ENRICHMENT OF MICROORGANISMS BY SUGAR CANE MOLASSES FOR POLYEHTYLENE DEGRADATION

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Abstract

Polythene does not dilute with soil, it remains within the soil surface, thereby causing harm to the soil character. Use of products made from polythene can have cancerous effect on human body. It is designed to be inert and stable so it is no surprise that it is slow to decompose. To overcome this problem microorganisms are able to degrade polythene and were isolated from garbage soil and enriched its growth with carbon sources like sugar cane molasses and polythene films, after six months of the incubation period the films were collected from the medium and its significant change in the surface of polythene film was observed and measured by FTIR which showed the introduction of carbonyl groups after natural weathering which decreased after microbial treatment. The results confirmed that microorganisms have the ability to degrade polythene efficiently when its growth enriched with sugar cane molasses at high concentrations (above 2.5%).

Keywords: *Polythene, Molasses, FTIR, Microorganisms*

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1. INTRODUCTION

Polyethylene is also called as polythene, it is a synthetic polymer made of long chain monomers of ethylene. They are seemingly ubiquitous in our world today (Joseph Greene *et al.,* 2006). They are made from inorganic and organic raw materials such as carbon, silicon, nitrogen, oxygen, chloride and hydrogen. Basic materials used for making polyethylene are extracted coal, oil and natural gas. They are defined as the polymer which become mobile on heating and thus can be cast into moulds and they are non-metallic mouldable compounds and the materials made from them can be pushed into almost any desirable shape and then retain that shape (Gnanvel *et al.,* 2012).

It has been widely used due to its versatile nature and effectiveness. Each year, about 500 billion to 1 trillion tones of polythene bags are consumed worldwide (Bhone Myint Kyaw *et al.,* 2012). After their wide applications and simultaneously increasing accumulation in the ambient environment causes a major threat to the living systems. To avoid this threat some physical and chemical methods were employed to degrade this polythene but it did not showed satisfactory results (Marina Paul das and Santosh Kumar., 2013). Microorganisms are using biological catalyst which can degrade either completely or incompletely these higher molecular compounds to lower one. The potentiality of microbes as agents for degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants. The microbes are using these degraded compounds as nutrient source that means carbon and energy source (Marina and Santhosh Kumar, 2013).

The present investigation is aimed to enrich the growth of microorganisms for degradation of polythene film; the growth of microorganisms was enriched with carbon sources like sugar cane molasses in different concentrations (2%, 2.5%, 3%, and 3.5%) and polythene sheet (3cm size). After six month of incubation period the surface changes made in polythene film was analyzed with the help of FTIR and it was used to evaluate the biodegradation of polythene film by compared with control and treated film with sugar cane molasses, before and after incubation. FTIR spectroscopy evidence was further used to confirm the biodegradation of polythene materials.

2. MATERIALS AND METHODS

2.1 Sample Collection

Soil sample and polythene bag materials were collected from the garbage of Musiri Taluk of Tiruchirappalli District, Tamil Nadu, India and stored in zip lock covers and then kept at 4^0C for further studies.

2.2 Serial Dilution Method

One gram of collected soil sample was weighed using electronic balance and then it was mixed with 99ml of distilled water and shaked well until it get dissolved, then 9 sterile test tubes were taken, each of which contains 9ml of sterile distilled water, 1ml of sample was transferred serially to all the tubes to make about of 10^{-1} to 10^{-8} dilution.

2.3 Media Preparation

100 ml of potato dextrose broth for fungi and nutrient broth for bacteria were prepared and sterilized in an autoclave at

15 lbs pressure for 15 minutes. Then it was allowed to cool at room temperature.

2.4 Inoculation of Microorganisms

From the serial dilution, 10^{-2} dilution was taken and added into 0.1 ml of the same dilution into 100 ml nutrient broth and potato dextrose broth separately and kept in rotary shaker for 7 days.

