World Journal of
Experimental Biosciences

Volume 4, Number 1: 53-57
ISSN: 2313-3937
2016

Research article

Use of immobilized lipase in cleaning up soil contaminated with oil

Jenan Atiyah Ghafil[1]*, Shatha Salman Hassan[1], Ayaid Khadem Zgair[1]

ABSTRACT

Thirty five isolates of *Pseudomonas* spp were obtained from seventy samples of soil contaminated with crude oil. The ability of lipase production by these isolates was screened. *Pseudomonas* ps 12 isolated from oil contaminated soil showed the highest lipase production (60 U/mg). This isolate was identified by VITEK-2 compact as a strain of *Pseudomonas aeruginosa.* Lipase extracted from *P. aeruginosa* ps 12 was immobilized by adsorption on solid surface included charcoal and sawdust. The effect of enzyme (free and immobilized) on degradation of oils was studied into polluted soils. It was observed that the immobilized enzyme were more effective in removing oil than the free enzyme. The analysis of the heavy crude oil fractions was performed by Fourier Transform Infrared Spectroscopic (FTIR) technique to check the changes in their structure before and after treatment with *P. aeruginosa* ps 12 lipase. The result revealed that the spectra of crude oil treated with *P. aeruginosa* after 96 h of incubation many compound was disappeared and CO_2 were released. This change indicated degradation of oil to simple structure and it is possible to use lipase producing from these isolates in treatment of soils and polluted environments to reduce the harm on soil.

Keywords: Crude oil, Immobilization, lipase, Soil.

Citation: Ghafil JA, Hassan SS, Zgair AK. (2016) Use of immobilized lipase in cleaning up soil contaminated with oil. *World J Exp Biosci* **4**: 53-57.

Received January 1, 2016; Accepted February 2, 2016; Published February 11, 2016.

INTRODUCTION

Lipases have received great attention as industrial biocatalysts in areas like oils and fats processing, detergents, baking, cheese making, surface cleaning, or fine chemistry. They can catalyze reactions of insoluble substrates at the lipid-water interface, preserving their catalytic activity in organic solvents [1]. Previous study reported the method of lipase purification and characterization [2]. Different bacterial species especially *Pseudomonas* sp were applied in bioremediation of crude oil and clean soil from this oil, the lipase produced from this bacterial plays a crucial role in biodegradation of crude oil [3]. This area required a lot of work to find the best way to clean the environment from crude oil pollution. The aims and objective of the study are obtaining active isolate of *Pseudomonas* sp. for lipids degradation (produce lipase) from Iraqi soil. Determination the effective environmental conditions on lipase production and immobilizing of bacterial cells and

***Correspondence**: Ghafil JA.jenanghafil@gmail.com
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article.

enzyme will be evaluated in current study. Ability of free and immobilized enzyme in soil bioremediation (oil degradation) were evaluated.

MATERIALS AND METHODS

Collection of samples for isolation of bacteria

Seventy samples of oil contaminated soils included 40 samples from Baghdad city, 20 samples from Kut city and 10 samples from Hilla city. Each sample was collected under aseptic condition and then transported to the laboratory for bacterial isolation.

Isolation of bacteria

One gm of each soil samples was added to 9 ml of distilled water and shacked to homogenize. Serial dilutions were prepared for each sample. Hundred microliter of each dilution was spread onto Rhan agar medium (Himedia, India) and incubated at 37 ^{o}C for 2 days. The bacterial colonies were purified by sub-culturing on nutrient agar medium until pure culture was obtained.

Morphological and microscopical examination

Size, shape and margin of the bacterial colonies on nutrient agar plate were studied and a loopfull of the culture was fixed on a slide, and stained by Gram stain to examine Gram reaction, shape, color, arrangement and regularity and spore forming were observed.

Identification of bacterial isolates by VITEK 2 compact device

VITEK 2 compact device in Ibn Balady hospital laboratories, Ministry of health was used to identify the isolates. This device contains 47-biochemical tests. For bacterial identification, Gram-negative card was used; it is a complete system for routine identification of most significant fermenting and non-fermenting Gram-negative bacilli. The standard method of manfacture's instructions of company was followed in preparation of solutions, incubation of samples before appling, card sealing and incubation, adjusting the optical system, applying the test reactions and analytical techniques.

