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Assessment of Petrol and Petroleum Derivatives Degradation by Aspergillus Niger

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ABSTRACT
A major environmental problem today is hydrocarbon contamination resulting from the petrochemical industry. Accidental release of petroleum commodities are of major concern in the environment. A hydrocarbon component belongs to the family of carcinogens and neurotoxin organic pollutants. Biodegradation refers to total mineralization of organic contaminants into carbon dioxide, water, inorganic compounds and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. In this work suitable degrading medium i.e., Bushnell Hass media is selected and Aspergillus niger, a fungi is used as degrading microorganism. A.niger is grown at different concentrations 2%, 4%, 6%, 8% & 10% of petrol sample and checked for the growth resistance in medium. Biodegradation of the petrol sample is carried out and is confirmed with change in OD, pH and GC analysis. There is reduction in the level of hydrocarbons showed in GC analysis of the sample when compared to standard petrol resulting in the breakdown of long chain hydrocarbons. Research was extended on Degradation studies about commercial polythene carry bags of low density polyethylene (LDPE) over a period of four weeks under laboratory conditions by using Aspergillus niger as degrading microorganism. SEM (Scanning electron microscopy) analysis confirmed the degradation by revealing the presence of pores. The size of the pores increased from nano scale to micro scale thereby showing porosity and fragility of the fungal degraded polythene surface.

Keywords: Biodegradation, Petrochemical, Growth Resistance, Degradation Potential, Aspergillus Niger

I. INTRODUCTION

The predominance of petroleum products in the world economy which creates the conditions for distributing hydrocarbon molecules and a huge volume of oily sludge. [1, 2] Oil spillage is the accidental discharge of crude oil contaminates the environment with liquid hydrocarbons. These spills are threatening to public health, drinking water and natural resources [3]. Crude oil is a naturally occurring compound mixture of hydrocarbons and non-hydrocarbon compounds which at proper concentration consists of a quantifiable toxicity to living organisms. The toxicity of crude oil or petroleum products varies depending on their constituents, concentration, environmental factors and on the biological state of the organisms at the time of the contamination [4]. Plastics are Petrochemicals, which are also called petroleum distillates, are derived from petroleum. Plastic pollution includes accumulation of plastic products in the environment that harmful effects on wildlife, wildlife habitat, or humans.[5] Plastics that act as pollutants are categorized into micro, meso, or macro debris, based on size.[6] The prominence of plastic pollution is correspond with plastics being

inexpensive and durable, which lends to high levels use of plastics by humans.[7] However, it is slow to degrade .plastic materials are used in the production of so many products, ultimately, become waste with staying power. .[8] Plastic pollution can unfavourably affect lands, waterways and oceans. Living organisms, particularly marine animals, also be affected through direct ingestion of plastic waste, or through exposure to chemicals within plastics that cause disruptions in biological functions. Humans are also affected by plastic pollution, such as through the disruption of the thyroid hormone levels. In 1950, when plastic was first mass produced, the report was found 2m tonnes were manufactured. That quantity has risen to 8.3bn in 2017 and is predicted to reach 34bn by 2050[9]. The dispensation of plastic debris is highly inconstant as a result of certain factors such as wind and ocean currents, coastline geography, urban areas, and trade routes. Plastics can also be used as agent for chemical contaminants such as persistent organic pollutants and heavy metals.[10] Landfill areas contain many different types of plastics. Chlorinated plastic can release unfavourable chemicals into the surrounding soil, which can then drain into groundwater or other surrounding water sources and also the ecosystem. [11]This can cause serious harm to the species that drink the water. In 2012, it was estimated that there was around 165 million tons of plastic pollution in the world's oceans [12]. The litter that is being delivered into the oceans is toxic to marine life, and humans. The toxins that are elements of plastic include Diethylhexyl phthalate lead, cadmium and mercury which are toxic carcinogen. Plankton, fish and finally the human race through the food chain, ingest these highly toxic carcinogens and chemicals. Ingesting the fish that contain these toxins can cause an increase in cancer, immuno disorders and birth defects. One of the study estimated that there are more than 5 trillion plastic pieces (categorized into the four classes of small micro plastics, large micro plastics, meso- and macro plastics) afloat at sea. [13]

