

Original article <https://doi.org/10.12980/jclm.5.2017J7-94>

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## Biodegradation of high density polyethylene using *Streptomyces* species

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### ARTICLE INFO

#### Article history:

Received 1 Jun 2017

Received in revised form 19 Jun 2017

Accepted 15 Aug 2017

Available online 21 Oct 2017

#### Keywords:

High density polyethylene

Biodegradation

*Streptomyces*

Kinetic modeling

Toxicity

### ABSTRACT

**Objective:** To investigate the biodegradation of high density polyethylene (HDPE) by *Streptomyces* species isolated from the soil of East Azerbaijan, Iran.

**Methods:** Powders of HDPE samples were prepared by grinding in different particle sizes of 212, 300, 420, and 500 microns. Each time 50 mg of a sample was poured to a liquid medium containing species. Samples were incubated for 18 days at 28 °C in a shaker-incubator and their degradation percentage was measured by weighting method. Produced metabolite at 18th day was analyzed by gas chromatography-mass spectrometry. Also a film of HDPE was subjected to biodegradation and after one month was analyzed by scanning electron microscope which showed degradation on the surface of the film.

**Results:** The results showed that *Streptomyces* species degraded 50 mg of HDPE sample with the size of 212 µm about 18.26%, 300 and 420 µm about 14.4%, and 500 µm about 13%. Kinetic modeling of biodegradation process showed that the reaction rate was first order with respect to concentration of HDPE. Based on gas chromatography-mass spectrometry results, no high toxic material was produced during biodegradation of HDPE.

**Conclusions:** The research showed that isolated *Streptomyces* sp. are capable of degradation of HDPE polymer with high degradation efficiency.

## 1. Introduction

Plastics are long chain synthetic polymer molecules. More than half a century ago, production of synthetic polymers started and they became an alternative for natural materials in almost every field. Nowadays, they are an indispensable part of our life. Basic materials for production of plastics are extracted from oil, coal, and natural gas[1]. Development and utilization of synthetic plastics have changed the nature of wastes in the past four decades. Currently, plastics have replaced natural material in different aspects of human life and become a necessary part

of the society. Although durability of polymers is one of their suitable properties, this property is also their major problem for the environment. In the past two decades, the rate of plastic deposition is tremendously increased and plastics are intruded into the marine environment. Floating plastics can be found everywhere in oceans from polar region to the equator and have become the most prevalent and stable pollutants of seas. Polyethylene is the most abundant non-degradable solid waste in the environment and recently is known as a main threat for marine life. About 64% of polyethylene is discarded into environment in a short time period after use. It is resistant to acids, alcohols, bases, and esters[2].

Biodegradation is a biological method for bioremediation of organic pollutants. This innovation employs metabolic varieties of microbes to remove dangerous pollutants. Biological degradation methods don't have some restrictions of conventional methods[3].

Polyethylene is a critical material in construction of key

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

infrastructures for different industries, which makes it necessary for understanding its degradation from both stability and integrity viewpoints[4]. There is general agreement that biodegradation process of polyethylene is very slow under normal conditions[4-8]. Utilization of this polymer by microorganisms is physically limited by its insolubility in aqueous solution, lack of functional groups, and high molecular weight[8]. Although there is enough evidence which urges that polyethylene is biodegradable, there is still lack of knowledge about complete metabolic pathways of the process and biodegradation is slower than other methods[4].

Yanga *et al.*[9] studied biodegradation of plastics in compost stored at temperatures of  $-20^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ , and  $20^{\circ}\text{C}$  for different periods in South Korea. It was expected that the number of viable cells at  $-20^{\circ}\text{C}$  to be lower than those at  $4^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ , because these cells may be under stress or even be killed due to the formation of ice crystals. But at  $20^{\circ}\text{C}$  the number of mesophilic *Actinomycetes* was fewer compared to the other two lower temperatures, which indicates that the number of thermophile bacteria and *Actinomycetes* stored in compost at  $-20^{\circ}\text{C}$  was lower as expected which means that thermophile bacteria were under higher stress than mesophilic bacteria in freezing conditions.

