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Biodegradation of Polyethylene LDPE plastic waste using Locally Isolated *Streptomyces sp.*

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Abstract
Plastics become widely spread in would wild , plastic are strong and flexible, light material that make be widely used in different field and different application in medical, agricultural and industrial and in food packaging , that resolve many of problems related with transport, and other things,as a result , plasticaccumulated in environment andcaused serial of environmental pollution, this pollution include all area in environment soil water and air , and the traditional way for pollution treatment are very difficultand highly cost and effect of human and animal health,there for, in this paper, we focused on pollution treatment by used microorganism's and *Streptomycessp*. as the bestmicroorganism s for their biodegradable ability for plastic waste and other pollution .in this paper different isolates of *Streptomycessp.* were screened for polyrthelene low densitypolyethylene(LDPE) biodegradation, the result showed that *Streptomyces* isolate (SSP2, SSP4, SSP 14) have best degradationefficiency for LDPEin different tests ; Measurement the dry weight loss of plastic stripes [polyethylene bags (g) and plastic cup(p)] after cultivation in ATCC medium and incubation at 25-30ºC in shaker incubator at 120 rpm, Spectrophotometric assay and determination of Bioemulsifer production yield after one months of incubation, the result showed that the loss in dry weight in LDPE stripes by *Streptomyces* isolate (SSP2, SSP4, SSP 14) are (6%, 9%, 15%) for (p) stripes and (8%, 11%, 19%) for (g) stripes respectively, and spectrophotometric assay recorded best results for LDPEdegradation, SSP2, recorded (0.08, 0.55),SSP4 (0.09, 0.65) and SSP 14 recorded (0.13, 0.70) for p and g respectively. Finally, the bioemulsifer production determination also recorded highest results that play important role in biodegradation process , the results recorded thatbioemulsifer production yield by (SSP2, SSP4, SSP 14) isolates are (5.74%, 7.24%, 11.84%) for (p) stripes, and (8.44%, 9.84%, 12.94%) for (g)stripes. from these result, the SSP14 isolates shed best result for LDPE degradation that prove that the *Streptomyces*have best biodegradable efficiency for many pollutant, that agreed with manyresearch in microbiological environmental science .

1. INTRODUCTION

Plastic are polymers of carbon along with hydrogen, nitrogen, sulphur, and other organic and inorganic components and are made from fossil fuel, Plastics are non-biodegradable, strong, durable, moisture resistant, light weight. (1). Flexibility, easy and low-cost production. .However, these traits assisted the application of plastics indifferent field in 19th and 20th centuries. (2). the use of plastics has used our life in many ways (3). The total use of plastic is growing at a rate of12% per year and about 0.15 billion tons of synthetic polymers are produced worldwide every year (4). Accumulation rate of plastic waste in the environment is 25 million tons/year (5).It used in all parts of economy. It used in agriculture, consumer goods, health and medicine (6), it widely used in transportation, food, clothing, medical and recreation industries, packaging, food industry (7).

Plastics can be characterized into some of the essentials classes such asnatural plastics, semi synthetic plastics, synthetic plastics,thermoplastics, thermosetting plastics. Plastics classified polythene (PE), propylene (PP), polystyrene (PS), polyurethane (PUR), nylon etc. Polyethylene eitherLDPE (low density polyethylene) or HDPE (high density polyethylene) (4).

A plastic is one of the keycauses of environmental pollution that takes thousand years for their efficient degradation, the accumulation of these plastic wastescauses serious threat to environment and wild life**,** the environmental pollution include air, water and soil. The plastic sheets or bags causes infertility of soil, preventing degradation of other typical substances, depletion of underground water source (8), And cause human health problems because they effect on human hormone. And considered carcinogenic for human and animal (9), It also effects on local animals like cattle.Recently, it demonstrated as a major source to marine life (8). These problems cannot be solved by land filling and incineration because it is not suitable, safeand expensive and incineration excites the release of environmentally harmful gases such as Nitrous Oxide, Sulphur dioxide, Carbon dioxide etc. (10).

