Comparative Biodegradation behaviour study of different Plastic wastes by marine microbes

Submitted by

RWIDDHI SARKHEL

[Class Roll No. **001710303005**]

[Exam Roll No. **M4BPE19006**]

[Registration no. **140625** of 2017-18]

Under the guidance of

Prof. Papita Das

Professor, Department of Chemical Engineering, Jadavpur University.

&

Prof. Avijit Bhowal

Professor, Department of Chemical Engineering, Jadavpur University

Submitted in the partial fulfilment for the degree of

MASTER OF BIOPROCESS ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

JADAVPUR UNIVERSITY

JADAVPUR

Kolkata-700032

INDIA

DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains survey of literature and original research work by the undersigned candidate, as a part of her course "**Master of Bioprocess Engineering"** studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name: **RWIDDHI SARKHEL**

Roll Number: **001710303005**

Thesis Title: **Comparative Biodegradation behaviour study of different Plastic wastes by marine microbes**

Signature with date

Comparative Biodegradation behaviour study of different Plastic wastes by marine microbes

Submitted by

RWIDDHI SARKHEL

[Class Roll No. **001710303005**]

[Exam Roll No. **M4BPE19006**]

[Registration no. **140625** of 2017-18]

A thesis submitted in partial fulfillment of the requirements of the degree of

Master of Bioprocess Engineering in Chemical Engineering

Examination Committee

_____

_____

_____

_____

CERTIFICATE OF APPROVAL

This is to certify that this thesis work entitled " **Comparative Biodegradation behaviour study of different Plastic wastes by marine microbes"** submitted by Ms. Rwiddhi Sarkhel is a bonafide thesis work carried out under my supervision and guidance and fulfilling the nature and standard required for the partial fulfillment of the degree of Master of Bioprocess Engineering in Chemical Engineering. The work embodied in this thesis has not been submitted elsewhere for a degree.

_____ _____

Prof (Dr) Papita Das
Thesis supervisor
Professor
Department of Chemical Engineering
Jadavpur University, Kol-32

Prof (Dr) Avijit Bhowal
Thesis supervisor
Professor
Department of Chemical Engineering
Jadavpur University, Kol-32

_____ _____

Prof (Dr) Debashis Roy
Head of the Department
Department of Chemical Engineering
Jadavpur University, Kol- 32

Prof (Dr) Chiranjib Bhattacharjee
Dean of Faculty and Engineering
Department of Chemical Engineering
Jadavpur University, Kol-32

ACKNOWLEDGEMENT

I would like to express my immense gratitude to the Chemical Engineering Department of Jadavpur University for assigning me a project on the topic "**Comparative Biodegradation behaviour study of different Plastic Wastes by marine microbes".**

I would like to express my sincere gratitude to Prof (Dr) Debashis Roy, the Head of the Department of Chemical Engineering for his continuous support and encouragement.

I would like to express my heartfelt gratitude to Prof. (Dr) Avijit Bhowal, Professor of Chemical Engineering Department of Jadavpur University for allowing me to carry out this work under him elegant supervision and guidance. I am deeply indebted to him for their encouragement and co-operation.

I would like to express my deepest gratitude to my guide Prof. (Dr) Papita Das, Professor of Chemical Engineering Department of Jadavpur University for her supervision, encouragement and mentoring. She has always been supporting, patient and inspiring. I have been very lucky to have her as my advisor. I am indebted to her for her constant encouragement and knowledge sharing throughout my research work.

I am appreciative of my senior Dr. Shubhalakshmi Sengupta, post-doctoral fellow, Department of Chemical Engineering, Jadavpur University for her help in conducting experiments and various other constructive suggestions during my research work.

My thanks to Sandipan Bhattacharya, research fellow and PhD scholar of Department of Chemical Engineering, Jadavpur University for their hearty cooperation during my project work

Signature of Candidate.....

Date:

Place: Jadavpur University

Kolkata-700032

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

PET – Polyethylene Terephthalate

PE- Polyethylene

Ac- Acetate

PVA – Polyvinyl Alcohol / Polyvinyl Acetate

PP – Polypropylene

SEM – Scanning Electron Microscopy

FTIR – Fourier Transform Infrared Spectroscopy

XRD – X-Ray Diffraction

PVA/c – Polyvinyl alcohol reinforced with cellulose

SGB- Sugarcane bagasse

Hr – Hour

Min – Minutes

Asp – Aspergillus sp

Vib – Vibrio sp

mL – Milliliters

C - Celsius

AIMS AND OBJECTIVES

Aim: To study the biodegradation behaviour of different polymers by marine microbes.

Objectives:

- To remove the contaminated wastes from the sample.
- Isolation and screening of fungal and bacterial species from marine and saline environment.
- Characterization of microbial strains from the isolates.
- To increase the effectiveness of the bio-degradation process by using bio-composites.
- Synthetic polymers are used because it has high durability and can resist biodegradation.
- To compare the growth of microbes on the polymer film samples by performing different characterizations and optimizations.
- To implement the biodegradation techniques like Composting, bioremediation

ABSTRACT

Plastics plays an important role in our daily life nowadays. They are mainly used for packaging purposes. Plastics in marine environment been exposed to oxidising conditions lead to weathering of polymers causing degradation. Microbial degradation is generally considered as a phenomenon where changes occur in organic compounds by various types of microbes, especially bacteria or fungi. In this work biodegradation of non-biodegradable synthetic polymers obtained from Plastic bottle waste, commonly known as PET and petroleum based degradable polymers like Polyvinyl Alcohol (PVA) and PVA reinforced with natural fibre like cellulose, which is extracted from sugarcane bagasse fibres, (PVA cellulose) has been carried out. In this comparable study, bacteria and fungi, isolated from the marine environment (Bacterial strains of *Vibrio sp* and fungal strains of *Aspergillus sp*) have been used for degrading all the polymer strips in laboratory conditions. These polymer film strips were degraded by the various characterization methods like Weight loss study, FTIR analysis, SEM analysis and XRD study. The results indicated that the polymers from plastic bottle waste sample lost 32%, PVA polymer films lost 38% and PVA cellulose films lost 42% of weight in a period of 6 weeks. Different batch studies varying temperature, pH, inoculum dosage studies were also performed which showed that Plastic bottle waste when degraded by bacteria gives a better result as compared to fungal degradation. Hence there are various polymer degradation methods available, but the eco-friendliest method is by using microbes.

Keywords: Biodegradation, microbes, plastic polymers, eco-friendly.

CHAPTER 1

BIODEGRADATION STUDY OF POLYMER FILM FROM PLASTIC BOTTLE WASTE, OR PET

1.INTRODUCTION

1.1 Marine Plastic Pollution

Plastic is an artificial organic chemical compound made of rock oil with properties ideally fitted to a good style of applications, as well as packaging, building and construction, unit and sporting goods, vehicles, electronics and agriculture. Plastic is cheap, lightweight, strong and malleable. A huge number of plastics, above 300 million tonnes are used for shopping purposes. The amount of plastics has increased wildly in the marine environment since 1950's. There is a growing concern about the risks and possible adverse effects of micro-plastics over worldwide (Lohr et al, 2017). Plastic contamination in marine environment reflects on the sources and effects of marine litter and the effects of policies and actions taken worldwide. Yet, so far, the effects of policies and other initiatives are still largely insufficient. There are 192 countries which borders the coast of the Atlantic, Pacific and Indian oceans, or Mediterranean and Black seas, producing 2.5 billion tonnes of waste since 2010. Of this, an estimated 275 million tonnes was plastic, and 31.9 million tonnes was mismanaged coastal plastic waste. A calculable eight million tonnes of this plastic waste enters the Ocean once a year. Plastic waste is being generated rapidly worldwide. Environmental contaminants are found to accumulate in the marine waters across the world. The contribution of plastic waste by UK, India and China is about 1 million tons, 4.5 million tons and 16 million tons, respectively (Kumar et al. 2011). India generates around 10 thousand tons of plastic waste (Puri et al. 2013). The yearly generation of plastic waste was assessed as 57 million tons in Europe in 2012. Reusing of plastics is considered practical and in fact attainable choice to handle plastic waste.

Bio-degradable plastics are promising under the right conditions, but these conditions are generally not found in the natural environment, and especially not in the Ocean. They are also energy intensive, expensive, and have the potential to make the problem of littering worse by encouraging people to think that it is okay to throw away valuable resources like plastics (Anthony et al, 2010). Furthermore, even in ideal conditions, biodegradability does not resolve critical issues such as entanglement, or ingestion by marine animals. Plastic pollution threatens food safety and quality, human health, coastal touristy, and contributes to global climatic change. There is associate degree imperative ought to explore the

employment of existing lawfully binding international agreements to handle marine plastic pollution. Recycling and apply of plastic product, and support for analysis and innovation to develop new product to exchange single-use plastics also are necessary to stop and cut back plastic pollution.

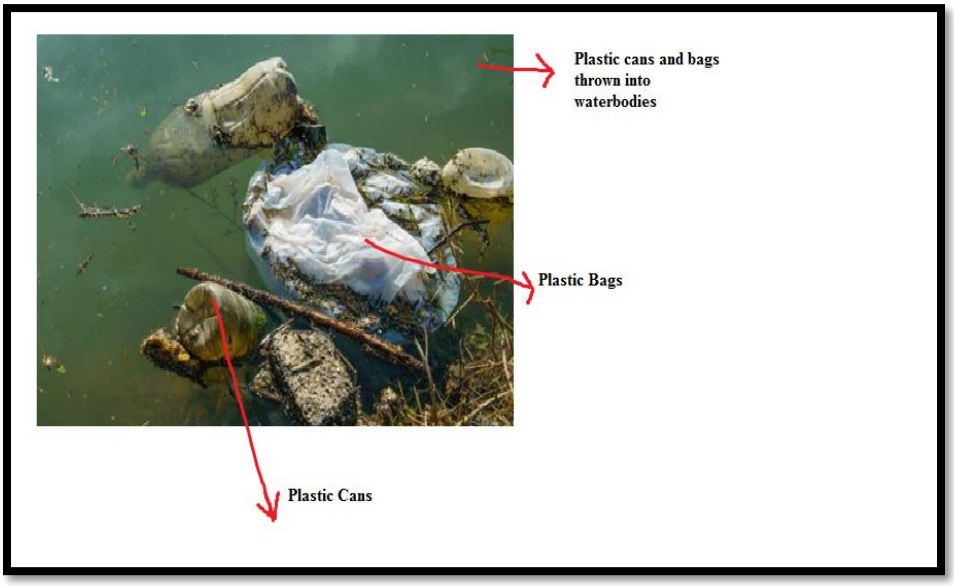


Fig 1: Marine plastic pollution

1.2 Plastic bottle waste film as PET

Plastic bottle waste obtained from a thermoplastic polymer or Polyolefins like Polyethylene Terephthalate (PET), one of the solid wastes nowadays is widely used for making films, fibers, etc. The study of plastic degradation was specified in using polymers and creating awareness for using plastic wastes on environmental hazards. Generally plastic bags and plastic polymers are quiet extensively used mainly for their cost effectiveness and for their large-scale production. The plastic degradation signifies that the response of plastic is greatest in environment and must be facilitated in large-scale production (Adane et al, 2011). Polyethylene-terephthalate, usually abbreviated as PET, is the most typical thermoplastic compound organic compound of the polyester family and is employed in fibers for wear, containers for liquids and foods, thermoforming for manufacturing, and together with fiber for engineering resins. Most of the world's PET

production is for artificial fibers (in way over 60%), with bottle production accounting for regarding half-hour of worldwide demand. In the context of textile applications, PET is said by common name PET. Polyester makes up concerning eighteen of world compound production,and isthe fourthproduced compound once polythene (PE), poly propylene (PP)and vinyl polymer (PVC). PET consists of polymerized units of the chemical ethylene terephthalate, of $C_{10}H_8O_4$ units. PET is commonly recycled. Depending on its process and thermal history, polyethylene terephthalate may exist both as an amorphous (transparent) and as a semi crystalline polymer. The structure of PET is as follows:

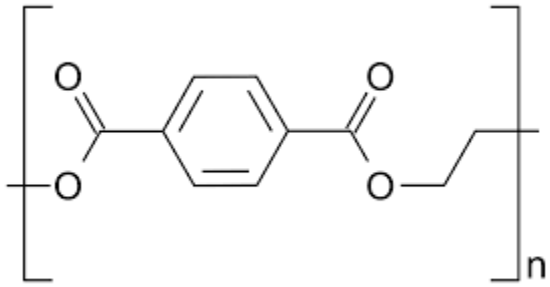


Fig 2: Structure of Polyethylene Terephthalate (PET)

PET is subjected to various types of degradations during processing. The degradations that occurs mainly are hydrolytic, and probably most important, thermal oxidation.

Physical Properties of PET:	
Density	1.38 g/cm^3
Melting Point	> 250 °C
Boiling Point	> 350 °C
Solubility	Water insoluble

Table 1: Physical properties of PET

1.3 Biodegradation of Polymers

Biodegradable polymers are defined as polymers comprised of monomers linked to one another through functional groups and have unstable links in the backbone. They are broken down into biologically accepted molecules that are metabolized. Biodegradable polymers represent a promising way to reduce the amount of plastics disposed off, reducing risk of pollution to enhance sustainable development of the environment (Raaman et al). Isolation and identification of polymer degrading microorganisms is important as this knowledge provides valuable information for their use in bioaugmentation processes. Numbers of test were used for the determination of biodegradation using various micro-organisms (Kumar et al, 2010). Many of the microbes can degrade polymer aerobically or some anaerobically (Singh et al, 2012). The degradation of plastic removal by the following microorganisms like *Aspergillus fumigatus and Penicillium sp* was found by the weight loss analysis (Shaha et al,2008). The microbes were investigated for the existence of naturally occurring PET and LDPE in waste dumping sites (Sabrina et al, 2018).

However, the degradation of polymers in marine environment by these types of microorganisms is very rare with a purely non degradable polymer so this novel research work is done. Biodegradation of polymer films obtained from Polyolefins like Polyethylene Terephthalate, was examined in marine rich environment with isolated microorganisms. In this study, many species of microorganisms were isolated from saline water of Bay of Bengal near Sunderban, West Bengal. Among which one bacterium and one fungus capable for degradation were used. The characteristic structure of Polyolefins like Polyethylene terephthalate (PET) films as a plastic bottle waste makes it non-susceptible to degradation since it consists of only long carbon chains.

Polymer degradation is a change in the properties of composition, tensile strength under the influence of environmental factors such as heat, light and chemicals (Mohanty et al).
Different mechanisms of biodegradation of polymer includes:

- Biodeterioration: Modification of properties of polymer due to the growth of microorganisms.
- Bio-fragmentation: Conversion of polymers to monomers by the action of micro-organisms.

- Assimilation: Microorganisms are supplied by necessary carbon, energy and nutrient sources from fragmentation of polymers and convert carbon of plastic to CO_2, water and biomass.

Based on biodegradability, polymers are classified as:

- Biodegradable polymers
- Non-biodegradable polymers.

The isolation and characterization of plastic polymer degrading bacteria from plastic waste was done. The bacterial strains were isolated from places of marine environment near Bakkhali and fungal strains from Sunderban area were contaminated with plastic wastes used to biodegrade the polymers (obtained from plastic bottles, commonly could be called as PET) in laboratory simulated conditions in order to study the biodegradation potential of the isolated microorganisms. Weight loss studies followed by FTIR, XRD and SEM analysis were carried out in order to study the biodegradation properties.

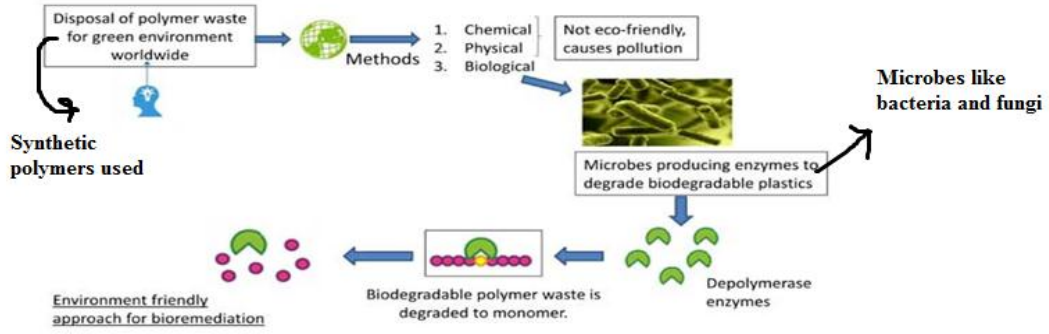


Fig 3: Bioremediation process from disposal of polymer waste.

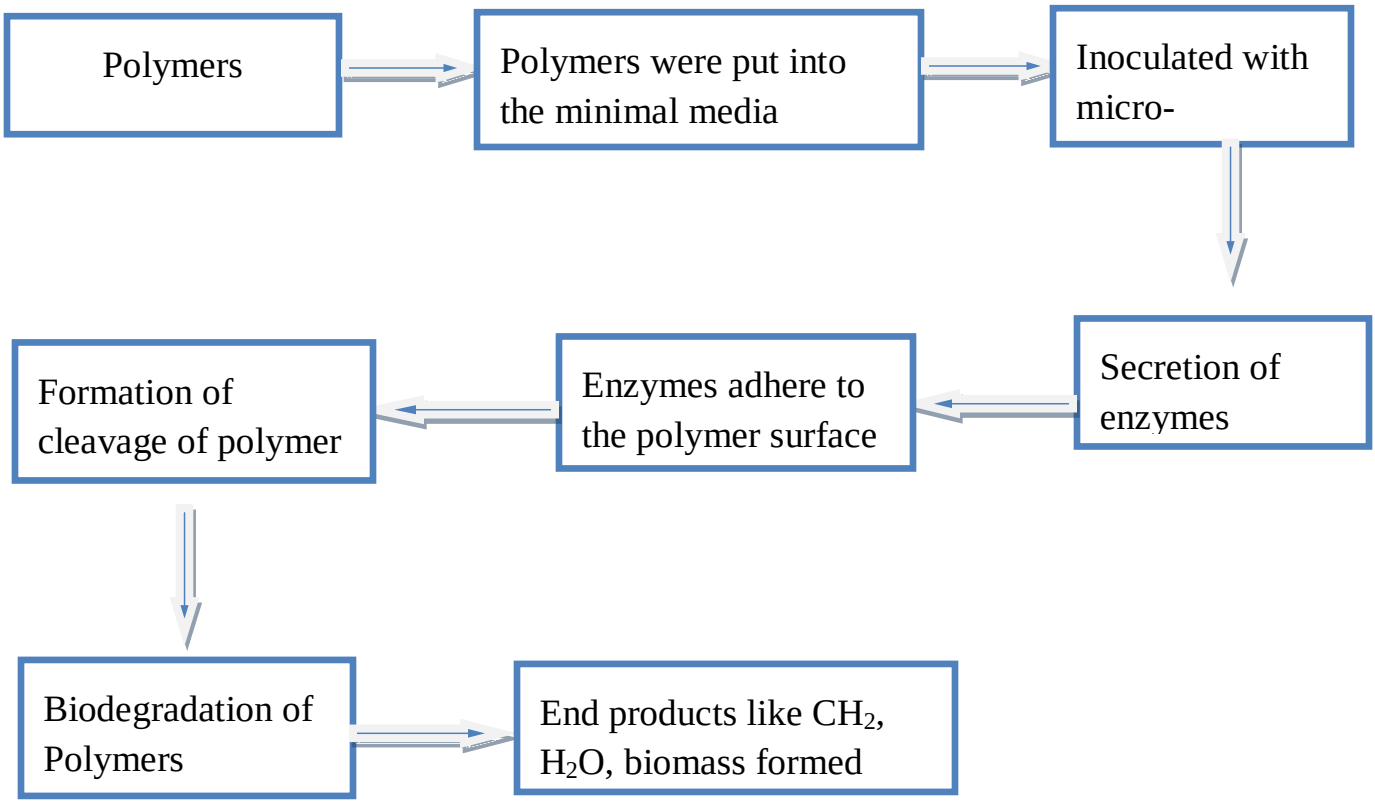


Fig 4: Schematic representation of a Polymer degradation

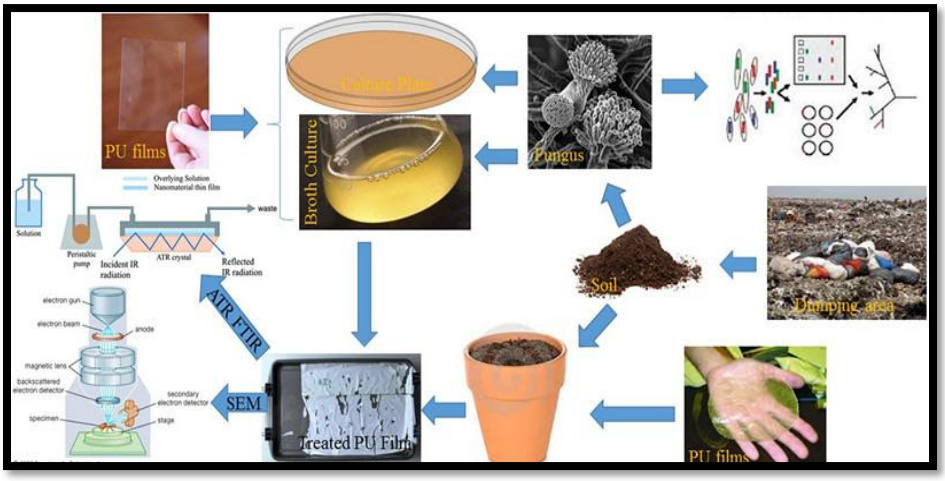


Fig 5: Biodegradation cycle in the environment using Polymer films

2. REVIEW OF LITERATURE

The decent variety and heap of heterotrophic micro-organisms related with the polythene degradation system was separated and distinguished by plating and recoloring system. Separated microbial strains were distinguished as Aspergillus sp and Vibrio sp. Transcendent microbial strains of both bacteria and fungi were chosen for polythene corruption under research facility conditions. Their viability on the degradation PET films of low-thickness polyethylene (LDPE) was examined over a time of about 6 weeks in a BOD incubator & shaker under lab conditions. Biodegradation was estimated regarding mean weight reduction, which was about 8 to 12% following a time of about 6 weeks. Further SEM (Scanning electron microscopy) investigation affirmed the corruption by uncovering the nearness of porosity and delicacy of the contagious degraded polythene surface. Aspergillus niger of 8% degradation appeared in one month. ***(Raaman, et al, 2012).***

In this review work was carried out on biodegradability of polymers by using microorganisms and the polymers are natural and synthetic types. Disposal methods were used for the biodegradation of polymers. Numbers of test were used for the determination of biodegradation of microorganism. Many of the microbes are active at aerobically or some anaerobically. In experimental work weight loss of polymer, physical, chemical properties were measured. **(Shah, et al., 2008).**

The study of plastic degradation specified that greatest of the respondent, nevertheless, of their experience, are in facility of banning of large-scale manufacture, circulation and use of these polymers, and are conscious of the opposing belongings of plastic container wastes on environmental hazards, and finally animal and human fitness. Nevertheless, plastic bags are quiet extensively used by the public additional than any other mostly due to their cheaper cost and large-scale production (**Adane, *et al*., 2011).**

The paper review found that *Aspergillius fumigatus* and *Penicillium sp*. were natural to the places of plastic removal and capacities indicates definite decomposability in regular situations and so similarly biodegradation in laboratory situations on artificial media. Conferring to this review bacteria cause highest degradation of polythene and plastics. **(*Singh, et al., 2012*).**

In this review work was carried out on biodegradability of polymers by using micro-organisms and the polymers are natural and synthetic types. Disposal methods were used for the biodegradation of polymers. Numbers of test were used for the determination of biodegradation of microorganism. Many of the microbes are active at aerobically or some anaerobically. In experimental work weight loss of polymer, physical, chemical properties were measured. **(*Shaha, et al., 2008*).**

In this research paper the isolation and characterization of polyethylene degrading bacteria from polyethylene garbage was done. Bacterial strains were successfully isolated from polythene garbage dumps. The identified bacterial species were *E. coli, Staphylococcus, Pseudomonas, Klebsiella and Bacillus*. The degradation was observed by changes in physical and optical characterstics. The percentage of Kathersan that *Pseudomonas* sp degraded the plastic up to 8.16% and 20.5% of degradation was observed anaerobically. The maximum degradation was observed in *staphyloccus* sp. The maximum amounts of polyethylene degradation by weight loss method was observed **(*Pande et al., 2014)***.

