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Biotic oxidation of polyethylene by using bio-surfactant produced by *B.licheniformis:* A novel technique.

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Abstract:

Polyethylene was incubated with bio-surfactant producing bacterium *B.licheniformis* for 2 months in a suitable media. Lower concentrations of NaCl were added to study its effect on bio-surfactants activity. Being amphiphilic, surfactant has the unique ability to decrease surface energy and this decrease measured through surface tension of the medium was 50%. Surfactant was able to oxidize both control (unoxidized) and pre-oxidized polyethylene during incubation. Oxidation level of control polyethylene sample increased in the presence of NaCl and oxidation level was higher in the presence of 1% of NaCl than that of 0.5% NaCl. Higher amount of surfactant was also produced, observed as comparatively low surface tension in the presence of NaCl. During the bio-oxidation of polyethylene, higher amount of unsaturated hydrocarbons were formed than carbonyl group. This oxidation was also observed through reduced crystalline property and cracked polyethylene surface under SEM. It was also observed that the oxidation product formed during oxidation by bio-surfactant was solubilising into the liquid media. For this rapid loss of oxidation product, deterioration of mechanical property of all the treated polyethylene samples was observed and this deterioration was highest in case of pre-oxidised polyethylene incubated with bio-surfactant for 2 months. In this study, a novel unique method of bio-oxidation of polyethylene by bio-surfactant was established.

A

1. Introduction:

The use of polyethylene films rise immensely in the last decade for its low cost, strong mechanical property with relatively lower thickness, multiple chemical resistances and

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longevity. Due to the absence of any polar groups in closely packed carbon-hydrogen backbone and high molecular weight, polyethylene is resistant towards abiotic oxidation and microbial attack under natural condition. Therefore, polyethylene once produced, does not degrade naturally, resulting in high amount of waste polyethylene accumulation (25 million tons per year) in the environment. This environmental pollution becomes one of the most important concerns of researchers and lots of studies have been done to find a way for degradation of polyethylene waste [1, 2]. Decades of researches have shown that biotic i.e. microbial degradation of polyethylene is the only way for environmental friendly degradation process. Microbial degradation is mainly achieved by formation of bio-film on the polyethylene surface. Lots of microbes i.e. *Lysinibacillus xylanilyticas, Aspergillus niger, Penicillium pinophilum, Rhodococcus ruber* have been used for microbial degradation of polyethylene[3, 4, 1]. To enhance microbial attachment to the polyethylene surface, hydrophobic property of polyethylene should be changed into hydrophilic one through abiotic oxidation [1]. U.V has been used in several studies for abiotic oxidation of polyethylene to introduce polar groups in the polymer backbone and thereby to reduce the hydrophobic property; for example, polyethylene containing pro-oxidant has been oxidised by using U.V. for 60 hour with the increase in the carbonyl index [1, 3, 5]. In another study, polyethylene mixed with pro-oxidant additives has been oxidised by U.V and highest oxidation level is achieved after 600 hour[2]. Another way to increase degradation rate, other than the pre-oxidation step is by increasing contact surface between polyethylene and water by adding surfactant in the biodegradation system.

Surfactants are wide varieties of surface active amphiphilic (both hydrophilic and hydrophobic part is present in the same molecule) molecules [6]. Surfactants have the ability to reduce surface tension and interfacial tension of the solution. Bioavailability of non-soluble/ hydrophobic material is enhanced by the reduction of surface tension. Surfactants also have the ability to enhance solubility of petroleum hydrocarbons. Surface tension reduction ability of surfactants can be used to increase biodegradation of polyethylene. In some of the polyethylene biodegradation studies chemical surfactant has been used into the system. Increased biodegradation of pre-oxidised polyethylene has been reported in soil after addition of surfactant into the system [7]. In another study, enhanced bio-film formation has been observed by adding tween 80 in the polyethylene biodegradation system containing *Pseudomonas aeruginosa* [8]. Another types of surfactant known as bio-surfactant, are produced by different microbes[9]. Main advantage of bio-surfactants over chemical surfactants is its environmental friendly nature and they are also bio-degradable. One such bio-surfactant producing bacterium is *Pseudomonas aeruginosa* and its bio-surfactant is called rhamnolipid [10, 11]. This specific bacterium has been used to study biodegradation of polypropylene and bio-film formation has been enhanced by rhamnolipid production [12]. Bio-surfactant producing bacteria *Bacillus pumilus*, *B. halodenitrificans*, *B. cereus* have been used for biodegradation of pre-oxidised polyethylene containing pro-oxidant and enhanced biodegradation has been reported [2]. There are mainly 6 types of bio-surfactants produced by bacteria, among which Lipopeptides are most important and effective bio-surfactants for high surface activity, mainly produced by different *Bacillus* strain [13, 6]. Such bio-surfactant producing bacterium is *Bacillus licheniformis,* isolated from oil reservoir [14]. This bacterium also has been used to enhance the oil recovery through the production of biosurfactant [15, 16, 5]. Bio-

surfactant produced by this bacterium is known as Lichenysin and is a lipopeptide, which is reported for its ability to reduce the surface tension[16]. *B.licheniformis* can be used for changing hydrophobic property of polyethylene into hydrophilic one by the action of bio-surfactant produced by this bacterium in the presence of polyethylene. For this treatment, a suitable growth medium should be used for optimum production and optimum activity of the bio-surfactant. Previously, it is reported that higher amount of bio-surfactant is produced by *B.licheniformis* in a growth medium without any trace of NaCl compared to the medium containing 0.5% NaCl [17]. But, in another study, 5% NaCl concentration in a growth medium is reported as optimum condition for maximum production of bio-surfactant [16].

