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Biodegradation of Cypermethrin by Two Isolates of *Pseudomonas aeruginosa*

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Abstract

Two local bacterial isolates were isolated from agriculture soil contaminated with cypermethrin from AlAbaeje village in Baghdad city, these fields were already sprayed with cypermethrin for past few years. Primary screening was done to test bacterial ability to growth and resistance to cypermethrin by using nutrient agar plates containing 500 mg L^{-1} cypermethrin as a sole source of carbon and energy, and incubation at 37 ºC for 72 hours. Secondary screening results showed that these two bacterial isolates have the ability to grow and resistance cypermethrin concentration till 3600 mg L^{-1} in solid MMSM. If we increase concentration above 3600 mg L^{-1} bacterial isolate can't tolerate and grow in it. According to VITEK 2 compact results the dominate isolate in soil contaminated with cypermethrin was *Pseudomonas aeruginosa* about 87.5%. The optimum conditions (pH, temperature and incubation period) for growth of two selected isolates and biodegradation of cypermethrin were examined. The results indicated that the best growth obtain at pH 7 under 30 ºC for 14 days. In lab experiment and under optimal conditions, cypermethrin biodegradation was measured by using FTIR and GC-MS analysis. The results shown that *P. aeruginosa* 1 was the best isolate for degrading cypermethrin with percentage 87.9% while *P. aeruginosa* 2 with degrading percentage 80%.

Keywords: Biodegradation**,** Cypermethrin**,** *Pseudomonas aeruginosa* , FTIR , GC-MS

التفكيك الحيوي للسيبرمثرين بوساطة عزلتين من *Pseudomonas aeruginosa*

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الخلاصة

تم عزل عزلتين بكتيريه محلية من ارضي زراعية ملوثه بمبيد السايبرمثرين من قرية العبايجي في مدينة بغداد، تستخدم هذه الارضي مبيد السايبرمثرين منذ عدة سنين. أجريت الغربلة الاولية لاختبار قدرة البكتريا على النمو ومقاومة مبيد السايبرمثرين بوساطة استعمال وسط مغذي الصلب يحتوي على المبيد بتركيز 500 مغ في اللتر كمصدر للكاربون والطاقة وحضنت في درجة حرارة 37 درجة مئوية لمدة 72 ساعة. نتائج الغربلة الثانية اظهرت ان هنالك فقط عزلتين لها القدرة على النمو ومقاومة السيبرمثرين بتركيز 3600 مغ في اللتر في وسط الاملاح المعدنية المحور الصلب. وعند زيادة التركيز الى اكثرمن 3600 مغ في اللتر لم تسطع اي عزلة بكترية مقاومته والنمو عليه. وطبقا لنتائج فحص جهاز VITEK 2 compact فان البكتريا السائدة كانت *Pseudomonas aeruginosa* بنسبة 87.5%. تم قياس الظروف المثلى (الاس الهيدروجيني و

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درجة الحرارة وفترة الحضن) اللازمة لنمو البكتريا و لتفكيك الحيوي لمبيد السايبرمثرين. وبينت النتائج ان أفظل نمو بكتري يحدث عن الاس الهيدروجيني 7 وتحتت درجة حرارة 30 درجة مئوية. في التجارب المختبرية وتحت الظروف المثلى تم قياس التفكيك الحيوي للسيبرمثرين بوساطة جهازي FTIR وGC–MS . واظهرت النتائج ان بكتريا1 *P. aeruginosa* كانت افضل في تفكيك السيبرمثرين بنسبة 87.9% بينما بكتريا *P. aeruginosa* 2 استطعت تفكيك المبيد بنسبة 80%.

Introduction

Pyrethroids insecticides are a class of lipophilic esters, with an alcohol and an acid moiety. Although less toxic and persistent than other groups of insecticides, they can still represent a problem. Pyrethroids display high affinity to Na+ channels and its binding to these channels causes a prolonged channel opening that may result in a complete depolarization of the cell membrane thus blocking neuronal activity [1]. The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin. Cypermethrin agricultural products can be applied at various stages of crop development [2].The use of cypermethrin has increased sharply especially in recent years with the restrictions or eliminations of highly toxic organophosphate pesticides, and it has become one of the dominant insecticides among retail sales to consumers [3]. cypermethrin has an extremely high toxicity to the aquatic environment with concentrations as low as 10 µg L^{-1}, destroying aquatic invertebrate life [4]. [5]reported that cypermethrin was found to have carcinogenic activity in both sexes of Swiss albino mice. Pyrethroids are responsible for respiratory effects, immunological or lymphoreticular effects, neurological effects, gastrointestinal effects, hematological effects and even cause death to the human being [6]. Cypermethrin persistence in environment varies from 14.6 to 76.2 days (half-life) depending on physicochemical properties of soil [7]. Microbial activity in soil also plays vital role in determining the fate and behavior of cypermethrin in soil.

Looking into the facts of toxicity and persistency of this pesticide, it is urgently required to develop some strategies to eliminate or detoxify cypermethrin and its metabolites from the environment. The biological degradation; involves the use of effective microorganism to degrade the complex pesticide into simple inorganic chemicals [8]. Moreover, this technology is less hazardous, environmentally friendly and economically viable and socially acceptable [9]. The native soil microbial consortia are superior and effective consortia for microbial degradation of pesticide than the non-native strains. Because, the native strains grow very good and have advanced adaptability in particular geographical region [10]. native microorganisms are highly adaptable and have the capability to degrade the recalcitrant compounds through evolution of new genes, which encode enzymes that can use these compounds as their primary substrates [11].