3. MATERIALS AND METHODS:

The materials used for this research study is as follows:

- Plastic bottle waste was procured from plastic bottles in the solid waste litter site near Bay of Bengal, Sunderban.
- Glutaraldehyde and ethanol obtained from Merck, Germany, was used for the process of fixation of the microbial isolates on the polymer film surfaces of Plastic bottle wastes.

- **Minimal Media:** Minimal media is the media that contain the minimum amount of nutrients attainable for colony growth, generally without the presence of amino acids, and are often used by microbiologists and geneticists to grow microorganisms.

  Media consisting of minimum amount of nutrients for the growth of the micro-organisms.

- Chemicals for Minimal media preparation: potassium dihydrogen phosphate (KH_2PO_4), sodium nitrate ($NaNO_3$), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), potassium chloride (KCl), ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) and ammonium chloride (NH_4Cl) was procured from Lobachemie, India.

- Composition consists of about 1g/l of potassium di-hydrogen phosphate, 2g/l of sodium nitrate, 0.5 g/l of magnesium sulphate hepta-hydrate, 0.5 g/l of potassium chloride, 0.01g /l of ferrous sulphate and 1 g/l of ammonium chloride.

METHODS:

3.1 Biological degradation of the polymer film sample by fungi & bacteria

Degradation Conduct of the polymer film samples (Plastic container waste, or PET) acquired from the waste litter locales or dirtied destinations close to rivers or other water-bodies , was inspected degradation rate through marine micro-organisms, in particular various types of recognized bacterial and contagious strains: Bacterial strains of Vibrio sp (GenBank promotion no: KY941137.1, strain PD6) (Bakkhali sp.) and Fungal strains of Aspergillus sp (GenBank increase no: MH119104.1). The microbes were isolated from the marine waters of Bay of Bengal, Sunderban near West Bengal. The polymer film samples were collected from Plastic bottles from the solid waste litters, generally known as PET acquired by cutting the mineral water bottle into square measured 1 cm in diameter. The polymer film samples of Plastic were taken out at 1, 2, 3, 4, 5 and 6 weeks respectively. Then the samples were ethanol washed, vacuum dried and in this manner degradation rate of the samples as for time was determined as a level of weight reduction, as a percentage of weight loss.

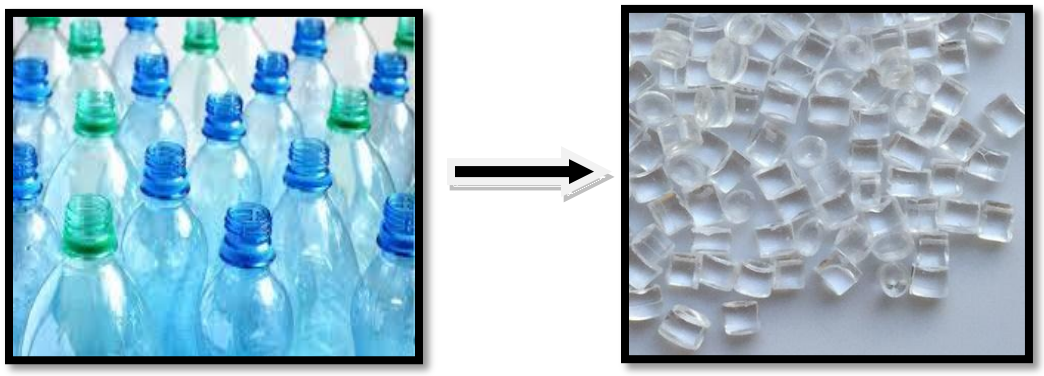


Fig 6: Plastic bottle waste from plastic bottles and cut into small films of 1 cm.

3.2 Characterizations:

3.2.1 Weight Loss Analysis

The polymer film samples acquired from Plastic bottle waste can be assessed by the system of Weight reduction analysis of the degraded polymer film samples. (Sain et al,2014) , estimating the rate of degradation at various time interims from the significant minimal media, Plastic bottle films were taken out from the conical flasks containing minimal media, with the assistance of forceps in the wake of keeping it for about 6 weeks and further, at a BOD shaker and incubator, at a temperature of around 36-37°C consequently ascertaining the level of weight reduction by the given condition, which is evaluated by the following equation,

Where, S1 is the initial weight of the polymer samples before biodegradation (before putting it in media) and S2 is the final weight of the polymer samples after biodegradation (after taking out from media) at different time intervals.

$$\%\, \boldsymbol{Weight\ Loss} = \frac{\boldsymbol{S1-S2}}{\boldsymbol{S1}} * \mathbf{100}$$

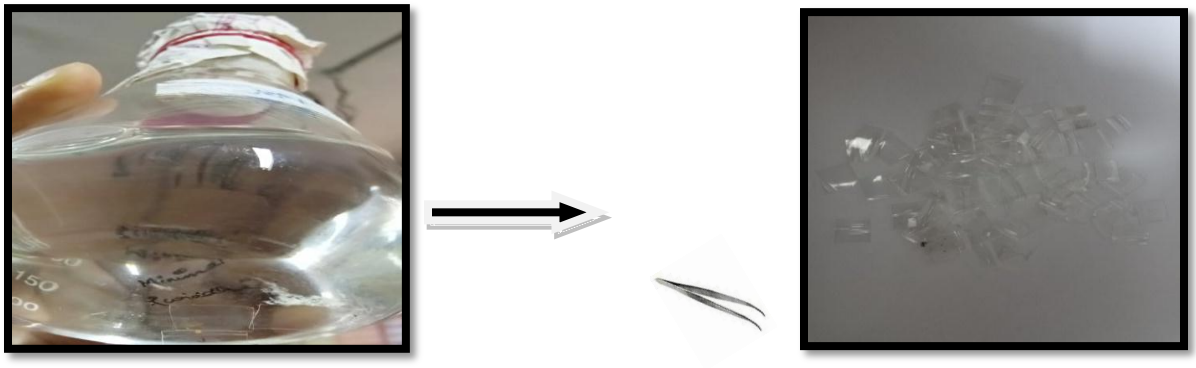


Fig 7: Plastic samples taken out from the conical flasks

3.2.2 SEM (Scanning Electron Microscopy) Analysis

The glutaraldehyde fixation of the micro-organisms (bacterial and fungal) on the polymer films of Plastic bottle waste was done by the following procedure:

- 2.5% glutaraldehyde was taken followed by 0.1 M PBS (Phosphate buffer saline) solution and dehydration by using varied concentrations of ethanol: 30%, 70% and 100% respectively.
- The incubation time for the polymer films was 10 mins (Sain et al, 2014) in each of the solution and then finally were dried and examined for SEM analysis (Carl Zeiss, Bangalore).
- SEM analysis was done to identify the morphological changes in the polymer structures.
- All the samples were kept under vacuum for some time and then platinum coated, hence polymer structures with fixed bacterial strains were observed on the film surfaces

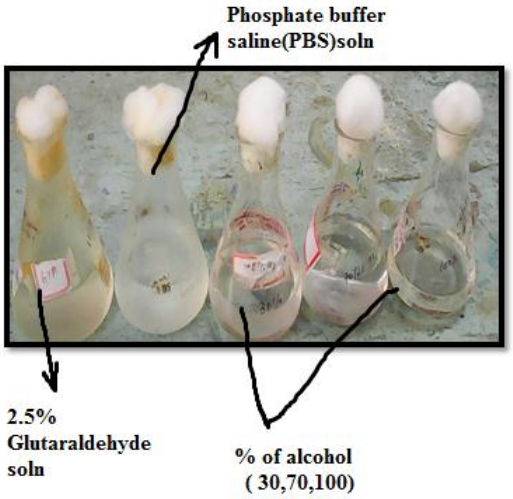


Fig 8: Solutions prepared for glutaraldehyde fixation before SEM analysis.

3.2.3 FTIR study

The polymer films of Plastic bottle waste before and after the biodegradation study using bacteria and fungi in the minimal media were subjected to FTIR analysis using Perkin Elmer Infrared Spectrophotometer (Spectrum 100).

3.2.4 XRD Analysis

The polymer films obtained from the Plastic bottle waste before and after the degradation using bacterial and fungal isolates were analyzed through X-ray Diffractometer at 30 kV and 30 mA. The XRD patterns of the sample was obtained (from fig 5) which analyses that the crystallinity of the polymer films has a decreasing trend with the increasing intensity. By using a diffractometer with Cu Ka radiation (k= 0.154 nm), X-ray diffraction (XRD) patterns of the samples were recorded in the range 2θ= 4–80°. The spectra were recorded with a 2θ step of 0.02°θ at a scanning rate of 2° θ/min.

3.3 Different Batch studies

3.3.1 Temperature study

The samples of polymer films obtained from Plastic bottle waste was treated with the bacterial and fungal strains and kept under an incubation period of 3 weeks at various temperatures: 25°C, 35°C and 45°C, to obtain the best growth at a specific temperature.

3.3.2 pH study

The polymer films obtained from the Plastic bottle wastes with the bacterial and fungal isolates after kept at an incubation period of 3 weeks were studied for different pH such as: 1,3,9,11 to find the best growth at optimized pH.

3.3.3 Inoculum dose study

The polymer films obtaining from the Plastic bottle wastes was inoculated with the different bacterial as well as fungal strains and kept for incubation for about 3 weeks with different inoculum volumes of 1ml, 3 ml, 5 ml and 7 ml per 75 ml of the minimal media respectively.

4. RESULTS AND DISCUSSIONS

4.1 Assessment of Weight loss study of polymers from Plastic bottle films

The plastic polymer samples have been effectually degraded by the micro-organisms (bacteria and fungi) and the growth of those isolates have been shown in Fig 9(A, and B). The degradation rate was studied from the analysis of percentage of weight loss with respect to the incubation time of 6 weeks from the subsequent graph as a function of biodegradation of the polymer samples with respect to the time calculated in weeks as explained in Fig.10(A, and B). The plastic bottle films showed a higher % of weight loss by the bacterial strains as compared to the fungal strains. This study reflects a higher weight loss of polymer degraded efficiently by the bacterial strains of *Vibrio sp* in comparison with the fungal strains of *Aspergillus sp.* This study reflects the weight loss of plastic films degraded with efficiency by the micro-organism strains of *Vibrio sp* compared with the strains of *Aspergillus sp*. Plastic film samples showed a good proportion of weight loss in 6 weeks (22% once degraded by fungi and 33% degraded by bacteria).

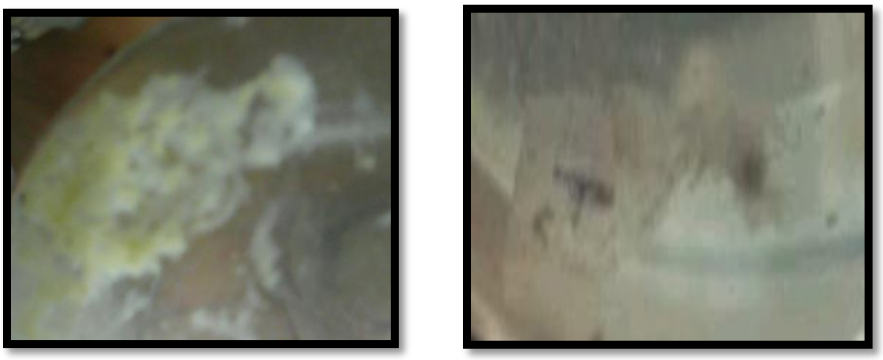


Fig 9(A, and B): Growth of bacteria and fungi on Plastic bottle film (PET)

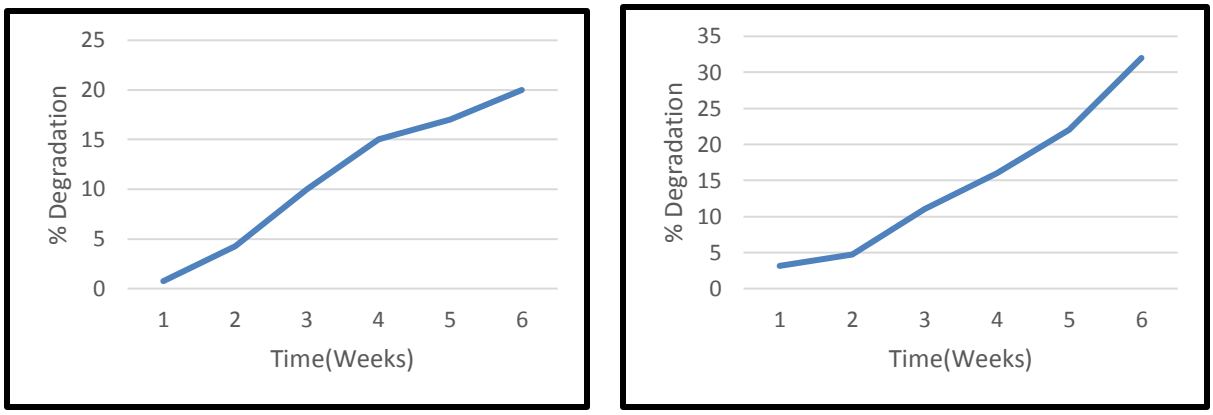


Fig 10 (A, B): Weight loss of PET films by fungal and bacterial strains.

4.2 FTIR (Fourier Transform Infrared Radiation) Analysis of the Plastic bottle films

The Fourier transform infrared light analyses the chemical compound present in the films of Plastic bottle waste before and after the degradation by microbial strains of bacterium and fungi. Based on the Fig 11 (A, and B), the main properties of the spectral bonds of the Plastic bottle waste has been recognised as PET since the peaks are decreasing, it shows that degradation has occurred within the chemical compound of the plastic samples. The transmittance of the samples at different wavenumbers represent: OH bond, (3600-3200 cm-1), C-O stretch (1150-1050 cm-1), organic compound alkene and C-H stretching (3100-2800 cm-1). The FT-IR information examines that the sample from Plastic bottle waste show no modification for the peaks attributed to -OCH3 cluster. This reveals that no degradation of this cluster occurred throughout biodegradation. The sample before degradation showed that identical peaks in wavenumber 250 cm^{-1} pictured identical cluster. The bonds at 769 and 806 cm^{-1} shows the ester stretching of the pet before degradation and after degradation, the C=O stretching started getting disappeared in the degraded samples, but the C-H bond intensifies more in the degraded polymer samples.

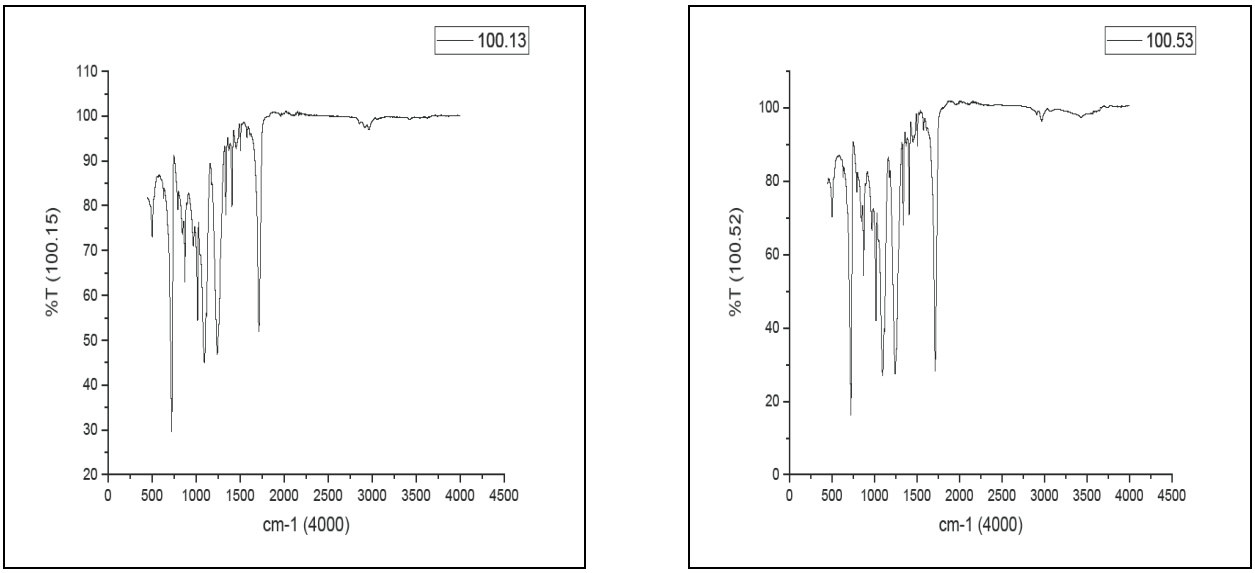


Fig 11 (A, B): FTIR spectrum of PET by fungal and bacterial isolates.

4.3 SEM (Scanning Electron Microscopy) Analysis of the degraded polymer film surfaces with glutaraldehyde fixation

After the comparison of microorganisms i.e. bacteria and fungi, bacteria sp from Bakkhali shows a far better degradation as compared to genus Aspergillus sp. Hence, SEM (Scanning Electron Microscopy) analysis has been performed for each of the microbial strains for analysing the composition and surface topography of the plastic bottle samples.

SEM analysis permits to look at different changes within the morphological structures of the plastic films at tiny scale, for viewing the changes within the structure of the samples undergoing degradation, pictures from the SEM were used. Surface morphology by scanning microscopy was resolute. This experiment determines the photographs of each the compound samples while not undergoing the degradation and the samples that underwent the biodegradation method. SEM analysis exhibited the microorganism activity of degradation on the polymer film samples represented in (Fig 12: A, B, C, D), the growth of the organism by the degradation method was clearly visible below SEM. The surface of plastic structure of the samples which underwent degradation method had lost its smoothness, and cracks were evident as compared to the samples before the degradation method is swish, and no cracks or holes may be seen. The samples

showed a big amendment within the structures. SEM pictures confirmed the biodegradation method of the compound samples.

(Fig 12) represents SEM images of the various surfaces of the compound films after and before degradation. The polymer films depicted as PET before degradation shows an electric sander surface compared to the films after degradation that shows roughness in surface. For more study and identification of biodegradation behaviour of the compound films, SEM analysis of the films with glutaraldehyde solution was carried out with EDS analysis.

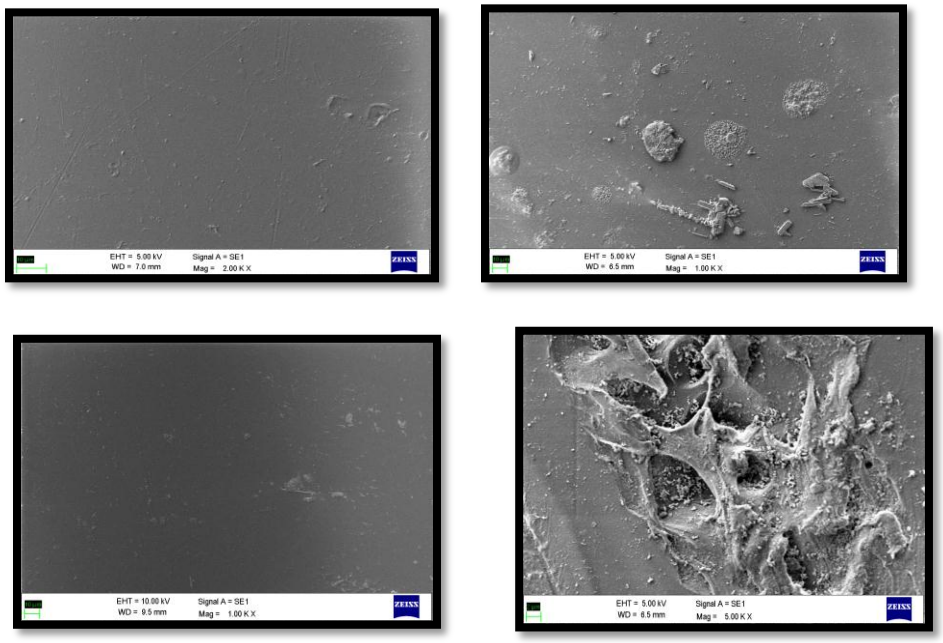


Fig 12 (A, B, C, D): SEM images of Plastic bottle waste or PET before and after degradation by bacterial and fungal strains.