2.5 Preparation of Sugar Cane Molasses in different Concentrations

2 ml, 2.5 ml, 3 ml, and 3.5 ml of sugar cane molasses were taken and then diluted with 98 ml, 97.5 ml, 97 ml and 96.5 ml of distilled water respectively and sterilized in an autoclave at 15lbs for 15 minutes and then kept at room temperature until it gets cool.

2.6 Inoculation of Bacterial and Fungal Cultures

1ml of well grown bacterial and fungal cultures were inoculated into different concentrations of sugar cane molasses as mentioned above and kept in shaker for 7 days incubation.

2.7 Inoculation of Polythene Strips into Sugar Cane Molasses:

Polythene bags were cut into small strips (each 3cm size) and inoculated it into different concentrations of sugar cane molasses and kept it for 6 month incubation.

3. ANALYSIS OF BIODEGRADATION

3.1 FTIR Spectroscopic Analysis

Fourier Transform Infrared Spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups (Milstein *et al*., 1994). After the incubation period the polythene film was removed from the media and washed with ethanol and followed by distilled water to remove the debris and undergone it into FTIR spectroscopy for analysis the surface changes made in polythene film.

4. RESULTS AND DISCUSSION

The growth of microorganisms in sugar cane molasses was visible by naked eye. FT-IR spectra are obtained by the films of four different polythene films in different concentrations of sugar cane molasses (2%, 2.5%, 3%, and 3.5%). It was found that some new peaks arose and de-arose after the period of degradation (Mahalakshmi *et al.,* 2012). FTIR spectroscopy is used as an analytical technique in many biodegradation studies (Kiatkamjornwong *et al*., 2007; Dirmal *et al*., 2007; Kirbas *et al.,* 1999., Arboleda *et al.,* 2004 and Naima Atiq *et al*., 2010). Synthetic polymers especially polyolefin, made up of only carbon and hydrogen atoms are generally less susceptible to microbial attack. Their inertness is probably due to a total lack of carbon-to-oxygen bonds (C=O, C-OR, C-OH), which are the sites of microbial enzymes act upon them (Motta *et al*., 2007 and Naima Atiq *et al.,* 2010)

Polystyrene structurally consists of the aliphatic chain with an aromatic ring attached to every other carbon atom. Styrene is the monomer of polystyrene and its degradation by bacteria and fungi is well established in the literature (Mooney *et al*., 2006 and Naima Atiq *et al*., 2010) The FTIR spectra of pre-treated polythene film in sugar cane molasses shows the following peak values:

The term degradation with respect to decomposition of polymeric materials has not been explicitly specified. The main problem is to determine the susceptibility of the polymer for degradation in the environment and the length of time during which process will last. Several methods can be used to estimate polymer deterioration. Frequently used methods rely on gravimetric, spectroscopic and microscopic techniques, mostly in combination with each other (Sudhakar *et al.,* 2008). A simple and quick way to measure the degradation of polymers is by determining the weight variable. However, this measurement itself cannot be a reliable indicative of material degradability, since both an increase in weight and a weight loss of polymer sample, not directly related to the breakdown of polymer chains, may occur. A good example is an increase in weight due to accumulation of microorganism, whereas loss of weight can be due to the vanishing of volatile and soluble impurities (Lucas *et al*., 2008).

Deterioration of polymers can be also evaluated by change in their rheological properties. Contrary to the weight measurement, these properties directly depend on molecular weight of polymers, their crystalline and the presence of branches and cross linking effects (Briassoulis *et al*., 2004).

Among them, FTIR spectroscopy is most widely used in determining the structural changes in macromolecules. Since it is known that degradation of polymers can proceed via both hydrolysis and oxidation, with this tool it is possible to estimate the extend of modification of the polymer main chain due to the action of abiotic or biotic factors. It is assumed, that the mechanism of polymer degradation can be determined by measuring the levels of ketone carbonyl, ester carbonyl and internal double bond absorbance peaks (Gilan *et al*., 2004; Jakubowicz *et al*., 2006; Sudhakar *et al*., 2008; Bożena, N *et al*., 2006)