Immobilization of bacterial cells and enzyme (lipase)

Sawdust and charcoal were used for the adsorption of enzyme or cells. To immobilize the enzyme, 5 ml of enzyme (partially purified) activity was 60 U and protein concentration was 0.01 mg, and then added to 10 gm of these carriers, mixed and left at room temperature for 24 h. To immobilize the bacterial cells, 5 ml of cells suspension (O.D 600=0.8) was added to 10 gm of these carriers, mixed and left at room temperature for 24 h. After incubation, the carriers were filtered by filter paper (Wattman No.1) and washed several times with 0.2 M Tris-HCl buffer pH 8.0 to remove the non – adsorbed enzyme or cells. A volume 3.6 ml of substrate solution was added to 0.3 gm of each carrier and incubated at 30 ^{o}C for 30 min. After incubation the activity and the remaining activity were estimated for the free and immobilized enzyme and cells.

Application of lipase in treatment of oil polluted soil

Treatment of oil polluted soil with immobilized cells and enzyme

Treatments of soil; five treatments of soil were applied. Fifteen gm of soil (autoclaved and un autoclaved) was polluted with 5 ml of heavy oil 150 and then mixed with 5 gm of immobilize lipase adsorbed on charcoal or sawdust and incubated at 30 ^{o}C for 96 h. Fifteen gm of soil (autoclaved and non autoclaved) was polluted with 5 ml of heavy oil 150 and then mixed with 5 gm of immobilize bacterial cells adsorbed on charcoal or sawdust and incubated at 30 ^{0}C for 96 h. Fifteen gm of soil (autoclaved and non autoclaved) polluted with 5 ml of waste oil and then mixed with 5 gm of immobilize lipase adsorbed on charcoal or sawdust and incubated at 30 ^{o}C for 96 h. Fifteen gm of soil (autoclaved and non autoclaved) was polluted with 5 ml of waste frying oil and then mixed with 5 gm of immobilize bacterial cells adsorbed on charcoal or sawdust and incubated at 30 ^{o}C for 96 h. The control in these experiments was autoclaved soil and oil (crude or waste) without treating with either immobilize bacteria or enzyme.

Extraction of oil from soil

After incubation period, 50 ml chloroform was added to each sample and mixed well to extract oil from soil. The samples were centrifuged at 6000 rpm for 30 min to precipitate the solid particles. The supernatants were separated and placed in Petri dishes then dried in an oven at 60 ^{0}C for 30 min to permit chloroform to evaporate. The weights of the residues were measured and the difference of oil content between before and after soil treatment was calculated to obtain the loss of oil.

Fourier Transform Infrared Spectroscopic (FTIR) analysis

In current study, FTIR technique was used to detect the degradation of oil in the polluted soil. The oil extracted from the polluted soil treated and untreated with the immobilized enzyme was analyzed by FTIR. The FTIR (Bruker, Germany) device was calibrated by the range of transmittance percents on the "Y" axes and the wave length (600-4000 cm) on the "x" axes [4].

RESULTS AND DISCUSSION

Isolation and identification of *Pseudomonas*

In the present study, thirty five *Pseudomonas* isolates were obtained from seventy samples collected from three Iraqi cities. Highest number of samples (40 samples) was collected from Baghdad; from these samples 20 *Pseudomonas* isolates were obtained. The 20 samples collected from oil contaminated area in Kut,

gave eight *Pseudomonas* isolates and from ten oil contaminated area in Hilla, seven *Pseudomonas* isolates were obtained. Many bacterial species are present in the soil normally and adapting to the soil conditions and almost the bacteria that degrade the oil present in the soil contaminated with oil, which help in clean the soil from oil products [5]. One of every dominant bacteria found in the oil contaminated soil is Pseudomonas [6].

Identification of bacterial isolates by VITEK 2 compact device.

Identification of bacterial isolate was carried out by VITEK 2 compact system. The result revealed that the isolate Ps12 was *P. aeruginosa*. VITEK 2 is a new automatic system for the identification and susceptibility testing of the most clinically and environmental important bacteria [7]. The VITEK 2 system was used in this study because it gives several advantages as compared with routine tests for identification of bacteria isolated from environmental samples since it provides rapid identification (only 3 h), a simple methodology, a high level of automation, and taxonomically updated databases. VITEK 2 system correctly identified 85.3 to 100% of *P. aeruginosa* strains. Previous study reported that VITEK 2 system can be used to identify all *S. maltophilia* isolates, 91.8% of *P. aeruginosa* isolates, and 76% of A. baumannii isolates [8].