Several bacterial strains such as *Pseudomonas aeruginosa* [12], *Streptomyces sp*. [13], *Stenotrophomonas sp*. [14] and *Serratia marcescens* [15] have been reported to degrade pyrethroid pesticides. Glutamyl Arylamidase pNA (AGLTp), which are intracellular enzymes used by *P. aeruginosa* for amino acid hydrolysis [16], and RESISTANCE O/129 comp. vibrio (O 129R) test , which are antimicrobial susceptibilities test [17], these two tests were measured by VITEK 2 compact device. The aims of current study, shown the efficiency and different in byproducts produced from biodegradation of cypermethrin by two isolates of *Pseudomonas aeruginosa* have different results in AGLTp and O 129R.

Materials and Methods:

Chemicals and Media

Commercial grade cypermethrin(10 g L^{-1},Bharat Insecticides Limited, India) was purchased from agricultural chemical dealers and dissolved in acetone to make a stock of 1000 mg L^{-1}. Stock solution was filter sterilized and kept in refrigerator for use. Modified Mineral Salt Media, Nutrient agar, Nutrient broth (pH 7) were used for the isolation and cultivation of pesticide degrading bacterial strains according to [18].

Screening and isolation of cypermethrin degrading bacteria

Soil samples were collected from different sites of agriculture soil contaminated with cypermethrin from AlAbaeje village in Baghdad city, Iraq. The samples were collected randomly from the superficial layer of soil (10-20 cm in depth), These fields were already sprayed with cypermethrin for past few years. These soil samples were used for the isolation of cypermethrin-degrading bacteria. 10

g of soil samples were added to 150 ml MMSM supplemented with 50 mg L^{-1} cypermethrin in 250 mL Erlenmeyer flasks, and incubation was carried out at 150 rpm and 30 ºC for five days [19]. After dilution, one ml of soil suspension (10^{-1},10^{-3},10^{-5} and 10^{-7}) was inoculated in nutrient agar plates supplemented with 50 mg L^{-1} cypermethrin [7], after 3 days of incubation, the discrete bacterial colonies were purified by repeated inoculating them on plates containing nutrients agar. Cypermethrin degrading bacterial cultures were screened from the isolated pure bacterial cultures by:

1- Primary screening by growing them on nutrient agar plates containing 500 mg L^{-1} cypermethrin as a sole source of carbon and energy as described by [13].

2- Secondary screening by growing them on solid modified mineral salt media containing cypermethrin as sole source of carbon and energy at different concentration ranging from (600 - 3600 mg L^{-1}) and incubated at 37 ºC for 24- 48 h The growth of bacterial isolates was examined according to formation of clear zones around the colonies [20].

Identification of cypermethrin degrading bacterial strain

The colonies developed on nutrient agar were studied in terms of their shape, colour, odour and their margin, also microscopic examination was achieved for G-stained slides to characterize G-negative bacteria from others. VITEK 2 compact device (Biomerienx, France) was only used to identify the isolates [21, 22]. Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card according to the Manufacturer's instructions [23]. The results were computerized and recorded by the vitek software after the bacterial suspension was loaded in the vitek cards.

Determination of optimum growth conditions

Optimum pH and Temperature

Overnight bacterial isolate (2%) with (OD=0.5) were seeded into the 250 ml Erlenmeyer flasks containing modified mineral salt medium (100 ml) supplemented with 100 mg l^{-1} of cypermethrin. The pH values of the medium were adjusted to a series of 5, 7 and 9. Three sets of flasks were used each having the unique value of pH. Flasks was inoculated in duplicates in a shaker incubator (160 rpm) at 25,30,35,40 ºC for 8 days, the growth of bacterial isolate was observed by measuring Optical Density (absorbance) at 600 nm in spectrophotometer after culturing for zero time, first, third, fifth and eighth day, respectively [24].

Optimum incubation period

After determine optimum pH and temperature the best incubation period for each isolate determine by inoculated 2% with (OD=0.5) overnight bacterial isolate in Erlenmeyer flask (250 ml) containing 100 ml of modified mineral salts medium, was adjusted to pH 7 and autoclaved at 121ºC for 15 min, then supplemented with100 mg L^{-1} of cypermethrin. The flasks were incubated in a shaker incubator (160 rpm) at 30^0C for different periods (1, 3, 5, 8, 10, 12, 14,16 days). Experiment carried out in duplicate and the growth of bacterial isolate was observed by measuring Optical Density at 600 nm in spectrophotometer [25].

Biodegradation Experiment

Erlenmeyer flasks (250ml) containing 100ml of the liquid modified mineral salts medium, the pH was adjusted to 7 and autoclaved at 121^0C for 15 min, supplement with 100 mg L^{-1} of cypermethrin. The flasks were inoculated separately with 2% (OD=0.5) from the selected isolates taken from the overnight cultures (one isolate for each flask) [26].

An experiment carried out in duplicate. The flasks were incubated in a shaker incubator with 160rpm at 30^0C for 14 days. After the incubation period, 5 ml of broth was taken for extraction and analysis of cypermethrin residues according to [27]. Extracted liquid was taken in eppendorff tube and stored at -20°C till analysed by FTIR and GC-MS.