4.4 XRD (X-Ray Diffraction) Study

This experimental study was performed to acquire the crystallinity of the Plastic polymer film samples by utilizing a x- ray beam diffractometer (at room temperature of about 35-37ºC). The structure of the polymer films has been dictated by the translation of the X-Ray diffraction designs given by the polymer films. A run of the mill diffractogram acquired for the present work by the polymer film is appeared in the Fig (13). Considering Fig 13, the intensity for the

crystallinity of the film sample is found to increment with the expanding pattern of the temperature estimated at 2θ.

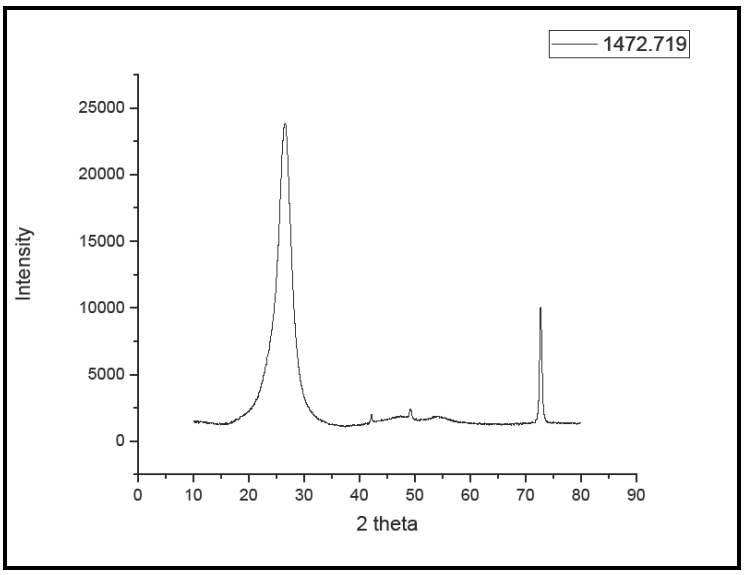


Fig 13: X-Ray diffraction pattern of Plastic bottle film sample (PET)

4.5 Analysis of the Batch Studies

4.5.1 Effect of temperature on the polymer films

The temperature assumes an essential role for solid waste management as it has impacts on the microbial development. The effect of temperature variety in degradation rate was investigated at temperatures from 25ºC - 45ºC with intervals of about 10ºC. The polymer films of Plastic bottles with the bacterial and fungal strains were held under a incubating time of 3 weeks at shifted temperatures: 25°C, 35°C and 45°C to break down the best and streamlined temperature for the development of smaller scale living beings on the polymer films. Typically, microorganisms develop best at 35ºC which is delineated by the diagram as shown in (Fig 14). Fig 14 gives the total investigation of the examination at different temperatures. 35°C has appeared better rate of degradation when compared with the temperatures of 25°C and 45º C. Contrasting outcomes of microbial growths, it assesses that bacterial degradation has a better outcome when compared with fungal.

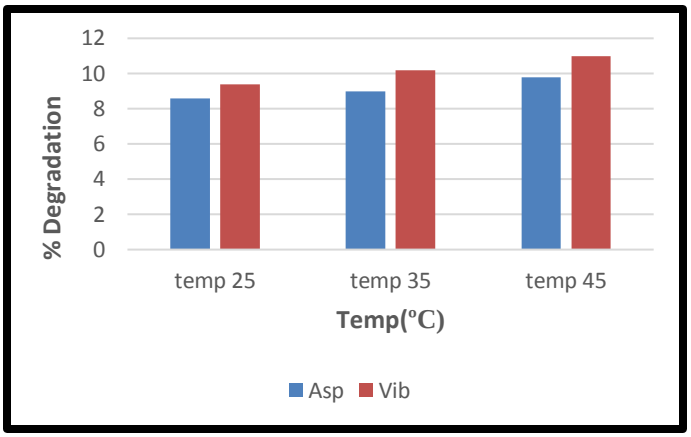


Fig 14: % degradation by the bacterial and fungal strain at different temperatures

4.5.2 Effect of pH on the polymer films by the microbial strains

Degradation of polymer film samples obtained from solid litter wastes of Plastic bottles, or PET utilizing microbial strains of bacterium (*Vibrio sp*) and fungus (*Aspergillus sp*) isolated from the marine sources close Bay of Bengal was performed. pH assumes a significant role in biodegradation as it is a vital situation factor for the development of organisms since it examines the hydrogen ion concentration. Impact of pH on polymer degradation was assessed at pH 1, 3, 9, and 11 subsequent to brooding the media containing polymer films for 3 weeks. In general fungi prefer more acidic conditions, whereas bacteria grow in a neutral to alkaline environment. Subsequently, this investigation evaluated the impact of pH on plastic polymer films at pH esteem that is highly alkaline. In this manner, resulting chart on impact of pH on plastic bottle waste biodegradation is shown in (Fig 15). In view of Fig 15, degradation rate indicates expanding pattern as pH increment. The corruption rate assessed that the best consequences of microbial development appears at basic pH.

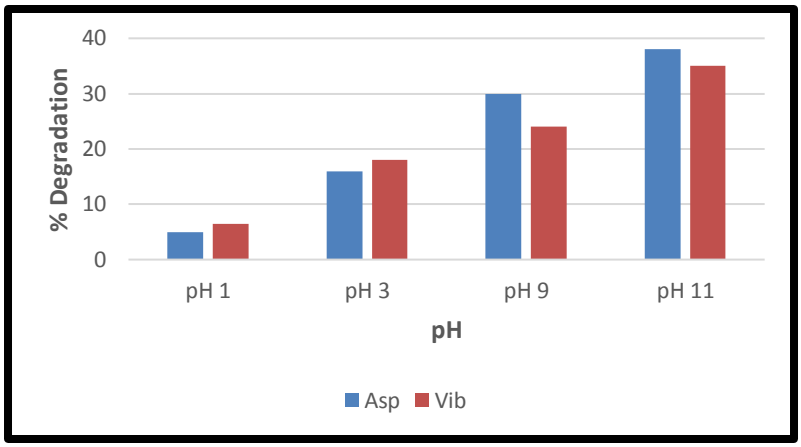


Fig 15: Degradation of polymer film by fungal and bacterial strain at different pH concentrations.

4.5.3 Effect of inoculum concentration dose on the polymer films

The volume of inoculum concentration assumes a viable role in the short-lived arrangement of biodegradation for the development of small- scale life forms. In some cases, little inoculum measurement might be insufficient to degrade and erode the polymers, while too enormous inoculum dose prompts poor degradation of the polymer films of plastic bottle waste. In this way, appropriate and proper amount of inoculum dosage is essential for the set-up of biodegradation systems. The plastic polymer films collected from bottle waste imperated with the distinctive bacterial strains (*Vibrio sp*) and fungal strains (*Aspergillus sp*) and kept for about 3 weeks in a BOD incubator and shaker culture with various inoculum concentrations of 1ml, 3 ml, 5 ml and 7 ml for each 75 ml separately. Thus, based on (Fig 16), it is assessed that bacterial strains have appeared higher inoculum dose focuses as for bacterial strains and the inoculum concentration measurement are directly connected with the degradation rate. The rate of degradation demonstrates an expanding pattern with the expansion of inoculum dose fixation.

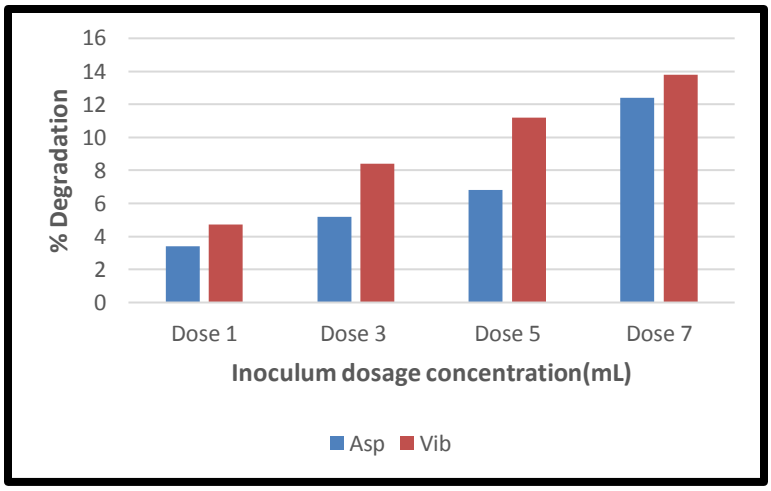


Fig 16: Rate of degradation by bacterial and fungal strains at different inoculum dosage concentrations.

5. CONCLUSION

Biodegradation of the polymer films from Plastic bottle waste, obtained from the solid litter waste near Bay of Bengal has been assessed. The present study deals with the influence of microorganisms (Bacterial strains of *Vibrio sp* from Bakkhali (GenBank accession no: KY941137.1, strain PD6) and Fungal strains of *Aspergillus sp*) (GenBank accession no: MH119104.1) on Plastic bottle films and its impact on degradation. The degradation has been evaluated by different characterizations: Weight Loss method, FTIR, XRD and SEM analysis. The results from the weight loss analysis indicates that the rate of degradation by Plastic bottle film strips by bacterial strains is more as compared to the degradation by fungal strains (the sample lost its weight by 22 % by fungal strains and by bacterial strains lost 35% of its weight) in 6 weeks. Different batch studies by varying temperature, pH, and inoculum dose as well as spectroscopic analysis (SEM, FTIR) has played an important role for the biodegradation process of the polymer films from Plastic bottle commonly can be called as PET. The surface profile of plastic polymer film after 6 weeks by both the microorganisms, observed under Scanning Electron Microscopy (SEM) showed the growth of microbes on the film surfaces, also the erosion and damage to the surface of the plastic film. This bacterium and fungus have the potential to be used for processing plastic waste in the future to reduce environmental damage. Different batch studies like analysis of temperature, pH and concentration of inoculum dose, performed also proved that the effect of bacterial strains on the degradation of plastic films is higher than that of fungal strains(Temp study: 9.8% by fungal strains, 11% by bacterial strains; pH study: 32% by fungal strains, 38% by bacterial strains; Inoculum dosage: 12.3 % by fungal strains and 13.6% by bacterial strains. Thus, the methods show that microbial degradation is an efficient way for different plastic polymers.

REFERENCES

1. Singh G, Singh A.K, Bhatt K, (December 2015), "**Biodegradation of Polyethene's By Bacteria Isolated from Soil",** (International Journal of Research and Development in Pharmacy and Life Sciences) 5**,** (2056-2062).

2. Sangale M.K, Shahnawaz M and Ade A, (2012), **"A Review on Biodegradation of Polythene: The Microbial Approach",** (Journal of Bioremediation and Biodegradation)10**,** (2155-6199).

3. Das Kumar S, (February 2015), **"An Approach to Low density Polyethylene Biodegradation by *Bacillus amyloloquefaciens*",** (Journal 3 Biotech) 5, (81-86).

4.Pandey P.K, Kass P, Soupir M. L, Biswas S, Singh V P, (May 2014), "**Contamination of Water resources By Pathogenic bacteria**", (International Journal of Biotechnology) 10, (451-568).

5. Ali S, Fariha Abdul H, Safia A**,** (January 2012), **"Biological Degradation of plastics: a comprehensive review**", (Journal of Biotechnology Advances) 26, (246-65).

6. Ghosh S K, Pal S, Ray S, (April 2013), **"Study of microbes having potentiability for biodegradation of plastics",** (International Journal of Environmental Science and Pollution), 20**,** (4339-4355).

7. Sain S, Sengupta S, Kar A, Mukhopadhyay A, Sengupta S, Kar Ray D, (January 2014), **"Effect of modified cellulose fibres on the biodegradation behaviour of in-situ formed PMMA/cellulose composites in soil environment: Isolation and identification of the composite degrading fungus** ", (Polymer Degradation and Stability), 9, (156-165).

8. La Mantia F.P., Morreale M, (January 2011) **"Green composites: A brief review",** (Composites), 42, (579-588).

9. N.A. Mostafa, Awatef A. Farag, Hala M. Abo-dief, Aghareed M. Tayeb, (2018), **"Production of biodegradable plastic from agricultural wastes",** (Arabian Journal of Chemistry), 11, (546-553).

10. Maiti S, Sain S, Ray D, Mitra D (November 2013), **"Biodegradation behaviour of PMMA/cellulose nanocomposites prepared by in-situ polymerization and ex-situ dispersion methods",** (Polymer Degradation and Stability), 98, (635-642).

11. Mohanty AK, Misra M, Drzal LT (2002**)," Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world**", (Journal of Polymers and the Environment), 10, (19-26).

12. Huang X, Brittain WJ (2001), **"Synthesis and characterization of PMMA nanocomposites by suspension and emulsion polymerization"**, (Macromolecules), 3, (3255-3260).

13. Sahoo PK, Samal R (2007), **"Fire retardancy and biodegradability of polymethylmethacrylate /montmorillonite nanocomposite"**, (Polymer Degradation and Stability) ,92, (1700-1707).

14. Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. and Uppara, P. V. (2008). "**Biodegradation of polyethylene and polypropylene"**, (Indian Journal of Biotechnology), 7, (9-22).

15. Augusta, J., Muller, R. and Widdecke, H. (1993). "**A rapid evaluation plate-test for the biodegradability of plastics",** (Applied Microbiologyand Technology), 39, (673-678).

16. Balasubramanian, V., Natarajan, K., Hemambika, B.,Ramesh, N., Sumathi, C. S., Kottaimuthu, R. and Rajesh Kannan, V. (2010). "**High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of gulf of Mannar, India"**, (Letters in Applied Microbiology), 51, (205-211).

17. Pranamuda, H., Tokiwa, Y. and Tanaka, H. (1995). "**Microbial degradation of an aliphatic polyester with a high melting point, poly (Tetramethylene Succinate)"**, (Applied and Environmental Microbiology), 61(5), (1828-1832).

18. Pranamuda, H., Tokiwa, Y. and Tanaka, H. (1997), "**Polylactide degradation by an amycolatopsis sp"**, (Applied and Environmental Microbiology), 63(4), (1637-1640).

19. Satlewal, A., Soni, R., Zaidi, M., Shouche, Y. and Goel, R. (2008). "**Comparative biodegradation of HDPE and LDPE using and indigenously developed microbial consortium"**, (Journal of Microbiology and Biotechnology), 18 (3), (477-482).

CHAPTER 2

BIODEGRADATION STUDY OF PVA FILM (POLYVINYL ALCOHOL)

1.INTRODUCTION

Biodegradation

Plastics can degrade by various mechanisms: thermal, chemical, photo and biological degradation. The degradation of plastics is a physical or chemical change in polymers that occurs as a result of environmental factors, like light, heat, moisture, chemical conditions or biological activity (Tokiwa et al, 2009). Biodegradation is a bio-chemical process that refers to the degradation and assimilation of polymers by living microorganisms, to produce degradation products (Raaman et al ,2012).

1.1 Biodegradation of Plastics

Biodegradation is defined as any physical or chemical change in a material caused by biological activity. Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics. Plastics are usually biodegraded aerobically in nature, anaerobically in sediments and landfills and partly aerobically in compost and soil. Carbon dioxide and water are produced during aerobic biodegradation, while anaerobic biodegradation produces carbon dioxide, water and methane (Fujita et al, 2004).

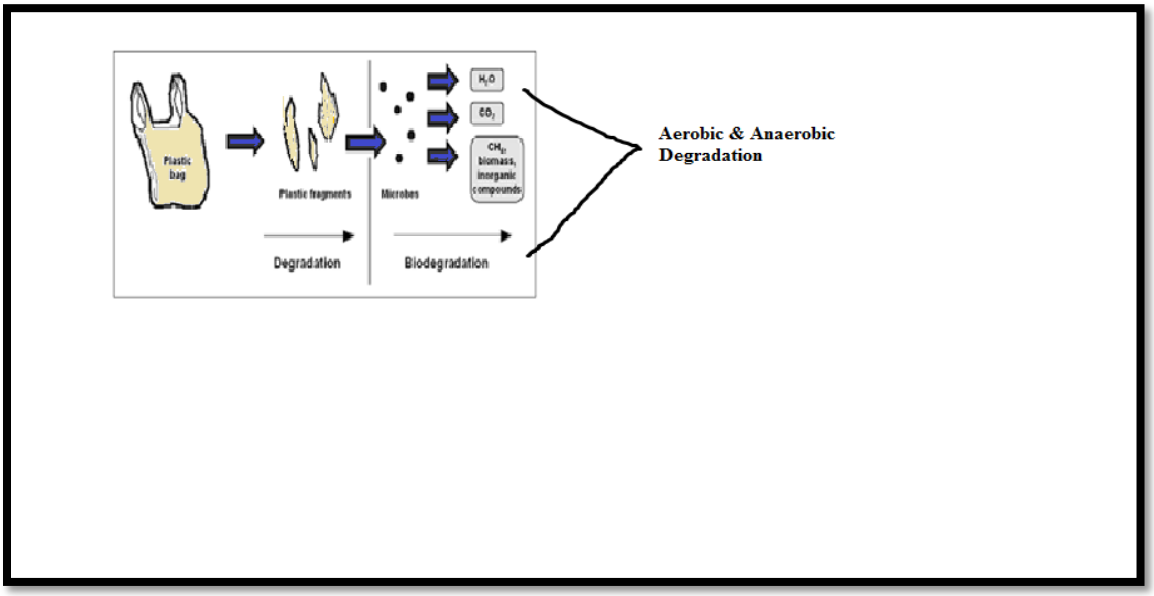


Fig 1: Process of Degradation to Biodegradation

1.1.1 Aerobic Biodegradation

Also known as aerobic respiration, aerobic biodegradation is an important part of the natural attenuation of contaminants in many hazardous waste sites. Aerobic microbes use oxygen as an electron acceptor and break down organic chemicals into smaller organic compounds. CO_2 and water are the by-products of this process (Archana et al, 2011).

The reaction mechanism:

C plastic + O_2 → CO_2 + H_2O + C residual + Biomass

1.1.2. Anaerobic Biodegradation

Anaerobic biodegradation is the breakdown of organic contaminants by microorganisms when oxygen is not present. It is also an important component of the natural attenuation of contaminants at hazardous waste sites. Some anaerobic bacteria use nitrate, sulphate, iron, manganese and carbon dioxide as their electron acceptors, to break down organic chemicals into smaller compounds.

The reaction mechanism:

C plastic → CH_4 + CO_2 + H_2O + C residual

Microorganisms are unable to transport the polymers directly through their outer cell membranes, into the cells where most of the biochemical processes take place, since polymer molecule are long and not water-soluble. In order to use such materials as a carbon and energy source, microbes developed a strategy in which they excrete extracellular enzymes that depolymerize the polymers outside the cells (Gu et al, 2003).

Anaerobic and aerobic biodegradation mechanism pathways are given in Figure 5. Extracellular and intracellular depolymerize enzymes are actively involved in biological degradation of polymers. During degradation, microbial exoenzymes break down complex polymers, yielding short chains or smaller molecules like oligomers, dimers and monomers. These molecules are small enough to be water-soluble and can pass through the semi-permeable outer bacterial membranes to be used as carbon and energy sources. This initial process of breaking down

polymers is called depolymerization; and when the end products are inorganic species (e.g., CO_2, H_2O, or CH_4), the degradation is called mineralization (Gu et al, 2004).