The microorganisms were isolated from the garbage soil was enriched with carbon source such as sugar cane molasses and polythene film, After six months of incubation period, the film was collected from the molasses medium and undergoes for surface study through FTIR its graph results were given below:

4.1 FTIR Analysis:

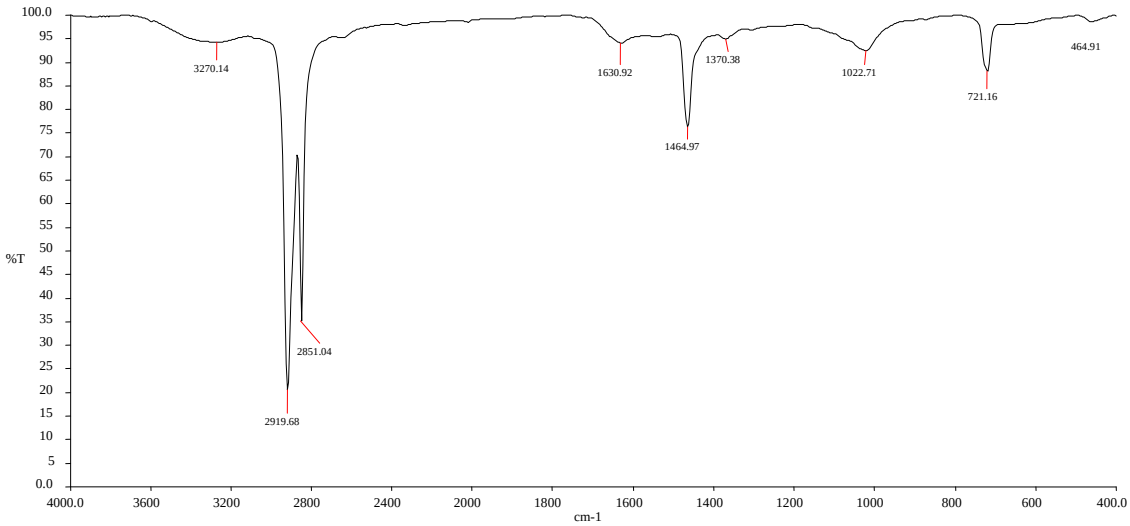

Fig.1. FTIR spectrum of control Polythene film after 6 months of incubation period

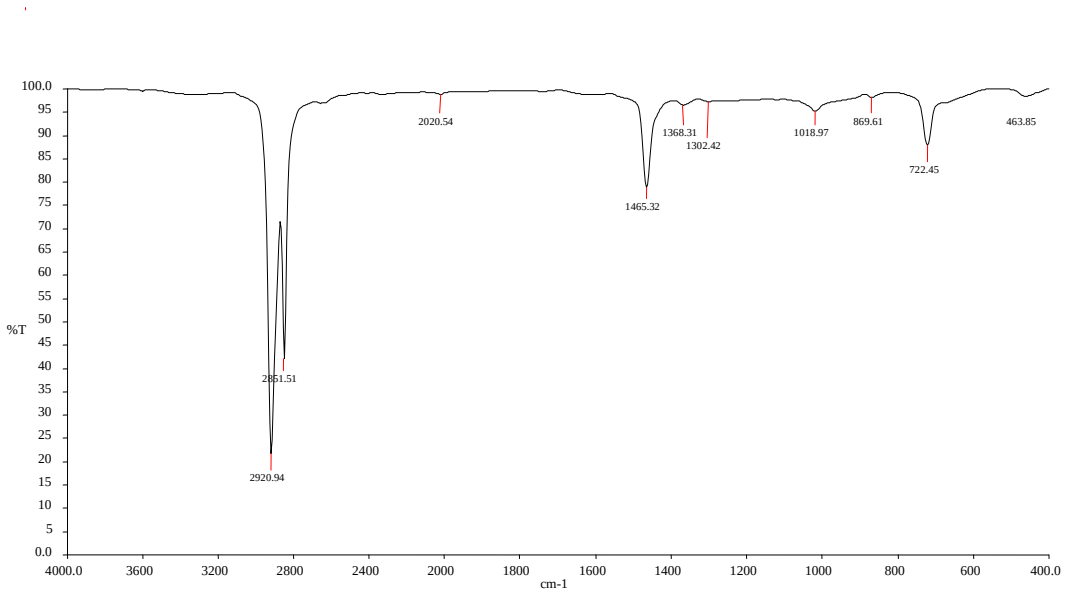

Fig.2. FTIR spectrum of Polyethylene film in 3.5% molasses after 6 months of incubation period

