

## OPTIMIZATION OF PARAQUAT DEGRADATION WITH MICROBIAL CONSORTIUM FROM CONTAMINATED SOIL USING STATISTIC METHOD

Mullika Teerakun<sup>1</sup>, Alissara Reungsang<sup>2</sup>, Monrodee Chaowarat<sup>3</sup>, and \*Piyawadee Saraphirom<sup>4</sup>

<sup>1</sup>Faculty of Agricultural Technology, Kalasin University, Thailand; <sup>2</sup>Faculty of Technology, Khon Kaen University, Thailand; <sup>3</sup>Faculty of Technology, Mahasarakham University, Thailand; <sup>4</sup>Faculty of Science and Technology, Rajabhat Mahasarakham University, Thailand

\*Corresponding Author, Received: 15 June 2019, Revised: 16 Nov. 2019, Accepted: 07 Feb. 2020

**ABSTRACT:** Paraquat is one of non-selective herbicides, widely used in Thailand and other countries that can be used to prevent and mitigate problems with weeds that have become resistant. It has a broad spectrum of weed knockdown herbicide which can be easily distributed in an aquatic environment due to its high solubility in water. The aim of the research is to study the optimization condition for biodegradation of paraquat by microbial consortium using an orthogonal array design in culture media. The microbial consortium which can degrade paraquat were isolated from cassava rhizosphere soil with a historic area of using paraquat in Kalasin, Thailand. Analysis of the 16S rDNA gene sequences compared with the database in Gen Bank demonstrated that microbial consortium showed the similarity with *Sphingomicrobium marinum* (97%), *Ferrovibrio xuzhouensis* (93%), *Azospirillum lipoferum* (93%), *Altererythrobacter xinjiangensis* (94%), *Xanthobacter autotrophicus* (92%) and *Azospirillum amazonense* (99%). To achieve the biodegradation experiments, orthogonal arrays design was investigated, with the three independent variables: glucose concentration (1-20 g/L), paraquat concentration (10-100 mg/L) and inoculum concentration (1-10%). The biodegradation consortium was done triplicate in 250 mL Erlenmeyer flask at 30°C 150 rpm for 35 days. Paraquat biodegradation was reported as biodegradation percentage. The results demonstrated that the optimum concentrations of glucose, paraquat, and inoculum for paraquat biodegradation were 1 g/L, 10 mg/L, and 5%, respectively. The paraquat removal efficiency of 95.69% was achieved under the optimal condition.

**Keywords:** Paraquat, Biodegradation, Orthogonal arrays design, Microbial consortium

### 1. INTRODUCTION

Nowadays, chemicals substances are widely used for agriculture in Thailand. One group of this chemical substance is pesticides. Pesticides have been a spread part of agriculture to protect crops and increase an agriculture yield [1]. The group of pesticides that commonly used for eliminating weed is paraquat. Paraquat (1,1-dimethyl-4,4-bipyridyl dichloride) is non-selective contact herbicide, quick-acting and killing green plant tissue on contact. It is classified as non-moving herbicides due to strong adsorption on soil particles. However, repeated use of paraquat over the years or the used in large quantities caused an accumulation of paraquat in environment by adsorption to the soil. The erosion and run-off water can cause the leaching of paraquat and spread to the river bed [2], [3]. In Thailand, it had been reported that paraquat is the main pesticide disseminated to the aquatic environment, for example, in the Chao Phraya River (0.28 and 0.40 µg/L), Pa Sak River (0.22 and 3.23 µg/L), Tha Chin River (0.01-0.79 µg/L), Namphong River

(0.02-26.8 ng/L) and Chanthaburi River (0.50-15.00 µg/L) which paraquat in the sample water from those rivers was found to have a higher residue content than other groups of pesticide. [4]-[8]. Paraquat is classified as a moderately toxic chemical for lethal toxicity, with a relatively low potential to bio-accumulate in the aquatic environment [9]. Therefore, the removal of paraquat from contaminated environment is necessary.