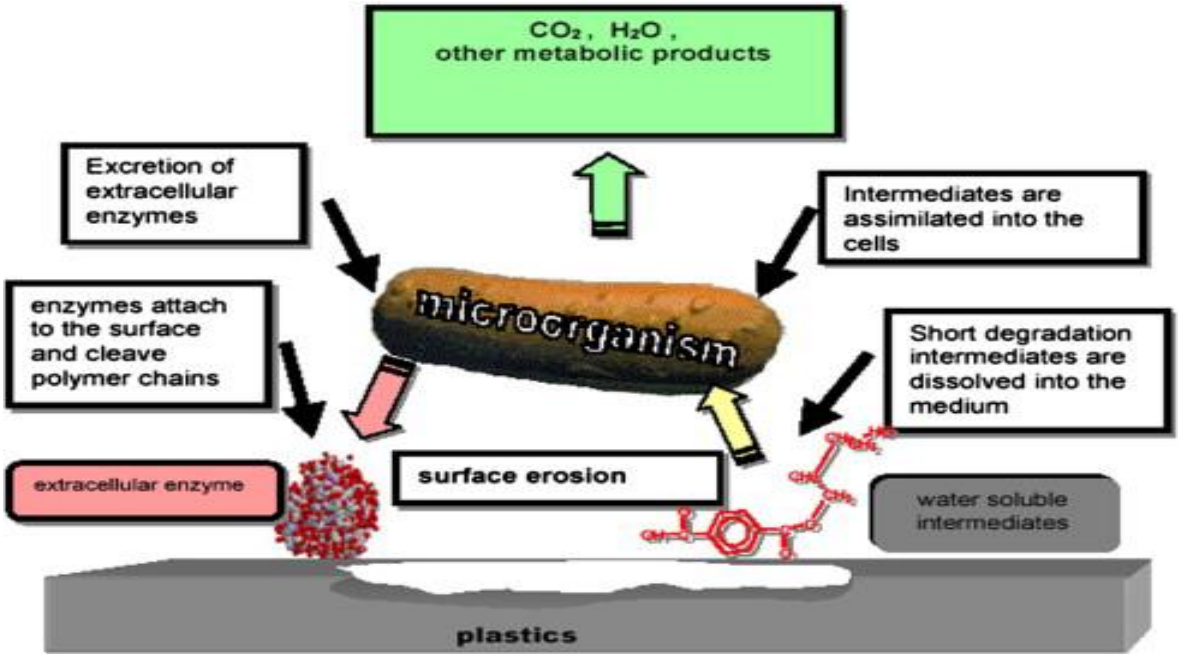


Fig 2: The General Mechanism of Plastic biodegradation under Aerobic Conditions (Gu et al)

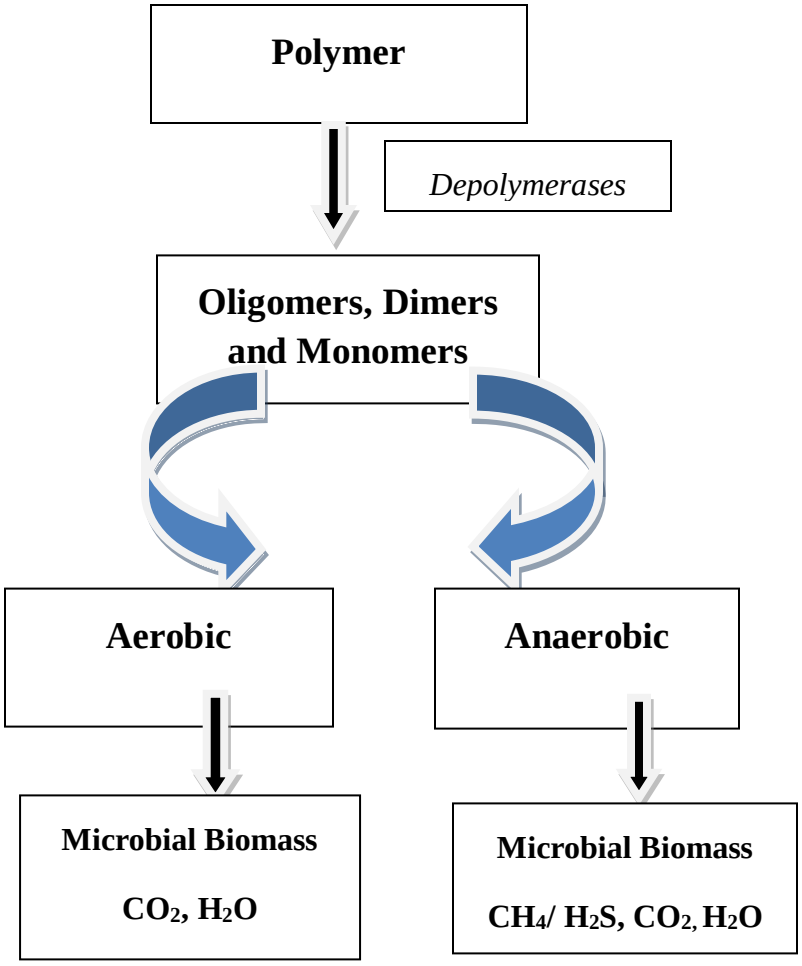


Fig 3: Reaction pathways during biodegradation of Polymers

1.2. Mechanism of Biodegradation

Biodegradation of polymers involves following steps:

1. Attachment of the microorganism to the surface of the polymer.
2. Growth of the microorganism, using the polymer as a carbon source.
3. Ultimate degradation of the polymer.

Microorganisms are able attach to a polymer's surface, if the latter is hydrophilic. Once the organism is attached to the surface, it can grow using the polymer as its carbon source. In the primary degradation stage, the extracellular enzymes secreted by the organism cause the main chain to cleave, leading to the formation of low-molecular weight fragments, like oligomers, dimers or monomers. These low molecular weight compounds are further used by the microbes

as carbon and energy sources. Small oligomers may also diffuse into the organism and get assimilated in its internal environment. (*Premraj et al, 2006*).

1.3. Factors Affecting Biodegradation of Plastics

The biodegradability of a polymer is essentially determined by the following physical and chemical characteristics:

1. The availability of functional groups that increase hydrophobicity (hydrophilic degradation is faster than hydrophobic).
2. The molecular weight and density of the polymer (lower degrades faster than higher).
3. The morphology of TM: amount of crystalline and amorphous regions (amorphous degrades faster than crystalline).
4. Structural complexity such as linearity or the presence of branching in the polymer.
5. Presence of simply breakable bonds like organic compound or organic compound bonds. Chain coupling (ester > ether > organic compound > urethane).
6. Molecular composition (blend).
7. The character and physical type of the compound (e.g., films, pellets, powder or fibers).
8. Hardness (Tg) (soft polymers degrade faster than hard ones)

1.4 About Polyvinyl Alcohol (PVA)

Poly (vinyl alcohol) (PVOH, PVA) is a water-soluble synthetic polymer. It has the idealized formula $[CH_2CH(OH)]$ n. It is employed in craft, textiles, and a variety of coatings. It is white (colorless) and odorless. It is typically provided as beads or as solutions in water. Unlike most vinyl polymers, PVA isn't ready by polymerization of the corresponding compound. The compound, vinyl alcohol, is unstable with respect to acetaldehyde. PVA instead is ready by initial polymerizing vinyl acetate, and the ensuing vinyl resin is regenerate to the PVA. Other precursor polymers square measure typically used, with formate, chloro-acetate groups instead of acetate.

The conversion of the polyesters is usually conducted by base-catalyzed transesterification with ethanol:

$$\mathbf{[CH_2CH(OAc)]\ n + C_2H_5OH \rightarrow [CH_2CH(OH)]\ n + C_2H_5OAc}$$

Preparation: The properties of the polymer depend on the amount of residual ester groups. Worldwide consumption of polyvinyl alcohol was over a million metric tons in 2006. Larger producers embody Kuraray (Japan, Europe, and USA) and Sekisui Specialty Chemicals (USA) however mainland China has put in many terribly massive production facilities within the past decade and presently accounts for 45% of world capacity. The North Korean manufacture Vinalon is from polyvinyl alcohol. Despite its inferior properties as a covering fiber, it is produced for self-sufficiency reasons, because no oil is required to produce it. PVA is an atactic material that exhibits crystallinity. In terms of microstructure, it is composed mainly of 1,3-diol linkages [CH_2-CH(OH)-CH_2-CH(OH)-] but a few percent of 1,2-diols [-CH_2-CH(OH)-CH(OH)-CH_2-] occur, betting on the conditions for the chemical change of the vinyl organic compound precursor. Polyvinyl alcohol has wonderful film forming, emulsifying and adhesive properties. It is additionally immune to oil, grease and solvents. It has high durability and adaptability, as well as high oxygen and aroma barrier properties. However, these properties are dependent.

1.4 Structure and properties of PVA

On humidity, in alternative words, with higher humidity additional water is absorbed. The water, that acts as a plasticizer, can then scale back its durability, however, increase its elongation and tear strength. PVA includes a freezing point of 230 °C and 180–190 °C (356-374 degrees Fahrenheit) for the totally hydrolyzed and partly hydrolyzed grades, respectively. It decomposes apace higher than 200 °C because it will endure transformation or undergo Pyrolysis at high temperatures. PVA is close to incompressible. The Poisson's ratio is between 0.42 and 0.48.

Physical Properties of PVA	
Density	1.19-1.31 g/cm3
Melting Point	230 °C
Boiling Point	228°C
Solubility	Water Soluble

Table 1: Physical Properties of PVA

1.5 PVA as a biodegradable polymer

Use of biodegradable, or single use disposable things as a replacement of synthetic plastics is gaining wide scale recognition thanks to its potential in overcoming, or a minimum of reducing problems related to the management of the post consumption of artificial or synthetic plastics waste (Tang and Alavi, 2011). Among the various others biodegradable polymers, Polyvinyl Alcohol (PVA) is attracting increasing attention for its application in the production of environmental-friendly plastic things (Corti et al., 2002; Qui and Netravali, 2012). PVA, a vinyl polymer where the most chain is joined by just one carbon-carbon linkage (-CH_2CHOH-) n. This linkage is the same as those of typical plastics, like polythene, plastic and vinylbenzene. PVA, a water- soluble polymer however additionally has thermo-physical property and might be shaped in numerous shapes like containers and films (Shimao et al).

Biodegradability of this material is mainly due to the presence of the hydroxyl radical, leading to its water solubility and susceptibility to oxidization (Kawai, 1995). Due to these properties, biodegradable plastic with polyvinyl alcohol gains commercially among other plastics and can be used in packaging and making agricultural mulch films (Bastioli et al., 1993). Also, varieties of plastic formulated with polyvinyl alcohol can be found in the market with many trade names like Akwa Tears, Alcotex, Alvyl, Aracet, Cipoviol, Celvol, Elvanol, Gelvatol, Ivalon, Solvar, Sumitex, Vinol, etc. Moreover, new and high production capacities of PVA-based plastics is opening in the Republic of Korea, India and South-East Asia (Flieger et al., 2003). Four major segments of PVA consumption embodies wrap sizing, paper coating, adhesives, films and use of biodegradable PVA things in mulching films, laundry luggage, etc. (Chiellini et al.,1999). Widespread application of this polymer as biodegradable plastic has induced analysis to know the nature of this polymer in undergoing microbial degradation.

The enrichment culture technique employs using PVA as the sole supply of carbon and energy and is the most extensively used methodology for isolation of those types of microorganisms. In the study, isolation of polyvinyl alcohol degrading microbial strains using the enrichment culture technique was applied from numerous samples collected from different polluted sites near Bay of Bengal, West Bengal.

In this present study, different types of micro-organisms (bacterial strains of *Vibrio sp* and fungal strains of *Aspergillus sp*) are used for the degradation of Polyvinyl Alcohol. However, the

degradation of polymers in marine environment by these types of microorganisms is very rare with a water soluble, and petroleum based degradable polymer, so this novel research work is done. Biodegradation of polymer films of PVA obtained from in situ polymerization, using solution casting technique, was examined in marine rich environment with isolated microorganisms. In this study, many species of microorganisms were isolated from saline water of Bay of Bengal near Sunderban, West Bengal. Among which one bacterium and one fungus capable for degradation were used.

2. REVIEW OF LITERATURE

Varieties of plastics and polymers are there but, in this research, synthetic biodegradable plastics of low-density polyethylene (LDPE) is involved for the study. The researchers and some scientists have developed some biodegradable plastics to use waste free plastic products. Polymers of decomposable entity can be degraded by microbes (such as bacteria or fungi) or organic chemical substance by break down of the molecular linkage in polymers. LDPE or synthetic plastic is one of the polymers till now, nearly impossible to be degraded in an easier or smoother way, therefore microbial strains required for LDPE degradation. In Current research twenty-five microbial strains were isolated from marine sources for plastic films. Out of twenty-five, one bacterium and one fungus identified as *Aspergillus* species and *Vibrio* species and the rest one was *Fusarium* species. Effectiveness of the microorganisms in polymer degradation was investigated by weight reduction, change in pH, temperature. The colonization of the micro-organisms was visualized by scanning electron microscope (SEM), whereas the surface chemical changes were confirmed by Fourier transform infrared spectroscopy (FTIR). Isolated bacteria and fungi showed the good biodegradation results for polymer Degradation (**Das, *et al*., 2014).**

Around 40% of the plastics other than the extension capacity of the films was lost by month 3 in either of soil types or marine systems. The loss in percentage for extension capacity(E) in polymer films was marginally higher in marine waters and loamy sand soil than in sandy soil. Although, it can decide that the decomposition of the bioplastic films under the study in marine environment degraded the films at higher than in the soil surfaces. (**Mostafa*, et al*., 2010).**

It is assessed in this review that there has remained a remarkable rise in attention of biodegradable materials for use in farming movement, packing of substantial, medicine, and other altered zones, this means that the interest for biodegradable polymers to use in bio-composites are highly increasing in demand. Many of the scientists are working on the modification and designing of plastic with a traditional material. This type of material is not hazardous to environment. Many of the biological resources may convert into decomposable polymers resources, with the best common existence starch and thin thread to take out from

different types of plants. The result is that decomposable plastic resources will decrease the necessity for artificial polymer manufacture at a cheap rate, so a decline in the progressive outcome on both environment and economy. This research paper intends to arrange for a small summary of effort that is under way in the zone of decomposable polymer study and improvement. (**Kumar, *et al.*, 2011).**

In this research paper the isolated bacteria used was *Pseudomonas stutzeri*. It was used for the biodegradability experiment of LDPE polymers and Polypropylene (PP). Tensile strength (TS) and extension of treated plastic films with and without the inoculum were studied experimentally at an interval of 15 days. The change in extension, tensile strength, and elongation were analyzed. There was a decline in the extension percentage, which was found to be maximum in polypropylene, 6.4 per cent more than in low density polyethylene and 13.3 per cent in comparison to control. Tensile strength was found to decrease with increase in time period on polypropylene (PP). Steep reduction in elongation was recorded in each the plastic films with inflated incubation time with a minimum a pair of 2.5 cm reduction discovered in polypropylene. The minimum per centage amendment within the strength was in LDPE on 15th day i.e.18 per cent and most was discovered in PP on forty fifth d, i.e. sixty-six per cent. Study indicates the increase in strength, many another times with reduction in delay, within the initial phases of degradation. This presence surveyed by a discount in ductile plus and extra decrease in extension (**Sharma, et al., 2003**).

Plastic, from the time of their origin became an essential a part of our life and in fashionable society. Artificial plastics are extensively employed in packaging of merchandise like food, pharmaceuticals, cosmetics, detergents and plenty of merchandise factory-made from plastics are a boon to public health, e g. disposable syringes and blood vessel baggage **(Halden, 2010**).

Production of plastic has inflated from zero.5 million tonnes in 1950 to 260 million tonnes in 2007. This increase in usage, particularly disposable things of packaging, makes up thirty seventh of all the plastic made (Plastic Europe, 2008). Packaging utility is that the biggest field wherever synthetic resin and its kind material square measure used. it's calculable that forty first of plastics square measure employed in packaging, which virtually half that volume is employed

to pack food merchandise (**O'Brien and Thompson, 2010**). LDPE polymer or low-density synthetic resin is that the most applied polyolefin in packaging, farming and agricultural utilizations (**Bastioli et al**.)

3. MATERIALS AND METHODS

The materials used for this research study is as follows:

- The commonly used polymer Polyvinyl Alcohol (PVA) was synthesized in laboratory.
- Glutaraldehyde and ethanol or ethyl alcohol obtained from Merck, Germany, was used for the process of fixation of the bacterial isolates on the polymer film surfaces.
- For minimal media preparation, potassium dihydrogen phosphate (KH_2PO_4), sodium nitrate ($NaNO_3$), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), potassium chloride (KCl), ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) and ammonium chloride (NH_4Cl) was procured from Lobachemie, India.
- Composition consists of about 1g/l of potassium di-hydrogen phosphate, 2g/l of sodium nitrate, 0.5 g/l of magnesium sulphate hepta-hydrate, 0.5 g/l of potassium chloride, 0.01g /l of ferrous sulphate and 1 g/l of ammonium chloride.

METHODS:

3.1 Preparation procedure of PVA polymer films

4 g of PVA granules were dissolved in 75 mL of water by heating at 95°C under moderate stirring for about 2 hrs. After 2 hrs of stirring when the solution becomes transparent, the PVA solution prepared, was casted on a sterile petri dish and then was cut into small uniform pieces to use further in the biodegradation process.

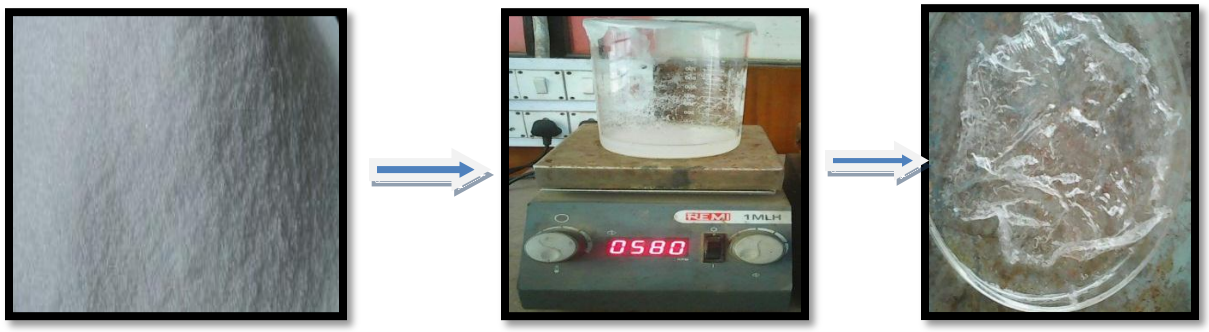


Fig 4: Preparation process of PVA film

3.2 Comparable biodegradation of PVA polymer film using fungal and bacterial strains

Biodegradability of the polymer film samples obtained from the solution casting method using PVA granules was examined by the degradation rate through different marine microbes, namely different species of identified bacterial and fungal strains: *Vibrio sp* (GenBank accession no: KY941137.1, strain PD6) and *Aspergillus sp.* (GenBank accession no: MH119104.1). The bacterial strains were isolated from the marine waters of Bay of Bengal near Bakkhali area, whereas the fungal strains were isolated from Sunderban area. The film sample of water soluble, petroleum-based polymer film of Polyvinyl Alcohol was obtained by the in-situ polymerization technique and then was cut into square sized 1 cm diameter. The polymer films samples were taken out at 1, 2, 3, 4, 5 and 6 weeks for PVA samples respectively, ethanol washed, vacuum dried at room temperature and thus rate of degradation of the samples with respect to time was evaluated as a percentage of weight loss.

3.3 Evaluation of Weight loss

The characterization of the PVA polymer films can be calculated by the weight loss study measuring the rate of degradation at different time intervals from the minimal media, PVA film samples were taken out after keeping it for 6 weeks at an incubator temperature of about 36-37°C hence calculating the percentage of weight loss by the given equation,

Where, M1 is the initial weight of the polymer samples before biodegradation (before putting it in media) and M2 is the final weight of the polymer samples after biodegradation (after taking out from media) at different time intervals.

$$\% \, \boldsymbol{Weight\ loss} = \frac{\boldsymbol{M1 - M2}}{\boldsymbol{M1}} * \mathbf{100}$$

3.4 Characterizations:

3.4.1 SEM Analysis

The fixation of bacterial and fungal isolates on the polymer films of PVA was done by the following procedure:

- 2.5% Glutaraldehyde (GTA), was taken followed by 0.1 M PBS (Phosphate buffer saline) solution.
- Dehydration by using varied concentrations of Ethanol or Ethyl alcohol: 30%, 70% and 100% respectively and the incubation time for the polymer films was 10 mins (Sain et al, 2014) in each of the solution and then finally were dried and examined for SEM analysis (Carl Zeiss, Bangalore).
- SEM analysis was done to identify the morphological changes in the polymer structures.
- All the samples were provided with vacuum and coated with platinum and polymer structures with fixed bacterial strains were observed on the film surfaces.

3.4.2 FTIR study

The polymer films of PVA before and after the biodegradation in the minimal media were subjected to FTIR analysis using Perkin Elmer Infrared Spectrophotometer (Spectrum 100).

3.4.3 XRD Analysis

The polymer films of PVA obtained from in-situ polymerization, before and after the degradation using bacterial and fungal isolates were analyzed through X-ray Diffractometer at 30 kV and 30 mA. The XRD patterns of the sample was obtained (from fig 5) which analyses that the crystallinity of the polymer films has a decreasing trend with the increasing intensity. By using a diffractometer with Cu Ka radiation (k= 0.154 nm), X-ray diffraction (XRD) patterns of the samples were recorded in the range 2θ= 4–80°. The spectra were recorded with a 2θ step of 0.02°θ at a scanning rate of 2° θ/min.

3.5 Batch Studies & Optimization

3.5.1 Temperature Study

The polymer films of PVA inoculated with the fungal and bacterial strains were kept under an incubation period of 3 weeks at varied temperatures: 25°C, 35°C and 45°C to obtain the best temperature for the growth of microbes.

3.5.2 pH Study

The PVA films obtained from solution casting technique were inoculated with the bacterial and fungal isolates and kept at an incubation period of 3 weeks and analysed for different pH: 1,3,9,11 to obtain the best optimised pH for the microbial growth on the surfaces of PVA films.

3.5.3 Inoculum dose study

The bio-polymer films of PVA inoculated with the fungal and bacterial strains were kept for incubation for 3 weeks in a BOD incubator & shaker with different inoculum concentrations of 1ml, 3 ml, 5 ml and 7 ml per 75 ml respectively.

4. RESULTS AND DISCUSSIONS

4.1 Weight loss study of the Polymer films obtained from casting solution of Polyvinyl Alcohol

The polymer film samples of PVA obtained from the solution casting method in laboratory conditions have been potentially degraded by the microbial strains (bacteria and fungi) and the growth of those microbial isolates have been shown in Fig 5(A, and B). The rate of degradation was studied from the evaluation of percentage of weight loss with respect to the incubation time of 6 weeks from the subsequent graph as a function of biodegradation of the polymer samples with respect to the time calculated in weeks as explained in Fig. 6(A, and B). The PVA polymer films showed a higher % of weight loss by the bacterial strains as compared to the fungal strains. This study reflects a higher weight loss of polymer degraded efficaciously by the bacterial strains of *Vibrio sp* in comparison with the fungal strains of *Aspergillus sp.* This study reflects the weight loss of polymer films of PVA degraded with efficiency by the micro-organism strains of *Vibrio sp* compared with the strains of *Aspergillus sp*. PVA film samples showed a good proportion of weight loss in 6 weeks (25% once degraded by fungi and 38% degraded by bacteria).

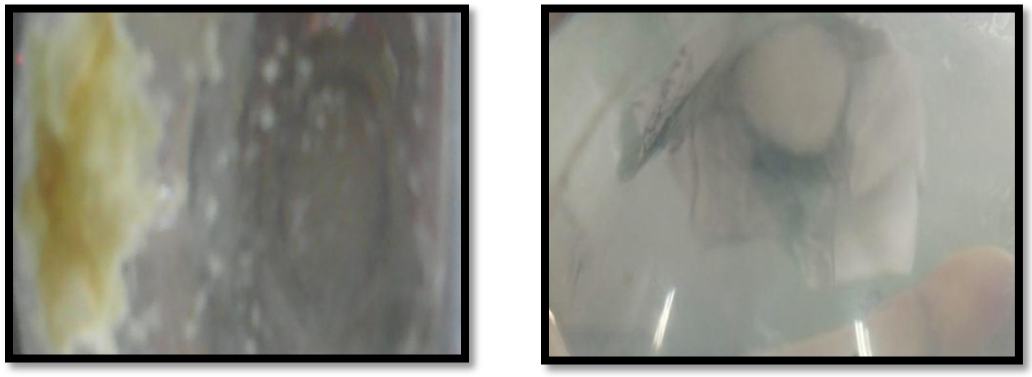


Fig 5(A, B): Colonies of bacteria and fungi on a PVA film.

