

## Sugarcane-Bagasse as Immobilizing Support for *Bacillus badius* ABP6 and its Use in Biodegradation of Atrazine

Hina Khatoon, J. P. N. Rai

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**Abstract** In the present study, the potential of sugarcane bagasse as bacteria-immobilizing support was investigated in atrazine biodegradation. The main purpose of this research was to study the biodegradation ability of atrazine by using free and immobilized bacterial cells. A novel bacterial isolate *Bacillus badius* ABP6 was applied in bacterial immobilization, which was capable of degrading over 70% of atrazine at initial concentrations ranging from 25 to 200 mg/L within 4 days. Scanning electron microscope (SEM) images showed that the bacterial cells were strongly absorbed and fully dispersed on bagasse surface after immobilization. Specially, 85.32 and 81.57% of atrazine was eliminated by bacteria immobilized on bagasse of 100 and 60 mesh sizes, respectively, which indicated that this immobilization was able to maintain a high degrading activity of the bacteria. Analysis of the degradation of atrazine was done with HPLC. In soil microcosm tests, over 80% of atrazine in soil was degraded within 14 days employing bagasse immobilized cells. The immobilized preparation demonstrated that atrazine could be degraded at a wide range of pH values (5.0–8.0) and temperatures (25–

35°C). These results showed that sugarcane bagasse might be a novel approach as bacteria-immobilizing support to enhance atrazine degradation by *Bacillus badius* ABP6 in contaminated soils. This research indicated the advantages of sugarcane-bagasse immobilized bacterial cells for biodegradation of atrazine herbicide with greater efficiency.

**Keywords** Sugarcane bagasse, Immobilization, Atrazine, *Bacillus badius* ABP6, Bioremediation.

### Introduction

The use of herbicides has contributed to over 40% of the pesticide consumption in the world market, which globally ensures crop cultivation and food production. Herbicide resistance and its side effects are the two main concerns, which are partially caused by the high levels of herbicide residues [1]. Moreover, inappropriate use of the herbicides potentially impact nontarget organisms in aquatic ecosystem and non-farming areas [2, 3]. Numerous studies reported the disposal of herbicide contaminants, which could be realized by approaches based on their physicochemical properties, for instance, physical absorption [4], chemical transformation, or plant mulch [5]. Microorganism-based remediation is a group of promising techniques introducing microbes with specific catabolic potential isolated from *in situ* microbial communities to degrade target contaminants [6, 7]. Compared with conventional strategies, it is permanent, more

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Hina Khatoon\*, J. P. N. Rai  
Department of Environmental Science, G. B. Pant University of Agriculture and Technology, Pantnagar (U. S. Nagar), Uttarakhand, India  
e-mail: hinakhatoon29@gmail.com  
\*Correspondence

cost-efficient, and non invasive to natural ecosystem [8, 9]. Therefore, biodegradation of chemical contaminants at environmentally relevant concentrations attracts interest in both industry and academia [10, 11]. Until now, research findings have proved that various microorganism species are capable of transforming herbicides into harmless inorganic compounds [12, 13].

However, the application of this technique is still limited since it could be easily inhibited by a variety of factors, including the activity of introduced organisms and environmental circumstances [14]. In order to improve the survival and activity of applied organisms, the immobilization method is introduced which plays an important role in the long-term stabilization and has been successfully applied in the remediation of heavy metal and oil contaminants [15-18]. In general, the activities of immobilized preparations are governed by the properties of both the enzyme and the support material which would largely improve the stability and activity of the immobilized composites [19]. The support can be a synthetic organic polymer, a biopolymer, or an inorganic solid. Water-insoluble polysaccharides such as cellulose, starch, agarose and chitosan have been widely used in constructing support platforms for enzyme immobilization [20]. Sugarcane bagasse is an abundant, eco-friendly, and low-cost lignocellulosic biomass which has been widely used in bioethanol production as a promising source of carbohydrates for microbial conversion [21, 22]. The manufacture procedure is conducted out by chemical pretreatments and microbial fermentation [23-25]. This technique demonstrates strong evidence that sugarcane bagasse is biocompatible with various microbes. Meanwhile, research studies showed recently that bagasse also possesses the potential in dye or phenol remediation as an absorbent for its natural porous structure [26, 27]. Therefore, the possibility of sugarcane bagasse serving as an immobilizing support was worthy of deep investigation, which would further develop the biotechnol utilization of bagasse. On the other hand, the structural and biocompatible advantages of sugarcane bagasse were expected to provide a breakthrough in contaminant bioremediation by active microbes. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino) - s - triazine] is the most commonly used herbicide in the world for