Bioremediation is the biological process or activity of the organisms to transform contaminants into less toxic compounds. This method is presently inexpensive and has greater efficiency in the removal of contaminants than conventional physicochemical methods [10]. Previous researches revealed efficient pesticide removal by microorganisms. *Brevibacterium frigoritolerans*, *Bacillus aerophilus*, and *Pseudomonas fulva* were isolated from aged phorate contaminated soil and could degrade phorate with the percentage of removal ranged between 97.65 and 98.31% [11]. Kafilzadeh et al. [12] isolated bacteria from sediments and water

samples from high agricultural activity areas. They belong to genus i.e. *Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, and *Bacillus*. *Bacillus* sp., *Micrococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Listeria* sp., *Proteus* sp., *Streptococcus* sp., and *Staphylococcus* sp. were found as the bacteria capable of utilizing the herbicide (paraquat dichloride) [13].

Design of Experiments techniques were widely used to optimize experimental parameters and enabled to determine simultaneously the individual and interactive effects of many factors that could affect the output results in the experiment [14]-[15]. A full factorial experiment tests all possible combinations of the factor levels and can, therefore, find the overall optimum setting. However, this process became excessive as the number of design parameters or levels increases [16]. An orthogonal array led to the reduction of variability in the process, compliance that is close to the desired result and a decrease in operating costs [17]. This technique has two major requirements consisting of the levels of any factor must occur with the same frequency and two factors, which each possible combination of levels must occur with the same frequency. If all factors have  $q$  levels, an orthogonal array is typically expressed as  $LM(qm)$ , where  $m$  is the number of factors and  $M$  represents the number of rows in the array [16].

The aim of this research was to isolate the bacterial consortium capable of paraquat degradation. The paraquat removal efficiency of the isolated bacterial consortium was also examined in the batch experiment with statistical experimental design. In addition, the optimal conditions for paraquat removal by isolated consortium were determined in order to achieve the highest paraquat removal efficiency.

## 2. MATERIALS AND METHODS

### 2.1 Soil Sampling

Cassava rhizosphere soil sample was collected from the field, with a history of paraquat application, in Kalasin province, Thailand and used as a source for paraquat degrading bacteria isolation. It was preserved in plastic bags and kept at 4°C to maintaining the activity of microorganisms until the usage.

### 2.2 Isolation of Bacterial Consortium Capable of Degrading Paraquat

Isolation of microbial consortium capable of degrading paraquat from rhizosphere soil was carried out by enrichment culture technique. Soil samples (10 g) were put into 250 mL Erlenmeyer flasks containing 90 mL of the mineral salt

medium [18] supplemented with 10 mg/L paraquat. Flasks were incubated at 30°C with shaking at 150 rpm. After 7 days, 10 mL of broth from flask was inoculated to fresh mineral salt medium containing 10 g/L of paraquat. This process was repeated until the paraquat removal on day 7 was less than 50% of the initial value. The isolated consortium was then harvested using the method previously described by Teerakun et al. [19]. The isolated consortium was used in further experiments. Bacterial community in the isolated consortium was analyzed by Denaturing gradient gel electrophoresis (DGGE) technique.

### 2.3 Analysis of Community Structure of Bacterial Consortium by DGGE Technique

The genomic DNA of the isolated consortium was extracted by using TIANamp Soil DNA Kit (TIANGEN Biotech (Beijing) Co., Ltd.). The 16S rRNA was amplified by the first polymerase chain reaction (PCR) with universal primer 1525r (5' AAGGAGGTGWTCCARCC 3') and 27f (5' GAGTTTGATCCTT GGCTCAG 3'). In the second PCR, primer 518r (5'GTATTACCGCGGCTGCTGG3') and 357f (5'CTCCTACGGGAGGC AGCAG3') with CG clamp (CGCCCGCCGCGC GCGGCGGGCGGGGCGGGGGCACGGGG GG) were used to amplify the fragment of V3 region of 16S rDNA product from first PCR. PCR products were stored at 4°C and analyzed on 1.0% agarose before DGGE analysis. The DGGE analysis of the PCR products was performed by electrophoresis (DGGE unit, V20-HCDC, Scie-Plas Limited, UK) according to the method previously described by Kongjan et al. [20]. The closest matches for partial 16S rRNA gene sequences were identified base on the sequence data of ribosomal database project (<http://rdp.cme.msu.edu/>). The Seq Match program and basic local alignment search tool (BLAST) with nucleotide database in the National Center for Biotechnology Information were employed [21].