Microorganisms are able to degrade the plastic waste with help of different physical factor such as temperature, moisture, pressure causeharmful to the polymers in a process called biodegradation (4).Microorganisms that able to degrade the common of the organic and inorganic materials as carbon source. Microorganisms produced many of degradingenzymesthat cause cleavage of the polymer chains into monomers and oligomers. Plastic wastes areabsorbed by microbial cell. Aerobic metabolism produces carbon dioxide and water. And anaerobic metabolism produces carbon dioxide, water, and methane as end products (6). Many microorganism are able to degraded plastic compounds such as fungi, algae, (11), bacteria,*ex. Bacillus , Pseudomonas, Klebsiella, Mycobacterium, Rhodococcus, Flavobacterium, Escherichia, Nocardia,* and *Azotobacter* (12), *Actinomycetes, that able to* degradation natural and synthetic plastics. Include *Streptomycessp. Streptomyces* are gram-positive soil filamentous bacteria belong to family *Streptomycetaceae* with a complex cycle of morphological differentiation,*Streptomyces* are major source for secondary metabolites having a variation of biological activities antibiotics, antitumor, immunosuppressive agents, and enzymes, and other microorganismsthat produced severalextracellular enzymes which leads of biodegradation of plastic.(13),

The main objective of the present study was toisolate *Streptomycessp*. degrading types ofpolyethylene (PE)Low Density Polyethylene (LPDE) from fivedifferent soil samples contaminated with plastic waste collectedfrom different locations in Iraq- Baghdad, and screening the *Streptomycessp*.For(LPDE) degradation, the degradation rate of *Streptomyces sp.* was measured separately by calculating percentageweight loss in plastic stripes. Spectrophotometric assay and determination bioemulsifer production yield in laboratory.

2. MATERIAL AND METHOD

A. Sample collection for isolation

For isolation of *Streptomyces sp.*degrading polyethylene (Low density polyethylene LDPE) , four soil sample were collected at depth 3-5cm from different sites in Baghdad | Iraq highly contaminated with plastic wastes), these samples well collected in sterile containers, and transported to the Microbiology laboratory in AL-Karkh University of science for analysis.

B. Isolation of (LDPE) degrading micro-organisms:

For isolation of *Streptomyces sp*. From soil sample, the soil was heated at 70c for 2h. Serial dilution was made by add 1gram of preheated soil to 9ml of sterile distilled water in falcon tube to yield a 10-fold dilution, and mixed well. Add 100µl of each dilution and cultivates on soy bean agar plates (composed of g/l, soybean 20g, manitol 20g and agar 30g, at pH 7.0. These plates were enhanced with 0.25% of Nystatin)each colony was isolated on its own plate by standard streaking method, and subculture many times to obtain pure culture of *Streptomyces*, finally these plates was incubated 30c for 14 day, after incubation the plates were observes their growth and morphological future (Colony color and shape, Colony diameter , Presence of spores, Spore surface Aerial spore mass color,produced f pigment) and make biochemical test for identification the isolated colonies (14), The biochemical tests conducted were sugar fermentation, nitrate reduction, oxidase, citrate and catalase tests. The bacterial isolates were identified based on the keys detailed by (15). The pure isolates well-kept as stock suspensions in 20% glycerol at -20 °C (16).

C. Screening the isolates for polyethylene (LDPE) biodegradation

For Screening of isolate for LDPE biodegradation, add LDPE powdered to mineral salt medium 0.1% (w\v), Mineral salts medium (ATCC medium)constants (g\l) its: (2g $NaNo_3$, 0.5 g $MgSO_4$, 0.5 g KCL, 0.01g $FeSO_4$, 0.14g KH_2PO_4, 1.2g K_2HPO_4, 0.02g yeast extract , 30g agar agar for solidifying media), the pH of media was adjusted to 7 and sterilized in autoclave at 121°C and pressure for 15 lbs./inch2 for 15 minutes. After sterilization 20 ml of media was poured in plate and left to solidifying, after this , the biodegradation of LDPE by was measured by clear zones method by making hole by Kroc Boral in plates duplicates, about 500µl of 48h of old seed culture of*Streptomyces* isolate was add to the holes and incubated at 25-30C for 2weeks, after this period the isolates were screening depending of produced largest clearance zones in plates (17) .

