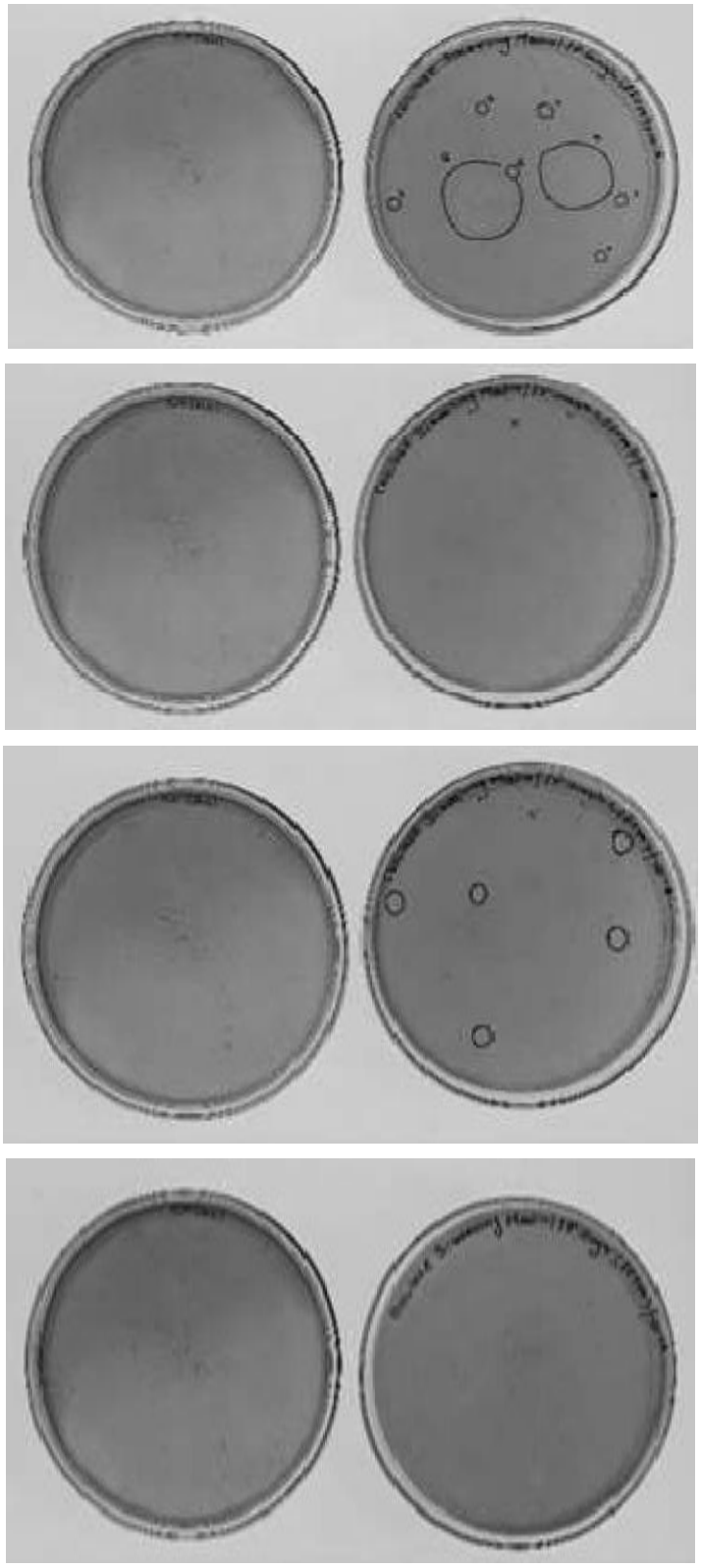
- Soil was collected from UPSIDC Industrial Area, located in Dist-Barabanki
- Collected sample (1 g) was led to serial dilution.
- The sample was diluted up to 10^{-11}
- 100 µl of the solution from concentration 10^{-6}, 10^{-8} and and10^{-10} was transferred into Petri dishescontaining carboxymethyl cellulose (CMC) agar media plates containing 0.5 g KH2PO4, 0.25 g
- MgSO4, 0.25 g cellulose and 2 g gelatin for the enhancement of the bacterial activity.
- Petri dishes were then incubated at 37 °C for overnight and preserved at 4 °C.

- Enzyme activity was confirmed by Congo red method.
- Bacterial isolates were inoculated in a basal salt medium containing filter paper for their cellulyticactivity test.



Isolation of Cellulase producing





Screening of Cellulase producing

Observation and result:

Morphology and Biochemical results of Isolate 1: CL002

S.No	Shape	Size	Structur e	Texture	Appearanc e	Colo r	Code
1	Round	Smal l	Round	Smooth	Creamy	Whit e	CL00 2

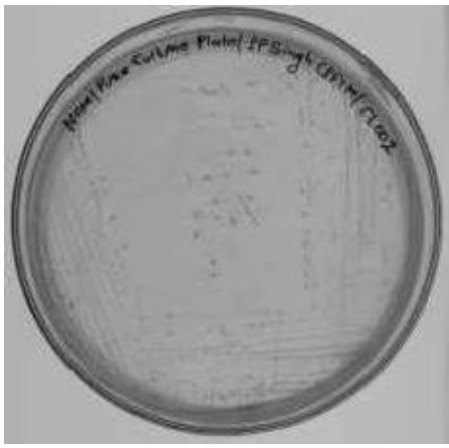
Table 1: Colony appearance of isolate 1 (CL002)

S.No	Test	Observation	Test Result	Inference
1	Catalase test	No Bubble formation	-ve	Not a Catalase producing Bacteria
2	Citrate utilization test	Color change	+ve	Capable of Fermenting Citrate
3	Gelatin Hydrolysis Test	Liquefaction of media	-ve	Not a Gelatinases producing bacteria
4	Starch Hydrolysis Test	halo zone	+ve	Amylase producing
5	Indole Test	Brown ring	-ve	Unable to decompose tryptophaneto indole
6	Gram’s staining	Purple Colour/round shape	+ve	Gram +ve; coccus
7	Carbohydrate Utilization Test	Glucose- Pink to orangecolour change	(A/NG)* Weak +ve	Glucose fermenting
		Lactose- Pink to orange	(A/NG)* Weak +ve	Lactose fermenting

		Sucrose- Pink to yellow	(A/NG)* Strong +ve	Sucrose fermenting
8	Methyl Red	Red colour ring appearance	+ve	Glucose fermenting with mixedacid formation
9	Voges Proskauer Test	No cherry red ring appearance	-ve	No acetyl methyl carbinol

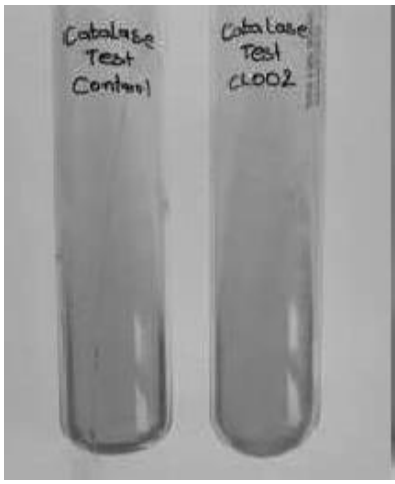
Table 2: Observation and Inference of Biochemical tests of isolate 1 (CL002)