### 2.4 Determination of Optimal Conditions for Degradation of Paraquat in Culture Media using Orthogonal Arrays Design

Degradation of paraquat by isolated consortium was investigated in 250 mL Erlenmeyer flask with a working volume of 150 mL. The experimental conditions were designed by orthogonal arrays design, with the three independent variables: glucose concentration (A; 1, 10 and 20 g/L), paraquat concentration (B: 10, 50 and 100 mg/L) and inoculum isolated concentration (C: 1, 5 and 10%) on paraquat biodegradation. All experimental runs were conducted in triplicate. The L9 (34) orthogonal test parameters were

showed in Table 1. The blank factor was a dummy and was used for error assessment.

Table 1 The  $L_9$  ( $3^4$ ) orthogonal design for the paraquat biodegradation.

Experimental run	A: glucose (g/L)	B: paraquat (mg/L)	Blank	C: inoculum (%)
1	20	100	2	1
2	20	50	1	10
3	20	10	3	5
4	10	50	3	1
5	1	50	2	5
6	1	100	3	10
7	1	10	1	1
8	10	10	2	10
9	10	100	1	5

## 2.5 Analysis of Paraquat Residue in Culture Media

The culture media was taken at the interval time and centrifuged for 15 min at 4,000 rpm. One milliliter of supernatant was filled in the glass tube with 1 mL of 2%  $\text{Na}_2\text{S}_2\text{O}_4$  in 0.3 M NaOH. Paraquat concentration was examined by Spectrophotometer UV-1800 (SHIMADZU, Japan) at 600 nm [22]. The paraquat recovery efficiency of this method was 93%.

## 3. RESULTS AND DISCUSSION

### 3.1 The Community Structure of a Bacterial Consortium Capable of Degrading Paraquat by DGGE

The community structure of a bacterial consortium capable of degrading Paraquat was analyzed by DGGE.

The fingerprints obtained from the bacterial community of isolated consortium demonstrated 6 differently visible bands as shown in Fig. 1. The sequences of the bands were identified base on 16S rRNA gene and exhibited a greater than 92% identity to the sequences deposited in the databases (Table 2) which were closet matched to 6 genera i.e., *Sphingomicrobium marinum*, *Ferrovibrio xuzhouensis*, *Azospirillum lipoferum*, *Altererythrobacter xinjiangensis*, *Xanthobacter autotrophicus*, and *Azospirillum amazonense*. *Sphingomicrobium* sp. is members of genus *Sphingomonas* which are often reported as the bacteria capable of degrading recalcitrant natural, anthropogenic compounds and different herbicides and pesticides [23]. Song et al. [24] reported cyhalothrin, synthetic pyrethroid pesticide, degradation by *F. xuzhouensis*. It was isolated from cyhalothrin contaminated wastewater. *Azospirillum* is of the genera that able to degrade organic and inorganic contaminants in soil [25]-[26] and were capable of biodegradation ethion in minimal salt media [27]. Romeh and Hendawi [28] found that *A. lipoferum* has capability of utilizing organophosphorus insecticides, chlorpyrifos, chlorpyrifos-methyl, cyanophos and malathion in mineral salts media as carbon and phosphorus source. *Azospirillum* sp. could tolerate pesticides such as monocrotophos and chlorpyrifos at low concentrations. However, the higher concentrations of these pesticides could cause antagonistic effects on *Azospirillum* sp. And decrease the ammonification process [29]. *X. autotrophicus* could degrade 2-chloroethanol (2-CE) that are metabolite of Tris (2-chloroethyl) phosphate into glycolic acid [30] and growing cells of *X. autotrophicus* strain GJ10 completely degraded 180  $\mu\text{M}$  of 2-CE within 24 h [31].

