Chapter

Bacillus megaterium Biodegradation Glyphosate

Nibal Khaleel Mousa, Abdul-Jabbar Ali and Maha Hussein

Abstract

The *Bacillus megaterium* ability was evaluated in this paper to degrade the Glyphosate. organophosphorus pesticides, The bacteria re-cultured that isolated from other researches of Baghdad soils and morphological identification and biochemical tests besides by selectivity media. The (5 and 25) ppm showed the highest growth results were within two days to two months on mineral salt media. The highest glyphosate degradation ratio % were (70) % per 25 ppm/two months. Incubation period Increasing led to highest glyphosate degradation ratio% at (25) ppm led to conclusion that bacteria digestive the pesticides as carbon and nitrogen sources and will be well harvest it form contaminated areas.

Keywords: bacterium, bioremediation, glyphosate, HPLC

1. Introduction

Glyphosate is an organophosphate which is a heterogeneous compound, utilized on the grasses leaves and broadleaf plants due its non-selectivity pesticides. In the seventies was the first registration in the United State by Monsanto (Roundup) [1]. Glyphosate stops a shikimic acid enzyme pathway which is essential for some microorganisms and plants. In trace analysis, Glyphosate is a difficult herbicide, has a good water solubility that causes difficulty in determination its physical and chemical properties [2].

The microorganism ability to eliminate pollutants is one of the bioremediation methods [3]. Bioremediation is applied as an auxiliary strategy due to its inoffensive to ecology, economic worth, reduce environmental poison, and validation [4–8]. Metabolic is one of the microorganism degradation methods across pesticides in soils, also catabolism strategies and the enzymes of co-metabolism [9]. Organic-pesticides fate in the ecology system can be marked by utilizing biodegradation as a major agent. The study purpose is to achieve and inspect the domestic bacterial separated on broken down different glyphosate concentrations that can be remaindered in soil and detect residues concentration from bacteria digestive via HPLC after extraction.

2. Material and methods

2.1 Materials

Chemosate. The trade name of "Glyphosate", bought from the local market. Materials were available and supply in Remediation Pollutants Center. The Mineral

Weight (g)	Compounds	Note
0.2	KH_2PO_4	Sterilized separately at 125 °C/25 min (part A)
0.5	K_2HPO_4	
1	$(NH_4)_2SO_4$	All mixed and added to part A of 1-liter flask, and adjust (pH 7.0 ± 0.3)
0.2	$MgSO_4{\cdot}7H_2O$	
0.2	NaCl	
0.05	$CaCl_2{\cdot}2H_2O$	
0.025	$FeSO_4{\cdot}7H_2O$	
0.005	Na_2MoO_4	
0.005	$MnSO_4$	

Table 1.
The mineral salt media.

Weight (g)	Compounds	Note
10	Glucose	• 1liter distillate water • adjust (pH 7.0 ± 0.3) • incubate28-30 °C/48 h.
0.5	Yeast extract	
0.25	$MgSO_4{\cdot}7H_2O$	
15	Agar	
0.1	$CaCl_2{\cdot}2H_2O$	

Table 2.
Sperber media structure.

Salt Media (MSM), was used in growing *B. megaterium* to investigate glyphosate degradation, **Table 1** [10]. As the only carbon source, Flasks (125 ml) were supplemented with Glyphosate. The Final Concentration of Glyphosate with 0.5 were (5 to 25) ppm from bacteria re- culture in comparison with control.

2.2 Re-growth and identification of *Bacillus megaterium*

Bacillus megaterium was growth and kept in incubation of our laboratory from other studies [3] and to re-identification by selective media, was by Sperber's Medium [10, 11], **Table 2**, By the spectrophotometer OD $_{600,}$ then the hydrolysis capacity measured for different period (2,5,7,14,21,30,60) days [12].