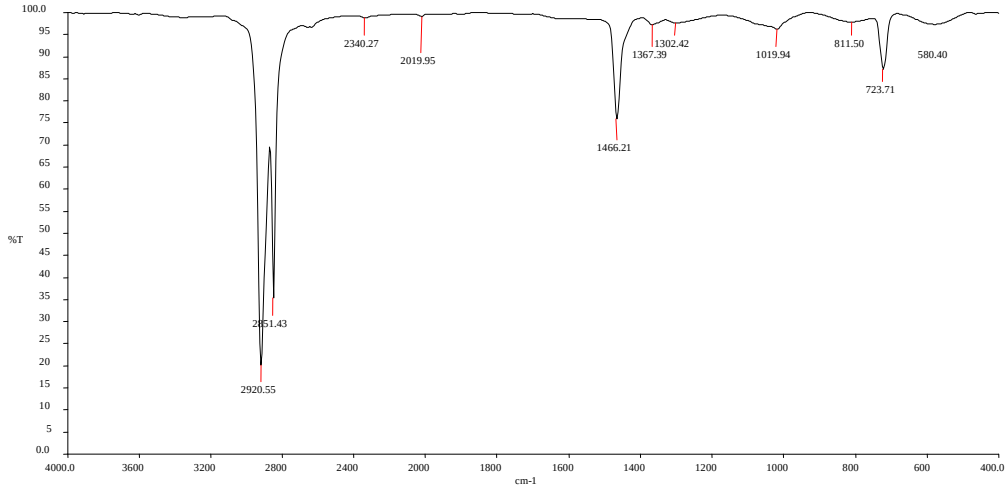

Fig.3. FTIR spectrum of Polyethylene film in 3% molasses after 6 months of incubation period

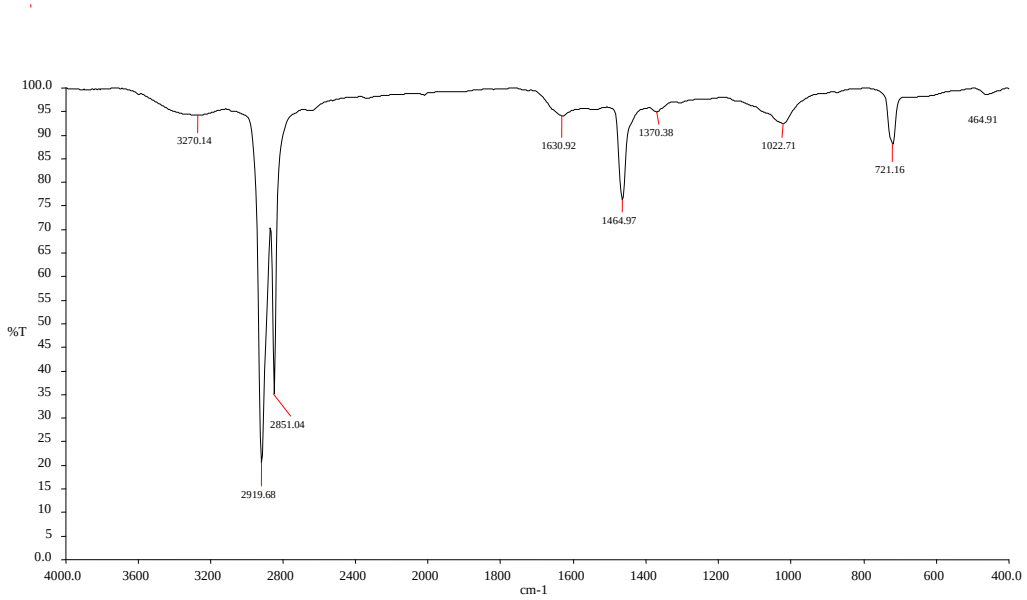

Fig.4. FTIR spectrum of Polyethylene film in 2% molasses after 6 months of incubation period