Determination of Cypermethrin Residual

Residual analysis of cypermethrin in media was performed according to [27]. Residual pesticide was extracted by adding 5 ml of culture broth to 20 ml of acetone in a flask. The mixture was filtered using Buchner funnel after shaking for 1 h and the obtained residue was filtered again by Millipore filter unit 8µm(Whatman Grade 540 Quantitative Filter Paper, Hardened Ashless) after washing thoroughly with 10 ml acetone. Filtrate was collected in a round-bottom flask [26]. The cypermethrin content was detected using GC-mass and the percentage of cypermethrin biodegradation was measured according to the following equation [28]:

$$\% \, \boldsymbol{of\ Biodegradation} = \frac{\boldsymbol{Peak\ area\ of\ Zero\ Time(T_0) - peak\ area\ of\ sample(T_1)}}{\boldsymbol{Peak\ area\ of\ Zero\ Time(T_0)}} \times \mathbf{100}$$

By FTIR

The use of Fourier transform infrared (FTIR) spectroscopy provided additional information to aid the interpretation of the biodegradation process. Analysis was carried out in labs of chemistry department/college of science/University of Baghdad. Measurement were performed using a Shimadzu FTIR-8400 S using KBr discs containing 2ml of test samples which are prepare performed according [27]. Ten scans were taken for each sample in the range 4000 to 400 cm^{-1}.the measure FTIR for samples done on zero time and fourteenth day of experiment [29].

By GC-MS

Cypermethrin compounds in soil samples and media were determined using gas chromatography - mass spectrometry (GC-mass). All analysis was carried out in labs of the environment and water directorate/ ministry of Science and Technology.

Statistical analysis

Least significant difference (LSD) was carried out using statistical package for social sciences (SPSS, Version 17.0). Analysis of variance (ANOVA), P – values, tests of significance, was carried out at 95% level of confidence using statistical package for social sciences. P – Values[* ($P \leq 0.05$)] were used to determine the significance levels between various treatments and data obtained during the experimental study [30].

Results and Discussion

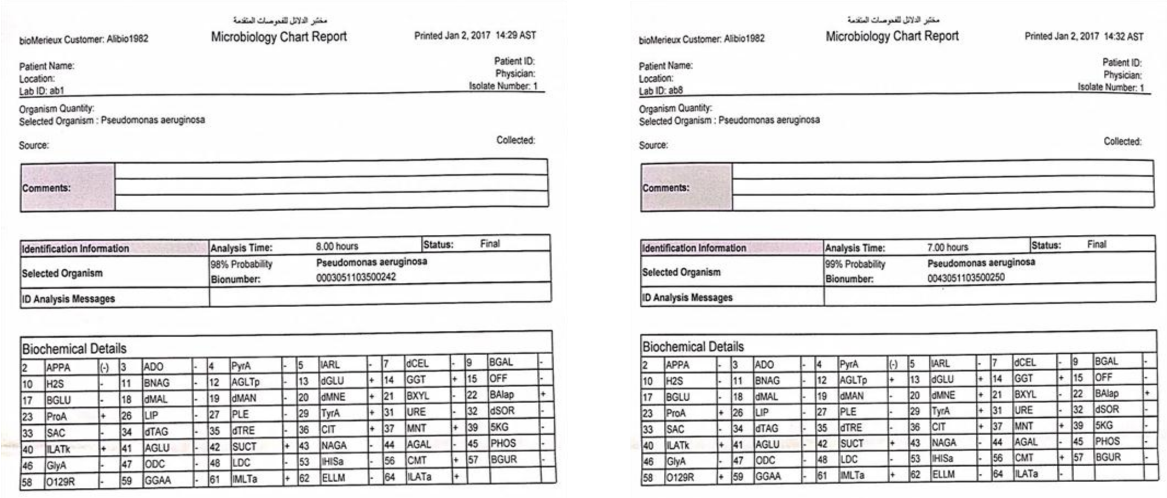
Isolation and characterization of cypermethrin degrading bacteria

Table 1: Morphological characteristic, Gram Stain , ID and probability value of bacterial isolates.

Characteristics / Isolates No.	Colony shape	Colony color	Cell shape	Gram stain	ID of isolate By Vitek 2 compact	probability value
1	Smooth convex	green	Rod	Negative	*Pseudomonas aeruginosa*	99%
2	Smooth	yellow	Rod	Negative	*Pseudomonas aeruginosa*	98%

In the present study two isolates of *Pseudomonas aeruginosa* were only selected due to their ability to utilize cypermethrin as a carbon source. Characteristic of Bacterial colonies growing on nutrient agar plates give in Table-1.

Two isolates from *Pseudomonas sp*. selected because it's dominate isolates and according to results of Vitek Figure-1 there are different in results between isolate 1 and 2 in AGLTp and O 129R test. Isolate 1, have green colour on nutrient agar, are positive for AGLTp and O 129R, while isolate 2 which have yellow colour on nutrient agar, are negative for AGLTp and O 129R test. So according to these tests there are genetic variation between isolate 1 and 2. In current study isolate 1 named as *P. aeruginosa* 1and isolate 2 named as *P. aeruginosa 2.*


مختبر الدلائل للفحوصات المتقدمة

Microbiology Chart Report

bioMerieux Customer: Alibio1982 Printed Jan 2, 2017 14:29 AST

Patient Name: Patient ID:
Location: Physician:
Lab ID: ab1 Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: Collected:

Comments:

Identification Information	Analysis Time:	8.00 hours	Status: Final
Selected Organism	98% Probability Bionumber:	Pseudomonas aeruginosa 0003051103500242	
ID Analysis Messages			

Biochemical Details

2	APPA	(-)	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	+			

مختبر الدلائل للفحوصات المتقدمة

Microbiology Chart Report

bioMerieux Customer: Alibio1982 Printed Jan 2, 2017 14:32 AST

Patient Name: Patient ID:
Location: Physician:
Lab ID: ab8 Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: Collected:

Comments:

Identification Information	Analysis Time:	7.00 hours	Status: Final
Selected Organism	99% Probability Bionumber:	Pseudomonas aeruginosa 0043051103500250	
ID Analysis Messages			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	(-)	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Figure 1- VITEK 2 compact identification results sheet for identified isolates 1 & 2.