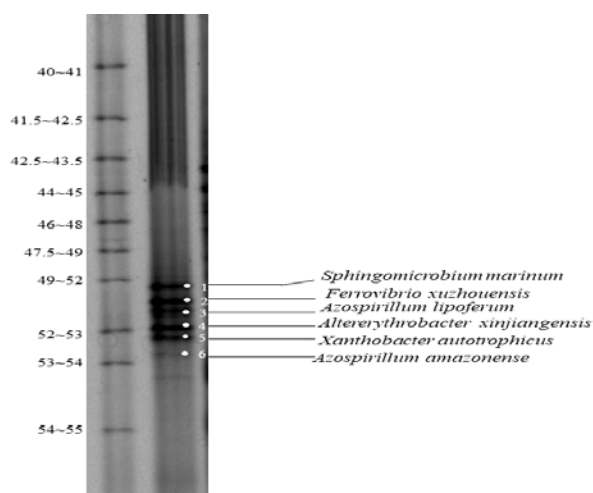


Fig.1 DGGE fingerprint of 16S rDNA fragments of paraquat biodegradation isolation consortium. The sequence of bands was searched in the GenBank with the BLAST program to determine the closest known relatives of the partial 16S rDNA sequences obtained (Table 2).

Table 2 Identification of dominant fragments in DGGE patterns in paraquat biodegradation isolation consortium.

Bands	Closet Identify Relative		
	Bacterial species	Accession No.	% Identity
1	<i>Sphingomicrobium marinum</i>	NR_135708.1	97%
2	<i>Ferrovibrio xuzhouensis</i>	NR_145543.1	93%
3	<i>Azospirillum lipoferum</i>	NR_116846.1	93%
4	<i>Altererythrobacter xinjiangensis</i>	NR_108901.1	94%
5	<i>Xanthobacter autotrophicus</i>	NR_074255.1	92%
6	<i>Azospirillum amazonense</i>	NR_104981.1	99%

### 3.2 The Orthogonal Experiment Results of Paraquat Biodegradation

Batch paraquat biodegradation (the experimental runs 1 to 9) was examined. The results of the orthogonal experiment of paraquat biodegradation at day 35 were shown in Table 3. The paraquat removal on the orthogonal experiments ranged from 70.02 to 97.87% (Table 3). Range analysis was applied to clarify the important sequence of glucose concentration (factor A), paraquat concentration (factor B) and inoculum concentration (factor C) in the orthogonal experiments (Table 4). The highest range value (R) of 13.63 was found for factor B, while the lowest range value of 7.79 was found for factor A. The bigger R-value of a factor represents a greater effect on the paraquat removal. According to the range, the order of influence on the paraquat removal was paraquat concentration > inoculum concentration > glucose concentration. Thus, the optimum condition for improving paraquat removal was determined as A1B1C2 corresponding to glucose concentration, 1 g/L; paraquat concentration, 10 mg/L and inoculum, 5%.

In order to verify the reliability of the results in flasks, an additional experiment with the corresponding parameters under the optimum nutrient condition A1B1C2 was performed in flask 250 mL (data not show). Under the optimum condition, the observed paraquat removal of 95.69% was obtained. This indicated that the statistical experimental design i.e. orthogonal experiment design is a useful tool for optimizing paraquat removal conditions.

The addition of glucose could lead to a higher degradation of pesticides [32]. This is in accordance with a study by Hashemi et al. [33] who reported that paraquat biodegradation

increased in the presence of glucose by *Achromobacter xylosoxidans* and *Streptomyces* sp. Kanissery and Sims [34] reported that the addition of glucose was enhanced the extent of mineralization herbicide at a low herbicide concentration.

Paraquat was used as a nitrogen source by microorganism [19], [35]-[36]. However, the paraquat concentration was impact affected on growth of the organisms. According to report of Andy et al. [37] found that the cell mass yield of the microbes decreased with increased concentration of the paraquat. Paraquat had a toxic effect on the microorganisms leading to the higher counts obtained from the lowest concentration of 25 mg/kg and the lowest counts from the highest concentration of 100 mg/kg [38].

Microbial population density is an important determinant in the biodegradation rate [39]. When the inoculum size is small and viable cell fraction is low, there may be a pseudo-lag phase, which is not a result of adaptation but of the size of inoculum [40]. In addition, higher inoculum sizes would contain a larger population of microorganism that would consume more oxygen. The optimal condition, especially in terms of the specific degradation rate of substrate, did not occur at initial optical density (OD) of 3 [41].

Table 3 Orthogonal experiment results of paraquat biodegradation at the remediation time of 35 days.