2.3 Glyphosate degradation ratio via *Bacillus megaterium*

The degradation ratio % investigated for (1-2) months, extracted through added equal volume each ethyl acetate and MSM which utilized as a reagent, for twice time, centrifuged at 3000 rpm/10 minutes, filtered then anhydrous sodium sulfate utilized as dry factor followed glass-fiber paper (Whatman GF/B) [10]. In Eq. (1), measured the degradation ratio:

$$P = \left(1 - \frac{C1}{C0}\right) x100\% \quad (1)$$

P = the rate of degradation of Glyphosate,

HPLC condition analysis	
UV–Vis detector	254 nm
Manual Injector Equipped	20-μL loop
Column/Stationary phase	C-18*
Mobile Phase	Acetic acid (1%) + methanol (6:4 (v/v).
Flow Rate	1.0 ml /min
Temperature	23–25 °C

**ZORBAX (5 μm, 150 mm × 4.6 mmi.d.)*

Table 3.
HPLC conditions.

C1 = Glyphosate dose in sample.
C0 = control [13].

2.4 Metabolite analysis

Each ethyl acetate extraction was analyzed by HPLC condition, **Table 3** [14], calculates the final concentration [15] of glyphosate used Eq. (2):

$$Pest.con = Asa\ x\ Cs\ /\ As\ x\ Csa \quad (2)$$

Pest.con = pesticide concentration in sample (mg/L)
Asa = sample peak area
Csa = sample concentration, mg/L
As = standard. peak.area
Cs = standard concentration, mg/L

3. Results and discussion

3.1 Re-growth and identification of *Bacillus megaterium*

Besides using selective media, Sperber Medium, **Table 4** shows the Test of Morphological, and **Table 5** represents the biochemical tests.

3.2 *Bacillus megaterium* hydrolyzes and bacteria growth

3.2.1 Growth of B. megaterium

In **Figure 1**, the results show that the highest *B. megaterium* growth was in (two months) for both (5, 25) ppm (0.164, 0.167) sequentially, while the 15 ppm show in 60 days, the highest growth is (0.215) in comparison with others when used glyphosate as carbon sources.

3.2.2 Degradation rate%

The highest degradation rate% for Glyphosate by *B. megaterium* in comparison among concentration was for both the 5-25 ppm in 60 days reached (70.01-70.9) %, **Figure 2**.

Morphological tests	
Spore shape	Rod-like/ flagella spores
Colonies	Round to irregular /yellow to brown or black after prolonged incubation
Motility	+
Gram stain	+
Aerobic	+
Temperature	3-20 °C/ 35-45 °C,optimun30°C
pH	5.7- 7

Table 4.
The tests of morphological.

Biochemical tests			
Catalase	+	Nitrate reduction / Degradation of tyrosine	+/−
Starch Hydrolysis	+	Casein hydrolysis	+
Citrate utilization	+	Indol/ Methyl Red	—
Esculin hydrolysis	+	Arginine dihydrolase	—
Gelatin hydrolysis	+	Tryptophan deaminase	—
Oxidase	+	Hydrolysis Urea	—

Table 5.
The tests of biochemical.