A: Acid producing; NG: Non-gas producing

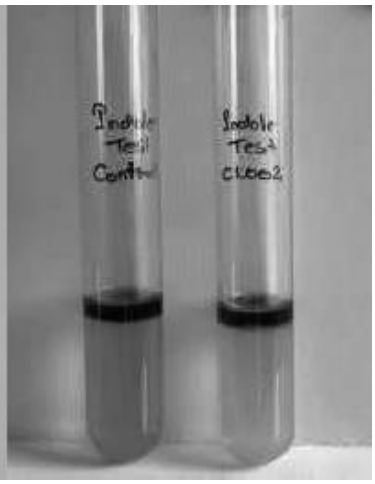


Pure Culture

Citrate Utilization Test

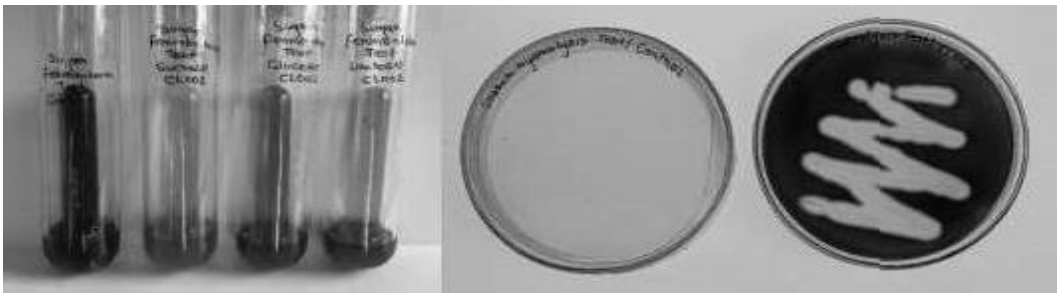


Catalase

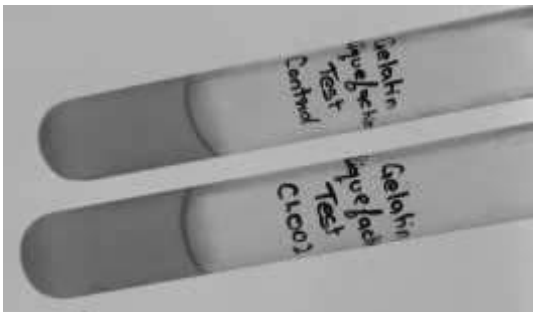


Test Indole Test

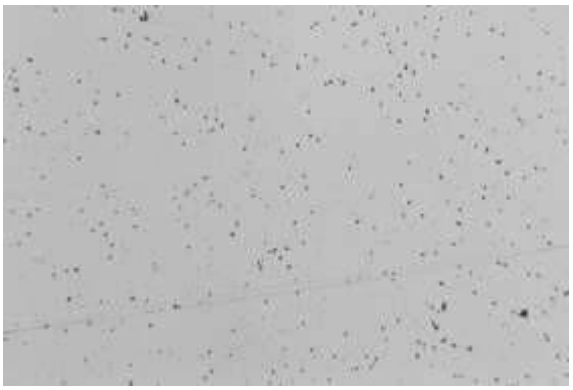
Methyl Red Test ***VP Test***



Sugar Fermentation Test ***Starch Hydrolysis Test***



Gelatin Liquefaction Test



Gram's Staining

Morphology and Biochemical results of Isolate 1: CL005

S.No	Shape	Size	Structure	Texture	Appearance	Color	Code
2	Round	Small	Round	Smooth	Creamy	White	CL005

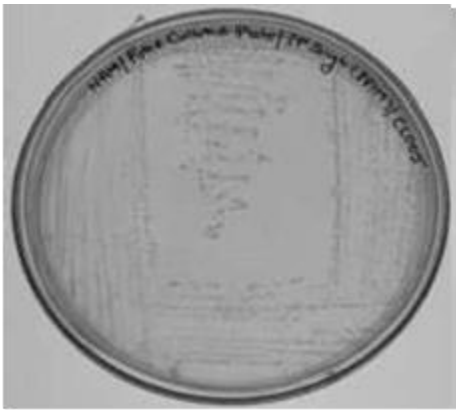
Table 3: Colony appearance of isolate 1 (CL005)

S.No	Test	Observation	Test Result	Inference
1	Catalase test	Bubble formation	+ve	Catalase producing
2	Citrate utilization test	No Colour change	-ve	Non-Fermentative
3	Gelatin Hydrolysis Test	No Liquefaction of media	-ve	Not a Gelatinases producing bacteria
4	Starch Hydrolysis Test	Halo zone	+ve	can hydrolyze starch
5	Indole Test	Cherry red ring	+ve	Has ability to decompose tryptophane to indole
6	Gram's staining	Purple colour/ round shape	+ve	Gram positive; Streptococcus
7	Carbohydrate Utilization Test	Glucose- Pink to yellow	(A/NG)* Strong +ve	Weak Glucose fermenting
		Lactose- Pink to yellow	(A/G)* Strong +ve	Weak Lactose fermenting
		Sucrose- No colour change	(NA/NG)* -ve	Non sucrose fermenting
8	Methyl Red	Red colour ring appearance	+ve	Glucose fermenting with mixed acid formation
9	Voges Proskauer Test	No cherry red ring appearance	-ve	No acetylmethyl carbinol

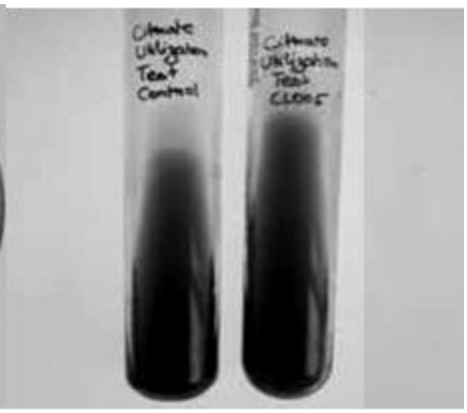
Table 4: Observation and Inference of Biochemical tests of isolate 1 (CL005)

***(A/NG): Acid producing/non-gas producing (A/G):** Acid producing/Gas producing

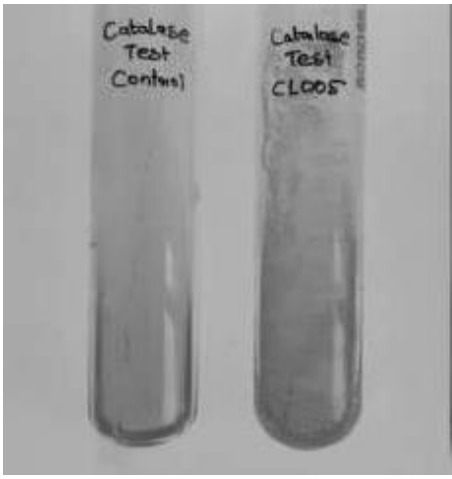
(NA/NG): Non-acid producing/non-gas producing