Harshvardhan and Jha[2], used sixty types of bacteria isolated from sea water for biodegradation of low density polyethylene. The weight loss of polyethylene after 30 days of incubation with M16, M27, and H1584 bacteria was 1%, 1.5%, and 1.75%, respectively.

Sunilkumar *et al.*[10], studied biodegradability of bio-composite films constructed from low density polyethylene and chitosan by inoculating the films with *Aspergillus niger* on a potato dextrose agar media. Chitosan, maleic anhydride, dicumyl peroxides, and oleic acid constituents in palm oil were used as biodegradable filler, coupling agent, free radical initiator, and Lewis catalyst, respectively. Samples were incubated for 21 days at  $25^{\circ}\text{C}$ . It was observed that biodegradation rate and hydrophilicity increase with increase in chitosan loading in the matrix. Plasticized samples showed higher biodegradation rate and hydrophilicity compared to the unplasticized samples.

El-Shafei *et al.*[11] investigated capability of fungi and *Streptomyces* species on biodegradation of disposable polyethylene bags containing 6% starch. They used 8 different isolated *Streptomyces* strains and two fungi, *Mucor rouxii* NRRL 1835 and *Aspergillus flavus* for biodegradation of polyethylene films which were incubated at  $30^{\circ}\text{C}$  and 125 r/min in yeast extract medium for up to 4 weeks. Active enzymes caused changes in the films' mechanical properties and weight.

Bonhomme *et al.*[12] degraded polyethylene with molecular weight of 4000–28000 into units with molecular weight of less than 1500 during 8 months in liquid mineral salt media using *Rhodococcus rhodochrous* ATCC 29672, and *Nocardia asteroides* GK 911 bacteria and *Cladosporium cladosporoides* ATCC 20251 fungus.

As many works done on biodegradation of different types of polyethylene polymer, but no significant work has been done

on biodegradation of high density polyethylene (HDPE) using *Streptomyces* species.

Balasubramanian *et al.*[13] used *Arthrobacter* and *Pseudomonas* bacteria for biodegradation of HDPE. The weight loss of HDPE samples was about 12% for *Arthrobacter* and 15% for *Pseudomonas* after 30 days of incubation. They used bacteria on film samples of HDPE before biodegradation.

Muthukumar *et al.*[14] studied biofouling and biodegradation of starch blended HDPE in Bay of Bengal for 6 months. The weight loss of HDPE sample was about 17% and the surface of polymer turned hydrophilic. 22 bacteria strains were identified and isolated from the biofilm. Sangeetha Devi *et al.*[15] isolated HDPE degrading various fungal strains from the polyethylene waste dumped marine coastal area and screened under *in vitro* condition. Based on weight loss and Fourier-transform infrared spectroscopy spectrophotometric analysis, they found that two fungal strains designated as VRKPT1 and VRKPT2 were efficient in HDPE degradation. A complete review of biodegradation of polyethylene could be found in the work of Sangale *et al.*[8].

The goal of this research was to use *Streptomyces* species isolated from the soil of North West of Iran for biodegradation of HDPE used for manufacture of bleach containers in both forms of powder and film.

## 2. Materials and methods

In this research, polyethylene samples were prepared from bleach containers. The grade of HDPE used for manufacture of these containers is HD 5620 EA whose properties are shown in Table 1[16].

**Table 1**

Physical properties of HDPE containers used for biodegradation.

Property	Unit	Value	Test method
MFI (190 °C/2.16 kg)	g/10 min	20	ASTM D 1238 – 7 condition E
Density	g/cm <sup>3</sup>	0.956	ASTM D 2839 – 69
Tensile strength at yield	MPa	22	ASTM D 638 – 72
Elongation at break	%	900	ISO R527 – Type 2 speed D
Flexural modulus	MPa	1000	ASTM D 790 – 71
Charity impact strength (Notched)	kJ/m <sup>2</sup>	10	ASTM D 256 – 73B
Hardness	Shore D	66	ASTM D 2240 – 75

MFI: Melt flow index.

Firstly, containers were sliced into smaller parts and then were ground using ultra centrifugal mill model ZM 200-Retsch (Germany). To achieve regular sizes, ground material was screened to particle sizes of 500, 420, 300 and 212 microns, respectively. Biological degradation tests were performed on prepared samples. Samples were firstly washed and dried at  $60^{\circ}\text{C}$  and then were sterilized at  $105^{\circ}\text{C}$  for 1 h within an autoclave (Autoclave, Kavooshmega Medical, Iran)[2].

