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Identification of Plastic Degrading Micro-Organisms from Soil Source of GHMC Dump Yard

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ABSTRACT: Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. Plastics that are biodegradable can be considered environment friendly, they have an increasing range of potential application and are driven by the growing use of plastics in packaging. In this study, the biodegradation of polythene bag was analyzed 1 month of incubation in liquid culture method. Microbial counts in the degrading materials were recorded up to 0.0278×109 per gram for total heterotrophic bacteria. The microbial species found associated with the degrading materials were identified as two Gram positive and five Gram negative bacteria. The microbial species associated with the polythene materials were identified as *Bacillus subtilis, Bacillus amylolyticus, Arthobacter defluvii*. The efficacy of microbes in the degradation of plastics were analyzed in liquid (shaker) culture method, among the bacteria *Bacillus amylolyticus* degrades plastic more in 1 month (30% weight loss/month) period compared to others and lowest degradation rate was observed in case of *Bacillus subtilis* (20% weight loss/month). This work reveals that *Bacillus amylolyticus* posses greater potential to degrade plastics when compared with other bacteria.

KEYWords: Biodegradation, plastics, degradation.

I. INTRODUCTION

Plastics are defined as the polymers (solid materials) which on heating become mobile and can be cast into moulds. They are non-metallic moldable compounds and the materials that are made from them can be pushed into any desired shape and sizes (saymour, 1989). Commonly plastics are used in many purposes including packaging, disposable diaper backing, agricultural films and fishing nets. Plastics and their use has become a part in all sectors of economy. Infrastructure such as agriculture, telecommunication, building and construction, consumer goods, packaging, health and medical are all high growth areas that ensures present demand for plastics. Plastic is the mother industry to hundreds of components and products that are manufactured and used in our daily life like automobiles parts, electrical goods, plastic furniture, defense materials, agriculture pipes, packages and sanitary wares, pipes and fittings, tiles and flooring, artificial leathers, bottles and jars, PVC shoes and sleepers hundreds of household items.

Plastics are used in packaging of products such as food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of plastics are used worldwide for packaging applications and the most widely used plastics used for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. At present the industry is split into organized and unorganized sectors. The organized sector produces quality products whereas unorganized sector is not capable of producing quality products, it produces low quality, cheap products through excessive use of plastic scrap.

Almost invariably, organic polymers mainly comprise plastics. The majority of these polymers are based on chains of carbon atoms alone or with sulfur, oxygen or nitrogen as well. The backbone is the part of the chain on the main "path" linking a large number of repeat units together. In order To customize the properties of a plastic, different molecular groups "hang" from the backbone (usually they are "hung" as part of the monomers before linking monomers together to form the polymer chain). This property of the polymer by repeating unit's molecular structure has allowed plastics to become an indispensable part of the twenty-first century world.

Plastics are usually classified by their chemical structure of the polymer's backbone and side chains. Important groups in these classifications include acrylics, silicones, polyesters, polyurethanes, halogenated plastics. Plastics can be classified by the chemical process that is used in their synthesis.

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II. MATERIALS AND METHODS

1) Sample collection
Plastic sample was collected from the dumped soil of Ghmc dump yard area.

2) Isolation
a] Serial dilution:
After the collection of plastic sample, these were taken and 1gm of this sample was cut into pieces and added to 9 ml of sterile water to make 1:10 dilution, adding 1ml of the 1:10 dilutionof 9ml of sterile water makes a 1:100 dilution and so on.

b] Total heterotrophic count:
C.F.U. /g= Number of colonies/ inoculum size (ml) X dilution factor

3) Identification
Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics by following Bergey's Mannual of Systematic Bacteriology (Kandlerand Weiss, 1986). All the isolates were subjected to Gram staining and specific biochemical tests.

COLONY MORPHOLOGY:
This was done to determine the morphology of selected strains on the basis of shape, size and colour.

4) Biochemical tests:
CATALASE TEST:
The catalase test was performed to detect the presence of catalase enzyme by inoculating a loopful of culture into tubes containing 3% of hydrogen peroxide solution. Positive test was indicated by formation of effervescence or appearance of bubbles, due to the breaking down of hydrogen peroxide to o_2 and h_2o.