D. Screening of isolates for LDPE biodegradation in laboratory

For degradation the plastic waste in laboratory, the following procedure will be madethat show ability of microorganisms to degraded the polyethylene (LDPE), Take two groups of LDPE source: first plastic cups (p) and second polyethylene bags (g) that used for food packaging from domestic shop, and cut in to small stripes 2cm and determined the weight before add to media, add 1g from each group to 100 ml of ATCC mediuminErlenmeyer flask (250) ml, and cultivation with inoculum of 2% of 48h *Streptomyces*culture broth that provisory screening for LDPE biodegradation. And make the control without the inoculum for each type of LDPE stripes (g and p). and incubated with flask was in shaker incubator at 30C ,120 rpm for 1 month (*6*). The biodegradation activity of *Streptomyces* isolates for LDPE groups was determined by three different methods: (1) measurement the dry weight of plastics stripes (2) spectrophotometric assay (3) bioemulsifer production.

1- Measurement the dry weight

After incubation period, the plastic stripes well harvested separately for each group and isolates and rinsed with70% methanol to remove of any cell depires anddistilled water respectively, and dried it at room temperature for 24 hours. Weight, put them on weighted filter paper and let to dried at room temperature for 24h. (17), the ratio of degradation in this method was calculated as follows:

$$\textbf{Weight loss } \% = \frac{\boldsymbol{wt1 - wt2}}{\boldsymbol{wt1}} \times \mathbf{100}$$

W1 (Initial weight) = weight before incubation.**W2** (final weight) = weight after incubation.

2- Spectrophotometric assay

The degradation ratio of *Streptomyces sp*. for LDPE groups was also estimated by measuring the cell growth by reading the optical density at 600 nm.The growths of isolates for two groups, 2ml from each incubated flask were taken and measured the OD at (600) nm in spectrophotometer devises at weekly intervals (i.e. day 0, day 7, day 14, and day 21, day 28) for accounting the rate of biodegradation (18).

3- Bioemulsifer production determination

Biosurfactants are biological active molecules produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi. Biosurfactants are amphiphiles, consisting of two parts, a polar (hydrophilic) moiety and a non-polar (hydrophobic) group. The hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides or proteins while the hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols (19).Bioemulsifer are wetting agent or petroleum derivatives that lower the surface tension between liquid and liquid or liquid and solid, [20].

Biosurfactant can be extracted from flask by centrifugation the culture media after extraction the stripes at (10.000 rpm, 15 min, 10˚C) to remove the cells, and using solvent chloroform and methanol at ratio (2:1). Respectively, the solvent are add to supernatant produced from centrifugation, and shaken for few minutes, and allowed to stand for 30 min until phase separation. The extract concentrated and then sodium sulphate anhydrous was added to remove water. The crud extract was obtained after removal of solvent and moisture by evaporation and drying in oven at 45 ˚C respectively for 24h (21). The yields of bioemulsifer produced are calculated using the following equation;

$$\textbf{Bioemulsifer yield} = \frac{\textbf{wt. 2} - \textbf{wt. 1}}{v} \times \textbf{100}$$

Wt. 1= the weight of plate before drying.**Wt. 2** = the weight of plate before drying.**V** = volume of sample.

3. Result and Discussion

A. Isolation and screening of isolates

For isolation *Streptomyces* sp. From soil isolated from different area in Baghdad | Iraq contaminated with different plastic waste, A total of 15 isolates of *Streptomycessp*. were isolated and screening for LDPE biodegradation by clear zone method, Among these isolates, 3 isolates (SSP 2, SSP 4, SSP14) of *Streptomyces* are able with efficiently to degraded the LDPE powdered that add to media with value reached to (**31**mm, **35**mm, **38**mm) respectively after incubation at 25-30ºC for 2 week, among these the isolates SSP14 are recorded the best reading reached to (**38**mm) as showed in the table 1 and figure 1. Usha et al., (6), proved that producing of clear zone in plate around the colonies is due to that un extracellular hydrolysing enzymes secreted by the target organism hydrolyse the plastic stripes in the medium into water soluble products thereby producing zones of clearance Around the colony.