Isolates firstly were sub-cultured from stock cultures on Mueller-Hinton agar containing (per liter): 2 g beef extract, 17.5 g acid hydrolysate of casein, 1.5 g starch, and 17 g agar. After incubation at  $30^{\circ}\text{C}$  for 1 day, *Streptomyces* species separated from the soil of

East Azerbaijan, which was stored in Biotechnology Department, East Azerbaijan Agricultural Education Center, Iran, was transferred to Mueller-Hinton broth medium, containing (per liter): 2 g beef extract, 17.5 g acid hydrolysate of casein, and 1.5 g starch. Once the culture reached to a proper turbidity, 400  $\mu\text{L}$  of the culture with 0.5 McFarland turbidity was used for biodegradation process in mineral medium (MM), containing (per liter): 0.64 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (Merck), 0.11 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck), 0.79 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (Merck), and 0.15 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck). The pH of the solution was adjusted about  $7.20 \pm 0.01$  using 0.1 mol/L  $\text{H}_2\text{SO}_4$  or 0.1 mol/L KOH by pH meter (654 pH meter Metrohm, Switzerland). Finally, the medium was sterilized by autoclave (Autoclave 87419, Iran Teb Zaeem, Iran) at  $121^\circ\text{C}$  for 20 min<sup>[17]</sup>. The experiments were performed in 250 mL Erlenmeyer flasks containing 200 mL MM supplemented with 50 mg sterilized HDPE as sole source of carbon. Two cultures including: (1) MM + HDPE + species isolate and (2) MM + HDPE were used as control mediums. To evaluate HDPE biodegradation potential of *Streptomyces* species, the samples were incubated within 180 r/min shaking incubator (VS 8480 SR, South Korea) at  $25^\circ\text{C}$  for 18 days.

For the assessment of kinetic model in an 18-day period, samples were analyzed firstly each 3 days and then each 6 days, and the amount of degraded polyethylene was determined. A fast and easy method for the assessment of HDPE biodegradation is to measure the mass of remained polyethylene. For this purpose, colonies of bacteria on remained polyethylene surface were removed using 2% sodium dodecyl sulfate solution. Then the medium was incubated for 4 h at  $50^\circ\text{C}$  and washed by distilled water. Polyethylene samples were separated on filter paper and washed by distilled water. Before final weighting, it was dried within an oven at  $60^\circ\text{C}$ <sup>[2,18]</sup>. Degradation percentage was calculated using the following formula:

$$\% \text{Biodegradation} = (mA_0 - mA) / mA_0 \times 100 \quad (1)$$

where  $mA_0$  and  $mA$  are initial and final masses of polyethylene sample, respectively. Each experiment was repeated three times to assure repeatability.

### 2.1. Analysis of products by gas chromatography-mass spectrometry (GC-MS)

At the end of 18th day of biodegradation, a sample with the highest degradation percentage was taken for GC-MS for each size of HDPE powder. Samples were centrifuged by a small centrifuge (Hettich EBA 20, Germany) with 10 000 r/min for 20 min. The surface liquid was extracted two times with toluene. For identification of secondary metabolites, GC-MS equipment (Model Agilent 6890, USA) coupled with a mass spectrophotometer (Model Agilent 5973, USA) was used. Extracted components were separated on HP-5ms capillary column ( $30 \text{ m} \times 0.25 \text{ mm}$  inner diameter  $\times 0.25 \mu\text{m}$  film, J&W Scientific, Folsom, CA) using temperature programming: initial temperature of  $40^\circ\text{C}$  was kept for 5 min and then it was raised up to a maximum of  $250^\circ\text{C}$  with a rate of  $5^\circ\text{C}/\text{min}$ . Initial injection was performed at  $280^\circ\text{C}$  within an oven<sup>[19,20]</sup>.