OXIDASE TEST:
The oxidase test was done with the help of commercially available disc coated with a dye N- tetramethyl paraphenylene diamine dihydrochloride (Himedia), to detect the presence of cytochrome 'c' oxidase which is responsible for the oxidation of the dye. Rubbing a small quantity of bacterial culture by means of a sterile toothpick on the disc causes formation ofpurple colour within 10-30 sec indicating positive reaction whereas no colour change indicates a negative reaction.

MANNITOL TEST:
This experiment is generally performed to determine whether the bacteria is capable of fermenting mannitol sugar or not. Whenever organisms ferment mannitol agar, the pH of media becomes acidic due to production of acids. The fermentation of the media form red to yellow which shows positive test result.

MOTILITY TEST:
The motility test was done to determine the motility of the organism. Bacterial cultures were stabbed into the motility test medium (Himedia) and were incubated at 37 C for 48 hrs. Turbitity and observation of growth besides the stab line indicated a positive reaction whereas clear visibility with growth indicated a negative reaction.

MALONATE UTILISATION:
Malonate utilisation test was performed to observe the utilisation of malonate present in the malonate test medium (Himedia). Malonate test medium contains Bromothymol blue as indicator. Sodium malonate is the carbon source and ammonium sulphate is the nitrogen source. Organisms, which are able to utilize malonate, release sodium dioxide. The resulting alkaline conditions cause the indicator to change from light green to blue. Colour of the medium changes from light green to blue if the test is positive. Medium remains in light green colour if the test is negative.

NITRATE REDUCTION TEST:
This test was done to test if microorganisms are able to convert nitrate to nitrite or not by adding 1-2 drops of sulphanilic acid and 1-2 drops of N,N-Dimethyl-Napthylanine reagent to the kit medium. Immediate development of pinkish red colour there on addition of reagent indicates positive reaction. Negative reaction could be observed if there is no change in the colour.

CITRATE UTILISATION TEST:
This test determines the ability of bacteria to convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle). Citrate is the only carbon source available to the bacteria in this media. If bacteria cannot use citrate, it will not grow. Positive result is seen if the bacteria grows and the media turns into bright blue colour as a result of an increase in the pH of the media.

CARBOHYDRATE UTILIZATION TEST:
For carbon utilisation pattern HiCarbo Kit(Part A, Part B, and Part C) (Himedia catalog no. KB009) was used. Bacteria produce products that are acidic in nature when they ferment certain carbohydrates. The carbohydrate utilisation tests are designed to detect the change in pH that occurs if fermentation of the given carbohydrate occurred. Acids lower the pH of the medium which causes the pH indicator (phenol red) to turn yellow. If the given carbohydrate is not fermented by bacteria then the media remains red.

5. Microbial Degradation of Plastics in Laboratory Condition:Determination of Weight Loss:
Pre-weighed discs of 1-cm diameter prepared from polythene bags were aseptically transferred to the conical flask containing 50 ml of culture broth medium, inoculated with different bacterial species. Control was maintained with plastic discs in the microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics was calculated.

III. RESULTS

Table no. 1: Colony morphology of the bacterial strain on the basis of serial dilution.

Dilutionno.	Sl. No.	Colony morphology	Source	Code
10^{-1}	1 2 3 4	Large round white Small round yellow Small round white Large irregular white	Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard.	PLRW PSRY PSRW PLIW
10^{-2}	1 2 3 4	Large round pale yellow Small round yellow Small round transparentLarge irregular white	Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard.	PLRP PSRY PSRT PLIW
10^{-3}	1 2	Large round white Small irregular yellow	Dumped plastic material from Ghmc dump yard. Dumped plastic material from Ghmc dump yard.	PLRWPSIW
10^{-4}	1	Large irregular white	Dumped plastic material from Ghmc dump yard.	PLIW