Optimization of growth conditions

Optimum pH and Temperature

Effect of pH and Temperature on growth of *Pseudomonas aeruginosa* 1

The results in Table-2 explains that there are significant difference ($p<0.05$) of bacterial growth at different pH and temperature degrees. Under 25 ºC, 30 ºC, 35 ºC and 40 ºC the best and significant means bacterial growth recorded at pH 7 were 0.218, 0.360, 0.224 and 0.133 nm respectively. Also under pH 5, pH 7 and pH 9 the best and significant means of bacterial growth recorded at 30 ºC as 0.205, 0.360 and 0.114 nm respectively. The highest mean value for P.aerogenosa growth recorded at 30 ºC and pH 7 was 0.360 nm, while lowest mean value recorded at 35 ºC and pH 9 was 0.022 nm. As shown in Figure-2.

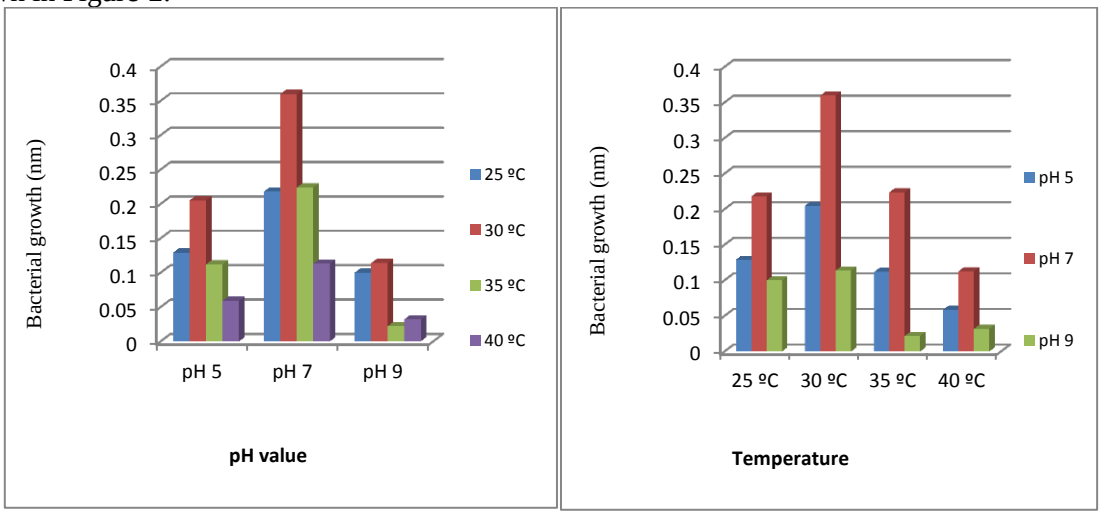


Figure 2-Mean value of *Pseudomonas aeruginosa 1* growth of at different pH and temperature value after 8 days of incubation.

Table 2- Mean value of *Pseudomonas aeruginosa 1* growth of at

Temp. \ pH	pH 5	pH 7	pH 9	LSD ≤ 0.05
25 ºC	0.129 ± 0.046 Bb	0.218 ± 0.062 Ab	0.100 ± 0.047 Bab	0.064
30 ºC	0.205 ± 0.051 Ba	0.360 ± 0.098 Aa	0.114 ± 0.114 Ba	0.126
35 ºC	0.112 ± 0.058 Bb	0.224 ± 0.100 Ab	0.022 ± 0.016 Bc	0.073
40 ºC	0.059 ± 0.021 Bb	0.113 ± 0.039 Ab	0.032 ± 0.032 Bb	0.035
LSD ≤ 0.05	0.074	0.122	0.070	

*Uppercase refer to comparison between means with same raw, lowercase refer to comparison between means with same column and similar characters refer to non-significant difference

Effect of pH and Temperature on growth of *Pseudomonas aeruginosa* 2

The results in Table-3 shown that there are significant difference ($p<0.05$) of bacterial growth under different pH and temperature degrees. Under 25 ºC, 35 ºC and 40 ºC the best and significant means bacterial growth recorded at pH 7 were 0.279, 0.215, and 0.166 nm respectively. While no significant difference was observe at 30 ºC under pH 5, 7 and 9. Also under pH 5, pH 7 and pH 9 the best and significant means of bacterial growth recorded at 30 ºC were 0.255, 0.410 and 0.253 nm respectively. The highest mean value 0.410 nm for *P. aeruginosa* 1 growth was recorded at 30 ºC and pH 7, while lowest mean value 0.051 nm was recorded at 40 ºC and pH 9. As shown in Figure-3.