### 2.2. Analysis of HDPE film using scanning electron microscope (SEM)

For the assessment of any structural changes of HDPE films, a film sample of HDPE was exposed to biodegradation by *Streptomyces* and after one month of treatment by species, surface morphology of film was analyzed using SEM (model VEGA II LMH, TESCAN Co., Czech Republic). After complete washing with sterile distilled water, the sample was mounted on copper stubs with gold paint. Gold coating was carried out using vaporization in vacuum to make the sample conductive. The image of the sample was compared with test sample<sup>[2,20]</sup>.

### 2.3. Kinetic model

In this research, reaction rate equation for biodegradation of powdered HDPE samples was obtained using experimental data. Because the only reactant of biodegradation is polyethylene and the reaction rate changes with time, the reaction kinetics may be first order with respect to polyethylene concentration. The rate equation for irreversible first order reaction is as follows:

$$-r_A = kC_A \quad (2)$$

where  $C_A$  is concentration of powdered HDPE within medium, and  $k$  is reaction rate constant. By writing mass balance equation for the batch process of biodegradation and integration with respect to time, the following equation will be obtained:

$$-\ln \frac{C_A}{C_{A0}} = kt \quad (3)$$

where  $C_{A0}$  ( $= 250 \text{ mg/L}$ ) is initial concentration and  $t$  is time. By plotting experimental data in the form of  $\ln C_A$  versus  $t$ , if the reaction kinetics is first order, the trend line of experimental data must have the slope of  $k$ .

Also, second order rate equation was used for the assessment of HDPE biodegradation kinetics:

$$-r_A = kC_A^2 \quad (4)$$

In this case, the following equation can be used for linear regression:

$$\frac{1}{C_A} - \frac{1}{C_{A0}} = kt \quad (5)$$

By plotting  $1/C_A$  versus  $t$ , if the reaction kinetics is second order, experimental data will fit a straight line with the slope of  $k$ .

In order to select the best model, two statistical parameters of coefficient of determination,  $R^2$ , and mean square of errors (MSE) were used which are defined as below:

$$R^2 = 1 - \frac{\sum_{i=1}^n (C_{Ai} - \bar{C}_A)^2}{\sum_{i=1}^n (C_{Ai} - \hat{C}_{Ai})^2}, \quad \bar{C}_A = \frac{\sum_{i=1}^n C_{Ai}}{n} \quad (6)$$

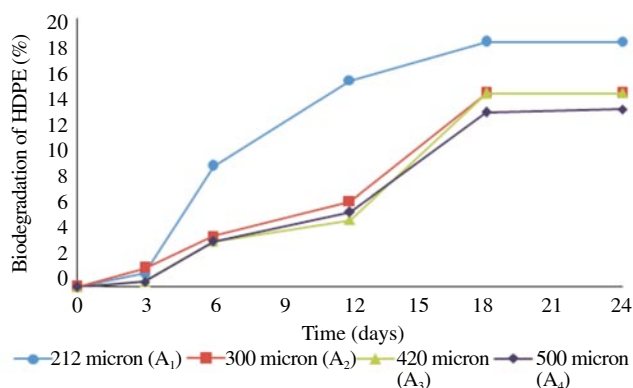
$$\text{MSE} = \frac{\sum_{i=1}^n (C_{Ai} - \hat{C}_{Ai})^2}{n} \quad (7)$$

where  $C_{Ai}$  and  $\hat{C}_{Ai}$  are experimental and calculated values of HDPE concentrations, respectively.

### 3. Results

#### 3.1. Biodegradation results

Figure 1 shows biodegradation percentages of HDPE with respect to time for different sizes calculated by equation (1). As it can be seen, biodegradation begins from the 3rd day for all samples. Biodegradation of sample A<sub>1</sub> was much higher than other samples, but its rate decreased after the 12th day. Biodegradation of samples A<sub>2</sub>, A<sub>3</sub>, and A<sub>4</sub> was almost the same, and sample A<sub>4</sub> had the lowest biodegradation value after the 15th day.



**Figure 1.** Biodegradation percentage of HDPE with respect to time for different particle sizes.

An experimental model was fitted on experimental data where degradation time,  $t$  (days), and size of particles,  $s$  (microns), were selected as input parameters and concentration of remained HDPE after biodegradation,  $C_A$ , was used as output of the model. The model was selected as second order because the levels used for input parameters was more than 2. After linear regression, the following model was obtained:

$$C_A = 218.8 - 2.435t + 0.1831s - 0.0387t^2 - 2.42 \times 10^{-4}s^2 + 2.93 \times 10^{-3}ts \quad (8)$$

ANOVA was applied on the above model whose results are shown in Table 2.

