Biodegradation of Atrazine herbicide with Bacillus encimensis ABP8 isolate immobilized on sugarcane baggase

[1]HINA KHATOON, [2]J. P. N. RAI

[1]Research Scholar, [2]Professor
Department of Environmental Science,
G.B. Pant University of Agriculture and Technology,
Pantnagar (U.S. Nagar), Uttarakhand, India

Abstract: The free cells of microorganisms generally not succeed to degrade pollutants; thus, extra treatments are alluring to make strides biodegradation. This study represents the capability of sugarcane-bagasse as immobilizing support for biodegradation of atrazine. A novel bacteria Bacillus encimensis ABP8 isolated from atrazine contaminated soil was immobilized on baggase, which was able to degrade over 75 % of atrazine with concentrations ranges from 25 to 200 mg/L in 10 days. Scanning electron microscope (SEM) images appeared that after immobilization the bacterial cells of Bacillus encimensis ABP8 were totally retained and entirely distributed on the surface of sugarcane bagasse. Bacteria immobilized on 72 and 16 mesh size bagasse resulted 84.35 % and 80.64 % of atrazine degradation, showing imperative part of immobilization, upgrading biodegradation activity. Experimental results showed that atrazine could be degraded at a wide range of physicochemical parameters viz. pH (5.0 to 8.0), temperatures (25 to 35 °C) and atrazine concentrations ranging 50 to 200 mg/kg in soil. Microcosm experiment exhibits over 70 % of soil atrazine degradation in 14 days with immobilized bacterial cells, underlines that sugarcane bagasse might be a better bacteria-immobilizing support to improve the biodegradation effectiveness of Bacillus encimensis ABP8 isolate against atrazine herbicide.

Keywords: Sugarcane bagasse. Immobilization. Bacillus encimensis ABP8. Atrazine. Biodegradation.

I. INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)- 1,3,5-triazine], a subsidiary of the S-triazines is the most regularly utilized herbicide in the world, controlling broad-leaf and lush weeds in sugarcane, maize, sorghum and other crops. It is very diligent in impartial environment that makes toxicity to various living organisms like algae, aquatic plants, insects, fishes, and mammals [1, 2]. Because of high mobility and persistence in soil, it has frequently been identified in surface and ground water at concentrations well over the reasonable limits and hence considered as a potential environmental contaminant and absolutely considered as most noticeably awful ground water contamiants [3].

Amongst the predominant physical, chemical, and biological strategies to expel atrazine from soil and water [4], the biological processes have preferences over others [5-7]. Compared with conservative procedures, it is permanent, cost-effective, environment friendly and non intrusive to common biological system [8, 9]. Hence, in both industry and academia the biodegradation of chemical contaminants at ecologically related concentrations draws in interest [10]. On the other hand, most recent researches have demonstrated that a various microorganisms are able to convert herbicides into nontoxic inorganic compounds [11,12], the enhancement in survival and movement of connected organisms, and their immobilization plays a vital part in long-term stabilization and effective application of the methodologies for remediation of different contaminants [13-15]. In general, the execution of immobilized arrangements is administrated by the properties of both the enzyme and the support material which would largely improve the stability and movement of the immobilized composites [16]. Water-insoluble polysaccharides such as cellulose, agarose, chitosan and starch have been broadly utilized in constructing support material for enzyme immobilization [17].

Sugarcane bagasse is an abundant, eco-friendly, and low-cost lignocellulosic biomass which has been broadly utilized in bioethanol generation as a competent source of carbohydrates for microbial tansformation [18, 19]. Various chemical pre-treatment procedures and microbial fermentation were conducted [20-22], which unequivocally delineates the biocompatibility of sugarcane bagasse by means of diverse organisms. Sugarcane bagasse also has the prospective role in remediation of dye or phenol, because of its natural porous structure [23, 24]. In this manner, the possibility of sugarcane bagasse serving as an immobilizing support was commendable of profound examination, which would further expand the utilization of bagasse in biotechnology. On the other hand, the basic and biocompatible focal points of sugarcane bagasse were anticipated to provide a step forward in contaminant biodegradation by dynamic organisms. In present study, the degradation of Atrazine by Bacillus encimensis ABP8 was examined with free and sugarcane baggase immobilized cells in varied small scale environmental condition which could pave way to plan superior procedure for atrazine biodegradation.

II.   MATERIALS AND METHODS

2.1. Chemicals

Analyical grade Atrazine (>98 % purity) and methanol was obtained from Sigma-Aldrich, USA. Atrazine was dissolved in methanol at a stock concentration of 100 mg/L and stored at 4 °C prior to use. To conduct this, all erstwhile analytical grade solvents and chemicals were purchased from Himedia Laboratories, India.

2.2. Collection of Soil samples

Atrazine contaminated surface soil samples at depth of 15 cm were collected from Norman E. Borlaug, Crop Research Centre, Pantnagar, India after 10 days of herbicide spray in the Maize fields. One portion of soil samples were air dried and sieved at 0.2 mm and utilized for assurance of physicochemical properties though, the second part was preserved at 4°C till the isolation of Atrazine degrading bacteria.

2.3. Determination of physico-chemical properties of the soil samples

Various soil properties including all physico-chemical parameters like sand, silt clay, soil pH, organic carbon (OC) content, available nitrogen (N), available phosphorous (P), and available Potassium (K) were determined and presented in Table(1).

Table 1. Physicochemical parameters of soil sample

S. No.	Physical and chemical characteristics of soil	
1.	pH	7.21
2.	Nitrogen %	226 kg/ha
3.	Phosphorous (kg/ha)	30.6 kg/ha
4.	Potassium (kg/ha)	243.3 kg/ha
5.	% Carbon	0.75 %
6.	% moisture	30.28 %
7.	Colour	Light brown
8.	Texture	Silt Clay Loam

2.4. Enrichment, isolation and identification of atrazine degrading bacteria.

For the isolation of atrazine degrading bacteria from contaminated soil, 5 g of Soil samples were occupied in Erlenmeyer flasks (250 ml). Then the minimal broth (50 mL) with 50 mg/L atrazine concentration was added. The enrichment was done by incubating at 30 °C on a rotary incubator (150 rpm). After 7 days, the broth culture (5mL) from flask was reinoculated into fresh minimal media (50 mL) concentrated with 100 mg/L atrazine under above mentioned circumstances. At that point, the same method was repeated twice upto 200 mg/L of atrazine. After that, 0.2 mL of final culture broth was pour plated on agar plates for isolation of a single colony. Each colony, considered as a diverse species, was more than once streaked on agar plates. To obtain pure culture of best atrazine degradation, streaking about more than 10 times and then screening was done. The atrazine-degrading ability of purified isolates was determined by minimum inhibitory concentration method and the most active strain was identified by 16S rRNA analysis as Bacillus encimensis ABP8.