Bioremediation is a process of use of microorganisms to detoxify the pollutants owing to their diverse metabolic capabilities is an advance method for the removal and degradation of many environmental pollutants including all the products of petroleum industry. In Addition to this, bioremediation technology is accepted to be non invasive and relatively cost-effective. Biodegradation by natural populations of microorganisms represents one of the chief mechanisms by which petroleum hydrocarbon pollutants can be removed from the environment and is cheaper than any other remediation technologies [14]. The success of oil spill bioremediation depends on one's ability to establish and maintain conditions to enhance oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various parameters that influence the frequency of oil biodegradation. One important parameter is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, optimal rates of growth and hydrocarbon biodegradation can be persisted by ensure that an adequate concentrations of nutrients and oxygen are present and the pH is between 6 and 9. The physical and chemical characteristics of the oil and its surface area are also important determinants of bioremediation success [15]. Biodegradability of polymers by microorganisms decreases with increase in molecular weight of the polymer. With increase in molecular weight, there is decrease in polymer solubility which is not favourable for microbial attack as the polymer needs to be incorporate into the bacterial cell membrane and broken down by cellular enzymes. Repeating units of polymers such as monomers, dimers and oligomers are easily degraded and mineralized. Biodegradation is enhanced by abiotic hydrolysis, photo-oxidation and physical disintegration. These processes magnify the surface area of the polymer and reduce its molecular weight; facilitating microbial degradation [16].

Aspergillus niger is a fungi, one of the most common species of the genus Aspergillus. Degradation of plant

cell wall polysaccharides is of major principle in the food and feed, beverage, textile, paper and pulp industries, as well as in several industrial production processes. Enzymatic degradation of these polymers has gains attention for many years and is becoming a more striking alternative to chemical and mechanical processes. Most of the fungi produce extracellular enzymes for the assimilation of compound carbohydrates makes possible the degradation of a wide range of pollutants. They also have the advantage of comparatively easy to grow in fermenters,thus suitable for large scale production. An additional advantage is the easy separation of fungal biomass by filtration due to its filamentous structure. Filamentous fungi are less sensitive to variations in nutrients, aeration, pH, temperature and have a lower nucleic content in the biomass. A number of industrial activities, such as chemical manufacture, oil and gas production that contains high concentration of salts, oil, organic acids, heavy metals and radionuclides have to be degraded. Therefore, the ability of halo tolerance to remediate pollutants in the presence of salt is useful for biological treatment without damage to the physically sensitive ecosystem [17]. This work is about laboratory study to investigate the efficiency of utilization petrol and plastics by A.niger in BH medium, for degradation process.

II. MATERIALS AND METHODS

2.1 Collection, pretreatment and weighing of plastic and petrol sample

Petrol are collected from petrol bunks in Bangalore in sterile container and subjected to Optimization of growth pattern, degradation process by Aspergillus niger, for resistance determination.40micron LDPE plastic covers were collected. These plastic samples are cut into 20mm X 20mm size. These cut plastic samples are weighed in weighing balance. Weight is noted down plastic samples are washed and treated with 99.9% ethanol. Weight percentage loss is calculated.

2.2 Determination of resistance of Aspergillus niger in petrol

Potato dextrose agar media was prepared and autoclaved at 121°C, 15 lbs pressure. 20ml of PDA media was poured into the petri plates and allowed for cooling. Different concentrations of petrol sample i.e. 2%, 4%, 6%, 8%, 10% were poured into different petri plates. These plates were inoculated with Aspergillus niger and were kept for incubation at room temperature for 5-6 days.

2.3 Optimization of growth pattern of Aspergillus niger with different temperature and pH

Potato dextrose agar media was prepared and autoclaved at 121°C, 15 lbs pressure. 10% petrol sample is added to 20ml of the media. These plates are placed at different temperatures 25°C, 27°C, 29°C, 31°C for incubation and at different pH 5, 6, 7, 8. Growth is monitored for 5-6 days.

2.4 Biodegradation studies for petrol

BH (Bushnell Haas) broth was prepared and autoclaved at 121°C, 15 lbs pressure.20 ml of BH broth was poured into conical flask. Different concentrations of petrol sample, 2%, 4%, 6%, 8%, and 10% were poured into media containing conical flasks. These flasks were inoculated with Aspergillus niger and were kept for incubation at room temperature for several days. pH meter was calibrated with the standard BH broth. Broth containing different concentrations of petrol samples were taken and tested for the pH and change in optical density. Readings are noted down and a graph of concentration in y-axis versus days in x-axis is plotted. pH and optical density of the fermented broth was determined after 0, 4, 8, 12 &16 days of treatment. Degradation efficiency of the isolates were analysed by GC Analysis.

