5.1 Introduction

Plastic, a synthetic polymer, are consumed in trillions of amount (about 140 million tons) annually as packaging materials (Caruso *et al*. 2015, Roy *et al.* 2008) which is increasing continuously day by day (Sekhar *et al*. 2016). Among plastics, high-density polyethylene (HDPE) (29%) as well as low density polyethylene (LDPE) (33%) are widely used in packaging industry due to its effectiveness and versatility (such as light weight, inexpensiveness, durability, easy processing etc.) (Rivard *et al*. 1995, Begum *et al*. 2015, Witt *etal*. 2001 & Muller *et al*. 2001). Low density polyethylene exhibits random branching resulting in low packing of chains, whereas high density polyethylene shows less branching and more linearity, which provides it a high packing density (Arutchelvi *et al*. 2008). LDPE constitutes 60 % of the aggregate plastics production of plastic bags and is most prevalent solid waste. Polyethylene is xenobiotic in origin and resistant to degradation in nature. Its hydrophobic character leads to a very slow degradation (Orhan and Büyükgüngör, 2000). (Hadad *et al*. 2005, Geresh *et al.* 2005, Sivan *et al.* 2005). Polyethylene wastes are normally discarded as landfill or thrown in water bodies to decompose/degrade (Priyanka *et al*. 2011). Its accumulation rate is 25 bmillion tons per annum, so its degradation is great challenge to the scientists (Sangale *et al.*2012, Orhan and Büyükgüngör 2000).

Recycling of polyethylene is one of the environmentally attractive solutions. Currently, a very small part of the plastics are recycled and the remaining goes to the burial sites (Bhardwaj *et al*. 2012). Landfills are less in number and are rarely satisfactory, whereas, incineration creates highly toxic fumes which are released into the environment causing air pollution (Bhatia *et al*. 2013). Potential hazardous emissions from incinerating polyethylene include hydrogen chloride, dioxin, cadmium, and fine

particulate matter. Polyethylene can release harmful chemicals into the surrounding soil, which can then seep into ground water or other surrounding water sources. This can cause serious harm to the species that drink this water. Studies indicate towards no clue of deterioration in polyethylene sheet incubated in moist soil for 12 years (Otake *et al*. 1995**)**. However, biodegradation could be the best alternative to combat this environmental pollution (Satlewal *et al*. 2008).

Biodegradability is defined as the propensity of a material to breakdown into its constituent molecules by natural processes (often microbial digestion). According to some reports, partial biodegradation of polyethylene could be achieved after UV irradiation (Cornell *et al*. 1995) thermal treatment (Awasthi *et al.* 2017, Albertsson *et al*.1998) and oxidation with nitric acid (Brown *et al*. 1997). The metabolites released after biodegradation are required to be non-toxic to the environment and redistributed through the carbon, nitrogen and sulfur cycles. Biodegradation is bio-chemical in nature in which enzymes released from microorganisms acts as a catalyst. The vulnerability of the polymers to microbial attack generally depends on enzyme availability, availability of a site in the polymers for enzyme attack, enzyme specificity for that polymer and the presence of coenzyme if required (Reich *et al.* 1971). Thermal or radiation treatments on polyethylene reduce the polymeric chain size and form oxidized groups such as carboxyl, carbonyl and hydroxyl which are more easily degraded by microorganisms (Albertsson et al. 1995). As oxidized groups modulate the microbial attachment by increasing the surface hydrophilicity (Tribedi *et al*. 2013), therefore, polyethylene degradation will be boosted if a more oxidized surface is used as a substrate. (Awasthi *et al*. 2017). These treatments modify the properties such as crystallinity level and morphological changes of the original polymer, and facilitate the polymer biodegradation (Lee *et al*. 1997).

Both natural and synthetic plastic are degraded by microorganisms such as bacteria and fungi (Gu *et al*. 2000a).The microbial degradation of polyethylene leads to a cleavage of the polymer chain into oligomers and monomers, which is promoted by certain enzymatic activities. Microbial cells absorb these water soluble degradation products produced enzymatically for use in their metabolism. Aerobic metabolism results in carbon dioxide and water (Starnecker *et al*. 1996), whereas anaerobic metabolism results in carbon dioxide, water and methane as the end products, respectively (Gu *et al*. 2000). In the present paper, biodegradation study of thermally treated HDPE is described, using an indigenous bacterial stain *Klebsiella pneumoniae* CH001. Bacterium *Klebsiella pneumoniae* is known for potential degradation of complex compound (Alberts *et al*. 2009). Olszanowski *et al* reported that *Klebsiella pneumoniae* degrades hydrocarbons from soil (Olszanowski *et al*. 2006).

Research on biodegradation of polyethylene by *Klebsiella pneumoniae* CH001 is scarce. It qualifies as a suitable microorganism for HDPE and LDPE degradation in our study. As PE accumulation in the environment is a serious threat, this isolate will be of major use in degradation. It is probably because of *Klebsiella pneumoniae* secretes lipase (Ali *et al*. 2015) tyrosinase, laccase and peroxidase enzymes (Kalme *et al*. 2007a, 2007b, Dhanve *et al*. 2008), which are capable of degrading polyethylene via groove formation. Also, the extracellular polymers released by this microorganism act as surfactants (Bandeira *et al.*2017) which facilitate the exchanges between hydrophilic and hydrophobic phases. These exchanges favour the penetration rate of microbial species.

5.2 Result and Discussions

5.2.1 Characterization of potential strain and phylogenetic analysis

The selection criteria of the bacterial culture were based on their effectiveness of polyethylene degradation in NB media based on their distinct morphology. The isolated bacterial strains were morphologically and biochemically characterized and identified on

the basis of 16S rRNA gene sequence analysis. A phylogenetic tree was constructed by software version 8 of Molecular Evolutionary Genetics Analysis (MEGA). The phylogenetic analysis was grounded on BLAST search applying 16S rDNA gene sequence demonstrated maximum homology (100%) with bacterium *Klebsiella pneumoniae* strain AANP1 with gene bank Accession Number: KY494861.1. Based on the cladistic analysis as well as homology valuation, it was concluded that the selected bacterial isolate could be regarded as *Klebsiella pneumoniae* CH001 (Fig.5.1). The sequence of *Klebsiella pneumoniae* strain CH001 has been deposited in NCBI with accession no. MF399051 (http://blast.ncbi.ntm.nih.gov).

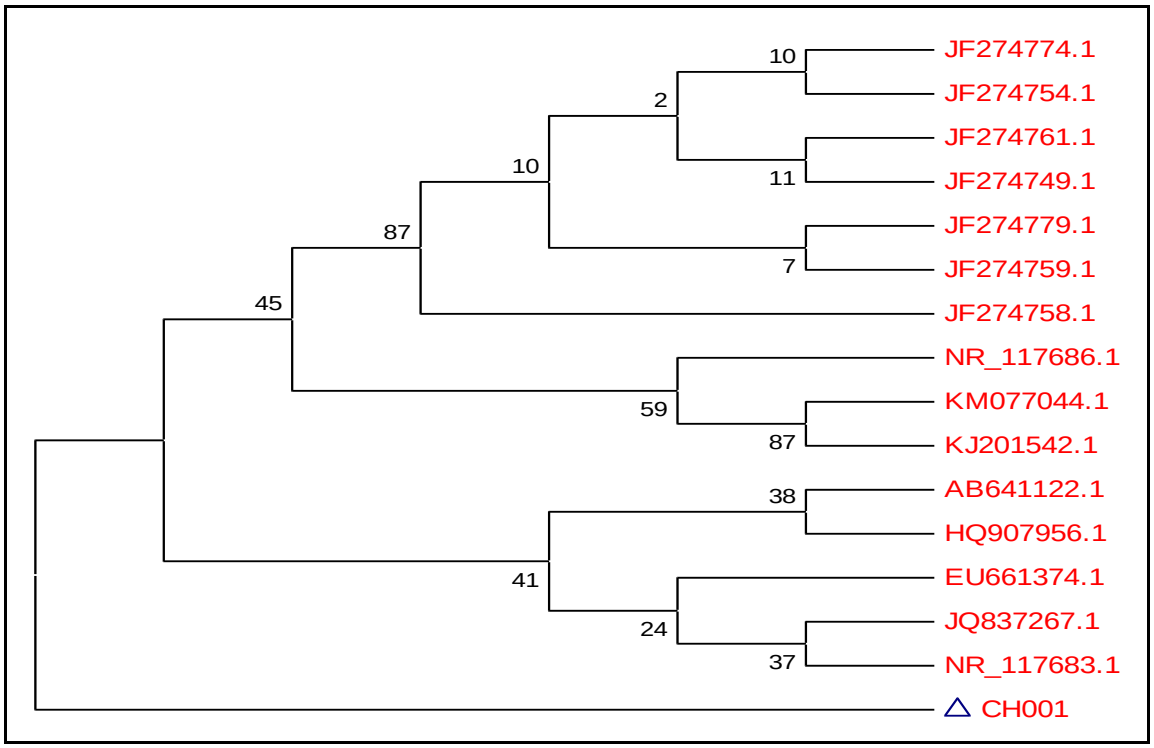

Fig.5.1 Phylogenetic tree of *Klebsiella pneumoniae* CH001

5.2.2 Measurement of weight loss

Klebsiella pneumoniae CH001 was found to degrade thermally pretreated HDPE and LDPE films up to 18.4± 0.3 and 8.4±0.3% respectively after 60 days of incubation

(Fig.5.2a-b). ANOVA result indicates that weight loss of HDPE ($F=2152$ and $P<0.001$) and of LDPE ($F=368$, $P<0.001$) with incubation time was significant. Moreover, the PE films were found to develop a biofilm on the surface within 15 days of incubation with *Klebsiella pneumoniae* CH001. It might be attributed to the fact that thermal pretreatment oxidizes polyethylene chain which leads to the carbonyl group formation leading to biofilm development on films. The biofilm reduces the hydrophobicity of the polymer, which resulted in the enhancement of the degradation rate in the present study. Similar results were also reported by Sudhakar *et al* where thermal pretreatment was found to increase HDPE degradation when incubated with *B. sphericus* i.e. 9% against the 3.5% in case of untreated HPDE (Sudhakar *et al*. 2008). Balasubramanian *et al* also observed a weight loss of 12% and 15% in HDPE after 30 days of incubation with *Arthrobacter* sp. and *Pseudomonas* sp. Respectively (Balasubramanian *et al.* 2010). This biodegradation rate is in agreement with the earlier reports ranging from 3.5% to 8.4% for polyethylene incubated in soil for 10 years (Albertsson and Karlsson 1990). This decrease in weight is in association to the others' findings (Singh *et al.* 2012). Tribedi *et al* reported 5 % weight loss of LDPE by *Pseudomonas* sp AKS2 after 45 days (Tribedi *et al* 2013).