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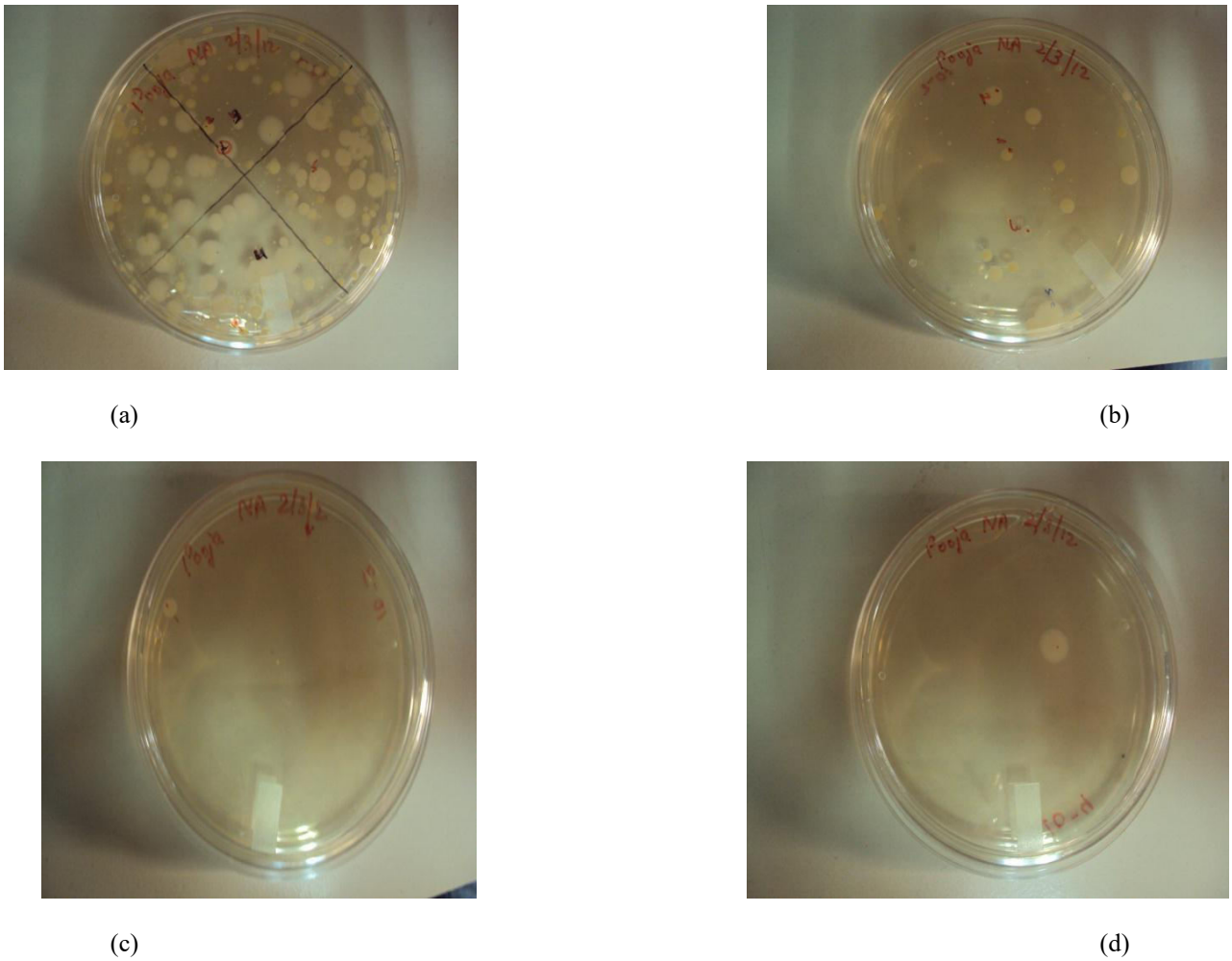
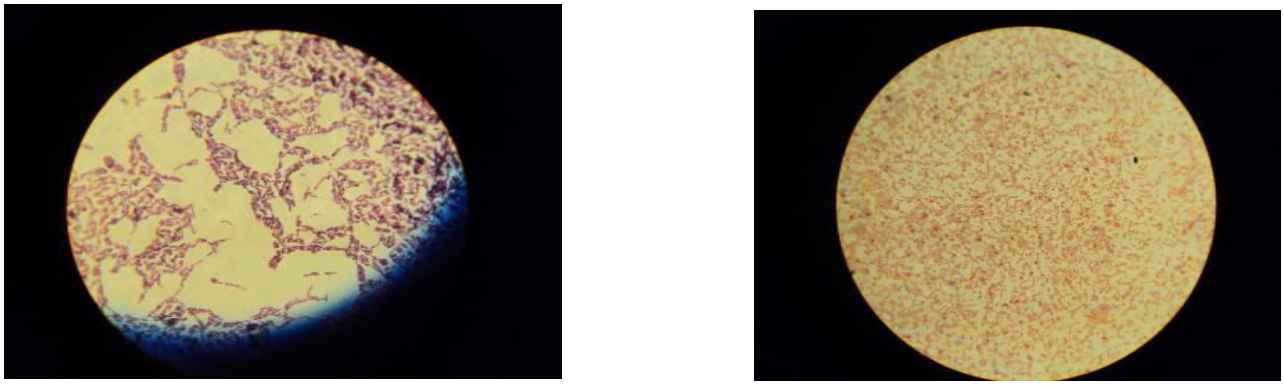