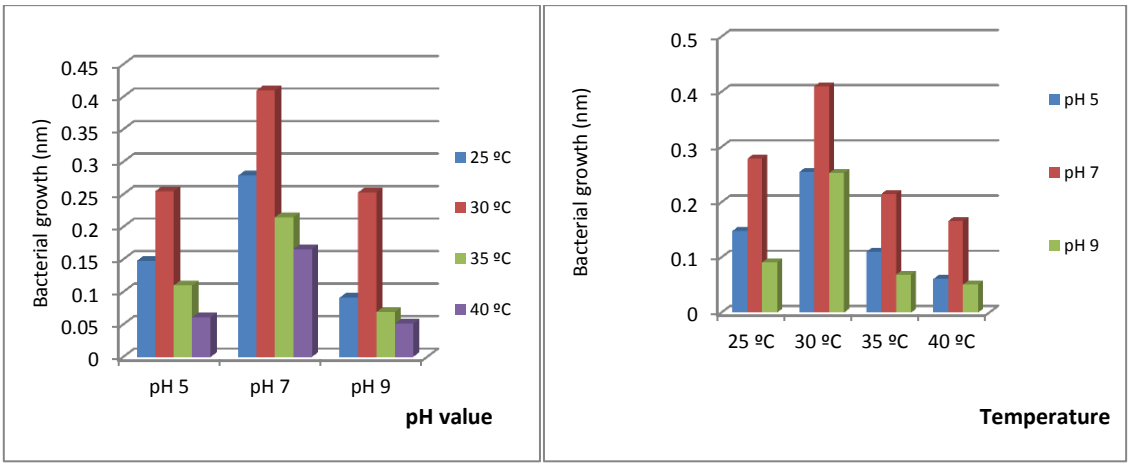


Figure 3- Mean value of *Pseudomonas aeruginosa 2*growth of at different pH and temperature value after 8 days of incubation.

Table 3- Mean value of *Pseudomonas aeruginosa 2* growth of at different pH and temperature value after 8 days of incubation.

pH / Temp.	pH 5	pH 7	pH 9	LSD ≤ 0.05
25 ºC	0.148 ± 0.049 Bb	0.279 ± 0.126 Ab	0.091 ± 0.028 Bb	0.122
30 ºC	0.255 ± 0.085 Aa	0.410 ± 0.157 Aa	0.253 ± 0.165 Aa	0.220
35 ºC	0.110 ± 0.056 Bbc	0.215 ± 0.079 Ab	0.069 ± 0.041 Bb	0.097
40 ºC	0.061 ± 0.021 Bc	0.166 ± 0.024 Ab	0.051 ± 0.048 Bb	0.053
LSD ≤ 0.05	0.086	0.119	0.138	

*Uppercase refer to comparison between means with same raw, lowercase refer to comparison between means with same column and similar characters refer to non-significant difference

So the pH 7 is optimum pH for growth of two selected bacterial isolates in MMSM contain cypermethrin, this finding is supported by [31], who reported that isolated *Pseudomonas* strain can grow and retain their degradation ability in a wide range of pH with optimum growth at pH around 7. Regarding temperature 30ºC is optimum for growths of two selected bacteria isolate. During Cypermethrin degradation, the direct correlation was found between temperature and microbial activity, significant removal occurred when biosimulator was operated at 28 - 30 ºC [32].

Effect of Incubation period on growth and biodegradation

The results in Table-4 showed significant difference ($p<0.05$) among means of bacterial growths for different incubation periods. The two selected bacterial isolates had highest and significant growth after 10 days of incubation. From day 1 to day 10 there are gradually increase in growth rate of two selected bacterial isolates after 10 days of incubation the growth start to decrease. The highest mean growth value 0.563 and 0.517 recoded for *P. aeruginosa* 2 and *P. aeruginosa* 1 respectively. Also there are significant difference ($p<0.05$) between mean growth of two selected isolates within same day and this difference start significantly after 3 days of incubation Figure-4.

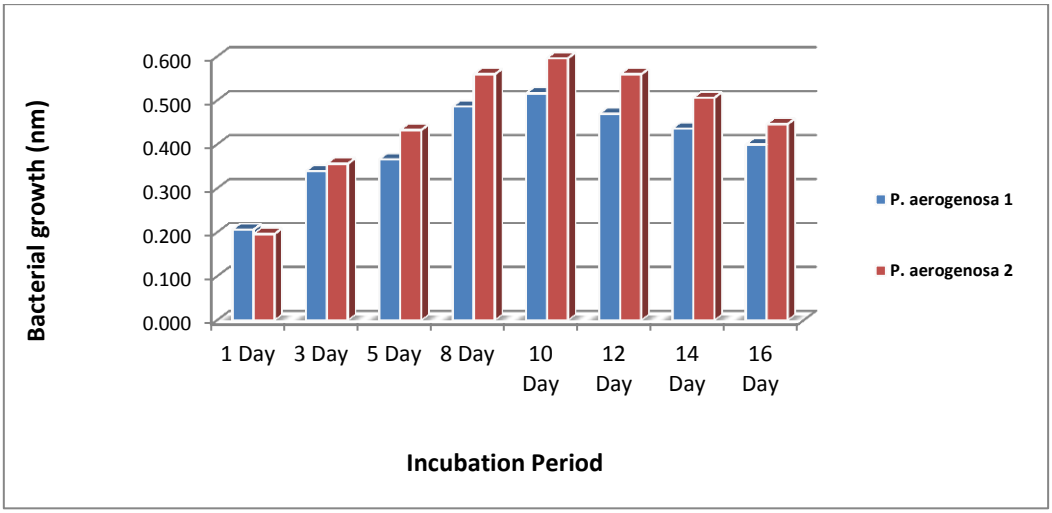


Figure 4- Mean value of Bacterial growth at different incubation periods.

Table 4-Mean values ± SD of Bacterial growth at 600nm at different incubation periods, and LSD value.