Production of protein enables the formation of a stable biofilm which possibly accounts for strong adhesion of *K. pneumoniae* to the polyethylene surface. The relatively high survival rate of the biofilm (tested for up to 60 days), formed by strain *Klebsiella pneumoniae* on polyethylene, in spite of the low carbon availability, further confirms its efficiency in utilizing polyethylene as a carbon and energy source.

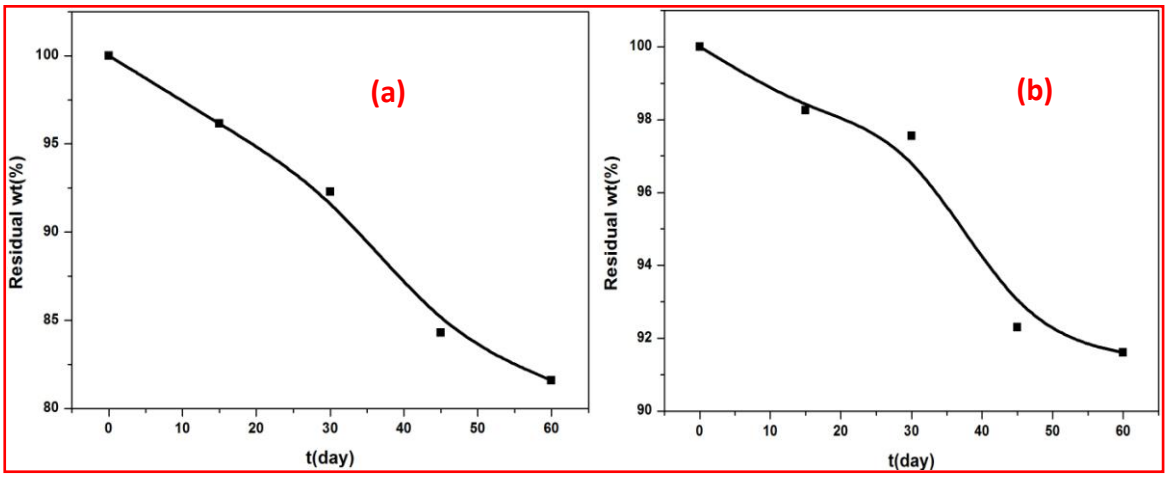

Fig. 5.2(a) Change in weight of HDPE and LDPE (b)

5.2.3 Measurement of change in tensile strength

In the present study, approximately 57% reduction in tensile strength of HDPE and 52% reduction in LDPE was observed after 60 days incubation (Fig5.3a-b). ANOVA result indicates that reduction in tensile strength of HDPE ($F=204$ and $P<0.001$) and LDPE ($F=187$, $P<0.001$) with incubation time is significant. Vijaya *et al.*2008 reported that HDPE and LDPE incubation with *Diplococcus* for 4 months there was 16-20% and 12-13% reduction in TS of HDPE and LDPE respectively. Similar results were also reported by Hanaa *et al*., Lee *et al*. and Nowak *et al*. they reported that biodegradation reduces the percentage elongation of polyethylene films (Hanaa *et al*. 1998, Lee *et al.* 1991 and Nowak *et al*. 2011). Our results are also in agreement with earlier studies by Jakubowicz *et al*. on thermally-treated-LDPE film, which showed a reduced elongation (%) after incubation with compost microorganism (Jakubowicz *et al*. 2006) and according to Sharma *et al* there was a reduction of 76% in tensile strength of LDPE by *pseudomonas stutzeri* (Sharma *et al.* 2004). According to Kyaw *et al* there was 76% reduction in tensile strength of LDPE in 120 days by *Pseudomonas aeruginosa PAO1*. (Kyaw *et al.* 2012)

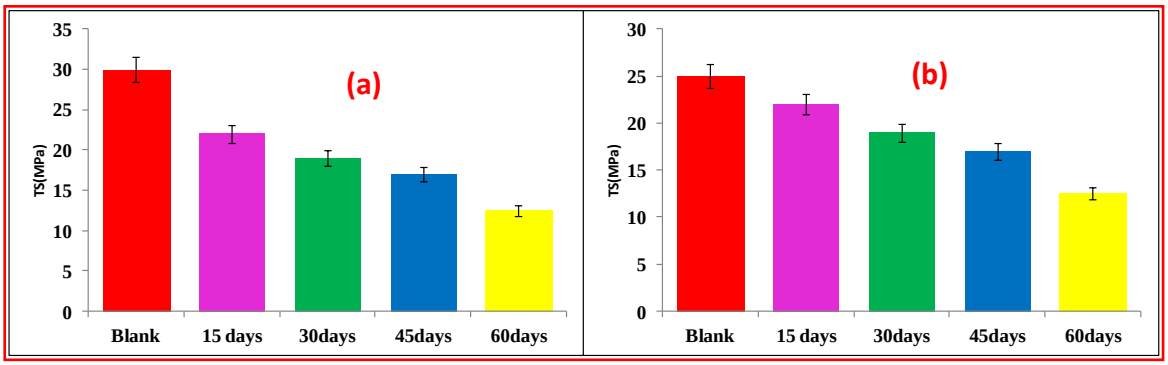

Fig.5.3 Change in TS of HDPE (a) and of LDPE (b) film

5.2.4 Measurement of change in pH

The initial pH of the media was 7.0 which was reduced to 5.7±0.4 with HDPE and 6.0±0.4 with LDPE (Fig5.4 a-b). ANOVA results indicate that reduction in pH of HDPE ($F=89.5$ and $P<0.001$) and of LDPE ($F=18.5$, $P<0.001$) incubated media with time was significant. The reduction in pH proves that the culture is still metabolically active and it is utilizing PE films for its growth. No change in pH of control sample was noticed. The reduction in pH not only confirms the usage of the polyethylene sheets as their source of carbon however, it also paves the way for the idea regarding the production of several monomers which have possibly been produced post-degradation (Awasthi *et al.* 2017, Arutchelvi *et al.* 2008 and Orhan *et al.*2000).

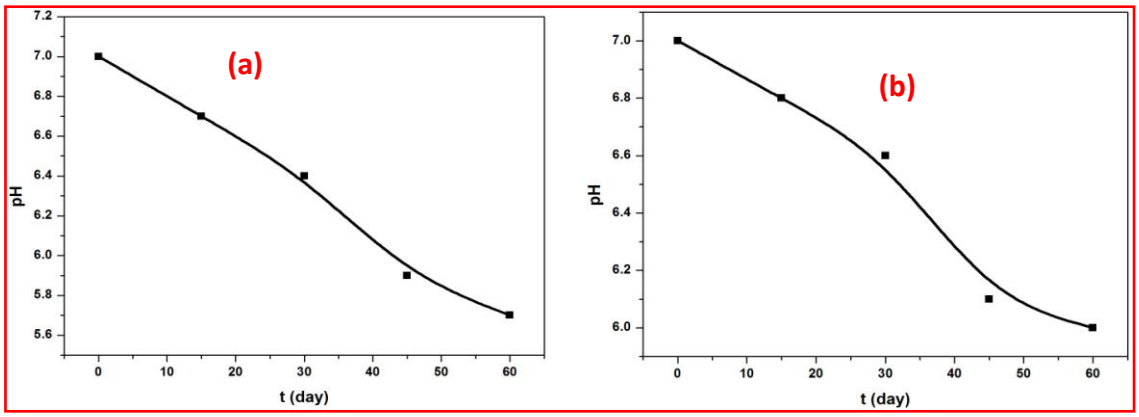

Fig.5.4 Change in pH of HDPE (a) & LDPE (b) film

5.2.5 Measurement of change in contact angle

Contact angle of blank HDPE and LDPE was 102.3±2.6 and 98.6±2.6° respectively (Fig. 5.5a & 5.6a). There was a decrease in contact angle of both the films after thermal treatment and it has been found to be 95.2±2.6 and 91.5±2.6 ° for HDPE and LDPE respectively (Fig.5.5b & 5.6b). Again there was a decrease in contact angle after 60 days incubation with bacterium and contact angle was 80.2±2.6 and 73.0±2.6° for HD and LDPE films (Fig.5.5c & 5.6c) respectively. There was no decrease in the contact angle for the control set of samples. Reduction in contact angle confirms decrease in hydrophobicity of the films and attachment of bacterial strain on the surface. Decrease in contact angle and increase in hydrophilicity has also been reported by Sudhakar *et al.* 2008. In a study by Yang *et al.* contact angle of blank polyethylene film was decreased from 97.2 ± 1.6 to 69.3 ± 3.8° and 67.1 ± 1.6° after biodegradation with *Enterobacter asburiae* YT1 and *Bacillus* sp YP1 respectively. (Yang *et al.*2014).