controlling broadleaf and grassy weeds and is quite persistent in neutral environment and toxic to various living organisms [28]. It is registered for use in agriculture as a selective pre- and postemergence herbicide in corn, sorghum, soybeans, wheat, pineapple, sugarcane and many other crops [29]. Because of its high mobility and persistence in soil and its massive application, atrazine has often been detected in surface and ground water at concentrations well above the permitted limits [30, 31]. It is considered as a potential environmental contaminant and considered also as one of the worst ground water pollutants [32, 33]. It is removed from soil and water by physical, chemical and biological methods. In general, biological treatment processes have advantages over physical and chemical treatment methods. It is cost effective and environmental friendly. Few works are available on biodegradation of atrazine [34-36]. Most of these works are carried out using pure species and/or anaerobic conditions. Hence, our work will focus on the degradation of atrazine using mixed cultures in aerobic condition. In the present study, we described the degradation of atrazine by *Bacillus badius* ABP6 and the bacterial immobilization using the eco-friendly material, sugarcane bagasse as a loading support. Moreover, we investigated the environmental effects and determined the metabolic products of atrazine in the bacterial degradation process. Finally, the bioremediation ability of immobilized composite was evaluated in soil conditions. Our findings demonstrate a feasible way of applying sugarcane bagasse and provide a better strategy for atrazine bioremediation.

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## Materials and Methods

### Collection of soil samples

Atrazine treated composite surface soil samples (0–15 cm) were collected from Norman E. Borlaug Crop Research Center, Pantnagar after 10 days of herbicide spray in the maize fields. One part of soil samples

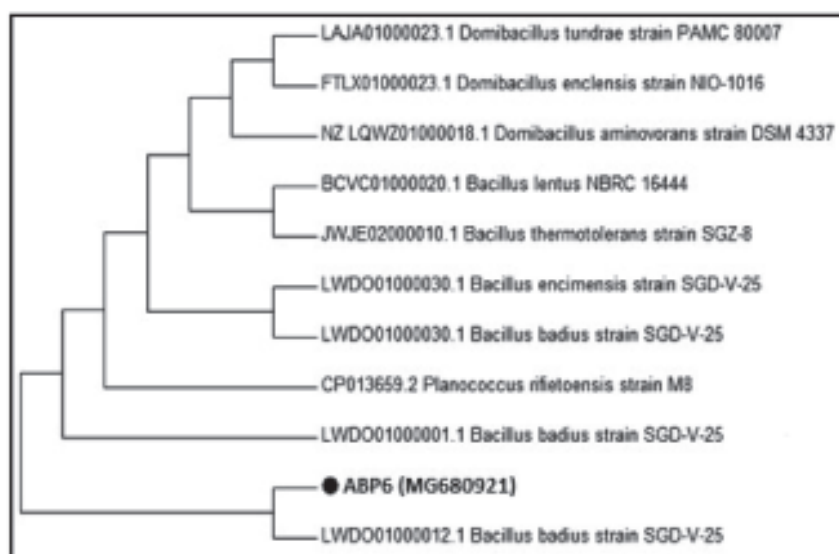


Fig. 1. Phylogenetic analysis of *Bacillus badius* ABP6 based on neighbor joining method.

were air dried and sieved at 5 mm and used for determination of physicochemical properties whereas, the second part was preserved at 4°C for isolation of atrazine degrading bacteria.

#### Chemicals

Atrazine (99%) was obtained from Sigma-Aldrich. The chromatographic grade methanol was purchased from Sigma-Aldrich, USA. Atrazine was dissolved in methanol at a stock concentration of 100 mg L<sup>-1</sup> and stored at 4°C prior to use. All other chemicals and solvents used in this study were of analytical grade. Composition of the different media used were broth minimal medium (g/L) KH<sub>2</sub>PO<sub>4</sub>, 1.0; K<sub>2</sub>HPO<sub>4</sub>, 1.0; NH<sub>4</sub>NO<sub>3</sub>, 1.0; MgSO<sub>4</sub> 0.2; CaCl<sub>2</sub> 0.02; Fe (SO<sub>4</sub>) 0.01. Nutrient agar plates contained (g/L) : Peptone 10; Yeast extract 5.0 and NaCl 5.0. Unless mentioned, the pH of the medium was adjusted at 7.0 [37].