	Mean± SD of bacterial growth		**LSD ≤ 0.05**
	P. aerogenosa 1	***P. aerogenosa 2***	
First Day	0.207 ± 0.015 Ag	0.197 ± 0.015 Ae	0.036
Third Day	0.340 ± 0.011 Af	0.357 ± 0.012 Ad	0.025
Fifth Day	0.367 ± 0.015 Be	0.433 ± 0.012 Ac	0.038
Eighth Day	0.487 ± 0.006 Bb	0.503 ± 0.010 Ab	0.015
Tenth Day	0.517 ± 0.006 Ba	0.563 ± 0.015 Aa	0.026
Twelfth Day	0.470 ± 0.010 Bb	0.540 ± 0.010 Aa	0.024
Fourteenth Day	0.437 ± 0.006 Bc	0.507 ± 0.021 Ab	0.031
Sixteenth Day	0.400 ± 0.010 Bd	0.447 ± 0.031Ac	0.041
LSD ≤ 0.05	0.018	0.029	

Uppercase refer to comparison between means with same raw, lowercase refer to omparison between means with same column and similar characters refer to non-significant difference

It is well known the increasing of incubation period which results in to increase viable counts [33] particularly on media with low nutrient concentrations, and due to depletion of contaminate concentration and produce of intermediates compounds and metabolic products that result in decrease the pH of media and then decreases the growth of bacteria [34].

Biodegradation of Cypermethrin

FTIR analysis of biodegraded cypermethrin

Between 600 and 1800 cm^{-1} In this region, the main absorption bands of cypermethrin are assigned to the carbonyl asymmetric stretching (1720- 1740 cm-1), C=C stretching of the aromatic rings (1450 and 1600 cm^{-1}), CH2 deformation in R–CH2–CN structure (1400-1450 cm^{-1}) and the (C=O) –O– stretching (1050-1095 cm^{-1}). The band at 1200-1275 cm^{-1}, caused by aryl–O of diphenyl ether, involves aryl–O stretch, out-of-phase C–O–C stretching and ring vibrations. An additional important representing band of the molecule is observed at 1125-1205 cm^{-1} and is related to the C–O stretching of the cyanate group (–O–C=N). The band at 910-990 cm^{-1} was assigned to the asymmetric wagging vibrations of the terminal dihalovinyl group, and the band at 810-910 cm-1 was assigned to the deformation vibrations of the cyclopropane ring [31].

In current study, we focused on change in transmission of cypermethrin bonds because according to Beer-Lambert Law "IR transmitted through a solution changes in an inverse logarithmic relationship to the sample concentration" [35].

The results in Table-5 appears clear differences in transmission of cypermethrin bands between zero time and after 14 days of biodegradation. For *P. aeruginosa* 1, it was clearly note formation new three peaks, which belong to Carbonyl asymmetric, CH2 deformation in R–CH2–CN structure and

Aryl–O of diphenyl ether. Peak at 1335 belong to N-O symmetric stretch (nitro compound), Peak 1649 belong to N-H amines group and 1689 belong to C=O (carboxylic acid) cannot be detected after 14 days of biodegradation. For *P. aeruginosa* 2, Four peaks formed during biodegradation which belong to Carbonyl asymmetric, CH2 deformation in R–CH2–CN structure, Aryl–O of diphenyl ether and (C=O) –O– Stretching. In addition, peaks at 1620 belong to N-H amines group and 1662 belong to -C=C- (Alkenes) cannot be detected after 15days of biodegradation Figures- (5, 6).

Table 5-Change in transmission of Cypermethrin 100 mg L^{-1} bands after treatment with*Pseudomonas aerogenosa 1 & 2* for 14 days

Band	Wave Number	Change in Bands transmission %			
		P. aeruginosa 1		*P. aeruginosa 2*	
		Zero time	Day 14	Zero time	Day 14
Carbonyl asymmetric stretching	1720- 1740 cm^{-1}	Zero	30	Zero	30
C=C stretching of the aromatic rings	1450-1600 cm^{-1}	24	60	4	70
CH_2 deformation in R–CH_2–CN structure	1400-1450 cm^{-1}	Zero	30	Zero	10
Aryl–O of diphenyl ether	1200- 1275 cm^{-1}	zero	28	Zero	Zero
C–O stretching of the cyanate group (–O–C=N)	1125-1205 cm^{-1}	38	66	8	76
(C=O) –O–Stretching	1050-1095 cm^{-1}	4	39	Zero	36
Terminal dihalovinyl group	910-990 cm^{-1}	34	68	10	76
Cyclopropane ring	810-910 cm^{-1}	12	51	2	54