2.5. 16S rRNA Gene Sequencing and Construction of Phylogenetic Tree

16S rRNA partial nucleotide sequence analysis of bacterial isolate was carried out by Chromous Biotech Pvt. Ltd., Bangalore, India. Alignment of the 16S rRNA partial gene sequence of bacterial isolate ABP8 was performed with sequences present in the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/blast/). A MEGA version 7.0 software package was used for Phylogenetic analysis. The neighborjoining (NJ) method was used for phylogenetic analysis (Fig.1). Submission of sequence was completed in the GenBank database with accession number MG680922. Homology search using BLAST revealed 98 % similarity of this sequence with 16S rRNA partial gene sequence of Bacillus encimensis strain (GenBank accession no. LWDO01000030.1), thus isolate was designated to be Bacillus encimensis ABP8.

2.6. Inoculation and degradation of atrazine

For atrazine degradation experiment 250-mL Erlenmeyer flasks were taken containing 50 mL of sterile minimal medium and inoculated with Bacillus species ABP8, then Incubation was done at 30°C and 150 rpm for 24 h. The densities of ABP8 isolate were measured at 600 nm wavelength with UV spectrophotometer and adjusted to approximately 1.0×10^8 colony-forming units (CFU) mL^{-1}. Incubation and inoculation of microorganisms on minimal medium containing Atrazine with concentration ranging 25, 50, 100, and 200 mg/L was done. Control without inoculation was also set. The degradation of atrazine was determined through high-performance liquid chromatography (HPLC) at intervals of 1, 2, 3, 4, and 5 days, respectively. Extraction was done with dichloromethane, and the organic layer was dehydrated, dried, and redissolved in methanol. After filtration, the samples were subjected to HPLC.
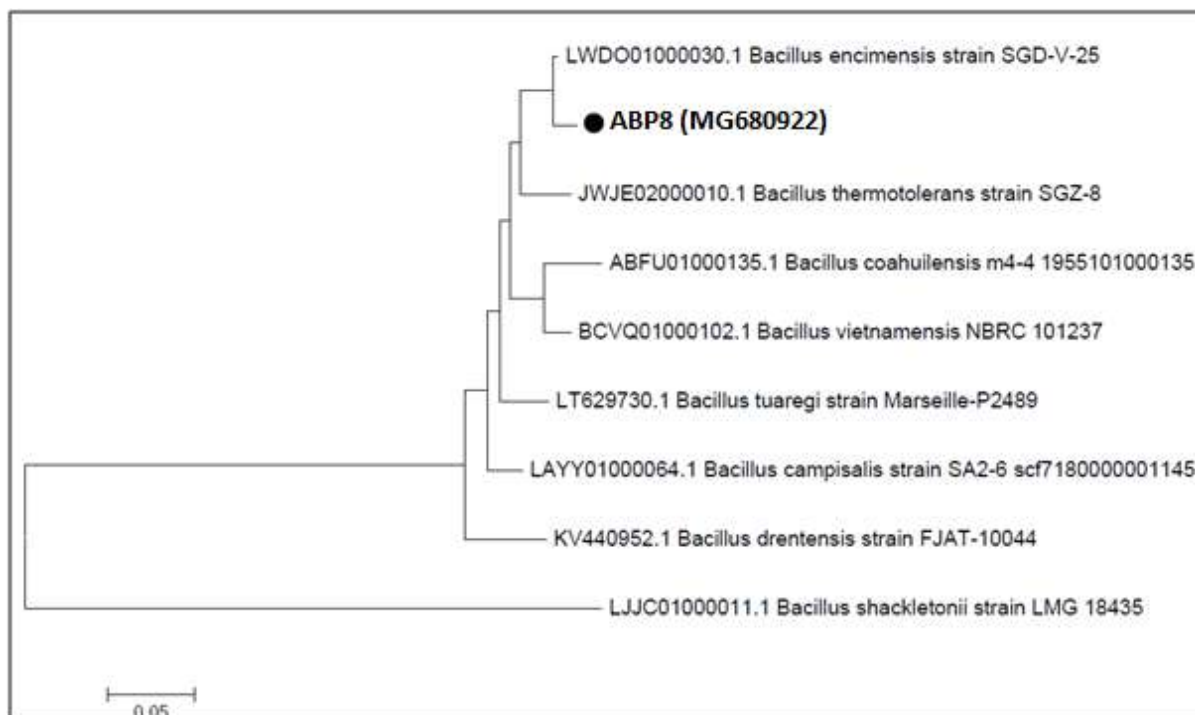
Fig.1. Phylogenetic analysis of Bacillus encimensis ABP8 based on neighbour joining method.

2.7. Sugarcane bagasse pretreatment

Sugarcane bagasse was procured from G. B. Pant University of agriculture and technology, Pantnagar, India (Fig.2). The bagasse was dried at 100 °C in an oven for 24 h and mechanically grinded into powder, followed by being sieved size to 10, 18, and 72 mesh (with the diameters of 800, 250, and 150 μm, respectively). Then, the material was washed with distilled water, dried at 100 °C, and stored in room temperature for further use.

2.8. Immobilization and degradation evaluation

In Erlenmeyer flask (250-mL) containing minimal medium supplemented with atrazine was mixed with the Bacteria suspension and incubated on a shaking incubator at 30 °C, 150 rpm for 7 days to obtain growth condition. Then, from broth culture, 2 mL of bacteria suspension (adjusted to 1×10^8 CFU mL^{-1}) was mixed with sugarcane-bagasse (0.1g) and putted on shaking incubator at 30 °C, 180 rpm for 24 h. The centrifugation (10 min, 4800 rpm) of immobilized mixture was done to obtain settled substance which was again dried at 20 °C and preserved for SEM. For the observation of morphological characters, the immobilized bacterial sample was subjected to scanning electron microscope (SEM). The growth activity detection was assessed to see immobilization effect on bacteria, for this immobilized mixture (0.1 g) was added in 50-mL minimal broth to determine Optical density (OD600) and CFU values at 24 and 48h. The medium, inoculated with the similar amount of bacterial culture without immobilization was setup as control and placed on a shaking incubator, respectively. Then, the degradability of immobilized bacteria was investigated by pertaining 0.1 g of bacteria-bagasse in 50-mL minimal medium supplemented with 50 mg/L of atrazine, and the amount of residues was determined by HPLC.

