

Modern Approaches in Chemical and Biological Sciences

Volume - 2



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Edited by
Hari Shankar Biswas
Sheikh Ahmad Izaddin Sheikh Mohd Ghazali
Sandeep Poddar
Amiya Bhaumik

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Editors



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Editorial

From time immemorial nature's molecular diversity has been extensively screened for new medications and for the discovery of leading compounds. The search for agents intended to combat infectious diseases has been of interest and has enjoyed a high degree of success. Chemistry, particularly chemical synthesis, continue to be key enabling technologies. The book "Modern Approaches in Chemical and Biological Sciences- Volume 2", spans the fields of chemistry and biology, to disseminate knowledge in the current advancements and challenges in this field for the development of multidisciplinary research. The book deals with the application of chemical techniques and analysis to the study and manipulation of biological systems. The book consists of collection of chapters exploring the foundations of chemistry and biology, reviewing some of the foremost technical and conceptual advances of the last decade, and shaping the future of this vibrant field.

Water cleanup is in urgent need but a very difficult task. Aquatic life is negatively impacted by a variety of industrial contaminants, including organic synthetic colours that combine with ground water. The first chapter by Dr. Moumita Roy describes the catalytic degradation of a variety of colours (cationic dyes and anionic dyes) in the presence of sodium borohydride using a polyaniline anchored palladium (PANI-Pd) catalyst (SB).

Natural food contaminants, such as pathogenic organisms and several other fungal and bacterial toxins, are typically of microbiological origin. Given that food is one of the most fundamental elements of living creatures, it is one of the most important concerns for public security. Dr. Kamala Mitra examines the noble nanoparticles that can be used precisely to verify the quality and safety of food.

Over the past few decades, it has become clear that reactions in micro-heterogeneous systems are of tremendous interest. Micelles and reverse micelles are two such micro-heterogeneous aggregates that are created by the surface-active components of surfactants. Since many reactions have been successfully carried out in reverse micelles and micelles as catalytic reactors over the past few decades, the chapter "Micelles and Reverse Micelles: a fascinating Micro-reactor" will focus on these systems.

Experimental chemists are currently interested in hypervalent iodine reagents, such as (diacetoxyiodo) benzene or DIB, (hydroxytosyloxyiodo) benzene or HTIB, iodosylbenzene, IBX, Dess-Martin Reagent, etc. Hypervalent iodine reagents have recently emerged as a suitable synthetic substitute for highly widespread chemical reactions. In the chapter "Recent Advances in Oxidative Transformation of Oximes with Hypervalent Iodine (III) Reagents," the author Dr. Harisadhan Ghosh reports on the fascinating chemistry of hypervalent iodine (III) reagents, which triggered a number of oxidation processes of aldoximes and ketoximes.

The term given to the acceleration of cyclization brought on by the substitution of hydrogen atoms on the carbons anchoring the two reaction centres with alkyl groups is the Thorpe-Ingold effect. Intramolecular reactions frequently advance more quickly than intermolecular reactions because of the more favorable entropy shift that occurs when the reaction reaches the transition state. In his article, Dr. Biswajit Panda focuses on the Thorpe-Ingold effect used to build strain-based three- and four-membered rings. The yield of both intramolecular and intermolecular cyclization can be efficiently accelerated and increased by the Thorpe-Ingold effect.

Oriented external electric field (OEEF) has catalytic activity in some organic reaction mechanisms and the ability to cleavage hemolytic bonds in a heterolytic way. OEEF is a budding area of research, waiting for eventual blossom. In the sixth chapter, the improvement of the kinetics and thermodynamics of chemical reactions, and its crucial role in the applied oriented external electric field has been reviewed.

Although the metabolic basis of their contents is unknown, microorganisms have been employed for millennia as biocatalysts to produce bread, wine, vinegar, and other common goods. Due to their stability, catalytic activity, simplicity of production processes, and wide application in industries and medicine, microbial enzymes have grown in popularity. The study by Dr. Baishali Pandit presents a current viewpoint on the usefulness and industrial applications of microbial enzymes.

The nitrogen cycle is a biogeochemical process through which nitrogen is changed into a variety of forms before returning to the atmosphere. The nitrogen is essential to life and its availability is crucial for sustaining the balanced of the biotic and abiotic components of our ecosystem. But human-mediated changes to the earth's nitrogen cycle are having a profoundly disturbed effect on ecosystems around the world. According to Dr. Suman Tamang's, implementing time-tested sustainable methods can help to reduce environmental destruction. Therefore, it is imperative that national and international policymakers to decrease these negative impacts through the expansion and widespread adoption of better-planned and more efficient methods of managing human activities.

The greatest organ of our body, the skin, maintains a healthy equilibrium with the surrounding living things. About one million microorganisms per square centimetre, including various bacterial, fungal, and viral species, reside on the skin. Dr. Sucheta Das and Mr. Shamba Chatterjee talk about the role of probiotics in skin health.

The fifth pandemic has been identified as COVID-19, a novel coronavirus illness. In their article, Suvroma Gupta et al., emphasise the virology and origin of the virus. The discussion then turns to the several virus mutations, with a special emphasis on the spike protein mutation. Additionally, talk about the various tests and how they are administered in India.

Without a doubt, natural ingredients play a very important part in pharmaceuticals. In this context, *Azadirachta indica* is fast becoming the focus of contemporary medicine. In her review article, Dr. Sonali Ray makes an effort to summarize the plant's antibacterial and antifungal capabilities and emphasizes the need for more analytical study to find new drugs.

The COVID-19 new coronavirus disease has been identified as the fifth pandemic since the 1918 flu pandemic, or about a century ago. A chapter deals with the origin, virology, and challenges of Severe Acute Respiratory Syndrome Coronavirus 2. The discussion then turns to the several virus mutations, with a special emphasis on the spike protein mutation. Dr. Debdeep Dasgupta and Ms. Lipika Das examine the immunopathogenesis of COVID-19. It is noteworthy that there are currently no such suitable COVID-19 therapies that have been shown to be effective. Designing a successful treatment for the infection requires a thorough understanding of the immune system's role in pathogenesis.

Warm Pacific equatorial waters periodically migrate southward during El-Ninos, which often occur around Christmas. El-Nino is associated with the Southern Oscillation, which influences the atmosphere and disturbs the global weather patterns. In his paper "El-Nino and It's Varied Impacts: A Review" Dr. Tarikul Islam Golder discusses the socioeconomic effects of El-Nino frequency on various countries.

The many Indian tribal cultures are particularly fond of ethnomedical herbs. Indian subcontinent tribes place a high value on their traditional healthcare system. The globe places a high value on maintaining their expertise of ethnomedicine. The exact applications of the ethnomedicinal plant species and their use during various seasons are covered by Dr. Amit Saha.

Strengthening aquaculture as an alternative to increase aquatic production as a result of competition brought on by the exploitation of natural resources like soil and water has been utilized by other production and development businesses. The author of the paper "Waste Production in Aquaculture" looked at a variety of aspects of aquaculture waste production, including its sources, components, and management practices in different cultural systems, with a focus on waste production from food, feedstuffs, fertilizers, pesticides, herbicides, and other sources, as well as to identify waste sources,

content, and potential hazards to both fish culture and the environment.

Therefore, the present book provides a platform for discussion about the potential changes and role of chemistry and biology in the academic and industrial fields. This will help in the innovation and invention of new molecules. The ultimate goal of this innovation is the creation of a better future. This platform also brings together all the researchers whose brainstorm on the crucial issues pertaining to theory process, and practice of innovation and entrepreneurship has created this vast array of chapters. The book is intended to provide more opportunities to the researchers in the areas of chemical and biological sciences and other related fields along with a forum for in-depth assessment of the challenges involved in this dynamic and fast-moving arena. This book emphasizes on the up-to-date research, development, and commercial aspects of recent advances in chemical and biological sciences. Hence this book will be valuable resource for researchers, academicians as well as professionals interested in the exceedingly interdisciplinary field of Chemical, Biological and Environmental sciences for sustainable growth and development.

Hari Shankar Biswas
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Foreword

The book "Modern Approaches in Chemical and Biological Sciences" volume-2 edited by Dr. Hari Shankar Biswas, Dr. Sheikh Ahmad Izaddin Sheikh Mohd Ghazali, Dr. Sandeep Poddar, and Dr. Amiya Bhaumik, spans the fields of chemistry and biology. The sole purpose of the book is to highlight the role of inclusive approach among various disciplines, and to disseminate current knowledge regarding advancements and challenges in these fields. The book consists of a collection of chapters exploring the mergers of chemistry and biology, reviewing some of the most notable technical and conceptual advances of the last decade and shaping the future of this exciting field. The book highlights possible scientific solutions to some of the current issues of daily life. For example, water cleanup: natural food contaminants, such as pathogenic organisms and several other fungal and bacterial toxins, are typically of microbiological origin. Micelles and reverse micelles are two such micro-heterogeneous aggregates that are created by the surface-active components of surfactants. The fascinating chemistry of hypervalent iodine (III) reagents, which triggered a number of oxidation processes of aldoximes and ketoximes. The yield of both intramolecular and intermolecular cyclization can be efficiently accelerated and increased by the Thorpe-Ingold effect. Oriented external electric field (OEEF) has catalytic activity in some organic reaction mechanisms and the ability to cleave hemolytic bonds in a heterolytic way. The nitrogen cycle is a biogeochemical process that converts nitrogen into various forms before returning it to the atmosphere. The role of probiotics in skin health has been described in another chapter.

The book also contains a chapter discussing the origin, virology, and challenges of Severe Acute Respiratory Syndrome Coronavirus 2. The discussion then turns to the several viral mutations, with a special emphasis on the spike protein mutation. The antibacterial and antifungal capabilities of the plants, ethnomedicinal plant species and their use during various seasons are also discussed in some of the chapters of this book. Moreover, the need for analytical study to find new drugs suggesting the adoption of better-planned and more efficient methods of managing human activities are included in this book. Management practices in different cultural systems along with the different aspects of aquaculture waste production, including its sources, components are also discussed in this book. A special significance has been given not only to the procedure of waste production from food, feedstuffs, fertilizers, pesticides, herbicides, and other sources but also to identify waste sources, content, and potential hazards to both fish culture and the environment.

Therefore, the present book provides a platform for discussion about the potential changes and role of chemistry and biology in the academic and industrial fields. I strongly believe that this book will serve as a valuable resource for researchers, academicians, as well as professionals interested in the exceedingly interdisciplinary field of chemical, biological, and environmental sciences for sustainable growth and development.

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Polyaniline Immobilized Palladium: An Efficient Catalyst for Reductive Degradation of Dyes

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ABSTRACT

Water remediation is a very challenging task and a need of the hour. Various industrial pollutants, primarily organic synthetic dyes, mix with ground water and have a negative impact on aquatic life. There are various techniques available to treat the polluted water. Reductive degradation of organic dyes is one of the most commonly used techniques. Herein, the catalytic degradation of a diversified range of dyes (cationic dyes and anionic dyes) using a polyaniline anchored palladium (PANI-Pd) catalyst in the presence of sodium borohydride (SB) is discussed in detail. PANI-Pd is easy to recover and efficiently recycle with minimum loss of activity. It was found in the study that the PANI-Pd (OAc)₂ catalyst is highly active in the reductive degradation of both cationic (MB) and anionic dye (MO). Furthermore, reaction time is also very short.

Keywords: *Dye degradation; Polyaniline; Palladium; Heterogeneous; Catalyst*

INTRODUCTION

Synthetic organic dyes liberated into waterbodies by a number of production companies such as clothing material, pulp and paper, pharmaceutical, agrochemicals and processed food are causing damages to aquatic life and in turn harming all other living organisms. Research suggests that most of these dyes are non-biodegradable, highly stable, toxic and carcinogenic. Some even cause reduced photosynthesis in plants (Weerasekara, 2017; Wong *et al.*, 2004; Gupta, 2009).

In view of the adverse effect of water pollution on mankind, pollution control agencies across the nation have enacted stringent legislation to control the permissible limit of such entities in the discharged effluent. To meet these regulatory guidelines, such hazardous chemicals, need to be controlled below a ppm (parts per million) level in the effluent.

Different techniques are employed for the complete or partial removal of these pollutants from the wastewater. These are broadly divided into the following types: physical methods like adsorption, coagulation; chemical methods utilizing both oxidation (by O₂, NaOCl, O₃, H₂O₂ etc.) (Singh & Arora, 2011), and reduction (by Na₂S₂O₄) (Li *et al.*, 2009), biological methods (Sudarjanto, Keller-Lehmann & Keller, 2006) and photochemical methods (Van der Zee & Villaverde, 2005). Diversified metal based catalysts (e.g. Au, Ag, Pt and Pd) are found to be extremely powerful towards reductive degradation of organic colorant molecules as metal can participate in the electron transfer process between NaBH₄ and pigments (Gupta, Singh & Sharma, 2011; Mallick, Witcomb & Scurrill, 2005; Khan, Lee & Cho, 2014).

Heterogeneous palladium-based catalysts demonstrated very efficient reductive degradation of different dye molecules as palladium is inherently highly active in reduction reactions. Moreover, being heterogeneous offers the added advantages of easy recovery and recyclability (Hu *et al.*, 2007; Wang *et al.*, 2009; Li *et al.*, 2015; Patra *et al.*, 2016).

Polyaniline (PANI) which is a conducting polymer, has very interesting properties such as inert nature, easy to prepare from readily available aniline, insolubility in common organic solvents as well as water,

extensive N=N and NH-NH functional groups and modular redox nature. All these qualities make PANI a good support for anchoring active metal catalysts (Choudary *et al.*, 2006). By utilizing these properties of PANI, several different metal catalysts were immobilized. PANI and their catalytic properties are explored in various types of coupling reactions as well as oxidation, reduction, etc. (Choudary *et al.*, 2006). Herein, PANI-Pd is explored for the degradation of different organic dyes, e.g. Methylene blue, methyl orange, etc. (Roy *et al.*, 2019) under a reductive environment.

METHODOLOGY

All the chemicals were purchased from commercial sources and used, except Aniline which was distilled before use. Particle size was analyzed by Transmission electron microscopy (TEM) and Pd content of the catalyst was analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Synthesis of Polyaniline Base (PANI)

To a solution containing concentrated H_2SO_4 (30mL) in water (750mL), purified aniline (9.8 g, 106 mmol) was added and the solution was stirred continuously at 0°C . Ammonium persulfate (24 g, 106 mmol) in water (250 mL) was added slowly within 4 h to the solution so obtained. Polyaniline-sulfate salt was recovered by filtration and it was washed with ample water followed by acetone. Then 1000 mL 1N sodium hydroxide solution was added to polyaniline-sulfate salt and stirred for 12 h at $25\text{--}30^\circ\text{C}$. Finally, it was filtered, washed with water, followed by acetone and dried under reduced pressure at $50\text{--}60^\circ\text{C}$ to obtain 8.37 g of PANI as black solid powder.

Synthesis of PANI-Pd(OAc)₂

PANI (500 mg) was added to a solution of palladium acetate (500 mg) in acetonitrile (40 mL) and agitated under an inert environment for 2 days. The solid so obtained was filtered and washed with acetonitrile and acetone respectively. Wet catalyst was dried for almost 1 day to afford PANI-Pd(OAc)₂ (600 mg). The catalyst was subjected ICP-AES analysis to get the palladium content (Pd 0.35 mmol g^{-1}).

Catalytic degradation of dyes

Estimated quantities of dye, NaBH_4 , and catalyst were mixed in water and decrease in absorption of the dye was monitored by UV-vis spectrophotometer at the λ_{max} of the dye.

RESULTS AND DISCUSSION

Study of catalytic degradation process

A typical degradation experiment was performed by adding 5 mg of PANI-Pd(OAc)₂ and aqueous solution of NaBH_4 (2ml, $2 \times 10^{-6}\text{ M}$) rapidly into an aqueous solution of MB (2ml, $1.5 \times 10^{-5}\text{ M}$). The resultant solution was then subjected to UV-vis spectral analysis at ambient temperature. The absorption intensities at $\lambda_{\text{max}} 664\text{ nm}$ were monitored to estimate the concentrations of methyl orange. At the same time, catalytic degradation of Rhodamine B and MO were also studied by measuring the absorption intensities at $\lambda_{\text{max}} 550\text{ nm}$ and 465 nm respectively.

Study of catalytic degradation of Methylene Blue (MB)

To explore the catalytic activity of PANI-Pd(OAc)₂ in the reduction of dyes, MB was chosen as a representative example because it is widely used as colorant for fabric and related material and cause of wide spread aquatic pollution. Reaction between MB and PANI-Pd(OAc)₂ is summarized in figure 1.

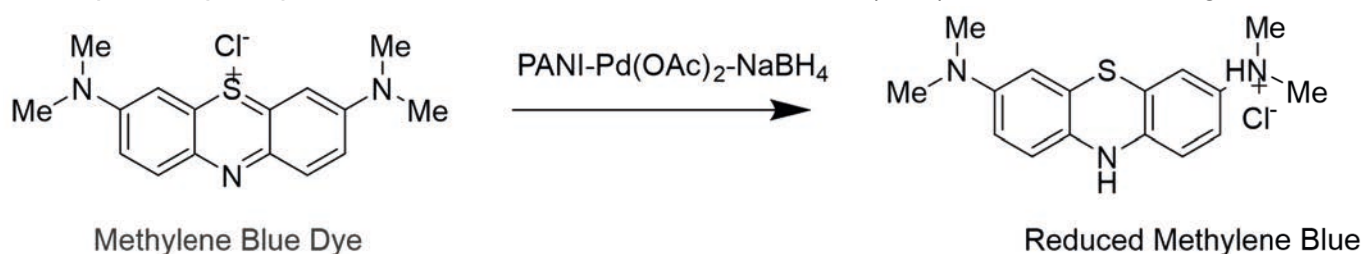


Figure 1: Reduction of MB in presence of PANI-Pd(OAc)₂ and SB

Optimization of the reaction condition was carried out by using various concentrations of SB while keeping MB concentration set at 2×10^{-6} M. It was found that at higher concentration of SB (1×10^{-4} M, 4×10^{-5} M, 2×10^{-5} M) and 5 mg of catalyst reaction was very fast and became difficult to follow properly. Finally, the SB concentration was optimized to be 1.5×10^{-5} M. Figure-2(a) depicts the decrease of absorption intensity of MB at 664 nm with time. The degradation process was observed to be complete within 8 min.

Control experiments were also carried out using SB or PANI-Pd(OAc)₂ or PANI alone failed to reduce the dye (Figure-2 (b), (c) & (d)).

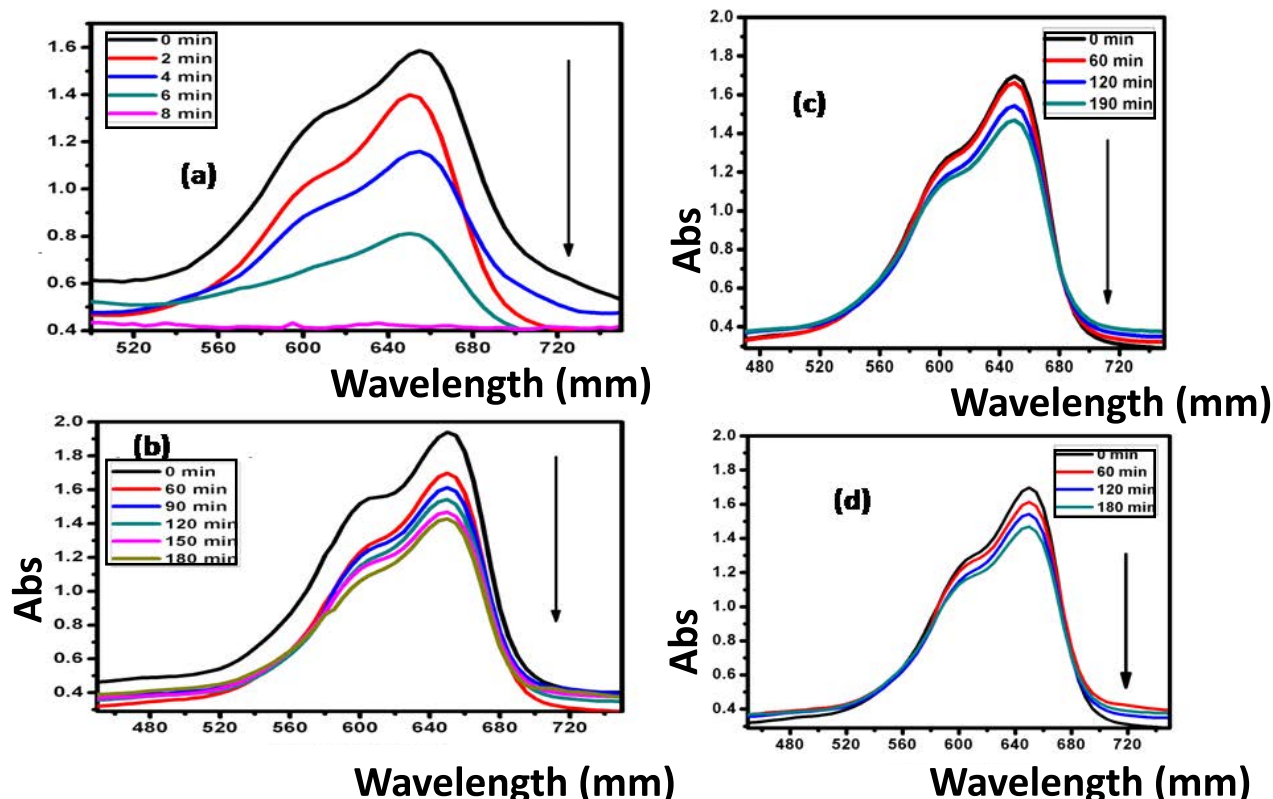


Figure 2: a) Degradation of MB in presence of PANI-Pd(OAc)₂ and SB; b) Degradation of MB with SB only; c) Degradation of MB with PANI-Pd(OAc)₂ only; d) Degradation of MB with SB and PANI-Base

The efficiency of the catalyst was also compared with homogeneous counterpart i.e. Pd(OAc)₂. It took five minute for the degradation of MB when treated with Pd(OAc)₂ (0.08 mg, corresponding to 5.0 mg PANI-Pd(OAc)₂). In the process precious palladium became non recoverable. Moreover, addition of another lot of MB and SB to the reaction mass lead to very slow degradation of MB and it was incomplete even after 3h. Agglomeration of Pd particles to form inactive palladium black could be the reason behind this phenomenon.

MB was also reacted with 5% Pd/C (50% wet, 5.0 mg) keeping all the reaction condition same. The absorption of MB measured at λ_{max} 664 nm ceased to zero after 30 min where as it took only 8.0 min for the PANI-Pd(OAc)₂. Superior catalytic efficiency of PANI-Pd(OAc)₂ over Pd(OAc)₂ or Pd/C highlights the positive effects of PANI by facilitating the e⁻ transport between borohydride, Pd and MB with the help of its elaborative π -electron network. Moreover, its extended -NH=N- functional moieties as well as quinoid-benzenoid structure might be helping to bring all the reacting species such as MB, NaBH₄ and Pd in close enough required for the reaction by H-bonding and or acid-base interaction. Detailed studies are needed to comment on the precise function of the PANI in the catalytic system.

Study of catalyst loading

The effect of the catalyst concentration was thoroughly checked by means of three experiments using

3.0 mg, 5.0 mg and 10.0 mg of catalysts maintaining fixed proportion of MB and SB (2×10^{-6} : 1.5×10^{-5}) at ambient temperature ($\sim 25^\circ\text{C}$). Degradation of MB was monitored spectroscopically by intensity drop in the absorption at λ_{max} 664 nm (Table-1). Higher loading of catalyst afforded faster degradation which could be attributed to the availability of more active palladium molecules.

Table 1: Consequence of different concentration PANI-Pd(OAc)₂ on reduction of MB in presence of SB

Quantity of PANI-Pd(OAc) ₂ (mg)	Time taken for complete degradation (sec)
3	1800
5	480
10	60

Study of pH

At different pH values the degradation of MB was studied using the MB at 2×10^{-6} M, and SB at 1.5×10^{-5} M at room temperature $\sim 25^\circ\text{C}$. pH of the reaction mixture was maintained with controlled addition of HCl (0.1 N) and NaOH (0.1 N) respectively. Without any interference pH of the reaction mass was recorded to be 8.7. Figure-3 clearly shows the variation of degradation rate with pH (Choi *et al.*, 2016).

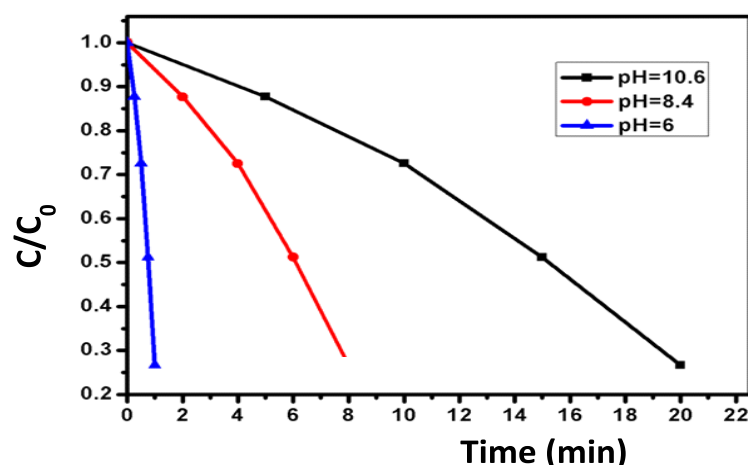


Figure 3: Degradation of MB with PANI-Pd(OAc)₂ and SB at various pH

Study of reductive degradation of methyl orange (MO)

The degradation of anionic dye using PANI-Pd(OAc)₂ catalysts was also investigated (figure 4) (Mondal, Adhikary & Mukherjee, 2015).

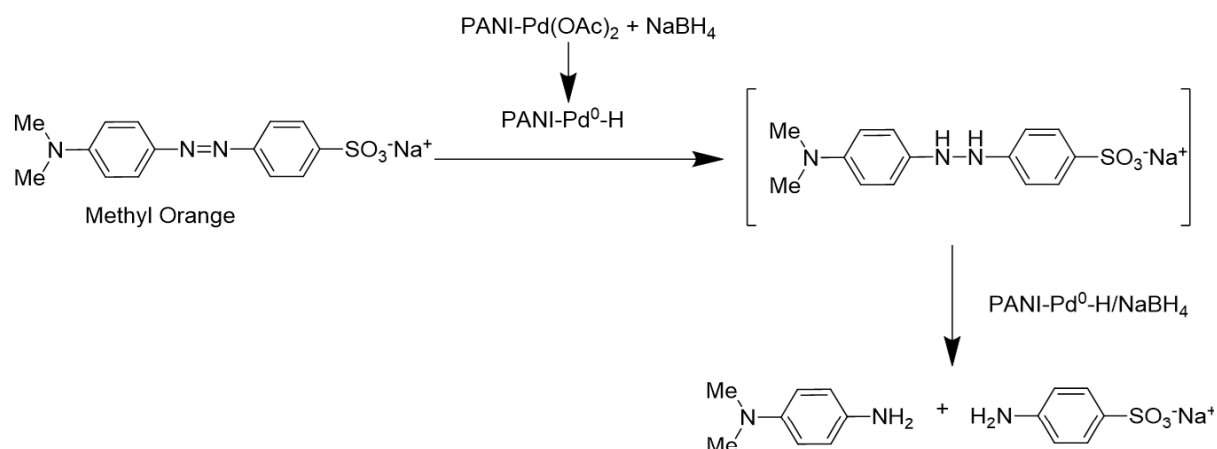


Figure 4: Reduction of MO using PANI-Pd(OAc)₂ and NaBH₄

MO was chosen for this purpose as it has wide application as indicator in acid-base chemistry as well

as colorant mainly for wool and silk. As the degradation of MO was very fast under identical condition for MB, concentration of SB was reduced significantly (2×10^{-6}) to capture the entire UV-picture of the degradation of MO. Decrease in the absorption of MO over time is depicted in Figure 5.

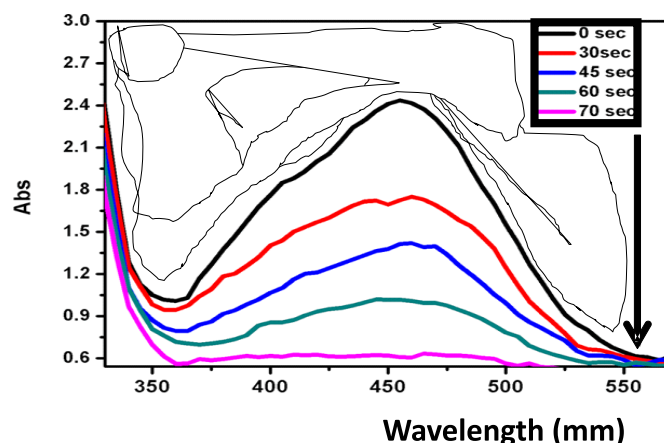


Figure 5: Degradation of MO in with PANI-Pd(OAc)₂ and SB

Study of catalyst recovery and recyclability

PANI-Pd(OAc)₂ catalyst was recovered by simple centrifugation technique after completion of reaction. It was then thoroughly washed with water followed by acetone and dried in air. Five consecutive cycles were performed using used catalyst obtained after each cycle and the result is shown in Table-2.

Table 2: Reusability of PANI-Pd(OAc)₂ in reductive degradation of MB

No of use	Time taken for complete degradation (min)	Palladium concentration (mmol g ⁻¹)
1 st	8	0.35
2 nd	15	0.33
3 rd	17	n.d.*
4 th	17	0.32
5 th	28	n.d.*

* n.d.: Not determined.

Catalytic activity after the first cycle was decreased and then it remained constant for the next three cycles. Finally, after the fourth cycle, the activity was found to be considerably reduced. Leaching of active Palladium was found to be nominal (From ICP-AES analysis, it is evident there is a decrease in palladium content by 5% for the used catalyst after 1st compared to the fresh catalyst) and the filtrate obtained after first use did not exhibit any catalytic activity towards reduction of MB after addition of SB. TEM analysis (Figure 6) of the recovered catalysts showed the formation of Pd-nanoparticles and repeated use led to agglomeration of the same (20-25 nm vs. >40 nm after the 4th cycle). Reduction in the catalytic activity probably due to different nature of palladium in fresh (Pd(II)) and recycled catalyst (Pd(0)). Moreover, agglomeration of Pd-nanoparticles along with deposition of B related materials and or degraded dye compounds on the catalytically active Pd made the catalyst less effective after the 4th recycle.

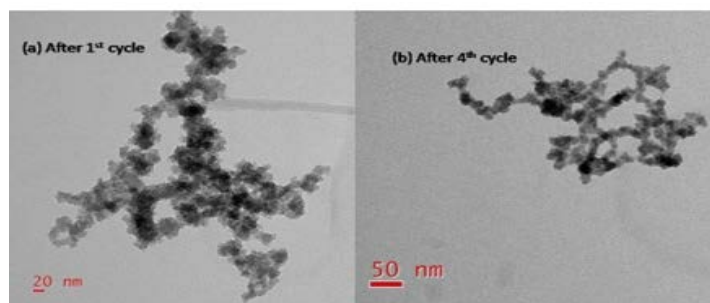


Figure 6: TEM photograph of PANI-Pd after first and fourth use

CONCLUSION

The PANI-Pd(OAc)₂ catalyst was discovered to be extremely active in the reductive degradation of both cationic (MB) and anionic dye (MO). In addition, the reaction was completed within a very small span of time, e.g., less than a few minutes. Easy recovery and recyclability offer further opportunities for larger applications.

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Applications of Noble Nanomaterials in Ensuring Food Safety: A Review of Recent Developments

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ABSTRACT

Compounds derived from natural sources, as well as industrial and agricultural waste, are examples of food contaminants. Food contaminants found naturally are mostly of microbiological origin, including pathogenic organisms and some other fungal and bacterial toxins. Food is among the most significant priorities for public security because it is one of the most basic components of living organisms. As a result, precautions are needed to make sure that foods are exempt from contaminants that enter the food supply via food handling, manufacturing, and dispersion. At the moment, people are increasingly concerned with the accuracy, convenience, and efficiency of food quality and safety testing. On the other hand, many traditional detection methods have drawbacks such as inconvenient operation, interference factors, and long detection times. Nanomaterials can also be used to monitor the lifespan of preserved foods. These can be developed to fluoresce when exposed to food pathogens, chemicals, or contaminants, acting as a sensor for detecting small traces of contaminants. As a result, functionalized nanomaterials with higher selectivity and sensitivity, such as pesticides, veterinary drugs, heavy metals, additives, and synthetic pigments, pathogenic bacteria, and mycotoxins, are widely used in food detection. This paper looks into how noble nanomaterials that can be placed precisely can be used to check the quality and safety of food.

Keywords: Food Contaminant; Pathogens; Noble Nanomaterial; Food Safety

INTRODUCTION

Numerous nanoparticles exist, including metallic NPs, polymeric NPs, and magnetic NPs. NPs can also have multiple functionalities, including hydrophobicity or hydrophilicity, and that has a significant impact on their implementations. Noble metal NPs (NMNPS) have a high multifunctionality due to their physical–chemical properties (Fratoddi *et al.*, 2018). Nanoparticles of noble metals, such as AgNPs, AuNPs, and PtNPs, have a high level of stability, are simple to synthesise, and can have their surface functionalization tuned (Neuschmelting *et al.*, 2018). NPs have two significant applications in food control: assessing toxic compounds (like mycotoxins, pesticides, and so on) and biologically active compounds (nutrients, antioxidant substances, proteins, etc.).

Recently, the application of noble metal nanoparticles as a substitute for traditional methods of assessment was proposed. NPs might be able to improve analytical precision, high accuracy, detection limits, and sample size, which would make a wider range of food applications possible.

Contaminants are biological or chemical components that are not deliberately added to food and could be noticeable as a result of different phases of production, manufacturing, or shipping. They are also capable of causing environmental pollution. Synthetic pollutants and xenobiotics that enter the environment, water and land can contaminate food. This, however, at low concentrations, has negative consequences for living creatures (Chen *et al.*, 2018). Food safety legislation has emerged as a top priority to impose strict control on the production of food, manufacturing, stockpiling, and xenobiotic tolerance to avoid the onset of toxic chemicals.

Traditional methods, for example, spectrophotometric or chromatographic methods, are typically used

to determine such species as food sources. As food manufacture and supply become more globalised, food pollution poses a number of threats from both natural and human activities (Akrami-Mohajeri *et al.*, 2018). This includes biological and chemical pollutants, drugs, external hazards, and microbial organisms. Foodborne organisms have emerged as a leading issue within the food industry. In spite of the progress in advanced analytics and the implementation of food laws, the prevalence of foodborne illnesses has not decreased.

Food nano packaging, nano sensing, nanostructured food ingredients, and nutrient delivery are just a few of the applications for nanomaterials in food manufacturing, as well as in nutritional science. Uncertainties and health issues, however, are emerging as a result of their probable cytotoxicity and hazards to the environment and health. Even successful nanotechnology applications in food are still rare. To overcome this barrier, novel nanomaterials with high effectiveness and security must be developed.

There has already been significant progress in nanotechnology in current years, including the creation of specifically designed nanostructures for analytical techniques. For food safety control, the viability of employing a wide range of inorganic nanomaterials like silver, gold, and Pt was investigated (Lukman *et al.*, 2018). A few of the most significant advancements with this field in recent years are described and analyzed in this review. The goal is to concentrate on effective applications of noble nanomaterials using modern techniques with a specific focus on detection of food additives and contaminants. Specifically, Au, Ag NPs delivering excellent results in order to detect food contaminants and pollutant is outlined here.

RESULTS AND DISCUSSION

Determination of Contaminants

Even after food handlers' endeavours, pathogen adaptation technologies enable foodborne pathogens to survive and thrive. Microbial contaminants seem to be the most commonly notified foodborne causative factors (Sugrue *et al.*, 2019). Innumerable foodborne epidemics have emphasized the threats of foodborne diseases, prompting the design and technology for execution of schemes (e.g., HACCP systems) to sharply and delicately identify biotoxins and food pathogens.

There are two types of food-borne biotoxins: intrinsic and extrinsic foodborne biotoxins. Bacterial endotoxins can be produced through epithelial autolysis, peripheral lysis, or cytolytic digestion. But bacterial exotoxins like mycotoxins, enterotoxins, and hemolysins are ejected from the interstitial spaces directly.

Mycotoxin Toxins generated by mould as well as other microscopic organisms that induce toxicity, both acute and chronic, are known as mycotoxins (trichothecenes, aflatoxins, fumonisins, and so on). Depending on the mode of activity, mycotoxins are categorized into four types: poisons that are cytotoxic, neurotoxins, and gastrointestinal allergens, and toxins that produce symptoms whenever ethyl alcohol is consumed (Ünüşan, 2019). Secondary metabolites of the genera *Penicillium*, *Aspergillus*, and *Fusarium* accumulate as mycotoxins, which are commonly observed in food.

Alternariol monomethyl ether (AME), a carcinogenic and mutagenic substance, is available in a diverse selection of fruits and vegetables, as well as cereals. In a recent article (Man *et al.*, 2018), a method for determining AME was developed. The colorimetric approach for immunosensor relies on AuNP aggregation and a monoclonal antibody was used to modify the properties that unify AME molecules in specimens competitively.

The use of gold nanorods with platinum coating (AuNR@Pt) for the quick and precise identification of staphylococcal enterotoxin B was observed. It was supported by immobilization of a toxin aptamer via a complementary DNA (cDNA) fragment. Ochratoxin A was detected using a hypersensitive surface-enhanced Raman scattering (SERS) aptasensor based on Au(core)@Au-Ag(shell) nanogapped nanostructures (Shao *et al.*, 2018). Different types of mycotoxin detection are summarised in the Table 1.

Table 1: Detection of Mycotoxin in Food stuffs.

Nanomaterial	Food	Type of detection	Procedure	References
AuNCs	Maize	AflatoxinB(1)	Fluorescence resonance energy transfer	(Khan <i>et al.</i> , 2019)
AuNRs	Maize	Aflatoxins and Zearalenones	Immunochromatographic assay	(Chen <i>et al.</i> , 2020a)
PtNPs		Zearalenone	Chronoamperometry	(Ji <i>et al.</i> , 2019)
AuNps		T-2 toxin	Chronoamperometry	(Zhong <i>et al.</i> , 2019)
AgNps	Redwine	Ochratoxin A	Electrochemical determination	(Zhang, Yang & Chen, 2019)
AuNps	Grapejuice	Ochratoxin A	Multicolor colorimetric detection	(Tian <i>et al.</i> , 2020)
AuNps	Maize	Deoxynivalenol	SERS	(Li <i>et al.</i> , 2019)
AgNPs	Red yeast rice	Citrinin	SPCC immunoassay	(Jiang <i>et al.</i> , 2020)

Carcinogenic Components

Carcinogenic compounds are indeed substances that have the potential to cause cancer in humans. A colorimetric method based on the aggregation of gold nanoparticles (AuNPs) by glutathione (GSH) was also created for detecting azodicarbonamide (ADA) in flour commodities. Melamine and nitrites are commonly found in food as a result of food preservation methods. Rajput (2018) recently established a melamine detection assay using AgNPs that is based on melamine's interaction with Ag⁺ ions. A gold nanoparticle/poly (methylene blue) (GNP/PMB)-modified pencil graphite electrode (PGE) was used to detect nitrites. This methodology was employed on samples of mineral water and commercial sausage.

Table 2: Detection of carcinogenic compound in Food stuff

Nanomaterial	Food	Type of detection	Procedure	References
AgNps	Flour	Azodicarbonamide	UV-vis spectrometry	(Chen <i>et al.</i> , 2021)
Ag NPs	Apricot	Cyanide detection	Voltammetric and Amperometric techniques	(Zhang <i>et al.</i> , 2020)
AuNps	Milk	Melamine	SERS	(Sun <i>et al.</i> , 2021)
AuNps	Apple juice	Thiram	SERS	(Sun <i>et al.</i> , 2021)
AuNps	Milk	Melamine	Optical absorbance	(Siddiquee <i>et al.</i> , 2021)

Pesticide

Pesticide residues on vegetables and fruits are among the most serious consumer concerns about food safety. For detection of atrazine present in apple juice. Surface enhanced Raman spectroscopy (SERS) in conjunction with AuNPs and for difenoconazole present in grape nanoparticle aggregates of core-shell Au@Ag (Ma *et al.*, 2018) has been used to detect two pesticides. In this research, a potential use of AgNPs/GO (Graphene Oxide) for the detection of pesticides in food was explored in this research, with promising results by Ma *et al.*, 2018.

Table 3: Detection of Pesticide in Food stuff

Nanomaterial	Food	Type of detection	Procedure	References
AgNps	Grapes Tomato	Chlorpyrifos	Surface-enhanced Raman scattering	(Subramaniam & Kesavan, 2022)
AuNps	Fruits and vegetable	Chlorpyrifos	Electrochemical Immuno - Sensing	(Talan <i>et al.</i> , 2018)
AuNps	Apple skin	Thiram, Malathion, Acetamiprid, Phosmet	Surface enhanced Raman spectroscopy	(Kabashin, Dubowski, & Geohegan, 2019)

Allergens and Drugs

Veterinary drugs employed in livestock for food production may end up with some residues in commonly consumed animal products like milk, meat, honey and eggs. As a result, numerous AuNPs applications for detection in food samples have been discovered (Rath *et al.*, 2019). Unauthorized veterinary drug use is now a major issue. To regulate the illegal use of unfamiliar drugs and drug residue mixtures in farm animals, new detection methods like metabolomics have now been established. This method works by monitoring metabolite changes in body tissues. This intriguing paper discusses the prospect of enhancing the designed immunoassay's signal. The reaction boosted assay responsivity and resulted in a visible colour shift from bright red to deep purple which can be seen also with bare eyes. This immunoassay has the potential to be used for simple detection on-site detection in ensuring food safety.

Some techniques also have been devised for antibiotic detection that are being exploited in the animal husbandry and may be discovered as remnants in food derived from animals. These AuNP-based techniques were designed to detect aminoglycoside antibiotics (Yan, Lai, Du & Xiang, 2018) and ceftriaxone in foods derived from animals for example eggs, milk, and meat. A voltammetry biosensor comprised of a carbon electrode with AuNP-coating was developed. It was used in conjunction with a sandwich immunoassay to recognize Peanut allergens in food products.

Table 4: Detection of Allergens and Veterinary Drug residue

Nanomaterial	Food	Type of detection	Procedure	References
AuNp	Kidney beans,	Lectins	Voltammetric immunosensor	(Sun <i>et al.</i> , 2019)
AuNP	Milk Shrimp	β -lactoglobulin Tropomyosin	Microfluidic paper-assisted analytical device (PAD)	(Tah <i>et al.</i> , 2018)
AuNp	Shellfish	Tropomyosin	Surface plasmon resonance (SPR)	(Zhou <i>et al.</i> , 2020)
AuNp	Fish	Parvalbumin	Enzyme-linked immunosorbent assay	(Wang <i>et al.</i> , 2020)
AuNp	Soybean and Sesame	Gly m Bd 28K 2S albumin	Hybridization chain reaction	(Yuan <i>et al.</i> , 2019)
AuNPs	Fish	Sulfadimethoxine	Fluorescence	(Chen <i>et al.</i> , 2020b)

Bacteria

Certain bacteria must be absent from food for safe consumption since some strains of bacteria are harmful to human health. They are capable of causing diarrhoea, fever, typhoid, hemorrhagic colitis and haemolytic uraemic syndrome. Method based on AuNP used lateral flow immunoassay to detect bacteria like Salmonella and E. coli in milk and water (Lukman *et al.*, 2018).

Table 5: Detection of Pesticide in Food stuff

Nanomaterial	Food	Type of detection	Procedure	References
AgNps	-----	<i>Salmonella</i>	SERS	(Wei, Li & Zhao, 2018)
AgNCs	Milk	<i>Staphylococcal enterotoxin A</i>	Fluorescence aptasensor detection	(Zhang, Sun & Cao, 2020)
AuNPs	Milk	<i>Salmonella Enterica</i>	Colorimetric ELISA	(Gao <i>et al.</i> , 2019)
AuNPs	Orange juice	<i>Staphylococcus Aureus</i>	SERS	(Wang <i>et al.</i> , 2021)

Bioactive Compound

Gluten A protein complex Gluten is found in several cereals that are made up of glutenin and gliadin, two proteins. It is indeed the main protein responsible for allergic reactions, and the majority of applications of nanoparticles are dependent on it. Numerous immunosensors using modified AuNPs have recently been designed in order to measure gliadin in samples of foodstuffs (Manfredi *et al.*, 2016). These are also entirely focused on the recognition of DNA.

Amino Acid In amperometric immunosensor monosodium glutamate (MSG) detection, the anti-glutamate antibody was encapsulated on the surface of the sensor, which was made with a carbon electrode decorated with gold nanoparticles and a nanocomposite of molybdenum disulfide/chitosan (Au@MoS₂/Ch). Li *et al.* used sensitive nanoprobe made of gold nanoparticles on graphene oxide to detect L-cysteine easily by the optical absorption method. A smartphone-based system was used in this method that performs analysis of multiple modes of hue-saturation-value and lightness, as well as red-green-blue (RGB), and cyan-magenta-yellow-black (CMYK) values (Li *et al.*, 2018).

Antioxidants Metabolic byproducts of several plants Antioxidants, widely present, particularly in vegetables, are also among the most essential natural compound groups. These have anticarcinogenic, antimicrobial, and antioxidant properties, which have been illustrated in vivo and in vitro experimental studies. Furthermore, their potential anti-cardiovascular and neurodegenerative consequences have recently been explored. Della Pelle *et al.* provided a colorimetric assay for phenolic compound identification. The said technique relies on the formation of gold nanoparticles by phenolic content found in endogenous fat. The intensity of phenolic compounds was associated with the formation of AuNP, which was governed by surface plasmon resonance (Della Pelle *et al.*, 2015). Functionalized AuNPs were also used to retrieve phenolic compounds derived from olive oil. This quick and sustainable method was improved by employing a response surface analysis and building a central composite design (CCD) of some parameters, among which was the amount of AuNPs or the time spent stirring NPs in oil. The agglomeration or morphological characteristics of AuNPs and AgNPs were also responsible for the development of antioxidant activity in beverages, including tea and lemon juice.