Pure Culture

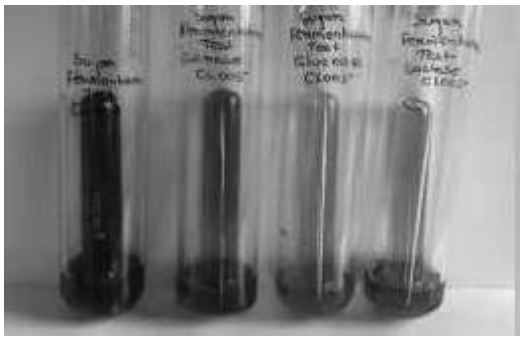


Catalase Utilization Test

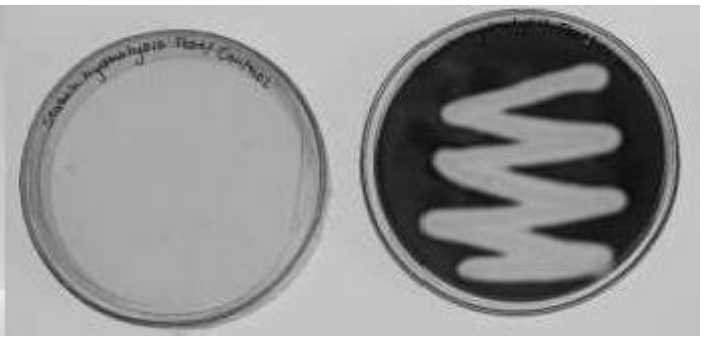


Catalase Test

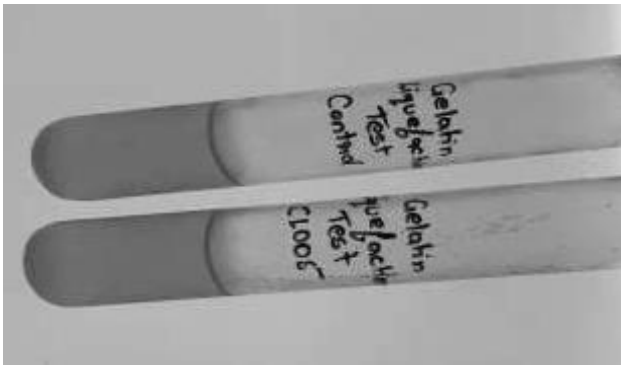
Indole Test



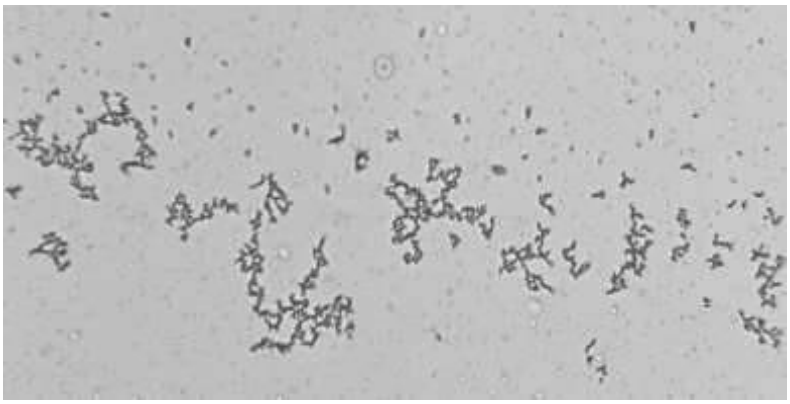
Sugar Fermentation Test



Starch Hydrolysis Test



Gelatin Liquefaction Test



Gram's Stainin

Test Method ubculturing of isolate

Procedure:

- For the isolation of bacteria, initially Nutrient Agar Media (NAM) was prepared as per thestandard composition and the media was autoclaved at 121C and 15psi for 15 minutes in autoclave.
- After the sterilization media was poured in sterile glass petri plates inside the Laminar airflow using the aseptic techniques, each plate was poured with 20ml of the culture media.
- The Plates were then allowed to solidify properly, then the media was inoculated with therespective bacterial isolate.
- After solidification, Flame sterilized the inoculating loop and allowed it to cool down.
- Touched the loop to a corner of culture plates and then streaked it into the plates.
- After streaking, inverted the plates and sealed them with cling tape and kept them in theincubator for 24-48 hours.

- Observed the plates after incubation period for the appearance of microbial colonies.

Citrate Utilization TestMedia Composition

S.No.	Composition	(g/lit)
1	Sodium Chloride	5
2	Sodium Citrate	2
3	Ammonium dihydrogen Phosphate	1
4	Magnesium phosphate	0.2
5	Bromothymol Blue	0.08
6	Agar	20
7	Distilled Water	1000ml

Procedure:

1. For the isolation of bacteria, initially Simmons citrate agar was prepared as per the standardcomposition and the media was autoclaved at 121^{o}C and 15psi for 15 minutes in autoclave.
2. After the sterilization, media was poured in sterile glass test tubes inside the Laminar air flow usingthe aseptic techniques
3. The test tubes then were placed in tilt position to make a slant and allowed to solidify properly, thenthe media was inoculated with the respective bacteria.
4. After solidification, Flame sterilized the inoculating loop and allowed it to cool down.
5. Touched the loop to a corner of culture test tube and then streak it into the test tube.
6. Streaked the slant from center of isolated colony and kept them in the incubator for 24- 48 hours.
7. Next day, after incubation, green and blue colours were obtained.
8. A positive result indicated by the formation of blue colour and negative result indicated by theabsence of colour change.

Catalase test

Composition of Nutrient agar

S.No.	Composition	(g/lit)
1	Yeast extract	1
2	Peptone	5
3	Sodium chloride	5
4	Agar	15
5	Distilled Water	1000 ml

Procedure:

1. For this test, nutrient agar (NAM) was used the media was prepared as per the standard compositionand autoclaved for sterilization.
2. After the test tubes cooled down and the agar was solidified completely, they were streak the tubeaseptically by taking the growth from 24 hours culture and incubated at 37°C for 24 hours
3. Then added 0.5ml H2O2 to the test tubes.
4. Place the tube against a white background and observe for immediate bubble formation.

Carbohydrate Fermentation Test

Phenol Red Carbohydrate Broth media composition

S.No.	Composition	Amount (g/lit)
1	Peptone	10
2	Sodium chloride	5
3	Yeast extract	1
4	Phenol red	0.018
5	Carbohydrate source	10
6	Distilled water	1000 ml

Procedure:

1. For this test, carbohydrate fermentation media broth media was used the media was prepared as perthe standard composition and autoclaved for sterilization.
2. After the media cooled down completely, they were inoculated the tube aseptically by taking thegrowth from 24 hours culture and incubated at 37°C for48 hours.
3. At the end of the incubation period, observe the observe the color change from red to yellow alongthe broth

Positive: Development of yellow color in the medium is indicative of a positive carbohydrate fermentation reaction.