Immobilization of bacterial cells and lipase

Lipase produced from *P. aeruginosa* Ps 12 was immobilized as enzyme and bacterial cells on solid surfaces by adsorption on charcoal and sawdust. The results illustrated in showed that the charcoal was more suitable than sawdust for immobilization of lipase enzyme and bacterial cells. The remaining activities of charcoal for bacteria and enzyme were 80 and 85 %, respectively, while the remaining activities of sawdust for bacteria and enzyme were 70 and 72 %, respectively. This may attributed to that charcoal constitutes more fine particles which offer large surface area for adsorption of enzyme or bacterial cells. Charcoal is widely used as a support for loading the plant fertilizers and growth factors [9].Charcoal supports have been also used in soil treatment for enzyme immobilization and in food industries for immobilizing amylo glucosidase for starch hydrolysis without any cross linking agent. It was reported that the charcoal was an excellent adsorbent with high adsorptive capacity and minimum fine particulate matter. Recent studies have provided evidence that wood-derived charcoal produced by fire can significantly stimulate plant growth. However, the mechanisms by which charcoal affects plant growth are poorly understood, and little is known about how charcoal are influenced by charcoal type, soil type and plant species [10]. Charcoal and sawdust were used in our study to immobilize the enzyme and bacterial cells because they are cheap, available materials; they have a good ability to adsorb materials, they are suitable for mixing with the soil and do not decompose by microorganisims [11].

Application of lipase: ability of immobilized enzyme to degrade heavy oil 150 and waste oil in soil.

The immobilized enzyme was used to determine its ability to degrade the oils in contaminated soil (Fig. 1). The result showed that immobilize lipase on charcoal is more efficient than immobilize bacterial cells in reducing the oil weight in the soil. In general, our study showed that the immobilized lipase was higher effective to degrade the oils than the immobilized bacteria because the lipase affect directly on oil while, the immobilized bacterial cells affect indirectly on oil as bacterial cells will produce lipase and then the lipase will degrade the oil. The results showed that the immobilized bacterial cells and lipase on charcoal were more effective to degrade the oils than the immobilized bacterial cells and lipase on sawdust. The ability of immobilized lipase and bacterial cells to degrade the waste oil was higher than the crude oil (**Fig 1 a**, **b**).

Treating soil by immobilized enzyme or immobilized bacterial cells is a good way to preserve and perpetuate the enzymatic efficiency and survival for long time [12]. The current demands of the world's biotechnological industries are enhancement in enzymes productivity and development of novel techniques for increasing their shelf life. These requirements are inevitable to facilitate large-scale and economic formulation.

Enzyme immobilization provides an excellent base for increasing availability of enzyme to the substrate with greater turnover over a considerable period of time. Several natural and synthetic supports have been assessed for their efficiency for enzyme immobilization.

Nowadays, immobilized enzymes are preferred over their free counterpart due to their prolonged availability that curtails redundant downstream and purification processes. Future investigations should endeavor at adopting logistic and sensible entrapment techniques along with innovatively modified supports to improve the state of enzyme immobilization and provide new perspectives to the industrial sector.

Microbial degradation targets the aliphatic or light aromatic fractions of oil. Several microbial species live on hydrocarbons and are responsible for the biodegradation of crude oil. Christoph et al., (2009) found that fertilizer application on oil spilled sites enhances the growth of hydrocarbon degrading microbes and in turn gives a better rate of oil biodegradation [13].

Fourier Transform Infrared Spectroscopic (FTIR) analysis

The analysis of the heavy crude oil fractions was performed by FTIR technique to check the changes in