Fig.2. Sugercane-baggase before pretreatment used as a bioprocess material for immobilization.

2.9. Effects of reaction conditions on atrazine degradation

The effects of reaction conditions including pH (4.0 to 8.0), temperature (20 to 40 °C), atrazine concentration (50 to 200 mg/kg), and the amount of dosage (5 to 20 g) on atrazine degradation were investigated. The effect of each factor was conducted in a single-factor experiment that only the tested factor was altered accordingly. The atrazine residues were measured after 2, 3, 5, 7, 10, and 14 days by HPLC.

2.10. Degradation of Atrazine in soil microcosm

We performed a soil microcosm study of three treatments with Bacillus encimensis ABP8 in 1L Erlenmeyer flask with three replicates. For treatment 1, about 200 g sterilized soil supplemented with 100 mg/kg concentrated atrazine was contained in flask. Treatment 2, with sterilized soil 200 g and bacterial inoculum (2×10^6 cells/g of soil) and atrazine at a concentration of 100 mg/kg. Treatment 3 contained sterilized soil 200 g, immobilized bacterial inoculum (2×10^6 cells/g of soil) and 100 mg/kg concentrated atrazine. To maintain soil moisture content the sufficient amount of water was added. In the Erlenmeyer flasks, the entire components in the three treatments were altogether blended, covered with cotton plug and incubated at 30 °C for 21 days. Samples were withdrawn after 1, 3, 5, 7, 10, 14, 21 days incubation and atrazine residues were measured by HPLC to estimate atrazine degradation. The samples were extracted with 5 g soil using 20mL methanol. Then samples were shaken for 1 h on an orbital shaker and filtered through Whatman filter paper. This procedure was repeated thrice and collected solvent extract was dried up using rotary flash evaporator by re-dissolving in 2mL methanol solvent for HPLC analysis. A Chromatogram of HPLC analysis for atrazine after 21 days degradation with bacterial isolate is shown in Fig. 3.
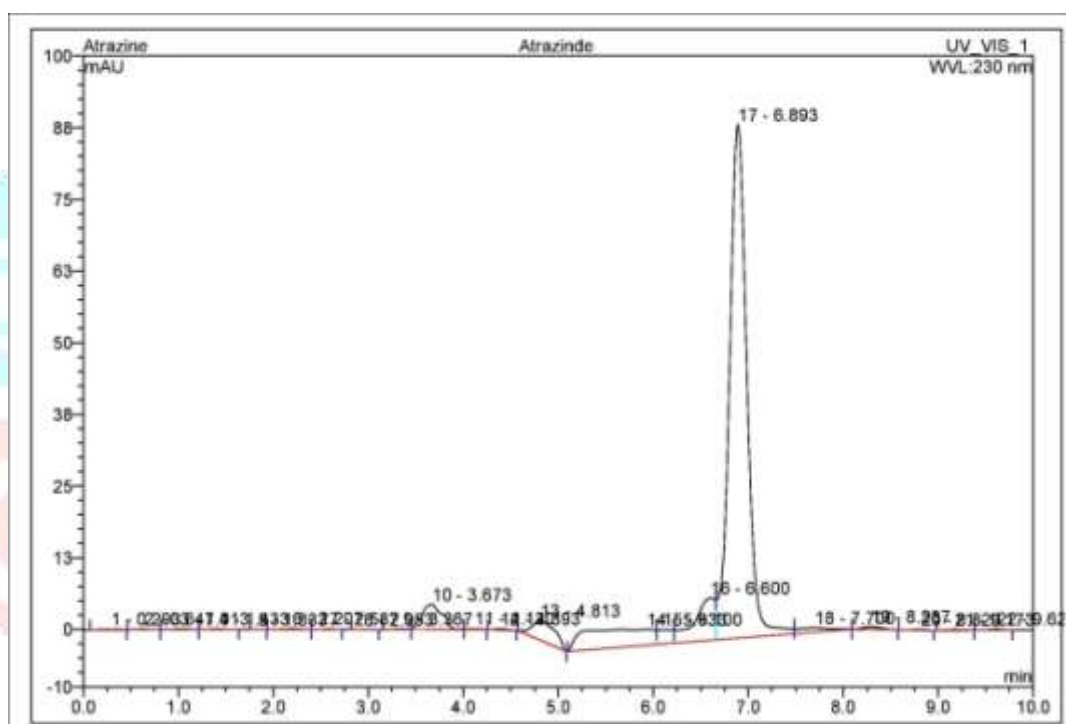

Fig.3. Chromatogram of HPLC analysis for determination of atrazine residues after degradation with bacterial isolate.

2.11. HPLC analysis of atrazine

The residual atrazine was analyzed on an Dionex ultimate 3000 HPLC equipped with a C18 reversed phase column (250×4.6 mm id, 5 μm), and the analytical method of which is listed in Table 2.

Table 2. HPLC analysis of atrazine.

Model :	Dionex ultimate 3000
Column :	C-18 Reverse Phase (250×4.6 mm id, 5 μm)
Detector :	UV- detector
Solvent :	Methanol: Water (80:20)
Flow rate :	1ml/min.
Retention time :	7 min

2.12. Data analysis

All of the experiments were carried out in three sovereign experiments, and the results were the means of three replicates. The degradation rate of atrazine was analyzed according to the following equation (Eq. (1)):

$$\rho = (A/A_o) \times 100 \qquad \text{.......} \qquad (1)$$

where, ρ is the Percent degradation of atrazine (%), A (mg/L) is the residual concentration of sample, and A_0 (mg/L) represents the concentration of control sample.

2.13. Statistical analysis

The experimental data were processed for calculating standard error of the means and multi-factorial analysis of variance as available in the SPSS statistical package (Stat Graphics Plus V. 11), and expressed at 0.05 probability level. The significance ($p<0.05$) of differences was treated statistically by one and two-way analysis of variance (ANOVA) and evaluated by post hoc comparison of means using lowest significant differences (LSD).