Bio-surfactant producing *Bacillus licheniformis* has not yet been used to study its effect on the oxidation of polyethylene. Oxidation of unoxidized polyethylene and chemical modifications of pre-oxidized polyethylene by the bio-surfactant produced by *B.licheniformis* is reported in this study in the presence or absence of NaCl during the incubation of polyethylene for 2 months.

2.Materials and Methods:

2.1.Test materials:

Daily used 0.01 mm thick, transparent colourless polyethylene bags were collected from the waste bins of Kolkata Municipal Corporation. Bags were then cut into rectangular pieces (5mm × 5mm) and washed vigorously with soap water and distilled water, consecutively to remove any debris and bio-material attached with the polyethylene surface. Rectangular pieces were then dried at 60°C overnight in a hot-air oven. These unoxidised polyethylene films were used as control polyethylene films.

For heat-U.V. treatment, rectangular pieces of polyethylene films were taken into a beaker and kept in custom made chamber where the temperature was kept at 60˚C under continuous U.V. light for 1 month. Wavelength of the U.V. light was within a range of 350 nm to 200 nm.

2.2.Microbial Culture:

Bacillus licheniformis JF2 (ATCC No. 39307, MTCC No. 2454) was used for bio-treatment study. This microbial culture was obtained from Institute of Microbial Technology, Chandigarh, India. Microbial culture was maintained in nutrient broth (Himedia). Bio-treatment was carried out in YPD medium containing 10 g of yeast extract, 20 g of glucose and 20 g of peptone in 1 litre of double distilled H_2O at 37°C for different time period. 0.5% and 1% of Sodium Chloride (NaCl) was added to the YPD medium incubated with *B.licheniformis* containing control polyethylene to study its effect on the stability of the bio-surfactant and its ability to reduce the surface tension. Control polyethylene samples incubated with *B.licheniformis* in the YPD growth medium without NaCl and with NaCl of 1%, 0.5% and U.V. treated polyethylene films incubated with *B.licheniformis* in YPD growth medium were kept for 2 months. After 1 and 2 months, samples from each case were harvested, washed and dried. For negative control, polyethylene samples were kept in YPD growth medium without any bacterial sp. All the samples were incubated at 37˚C and in triplicate.

Surface tension (σ) of microbial culture medium was measured by stalagmometer at 25°C at day zero and at

different intervals of time[18]. Surface tension was calculated by following formula.

$$\sigma_{water} \times \frac{weight\ of\ N\ drops\ of\ solvent}{weight\ of\ N\ drops\ of\ water} = \sigma_{solvent}$$

σ_{water} is the surface tension of distilled water at 25°C and $\sigma_{solvent}$ is surface tension of solvent. N is the number of drops and this was same for both water and solvent. Every measurement was done in triplicate.

2.3. Characterization of Polyethylene:

FTIR analysis was carried out with ATR-FTIR (model alpha, Bruker, Germany) spectrometer, scanning from 4000 cm^{-1} to 500 cm^{-1} at room temperature. The resolution was set at 4 cm^{-1} with 42 scans per spectrum. Carbonyl index (C.I.) and double bond index (D.B.I.) were calculated using the ratio of absorbance frequency of the carbonyl peak (1740 cm^{-1}) and double bond (1650 cm^{-1}) to that of the CH_2 group bending frequency (1465 cm^{-1}) respectively.

All polyethylene samples were sputter coated with gold layer by a Hitachi sputter coater (model-E1010 Ion Sputter), Japan. Photomicrographs were observed under scanning electron microscope (EVO 18, Carl Zeiss, Germany).

X-ray diffraction study of all types of polyethylene samples were recorded with an X-ray diffractometer (PANalytical, Netherlands) at an angle of 2θ from 3° to 50° and a fixed scan rate of 1° min^{-1}. Percentage (%) of crystallinity was calculated by using the following formula.

$$\%Crystallinity = \frac{Area\ under\ crystalline\ peaks}{Total\ \ Area\ under\ all\ \ peaks} \times 100\%$$

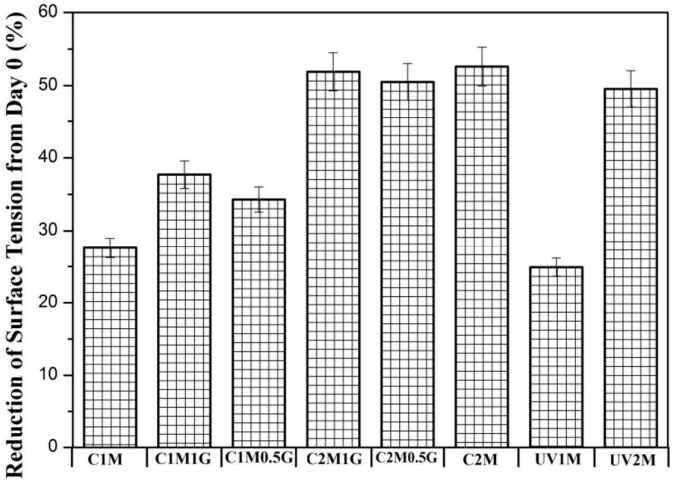

Figure 1: Surface reductions. **C1M**-control polyethylene incubated for 1 month with *B.licheniformis* without NaCl ; **C1M1G**-control polyethylene incubated for 1 month with *B.licheniformis* in the presence of 1% of NaCl; **C1M0.5G**- control polyethylene incubated for 1 month with *B.licheniformis* in the presence of 0.5% of NaCl; **C2M1G**- control polyethylene incubated for 2 months with *B.licheniformis* in the presence of 1% NaCl; **C2M0.5G**- control polyethylene incubated for 2 months with *B.licheniformis* in the presence of 0.5% of NaCl; **UV1M**- UV treated polyethylene incubated for 1 month with *B.licheniformis*; **UV2M**- UV treated polyethylene incubated for 2 months with *B.licheniformis*.