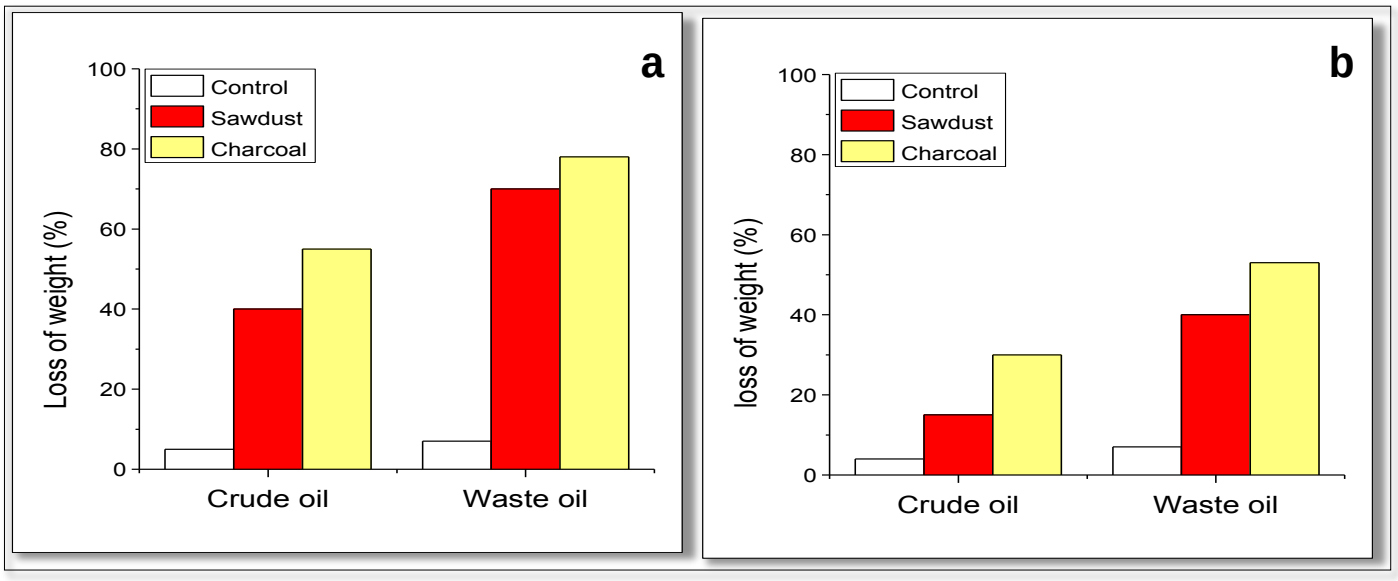

Fig 1. Ability of immobilized enzyme (a) and bacteria (b) on charcoal and sawdust to degrade crude oil and waste oil in autoclaved polluted soil.

hydrocarbon structure before and after treatment of crude oil with *P. aeruginosa* lipase. Results showed that, the spectra reflected more pronounced alterations than the control sample after degradation (**Fig 2** and **3**), the control sample contained the absorption bands of aliphatic (CH2) rocking and aromatic C-H bending at (600-900 cm-1), (C–H) and (C–C) stretching (2873-2959 cm−1) of alkanes and saturated aliphatic compounds and (C–H) deformation (1378-1500 cm−1) of aliphatic CH2 and CH3 groups, and absorption band of C=C for aromatic ring [14]. The spectra of crude oil treated with *P. aeruginosa* Ps12 after 7 days indicated many changes