III. RESULTS

3.1. Morphological observation and degradation assessment

Sugarcane bagasse served as a support material for immobilization of bacteria, because of its natural porous structure, which provides large surface area and high loading volume for cell absorption. For morphological observation, the samples of pure bagasse and immobilized microbes after immobilization were scanned by SEM. The scanning image of bagasse particles before immobilization were presented in Fig. 4a, in this the shape of initial bagasse particles was irregular columnar with porous vascular tissue, which provides a large area for free bacteria to get in touch with baggase surface.
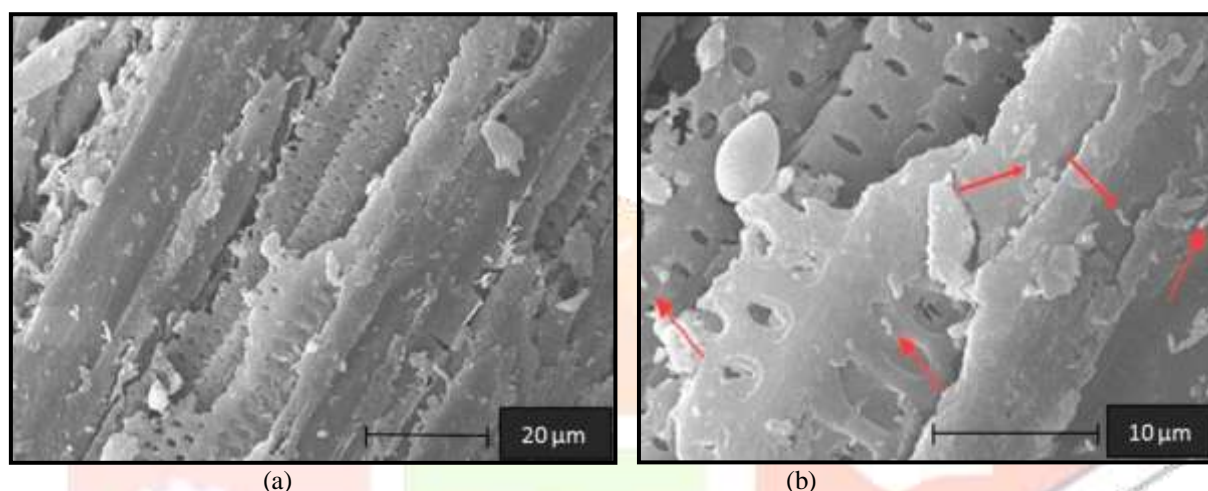

<div align="center">(a)   (b)</div>

Fig. 4. SEM images shows (a). The Sugercane-baggase surface before immobilization, (b). The appearance of vascular bundle surface after immobilization, and the arrows represent the locations of bacteria absorbed on the surface of sugarcane baggase.

Though, after biomass stacking as appeared in Fig. 4b, the surface and shape of sugarcane-bagasse were not significantly adjusted. Which appeared congruity with the dispute that immobilization of enzymes interior the permeable structure of a solid may allow to have the enzyme molecules completel scattered [25]. As such, dynamic microbes were emphatically retained and haphazardly scattered on the surface and interior the vascular bundles, demonstrating that the applied bagasse was biocompatible with dynamic microbial cells and formation of a steady bacteria-bagasse composite takes place. Bacterial development was observed by the optical density (OD600) and CFU values (Table 3). Bagasse, which was already classified in 10, 16, and 72 mesh (with particle size of 1700, 1000, and 200 µm, separately), was independently served as carrier candidates.

Table 3. The growth of immobilized bacteria and the degradation of atrazine at initial concentration of 50 mg/L

Size	Value of OD600			Bacteria counts (CFU mL^{-1})			Degradation rate
	0hr	12hr	24hr	0hr	12hr	24hr	(%)
10 mesh	0.06±2.2c	0.36±2.0c	0.46±1.3c	4.5×10^5	2.7×10^6	8.5×10^7	39.03±1.2
16 mesh	0.05±1.3b	0.27±1.3a	0.41±1.8a	3.8×10^5	7.3×10^6	6.4×10^8	80.64±0.7
72 mesh	0.04±0.9b	0.33±1.9b	0.45±0.3b	3.3×10^5	6.5×10^6	5.2×10^8	84.35±1.3
Control	0.02±1.0a	0.35±0.4b	0.42±1.6a	6.2×10^5	7.5×10^6	5.8×10^8	63.92±0.6

As compare to control group, the immobilized microbes were feasble and competent of multiplying after immobilization approach since the values of CFU and OD600 were persistently expanding. Altering the medium to 50 mg/L of atrazine, the degradation rates with immobilized bagasse in 72 and 16 mesh come to 84.35 and 80.64 %, respectively, proposing that the atrazine degradation capacity was still held after immobilization. Due to the greater measure and littler surface zone, microscopic organisms immobilized in bagasse of 10 mesh showed low degradation rate (39.03 %), showing that this measure of bagasse

might not be suitable. Furthermore, it also recommended that the littler estimate of carrier particles could considerably make strides biomass stacking for their bigggger surface ragion, subsequently moving forward degradation of target chemical. In this manner, bagasse milled into 16 and 72 mesh could be more suitable as carriers for the way better capacities in terms of cell development and biodegradation.

3.2. Atrazine degradation by Bacillus encimensis ABP8 in broth culture:

The rate of atrazine dedradation by bacterial isolate enhanced with increase of incubation time i.e. from 1 to 10 days as a result atrazine residues in broth cultures was found almost negligible after 10 days (Fig. 5). The degradation rate reached above 40 % after 3 days, and 50 % after 5 days at the initial concentration of 25, 50, 100, and 200 mg/L, suggesting that the soil bacterial strain was competent of metabolizing rapidly, particularly from lower concentration of atrazine. This attribute further signifies the efficient performance of Bacillus encimensis ABP8.