All samples were observed under AFM (Bruker- Germany, model: Innova) without doing any kind pre-treatment. Depth analysis of the surface of treated polyethylene was calculated by 'Nanoscape anaylsis' software.

Tensile property of all polyethylene samples were tested by a tensile tester (Tinius Olsen H5KT, ASTM D638 standard) at 23°C with a cross head speed of 20 mm/min. All eight samples were cut into rectangular shape of 100 mm in length and 10 mm in width and 0.01 mm thick.

3. Results and Discussion:

3.1. Surface Tension:

Reduction of surface tension from day 0 in percentage is presented in Figure 1. Minimum surface tension is achieved after 2 months of incubation with *B.licheniformis* with both control and pre-oxidized polyethylene with or without NaCl. The surface tension reduction from day 0, after 2 months of incubation with *B.licheniformis*, are 51.9% in case of YPD medium with control polyethylene and 1% of NaCl, 50.5% in case of YPD medium with control polyethylene and 0.5% of NaCl, 52.6% in case of YPD medium with control polyethylene and without NaCl and 49.6% in case of YPD medium with UVPE.. After 1 month, minimum surface tension is achieved in case of YPD medium incubated with *B.licheniformis* in the presence of 1gm of NaCl and control polyethylene. Presence of surface active molecules can be indirectly predicted from this reduction of surface tension. In a previous study, surface tension reduction from 70mN/m to 58.8 mN/m in the presence of pro-oxidant containing polyethylene by *B. pumilus, B. halodenitrificans* and *B. cereus* is reported [2]. But in the present study, the minimum surface tension achieved in the presence of control polyethylene (unoxidised) and UVPE by *B. licheniformis* is much lower than the previously reported one. NaCl was added in the growth medium to observe its effect on surface tension reduction ability of bio-surfactant. It is clear from the above mentioned result that after 1 month, lowest surface tension is observed in case of growth medium containing 1% of NaCl. As reported by previous studies, lower concentration of NaCl stabilizes the bio-surfactant and increases its activity; this may be the reason for observed comparatively lower surface tension by the bio-surfactant produced by

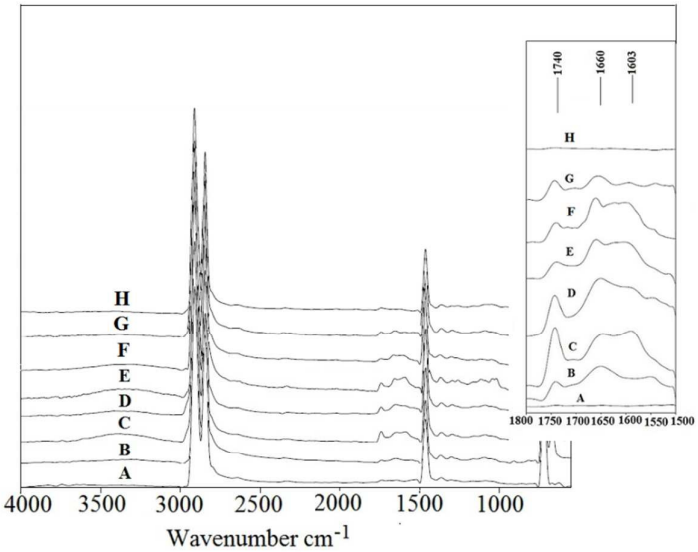

Figure 2: FTIR spectra of treated control (unoxidized) polyethylene. A-control PE, B-C1M; C-C1M1G; D-C1M0.5G; E-C2M1G; F-C2M0.5G; G-C2M; H-negative control.

B.licheniformis in the presence of 1% of NaCl[16]. After 2 months, minimum surface tension achieved which is slightly higher than reported minimum surface tension by *B.licheniformis*, is almost same in the all the cases of YPD medium incubated with *B.licheniformis* with control and pre-oxidized polyethylene with or without any NaCl[16]. May be for this reason presence of NaCl do not affect surface tension reduction ability of bio-surfactant after 2 months of incubation, though presence of NaCl stabilizes bio-surfactant produced by *B.licheniformis* in the presence of polyethylene after 1 month of incubation. In case of negative control, polyethylene kept in YPD medium without any bacteria, no change in the surface tension is observed during 2 month of bio-treatment time (Figure 13).

3.2.Characterization of Polyethylene Incubated with *B.lichenformis*:

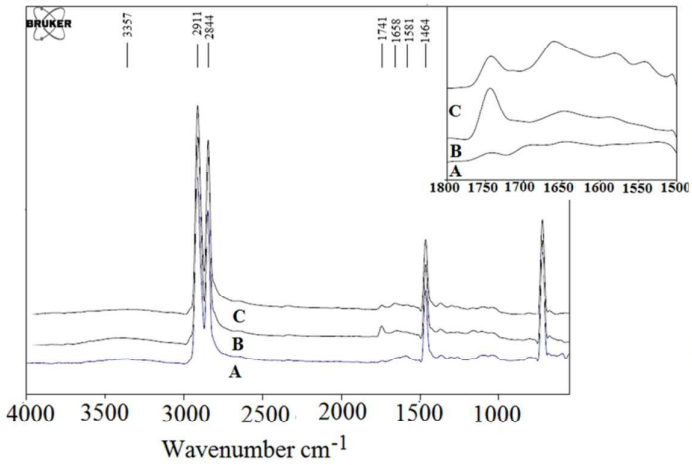

Figure 3: FTIR spectra of treated UVPE (Preoxidized) polyethylene. A-UVPE , B-UV1M, C-UV2M,