From the  $P$ -values with confidence level of 95%, it was obvious that significant parameters in the model were degradation time and size of particles. Thus, first order model could predict the results precisely.

**Table 2**

ANOVA for the model obtained by linear regression of experimental results.

Term	$F$ -value	$P$ -value
$t$	9.10	0.009
$s$	4.29	0.057
$t^2$	1.43	0.252
$s^2$	3.92	0.068
$t.s$	3.97	0.066
$R^2$ for this model	93.05	

#### 3.2. Calculation of biodegradation reaction rate constant

Using experimental results shown in Figure 1, reaction rate constants were found for both first and second order reaction rate kinetics using equations (2) and (4) for 18 days. Table 3 shows

results of calculations for different sizes. In order to select the best model for reaction kinetics, three parameters of coefficient of determination,  $R^2$ , MSE, and initial concentration,  $C_{A0}$ , were used. Because  $C_{A0}$  was 250 mg/L for all experiments, thus any model capable of predicting this value more precisely and having the highest value of  $R^2$  and lowest value of MSE was selected as the best kinetic model for the process. It was obvious from the following results that the first order model was the best one for all cases.

Figure 2 shows comparison of experimental and calculated biodegradation results using first order kinetics for different particle sizes till the 18th day from the start of the experiments. As it can be seen, first order model fitted experimental results very well.

**Table 3**

Biodegradation reaction rate constant for first and second order kinetics.

Sample	First order				Second order			
	$k \times 10^3$	$R^2$	MSE	$\hat{C}_{A0}$ (mg/L)	$k \times 10^5$	$R^2$	MSE	$\hat{C}_{A0}$ (mg/L)
A <sub>1</sub>	12.2	0.94	32.89	250.06	5.40	0.95	29.35	250.58
A <sub>2</sub>	8.27	0.94	15.92	252.42	3.57	0.93	18.44	252.93
A <sub>3</sub>	8.24	0.89	32.41	253.81	3.55	0.88	36.02	254.39
A <sub>4</sub>	7.57	0.93	15.77	253.00	3.24	0.92	17.83	253.44

$\hat{C}_{A0}$ : Calculated value of  $C_{A0}$

#### 3.3. Results of the analysis of produced metabolites with GC-MS

GC-MS analysis was performed on the samples taken from the medium at the end of biodegradation process to analyze products of biodegradation and to assess their toxicity. Table 4 shows products and their toxicity. As it is evident, no high toxic compounds were observed in the metabolites.

**Table 4**

Products of HDPE biodegradation identified by GC-MS and their toxicity.

Compound	Toxicity (LD <sub>50</sub> mg/kg)	Reference
Arsenous acid, tris(trimethylsilyl) ester	-	-
Benzene dicarboxylic acid (phthalic acid)	7900	[21]
Tetradecanoic acid (myristic acid)	> 10000	[22]
Eicosane	> 2000	[23]
Heneicosane	-	-
Docosane	> 2000	[23]
Tricosane	1600	[24]
Tetracosane	43000	[24]
Hexacosane	1600	[25]
Benzoic acid	1700	[21]
Hexadecanol	2510	[26]
1,3,6-Octatriene	-	-

#### 3.4. SEM results

In order to investigate the effect of *Streptomyces* species on biodegradation of HDPE films, a film of HDPE was prepared for this purpose and was treated by the bacteria. After one month of treatment, the film was analyzed by SEM. Figure 3 shows SEM result after treatment by species. As it can be seen, digestion on the film was significant and erosion was visible on its face.