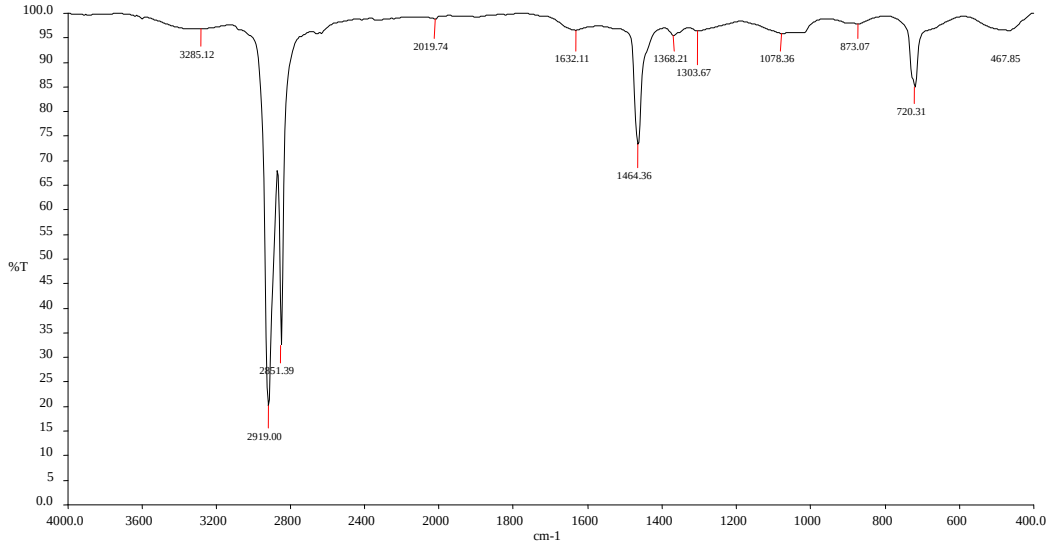

Fig.5. FTIR spectrum of Polyethylene film in 2.5% molasses after 6 months of incubation period

The non-hydrogen bonded hydroxyl group can be observed in the range of 3659.26 cm-^{21} is slightly decreasing into 3270.14 and 3285.12 cm-^{21} because of the action of the selected microorganisms. A band around 1461-1466 cm^{-1} reveled a bending deformation and another band at 720-724 cm^{-1} indicates a rocking deformation. The carbonyl band corresponds to the ketone and ester carbonyl groups and it is a typical product of oxidative degradation of polythene (Gilan *et al*. 2004, Hadad *et al*., 2005, Ibiene., 2013).

The methane C-H exhibits C-H stretching and bending vibration at 2890 and 1340m^{1} respectively and are weak having no practical utility in structural elucidation, The Methylene group, however exhibits the two bands near 2924 and 2850 cm^{-1} respectively with slight modification made by microorganisms.

An isolated hydrogen in m-DI, unsymmetrical tri-symmetric tetra-, un -symmetric tetra and Penta substituted benzene exhibits absorption in the region 890-835 cm^{-1}. The C-H out of plane bending vibration is strongly coupled vibrations and occurs in the region 900-667 cm^{-1}. These extremely intense absorptions are used to assign the position of substituent on the aromatic ring.

The band 723.60 (-CH=CH-) cis is slightly decreased into 722,723,721,720 cm^{-1} respectively due to microbial degradation , However it attack the surface only but not break the bond completely. When examined in the solid state, they exhibit only one band at 1630-1634 cm^{-1}, which is split into amide I and II bands when the spectra are measured as dilute solutions. So the band is easily breaking by microorganisms, the alkane, aromatic, aliphatic compound peak values and alkyl carbonyl peak value also get decreased (Fig.1 compared with other four figures).