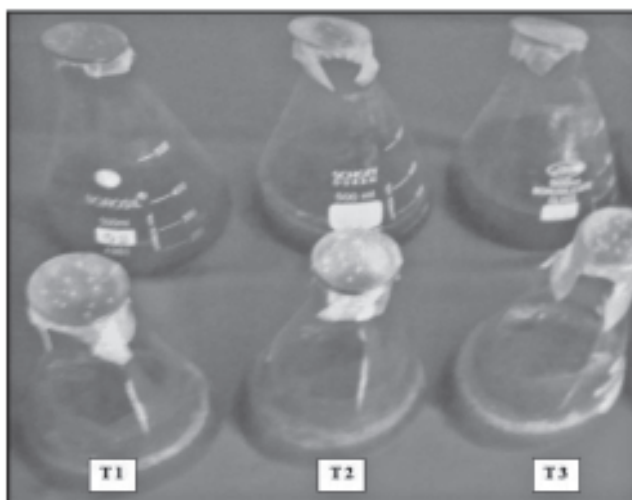
#### Enrichment, isolation and identification of micro-organisms

Surface soil (0–15 cm) from fields treated with atrazine was used for the isolation of bacteria. Soil samples (5 g) in triplicate were taken in 250 mL. Erlenmeyer

flasks containing 50 mL of the minimal broth and 50 mg/L atrazine. The enrichment culture was incubated at 30°C on a rotary shaker at 150 rpm. After 7 days, 5mL of broth culture from each flask were reinoculated to 50 mL of fresh media containing atrazine at 100 mg/L and were cultured under the same conditions. Then, the same procedure was repeated two more times 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 different species, was repeatedly streaked on agar plates. Pure cultures were obtained by streaking for more than 10 times and were screened for atrazine degradation before using for subsequent study. The atrazine-degrading ability of purified isolates was determined respectively at a wide range of atrazine, and the most active isolate was identified by 16S rRNA analysis as *Bacillus badius* ABP6.

#### 16S rRNA gene sequencing and construction of phylogenetic tree

16S rRNA partial nucleotide sequence analysis of bacterial isolate was carried out with the help of Chromous Biotech Pvt Ltd, Bangalore, India. Alignment of the partial 16S rRNA gene sequence of bacterial



**Fig. 2.** Soil microcosm treatments: T<sub>1</sub> (Control), T<sub>2</sub> (sterilized soil and free bacterial cells), T<sub>3</sub> (sterilized soil and immobilized bacterial cells).

isolate was performed with sequences present in the GenBank database using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Phylogenetic analysis was performed using MEGA version 7.0 software packages. The neighbor-joining (NJ) method was used for phylogenetic analysis and the robustness of the tree topology was assessed by bootstrap analysis, with 1,000 resembling replicates. Sequence was deposited in the GenBank database under accession number MG680921. Homology search using BLAST revealed 99% similarity of this sequence with 16S rRNA gene sequence of *Bacillus badius* strain (GenBank accession no. LWDO01000012.1), giving the phylogenetic relationship of this bacterial isolate with several *Bacillus* species (Fig. 1), thus isolate was designated to be *Bacillus badius* ABP6.

#### Inoculation and degradation of atrazine

Before each experiment, the isolate was thawed and grown in 250-ml. Erlenmeyer flasks containing 50 mL of sterile minimal medium. The flasks were placed on a platform shaker at 150 rpm and 30°C for 24 h. Cells were harvested by centrifugation (10 min, 4800 rpm). Sterilized minimal media were inoculated at 25, 50, 100 and 200 mg L<sup>-1</sup> of atrazine, respectively and incubated at 30°C and 150 rpm on a rotary shaker. Controls

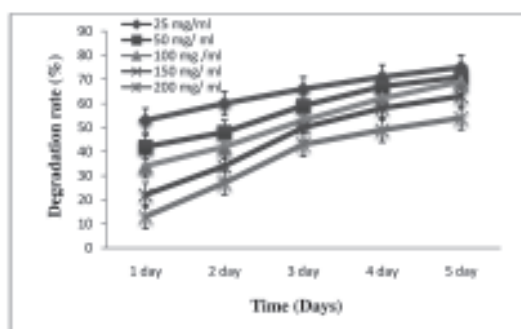
without inoculation were set. The degradation of atrazine was determined by measuring its residues at intervals of 1, 2, 3, 4 and 5 days, respectively, by high-performance liquid chromatography (HPLC). Specifically, atrazine in culture medium was extracted by dichloromethane and the organic layer was dehydrated, dried and redissolved in methanol. After filtration with a 0.45- $\mu$ m membrane, the samples were subjected to HPLC.

#### Sugarcane bagasse pretreatment

Sugarcane bagasse was procured from G. B. Pant University of Agriculture and Technology, Pantnagar, India. The bagasse was dried at 100°C in an oven for 24 h and mechanically grinded into powder, followed by being sieved size to 20, 60 and 100 mesh (with the diameters of 800, 250 and 150  $\mu$ m, respectively). Then, the material was washed with distilled water, dried at 100°C and stored in room temperature for further use.