Table 6: Detection of Bioactive Compounds

Nanomaterial	Food	Type of detection	Procedure	References
AuNPs AgNPs	Lemon juice Tea	Flavonoids and polyalcohols.	Colorimetric sensor array	(Bordbar <i>et al.</i> , 2018)
Au@Ag nanobox	Green tea	Polyphenols	Localized surface plasmon resonance	(Wang <i>et al.</i> , 2018)
Ag NPs	Corn flour	Gliadin	Enzyme-linked immunosorbent assay	(Mercadal <i>et al.</i> , 2018)

CONCLUSION

Deeper insights exploration on material stability, physicochemical properties is indeed required in the coming years. Bulk manufacturing activities and growing quite automated techniques regarding

application also to be realized. Greener and more ecofriendly materials should be designed and developed. Pollution from heavy metal ions in the eco system has become much pervasive as industry develops. Since these contaminants enter food sources, aquatic animals and plants, started to accrue in the food web, they have a negative impact on human health. As a result, detection of heavy metal is an essential aspect of food quality and safety sensing. Several agricultural productions are conveniently affected by various fungal pathogens resulting in food material adulterated with mycotoxins. The significance of mycotoxin is that it causes drastic physical problems also at trace levels. Analytical methods that are more responsive, sophisticated, productive, and cost-effective need to be developed in the future to ensure food safety, reliability, and greater transparency without jeopardising dietary, functional, or sensory properties in accordance with relevant legislation and customer expectations.

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Micelles and Reverse Micelles: a Fascinating Micro-reactor

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ABSTRACT

Reactions in micro-heterogeneous systems have been found to be of great interest in the past few decades. Two such micro-heterogeneous aggregates are micelles and reverse micelles, which are formed by surface active agents or surfactants. In numerous chemical reactions, the use of these systems as microreactors increases the rate of the reaction up to 10-100 folds. This chapter will be concentrated on micelles and reverse micelles as catalytic reactors since, in the past few decades, numerous numbers of reactions have been successfully carried out in these systems.

Keywords: *Micelles; Reverse Micelles; Catalysis; Micro-Reactor*

INTRODUCTION

Nature usually prefers self-assembled structures for doing millions of reactions in an efficient manner. Numerous reactions occur on the surface of the cell membrane, DNA, RNA, in the active site of enzymes, etc. All are examples of self-organisation of small units into large supramolecular structures. The process can be mimicked in synthetic supramolecular agglomerates to do the desired reaction in an inefficient manner. Molecular self-assembly is a process in which small molecules spontaneously form larger superstructures by interacting with each other through various attractive forces, which are generally weak non-covalent interactions like van-der Waal force, Coulombic force, hydrophobic interactions, and H-bonding. Cyclodextrins, crown ethers, cryptands, as well as surfactant aggregates like micelles and reverse micelles can be considered as efficient reaction sites or reactors. Among these ordered structures, micelles and reverse micelles are formed by the interactions of small surfactant molecules.

Surfactants are the basic building blocks of the micellar and reverse micellar superstructures. Surfactant is a short form of "surface active agent" that actually means "active at the surface." Surfactant molecules contain two distinct parts: a water-insoluble hydrophobic part and a water-soluble hydrophilic part, which may or may not possess charges (Fendler, 1982). Surfactants are mainly of four types. i) cationic surfactant; the cationic portion of the surfactant remains in the hydrophobic unit ii) anionic surfactant; the anionic portion of the surfactant remains in the hydrophobic unit ii) Non-ionic surfactant; there is no formal charge in the molecule but the molecule contains an uncharged hydrophilic part and a hydrophobic part. iv) amphoteric surfactants; both cation and anion exist in the same part of the molecule (Figure 1).

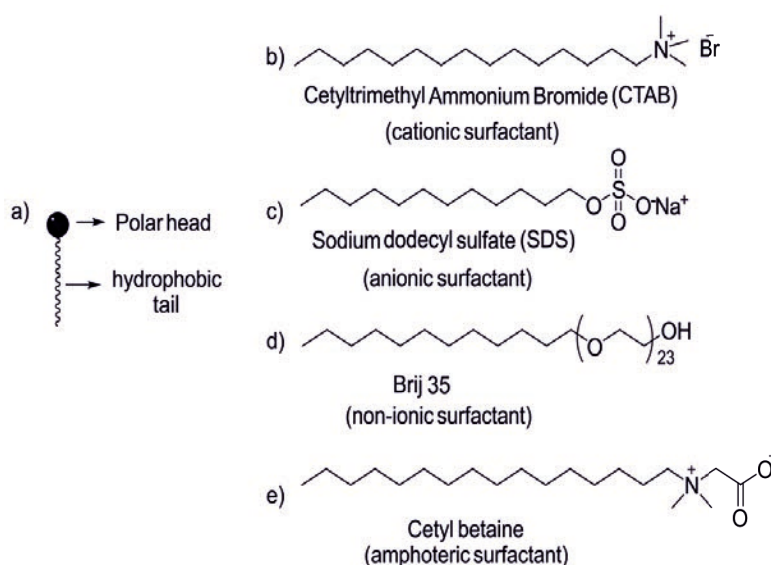


Figure 1: a) Representative structure of a surfactant b) Cationic surfactant c) Anionic surfactant d) Non-ionic surfactant e) Amphoteric surfactant

The simplest forms of supramolecular structures are micelles. When surfactant molecules are dissolved in water, they orient themselves in such a way that the hydrophilic part of the molecule remains in contact with water and the hydrophobic part stays away from water to avoid unfavourable interactions. In this way, a sphere is formed where the hydrophobic parts of many molecules form the hydrophobic core and the hydrophilic parts of those molecules remain in the outer layer of the sphere so that this layer can remain in contact with water (Figure 2). These aggregates are called micelles (Fendler, 1982). For every surfactant, at a particular temperature, there is a minimum concentration above which the micellization process starts. This minimum concentration of the surfactant is called 'critical micellar concentration'(CMC) of that surfactant at that particular temperature. Below CMC, the individual molecules are dissolved like a normal electrolyte. Above CMC, the concentration of surfactant molecules becomes sufficient to form the spherical structure, i.e., micelles. The special feature of micellar solution is that it can solubilise both hydrophilic and hydrophobic materials since micelles have both hydrophobic and hydrophilic domains for solubilisation; a hydrophobic core and a hydrophilic outer layer.

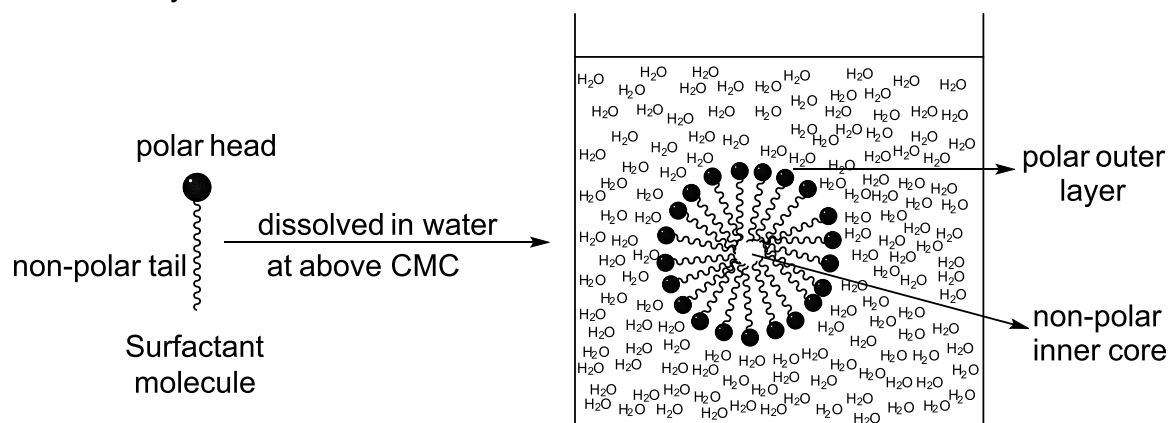


Figure 2: Cross section of micelle structure

LITERATURE REVIEW

In the past few decades, these micellar aggregates have been used efficiently for several chemical transformations in water, and in most of these cases, the rate of the reactions is found to be higher than the reaction with water alone (Paprocki *et al.*, 2018). It has also been found that some reagents cannot do a particular reaction in normal conditions, whereas the reaction can go efficiently in the presence of micellar solution (Bhat & Shairgojray, 2020). These reactions will be discussed later in detail.

Another class of fascinating supramolecular aggregate is reverse micelles. It has been seen that when surfactant molecules are dissolved in bulk water, they form a supramolecular sphere like aggregate where the hydrophobic parts of many molecules form the inner hydrophobic core and the hydrophilic parts form an outer hydrophilic layer which remains in contact with water (Figure 3). But when the

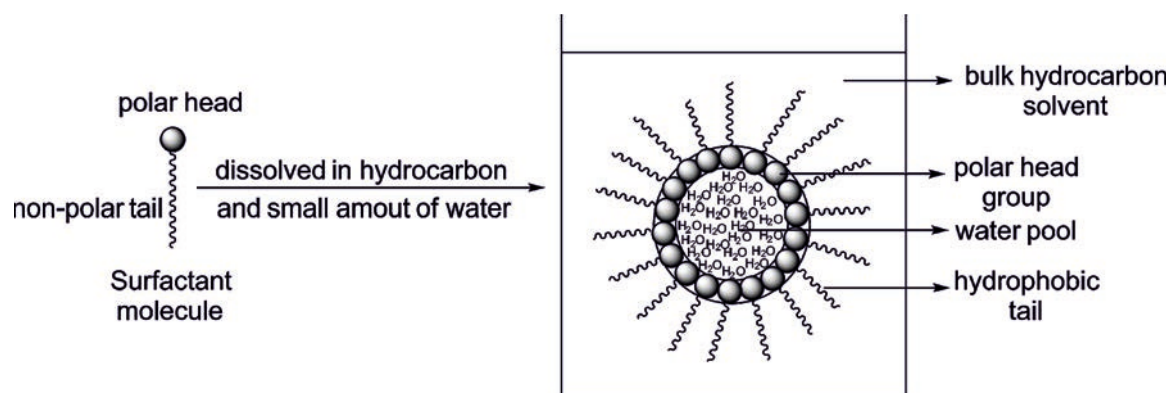


Figure 3: Cross section of reverse micelle structure

medium is changed from bulk water to bulk non-polar solvent in the presence of a small amount of water, the polar heads aggregate themselves into nanometre-scale tiny water droplets where the hydrophobic parts of the molecules are oriented towards the bulk non-polar medium and the hydrophilic parts towards the water droplets, which are known as water pools (Arsene *et al.*, 2021). Thus, the surfactant molecules form the interface between the polar aqueous part and the non-polar oily part. These spherical aggregates are called reverse micelles or water-in-oil microemulsions (Schulman & Prince, 1959).

The important characteristic of reverse micellar solutions is that a large number of bio-molecules, including proteins, enzymes, and nucleic acids, can easily be solubilised without losing their bioactivity (Menger & Yamada, 1979). Actually, these bio-molecules are solubilised in the water pool of the reverse micellar solution and hence they can preserve their biological activity (Mitsou, Xenakis & Zoumpantioti, 2017).

DISCUSSION

In micellar medium, many reactions have been performed in recent years. The demand for the micellar system as a reaction medium has increased in recent times since it also meets the criteria of 'green chemistry' as the solvent used in this system is water, which is a green solvent. In the naked eye, the micellar system seems to be homogeneous, but in reality, it is a microheterogeneous system and the absorbed reactants remain in a micro heterogeneous two-phase system, i.e., oil (hydrophobic inner core) and water. In most of the cases, the rate of the reaction was found to be increased. That's why the reaction in the micellar system is called 'micellar catalysis' when the rate of the reaction increases with respect to water.

Micelle is unique as a micro- or nanoreactor because the reactants are so close together in the polar and nonpolar regions. The effect of micellar structure on reaction rate is due to the following factors (Castanho, Brown & Prieto, 1992).

- 1) The micellar core has a lower dielectric constant than bulk water. Therefore, the different positions of the substrates can create a solvent effect on the reaction rate.
- 2) The polar head group of the surfactant molecule can stabilise the transition state of the reaction, which actually accelerates the rate of the reaction.
- 3) The hydrophilic reactants are concentrated more to the bulk water phase through interactions with the highly polar micelle surface. These causes the close proximity of the reactants, which effectively increases the bimolecular collisions between the reactants, leading to an increased rate of bimolecular reactions.

Reactions in micellar solution:

a) Hydrolysis of ester:

It has been found that the micelles formed by cationic surfactants catalyse the reaction of neutral substrate with anionic reagents like hydroxide (OH^-), fluoride (F^-), thiolate (R-S^-), etc. Hydrolysis rates of p-nitrophenyl esters, for example, are found to be 2.3–16.5 times faster in the presence of a small amount of quaternary ammonium ion exchange latex particles than in water alone (Miller *et al.*, 2000). It has been found that the acetates are absorbed strongly by the micellar polymer particles and the concentration of OH^- inside the polymer particles is 10 times higher than the bulk water. These two factors are responsible for the higher reaction rate in the presence of cationic polymeric

micellar particles (Figure 4).

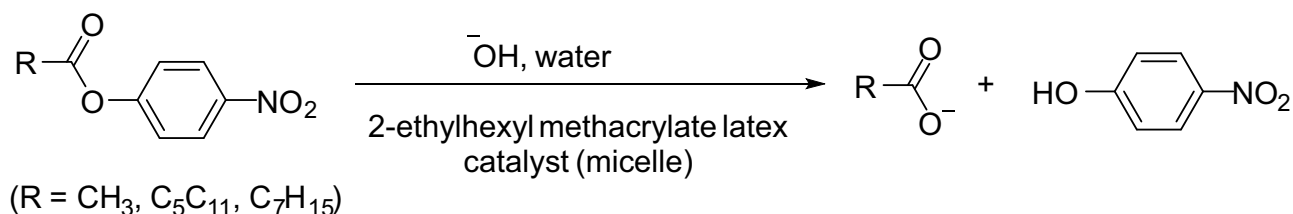


Figure 4: Reaction in polymeric cationic micellar solution

Another ester hydrolysis reaction has been carried out in non-ionic micelles with phosphoryl nitrophenyl esters (Figure 5).

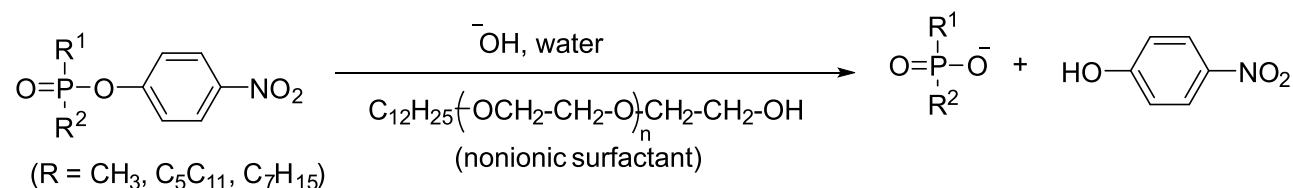


Figure 5: Reaction in nonionic micellar solution

In this case, the rate of the reaction is accelerated due to the formation of alkoxide at the terminal -OH group of the surfactant, which is responsible for easy hydrolysis (Foroudian, Gillitt, & Bunton, 2002).

b) Oxidation:

The rate of chromic acid oxidation of malic acid to oxaloacetic acid was found to be twice as high in CTAB micellar medium than in water alone (Figure 6). Here, the bromide (Br⁻) counterion of cationic micelles is exchanged with an HCrO₄⁻ ion, which increases the local concentration of oxidants and hence increases the rate of the reaction. This acceleration of the rate of the reaction was found to be absent when the CTAB is replaced by a nonionic surfactant (Morshed & Khan, 2004). This proves that the reaction occurs by ion exchange of Br⁻ with HCrO₄⁻ at the hydrophilic outer layer of the micelles.

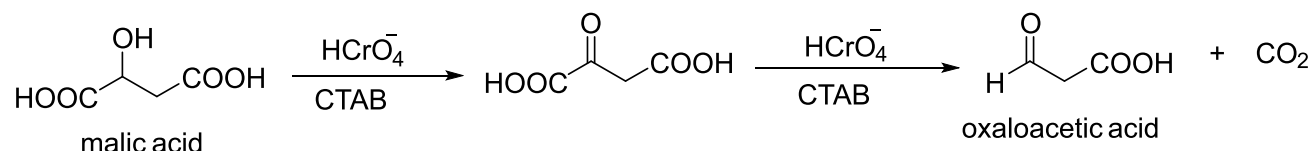


Figure 6: Oxidation of malic acid in a CTAB micellar medium

c) Reduction of esters: NaBH₄ can not reduce esters in ambient reaction condition. This is because the electrophilicity of carbonyl carbon of the -COO₂ Et group is less than that of aldehydes and ketones due to the resonance of C=O with the -OR group in esters. Also, the reactivity of NaBH₄ is less than the other metal hydride reducing agents like LiAlH₄. NaBH₄ can reduce the ester groups only at very high temperatures or at very high concentrations of NaBH₄. A very efficient method of reduction of ester with NaBH₄ at room temperature has been developed using micellar catalytic reaction medium (Das, Roy & Das, 2004). It has been found that when the reaction is done in CTAB micellar medium, a large amount of the reduction product is made. The enhanced yield in the micellar medium is due to an increase in the local concentration of NaBH₄ at the micellar surface where the reaction actually takes place. Thus, micellar solution acts as a catalyst in the reaction (Figure 7).

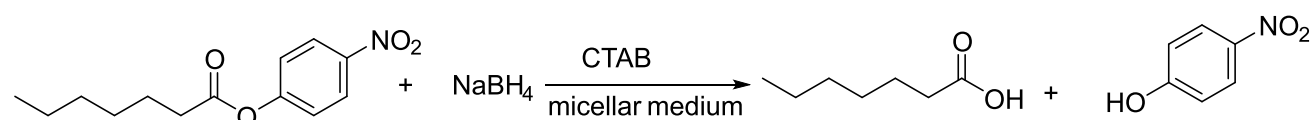


Figure 7: Reduction of ester in CTAB micellar medium

d) Diels-Alder Reactions in Micellar Media: A huge number of Diels-Alder reactions in micellar medium has been done in past few years. Recently, Diels-Alder reaction in micellar medium has been used for the synthesis of pharmacologically relevant 4-amidyl-2-methyl-1,2,3,4-tetrahydroquinolines (THQs) (Arenas, Bonilla & Kouznetsov, 2013). It has been found that the best yield is obtained by the presence of CTAB or sodium dodecyl sulphate (SDS) surfactant solution above its critical micellar concentration (CMC). Here, micelles catalyse the reaction. The explanation for the micellar catalysis is that the micelle has the ability to arrange of stable catalytically active groups which together function as a nanoreactor (Figure 8).

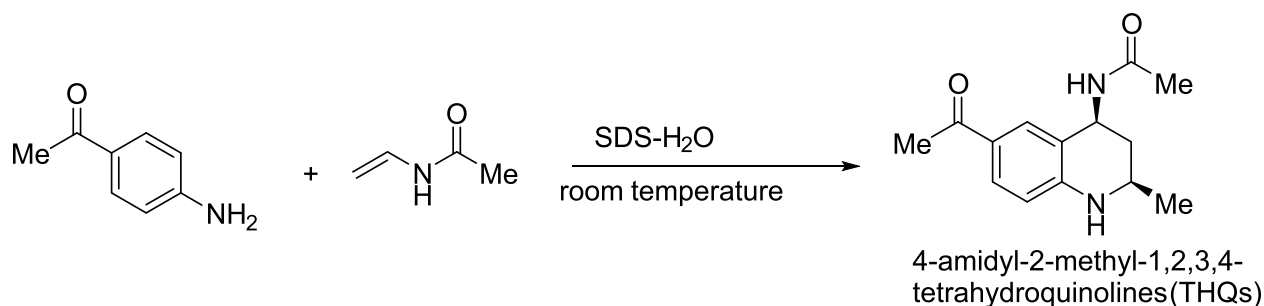


Figure 8: Diels-Alder reaction in a SDS micellar medium

Reactions in reverse micellar solution:

Reverse micelles are also a very good microreactor for chemical transformation, especially enzyme-catalysed reactions. As already mentioned above, biomolecules like enzymes, DNA, RNA, etc. can preserve their activity when they are dissolved in the water-pool of the reverse micellar system. The examples of some reactions which have been done in reverse micelles are discussed below.

a) Reduction: Ketones have been reduced by NaBH_4 in reverse micellar solution (Zhang & Sun, 1996). Here the chiral surfactant has been taken for the formation of reverse micelles and, surprisingly, it is found that the product shows some degree of enantiomeric excess (Figure 9).

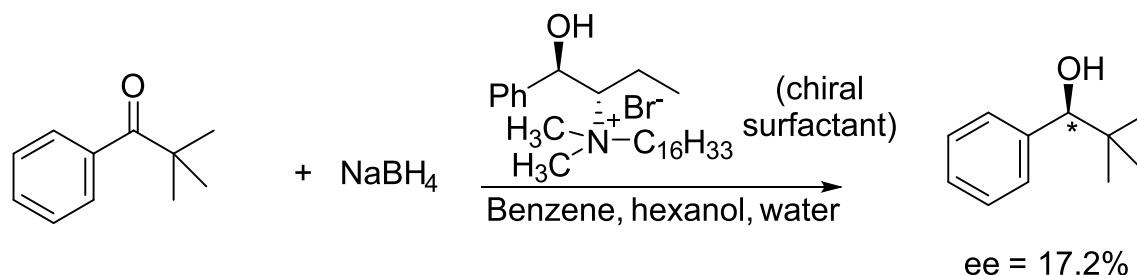


Figure 9: Stereoselective reduction of prochiral ketone in a chiral reverse micellar medium

b) Oxidation: oxidation of toluidine blue by periodate was done in water and in CTAB based reverse micelles. It has been found that the reaction in reverse micelles is 40 times faster than that in water under the identical reaction conditions (Figure 10). In both the solvent systems, the reaction obeys the first order kinetics with respect to both the reactants. The pronounced acceleration of the reaction rate in the reverse micellar system is due to the lower micropolarity of water as well as the higher effective concentration of the substrate and the reactant in the bound water of the water-pool (Nagalakshmia, Shyamala & Rao, 2018).

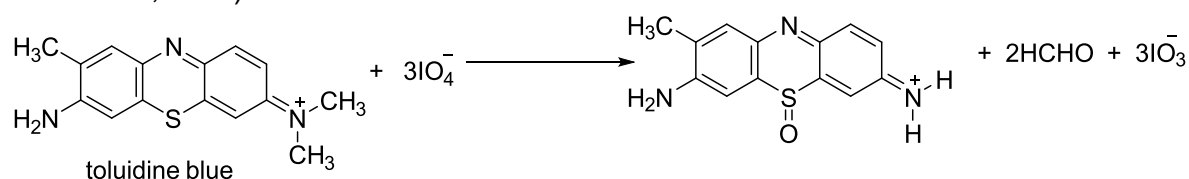


Figure 10: Oxidation reaction in a CTAB based reverse micellar medium

Another reaction of enantioselective sulfoxidation in reverse micelles has been done in the water/CTAB/diacetyltartaric acid/toluene system (Figure 11). Here, prochiral dialkyl sulphides are oxidised with iodoxybenzene (PhIO₂) in the mentioned reverse micelles to get a quantitative yield of sulfoxide with an enantiomeric excess (ee) of up to 72%. Here, the chirality of tartaric acid creates the chiral environment in the water-pool, which is actually responsible for enantioselectivity (Tohma *et al.*, 1999).

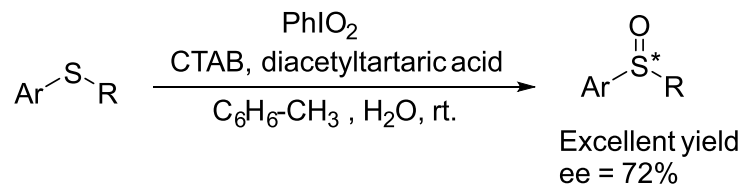


Figure 11: Enantioselective sulfoxidation of prochiral prochiraldialkyl sulphides in a chiral reverse micellar medium

c) Esterification: Lot of esterification reactions have been done by enzyme lipase in reverse micelles (Hayes & Gulari, 1990). In an example, the fatty alcohol and fatty acid are esterified in water/AOT/isooctane reverse micelles (Figure 12). It has been found that the enzyme activity depends on the type and concentration of substrate, H₂O/AOT ratio (w).

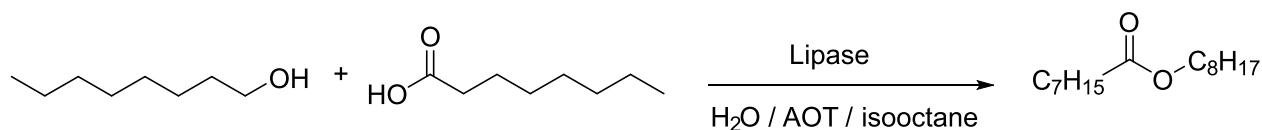


Figure 12: Lipase catalysed esterification of fatty acid and alcohol in reverse micellar medium

CONCLUSION

It has been found that the micellar and reverse micellar solutions serve as fascinating nano/microreactors for a huge number of reactions. In most of the cases, the rate of the reaction was found to be higher than that in water alone. In some cases, stereoselective reactions have also been carried out in chiral micelles or chiral reverse micelles. Faster reaction rates, ambient reaction conditions, and stereoselectivity make these systems highly useful for many organic transformations.

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Recent Advances in Oxidative Transformation of Oximes with Hypervalent Iodine (III) Reagents

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ABSTRACT

In the present book chapter, the fascinating chemistry of hypervalent iodine (III) reagents promoted various oxidation reactions of aldoximes and ketoximes have been described. When organo-iodine (III) reagents are reacted with aliphatic or aromatic aldoximes, they usually furnish nitrile oxides, which are useful synthetic intermediates. But in slightly different reaction conditions, hypervalent iodine (III) reagents oxidise the aldoximes to N-acetoxy amides or hydroxamic acids as the major isolable products instead of the anticipated nitrile oxide or its dimerized product oxadiazole-N-oxides. On the other hand, oxidation of ketoxime using hypervalent iodine (III) usually produces the parent ketone or it undergoes Beckmann Rearrangement. The synthetic utilities of these transformations have been summarized here.

Keywords: Hypervalent Iodine; Oxidation; Aldoxime; Ketoxime; Nitrile Oxide

INTRODUCTION

Currently, hypervalent iodine reagents, e.g., (diacetoxyiodo) benzene or **DIB**, (hydroxytosyloxyiodo) benzene or **HTIB**, iodosylbenzene, **IBX**, Dess-Martin Reagent, etc. (Figure-1) have gained the attention of experimental chemists. Recently, hypervalent iodine reagents have become an alternative useful synthetic reagent for very common organic transformations. This is because of their commercial availability, non-toxic and user-friendly nature. Due to their environmentally benign character, these reagents are valuable from the *Green Chemistry* point of view. The distinctive reactivity pattern of these non-metallic oxidizing reagents is revealed in their distinguishing nature in various organic transformations, which are very tough or sometimes unachievable using other oxidizing agents (Yoshimura & Zhdankin, 2016).

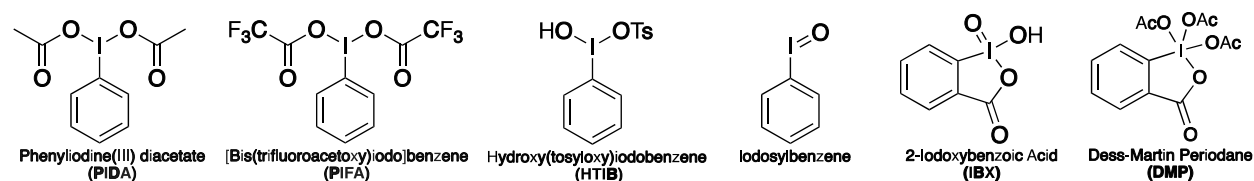


Figure 1: Frequently used hypervalent iodine reagents

The general reactivity relationship and stability of hypervalent iodine compounds can be explained through the character of the hypervalent bond of the compound. The hypervalent iodine compound contains the hypervalent bond, which is in fact a 3c-4e (three-center-four-electron) bond in the apical orientation (Figure 2). The hypervalent bonds have a longer bond length than typical bonds. That is why, the hypervalent bonds can be easily cleaved. The cleavage causes iodine (III) species with 10 electrons to be reduced to iodine (I) with a stable 8 electron structure. Because of this, hypervalent iodine (III) can be used as a very good metal-free oxidizing agent in organic synthesis (Reed & Schleyer, 1990).

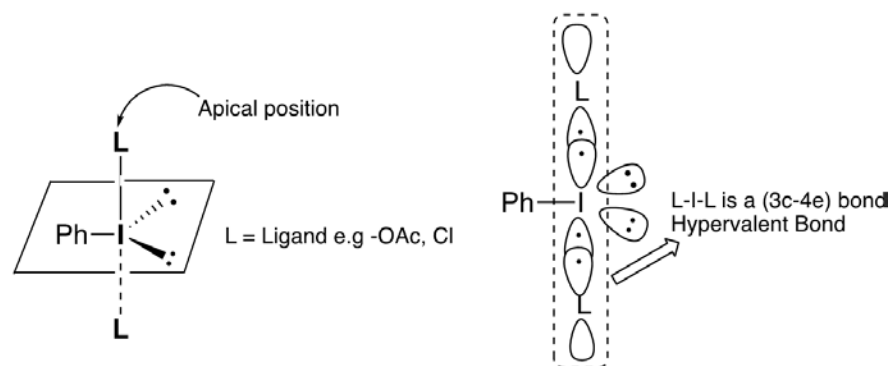


Figure 2: Bonding nature of Organo-iodine(III) reagent

LITERATURE REVIEW

Iodine (III) compounds have been utilised for the oxidation of organic nitrogen compounds, e.g. hydrazones and acid hydrazides. On treatment with hypervalent iodine (III) reagents, various hydrazones and N', N'-dialkylhydrazides are cleaved to the corresponding parent ketones and carboxylic acids (Wuts & Goble, 2000). In the case of ketoxime, the oxidation product is either the deprotection of the parent ketone or a Beckmann Rearrangement product. But, in the case of aldoximes, the oxidation result is either deprotection of the parent aldehyde or oxidation to carboxylic acid or the formation of nitrile oxide. Here, the oxidation outcome depends on the hypervalent iodine agent employed and the reaction conditions chosen for the transformation (Yoshimura & Zhdankin, 2017).

(A) Oxidative transformation of aldoximes using Organo-Iodine(III) reagents:

The oxidation of aldoximes using organo-iodine(III) reagents can generate various products such as nitrile oxide, the parent aldehyde or carboxylic acid (Figure 3). The outcome of the reaction depends on the reaction conditions and iodine(III) species chosen as the oxidant.

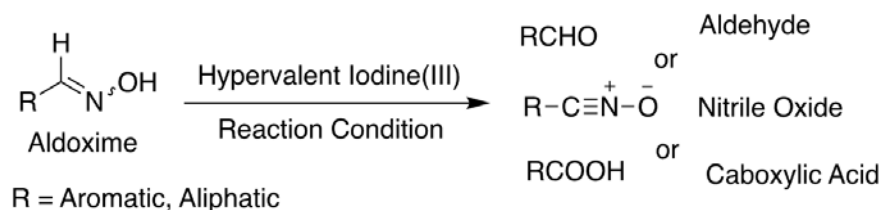


Figure 3: Hypervalent Iodine(III) induced oxidation of aldoximes

Among all these products, nitrile oxide is the most valuable synthetic intermediate. The chemistry of nitrile oxide is attractive because of its immense biological and synthetic importance. Nitrile oxide is the main precursor for the synthesis of the compound 5-aminoisoxazoles, which are of vast biological interest. 5-aminoisoxazole has fungicidal, anthelmintic, and bactericidal properties, and these are also useful for the treatment of various cerebrovascular disorders (Saad, Vaultier, & Derdour, 2004). Nitrile oxides are an important class of synthetic intermediates because of their ability to undergo 1,3-dipolar cycloaddition reactions with alkenes or alkynes, giving heterocyclic compounds, mainly isoxazolines and isoxazoles (Figure 4). Organo-iodine (III) reagents are considered to be the best reagents of choice for the generation of nitrile oxides from aldoximes (Yoshimura *et al.*, 2020).

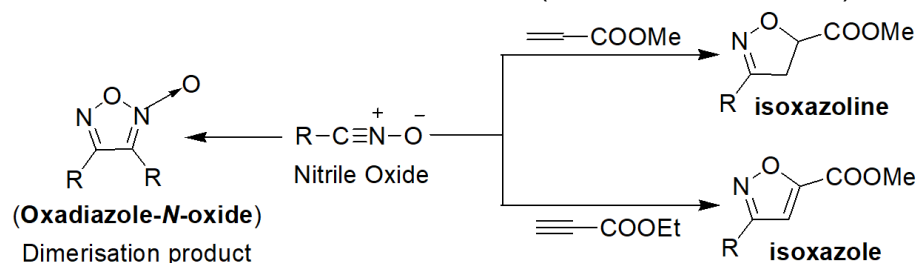


Figure 4: 1,3-Dipolar additions reaction of nitrile oxide

Yoshimura *et al.* (2013) reported a novel strategy for the cyclization reaction of aldoxime and an alkene or alkyne (Figure 5). They have used a catalytic amount of organo-iodine (III) reagent in the presence of stoichiometric oxone as a terminal oxidant in hexafluoroisopropanol (HFIP) and an aqueous methanol solvent mixture. Initially, a nitrile oxide intermediate is formed in this reaction. Then, the nitrile oxide reacts with various alkenes and alkynes in a 1,3-dipolar cycloaddition reaction fashion, to produce theisoxazolines and isoxazoleskeletons, respectively (Figure 5).

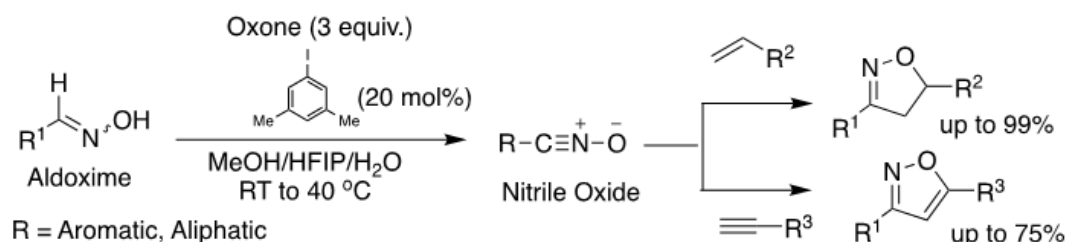


Figure 5: Use of catalytic hypervalent iodine for oxidative cyclization reaction

Hou, Lu and Liu (2013) demonstrated the iodobenzene diacetate (DIB) mediated synthesis of benzo[d]isoxazole-4,7-diols through [3+2] cycloaddition reaction in aqueous medium and *one-pot methodology* (Figure 6). In this transformation, nitrile oxide and benzoquinone are found to be formed as the intermediates. This method was also extended to synthesize another three types of compound e.g. benzodiisoxazole-4,8-diols, isoxazolo[5,4-a]phenazines, and indazole-4,7-diols.

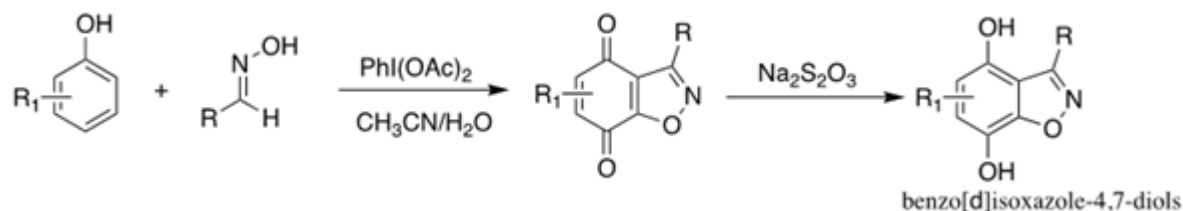


Figure 6: Preparation of Benzo[d]isoxazole-4,7-diols through [3 + 2] cycloadditive reaction

A plausible mechanism of this conversion involves the intermediate formation of nitrile oxide and *para*-benzoquinone by DIB promoted oxidation in one-pot and subsequent [3+2] cycloaddition reaction among them (Figure 7).

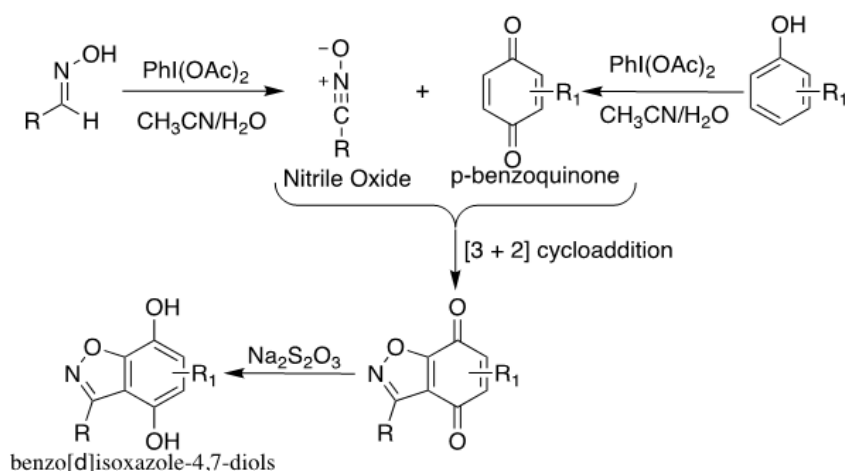


Figure 7: Mechanism of formation of Benzo[d]isoxazole-4,7-diols through [3 + 2] cycloaddition reaction

In a report by Yoshimura *et al.* (2017), they have demonstrated an efficient synthesis of a heterobicyclic scaffold fused with an isoxazoline moiety through an oxidative [3+2] cycloaddition reaction using

HTIB as the mild oxidant (Figure 8). Aldoximes and sulfur and phosphorus-containing heterocyclic alkenes were taken as the starting materials. This oxidative cyclization reaction proceeds via initially formed nitrile oxides intermediate from aldoximes mediated by HTIB reagent. Then, the nitrile oxides intermediate undergoes intermolecular 1,3-dipolar cyclization reaction with 1-propene-1,3-sulfone or cyclic phospholene-oxideto furnish the respective isoxazoline-ring fused heterobicyclic scaffolds in satisfactory yields (Figure 8).

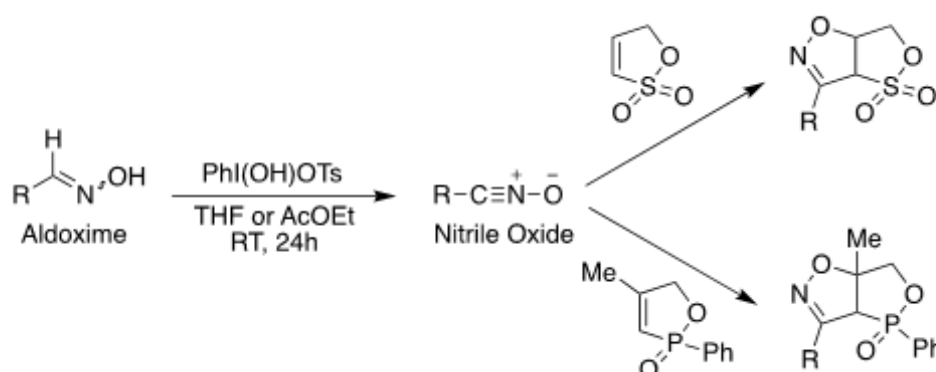


Figure 8: Synthesis of Isoxazoline-fused heterobicyclic compound

Nakamura *et al.* (2018) disclosed a mild and eco-friendly synthetic technique for oxidative transformation of aldoximes to carboxylic acids using the hypervalent iodide(III) reagent HTIB (Figure 9). They have shown that treatment of a series of substituted aldoximes with 2.2 equivalent of PhI(OH)OTs as an oxidant in DMSO-H₂O solvent mixture furnishes the corresponding carboxylic acid in excellent yield.

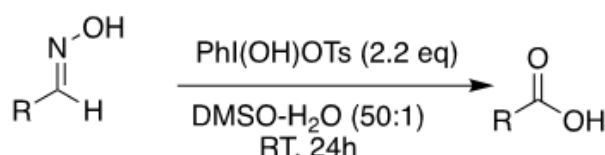


Figure 9: HTIB mediated synthesis of carboxylic acids from aldoximes

They have established the mechanism of this conversion by performing a few controlled experiments. The intermediate of this transformation is hydroxamic acid, which undergoes ligand exchange with tosylate ligand on HTIB. After that, liberation of iodobenzene and water from the intermediate provided the acyl-nitroso compound, which ultimately undergoes hydrolysis to produce carboxylic acid (Figure 10).

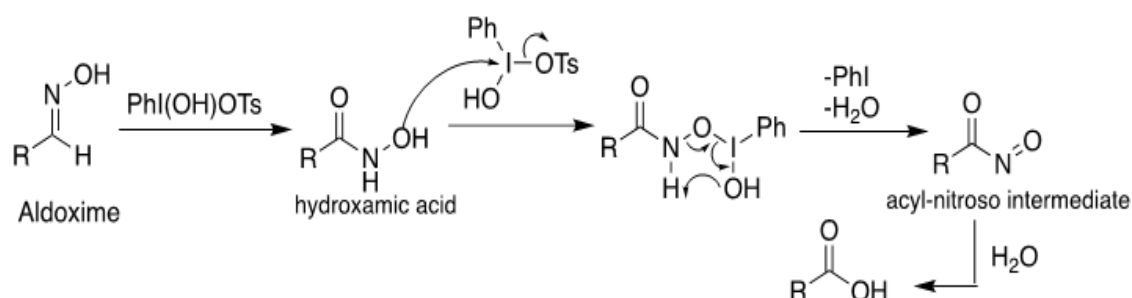


Figure 10: Mechanism of formation carboxylic acids from aldoximes promoted by HTIB

Professor Patel's research group found out a different outcome during the studies of organo-iodine(III) induced oxidation reaction of aldoximes (Patel & Ghosh 2010). They have detected the generation of N-acetoxy benzamide from aromatic aldoximes [Figure 11, (a)] on reaction with (diacetoxyiodo)benzene. When they performed the same reaction with Koser's reagent (HTIB),

the product isolated was N-hydroxy benzamide or hydroxamic acid [Figure 11, (b)].

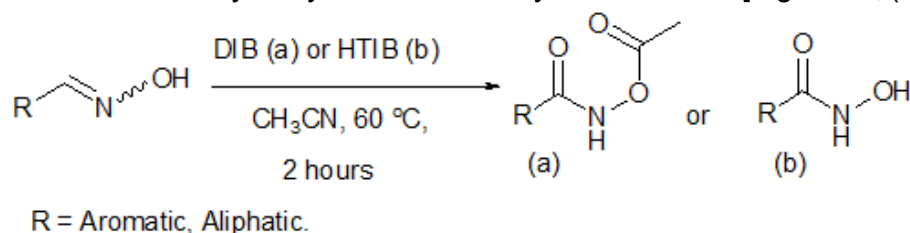


Figure 11: DIB and HTIB mediated oxidation of aldoximes

When 1 equivalent of benzaldehyde oxime was treated with 1.1 equivalent of (diacetoxyiodo)benzene in acetonitrile solvent at a temperature of 60 °C, N-acetoxy-benzamide was isolated in 78% yield as the final product. It should be mentioned here that, in this reaction condition, neither the benzonitrile oxide nor its dimerised adduct were isolated from the reaction mixture. This observation contradicts an earlier report (Das *et al.*, 2004) where in CH_2Cl_2 reaction solvent and in the lack of any added alkenes, a nitrile oxide dimerised adduct (oxadiazole-N-oxides) is reported to be formed. The only difference between the earlier report by Das *et al.* and the present method by Patel and Ghosh (2010) is the use of the solvent acetonitrile instead of dichloromethane.

The proposed mechanism for this transformation is shown in Figure-12. First, benzonitrile oxide (X) (Figure 12), which is expected to be the intermediate for this kind of transformation, is formed from the aldoximes. Then, the nitrile oxide intermediate (X) is attacked by the insitu liberated acetate anion from (diacetoxyiodo)benzene, providing a C-acylated intermediate (Y). Finally, the intermediate (Y) undergoes intramolecular rearrangement to give the expected N-acetoxy-arylamide (Figure 12).

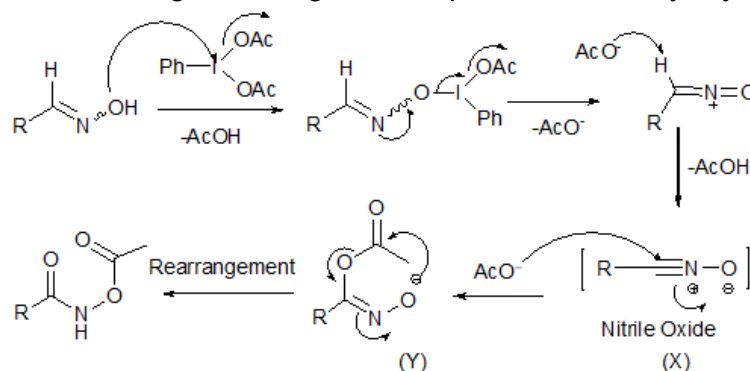


Figure 12: Mechanism of N-Acetoxy amides formation promoted by DIB

A few controlled experiments were carried out to establish the proposed reaction mechanism. The reaction was conducted in the presence of propionic acid (1 equivalent) in the standardised reaction condition. Surprisingly, formation of *N-propionyloxy*-benzamide was observed along with *N-acetoxy*-benzamide in the ratio of 40:60 (Figure 13). From this experiment, the authors were able to establish two of their arguments: (1) benzonitrile oxide is formed as the intermediate and (2) acetate or propionate attack the intermediate benzonitrile oxide in an inter-molecular pathway (Figure 13).