2.5 Biodegradation studies for Plastic

BH broth was prepared and autoclaved at 121°C, 15 lbs pressure.50 ml of BH broth was poured into conical flask. Pre-treated Plastic sample was put into the conical flask aseptically. Conical flask was inoculated

with Aspergillus niger and was kept for incubation at room temperature for 4weeks.After incubation. Initial weight of the sample is taken before degradation process. Final weight of the sample after degradation process is taken after 4 weeks of incubation at room temperature in BH (Bushnell Haas) media. Weight percentage loss is calculated using the formula.

Weight % loss= initial weight – final weight.
100

Degradation efficiency is measured by SEM analysis.

III. RESULTS

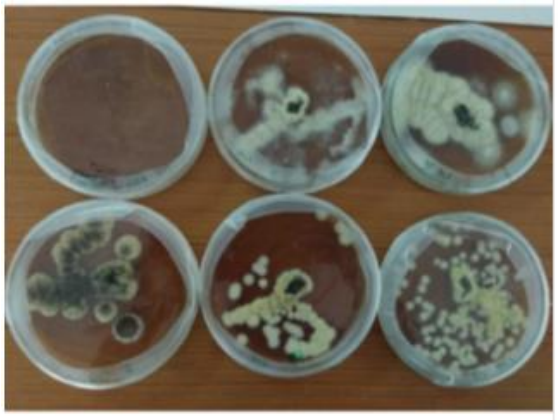


Figure 1. Growth of Aspergillus niger in PDA Media containing 2%, 4%, 6%, 8%, 10% of petrol.

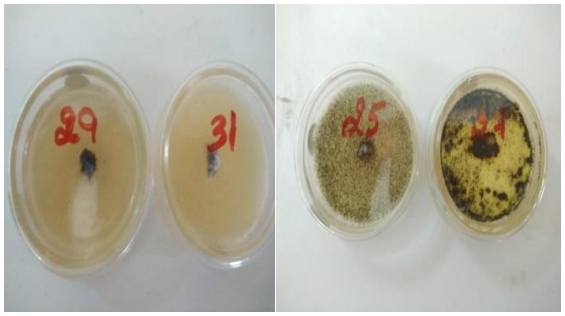
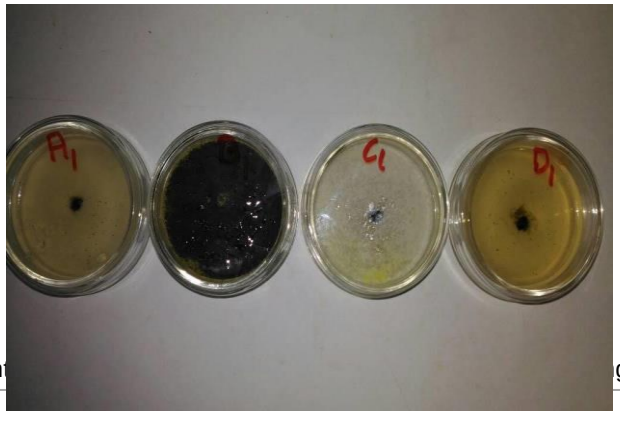


Figure 2. Growth of A.niger in PDA media containing 10% petrol sample at different temperatures(25°C, 27°C, 29°C, 31°C) of incubation.



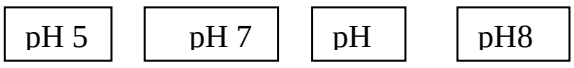
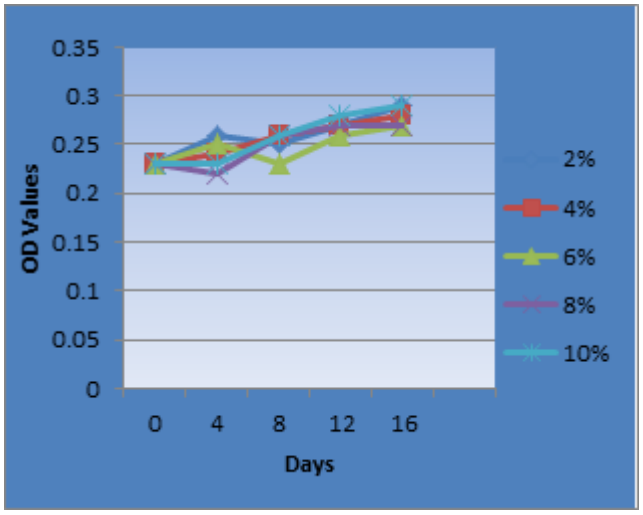
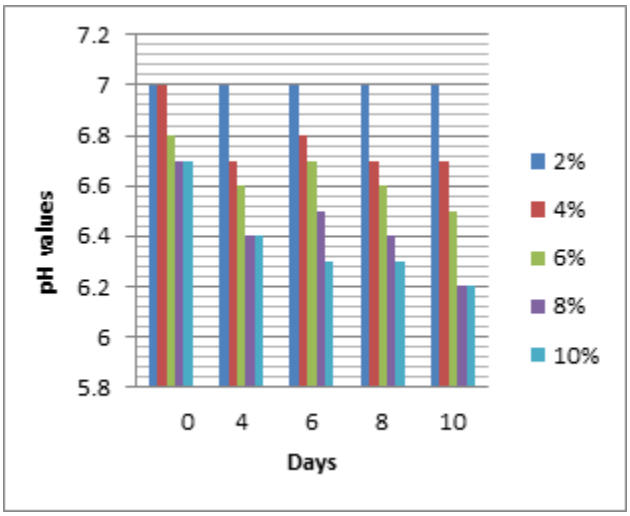