#### Immobilization and degradation evaluation

Bacteria suspension was previously grown in 250-mL Erlenmeyer flasks placed on a rotary shaker at 30°C, 150 rpm for 7 days. Then, 2 mL of bacteria sus-



**Fig. 3.** Degradation of atrazine at different initial concentrations by free cells of *Bacillus badius* ABP6 IN minimal broth.

pension was mixed with 0.1 g of bagasse and placed on a rotary shaker at 180 rpm and 30°C for 24 h. The immobilized mixture was harvested by centrifugation (10 min, 4800 rpm), washed twice in 0.9% N-saline, and vacuum dried at 20°C. The sample of immobilized bacteria was subject to scanning electron microscope (SEM) for the morphological observation. The effect of immobilization on bacteria was assessed by growth activity detection. A 0.1 g of bacteria-bagasse was added in 50-mL minimal broth and the values of OD 600 were determined and set as zero levels. The medium that inoculated the same amount of bacteria was set as control group. Placing the inoculated cultures to a rotary shaker, OD600 were determined in 24 and 48 h, respectively. Then, the degradation ability of immobilized bacteria was investigated by applying 0.1 g of bacteria-bagasse in 50-mL minimal medium containing 50 mg L<sup>-1</sup> of atrazine and the amount of residues was determined in 3 days by HPLC.

#### Effects of reaction conditions on atrazine degradation

The effects of reaction conditions on atrazine degradation, including pH (4.0 to 8.0), temperature (20 to 40°C), initial concentration of atrazine (50 to 150 mg/kg) and the amount of microbial preparation (5 to 20 g), were investigated. The effect of each factor was conducted in a single-factor experiment that only the tested factor was changed accordingly. The atrazine residues were measured in 2, 3, 5, 7, 10 and 14 days, respectively, by HPLC.

**Table 1.** HPLC analysis of atrazine.

Model	:	Dionex ultimate 3000
Column	:	C-18 Reverse Phase (250 × 10 × 4.6 mm ID)
Detector	:	UV-detector
Solvent	:	Methanol : Water (80 : 20)
Flow rate	:	1 ml / min
Retention time	:	7 min

#### Atrazine degradation in soil microcosm

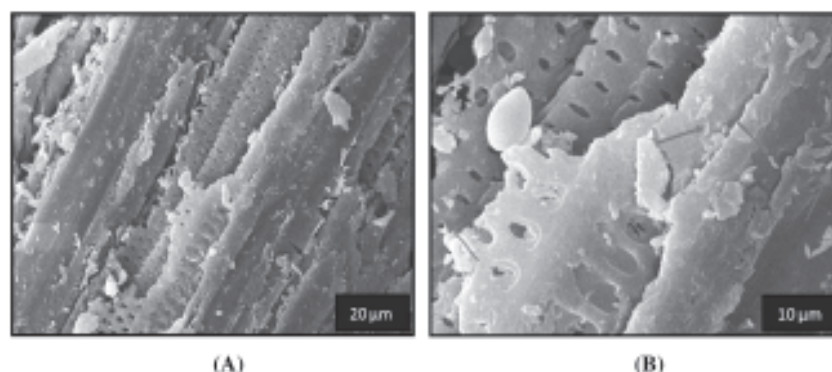
We performed a microcosm study of three treatments with *Bacillus badius* ABP6 in 1L capacity cylindrical glass jars. Treatment 1 contained sterilized soil 200 g and atrazine at a concentration of 100 mg/kg. Treatment 2 contained sterilized soil 200g, bacterial inoculum at the concentration of 2×10<sup>6</sup> cells/g of soil and atrazine at a concentration of 100 mg/kg. Treatment 3 contained sterilized soil 200g, immobilized bacterial inoculum at the concentration of 2×10<sup>6</sup> cells/g of soil and atrazine at a concentration of 100 mg/kg. An adequate amount of water was added to maintain soil moisture content. All the components in the four treatments were thoroughly mixed and glass jars were covered with perforated aluminium foil and incubated at 30°C for 21 days. Samples were withdrawn after 1, 3, 5, 7, 10, 14, 21 days incubation to estimate atrazine degradation (Fig. 2).

#### Extraction of atrazine from microcosm treatments

Atrazine was extracted from each 5g soil samples using 20mL methanol. The samples were shaken for 1 h on an orbital shaker and then filtered through Whatman filter paper. This procedure was repeated thrice. Collected solvent extract was dried up using rotary flash evaporator and re-dissolved in 2mL solvent methanol for HPLC analysis.

#### HPLC analysis of atrazine

The residual atrazine was analyzed on an Dionex ultimate 3000 HPLC equipped with a C18 reversed phase column (4.6 nm × 250 mm, 10 μm) and other information are listed in Table 1.



**Fig. 4.** SEM images of sugarcane bagasse before (A) and after (B) immobilization. (A). The appearance of vascular bundle surface of bagasse before immobilization, (B). The appearance of vascular bundle surface after immobilization and The arrows point the locations of bacteria absorbed on the support surface of sugarcane bagasse.