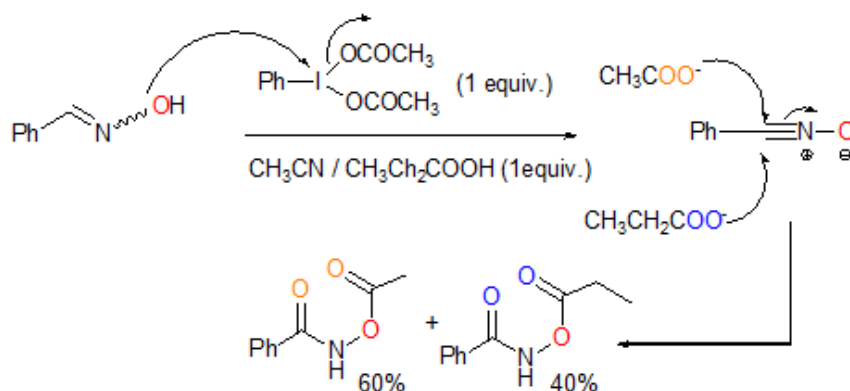


Figure 13: Inter molecular nature of the mechanism

Professor Tanaka's research group demonstrated that iodosylarene (PhIO) oxidises aldoxime to oxadiazole-*N*-oxide, which is actually the dimerised adduct of intermediate nitrile oxide. They isolated oxadiazole-*N*-oxide from the reaction mixture and characterised it (Figure 14, Path-B) (Tanaka *et al.*, 2002). Therefore, it can be understood that in the absence of a suitable alkene or nucleophile, the intermediate nitrile oxide undergoes dimerisation to form oxadiazole-*N*-oxide. But, in the presence of an appropriate external nucleophile, e.g. AcO⁻ or OH⁻ group, the intermediate nitrile oxides produce the corresponding *N*-acetoxy-benzamides or *N*-hydroxy-benzamides through an oxidative rearrangement process (Figure 14, Path-C). In that case, no dimerised product formation was observed. The reactivity pattern of nitrile oxide has been summarised below (Figure 14, Path-A, B, C).

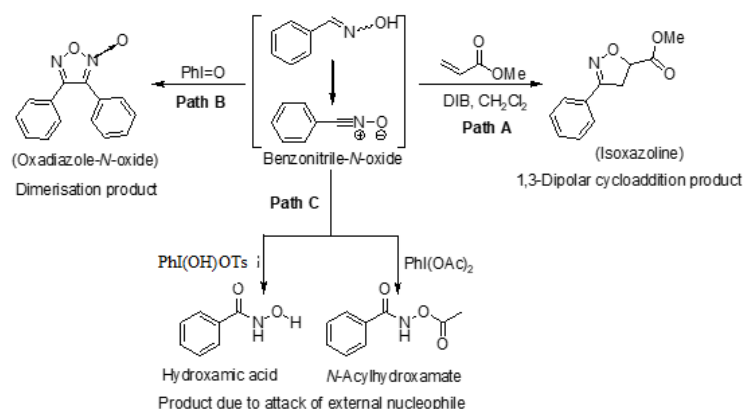


Figure 14: Difference in reactivity of nitrile oxide in various conditions

N-acetoxy amide is a highly significant organic scaffold. So, the authors applied the developed methodology to various aldoximes for the synthesis of a wide array of *N*-acetoxy amides. The authors reported the preparation of *N*-acetoxy amides with electron donating substituents as well as electron withdrawing substituents in the aromatic moiety (Figure 15). All the products were reported to be formed with DIB reagent in acetonitrile medium at 60°C within 2 hours in good yields.

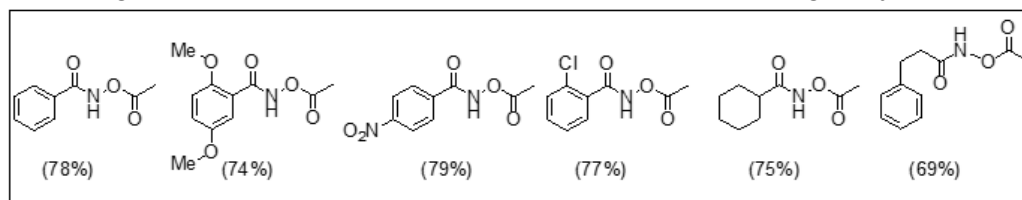


Figure 15: Various *N*-Acetoxy amides prepared

Aldoximes with both electron donating (e.g. Me, OMe) and electron withdrawing (e.g. Cl, NO₂) substituents in the aromatic moiety react well with DIB. In a competing reaction, when an equimolar mixture of two aldoximes with different electronic natures, e.g. 4-methyl-benzaldehyde oxime and 4-nitro-benzaldehyde oxime, were reacted with one equivalent of DIB, the ratio of the corresponding acetoxy product formed after two hours was found to be 3:1. This demonstrates the higher reactivity for the substrates having an electron donating substituent in comparison to the substrates having an electron withdrawing substituent in the aromatic moiety (Figure 16).

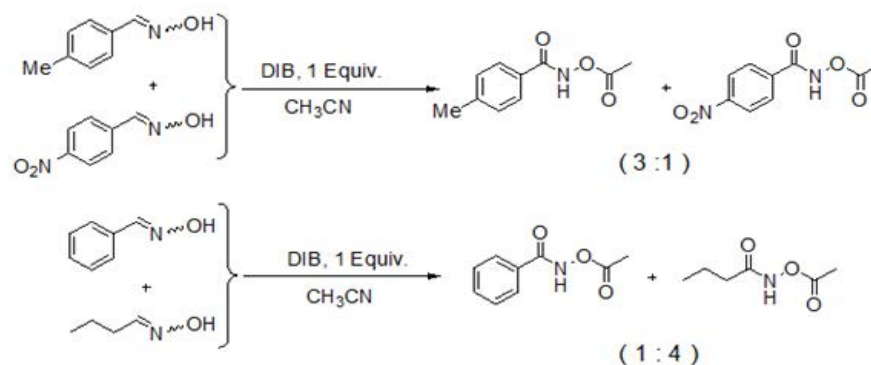


Figure 16: The reactivity difference of aliphatic and aromatic aldoximes

The most interesting reactivity difference was observed when a competing reaction was performed with a (1:1) mixture of aliphatic and aromatic aldoxime. Benzaldehyde and n-butyl aldehyde aldoxime were used for this purpose (Figure 16). The proportion of the corresponding acetoxy product produced after two hours was analysed as (4:1). This observation proves that the aliphatic aldoxime has faster reactivity compared to the aromatic aldoxime.

HTIB or Koser's Reagent, was also tested for the oxidation of benzaldehyde oxime. The product isolated was *N*-hydroxybenzamide, or hydroxamic acid. The authors proposed that the –OH nucleophile, which is generated from the Koser's Reagent, reacts with the intermediate nitrile oxide, generating *N*-hydroxy benzamide (Figure 12). Chloroform was found to be better than acetonitrile as a solvent for the preparation of *N*-hydroxy amide.

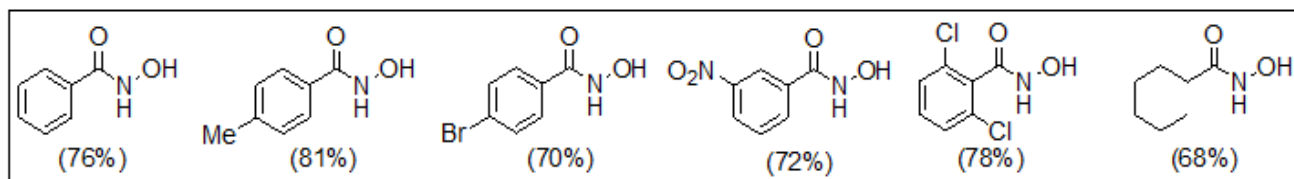


Figure 17: *N*-Hydroxy amides prepared

Various *N*-hydroxy amide having electron donating or withdrawing substituents in the aromatic ring can be successfully synthesized from the respective starting material oximes, in satisfactory yield as shown in Figure 17.

Based on the above results, Professor Patel's Research Group developed a simple and eco-friendly methodology to construct various aromatic amine via a reaction of aromatic aldoxime with HTIB in an alkaline sodium hydroxide medium and DMSO solvent (Ghosh *et al.*, 2011). They were successful to conduct the whole process in one-pot (Figure 13).

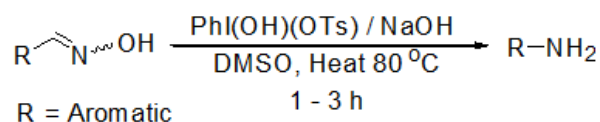


Figure 18: Preparation of amine from aldoxime in one-pot

At first, Koser's reagent converts the aldoxime to the intermediate *N*-hydroxy amide (hydroxamic acid) as described earlier. Then, the hydroxamic acid undergoes base (sodium hydroxide) mediated Lossen rearrangement which generate corresponding amine as the desired product (Figure 19). Various aromatic amines, which are challenging substrate to be synthesised by the reduction method from the corresponding nitro compounds, can be prepared using the present methodology.

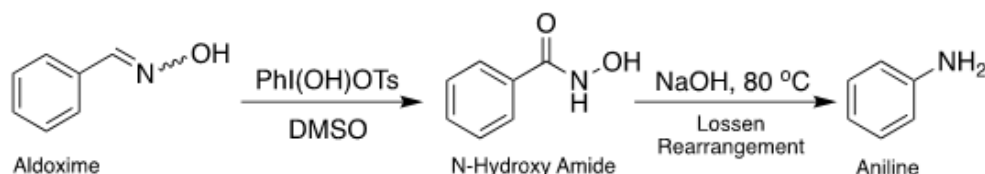


Figure 19: Synthesis of aniline from aldoxime in one-pot using HTIB reagent

In a standard reaction procedure, 1.4 equivalents of Koser's reagent were poured into a stirring mixture of 1 equivalent of benzaldehyde oxime and dimethylsulfoxide solvent. The resultant mixture was stirred at ambient temperature for 30 minutes. Then, the reaction mixture was heated at 80°C for one hour to generate the hydroxamic acid. Finally, 1.5 equivalents of sodium hydroxide were mixed into the reaction mixture and heating was continued for another 1.5 hours to produce the aniline (Figure 19). The authors found out that DMSO was the best solvent among all the solvents examined to achieve optimum conversion for the product.

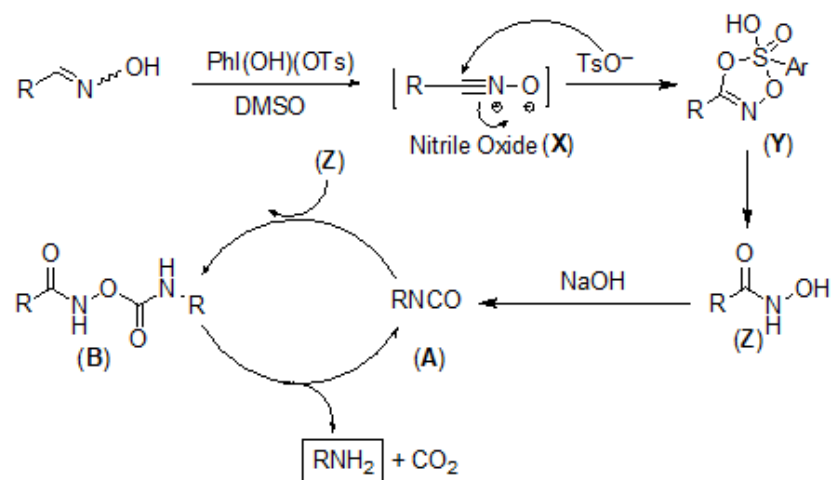


Figure 20: Plausible mechanism for formation of the amine from aldoxime

Generation of the aromatic amine from aromatic aldehyde oxime can be justified through the above plausible reaction mechanism (Figure 20).

The usefulness of the developed methodology have been demonstrated by synthesising various aromatic amines (Figure 21). Substituted amine which contain allyloxy- and propargyloxy- group is difficult to prepare using usual reduction method from nitro arene, because allyloxy- or propargyloxy- group does not tolerate the nitration condition or reduction condition.

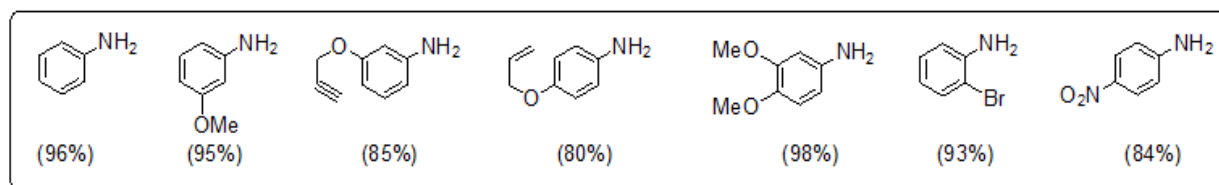


Figure 21: Various amine derivative synthesized

(B) Hypervalent Iodine Mediated Oxidation of Ketoximes:

In Moriarty, Prakash and Vavilikolanu (1986) studied the oxidation reaction of ketoxime with iodosobenzene diacetate (Moriarty, Prakash & Vavilikolanu, 1986). They found out that hypervalent iodine cleaved ketoxime and produced the corresponding ketones under neutral conditions in good yields.

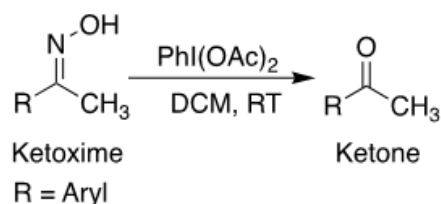


Figure 22: Oxidation reaction of ketoxime with iodosobenzene diacetate

The authors noticed evolution of N_2 gas during the reaction. Based on this observation, they proposed a probable mechanism for this organic conversion where generation of a nitroso intermediate was suggested (Figure 23).

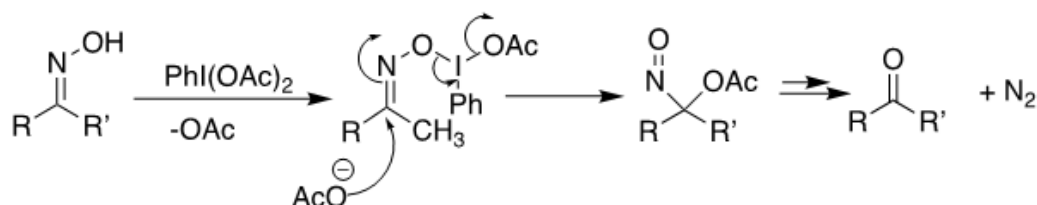


Figure 23: Mechanism of cleavage ketoxime with iodosobenzene diacetate

Very recently, Oishi *et al.* reported a novel Beckmann rearrangement reaction employing $\text{PhI}(\text{OAc})_2$ and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ starting from various aliphatic and aromatic ketoximes (Figure 24). In this methodology, the Lewis acid $\text{BF}_3 \cdot \text{Et}_2\text{O}$ was used for the pre-activation of organo-iodine (III) species.

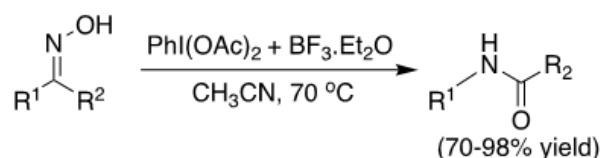


Figure 24: Beckmann rearrangement mediated by hypervalent iodine(III) reagent

The authors proposed a plausible reaction mechanism for this transformation. First, a pre-activation of the reagent, diacetoxy iodobenzene, occurs by the Lewis acid. Then, the activated diacetoxy iodobenzene triggers the hydroxy group of the ketoxime by substitution with an acetoxy group. Finally, the intermediate (X) (Figure 25) undergoes rearrangement with the elimination of iodobenzene and the acetoxy group to generate the cation which subsequently reacts with H_2O to afford the amide.

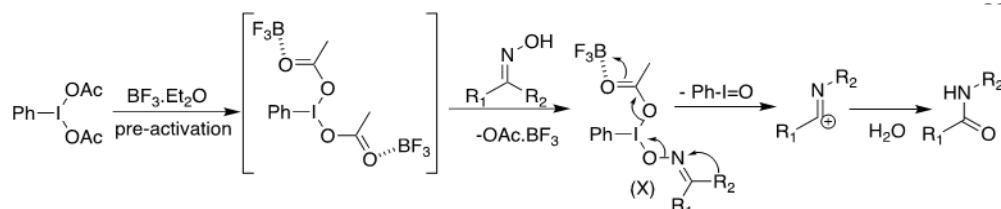


Figure 25: The mechanism of Beckmann rearrangement initiated by iodine(III) reagent

CONCLUSION

The oxidation results of various aldo/ketoximes induced by organo-iodine(III) reagents have been summarised in this book chapter. Aldoximes are oxidised with iodine(III) species to create nitrile oxide intermediate which is a vital synthon that undergoes 1,3-dipolar cycloaddition reaction to produce a series of valuable organic scaffolds, e.g. isoxazolines and isoxazoles. Hypervalent iodine (III) reagents DIB or HTIB oxidise both aromatic and aliphatic aldoximes to produce the corresponding *N*-acetoxy or *N*-hydroxy amides in satisfactory yield instead of the anticipated nitrile oxides or its dimerised adduct. Hydroxamic acid can be generated using hypervalent iodine(III) reagent HTIB in DMSO solvent. The *in situ* generated hydroxamic acids undergo an alkali-initiated rearrangement process to afford various aromatic amines in *one-pot*. Oxidation of ketoxime with hypervalent iodine(III) reagent regenerates the parent ketone in normal reaction conditions. But in the presence of Lewis acid activator, the ketoxime undergoes Beckmann Rearrangement while reacting with hypervalent iodine(III) reagent. So, in conclusion, oxidation of oxime compounds using organo-iodine(III) reagents leads to many interesting results and it is expected that this specific reactivity of these reagents will lead to many more fascinating organic transformations in the future.

ACKNOWLEDGEMENT

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Thorpe-Ingold Effect Assisted Strained Ring Synthesis

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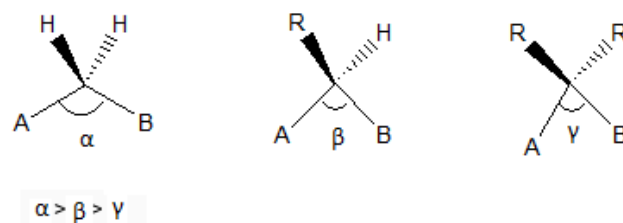
ABSTRACT

The Thorpe-Ingold effect (also known as the gem-dialkyl effect) is the term assigned to the acceleration of cyclization caused by the substitution of hydrogen atoms on the carbons anchoring the two reaction centres with alkyl groups. Because of the more favourable entropy shift that happens when the reaction reaches the transition state, intramolecular reactions often proceed faster than intermolecular reactions. The formation of five- and six-membered rings, among other things, benefits from these entropic effects. Alternatively, depending on the situation, increased ring strain can be countered and cyclization retarded in the formation of three- and four-membered rings. To overcome the unfavourable enthalpic parameters, alkyl substituents inserted onto the acyclic carbon chain that connects the two reacting junctions can be employed to improve the rate of cyclization. This article focuses on the application of the Thorpe-Ingold effect in the construction of strain three- and four-membered ring systems. To sum up, the Thorpe-Ingold effect can effectively speed up and increase the yield of both intramolecular and intermolecular cyclization.

Keywords: Thorpe-Ingold Effect; Gem-Dialkyl Effect; Strained Molecules; Gem-Dimethyl Effect

INTRODUCTION

The Thorpe-Ingold effect, also known as the gem-dimethyl effect or angle compression effect, refers to the promotion of cyclization through gem-di-substitution on the molecular chain (Jung & Piizzi, 2005). This effect was first proposed by Beesley, Ingold and Thorpe (1915), that is, when the hydrogen on the methylene group in the molecular chain is replaced by a bulky alkyl group, the internal angle (bond angle) is compressed (Ingold, 1921), and the two reactive groups at the end of the molecular chain are compressed close to each other for intramolecular cyclization (Ingold, Sako & Thorpe, 1922) (Figure 1).



α, β, γ are bond angles

Figure 1: Thorpe-Ingold effect

It was later found that the Thorpe-Ingold effect is very important in explaining the change in bond angle (Lightstone & Bruice, 1994). Small five- and six-membered rings with unchanged bond angles are not perfect (Von Ragué Schleyer, 1961), and it is difficult to explain the increase in bond angle changes problems with yield and rate of formation of macrocycles (Galli *et al.*, 1979).

Bruice and Pandit (1960a) proposed the reaction rotamer effect to explain the effects of dialkyl substitution (Figure 2). When the base is present, the molecule mainly exists in the trans (anti) conformation; after the geminal dialkyl group is substituted, the higher energy ortho-crossing (gauche) is favourable for cyclization (Bruice & Pandit, 1960b). The conformation ratio is increased,

and the cyclization is easy. The Thorpe-Ingold effect is examined in this article for intramolecular and intermolecular cyclization to produce three- to six-membered ring products.

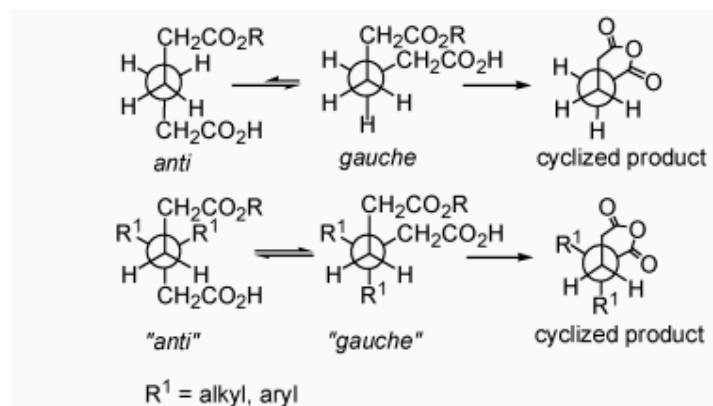


Figure 2: Reactive Rotamer Effect

Jung and Gervay (1991) studied the bond angle compression effect and the reaction rotamer effect in geminal dialkyl substitution by intramolecular D-A cycloaddition (Figure 3). Substrates 1d and 1e substituted with a spiro ring were designed, the internal angle β of the spiro ring substitution position was increased, and the cyclization would be dominated by the reaction rotamer effect.

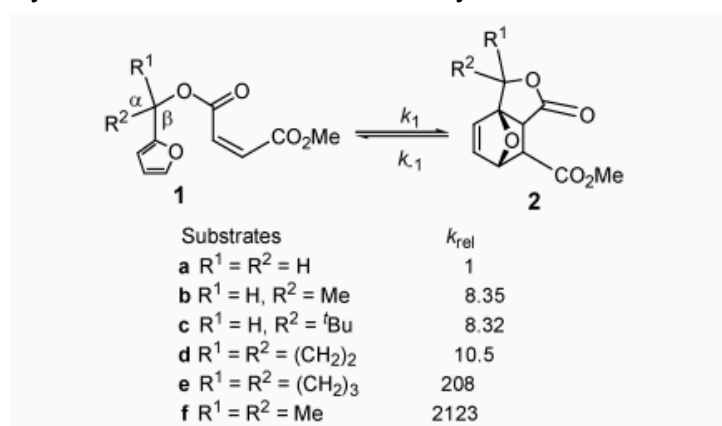


Figure 3: Bond angle compression effect and the reaction rotamer effect

As a result, the unsubstituted substrate 1a had the slowest cyclization rate, and the cyclization rates of monomethyl substituted 1b and mono-tert-butyl substituted 1c increased slightly but were largely unaffected by the volume change of the monosubstituent. The cyclization rate of geminal dimethyl substituted 1f is significantly increased, more than 2000-fold higher than that of 1a. The cyclization rates of 1d and 1e with enlarged internal angles of the substitution positions also increased, and the cyclization rate of 1e was approximately 208 and 25 times that of 1a and 1b, respectively. It is therefore believed that at least in cyclization to form a five-membered ring system, the effect of reaction rotamers should be fully considered.

The enthalpy and entropy changes of some reactions are also major contributors to the Thorpe-Ingold effect. Allinger and Zalkow (1960) calculated the thermodynamic properties of the geminal dimethyl effect in the cyclization of n-hexane to cyclohexane. When the hydrogen at the 2-position of n-hexane is replaced by a gem-dimethyl group, the cyclization rate can be increased by about 100 times, and when the hydrogen at the 3-position is replaced by a gem-dimethyl group, the cyclization rate is increased by about 1000 times. The authors believe that the enthalpy change or entropy change, or a combination of the two, caused by gem-dimethyl substitution results in a decrease in non-bonding interactions and rotational entropy, which increases the rate of intramolecular cyclization.

In 2009, Karaman studied the kinetic and thermodynamic properties of gem-disubstituted bromobutylamine by calculation and showed that the rate constant is only determined by the free energies of the ground and transition states (Figure 4). Although the initial studies were reported by Brown and Van Gulick in 1956, for the geminal alkyl effect on the rates of ring closure of bromobutylamines.

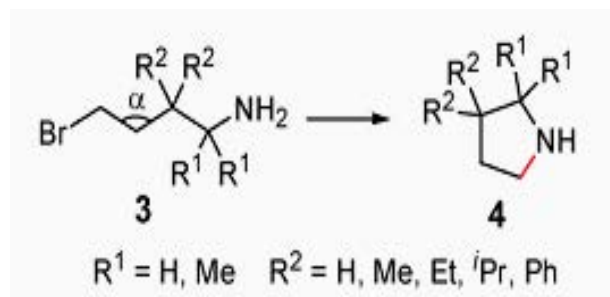


Figure 4: Cyclization of gem-disubstituted bromobutylamine

In 2010, Kostal and Jorgensen used quantum and statistical mechanics calculations to study the reactions of 2-chloroethanol, 1-chloro-2-propanol, and 2-methyl-1-chloro-2-propanol in the gas phase and liquid phase under base catalysis, respectively. Generate the corresponding epoxide. The results show that the C-C-C bond angle in the main chain does decrease with the increase of the substituted methyl groups. Gas-phase MP2 and CBS-Q calculations show that the energy changes in the reaction process of the three chloroethanol derivatives are very small; considering continuous hydration or MC/FEP calculations can accurately reproduce the reaction in the presence of water, thus It is speculated that the bond angle compression effect in the cyclization of chlorohydrin is mainly caused by the solvent effect.

In a nutshell, the Thorpe-Ingold effect may be affected by many factors, such as solvent, auxiliary agent, and substituent group. Therefore, these factors should be fully considered when considering the role of the Thorpe-Ingold effect in the reaction.

Although the essence of the Thorpe-Ingold effect has not yet been explained by a perfect theory, it is indeed a good method to apply the Thorpe-Ingold effect to effectively promote intramolecular cyclization. This article focuses on the study of the Thorpe-Ingold effect for intramolecular and intermolecular cyclization to form three- to six-membered ring products.

LITERATURE REVIEW

Synthesis of three-member ring products

Generally, intramolecular reactions go on more quickly than their intermolecular analogues due to the more favourable entropy change that occurs when the reaction enters the transition state. These entropic effects lead to rate enhancements in the production of five- and six-membered rings, among other things. As an alternative, enhanced ring strain may be counteracted and cyclization may be delayed in the development of three- and four-membered rings, depending on the situation. To get around the unfavourable enthalpic conditions, however, it is possible to use the faster rate of cyclization caused by adding alkyl substituents to the acyclic carbon chain that connects the two reacting junctions.

Synthesis of cyclopropane derivatives

Coscia and Lambert (2009) studied the palladium catalyzed intermolecular cyclopropanation reaction of gem-dialkyl substituted 3-oxo-7,9-decadienoate 5 to reveal the effect of the various gem-dialkyl groups. They have found that gem-dialkyl groups are necessary for the Pd(OAc)₂ catalyzed

propanation reaction to obtain the targeted bicyclic product **6** (Figure 5).

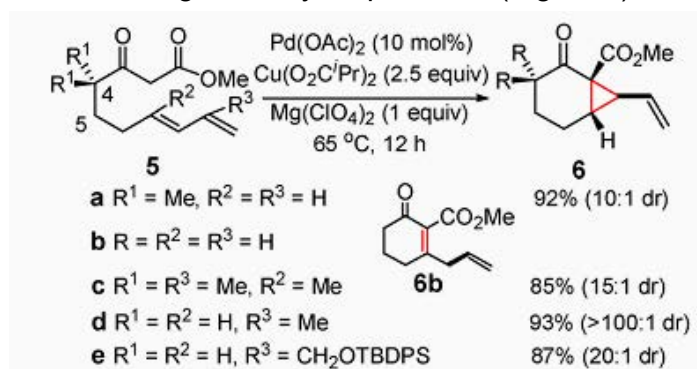


Figure 5: Thorpe-Ingold effect assisted cyclopropanation

If the substrate is an unsubstituted ketoester, it is prone to undergoing Saegusa-Ito type oxidation to give conjugated cyclohexenone (**6b**). Here, the Thorpe-Ingold effect not only promotes cyclization but also promotes the reaction in a specific direction. There are methyl groups (**5c** and **5d**) and tert-butyl-diphenylsilyloxymethylene (**5e**) substitutions on the conjugated diene bonds, and the target products (85%–93%) can be obtained in high yields. When the geminal dimethyl group was at the fifth position, the yield decreased by 40%–59%. This shows that the position of the geminal disubstituted group on the molecular chain has some effect on the intramolecular cyclization, which may work through steric hindrance.

Ethylene oxide derivatives

β-Halohydrin cyclization

In 1933, Nilsson and Smith studied the cyclization of *o*-chloroethanol **7** to form the corresponding epoxide **8**, which was the first example of using the Thorpe-Ingold effect to explain the substitution-promoted intramolecular cyclization. The results show that the cyclization rate increases differently when different numbers of hydrogens are substituted in different methylene groups of *o*-chloroethanol (Figure 6). The cyclization rate was increased by about 250-fold when either the 1- or 2-carbon hydrogen was replaced by a geminal dimethyl group. When all four hydrogens in both methylene groups of *o*-chloroethanol were replaced by methyl groups, the cyclization rate increased more than 104-fold. The Thorpe-Ingold effect greatly accelerates the rate of intramolecular cyclization.

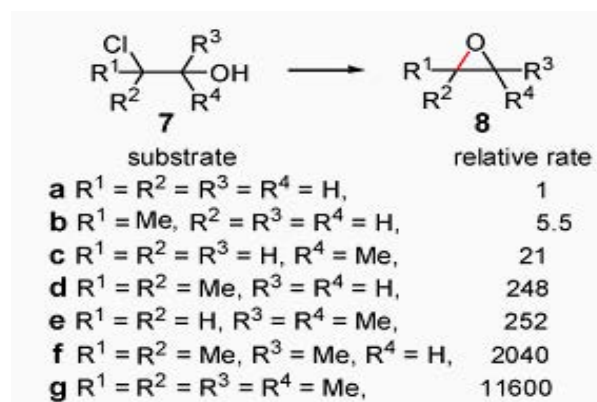


Figure 6: Thorpe-Ingold effect assisted epoxidation

Free radical reaction

The free radical reaction can easily form cyclopropane and cyclobutane, which are difficult to obtain by other methods, and the Thorpe-Ingold effect also greatly promotes the free radical cyclization reaction. In 1974, when Bloodworth *et al.* (1974) studied the rate constant of the formation of ethylene oxide from β-bromoperoxide by free radicals, they found that there was a geminal dialkyl effect in the process of homolytic cyclization (Figure 7). The corresponding ethylene oxide **10** was obtained by

reacting β -bromoperoxide **9** with hexamethylditin in a benzene solution at 25 °C using di-tert-butyl bis-nitric acid as an initiator. The results show that with the increase of substituted methyl groups, the cyclization rate increases significantly, and this study is the first to demonstrate that the Thorpe-Ingold effect also plays a role in the homolysis reaction.

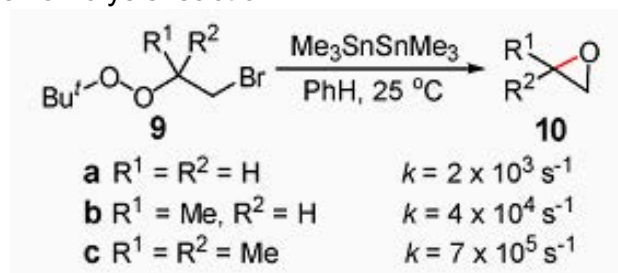


Figure 7: Thorpe-Ingold effect assisted epoxidation through radical reaction

The same research group also studied a system similar to the homolysis—that is, the cyclization of chloroethanol **11** to form ethylene oxide **10** (Figure 8), and the reaction rate ratio of compounds **11a-c** is about 1:20:250. Due to the geminal substitution, on the one hand, the tension of forming the small ring is reduced, and on the other hand, it also plays the role of stabilizing the small ring. Thus, the presence of geminal disubstituents increases the rate of radical cyclization.

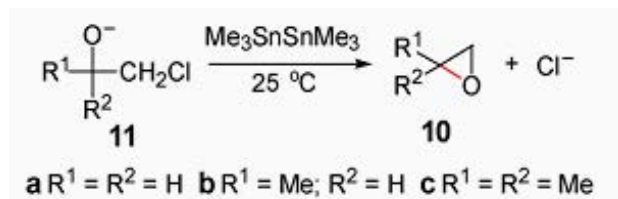


Figure 8: Thorpe-Ingold effect assisted epoxidation of chloroethanol

Synthesis of four-member ring products

Cyclobutane derivatives

Radical reaction

When studying the formation of cyclobutane by free radical reaction, Jung *et al.* found that geminal disubstitution can improve the cyclization efficiency (Figure 9) (Jung, Trifunovich & Lensen, 1992; Jung & Kiankarimi, 1995,1998; Jung & Marquez, 1997; Jung, Marquez & Houk, 1999). When R is ethoxy and ethoxycarbonyl, cyclobutane product **14** is mainly obtained; when R is methyl, only 25% of cyclobutane product **14** is obtained; and when R is hydrogen, only reduction product **13** is obtained. When the two Rs are O(CH₂)₃O and O(CH₂)₂O, as the internal angle of the substitution position increases, it is not conducive to cyclization, and the product changes from cyclobutane **14** as the main product to the reduction product **13** as the main product. Electronic effects and steric hindrance together affect the selectivity of the reaction (Jung, 1999).

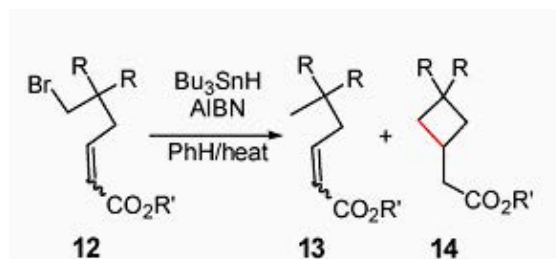


Figure 9: Thorpe-Ingold effect assisted cyclobutane formation via radical reaction

Metal-catalyzed cyclization

Chaumontet *et al.* (2008) studied the palladium catalyst system to activate CH on methyl to

synthesize benzocyclobutene (BCB) **16** (Figure 10).

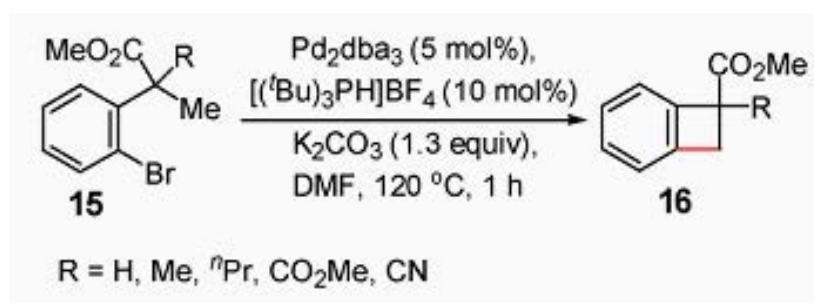


Figure 10: Thorpe-Ingold effect assisted formation benzocyclobutanes

When R = H, no target product was formed even if the reaction time was extended to 4 h. Substituted BCB, **16** can be obtained in higher yields when R is an alkyl group, an alkoxy carbonyl group, or a nitrile group. When R = Me, the corresponding BCB can be obtained regardless of whether there are electron-withdrawing or electron-donating groups on the benzene ring, with yields ranging from 44% to 92%. The results show that after geminal di-substitution at the benzylic position, the dominant conformation for ring formation is dominant. After palladium is added to the C–Br bond in an oxidative way, the C–H bond on the methyl group can be activated to make benzocyclobutene (BCB).

Oxetane derivatives

Shiina *et al.* (2012) studied 2-methyl-6-nitrobenzoic anhydride (MNBA) to assist in the total synthesis of orlistat and found that there is no substituent on the molecular chain of β -hydroxycarboxylic acid, the yield is very low, and only a small amount can be obtained by increasing the temperature of Cyclobutyrolactone (Figure 11).

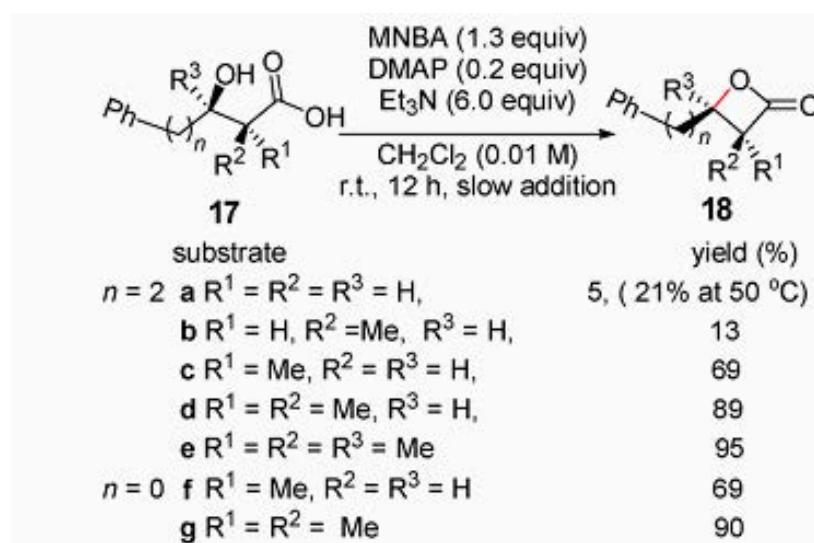


Figure 11: Thorpe-Ingold effect assisted esterification of β -hydroxyacids

The introduction of substituents at the α and β positions of the carboxylic acid can improve the yield. Compared to **17b** and **17c**, it is more favourable for cyclization when the methyl group at the α -position of the carboxylic acid and the hydroxyl group at the β -position are in trans, because when the α -position of the carboxylic acid is substituted by a methyl group, the internal angle decreases, which is favourable for the cyclization reaction. The methyl group of the formula structure has a large steric hindrance with the hydroxyl group, which is not conducive to cyclization. The benzene ring (**17f**) connected to the same carbon as the hydroxyl group has no effect on the esterification yield. The α -position of the carboxylic acid was substituted with a geminal dimethyl group (**17d**, **17e**, **17g**), the internal angle was further reduced, the energy required for the reaction was reduced, and high yields of β -lactones were obtained.

The methyl substitution (17e) on the hydroxyl carbon is also helpful because it lowers the energy needed for the reaction, which helps improve the yield even more.

DISCUSSION

The Thorpe-Ingold effect is not only limited in the reactivity of the substrate molecules but also effective for ligand binding with metal salts. The Thorpe-Ingold effect caused by gem-dialkyl substitution of the ligand framework led to a chelation effect that produced excellent yields and selectivities for maintaining the nucleophile configuration. While working on optimising the ligand to increase the branched product ratio, an intriguing gem-dialkyl effect was found in the ligand backbone. Traditionally, the Thorpe-Ingold effect has been used to describe how gem-dialkyl substitution causes a conformational restriction that speeds up the process by bringing the two active hemispheres of the molecules into proximity (O'Neill, Riesebeck & Cornella, 2018). The Thorpe-Ingold effect caused by gem-Dialkyl replacement of the ligand backbone led to a chelation effect that produced excellent yields and selectivities for maintaining the nucleophile configuration. Recent developments in using the Thorpe-Ingold effect concept to explain certain synthetic chemistry, including organosilicon compounds, were addressed by Luh and co-workers, with a focus on the photophysical characteristics of some dialkylsilylene spaced conjugated chromophores (Luh & Hu, 2010). In these silicon-containing copolymers, the silicon moieties are regarded as insulating tetrahedral spacers. The silicon substituents are easily adjustable, and their steric environment may determine the orientation (or helicity) of the copolymers and, consequently, their photophysical characteristics. The Thorpe-Ingold effect may be used to understand this substituent impact on silicon.

CONCLUSION

In conclusion, the Thorpe-Ingold effect can effectively promote intramolecular and intermolecular cyclization and improve the reaction rate and yield. Moreover, this effect is applicable to various systems and reaction types, and the effect is obvious. Therefore, the Thorpe-Ingold effect can be utilized in the cyclization reaction to improve the synthesis efficiency. It is believed that there will be more theoretical and experimental studies on this effect in the future, and improving the understanding of the essence of this effect will be beneficial to its application in synthesis.

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Effects of Oriented External Field in Organic Reaction

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ABSTRACT

In this chapter, the improvement of the kinetics and thermodynamics of chemical reactions will be reviewed, which have a crucial role in the applied oriented external electric field (OEEF). Also, it is studied that OEEF has catalytic activity in some organic reaction mechanisms and the ability to cleavage hemolytic bonds in a heterolytic way. The use of the Nitroxide radical in the presence of time-dependent fluorescence spectroscopy, the use of Radical mediated Polymerization, and so on are also being investigated. Again, OEEF has a budding area of research, waiting for eventual blossom. In this context, the chemical bond breaking, bond dissociation energy, product selectivity, and how OEEF catalyzes the reaction will be discussed, as well as an overview of fluorescent spectroscopy.

Keywords: Reaction Mechanism; Nitroxide Radical; External Orientation Field

INTRODUCTION

The electron transfer reaction facilitated molecular geometry changes, bonding, and bonding interaction in the presence of an external electric field, affecting homonuclear bonds, heteronuclear bonds, bonds, as well as bond dissociation energy, spin state crossover, and methyl transfer (Menshutkin Reaction). A quantum mechanical concept governs the principle of the external electric field (EEF). EEF along the 'Bond axis' and the 'Reaction axis' facilitated bond breaking and electron pairing changes from reactant to product, respectively. Oriented external electric field (OEEF) provides chemical reaction regioselectivity and allowed the forbidden orbital mixing (Shaik *et al.*, 2018). In we consider a Hydrogen atom, where quantum level $n=2$ is considered, hydrogen possesses four degenerate orbitals that are $2s$, $2p_x$, $2p_y$, $2p_z$ (Shaik *et al.*, 2018). In absence of external electric field all the four levels are degenerate that is they are mutually orthogonal to each other. If OEEF applies along Z-axis $2p_x$, $2p_y$ levels/states remain the same as there is no z-component in $2p_x$, $2p_y$ states split widely, one above and another below the $2p_x$, $2p_y$ orbitals, where $2s$ and $2p_z$ Show mixed hybrid orbitals. Removal of forbiddance is due to the expectation value of the orbital interaction is no longer zero in presence of the z-oriented field.

As the F_z (electric field operator) and z axis have the same symmetry. In this case, it will discuss the space field effect. In presence of OEEF along the z-direction bond axis develops some charge polarization due to the hybridization of σ_g bonding and σ_u^* antibonding orbitals of the bond. This mixing is forbidden in absence of field. At particular field strength the charged species is stabilized, and this stabilization is proportional to the dipole moment of the ion pair.

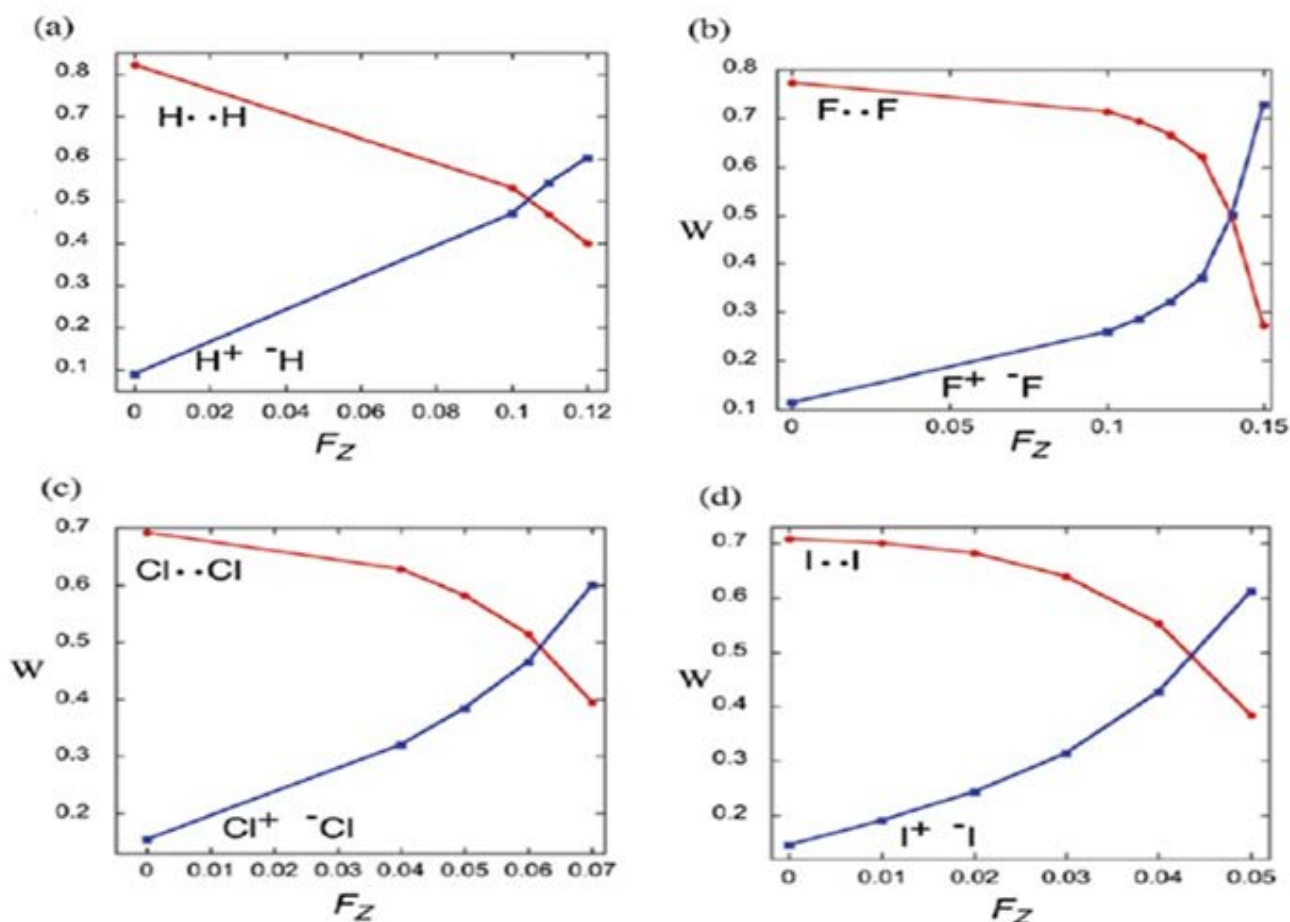
LITERATURE REVIEW

The homonuclear bond cleavage:

The homonuclear bonds undergo heterolysis under increasing OEEF along the bond axis. All bonds elongated and dissociated into ions. i.e $X^+ X^-$ at a certain electric field. This dissociation basically based upon two parameter i.e the difference between ionization energy (IE) and electron affinity (EA) and this difference determines the polarizability of the bond. Now the weight of ionic and covalent structure will be considered corresponding to the bond wave function. In absence of electric field along z axis $F_z=0$ then the bonds are primarily covalent with increasing F_z (i.e electric field along the z- direction)

covalent ionic crossover happens. OEEF can control a variety of named reaction called Diel's alder reaction (Yu *et al.* 2021), Menshutkin reaction etc.