Negative: No color change is indicative of a negative carbohydrate fermentation reaction

Voges Proskauer Test

VP Broth media composition

S.No.	Composition	Amount (g/lit)
1	Buffered Peptone	7.0
2	Glucose	5.0
3	Dipotassium Phosphate	5.0
4	Distilled Water	1000 ml

Procedure:

1. For this test, carbohydrate MRVP broth was used. The broth was prepared as per the standardcomposition and autoclaved for sterilization.
2. After the broth cooled down completely, it was inoculated using organism taken from an 18-24hours pure culture.
3. Incubate aerobically at 37 degrees C. for 24 hours.
4. Following 24 hours of incubation, aliquot 2 ml of the broth to a clean test tube.
5. Re-incubate the remaining broth for an additional 24 hours.
6. Add 6 drops of 5% alpha-naphthol, and mix well to aerate.
7. Add 2 drops of 40% potassium hydroxide, and mix well to aerate.
8. Observe for a pink-red color at the surface within 30 min. Shake the tube vigorously during the30-min period.
9. At the end of the incubation period, observe the observe the color change from red to yellow alongthe broth
10. Positive: the development of yellow color in the medium is indicative of a positive carbohydratefermentation reaction.
11. Negative: No color change is indicative of a negative carbohydrate fermentation reaction

Methyl Red Test

MR Broth media composition

S.No.	Composition	Amount (g/lit)
1	Buffered Peptone	7.0
2	Glucose	5.0
3	Dipotassium Phosphate	5.0
4	Distilled Water	1000 ml

Procedure:

1. Prior to inoculation, allow medium to equilibrate to room temperature.
2. Using organisms taken from an 18–24-hour pure culture, lightly inoculate the medium.
3. Incubate aerobically at 37 degrees C. for 24 hours.
4. Following 24 hours of incubation, aliquot 1ml of the broth to a clean test tube.
5. Reincubate the remaining broth for an additional 24 hours.
6. Add 2 to 3 drops of methyl red indicator to aliquot.

7. Observe for red colour immediately.

Indole Test

Indole Broth media composition

S.No.	Composition	Amount (g/lit)
1	Peptone	10.0
2	Sodium Chloride	5.0
3	Tryptophan	1.0
4	Distilled Water	1000 ml

Procedure:

1. Take a sterilized test tubes containing 4 ml of tryptophan broth.
2. Inoculate the tube aseptically by taking the growth from 18 to 24 hrs culture.
3. Incubate the tube at 37°C for 24-28 hours.
4. Add 0.5 ml of Kovac's reagent to the broth culture.
5. Observe for the presence or absence of ring.

Starch Hydrolysis Test

Media Composition

S.No.	Composition	(g/lit)
1	Peptic digest of animal tissue	5
2	Sodium Chloride	5
3	Yeast Extract	1.5
4	Beef Extract	1.5
5	Starch soluble	2.0
6	Agar	15
7	Distilled water	1000ml
	Final pH	7.4±0.2

Procedure

1. Using a sterile technique, make a single streak inoculation of organism to be tested into the centerof labeled plate.
2. Incubate the bacterial inoculated plates for 48 hours at 37°C.
3. Following incubation, flood the surface of the plates with iodine solution with a dropper for 30seconds.
4. Pour off the excess iodine.
5. Examine for the clear zone around the line of bacterial growth.

6. Positive result: A clear zone around the line of growth after addition of iodine solution indicates thatthe organism has hydrolyzed starch.

Gelatin Hydrolysis MethodMedia composition

S.No.	Composition	(g/lit)
1	Enzymatic digest of gelatin	5
2	Beef extract	3
3	Gelatin	120
4	Distilled water	1000 ml
	pH	6.8

Procedure

1. Inoculate the gelatin deep with 4 to 5 drops of a 24-hour broth culture.
2. Incubate at 35°-37°C in ambient air for up to 14 days.

Note: Incubate the medium at 25°C if the organism grows better at 25°C than at 35°C.

3. Alternatively, inoculate the gelatin deep from a 24-hour-old colony by stabbing four or fivetimes, 0.5 inch into the medium.
4. Remove the gelatin tube daily from the incubator and place at 4°C to check for liquefaction.
5. Refrigerate an un-inoculated control along with the inoculated tube. Liquefaction is determined only after the control has hardened (gelled).

Note: Do not invert or tip the tube, because sometimes the only discernible liquefaction occurs at thetop of the deep where inoculation occurred.

Gram Staining

Reagents Used in Gram Staining

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counterstain

Procedure

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loopful of sample.
3. Air dry and heat fix

4. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
5. Flood the gram's iodine for 1 minute and wash with water.
6. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.
8. Air dry, blot dry and Observe under Microscope.

Test Report:

2Test

Enzyme production; Enzyme Assay; Optimization for Cellulose production (Ph, Temperature, Carbon source, Nitrogen source, Agro-based waste material); Production of enzyme using optimized condition; Ammonium sulphate precipitation; Lowry's assay; Application of Cellulase enzyme

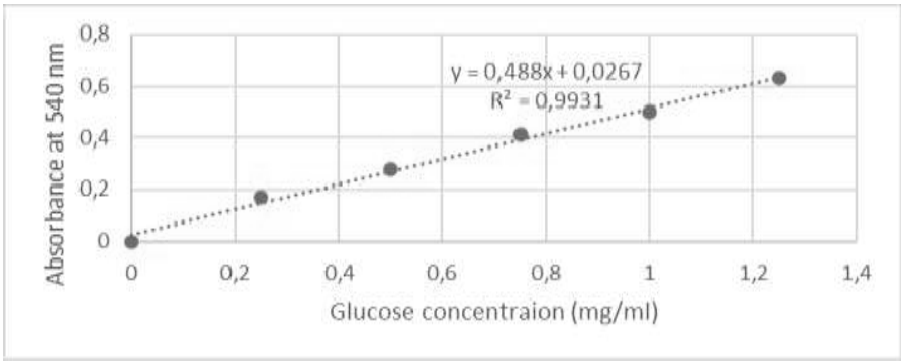
Test Sample: CL002 and CL005

Test Methods: Spectrophotometric assay (PC Based UV-Vis Spectrophotometer Systronic 2202)

Result and Observation:

Table: Absorbance observed at 540 nm for glucose solution at different concentrations

S.No.	Concentration (mg/ml)	Absorbance at 540 nm
1	0	0
2	0.25	0.17
3	0.5	0.28
4	0.75	0.41
5	1	0.5
6	1.25	0.63



Graph: Calibration graph of Glucose for Enzyme activity Assay

Table: Result recorded for Optimization of pH- Glucose released and enzyme activity (U/mL) forisolates CL002 and Cl005

S.No	Parameter	Sample	pH	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	pH	CL002	7	0.11	0.171	0.126
2		CL002	8	0.38	0.724	0.536
3		CL002	9	1.21	2.425	1.795
4		CL002	10	1.28	2.568	1.901
5		CL002	11	0.27	0.499	0.369
1		CL005	7	0.09	0.130	0.096
2		CL005	8	0.17	0.294	0.217
3		CL005	9	1.35	2.712	2.007
4		CL005	10	1.33	2.671	1.977
5		CL005	11	0.93	1.851	1.370

Table: Result recorded for Optimization of Temperature- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005

S.No.	Parameter	Sample	Temp (°C)	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Temperature	CL002	35	1.11	2.220	1.643
2		CL002	40	1.28	2.568	1.901
3		CL002	45	0.75	1.482	1.097
4		CL002	50	0.54	1.052	0.778
5		CL002	55	0.47	0.908	0.672
6		CL002	60	0.39	0.744	0.551
1		CL005	35	1.31	2.630	1.946
2		CL005	40	1.45	2.917	2.159
3		CL005	45	0.88	1.749	1.294
4		CL005	50	0.65	1.277	0.945
5		CL005	55	0.41	0.785	0.581
6		CL005	60	0.37	0.703	0.521