Table 1, Screening of isolates for polyethylene LDPE powdered in ATCC .Medium

No	Isolates	LDPE biodegradation by (Clear zone method)
1	SSP 1	25 mm
2	**SSP 2**	**31mm**
3	SSP 3	22mm
4	**SSP 4**	**35mm**
5	SSP 5	15mm
6	SSP 6	20mm
7	SSP 7	12mm
8	SSP 8	19mm
9	SSP 9	27mm
10	SSP 10	25mm
11	SSP 11	20mm
12	SSP 12	15mm
13	SSP 13	19mm
14	**SSP 14**	**38mm**
15	SSP 15	9mm

Figure 1, Clear zone method of *Streptomyces* isolates (SSP2, SSP4, and SSP 14) for polyethylene LDPE powdered in ATCC Medium pH7, at 25-30º C.

B. Morphological and biochemical Characterization polyethylene (LDPE) degrading *Streptomyces* isolates.

The selected isolates (SSP 2, SSP 4, and SSP14) were chosen examined for it's microscopically and morphologically and biochemical test for their higher degradability. The microscopic view of isolates showed Gram-positive filaments with non-motile, The colonies of the isolates showed different morphological abilities, the colony color ranged from (light gray to white and yellow) respectively when cultured on soybean agar plates, and the colony shape of all isolates are circular, the diameter of three isolates are (3 mm, 2 mm, 2 mm) respectively, and all *Streptomyces sp.* have Aerial mycelium spore , the surface of this spores ranged from rough to flexuous, and color are ranged from gray for SSP2 and golden gray for SSP4 and gray for SSP14, the pigment production show that the substrate mycelium for these isolates ranged from dark yellow to green and all three isolates are no producing another pigment. The results shown These isolate was capable of assimilating all 6 sugars tested, and depending on biochemical test, the result shown that these three isolates have positive result

for Oxidase, Urease,Starch hydrolysis tests and negative for Gelatine hydrolysis test as shown in Table 2. This result is agreed with laidi *et al*., (15) for Taxonomy, identification and biological activities of a novel isolate of *Streptomyces tendae.*

Table 2, Biochemical tests and Morphological characteristic for *Streptomyces* isolates (SSP2, SSP 4, and SSP 14) in Soy bean agar Medium

No	Characteristic	SSP 2	SSP4	SSP14
1	Colony color	Light gray	White	yellow
2	Colony shape	Circular	Circular	circular
3	Colony diameter	3mm	2mm	2mm
	Presence of spores	Aerial mycelium	Aerial mycelium	Aerial mycelium
5	Spore surface	Rough	Flexuous	rough
6	Colour of spore mass	Gray	Golden gray	Gray
7	Pigment production			
	a. Substrate mycelia	Dark yellow	Dark yellow	green
	b. Diffusible pigment	-	-	-
	c. Melanin pigment formation	-	-	-
8	Biochemical test			
	Oxidase test	+	+	+
	Gelatine hydrolysis test	-	-	-
	Urease test	+	+	+
	Starch hydrolysis test	+	+	+
9	Sugar hydrolysis test			
	a. Glucose	+	+	+
	b. Sucrose	+	+	+
	c. Fructose	+	+	+
	d. Maltose	+	+	+
	e. Manitol	+	+	+
	f. Xylose	+	+	+
	g. Rahminose	+	+	+

C. Screening of isolates for LDPE biodegradation in laborator

The three isolates (SSP 2, SSP4, SSP 114) that shown higher result in clear zone method were screened for their degradability in laboratory condition for compressive between them , these isolates showed higher degradability for polyethylene LDPE stripes in different tests that determine the degradation ratio of plastic waste, . The degradation efficiency of this isolates for polyethylene stripes was determined by the following tests:

1- Measurement the dry weight

The polyethylene LDPE stripes (t, g) that add to the flask and incubated at 25-30 c at 120 rpm, the polyethylene LDPE stripes are harvested and calculated the weight loss of it after one month of incubation with *Streptomyces* isolates (SSP2, SSP 4, SSP14). As in table 3, The result showed that the isolates SSP 14 recorded highest loss in weight of stripes with value ranged 15% for (p) stripes and 19% for (g) stripes following by the isolates SS4 that also recorded higher loss in weight (9%, 11%) for p and g respectively and finely isolates SSP2 that recorded also good loss in weight(6 %, 8%) for p and g stripes respectively, that's mean that, the loss in the weight due to microbial activity and these isolates when cultured in mineral salt medium for one month , it utilized this stripers as carbon ,nitrogen and energy source during the long exposure in situ, Once the organisms become attached to the surface, it degraded it and cleaves it in to low-molecular weight fragments, oligomers, dimers or monomers.

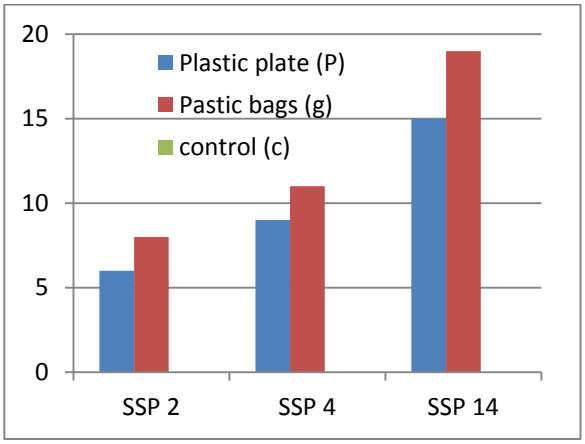

Figure 2, dry weight measurement test for biodegradation of polyethylene (LDPE) stripes (p, g) by*Streptomyces* sp. (SSP2, SSP 4, SSP 14)

Omar Saad Jumaah, *(22)* also used the weight method for polyvinyl alcohol (PVA) plastic bags by using the different isolate of pseudomonas and bacillus *(Bacillus amylolyticus, Bacillus firmus Bacillus subtilis, Pseudomonas putida, Pseudomonas fluroscence),* microbes cause greatest degradation of polythene and plastics. Among the bacteria, the*Pseudomonas putida* followed by *Bacillus subtilis, Bacillus amyloliticus Bacillus firmus, Pseudomonas fluroscence,* having greater degradation ability. This result was also agreed with Usha et al., (6) that reported the weight loss form *Streptomyces*species is 46.16% of polythene and 35.78% of plastics.

2- Spectrophotometric assay

The growth of screening isolates also be measured by spectrophotometric assay, the growth of isolates was measured at 600 nm in spectrophotometer devises, after one month of incubation these isolates are able to grow with presence of nutrition sources, among these isolates the isolates SSP14 are more active than other isolates and their growth value of it recorded (0.04- 0.04- 0.06-0.08-0.13) for plastic cups, and recorded best result for polyethylene bags that ranged from (0.15- 0.27- 0.57 -0.63- 0.70) in intervals day as showed in table 4 and and figure (3, 4, & 5).

Table 3, Result of dry weight measurement test for biodegradation of polyethylene (LDPE) stripes by *Streptomyces* isolates (SSP2, SSP 4, SSP 14)

No	Isolates	Plastic plate (p)				Polyethylene bags (g)			
		Initial wt. (mg)	Final wt. (mg)	Difference	Weight Loss/month (in %)	Initial wt. (mg)	Final wt. (mg)	Difference	Weight Loss/month (in %)
1	**SSP 2**	100	94	6	**6 %**	100	92	8	**8 %**
2	**SSP 4**	100	91	9	**9 %**	100	89	11	**11 %**
3	**SSP 14**	100	85	15	**15 %**	100	81	19	**19 %**
4	**Control**	100	100	0	**0%**	100	100	0	**0 %**