Ionic structure becomes more prominent with the increase in the electric field and overrides the weight of covalent structure. At particular field strength the bond becomes ionic and heterolyze without barriers. (Shaik, Mandal & Ramanan, 2016) For example, H-H, F-F, Cl-Cl, I-I bond will dissociate at field strength stronger than 0.1 au. (Shaik *et al.*, 2018). Off course the difference between IE and EA and hence corresponding polarisability determines the actual critical field at which the bond heterolysis occurred. Na-Na, Li-Li bonds needs much lower fields to dissociate into ions. The figure 1 explained the weight of covalent-ionic structure for bonds under Z-OEEF along the bond axis.



Source: Shaik *et al.*, 2018

Figure 1: Plots of weight of covalent-ionic structure for bonds under Z OEEF along the bond axis

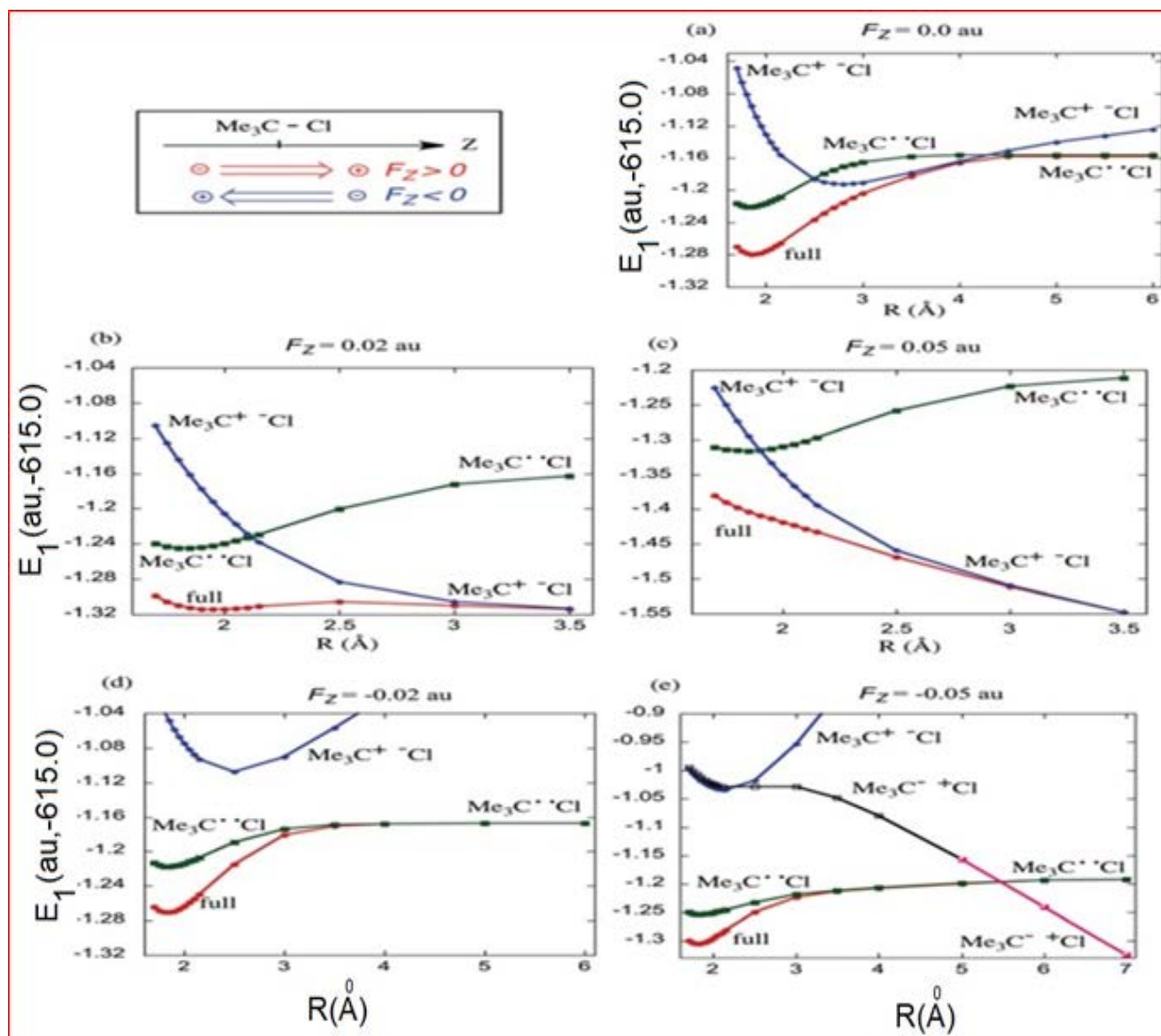
π bond cleavage in presence of OEEF:

In 1859, Breakage of π in OEEF has completely different consequence than in σ bond. When π -bond of ethylene $CH_2=CH_2$ is broken, due to the rotation of C-C bond the C-C bond elongates so that, the dipole moment that is created by the respective ionic structure varies significantly. Experimentally, it is observed that ionic structure ($CH_2^+CH_2^-$) in the π bond wave function mildly increases by OEEF. But huge amount of field is required to lower the π ionic structure. If H is replaced by F in ethylene, the positive charge on C that is in ($CF_2^+CF_2^-$) is stabilized by for its π donation. But huge amount of field in laboratory is impractical (Shaik *et al.*, 2018).

Heteronuclear bond cleavage in presence of orientation external electric field:

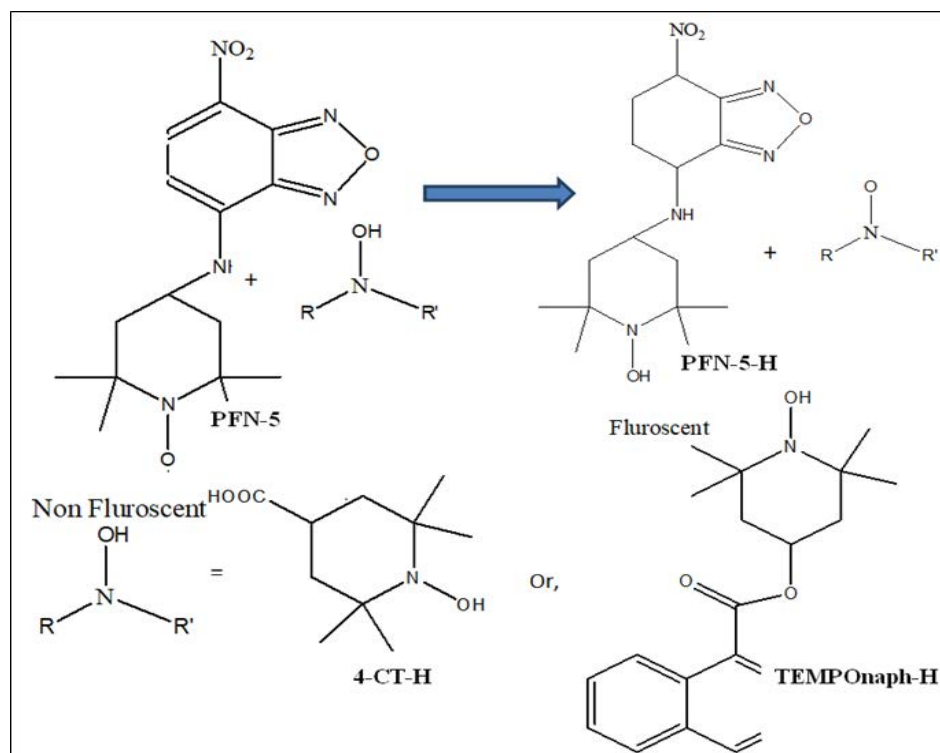
Here, three different types of heteronuclear bonds, i.e., H-Cl, CH_3Cl , Me_3CCl will be considered. In

absence of F_z the covalent bond exists. When F_z field is along the bond axis, H-Cl undergoes bond elongation, then the bond dissociates into $H^+ + Cl^-$ and F_z field applied in the negative direction, the bond shrinks and breaks into opposite ions, $H + Cl^+$. Similarly, the C-Cl bond of CH_3Cl elongates under applied field and breaks into ions $CH^+ + Cl^-$. When the opposite direction is applied, the ions break into opposite ions $CH_3^+ + Cl^-$. In the case of Me_3CCl , it dissociates, when the electric field is applied into $Me_3C^+ + Cl^-$, whenever the external electric field is applied along the negative Z direction, the ions split into opposite ions i.e. $Me_3C^- + Cl^+$. The figure 2 indicates that the valence bond curves of covalent and ionic structure of C-Cl bond in Me_3CCl . (a) a field free situation, (b) and (c) $F_z > 0$ and (d), (e) $F_z < 0$. The valence bond structures are colored: Green shows covalency, blue shows ionic ($F_z > 0$), Black also shows ionicity but ($F_z < 0$) and red denotes the full wave function.



Source: Shaiket al., 2018

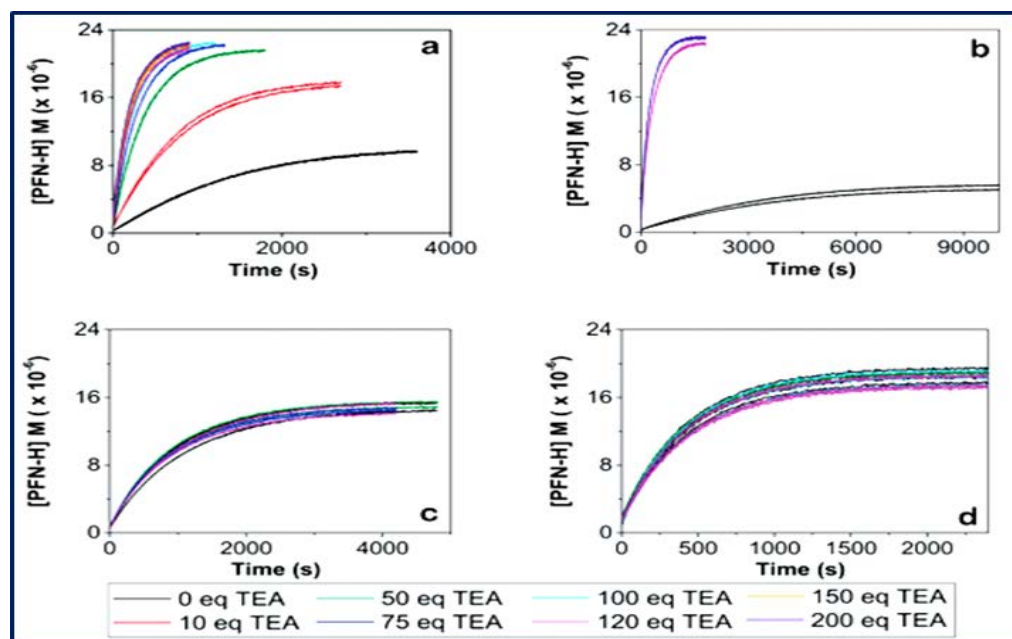
Figure 2: Valence bond curves of covalent and ionic structure of C-Cl bond in Me_3CCl . (a) a field free situation, (b) and (c) $F_z > 0$ and (d), (e) $F_z < 0$. The valence bond structures are colored: Green shows covalency, Blue shows ionic ($F_z > 0$), Black also shows ionicity but ($F_z < 0$) and red denotes the full wave function. i.e., $\psi = C_1\phi_1 + C_2\phi_2$.



Source: Gryn'ova & Coote, 2016

Figure 3: PFN-5H, 4-CT-H, PFN-5, 4-CT

Now Authors have studied the Fluorescence intensity versus Time curve which indicate in the figure 4, Here PFN-5 molecule is Non fluorescent, but PFN-5H molecule is Fluorescent. Fluorescence intensity is shown up to when COOH group of 4-CT molecule is there after a certain time there is no COOH group then a saturation will be seen in the curve with the addition of the base TEA. As no acid remains, it is only noticed the saturated curve of the base. Another study has done with changing the medium of the solvent (Gryn'ova & Coote, 2016).



Source: Klinska et al., 2015

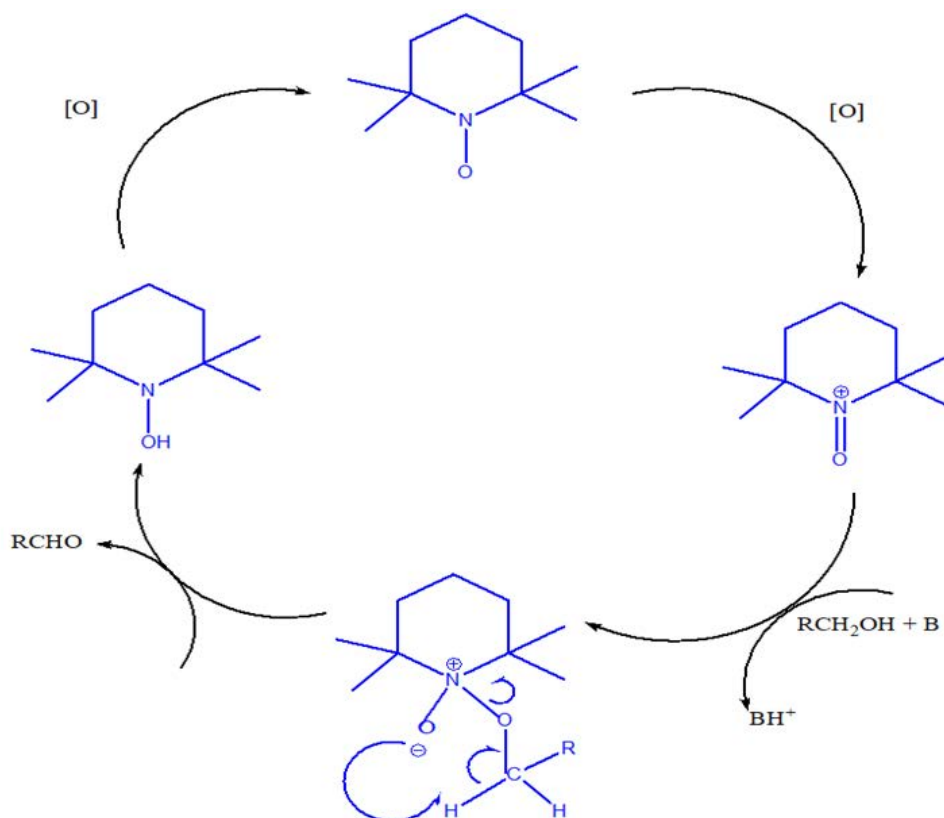
Figure 4: Concentration of PFN-5H vs Time for the addition of TEA at RT, (a) PFN-5 or 4-CT-H in acetonitrile at 25°C (b) the same system at 10°C (c) PFN-5 or 4-CT in acetonitrile at 25°C and (d) PFN-5/TEMPONE-H control in dichloromethane at 25°C

This type of fluorescent intensity vs Time curve surging has broad application in the field of chemistry. The proposed molecule in this study has the same type of property. There have a scope to study that molecule's fluorescent intensity vs time curve.

RESULTS AND DISCUSSION

Application of Nitroxide radical in presence of OEEF:

The figure 5 indicates the scheme. Structure (III) is the polar structure, as N contains the positive charge where O contains the negative charge. Hence, if OEEF is applied in a particular direction (i.e., in a positive direction), this structure will be more stabilized, and the reaction will proceed in a forward direction. Although the TEMPO molecule acts as a catalyst here) OEEF also catalyses the reaction more. By this process, Aldehyde is easily obtained from Alcohol in the presence of a base.



Source: Klinska et al., 2015

Figure 5: Formation of aldehyde from alcohol by using catalyst TEMPO, where OEEF also catalyse the reaction to some extent.

Kinetic Study of reactions accelerated by OEEF:

Polarization is enhanced by resonance stabilization in presence of OEEF which acts as a catalyst (Joy, Stuyver & Shaik, 2020). Where TS is more polar than reactant, It will be needed to apply $F_z > 0$ hence more polarity will stabilize the TS than the reactant and the reaction will be faster, the energy barrier of the reaction can be lowered by this process, and as a consequence, the kinetics of the reactions can be controlled. Importantly, in this case the charges are not optimal for the stabilization of the charge separated resonance contributors to the transition state. When the same charges are placed, they appear in the substrate bound crystal structure, the effects reinforce each other, observe significant catalytic activity. To explore the net effect of charge on radical reaction one can consider a series of Hydrogen transfer reaction and investigate the effect of charge on the TS axis of symmetry

The polar effects of radical reactions will now be considered in the context of standard physical organic chemistry.

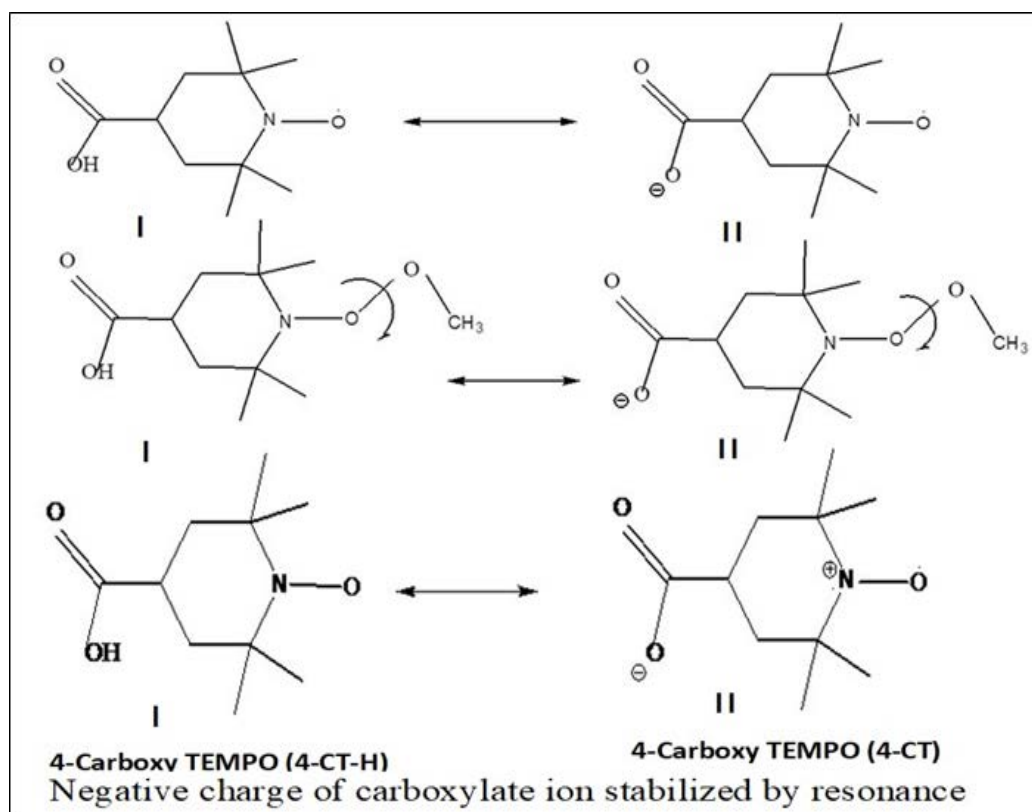
There are three types of polar effects are there. Firstly, the interaction between a charged functional group and a radical. This interaction additionally stabilized by resonance. Resonance is restricted when charged functional group is absent or where this charged functional group is replaced by point charge.

Second type of interaction is that where the radical itself is stabilized by valence bond in absence of charge. Here resonance stability can be enhanced by protonation or deprotonating (Gryn'ova & Coote, 2016).

The third type of interaction is the subject matter of this study. Here the resonance stabilization of radical is enhanced or disrupted not via direct orbital interaction but through space electric field effects. It will be said that the third type of polar effect does not require direct orbital overlap and is replicated by replacing the charged functional group with an equivalent external electric field.

Following the above concept, the following mechanism will be proposed, in which resonance is stabilised via space orbital interaction. In the presence of the charge transfer resonance contributor of electrostatic stabilization, the carboxylic acid group leads to significant of the nitroxide radical (which is formed due to breakage of the peroxide bond).

This type of radical formation can be enhanced from other previous method by the addition of peroxide bond which can replace the any electron donating group like CH_3 , C_2H_5 etc. Figure 6 explain the 4-CT molecule stabilize through space interaction, 4-CT-H where OH group is present.



Source: Shaik, Mandal & Ramanan, 2016

Figure 6: 4-CT molecule stabilize through space interaction, 4-CT-H where OH group is present

Bond dissociation energy is lowered in case of (B) Structure due to presence of through space resonance stabilization of carboxylate ion and NO (Nitroxide radical) this is due the effect of deprotonation of the carboxylic group and resonance stabilization is enhanced.

The gas phase room temperature bond dissociation Gibbs free energy decreases from 261.1 to 242.2KJ/mol due to deprotonation of the carboxylic acid group. It is known that with the increase in

proton donation, the medium will be more acidic. Hence this system is called "pH switch".

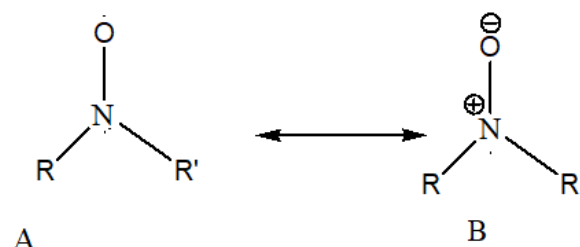
Application of Radical mediated Polymerization:

Nitroxide radical has a wide variety of practical applications. Nitroxide radicals are utilized as spin trap can act as a control agent for free radical polymerization and active species when hindered amine light stabilizers used as a radical trapping antioxidant. It has an application in the redox mediators for dye sensitized solar cell and organic batteries and useful in a variety of imaging and structure determination application. External conditions could be greatly enhanced the utility of nitroxide radicals to manipulate the stability of it. Nitroxide mediated polymerization for the application such as sequence control, block polymerization and end group synthesis can be done by simple pH change (Klinska *et al.*, 2015).

Application of this nitroxide Radical in the field of Chemistry:

Nitroxide radical has a wide variety of practical application. It can act as a effective traps for carbon centered radicals during the self-terminated product formation. Most importantly it can act as a free radical polymerization and are the active species when hindered amine light stabilizers are used as radical trapping antioxidant. In addition they show a redox mediator for dye sensitized solar cells and organic batteries. Nitroxide has the ability to act as a "Radical protecting groups" (Klinska *et al.*, 2015).

By changing the pH in Nitroxide mediated polymerization, it will be able to tune the stability of the radical and can apply it in sequence control, block copolymer synthesis or end group exchange. Figure 7 indicates the two different resonance structures of the nitroxide.



Source: (Klinska *et al.*, 2015)

Figure 7: Resonance contributing structure is B

Here Structure B is more polar than that of structure A. Its effect is strongly dependent on the polarity of the medium, experimental studies shows that while the gas phase pH switches on radical stability should be largely preserved in low polar solvent such as Toluene. The electrostatic pH switches are effectively quenched in water. As in the gas phase solute –solvent interaction is less.

CONCLUSION

OEEF has a budding area of research, waiting for eventual blossom. In this context, the chemical bond breaking, bond dissociation energy, the product selectivity, how OEEF catalyze the reaction, and an overview of fluorescent spectroscopy will be discussed. OEEF can control a variety of named reactions called Diel's alder reaction, Menshutkin reaction, etc. Analysis of radical stabilization in presence of an external electric field can be a potentially useful tool for manipulating the chemical process. By changing the orientation, the ionic charge of the particular ion will be manipulated.

As a consequence, polarizability changes. It can vary from case to case, as it is discussed in the introductory part. The proposal of radical stability through space in the presence of EEF polarity becomes a major parameter in determining the reaction's forward mechanism and future. Although this type of approach has some drawbacks, that is, the field associated with charged functional groups is progressively quenched by polar solvent and lower polarity or lower field is required for the optimum stabilization. All the possibilities are waiting for experimental data, which can be done by computational methodology, which has immense potential in this field.

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Current Perspective on Relevance and Applications of Microbial Enzymes in Industries: A Review

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ABSTRACT

Microorganisms' biocatalytic capacity has been used to make bread, wine, vinegar, and other familiar items for generations, although the biochemical foundation of their constituents is unknown. Microbial enzymes have gained appeal for their widespread application in industries and medicine due to their stability, catalytic activity, and ease of production procedures and optimization. Enzymes' usage in a variety of sectors (e.g., food, agriculture, chemicals, and medications) is fast rising due to its shorter processing time, low energy input, cost efficiency, nontoxic, and environmentally benign features. Toxic chemical substances in industrial and residential wastes (nitriles, amines, phenolic compounds and so on) can also be degraded or transformed by microbial enzymes.

Keywords: *Biocatalysts; Microorganisms; Enzymes; Industries; Non-Toxic; Eco-Friendly*

INTRODUCTION

Enzymes are biological substances or biological macromolecules generated by living organisms that serve as a catalyst for a certain biochemical process. These operate as chemical catalysts in chemical processes, speeding up biological and biochemical reactions both within and outside the cell. Natural enzymes have been employed in the production of items such as linen, leather, and indigo since ancient times. The invention of fermentation techniques was primarily focused at the manufacture of enzymes using specially chosen strains, allowing for the large-scale production of pure, well-characterized enzymes. This breakthrough allowed enzymes to be integrated into real commercial goods and processes, such as in the detergent, textile, and starch sectors. Recombinant DNA technology has helped to enhance manufacturing processes and commercialize enzymes that were previously unavailable (Le Roes-Hill & Prins, 2016). Furthermore, technological advances such as protein engineering and guided evolution altered the marketing of industrially significant enzymes. This advancement in biotechnology is resulting in the development of novel enzymes with new activity and adaptability to new environments, resulting in their increased usage in industrial applications. As a result, enzymes have a wide variety of uses in several sectors, including food, textiles, medicine, dairy, and others. The competence of the genes can be changed to make these new enzymes applying contemporary biotechnology and protein engineering. The goal of this study is to highlight the existing function of microbial enzymes, as well as the current state of their utilization in many sectors and biotechnological potential for future development. Industrial, pharmaceutical and biotechnological processes all rely heavily on enzymes procured from microbial sources. They are profusely used in industries like detergents, textiles, pulp and paper, biofuels and others.

LITERATURE REVIEW

Textile industry

Enzymes are being used more and more in the textile industry to create cleaner processes and reduce raw material consumption and waste generation (Araujo, Casal & Cavaco-Paulo, 2008). Reducing starch size, dissolving glue between the fibre core and the waxes, finishing denims, decomposing

residue hydrogen peroxide after cotton bleaching, wool treatment, and biopolishing may all be done with enzymes including cellulase, amylase, catalase, pectinase, and protease (Aiyer, 2005).

Table 1: Some enzymes used in textile industry with their sources and applications

Enzyme	Source	Applications
Catalase	<i>Aspergillus sp.</i>	Bleach termination
Pectate lyase	<i>Bacillus sp.</i> , <i>Pseudomonas sp</i>	Bioscouring
Cellulase	<i>Aspergillus niger</i> , <i>Penicillium funiculosum</i>	Softening of cotton, finishing of denim
Lipase	<i>Candida antarctica</i>	Denim processing
Amylase	<i>Bacillus licheniformis</i> and other <i>Bacillus sp.</i>	Desizing
Laccase	<i>Bacillus subtilis</i>	Dyeing and bleaching of fabric
Protease	<i>Aspergillus niger</i> , <i>Bacillus subtilis</i>	Processing of wool and silk
Cutinase	<i>Thermomonospora fusca</i> , <i>Pseudomonas mendocina</i> , <i>Fusarium solani pisi</i>	Processing of cotton fiber
Ligninase	<i>Trametes versicolor</i> , <i>Phlebia radiata</i>	Wool processing
Collagenase	<i>Clostridium histolyticum</i>	Wool processing

Feed Industry

Cellulases are low-cost lignocellulosic biomass conversion systems which can further be utilized for generation of biofuels and other products (Choct, 2006). Cellulases and hemicellulases from *Trichoderma reesei* and *Caldicelluloseruptor bescii* efficiently degrade the complex carbohydrates of plants into simple sugars. Feed enzymes improve nutrient digestibility and breakdown undesirable feed components (Kanafusa-Shinkai *et al.*, 2013). Galactosidases, phytases, glucanases, proteases, polygalacturonases, amylases and xylanases are important feed enzymes.

Food Processing

Fungal amylase, lipase, invertase, glucose isomerase, glucoamylase, all are utilised in vegetable fermentations, fruit juices, dairy enrichment, candy, baked goods and jam production (Aravindan, Anbumathi & Viruthagiri, 2007; Aruna, Shah & Birmole, 2014; Camacho & Aguilar, 2003; Grassin & Fauquembergue, 1996; Jooyendeh, Amarjeet & Minhas, 2009; Law, 2009, Al-Maqtari, Waleed & Mahdi, 2019, Panda & Gowrishankar, 2005; Zhu *et al.*, 2017). The FDA has authorised four recombinant proteases for use in cheese manufacturing. Fructose syrup is generated by xylose isomerase. Food additives such as lysozyme and invertase are used. *Bacillus subtilis*, *Bacillus licheniformis*, *Aspergillus oryzae* and *Aspergillus niger* are approved by FDA as "Generally Recognized As Safe" (GRAS) for food processing. Amylases, glucanases, arabinoxylans, proteases, beta-glucanases, pullulanases, amyloglucosidase, and acetolactate decarboxylase are used in the production of alcoholic beverages (Blanco *et al.*, 2014; Choi, Ahn & Kim, 2015; Seo *et al.*, 2016).

Table 2: Some enzymes used in food processing with their sources and applications

Enzymes	Sources	Industrial Applications	Functions
Protease	<i>Aspergillus usarii</i> , <i>Aspergillus niger</i> , <i>Bacillus subtilis</i> <i>Aspergillus flavus</i> , <i>Alcaligenes faecalis</i> , <i>Chrysosporium keratinophilum</i>	Brewing Tenderization of meat Milk coagulation Enhanced bread quality	Restrict haze formation in beverage industry
α -Amylase	<i>Bacillus amyloliquefaciens</i> , <i>Bacillus stearothermophilus</i> , <i>Bacillus licheniformis</i> , <i>Streptomyces</i> , <i>Rhizopus</i>	Baking, brewing, starch liquefaction, Bread quality improvement, Clarification of fruit juice	Flour adjustment, bread softness in baking. Starch hydrolysis in beverage industry
Glucoamylase	<i>Aspergillus niger</i> , <i>Aspergillus awamori</i> , <i>Rhizopus oryzae</i>	Beer production Manufacture of glucose and fructose syrups	
Lactase (β -galactosidase)	<i>Kluyveromyces lactis</i> , <i>Kluyveromyces fragilis</i>	Used to reduce Lactose intolerance, Ingredients of Prebiotic food	
Lipase	<i>Aspergillus niger</i> , <i>A. oryzae</i> , <i>Penicillium camemberti</i> , <i>Rhizopus miehei</i> , <i>Candida antarctica</i> , <i>Candida cylindracea</i> <i>Ay30</i> , <i>Candida rugosa</i> , <i>Pseudomonas sp.</i> , <i>Helvina lanuginosa</i> , <i>Geotrichum candidum</i>	Cheese flavour development, Cheddar cheese production	
Phospholipase	<i>Fusarium oxysporum</i> , <i>Bacillus licheniformis</i>	Development of flavour of cheese, lipolyzed milk fat production	
Esterase	<i>Lasiodiplodia theobromae</i>	Flavour and fragrance augmentation in fruit juice, De-esterification of dietary fibre.	
Cellulase	<i>Aspergillus sp.</i> , <i>Trichoderma sp.</i> , <i>Bacillus sp.</i> , <i>Paenibacillus sp.</i>	Enhancement of flavour of fruit juice	
Pectinase		Enhancement of flavour of fruit juice	
Glucose oxidase	<i>Aspergillus niger</i> , <i>Penicillium glaucum</i> , <i>Aspergillus niger</i> , <i>Penicillium adametzii</i> .	Increase in shelf life of food, enhancement of flavour of food	
Laccase	<i>Funalia trogii</i> , <i>Bacillus licheniformis</i>	Removal of polyphenol from wine, used in baking	
Xylanase	<i>Streptomyces sp.</i> , <i>Bacillus sp.</i> , <i>Pseudomonas sp.</i> <i>Aspergillus sp.</i> , <i>Fusarium sp.</i> , <i>Penicillium sp.</i>	Enhancement of flavour of fruit juice, Enhancement of quality of Beer	

Peroxidase	<i>Phanerochaete chrysosporium</i> , <i>Streptomyces viridosporus</i> T7A	Food flavour, colour, and nutritional quality enhancement	
α -Acetolactate dehydrogenase	<i>Brevibacillus brevis</i>	Beer maturation and shortening	
Asparaginase		During baking, the generation of acrylamide is reduced.	
Debittering enzymes - naringinase	<i>Penicillium</i> , <i>Rhizopus</i> , <i>Circinella</i> , <i>Trichoderma</i> , <i>Fusarium</i> , <i>Aspergillus niger</i> , <i>Eurotium</i> , <i>Bacteriodes distasonis</i> , <i>Bacillus</i> <i>sp.</i> , <i>Thermomicrobium roseum</i> , <i>Burkholderia cenocepacia</i> , <i>Pseudomonas paucimobilis</i> .	Enhancement of wine scent and removal of bitter flavour in fruit juice	
Catalase	<i>Bacteroides fragilis</i> , <i>Enterococcus faecalis</i> , <i>Bacillus maroccanus</i> , <i>Pyrobaculum calidifontis</i>	Preservation of food, Employed in production of cheese for hydrogen peroxide removal from milk	

Pulp and paper industry

The use of microbial enzymes in this industry decreases processing time, energy usage, and chemical use (Fu, Chan & Minns, 2005). Xylanase, ligninase, laccase, mannase, amylase, lipase, cellulase, hemicellulase, and esterase breakdown starch to reduce viscosity, making it easier to size, deink, and coat paper (Farrell, Hata & Wall, 1997; Gutiérrez, Del Río & Martínez, 2009; Pasha, Anuradha & Subbarao, 2013; Patrick, 2004; Srivastava & Singh, 2015). Lipases lower pitch, lignin-degrading enzymes remove lignin to soften paper for esterification and cellulases and hemicellulases aid in water drainage, fibre smoothing, and ink removal.

Table 3: Some enzymes used in pulp and paper industry with their sources and applications

Enzyme	Source	Applications
Laccase	<i>Bacillus subtilis</i>	Non-chlorine bleaching, delignification
Protease	<i>Bacillus subtilis</i>	Biofilm removal
Amylase	<i>Bacillus licheniformis</i>	Deinking, drainage improvement
Lipase	<i>Candida antarctica</i>	Pitch control
Mannase		Degrades glucomannan to improve brightness in paper
Ligninases		Removal of lignin and hemicellulose
Xylanase	<i>Aureobasidium pullulans</i> , <i>Trichoderma reesei</i> , <i>Thermomyces lanuginosus</i> ,	Improvement of Bleaching
Cellulase	<i>Bacillus sp.</i> , <i>Aspergillus niger</i>	Deinking, drainage improvement

Polymer industry

The ecologically friendly in vitro microbial enzyme catalyses production of biodegradable polymer and has various benefits over traditional chemical techniques. Biopolymers are ecologically benign materials, made from renewable carbon sources and decompose after use into renewable nutrient resources to be recycled in the environment again. To synthesise materials in situ via polymerization

Relevance and applications of microbial enzymes

processes, peroxidases, transglutaminases, lipases, and laccases tend to produce cross-links in biopolymers (Lang & Cotteret, 2004; Vroman & Tighzert, 2009).

Table 4: Some enzymes used in polymer industry with their sources and applications

Enzyme	Source	Applications
Glucose oxidase	<i>Aspergillus niger</i> , <i>Penicillium chrysogenum</i>	Polymerization of anilines
Lipase	<i>Candida antarctica</i>	lactone polycondensation and polymerization (ring opening), carbonates
Transglutaminase	<i>Streptomyces mobaraensis</i>	Crosslinking of protein
Tyrosinase	<i>Trichoderma reesei</i>	lignin and chitosan polymerization
Laccase	<i>Trametes hirsuta</i>	Bisphenol polymerization

Detergent industry

Microbial enzymes such as lipase, protease, cellulase, amylase, mannanase and peroxidase are added to detergents to catalyse chemical bond breakdown under high temperature (60°C) and highly alkaline (pH 9–11) conditions. These enzymes help remove protein stains, insoluble starch in dishwashing, oils and fats, and they also help detergents work better (Hasan *et al.*, 2010; Keshwani, Malhotra & Kharkwal, 2015).

Table 5: Some enzymes used in detergent industry with their sources and applications

Enzyme	Source	Applications
Protease	<i>Aspergillus oryzae</i> , <i>Bacillus subtilis</i>	Protein stain removal
Amylase	<i>Aspergillus sp.</i> , <i>Bacillus subtilis</i>	Carbohydrate stain removal
Cellulase	<i>Aspergillus niger</i> , <i>Bacillus sp.</i>	Colour clarification
Lipase	<i>Aspergillus oryzae</i> , <i>Aspergillus flavus</i>	Fat stain elimination
Mannanase	<i>Bacillus sp.</i>	Mannan spot removal
Cutinase	<i>Fusarium solani f. pisi</i>	Triglyceride removal

Leather industry

The use of enzymes as chemical alternatives in leather manufacturing has been shown to improve leather quality while also lowering pollution (De-Souza & Gutterres, 2012; Money, 1996). In this industry, alkaline lipases from *Bacillus* strains that thrive in very alkaline conditions are employed in conjunction with other alkaline or neutral proteases (Choudhary, Jana & Jha, 2004).

Table 6: Some enzymes used in leather industry with their sources and applications

Enzyme	Source	Applications
Amylase	<i>Aspergillus sp.</i> , <i>Bacillus subtilis</i>	Fiber splitting
Alkaline protease	<i>Alcaligenes faecalis</i>	Removal of hair, bating
Neutral Protease	<i>Bacillus subtilis</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>	Removal of hair, soaking
Lipase	<i>Aspergillus oryzae</i> , <i>Aspergillus flavus</i>	Removal of grease

Cosmetic industry

Retinoids (vitamin A and derivatives) are widely used in cosmetics and medications, including skin care products. The manufacture of water-soluble retinol derivatives is accomplished with immobilised lipases (Cho, Cho & Han, 2007). Lipases are employed in the creation of hair waves and have also been utilised as components in topical anti-obesity treatments or as an oral administration (Babizhayev, 2006).

Table 7: Some enzymes used in cosmetic industry with their sources and applications

Enzyme	Source	Applications
Endoglycosidase	<i>Mucor hiemalis</i>	Teeth and gum tissue care
Superoxide dismutase	<i>Corynebacterium glutamicum</i> , <i>Lactobacillus plantarum</i>	Free radical scavenging, skin care
Laccase	<i>Bacillus subtilis</i> , <i>Trametes versicolor</i>	Hair dye
Protease	<i>Bacillus subtilis</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	Removal of dead skin
Lipase	<i>Aspergillus flavus</i> , <i>Aspergillus oryzae</i>	Skin care

Waste management

Enzymes are widely used in management of waste, and a multitude of enzymes are involved in the breakdown of harmful substances found in industrial effluents and household garbage. Amyloglucosidases, amylases, amidases, glucoamylases, cellulases, lipases, pectinases, nitrile hydratases and proteases are some of the enzymes used in waste treatment (Kuhad, Gupta & Singh, 2011). They are used to encourage the removal of hazardous compounds from industrial effluents as well as the recycling and reuse of garbage (Masse, Kennedy & Chou, 2001).

Table 8: Some enzymes used in waste management with their sources and applications

Enzyme	Source	Applications
Amyloglucosidase	<i>Aspergillus niger</i>	Hydrolysis of starch for bioremediation
Amidase	<i>Rhodococcus erythropolis</i>	Wastes containing nitriles are degraded.
Cutinase	<i>Fusarium solani f. pisi</i>	Plastic degradation, Polycaprolactone
Amylase	<i>Bacillus licheniformis</i> , <i>Aspergillus sp</i>	Vegetable waste bioremediation
Lipase	<i>Aspergillus oryzae</i> , <i>Candida tropicalis</i>	Hydrocarbons from crude oil are degraded.
Protease	<i>Chrysosporium keratinophilum</i>	Keratinic waste bioremediation
Manganese peroxidase	<i>Phanerochaete chrysosporium</i> , <i>Coprinus cinereus</i>	Phenolic molecules are degraded.
Laccase	<i>Trametes versicolor</i>	Waste comprising olefin units, polyurethane, and phenolic chemicals is degraded.
Oxygenase	<i>Pseudomonas sp.</i> , <i>Rhodococcus sp.</i>	Halogenated pollutants are degraded.
Nitrile hydratase	<i>Rhodococcus sp.</i>	Wastes containing nitriles are degraded.
Lignin peroxidase	<i>Phanerochaete chrysosporium</i> , <i>Coprinus cinereus</i>	Phenolic molecules are degraded.

Therapeutic applications of microbial enzymes

Anticoagulants, oncolytics and thrombolytics are only a few of the applications for therapeutic enzymes, as well as substitutes for metabolic deficits (Kaur & Sekhon, 2012; Mane & Tale, 2015). Proteolytic enzymes have anti-inflammatory properties.

Table 9: Some enzymes used as therapeutic agents with their sources and applications

Applications	Source	Enzymes
Antibiotic synthesis	<i>Penicillium sp.</i>	Penicillin oxidase, rifamycin B oxidase
Antitumor	<i>Pseudomonas acidovorans</i> , <i>Escherichia coli</i> , <i>Acinetobacter</i> , <i>Beauveria bassiana</i>	L-tyrosinase, L-Asparaginase, galactosidase L-glutaminase
Antioxidants	<i>Lactobacillus plantarum</i> , <i>Corynebacterium glutamicum</i>	Superoxide dismutases, glutathione peroxidases, catalase
Anticoagulants	<i>Streptococcus sp.</i> , <i>Bacillus subtilis</i>	Streptokinase, urokinase
Anti-inflammatory	<i>Mycobacterium sp.</i> , <i>Lactobacillus plantarum</i> , <i>Corynebacterium glutamicum</i> , <i>Nocardia sp.</i>	Superoxide dismutase, Serrapeptase
Antiviral	<i>Saccharomyces cerevisiae</i>	Ribonuclease, Serrapeptase
Resistance to Antibiotics	<i>Citrobacter freundii</i> , <i>Klebsiella pneumonia</i> , <i>Serratia marcescens</i>	Lactamase
Skin ulcers	<i>Clostridium perfringens</i>	Collagenase
Detoxification	<i>Pseudomonas aeruginosa</i>	Laccase, rhodanese
Cyanide poisoning	<i>Sulfobacillus sibiricus</i>	Rhodanase
Digestive disorders	<i>Candida lipolytica</i> , <i>Bacillus spp.</i> , <i>Aspergillus oryzae</i>	Amylase, lipase
Gout	<i>Aspergillus flavus</i>	Uricase

Application in molecular biology

DNA ligases, restriction enzymes, and polymerases are used in genetic engineering to alter DNA for restriction digestion and polymerase chain reactions. In addition, they can be employed in forensic science (Le Roes-Hill & Prins, 2016).

DISCUSSION

Industry-produced microbial enzymes are derived from a variety of microorganisms, including bacteria, fungus, and yeasts (Kirk, Borchert & Fuglsang, 2002). Enzymes made in factories with the aid of microbes were discovered to have high biological activity. Enzymes are cost-effective and can be generated in big quantities (Gurung *et al.*, 2013; Singh *et al.*, 2016; Zhang, 2011). In compared to plant and animal sources, the isolation and purification of enzymes from microbial sources is easier. Microbial sources can produce a wide range of enzymes under a variety of environmental circumstances in a short amount of time and space. To increase the number of enzymes generated by microbial sources, genetic modification is used (Adrio & Demain, 2014). Traditional means to Reducing starch size, dissolving glue between the fibre core and the waxes, finishing denims, decomposing residue hydrogen peroxide membrane filtering techniques combined with downstream processing. Thus, industries are keenly interested in employing enzymes for making the procedure less complex, energy saving and moreover to keep the environment pollution free.

CONCLUSION

Microbial enzymes may be used to clean water sanitization systems, for decontamination of effluents from petrochemical industries, paper and pulp industry, food, detergent, pharmaceutical, and paper

sectors textiles, for bioremediation of herbicides, as a medical diagnostic tool, as a cleaning agent for water purification systems, as cosmetic constituents, as a catalytic agent in drug manufacturing and as pesticides. Because of their environmentally benign nature, effective procedure regulation, little purifying expenses, high yield, and process safety, enzymatic hydrolysis and enzyme-based technologies are now favoured over chemical processes.

Different fermentation processes, such as solid-state and submerged fermentations, can manufacture microbial enzymes more effectively than plant and animal enzymes. It is also simple to mass-produce microbial enzymes. Various molecular and biological techniques can be used to modify microbial enzymes. Overexpression of microbial enzyme genes can result in hyperproduction of enzymes with high specific activity. Many microbial enzymes are still to be discovered, and there are numerous prospects for developing greater commercial applications of microbial enzymes, for most of the industries.