Fig.5.5 Contact angle of blank (a), thermally treated (b) and biodegraded (c) HDPE film

Fig.5.6 Contact angle of blank (a), thermally treated (b) and biodegraded (c) LDPE film

5.2.6 Surface morphology

5.2.6.1 Scanning electron microscopy (SEM)

In our study, the HPDE and LDPE films showed rough surface with a number of cracks and grooves after 60 days incubation with bacterial culture. We observed some localized degradation of the films around the bacterial biofilm in the the SEM micrographs (Fig 5.7(a-d) & 5.8(a-d)). While untreated film retained a smooth surface under the same conditions. Abrasion of the surface can be seen but interestingly, though microoganisms spread surrounding the ruptures which results from crack propagation, not a one grow within the ruptures indicating that most of the nutrients of low molecular weight move to the surface from the (oxidised) layers of the polymer (Bonhomme *et al*. 2003). It indicates that bacteria secrete enzymes capable of degrading polyethylene, which result in grooves formation Bacterial films showed a cell-like molded pattern in the polyethylene. Such shapes have previously been noticed for biodegradable polymers, e.g. poly-*β*-hydroxybutyrate (Otake *et al*. 1995). In an another study SEM images showed an alteration in surface topology for PE films that were treated with bacterium *Enterobacter* sp. Skariyachan *et al*. (2014 & 2016). Yang *et al* reported that the surfaces of the controls remained smooth, without any defects, while after inoculation with strains YT1 and YP1, cavities on the surfaces of the PE sheets developed having maximum depths of approximately 0.3 and 0.4 μm respectively (Yang *et al* 2014).We can see from the figure 5.7 (a-d) and 5.8 (a-d) that density of biofilm is less on the PE surface after 15 days bio incubation with bacterial isolate, it increases with increase in the incubation period. Bacterial biofilm is maximum after 60 days on the surface of the film.

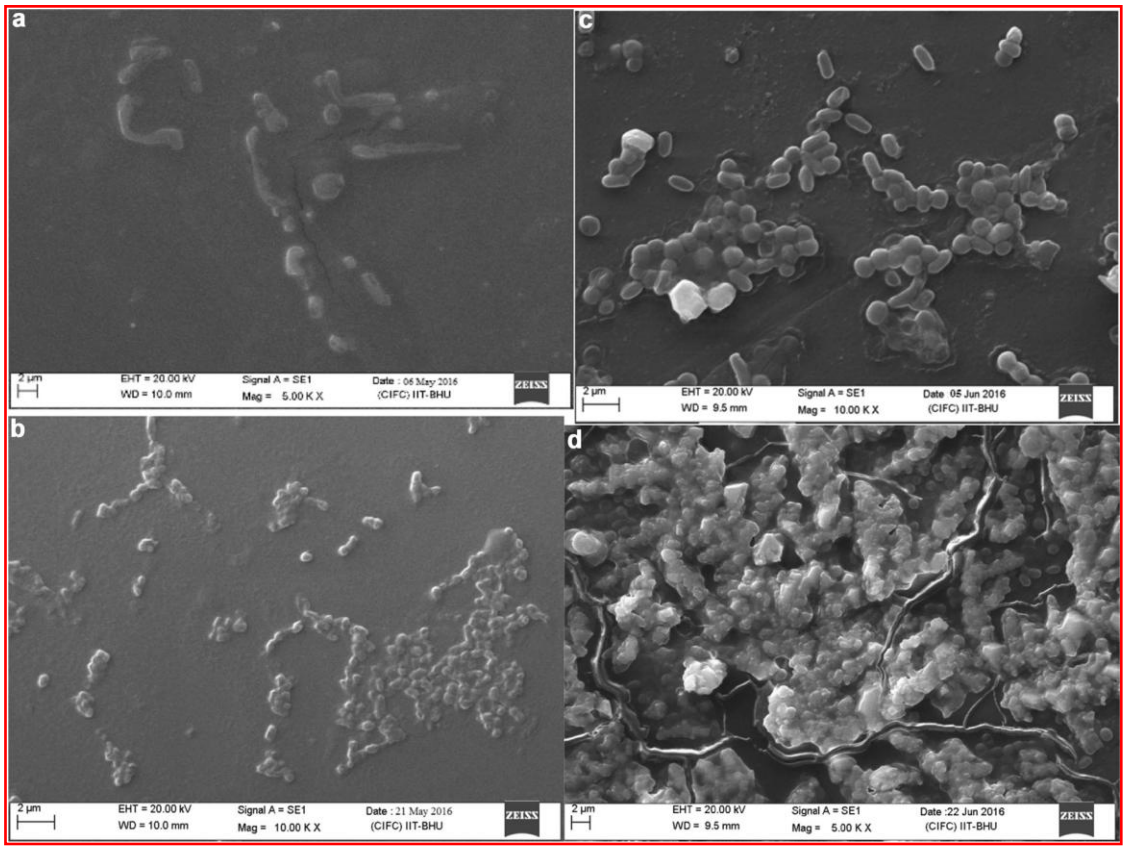

Fig.5.7 SEM image of HDPE after 15 days (a), 30 days (b) 45 days (c) & (d) 60 days

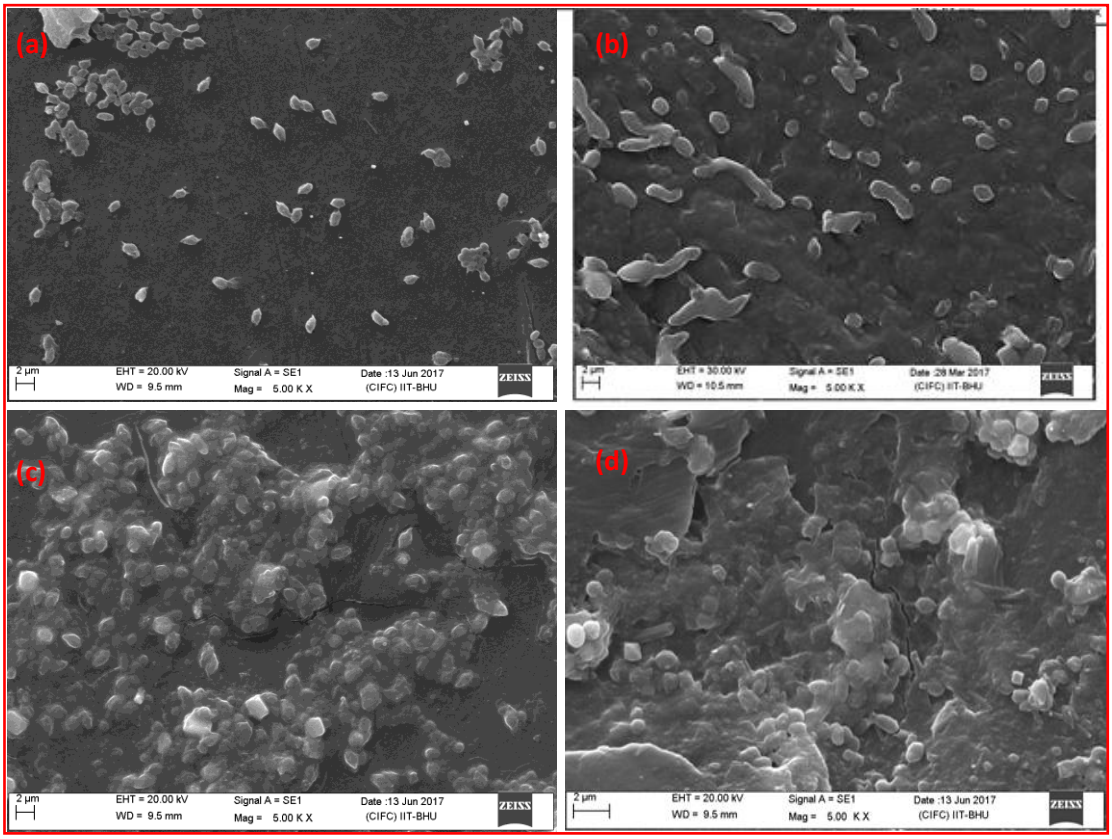

Fig.5.8 SEM image of LDPE film after 15 days (a), 30 days (b), 45 days(c) & (d) 60 days

5.2.6.2 Atomic force microscopy (AFM)

The change in surface roughness was studied by Atomic force microscopy. It was observed that roughness was increased after microbial incubation for 60 days (Fig. 5.9 a-b & 5.10 a-b). Roughness of HDPE increased from 32.2nm to 46.5 and of LDPE from 30.1 nm to 40.2 nm .These alterations are similar to SEM results. AFM results are consistent with the reports by Tribedi *et al* according to their study surface of the LDPE film was altered after treatment with *Pseudomonas* sp. AKS2 and cavities were generated. Similar results were obtained by yang *et al*. (Tribedi *et al*. 2013, Yang *et al*.2014 and Awasthi *et al*. 2017).