Table 4, Result of spectrophotometric assay for biodegradation of polyethylene (LDPE) stripes (p, g) by*Streptomyces*isolates (SSP2, SSP 4, and SSP 14)

No	Isolates	At 0 day		After 7day		After14day		After21day		After28 day	
		p	G	P	G	p	G	P	G	p	g
1	SSP 2	0.03	0.10	0.05	0.15	0.05	0.30	0.06	0.41	0.08	0.55
2	SSP 4	0.03	0.11	0.05	0.19	0.06	0.49	0.06	0.60	0.09	0.65
3	SSP14	0.04	0.15	0.04	0.27	0.06	0.57	0.08	0.63	0.13	0.70

Table 5, Result of Bioemulsifer production yield by polyethylene (LDPE) stripes (p, g) degrading *Streptomyces* isolates (SSP2, SSP 4, SSP 14)

No	Isolates	Sample volume	Plastic plate (p)			Polyethylene bags (g)		
			W1	W2	Yield %	W1	W2	Yield%
1	SSP 2	100	23.4897	24.0637	5.74%	26.5818	27.4258	8.44%
2	SSP 2	100	29.5631	30.2871	7.24%	30.1085	31.0925	9.84%
3	SSP14	100	24.9528	26.1368	11.84%	31.5700	32.8640	12.94%

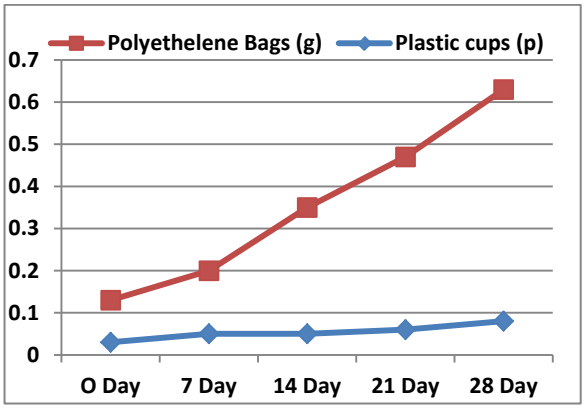

Figure 3, Growth of *Streptomyces* isolates SSP2 in ATCC medium containing polyethylene (LDPE) stripes (p, g) incubated at 25-30° C, pH7 at 120 rpm.

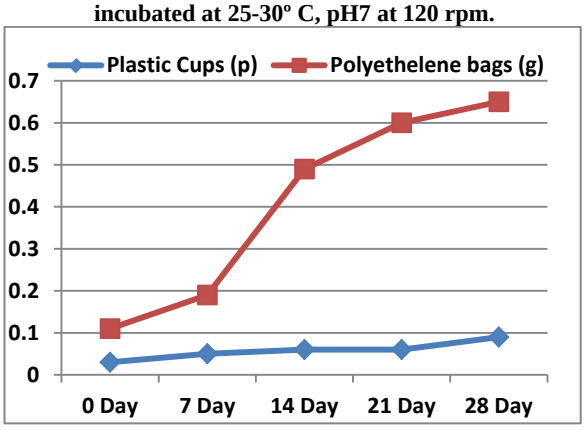

Figure 4, Growth of *Streptomyces* isolates SSP4 in ATCC medium containing polyethylene (LDPE) stripes (p, g) incubated at 25-30° C, pH7 at 120 rpm.

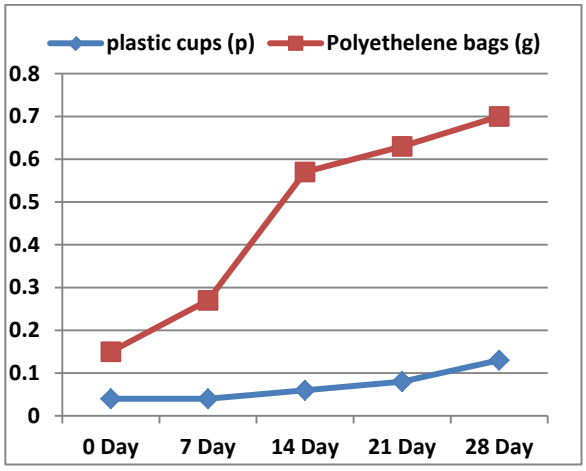

Figure 5, Growth of *Streptomyces* isolates SSP 14 in ATCC medium containing polyethylene (LDPE) stripes (p, g) incubated at 25-30° C, pH7 at 120 rpm.