Table: Result recorded for Optimization of Carbon source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005

S.No.	Parameter	Sample	Carbon source	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Carbon source	CL002	Starch	1.15	2.302	1.704
2		CL002	Glucose	1.93	3.900	2.887
3		CL002	Maltose	1.04	2.076	1.537
4		CL002	Lactose	0.91	1.810	1.340
5		CL002	Fructose	0.83	1.646	1.218
1		CL005	Starch	1.85	3.736	2.765
2		CL005	Glucose	2.01	4.064	3.008
3		CL005	Maltose	1.54	3.101	2.295
4		CL005	Lactose	1.6	3.224	2.386
5		CL005	Fructose	1.22	2.445	1.810

Table: Result recorded for Optimization of Nitrogen source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005

S.No.	Parameter	Sample	Nitrogen source	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Nitrogen source	CL002	Yeast extract	1.86	3.757	2.780
2		CL002	Peptone	1.73	3.490	2.583
3		CL002	Urea	1.66	3.347	2.477
4		CL002	Ammonium Sulphate	1.41	2.835	2.098
1		CL005	Yeast extract	1.62	3.265	2.416
2		CL005	Peptone	1.84	3.716	2.750
3		CL005	Urea	1.33	2.671	1.977
4		CL005	Ammonium Sulphate	1.31	2.630	1.946

Table: Result recorded for Optimization of Agri-waste substrate- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005

S.No.	Parameter	Sample	Agri-waste substrate	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Agri-waste substrate	CL002	Groundnut cake	0.64	1.257	0.930

2		CL002	Coconut cake	0.52	1.011	0.748
3		CL002	Soy cake	0.41	0.785	0.581
4		CL002	Wheat bran	0.78	1.544	1.142
1		CL005	Groundnut cake	0.34	0.642	0.475
2		CL005	Coconut cake	0.21	0.376	0.278
3		CL005	Soy cake	0.27	0.499	0.369
4		CL005	Wheat bran	0.73	1.441	1.067

Table: Result recorded for Optimization of Different concentration of Optimized C-source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005

S.No.	Parameter	Sample	% C-source (Glucose)	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Concentration of C-Source (Glucose)	CL002	1	0.15	0.253	0.187
2		CL002	2	0.18	0.314	0.232
3		CL002	3	1.63	3.285	2.432
4		CL002	4	1.72	3.470	2.568
5		CL002	5	1.82	3.675	2.720
1		CL005	1	0.09	0.130	0.096
2		CL005	2	0.07	0.089	0.066
3		CL005	3	1.15	2.302	1.704
4		CL005	4	1.47	2.958	2.189
5		CL005	5	1.59	3.203	2.371

Enzyme Activity Calculation At Optimized Condition

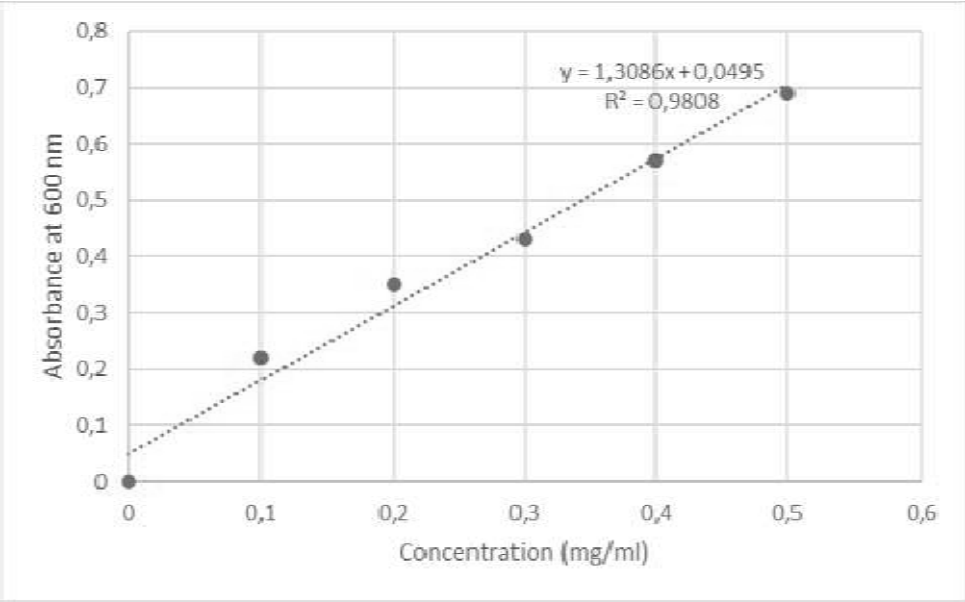
Table: Enzyme activity (U/mL) for isolates CL002 and Cl005 under Optimized conditions

S.No.	Sample code	Conditions	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	CL002	pH-10; 40°C; N-source- yeast extract	2.24	4.699	3.478
2	CL005	pH-9; Temp-40°C; N-source- peptone	2.15	4.351	3.220

Protein Estimation using Lowry's Assay

Table: Absorbance observed at 600 nm for BSA solution at different concentrations

S.No.	Concentration (mg/ml)	Absorbance at 600 nm
1	0	0
2	0.1	0.22
3	0.2	0.35
4	0.3	0.43
5	0.4	0.57
6	0.5	0.69



Graph: *Calibration graph of BSA for Protein estimation Assay*

Table: *Protein concentration observed in crude enzyme extracted from isolates*

S.No.	Sample code	Absorbance at 600 nm	Protein concentration (mg/ml)	Enzyme activity (U/mL)	Specific activity (U/mg)
1	CL002	1.32	0.971	3.478	3.582
2	CL005	1.14	0.833	3.22	3.864

Test Method

Enzyme Production Medium

In a conical flask with a capacity of 100 mL, ten mL of medium were withdrawn. After being sterilized in an autoclave at a temperature of 121°C for fifteen minutes, the flasks were allowed to cool before being inoculated with a bacterial culture that had grown overnight. After being inoculated, the medium was shaken for 24 hours at 37°C inside an incubator. After the fermentation process was complete, theculture medium was

centrifuged at 5000 rpm for 15 min in order to obtain the crude extract, which wasused as an enzyme source.

Production Medium (G/L)

S.No.	Composition	Amount (g/L)
1	glucose	0.5 gm
2	peptone	0.75 gm
3	FeSO4	0.01 gm
4	KH2PO4	0.5 gm
5	MgSO4	0.5 gm

Ammonium Sulphate Precipitation

- Crude Enzyme was precipitated using Salting out method using Ammonium Sulphate. Desired saturation range of ammonium sulphate was 40-80%, therefore precipitation was conducted in three ranges of 0-20%,20-40% and then 40-80%.
- Precipitation was conducted by mixing calculated amount of ammonium sulphate salt in the extracted crude enzyme. The amount of ammonium sulphate and crude enzyme used were as follows:
- Initially, 20 ml of crude enzyme was taken and mixed with 2.30gm of ammonium sulphate crystalsfor 0-20% saturation. Stirred for 1 hour to fully equilibrate.
- Centrifugation was done at 10,000g for 15 minutes to pellet out protein.
- Added more saturated ammonium sulfate or solid ammonium sulfate to make next concentration,repeat stirring and centrifugation.
- Next, for 21.25ml of solution 2.61gms of ammonium sulphate crystals for 20-40% saturation.Stirred for 1 hour to fully equilibrate.
- Centrifugation was done at 10,000g for 15 minutes to pellet out protein.
- Then finally, for 22.67ml of solution 6.43gm of ammonium sulphate crystals for 40- 80%saturation. Stirred for 1 hour to fully equilibrate.
- Centrifugation was done at 10,000g for 15 minutes to pellet out protein.
- Pooled and dissolved pellets obtained at three stages in PBS and moved to the next step to furtherdialyze out the Ammonium Sulfate.