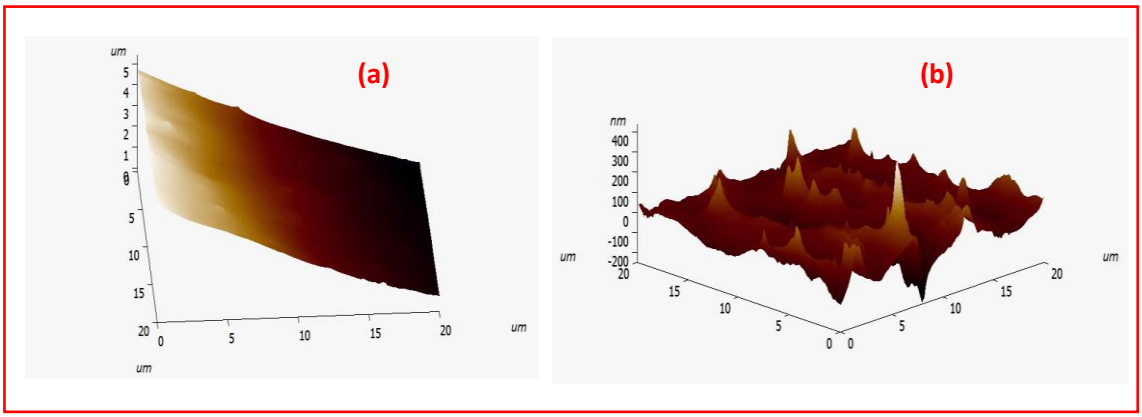

Fig.5.9 AFM images of blank (a), biodegraded (b) HDPE film

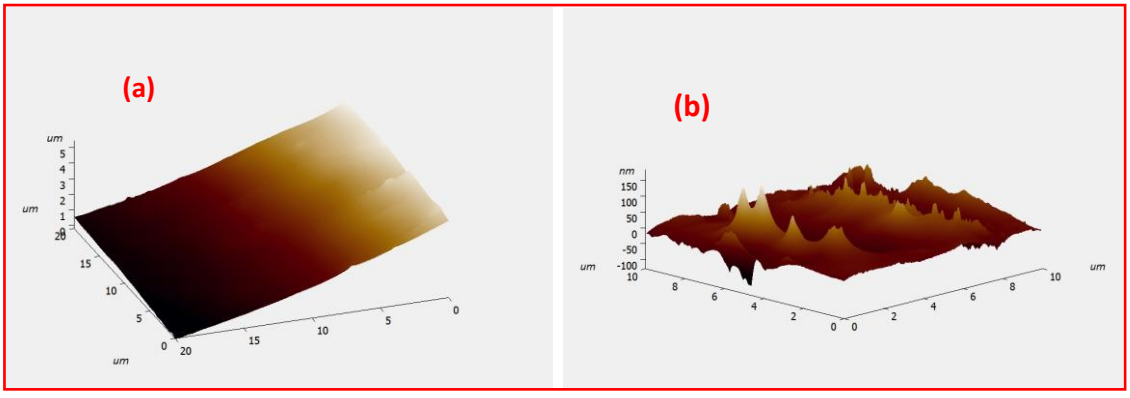

Fig.5.10 AFM images of blank (a), biodegraded (b) LDPE film

5.2.7 Fourier Transform Infrared Spectroscopy (FTIR)

The changes in bond scission, chemical transformation and formation and disappearance of new functional groups are the areas of interest that help us determine whether any changes in the chemical structure of the polyethylene has taken place or not ((Usha *et al*.2011 & Suresh *et al*.2011) was determined with the help of FTIR. In the present study, increase in carbonyl group (at 1720cm^{-1} for HDPE and at 1760cm^{-1} for LDPE) was observed for all samples during the thermal pretreatment at 70 °C for 10 days (Fig 5.11b & 5.12b). It is consistent with some previous studies Albertsson *et al*. Karlsson and Albertsson, Khabbaz *et al*. who reported that ester- and keto-carbonyls are the major products formed during abiotic oxidation of polymer under thermal oxidation or after oxidoreductase activity (Albertsson *et al*. 1987, Karlsson and Albertsson 1998, Khabbaz *et al*. 1998, 1999). It could be due to the formation of loose HDPE and LDPE chain fragments during the heat treatment. It is assigned to the C=O stretching vibration of a ketone group, which grows in intensity with prolonged aging. The disappearance/or utilization of carbonyl groups (i.e. 1720 cm^{-1} and 1760 cm^{-1}) might be due to the enzymatic attack of *Klebsiella pneumoniae.* It underwent higher oxidative products leading to the formation of carboxyl and double bonds when exposed to *Klebsiella pneumonia* CH001. Gajendiran *et al*, Das *et al*, and Balasubramanium *et al*. reported that carbonyl groups once formed can further be attacked by microorganisms, which results in decrease in carbonyl content (Gajendiran *et al*. 2016, Das *et al* 2015 and Balasubramanium *et al*. 2010).As the rate of degradation increases with the passage of time, the peaks get broader as several monomeric and oxidative forms of the polyethylene gets produced (Bonhomme *et al*.2003).

Bhatia *et al*. has carried out a similar work with a bacterial consortium, and a related shift in the peaks was observed. These peaks were observed due to the vibrations in the stretching of the O-H bond in alcohols and phenols. An absorbance range of 3500–3200 cm^{-1} corresponds to the presence of alcohols and phenols (Bhatia *et al*. 2014). It is consistent with Dolezel (1967) who also observed that the amount of carbonyl groups decreased with prolonged exposure to a microbial environment. Albertsson *et al*. and Weiland *et al.* also observed a reduction in the carbonyl group after 150 days of incubation with mixed fungal culture (Albertsson *et al*. 1987 and Weiland *et al*. 1995).

It is attributed to the fact that oxidized polyethylene molecules are hydrolyzed by the extracellular enzymes and fatty acids are produced which is further metabolized by ß-oxidation (Albertsson *et al*. 1987). It is confirmed with the new absorption bands at 3375cm^{-1} in biodegraded HDPE (Fig.5.11c) and at 3320cm^{-1} in biodegraded LDPE (Fig.5.12c) as observed in the present study due to the formation of carboxylated compounds.

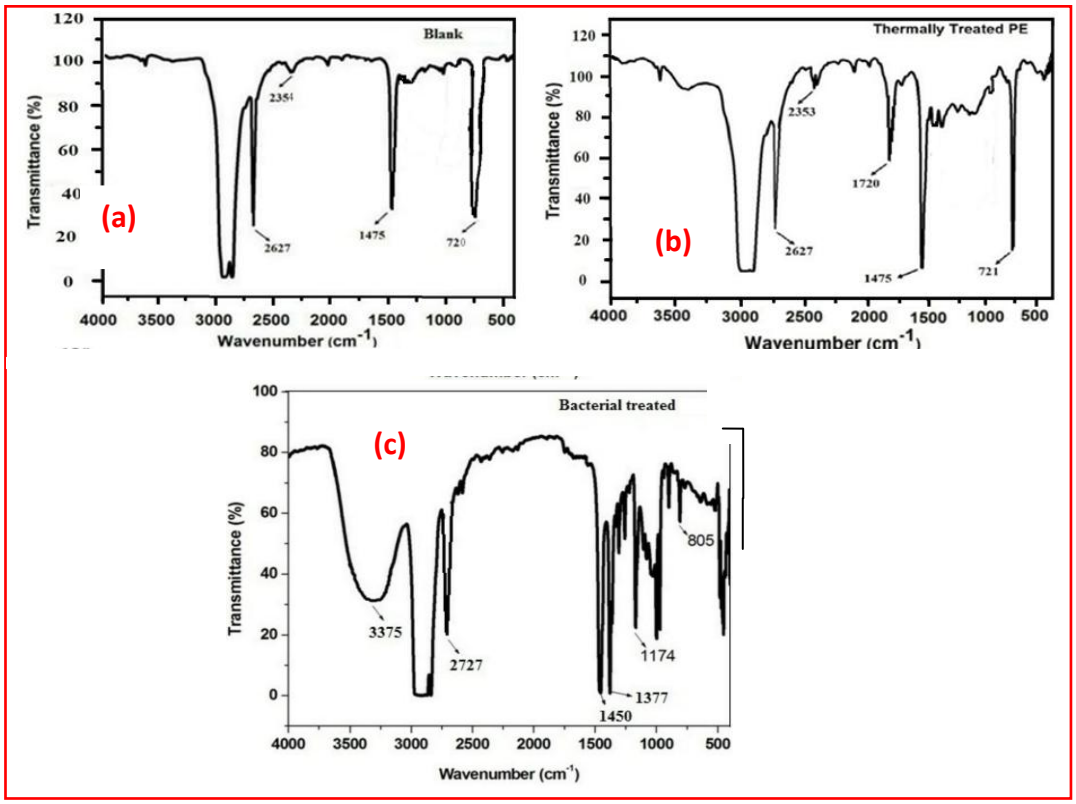

Fig.5.11 FTIR of blank (a), thermally treated (b) and biodegraded HDPE film(c)