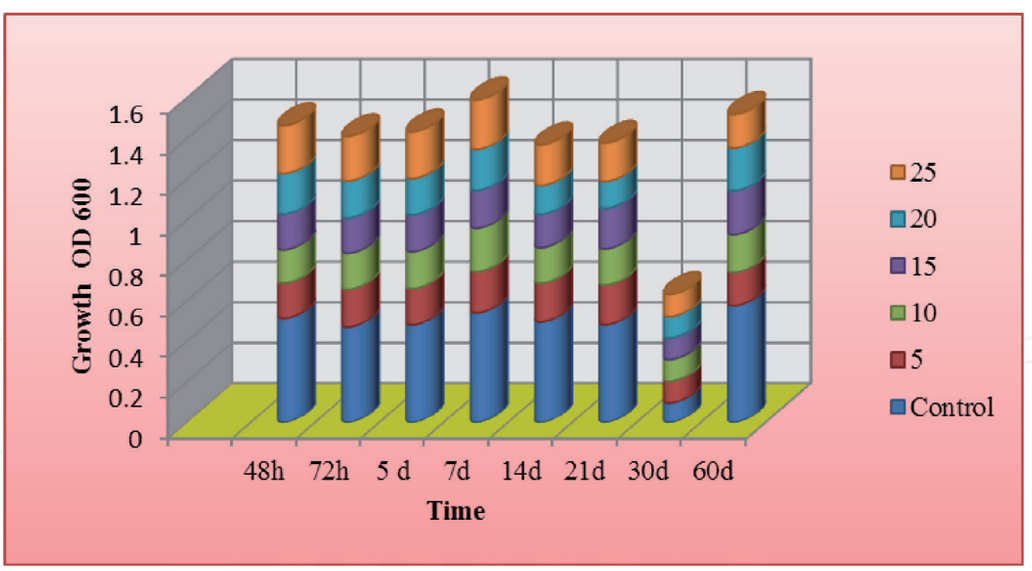


Figure 1.
Culture of B. megaterium *on Mineral Salt Media with Glyphosate.*

3.2.3 Glyphosate residues by HPLC test

The *B. megaterium* growth on different concentration in MSM at 30 °C, in comparison with control in **Figures 3** and **4**. Decreasing in glyphosate in 30 days for concentration (5,10) ppm (7, 8)% is the best peak area, while the 25 ppm showed 28%, while the results glyphosate peak area two months incubation, led to (20, 15) ppm in comparative with control. When comparing among HPLC, **Figures 5** and **6**

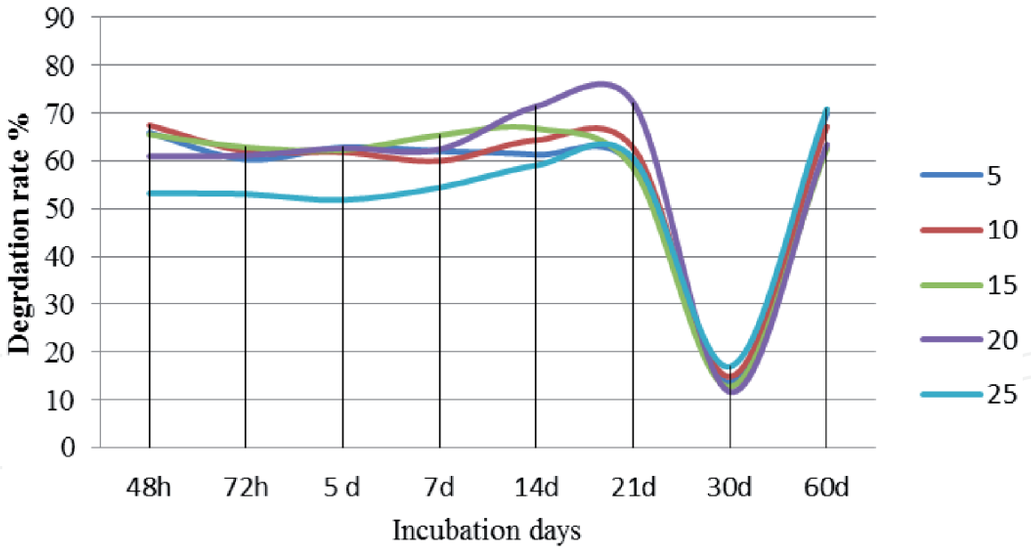
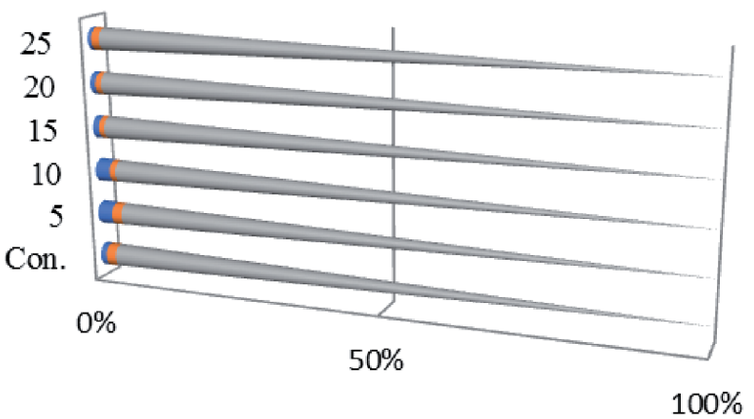


Figure 2.
The degradation rate of Glyphosate in MSM in Comparative with control.