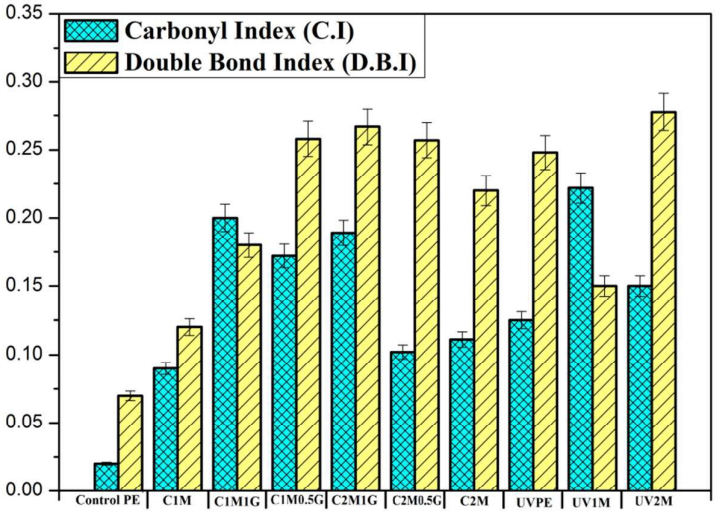

Figure 4: Carbonyl index and double bond index of treated and untreated polyethylene.

242 Control polyethylene films were incubated with *B.*
243 *licheniformis* for 1 (C1M) and 2 (C2M) months in YPD
244 medium without NaCl. Control polyethylene samples were
245 also incubated with *B.licheniformis* in YPD medium
246 containing 1% (C1M1G) and 0.5% (C1M0.5G) of NaCl for 1
247 month and 2 months (C2M1G for 1% NaCl and C2M0.5G for
248 0.5% NaCl) separately. To study the effect of surfactant on
249 pre-oxidized polyethylene sample, oxidized polyethylene
250 samples by U.V. were also incubated with *B. licheniforrmis*
251 for 1 (UV1M) and 2 (UV2M) months in YPD medium.
252 Polyethylene incubated in YPD growth medium without any
253 bacterial sp. was also characterized. No bio-film formation
254 was observed on the treated polyethylene sample, incubated
255 with *B.licheniformis*.

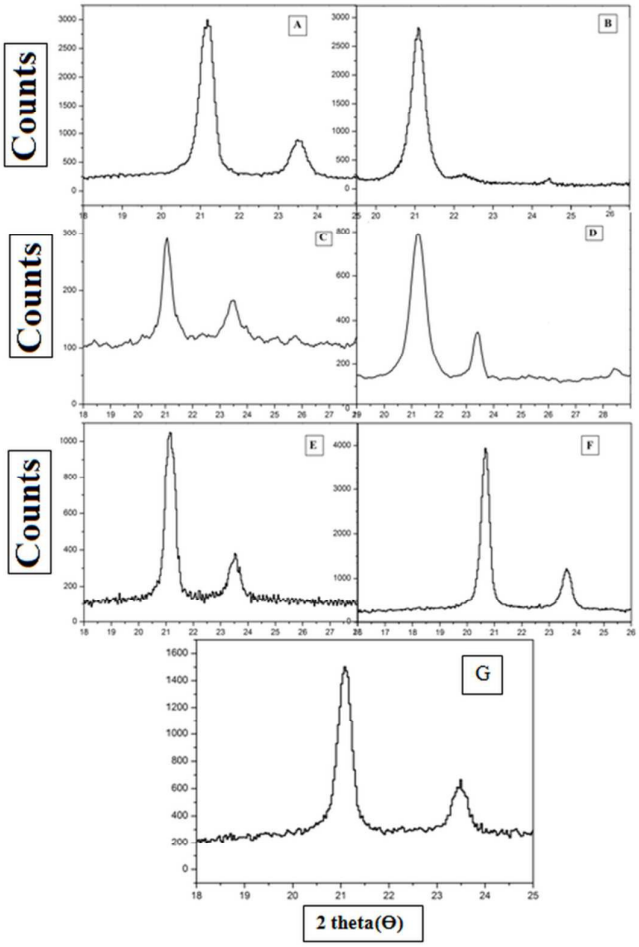

Figure 5: XRD spectra A-control PE, B-C1M, C-C1M1G, D-C1M0.5G, E-C2M, F-C2M1G, G-C2M0.5G

256 **3.2.1. FTIR Analysis:**

257 FTIR spectra of treated control (unoxidized)
258 polyethylene samples are compared to the untreated and
259 negative control one in Figure 2. Appearance of new peaks in
260 the 1800-1500 cm^{-1} region can be observed in all types of
261 treated control polyethylene samples. Another peak at 3400
262 cm^{-1} appears in case of C1M1G, which is due to –OH group
263 formation. Peaks at 1800-1500 cm^{-1} region are resulted by
264 overlapping of 1740 cm^{-1} (for ketones), 1730 cm^{-1} (for
265 aldehydes) and 1660 cm^{-1} for the formation of unsaturated