3.3. Effects of reaction conditions on atrazine degradation

Four Physicochemical parameters that might influence the atrazine-degrading activity of the microbial composite were explored with experiments. As appeared in Fig. 6 (a), the maximum (53%) degradation was found at pH 7.0 after 5 days; whilst at lower and higher pH values the degradation rates were around 48%. The impact of temperature (in fig 6. b) delinated that over 40 % atrazine degradation accomplished after 5 days with temperature range from 25 to 35 °C. In any case, the degradation execution essentially diminished at temperature >35 °C. The impact of the amount of immobilized microbial dosage applied on atrazine degradation (in Fig. c) revealed that in 7 days, 62.20 and 64.21 % of atrazine was degraded with 10 and 20 g of dosage, respectively, whilst at 5 g dosage the degradation was only 58.19%. The change of percent degradation rate with the increment of atrazine initial concentrations is presented in Fig. 6(d), which clearly depicted that this microbial preparation possessed great degradation ability at low concentrations of atrazine < 200 mg/kg. To figure out, this bacteria-bagasse preparation can be applied for biodegradation at variable environments i.e. 5.0 to 8.0 pH, 25 to 35 °C temerature and with dosage of 10 g, especially at low concentrations 50 to 200 mg kg^{-1} of atrazine.
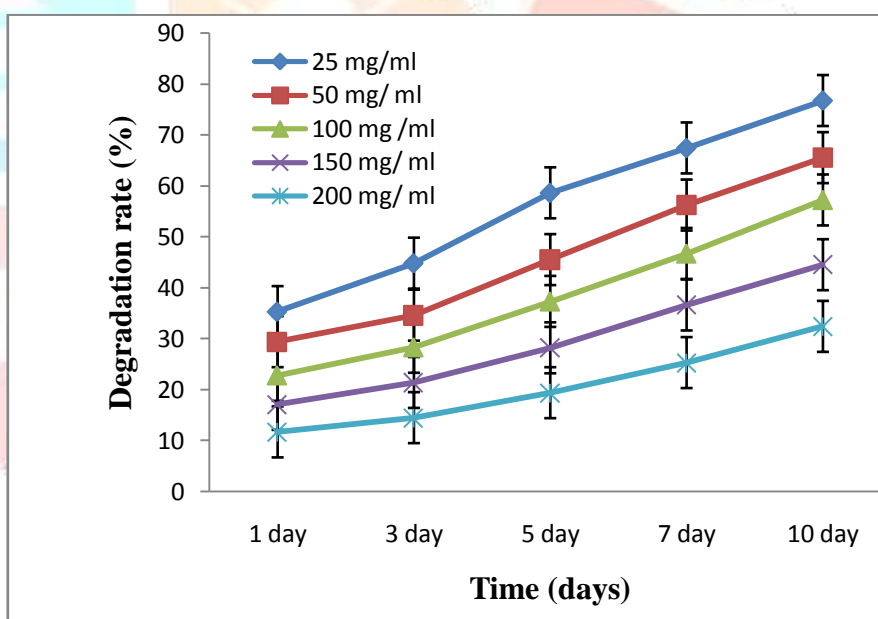

Fig.5. Degradation of atrazine at different initial concentrations by free cells of Bacillus encimensis ABP8 in Minimal broth. Values are means of three replicates with standard deviation.

3.4. Atrazine biodegradation in soil microcosm:

In the soil microcosm the biodegradation of atrazine after 21 days was 33.88 % atrazine in treatment 1 (control) and in Treatment 2 containing sterilized soil amended with bacterial inoculum and atrazine, the percent degradation was 47.84 %, whilst in Treatment 3, which contained sterilized soil improved with immobilized bacterial cells and atrazine, showed maximum 72.24 % atrazine degradation (Fig. 7).
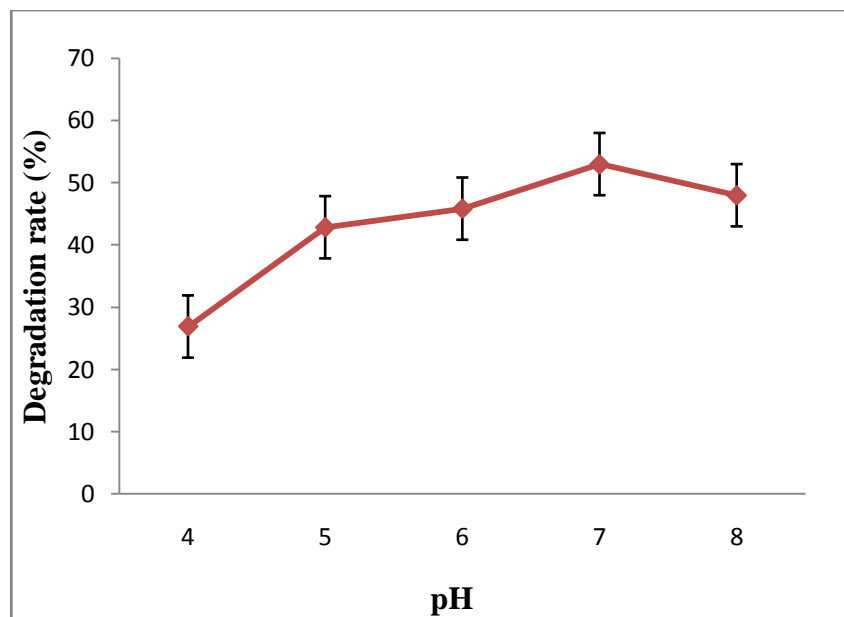
Fig. 6(a). Effects of pH on biodegradation of soil atrazine. Values are means of three replicates with standard deviation.
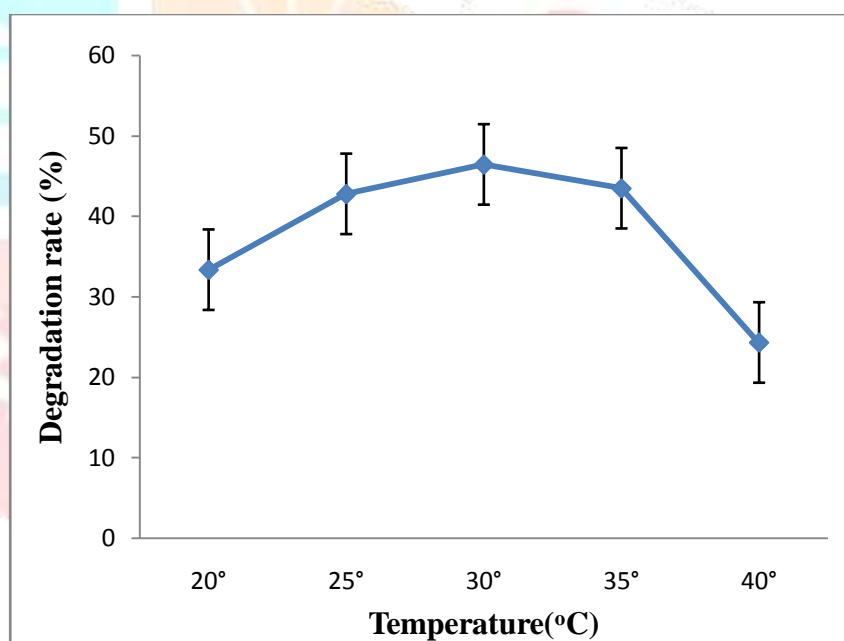