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Human Impacts on Nitrogen Cycle

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ABSTRACT

Human beings have played the role of a major force in bringing about a significant change in our global ecosystems. In fact, among the various attributes of our ecosystems, anthropogenic activities have been crucially affecting the biogeochemical cycles. The human-mediated alterations of the earth's nitrogen cycle are having a very perturbed impact upon the world's ecosystems as the nitrogen element is key to living beings and its attainability plays a critical part in maintaining the balanced performance of the biotic and abiotic components of our ecosystem. Human activities such as the use of fossil fuels, the use of nitrogen-based fertilizers, and nitrogen-producing industrial and agricultural pursuits have significantly increased the nitrogen content of our ecosystem. The pollution caused due to the addition of excess nitrogen to the different constituents of the ecosystems alters both their ecological operations as well as the living communities they support. Other than the increase in the supply of nitrogen by human activities, the global intensification of the drifting of nitrogen in its various forms in the environment has also been observed. Because of this increased mobility, excess nitrogen introduced to the ecosystem initiates serious and long-term environmental consequences. The data obtained from the latest research studies indicates that the huge amount of nitrogen introduced on account of human activities also contributes to climate change. However, such environmental desecration can be minimized by the adoption of time-honored sustainable practices. Therefore, it is high time that state and global decision-making should strive to bring down these detrimental effects through the growth and extensive distribution of more well-planned and effective management practices of human activities that reduce them.

Keywords: *Nitrogen Cycle; Nitrogen Fixation; Nitrogen Saturation; Acidification of Water; Eutrophication of Water*

INTRODUCTION

Nitrogen (N_2) forms one of the chief and primary nutrients present in our ecosystem, which is extremely necessary for the continuity of life. Nitrogen forms the essential constituent of many biological molecules in the living world, including proteins, DNA, and chlorophyll. The intricate interplay of the biological and geochemical processes (like the other nutrient elements: carbon, sulphur, phosphorus) maintains the continuous circulation of nitrogen through all the components of the ecosystem for the sustenance of life and the environment. Nitrogen, like any other nutrient element, also moves from one component to the other in the environment in a closed loop of "cycle", which is termed the Nitrogen Cycle. The cycle has been in operation on Earth for billions of years, converting the non-biological form of nitrogen in the atmosphere into various biologically useful forms used by living organisms. This particular conversion is called Nitrogen Fixation. According to the information from the Science paper, the nitrogen cycle has undergone several major changes since pre-biotic times. The geological processes like volcanic processes and lightening were the key factors in regulating the cycle, and then gradually the anaerobic organisms also took over, as the biological activities commenced. It was about 2.5 billion years ago that the appearance of molecular oxygen (O_2) on the earth, as a result of microbial processes, led to the evolution of the modern nitrogen cycle.

The supply of nitrogen in the terrestrial and aquatic ecosystems forms the essential factor in controlling the character and variety of autotrophic plants, the biological processes of heterotrophic consumers

and the indispensable ecological processes. On account of heavy amounts of nitrogen addition, the ecosystems get polluted, altering both the operation of ecological processes and the living communities they sustain.

With the advent of the 20th century, anthropogenic activities and their effects on the nitrogen cycle started to rise extremely high, driven by extensive industrialization, farming, and agricultural practises to meet the demands of global production, consumerism, and Gross Domestic Production (GDP). Among the various pursuits of humans, the chief culprit has been the burning of fossil fuels-which brings about the release of nitrogen oxides into the atmosphere, which in turn combines with other elements of the atmosphere to form smog and acid rain-affecting the nitrogen cycle. The massive effect of human activities on the global nitrogen cycle started to be felt and observed from the mid-1900s onwards. Along with the burning of fossil fuels, the making of fertilizers by humans has significantly contributed to altering the fixed nitrogen present in the ecosystems of the earth. The prediction of some environmental scientists is that by 2030, the nitrogen fixation brought about by human activities would surpass the amount of nitrogen that is fixed by microbes. The unprecedented surge in nitrogen that remains available has altered the ecosystems by augmenting primary production, carbon shortage and eutrophication. Ecologists and environmentalists have focused their attention on the importance of nitrogen in ecosystems and the significant impact of human activities on nitrogen and its transformations, emphasising the development of technology and management to pave the way for sustainable living and the protection of our ecosystems and environment (Bernhard, 2010).

Local human activities are chiefly responsible for the augmentation of global nitrogen on a large scale. Besides, human activities mediated increased supplies of nitrogen have also enhanced the flow of nitrogen in its various forms globally through the different constituents of the environment. The increased mobility of surplus nitrogen resulting from human activities has a grave and long-term environmental consequence. It is truly a painful lesson to realize that the things human beings depend on to make their lives more comfortable can also kill them. Human addiction towards fossil fuels is the obvious example, but at the same time, they are addicted to nitrogen too, and it now appears that the combination of climate change and nitrogen pollution is multiplying the possibility of wrecking the world around them. (Bernhard, 2010).

LITERATURE REVIEW

What is nitrogen cycle?

Nitrogen plays a very vital role in the lives of every living creature. It forms the most abundant element in our atmosphere, existing as dinitrogen gas (N_2). But nitrogen remains mostly unreachable to the living components of the ecosystems in this particular form, often limiting its primary productivity. It is only the conversion from the dinitrogen gas form of nitrogen to ammonia (NH_3) that makes the availability of nitrogen to primary producers possible. The inorganic form exists freely in the environment and the organic form exists in living beings as the transfer of nitrogen takes place through the food chain from one organism to another by the process of eating and being eaten. Thus, the nitrogen cycle brings about the various transformations of nitrogen in the ecosystem, making it available to all the living organisms. The major processes of nitrogen transfer involve nitrogen fixation, nitrification, denitrification, and ammonification. The conversion of nitrogen takes many forms through the biogeochemical cycle called the Nitrogen Cycle, with nitrogen being sequentially transferred from the atmosphere to the soil, then to organisms, and again back into the atmosphere. The transformation of nitrogen into its various forms takes place in the environment, which helps to maintain a balance in the ecosystem (Bernhard, 2010).

Three major steps of Nitrogen cycle:

Nitrogen Fixation – Nitrogen is made available to living organisms for the building of proteins, DNA, and other key biological compounds. In this way, N_2 is converted into a biologically available form, and this process is called "nitrogen fixation." This particular process is carried out by a selected group of

prokaryotes that carry out this energy demanding process. Although the maximum amount of nitrogen is fixed by the prokaryotes, some nitrogen can be fixed abiotically by lightning, which supplies energy for mediating the reaction of N_2 with oxygen, producing oxides of nitrogen (NO , NO_2). These nitrogen oxides enter the soil through rain and snow. Some of the prokaryotes that bring about nitrogen fixation are free-living, while others live symbiotically (*Rhizobium*) in close association with the host plant to carry out the process. Though the prokaryotes manifest a diversity of phylogenetic and physiological differences, they all have a similar enzyme complex called Nitrogenase that catalyzes the reaction, bringing about the reduction of N_2 to NH_3 (ammonia). Due to its high sensitivity to oxygen, the enzyme gets deactivated in the presence of oxygen. Symbiotic bacteria make this nitrogen available to their host plants by converting the inert nitrogen to its usable form – such as nitrites and nitrates.

Nitrification – This particular step occurs in the soil, which involves the conversion of ammonia to nitrites (NO_2^-) and nitrates (NO_3^-). Some of the soil prokaryotes (nitrobacter) convert the nitrites into nitrates and others (nitrosomonas) convert ammonia into nitrites. Both these prokaryotes can act only in the presence of oxygen. Nitrate is a more stable molecule than nitrite and is the chief source of nitrogen for primary producers. The nitrate form is mainly absorbed and assimilated by plants and animals directly.

Denitrification - In this particular stage of the nitrogen cycle occurring in soil and aquatic habitats, the nitrate is converted into nitrogen gas. Denitrification is carried out with the aid of diverse groups of prokaryotes (*genera Bacillus, Paracoccus, and Pseudomonas*). This particular step is important because it takes out nitrogen (nitrate) that had remained fixed within the living biomass of the ecosystem, returning it back to the air in the biologically inert form (N_2). Dinitrogen (N_2) gas, which forms the end product of denitrification, also exists with the other forms of nitrogen. Among those forms is nitrous oxide (N_2O), which acts as an important greenhouse gas, undergoing reaction with ozone and causing air pollution.

Ammonification – Here, the nitrogen present in the organic form (amino acids, DNA) of the biotic organisms is released back into the ecosystem as ammonia. Generally, when the living organisms die or excrete their organic wastes, the various fungi and prokaryotes of the detritus food chain start decomposing and breaking down the organic nitrogen, transforming it into ammonia, which finally becomes available to the plants and microorganisms for growth.

- **Significance of Nitrogen cycle-**

Nitrogen is a critical component for the survival of life on Earth. cycling is very essential for maintaining a productive and healthy ecosystem, keeping it in a stringently regulated manner. Nitrogen forms the significant component of many cells and biological operations, playing a very significant part in the sustenance of life in our ecosystem (Bernhard, 2010).

Quantity of nitrogen in normal environment:

Nitrogen, as it comes in many forms in our environment, is significant to primary producers for their survival. Plants cannot use the Di-nitrogen (N_2) or atmospheric form of nitrogen, but it can be found in significant quantities in various organic and inorganic forms on the earth's crust. Nitrogen present in its organic configurations constitutes a very huge share of the total nitrogen content present on land. The amount of nitrogen produced by the nitrogen fixing bacteria approximately stands to 32-53 Tg/year (Galloway *et al.*, 1995).

- **Environmental issues of surplus nitrogen levels**

Nitrogen entering in huge amounts to our environment – generally resulting out of diverse human activities –pollute the atmosphere and water bodies extensively. The impact of nutrient mediated pollution has also been manifested in many water bodies like - streams, rivers, lakes, bays and coastal waters for the past several decades, creating a detrimental impact on our environment, health and economy.

The introduction of unwanted and excess quantity of nitrogen triggers the burgeoning of aquatic plants and algae, which in turn causes the clogging of water intakes, utilization of dissolved oxygen on account of their decomposition and obstructing the entry of light to the bottom levels of water. Eutrophication of water bodies produces dense formations of algae on the surface of water bodies, which on certain instances causes the killing of aquatic animals of the water bodies, and can even proceed to the "killing" a lake by causing oxygen deprivation. The effect on the efficiency of respiration of aquatic organisms is also observed, having a massive effect in the reduction of diversity of aquatic organisms (Galloway *et al.*, 1995).

- **Human mediated fixation of nitrogen**

Human activities since the past century have considerably augmented the fixation of terrestrial nitrogen at a significant rate, pragmatically causing the annual transfer of nitrogen to be doubled, converting the vast but unavailable pool of nitrogen present in the atmosphere to the forms that have been made available biologically. The major sources causing such a magnified supply include the manufacturing of nitrogen fertilizers by industrial processes, the kindling of fossil fuels; and the raising of agricultural crops like soybeans and peas that host nitrogen-fixing bacteria by forming a symbiotic relationship with them. Besides, anthropogenic activities are speeding up the process of liberation of nitrogen from sources like soils and organics where it has remained locked up for a very long term. Different human activities continue to increase the amount of nitrogen oscillating between the biotic and abiotic components of the ecosystem. The excessive amount of nitrogen pollutes the ecosystem and alters the ecologically functioning processes and the living communities supported by them.

The primary human activities blamed for the increase in nitrogen on the planet are on a local scale, ranging from the production and use of nitrogen fertilizers to the combustion of fossil fuels. However, it is not only the increase in supply that has been achieved by the effect of human activities, but they have also magnified the mobilization of nitrogen in its various forms through the different constituents of the ecosystem. Because of this increased mobility, the huge amount of nitrogen injected into our ecosystem through the activities mediated by human beings has grave and extended environmental consequences for large realms of the Earth. Global nitrogen fixation contributes to about 413 Tg of nitrogen (N) to ecosystems sustained in terrestrial and marine environments per year, of which human affairs account for about 210 Tg of N, which amounts to almost half of the global nitrogen fixation.

According to graphical data from a report published in *Issues in Ecology* in 1997, the research work carried out by professor Peter Vitousek and his colleagues, the human activities contributing to the generation of nitrogen like burning of biomass and organic matters mediate the transfer of more than 40 Tg annually; the conversion of forests and grasslands into agricultural lands adds about 20 Tg per year. In fact, human activities inject approximately 140Tg of nitrogen into the global nitrogen cycle each year (Erisman *et al.*, 2013).

RESULTS

Human activities impacting the Nitrogen cycle:

- **Production and utilization of Nitrogen Fertilizer**

The human mediated fixation of nitrogen by industrial processes to apply as fertilizer currently amounts to approximately 80 Tg per year and represents the largest share of the contribution made by humans of newly added nitrogen to the global cycle. This quantum excludes organic nitrogen fertilizers and manures, which are the transfer of nitrogen which has already been fixed, from one place to another rather than the fixation of new nitrogen. The industrial method of nitrogen fixation was first developed for the first time in Germany during World War I, and since the 1940s its growth has been taking place enormously. In recent years, the tremendous expansion of production has been found to be remarkable to meet the demand of a growing capitalist economy. The application of the quantity of fixed nitrogen produced by industrial methods to the crops from 1980 to 1990 has surpassed all the industrial fertilizer that has been applied in human history. The fertilizer produced industrially during the

late 1970s was mostly utilized in developed countries. In the present scenario, the use of fertilizer among the developed countries has been brought under control, but its application in the developing countries has been observed to rise greatly. The increase in human population growth and an increasing trend of urbanization have created an ever-increasing demand for food production to satiate hunger, ensuring a parallel increase in industrial fertilizer production to continue for decades (Erisman *et al.*, 2013).

- **Propagation of Crops**

About one third of the Earth's terrestrial surface is dedicated to the utilities of agriculture and pastoralism, as a result of which diverse natural vegetation with monocultures of leguminous crops (soybeans, peas, beans, lentils) has replaced large extents of land comprising of natural vegetation manifesting huge diversities and forages has been brought about by humans because of the demand for protein-rich food. Since these plants house symbiotic nitrogen-fixers, a good amount of the nitrogen is obtained from the atmosphere directly by them, which greatly enhances the rate of nitrogen fixation that had been occurring earlier on those lands. Substantially, a good amount of fixation of nitrogen also occurs in the cultivation of some non-legume plants, like rice. All of these represent the newly introduced stocks of biologically available nitrogen generated as a consequence of human activities. The quantum of nitrogen that the crops fix is more difficult to analyze than the production of nitrogen mediated by the industries. The estimates have ranged from 32 to 53 Tg per year (Erisman *et al.*, 2013).

- **Fossil Fuel Burning**

Humans have endeavored for the production of goods and commodities to cater to the call of consumerism and economic growth by the burning of fossil fuels, as a result of which the nitrogen that remained locked in the geological sources got released into the atmosphere in the form of its gases. The release of nitric oxide gas into the atmosphere as a result of the combustion of fossil fuels causes smog and acid rain, as well as contributing to the global reactive nitrogen load. Nitrous oxide (N₂O) is also the primary greenhouse gas, causing the greenhouse effect and climate change. The combustion activities occurring at high temperatures even fix a trifling amount of atmospheric nitrogen directly. The functioning of automobiles, factories, industries, power plants, and other combustion processes causes the emission and introduction of fixed nitrogen to our environment. That amounts to more than 20 Tg per year in the atmosphere. All of these are considered newly fixed nitrogen since they have remained locked up for millions of years under the influence of geologically high temperature and pressure, and would continue to remain locked up indefinitely if human actions don't allow their liberation (Erisman *et al.*, 2013).

- **Mobilization of Stored Nitrogen**

Other than increasing fixation and release of nitrogen from geological reservoirs, nitrogen liberation from long-term biological storage pools like terrestrial organic matter contributes further to the enhancement of biologically available nitrogen. The draining of wetlands also mediates mobilization of 10 Tg per year or more of nitrogen by facilitating the oxidation of organic matter present in the soil; land clearing for crops also mobilizes 20 Tg per year from soils. Taken together, these human activities cause significant changes in the earth's nitrogen cycle (Galloway *et al.*, 1995).

Human versus Natural Nitrogen Fixation:

The terrestrial ecosystems experience the introduction of approximately 140 Tg of new nitrogen each year due to the production of legume crops and fertilizer and also fossil fuel burning, which is equated to the upper estimates of nitrogen naturally fixed by ecosystem organisms. Various other human activities also liberate half that much nitrogen and make it available to our environment. All the ground level studies point to the conclusion that the transfer of nitrogen from the atmosphere into the terrestrial biological nitrogen cycle has been doubled by human activities. This amount of excess nitrogen is distributed in an uneven manner across the Earth's surface, where the northern hemisphere gets

profoundly affected while the southern hemisphere receives a very small amount of direct input of human generated nitrogen (Erisman *et al.*, 2013).

Ecological consequences of human mediated changes of nitrogen cycle:

Human activities have significantly influenced the nitrogen cycle. The burning of fossil fuels, the application of nitrogen-based fertilizers, and other activities dramatically increase the amount of nitrogen that remains available biologically in an ecosystem. Huge changes in the amount of nitrogen available bring about severe changes in the nitrogen cycle in both aquatic and terrestrial ecosystems. The exponential increase of industrial nitrogen fixation and other human activities has been found to double the amount of global nitrogen fixation since the 1940s.

The introduction of nitrogen in the terrestrial ecosystem can lead to a number of alterations in our environmental components, like nutrient imbalance in trees, forest health degradation, and the diminishing of biodiversity. A change in carbon storage has also been experienced with an increase in nitrogen availability, thus ramifying the impacts upon other processes than just the nitrogen cycle. The extensive application of fertilizers in agricultural systems to achieve the increment of plant production, generally in the nitrate forms, leaches the soil out, which enters the water bodies, and ultimately makes its presence in our drinking water.

Nitrogen applied to agricultural and urban areas ultimately makes its way to the water bodies (rivers and near shore coastal systems). An increase in the amount of nitrogen in coastal marine ecosystems often leads to the condition of anoxia (no oxygen) or hypoxia (low oxygen), creating alterations in the biodiversity, changes in the food web structure, and general habitat degradation. A common consequence of nitrogen getting elevated is a rise in harmful algal blooms. Certain types of dinoflagellates (algal blooms) have been associated with a high level of mortality in fish and shellfish due to their toxicity. Even without any catastrophic effects on the ecosystem, nitrogen addition may lead to overall changes in ecosystem function. Results of many field studies have also indicated that changes in the nitrogen cycle can also bring about a higher risk of parasitic and infectious diseases among humans and wildlife. Furthermore, increased nitrogen levels in aquatic systems cause acidification of freshwater ecosystems (Hu, 2018).

Nitrogen saturation and ecosystem functioning:

The natural deficiencies of nitrogen in an ecosystem at a particular point are fully relieved, but still growth of the plants get restricted due to the shortage of other resources such as phosphorus, calcium, or water. When no further response from the vegetation are manifested towards further inclusions of nitrogen, it clearly indicates that the ecosystem has reached the complete nitrogen saturation stage. Theoretically, completely nitrogen-saturated ecosystems and its components are unable to utilize or hold the new nitrogen deposits anymore, as a result of which the excess nitrogen gets dispersed to streams, groundwater, and the atmosphere. Many harmful consequences for the ecosystem's health and functioning has been accounted for nitrogen saturation. The impacts of such consequences became apparent for the first time in Europe about two decades ago when observation by the scientists in some lakes and streams found that as a result of significant increase in nitrogen concentration extensive yellowing and loss of needles in spruce and other conifer forests was seen. In a similar manner a number of field experiments conducted in the U.S. and Europe have come up with the revelation of a complex outpouring of effects brought about by the excess nitrogen in forests soils. Due to the building up of ammonium in the soil, it gets converted to nitrate by bacterial action, a process that causes the release of hydrogen ions causing the acidification of soil. The building up of nitrate enhances the emission of nitrous oxides from the soil and also enhances draining away of highly water-soluble nitrate into terrestrial and ground water bodies. The swiping away of the negatively charged nitrates also results in the carrying of positively charged alkaline minerals such as calcium, magnesium, and potassium. Therefore, the modifications of the nitrogen cycle caused due to the impact of human activities decreases the fertility of soil by greatly accelerating the loss of calcium and other nutrients which are vital for the growth of plants. Trees growing in soils well-stocked with nitrogen

but deficient in calcium, magnesium, and potassium expresses nutrient imbalances in their roots and leaves. This also leads to the reduction of their photosynthetic rate and efficiency, resulting in abnormalities in their growth and development and even deaths (Hu, 2018).

Impacts on the atmosphere:

Alterations in the nitrogen cycle have been the major outcome of human-driven sources, like the increased emissions of nitrogen-based trace gases such as nitrous oxide, nitric oxide, and ammonia (NH_3), mediating its significant effect on the chemistry of the atmosphere at the regional and global level. The trace amounts of nitrogen gases both in the airborne and in the deposited form cause environmental effects, like the nitrous oxide, which remains sustained for a long time in the atmosphere and contributes to the human-driven rise of the greenhouse effect, which adds to the warming of the Earth's climate. Nitric oxide also forms an important precursor of acid rain and photochemical smog. Essentially all of the more than 20 Tg per year of fixed nitrogen released from automobile exhausts and other emissions from fossil fuel burning is released into the atmosphere as nitric oxide. Intensive fertilization of agricultural soils also increases the rate at which nitrogen in the form of ammonia is released into the atmosphere. The speeding up of the breakdown of ammonium and nitrates by the microbes in the soil escalates the release of nitrous oxide (National Science Foundation, 2010).

- **Nitrous Oxide**

Nitrous oxide plays a significant role in heat-trapping in the atmosphere. It does so by partly absorbing the departing radiant heat energy from the Earth's surface in the form of infrared wavelengths, which goes uncaptured by other major greenhouse gases, like water vapour and carbon dioxide. The contribution of nitrous oxide remains to a small extent to the overall greenhouse warming, by absorbing and reflecting this leftover heat back to the earth's surface. Besides remaining unreactive, the nitrous oxide remains sustained for a long period of time in the lower atmosphere. More importantly, its ascent into the stratosphere triggers the reactions that deplete and thin the stratospheric ozone layer, which forms a protective covering surrounding the Earth, shielding it from the incoming harmful ultraviolet radiation. The present concentration of nitrous oxide in the atmosphere is increasing at a rate of two-to three tenths of a percent per year. Fossil fuel burning and agricultural fertilization have been considered to be the major sources of such an increase. A consensus has been reached, clearly indicating that a wide array of human-driven sources has been systematically contributing to enhancing the terrestrial nitrogen cycle (Hu, 2018).

- **Nitric Oxide and Ammonia**

Both nitric oxide and ammonia are extremely reactive in nature, unlike nitrous oxide and therefore they are much short lived. On account of this the detection of changes in their atmospheric concentrations can be only be manifested at regional scales only. Varied critical roles are played by Nitric oxide in atmospheric chemical interactions, including catalysis of the formation of photochemical (or brown) smog. The nitric oxide and oxygen react with hydrocarbons emitted from the automobile exhausts, in the presence of sunlight, leading to the formation of ozone, the most dangerous component of smog. The ozone present in the ground level causes serious detrimental effects on human health and the health and productivity of crops and forests. The oxides of nitrogen and sulphur gets transformed into nitric acid and sulfuric acid in the atmosphere, forming the major components of acid rain. Among the different sources contributing to the nitric oxide emissions, the Combustion is the dominant one. Fossil fuel burning alone contributes the amount of more than 20 Tg per year of nitric oxide. Forest burning by humans add about 10 Tg and global emissions of nitric oxide from soils, amounts to a total 5 to 20 Tg per year. The worldwide nitric oxide emissions produced on account of the human activities stands to about 80% or more, which is manifested in many regions with increased smog and acid rain. Compared to nitric oxide, ammonia functions as the chief acid-neutralizing agent in the atmosphere, with its antagonistic effect on the acidity of aerosols, cloud water, and rainfall. The proportion of global ammonia emissions caused by human activities stands to about 70%. The volatilized ammonia

emanating from fertilized fields contributes an estimated quantum of 10Tg per year; ammonia released from domestic animal wastes forms about 32 Tg; and forest burning to some 5 Tg (National Science Foundation, 2010).

Effects on the carbon cycle:

The elevation of atmospheric nitrogen emissions has increased the deposition of nitrogen on terrestrial and aquatic bodies. The fertilizer effect of nitrogen chiefly triggers the growth of plants, but the deposition may be influencing the atmosphere indirectly, causing a change in the global carbon cycle. The overgrowth of plants and the accumulation of plant material over the earth's crust have been effectively limited historically by a very limited supply of nitrogen, particularly in the regions experiencing temperate and boreal conditions. The activities of human beings have also expanded nitrogen deposition over much of the earth's surface area. Carbon dioxide emissions on account of human activities such as fossil fuel burning and deforestation have exceeded by more than 1000Tg, which stands to be equivalent to the amount of carbon dioxide accumulating in the atmosphere each year. Empirical studies in Europe and America indicate that a greater extent of the extra nitrogen retained by natural ecosystems like forests, wetlands, and tundra stimulates the absorption and storage of carbon. On the other hand, this nitrogen also stimulates microbial decomposition, thus releasing carbon from the organic matter of the soil. During the balanced condition, however, the uptake of carbon by the new plant growth appears to exceed the carbon losses, especially in forest ecosystems. Numerous attempts made by many research institutes to calculate the amount of carbon that can be stored in terrestrial vegetation have come up with estimates ranging from 100 to 1300 Tg per year. However, the number has shown a gradual increase in more recent analyses as the magnitude of human-driven changes in the nitrogen cycle has become more prominent (National Science Foundation, 2010).

Effects on biodiversity:

Limitations in the supply of biologically available nitrogen in the natural ecosystems mediate the best functioning and adaptation of many native plant species. The introduction of a new supply of nitrogen into the ecosystems causes a major deviation in the composition of dominant species and also a marked depletion in overall species diversity. From the various empirical studies, it has been deduced that quite a number of plant species experience a decline of more than fivefold as a result of the highest fertilization rate. Besides, the rich species composition of the earth and the biological diversity of the landscape get reduced because of the resemble of the plant communities occupying more fertile soils by the modified plant communities. The distinctive species composition adapted to sandy, nitrogen-poor soils is also getting lost from the region. Biodiversity loss caused by nitrogen accumulation also has an impact on other ecological processes (Vitousek, 1997).

Effects on the aquatic ecosystem:

The various responses observed in aquatic ecosystems lead to nitrogen enrichment, which involves NO_3^- loading from N-saturated terrestrial ecosystems, resulting in acidification and eutrophication of downstream fresh and marine water systems. The acidified freshwater also causes mortality of pH-sensitive fish species on account of aluminium toxicity. Since there is a limitation of nitrogen in marine systems, the unrestricted N inputs can result in the degradation of water quality due to the blooming of toxic algae, deficiency of oxygen, decrease in biodiversity, loss of biodiversity and fisheries.

- **Acidification of freshwaters**

The deposition of N_2 from the atmosphere in terrestrial landscapes undergoes microbial transformations through the soil, which results in surface water acidification and biodiversity loss. The NO_3^- and NH_4^+ that pass from terrestrial and atmospheric systems acidify freshwater systems when the buffering capacity is insufficient due to soil acidification. The introduction of reactive nitrogen from agricultural activities, animal raising, fertilizer application, and other sources has raised the nitrate concentration in the waterways of most industrialized nations. Recent empirical studies report that the

concentration of nitrate in 1000 Norwegian lakes and land has doubled in less than a decade. In the United States alone, 20% of groundwater sources were found to exceed the World Health Organization's limit of nitrate concentration in fresh water. Such high concentrations cause "blue baby disease," where nitrate ions weaken the capacity of the blood to carry oxygen. Exposure of reproductive tissues to high concentrations of nitrates augments the chances of some cancers like Saturday bladder and Ovarian cancer (Vitousek, 1997).

- **Eutrophication of marine ecosystems**

Anthropological activities like urbanization, deforestation, and agriculture have significantly contributed to the sedimentation and introduction of nutrients to coastal waters via rivers. A high increase in the primary production of estuarine and coastal systems has been manifested on account of high nutrient inputs. The increased primary production leads to increased carbon flow to the bottom waters as the decaying organic matter is absorbed and consumed by aerobic bacteria. As a result, the oxygen consumption in water is greater compared to the dissemination of oxygen from the surface waters. In addition, the toxins produced by certain algae blooms can act as neuromuscular or organodamaging compounds. These algal blooms can be detrimental to humans as well as other marine life (Vitousek, 1997).

- **Impact on water quality**

The conversion of nitrogen to the nitrate form in the soil provides an extreme concern for water quality, as the mobile attribute of nitrate makes it move and flow easily along with water. The effect of nitrates on water quality has been mostly observed in ground water, but sometimes they make their way into the surface water too, such as ponds, streams, and rivers. Besides the formation of nitrate in the soil by natural biological processes, the nitrates may also arrive from animal manure and nitrogen fertilizers. The underlying soil as well as the depth of groundwater determine the entry of nitrates into the ground water.

High levels of nitrates also cause serious health problems in humans, such as anoxia or internal suffocation in newborns. The most commonly observed symptom of nitrate poisoning in babies is the appearance of a bluish colour on the skin, particularly around the baby's eyes and mouth. These symptoms of nitrate toxicity are commonly referred to as the "blue baby" syndrome (Vitousek, 1997).

DISCUSSION

Nitrogen (N_2), goes through the environmental metabolism which involves the conversion of N_2 into chemically diverse reactive forms. Some of them play a crucial role for life itself and some are responsible for dangerous nitrogen pollution. The reporting from 2018-2019 Frontiers alerts that the human activities have been creating diverse forms of nitrogen species which are creating a concerned effect on living beings, ecosystems and climate (de Vries, 2021). The chief concern over the problem still goes unacknowledged to a wide extent outside scientific borders. The degradation of water and air quality, greenhouse-gas alterations, decline in ecosystems and biodiversity has been significantly recognized by The European Nitrogen Assessment as the most significant threat of nitrogen to the environment (Mellilo, 2021). Accelerating demand of human needs in agriculture, transport, industry and energy sectors have led to a high level of nitrogen pollution and related greenhouse gas emissions. It is high time that a well-managed and proper steps have to be taken in our society from individual to governmental and administrative levels for a healthy maintenance of N_2 balance in our ecosystems, so that a balanced sustained life forms can be perpetuated with organized preservation of our environment (National Science Foundation, 2010).

CONCLUSION

Since the past century the human activities have increased twice than the natural annual rate of nitrogen fixation and its entry into the terrestrial and soil nitrogen cycle, and it is going on accelerating substantially. Very concerned environmental circumstances have already started to emerge empirically. The levels of the nitrous oxide (greenhouse gas) and the nitrogen precursors of smog and

acid rain are significantly augmenting in the atmosphere. The acidification of soil in many regions have resulted in its stripping of nutrients that are essential for continued fertility. Besides, the water bodies present in these regions are also getting acidified, resulting in the transportation and delivery of excess nitrogen into the estuaries and coastal waters.

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Role of Probiotics on Skin Health

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ABSTRACT

Like the internal digestive system, the largest organ of our body, the skin, maintains a healthy balance with the natural inhabitants of it. Skin act as a host for several microorganisms (about one million per square centimetre) including different types of bacteria, fungi, and viruses. This microbiome takes active role in the fighting with infection, providing protection against environmental damage, regulation of pH and in keeping the skin hydrated and healthy. Probiotics are live and healthy microorganisms that can be consumed directly to restore the pH levels and the balance in the guts. They act like good bacteria and are important to keep away the “bad” bacteria from multiplying in different organ of human body. They can also be applied on the skin to restore the skin's ecosystem, pH and immunity. Again, long term exposure to solar radiation may induce UV damaged skin cancer. The utilization of probiotic bacteria in skin cancer research may help in development of new skin cancer prevention and treatment options.

Keywords: *Lactic Acid bacteria; Probiotics; Bacteriocin; Skin Cancer*

INTRODUCTION

Skin, the widely spanned organ of the body is always exposed to external environment, so it is very much prone to attack by several groups of pathogenic micro-organisms. This is the basis of most of the dermatological disorders. According to the Global Burden of Disease (GBD), skin and subcutaneous diseases were substantial reason of non-fatal disease encumbrance globally in 2010 and 2013 and accountable for 42.9 million (95% UI 28.6–63.4) disability-adjusted life years (DALYs) and 4.86 billion (4.68–5.06) incidences globally in 2019. This number comprises of death and disability triggered by acne, alopecia, bacterial skin infections, decubitus ulcer, fungal skin diseases, pruritus, psoriasis, scabies, urticaria, viral skin diseases, and other skin diseases. Disability burden is calculated using DALYs, which is the sum of years lost following a disease or untimely deaths (YLLs) and years lived with disability (YLDs). For calculation of GBD, over 7,000 researchers beyond 156 countries, collected the data of early deaths and information of disability about over and above 350 diseases and injuries in nearly 200 countries on the basis of age and sex, from 1990 to the present. According to GBD, 2017, Asian countries also have a high incidence of skin problem that are often associated with itching (i.e., psoriasis, contact dermatitis, atopic dermatitis, and pruritus) (Urban *et al.*, 2021). Acne vulgaris, scabies, cutaneous leishmaniasis are also common in Asian countries. In India last few decades due to increased pollution, global warming, skin cancers are increasing at a faster pace. As a developing country with high population size the frequency of infectious skin disease is also very high in India. There were 4.07 million (95% uncertainty interval 2.65–6.19 million) years subsisted with disability as a result of skin and subcutaneous diseases in 1990, which amplified to 6.26 million (95% uncertainty interval 4.12–9.35 million) in 2017 (Kavita *et al.*, 2021). The incidence of skin infection is increasing day by day due to emergence of multidrug-resistant microbial strains and the growing numbers of immune-compromised patients owing to immunosuppressive therapy, malignancy, transplant interventions, or HIV/AIDS (Esposito *et al.*, 2016). Around 50% of these were fungal skin diseases and rest are bacterial, viral and parasitic (Johnson *et al.*, 2018). In a recent report a group of scientists had shown that several skin disorders are associated with coronavirus, SARS-CoV-2 infection (Akl *et al.*, 2021). Some are linked with the viral infection directly and some are happening due to an upsurge in PPE usage and hygiene actions (hand sanitizer gels, sprays, frequent hand washing with soaps etc.)

(Darlenski & Tsankov, 2020). Among the bacteria involved in skin infections, role of *Staphylococcus aureus* (including methicillin-resistant *S. aureus*/ MRSA strains), *Staphylococcus epidermidis*, *Cutibacterium acnes*, *Propionibacterium acnes* and beta-hemolytic Streptococci, Gram negative *Klebsiella* sp., *Pseudomonas* sp. are well studied. The common fungal diseases are fungal nail infection, *Candidiasis*, *Blastomycosis*, *Mucormycosis*, *Aspergillosis* etc. In recent study it has also been found that prolonged infection by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species* increased the propensity to cancer development, due to weakened immune system. During this 21st century with the advent of all modern techniques, equipment, scientific knowledge, treatment system, cancer remains one of the major causes of human death worldwide. Though incidence of malignancies in skin cancer is very less, worldwide increased cases of skin cancer has been observed during last few decades. In India people mostly suffer from squamous cell carcinoma (SCC). Malignant melanoma and Basal cell carcinoma (BCC), which is one type of Non-Melanomatous Skin Cancers (NMSC), are the other types of skin cancer found in India. Recent studies shows that due to ozone layer depletion higher exposure to Ultraviolet B radiation (UVB 290-320 nm), presence of arsenic in drinking water as well as in insecticides, pesticides, herbicides and poultry feeds, presence of other harmful pollutants like coal tar and various hydrocarbons are the important causing agent for increased cancer cases in India (Lal *et al.*, 2016). Conventional treatment system of cancer relies on either surgery or radiotherapy or chemotherapy or a combination therapy. Generally small molecule drugs like tyrosine kinase inhibitors and human or humanized proteins (e.g., monoclonal antibodies) are mostly used in chemotherapy (Nguyen & Nguyen, 2016). But long-term usage of these chemotherapeutic drugs makes the cancer cells resistant and due to non-specificity, the nearby healthy cells are affected heavily. Most of the cases antibiotics are prescribed along with the anticancer drugs to prevent infections. But due to emergence of antibiotic resistance microbial infections remain uncontrolled. So, an alternate approach is very much necessary for cancer cell specific and deal with the side effects of chemotherapeutic drugs.

LITERATURE REVIEW

Probiotics-the friendly microbes

Foods derived from microbial activity were common since the dawn of human civilization and fermented milks were possibly the first of its kind to have active micro-organisms. In 1908 Elie Metchnikoff, father of modern probiotics, observed a very interesting phenomenon showing regular intake of lactic acid bacteria in fermented dairy products, for instance yogurt, was related with enhanced health and longevity in Bulgarian peasant populations. Later few years there was an immense interest in search for the role of lactic acid bacteria as probiotics. Lilly and Stillwell originally coined the term 'probiotics' representing 'substances secreted by one organism which stimulate the growth of another' (Lilly & Stillwell, 1965). Later United Nations Food and Agriculture Organization and the World Health Organization (FAO/WHO, 2001) adopted an alternative definition of probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Upon ingestion the probiotic strain should show some beneficial effect on the host such as prevention of colonization of harmful micro-organisms in intestine, alleviation of lactose intolerance, relief of constipation, antitumor or anti-carcinogenic effect, improvement of growth rate and feed utilization of animals, improvement of balance of the intestinal micro-flora, maintaining a chronic and immunological balanced inflammatory response, maturation of immune system, anticholesterolemic effect etc. Scientific reports have already shown that use of probiotics is a sound choice in prevention and therapy of antibiotic associated diarrhoea, against traveller's diarrhoea, irritable bowel syndrome (IBS), IBD, lactose intolerance, peptic ulcers, allergy and autoimmune disorders etc. A variety of micro-organisms including lactic acid bacteria, *Bifidobacteria*, *Saccharomyces*, enteric, *Streptococci* and some other beneficial microorganisms have been targeted as potential probiotics with therapeutic application. A clinical study unveiled that prenatal administration of a probiotic strain of *Lactobacillus rhamnosus* GG (LGG) declines the development of atopic eczema in children (Foolad *et al.*, 2013; Kuitunen, 2013) following the anti-inflammatory properties of the above-mentioned bacterium.

Another study reported LGG intake by children with atopic dermatitis enhances the production of the anti-inflammatory cytokine IL-10 (Pessi *et al.*, 2013). Additionally, it has been seen that oral administration of *L. casei Shirota* strain to mice inhibited specific IgE production (Lim *et al.*, 2009). A double-blind, randomized, placebo-controlled trial by Abrahamsson *et al.*, (2007), have experienced that infants treated with *L. reuteri* ATCC 55730 strain had a reduced amount of IgE-associated eczema. It is known that, Lactic acid bacteria (LAB) are a cluster of associated bacteria that yield lactic acid as a result of carbohydrate fermentation. The genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are vital members of this group. From ancient time they are present in different types of fermented foods like curd (*Lactobacillus* spp., *Lactococcus* spp.), yogurt (*Streptococcus* spp. and *Lactobacillus* spp.), cheeses (*Lactococcus* spp.), sauerkraut (*Leuconostoc* spp., *Lactococcus* spp.), sausage (*Pediococcus*) etc. They are Gram positive bacteria with GC content less than 50% in their DNA. Most LAB are non-spore forming rods or cocci as well as aero-tolerant anaerobes lacking cytochromes and porphyrins which makes them catalase and oxidase negative. This group of bacteria can be used for control of surface infecting pathogens as they produce several antimicrobial compounds like lactic acid, hydrogen peroxide, di-acetyl along with bacteriocins. Bacteriocins are synthesized in the ribosome small peptides and showed high antimicrobial activity to closely related species. They are non-toxic to eukaryotic cells and in addition bacteriocins also exert direct activity toward bacterial pathogens.

Bacterial therapy of cancer-a newer approach

The idea of using bacteria or the bacterial proteins in cancer therapy is one of the alternate approaches and it started after observing some practical incidences where bacterial infection helps in regression of tumor /cancer. At the end of 1890, American physician, Coley witnessed the liaison between bacterial infection and cancer regression and became pioneer in the field of immunotherapy by developing the first killed bacterial vaccine named as "Coley's toxin". Bacteria release varieties of proteins, peptides, toxins, enzymes, immunotoxins etc. as their survival strategy. Antimicrobial peptides (AMPs) obtained from different groups of bacteria can be investigated for their anti-cancerous property and can be applied alone for both for treatment of skin infection as well as skin cancer (Coley, 1891 and 1893). They can also be combined with conventional chemo-therapeutic drugs (Felicio *et al.*, 2019).

Characteristics like low toxicity, good solubility, high penetration to cells, high specificity, less interaction time with target cells which lower the chances of generation of resistance, membrane-permeabilizing ability make them attractive antimicrobial as well as anti-cancerous therapeutic agent (Woo *et al.*, 2022). Though most of the contemporary research on probiotics and cancer is restricted to gastrointestinal tumors, some recent evidences showed that the consumption of oral probiotics improve indirectly various skin disorders comprising atopic dermatitis, acne, and psoriasis. According to Weill *et al.* (2013), probiotics can directly regulate the cutaneous immune system and reinstate the homeostasis via the gut–skin axis. But a very less information is found about the effect of direct topical application of probiotics on skin cancer till date. Bacteriocin, one AMP mainly from probiotic bacteria, has both the antimicrobial as well as anticancer property. Bacteriocins are small peptides manufactured by ribosomes in several Gram-positive and Gram-negative bacteria. Most of them have GRAS status. Long time they have been used in food industry as natural preservatives. Due to their cationic and amphipathic nature, they are less toxic to mammalian cells, membrane dissolution ability, immuno-modulatory power and most importantly, their antimicrobial activities can be used directly topically to treat skin infections as well as in skin cancer. They can be formulated as ointments, combined with cosmetics, applied directly as anticancer agent or applied in conjunction with conventional cancer drugs. There are several evidences that show the topical application of bacteriocin decreases inflammation, colonization of infecting pathogen and even improve the acne, psoriasis.

From a work published by RocíoLópez-Cuellar *et al.* (2016), it is analyzed that though at the beginning of 21st century research on bacteriocin from Lactic acid bacteria was confined to food science, in the last decade about 37% of the published research was portrayed mostly on its biomedical applications.

During this time, the two main problems faced by the scientists worldwide are multi drug resistance and non-specificity of the applied drugs. In search of alternatives to complications like cancer, systemic infections, oral-care, vaginal infections, contraception and skincare, recent research is focused on therapeutic application of Anti-Microbial Proteins (AMPs). Huang *et al.* (2021) highlights the potential of bacteriocins as novel therapeutic treatments in microbe infection, cancer, and immune system in human body. Cesa-Luna *et al.* (2021) also reveal the advance usage of bacteriocin as biocontrol agent in medical and agricultural field. Along with these, they discussed about their mode of action and role of bacteriocins to modulate the signaling in host-associated microbial populations. Another report by Ovchinnikov *et al.* (2020) showed that two broad-spectrum bacteriocins, garvicin KS and micrococcin P1 act synergistically and with penicillin G, killed methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro*. They compared the effect of this formulation to Fucidin antibiotic cream, normally employed in skin infection treatments, and found its superiority in preventing resistance development. Kober *et al.* (2015) discussed the contribution of probiotics in acne and rosacea treatment, and protection against aging and photodamage.

Nguyen and Nguyen (2016) showed that Azurin a bacteriocin from *Pseudomonas aeruginosa*, can precisely penetrate human cancer cells and induce apoptosis. The study also mentioned about eight recognized bacteriocins from human gut pathogenic and commensal bacteria having functional properties quite like azurin and p28-azurin, with the help of bioinformatics approaches. Review article of Felgner *et al.* (2017) revealed the clinical trials performed during the last 150 years on bacteria mediated tumor therapy (BMTT). The dual activity of Antimicrobial proteins (AMPS) was also established. Rodrigues *et al.* (2019) reported that some infectious agents like *Streptococcus pneumoniae*, *Stomatococcus mucilaginosus*, *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Candida* spp., *Helicobacter pylori*, Hepatitis B and C, and Human Papilloma Viruses (HPV), related to the progress of cancer and bacterial proteins and peptides can be used as a treatment strategy against them. The preferable characteristics of proteins and peptides from bacterial sources that revealed activity against microbial infections and cancer, along with their efficacy *in vitro* and *in vivo* are also discussed.

The report of Benítez-Chao *et al.* (2021) gave emphasis on the necessity to increase *in vivo* testing of bacteriocins and advance the *in vivo* models that both evaluate the efficacy of bacteriocins as antimicrobial agents and introspect probable toxicity and side effects, which are crucial factors to define their achievement as prospective therapeutic agents in the fight against infections triggered by multidrug-resistant microorganism. In a recent report by Khalfallah *et al.* (2021), antimicrobials from two *Streptococcus salivarius* strains and one *Lactobacillus plantarum* were used to develop a plaster/bandage for the application to diseased skin. The anti-cancer activity of bacteriocin to other types of organs also reported. From the work of Al-Madboly *et al.* (2020), it is proved that enterocin (LNS18) from *Enterococcus* species can be used in treatment of liver cancer. Application of Nisin in head and neck squamous cell carcinoma (HNSCC) is well documented (Shin *et al.*, 2016). It has also shown activity in oral cancer.

Recently, Le Noci *et al.* (2018) revealed that probiotic aerosol therapy inhibits lung melanoma metastasis *Lactobacillus rhamnosus* GG strain. It can inhibit cancer progression and encourages apoptosis in mouse colon cancer HGC-27 and human colon cancer Caco-2, DLD-1, and HT-29 cells as well (Orlando *et al.*, 2012). Application of prebiotics along with probiotics that is application of symbiotic (*Lactobacillus rhamnosus* + *Lactobacillus acidophilus* + inulin) had shown much more effectiveness in lowering the oxidative stress than the application of probiotics alone (Verma & Shukla, 2014). Sharma and Shukla (2016) also told that metabolites from probiotics can be applied independently or in conjunction with others in colon cancer or for other diseases. Sharaf *et al.* (2018) reported that application of probiotics (*Lactobacillus acidophilus* and *Lactobacillus rhamnosus* GG) in combination with celecoxib, a selective cox-2 inhibitor diminishes the incidence risk of colon cancer. Cancer patients receiving chemotherapy can be given probiotics as an adjunct therapy (Sankarapandian *et al.*, 2022).

Apoptosis-like morphological changes was observed after application of Enterocin 12a, a bacteriocin

from a vaginal isolate *Enterococcus faecium* 12a (Sharma *et al.*, 2021). The concept of gut-brain-skin axis and role of microbiomes in spreading cancer is also revealed (Misra & Raghuwanshi, 2022). In a similar study, bi-directional communication between gut and lung (termed as Gut–Lung axis) and role of probiotics in lung diseases is also established (Rai, 2021). The gut microbiota has a noteworthy effect on the skin good health as it is accompanying with various chronic inflammatory skin disorders, including acne, rosacea, atopic dermatitis, and psoriasis (Divyashri *et al.*, 2021). Probiotics may offer health benefits during vaginosis. This was reported by Hattiholi *et al.* (2021). From the report of Upadhaya *et al.* (2021), it can be seen that probiotics are useful in the prevention and cure of cancer through microbiota and AMPs, following the mechanisms like immune modulation, condensed bacterial translocation, enhanced gut barrier function, anti-inflammatory, anti-pathogenic activity, reduced tumour formation, reduced metastasis, and others.