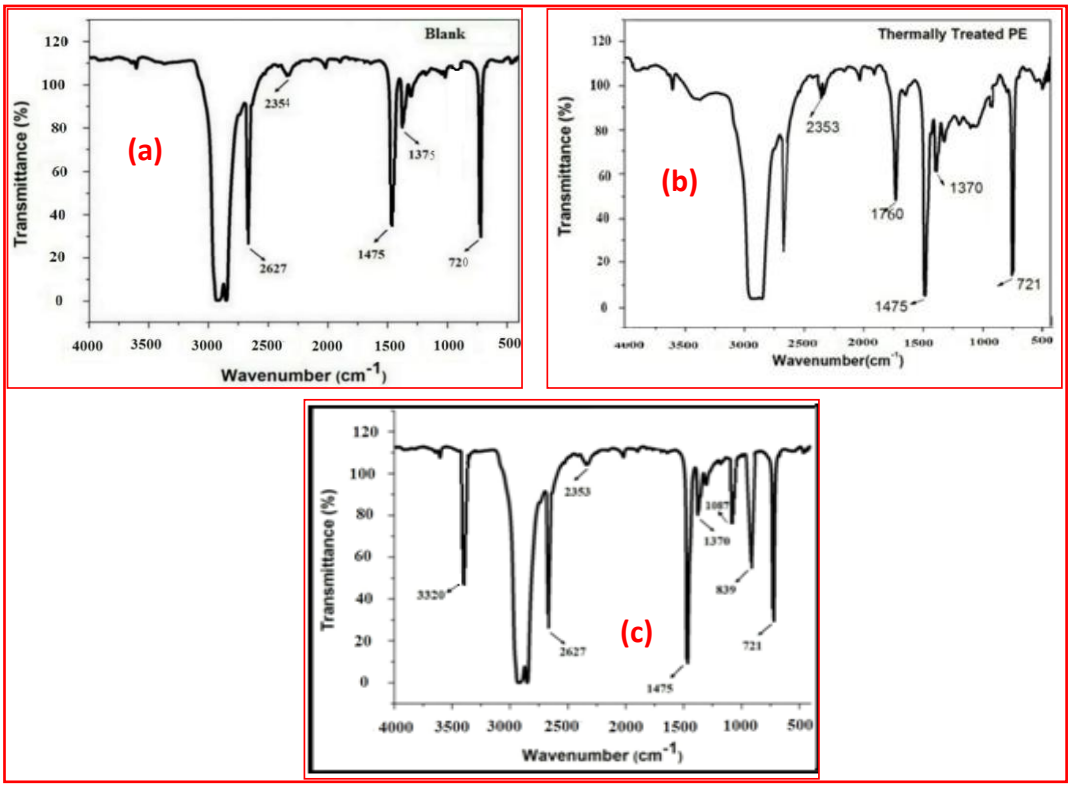

Fig.5.12 FTIR of blank (a), thermally treated (b) and biodegraded (c) LDPE film

5.2.8 Gas chromatography mass spectroscopy (GC -MS)

In order to find the intermediates formed during PE biodegradation GC-MS analysis was done. Compounds present in the largest amount in blank sample of HDPE are 1-trimethyl silylmethanol, Cyclopropanyl1,1-dimethyl- (Fig.5.13a) at 18.0 and 27.6 retention time respectively. After biodegradation cyclopropanetetradeconic acid, benzene dicarboxylic acid, acetic acid, hexanoic acid, octadeconoic acid, butanoic acid, oxamimidic acid, undecanonic acid, docosanoic acid, tetradecanoic acid and low molecular weight alkanes and alkenes like 2-Butene, 2-methyl, Ethene-1,2-dichloro-(2)-1,1 di, acetic acid ethyl ester, Methane, trichloro- are formed which are further consumed by *K. pneumoniae* (*cf* fig.5.13b).Presence of carboxylic acids, was consistent with FT-IR analysis (*cf* fig. 5.11c &5.12c). In blank LDPE there are cyclopropanol-1, 1-dimethyl, 2-propanone, hexanal and methane dichloro (fig.5.14a). Compounds observed after biodegradation are carboxylic acids: n-decanoic acid, docosanoic acid, undeconic acids, n-decanoic acid, hexadecanoic acid, propanoic acid, oleic acid, oxalic acid. 2-butene, 2-methyl, ethane dichloro, ethane trichloro and acetic acid ethyl ester (fig.5.14b). These results are consistent with the findings of Pramila *et al.* and Mahalakshmi *et al* (Pramila *et al.* 2015, Mahalakshmi *et al*. 2012). Carboxylic acids underwent further degradation by *Klebsiella pneumoniae* sp. and alkanes are produced such as ethane, pentane, decane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, undecane, dodecane, and nonadecane. This is similar with the results of Albertsson *et al.* who observed that the long chains of PE films were cut into small pieces as alkanes and carboxylic acids (Albertsson *et al.* 1987).

Koutny *et al*. reported the release of low molecular weight compounds to media from thermo- and photo-oxidized HDPE (Koutny *et al.* 2006). They observed that these

substances were consumed completely by *Rhodococcus rhodochrous* strain during 4 days of incubation. Konduri *et al.* observed that alkanes and carboxylic acids (i.e. ethane, pentane, decane, acetic acid, n-deconic acid, propanoic acids, oleic acids, and oxalic acids were oxidized/degraded by *A. terreus* MF12 by β- oxidation (Konduri *et al.* 2010).