Experimental run	A: glucose (g/L)	B: paraquat (mg/L)	C: inoculum (%)	Paraquat removal (%)
1	20	100	1	70.02
2	20	50	10	91.28
3	20	10	5	95.15
4	10	50	1	87.88
5	1	50	5	97.87
6	1	100	10	86.72
7	1	10	1	95.23
8	10	10	10	93.96
9	10	100	5	86.70

Table 4 The range analysis of L<sub>9</sub> (3<sup>4</sup>) orthogonal experiment for paraquat biodegradation.

	A: glucose	B: paraquat	Blank	C: inoculum
K1	279.82	284.34	273.21	253.13
K2	268.54	277.03	261.85	279.72
K3	256.45	243.44	269.75	271.97
k1	93.27	94.78	91.07	84.38
k2	89.51	92.34	87.28	93.24
k3	85.48	81.15	89.92	90.66
R	7.79	13.63	3.79	8.86
Q	A1	B1		C2

#### 4. CONCLUSION

Microbial consortium capable of degrading paraquat was isolated from cassava rhizosphere soil. The microbial analysis using the PCR-DGGE technique demonstrated 6 differently visible bands identified as *S. marinum*, *F. xuzhouensis*, *A. lipoferum*, *Al. xinjiangensis*, *X. autotrophicus* and *A. amazonense*. The orthogonal arrays design results indicate that the optimum conditions for paraquat removal were 1 g/L glucose concentration, 10 mg/L paraquat concentration, and 5% inoculum concentration possessing paraquat removal efficiency of 95.69%.

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#### 6. REFERENCES

- [1] Özkara A., Akyil D. and Konuk M., Pesticides, Environmental Pollution, and Health, Environmental Health Risk - Hazardous Factors to Living Species, Larramendy M.L. and Soloneski S., Ed. Intech Open, 2016, pp. 3-27.
- [2] Kajitvichyanukul P., Complete report of research plan on integrated research to strengthen local authorities in managing and preventing toxic contamination on Nan watershed, National Research Council of Thailand, Bangkok, 2012. [in Thai]
- [3] Yimpoolsap S., Hungspreug N. and Anurugsa B., The Effects of Paraquat Used in Upland Rice and Maize Fields on Biomass of Attached Algae, Thammasat International Journal of Science and Technology, Vol. 19, No. 1, 2014, pp. 32-38.
- [4] Wetchayanon M., Kungsakulniti N., Charoenca N. and Tangbanluekan C., Health risk assessment of contaminated pesticides in the main rivers of Thailand, in The National and International Graduate Research Conference, 2017, pp. 880-890.
- [5] Komarova T., Iwai C.B., Somparn A., Tokhun N., Mueller J., Kennedy I. and Noller B., Pesticide distribution in the Namphong river NE Thailand arising from land use and other practices, International Journal of Environmental and Rural Development, Vol. 6, No. 1, 2015, pp. 175-180.
- [6] Pataranawatab P., Kitkaewab D. and Suppaudomab K., Paraquat contaminations in the Chantaburi River and vicinity area, Chantaburi province, Thailand, Journal of Science, Technology, and Humanities, Vol. 10, No. 1, 2012, pp. 17-24.
- [7] Leadprathom N., Parkpain P., Satayavivad J., Delaune R.D. and Jugsujinda A., Transport and deposition of organochlorine pesticides from farmland to estuary under tropical regime and their potential risk to aquatic biota, J. of Environmental Science and Health Part B, Vol 44, No. 3, 2009, pp. 249-261.
- [8] Leadprathom N., Toxicity of paraquat to aquatic animal, King Mongkut's Agricultural Journal, Vol. 31, No. 2, 2013, pp. 95-101.
- [9] Sawasdee B., Phuthonghin P. and Kunapratom S., Acute Toxicity of Paraquat and Glyphosate on embryonic development of Climbing Perch (*Anabas testudineus*), Prawarun Agricultural Journal, Vol. 13, No. 1, 2016, pp. 70-78.
- [10] Letti L.A.J., Vítola F.M.D., de Melo Pereira G.V., Karp S.G., Medeiros A.B.P., da Costa E.S.F., Bissoqui L. and Soccol C.R., Solid-state fermentation for the production of mushrooms, In Current Developments in Biotechnology and Bioengineering, Pandey A., Larroche C. and Soccol C.R., Ed. Elsevier, 2018, pp. 285-318.
- [11] Jariyal M., Jindal V., Mandal K., Gupta V.K. and Singh B., Bioremediation of organophosphorus pesticide phorate in soil by microbial consortia, Ecotoxicol. Environ. Saf., Vol. 159, 2018, pp.310-316.
- [12] Kafilzadeh F., Ebrahimnezhad M. and Tahery Y., Isolation and identification of endosulfan-degrading bacteria and evaluation of their bioremediation in Kor River, Iran, Osong. Public. Health. Res. Perspect., Vol. 6, No. 1, 2015, pp. 39-46
- [13] Ozoude T.O., Etok C.A., Eleanya E.U. and Agwa O.K., Monitoring the bio-utilization of herbicide (paraquat dichloride) by some bacterial species isolated from soil in Calabar, Cross-River State, Nigeria, Journal of Applied Life Sciences International, Vol. 16, No. 1, 2018, pp. 1-6.
- [14] Wang T.Y. and Huang C.Y., Improving forecasting performance by employing the Taguchi method, European Journal of Operational Research, Vol. 176, No. 2, 2007, pp. 1052-1065.
- [15] de Freitas A.P.B.R., de Freitas L.V., Loures C.C.A., dos Santos M.A.R., Ricardo G.D.,