(a) (b)

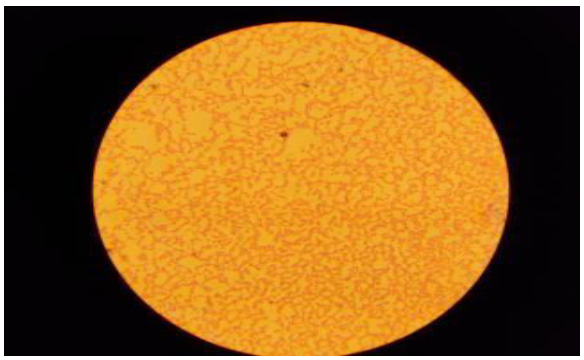
(c) (d)

Fig 2: colony morphology of the strains on the basis of serial dilutiona):10^{-1}, (b):10^{-2}, (c):10^{-3}, (d):10^{-4}

The code stands for the morphological characteristics of the bacterial strain: **LRW**- LargeRound White, **SRY**- Small Round Yellow, **SRW**- Small Round White, **LIW**- Large Irregular White, **LRP**- Large Round Pale, **SRT**- Small Round Transparent, **SIY**- Small Irregular Yellow.



A B



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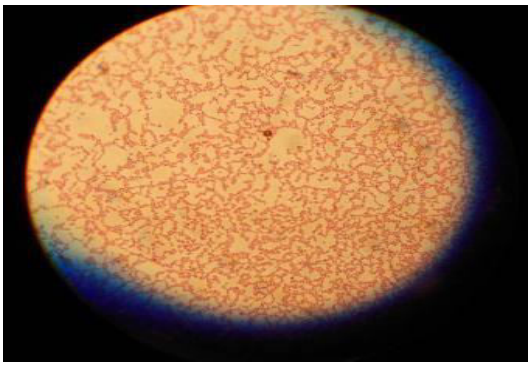

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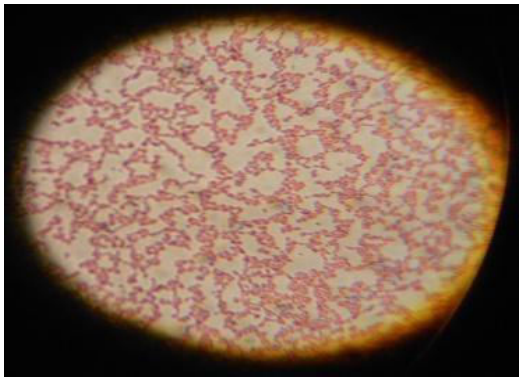

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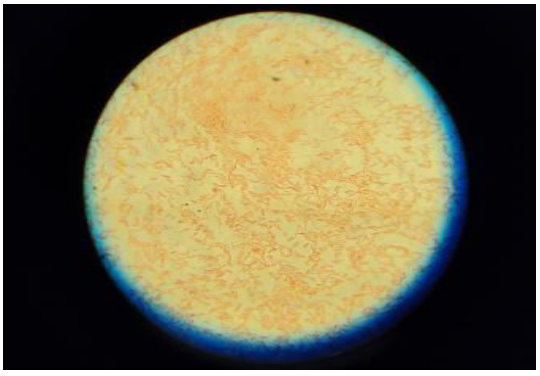

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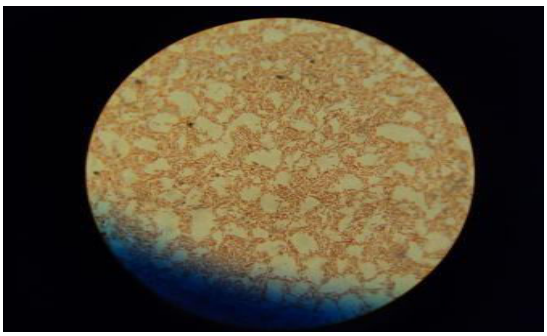

Fig 3: Gram staining of seven selected strains (A-G) on the basis of colony morphology.