hydrocarbons (-C=C-). After 2 months of incubation of *B.licheniformis* in YPD medium, in case of C2M1G (1% of NaCl), C2M0.5G (0.5% of NaCl) and C2M (without NaCl) peak intensity at 1660 cm^{-1} (for unsaturated hydrocarbons) increase than that of the C1M1G, C1M0.5G and C2M respectively. In case of C2M peak intensity of 1740 cm^{-1} (for ketones) increases slightly from C1M and in case C2M1G and C2M0.5G peak intensity of 1740 cm^{-1} (for ketones) decrease from that of the C1M1G and C1M0.5G respectively (Figure 2). This indicates that more unsaturated hydrocarbons are formed compared to the ketone groups. In case of treated UVPE (Figure 3), after 1 and 2 months, peak intensity of 1800-1500 cm^{-1} region increase. In case of UV1M, an increase in the intensity of peak at 1740 cm^{-1} is observed due to the formation of ketones. But after 2 months, intensity of peak at 1740 cm^{-1} decreases and the intensity of peak at 1660 cm^{-1} increases. Carbonyl Index (C.I) and Double bond Index (D.B.I) of treated polyethylene films are compared to that of the untreated polyethylene i.e control PE and UVPE in Figure 4. Both of C.I. and D.B.I. of treated polyethylene films increases from that of the untreated polyethylene (control and UVPE) films. Formation of unsaturated hydrocarbons is much higher than that of the C=O bonds in case of all the treated control polyethylene except C1M1G as observed from FTIR spectra and C.I.-D.B.I graph (Figure 2, 4). But after 2 months, increase of D.B.I is observed in case of C2M1G, C2M0.5G and C2M. In case of UVPE, after 1 month, formation of C=O group is comparatively higher than formation of unsaturated hydrocarbons. But after 2 months, in case of UV2M, amount of C=O group is comparatively low than amount of unsaturated hydrocarbons. From this result, it is evident that the polar group are formed in the polymer backbone. After oxidation by means of U.V and heat, polyethylene shows similar changes observed through increased carbonyl index [1]. Bio-surfactant being an amphiphilic molecule has the ability to increase the solubilisation of hydrocarbons. Hydrophobic part of the bio-surfactant remains attached with the polyethylene surface with hydrophilic part protruding towards the aqueous solution. This phenomenon enhances polyethylene's availability to the dissolved oxygen, which further results in the oxidation of polyethylene. In case of pre-oxidized polyethylene samples, oxidation rate is higher than control polyethylene. Previously formed oxidation product present in pre-oxidized polyethylene may be helping in the bio-surfactant initiated oxidation. But after 2 months, already

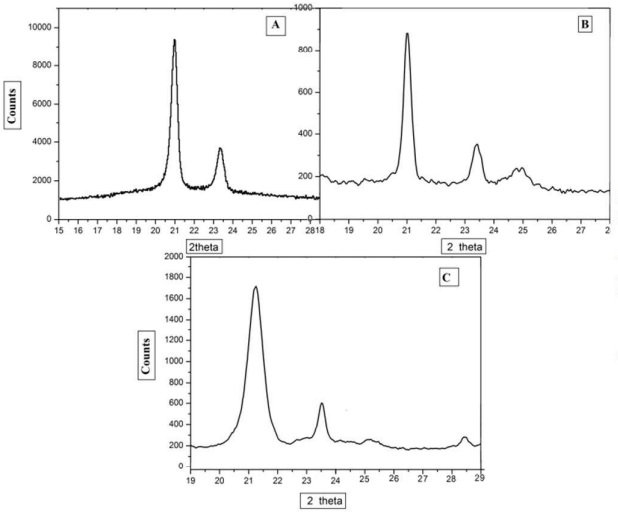

Figure 6: XRD spectra A-UVPE, B-UV1M, C-UV2M.

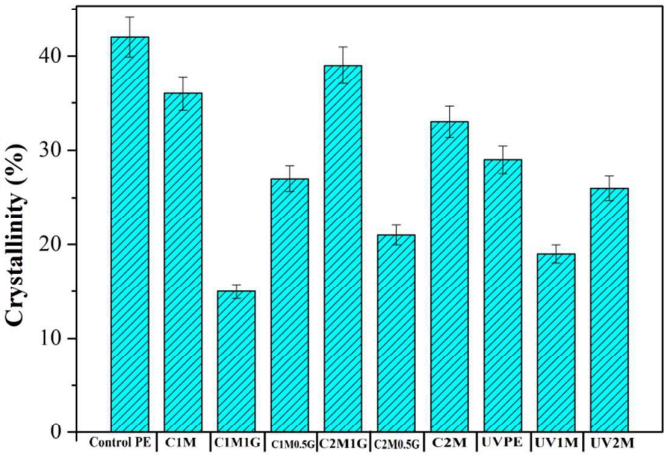

Figure 7: Crystallinity (%) of treated and untreated polyethylene

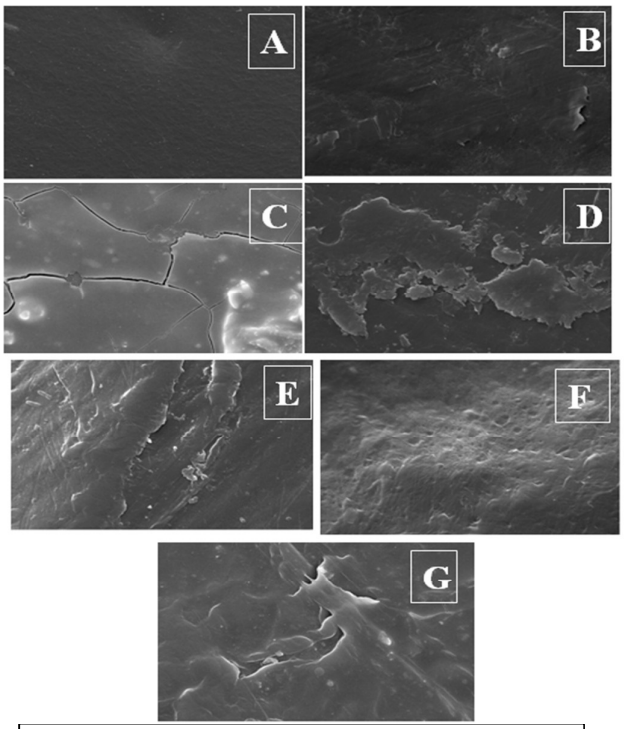

Figure 8: SEM Image A-Control PE, B-C1M, C-C1M1G, D-C1M0.5G, E-C2M, F-C2M1G. G-C2M0.5G

formed oxidation product may get solubilised into the aqueous medium, resulting in a decrease of the intensity of the peak at 1740 cm^{-1}. Another reason for this phenomenon can be conversion of carbonyl groups into double bonds due to its further oxidation. Similar phenomenon is also reported in another study, where the conversion of carbonyl groups into double bonds has been observed during degradation process by *Lysinibacillus sp*. In this study, 42% reduction of carbobyl index and 200% increase of double bond index is reported after 18 weeks of incubation with lysinibacillus sp with U.V irradiated films[3]. But this reduction in carbonyl index is 32% and increase in double bond index is 85% in case of UVPE incubated in the presence of bio-surfactant for 2 months which is much higher than previously reported data. In another study, such 75% reduction of carbonyl index of pre-oxidized polyethylene containing pro-oxidant by bacteria strain in 21 days is reported. Formation of unsaturated hydrocarbons after bacterial treatment is also reported in GC-MS studies[2].