- Marins F.A.S., dos Santos M.S.A. and Silva M.B., Taguchi Method Applied to Environmental Engineering, Design of Experiments – Applications, Silva M.B., Ed. Intech Open, 2013, pp. 79-92.
- [16] Lee C.T. and Kuo H.C., A Novel algorithm with orthogonal arrays for the global optimization of design of experiments, Appl. Math. Inf. Sci., Vol. 7, No. 3, 2013, pp. 1151-1156.
- [17] de Freitas A.P.B.R., de Freitas L.V., Loures C.C.A., Gonçalves L.G. and Silva M.B., Response surface method and Taguchi Orthogonal Array applied to phenolic wastewater by advanced oxidation process (AOP), American Journal of Theoretical and Applied Statistics, Vol. 3, Issue 6-1, 2014, 35-41.
- [18] Ansari S.M., Dehghani M. and Samaei M.R., Isolation of atrazine degrading bacteria in semi-salinity medium, Journal of Health Sciences and Surveillance System, Vol. 4, No. 3, 2016, pp. 121-128.
- [19] Teerakun M., Saraphirom P. and Reungsang A., Optimization of paraquat degradation by microbial consortium from rhizosphere soil, EAU Heritage Journal Science and Technology, Vol. 11, No. 2, 2017, pp. 90-99.
- [20] Kongjan P., O-Thong S., Kotay M., Min B. and Angelidaki I., Biohydrogen production from wheat straw hydrolysate by dark fermentation using extreme thermophilic mixed culture, Biotechnology and Bioengineering, Vol. 105, No. 5, 2010, pp. 899-908.
- [21] Suksong W., Jehlee A., Singkhala A., Kongjan P., Prasertsan P., Imai T. and O-Thong, S., Thermophilic solid-state anaerobic digestion of solid waste residues from palm oil mill industry for biogas production, Industrial Crops and Products, Vol. 95, 2017, pp. 502-511.
- [22] Lima T.L., Nicoletti M.A., Munhoz C., De Abreu G.R., Magalhães J.Z., Ricci E.L., Waziry P.A.F., Alves da Costa J.N., Antônio A.C.N. and Fukushima A.R., Determination of paraquat in several commercially available types of rice, Food and Nutrition Sciences, Vol. 09, No.12, 2018, pp. 1368-1375.
- [23] Stolz A., Molecular characteristics of xenobiotic-degrading Sphingomonads, Appl Microbiol Biotechnol., Vol. 81, No. 5, 2009, pp. 793-811.
- [24] Song M., Zhang L., Sun B., Zhang H., Ding H., Li Q., Guo S. and Huang X., *Ferrovibrio xuzhouensis* sp. nov., a cyhalothrin-degrading bacterium isolated from cyhalothrin contaminated wastewater, Antonie van Leeuwenhoek, Vol. 108, No. 2, 2015, pp. 377-382.
- [25] Zhuang X., Chen J., Shim H. and Bai Z., New advances in plant growth-promoting rhizobacteria for bioremediation, Environ. Int., Vol. 33, No. 3, 2007, pp. 406-413.
- [26] Hong S.H., Ryu H., Kim J. and Cho K.S., Rhizoremediation of diesel-contaminated soil using the plant growth-promoting rhizobacterium *Gordonia* sp. S2RP-17, Biodegradation, Vol. 22, No. 3, 2011, pp. 593-601.
- [27] Foster L.J., Kwan B.H. and Vancov T., Microbial degradation of the organophosphate pesticide, Ethion, FEMS Microbiol Lett., Vol. 240, No. 1, 2004, pp. 49-53.
- [28] Romeh A.A. and Hendawi M.Y., Bioremediation of Certain Organophosphorus Pesticides by Two Biofertilizers, *Paenibacillus (Bacillus) polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck), J. Agr. Sci. Tech., Vol. 16, 2014, pp. 265-276.
- [29] Venieraki I., Kefalogianni I., Pergalis P., Chatzipavlidis I., Dimou M. and Katinakis P., Isolation of *Azospirillum* and *Pseudomonas diazotrophs* from wheat rhizosphere in Greece, Proc. Natl. Acad. Sci., Vol. 105, No. 21, 2008, pp. 7564-7569.
- [30] Takahashi S., Miura K., Abe K. and Kera Y., Complete detoxification of tris (2-chloroethyl) phosphate by two bacterial strains: *Sphingobium* sp. strain TCM1 and *Xanthobacter autotrophicus* strain GJ10, J. Biosci. Bioeng., Vol. 114, No. 3, 2012, pp. 306-311.
- [31] Takahashi S., Abe K. and Kera Y., Microbial degradation of persistent organophosphorus flame retardants, Environmental Biotechnology - New Approaches and Prospective Applications, Petre M., Ed. Intech Open, 2013, pp. 91-122.
- [32] Hindumathy C.K. and Gayathri V., Effect of pesticide (chlorpyrifos) on soil microbial flora and pesticide degradation by strains isolated from contaminated soil, Journal of Bioremediation and Biodegradation, Vol. 4, No. 2, 2013, pp. 1-6.
- [33] Hashemi S.S., Aliasgharzad N., Khakvar R. and Oustan S., Isolation and identification of paraquat degrading bacteria from Tabriz soil, Journal of Soil Biology, Vol. 3, No. 2, 2015, 117-127.
- [34] Kanissery R.G. and Sims G.K., Biostimulation for the enhanced degradation of herbicides in soil, Applied and Environmental Soil Science, Vol. 2011, Article ID 843450, 2011, pp. 1-10.
- [35] Gondar D., López R., Antelo J., Fiol S. and