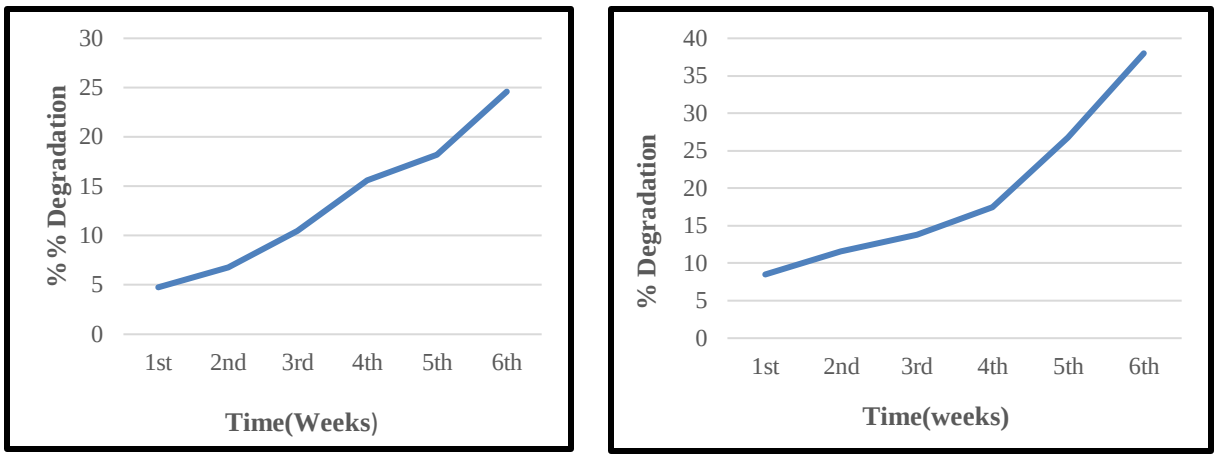


Fig 6(A, B): Weight Loss Study of PVA films by fungal and bacterial strains.

4.2 FTIR Analysis of the PVA films

The Fourier transform infrared radiation analyses the pure polymer films of PVA before and after the degradation. It clearly reveals the major peaks associated with Polyvinyl alcohol (PVA). For instance, As shown in the Fig 7(A, B, C), the main properties of the spectral bonds of PVA are:

It is observed that the band of aldehyde group (ν =1740-1720 cm^{-1}), C–H broad alkyl stretching band (ν = 2850-3000 cm-1) and typical strong hydroxyl bands for free alcohol (nonbonded –OH stretching band at ν = 3600-3650 cm-1), and hydrogen bonded band (ν = 3200-3570 cm-1). Intramolecular and intermolecular hydrogen bondings are expected to occur among PVA chains due to high hydrophilic forces. An absorption peak verified at (ν = 1142 cm^{-1}), has been used for assessing PVA structure as it is a semi-crystalline synthetic polymer able to form some domains depending on several process parameters. The chemical shift determines the orientation and the structure of a plastic polymer. The FTIR data analysis shows that the PVA film sample showed no change for the peaks attributed to -OCH_3 group. It can be observed that two important peaks at (ν = 2860 and 2730 cm^{-1}), C–H stretching are related to aldehydes, a duplet absorption with peaks attributed to the alkyl chain. By crosslinking PVA with GA the O-H stretching vibration peak (ν = 3330-3350 cm-1) was decreased when compared to pure PVA (Fig 7). This result suggests that the hydrogen bonding becomes weaker in crosslinked PVA than in pure PVA because of the diminution in the number of OH groups. The relative increase of the C=O band at approximately ν = 1720 cm-1 indicates that the aldehyde groups of GA did not completely react

with O-H groups of PVA chain. In addition, the C-O stretching at approximately 1100 cm-1 in pure PVA is replaced by a broader absorption band (from ν = 1000 to 1140 cm-1), which can be attributed to the ether. This revealed that no degradation of this group occurred during biodegradation. The sample before degradation showed that the same peaks in wavenumber 250 cm^{-1} represented the same -OH group. The bonds at 769 and 806 cm^{-1} shows the ester stretching of the pet before degradation and after degradation the C=O stretching started getting disappeared, but the C-H stretch got intensified more in both the polymer samples.

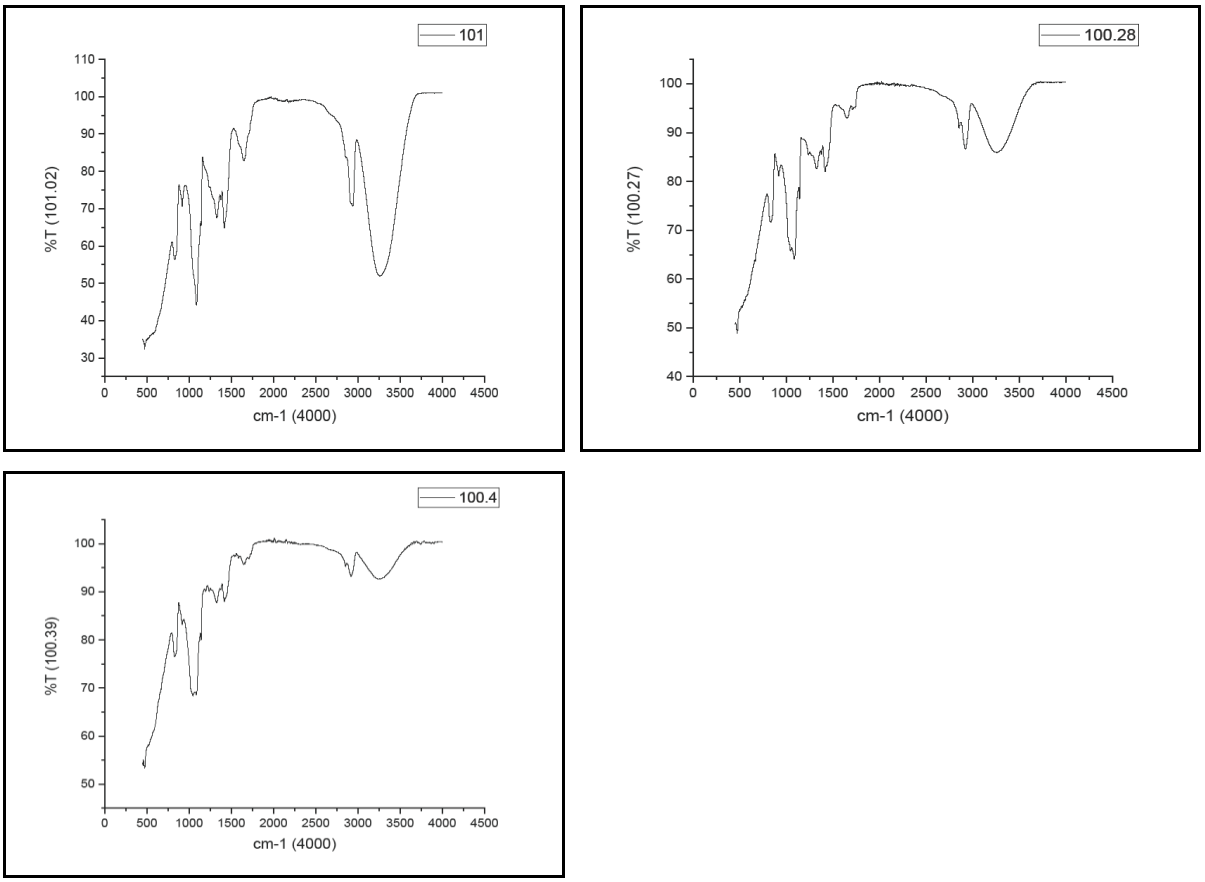


Fig 7(A, B, C): FTIR of PVA film sample before and after degradation by bacterial and fungal strains.

4.3 SEM Analysis of the degraded polymer film surfaces fixed with glutaraldehyde

After the comparison of bacterial and fungal strains, we can notice that bacterial strains of *Vibrio sp* from Bakkhali shows a better degradation as compared to fungal strains of *Aspergillus sp* from Sunderban area. Hence, we performed the SEM (Scanning Electron Microscopy) analysis for both the strains.

SEM analysis allows to examine different changes in the morphological structures of the material at small scale. In order to examine the changes in the structure of the samples undergoing degradation, images from the SEM were used. Surface morphology by scanning electron microscopy was determined. This experiment determines the images of both the polymer samples without undergoing the degradation and the samples which underwent the biodegradation process. SEM analysis exhibited the microbial activity of degradation on the polymer samples (Fig 8: A, B, C, D). The growth of the microorganism by the degradation process was clearly visualized under SEM. The surface of plastic structure of the samples after the degradation process had lost its smoothness, and cracks were evident as compared to the samples without degradation process which is smooth, and no cracks or holes can be seen. The samples showed a significant change in the structures. SEM images confirmed the biodegradation process of the polymer samples.

Fig 8 represents SEM images of the different surfaces of the PVA polymer films after and before degradation. The films before degradation shows a smoother surface compared to the films after degradation which shows a rougher surface. For further study and identification of biodegradation behaviour of the polymer films, SEM analysis of the films with glutaraldehyde solution was carried out.

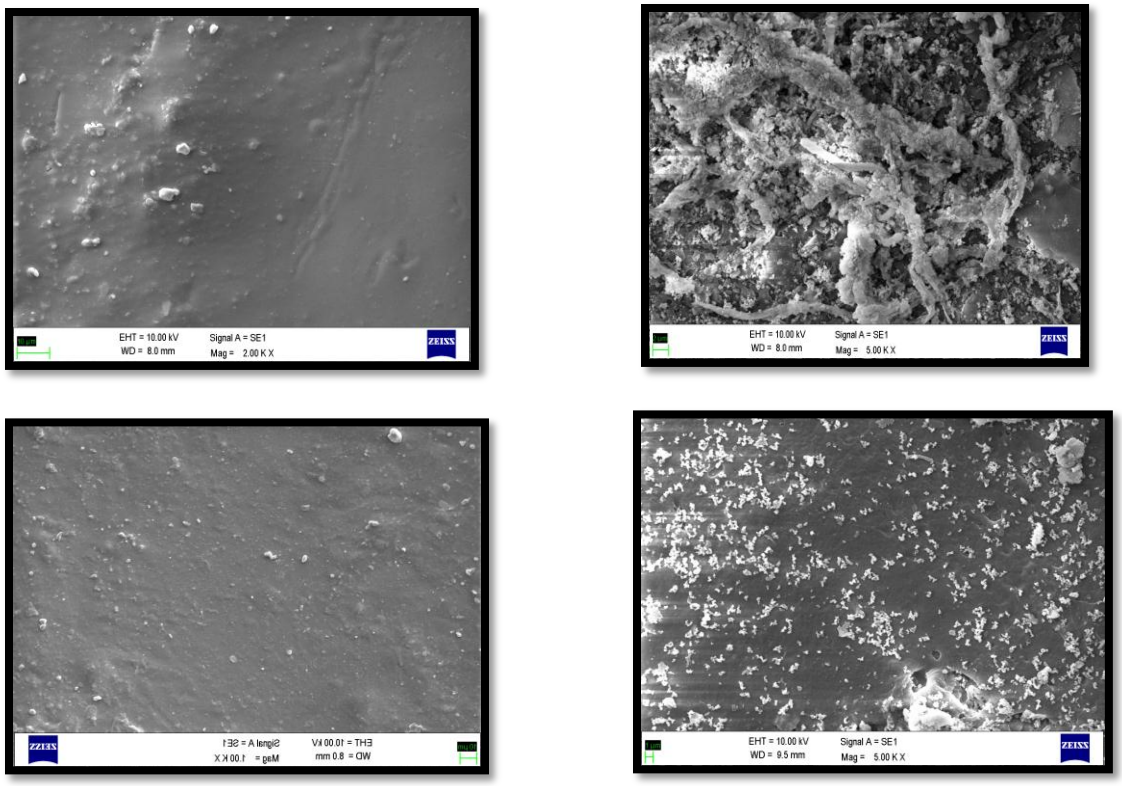


Fig 8 (A, B, C, D): SEM images of PVA before and after degradation by fungal and bacterial colonies.

4.4 XRD Analysis

This study was performed to obtain the crystallinity of the polymer films of PVA by using an x-ray diffractometer (at room temperature). The crystal structure of the polymer films has been determined by the interpretation of the X-Ray diffraction patterns given by the films. A typical diffractogram obtained for the present work by the polymer film is shown in the Fig (9). Based on Fig 9, the intensity for the crystallinity of the sample is found to increase with the increasing trend of the temperature measured at 2θ.

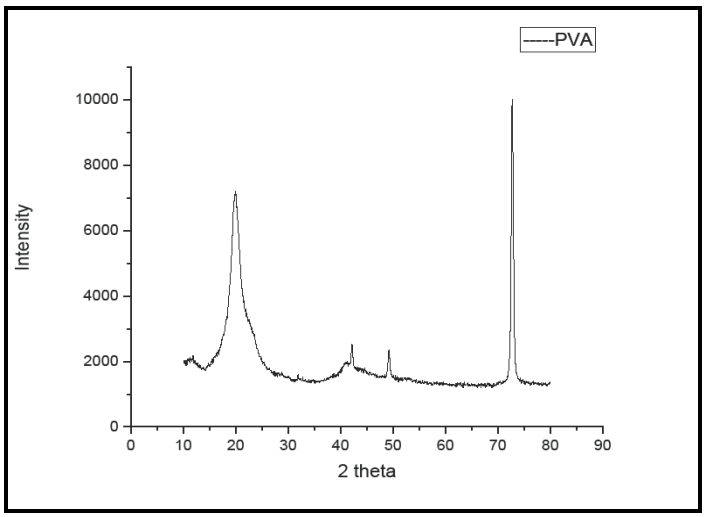


Fig 9: XRD Diffractogram of PVA sample

4.5 Analysis of Different Batch Studies

4.5.1 Effect of temperature on the PVA polymer films

The temperature plays a necessary role for waste management as it has effects on the microbial growth. The impact of temperature variation in degradation rate was analyzed at temperatures from 25ºC - 45ºC with intervals of 10ºC. The polymer films of Polyvinyl Alcohol (PVA) with the bacterial and fungal strains were kept under an incubation period of 3 weeks at varied temperatures: 25°C, 35°C and 45°C to analyse the best and optimised temperature for the growth of micro-organisms on the polymer films. Normally, microbes grow best at 35ºC which is depicted by the graph represented in (Fig 10). Fig 10 gives the complete analysis of the experiment at various temperatures. 35°C has shown the better rate of degradation as compared to 25°C and 45º C. Comparing results with bacteria and fungi, it evaluates that bacterial degradation has shown a better result as compared to fungal.

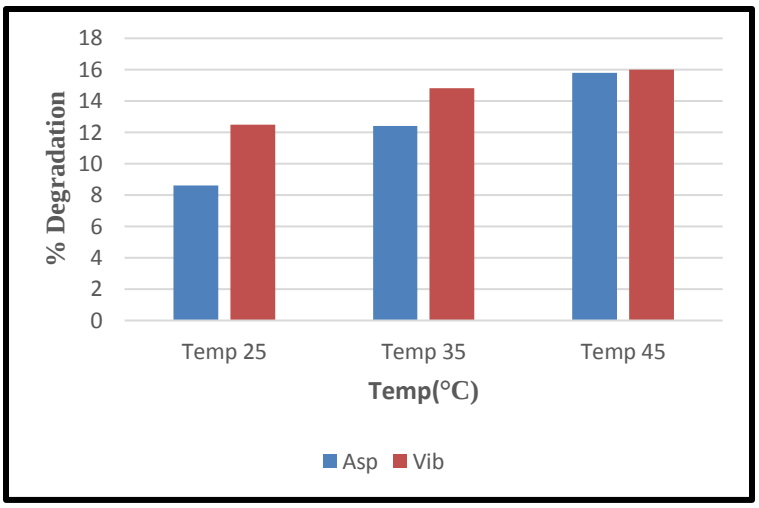


Fig 10: % Degradation of PVA films by the bacterial and fungal strain at different temperatures.

4.5.2 Effect of pH on the PVA polymer films by the microbial strains

Degradation of polymer films of Polyvinyl Alcohol, or PVA using microbial strains of bacteria *(Vibrio sp)* and fungus *(Aspergillus sp)* isolated from the marine sources near Bay of Bengal was used for the purpose of pH. pH plays a very important role in biodegradation as it is a crucial environment factor for the growth of microbes since it analyses the hydrogen ion concentration. Effect of pH on polymer degradation was evaluated at pH 1, 3, 9, and 11 after incubating the media containing polymer films for 3 weeks. In general fungi prefer more acidic conditions, whereas bacteria grow in a neutral to alkaline environment. Thus, this study assessed the effect of pH on PVA degradation at pH value that is highly alkaline. Thus, subsequent graph on effect of pH on PVA biodegradation is shown in (Fig 11). Based on Fig 11, degradation shows increasing trend as pH increase. The degradation rate evaluated that the best results of microbial growth shows at alkaline pH.

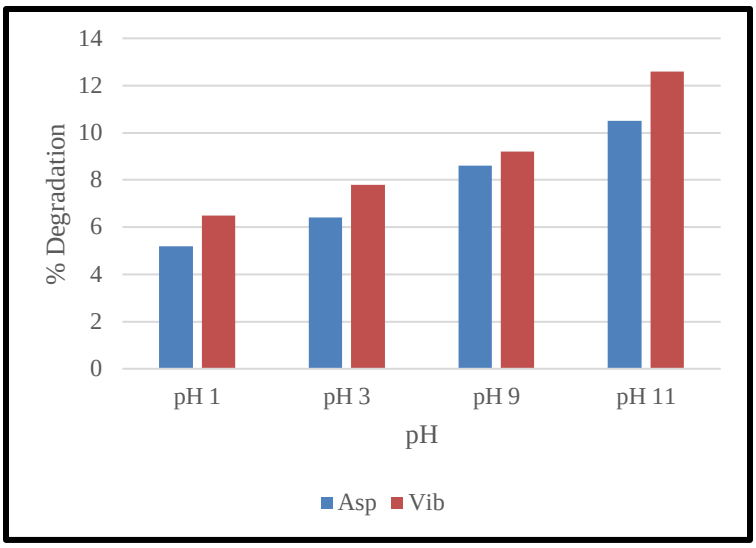


Fig 11: Degradation of PVA polymer films by fungal and bacterial strain at different pH concentrations.

4.5.3 Effect of inoculum dose on the PVA polymer films

The volume of inoculum concentration plays an effective role in the perishable system of biodegradation for the growth of micro-organisms. Sometimes, small inoculum dosage may be deficient to degrade the polymers, while too large inoculum dosage leads to poor degradation of the polymer. Therefore, appropriate and acceptable inoculum amount is vital for the set-up of biodegradation systems.The plastic polymer films of bottle waste inoculated with the different bacterial strains (*Vibrio sp)* and fungal strains *(Aspergillus sp)* and kept for 2 weeks in a BOD incubator with different inoculum concentrations of 1ml, 3 ml, 5 ml and 7 ml per 75 ml respectively. Hence, based on Fig 12, it is evaluated that bacterial strains have shown the higher inoculum dosage concentrations with respect to fungal strains and the inoculum dosage concentrations are directly associated with the degradation rate. The rate of degradation shows an increasing trend with the increase of inoculum dosage concentration.

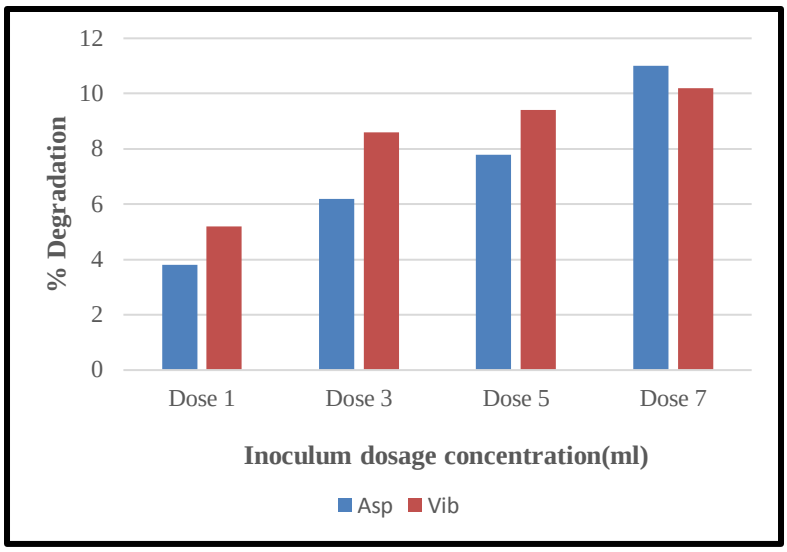


Fig 12: Rate of degradation of PVA films by bacterial and fungal strains at different inoculum dosage concentrations.

5. CONCLUSION

Biodegradation of the polymer films from polymerization of Polyvinyl Alcohol (PVA) in laboratory conditions, obtained by solution casting method has been assessed. The present study deals with the influence of microorganisms (Bacterial strains of *Vibrio sp* from Bakkhali (GenBank accession no: KY941137.1, strain PD6) and Fungal strains of *Aspergillus sp*) (GenBank accession no: MH119104.1) on PVA polymer films and its impact on degradation. The degradation has been evaluated by different characterizations: Weight Loss method, FTIR, XRD and SEM analysis. The results from the weight loss analysis indicates that the rate of degradation by PVA film strips by bacterial strains is more as compared to the degradation by fungal strains (the sample lost its weight by 25% by fungal strains and by bacterial strains lost 38% of its weight) in 6 weeks. Different batch studies by varying temperature, pH, and inoculum dose as well as spectroscopic analysis (SEM, FTIR) has played an important role for the biodegradation process of the polymer films from Plastic bottle commonly can be called as PET. The surface profile of plastic polymer film after 6 weeks by both the microorganisms, observed under Scanning Electron Microscopy (SEM) showed the growth of microbes on the film surfaces, also the erosion and damage to the surface of the polymer PVA film. This bacterium and fungus have the potential to be used for processing plastic waste in the future to reduce environmental damage. Different batch studies like analysis of temperature, pH and concentration of inoculum dose, performed also proved that the effect of bacterial strains on the degradation of plastic films is higher than that of fungal strains(Temp study: 15% by fungal strains, 16.5% by bacterial strains; pH study: 10.2% by fungal strains, 12.6% by bacterial strains; Inoculum dosage: 9.8% by fungal strains and 11.5% by bacterial strains. Thus, the methods show that microbial degradation is an efficient way for different plastic polymers.