No change in peak intensity is observed in case of negative control from the untreated control polyethylene.

3.2.2.XRD analysis:

XRD spectra of biologically treated samples are compared to the untreated control polyethylene sample in the Figure 5. In Figure 6, XRD spectra of biologically treated UVPE samples are compared to the untreated UVPE sample. Peaks at 21˚ and 23.5˚ are the characteristic peaks of semi-crystalline polyethylene molecule. Crystallinity in percentage (%), calculated from these XRD spectra of biologically treated polyethylene samples are represented in Figure 7. After biological treatment, crystallinity (%) of control polyethylene samples incubated with 1% and 0.5% of NaCl and UV treated polyethylene samples decrease from that of the untreated control polyethylene and UVPE samples after 1 month of bacterial incubation. Lowest crystallinity (%) is observed in case of C1M1G. After 1 month of bacterial treatment crystallinity decrease and 2 months crystallinity increase as observed in XRD analysis of C2M1G (Figure 7). In case of UVPE, similar trend is observed. But in case of control polyethylene incubated with *B.licheniformis* without NaCl and with 0.5% of NaCl, after 1 and 2 months crystallinity decrease from the untreated control polyethylene (figure 7). During the

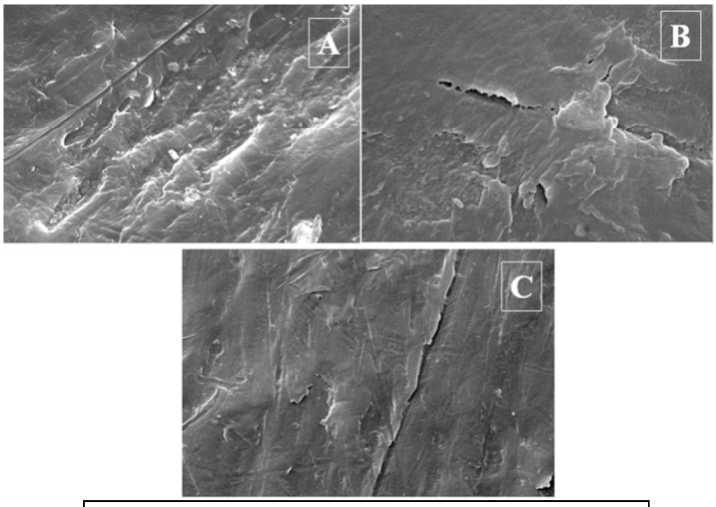

Figure 9: SEM Image A-UVPE, B-UV1M,C-UV1M.

process of oxidation by natural aging or by U.V., the crystallinity of polyethylene is found to increase during initial phase followed by decrease in the second stage. Increase in crystallinity during abiotic oxidation is observed with the increase in oxidation level [19, 20, 21]. Mainly Short hydrocarbon chains produced during the process of oxidation, can initiate secondary crystallization, due to which crystallinity increases consequently. Such increase due to secondary crystallization is a common phenomenon in oxidation by natural aging. Such phenomenon of increase in crystallinity (55%) after oxidation of polyethylene by natural aging and accelerated aging is reported by Benitez et al[22]. Solubilisation of oxidised amorphous part into aqueous medium can also result in the increase in crystallinity as reported by Sepulveda et al [2, 4, 19, 20, 21]. And during second stage decrease in the crystallinity is observed to further oxidation of crystalline phase. But in this study, reduction of crystallinity is observed during initial stage followed by increase in crystallinity during second stage of oxidation. Attached surfactant on the polyethylene samples in this bio-surfactant initiated oxidation can cause structural irregularities in crystalline structure, leading to the reduction in crystallinity during initial stage. But following increase in crystallinity can be due to formation of short hydrocarbon chain initiating secondary crystallization. Solubilisation of amorphous oxidation product formed during 1 month incubation of *B.licheniformis* can cause the increase in the crystalline region, therefore increasing crystallinity after 2 months of incubation. Reduction of crystallinity even after 2 months in case C2M, can be lower oxidation level even after 2 months as observed in FTIR spectra. Another reason can be lower amount of solubilisation of amorphous region into aqueous media.

3.2.3.Morphological analysis:

3.2.3.1Scanning Electron Microscope:

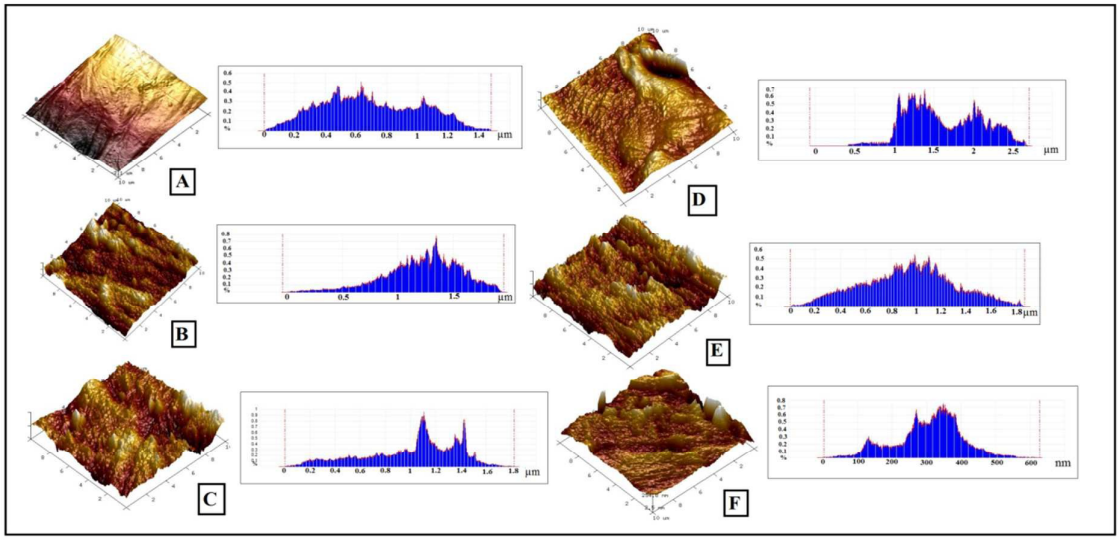

Figure 10: AFM image. A-Control PE, B-C1M, C-C1M1G, D-C1M0.5G, D-C2M,E-UVPE, F-UV2M

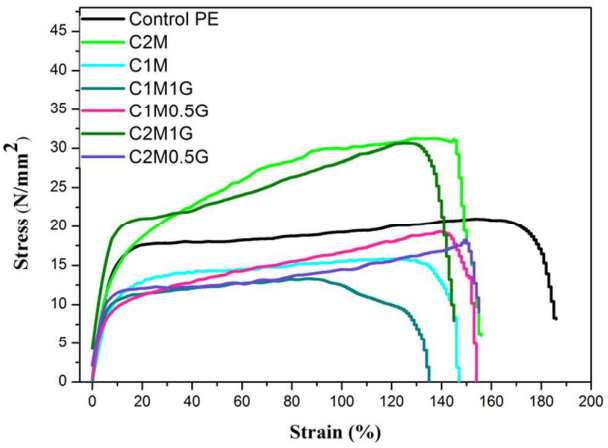

Figure 11: Stress Vs Strain graph of treated and untreated control polyethylene

Surface morphology of biologically treated and untreated control polyethylene is observed under SEM and images are represented in Figure 8. Images of surface morphology of biologically treated and untreated UVPE are represented in Figure 9. Surface morphology of all biologically treated polyethylene is rough and severely cracked. This kind of crack formation on the surface is usually observed in case of oxidized polyethylene samples resulted from natural contractions. The cracks are formed due to cross linking during oxidation or due to loss of oxidation product by solubilisation into bacterial mediumfrom the surface of polyethylene [2, 3, 24].

3.2.3.2Atomic Force Microscope:

Contact mode AFM height images of biologically treated and untreated polyethylene samples are represented in Figure 10. The AFM graph shows the amount and depth of cavity formed during treatment on the polyethylene surface. Depth of the cavity formed during biological treatment increases and more cavity forms on the surface of polyethylene in the case of C1M sample. This cavity formation process becomes slower after 1 month. After 2 months of biological treatment, deeper cavity formed on the surface of C2M but the amount is much less than C1M. As it is already reported that amorphous region is readily available for oxidation than crystalline region of semi-crystalline polyethylene molecule. These cavity forms due to formation of nodule made of little or unoxidized crystalline part of polyethylene molecule and due to solubilisation of oxidation product into liquid media, resulted during oxidation of amorphous region by bio-surfactant[18, 24]. During 1 month of biological treatment, in case of C1M, amorphous region is oxidised. After 2 months, oxidation process proceeds further oxidizing amorphous region. But, in case of C2M, depth of the cavity increase rather than amount of cavity formed on the polyethylene surface. Also, C2M oxidize more than solubilised less in amount which can be reason for slight increase in depth of the cavity than C1M. Depth of the cavity is higher in case of the C1M0.5G than that of the C1M1G. In case of the C1M1G, both amorphous region and crystalline region is oxidised, from where oxidation product solubilises into liquid mediumforming lesser deep cavity. This can also be observed through reduce crystallinity through XRD data [Figure 7]. For these reason, depth of the cavity is less in case of C1M1G. In case of C1M0.5G, only amorphous region is oxidised leaving crystalline region very little or unoxidized

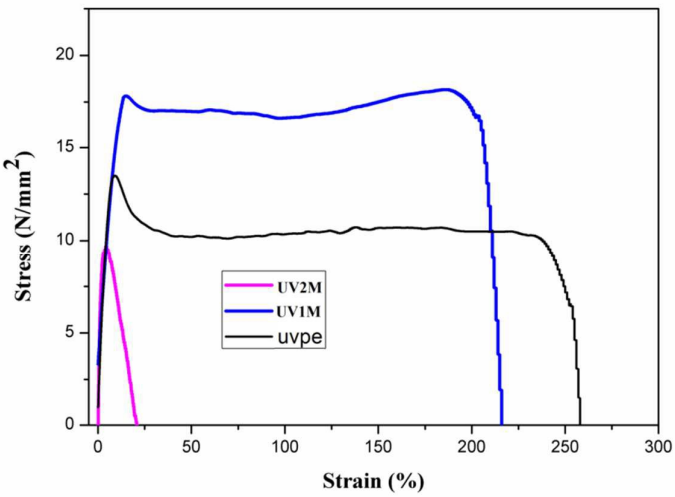

Figure 12: Stress Vs Strain graph of treated and untreated U.V. treated polyethylene