Fig. 6(b). Effects of temperature on biodegradation of soil atrazine. Values are means of three replicates with standard deviation
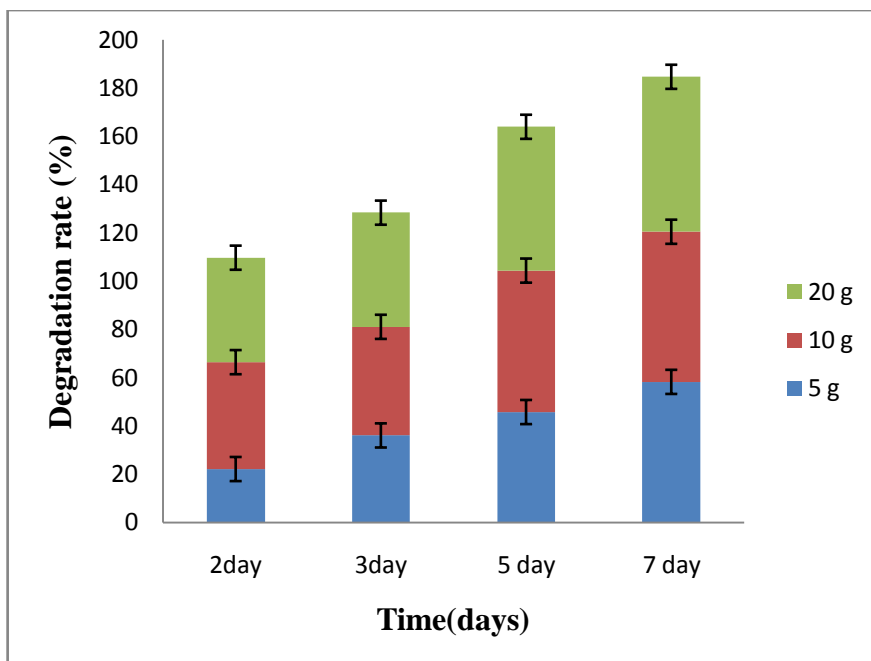
Fig. 6(c). Effects of dosage on biodegradation of soil atrazine. Values are means of three replicates with standard deviation.
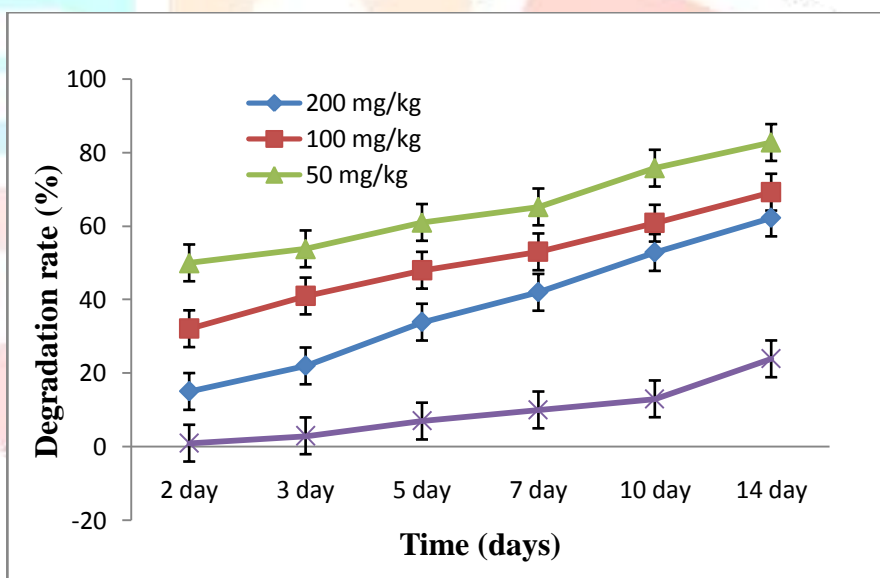

Fig. 6(d). Effects of initial concentrations on biodegradation of atrazine in soil. Values are means of three replicates with standard deviation
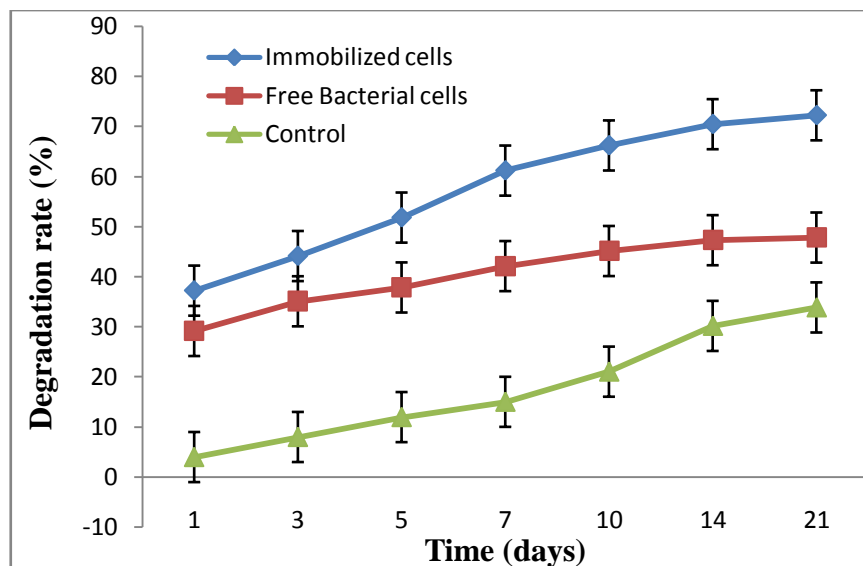
Fig. 7. Biodegradation of atrazine in soil microcosm by immobilized and free Bacterial cells.
Values are means of three replicates with standard deviation.

IV. DISCUSSION

For the most part, herbicides applied in culturing lands are included in sorption, desorption, transport, volatilization, and alteration procedures [26, 27]. Based on the understanding of these physicochemical or biochemical behaviors, superior procedures could be gotten for compelling utilization of dynamic compounds as well as remediation of herbicide-contaminated locales. Biodegradation includes the utilization of living microorganisms or their enzymes to detoxify pollutants, which has been by the large considered as a successful and cost-effective method for the removal of contaminant [28]. It was detailed that different microbes had the capacity of efficiently degrading atrazine including Chelatobacter heintzii [29], Enterobacter (E. cloacae), Bacillus (B. cereus and B. anthracis), Pseudomonas (P. aeruginosa, P. balearica, P. indica and P. otitidis), Ochrobactrum (O. intermedium) and Providencia (P. vermicola) [30] and P. fluorescence + P. putida [31].