REFERENCES

1. Singh G, Singh A.K, Bhatt K, (December 2015), "**Biodegradation of Polyethene's By Bacteria Isolated from Soil",** (International Journal of Research and Development in Pharmacy and Life Sciences) 5**,** (2056-2062).

2. Sangale M.K, Shahnawaz M and Ade A, (2012), **"A Review on Biodegradation of Polythene: The Microbial Approach",** (Journal of Bioremediation and Biodegradation)10**,** (2155-6199).

3. Das Kumar S, (February 2015), **"An Approach to Low density Polyethylene Biodegradation by *Bacillus amyloloquefaciens*",** (Journal 3 Biotech) 5, (81-86).

4.Pandey P.K, Kass P, Soupir M. L, Biswas S, Singh V P, (May 2014), "**Contamination of Water resources By Pathogenic bacteria**", (International Journal of Biotechnology) 10, (451-568).

5. Ali S, Fariha Abdul H, Safia A**,** (January 2012), **"Biological Degradation of plastics: a comprehensive review**", (Journal of Biotechnology Advances) 26, (246-65).

6. Ghosh S K, Pal S, Ray S, (April 2013), **"Study of microbes having potentiability for biodegradation of plastics",** (International Journal of Environmental Science and Pollution), 20**,** (4339-4355).

7. Sain S, Sengupta S, Kar A, Mukhopadhyay A, Sengupta S, Kar Ray D, (January 2014), **"Effect of modified cellulose fibres on the biodegradation behaviour of in-situ formed PMMA/cellulose composites in soil environment: Isolation and identification of the composite degrading fungus** ", (Polymer Degradation and Stability), 9, (156-165).

8. La Mantia F.P., Morreale M, (January 2011) **"Green composites: A brief review",** (Composites), 42, (579-588).

9. N.A. Mostafa, Awatef A. Farag, Hala M. Abo-dief, Aghareed M. Tayeb, (2018), **"Production of biodegradable plastic from agricultural wastes",** (Arabian Journal of Chemistry), 11, (546-553).

10. Maiti S, Sain S, Ray D, Mitra D (November 2013), **"Biodegradation behaviour of PMMA/cellulose nanocomposites prepared by in-situ polymerization and ex-situ dispersion methods",** (Polymer Degradation and Stability), 98, (635-642).

11. Mohanty AK, Misra M, Drzal LT (2002**)," Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world**", (Journal of Polymers and the Environment), 10, (19-26).

12. Huang X, Brittain WJ (2001), **"Synthesis and characterization of PMMA nanocomposites by suspension and emulsion polymerization"**, (Macromolecules), 3, (3255-3260).

13. Sahoo PK, Samal R (2007), **"Fire retardancy and biodegradability of polymethylmethacrylate /montmorillonite nanocomposite"**, (Polymer Degradation and Stability) ,92, (1700-1707).

14. Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. and Uppara, P. V. (2008). "**Biodegradation of polyethylene and polypropylene"**, (Indian Journal of Biotechnology), 7, (9-22).

15. Augusta, J., Muller, R. and Widdecke, H. (1993). "**A rapid evaluation plate-test for the biodegradability of plastics",** (Applied Microbiologyand Technology), 39, (673-678).

16. Balasubramanian, V., Natarajan, K., Hemambika, B.,Ramesh, N., Sumathi, C. S., Kottaimuthu, R. and Rajesh Kannan, V. (2010). "**High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of gulf of Mannar, India"**, (Letters in Applied Microbiology), 51, (205-211).

17. Pranamuda, H., Tokiwa, Y. and Tanaka, H. (1995). "**Microbial degradation of an aliphatic polyester with a high melting point, poly (Tetramethylene Succinate)"**, (Applied and Environmental Microbiology), 61(5), (1828-1832).

18. Pranamuda, H., Tokiwa, Y. and Tanaka, H. (1997), "**Polylactide degradation by an amycolatopsis sp"**, (Applied and Environmental Microbiology), 63(4), (1637-1640).

19. Satlewal, A., Soni, R., Zaidi, M., Shouche, Y. and Goel, R. (2008). "**Comparative biodegradation of HDPE and LDPE using and indigenously developed microbial consortium"**, (Journal of Microbiology and Biotechnology), 18 (3), (477-482).

CHAPTER 3

BIODEGRADATION STUDY OF A BIOPOLYMER PVA COMPOSITE FILMS REINFORCED WITH NANOCELLULOSE

1.INTRODUCTION

1.1 Natural fibers

Natural fibers have grabbed the eye of numerous in light of the fact that they are inexhaustible and liberally accessible assets. They have been considered as an elective material to supplant the inorganic fillers and strands because of genuine natural issues. Natural fibers are normally naturally occuring polymers in the environment which can be available in grasses, leaves, stalks of plants or even creatures. In the composite business, they are normally alluded to as plant filaments and further classified into wood or non-wood sources (Adane et al, 2010). They are likewise alluded to as lignocellulose filaments since lignin and cellulose are the primary parts in their structure.

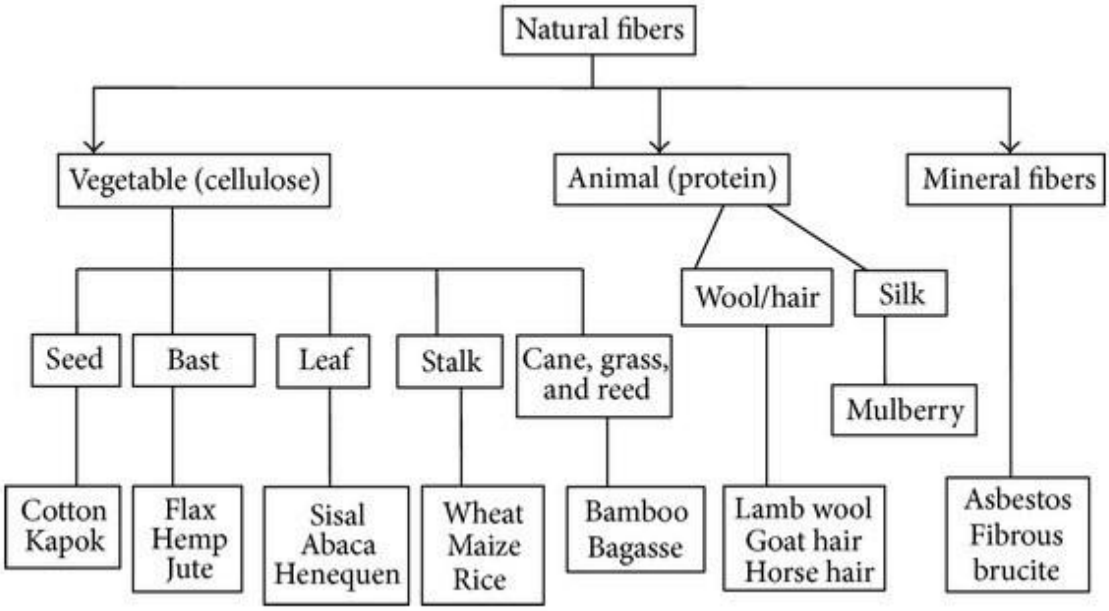


Fig 1: Schematic representation of types of natural fibers

Fig 2: Natural fibers like jute, sugarcane, cotton, wool

1.2 Agro-wastes

Other than the natural fibers, agricultural or Agro-wastes, for example, rice husks, squanders from elastic plants, cocoa development, cultivation of sugarcane and oil palm development have additionally been considered. In Malaysia, the oil palm industry is the greatest biomass maker. It was evaluated a sum of 17 million tons of void organic product groups squander were created every year. The losses from palm oil (overabundance fiber, void organic product clusters and shell) have been used nearby to give vitality to the plant and power fares to the framework. For low-weight frameworks with an accepted change rate of 2.5 kg of palm oil squander per kW/hr, possibly 7000 GW/hr could be created (Azlina et al,2006). The Malaysian government emphatically advances the employments of palm diesel as a swap for non-renewable energy source. The biofuel strategy system has been drafted by the administration to energize the utilization of biofuels.

Advancing the utilization of Agro-wastes in composites may tackle some portion of the worldwide farming reject issue. Enormous heaps of bio agricultural wastes could be changed over into reasonable value-added items which enable them to be increasingly important for more extensive applications. In addition, they are sustainable, shabby, totally or incompletely recyclable, and biodegradable. In car businesses, the assortment of bio-based car parts at present underway is bewildering. For instance, DaimlerChrysler is the greatest defender with up to 50 parts in its European vehicles being delivered from bio-based materials. The properties of some natural fibers are firmly identified with the idea of cellulose and its crystalline properties. For instance, filaments with higher cellulose content have noteworthy explicit mechanical properties yet in addition will in general be more combustible than those with higher hemicellulose content.

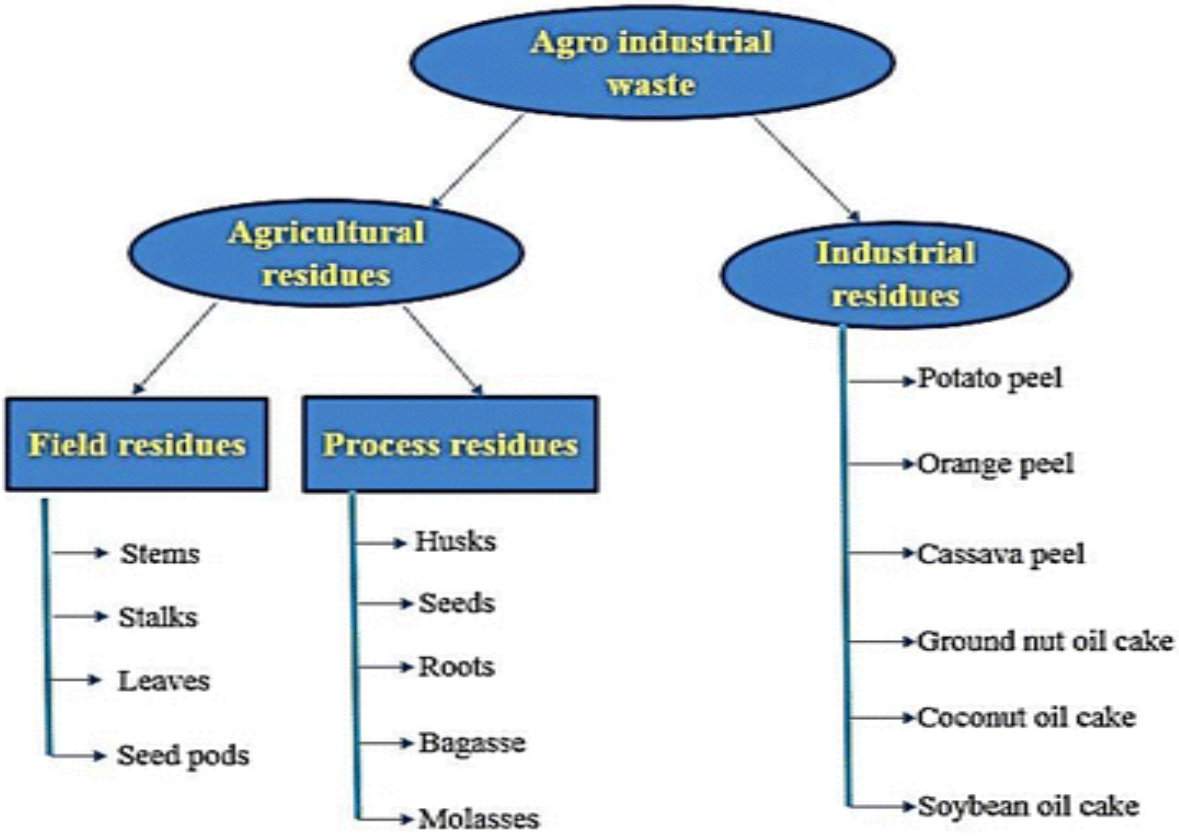


Fig 3: Types of Agro-wastes

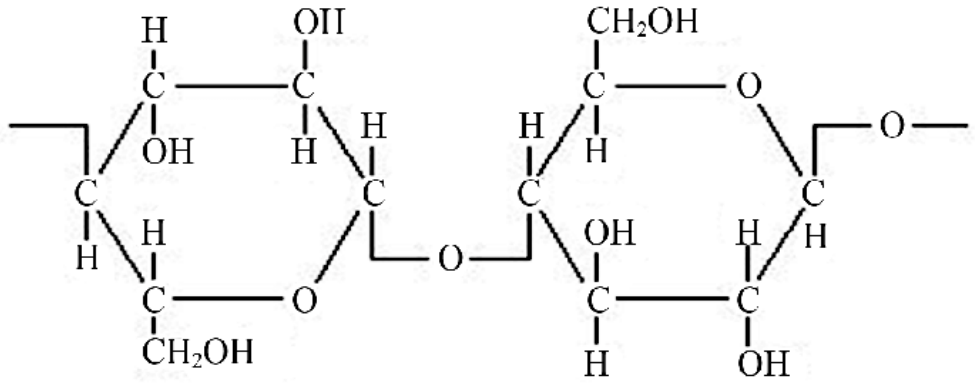
1.3 Reinforcement of PVA with cellulose making a biopolymer

For amendment and reinforcement purposes, cellulose is extracted from the natural fibers and used to create composites because of its progressive structure and semi-crystalline nature (Dufrene et al2013). In spite of their points of interest, the real test of normal filaments is the trouble fabricating them into the ideal structure or film since they can't be softened or disintegrated in a typical dissolvable because of the solid intermolecular hydrogen holding, high level of polymerization, and high crystallinity degree(Chang et al, 2015). Related to the totally green ecological strategy, it is desirable over include the characteristic strands in biodegradable polymers, for example, polyvinyl liquor (PVA) to create eco-manageable composites.

PVA is a most attractive polymer amongst the most encouraging instances of biodegradable network polymers utilized in mulch films. These polymers have great potential as biodegradable

networks in condition agreeable composites, in contrast with carbon filaments composites or any non-biodegradable, recyclable fillers. PVA is generally utilized in agrarian mulch film or biodegradable bundling. For some inventive and ecologically cognizant makers, composite comprises of PVA, a biopolymer, with characteristic filaments, that will additionally improve PVA biodegradability and physical properties, is decision of eco-reasonable materials.

In contrast to the greater part of the polymers, PVA can't experience polymerization from its very own monomer, be that as it may, through polyvinyl acetic acid derivation (PVAc) because of the unsteadiness of vinyl monomer. Consequently, PVA can just be acquired through saponification procedure from PVAc or alcoholysis by responding PVAc with methanol (Ghosh et al, 2013). PVA can be hydrolyzed from PVAc into two unique evaluations, completely or halfway hydrolyzed dependent on their applications. The level of hydrolysis shows the quantity of lingering acetic acid derivation bunches that are available in the polymer in which saponification has not taken place (Goldsmith et al). The level of hydrolysis will in the long run influence the properties of PVA including its solubility (Ching et al). The structure of cellulose is as per the following:



Cellulose

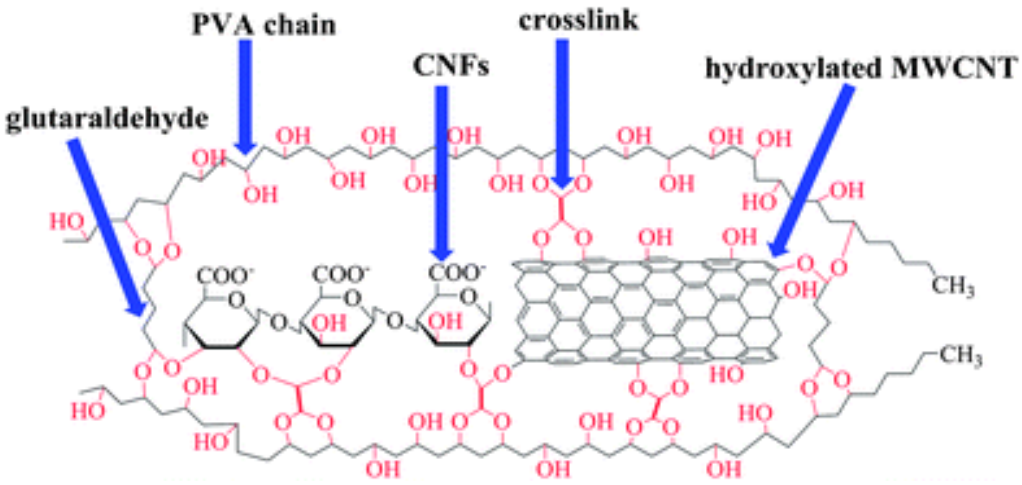


Fig 4(A, B): Structure of Cellulose and structure of PVA With Cellulose

Physical Properties of Cellulose	
Density	1.5 g/cm^3
Melting Point	260–270 °C
Solubility	Water soluble

Table 1: Properties of Cellulose

1.4 Preparation of PVA with nanocellulose fibrillated biocomposites

The preparation method for nanocellulose fortified PVA are generally arrangement throwing, in situ polymerization or electrospinning. (Peresin et al) created CNC (from ramie filaments) fortified PVA nanofibers in composite mats by means of electrospinning technique. The most extreme breadth of electro spun filaments is around 290 nm. Peresin et al. announced a 3 folds increment away modulus of PVA with the expansion of CNCs. Generally speaking, the smooth nonwoven mats with homogeneous nanofibers were acquired. The solid linkage between CNC stage and PVA continual stage was illustrated. The arrangement cast PVA/CNC composites by Gonzalez et al was explained. likewise indicated improved mechanical properties and obstruction properties, attributable to the predominant support property of CNC, well scattering and conveyance of CNC in PVA and phenomenal attachment between them. The ideal fixation in

PVA composites is 3 wt % of CNC. Their brilliant boundary properties against bacterial infiltration propose their reasonableness as twisted dressing to shield the injury from disease and in this manner quicken the recuperating procedure.

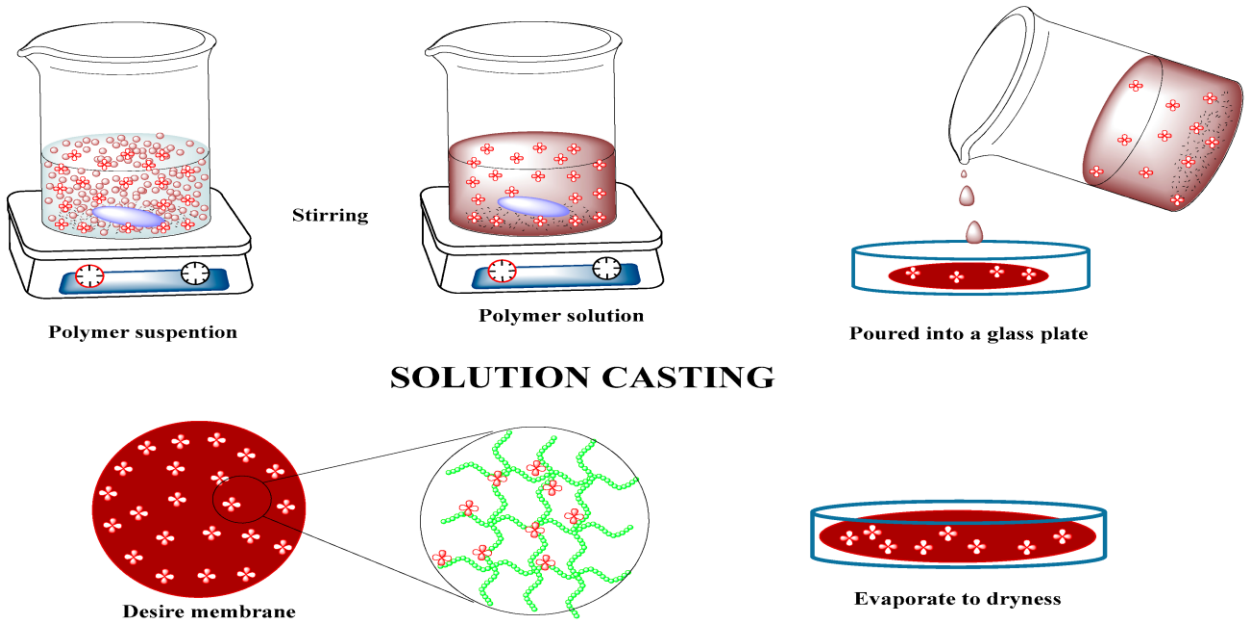


Fig 5: Solution casting method of PVA with cellulose

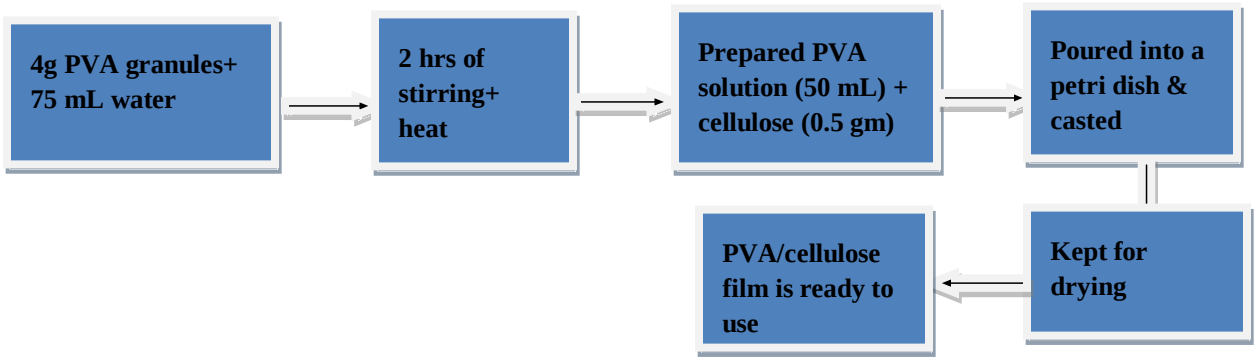


Fig 6: Schematic representation for the Preparation of PVA/cellulose film

The capacity of bonding between the polymer matrix and reinforced material is one of the key factors in choosing the properties of a composite material (Baker et al). The composites dependent on PVA and cellulose is likely to create materials with fantastic mechanical properties since the two materials are polar polymer. Be that as it may, direct consolidation of immaculate cellulose filaments into a PVA lattice may not get a composite with great mechanical properties, despite their similarity. This is on the grounds that most of the hydroxyl bonds in cellulose atoms have effectively shaped either intra-or between sub-atomic hydrogen bonds inside one another. Consequently, they are not ready to shape solid connection with PVA, prompting unacceptable mechanical execution of the composites (Ali et al).