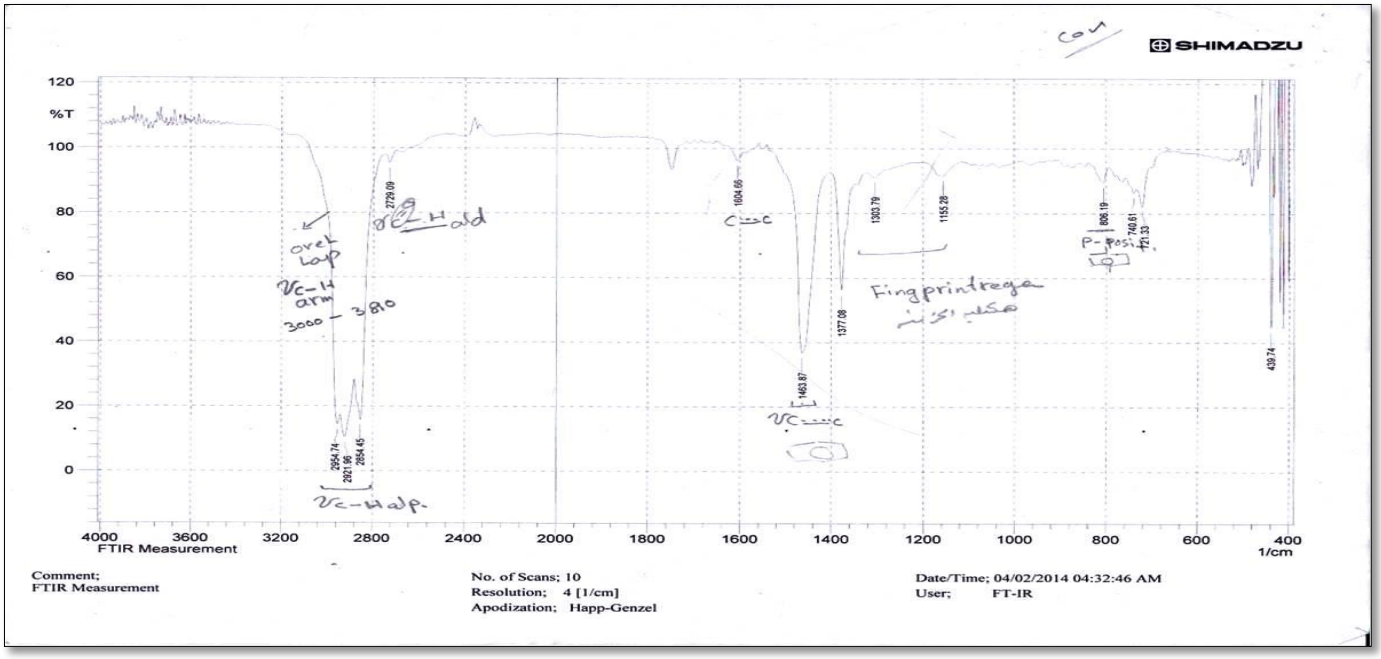

Fig 2. FTIR analysis of oil extracted from contaminated soil with crude of post treated with free enzyme (lipase). FTIR analysis of the extract showed several peaks of bonds.

In crude oil. By comparing the results with the control sample, a new absorption bands appeared in the range of wave number (2340-2362 cm−1), which refer to the C-O stretching and accompanied by release of CO2 from the oxidation of hydrocarbons. The structure of the crude oil converted from complex to more simple structure because of breakdown of bonds, thus many groups of structure was disappeared.