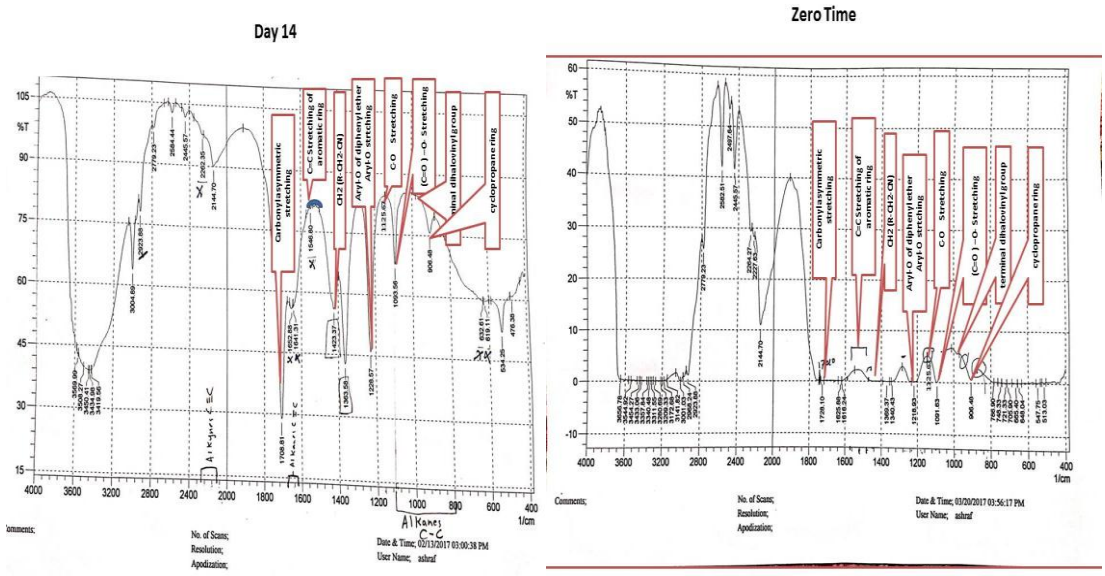


Figure 5- FTIR Results of Cypermethrin 100 mg L^{-1} after treatment with *Pseudomonas aerogenosa* 1 for 14 days.

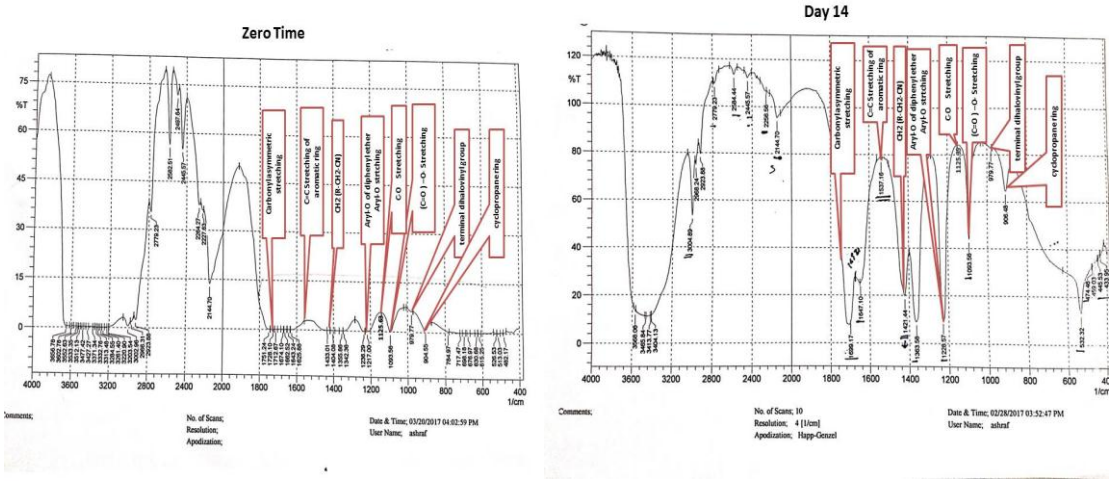


Figure 6- FTIR Results of Cypermethrin 100 mg L^{-1} after treatment with *Pseudomonas aerogenosa* 2 for 14 days

Changes in peak pattern of cypermethrin under bacterial treatment as compared to zero time were observed in above table, which indicate to ability of P.aerogenosa 1&2 to increase transmission of cypermethrin bands, which indicate that decrease in concentration of compound which composed from these bands according to Beer-Lambert Law. Difference in the FTIR spectrum of zero time and after 14 days of pesticide aqueous solution indicates degradation of the organic bonds of the pesticide, also Stretching in C=C chloroalkenes, ring vibration of benzene, CH2 deformation in R–CH2–CN structure and (C=O)–O–stretching in cypermethrin were reported by [36] using *Fusarium sp* which approve occurrence of biodegradation.

GC-MS analysis of biodegraded cypermethrin

The results of analysis of MMSM containing cypermethrin at concentrations 100 mg L^{-1} by GC-MS treated separately with each one of two selected isolates showed the presence of cypermethrin and its Intermediate metabolites. These compounds were identified based on their retention time and molecular weight with those of corresponding authentic compounds in the database, also the comparison with standard library of Wiley Registry of Mass Spectral Data version-7 confirmed the matching of mass/charge ratio v/s relative intensity.

The results showed variation in bacterial capacity for degradation of cypermethrin and also variation in intermediate metabolites after 14 day of incubation, the percentage of its degradation were 87.9 % for *P. aerogenosa* 1 and 80% for *P. aerogenosa* 2 according to biodegradation equation [28].

$$\textbf{\% of Biodegradation by } \textbf{\textit{P. aerogenosa 1}} = \frac{698539-83825}{698539} \times 100 = 87.9\ \%$$

$$\textbf{\% of Biodegradation by } \textbf{\textit{P. aerogenosa 2}} = \frac{698539-139708}{698539} \times 100 = 80\ \%$$

P. aeruginosa 1 in current study provides the evidence of efficient degradation pathway of cypermethrin. The bacteria converted cypermetrin into smaller molecular weight compounds which can further be mineralized under natural environmental conditions. Intermediates compounds formed from cypermethrin biodegradation by *P. aerogenosa* 1are present in Table-6.