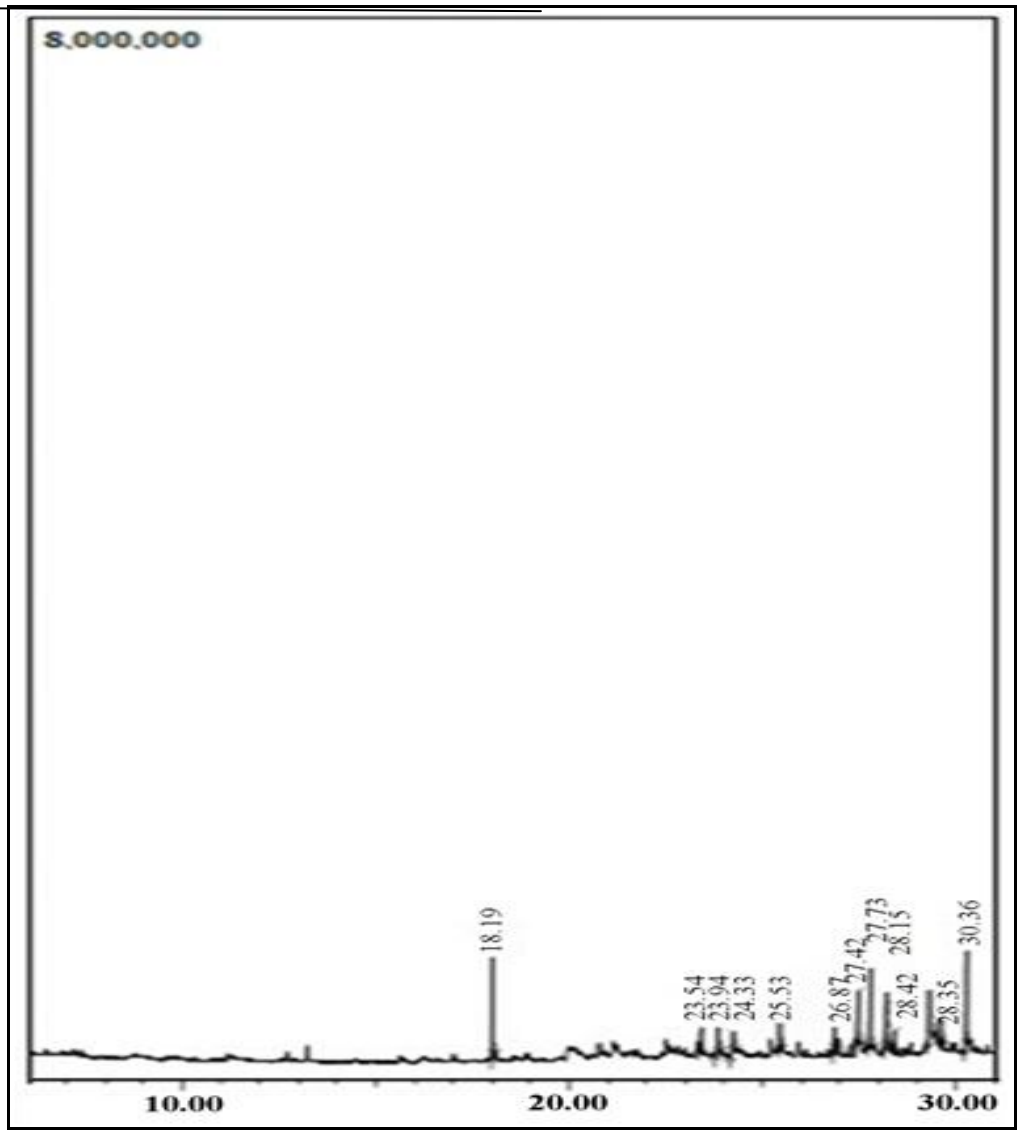

Fig. 5.13(a) GCMS of blank HDPE

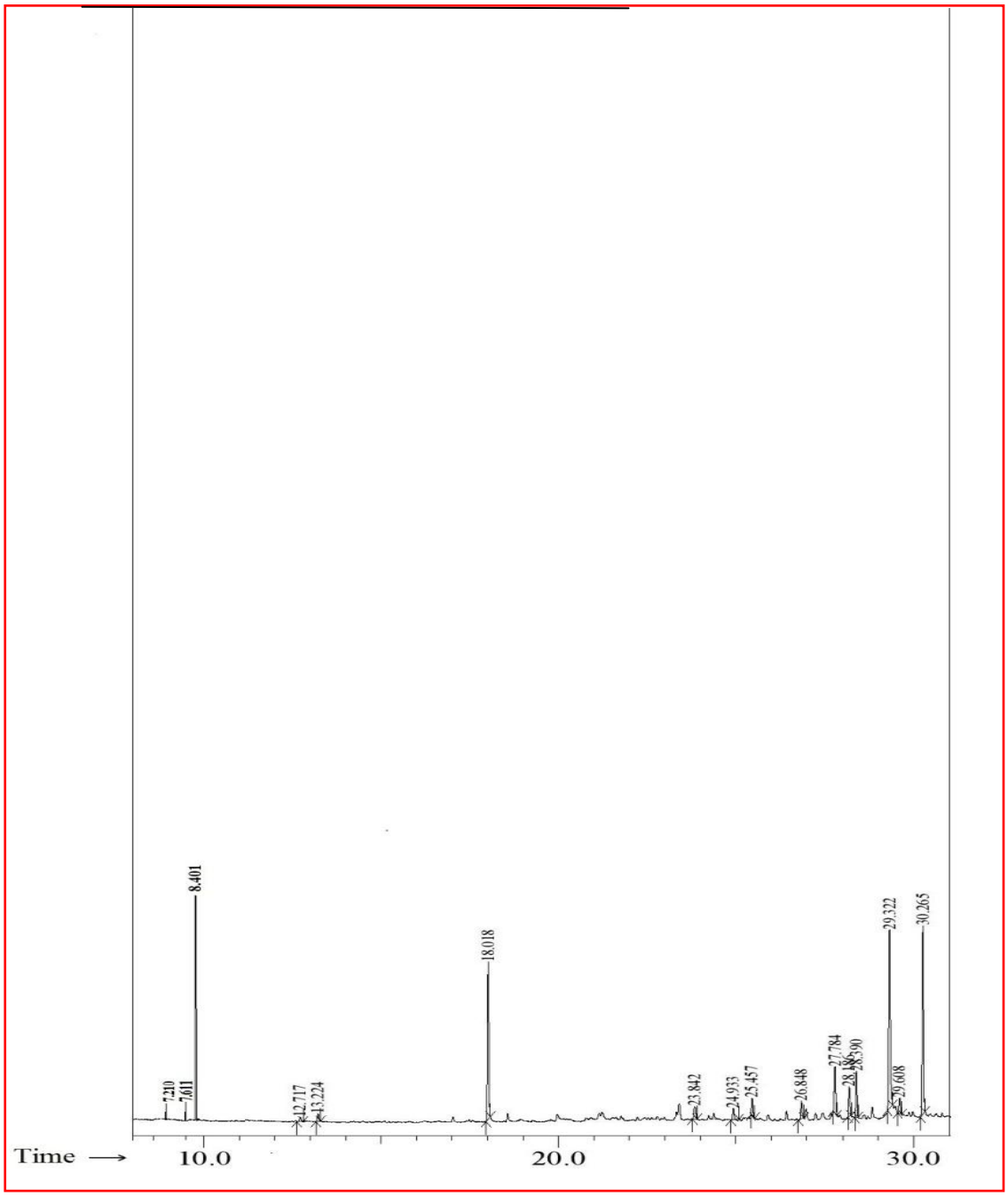

Fig. 5.13(b) GCMS of HDPE after biodegradation

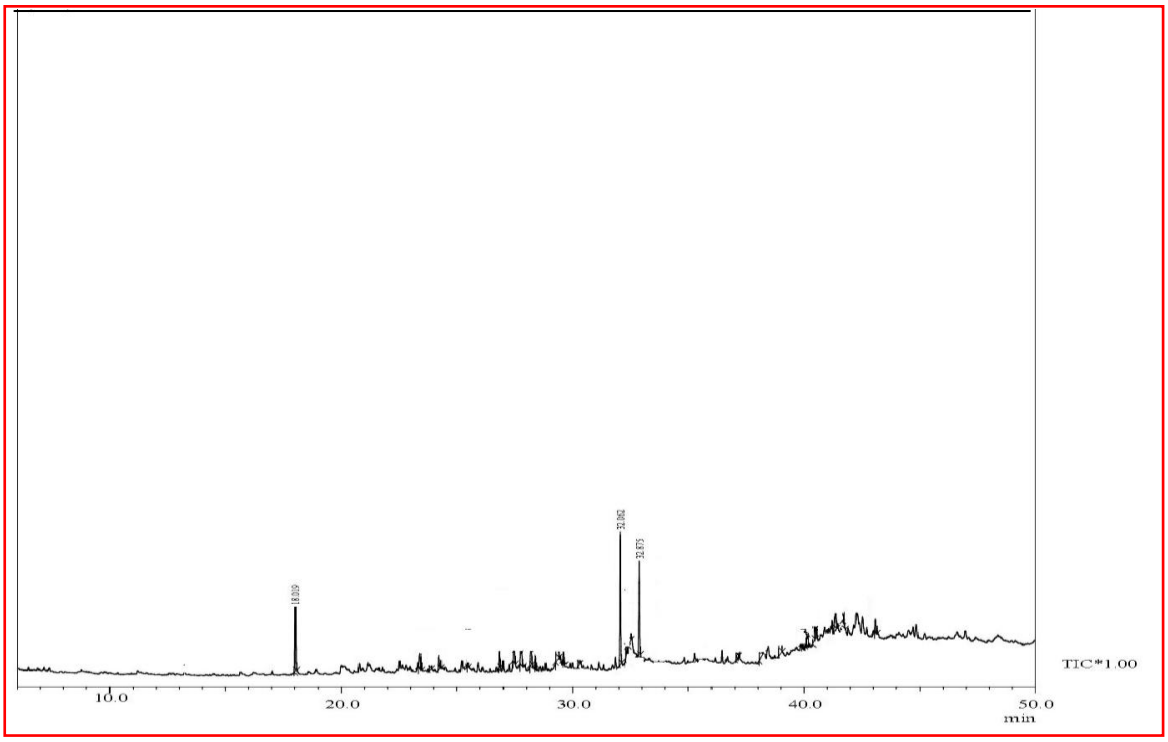

Fig. 5.14(a) GCMS of blank LDPE

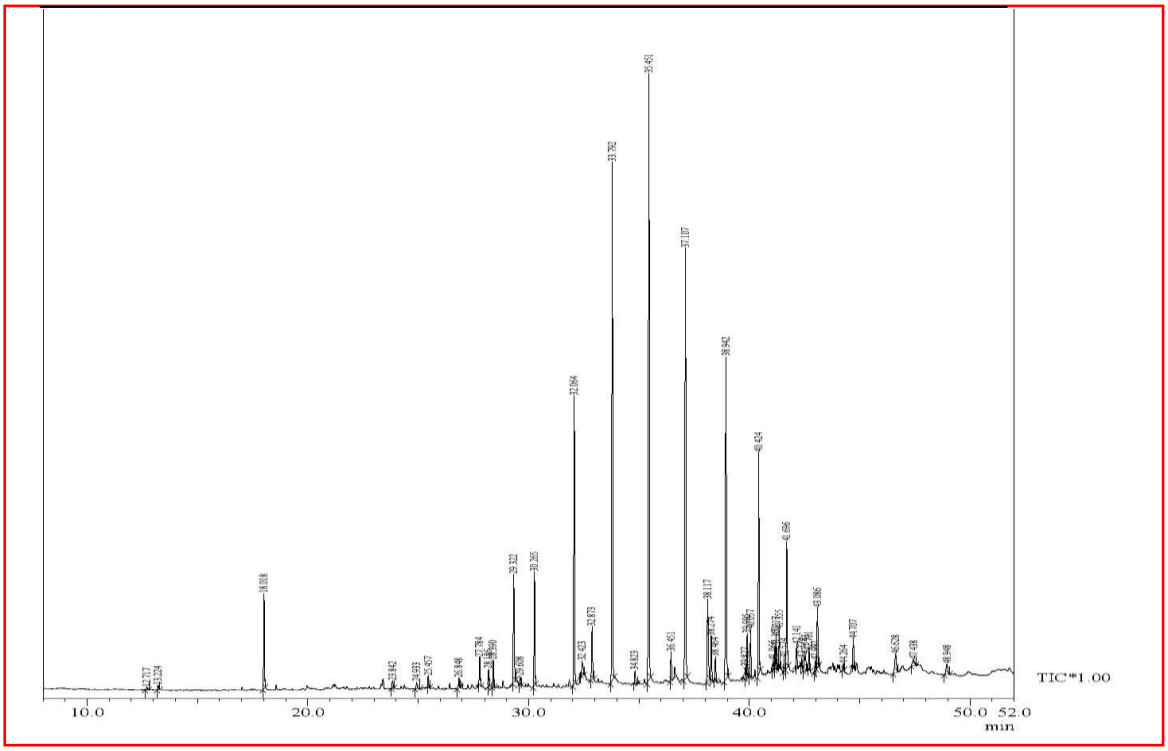

Fig. 5.14(b) GCMS of LDPE after biodegradation

5.2.9 Optimization of physico-chemical parameters

5.2.9.1 Optimization of pH

Growth and enzymatic activities of a microorganism very much depends on pH. So study of biodegradation by *K. pneumoniae* CH001 was carried out at different pH values (from 3 to 10). Figure 5.15(a-b) shows the effect of pH on degradation of HDPE and LDPE films respectively. It was observed from the figures that minimum degradation achieved at pH 3 was below 1% (i.e. 0.7±0.3% and 0.3±0.3%) whereas degradation efficiency improved drastically when pH was increased from 5.0, wt loss of 18.4 ±0.3 and 8.4± 0.3% for HDPE and LDPE respectively at pH 6.5 was found. Wt. loss of 17.6±0.3% for HDPE and 5.9±0.3% for LDPE was observed at pH 7.0.With further increase in pH efficiency of degradation again reduced, and only 2.6±0.3, 1.7±0.3% at pH 9 and 2.3±0.3, 1.0±0.3% wt loss was observed at pH 10 for HDPE and LDPE respectively. We can see that wt loss at alkaline pH range is more in comparison to that of acidic range. This might be due to the conducing environment for the growth of bacterial strain in alkaline environment.