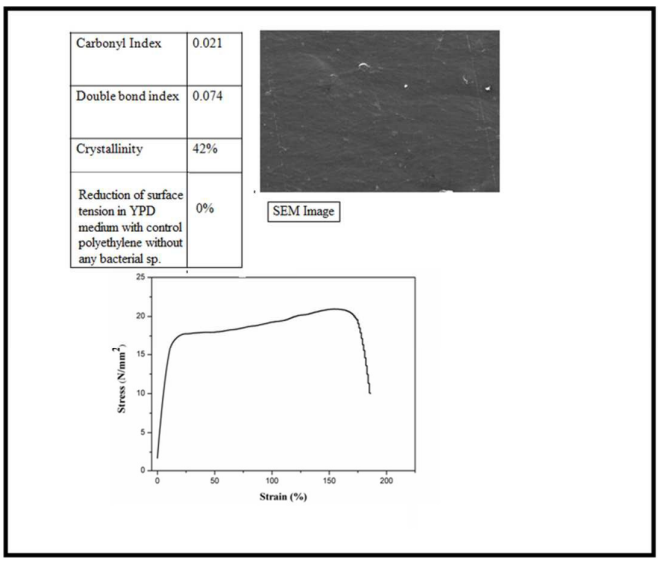

Figure 13: Analysis of Negative control

which creates nodules on the surface with deeper cavity. This result is also in accordance with the observed crystallinity level in Figure 7. This little or unxidized crystalline part of C1M0.5G is further oxidized during 2 months of bacterial incubation, in case of C2M0.5G. Reduction of crystallinity of C2M0.5G may be resulted due to this phenomenon. Crystallinity (%) of C1M0.5G is slightly lower from untreated control polyethylene. Although in both C2M and C1M0.5G cases, only amorphous region is oxidised, but deeper cavity is formed in case of C1M0.5G as oxidation level was higher in case of C1M0.5G than that of the C2M. In case of UV2M, after 2 months, due to loss of oxidation product from amorphous and crystalline region, cavity depth is very less than other treated polyethylene samples. After 1 month, both crystalline and amorphous region is oxidised as observed through reduced crystallinity from Figure 7. After 2 months, in case of UV2M, oxidation product formed during U.V treatment and during 1 month of incubation of UVPE with surfactant, solubilised in to the liquid mediumresulting lesser deep cavity. This phenomenon can be co-related with the slightly increased crystallinity level.

3.2.4. Mechanical analysis:

In Figure 11, stress versus strain curve of treated and untreated control polyethylene is plotted. Although, mechanical property of polyethylene samples do not depend on the oxidation level, but changes in tensile property is observed in case of treated control polyethylene samples. It is previously reported that elongation at break directly depends on carbonyl index of the polyethylene sample [25]. This phenomenon is observed in case of C1M1G which shows highest reduction in elongation at break. This reduction in elongation at break of C1M1G can be correlated with its highest C.I. among all treated control polyethylene samples after 1month of bacterial incubation [Figure 4]. Higher C.I. value is related to higher oxidation level and chain breakage during oxidation is another possible reason for loss of mechanical property as observed in these cases through the loss of elongation at break. In case of mechanical property, control polyethylene incubated with *B.licheniformis* in YPD growth medium with 1% of NaCl for 2months (C2M1G), with 0.5% of NaCl (C2M0.5G) and without NaCl for 2months (C2M) shows similar effects (Figure 11). Similar trend of increasing elongation at break during bacterial treatment is also reported by Lee et al[5].

Stress versus Strain graph of treated and untreated UVPE is plotted in Figure 12. After 1 month of biological treatment, elongation at break of UV1M decreases. But this decrease in elongation at break is major in case of UV2M. This polyethylene sample shows total loss of tensile property. Higher oxidation rate and structural modification can be a reason for this loss of tensile property. Oxidation products formed during U.V treatment and during two months

of bacterial incubation dissolve into the liquid media, as observed through SEM and depth analysis by AFM; this is another reason for the loss of mechanical property.

Negative control polyethylene, kept in YPD medium without any added bacterial sp., does not show any change of chemical, physical or mechanical property during 2months of bio-treatment (Figure 13).

Polyethylene is oxidized in the presence of the bio-surfactant produced by *B.licheniformis*. In the presence of NaCl, oxidation level is higher. During 2 months incubation, already formed oxidation products get solubilised into the aqueous medium which is a characterized property of surfactant. This property is also enhanced in the presence of NaCl. Therefore, NaCl present in the medium can stabilise and enhance activity of bio-surfactant as reported earlier. Similar level of oxidation of polyethylene films in high density form is reported by Ojeda et al by natural weathering in 161 days; this can be achieved in 60 days by bio-surfactant initiated oxidation process [26]. Mostly natural weathering or accelerated weathering is used for oxidation of polyethylene. If polyethylene is mixed with pro-oxidant additives, then the oxidation process by this method is very effective and fast. But, in case of polyethylene films without pro-oxidant or commercial polyethylene with added antioxidant, oxidation by this process can take 9 months to 1 year. Although pro-oxidant initiates the process of oxidation very fast, but this process is not that much economical. On the other hand, bio-surfactants are environmental friendly and more effective than above mentioned processes. In addition, it can easily be isolated after polyethylene treatment and can be used for any other application. Also solubilisation of oxidation products into aqueous medium can further be resulted into degradation process with weight loss if observed for long duration.

4. Conclusion:

Continuous production of bio-surfactant by *B.licheniformis* in the presence of polyethylene proved to be an effective process of oxidation of polyethylene. The presence of lower concentration of NaCl in ypd growth mediumnot only stabilized the bio-surfactant produced but also enhanced its activity as observed through higher level of oxidation of polyethylene in the presence of lower concentration of NaCl. In case of pre-oxidized polyethylene, oxidization and solubilisation of oxidation products into aqueous mediumwas also observed.

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