#### Data analysis

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

$$\text{Percent (\%)} \text{ Atrazine degradation} = (A_s/A_c) \times 100\% \dots (1)$$

Where,  $A_s$  is the peak area of sample and  $A_c$  is the peak area of the control.

#### 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-, two-way analysis of variance (ANOVA).

### Results

#### Atrazine degradation by *Bacillus badius* ABP6 in minimal broth

With the increase of incubation time, degradation rates considerably grew and atrazine residues in liquid cul-

tures were scarcely detected in 5 days (Fig. 3). Specifically, the degradation rates reached 43–66% within 3 days and 54–75% of atrazine was degraded by free cells in 5 days at the initial concentration of 25, 50, 100 and 200 mg L<sup>-1</sup>, respectively, suggesting that this bacterial isolate was able to metabolize atrazine in a wide range. In addition, the degradation performance was significantly good when atrazine concentrations were at relatively low levels. For instance, 75% of atrazine at initial 25 mg L<sup>-1</sup> was rapidly eliminated in 5 day, while the degradation rates of 200 mg L<sup>-1</sup> concentrated atrazine was about 54% during this time. Therefore, results above indicated the wide availability of *Bacillus badius* ABP6, which would enlarge its application scale in practical bioremediation.

#### Morphological observation and degradation evaluation

Due to its natural porous structure, sugarcane bagasse was served as a support candidate for bacteria immobilization, which provided large surface area and high loading volume for cell absorption. After been immobilized, the samples of immobilized microbes and pure bagasse were scanned by using SEM and their morphological characters were observed. Figure 4A shows the scanning image of bagasse particles before immobilization. The shape of initial bagasse particles was irregular columnar and porous vascular tissue was found, which provided a large area for free

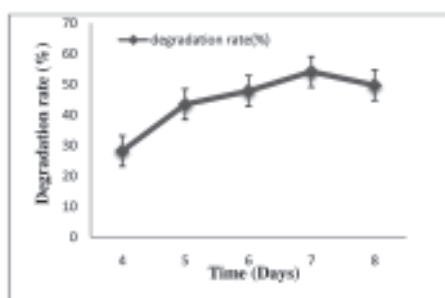


Fig. 5(A) Effects of pH on biodegradation of soil atrazine.

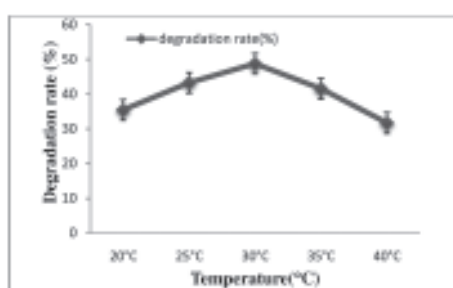


Fig. 5(B) Effects of temperature on biodegradation of soil atrazine.

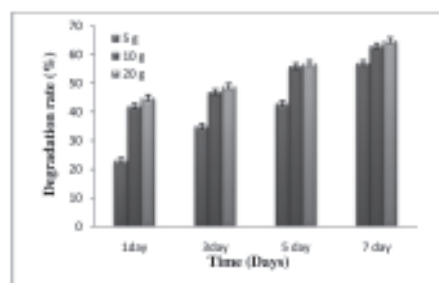


Fig. 5(C). Effects of dosage of bagasse on biodegradation of soil atrazine.

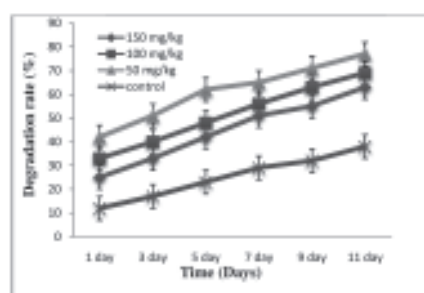


Fig. 5(D). Effects of initial concentrations on biodegradation of soil atrazine.

**Fig. 5 (A).** Effects of pH on biodegradation of soil atrazine. **Fig. 5 (B).** Effects of temperature on biodegradation of soil atrazine.

**Fig. 5 (C).** Effects of dosage of bagasse on biodegradation of soil atrazine. **Fig. 5 (D).** Effects of initial concentrations on biodegradation of soil atrazine.

bacteria to contact and load. Due to the mechanical comminution, the vascular walls were partially broken into fragments, but no adverse consequences were revealed so far. Served as a support, the shape and surface of bagasse were not dramatically modified after biomass loading as shown in Fig. 4B. It was reported that immobilization of enzymes inside the porous structure of a solid may permit to have the enzyme molecules fully dispersed [38]. Hence, active bacteria were strongly absorbed and randomly dispersed on the surface and inside the vascular bundles, indicating that the applied bagasse was biocompatible with active cells and a stable bacteria-bagasse composite was formed.