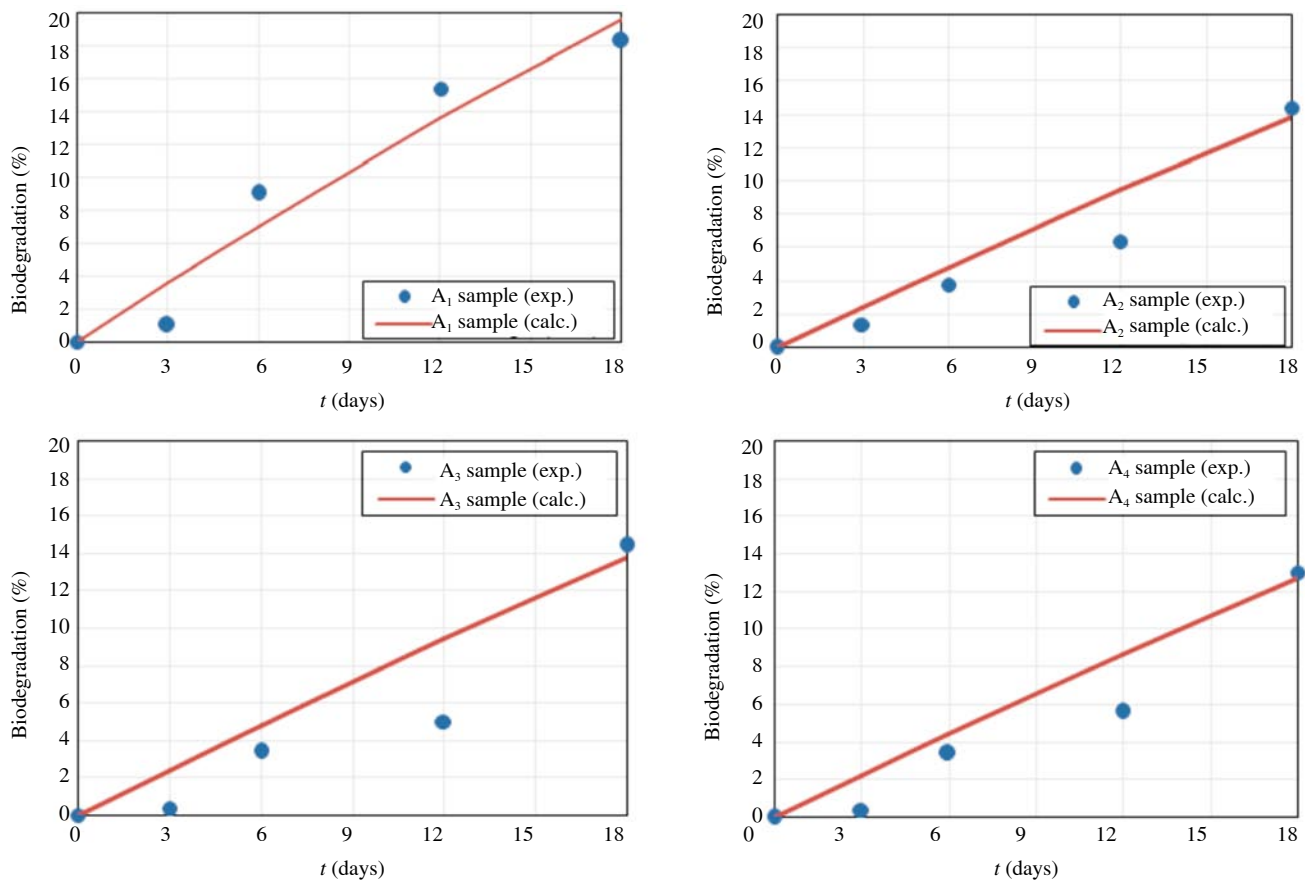


Figure 2. Comparison of experimental and calculated biodegradation percentage using first order kinetic model for different particle sizes.

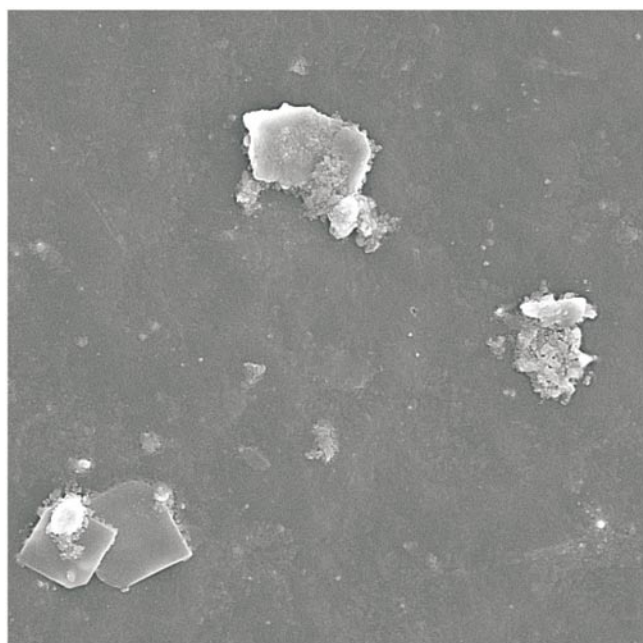


Figure 3. SEM image taken from HDPE film sample after treatment.