Table 6- Retention time, Peak area and M. wt. of intermediates metabolites formed from cypermethrin at 100 mg L^{-1} biodegraded by *P. aerogenosa* 1

NO	Retention time	Peak Area	M. wt.	Intermediate metabolites
1	4.973	120459	106	1,3-Dimethylbenzene
2	9.07	448468	142	2-Methylnonane
3	10.966	905981	94	Phenol

4	10.968	816099	138	4-Hydroxybenzoate
5	11.809	477692	314	1,2-Benzenediol,
6	12.251	3409699	188	1,8-Cyclotetradecadiyne
7	12.522	426879	138	4-Isopropenylcyclohexanone
8	15.191	1160967	148	4-Propylbenzaldehyde
9	15.43	1138664	150	Phenol, M-tert-butyl-
10	15.963	927213	226	3-Methylpentadecane
11	16.331	8487199	198	n-Tetradecane
12	16.357	1175678	164	2-Tert-pentylphenol
13	16.881	600633	220	Butyric acid
14	17.751	991174	186	1-Dodecanol
15	20.568	8882501	225	a-Hydroxy-3- phenoxy- benzeneacetonitrile
16	20.571	703191	198	3-Phenoxy-benzaldehyde,
17	21.749	1125347	270	Isopropyl myristate
18	22.787	2281725	298	Hexadecanoic acid, methyl ester
19	24.895	524846	282	Oleic Acid
20	25.76	2099301	270	Isoamyl laurate
21	30.543	882673	256	Phenyl ester of o-phenoxy benzoic acid
22	30.801	83825	415	Cypermethrin
23	30.943	3049045	236	3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate

Cypermethrin could be metabolized by *P. aerogenosa1* into two major compounds (ά -hydroxy-3-phenoxy- benzene acetonitrile and 3-(2, 2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate). ά-hydroxy-3- phenoxy- benzene acetonitrile is unstable and spontaneously transformed to yield 3-phenoxy benzaldehyde [31, 37]. 3- phenoxybenzaldehyde has antimicrobial activity, but does not affect producing culture and enhances biodegradation in soil or media [38]. 3-phenoxybenzaldehyde is transformed into 4-propylbenzaldehyde which again converts to 4-hydroxybenzoate. Afterward 4-hydroxybenzoate was metabolized by *P. aerogenosa1* 1 to form phenyl ester of o-phenoxy benzoic acid. Two intermediate metabolites (3-phenoxybenzoic acid and 3-phenoxybenzaldehyde) are the key metabolites of pyrethroids [39].

The GC-MS chromatogram revealed that primary metabolites of degraded cyermethrin by *P. aerogenosa* 2 are presented in Table-7.

Table 7- Retention time, Peak area and M. wt. of intermediates metabolites formed from cypermethrin at 100 mg L-1 biodegraded by *P. aerogenosa* 2

NO	Retention time	Peak Area	M. wt.	Intermediate metabolites
1	4.083	169199	160	Pentanedioic acid, 2,4-dimethyl,
2	4.973	100459	106	1,3-Dimethylbenzene
3	7.801	630684	156	Undecane
4	10.966	886936	94	Phenol

5	10.968	797076	138	4-Hydroxybenzoate
6	14.876	2154411	330	Hexadecanoic acid, ethyl ester
7	15.063	201138	186	1,6-Heptadiene, 2-methyl-6-phenyl-
8	15.43	1083039	150	Phenol, M-tert-butyl-
9	16.357	1151633	164	2-Tert-pentylphenol
10	16.753	40545330	282	cis-Vaccenic acid
11	17.751	983151	186	1-Dodecanol
12	18.848	527067	314	Phenanthrene, 7-ethenyl
13	19.503	1246673	281	9-Octadecenamide, (Z)
14	20.678	8739346	348	Phthalic acid -isobutyl 2-pentyl ester
15	20.881	738112	335	Phthalic acid-butyl 4-octyl ester
16	22.103	1106844	290	2-Propenic acid-3-(4- methoxyphenyl)-2-ethylhexyl ester,
17	22.806	2343703	474	Azuleno(7,9- Dihydroxy-6,9a-dimethyl3-methylenedecarhydroazuleno[4,5-b]furan-29(3H)-one)
18	24.895	386691	282	Oleic Acid
19	30.801	139708	415	Cypermethrin
20	30.955	2911432	86	Cyclopropane carboxyc acid

From the results in Table-6, it can be concluded that the *P. aerogenosa* 2 were effective for breakdown of contaminated compound like cypermethrin , and obtained primary metabolites were less hazardous to the environment than cypermethrin. The GC-MS result showed that the ester metabolite was found during the biodegradation of cypermethrin pesticide which can eventfully disintegrate into simpler compounds, which would be mineralized further as nutrient and inorganic forms. The ester metabolite is ecofriendly for aquatic life and soil environment. The various researchers also showed that *Pseudomonas aeruginosa* was more potent against the cypermethrin degradation [20, 40].

Pollution by pyrethroids has become a very important problem in pesticide-treated areas. Currently, one of the major environmental problems is pesticide contamination caused by activities related to agricultural applications. Different mechanical and chemical methods are rarely used to remove pesticides from contaminated fields due to limited efficiency and the expense. Biodegradation is an encouraging process for the treatment of pesticide-contaminated areas due to the rapid and complete mineralization of pesticides. An isolated bacterial strain might be useful for bioremediation of cypermethrin-polluted soil and water environments.

Conclusion

P. aerogenosa 1 and P. aerogenosa 2 have been proved to possess good degradation performance on cypermethrin. The Intermediate metabolites result from two selected isolates were not only proficiently to degrade cypermethrin but also converted the metabolites compound into nontoxic forms. The difference between *P. aerogenosa 1 and P. aerogenosa 2* in AGLTp and O129 R test, which maybe result from genetic variation of these two isolates led to difference in biodegradation capability and intermediate metabolites formed from cypermethrin biodegradation.

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