Figure 3. Growth of A.niger in PDA media containing 10% petrol at different pH (5, 6, 7, 8).



Graph 1. Changes in OD during degradation of petrol by A.niger



Graph 2. changes in pH of the medium during the degradation of petrol by A.niger

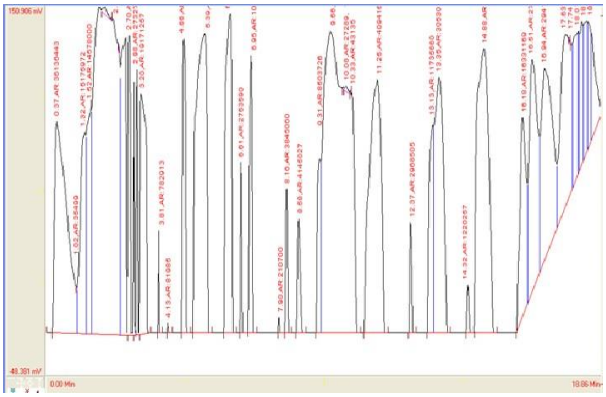


Figure 4 (a). Chromatograph of untreated petrol sample after 16 days of incubation at room temperature.

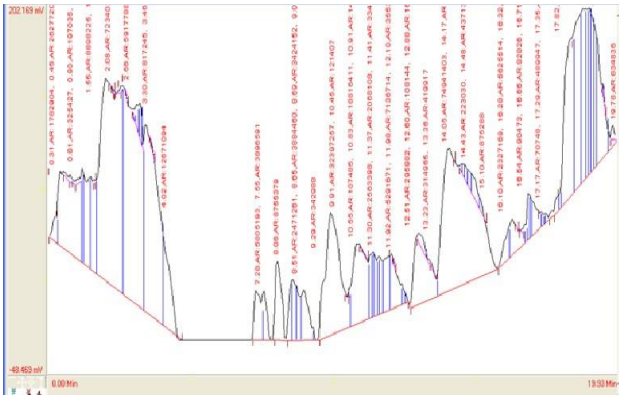


Figure 4(b). Chromatograph of treated petrol sample after 16 days of incubation at room temperature.

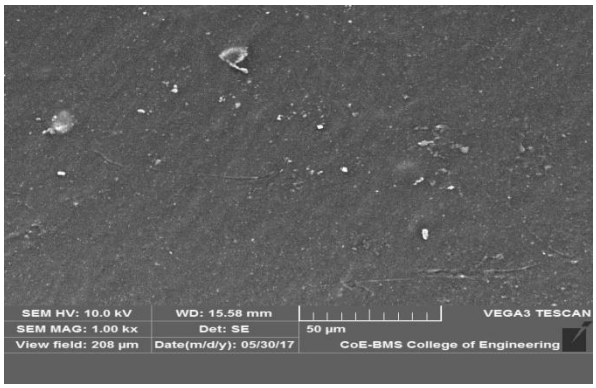


Figure 5 (a). SEM Analysis of untreated plastic (blank)