CONCLUSION

This review attempts to mention about some recent observations that evidenced the potency of both oral and topical probiotics in preventing and treating skin diseases in addition to skin cancer. Due to antibiotic resistance and side effects of chemo and radiotherapy of cancer, combinational therapy can be encouraged as an alternative approach. Past few years' number of in vivo tests has increased in search of newer peptides or proteins with antimicrobial and antitumoral activity. Probiotics are source of varieties of such proteins and peptides. They can also be modified to decrease or eliminate their cytotoxicity and can be very target specific. Besides they can be applied directly with the conventional therapeutics which reduces the cost of the treatment.

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Treatment for COVID-19: Current Therapy and Challenges

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ABSTRACT

The novel coronavirus disease, COVID-19, has been documented as the fifth pandemic since the flu pandemic of 1918, around a hundred years ago. It was first reported in Wuhan province in China. The coronavirus was officially named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The cumulative death toll during this pandemic is 6,029,852, with an approximate death rate of 1.33%. This article focuses on the origin, and the virology of the virus. Then it focuses on various mutations occurring in the virus, with a special note on the mutation in spike protein. A mention of worldwide death statistics is then discussed. Several tests and how they are conducted in India are discussed thereafter. After that, various platforms for vaccine development alongside their manufacturing efficiency are discussed. It then focuses on remedies concerning vaccines both at the global and national level, with a special note on major manufacturers in India. The pros and cons of many new generation vaccines in the clinical or preclinical stage are also illustrated to some extent. Finally, the article focuses on several other challenges being faced or which can be faced in the future.

Keywords: COVID-19; SARS-CoV-2; Origin; Death statistics; New generation vaccine; Challenges

INTRODUCTION

The world is currently facing a pandemic of a deadly virus known as SARS-CoV-2 or nCoV-19, better known as COVID-19. This particular disease has led humankind to face a sociological, psychological, and economic crisis. Extensive measures like testing, social distancing, and isolation of the infected individuals are required to impede further spread and suffering. As of March 12th, 2022, the virus is responsible for more than a total of 6 million deaths. The coronavirus 2019 has caused an intimidating health emergency throughout the globe.

Origin of SARS-CoV-2

Tracing back to the emergence of SARS-CoV-2, this contagious disease was first observed as unexplained cases of pneumonia in Wuhan City, Hubei Province, China in December 2019. Later on, on February 11, 2020, the World Health Organization (WHO) declared those pneumonia cases as COVID-19 (CoronaVirus Disease 2019). As the epidemic in Wuhan was witnessed in the early weeks, an association was noted in the early cases, and the prime spot was the Wuhan Huanan Seafood Wholesale Market (also referred to as the Huanan Market), where cases were mainly reported among the operating dealers and vendors (Yang *et al.*, 2020). At the initial stage of transmission, 27 patients out of 41 patients infected were connected with the Wuhan wet food market. Huanan Market is known globally for its wide variety of wild animals, which are predominantly sold as aquatic products and seafood, as well as some farmed wild animals and live wild animals being slaughtered. The wet food markets are hubs for close contact between humans and animals taken as food, which results in the transmission of microbes from animals to humans (Cruz *et al.*, 2020). Therefore, this market was initially suspected as the epicentre of the epidemic. Due to the outbreak of COVID-19, the Chinese government took action to close many wet markets and temporarily ban wildlife trade. People around the world and several health organisations are urging the Chinese government to make this ban permanent. This kind of market can again lead to another pandemic. The wild farm animals should be vaccinated before being sold in the market. Monitoring of hygiene and sanitization of the wet market is mandatory. Steps have to be taken by the government to centralise slaughtering instead of many wet

markets. On January 30th, 2020, the International Health Regulations Emergency Committee of WHO deemed the virus as a "public health emergency of international concern". Within two months of the epidemic on March 11th, 2020, WHO declared COVID-19 as a "global pandemic" (Cruz *et al.*, 2020).

LITERATURE REVIEW

History of SARS-CoV-2

The world is now affected by the coronavirus disease 2019, i.e., COVID-19, which is recorded as the fifth pandemic after (i) the Spanish flu pandemic in 1918 (H1N1), (ii) 1957 Asian flu (H2N2), (iii) 1968 Hong Kong flu (H3N2), and (iv) the 2009 Pandemic flu (H1N1), which was the cause of an estimated death of 50 million, 1.5 million, 1 million, and 300,000 respectively.

The coronavirus is a member of the family of severe respiratory viruses, which was discovered first in the 1960s (Cruz *et al.*, 2020). Before SARS-CoV-2 emerged, there were two more viruses from the same family that had emerged, namely, SARS-CoV-1 in 2002 and MERS-CoV in 2013.

In 2002-2004, there was an outbreak of SARS (severe acute respiratory syndrome) whose causative agent was SARS-CoV-1 where masked palm civets were considered as intermediate hosts. And during the outbreak of MERS (Middle East Respiratory Syndrome) in 2013, the intermediate hosts were the dromedary camels.

Some studies and evidence suggest that SARS-CoV-2 shows zoonotic origins, i.e., it emerged from bats to pangolins and then infected humans. But the exact natural reservoir is unknown yet. Most of the viruses that are related to pandemics originate from animals, like some viruses that cause flu are from birds and pigs, while Ebola originated from bats (Cruz *et al.*, 2020).

All human coronaviruses are known to have animal origins, i.e., natural hosts. Bats have been identified as the main host of various zoonotic viruses (e.g. Nipah virus, SARS-CoV, MERS-CoV, and Hendra Virus), which also include coronaviruses with considerable genetic diversity (Latinne *et al.*, 2020).

Virology of SARS-Cov-2

Coronavirus is a member of the Coronaviridae family, whose subfamily is Coronavirinae. SARS-CoV-2 is a member of the same Coronaviridae family as SARS-CoV-1, which on further classification falls under the alpha, beta, gamma, and delta coronavirus genus and order is Nidovirales (Samudrala *et al.*, 2020). The World Health Organization has said that these four versions are worrying (Duong, 2021).

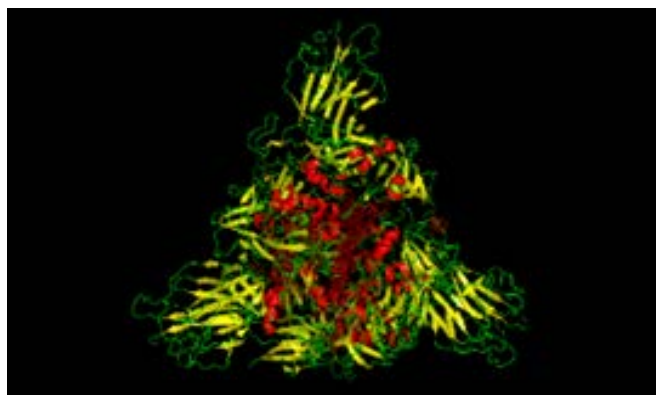
SARS-CoV-2 is spherically enveloped, contains a positive-sense, single-stranded RNA genome, and has a diameter of approximately 120 nm. The major known viral RNA genome has a length of 27 to 32 kb. The RNA genome present in SARS-CoV-2 contains a 5' methyl-guanosine cap, a poly (A)-tail, and 29,903 nucleotides according to WH-Human1 coronavirus (WHCV) (Chan *et al.*, 2020; Cruz *et al.*, 2020; Wu *et al.*, 2020a). Bats are considered to be the natural reservoir of all three of these viruses. And infections mainly occur through the intermediate host, like in the case of SARS-CoV-2, where pangolins are the primary suspects as an intermediate host (Andersen *et al.*, 2020). All coronaviruses are RNA viruses, which benefit them by mutation and homologous and non-homologous recombination, resulting in the expansion of their host range. Some club-like projections are present on the peripheral region of COVs, which are known as "spikes" (protein spikes). So, the name comes from the Latin word "corona," which means "crown," because the structure looks like a crown. The genome of SARS-CoV-2 is comprised of a 5' untranslated region including a 5' leader sequence, an open reading frame (ORF) 1a/ab encoding nonstructural proteins (nsp) for replication, and four structural protein components including (i) spike (S) (ii) Envelope (E) (iii) Membrane (M) (iv) Nucleocapsid as well as several accessory proteins such as ORF 3a, 6, 7a/b, and 8; and a 3' untranslated region and transcribes nine subgenomic RNAs. The glycoprotein spike facilitates binding to the transmembrane angiotensin-converting enzyme (ACE) 2 in the host receptor and that is also dependent on S protein priming by the transmembrane serine protease TMPRSS2. The S protein is the primary determinant of transmissibility and pathogenicity. The S protein is also therefore considered as the primary target of vaccine design for its ability to neutralise antibodies (Chan *et al.*, 2020; Gao *et al.*, 2020; Salvatori *et al.*, 2020; Shang *et al.*,

2020; Walls *et al.*, 2020; Wang *et al.*, 2020; Wu *et al.*, 2020b). A similar panel of mammalian cell lines can also be infected with SARS-CoV-2. The host protease can cleave the S protein into two subunits, i.e., subunit-1 and subunit-2 (S1 and S2), which are responsible for recognition of receptors and membrane fusion, respectively. The S1 subunit can again be classified into N Terminal Domain, i.e., NTD, and C Terminal Domain, i.e., CTD. In SARS-CoV-2, the CTD of S1 shows strong affinity for human ACE2 (hACE2). The CTD is the key region of the receptor binding domain (RBD) within SARS-CoV-2 that interacts with the hACE2 receptor with higher affinity (Wang *et al.*, 2020; Wrapp *et al.*, 2020). In the host cell, the putative life cycle of SARS-CoV-2 begins with S protein and hACE2 receptor binding. A change in the structure of S protein, which is conformational in nature, facilitates viral envelope fusion with the cell membrane with the help of the endosomal pathway. The viral RNA genome is released, after the fusion, into the cytoplasm and it is translated into viral replicase polyproteins, i.e., pp1a and 1ab, which can be cleaved further into small products by virus encoded proteinases. A series of subgenomic mRNAs is transcribed by polymerase by discontinuous transcription. Viral subgenomic proteins are then formed by the translation of subgenomic mRNAs. The S, E, and M proteins enter the ER and the Golgi apparatus, where they form a nucleoprotein complex by combining the N terminal with a positive-stranded genomic RNA. at the golgi-Endoplasmic reticulum intermediate compartment, the viral envelope is assembled with structural proteins and the nucleoprotein complex. And then the newly assembled viral particles get released from the infected cell.

SARS-CoV-2 is genetically close to other coronavirus strains like SARS-CoV and MERS-CoV. The SARS-CoV-2 has a high homology (79.6%) with the SARS-COV from 2002-2004 (Zhu *et al.*, 2020). It shares 96.2% homology with a sequence of strains of coronavirus (RaTG13). This RaTG13 is the closest known sequence to SARS-CoV-2.

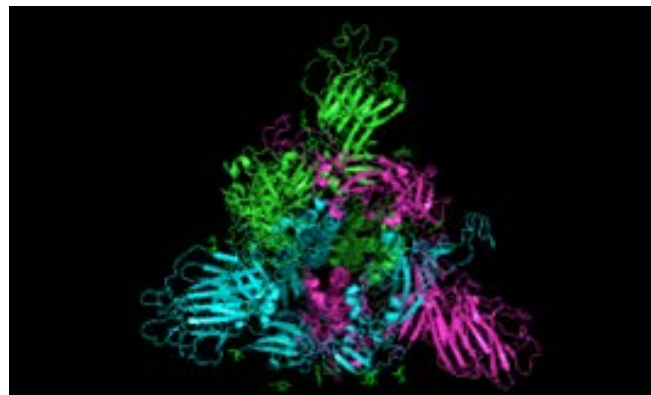
Spike Protein Mutations

As it is known, starting from the first wave, there were a lot of changes in the structure of the spike protein of SARS-CoV-2. The mutations in the spike protein led to the development of different variants like delta variant and omicron. The images and table below offer an insight.



Source: Wong *et al.*, 2022

Figure 1: Spike Protein of delta variant



Source: Ye, Liu & Li, 2022

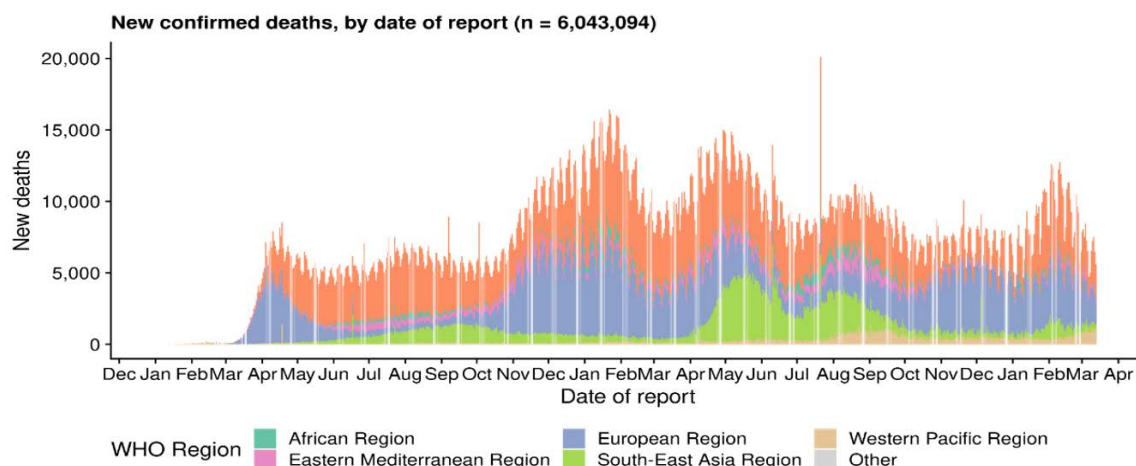
Figure 2: Spike Protein of omicron variant

Table 1: Mutation and its effects

Variant	Site of Mutation	Site of Infection	Infected Age Group
Wuhan-Hu-1		Lungs	Older
Delta	A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, L212V, ins213-214RE, V215P, R216E, Δ213-214, L452R, T478K, D614G, P681R, D950N	Lungs (Majorly)	Children, younger and middle age
Omicron	K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	Pharynx, Larynx, Sinus	Older

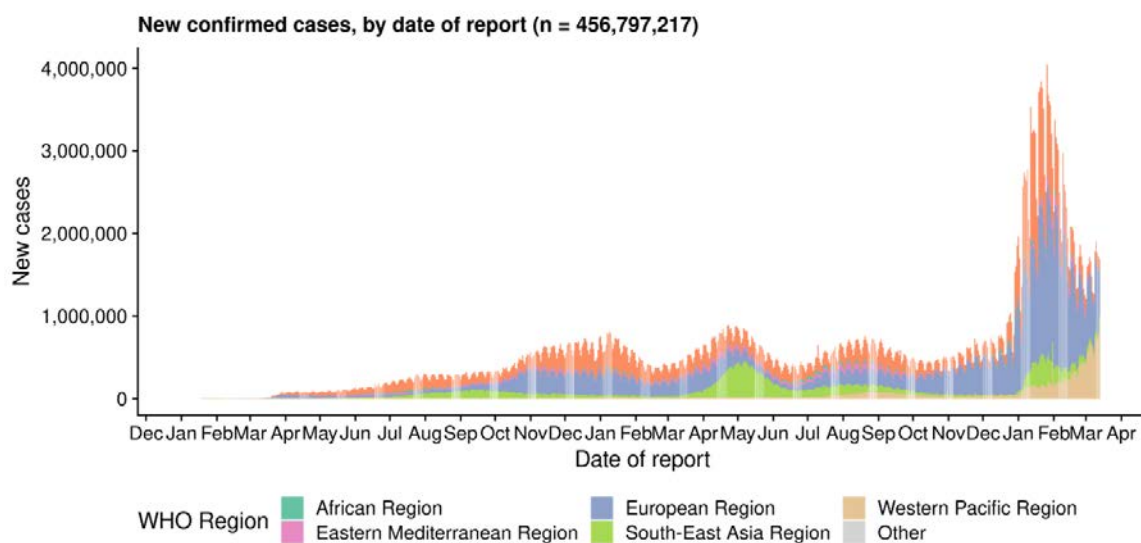
Statistics

WHO has made public the information about cases and death and other necessary information globally and nationally. Here some graphs are provided for an insight into the loss which people are facing due to COVID-19, both globally and nationally.



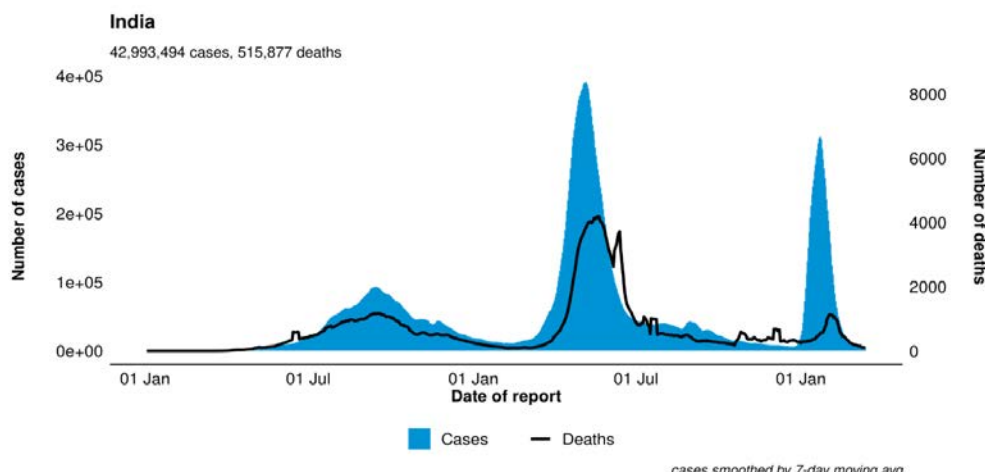
Source: <https://worldhealthorg.shinyapps.io/covid/>

Figure 3: New confirmed deaths worldwide



Source: COVID Intel database

Figure 4: New confirmed cases worldwide



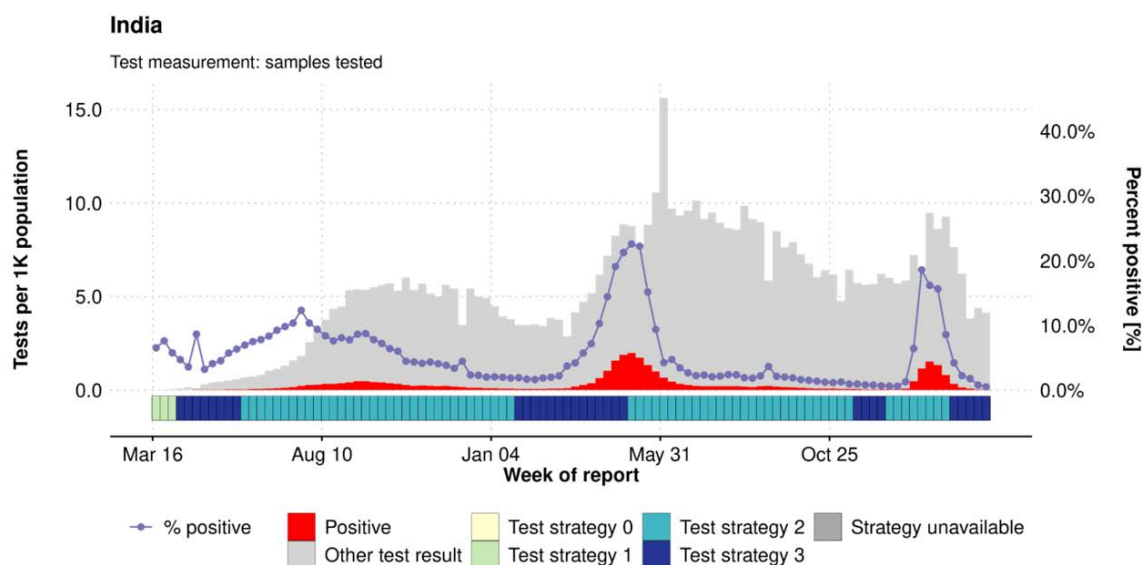
cases smoothed by 7-day moving avg

Source: <https://worldhealthorg.shinyapps.io/covid/>

Figure 5: Cases and deaths in India

Test Related to Covid-19

Seeing the mortality rate and morbidity rate, test and treatment has really become an important issue to combat COVID-19. There are certain kinds of tests like 'swab test' where samples of saliva and mucus are taken from the mouth and nose to diagnose COVID-19. Some other tests like RT-PCR, antibody testing was also performed. Even certain drugs like Lopinavir, Ribavirin were also tested but they didn't turn out to be so effective to fight against SARS-CoV-2. Below is a chart showing various test strategies in INDIA



Source: <https://worldhealthorg.shinyapps.io/covid/>

Figure 6: Testing strategies in India. Test strategy 0 depicts no strategy. Test strategy 1 depicts only those who both (a) have symptoms AND (b) meet specific criteria. Test strategy 2 depicts anyone showing symptoms. Test strategy 3 depicts open public testing (i.e. available to asymptomatic people)

The only strategy to combat this deadly virus is by vaccinating the people. Discussion of some of the handful of vaccines manufactured by some of the countries are in the following section.

Platforms of Vaccines

Various novel approaches are now being taken for the development of new-gen vaccines. Gavi, the vaccine alliance, has taken an active part in informing how these novel approaches work on a public level. Below, four basic approaches and their manufacturing efficiency are discussed. The number of candidates can be found in the COVID-19 Vaccine Tracker and Landscape (WHO, 2022).

Protein Subunit

Protein subunit vaccines contain protein fragments and/or polysaccharide chains. The fragments and other molecules are studied carefully to produce the desired immune response. By restricting the access of the immune system to the whole pathogen, side effects are minimised. These are generally produced using living organisms. The organisms also require a certain substrate and culture medium, alongside strict hygiene to avoid contamination. The manufacturing process is also precise and depends on the subunits to be used. So generally, these are expensive. As of March 11th, 2022, 48 vaccine candidates enlisted in the WHO candidate list are protein subunit based.

Viral Vector

Viruses invade the host cells and replicate themselves in order to survive. This makes the infection in the host cell. They make new viruses by hijacking ribosomes. The virus particles contain antigens which cause the immune response. This is the underlying principle for viral vector-based vaccines. In this case, the host cells only receive the codes to produce antigens. Thus, the immune response is invoked

by the host cell. The carrier which carries the code is known as the vector. Viral vectors are generally attached to substrates. But this is also a major drawback for large scale manufacturing. Suspension cell lines are now being made for producing vectors in bioreactors. Additionally, assembling vectors is a complex process that requires testing before each step and has a high chance of contamination. Thus, it obviously increases the cost. As of March 11th, 2022, 21 non-replicating and four replicating vaccine candidates enlisted in the WHO candidate list are viral vectors.

Nucleic Acid

These can be of mainly two types, depending on the type of nucleic acid used. The nucleic acid is injected into the bacterial plasmid. This gene is stored in the bacteria as it may be helpful in the survival of the organism. In the case of DNA, the antigen is injected into muscle cells. But one challenge is bypassing the cell membrane, as the ribosome, which translates the gene, is situated inside the cell. On the other hand, in the case of RNA, the antigen is encoded in mRNA or self-amplifying RNA (saRNA). It can be injected by itself or encapsulated in nanoparticles. Once inside the cell, antigens are produced and surfaced. This causes the immune response, including killer T cells. Once the pathogen's genomes are sequenced, it is relatively fast to build one nucleic acid-based vaccine. Moreover, both kinds of vaccines can be produced in the same facility, reducing the costs even more. As of March 14th, 2022, 16 DNA-based and 26 RNA-based vaccine candidates enlisted in the WHO candidate list are nucleic acid-based.

Whole virus

Live attenuated vaccines use the lab-weakened version of the original pathogen. Once inside the cell, they cause the natural immune response and may cause slight symptoms of the original disease. In the case of inactivated virus vaccines, the pathogen or part of it is injected. But their genetic material is destroyed prior to injection. As inactivated viruses contain destroyed genetic material, they are considered safe compared to live attenuated vaccines. Different vaccines will contain different substrates and culture media to grow the virus. Additionally, there will be a high chance of contamination. Eliminating this will certainly increase the cost of manufacturing. As of March 14th, 2022, 21 inactivated virus and 2 live attenuated virus vaccine candidates are enlisted in the WHO candidate list.

An Insight to Vaccines

Covishield

The Oxford-AstraZeneca vaccine was first prepared in the United Kingdom (UK), which is also known by its local name 'Covishield'. It is also called AZD1222. In India, the covishield vaccine was prepared by the 'Serum Institute of India (Pune)'. This vaccine is actually based on viral vector based technology. The covishield vaccine mainly constitutes disabled adenovirus having certain segments of coronavirus, L-histidine hydrochloride monohydrate, polysorbate 80, ethanol, aluminium hydroxide gel, L-histidine, magnesium chloride hexahydrate, sodium chloride, EDTA (also called disodium edetate) (Phiddian, 2022). The method of administration of the vaccine is intramuscular injection. Here, ChAdOx1 (also known as modified chimpanzee adenovirus) is used as a vector. The covishield vaccine is actually a replication-lagging simian adenovirus vector which constitutes a full length codon-optimised coding sequence of the S protein of SARS-CoV-2 having a tPA (a.k.a. plasminogen activator) leader sequence. Since in adenovirus, many vital genes are eliminated and restored by the gene with no contribution from the S protein of SARS-CoV-2 virus, that is why the adenovirus does not undergo replication. This vaccine contains the incapacitated genome of an adenovirus which contains the genetic material of the S protein of SARS-CoV-2. After the vaccine is given, the S protein that is formed primes the immune system to attack the SARS-CoV-2 virus if it later attacks the body. Covishield vaccine is stored in refrigerated conditions at 2°C to 8°C.

The Covishield vaccine is given in 2 doses, and the time gap between the 2 doses is stretched from 4-6 weeks to 4-8 weeks. After the vaccination, the adenovirus vector enters the cells. Then it releases its genetic material, which is transferred to the nucleus of the cell. After that, the cell undergoes transcription into mRNA and proteins (by translation). Since it is known that S protein is the protein of

interest, an exterior protein which sets up the SARS-CoV-2 virus enters the cell machinery through the ACE2 (angiotensin-converting enzyme 2) enzymatic domain. By preparing this, the vaccine provokes the immune system to attack the SARS-CoV-2 virus through T-lymphocyte cells (or T cells) and antibodies if this virus attacks the body in the future.

Covaxin

The Covaxin vaccine is manufactured by Bharat Biotech. Covaxin contains the whole virion of inactivated SARS-CoV-2, which makes it quite different from Covishield. It is not capable of replication and constitutes some crucial ingredients like aluminium hydroxide gel, TLR 7/8 agonist, 2-Phenoxyethanol, phosphate buffer saline as mentioned (Firdous, 2021). Like COVISHILD, it is also stored at the same temperature. After the covaxin is given inside the body, certain antigen-presenting cells shred the inactivated virus apart and then it splits into certain pieces flaunted on its surface. Now certain helper T cells get exposed to those fragments. Then the T cells get activated if they fit properly on the fragments, and it also assists other immune cells to respond to the vaccine. When these helper T cells get activated against the coronavirus, it can attach to the fragments and, due to this, B cells also get activated. It basically multiplies and oozes out the antibodies that have similar shapes as their surface protein. The immune system responds when any live SARS-CoV-2 virus infects the body once the covaxin is injected. These antibody-producing B-lymphocyte cells get attached to those invaders. So, these antibodies prevent the virus by targeting the S protein. On March 3rd, 2021, it was found that the covaxin had an efficiency of around 80.6%.

Sputnik V

Sputnik V is also a viral vector vaccine that was prepared by the Gamaleya Research Institute of Epidemiology and Microbiology'. This vaccine was also prepared from adenovirus and then the gene of S protein was added to two types of adenoviruses, Ad26 and Ad5. These were made in such a way so that they could invade the cells. When the Sputnik V is injected, the adenovirus gets attached to the peripheral region of the proteins. Then the adenovirus goes into the nucleus of the cell; after that, it pushes its genetic material into the nucleus. The adenovirus is engineered in such a way so that it doesn't replicate, but a gene of S protein could be copied into mRNA molecules. After reading its sequences, the mRNA leaves the nucleus of the cell, and then it starts to accumulate the S protein. Some of these proteins made by cells form spikes which get transferred to the surface. Now these proteins are split up into small pieces by the vaccinated cells present on the surface. After that, the immune system recognises the extended S protein and fragments of S protein. By switching the alarm system of the cell, the adenovirus exasperates the immune system.

Moderna mRNA-1273

The Moderna mRNA-1273 vaccine is based on mRNA and helps to fight against COVID-19. Here the mRNA gives all the information to those host cells which helps in the formation of the protein S antigen, which is very much outlandish to SARS-CoV-2. So, in order to keep that information in the memory immune cells, the protein of S-antigen allows the body to generate an immune response. After certain clinical trials, it was found that the efficiency of the Moderna mRNA-1273 vaccine was around 94% for those patients who received two doses of vaccines (that is, a full series of vaccines) and with a negative baseline SARS-CoV-2 status. This vaccine is stored in a freezer at -25°C to -15°C.

Pfizer-Biontech

Pfizer and Biontech collaborated with each other and are now working on their COVID-19 vaccine candidate. The Pfizer vaccine mainly consists of modRNA (or nucleoside modified mRNA), which encodes a mutated form of the S protein of SARS-CoV-2 encapsulated in lipid nanoparticles. Along with the mRNA molecule, the vaccine comprises of many ingredients like sucrose, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), ALC-0315, ALC-0159, cholesterol, dibasic sodium phosphate dihydrate, monobasic potassium phosphate, potassium chloride, sodium chloride, sucrose etc. This vaccine is also given by intramuscular injection. This vaccination is given in 2 doses, with 3 weeks' aside. After certain clinical trials, it was found that the Pfizer vaccine has an efficiency rate of around 91.3% to fight the SARS-CoV-2 virus. Some researchers found that the modRNA had a sequence of

around 4,284 nucleotides. It is mostly made up of a 5' cap, a 5' UTR (untranslated region) made from the sequence of human-alpha globin, a signal peptide, and two proline substitutions.

Booster Doses

It was found that when 2 doses of the Moderna mRNA-1273 or BNT162b2 were given, they were unable to provide sufficient protection against the omicron variant, but after giving the booster dose, there was a hike in the rate of protection against omicron. This dose basically accelerates the neutralizing antibody. A single booster was also given by Pfizer-BioNTech to the 18-year-old and older age groups who had already taken the primary doses. So, it was found that these booster doses showed quite encouraging results for preventing the infection caused by Omicron. Booster doses showed protection of around 90%.

Problems with Traditional Vaccines

There are many kinds of vaccines available on the market. The vaccine can treat, or at the very least try to protect by increasing the immunity. But SARS-CoV-2 is a new virus. The vaccines available now are not able to treat it. The development of a conventional vaccine is time-consuming. Normally, the average period for vaccine production is 12–15 years. Classical vaccines use the antigen itself. For this reason, a huge amount of antigen, the key element, as well as human subjects with consent are needed. Traditional vaccines have fought well against highly contagious diseases, viz., measles. But in the case of COVID-19, no cure is still available. So, people are not willing to volunteer as test subjects. Again, time is short, and thus, conventional vaccines are not an alternative in this case.

Advantages and Disadvantages of New Generation Vaccines

Many new generation vaccines have emerged that can be used, though further research is required. This part will explore those kinds along with their advantages and disadvantages, respectively.

Recombinant Protein Vaccines

In this case, a part of a whole protein or fragment of proteins, such as the RBD or fusion of RBD with carrier or other elements, can be used. It has been shown that animals can be immunized with the help of recombinant protein vaccines. The main disadvantage with these kinds of vaccines is that they can only provide partial immunity and thus need auxiliary elements, termed adjuvants, to boost immunity. The vaccine candidate 'NVX-CoV2373', for COVID-19, uses Matrix-M as an adjuvant.

Viral Vector-Based Vaccines

In this case, the antigen is cloned in a virus unit unable to reproduce itself in the host body. The viral vectors stimulate the same kind of reaction in the body that would otherwise be produced only while encountering the toxic virus. By this means, the vaccine actually "trains" the body on what to do while encountering the virus and thus delivers protection against the virus and an immunity boost. Adenovirus is being used in the case of COVID-19.

Bacterial Vector-Based Vaccines

As the name suggests, the vector used in this case is bacteria. LAB (Lactic acid bacteria) is very promising in this case. Symvivo's vaccine candidate for COVID-19, bacTRL-Spike, uses LAB along with the S protein, and is currently in the clinical trial phase.

Plasmid DNA Vaccines

Plasmid DNA vaccines do provide a higher safety profile because the use of live viruses is eliminated. Again, double-stranded DNA is much more stable than RNA, protein, or even the virus itself, and can be frozen or put into a cryo period for a long time. But the main drawbacks of this kind are low transfection efficacy. INO-4800, Inovio's COVID-19 vaccine candidate, uses a hand-held electroporation device, known as CELLECTRA. The vaccine is injected intradermally with electrodes. The electric pulses open up the cell membrane for the plasmid to enter without any defect. But adequate infrastructure and cost will be vital points for this on a bigger scale.

Messenger RNA Vaccines

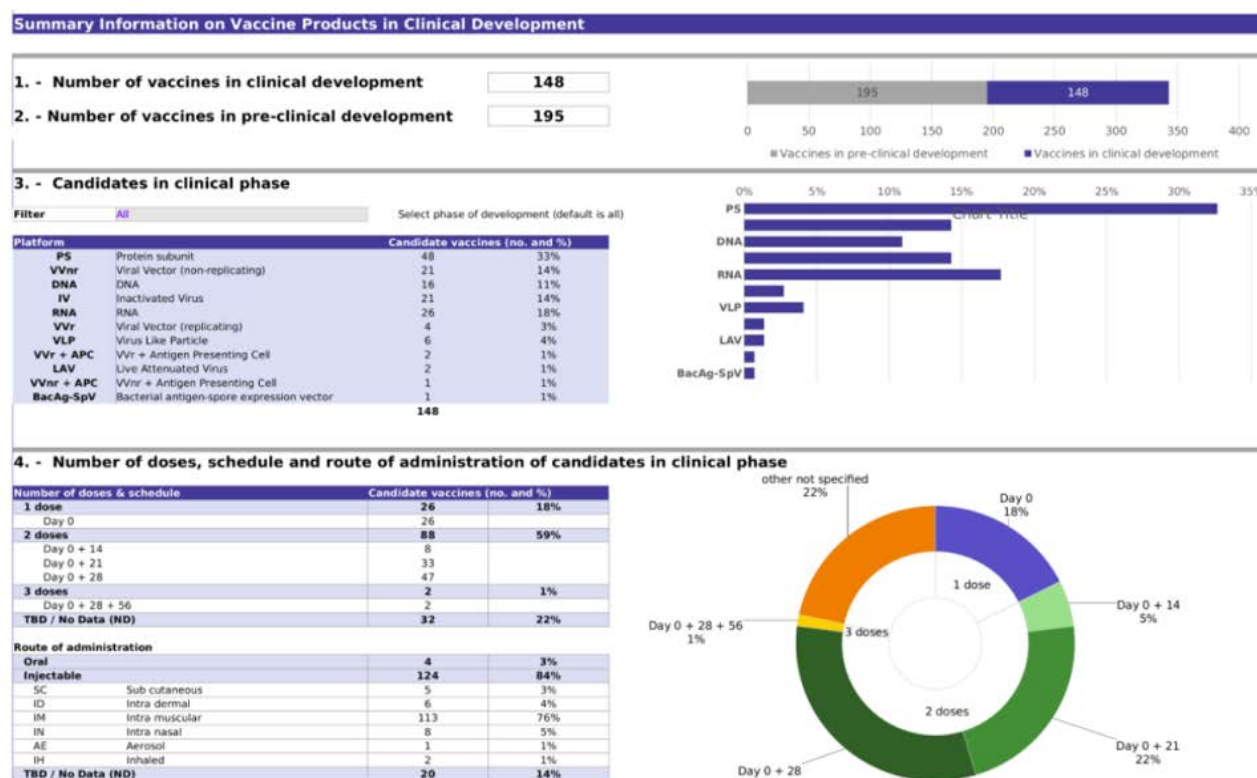
These are the newest generation of vaccines. All the components of an m-RNA vaccine can be

"chemically synthesised". The elimination of any living agent from the virus or antigen makes it the safest of all. Again, the manual synthesis gives a large number of quality checks for large productions. And it prevents any chance of transmission from the production facility, by human or non-human means, especially for high-risk pathogens like ebola. Generally, mRNA molecules have low apparent transfection efficacy. Lipid nanoparticles (LNP) are added to incorporate the mRNA for transfection. The mRNA-1273 vaccine candidate for COVID-19 from Moderna is an LNP-encased mRNA vaccine (Baden *et al.*, 2021).

Trained Immunity-Based Vaccines

Traditional and conventional vaccines provide a safe guard only against the toxic virus. But trained immunity-based vaccines (TIV) provide protection against unwanted pathogens from entering the bodies, thus keeping one healthy. Currently, BCG, a vaccine against tuberculosis, is under clinical evaluation (Jirjees, Bashi, & Al-Obaidi, 2021) for its power to suppress SARS-CoV-2. Still, the exact cause is unknown. And that's preventing it from being used on a larger scale, as the long-term side effects, if any, are still unknown.

For the lack of space, all kinds of vaccines and their detailed discussion can't be done here. One can find a detailed table and discussion on the website of WHO about various vaccines (WHO, 2022). Some relevant charts are provided below (data taken from WHO draft-landscape as of March 11th, 2022).



Source: <https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>

Figure 7: A glimpse of vaccines in clinical and pre-clinical development and their platforms and doses

Challenges

In the following part, it will be discussed in detail the challenges being faced or likely to be faced in the future. These will mostly contain challenges imposed by various limiting entities or on social or economic grounds. The challenges being faced, or which can be faced in the future, are

Data collection and management

The very first challenge to preventing COVID-19 is accessing data. If any person comes into the

COVID cluster, chances are high that she/he will get infected. But without adequate data, the spread of disease or insight into the actual situation or the trend of infection is impossible. GIS (Geographic Information System) was used by India, as well as many other countries. Under immense pressure, the Govt. of India launched the AarogyaSetu app. It is developed under the Ministry of Electronics & IT. The app uses Bluetooth and GPS capabilities. It keeps records by analysing nearby users of the app. Thus, it forms the cluster, and by analysis of data, it can be used to determine the spatial propagation pattern, spatial and temporal transfer of the COVID-19 hotspots. It was noted through the information maps that, almost in all cases at the city level, the concentration of infected people occurred in clusters. In the age of artificial intelligence and social media, the government of India, along with the state governments, provided the AarogyaSetu app and WhatsApp and FB chat bots.

Market competition

Sooner or later, a cure for COVID-19 will be found. But right now, who holds the vaccine (possibly) holds either the greatest cure of recent times or the most money-making agent in recent history. One should not treat the vaccine as an agent of profit. On November 9th, 2020, Pfizer and BioNTech shared their primary findings about the efficacy of their candidate. Nine days later, they announced 95% efficacy. On November 16th, Moderna announced their candidate's efficacy to be 95%. Two days later, Oxford and AstraZeneca reported their safety and immunogenicity in a larger context (Mills & Salisbury, 2021). It should be taken into account that there should be more openness about the test and how the vaccine is distributed and used.

Funding

It has already encountered one outbreak in early 2000. The National Institute of Allergy and Infectious Diseases of the United States contributed approximately \$100 million (Prudêncio & Costa, 2020). But it dissolved in 4 years. No further funds were organized, and thus the SARS-CoV-1 research was finished. Unfortunately, the low priority for long-term medical research is not new. As one researcher points out, in the post-COVID-19 era, without any hesitation or political controversy, research funds should be provided for adequate, fair, and sustainable research. Only by that means, will it ensure healthy lives for humanity. The global economy is now at a pivot point, as it was after the last SARS encounter. An unstable economy points towards chaos in every level of society and government. To get the economy back to where it was, scientific models should be used, and funding for long-term research on these kinds of high-risk pathogens should be given in a sustainable way.

Quality control

With many new technologies at the disposal, the challenge is not to make a vaccine but to provide it to people. As time is the key factor here, testing for standardizing the vaccine isn't a possible approach. There is a need to test the vaccines on human subjects only by deliberately infecting them with the pathogen. But no cure for this has been found till now, and thus inhibits the way. The Goldilocks approach (Billings & Bernacki, 2014) may be used for this purpose.

Route of administration

The route of administration for a vaccine is important. SARS-CoV-2 is an airborne virus. It attacks through the air that is breathed. The vaccines currently in progress are injected into the blood. But it would be better if they could be intranasal or inhalation or oral (Wu, 2020). Again, many people are trypanophobic in nature. They will be encouraged if the vaccine is intranasal, etc. in nature.

Thermostability

Thermostability is now the most problem causing aspect. Current vaccines are stable within 2°C to 8°C. But, for a vaccine drive of this magnitude, this can cost up to 80% of the cost of a vaccine. And also, maintaining a cold chain is still very costly and immobile for both developed and developing countries. A lack of adequate knowledge about the cold chain may result in serious consequences while transporting the vaccines.

Priority of being vaccinated

Due to the scarcity of supplies and the urgency of the situation, a decision on who to vaccinate first must be made. A clear distinction on the basis of age has been shown. Many countries have relied upon various strategies. In India, as published by the Ministry of Health and Family Welfare (MoHFW), the government of India has worked on priority groups. The government decided that the first group would include healthcare and front-line workers. The second group will include people over 60 years of age and people aged between 45 and 59 years of age with comorbid conditions. At last, as of March 1st, 2021, all eligible candidates over 18 have been vaccinated. Vaccination for candidates under 18 has started from January 3rd, 2022.

Fast track vaccination

COVID-19 vaccines are and will be introduced to the market in a very short time. There can be negligence in manufacturing them or transporting them. Even, for several vaccines, the dosage provided is different. It is very difficult to make people aware of the remaining dosage after day 0. In India, this is being done by the Aarogyasetu app. Distributing vaccines in clusters in such a short time is also equally risky, as studies on them are still going on.

Supply chain logistics

Thermostability is already discussed above. Asian countries will find it very hard to cope with this problem with poor and inadequate infrastructure (Haque & Pant, 2020). Production of not only vaccines but also glass vials, etc., will be subjected to a sudden boost. The world economy is now facing a challenge. Boosts of this kind will only make the situation worse. WHO has arranged the logistics on a global level. WHO is working with CEPI and GAVI to manufacture a vaccine, alongside UNICEF as the delivery partner. Approximately 80 rich nations have signed on for COVAX, the global vaccine (Haque & Pant, 2020). Money will be invested for fair manufacturing and distribution purposes.

SARS-CoV-2 genetic instability

SARS-CoV-2 undergoes certain mutations in a small amount of time. Five prominent signatures have been reported as of 2020. They are all reported from various geographic locations. They differ from the viruses in Wuhan in genetic aspects. Many vaccines use the viral S protein, which has, till now, been found to be resistant to mutation. Certainly, this helps to some extent. But, after the introduction of effective vaccines, mutant viruses may appear with selective advantage, posing a threat (Forni & Mantovani, 2021).

The unknowns

There can be various other problems. People don't know them yet. They don't know the effects of vaccines in the long term. Besides, there can be political arguments over the possession and distribution of vaccines. The impact on the climate due to the waste generated by manufacturing vaccines is still unknown. The fate of used equipment during vaccination is also unclear. Lastly, we should be extremely cautious as this pathogen can be used as a bioweapon, like ebola (Gunaratne, 2015).

CONCLUSION

Viruses that have evolved from the conventional to the modern age of science pose a significant threat to humanity. History shows that in every century, one or more viruses have evolved and caused a significant effect on the fate of humanity. On one hand, people face a significant loss of human lives and resources; on the other hand, unrestful socio-economic conditions. They began to question the own knowledge and morality in various aspects. But on the other hand, science and technology are greatly beneficial as there is much more activity going on in this kind of time. The coronavirus pandemic is a similar kind of situation. Many lives have been lost, and the whole world is in mourning over it. But in contrast, science and technology related to microbiology and medical professions have

experienced a certain boom. Many new generation vaccines are on their way, which would otherwise require a lot of time and ethical controversy. The vaccine drive for coronavirus over the globe is a mammoth task. Politics and private interests should not hamper the vaccine drive, which is manufacturing them and providing them to the people at a nominal cost. This mammoth task must be achieved through altruistic partnerships between industry, governments, and international organizations for the universal benefit of human health. It may take some more time and could cost more lives, but it is certain that people will find a cure for COVID-19.

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Antibacterial and antifungal activities of *Azadirachta indica* A. Juss (Neem): A review

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ABSTRACT

The role of natural products in pharmaceuticals is undisputedly very significant. In this vista, *Azadirachta indica* is becoming the cynosure of modern medical science. Traditionally, this plant has been widely utilized for a diverse variety of ailments in the animal world as well as in the plant forum. An array of phytochemicals present in neem plants has been attributed to their pharmacological activities. This review attempts to summarise the antibacterial and antifungal properties of the plant and emphasises the need for further analytical research for new drug discoveries.

Keywords: *Azadirachta indica*; Neem; Antibacterial; Antifungal; Azadirachtin

INTRODUCTION

Azadirachta indica, commonly known as "Neem", belongs to the family Meliaceae under the order Rutales. It is native to the Indian sub-continent and is also commonly found in other tropical and subtropical regions of the world. This is a fast growing tree that reaches over 20 metres in height and has an erect trunk. It has pinnately compound leaves, bears axillary panicle inflorescences, and fruits as drupes.

This valuable species has been used in traditional remedies since a long time ago and has been documented in various literature related to Ayurveda, Homeopathy, and Unani medicine. The multifaceted pharmacological attributes of this plant have attracted worldwide attention and it is now extensively utilized in modern medicine.