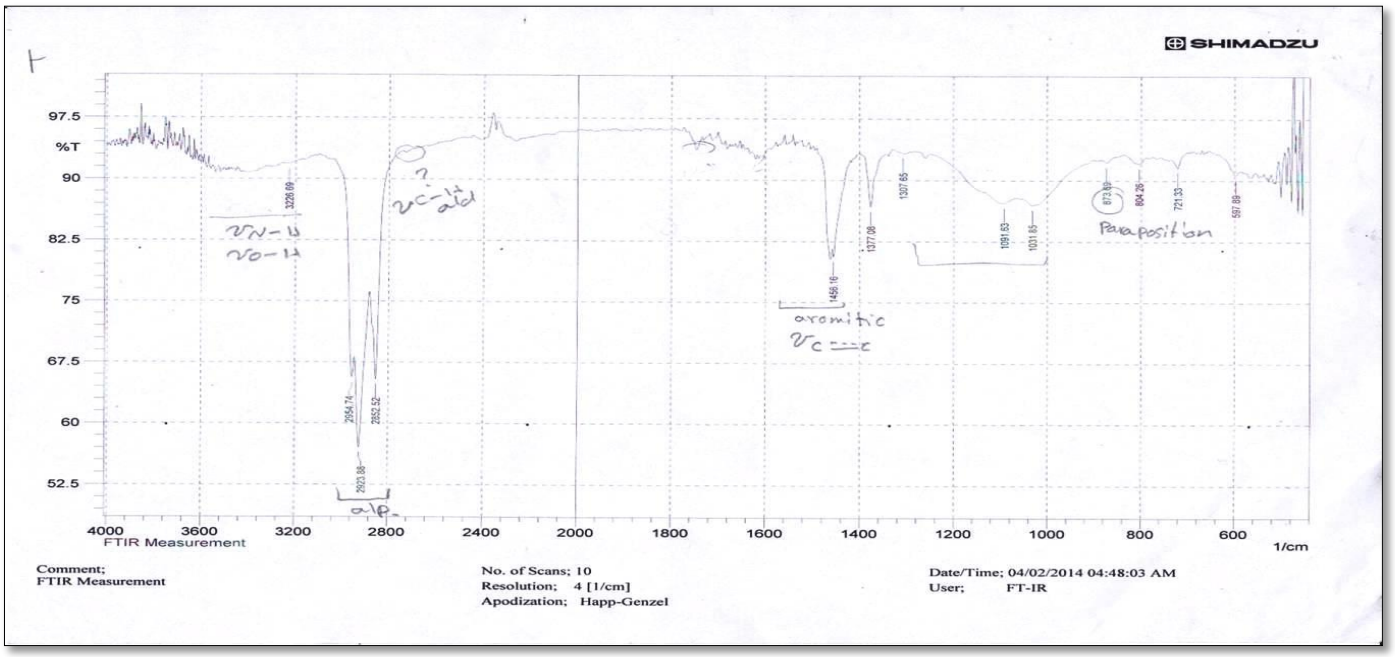

Fig 3. FTIR analysis of oil extracted from contaminated soil with crude oil post treated with immobilized enzyme (lipase). FTIR analysis of the extract showed several peaks of bonds.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCE

1. **Gupta R, Gupta N, Rathi P.** (2004) Bacterial lipases: an overview of production, purification and biochemical properties. *Appl Microbiol Biotechnol* **64**: 763–781.
2. **Ghafil JA, Hassan SS.** (2014) Effect of cultural conditions on lipase production from *Pseudomonas aeruginosa* isolated from Iraqi soil. *World J Exp Biosci* **2**: 13-18.
3. **Chuks UK, Agha NC, Ogbulie JN.** (2008) Lipase activities of microbial isolates from soil contaminated with crude oil after bioremediation. *Afr J Biotechnol* **7**: 2881-2884.
4. **Hamm P, Lim MH, Hochstrasser RM.** (1998) Structure of the amide I band of peptides measured by femtosecond nonlinear-infrared spectroscopy. *J Phys Chem B* **102**: 6123.
5. **Cesarini S, Infanzón B, Javier FI, Diaz P.** (2014) Fast and economic immobilization methods described for non-commercial *Pseudomonas* lipases. *BMC Biotechnol* **14**:27.
6. **Kanwar L, Goswami P.** (2002) Isolation of a *Pseudomonas* lipase produced in pure hydrocarbon substrate and its applications in the synthesis of isoamyl acetate using membrane-immobilized lipase. *Enzyme Microb Technol* **31**:727–735.
7. **Providencia J, Maria DC, Luis MM, perea J.** (2001) Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram- negative rods frequently isolated from clinical samples. *J Clin Microbiol* **39**:3247–3253.
8. **Ece G, Erac B, Limoncu MH, Baysak A, Oz AT, Ceylan KC.** (2014) *Stenotrophomonas maltophilia* Pseudo-outbreak at a University Hospital Bronchoscopy Unit in Turkey. *West Indian Med J* **63**:59-61.
9. **Tomotani EJ, Vitolo P.** (2006) Method for immobilizing invertase by adsorption on dowex anionic exchange resin. Braz *J Pharm Sci* **42**: 245-249.
10. **Pluchon N, Gundale MJ, Nilsson M-C, Kardol P, Wardle DA.** (2014) Stimulation of boreal tree seedling growth by wood-derived charcoal: effects of charcoal properties, seedling species and soil fertility. *Funct Ecol* **28**: 766-775.
11. **Mahmoud DAR.** (2007) Immobilization of Invertase by a New Economical Method Using Wood Sawdust Waste. *Aust J Basic Appl Sci* **1**: 364-372.
12. **Ramachandra M, Jayadev VB, Muniswaran PKA.** (2002) Hydrolysis of oils by using immobilized lipase enzyme: a review. *Biotechnol Bioprocess Eng* **7**: 57-66.
13. **Christoph G, Gunnar G, Kenneth N, Peter N.** (2009) Microbial consortia in mesocosm bioremediation trial using oil sorbents, slow-release fertilizerand bioaugmentation. *FEMS Microbiol Ecol* **69**: 288–300.
14. **Singh BG, Thomas E, Sawant SN, Takahashi K, Dedachi K, et al.** (2013) Radical cations of aromatic selenium compounds: role of Se···X nonbonding interactions. *J Phys Chem A* **117**:9259-65.

Author affiliation

1. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*