One of the potential ways to deal with further reinforce the properties of composite is cross-linking the structure of the polymers with the desired materials to build their similarity. Cross-connecting is perceived as a marvel where a multifunctional compound is responded to the hydroxyl bonds in PVA, shaping the system through synthetic bonds. The impact of cross-connecting specialists has been accounted for widely.

1.5 Biodegradability of PVA/c bio composites

It is realized that PVA highly degrades when presented to humid atmosphere. In any case, Chiellini et al pointed out that PVA could be biodegraded by specific microorganisms. Such PVA-corrupting microorganisms are restricted (55 species including microscopic organisms, parasites, yeast and form) contrasted with polyesters family, for example, polylactic corrosive (PLA), polyhydoxyalkaoates (PHA), etc. For a material to biodegrade, there must be presence of some comparative parts between the principle chain of polymer and normal happening substances (Kumar et al). It is recommended that PVA should mix with other biodegradable materials to additionally upgrade its biodegradability. Regular filaments contain cellulose, hemicellulose and lignin, yet in addition contain different segments including pectin's, oil and waxes. The normal strands are defenseless to biodegradation in an outside domain relying upon the natural conditions (dampness, temperature and UV-radiation) and microbial exercises (Moo et al). It is imperative to think about the biodegradability of PVA/regular fiber composites especially for mulches or manure bearer applications.

2. REVIEW OF LITERATURE

In this paper, the study was on decomposable polymers for sustenance pressing material. The most elevated goal was to introduce components actuating polymer degradation and biodegradation in various situations. It was notable that biodegradable polymers are an insufficient substitute for traditional polymers and its disintegration emphatically relies upon corruption circumstance. It was likewise seen that there are various procedures in various nations to affirmation of biodegradable polymer materials, this is very confusing its application than advances it (***Guzman et al,2011***).

This review found that deterioration of polycyclic sweet-smelling of aromatic compound of hydrogen and carbon from free living microorganisms in soil or water techniques were functional to review design bio-diversities. The dominating free alive species, *Brevundimonas (Pseudomonas) diminuta, Caulobacter species., Mycoplana bullata, Acidovorax species*. Furthermore, Pseudomonas aeruginosa, can be seen by fluorescence in situ hybridization (FISH). (***Chang, et al***).

The biodegradable PVA/natural fibre like cellulose composites are items that are environment friendly, they can be connected in different territories, especially for nourishment binding material and mulch films. PVA is a proficient fastener for strong particles, including colours, artistic materials, concrete based materials, mortar, plug, packed waste items, nonwoven textures, and strips. PVA/pinewood saw dust composites was prepared by hot press moulding for particleboard application. The elasticity, shore hardness, and tractable modulus are influenced by the preparing conditions. The ideal properties were acquired when the composite particleboard was squeezed at 140°C for about 10–12 min. The use of such PVA/wood composites in particleboard may give long haul advantage in foundation from the financial and natural perspective. Packaging materials as a rule request certain obstruction properties just as mechanical opposition. Filaments tend to ingest moisture, oil, and oil from environment. ***(Li, et al).***

In the medical field, PVA based nanocomposites were discovered appropriate for tissue building applications, for example, frameworks, sedate conveyance, restorative embeds, and wound dressing (***Rahman et al.)***.

The likelihood of PVA/nanocellulose as wound dressing, such nanocomposites may respond to coordinate the indigenous habitat dampness of an injury surface and in the long run lead to quickened wound mending. The electro spun PVA/nanocellulose containing stryphnodendron astringents bark separate with improved natural properties may be appropriate for restorative inserts (***Gonzalez et al***).

Exceptionally permeable frameworks with great pore-interconnectivity were created dependent on ovalbumin/PVA strengthened with unmodified and – NH2 adjusted nanocellulose cross-connected with glutaraldehyde by means of stop drying process (***Kumar et al***). Another fascinating use of PVA/cellulose composites is utilized as taste sensor. The layers demonstrated trademark reaction designs for natural acids, mineral acids, salts, severe substances, sweet substances, and umami substances.

Natural fibers acquired from feasible sources or available from Agro-wastes endure an enormous changeability extending from the age and gathering time of plant, atmosphere and geological conditions, variety in preparing techniques, etc. Thus, a conceivable variety in the nature of end products has developed. These finished results may have non-uniform properties relying upon the storage and transport conditions. In this manner, the test falls into material plan where the materials must be basically and practically stable amid capacity however degradable after use. (***Zheng et al***)

When hydrophilic natural fibers and PVA are joined, it is inescapable to consider their poor moisture resistance because of the nearness of hydroxyl bindings. These natural fibers with high moisture take-up will influence the holding among strands and framework which lead to low mechanical properties. The long-haul sturdiness of these composites, especially for load-bearing application, (for example, particleboards), is a noteworthy concern.

Much work has tended to the poor dampness opposition of PVA/common strands and recommended compound alterations or use of specific coatings to decrease their dampness take-

up. As work keeps on improving the composites' properties, it is imperative to assess their impact on the earth since substance reagents are included. Furthermore, high beginning expense for certain strategies ought to be considered (***Rahman et al***)

3. MATERIALS AND METHODS

The materials used for this research study is as follows:

- Sugarcane Bagasse was procured from local markets near Jadavpur for the cellulose extraction.
- Sodium Chlorite, Sodium Sulphate and Concentrated Sulphuric acid were used for cellulose preparation.
- The commonly used polymer Polyvinyl Alcohol (PVA) was synthesized in laboratory. Glutaraldehyde and Ethanol, or Ethyl alcohol obtained from Merck, Germany, was used for the process of fixation of the bacterial isolates on the polymer film surfaces.
- For minimal media preparation, potassium dihydrogen phosphate (KH_2PO_4), sodium nitrate ($NaNO_3$), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), potassium chloride (KCl), ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) and ammonium chloride (NH_4Cl) was procured from Lobachemie, India.
- Composition consists of about 1g/l of potassium di-hydrogen phosphate, 2g/l of sodium nitrate, 0.5 g/l of magnesium sulphate hepta-hydrate, 0.5 g/l of potassium chloride, 0.01g /l of ferrous sulphate and 1 g/l of ammonium chloride.

METHODS:

3.1 Extraction of Sugarcane bagasse fibres

The extracted sugarcane was collected from the local markets near Jadavpur, and then was washed, oven dried or sun dried for 1-2 days, cut into small pieces, grinded and thus the crushed sugarcane bagasse fibres were obtained for the extraction of cellulose.

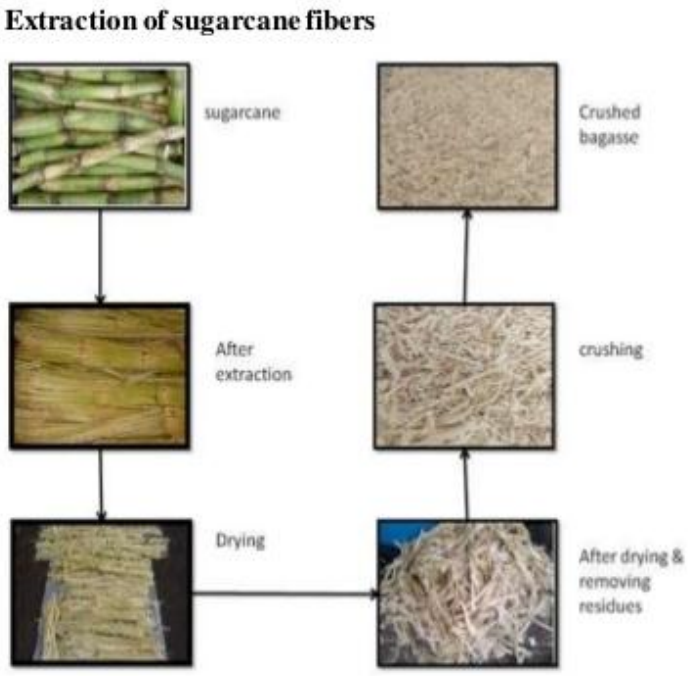


Fig 7: Grinded Sugarcane bagasse fibers from Sugarcane

3.2 Nano-Cellulose preparation from Sugarcane Bagasse

Cellulose Nano-fibers (CF) was prepared by chemical treatment of raw sugarcane bagasse fibers with 0.7% sodium chlorite ($NaClO_2$) solution at pH 4 for continuous stirring of 2 hrs., treated with 2 % sodium sulphate ($NaHSO_3$) for 15 mins, filtered, dried and then treated with 17.5% NaOH solution for 15 mins to remove the lignin fractions from sugarcane bagasse, followed by

acid hydrolysis, 47% H_2SO_4 for constant stirring of 3 hrs.at 50°C. A dry cellulose powder with a mix of micro and Nano- cellulose fibers was obtained after lyophilization/ freeze drying.

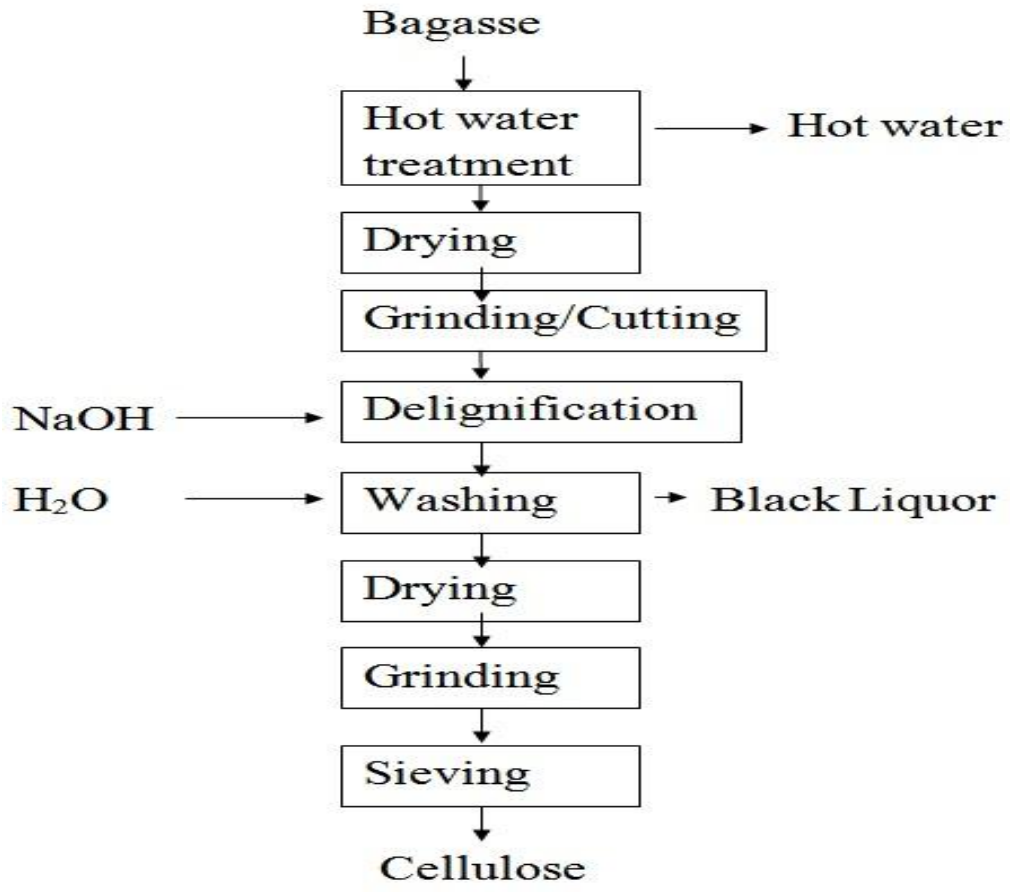


Fig 8: Schematic of Cellulose extraction from sugarcane bagasse fibers

Fig 9: Nanocellulose extraction from Raw sugarcane bagasse fibers

3.3 Preparation of PVA

4 g of PVA granules were dissolved in 75 mL of water by heating at 95°C under moderate stirring for about 2 hrs. After 2 hrs, the PVA solution was casted on a sterile petri dish and then was cut into small uniform pieces to use in the biodegradation process.

3.4 Preparation of PVA/cellulose composites by solution casting method

PVA/cellulose composites were prepared by solution casting method. 0.5 gm of cellulose was taken into 50 mL of PVA and kept continuous stirring for about 2 hrs., was sonicated for 30 mins. The solutions were then casted on separate glass petri dishes of same dimensions to prepare solution cast films.

Fig 10: PVA cellulose composite film

3.5 Comparable biodegradation of PVA/cellulose polymer film using fungal and bacterial strains

Biodegradability of the polymer film samples obtained from the solution casting method using PVA granules and raw sugarcane bagasse was examined by the rate of degradation through different marine microbes, namely different species of identified bacterial and fungal strains: *Vibrio sp* (GenBank accession no: KY941137.1, strain PD6) and *Aspergillus sp*. The bacterial strains were isolated from the marine waters near Southern Bengal near Bakkhali area, whereas the fungal strains were isolated from Sunderban area. The film sample of water soluble,

petroleum-based polymer film of Polyvinyl Alcohol reinforced with cellulose was obtained by the in-situ polymerization technique and then was cut into square sized 1 cm diameter. The polymer films samples were taken out at 1, 2, 3, 4, 5 and 6 weeks for PVA samples respectively, ethanol washed, vacuum dried at room temperature and thus rate of degradation of the samples with respect to time was evaluated as a percentage of weight loss

3.6 Evaluation of Weight loss

The characterization of the polymer films can be calculated by the weight loss study measuring the rate of degradation at different time intervals from the minimal media, PVA film samples were taken out after keeping it for 6 weeks and PVA was taken out after keeping it for 6 weeks at an incubator temperature of about 36-37°C hence calculating the percentage of weight loss by the given equation,

Where, M1 is the initial weight of the polymer samples before biodegradation (before putting it in media) and M2 is the final weight of the polymer samples after biodegradation (after taking out from media) at different time intervals.

$$\%\text{Weight loss} = \{(M1-M2)/M1\} * 100$$

3.7 Characterizations:

3.7.1 SEM Analysis

The fixation of bacterial isolates on the polymer films was done by the following procedure:

2.5% glutaraldehyde was taken followed by 0.1 M PBS (Phosphate buffer saline) solution and dehydration by using varied concentrations of ethanol: 30%, 70% and 100% respectively and the incubation time for the polymer films was 10 mins (Sain et al, 2014) in each of the solution and then finally were dried and examined for SEM analysis (Carl Zeiss, Bangalore). SEM analysis was done to identify the morphological changes in the polymer structures. All the samples were

provided with vacuum and coated with platinum and polymer structures with fixed bacterial strains were observed on the film surfaces.

3.7.2 FTIR study

The plastic polymer films before and after the biodegradation in the minimal media were subjected to FTIR analysis using Perkin Elmer Infrared Spectrophotometer (Spectrum 100).

3.7.3 XRD Analysis

The polymer films obtained from the polymer films of PVA cellulose(PVA/c) before and after the degradation using bacterial and fungal isolates were analyzed through X-ray Diffractometer at 30 kV and 30 mA. The XRD patterns of the sample was obtained (from Fig 15) which analyses that the crystallinity of the polymer films has a decreasing trend with the increasing intensity. By using a diffractometer with Cu Ka radiation (k= 0.154 nm), X-ray diffraction (XRD) patterns of the samples were recorded in the range 2θ= 4–80°. The spectra were recorded with a 2θ step of 0.02°θ at a scanning rate of 2° θ/min.

3.7.4 Testing of Tensile Strength

The tensile strength or Elasticity of a polymer decides the capacity to oppose breaking under pliable pressure (stress). It is amongst the best and broadly estimated properties of polymeric materials utilized for basic applications. The force per unit area (MPa or psi) required to break a polymer in such a way is known as a definitive rigidity or elasticity at break. The rate at which a polymer sample is pulled apart in the assessment reason can run from 0.2 to 20 inches for each moment. The malleable testing machine pulls the sample from the two finishes and measures the power required to pull it separated and investigations the sum the example extends before breaking. The analogous test to perform tensile testing in the ISO framework is ISO 527.

3.8 Different Batch Studies

3.8.1 Temperature study

The polymer films of PVA/c with the bacterial and fungal strains were kept under an incubation period of 3 weeks at varied temperatures: 25°C, 35°C and 45°C.

3.8.2 pH study

The polymer films of PVA/c with the microbial isolates after kept at an incubation period of 2 weeks were studied for different pH: 1,3,9,11.

3.8.3 Inoculum dose study

The polymer films of PVA/c inoculated with the bacterial and fungal strains were kept for incubation for 2 weeks with different inoculum concentrations of 1ml, 3 ml, 5 ml and 7 ml respectively.

4. RESULTS AND DISCUSSIONS

4.1 Weight loss study of the polymer sample of PVA reinforced with a natural fibre cellulose

The polymer film samples of PVA reinforced with cellulose, obtained from the solution casting method in laboratory conditions have been potentially degraded by the microbial strains (bacteria and fungi) and the growth of those microbial isolates on the polymer film samples have been shown in Fig 11(A, and B). The rate of degradation was studied from the evaluation of percentage of reduction of weight with respect to the incubation time of 6 weeks from the subsequent graph as a function of biodegradation of the polymer samples with respect to the time calculated in specific number of weeks as explained in Fig. 12(A, and B). The PVA polymer films showed a higher % of weight loss by the bacterial strains as compared to the fungal strains. This study reflects a higher weight loss of polymer degraded efficaciously by the bacterial strains of *Vibrio sp* in comparison with the fungal strains of *Aspergillus sp.* This study reflects the weight loss of polymer films of PVA degraded with efficiency by the micro-organism strains of *Vibrio sp* compared with the strains of *Aspergillus sp*. PVA film samples showed a good proportion of weight loss in 6 weeks (26% once degraded by fungi and 42 % degraded by bacteria).

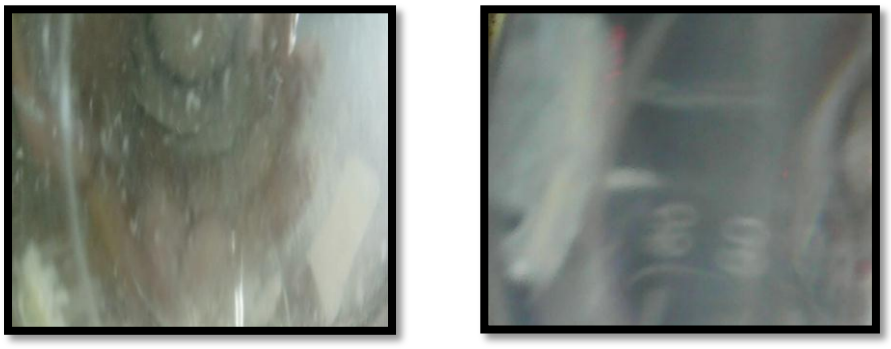


Fig 11(A, B): Growth of Colonies of bacteria and fungi on a PVA/cellulose film.

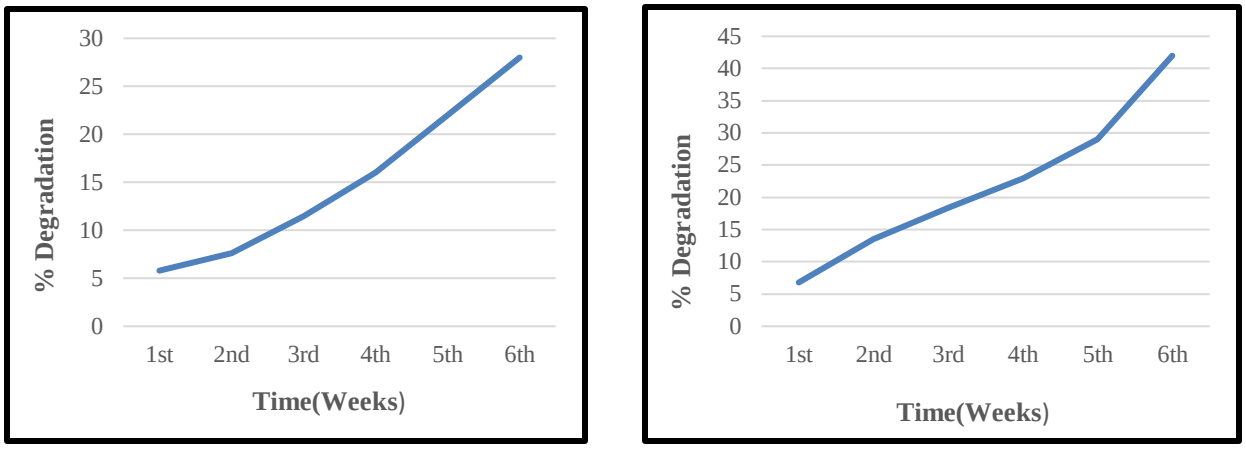


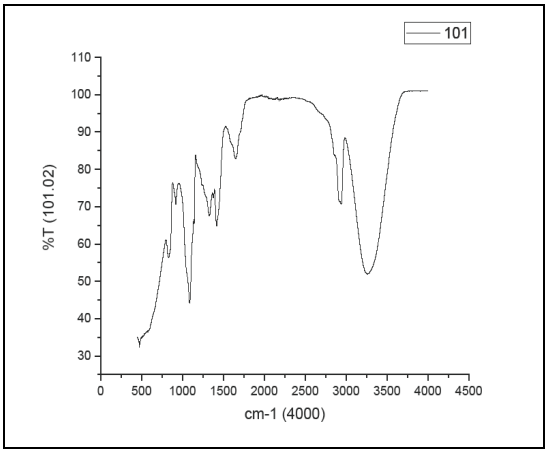
Fig 12(A, B): Reduction of weight representation of a PVA/cellulose film by fungal and bacterial strains.