Bacterial count

Table no 3: Total heterotrophic bacterial count:

Dilution	Number of colonies	Inoculums size (inmL)	CFU/g
10^{-3}	278	0.1	0.0278×10^{9}

Table no. 4: RESULT OF BIOCHEMICAL TEST

Sl no:	Catalase test	Oxidasetest	Mannitoltest	Motilitytest	Citrate utilisationtest	Nitrate reductiontest	Malonate utilisationtest	Gas production from glucose
1	+ve	+ve	+ve	Non- motile	+ve	-ve	-ve	-ve
2	+ve	+ve	+ve	Non- motile	-ve	-ve	+ve	+ve
3	+ve	+ve	+ve	Non- motile	+ve	-ve	-ve	+ve
4	+ve	+ve	+ve	Non- motile	-ve	+ve	-ve	+ve
5	+ve	+ve	+ve	Non- motile	-ve	-ve	-ve	-ve
6	+ve	+ve	-ve	Non- motile	-ve	+ve	+ve	-ve
7	+ve	+ve	+ve	Non- motile	-ve	-ve	-ve	-ve

- Biochemical tests show, catalase and oxidase test result of all the strains were found tobe positive.
- Mannitol test of the strains were also found positive excluding strain no.6, Motility testshows all the strains are non-motile.
- Citrate test of strain no 1 and 4 were found positive and rest of them showed a negativeresult.
- Nitrate reduction test of strain no.4 was found positive and rest of them showed anegative result.
- Malonate test shows only strains 4 and 6 gave a positive result.
- The test named gas production from glucose shows strains 2, 3 and 4 showed a positiveresult.

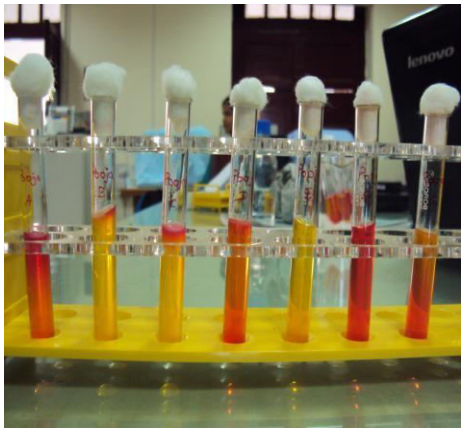

Fig 4: Mannitol-Motility test.

Fig 5: Citrate utilisation test.

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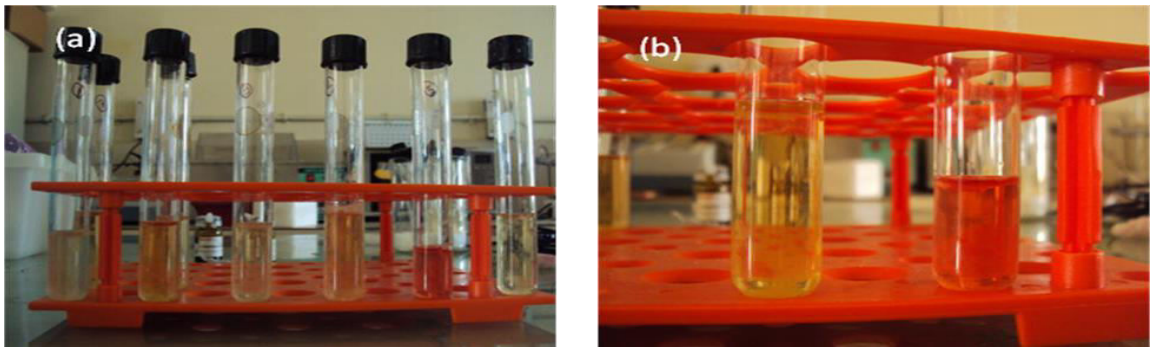

Fig 6 a and b: Nitrate reduction test.