- Arce F., Adsorption of paraquat on soil organic matter: Effect of exchangeable cations and dissolved organic carbon, J. Hazard. Mater., Vol. 235-236, 2012, 218–223.
- [36] Devashree Y., Dutta B.K., Paul S.B. and Choudhury S., The effect of paraquat and fipronil on the soil and rhizosphere microflora of tea (*Camellia sinensis* (L) O. kuntze), International Journal of Innovation and Applied Studies, Vol. 7, No. 4, 2014, 1534–1543.
- [37] Andy I.E., Edu G.S., Bassey I.U., Markson A.A., Umana E.I. and Udo S.E., Biodegradation of paraquat, Journal of Biopesticides and Environment, Vol. 1, 2015, pp. 80-85.
- [38] Obuotor T.M., Yahaya P.B., Akinloye O.A. and Sakariyau A.O., Biodegradation of paraquat dichloride-contaminated soil with fermented corn steep, Uniosun Journal of Sciences, Vol. 1, Issue 1, 2016, pp. 67-73.
- [39] Das N. and Chandran P., Microbial degradation of petroleum hydrocarbon contaminants: an overview, Biotechnology Research International, Vol. 2011, Article ID 941810, 2011, pp. 1-13.
- [40] Ukpaka C.P., Application of polynomial, exponential techniques to monitor and predict the biokinetics of aromatic hydrocarbon degradation using *Pseudomonas* sp., SWISS J. Appl. Sci., Vol. 2, 2013, pp. 10-30.
- [41] Dong S., Li J. and Shim H., Bioremoval of trichloroethylene/toluene mixture by *Burkholderia vietnamiensis* G4 from Water, Research Journal of Chemistry and Environment, Vol. 17, Issue 9, 2013, 32-37.
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