4.2 FTIR Analysis of the PVA/cellulose films

The Fourier transform infrared radiation analyses the polymer films of plastic bottle waste and PVA before and after the degradation. As shown in the Fig 13(A, B, C), the main properties of the spectral bonds of the polyvinyl alcohol reinforced with a natural fibre cellulose are:

The peak at wavelength 1647 cm-1 (C=C stretching vibration of aromatic ring) in sample was sharp and larger as compared to the control. Similarly, the small peak between wavelength 1956 cm-1 and 2358 cm-1 (corresponding to O-H) disappeared in the sample spectrum, which was present in control (Fig.13). FTIR spectroscopy was used to examine the structural changes in the melt blends of PVA polymer with cellulose (Singh *et al.*, 2004). Biodegradation of PVA reinforced with cellulose brought some structural changes in the FTIR spectra of the polymer.

The FTIR analysis of Cellulose blended PVA film showed that the peak at wavelength 3245 cm^{-1} present in the pure sample, which was absent in the degraded sample. The length of the peak at wavelength 2359 cm-1 (corresponding to O-H) was sharp in pure sample and was broader in the sample attributed to degradation. Similarly, the sharp peak at wavelength at 987.4 cm-1 (corresponding to C-O) in control was broad in sample spectrum showed C-O-C bond stretches (Fig. 13). Our results are in accordance with the studies of (Raman *et al*,2001). The FTIR spectra of poly (vinyl alcohol) showed a sharp decrease in the bands and peaks of the polymer.



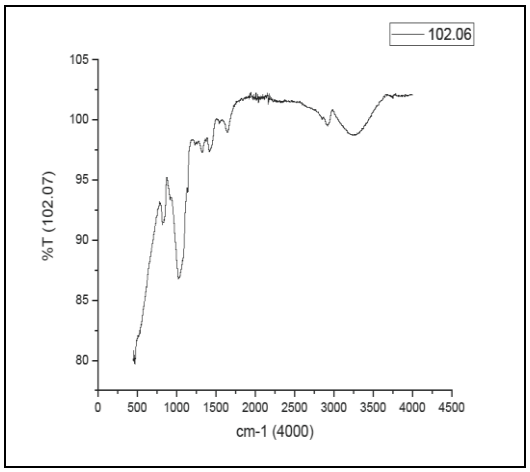


Fig 13(A, B, C): FTIR of PVA film sample before and after degradation by bacterial and fungal strains.

4.3 SEM Analysis of the degraded polymer film surfaces fixed with glutaraldehyde

After the comparison of bacterial and fungal strains, it has been viewed that bacterial strains of *Vibrio sp* from Bakkhali shows a better degradation as compared to fungal strains of *Aspergillus sp* from Sunderban area. Hence, we performed the SEM (Scanning Electron Microscopy) analysis for both the strains for evaluation of changes in composition and structural topography of the samples.

SEM analysis allows to examine different changes in the morphological structures of the material at small scale. In order to examine the changes in the structure of the samples undergoing degradation, images of the polymer surfaces from the SEM were used. Surface morphology by scanning electron microscopy was determined. This experiment determines the images of both the polymer samples of PVA cellulose without undergoing the degradation and the samples which underwent the biodegradation process. SEM analysis exhibited the microbial activity of degradation on the polymer samples of PVA cellulose as depicted in (Fig 14: A, B, C,

D). The growth of the microorganism by the degradation process was clearly visualized under SEM. The surface of polymer structure of the samples after the degradation process had lost its smoothness, and cracks were evident as compared to the samples without degradation process which is smooth, and no cracks or holes can be seen. The polymer samples of PVA cellulose showed a significant change in the structures. SEM images confirmed the biodegradation process of the polymer samples.

Fig 14 represents SEM images of the different surfaces of the polymer films after and before degradation. The films before degradation shows a smoother surface compared to the films after degradation which shows a rougher surface. For further study and identification of biodegradation behaviour of the polymer films, SEM analysis of the films with glutaraldehyde solution was carried out.

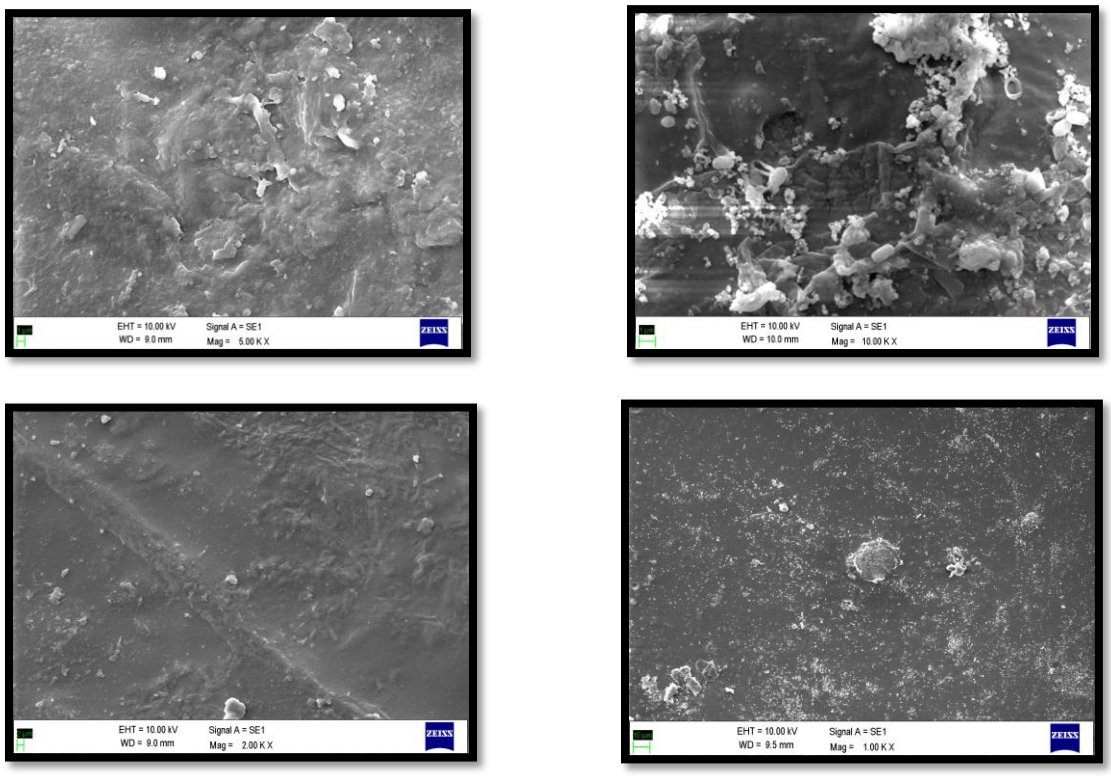


Fig 14 (A, B, C, D): SEM images of PVA/c before and after degradation by fungal and bacterial colonies.

4.4 XRD Analysis

This study was performed to obtain the crystallinity of the polymer films of PVA/c by using an x-ray diffractometer (at room temperature). The crystal structure of the polymer films has been determined by the interpretation of the X-Ray diffraction patterns given by the films. A typical diffractogram obtained for the present work by the polymer film is shown in the Fig (15). Based on Fig 15, the intensity for the crystallinity of the sample is found to increase with the increasing trend of the temperature measured at 2θ.

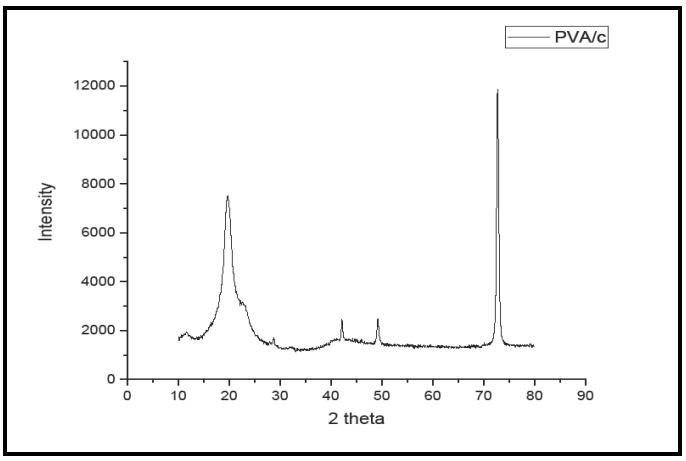


Fig 15: XRD Diffractogram of a PVA biopolymer reinforced with cellulose

4.5 Tensile strength Analysis

The mechanical properties of unmodified PVA polymer and PVA with nanocellulose composite films were performed by a tensile test. The analysis of tensile strength has been done by comparing the two bio-polymer films: one is PVA and the other is PVA reinforced with cellulose. It is noticed that the tensile strength of PVA cellulose is declining as compared to the pure PVA samples because of the biodegradation process. As we reinforce the PVA polymer with a natural fibre of cellulose, the tensile strength of the material decreases.

4.6 Assessment of Different Batch Studies

4.6.1 Effect of temperature on the polymer films

The temperature plays a necessary role for waste management as it has effects on the microbial growth. The impact of temperature variation in degradation rate was analyzed at temperatures from 25ºC - 45ºC with intervals of 10ºC. The polymer films of Polyvinyl Alcohol (PVA) reinforced with natural fibre cellulose, with the bacterial and fungal strains were kept under an incubation period of 3 weeks at varied temperatures: 25°C, 35°C and 45°C to analyse the best and optimised temperature for the growth of micro-organisms on the polymer films. Normally, microbes grow best at 35ºC which is depicted by the graph represented in (Fig 16). Fig 16 gives the complete analysis of the experiment at various temperatures. 35°C has shown the better rate of degradation as compared to 25°C and 45º C. Comparing results with bacteria and fungi, it evaluates that bacterial degradation has shown a better result as compared to fungal.

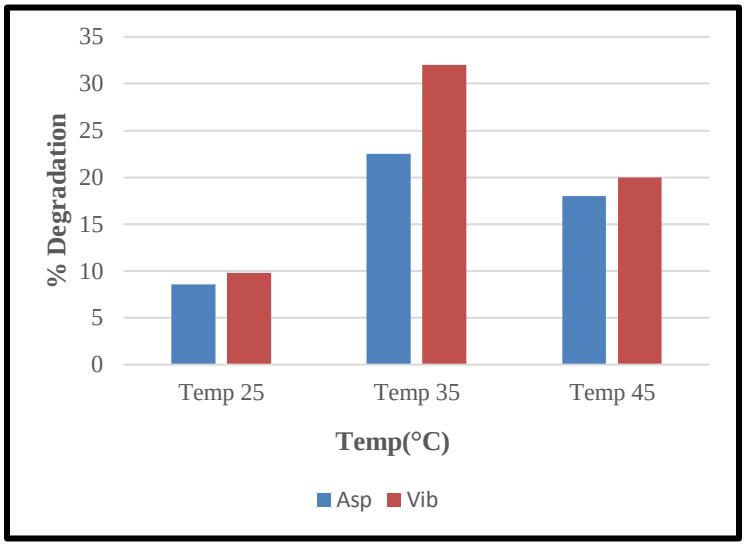


Fig 16: % Degradation of PVA/c biopolymer films by the bacterial and fungal strain at different temperatures.

4.6.2 Effect of pH on the polymer films by the microbial strains

Degradation of polymer films of Polyvinyl Alcohol with reinforcement of cellulose natural fiber, or PVA/c using microbial strains of bacteria *(Vibrio sp)* and fungus *(Aspergillus sp)* isolated from the marine sources near Bay of Bengal was calculated. pH plays a very important role in biodegradation as it is a crucial environment factor for the growth of microbes since it analyses the hydrogen ion concentration. Effect of pH on polymer degradation was evaluated at pH 1, 3, 9, and 11 after incubating the media containing polymer films for 3 weeks. In general fungi prefer more acidic conditions, whereas bacteria grow in a neutral to alkaline environment. Thus, this study assessed the effect of pH on PVA/cellulose degradation at pH value that is highly alkaline. Thus, subsequent graph on effect of pH on PVA/cellulose biodegradation is shown in (Fig 17). Based on Fig 17, degradation shows increasing trend as pH increase. The degradation rate evaluated that the best results of microbial growth shows at alkaline pH.

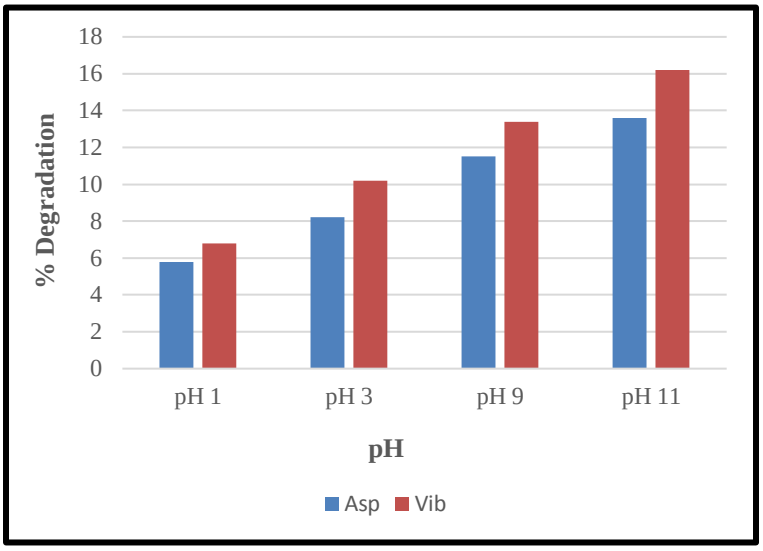


Fig 17: Degradation of PVA/cellulose polymer film by fungal and bacterial strain at different pH concentrations.

4.6.3 Effect of inoculum dose on the polymer films

The volume of inoculum concentration plays an effective role in the perishable system of biodegradation for the growth of micro-organisms. Sometimes, small inoculum dosage may be deficient to degrade the polymers, while too large inoculum dosage leads to poor degradation of the polymer. Therefore, appropriate and acceptable inoculum amount is vital for the set-up of biodegradation systems.The plastic polymer films of bottle waste inoculated with the different bacterial strains (*Vibrio sp)* and fungal strains *(Aspergillus sp)* and kept for 2 weeks in a BOD incubator with different inoculum concentrations of 1ml, 3 ml, 5 ml and 7 ml per 75 ml respectively. Hence, based on Fig 18, it is evaluated that bacterial strains have shown the higher inoculum dosage concentrations with respect to fungal strains and the inoculum dosage concentrations are directly associated with the degradation rate. The rate of degradation shows an increasing trend with the increase of inoculum dosage concentration.

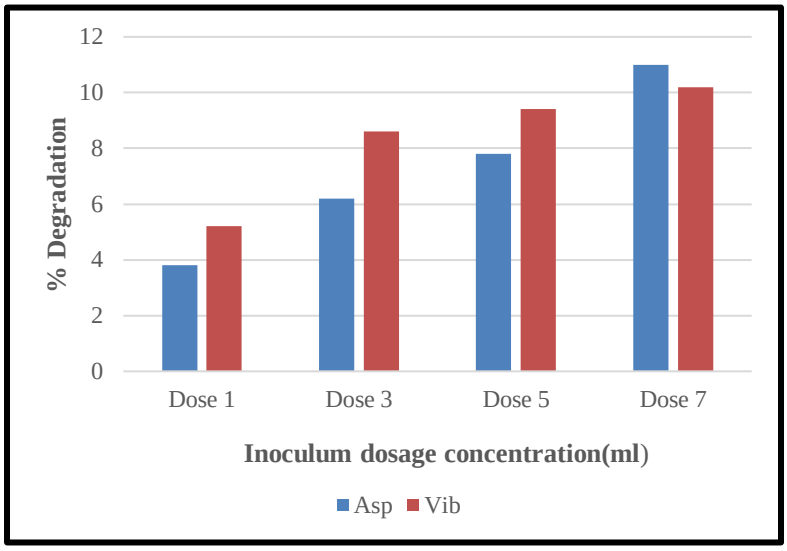


Fig 18: Rate of degradation by bacterial and fungal strains at different inoculum dosage concentrations

5. CONCLUSION

Biodegradation of the polymer films of PVA (Polyvinyl Alcohol) reinforced with a natural fiber cellulose prepared by the solution casting method, and further ultra-sonification in laboratory conditions was assessed. The present study deals with the influence of microorganisms (Bacterial strains of *Vibrio sp* from Bakkhali (GenBank accession no: KY941137.1, strain PD6) and Fungal strains of *Aspergillus sp*) (GenBank accession no: MH119104.1) on Plastic bottle films and its impact on degradation. The degradation has been evaluated by different characterizations: Weight Loss method, FTIR, XRD and SEM analysis. The results from the weight loss analysis indicates that the rate of degradation by Plastic bottle film strips by bacterial strains is more as compared to the degradation by fungal strains (the sample lost its weight by 22 % by fungal strains and by bacterial strains lost 35% of its weight) in 6 weeks. Different batch studies by varying temperature, pH, and inoculum dose as well as spectroscopic analysis (SEM, FTIR) has played an important role for the biodegradation process of the polymer films from Plastic bottle commonly can be called as PET. The surface profile of plastic polymer film after 6 weeks by both the microorganisms, observed under Scanning Electron Microscopy (SEM) showed the growth of microbes on the film surfaces, also the erosion and damage to the surface of the plastic film. This bacterium and fungus have the potential to be used for processing plastic waste in the future to reduce environmental damage. Different batch studies like analysis of temperature, pH and concentration of inoculum dose, performed also proved that the effect of bacterial strains on the degradation of plastic films is higher than that of fungal strains(Temp study: 9.8% by fungal strains, 11% by bacterial strains; pH study: 32% by fungal strains, 38% by bacterial strains; Inoculum dosage: 12.3 % by fungal strains and 13.6% by bacterial strains. Thus, the methods show that microbial degradation is an efficient way for different plastic polymers.

REFERENCES

1. Singh G, Singh A.K, Bhatt K, (December 2015), "**Biodegradation of Polyethene's By Bacteria Isolated from Soil",** (International Journal of Research and Development in Pharmacy and Life Sciences) 5**,** (2056-2062).

2. Sangale M.K, Shahnawaz M and Ade A, (2012), **"A Review on Biodegradation of Polythene: The Microbial Approach",** (Journal of Bioremediation and Biodegradation)10**,** (2155-6199).

3. Das Kumar S, (February 2015), **"An Approach to Low density Polyethylene Biodegradation by *Bacillus amyloloquefaciens*",** (Journal 3 Biotech) 5, (81-86).

4.Pandey P.K, Kass P, Soupir M. L, Biswas S, Singh V P, (May 2014), "**Contamination of Water resources By Pathogenic bacteria**", (International Journal of Biotechnology) 10, (451-568).

5. Ali S, Fariha Abdul H, Safia A**,** (January 2012), **"Biological Degradation of plastics: a comprehensive review**", (Journal of Biotechnology Advances) 26, (246-65).

6. Ghosh S K, Pal S, Ray S, (April 2013), **"Study of microbes having potentiability for biodegradation of plastics",** (International Journal of Environmental Science and Pollution), 20**,** (4339-4355).

7. Sain S, Sengupta S, Kar A, Mukhopadhyay A, Sengupta S, Kar Ray D, (January 2014), **"Effect of modified cellulose fibres on the biodegradation behaviour of in-situ formed PMMA/cellulose composites in soil environment: Isolation and identification of the composite degrading fungus** ", (Polymer Degradation and Stability), 9, (156-165).

8. La Mantia F.P., Morreale M, (January 2011) **"Green composites: A brief review",** (Composites), 42, (579-588).

9. N.A. Mostafa, Awatef A. Farag, Hala M. Abo-dief, Aghareed M. Tayeb, (2018), **"Production of biodegradable plastic from agricultural wastes",** (Arabian Journal of Chemistry), 11, (546-553).

10. Maiti S, Sain S, Ray D, Mitra D (November 2013), **"Biodegradation behaviour of PMMA/cellulose nanocomposites prepared by in-situ polymerization and ex-situ dispersion methods",** (Polymer Degradation and Stability), 98, (635-642).

11. Mohanty AK, Misra M, Drzal LT (2002**)," Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world**", (Journal of Polymers and the Environment), 10, (19-26).

12. Huang X, Brittain WJ (2001), **"Synthesis and characterization of PMMA nanocomposites by suspension and emulsion polymerization"**, (Macromolecules), 3, (3255-3260).

13. Sahoo PK, Samal R (2007), **"Fire retardancy and biodegradability of polymethylmethacrylate /montmorillonite nanocomposite"**, (Polymer Degradation and Stability) ,92, (1700-1707).

14. Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. and Uppara, P. V. (2008). "**Biodegradation of polyethylene and polypropylene"**, (Indian Journal of Biotechnology), 7, (9-22).

15. Augusta, J., Muller, R. and Widdecke, H. (1993). "**A rapid evaluation plate-test for the biodegradability of plastics",** (Applied Microbiologyand Technology), 39, (673-678).

16. Balasubramanian, V., Natarajan, K., Hemambika, B.,Ramesh, N., Sumathi, C. S., Kottaimuthu, R. and Rajesh Kannan, V. (2010). "**High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of gulf of Mannar, India"**, (Letters in Applied Microbiology), 51, (205-211).

17. Goldschmidt, A.; Streitberger, H.J, (2003), ("**BASF Handbook on Basics of Coating Technology"**); Vincentz Network:Hannover, Germany.

18. Ng, T.S.; Ching, Y.C.; Awanis, N.; Ishenny, N.; Rahman, M.R, (2004), **"Effect of bleaching condition on thermal properties and UV-transmittance of PVA/cellulose bio composites**". (Mater. Res. Innov)., 18, (400–404).

19. Pranamuda, H., Tokiwa, Y. and Tanaka, H. (1997), "**Polylactide degradation by an amycolatopsis sp"**, (Applied and Environmental Microbiology), 63(4), (1637-1640).