### 3.5. Results of kinetic modeling

The results of kinetics modeling showed that the rate of biodegradation of HDPE obeyed a first order kinetics with respect to the HDPE concentration in the medium. Also it was shown that the reaction rate increased with decrease of sample particle size. Dependency of reaction rate constant on samples particle size is

shown in Figure 4. The dependency of rate constant on temperature was not investigated because the range of applicable temperature change in biological processes was narrow and rate constant could be assumed to be constant over this narrow range.

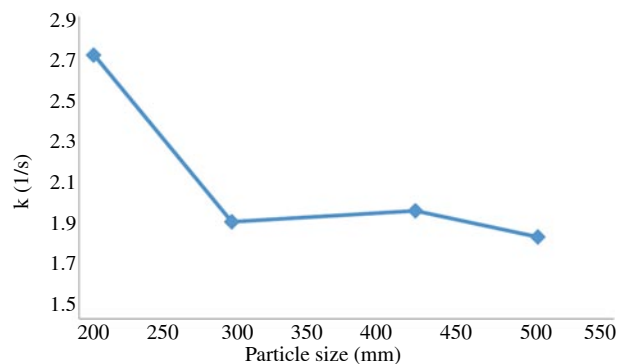


Figure 4. Changes of biodegradation reaction rate constant with respect to samples particle size.

## 4. Discussion

Based on the results of biodegradation shown in Figure 1, it can be concluded that the amount of biodegradation for finer particles is higher. After the 18th day, biodegradation percentage remains constant for all samples because of saturation of the medium by cells, reduction of carbon source, death of cells, and production of inhibitory metabolites in the medium.

Results showed that biodegradation of HDPE increased by decreasing particles size. But it didn't change sharply for particle sizes larger than 300 microns. Thus the optimum particle size between all investigated sizes is 212 microns. Sizes finer than 212 including nano-scale sizes may be investigated. But generation of

particles with nano-scale sizes could increase degradation cost.

An advantage of biological degradation is that degradation products are not highly toxic and harmful which was examined by GC-MS (Table 4). Thus, utilization of biological methods is a promising method for degradation of environmentally harmful materials.

SEM results (Figure 3) obtained after biodegradation of a sample film of HDPE showed good digestion on film surface, but it is not efficient for entire degradation of HDPE. Therefore, mechanical treatment of HDPE containers and grinding them into micro-scale sizes are necessary before treatment by biological methods.

In this work, biodegradation of HDPE was investigated using *Streptomyces* species separated from the soil of East Azerbaijan, Iran. Samples of HDPE were ground to four different sizes of 212, 300, 420, and 500 microns in order to investigate the effect of particle size on biodegradation. Results showed that biodegradation percentage remained constant after 18 days for all samples. Loss of mass was measured in each three repeats for all samples. Maximum biodegradation percentage was obtained for the sample with particle size of 212 microns which was about 18.2%. The results of experiments were reasonable compared to the literature [1,10,18] which proves high performance of *Streptomyces* species compared to the other microorganisms.

In order to identify secondary metabolites produced during biodegradation, the sample with the highest degradation percentage was analyzed by GC-MS which showed that all produced metabolites are safe for the environment. Also a film sample of HDPE was exposed to biodegradation and after one month of treatment with isolates was analyzed by SEM. Comparison of SEM result with control sample and previous works [18] showed that the biodegradation is acceptable.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

This paper is supported by Biotechnology Department, East Azerbaijan Agricultural Education Center, Agricultural Research, Education and Extension Organization, Tabriz, Iran, and University of Tabriz, 29 Bahman Blvd., Tabriz, Iran.

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