The virtue of the medicinal properties of the neem plant is attributed to the presence of a diverse and complex range of bioactive compounds. Each specific part of the plant is a significant source of important phytochemicals, belonging to the classes of terpenes and limonoids. More than 140 biological compounds have been identified and isolated to analyse the properties they possess. Azadirachtin is the most important limonoid or teranotriterpenoid. Nimbidin is the crude principle that is extracted from the seed kernels of neem and from this crude base, some more teranotriterpenes like nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid have been reported to be isolated (Kudom, Mensah & Botchey, 2011; Ojha, 2016). Gedunin and mahmoodin are also isolated from seed oil. Other compounds extracted from the fresh leaves of neem are quercetin and β -sitosterol.

These various bioactive constituents isolated and identified from the different parts of the plant are responsible for the wide range of therapeutic properties like anti-inflammatory, immunomodulatory, anti-diabetic, hepatoprotective, neuroprotective, antiulcer, antimicrobial, antioxidant, antimutagenic, anticarcinogenic, and wound healing (Alzohairy, 2016). A large number of workers have contributed to review and research of the bioconstituents and the biological activities of the plant for decades together (Biswas *et al.*, 2002; Subapriya & Nagini, 2005; Arora, Singh & Sharma, 2008; Atawodi & Atawodi 2009; Nishan & Subramaniam, 2014; Kumar, Mehta & Pathak, 2018).

This review concentrates and enumerates the reported evidence of the antibacterial and antifungal activities of *Azadirachta*.

LITERATURE REVIEW

Antibacterial Activity:

Several researchers have long demonstrated the antimicrobial properties of neem extract. Aqueous and alcoholic extracts of the plant parts were subjected to different assays to understand their activity against bacteria.

Khan and Wassilew (1987) documented the antibacterial activity of the extracts of the leaves, seeds, and bark of neem. The extracts exhibited inhibition against a broad spectrum of gram-negative and gram-positive microorganisms, which included *Mycobacterium tuberculosis* and strains that are resistant to streptomycin. The extracts also showed inhibition against *Vibrio cholera*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis* and *M. pyogenes* in *in vitro* conditions.

Reports of nimbolide showing effectiveness against *Staphylococcus aureus* and *S. coagulase* have been given by Schmutterer (1995).

Govindachari *et al.* (1998) first reported about the polyphenolic flavonoids - Quercetin and β -sitosterol, which were purified and isolated from fresh neem leaf extract and known to have antifungal and antibacterial properties.

Various extracts of neem were subjected to show antibacterial activity against certain bacteria, like *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Escherichia coli* and *Myxobacteria*, which are pathogenic to fishes.

Alzoreky and Nakahara (2003) reported the antibacterial action of neem extract against *Bacillus cereus*, *Escherichia coli* and *Salmonella infantis*.

A number of reports on the efficacy of the mouth rinses with the formulations of the extracts of different parts of the neem plant have been put forward by many workers. These neem extract-based preparations are observed to have positive results in treating chronic gingivitis and dental plaque (Botelho *et al.*, 2008; Chatterjee *et al.*, 2011).

Aslam *et al.* (2009) showed that the phytoconstituents in neem could stop the growth of *Staphylococcus aureus*. The crude flavanoids were found to be the most effective phytoconstituents.

Susmitha *et al.* (2013) reported the antibacterial activity of aqueous leaf extracts of neem against the two human pathogens - *Escherichia coli* and *Salmonella sp.* According to Farzana, Zerine, and Kabir (2014), methanolic extract of neem showed inhibition against *Vibrio cholera* and *Klebsiella*, and only the boiled water extract of the leaves could inhibit *Klebsiella* and *Escherichia coli*.

Raut, Sawant and Jamge (2014) worked on the antibacterial activity of neem on the gram negative bacteria of *Salmonella typhi*, *Vibrio cholera* and *Escherichia coli* and gram positive bacteria like *Bacillus subtilis*. The agar diffusion well method was used to determine the activity of the extracts of different plant parts. It was reported that the leaf and bark extracts worked effectively against *Vibrio cholera* and *Bacillus subtilis*.

Standardized disc diffusion and microdilution tests were subjected to assaying the biological activity of neem oil against forty-eight isolates of *Escherichia coli*. This assay performed by Del Serrone, Toniolo and Nicoletti (2015) showed that the neem oil extract showed antibacterial activity against all the isolates.

Ethanol extract of the neem leaves was shown to exhibit growth-inhibitory effects on Methicillin-resistant *Staphylococcus aureus* biofilm and the formation of planktonic aggregation as well as anthelmintic activity against *Schistosoma mansoni* worms (Quelemes *et al.*, 2015).

Mandal and Mandal (2011) reported the bactericidal activity of neem seed extract against a wide spectrum of gram-negative as well as gram-positive pathogens. This property has been reported to be due to the crude principal nimbidin present in the seed oil.

Similar effects of crude seed extracts were observed against the pathogenic bacteria that cause human eye and ear infections, including *Staphylococcus aureus*, *S. pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* (El-Mahmood, Ogbonna & Raji, 2013).

Aqueous extract of the leaves of *Azadirachta indica* along with silver salt has been used to prepare silver nanoparticles, and these nanoparticles are subjected to test the antimicrobial efficiency of the neem extract. Different concentrations of silver nanoparticles—ranging from 0, 2, 4, 8, 10 and 12 µg/ml—were added to the agar substrate plates with the bacterial colonies of *E. coli* and *S. aureus*, as obtained from the soil samples. The antibacterial activity induced by these particles of neem extract is by changing the permeability of the cell membrane and degrading the enzymes in the bacteria (Verma & Mehata 2016). These silver nanoparticles can be prepared by altering certain environmental parameters such as temperature and pH, as well as the concentration of reactants and reaction time during the process of production. These altered green silver nanoparticles of neem extract showed elevated levels of growth inhibition against the soil-borne bacterial colonies.

The most commonly found microorganism in the infected root canals of both primary and permanent teeth is *Enterococcus faecalis*, a facultative anaerobic gram-positive coccus. *E. faecalis* is very resistant and survives even in its stages of starvation (Bhardwaj, Ballal & Velmurugan, 2012). Among many irrigants, chlorhexidine is found to be very effective as a broad-spectrum antimicrobial agent and can remove both gram-positive and gram-negative bacteria from inaccessible areas of teeth. Chlorhexidine gluconate is also equally efficient in disinfecting the teeth. But there are a number of undesired side effects like staining of teeth, alteration of taste and even developing microbial resistance after continuous use. Due to these effects, there is a need for herbal alternatives without creating ill effects with their usage. Chandrappa *et al.* (2015) made an ethanolic extract of mature neem leaves and demonstrated its activity against *Enterococcus faecalis*. Considerably better inhibitory action against the bacteria was reported by the neem extract as compared to 2% chlorhexidine solution.

Similar results were earlier reported by Vinothkumar *et al.* (2013), who found the high efficacy of neem extract against *E. faecalis* in comparison to 5.25% sodium hypochlorite. Ghonmode *et al.* (2013) also found that neem showed high effectiveness against *E. faecalis* compared to 3% sodium hypochlorite.

Ghosh *et al.* (2016) also reported the phytochemicals derived from neem to be potential contributors to several biological activities of the plant. The plant's essential oil has strong antibacterial properties against *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, and *P. putida*, which can cause food to go bad.

The compound, mahmoodin, a deoxygedunin which is purified and isolated from the oil of neem seed, is shown to exhibit moderate antimicrobial action against certain strains of human pathogenic bacteria (Alzohairy, 2016).

The other compounds that are reported to have contributed to the antibacterial property of neem are nimbidin, nimbin, nimbolide, gedunin, margolone, and cyclic trisulfide (AlAkeel *et al.*, 2017).

One of the most rampant oral diseases is periodontitis, which is a polymicrobial infection caused by anaerobic bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Heyman *et al.* (2017) investigated and reported a persisting bacteriostatic action of the neem extract against these two species, and the antioxidant activity was also observed to be in play in the region of inflammation.

Evident bactericidal activity against *Helicobacter pylori* is established by the usage of the neem oil extract, where it exhibited properties like increasing acidity at low pH and the ability to kill non-growing bacteria. *H. pylori*, a potent pathogen that causes gastric disorders, can be prevented from infecting by administering a formulated dose of neem extract (Blum, Singh, & Merrell, 2019).

Antifungal activity:

The effectiveness of neem against certain phytopathogenic and human infecting-fungi has been

reported and proved by various workers. Several reports documented the effective use of neem extract preparations in controlling the growth of fungi causing athlete's foot, ringworm, and those occurring in the bronchi, lungs, mucous membrane in the case of mouth cavity thrush, intestinal cavity, etc.

The fungistatic and fungicidal properties of neem stem bark extract against *Aspergillus spp.* and *Candida spp.* was investigated by Fabry, Okemo and Ansorg (1996).

Sphaerotheca fuliginea (Podosphaera fuliginea) is the causal organism of the powdery mildew on cucurbits. Reduction of conidial germination of the pathogen by 11% was reported when subjected to neem seed extracts; thereby establishing the antifungal activity of neem (Coventry & Allan 2001).

Neem seed and leaf extracts were administered to dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum nanum*. At 15µg/mL concentration, the seed extract was found to be efficient in disrupting the growth pattern of the fungi tested. This was reported by Natarajan, Venugopal and Menon (2003).

Mondall *et al.* (2009) investigated the antifungal activity of neem extract on the two saprophytic fungi, *Rhizopus* and *Aspergillus*. The study showed growth inhibition of both the fungi with the effect of crude aqueous and alcoholic extracts of neem leaves of varying ages. *In vitro*, it was also found that the ethanolic extract of the leaves stopped the growth of both types of fungi better, and that this was true even for the extract of older leaves.

Reports of antifungal activity against phytopathogens such as *Sclerotium rolfsii* by the usage of neem cake extract and against powdery mildew of balsam by using foliar spray of neem cake extract were given by Singh *et al.* (2010). Bhonde, Deshpande and Sharma (1999) documented the other pathogenic fungi whose growth is effected by the extract of neem cake are *Fusarium oxysporum*, *Alternaria solani*, *Curvularia lunata* and *Helminthosporium sp.* Absolute growth inhibition of *H. pennisetii* by using neem cake extract at 0.6% drug concentration. This is achieved by inhibiting spore germination of the sporulating fungus. It inhibited *Colletotrichum gloeosporioides f. sp. mangiferae* as well (Kumari *et al.*, 2013).

Candida albicans is the most commonly persisting fungal species in root canal infections and other dental infections like candidiasis. For successful pathogenesis by the fungus, adhesion to the target tissue is the most important prerequisite for colonization. The neem extract has been tested and found to be a promising candidate for inhibiting fungal colonisation in the substratum. The neem leaf extract works in an antiadhesive mode for the fungus, with effects on its hydrophobicity and biofilm formation (Polaquini *et al.*, 2003).

Gas Chromatography and Mass Spectroscopy analysis was performed for n-Hexane extract of *Azadirachta indica* leaves. 45 biocompounds were identified and 33 of them were reported to have antifungal activity. The study was observed to demonstrate the activity against *Candida albicans* (Akpuaka *et al.*, 2013).

Rhizoctonia solani and *Sclerotium rolfsii* are phytopathogenic fungi which were subjected to nano emulsions of neem oil along with citronella essential oil. The nano emulsions were developed and characterised using Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM). The standardization of composition of the nano emulsions with their antifungal action against the two fungi was a pioneering report (Ali *et al.*, 2017).

All these reports of the antibacterial and antifungal properties of *Azadirachta indica* dictate the medicinal novelty of the plant in its entirety, where each part of the plant is a reservoir for novel compounds possessing immense biological significance.

CONCLUSION

For the enormous benefits that the neem plant caters to in various areas of health, this plant has rightly been labelled as 'Sarvarogharini' in the Charak Sanhita of Ayurvedic literature. This implies

that, traditionally, the plant has been hailed as the complete and perdurable component for curing any sickness. The variety of phytoconstituents present in the plant has rendered significant therapeutic values like antibacterial and antifungal properties. Scientific advances in the field of modern medicine have augmented the plant's utility in different pharmacological areas. *Azadirachta indica* is now the epicentre of medical research, and more upgraded exploration and analysis of its natural biocompounds would significantly benefit humankind therapeutically.

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The COVID-19 Summary: Immunological Facts and Figures

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ABSTRACT

The recent Coronavirus disease (COVID-19) has disrupted health as well as economic status throughout the globe. In December 2019, a sudden outbreak of pneumonia originating from the Wuhan city of Hubei province, China, turned into the COVID-19 pandemic worldwide. The infectious agent was later identified and characterised as being part of the Coronavirus family and was named as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy. From several pieces of evidence, major similarities with the genome of bat CoVs, the origin of the virus was hypothesized to be zoonotic. Individuals affected by COVID-19 usually show common symptoms, including fever, dry cough, dyspnoea, hypoxemia, and pneumonia. Recent studies have revealed the role of the immune system in the pathogenesis of COVID-19 as the virus induces not only the host innate immunity but also impairs adaptive immunity and induces an uncontrolled inflammatory response, leading to disruption of normal immune homeostasis, tissue damage, and finally death by respiratory failure. In this chapter, it discusses the immunopathogenesis of COVID-19. Up to date, there are no such appropriate treatments with proven efficacy available for COVID-19. Proper understanding of the involvement of the immune system in pathogenesis is important to designing an effective therapy against the infection.

Keywords: COVID-19; Pathogenesis; Innate and Adaptive Immunity; Immune Dysregulations; Lymphopenia; Leukocytosis; Macrophage's Response; Cytokine Storm; Silent Hypoxia

INTRODUCTION

In December 2019, a sudden outbreak of pneumonia broke out in the city of Wuhan in the Hubei province of China. The infectious agent was soon identified as a member of the Coronavirus family. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the name given by the International Committee on Taxonomy to the deadly coronavirus stains. Interestingly, the outbreak was a continuation of a similar incidence in 2002-2003 in some parts of the world (Cherry, 2004). Later, on February 11, 2020, the World Health Organization (WHO) finally named the disease as COVID-19, short for "CoronaVirus Disease 2019" (Sun *et al.*, 2020).

The source of COVID-19 is still controversial. Although various pieces of evidence are found that support the zoonotic origin of the virus, probably from the wildlife food market in the Wuhan district of China (Wassenaar & Zou, 2020). However, whole-genome sequencing of SARS-CoV-2 revealed many more similarities with the genome of a virus harboured in the bat species *Rhinolophus affinis* (Li *et al.*, 2021).

LITERATURE REVIEW

The Coronavirus

Viruses are sub-microscopic particles that have the capacity to infect animals, plants, and even bacteria. They are found in every ecosystem on Earth. Coronaviruses are characterised as a diverse family of enveloped, positive-sense, single-stranded RNA viruses. The family is so named because of the large spike protein molecules that are present on the outer surface of the virus, offering a crown-like structure to the virion. The genome of the coronavirus family of the order Nidovirales is the largest

genome found among RNA viruses known till date (Gorbalenya *et al.*, 2006). Their host range includes wildlife animals such as avians such as bats and mammals such as camels, pigs, and humans (Damas *et al.*, 2020).

Structure and genomic characteristics of SARS-CoV-2

SARS-CoV-2, the responsible viral pathogen for COVID-19 is an enveloped, single-stranded, positive-sense RNA virus. They are spherical in shape, with a diameter of 60-140 nm. The virus particle is made up of roughly four structural proteins, including Nucleocapsid protein (N), Envelope protein (E), Spike proteins (S) and Membrane protein (M), [Figure 1]. Spike proteins (S) are the outermost proteins that protrude from the outer fatty layer, so-called envelope, and are involved in the binding of the virus with the host cell receptor. The envelope proteins (E) and membrane proteins (M) are inserted into the lipidic envelope. Nucleocapsid protein (N) is the only protein present inside the virion that associates with the genomic RNA through electrostatic interaction. The functions of each structural protein are summarized in [Table 1]. (Bhardwaj *et al.*, 2022).

Table 1: Structural proteins encoded by SARS-CoV-2 genome and their functions

Structural protein	Function
N protein	<ul style="list-style-type: none"> •Impairs IRF3 phosphorylation and nuclear translocation •Prevents STAT1/STAT2 phosphorylation •Prevents inhibition of viral mRNA translation •Prevents GSDMD cleavage by caspase-1 •Disassembles and prevents formation of stress granules
E protein	<ul style="list-style-type: none"> •Forms an ion channel and participates in virion assembly
M protein	<ul style="list-style-type: none"> •Essential for the incorporation of viral components during virion assembly •Impairs MAVS self-association and association with SNX8
S protein	<ul style="list-style-type: none"> •Binds to the host receptor ACE2 and mediates fusion and entry

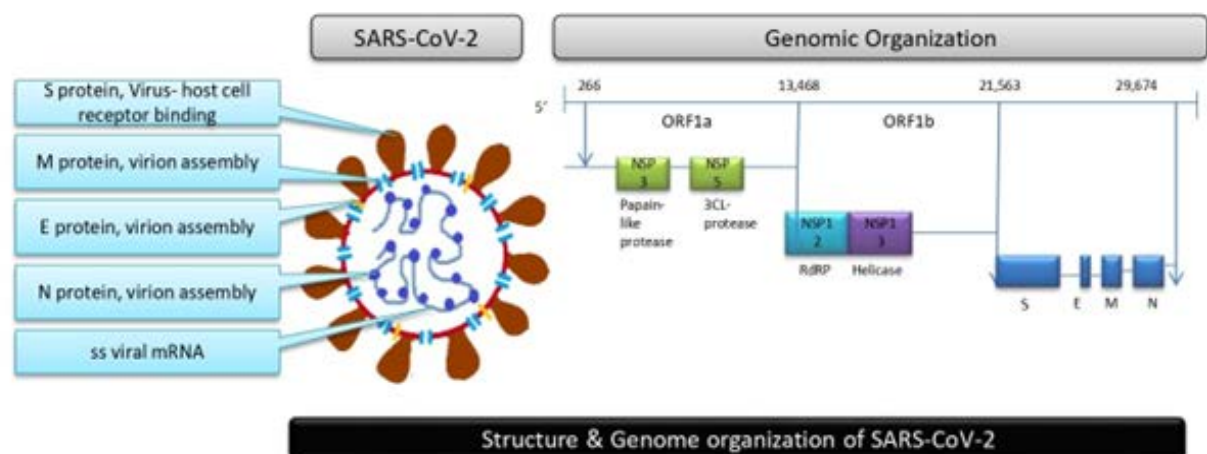


Figure 1: Structure and Genome organization of SARS-CoV-2

SARS-CoV-2 has shown genomic similarities with SARS-CoV and thereby is placed under the family Coronaviridae, order Nidovirales, genus Beta (β) coronavirus and subgenus Sarbecovirus. The +ve ssRNA genome of SARS-CoV-2 is about 26-32Kbp long. The genome comprises 12 open reading frames (ORFs) at the 5' end of the viral genome. Two overlapping ORFs, 1a and 1b, are present, occupying approximately two-thirds of the genome and encoding the RNA polymerase and other non-structural proteins of the virus. Other structural proteins are encoded by the remaining one-third of its genome, which runs from the 5' to the 3' terminal (Gómez *et al.*, 2021). Interestingly, as the SARS-CoV-

2 and other RNA viruses have demonstrated, the host immune system can eventually be escaped with potential drug resistance due to mutation. For example, the genes encoding the spike protein and RNA dependent RNA polymerase are major hotspots of mutation as conferred in such viruses (Pachetti *et al.*, 2020).

Different types of Coronavirus infection-

Among the seven coronaviruses identified, SARS-CoV-2 is the most common cause of human infection. The other six coronaviruses include SARS-CoV, NL63, MERS-CoV, HKU1, OC43, 229E, among which SARS-CoV and MERS-CoV cause various life-threatening respiratory diseases, whereas the others cause mild symptoms(Bhardwaj *et al.*, 2022).

Table 2: Different CoVs illnesses

Human coronavirus	Illness
SARS-CoV-2	COVID-19
SARS-CoV	Severe Acute Respiratory Syndrome (SARS)
MERS-CoV	Middle East Respiratory Syndrome(MERS)
HCoV-NL63	Mild symptoms
HCoV-229E	
HCoV-OC43	
HKU1	

The novel coronavirus (SARS-CoV-2), SARS-CoV (2002), and MERS-CoV, are major pathogens belonging to the family of Coronaviridae that primarily target the human respiratory system. In 2002, an epidemic caused by SARS-CoV, another beta coronavirus, began in South China. In 2012, the first case of MERS-CoV was reported in Saudi Arabia, whereas, in December 2019, COVID-19 infection emerged from Wuhan, China. The SARS-CoV like coronavirus derived from bats shares 88% sequence similarity with SARS-CoV-2, whereas the MERS-CoV viruses are 50% identical to the novel SARS-CoV-2 falling under the genus β -CoV(Abdelrahman, Li & Wang,2020).

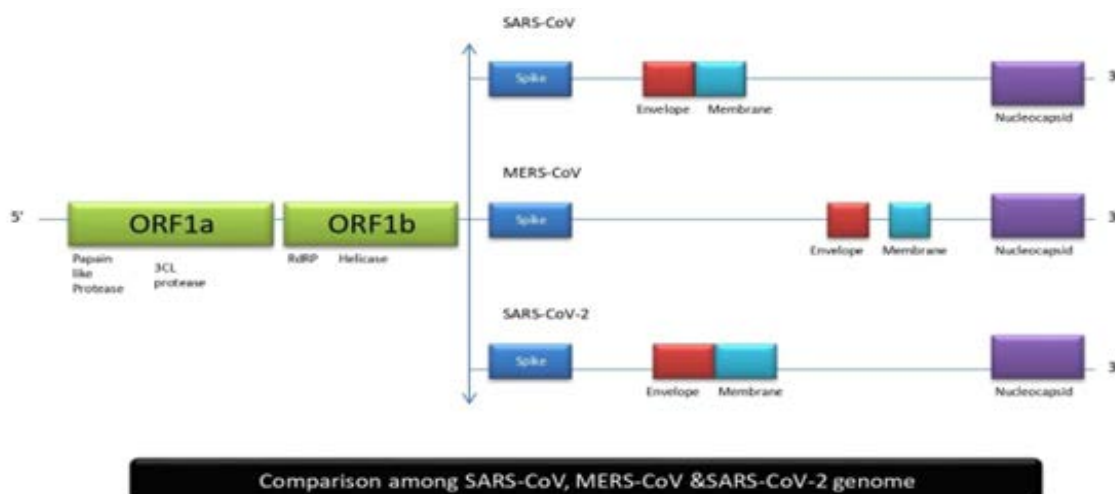


Figure 2: Comparison among SARS-CoV, MERS-CoV& SARS-CoV-2 genome

Notably, slight although significant differences can be found in terms of sequence, nucleotide number, gene order, and expression method among SARS-CoV, MERS-CoV, and SARS-CoV-2 falling under the same serogroup. For example, genes encoding the S protein, NSP(non-structural protein)2, NSP(non-structural protein)3, and receptor-binding domain (RBD) are prone to a few amino acid substitutions as reported in the novel SARS-CoV-2. The strain also showed significantly higher binding efficiency (10–20 times) of ACE2 and S protein in several studies as compared to the

previously known SARS-CoV. Therefore, the enhanced pathogenicity is believed to be due to the series of mutations resulting in higher binding efficiency to host cells as reported in recent studies (Zheng & Song, 2020; Nguyen *et al.*, 2020). Notably, the MERS-CoV binds specifically to the Dipeptidyl Peptidase 4 (DPP4) receptor to gain access to its host cells, whereas the SARS-CoV-2, similar to the SARS-CoV, exploits the Angiotensin-converting enzyme 2 (ACE2) receptor (Meyerholz, Lambertz & McCray Jr, 2016).

Life Cycle of Coronavirus (SARS-CoV-2)-

To understand the pathogenesis of a virus, a clear concept of its life cycle is necessary. Like other viruses, the novel coronavirus (SARS-CoV-2) also follows six general steps to complete its life cycle: attachment, penetration, uncoating, gene expression and replication, assembly and release [Figure-3]. A clear understanding of the life cycle of SARS-CoV-2 will help to understand how the virus elicits immune responses in the host body, and thereby the immunopathogenesis of SARS-CoV-2 will be divided into.

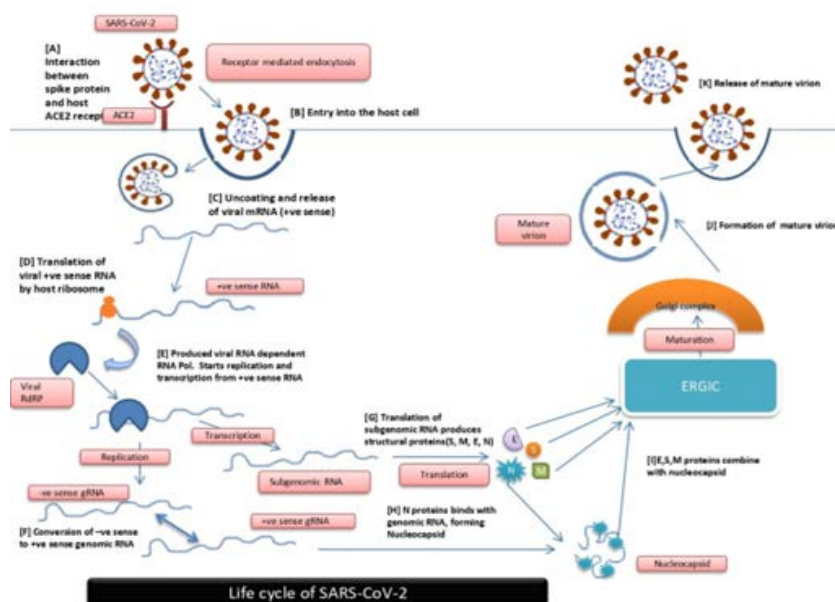


Figure 3: Life cycle of SARS-CoV-2

Entry of virus:

As stated earlier, SARS-CoV-2 infects the respiratory system by reaching the lungs via the naso-oral cavity. In the respiratory tract, it mainly invades mucus-producing ciliated epithelial cell lines or endothelial cells of the lungs. The primary virion-cell surface attachment is promoted by the mammalian cell attachment factor such as heparan sulphate. The entry of the virus is accomplished by the interaction between the viral spike protein's receptor-binding domain (RBD) and the host cell receptor Angiotensin-converting enzyme-2 (ACE2) (Clausen *et al.*, 2020). Angiotensin-Converting Enzyme 2 (ACE-2) is a type of membrane receptor that is expressed in almost all tissues of the human body, especially in capillary-rich organs such as kidneys, lungs etc.

The spike protein of the virus consists of two domains, S1 and S2. The receptor-binding domain (RBD) is present on the S1 subunit and, therefore, the S1 subunit binds to the ACE2 receptor. Hence, this subunit is involved in the determination of tissue tropism. The fusogenic activity of the virus-cell membrane is governed by two tandem domains, heptad repeats (HR1,2), that are present in the S2 region of the S protein. The interaction between the spike protein and the host receptor changes the conformation of the spike protein, followed by its activation and proteolytic cleavage at the S2 subunit. The processing includes priming of S1 and S2 subunits (those are non-covalently attached) followed by cleavage within the S2 subunit. These processing steps are manifested by the host's membrane protease or by the enzymes at the low pH of the endosome (in the case of endosome mediated entry), resulting in membrane fusion and thereby facilitating virus entry inside the cell (Benton *et al.*, 2020).

Once the virus reaches the host cell via membrane fusion, the +ve sense ssRNA genome is released into the cytoplasmic compartment, finishing the uncoating step of infection.

Gene expression and replication:

As SARS-CoV-2 contains a positive-sense RNA molecule, it can directly be used as a translational template by host cellular machinery. Therefore, after uncoating of the viral genome in the host cell cytoplasm, the virus hacks host translational machinery and starts expressing the viral mRNA. The translation of ORF-1a and ORF-1b results in the expression of two large polyproteins, pp1a and pp1b, respectively. These polyproteins are further cleaved into 16 non-structural proteins (NSP1-16) by three distinct functional proteases, thereby forming the viral RNA-dependent RNA polymerase core complex and other accessory proteins for virus assembly. An uninterrupted replication-transcription event results in the formation of sub-genomic mRNAs. Those are eventually translated into numerous structural and accessory proteins thereafter (Beig Parikhani *et al.*, 2021).

Assembly and release:

In post-synthesis, the resulting accumulation of structural proteins, such as the E proteins, is incorporated into the rough endoplasmic reticulum or Golgi membrane. The nucleocapsid is formed by the combination of +ve ssRNA with the capsid protein (N) and the subsequent assembled virus particles pass through the ER-Golgi Intermediate Compartment (ERGIC) and are finally matured for release. At this stage, particle-loaded vesicles are fused with the cell membrane. The new virions are ready to infect the neighbouring healthy cells. They are also released into the surrounding environment via respiratory droplets as infecting particles (Beig Parikhani *et al.*, 2021).

Pathogenesis of COVID-19:

SARS-CoV-2 reaches the lungs through the naso-oral cavity after inhalation of bioaerosols. It enters the epithelial cell line through the ACE2 receptor. The infection of SARS-CoV-2 can be divided into two stages. First, one to two days is the asymptomatic stage when the virus enters and multiplies in the cells of the upper respiratory tract. Thereafter, the virus begins to move through the naso-oral tracks towards the lower respiratory tract. As the virus first infects the upper respiratory tract, a mucus immune response starts to work, but the intensity of the viral infection becomes so high that it fails to eliminate the infection. However, when a virus particle reaches the lower respiratory tract, it provokes a strong immune response [Figure-4]. As discussed earlier, after invasion into the epithelial cell line, viral genome uncoating occurs, followed by gene expression and replication, thereby commencing the infection (Shah *et al.*, 2020).

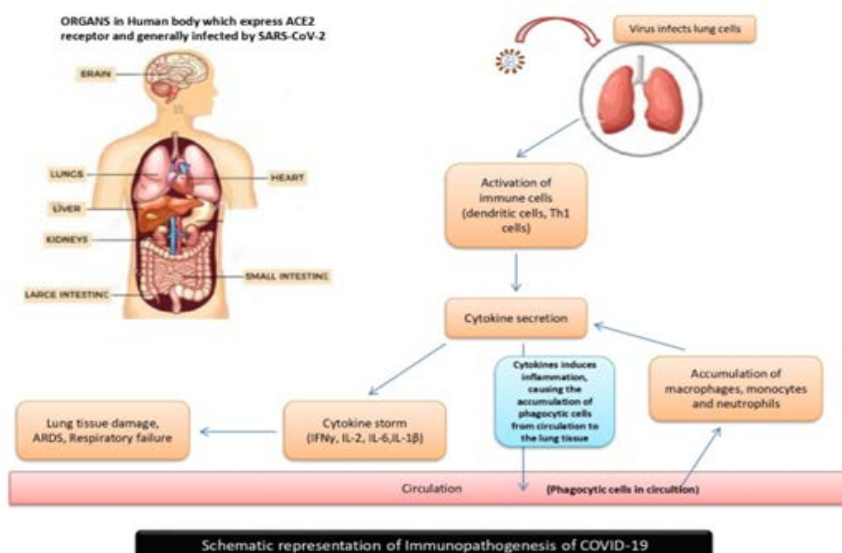


Figure 4: Schematic representation of Immunopathogenesis of COVID-19

Innate immunity

Virus infection first stimulates the innate immune system into the host. Pathogen associated molecular patterns (PAMP) are recognized by the pattern recognition receptor (PRR), present on the innate immune cells and triggers their activation. In the endosome, there are specific types of pattern recognition receptors (PRR) that associates with SARS-CoV-2 viral genomic ssRNA, ds intermediate RNA formed during viral replication, viral spike protein, viral membrane protein and other associated viral proteins. These PAMP molecule includes Toll-like receptors (TLR3, TLR8, TLR7, and TLR9) or the cytosolic RNA sensor, RIG-I/MDA5 or the secretory type PRR like Mannose-binding lectin (MBL) and C-reactive protein (CRP) (Chatterjee, Saha & Munoz, 2020). This recognition activates downstream cascade molecules and thereby provoking the transcription of type1 interferon molecules and other proinflammatory cytokines, especially IL-6. Notably, a potent innate immune response against viral infection and effective adaptive immune response is induced by Interferon (IFN) type I essentially mediated by the JAK-STAT pathway. The viral replication is thereby suppressed due to the accumulation of type I IFN resulting phagocytosis of antigens by the macrophages (Bouayad, 2020).

Strikingly the virus can survive within the host cell in the case of patients with disorder in JAK-STAT signalling pathway or any blockage in IFN production or even in the case of altered expression of macrophages. SARS-CoV-2 usually goes through these types of mechanisms to evade the host immune system. Usually, suppression of antiviral type 1 IFN response attributes in evasion of host innate immune response by the SARS-CoV-2. It has been shown that several protein-mediated mechanisms are involved that behave in antagonistic ways to the expression of type1 IFN.

- SARS-CoV-2 shields viral mRNA from the cellular sensory molecules to prevent IFN production.
- SARS-CoV-2 prevented IFN production by disrupting the stimulator of IFN genes.

Thus, the virus infection evades innate immune response by suppressing type1 IFN activity but the infection triggers a robust production of pro-inflammatory cytokines that leads to an influx of neutrophil and monocytes.

Adaptive immunity

Adaptive immune responses are mainly regulated by two types of lymphocyte cells: T lymphocyte and B lymphocyte. The antigen presenting cells (APCs) majorly regulate the T-cell response. In the case of viral infection, viral polypeptides and mRNA act as endogenous antigens and, after being processed, they will be presented towards T_H cells by MHC class I molecules of APCs. According to their presentation, CD8+ cytotoxic T cells and antibodies secreted by activated B cells will eventually act on the elimination of pathogens (Owen, Punt & Stranford, 2013).

Antigen presentation in SARS-CoV-2

In hosts such as humans, the Major Histocompatibility Complex (MHC) is known as the Human Leukocyte Antigen (HLA) complex. This system is controlled by genes located on chromosome 6, encoding cell surface molecules specialized to present antigenic peptides to the T_H cell receptor. With the binding of antigen, T_H cells get activated and they produce cytokines, thereby triggering the cytotoxic effect of T_C cells. However, in some cases of SARS-CoV-2, the involvement of MHC class 2 molecules has also been documented. Alleles of HLA such as HLA-DRB1*1202, HLA-B*0703, HLA-B*4601 and HLA-Cw*0801 are found to be more prone to be susceptible to coronavirus whereas alleles including HLA-Cw1502, HLA-DR0301 and HLA*0201 are involved protection against SARS infection (Khosroshahi *et al.*, 2021).

On the other hand, neutralizing antibodies, mainly IgG and IgM are produced in the B-cell mediated humoral immune response. The viral spike (S) and nucleocapsid (N) proteins are the sole target for most of the circulating antibodies in the case of CoVs infection. Several studies performed on patients with COVID-19 have revealed the antibody level in their serum by using magnetic chemiluminescence

enzyme immunoassay (MCLIA) [Figure-5]. The studies revealed IgM happens to be the first isotype to be detected in the serum followed by followed by IgA after 2-3 weeks of onset of symptoms. This declines gradually with the rise in level of IgG antibodies that can be detected even after several months post symptom onset (PSO) (Wu *et al.*, 2021).

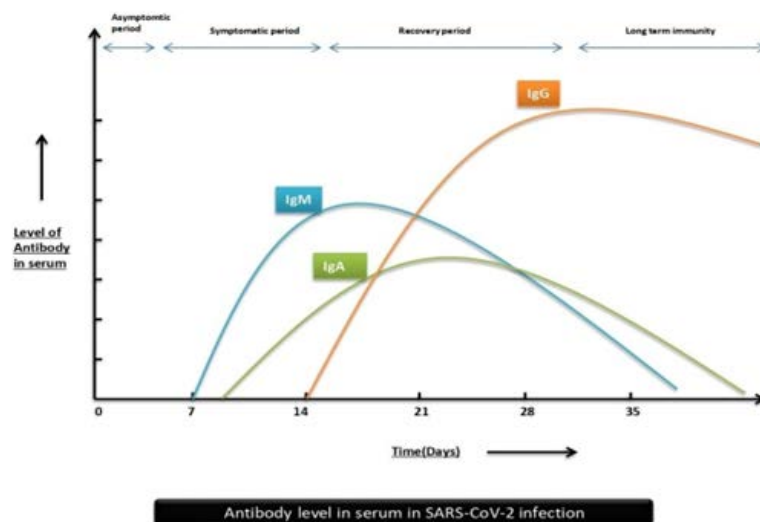


Figure 5: Antibody level in serum in SARS-CoV-2 infection

Dysregulation of immune cells/molecules in COVID-19:

SARS-CoV-2 infection destroys the intervening regulation between various immune cells and molecules, thereby leading to damage to immune homeostasis [Figure-6]. The SARS-CoV-2 infection stimulates an unrestrained innate immune response resulting in the production of an excess amount of pro-inflammatory cytokines, IFN- γ , macrophage colony-stimulating factor (MCSF) etc. that are associated with disease severity, causing tissue damage in the lungs and resulting respiratory failure. The common immune dysregulation associated with COVID-19 infection includes lymphopenia, leukocytosis, cytokine storm, ARDS (silent hypoxemia), and changes in macrophage behaviour, as described in the following section [Table-3].

Table 3: Responses of various immune cells during COVID-19:

Immune cells	Response to SARS-CoV-2 infection
Monocytes and Macrophages	<ul style="list-style-type: none"> • abundance of macrophages in the lungs • Macrophages produce proinflammatory cytokines such as IL-2, IL-6, IL-7, IL-1β and TNF-α • Delayed or suppressed type 1 IFN response
Dendritic cells	<ul style="list-style-type: none"> • Produce excess amount proinflammatory cytokines and chemokines (TNF-α, IL-6, RANTES, IP-10, MCP-1, MIP-1α, CCR1, CCR3 and CCR5) • Infection of dendritic cells with SARS-CoV-2 suppresses MHC-I expression that causes further delay in the expression of IFN-α • Increased expression of TRAIL (TNF related apoptosis inducing ligand) which leads to apoptosis of lymphocytes on their interaction with Dendritic cells, thereby adaptive immunity gets exhausted.
Natural killer cells	<ul style="list-style-type: none"> • SARS-CoV-2 infection depletes NK cell population • High level of IL-6 cytokine during SARS-CoV-2 infection inhibits cytotoxicity of NK cells

Mast cell	<ul style="list-style-type: none"> • SARS-CoV-2 infection induces mast cells to secrete proinflammatory cytokines like IL-1, IL-3 and IL-6, histamine and protease. • Mast cells upregulate RAS activity in airways • Mast cells secrete serine proteases.
Neutrophils	<ul style="list-style-type: none"> • Increased in number • Increased neutrophil to lymphocyte ratio • Increased NET release which leads to ARDS, thrombosis, coagulation and worst oxygenation conditions observed in COVID-19 patients
Eosinophil	<ul style="list-style-type: none"> • Gets depleted upon SARS-CoV-2 infection.
Basophil	<ul style="list-style-type: none"> • In severe COVID-19 patients number of basophils gets decreased.
T cell	<ul style="list-style-type: none"> • In SARS-CoV-2 infection the population of CD4+ T cells and CD8+ T cells get depleted, the state is called Lymphopenia. Therefore, the adaptive immune response becomes exhausted completely.
B cell	<ul style="list-style-type: none"> • Delayed antibody response.

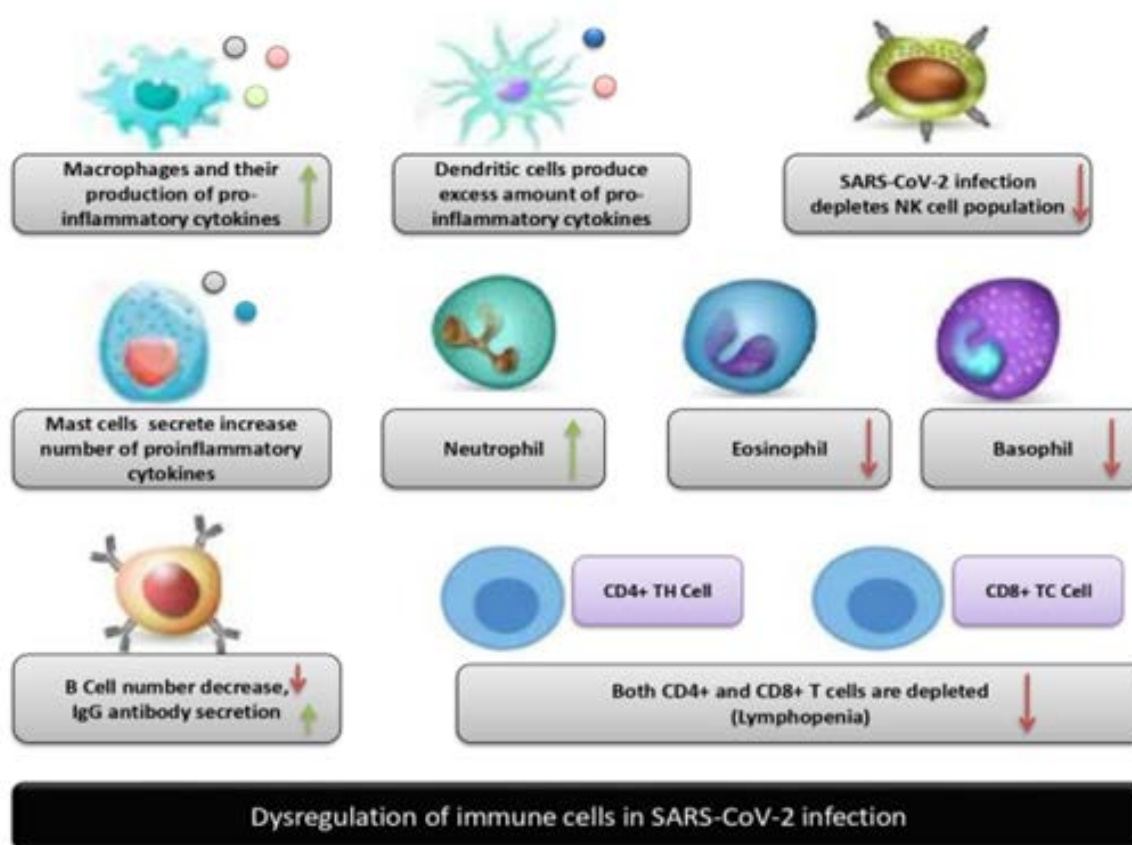


Figure 6: Dysregulation of immune cells in SARS-CoV-2 infection

Lymphopenia:

In severe cases of patients with COVID-19, lymphopenia is one of the major symptom observed among patients. The serious outcome involves significant reduction (<20%) of lymphocyte percentage in patient's serum [Figure-7]. Arguably the lymphocyte depletion in COVID-19 patients involves several potential mechanisms.

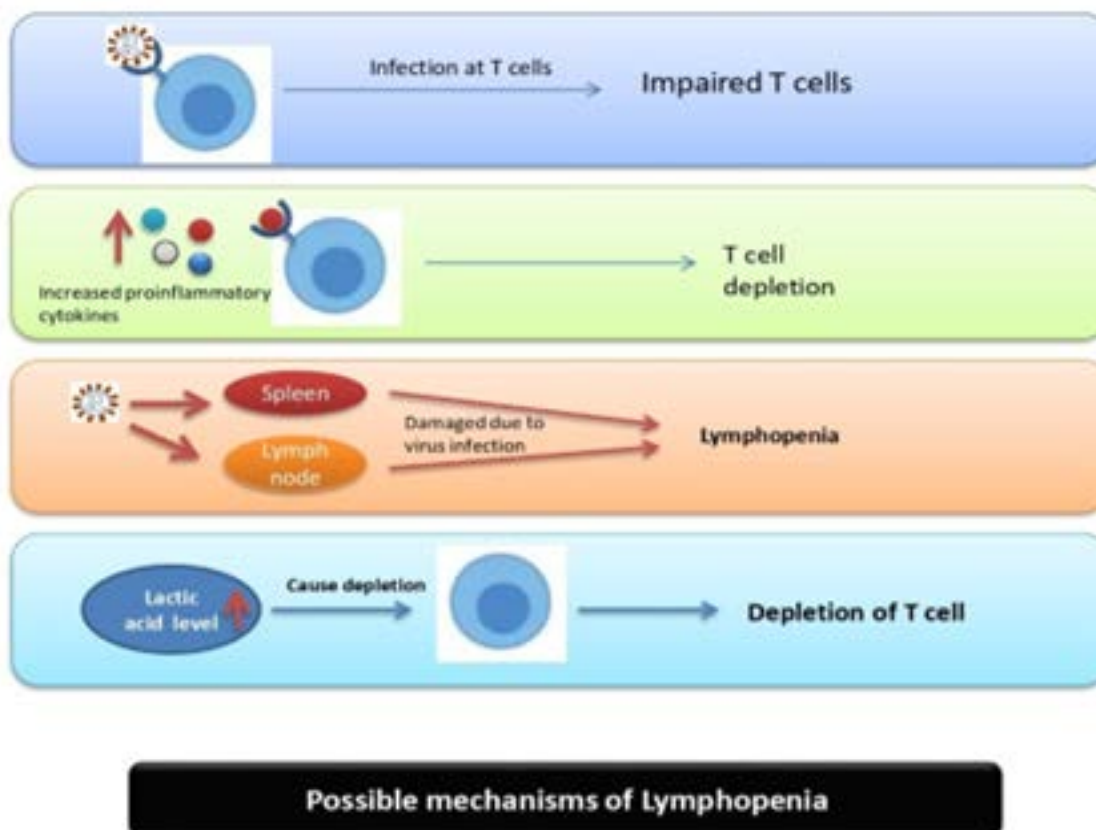


Figure 7: Possible mechanisms of Lymphopenia

(a) It is observed that level of proinflammatory cytokines (IL-6, IL-10 and TNF- α) in serum is inversely proportional with number of lymphocytes in peripheral blood.

(b) Notably the lymphatic organs including spleen and lymph node are directly destroyed by the process of spleen atrophy and necrosis of lymph node respectively by the SARS-CoV-2 virus inducing lymphopenia.

(c) Apoptosis of lymphocytes can be observed by upregulation of Fas expression in the cases of some COVID-19 patients.

(d) Interestingly, entry of SARS-CoV-2 into the lymphocytes and corresponding infection of T-cells and macrophages are often regulated by expression of ACE2 receptor essentially on the T-cells.

(e) The proliferation of lymphocytes is inhibited by increased level of lactic acid as detected in the blood of patients with severe COVID-19 (Yang *et al.*, 2020).