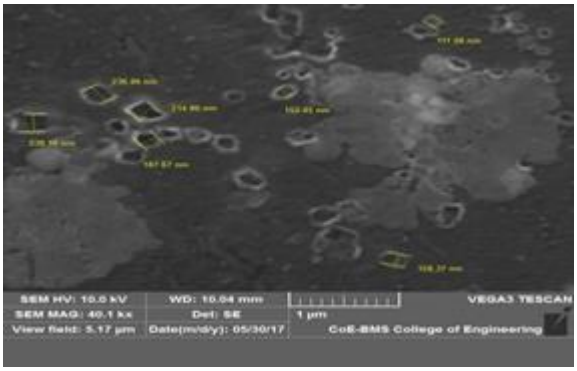


Figure 5(b). SEM analysis of treated plastics (two Weeks incubated)

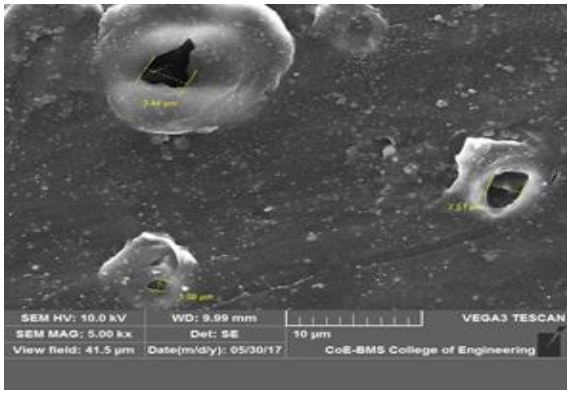


Figure 5(c). SEM Analysis of treated plastics (four Weeks incubated)

IV. DISCUSSION

In previous review it was reported that a group of fungi such as Aspergillus, Penicillium were found to be degraders of petroleum hydrocarbons (18).This study represents efficiency of Aspergillus niger in degradation of petrol and petroleum derivities.Aspergillus niger was grown at different concentrations of petrol to check for its growth resistance in the presence of petrol along with media. Fig 1 shows that the growth was found in each concentration of petrol, indicating that Aspergillus niger utilizes the petroleum hydrocarbons as substrate for their survival and growth This is the first step to prove, Aspergillus niger efficiency in biodegradation process. Present study also confirmed about the physiological conditions to support for the growth of Aspergillus niger in presence of 10%petrol. Which reveals that at pH 7 and at temperature 27°C the growth of Aspergillus niger was found to be maximum (fig2&3).Further Biodegradation studies were carried out by taking BH broth with Different concentration of petrol. Growth was observed in each concentration of petrol As BH broth does not contain carbon source, Petroleum hydrocarbons are used as a substrate for their growth.Variations in OD was recorded every 4 days at 600nm from the day of inoculation and graph 1 shows an increase in growth rate of Aspergillus niger in the deteriorating media such as BH broth. There is also a change in pH of the broth from 7 to 6.4 gradually resulting in the acidic environment indicating the degradation of petrol (graph2). This is due to the carbon dioxide that is released into the broth after the breakdown of long chain hydrocarbon in the petrol due to the enzyme activity of Aspergillus niger. Efficiency of degradation also confirmed by gas chromatographic analysis of untreated petrol sample (figure 4a) and treated petrol sample obtained from filtered fermented broth. Comparison of the chromatograph of treated petrol and untreated petrol indicates that breakdown of

longer chain hydrocarbons into shorter chain hydrocarbons resulting in the degradation of petrol (Figure 4b).Further the degradation studies was carried out for plastics, a petrochemical. Effective Microorganisms plays a significant role in biodegradation of non biodegradable synthetic plastics [19].The weight difference in the plastic sample shows 10% variation from initial to final weight showing the weight loss after treatment. Degradation process can be observed with the help of scanning electron microscope (SEM). SEM analysis of the plastic sample for blank has no significant pores (figure 5a). But after 2 weeks of incubation, formation of pores has occurred with a size range of ~112nm to ~240nm (fig5b). After 4 weeks of incubation the pore size has increased to micro scale up to the range of ~2.5 µ m to ~3.44 µ m (figure 5C). SEM result helps us analyze that due to the enzyme activity of the A.niger long chain polymers are able to break down into shorter chains and this could be observed physically through the pores formed. The increase in the pore size shows degradation of the plastic sample.

V. CONCLUSION

Aspergillus niger has ability to resist tested petrol concentration. Records of changes in OD,pH along with GC analysis reports confirms its ability of petrol degradation .further studies on Aspergillus niger efficiency in degradation also confirmed for collected plastics samples in laboratory condition by SEM analysis reports.SEM analysis reveals the presence of porosity and delicacy of fungal degraded plastic surface.

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