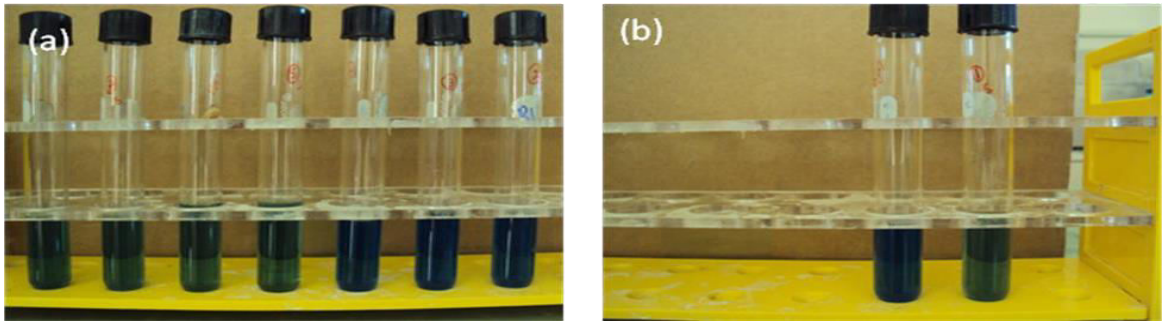

Fig 7 a and b:Malonate utilisation test.

Table no. 5: Result of carbohydrate test: (a)

Sl. No.	Carbohydrate	Isolate 1	Isolate 2	Isolate 3
1	Lactose	+ve	-ve	-ve
2	Xylose	+ve	-ve	-ve
3	Maltose	+ve	-ve	-ve
4	Fructose	+ve	-ve	-ve
5	Dextrose	+ve	-ve	-ve
6	Galactose	+ve	-ve	-ve
7	Raffinose	+ve	-ve	-ve
8	Trehalose	+ve	-ve	-ve
9	Melibiose	+ve	-ve	-ve
10	Sucrose	+ve	-ve	-ve
11	L-Hrabinose	+ve	+ve	-ve
12	Mannose	+ve	+ve	+ve

Sl. No.	Carbohydrate	Isolate 1	Isolate 2	Isolate 3
13	Inulin	-ve	-ve	-ve
14	Sodium galactose	-ve	+ve	-ve
15	Glycerol	-ve	-ve	-ve
16	Salicin	+ve	+ve	+ve
17	Dulcitol	-ve	-ve	+ve
18	Inositol	-ve	-ve	+ve
19	Sorbitol	-ve	-ve	+ve
20	Mannitol	-ve	-ve	+ve
21	Adonitol	-ve	-ve	+ve
22	Arabitol	-ve	-ve	+ve
23	Erythritol	-ve	-ve	+ve
24	α methyl Dglucoside	-ve	-ve	+ve

(c)

Sl. No.	Carbohydrate	Isolate 1	Isolate 2	Isolate 3
25	Rhamnose	-ve	-ve	-ve
26	Cellobiose	+ve	-ve	-ve
27	Melezitose	-ve	-ve	-ve
28	α methyl- Dmamoside	-ve	-ve	-ve
29	Xylitol	+ve	-ve	-ve
30	ONPG	+ve	+ve	+ve
31	Esculin hydrolysis	+ve	+ve	+ve
32	D-Arabinose	-ve	-ve	-ve

33	Citrate utilisation	+ve	+ve	-ve
34	Malonateutilisation	+ve	+ve	-ve
35	Sorbose	-ve	-ve	-ve

Table no.6: Result of degradation of plastic sample by bacteria after 1 month:

Strain no.	Initial wt (mg)	Final wt (mg)	Difference	Weight loss/month (in %)
1	50	40	10	20
2	50	35	15	30
3	50	38	12	24
4	50	37	13	26
5	50	39	11	22
6	50	37	13	26
7	50	38	12	24

IV. CONCLUSION

The bacteria were identified to be *Bacillus Subtilis, Bacillus Amylolyticus and Arthobacter defluvii.Bacillus amylolyticus* degrades plastic more than that of other bacteria.*Bacillus subtilis* has less capacity to degrade plastic as compared to other bacteria.The isolated microbes were native to the site of polyethylene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.

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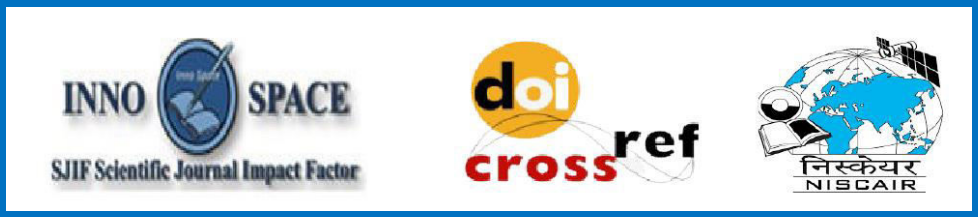

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