Leukocytosis:

The condition of excess accumulation of neutrophils in the blood of patients is known as neutrophilia. Strikingly, infection of secondary microbes leads to an increased level of neutrophils in the blood, often leading to an abnormal number of granulocytes (neutrophils) and monocytes. The presence of excess pro-inflammatory cytokines and impaired lymphocytes in patients with COVID-19 might be easily attributed to an abnormal number of granulocytes, especially neutrophils. Interestingly, the Neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio remained higher in the patients with COVID-19. Neutrophil upregulation in patients with COVID-19 is closely associated with lymphopenia where these cells provide the first line of defence by killing microbes via phagocytosis and degranulation. This often induces neutrophil recruitment to the tissue site where the infection has been initiated (Jimeno *et al.*, 2020).

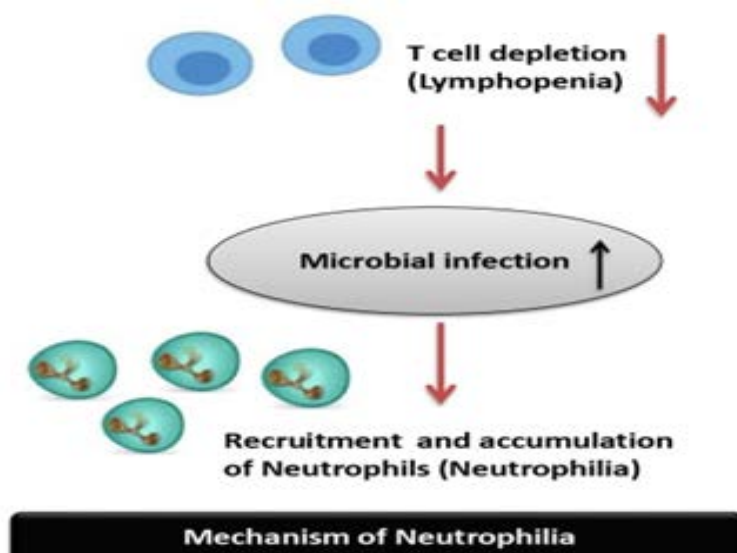


Figure 8: Mechanism of Neutrophilia

Monocyte/Macrophage phenotypic changes:

The macrophage activation syndrome is a dysregulated response to the cells, damaging the host in the case of SARS-CoV-2 infection. A profound alteration of activation status in the monocyte/macrophage system can be observed in patients. However, the absolute monocyte counts remain the same in severe COVID-19 cases. Additionally, higher numbers of inflammatory monocyte derived macrophages can be observed with significant depletion in tissue resistant alveolar macrophages. (Zhang *et al.*, 2021).

Cytokine Storm:

Cytokines are chemical mediators produced by several immune cells like innate macrophages, dendritic cells, NK cells, and T and B lymphocytes as an essential component of the inflammation reaction. Large amounts of pro-inflammatory cytokines can be detected in COVID-19 infection, causing an aggressive inflammatory response known as "cytokine storms". [Figure 9] Excessive inflammatory reaction events can be observed in patients with COVID-19, resulting in lung injury. The pro-inflammatory cytokines such as IL-6, IL-1 β activates the signalling pathways, resulting in the elimination of infection by recruitment of leukocytes and a few plasma proteins to the infection sites, exerting effector function.

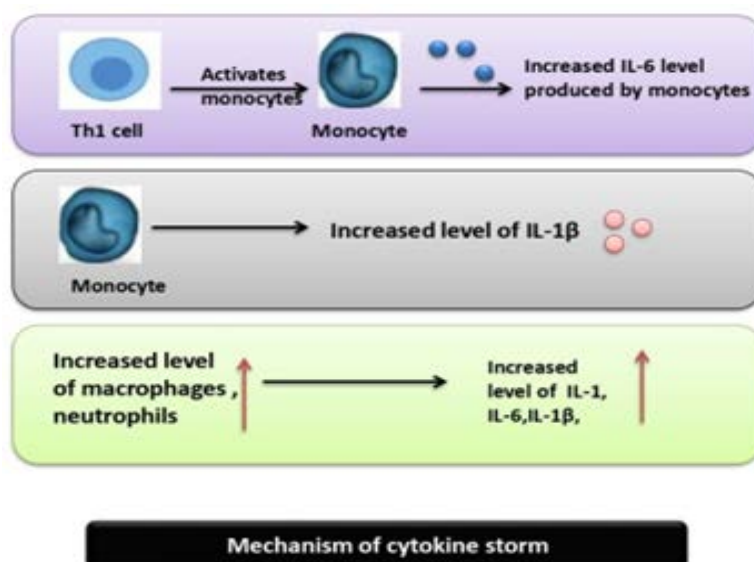


Figure 9: Mechanism of cytokine storm

Three types of the most important proinflammatory cytokines that are involved in the innate immune

response are IL-1, IL-6, and TNF- α , produced by tissue macrophages, mast cells, etc. The sudden increase in the cytokine's level results in the unchecked influx of various immune cells like neutrophils, T cells, and macrophages from the circulatory system to the infected tissue, which ultimately exhibits a number of consequences, including damage of the vascular barrier and capillary, diffuse alveolar damage, multi-organ failure, and finally leading to the death of patients (Ragab *et al.*, 2020).

Silent Hypoxia: Silent hypoxia is a critical condition where an individual has an alarmingly lower (~ 50-80%) oxygen saturation level than the expected value (>95%). However, the question might arise, why is this called "silent"? In normal hypoxia, the respiratory rate is elevated to the point where the condition is termed as dyspnea. But in cases of COVID-19 patients, the affected individual doesn't have any signs of distress even at the low oxygen saturation level. The underlying patho-mechanism behind this condition in SARS-CoV-2 infection is still under research. However, the prevalence of silent hypoxia in COVID-19 patients ranges from 20–40%.

Several investigations support that unrestrained induction of innate immunity leads to the production of a high level of inflammatory response, which ultimately results in tissue damage. Due to excessive lung tissue damage, alveolar air sacs collapse, resulting in a low oxygen supply in the blood.

Interestingly, the Hypoxia-inducible factor (HIF-1) is a dimeric transcription factor that acts as a master regulator of oxygen homeostasis in cells. The factor consists of two subunits of HIF-1 α and HIF-1 β . This factor is activated during conditions of reduced oxygen levels in patients. HIF-1 α subunit has a potent effect on the expression of the ACE2 receptor gene. As stated earlier, ACE2 is the key receptor through which SARS-CoV-2 enters the host cell. Therefore, the hypoxic state has been found to stimulate the overexpression of the ACE2 receptor, thereby worsening COVID-19 infection that ultimately results in respiratory failure and death [Figure 10] (Rahman *et al.*, 2021).

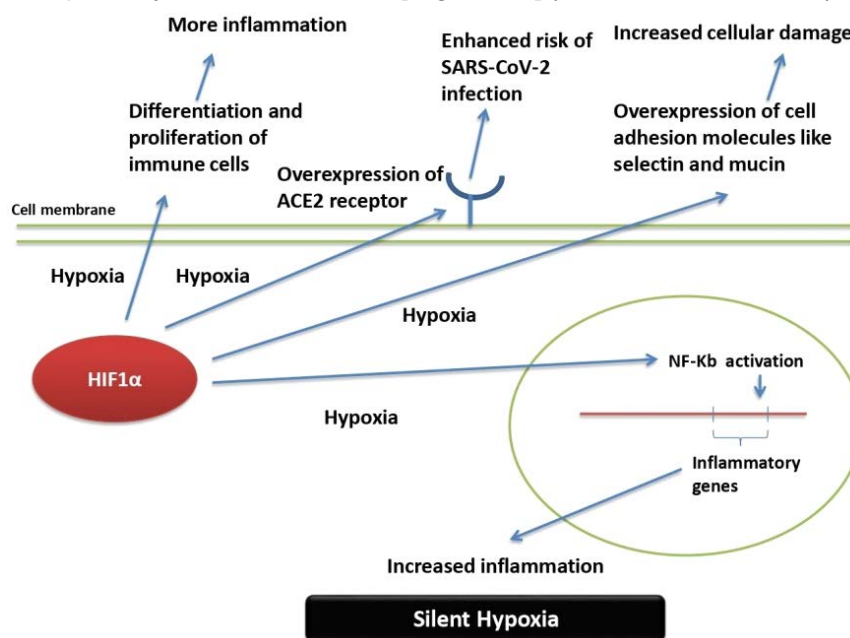


Figure 10: Silent Hypoxia

CONCLUSION

After the sudden emergence of the SARS-CoV-2 virus in 2019, a lot of papers and publications have been made to clarify the interactions between the pathogen and the host during the infection process. A clear image of this complex interaction is truly necessary to design an effective strategy against the infection and to move the present status of immunotherapies one step forward. Exact information on the virus's life cycle, genetic features, and interaction with specific host cellular biomolecules is critical for understanding and identifying new antiviral targets, and thus designing new therapies.

Finally, after quite a long journey through the pandemic situation, the infection rate finally slowed down.

However, the information and the data are being documented by the interests of several groups of researchers, which will be translated into effective strategies and will be helpful for the world to combat future viral outbreaks.

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El-nino and It`s Varied Impacts: A Review

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ABSTRACT

El-Nino is a periodic flow of warm Pacific equatorial waters southward, usually around Christmas time. El-Nino is linked to the Southern Oscillation and it affects the atmosphere, disrupting weather condition of the world. Its disastrous effects have resulted in drought in Australia and Papua New Guinea, a delayed monsoon in South-East Asia leading to massive forest fires choking smog, storms on the Pacific coast of South and Central America, drought in Southern Africa, and threat of flood in Peru and California. Its increasing sensitivity and frequency through the 1980s and 1990s, suggests that El-Nino is affected by an increase of heat trapping greenhouse gases in the upper atmosphere. Global Warming could make the El-Nino a permanent feature of world`s weather system. El-Nino and Southern Oscillation largely affect developing countries that are largely depending upon fishery and agriculture for employment, foreign exchange, and food supply. Global warming increases severity and frequency of El-Nino which has great socio-economic impact on these countries.

Keywords: *El-Nino; Southern Oscillation; Smog; Greenhouse Gas; Global Warming; Forest Fires; Flood*

INTRODUCTION

It is known that there are many anthropogenic factors in the climate, especially CO₂ and greenhouse gases such as methane (CH₄), CFC, and N₂O as part of the daily activities. However, there are many natural processes that affect local climate, regional climate and global conditions. Other effects on the climate are the result of fluctuations and inconsistencies with the world's most complex seawater. This fluctuation is known as oscillation and the most popular oscillation is El Nino (Kunzer *et al.*, 2009; Mc Phaden, 1993). Understanding El Nino requires knowledge of a wide range of data from most studies.

LITERATURE REVIEW

El Nino

El Nino is a natural phenomenon that involves a change in the temperature of oceans in the Pacific and the equator, along with changes in the atmosphere. This situation has a profound effect on climate patterns in various parts of the world. Scientific advances in understanding and modeling of El Nino have improved forecasting skills one to nine months in advance, helping the public to prepare for similar dangers such as heavy rain, floods and droughts. The value of this forecast could translate into hundreds of millions, if not billions, of dollars in potential savings.

The cycle begins when warm water in the western Pacific Ocean flows eastward toward the equator toward the coast of South America. Most of the hot water lakes are near Indonesia and the Philippines. During El Nino, the warm Pacific water resides on the northwest coast of South America.

There is the opposite of El Nino, called La Nina. This refers to a time when the eastern tropical Pacific waters are much colder than normal and commercial winds are blowing harder than usual. Together, El Nino and La Nina are part of the complexity of the space system in the ocean called the El Nino Southern Oscillation or ENSO cycle (Shaw & Moore, 1988), which also has a neutral phase.

La Nina

La Nina is an integral part of the El Nino spacecraft as part of the El Nino - Southern Oscillation. The

name La Nina comes from Spanish, meaning "little girl", which is similar to El Nino meaning "little boy". It has previously been called anti-El Nino, and El Viejo (meaning "old"). During La Nina, sea temperatures across the equatorial Eastern Central Pacific Ocean drops below normal by 3° to 5° C.

Impact of La Nina on global climate

La Nina has an impact on the global climate and disrupts normal climatic patterns, which can lead to severe storms in some areas and droughts in other parts.

Causes of El Nino

The name given to a change in the current flow of the Pacific Ocean near the equator is known as El Nino. El Nino is a Spanish word meaning 'Boy' because it happens at Christmas time - it repeats every five to seven years (Caviedes, 2001).

The following are the features of the Pacific Ocean before the El Nino events:

- The Pacific tropical is characterized by continuous western trade winds. The western wind, also known as the commercial wind, blows warm water from the ocean floor east to west. As a result, warmer waters form off Asia's west coast.
- On the other hand, cold water is pushed upward on the ocean's eastern shore, near the center of South America. As a result, the equatorial Pacific has a temperature gradient, with warm water in the west and cold water in the east. Warm water in the west warms the air, causing a rise in warmer air, which leads to inclement weather such as rain and thunderstorms. The rising warm air causes the east-west flow to spread across the Pacific, bringing warm, wet air rising to the west, and a cool dry air flowing down the east (Walker, 1988).
- All these natural factors contributed to the strengthening of the eastern winds, which resulted in a windward movement that propelled them into the Pacific.

This self-sustaining flow of air in the Pacific continues until slow changes occur in the ocean around the equator and cause a series of El Nino-like phenomena:

- When conditions are favourable, the trade winds weaken, causing less warm surface water to be pushed westward and less cold water to be drawn to the surface in the east. Parts of the ocean that are cold during the normal self-perpetuating cycle warm up, cancelling out the normal temperature difference between east and west in the equatorial Pacific.
- Rainfall patterns over the equatorial Pacific are changing as trade winds weaken and warm water moves.
- With the ocean temperature evened out, the warmest waters are closer to the centre of the ocean, resulting in the cloudy, rainy weather that is typical of the centre of the ocean.

All of these events cause abrupt changes in temperature and weather all over the world.

El Nino affects condition

El Nino is occurring every five years and it is a common occurrence due to atmospheric warming caused by winds, leading to the spread of this global warming. It alters atmospheric pressure with the effects of rainfall, wind patterns, sea surface temperatures and can sometimes have positive effects and, sometimes having a detrimental effect on climate limits. In Europe, for example, El Nino reduces the incidence of hurricanes in the Atlantic. The start of the El Nino program was seen in North America in the previous winter. El Nino includes:

- Low temperatures in winter in western Canada and northwestern USA.
- Rainfall increases in the Gulf Coast including Florida.
- The dry season is in the middle of Ohio and the Pacific Northwest.

Effects of El Nino on weather

Major effects of El Nino occur in tropical and subtropical areas. The following are some of the effects El Nino has on climate in this part of the world:

- In South America, the west coast is experiencing an increase in flood danger, while the east coast is experiencing an increase in drought risk (Marengo *et al.*, 2009). Inferred Oceanic Kelvin/Rossby Wave Influence on North American West Coast, Pacific post-El Niño heat transfer mechanism associated with oceanic Kelvin/Rossby waves that potentially affect atmosphere (Lee & Chelton, 1998)
- Drought is becoming more likely in eastern countries like India and Indonesia. Rainfall is prevalent in the eastern Pacific (west coast of South America) and extreme weather is common in the western hemisphere, including India and Indonesia. As a result, all of the excess heat on the Pacific Ocean's surface, as well as the heat released into the atmosphere, has resulted in temporary global warming (Glantz *et al.*, 2001).
- The influence of El Nino is on the weather peaks in December and can linger for a few months after that.
- After El Nino, trade with the winds of the east, a cycle of self-assertion becomes the norm. However, in the case of La Nina the effects are reversed, and the trade winds intensify, making the normal cycle much larger and having a dynamic El Nino effect.
- Atmospheric CO₂ levels increase as the effects of El Nino increase with greenhouse gases. This warms and dries the tropical environment, reducing their carbon intake and intensifying forest fires.

Economic effects of El Nino

- Rice production is declining in Asian countries. With increasing drought on the west side of the equatorial Pacific, GDP in the region is often falling during the El Nino cycle. Water is needed to harvest the rice. Drought is having a major impact on rice production in countries that harvest rice. As rice is an important crop in many of these Asian countries, El Nino has a negative impact on the economies of these countries.
- In non-Asian countries harvesting other crops, such as wheat is reduced due to drought. For the farmers and harvesters of these crops El Nino has a very negative impact on the economy, such as rice production and exports to Asian countries (Fagan, 1999).
- Fish harvesting in coastal countries such as Ecuador and Peru is difficult, as the fish in the water near these countries usually disappear during the months of December and January (Philander, 1990).
- In general, the severe El Nino climate change is wreaking havoc on the economies of several countries around the equator. While typical years have a steady climate, resulting in predictable market results, El Nino years have a drastic change in climate, resulting in harsh weather on both sides of the Pacific.

DISCUSSION

During the winter, El Nino promotes warmer conditions across the Indian subcontinent. It causes dry conditions and low rainfall in the summer. In Australia, it causes drought. La Nina, on the other hand, resulting in a better position in India than typical Australia, on the other hand, has seen floods (Li *et al.*, 2013).

- India has previously received high rainfall during El Nino events in 2002 and 2009, as well as frequent rainfall during El Nino events in 1994 and 1997.
- Analysis by the Indian Meteorological Department shows that, in the 18 El Nino years from 1880 to 2006, twelve were in short supply of rain or less - common in India. This means that, for the third time,

there was no convergence, and that led to surprisingly incorrect weather forecasts.

- Recent research aimed at finding a stronger link shows that not all El Nino's cause drought and heat only in central Pacific correlates and drought in India while warming in the eastern pacific means a common storm (Hayes *et al.*, 1991).
- Indian meteorological department, the official forecasting agency, has set to issue its first long range forecast of summer temperatures on 2015. Andhra Pradesh and Telangana witnessed a severe heat wave on 2015. In 2016, the winter temperatures have been markedly above normal in most part of the country. Due to negative effect of this phenomenon thousands of people lost their lives in Andhra Pradesh.

CONCLUSION

El Nino is a natural occurrence that has no control over the weather or the lives of people all around the planet. However, because it is usually visible to meteorologists, individuals in the afflicted countries should take precautions to avoid further harm.

People in El Nino-affected areas must take the required precautions to safeguard themselves, whether they are planning for floods or droughts. This might result in additional flooding during a drought or ensure that their homes are flood-proof. Due to the impact on many key crops in these nations, further precautionary measures could include storing these crops in anticipation of drought or damage caused by excessive rainfall.

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Ethnomedicinal Plants used in Different Seasons by Tribals of Asansol Raniganj Coalfield area of West Bengal, India

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ABSTRACT

Ethnomedicinal plants are very popular among the various tribal communities in India. An ethnomedicinal study was conducted in the Asansol-Raniganj coalfield area of West Bengal during the period 2012–2015. It was found that this area is rich in both mineral resources and ethnomedicinal plant resources. The specific use of the ethnomedicinal plant species and their use in different seasons were noted. The preference for ethnomedicine by the tribals of the Asansol-Raniganj coalfield area strengthens the importance of documentation of these ethnomedicinal plants.

Keywords: *Ethnomedicinal; Asansol; Raniganj; Seasons*

INTRODUCTION

Tribals in the Indian subcontinent have given great importance to their traditional healthcare system. The preservation of their ethnomedicinal knowledge is of great importance to the world. This acquired knowledge must be given proper recognition and these important natural resources should be properly conserved before they are lost from the ecosystem (Jain & De, 1964). The Asansol-Raniganj coalfield area is located in the western part of the Burdwan district of West Bengal. The Sanhtals are the most dominant tribe in this area in terms of population. Other tribes found in this area are Munda, Mali, Modikor, Ho, etc. These tribes have a rich ethnomedicinal knowledge which is passed from one generation to the next by means of oral communication. Ethnomedicinal knowledge plays a vital role in drug discovery (Dey & De, 2010). Therefore, it must be documented before it is lost from the society due to a lack of interest in the younger tribal population. The objective of the study was to systematically document the use of ethnomedicinal plants in different seasons by the tribals of the Asansol-Raniganj coalfield area of West Bengal.

METHODOLOGY

The Asansol Raniganj coalfield area is located in the Eastern coalfields. The study was done in the tribal localities of Sripur, Kalipahari Chanda, Nigha, Tirat, and Bansra of the Asansol Raniganj coalfield area from March 2012 to June 2015 in different seasons to collect the ethnomedicinal plants in their flowering condition. The data was gathered through oral communication with approximately twenty tribal medicinal practitioners and elderly people with expertise in ethnomedicinal knowledge, as suggested by Jain (1964), Martin (1995), and Maundu (1995). The tribal communities were repeatedly consulted so as to collect authentic data and no important information regarding these ethnomedicinal plants was missed. The plants used by the tribals were verified by the taxonomists and forest officers in the study area to avoid misidentification and kept for future reference. To collect the ethnomedicinal data in different seasons, the volunteers were also associated during the survey so that the language of the different tribal communities did not become a barrier in collecting the data in detail. The views of informants on Prior Informed Consent (PIC) and intellectual property rights (IPR) were taken on this ethnomedicinal knowledge. No conflict of interest was noted in this ethnobotanical study. All the safety measures were taken and the vital knowledge of the tribals about the ethnomedicinal plants was carefully documented.

RESULTS AND DISCUSSION

The Asansol-Raniganj coalfield area has a rich vegetation of medicinal plants and it is utilized by tribals and people working in collieries in different seasons. In this study, the usefulness of different ethnomedicinal plants towards curing various diseases was documented, which can be easily afforded by the common people. Poor economic conditions, religious faith, and lack of medical facilities in this area induce the people to utilize these plants of ethnomedicinal importance. The tribal medicinal practitioners have a rich knowledge of these ethnomedicinal plants, but the information given by them is verified by repeated consultations. There are chances of wrong identification of these ethnomedicinal plants due to the adoption of modern systems of medicine by the tribals of this area (Saha & Sahu, 2012). Individual and group interviews were conducted to get vital information about the use of ethnomedicinal plants in various seasons of the year. The use of various ethnomedicinal documents from various case studies of patients was correlated with the uses reported by other workers in different parts of India, such as Chakraborty and Bhattacharjee (2006) and Dey and De (2010) from east India; Maheshwari, Singh and Saha (1980) from north India; Kumar, Suman, and Dash, (2004) from central India; Jain, Jain, and Singh, (2009) from the north-west region; Jagtap *et al.*, (2009) and Jothi, Benniamin and Manickam (2008) from west India and Ramachandran and Nair(1981) and Ganesan, Venkateshan and Banumathy (2006) from southern India. Similar uses are noted in many ethnomedicinal plants in different parts of India. Table 1 shows the seasonal correlation of disease and ethnomedicinal plants used by tribals and other rural people in the Asansol-Raniganj coalfield area.

Table 1: Seasonal Correlation of Disease and Ethnomedicinal Plants

Disease	Name of the ethnomedicinal plant	Local Name	Family	Parts used	Time of Availability
1.Boils	Artocarpus lakoocha Roxb..	Dahua	Moraceae	Bark	August-March
	Euphorbia antiquarum L.	Tinshira	Euphorbiaceae	Leaf	Throughout the year
	Hibiscus syriacus L.	Sada joba	Malvaceae	Flowers	Throughout the year
	Tephrosia purpurea(L) Pers	Kulathia	Papilionaceae	Whole plant	April-October
2.Constipation	Cassia fistula L.	Nurnic	Caesalpiaceae	Fruits/Leaves	April-August
	Ricinus communis L.	Rerhi	Euphorbiaceae	Bark, Roots, Seeds	March-September
	Syzygium cumini(L.) Skeels.	Jam	Myrtaceae	Fruit	April-September
3.Diarrhoea	Agele marmelos	Bel/ Sinjo	Rutaceae	Fruit	March-September
	Celosia argenteaL.var.cristata(l.)Kuntze	Morgajhuti	Amaranthaceae	Flowers	May-August
	Cassia fistula L.	Nurnic	Caesalpiaceae	Leaves	April-August
	Cissampelos pareira L.	Telomalla	Menispermaceae	Root, Leaves	August-November
	Cyperus rotundus L.	Mothaghas	Cyperaceae	Tuber	Throughout the year
	Vernonia cinerea L.	Sonnraj	Asteraceae	Whole plant	July-November

Ethnomedicinal plants used by tribals

4. Dysentery	Boerhaavia diffusa(L.)Poir	Punarnaba	Nyctaginaceae	Roots	January- April
	Fleminga strobilifera L.W.T Aiton	Chorchabuk	Fabaceae	Root	October - December
	Helicteres isora L.	Atmora	Steruliaceae	Seeds, Fruit, Root	April- December
	Musa paradisiaca L.	Kela ba	Musaceae	Fruit	Throughout the year
	Urginea indica Kunth	Bir Payaj	Liliaceae	Bulbs	April- September
5. Jaundice	Achyranthes aspera L.	Chirchiti	Amaranthaceae	Stem	October- January
	Croton bonplandianum Baill.	Churchuri	Euphorbiaceae	Seeds	September- December
	Alangium salvifolium Lamarck	Akarkanta	Alangiaceae	Root, Bark	April-October
6. Indigestion	Allium sativum L..	Rasun	Lilaceae	Leaves and bulb	Throughout the year
	Calotropis gigantea L.R.Br	Akanda	Asclepiadaceae	Root	Throughout the year
	Semecarpus anacardium L.	Bhela	Anacardiaceae	Seeds	April-October
7. Stomach ache	Cajanus cajan L.	Arhar	Fabaceae	Leaf	Throughout the year
	Ficus racemosa L.	Lowa	Moraceae	Receptacles	March- September
	Boerhaavia diffusa(l.) nom .cons.	Punarnaba	Nyctaginaceae	Whole plant	April- October
	Vernonia cinerea L.	Sonnraj	Asteraceae	Whole plant	July- November
8. Vomit	Euphorbia hirta L.	Pusi toa	Euphorbiaceae	Whole plant	Throughout the yea
	Rubia cordifolia L.	Sajisthan	Rubiaceae	Root	August- December
9. Cough and Cold	Allium sativum L.	Rasun	Lilaceae	Leaves and bulb	Throughout the year
	Amaranthus viridis L.	Bon notey	Amaranthaceae	Leaf	December- April
	Bacopa monnieri L.	Brahmi	Scrophulariaceae	Leaves, stem, flower	July- September
	Bauhinia purpurea L.	Raktakanch an	Caesalpiniaceae	Root, leaf	September- November
	Brassica campestris L.	Turi/ Sarson	Cruciferae	Seed	November - April
	Chrozophora plicata A. Juss.	Pango nari	Euphorbiaceae	Root	February- August

Ethnomedicinal plants used by tribals

	<i>Euphorbia hirta</i> L.	Pusi toa	Euphorbiaceae	Whole plant	April-October
	<i>Euphorbia nerifolia</i> L.	Masa	Euphorbiaceae	Leaf	April-September
	<i>Glycyrrhiza glabra</i> L.	Ultat	Fabaceae	Root	Throughout the year
	<i>Madhuca longifolia</i> (J. Konig) J.F. Macbr	Mahua	Sapotaceae	Flower, fruit, seed	May - September
	<i>Hibiscus rosa sinensis</i> L.	Joba	Malvaceae	Petals	Throughout the year
	<i>Hibiscus syriacus</i> L.	Sada joba	Malvaceae	Seeds	Throughout the year
	<i>Urginea indica</i> Kunth	Bir Payaj	Liliaceae	Bulbs	April-September
10. Headache	<i>Achyranthes aspera</i> L.	Chirchiti	Amaranthaceae	Stem	October-January
	<i>Aloe vera</i> (L.)Burm.f.	Ghritkumari	Liliaceae	Leaf pulp	Throughout the year
	<i>Alstonia scholaris</i> R Br.	Chatnia	Apocynaceae	Bark	March - August
	<i>Cynodon dactylon</i> (L.) pers.	Dhurba	Poaceae	Whole plant	Throughout the year
	<i>Hibiscus syriacus</i> L.	Sada joba	Malvaceae	Seeds	Throughout the year
	<i>Vitex negundo</i> L.	Boan	Verbenaceae	Leaf	Throughout the year
11.Toothache	<i>Allium sativum</i> L.	Rasun	Liliaceae	Bulb	Throughout the year
	<i>Alstonia scholaris</i> R Br.	Chatnia	Apocynaceae	Latex	March - August
	<i>Cassia fistula</i> L.	Nurnic	Caesalpiniaceae	Leaves	April-August
	<i>Calotropis gigantea</i> L.R.Br	Akanda	Asclepiadaceae	Latex	Throughout the year
	<i>Euphorbia nerifolia</i> L.	Masa	Euphorbiaceae	Latex	April-September
	<i>Madhuca indica</i> Gmel.	Mahua	Sapotaceae	Root,bark	May - September
	<i>Solanum sisymbriifolium</i> Lamk.	Katari	Solanaceae	Fruits	June-September
12.Gout	<i>Clerodendrum viscosum</i> Vent.	Ghentu	Verbenaceae	Leaf	April-August
	<i>Euphorbia antiquorum</i> L.	Etkec	Euphorbiaceae	Stem,Leaf	Throughout the year
	<i>Piper longum</i> L.	Ralli	Piperaceae	Fruits, Root	June -October
	<i>Solanum nigrum</i> L.	Kakmachi	Solanaceae	Whole plant	April -October
	<i>Vitex negundo</i> L.	Boan	Verbenaceae	Leaf	Throughout the year

CONCLUSION

The ethnomedicobotanical study in the Asansol-Raniganj coalfield area was conducted with special emphasis on ethomedicinal plants to cure various ailments in different seasons. It was found that a well

developed ethnomedicinal system exists in the study area and this knowledge, which is an important part of the heritage and culture, needs to be scientifically documented before it is lost from the society. These herbal drugs prescribed by traditional medicinal practitioners should be administered at the proper time and dosage to achieve the desired effects. The discovery of the active compounds of these ethnomedicinal plants may help pharmacologists in drug development which can be easily afforded by the common man. Research on these plants must be encouraged to identify the active constituents that can be incorporated into modern drugs so that one can get the benefit of both traditional and modern practices for the welfare of mankind.

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Waste Production in Aquaculture

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ABSTRACT

Due to rivalry resulting from the exploitation of natural resources such as soil and water by other production and development businesses, strengthening aquaculture has emerged as an alternative to boosting aquatic production. However, enhanced waste disposal from aquaculture production systems is required for resilience, as is extra inclusion, such as fish and food, in each cultural region. Polluted aquatic animal products have sparked public outrage and jeopardised the long-term viability of aquaculture operations. The need to boost aquatic product output cannot be overstated, and as a result, cultural systems must be developed that will increase fish production through effective waste management, reducing environmental deterioration caused by marine pollution and ensuring its sustainability. This paper examined the various aspects of waste production from aquaculture, including its sources, components, and management practises in various cultural systems, with a focus on waste production from food, feedstuffs, fertilisers, pesticides, herbicides, and other sources, as well as to identify waste sources, content, and potential hazards to both fish culture and the environment. To ensure the development of enhanced and sustainable aquatic animals, waste management proposals in diverse cultural systems were produced.

Keywords: *Aquaculture; Biostats; Natural Resources; Waste management*

INTRODUCTION

Aquaculture is gaining importance day by day as the study of crustacea, mollusca, and fishes has made much progress. It is required to know the background concentration of nutrients in receiving waters, as well as the emission of nutrients from the farm per unit of time and the water retention duration in a given area, in order to determine the environmental impact of aquaculture effluents. Though the forms of waste produced in aquaculture farms are essentially the same, the quality and quantity of the components vary based on the species cultured and the culture procedures used. Much of the material accessible is about extensive salmonoid culture systems, and to a significant extent, pens and cages in temperate climates. It was revealed that the poison is harmful to a variety of marine creatures and causes molluscs to fail to reproduce or grow abnormally (Thain & Waldock, 1986).

LITERATURE REVIEW

Aquaculture waste:

Farming of fish, crustaceans and bivalves generates waste in the form of particulate and soluble organic materials. The nature and amount of effluents vary with the design, species cultured and level of intensification. In general waste products (faeces & excreta) and uneaten food (including pseudofaeces) are the major sources of inorganic and organic nutrients (predominantly organic carbon & nitrogen). The water column is the recipient for the dissolved material, while the largest proportion of solid wastes released settles to the seabed within the culture environment or in the immediate vicinity of the farm.

Classification of aquaculture waste products:

Aquaculture waste products are mainly of the following three types:

- a. Residual food and faecal matter

Waste Production in Aquaculture

b. Metabolic by-products

c. Residues of biocides and biostats

Other types of waste products:

1. Wastes can accumulate in land-based farms that use fertilisers to produce food organisms as a result of sedimentation, disintegration, and underutilization.
2. Tidal water fish ponds may receive additional organic matter in the form of suspended particles and dissolved nutrients as a result of inflows.
3. The use of therapeutically chemicals and hormones in aquaculture farms also yields waste.

Waste product composition:

The waste products contain organic carbon and nitrogen (carbohydrates, lipids, and protein), ammonia, urea, bicarbonate, phosphate, vitamins, therapeutants, and pigments.

The following are the components of aquaculture feed:

Feed comes in a variety of forms, including dry, moist, and wet. The content of commercially available feeds varies significantly, but all contain protein, carbs, and fat, as well as some supplements such as vitamins, therapeutants, and pigments. Håkanson (1988) gave typical values of composition of three types of typical salmon feeds in Nordic countries, which are shown in table 1.

Table 1: Composition of three types of typical salmon feeds in Nordic countries

Composition(g/Kg)	Moist Pellets	Dry Feed (Lower Energy)	Dry Feed (High Energy)
Dry Matter	325	900	900
Protein	170	500	450
Fat	60	120	240
Carbohydrates	50	150	100
Nitrogen	27	80	72
Phosphorous	4	15	10
Gross Energy (MCal/Kg)	1.3	4.6	5.2

Source: Pillay, 2004

Metabolic waste products derived from feed:

The production of faecal and excretory waste is obviously influenced by farm stock density. Experiments with rainbow trout have revealed that the amount of solid and dissolved organic metabolic products is proportional to the amount of food consumed (Pillay, 2004). Faecal output was estimated to be roughly 260g per kg of food ingested, or around 26% of total food consumption. Diets with fewer proteins and a larger percentage of carbohydrates have been found to be more digestible. It is estimated that 80 percent of shrimp feed is expelled as metabolites, surplus nutrients, faeces, or used for maintenance (Primavera, 2006).

Only 27.2 kg of the 122.9 kg consumed nitrogen is retained, with the remaining 78 percent lost as faecal and excretory nitrogen (Pillay, 2004). If the nitrogen content of the faeces is 4%, 68 to 86 percent of the nitrogen consumed by fish is voided as soluble ammonium and urea, resulting in 32 kg of

ammonium production per tonne of food supplied (Pillay, 2004). Ammonium excreted per tonne of fish in land-based trout farms is estimated to be 45 kg in Denmark and 55.5 kg in the UK (Pillay, 2004).

Effects

- i. Bicarbonate is produced as a by-product of respiration and is expelled through the gills. Despite the fact that it is alkaline, it is unlikely to have a substantial impact on the pH of sea water due to the buffering effect of sea water.
- ii. Phosphate produced on the farm is in particle form and has been shown to have no impact on the ecosystem in marine water.
- iii. Eutrophication is caused by an increase in the amount of dissolved inorganic phosphates in fresh water.

Waste production from food and feedstuffs:

In a mussel farm, the build-up of faecal matter and debris under a raft might vary significantly. If a raft of mussels consumes 180 tonnes of organic matter, 100 tonnes is returned to the sea (Pillay, 2004), and the mussels add to the sediments to be digested at the sea bottom in the mussel bed.

In all types of Bivalvia cultivation, the accumulation of faeces and pseudofeces can be exceedingly high. The output of faeces and pseudofaeces from a typical oyster raft in Hiroshima Bay, Japan, housing 420000 oysters over a nine-month period was estimated to be 16 tonnes (Pillay, 2004).

Phaeopigments formed by the breakdown of chlorophyll as a result of the mussels feeding on phytoplankton were estimated to discharge 10 times more under the mussel than in area far away from the farm. The organic carbon produced by oysters grown on rocks in France fluctuates periodically from 7.6 to 99 grammes per square metre per day, which is connected with phytoplankton seasonal change (Ottman & Sornin, 1985).

Effects

- i. High rates of oxygen uptake come from the bacterial breakdown of organic materials.
- ii. Bacterial breakdown frequently results in a reduced environment and the generation of hydrogen sulphide (H₂S), which is poisonous to oysters. (Ito & Imai, 1955; Pillay, 2004).
- iii. It also increases Biological Oxygen Demand (BOD).

Feed loss:

In an aquaculture farm, feed generated wastes comprise not just faeces and other excretory products, but also unfinished feed. Feed loss is determined by a variety of factors, including the stock's feeding habits, feed water stability, feed distribution method, and feeding timing. The percentage of unconsumed feed in Danish trout farms is 10–30% waste fish, 5–10% moist pellets, and 1–5% dry feed (Drusano *et al.*, 1982; Pillay, 2004). These figures tend to be based on pond and trunk farm measurements. Feed losses are expected to be higher in marine or freshwater cage farms. Pillay (2004) determined a wastage of 27 percent and 31 percent for dry and moist meals in freshwater, respectively.

Fertilizer derived:

Pond farms, where the addition of organic or inorganic fertilisers is either the only or partial method of food generation for the cultured stocks, are an important aspect of aquaculture in tropical and subtropical locations.

The content of animal dung is highly diverse and is influenced by the animal's species, the type of food it eats, how the manure is handled and stored, and the weather. Organic matter ranges between 15% and 34%, total nitrogen between 0.3 and 1.7 percent, and phosphate (P₂O₅) between 1.25 and 2.96

percent (Pillay & Dill, 1976). Animal manure increases heterotrophic bacteria development in the water body.

Despite the fact that organic fertilisers are favoured in aquaculture, many farms utilise inorganic fertilisers alone or in conjunction with organics. The primary nutrients, nitrogen, phosphorous, and potassium (NPK) are all included in inorganic fertilizers. Nutrients including calcium, magnesium, and sulphur, as well as trace minerals like copper, zinc, boron, manganese, iron, and molybdenum, may be present. Fertilizer is sprayed in quantities that the ponds can quickly use to produce the desired food organisms. Dosage must be regulated according to feeding intensity to avoid the formation of an algal bloom, which might cause unfavourable conditions.

Residues of Biocides and Biostats:

Chemicals and other poisonous compounds are often employed to control predators, pests, and weeds on land-based farms, particularly pond farms, as part of pond preparation before stocking with larvae, fry or fingerlings.

Pesticides

To rid aquaculture water of pests and predators, tea seed cake, derris powder, and rotenone are commonly employed. Tea seed cakes have a saponin content ranging from 10–15%. After lowering water, a dose of 216 kg of tea seed cake and 144 kg of quick lime is placed at the pond bottom per hectare. Within 2-3 days, the toxicity is gone. Rotenone, in the form of derris powder, is used for the same purpose, and the toxicity lasts for around 48 hours (Wood *et al.*, 2005). Under tropical conditions, even at doses of up to 20 ppm, toxicity ceases after 8 to 12 days (Alikunhi, 1957; Jhingran, 1986).

DDT, endrin, aldrin, and 2,4-D are examples of non-selective poisons used as pesticides to eliminate pests and predators. Snails and polychaete worms are controlled with bayluscide and nicotine, while crabs infesting pond dikes are controlled with BHC and the insecticides 'sevin`.

Herbicides:

Various types of herbicides are used to eradicate emergent, floating, and submerged weeds in tropical areas. Herbicides like 2,4-D and Diquat are extensively used for controlling floating and emergent weeds. Copper sulphate and simazine are used to control submerged weeds and have a long-term impact on pond productivity. Anhydrous ammonia is used to kill densely growing underwater weeds and has a nutrient-like effect on the water.

Environmental Impact:

Even though the toxicity of all pesticides used to manage weeds, pests, and predators in tropical pond farms is unknown, the culture procedures make it unlikely that they will have a significant environmental impact. It is assumed that farm waste releases will have long-term consequences. Dipterex, an insecticide, has been discovered to be rapidly hydrolyzed in tropical pond conditions (Haque & Barua, 1988; Beveridge & Phillips, 1993).

Chemical use has a greater direct influence on the ecosystem in open-water aquaculture, such as mollusk farming in coastal areas and cage and pen finfish farming. Pesticides such as Neguvon and Nuvon have had deadly effects on crustaceans in the vicinity of farms in certain conditions, such as salmon net-pin farming in Norway, where pesticides such as Neguvon and Nuvon are used to eliminate fish lice (Egidius & Moster, 1987). Because of their high biological effectiveness, even in ultra-micro amounts, pesticidal compounds are among the most challenging to analyse. They are frequently beyond chemical detection limits, necessitating the use of a mix of bioassay and chemical approaches to investigate them (Walker 1976; Stickney 1979).

Tributyltin (TBT) is an antifoulant that has been investigated in terms of its environmental impact. Because of their high toxicity to fouling species, low toxicity to humans, and lengthy antifouling protection, tributyltin (TBT) compounds are commonly utilised on pleasure boats, harbour

construction, and marine cages. TBT's toxicity was originally discovered on oyster beds in regions frequented by pleasure boats in France (Arcachon Bay). Poor spawn output, aberrant larvae, and shell deformity were detected, and tributyltin (TBT) was suspected of being the cause (Alzieu & Heral, 1984; Kan-atireklap *et al*, 1997). Following research, it was discovered that the toxin has a deleterious effect on various types of marine life, and that it causes reproductive failure or growth anomalies in molluscs (Cardwell & Sheldon, 1986; Paul & Davies, 1986; Thain & Waldock, 1986). Recent research has found that tributyltin (TBT) can kill caged fish and accumulate in their tissues.

How Production Dynamics Affect Aquaculture Waste Generation:

Aquaculture production is based primarily on three structural elements: the cultural elements, the cultural environment, and the cultural species and management practices. Production dynamics are defined as the way these structural elements change over time, how they are controlled and their inter-relationship. The functioning of the cultural environment involves chemical, biological, and physical processes influenced by external conditions (i. e., climatic factors & management practices).

Recent studies focusing on the dynamics of marine shrimp culture systems have shown that quality variables like dissolved oxygen (DO), pH, and temperature may change significantly over a 24-hour period due to respiration and photosynthesis. In addition, seasonal variations in rainfall may affect salinity, which decreases during the rainy season and increases during the dry season. Water transparency, pH, and dissolved oxygen concentration may also fluctuate over the growth cycle, exhibiting a decline pattern. This is mainly a result of an increased accumulation of uneaten food, which leads to higher bacterial growth. In semi-intensive shrimp ponds, polychaete populations may be drastically reduced by the end of the rearing period due to the shrimp's predation pressure.

The cultured animal may show changes in its feeding behaviour and physiology over the growth cycle. For example, some *Penaeus* species may change their diet over the rearing period, which often involves a shift from detritus-based sources to more animal-based sources at a larger body size.

DISCUSSION

Can aquaculture waste be reduced?

Several alternatives have been proposed for reducing aquaculture waste material entering coastal water. These include:

- The treatment of effluents through the construction of sedimentation ponds for decantation and biological oxidation of organic matter.
- Polyculture or integrated culture of filter-feeding organisms such as oysters, mussels, seaweed with fish or shrimp (Primavera, 2006).
- Feeds should be allocated according to the cultured animal's spatial distribution in the culture environment.
- The quantity of external feed inputs should be balanced with the biomass of naturally occurring food and the on-genic patterns in feed intake by the cultured animal (Dauda *et al.*, 2018).
- A uniform feed distribution favours a higher level of food intake among the cultured species that display homogenous spatial distribution patterns.
- Feed particle size should be increased in response to the handling efficiency of the cultured animal.
- Before being fed, the feed should be sieved to eliminate dust and broken pellets, and it should be provided effectively to guarantee that there is little or no waste from uneaten feed. (Dauda *et al.*, 2018).

Waste is processed in aquaculture and allied businesses, which is thought to constitute a substantial threat to the ecosystem. Appropriate technologies should be employed to reduce pollution. The conversion of these wastes, as well as the simultaneous collection of critical materials before disposal, has become the primary goal of fishery management. Fish wastes can be used to produce valuable products like biogas and biofertilizer (Pędziwiatr, 2017).

Evaluate the assessment approaches for local versus regional scenarios, for regions with varied farm density and hydrodynamic conditions, using modelling and satellite monitoring (Olsen, Holmer & Olsen, 2015).

Most solids may be removed with minimal labor in circular tanks with appropriately built inlets, drains, and filters. Filters such as drums, discs, beads, and sand catch etc remove particles as small as 60 microns from water. Artificial shallow wastewater treatment systems (ponds or canals) that have been planted with aquatic plants and rely on natural processes to clean wastewater are known as constructed wetlands. Constructed wetlands have an advantage over alternative treatment systems because they function with minimal energy (Miller & Semmens, 2002).

CONCLUSION

Many of the environmental issues relating to aquaculture waste production in the tropics are still speculative because definitive studies are not yet available. Research is required to define the sustainable carrying capacity of coastal areas in terms of supporting aquaculture production and assimilating wastes. It is argued that in some coastal areas of poor productivity, aquaculture effluents can actually be beneficial and that coastal environments have a high capacity to flush nutrients out into adjoining water. Such attributes, however, should not be over-emphasized. In many cases, effluents are discharged in small areas with limited water supplies, poor water exchange, and heavy farming. In general, mismanagement and intensification are recognized as the main causes of organic loading in the aquaculture system and in adjacent coastal areas.

The dynamic nature of tropical aquaculture systems has limited attempts to further improve farm management techniques associated with waste production. Alternatives, such as the treatment of waste through sedimentation ponds and polyculture with filter-feeding organisms, have not yet been widely accepted due to constraints related to the requirement of a large proportion of the farm area and problems associated with diversification of production. Therefore, the amount and effects of aquaculture waste discharged into coastal areas are highly dependent on the dynamics of the culture environment and the cultured species, which, on the other hand, can influence the effectiveness of management practices. This implies that in the short term, effective reduction of aquaculture waste can only be achieved after a detailed description and quantification of the cultured species' feeding patterns and their relationships with the culture environment have been made. This will allow the development of user-friendly simulation programs that combine the various components of the culture system, assisting farmers in the complex decision-making process of feed management.

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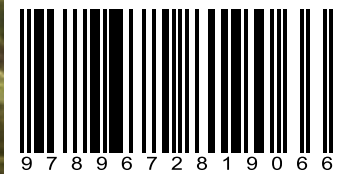
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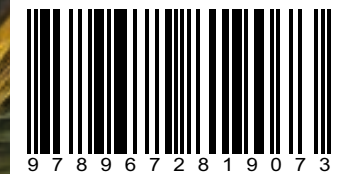
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