Bacterial growth was monitored by the amount of OD600 (Table 2). Bagasse, which was previously classified in 20, 60 and 100 mesh (with particle diameters of 800, 250 and 150  $\mu\text{m}$ , respectively), was sepa-

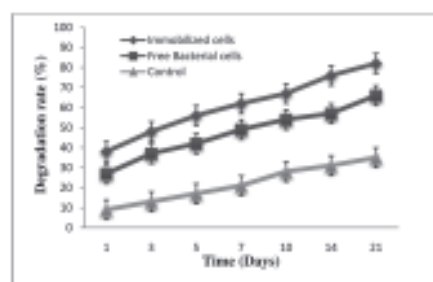
rately served as carrier candidates. Comparing with the control group, the immobilized bacteria were viable and capable of multiplying after immobilization procedure since the values of OD600 were continuously increasing. Adjusting the medium to 50 mg/L of atrazine, the degradation rates of immobilized bagasse in 100 and 60 mesh reached 85.32 and 81.57%, respectively, suggesting that the ability of degrading atrazine was still retained after immobilization. Due to the bigger size and smaller surface area, bacteria immobilized in bagasse of 20 mesh showed low degradation rate (43.23%), indicating that this size of bagasse might not be appropriate. Additionally, it also suggested that the smaller size of carrier particles could significantly improve biomass loading for their larger surface area, thereby improving degradation of target chemical. Therefore, bagasse milled into 60 and 100 mesh could be more appropriate as carriers for the better abilities in terms of cell growth and biodegradation.

**Table 2.** The growth of immobilized bacteria and the degradation of atrazine at initial concentration of 50 mg/L.

Size	Value of OD600			Degradation rate (%)
	0h	12h	24h	
20 mesh	0.04 ± 1.0	0.28 ± 1.5	0.50 ± 1.2	43.23 ± 1.4
60 mesh	0.05 ± 0.9	0.32 ± 1.2	0.53 ± 0.8	81.57 ± 1.2
100 mesh	0.06 ± 1.3	0.35 ± 1.0	0.57 ± 1.1	85.32 ± 1.0
Control	0.03 ± 1.2	0.25 ± 0.7	0.49 ± 1.0	42.18 ± 0.9

#### Effects of reaction conditions on atrazine degradation

Four parameters that might affect the atrazine-degrading activity of the microbial composite were investigated through single-factor experiments. The effect of pH was evaluated first. As shown in Fig. 5 (A), the degradation rate was about 54.13% at pH of 7.0 in 5 days ; however, when the treatments were tested at lower pH value (4.0) or at higher pH value (8.0), the degradation rates were around 28.21 and 49.72%. Temperature influences were assessed next. Fig. 5(B) displays that degradation rates achieved over 40% with slight fluctuation in the range of 25 to 35°C within 5 days, suggesting that this microbial preparation can function more effectively in warm situations. However, the degradation performance was significantly decreased at higher temperature (over 40°C) and only 31.68% of atrazine was removed within the same test period. The effect of the amount of immobilized microbial dosage applied on atrazine degradation (in Fig. 5C) exposed that in 7 days, 63 and 65% of atrazine was degraded with 10 and 20g of dosage, respectively, whilst at 5g dosage the degradation was only 57%. The change of percent degradation rate with the increase of atrazine initial concentrations is presented in Fig. 5(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 temperature and with disage of 10g, especially at low concentrations 50 to 200 mg kg<sup>-1</sup> of atrazine.



**Fig. 6.** Biodegradation of atrazine in soil microcosm by immobilized and free cells of Bacteria.

**Fig. 6.** Biodegradation of atrazine in soil microcosm by immobilized and free cells of bacteria.

#### Biodegradation of atrazine in soil microcosm:

As a result of biodegradation after 21 days, 35% disappeared from treatment 1 (control). Treatment 2, which contained sterilized soil amended with bacterial inoculum (free cells) and atrazine, showed 66% of atrazine degradation. Treatment 3, which contained sterilized soil augmented with immobilized bacterial cells and atrazine, showed maximum 82% atrazine degradation (Fig. 6).

#### Discussion

Atrazine is a common pollutant of surface water, ground water, and soil and the biodegradability of atrazine was found to be low [39]. It is also proved that atrazine has toxic effects on algae, aquatic plants, insects, fishes and mammals [40, 41]. Generally, herbicides applied in tillage lands are involved in sorption, desorption, transport, volatilization and transformation processes [42, 43]. Based on the understanding of these physicochemical or biochemical behaviors, better strategies are obtained for effective utilization of active compounds as well as remediation of herbicide-contaminated sites. Bioremediation involves the use of living microorganisms or their enzymes to detoxify pollutants, which has been generally considered as an effective and cost-efficient technique for contaminant clean-up [44, 16]. It was reported that various bacteria demonstrated the ability of efficiently degrading atrazine including *Arthrobacter* [25], *Rhodococcus opacus* sp., *Acinetobacter* sp.,

*Norcardioides* sp., *Streptomyces* sp., *Rastomia* sp., *Pseudomonas aeruginosa*, *Clavibacter michiganense*, *Chelatobacter heintzii* [45], *Enterobacter cloacae*, *Bacillus megatherium*, *Alcaligenes faecalis*, *Agrobacterium tumefaciens*, *Klebsiella ornithinolytica* and *P. fluorescence* + *P. putida* [46].