The increase in growth due efficiency of this isolates to degraded the polyethylene stripes and used as carbon and energy source, this result was greed with Joiti singh *et al*., (23) for used three strain of pseudomonas *putida* and PE7 and *Pseudomonas aurginosa* P. E10 and Bacillus *Amylique faciens* P. E 5 , preserved in increase in bacterial growth that's proved un efficiency of this strain for LDPE degradation at intervals day.

Rajashree Patil and Bagde, (18) also used the spectrophotometric assay for Determination of PVA degradation and proved in increase in PVA degradation by isolates of *Bacillussp.*That shown (65%) of degradation and *Pseudomonas sp*. 42% of PVA

degradation as determined by spectrophotometric assay in period of 1 month.

3. Bioemulsifer production determination

Bioemulsifer are surface active molecule produced during the biodegradation of plastic (23). microorganism produced it to enhance se biodegradation of polymers (i.e. plastic stripes p, g), the isolates SSP 14 recorded best value for bioemulsifer production during their growth in culture medium that reach to (11.84%) for plastic cups and 12.94% for Polyethylene pages , followed by SSP 4 that recorded (7.24%, 9.84%) and SSP 2 that recorded (5.74%,8.44%) for p and g respectively, as in Table5. These microorganisms grow on the surface of plastic stripes and forming biosurfactant, Cell surface hydrophobicity of these organisms. Oda *et al*., (24), studied it was found to be an important factor in the formation of Polycaprolactone depolymerise produced by the biofilm on the polythene surface, which consequently bacterium *Alcaligenes faecalis*. He isolated several enhanced biodegradation of the polymers.Khopade*et al*., (25), improved that *Streptomyces* is source of biosurfactant production.

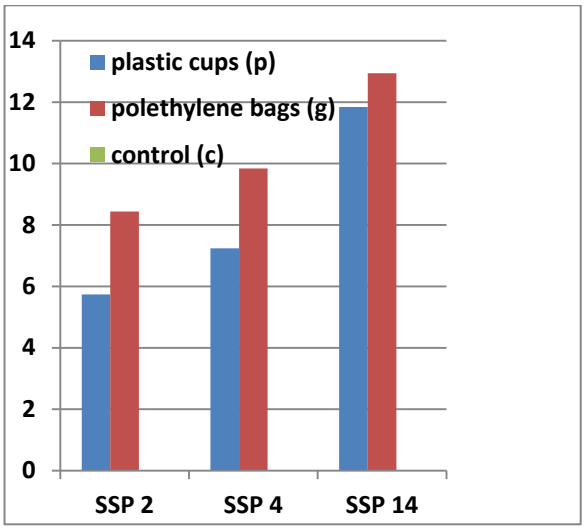

Figure 6, Bioemulsifer production yield by polyethylene (LDPE) stripes (p, g) degrading*Streptomyces* isolates (SSP2, SSP 4, SSP 14)

Streptomyces sp. have higher degradation ability from other microorganisms microbe or fungi this for polyethylene plastic waste this can agreed with Usha *et al*., (6), that studied the biodegradation efficiency of different microorganisms Efficacy of the microbes in degradation of polythene and plastics were among the bacteria *Pseudomonas sp*. degrade 37.09% of polythene and 28.42% of plastics Among the fungal species 20.96% of polythene and 16.84% of plastics and *Streptomyces* species 46.16% of polythene and 35.78% of plastics. This work showed that the *Streptomyces sp*. Possess greater potential to degrade polythene and plastics when compare with other bacteria and fungi. That shows efficiency of *Streptomycessp*. In degradation of LDPE stripes in ratio (46.7%) and 37.09% for LDPE powdered.

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