Dialysis

- Dialysis tubing was carefully cut of 10 cm using sterile scissor. After cutting the proper length ofthe dialysis bag, it was activated by keeping in a beaker filled with distilled water at 80℃.
- Now tie the membrane at one end with thread (make sure no leakage is there by tying it very tightlywith the thread)

- Now add the precipitated enzyme in the tubing using sterile micropipette tip. Once added, press thefree end of tubing using finger to prevent entrapment of any bubbles.
- Again, turn the free end of tubing and tie with another piece of thread very tightly.
- The dialysis bag was then suspended in the dialysis buffer**. A stir bar was placed in the dialysisbuffer and kept for stirring.
- Dialysis buffer was changed thrice during the process and stored at 4℃ overnight after third timechanging the buffer.
- After completion of the incubation period, samples were collected from dialysis bag and centrifugedat 10000 rpm for 10 mins.
- After centrifuging, the supernatant was collected and store at -20℃

Enzyme Assay

The method developed by Miller (Miller *et al*., 1959) was used to determine the level of cellulase activity. In a nutshell, a reaction mixture that consisted of 0.2 mL of crude enzyme solution and 1.8 mL of 0.5% carboxymethyl cellulose (CMC) in 50 mM sodium phosphate buffer (pH 7) was incubated at 37 °C in a shaking water bath for 30 minutes. This was done in order to achieve the desired results. Thereaction was stopped when 3 mL of DNS reagent was added to the mixture. After that, the colour was developed in the mixture by bringing it to a boil for five minutes. The optical density (OD) of the samples was measured at 575 nm in comparison to a blank that consisted of all the reagents except for the crude enzyme. The enzyme activity was calculated in terms of the micromoles of glucose units released in 1 minute using the standard graph of glucose. U/ml = Released glucose concentration (mg/ml)

* Reaction volume (ml) * Dilution factor * 1000/ Incubation time (min) * volume of enzyme (ml) * Mol. Wt. of glucose (mg/mol)

Where,

Reaction volume= 5.0 ml

Enzyme volume= 0.2ml

DF=160 (Enzyme pellet= 12.5 mg;Buffer used for dilution= 2 ml)

Mol wt= 180156 mg/mol

Process Optimization for Maximum Cellulase Production

pH

After taking flasks with broth that already contains the optimal concentration of substrate and carbon source, the pH of the broth is adjusted to 7.0, 8.0, 9.0, 10.0, and 11.0 in

various flasks using 1 N HCl and 1 N NaOH, and then the broth is sterilised. The cultures are then inoculated before being placed in an incubator at 37C temperature. At the completion of the incubation period, the cell-free culture filtrateis extracted, and it is subsequently put to use as a source of enzyme for enzymatic activity determination.

Temperature

Production medium with a pH of 7 was inoculated with a selected bacterial strain that had been grown overnight. For a period of twenty-four hours, the broth was heated to 35, 40, 45, 50, 55, and 60 degreesCelsius at various intervals. At the ending of the incubation period, the cell-free culture filtrate is extracted, and it is subsequently put to use as a source of enzyme for enzymatic activity determination.

Carbon Sources

The effects of a variety of carbon sources, including starch, glucose, maltose, lactose, and fructose, were investigated in the production medium at concentrations ranging from 1% to 5%.

Nitrogen Sources

By substituting 0.5% of the peptone in the production medium with one of many other nitrogen sources, such as yeast extract, peptone, urea, or ammonium sulphate, the effects of these nitrogen sources on enzyme production were analysed.

Agro-Based Waste Material

In order to determine whether or not agro-based waste is suitable for use as a substrate in the productionof enzymes, many different types of substrates, including groundnut cake, coconut cake, soy cake, and wheat bran, are placed in the growing medium while it is submerged. After twenty-four hours, the enzyme synthesis is evaluated by measuring the enzyme activity.

- Production of enzyme using optimized condition

Enzyme production was done using optimized condition as described above

- Lowry’s assay protein content

Determination Reagents:

F.C (Folin Ciocalteu) reagent Bovine Serum Albumin (1mg/ml)

Reagent A – 2% Na2Co3 + 0.1N NaOH.Reagent B - 2% CuSO4.5H2O.

Reagent C – 2% Potassium Sodium Tartarate

Reagent D – 99ml + 0.5 ml reagent B + 0.5 ml Reagent C.

Standard protein solution: Prepare BSA solution of concentration 1mg/ml.

Procedure:

Arrange the clean dry test tube on the stand and label them as per the table shows. Add the componentsto each labelled tube according to the information in the table below.

Table: Final components in the tube for Lowry estimation of protein

S.No.	Testtube name	Solution (□l)	Distilled water (□l)	Reagent D	Incubation time	FC reagent	Incubation time	OD at 630nm
1	Blank	0	1000	5 ml	10 minutes in Dark at RT	0.5 ml	30 Minutes in dark at RT	
2	1 std.	100 BSA	900	5 ml		0.5 ml		
3	2 std.	200 BSA	800	5 ml		0.5 ml		
4	3 std.	300 BSA	700	5 ml		0.5 ml		
5	4 std.	400 BSA	600	5 ml		0.5 ml		
6	5 std.	500 BSA	500	5 ml		0.5 ml		
7	Sample	100 sample	900	5 ml		0.5 ml		

Add 5mL of reagent D to each tube including the blank. Mix well and allow it to stand for 10min. Then add 0.5mL of reagent D, mix well and incubate at room temp. in the dark for 30min. Blue color is developed. Take the reading at 660nm. Draw a standard graph and calculate the amount of protein in the sample.

Miller G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959;31(3):426–428.