In this study, we selected a highly effective isolate of *Bacillus* species for atrazine degradation. Experimental results showed that bacteria *Bacillus badius* ABP6 was able to utilize as carbon source. Compared to the treatments with higher initial concentrations, the degradation at lower concentrations was more significant and efficient, which enabled the bacterial isolate to function in situations with low residues. Research studies have proved that relatively low levels of herbicide residues would affect and cause a chain of effects in ecosystem [3, 47].

In practical application, the degradation performance was not stable and effective as tested in laboratory since the direct use of free cells was influenced by multiple factors. Using immobilized microbes may provide a possible solution, which has been proved repetitively as an effective approach for pollution remediation [15, 17]. It is reported that the activity of immobilized composite is vastly affected by the properties of support material [19, 20]. Therefore, choosing a stable and economical support with good biocompatibility is the key to the immobilized composite and then sugarcane bagasse was employed as a support candidate. Additionally, it is believed that the immobilization methodology should be a relatively simple operation that does not require a highly pure enzyme preparation or an expensive support that may not be commercially available [20].

In general, sugarcane bagasse is produced in a large quantity by the sugar industry in countries like Brazil, India and China. As an industrial waste, partial residue is used in bioethanol production while the remainder is stockpiled [21]. Great interests exist in exploring other methods that offer economic, environmental and strategic advantages in bagasse biotechnology. In our study, the potential of bagasse as an immobilization support was validated as expected. From SEM images, bacteria were fully dispersed on

the surface of bagasse particle and their degrading activity retained after immobilization. To our best knowledge, this is the first report about sugarcane bagasse used in bacterial immobilization for herbicide bioremediation. However, it was obviously observed that density of absorbed cells was low, which might be caused by insufficient cells, multistep elution, or the properties of support material. Optimization of experimental skills would improve the quantity of loading microbes ready for immobilization. Unfortunately, research studies focused on sugarcane bagasse as supports for bacteria immobilization are rare. Therefore, the exploration and improvement of bagasse can be referred to the mature development of other porous structured materials, for instance, mesoporous silica nanoparticles (MSNs), which serve as ideal candidates in biotechnological field. It was explained that the adsorption and release of biomolecules in MSNs are effectively altered by particle size, pore size, surface area and surface functionalization, which are also considered as the four major parameters that the further optimization of bagasse. The adaptability and availability were the two main challenges in this microbial preparation design. Hence, we tested the effects of multiple factors on the degradation performance which could be easily impacted in a real soil environment [48].

## Conclusion

Persistent use of herbicides has become a growing threat to human and environment health. Although new products with low residues have been launching every year, the accumulation of herbicides is still a hot issue and effective solutions for residue disposal are urgently required. Utilization of active microorganisms is an efficient and economical method, while microbial immobilization breaks the barriers of limited use of microbes *in situ*. In our study, sugarcane bagasse was employed in herbicide bioremediation as loading supports. We presented evidence that the natural porous structures of bagasse could provide large surface area and high pore volume for bacteria loading and dispersing. To enhance its stability and efficiency, however, optimization of immobilization skills and physicochemical modification of bagasse particles could be promising strategies in future research studies.