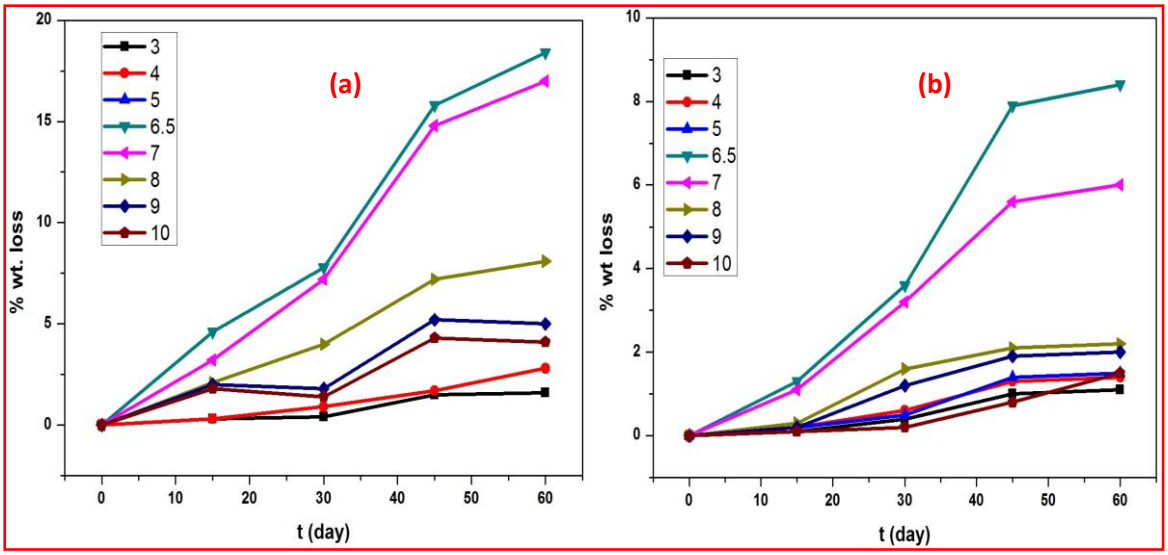

Fig.5.15 Effect of various pH on biodegradation of HDPE (a) & LDPE (b) by *K. Pneumoniae* CH001 in Nutrient Broth. Conditions: shaking condition, 10% inoculum size

5.2.9.2 Optimization of temperature

Temperature has a very significant role in growth and efficiency of bacterial strain. So, the performance of *K. pneumoniae* CH001 was evaluated in the temperature range of 25–50°C for degradation as shown in Fig. 5.16(a-b). It is clear from the figures that approximately 2.5 and 0.4% wt loss was obtained at 25°C for HDPE and LDPE respectively. However, with increase in temperature from 25 to 37°C there was an enhancement in the percent wt loss by bacterial culture, and 18.2 and 8.3% wt. loss of HDPE and LDPE respectively was observed at 37°C. With further increase in temperature, efficiency started decreasing resulting only 2.5 and 0.5% wt loss at 50°C. The lessened degradation efficiency beyond 37°C is possibly due to lowered metabolic activity of the microbe. The wt loss rate depends on temperature because at very low and high temperature (extreme conditions) inactivation of enzymes takes place.

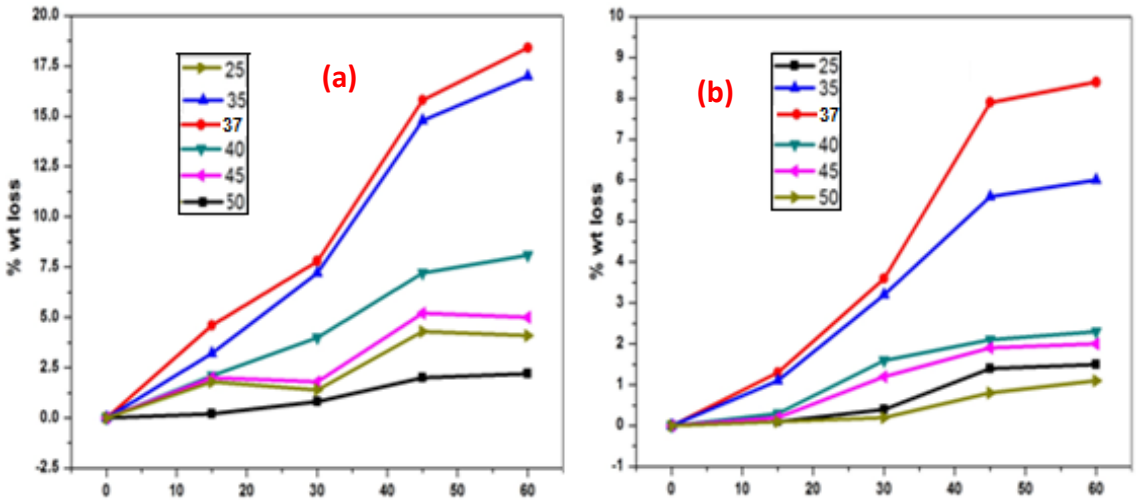

Fig.5.16 Effect of various temperatures on biodegradation of HDPE (a) & LDPE (b) by *K. pneumoniae* CH001 in Nutrient Broth. Conditions: shaking condition, pH 6.5, 10% inoculum size

5.2.9.3 Optimization of agitation speed

Figure 5.17(a- b) shows the effect of shaking conditions on degradation of HDPE and LDPE films. At low agitation speed (i.e.90 rpm) 3.1±0.3 and 1.0±0.3 % wt loss was observed for HDPE and LDPE respectively. It might be due to lower agitation speed results in the low amounts of dissolved oxygen in the cultivation medium leading to poor growth and decreased production of the enzymes. Thus it was demonstrated that agitation speed plays an important role in enhancing enzyme secretions because it alters the cell permeability. Wt loss was increased with increase in agitation speed upto 110rpm and maximum wt loss i.e. 18.2±0.3 and 8.1±0.3% was observed for HDPE and LDPE respectively (at 110rpm). Again there was decrease in wt loss at higher agitation (at 130 and 140 rpm). One possible explanation is that there are greater mechanical or shear forces at high agitation speeds, causing to a higher rate of cell destruction and lowering enzyme production (Venkatadri & Irvine 1990). Agitation speed higher or lower than 110 rpm resulted in decreased enzyme production and less bacterial activity.

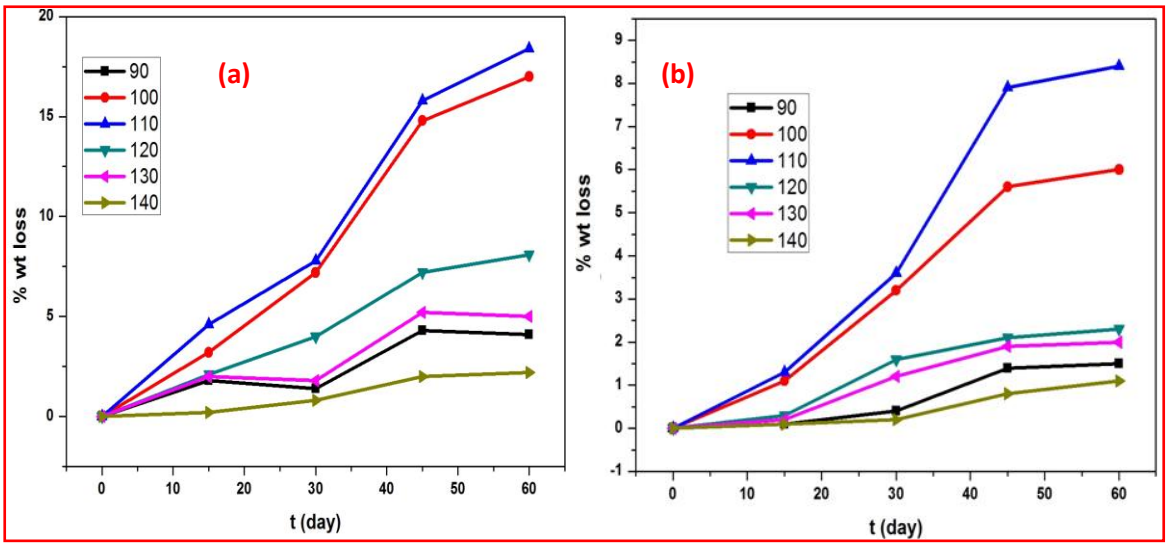

Fig.5.17 optimization of agitation speed for the biodegradation of HDPE (a) & LDPE (b) by *K. Pneumoniae* CH001 in Nutrient Broth. Conditions: Temp. 37°C, pH 6.5, 10% inoculum size.