Test Report: 3

Test: Application of Cellulase enzyme on General Biomedical waste

Test Sample: CL002 and CL005; Cotton swabs (CS), Dressings and bandages (DB), Plaster casts (PC),Discarded gloves (DG), Tissue and bits of papers (TP)

Test Methods: Spectrophotometric assay (PC Based UV-Vis Spectrophotometer Systronic 2202)

Result and Observation

% Degradation efficiency of Crude cellulase enzyme on general biomedical waste material

Table 1: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable temperature

Cotton swabs -CS; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers - TP

Table 2: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable pH

Parameter	**Sample**	**CL002**			**CL005**		
pH							
		Initial wt. (mg)	final wt. (mg)	%Degradation	Initial wt. (mg)	final wt. (mg)	%Degradation
		3					
	CS	25	17.8	28.8	25	14.3	42.8
	DB	25	23.5	6	25	21.4	14.4
	PC	25	14.7	41.2	25	17.64	29.44
	DG	25	25	0	25	25	0
	TP	25	14.9	40.4	25	14.9	40.4
		5					
	CS	25	17.3	30.8	25	18.6	25.6
	DB	25	16.3	34.8	25	14.3	42.8
	PC	25	20	20	25	18.33	26.68
	DG	25	25	0	25	25	0
	TP	25	16.7	33.2	25	15.8	36.8
		6					
	CS	25	24.7	1.2	25	12.9	48.4
	DB	25	23.3	6.8	25	17.1	31.6
	PC	25	20.9	16.4	25	11.7	53.2

		DG	25	25	0	25	25	0

Parameter	**Sample**	**CL002**			**CL00 5**		
Temperature		Initial wt. (mg)	final wt. (mg)	%Degradation	Initial wt. (mg)	final wt. (mg)	%Degradation
		30					
	CS	25	18.6	25.6	25	20.6	17.6
	DB	25	23.4	6.4	25	22.4	10.4
	PC	25	23.9	4.4	25	21.9	12.4
	DG	25	25	0	25	25	0
	TP	25	11	56	25	13.41	46.36
		35					
	CS	25	17.3	30.8	25	19.81	20.76
	DB	25	21.5	14	25	19.3	22.8
	PC	25	20.7	17.2	25	21.5	14
	DG	25	25	0	25	25	0
	TP	25	13.6	45.6	25	13.3	46.8
		40					
	CS	25	6.4	74.4	25	11.56	53.76
	DB	25	17.4	30.4	25	15.4	38.4
	PC	25	13.2	47.2	25	11.11	55.56
	DG	25	25	0	25	25	0
	TP	25	9.6	61.6	25	8.6	65.6
		45					
	CS	25	15.5	38	25	16.41	34.36
	DB	25	16.7	33.2	25	16	36
	PC	25	19	24	25	20.41	18.36
	DG	25	25	0	25	25	0
	TP	25	11.3	54.8	25	14.1	43.6
		50					
	CS	25	17.3	30.8	25	18.2	27.2
	DB	25	18.6	25.6	25	18.5	26
	PC	25	19.3	22.8	25	21.4	14.4
	DG	25	25	0	25	25	0
	TP	25	22.3	10.8	25	19.3	22.8

	TP	25	17.9	28.4	25	10.3	58.8
		7					
	CS	25	20.6	17.6	25	21.1	15.6
	DB	25	18.1	27.6	25	24.6	1.6
	PC	25	21.09	15.64	25	24	4
	DG	25	25	0	25	25	0
	TP	25	17.7	29.2	25	20.5	18
		8					
	CS	25	19	24	25	19.33	22.68
	DB	25	19.7	21.2	25	18.5	26
	PC	25	18.5	26	25	18.5	26
	DG	25	25	0	25	25	0
	TP	25	18.7	25.2	25	17.2	31.2

Cotton swabs -CS; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers - TP

Table 3: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable agitation speed

Parameters	**Sample**	**Initial wt. (mg)**	**final wt. (mg)**	**%Degradation**	**Initial wt. (mg)**	**final wt. (mg)**	**%Degradation**
Agitation speed	100						
	CS	25	23.3	6.8	25	20.13	19.48
	DB	25	21.56	13.76	25	20.41	18.36
	PC	25	22.5	10	25	20.02	19.92
	DG	25	25	0	25	25	0
	TP	25	23.8	4.8	25	19.8	20.8
	150						
	CS	25	12.4	50.4	25	13.5	46
	DB	25	13.7	45.2	25	12.7	49.2
	PC	25	16	36	25	15.21	39.16
	DG	25	25	0	25	25	0
	TP	25	9.07	63.72	25	12.08	51.68
	200						
	CS	25	17.9	28.4	25	19.45	22.2
	DB	25	17.9	28.4	25	21.4	14.4

	PC	25	15.4	38.4	25	16.33	34.68
	DG	25	25	0	25	25	0
	TP	25	16.3	34.8	25	18.4	26.4
	250						
	CS	25	21.05	15.8	25	22.5	10
	DB	25	23.8	4.8	25	22	12
	PC	25	23.1	7.6	25	23.5	6
	DG	25	25	0	25	25	0
	TP	25	24.5	2	25	24.8	0.8
	300						
	CS	25	21.3	14.8	25	20.4	18.4
	DB	25	23	8	25	21.4	14.4
	PC	25	23.4	6.4	25	22.5	10
	DG	25	25	0	25	25	0
	TP	25	23.7	5.2	25	21.6	13.6

Cotton swabs -CS ; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers – TP

Table 4: % Degradation efficiency of Crude cellulase enzyme from isolates CL002 and CL005 on general biomedical waste material at variable proportion of crude enzyme

Parameters	**Sample**	**Initial wt. (mg)**	**final wt. (mg)**	**%Degradation**	**Initial wt. (mg)**	**final wt. (mg)**	**%Degradation**
Concentration		2					
	CS	25	19.6	21.6	25	20.5	18
	DB	25	18.33	26.68	25	14.8	40.8
	PC	25	21.5	14	25	17.5	30
	DG	25	25	0	25	25	0
	TP	25	17	32	25	15.9	36.4
		4					
	CS	25	18.75	25	25	24.3	2.8
	DB	25	18.1	27.6	25	21.5	14
	PC	25	21.26	14.96	25	20.3	18.8
	DG	25	25	0	25	25	0
	TP	25	15.3	38.8	25	19.7	21.2

		6					
	CS	25	13.4	46.4	25	15.5	38
	DB	25	11.84	52.64	25	13.56	45.76
	PC	25	11.36	54.56	25	17.2	31.2
	DG	25	25	0	25	25	0
	TP	25	10.41	58.36	25	11.71	53.16
		8					
	CS	25	14.7	41.2	25	17.9	28.4
	DB	25	15.03	39.88	25	15.01	39.96
	PC	25	15.9	36.4	25	18.3	26.8
	DG	25	25	0	25	25	0
	TP	25	12.2	51.2	25	14.5	42
		10					
	CS	25	23.65	5.4	25	20.35	18.6
	DB	25	21.42	14.32	25	20.1	19.6
	PC	25	21.5	14	25	21.5	14
	DG	25	25	0	25	25	0
	TP	25	21.9	12.4	25	19.9	20.4

Cotton swabs -CS ; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers - TP

Table 5: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable temperature

Parameter	**Sample**	**CL00 2**		**CL00 5**	
		Absorbance	Concentration (mg/ml)	Absorbance	Concentration (mg/ml)
Temperature		30° C			
	CS	0.910	1.810	1.180	2.363
	DB	0.070	0.089	0.170	0.294
	PC	0.040	0.027	0.960	1.913
	DG	0.030	0.007	0.040	0.027
	TP	1.210	2.425	1.040	2.076
		35° C			
	CS	0.940	1.872	0.760	1.503

	DB	0.160	0.273	1.180	2.363
	PC	0.190	0.335	0.810	1.605
	DG	0.030	0.007	0.030	0.007
	TP	1.150	2.302	0.810	1.605
		40° C			
	CS	1.240	2.486	1.120	2.240
	DB	0.860	1.708	1.040	2.076
	PC	1.250	2.507	1.280	2.568
	DG	0.040	0.027	0.030	0.007
	TP	1.390	2.794	1.350	2.712
		45° C			
	CS	1.040	2.076	0.170	0.294
	DB	0.880	1.749	0.050	0.048
	PC	0.840	1.667	0.090	0.130
	DG	0.030	0.007	0.030	0.007
	TP	1.190	2.384	0.200	0.355
		50° C			
	CS	0.950	1.892	0.230	0.417
	DB	0.910	1.810	0.210	0.376
	PC	0.750	1.482	0.210	0.376
	DG	0.030	0.007	0.740	1.462
	TP	0.120	0.191	0.090	0.130