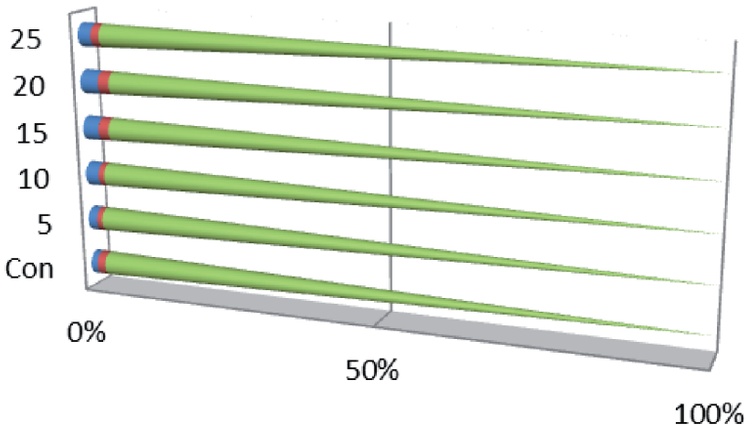


	Con.	5	10	15	20	25
Retention Time	4.9	5.7	6.2	6.2	5.9	5.3
peak height	7.792	3.792	2.437	5.319	6.141	10.988
peak area	443.557	212.476	230.015	535.95	612.048	778.009

Figure 3.
Peak height, peak area and retention time of Glyphosate after incubation B.M. 30 days in MSM.

improved that the *Bacteria* degradation ratio% positive results with all glyphosate concentrations via incubating two months incubation. However, the best was for (5, 25) ppm for each degradation ration% and the HPLC.

Organophosphorus pesticides microbial degradation and the bioremediation progress development for contaminated soils depend on the introduction of microbe's biodegrading [16]. *B. megaterium* shows the degradation rate% and highest growth in two months cultivation time for both five and twenty five concentration. The degradation pesticide ability like Chlorpyrifos (600 mg-L1) via *B. megaterium* in ten days incubation was 81% [17] and 73% for 20 ppm during three weeks. On the other hand, *Bacillus megaterium* 99% in seven days, degradation ratio towards atrazine (50 ppm) and Chlorpyrifos in (1-2) weeks [18, 19]. Monocrotophos (MCP), 83% degradation ratio reached led sub-products to CO_2, NH_4, and (HPO_3) [20]. Each bacterium produces enzymes to analyze



	Con	5	10	15	20	25
Retention Time	5.5	5.8	5.6	5.4	5.5	6.1
Peak Heght	6.898	3.794	2.414	3.486	3.441	3.903
Peak Area	463.7	372.577	236.264	188.52	170.858	260.242

Figure 4.
Peak height, peak area and retention of Glyphosate after incubation B.M. 60 days in MSM.