5.2.10 Kinetics of biodegradation of PE films by *K. pneumoniae* CH001

Increase in the cell growth and decrease in the weight of the polyethylene films was studied at different time intervals. It has been observed that there was increase in the cell biomass with time and this increase was complemented by decrease in the weight of polyethylene films. The bacterial cells were viable upto 60 days on the PE surface utilising it as a carbon source, after 60 days there was very negligible loss in weight of PE films. This confirms that fungus utilises the films as carbon source for its growth.

Cell growth kinetics in a batch process can be represented by the eqn. :

$$dX/dt = \mu X_0 \quad \text{.....Eqn. (5.2)}$$

here X_0 represents the initial biomass, X is the biomass at different time (days) intervals, μ is the specific growth rate. μ for different PE films were determined from the slope of $\ln(X/X_0)$ against time, during the exponential growth phase as shown in Figure 5.18(a) and 5.19(a). The results show that the specific growth rate for HDPE is 0.0216 day^{-1} and for LDPE is 0.0161 day^{-1}. A fair fitting was obtained as can be seen in Fig. 5.18(a-b) and 5.19(a-b). The parameters with their values are shown in Table 5.1 and 5.2 with r^2 (regression coefficient) of 95%. It has been observed that there was decrease in the weight of the HDPE as well as LDPE films with time. Slope of the graphs (fig 5.18b and 5.19b) were calculated and found to be 9×10^{-5}gday^{-1} and 4×10^{-5}gday which represent degradation rate constant for HDPE and LDPE films respectively. The value of specific growth rate constant and degradation constant has been presented in table 5.1(a-b).

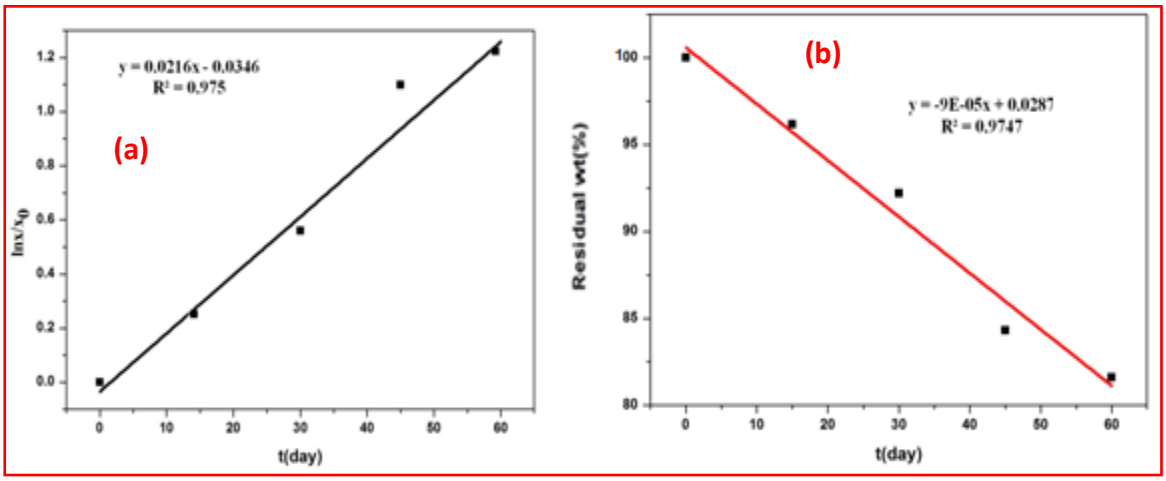

Fig.5.18 Increase in cell growth (a) and decrease in weight of HDPE film (b) with time

Table 5.1(a) Rate constants for cell growth and degradation of HDPE film

k(gday^{-1})	μ(day^{-1})	r^2(film)	r^2 (Bacterial growth)
9×10^{-5}gday^{-1}	0.0216	0.9747	0.9750

The values are the means of three replicates with the standard deviation which was within 5% of the mean

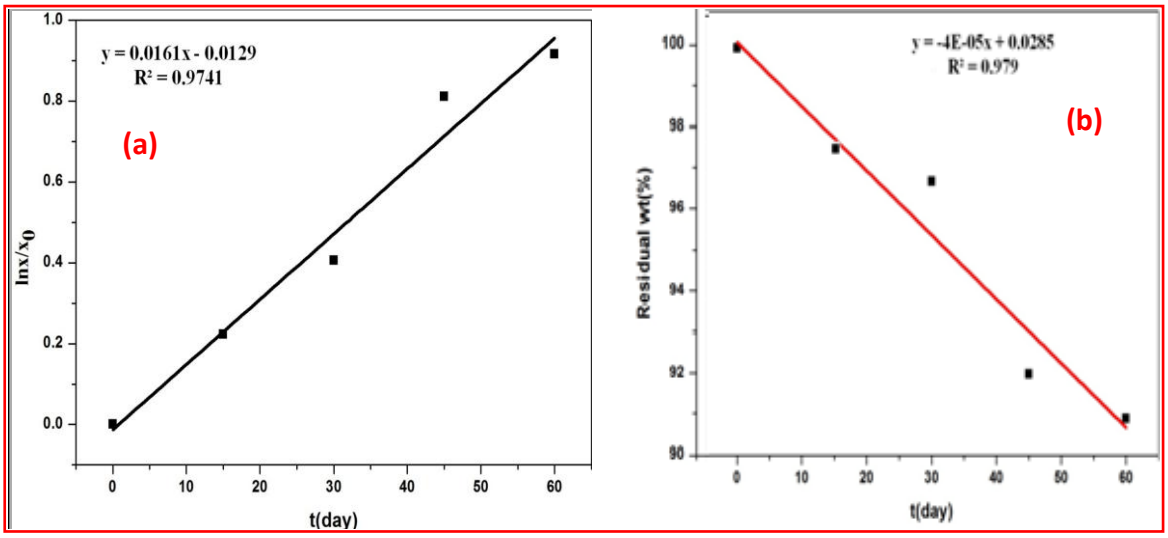

Fig.5.19 Increase in cell growth (a) and decrease in weight of LDPE film (b) with time

Table 5.1(b) Rate constant for cell growth and degradation of LDPE film

k ($gday^{-1}$)	μ(day^{-1})	r^2(film)	r^2 (Bacterial growth)
4×10^{-5}gday	0.0161	0.9790	0.9741

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

From the above graphs it is clear that growth of bacterium is maximum between 30 and 45 days which is complemented by maximum decrease in weight of polyethylene films (LDPE and HDPE) during the same period. It confirms that bacterium is utilising the polyethylene films for its growth as a carbon source. After 60 days growth of bacteria decreases due to exponential phase is followed by death phase and corresponding rate of polyethylene degradation also ceases.

5.2.11 Enzyme Assay

K. pneumoniae CH001 shows positive test for laccase and manganese peroxidase enzymes. These two enzymes are mainly responsible for the biodegradation of HDPE and LDPE. So the activity of laccase and manganese peroxidase was measured at regular time interval. There was a regular pattern of increase in enzymatic activity of laccase and manganese peroxidase. Maximum activity was found after 60 days of incubation. Activity of manganese peroxidase (0.00117 IU/ml) was more compared to laccase activity (0.00075 IU/ml) (Table 5.2a-b) for HDPE. Similar pattern was found with LDPE as activity of manganese peroxidase (0.00109 IU/ml) was higher than laccase (0.00059 IU/ml) after 60 days of incubation (Table 5.3a-b). Results obtained by the experiments performed by Sowmya *et al.* for the biodegradation of polyethylene by *Bacillus cereus* were similar to our findings (Sowmya *et al.*2014).

Table 5.2(a) Enzyme activity of Laccase for HDPE by *K. pneumoniae* CH001

Days	15	30	45	60
EnzymeActivity(Laccase) IU/ml	0.00023±0.0001	0.00039±0.0004	0.00060±0.0002	0.00075 ± 0.0003

Table 5.2(b) Enzyme activity of Manganese peroxidase for HDPE by *K. pneumoniae* CH001

Days	15	30	45	60
Enzyme Activity IU/ml	0.00035±0.0003	0.00069±0.0001	0.00106±0.0004	0.00117±0.000

Table 5.3(a) Enzyme activity of Laccase for LDPE by *K. pneumoniae* CH001

Days	15	30	45	60
Enzyme Activity (IU/ml)	0.00017±0.0001	0.00027±0.0004	0.00041±0.0002	0.00059±0.0003

Table 5.3(b) Enzyme activity of Manganese peroxidase for LDPE by *K. pneumoniae* CH001

Days	15	30	45	60
Enzyme Activity (IU/ml)	0.00026±0.0003	0.00052±0.0001	0.00089±0.0004	0.00109±0.0002

5.3 Conclusion

In the present study, lab isolate bacterial strain, *K. pneumoniae* CH001 (ITCC no. MF399051) has the potential of not only forming the biofilm to the surface of high density and low density polyethylene but also availing it as the source of carbon for its

growth. The degradation has been confirmed by weight loss, morphological changes in HDPE and change in functional groups. Even though it is a slow technique, the study gives an insight into the process of biodegradation of PE films. Further, the knowledge of the enzyme device of *K. pneumoniae* CH001 provides an insight to their role in biodegradation process. Efforts on elucidating the pathway for degradation of high and low density polyethylene required to develop a new bioremediation strategy using this bacterium.