Present study explained, an exceedingly successful strain from Bacillus species was chosen for atrazine degradation. Results comes out shown that bacteria Bacillus encimensis ABP8 was able to use atrazine as carbon and nitrogen source. As Compare to the treatments with higher introductory concentrations, the degradation at lower concentrations was more noteworthy and proficient; this empowered the strain to operate in circumstances with low residues. This is imperative and useful not at it were for intensifying the application scale of this strain but moreover for improving the harm to environment caused by pesticide residues beneath caution levels. Prior studies have demostrated that generally low levels of herbicide residues would influence and cause a chain of impacts on biological system [32, 33]. In this manner, degrading atrazine in low levels must be an advantage for Bacillus encimensis ABP8. In viable application, the degradation execution was not steady and compelling as tried in research facility since the coordinate utilize of free cells was affected by numerous variables. Utilizing diverse immobilized microbes may give a conceivable arrangement, which has been demonstrated tediously as an compelling approach for contamination remediation [13, 14]. It is detailed that the movement of immobilized composite is immensely influenced by the properties of support material [16, 17]. Therfore, choosing a steady and economical support with great biocompatibility is the key to the immobilized composite, as such sugarcane bagasse liberally accessible in countries like Brazil, India, and China as a support candidate. As an industrial squander, fractional buildup of baggase is utilized in bioethanol generation while the left over portion is stockpiled [18].

Awesome interface exist in investigating other strategies that offer economic, environmental, and vital points of interest in bagasse biotechnology. Present study explained that the potential of bagasse as an immobilization support was approved as anticipated. SEM images represented that bacteria were completely scattered on the surface of bagasse particles, and their degrading movement held after immobilization. Optimization of test aptitudes would process the amount of stacking microbes prepared immobilization, consequently, optimization of immobilizing support ought to be picked up more consideration. Moreover it is accepted that the immobilization technique should be a generally basic process that does not require a profoundly unadulterated enzyme preparation or a costly support that may not be commercially accessible [17].

The flexibility and accessibility were the two fundamental challenges in this microbial arrangement plan. Consequently, we tried the impacts of different variables on the degradation execution, which could be effectively affected in a genuine soil environment. pH is one of the key component impacting the amount of protein adsorbed onto the surface of a test support[34]. On this viewpoint a few discoveries affirms that the maximum adsorption of protein occurs at or near the iso-electric point of the protein [35]. Experiment results shown that this immobilized preparation can be utilized in shifted situations to accomplish proficient execution. Interestingly, present study clearly illustrated that in both research facility and field conditions atrazine degradation propensities, expanded gradually during first 5 days, while it raised significantly afterward, which could be conceivably due to the diauxic growth of degrading bacteria, a common incident observed in a medium having carbohydrates mixture [36].

V. CONCLUSION

Determine utilize of herbicides has gotten to be a key risk to human wellbeing and environment. In spite of the fact that modern items with low residues are being propelled each year, the amassing of herbicides is still an unanswered issue, which calls for a successful arrangement for residue clearance. Utilization of dynamic microorganisms is an efficient and economical strategy, while microbial immobilization gives a breakthrough in restricted utilize of microbes in in-situ. In the present study, sugarcane bagasse was utilized in herbicide biodegradation as fabulous stacking supports for bacterial immobilization. Conformations clarified that the normal permeable structures of bagasse could give expensive surface area and large pore volume for bacteria stacking and dispersing. Hence upgrade its stability and efficiency to degrade atrazine. Encourage optimization of immobilization skills and physicochemical modification of bagasse particles could be promising methodologies setting the issues pertaining to the degradation of herbicides and other recalcitrant chemicals.

VI. ACKNOWLEDGEMENTS

REFERENCES

[1]   Topp, E., Mulbry, W., Zhu, H., Nour, S., Cuppels, D. 2000. Characterization of s-triazine herbicide metabolism by a Nocardioides sp. isolated from agricultural soils. Applied and Environmental Microbiology, 66 (8): 3134–3144.

[2]   Ghosh, P.K., Philip, L. 2006. Environmental significance of atrazine in aqueous systems and its removal by biological processes: an overview. Global Nest journal, 8: 71–90.

[3]   Kaushik, G., Satya, S., Naik, S.N. 2009. Food processing a tool to pesticide residue dissipation - A review. Food Research International, 42: 26-40.

[4]   Page, D., Dillon, P., Mueller, J., Bartkow, M. 2010. Quantification of herbicide removal in a constructed wetland using passive samplers and composite water quality monitoring. Chemosphere, 82: 394–399.

[5]   Dash, H.R., Mangwani, N., Chakraborty, J., Kumari, S., Das, S. 2013. Marine bacteria: potential candidates for enhanced bioremediation. Applied Microbiology and Biotechnology, 97: 561–571.

[6]   Ramos, J., Marqués, S., van Dillewijn, P., Espinosa-Urgel, M., Segura, A., Duque, E., Krell, T., Ramos-González, M., Bursakov, S., Roca, A., Solano, J., Fernádez, M., Niqui, J.L., Pizarro-Tobias, P., Wittich, R. 2011. Laboratory research aimed at closing the gaps in microbial bioremediation. Trends in Biotechnology, 29: 641–647.

[7]   Singh, B., Kaur, J., Singh, K. 2012.Microbial remediation of explosive waste. Critical Reviews in Microbiology, 38:152–167.

[8]   Fuentes, S., Méndez, V., Aguila, P., Seeger, M. 2014. Bioremediation of petroleum hydrocarbons: catabolic genes, microbial communities, and applications. Applied Microbiology and Biotechnology, 98: 4781–4794.

[9]   Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., Naidu, R. 2011. Bioremediation approaches for organic pollutants: a critical perspective. Environmental international, 37: 1362–1375.

[10]   Xiao, Y., Chen, S., Gao, Y., Hu, W., Hu, M., Zhong, G. 2015. Isolation of a novel beta cypermethrin degrading strain Bacillus subtilis BSF01 and its biodegradation pathway. Applied Microbiology and Biotechnology, 99: 2849–2859.

[11]   Ermakova, I.T., Kiseleva, N.I., Shushkova, T., Zharikov, M., Zharikov, G.A., Leontievsky, A.A. 2010. Bioremediation of glyphosatecontaminated soils. Applied Microbiology and Biotechnology, 88: 585–594.

[12]   Sandoval-Carrasco, C.A., Ahuatzi-Chacón, D., Galíndez-Mayer, J., Ruiz- Ordaz, N., Juárez-Ramírez, C., Martínez-Jerónimo, F. 2013. Biodegradation of a mixture of the herbicides ametryn, and 2,4- dichlorophenoxyacetic acid (2,4-D) in a compartmentalized biofilm reactor. Bioresource Technology, 145: 33–36.