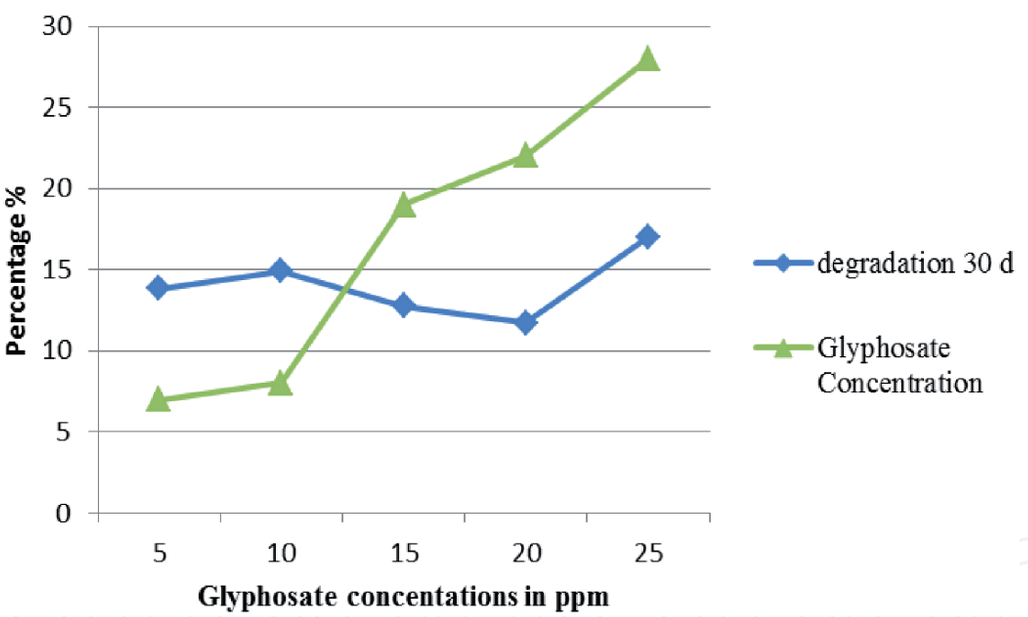


Figure 5.
Comparative among the degradation ratio% and Glyphosate concentrations via HPLC in 30 days incubation on MSM.

compounds and supplying its self with elements in need for re-build enzyme and cell body. One of *Bacillus megaterium* enzyme is β-Amylase [21] that consist from carbon and nitrogen mainly, so the bacteria most often organic-phosphors compounds, Glyphosate used to supply a single element (carbon, phosphorus or sulfur) and [22]. Due to that the concentration of glyphosate generally reduces along with bacteria growth. Phosphate ester and phosphonate are the analysis hydrolysis results of phosphorus. The esters groups have they have many vulnerable to hydrolysis sites. Beside hydrolysis, the oxidation also one of the major reactions, and the alkylation,dealkylation [23]. Detoxification is one of microbial degradation principles via hydrolysis phosphor bonds with oxygen (P-O-alkyl, P-O-aryl [24].

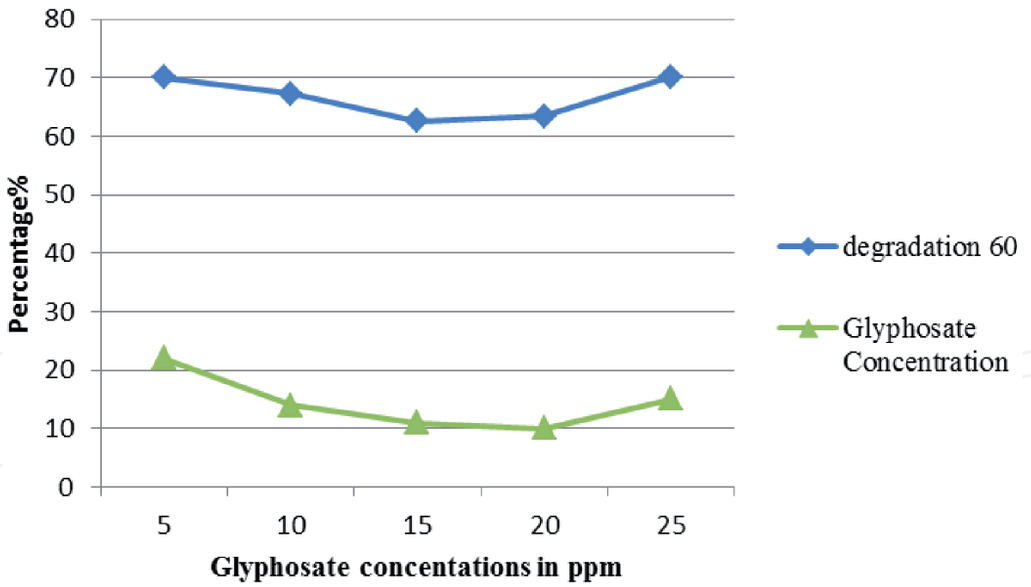


Figure 6.
Comparative among the degradation ratio% and Glyphosate concentration via HPLC in 60 days incubation on MSM.

4. Conclusion

In this study, *Bacillus megaterium* improved the best results for growth was in 48 h while in two months, had the same growth each five and twenty five concentration. The degradation rate % ability was the best in (5,25) ppm/two months reached (70-71)%. The Glyphosate degradation ratio% increasing equally with the increasing the incubation to two months,the best was for five and twenty five ppm, each the HPLC and Degradation ratio%. The conclusion is the *B. megaterium* utilized Glyphosate as supplier for elements sulfur, carbon, nitrogen and phosphorus and cultivated highly from culture could be well exploited for biodegradation from its pollutants sites.

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