Cotton swabs -CS ; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers – TP

Table 6: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable pH

Parameter	**Sample**	**CL002**		**CL005**	
		Absorbance	**Concentration (mg/ml)**	**Absorbance**	**Concentration (mg/ml)**
pH		3.000			
	CS	0.940	1.872	1.180	2.363
	DB	0.050	0.048	0.170	0.294
	PC	1.120	2.240	0.960	1.913
	DG	0.030	0.007	0.040	0.027

	TP	1.040	2.076	1.040	2.076
		5.000			
	CS	0.930	1.851	0.760	1.503
	DB	0.910	1.810	1.180	2.363
	PC	0.630	1.236	0.810	1.605
	DG	0.030	0.007	0.030	0.007
	TP	0.870	1.728	0.810	1.605
		6.000			
	CS	0.040	0.027	1.120	2.240
	DB	0.080	0.109	1.040	2.076
	PC	0.170	0.294	1.280	2.568
	DG	0.030	0.007	0.030	0.007
	TP	0.940	1.872	1.350	2.712
		7.000			
	CS	0.200	0.355	0.170	0.294
	DB	0.940	1.872	0.050	0.048
	PC	0.160	0.273	0.090	0.130
	DG	0.030	0.007	0.030	0.007
	TP	0.910	1.810	0.200	0.355
		8.000			
	CS	0.870	1.728	0.230	0.417
	DB	0.720	1.421	0.210	0.376
	PC	0.760	1.503	0.210	0.376
	DG	0.030	0.007	0.740	1.462
	TP	0.890	1.769	0.090	0.130

Cotton swabs -CS; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers - TP

Table 7: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable agitation speed

Parameter	**Sample**	**CL002**		**CL005**	
		Absorbance	Concentration (mg/ml)	Absorbance	Concentration (mg/ml)
Agitation speed (rpm)		100 rpm			
	CS	0.08	0.109	0.63	1.236
	DB	0.14	0.232	0.57	1.113

	PC	0.11	0.171	0.75	1.482
	DG	0.03	0.007	0.04	0.027
	TP	0.05	0.048	0.78	1.543
		150 rpm			
	CS	1.290	2.589	1.24	2.486
	DB	1.130	2.261	1.28	2.568
	PC	0.940	1.872	0.98	1.953
	DG	0.030	0.007	0.03	0.0067
	TP	1.340	2.691	1.27	2.548
		200 rpm			
	CS	0.940	1.872	0.86	1.707
	DB	0.950	1.892	0.47	0.908
	PC	0.970	1.933	0.67	1.318
	DG	0.030	0.007	0.04	0.0272
	TP	0.910	1.810	0.92	1.830
		250 rpm			
	CS	0.150	0.253	0.11	0.170
	DB	0.060	0.068	0.14	0.232
	PC	0.120	0.191	0.06	0.068
	DG	0.030	0.007	0.04	0.027
	TP	0.080	0.109	0.04	0.027
		300 rpm			
	CS	0.130	0.212	0.77	1.523
	DB	0.140	0.232	0.84	1.666
	PC	0.110	0.171	0.89	1.769
	DG	0.030	0.007	0.89	1.769
	TP	0.120	0.191	0.85	1.687

Cotton swabs -CS; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissue and bits of papers – TP

Table 8: Sugar estimation in degradation reaction from crude cellulase enzyme of isolates CL002 and CL005 on general biomedical waste material at variable proportion of crude enzyme

Parameter	Sample	CL002		CL005	
		Absorbance	Concentration (mg/ml)	Absorbance	Concentration (mg/ml)
Concentration (%)		2 %			
	CS	0.790	1.564	0.58	1.133
	DB	0.810	1.605	0.93	1.851
	PC	0.110	0.171	0.66	1.298
	DG	0.030	0.007	0.04	0.027
	TP	0.870	1.728	0.69	1.359
		4.0 %			
	CS	0.740	1.462	0.09	0.129
	DB	0.930	1.851	0.17	0.293
	PC	0.140	0.232	0.6	1.175
	DG	0.030	0.007	0.03	0.0067
	TP	0.970	1.933	0.85	1.687
		6.0 %			
	CS	1.240	2.486	0.71	1.400
	DB	1.330	2.671	0.23	0.416
	PC	1.370	2.753	0.68	1.339
	DG	0.030	0.007	0.03	0.0067
	TP	1.340	2.691	1.29	2.588
		8.0 %			
	CS	1.170	2.343	0.95	1.892
	DB	1.150	2.302	0.97	1.933
	PC	0.960	1.913	0.87	1.728
	DG	0.030	0.007	0.03	0.0067
	TP	1.300	2.609	0.95	1.892
		10.0 %			
	CS	0.060	0.068	0.88	1.748
	DB	0.110	0.171	0.74	1.461
	PC	0.100	0.150	0.81	1.605
	DG	0.030	0.007	0.9	1.789
	TP	0.090	0.130	0.74	1.461

Cotton swabs -CS; Dressings and bandages – DB ; Plaster casts – PC ; Discarded gloves – DG ; Tissueand bits of papers - TP

Test Method

- Cellulase from isolates CL002 and CL005 were used for the Degradation assessment in generalbiomedical waste.
- The samples included general wastes of health care [a] Cotton swabs (CS) [b] Dressings and bandages (DB) [c] Plaster casts (PC) (d) Discarded gloves (DG) [e] Tissue and bits of papers (TP). The samples were collected in polythene bags and cut into small pieces, and aliquoted fordifferent experimental set up.
- Crude enzyme was dissolved in 50 mM sodium acetate/NaOH buffer (pH 4.5) to reach concentration of 3.0 U/ml for both isolates and then mixed with 25 mg (Initial weight-W1) of waste materials and incubated at 50°C for 2 h.
- After incubation the tubes were centrifuged for 15 min using and the supernatants were transferred into clean test tubes with the concentration of the produced sugars determined by the DNS method. All absorbance readings were taken using the Double beam UV Vis spectrophotometer. For the concentration determination, the glucose standards were used as inmentioned in earlier report.
- The precipitate of papers collected after centrifugation was rinsed with distilled water, oven dried and weighed (Final weight-W2).
- Degradation efficiency was calculated using formula

Degradation Efficiency (%) = (1-W2/W1) *100 where W2 and W1 are the weight of sample after reaction and the initial weight of the waste in experimental set up.

Conclusion

In conclusion the three different cellulolytic bacteria such as *Bacillus cereus*, *Bacillus subtilis* and *Bacillus thuringiensis* have been isolated. *Bacillus cereus* showed maximum cellulolytic activity compared to other two isolated bacteria. *Acacia arabica* pod shows maximum enzyme activity comparatively other pods. Optimization of different physico-chemical parameter of the production medium is required to get the maximum yield of the enzyme. Further studies were in progress to get high yield production, purification and application of cellulase.

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