[13]   Piccirillo, C., Pereira, S.I.A., Marques, A.P.G.C., Pullar, R.C., Tobaldi, D.M., Pintado, M.E., Castro, P.M.L. 2013. Bacteria immobilisation on hydroxyapatite surface for heavy metals removal. Journal of Environmental Management, 121: 87–95.

[14]   Stelting, S., Burnz, R.G., Sunna, A., Visnovsky, G., Bunt, C.R. 2012. Immobilization of Pseudomonas sp. strain ADP: stable inoculants for the bioremediation of atrazine. Applied Clay Science, 64: 90–93.

[15]   Xu, Y. and Lu, M. 2010. Bioremediation of crude oil-contaminated soil: comparison of different Colloid Polym. Science, 293(11): 3381–3391.

[16]   Ema, T., Miyazaki, Y., Kozuki, I., Sakai, T., Hashimoto, H., Takada, J. 2011. Highly active lipase immobilized on biogenous iron oxide via an organic bridging group: the dramatic effect of the immobilization support on enzymatic function. Green Chemistry, 13: 3187–3195.

[17]   Sheldon, R.A. 2007. Enzyme immobilization: the quest for optimum performance. Advance Synthesis and Catalysis, 349: 1289–1307.

[18]   Cardona, C.A., Quintero, J.A., Paz, I.C. 2010. Production of bioethanol from sugarcane bagasse: status and perspectives. Bioresource Technology, 101: 4754–4766.

[19]   Geddes, C.C., Nieves, I.U., Ingram, L.O. 2011a. Advances in ethanol production. Current Opinion in Biotechnology, 22: 312–319.

[20]   Geddes, C.C., Mullinnix, M.T., Nieves, I.U., Peterson, J.J., Hoffman, R.W., York, S.W., Yomano, L.P., Miller, E.N., Shanmugam, K.T., Ingram, L.O. 2011b. Simplified process for ethanol production from sugarcane bagasse using hydrolysate-resistant Escherichia coli strain MM160. Bioresource Technology, 102: 2702–2711.

[21]   Rabelo, S.C., Carrere, H., Maciel Filho, R., Costa, A.C. 2011. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresource Technology, 102: 7887–7895.

[22] Wang, X., Yomano, L.P., Lee, J.Y., York, S.W., Zheng, H., Mullinnix, M.T., Shanmugam, K.T., Ingram, L.O. 2013. Engineering furfural tolerance in Escherichia coli improves the fermentation of lignocellulosic sugars into renewable chemicals. Proceedings of National Academy of Sciences of the Unites States of America, 110: 4021–4026.

[23] Basak, B., Bhuniab, B., Dey, A. 2014. Studies on the potential use of sugarcane bagasse as carrier matrix for immobilization of Candida tropicalis PHB5 for phenol biodegradation. International Biodeterioration and Biodegradation, 93: 107–117.

[24] Waghmare, P.R., Kadam, A.A, Saratale, G.D., Govindwar, S.P. 2014. Enzymatic hydrolysis and characterization of waste lignocellulosic biomass produced after dye bioremediation under solid state fermentation. Bioresource Technology, 168: 136–141.

[25] Mateo, C., Grazu, V., Palomo, J.M., Lopez-Gallego, F., Fernandez-Lafuente, R., Guisan, J.M. 2007. Immobilization of enzymes on heterofunctional epoxy supports. Nature Protocols, 2: 1022–1033.

[26] Lima, D.L.D., Schneider, R.J., Scherer, H.W., Duarte, A.C., Santos, E.B.H., Esteves, V. 2010. Sorption–desorption behavior of atrazine on soils subjected to different organic long-term amendments. Journal of Agricultural and Food Chemistry, 58: 3101–3106.

[27] Wong, F. and Bidleman, T.F. 2011. Aging of organochlorine pesticides and polychlorinated biphenyls in muck soil: volatilization, bioaccessibility, and degradation. Environment Science and Technology, 45: 958–963.

[28] Arora, P.K., Srivastava, A., Singh, V.P. 2014. Bacterial degradation of nitrophenols and their derivatives. Journal of Hazardous Materials, 266: 42–59.

[29] Rousseaux, S., Hartmann, A., Lagacherie, B., Piutti, S., Andreux, F., Soulas, G. 2003. Inoculation of an atrazine-degrading strain, Chelatobacter heintzii Cit1, in four different soils: effects of different inoculums densities. Chemosphere, 51: 569 - 576.

[30] El-Bestawy, E., Sabir, J., Mansy, A.H., Zabermawi, N. 2013. Isolation, identification and acclimatization of Atrazine-resistant soil bacteria. Annals of Agricultural Sciences, 58(2): 119–130.

[31] Chegini, S., Sani, B., Darvishi, H.H. 2015. Atrazine biodegradation by stimulating the activity of soil bacterial population in maize field. International Journal of Biosciences, 6(1): 293-297.

[32] Lewis, S.E., Brodie, J.E., Bainbridge, Z.T., Rohde, K.W., Davis, A.M. Masters, B.L., Maughan, M., Devlin, M.J., Mueller, J.F., Schaffelke B. 2009. Herbicides: a new threat to the great barrier reef. Environmental Pollution, 157: 2470–2484.

[33] Ricart, M., Barceló, D., Guasch, H., de Ald, M.L., Romaní, A.M., Vidal, G., Villagrasa, M., Sabater, S. 2009. Effects of low concentrations of the phenylurea herbicide diuron on bioflim algae and bacteria. Chemosphere, 76: 1392–1401.

[34] Popat, A., Hartono, S.B., Stahr, F., Liu, J., Qiao, S.Z., Lu, G.Q. 2011. Mesoporous silica nanoparticles for bioadsorption, enzyme immobilisation, and delivery carriers. Nanoscale, 3: 2801–2818.

[35] Meissner, J., Prause, A., Bharti, B., Findenegg, G.H. 2015. Characterization of protein adsorption onto silica nanoparticles: influence of pH and ionic strength. Journal of Hazardous Materials, 183: 395–40.

[36] Cohen, G.N. 2014. Microbial biochemistry, 3rd edn. Springer, New York.

[37] Kulkarni, S.V., Markad, V.L., Melo, J.S., D'Souza, S.F., Kodam, K.M. 2014. Biodegradation of tributyl phosphate using Klebsiella pneumonia sp. S3. Applied Microbiology and Biotechnology, 98: 919–929

[38] Kumar, S., Gummadi, S.N. 2011. Metabolism of glucose and xylose as single and mixed feed in Debaryomyces nepalensis NCYC 3413: production of industrially important metabolites. Applied Microbiology and Biotechnology, 89: 1405–1415.