Then the carbonyl index of the film, showing an increase after exposure and decreased, however some band revealing a bending deformation, and another band indicates a rocking deformation due to microbial growth. Because the microorganisms enriched with molasses has the ability to break the bond. So far that long polymer chains were likely cut into shorter pieces because of the action of enzymes secreted by the microorganisms. Because the films became fragile and lighter in weight indicates the preliminary stages of microbial decomposition, consisting of a reduction in molecular weight. So far that molasses enrich the growth of microorganisms at high concentrations (3.5%) and that microorganisms degrade the polyethylene film in smaller level.

5. CONCLUSIONS

Sugar cane molasses was prepared in four different concentrations (2%, 2.5%, 3%, and 3.5%).When compared to control the polythene strip in 2% and 2.5% concentrations, the microorganisms began to grow by utilizing the carbon source from sugar cane molasses and degrade, so far that the band value get decreased in point level and some new band arose but in 3.5% some band de-arose and some band get decreased very well, because in this concentrations the band value get decreased very well and shows that microorganisms utilize the carbon source very well and degrade the strips in surface level and analyze its surface changes through FTIR result. The results of FT-IR values showed the ability to degrade the selected microorganisms to modify and colonize both types of PE as the carbon source, and demonstrated the important role of these isolates in the PE biodegradation process.

Finally we assume that, the microorganism has the ability to degrade the polythene film when enriched with high amount of carbon source such as polythene and sugar cane molasses. The results of this investigation showed that in the near future, these microorganisms can be used to reduce the quantity of polythene waste, which is rapidly accumulating in the environment. On basis of this study the overall results of the experiment revealed that by increasing the concentration of carbon source such as sugar cane molasses (above 2%) enhance the growth of microorganisms that had the ability to degrade the polythene films.

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REFERENCES

[1] Atefeh, E., Ahmad, A.P., Hossein, A.A., Farzin, S and Ensieh, E (2013): Biodegradation of low density polyethylene by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in soil, *PLoS one* 8(1): 1-10.

[2] Baljit, S and Nisha, S (2007): Mechanistic implications of plastic degradation, *Science direct* 93(2008): 561-584.

[3] Bhone, M.K., Ravi, C., Meena, K.S., Chu, S.L., Kishore., R and Sakharkar (2012): Biodegradation of low density polythene(LDPE) by *Psedomonas* Species, *Indian journal of Microbiology* 52(3): 411-419.

[4] Bożena, N., Jolanta, P and Jagna, K (2012): Biodegradation of Pre-Aged Modified Polyethylene Films, *ISBN:* 978-953-51-0092-8.

[5] Gnanavel, G., Mohana Jeya, V.P., Thirumarimurugan, M and Kannadasan, T. (2012): Degradation of Plastics using microorganisms, *International journal of pharmaceutical and Chemical sciences* 1(3): 691-693.

[6] Josseph, G. (2006): Biodegradaiton of compostable plastics in cow manure compost environment, *GPEC*: 1-5.

[7] Jag, M (2011): Organic spectroscopy Principles and applications, *Narosa Publishing House* 2(7): 1-543.

[8] Marina, P.A and Santosh, K (2013): A comparative study of microbial growth and substrate utilization kinetics, *International journal of pharmacy and technology* 5(2): 5567-5573.

[9] Mahalakshmi, V., Abubakker, S., Niren(2012): Analysis of Polyethylene Degrading Potentials of Microorganisms Isolated From Compost Soil, *International Journal of Pharmaceutical & Biological Archives* 3(5):1190-1196.

[10] Ibiene, A.A., Stanley, H.O and Immanuel, O.M (2013): Biodegradation of polyethylene by bacillus sp. Indigenous to the Niger delta mangrove swamp, *Nigerian Journal of Biotechnology* 26(2013): 68-79.

[11] Naima, A., Safia, A., Ishtiaq, M.A., Saadia, A., Bashir, A and Geoffery, R (2010): Isolation and identification of Polystyrene biodegradaing bacteria from soil, *African Journal of Microbiology Research* 4(14): 1537-1541.