## References

1. Bruggeman AJ, Kuehler D, Weeks DP (2014) Evaluation of three herbicide resistance genes for use in genetic transformations and for potential crop protection in algae production. *PL Biotechnol J* 12 : 894—902.
2. Jacobsen CS, Hjelmsø MH (2014) Agricultural soils, pesticides and microbial diversity. *Curr Opin Biotechnol* 27 : 15—20.
3. Lewis SE, Brodie JE, Bainbridge ZT, Schaffelke B (2009) Herbicides: A new threat to the great barrier reef. *Environm Poll* 157 : 2470—2484.
4. Legroui A, Lakraimi M, Barroug A, De-Roy A, Besse JP (2005) Removal of the herbicide 2, 4-dichlorophenoxy acetate from water to zinc- aluminium-chloride layered double hydroxides. *Water Res* 39 : 3441—3448.
5. Page D, Dillon P, Mueller J, Bartkow M (2010) Quantification of herbicide removal in a constructed wetland using passive samples and composite water quality monitoring. *Chemosphere* 82 : 394—399.
6. Dash HR, Mangwani N, Chakraborty J, Kumari S, Das S (2013) Marine bacteria : Potential candidates for enhanced bioremediation. *Appl Microbiol Biotechnol* 97 : 561—571.
7. Ramos J, Marqués S, van Dillewijn P, Espinosa-Urgel M, Wittich R (2011) Laboratory research aimed at closing the gaps in microbial bioremediation. *Trends in Biotechnol* 29 : 641—647.
8. Fuentes S, Méndez V, Aguila P, Seeger M (2014) Bioremediation of petroleum hydrocarbons: Catabolic genes, microbial communities and applications. *Appl Microbiol Biotechnol* 98 : 4781—4794.
9. Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: A critical perspective *Environm Int* 37 : 1362—1375.
10. Chen S, Chang C, Deng Y, An S, Dong YH, Zhou J (2014) Fenpropathrin biodegradation pathway in *Bacillus* sp. DG-02 and its potentials for bioremediation of pyrethroid-contaminated soils. *J Agric Food Chem* 62 : 2147—2157.
11. 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. *Appl Microbiol Biotechnol* 99 : 2849—2859.
12. Ermakova IT, Kiseleva NI, Shushkova T, Zharikov M, Zharikov GA, Leontievsky AA (2010) Bioremediation of glyphosate contaminated soils. *Appl Microbiol Biotechnol* 88 : 585—594.
13. Sandoval-Carrasco CA, Ahuatzí-Chacón D, Galindez-Mayer J, Ruiz-Ordaz N, Juárez-Ramírez C, Martínez-Jerónimo F (2013) Biodegradation of a mixture of the herbicides ametryn, and 2, 4-dichlorophenoxy acetic acid (2, 4-D) in a compartmentalized biofilm reactor. *Bioresour Technol* 145 : 33—36.
14. Perelo LW (2010) Review: *in situ* and bioremediation of organic pollutants in aquatic sediments. *J Hazard Mater* 177 : 81—89.
15. Piccirillo C, Pereira SIA, Marques APGC, Pullar RC, Tobaldi DM, Pintado ME, Castro PML (2013) Bacteria immobilization on hydroxyapatite surface for heavy metals removal. *J Environm Manag* 121 : 87—95.
16. Singh R, Paul D, Jain RK (2006) Biofilms: Implications in bioremediation. *Trends Microbiol* 14 : 389—396.
17. Stelting S, Burnz RG, Sunna A, Visnovsky G, Bunt CR (2012) Immobilization of *Pseudomonas* sp. strain ADP: A stable inoculants for the bioremediation of atrazine. *Appl Clay Sci* 64 : 90—93.
18. Xu Y, Lu M (2010) Bioremediation of crude oil-contaminated soil: Comparison of different colloid polym. *Sci* 293 : 3381—3391.
19. 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 Chem* 13 : 3187—3195.
20. Sheldon RA (2007) Enzyme immobilization: The quest for optimum performance. *Adv Synth Catal* 349 : 1289—1307.
21. Cardona CA, Quintero JA, Paz IC (2010) Production of bioethanol from sugarcane bagasse: Status and perspectives. *Bioresour Technol* 101 : 4754—4766.
22. Geddes CC, Nieves IU, Ingram LO (2011a) Advances in ethanol production. *Curr Opin in Biotechnol* 22 : 312—319.
23. Geddes CC, Mullinnix MT, Nieves IU, Peterson JJ, Ingram LO (2011b) Simplified process for ethanol production from sugarcane bagasse using hydrolytase-resistant *Escherichia coli* strain MM160. *Bioresour Technol* 102 : 2702—2711.
24. Rabelo SC, Carrere H, Maciel Filho R, Costa AC (2011) Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. *Bioresour Technol* 102 : 7887—7895.
25. Wang X, Yomano LP, Lee JY, York SW, Zheng H, Mullinnix MT, Shanmugam KT, Ingram LO (2013) Engineering furfural tolerance in *Escherichia coli* improves the fermentation of lignocellulosic sugars into renewable chemicals. *Proc Nat Acad Sci Unites States Am* 110 : 4021—4026.
26. 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. *Int Biodeteriorat Biodegrad* 93 : 107—117.
27. Waghmare PR, Kadam AA, Saratale GD, Govindwar SP (2014) Enzymatic hydrolysis and characterization of waste lignocellulosic biomass produced after dye bioremediation under solid state fermentation. *Bioresour Technol* 168 : 136—141.
28. Tomlin CDS (2012) The pesticide manual. 16<sup>th</sup> (edn.), Crop Prot Publ, British Crop Prot Council. The Royal Soc of Chem.
29. Pathak RK, Dikshit AK (2012) Atrazine and its use.

- Int J Res Chem Environ 2 : 1—6.
30. Scribner EM, Battaglin WA, Goolsby DA, Thurman EM (2000) Changes in herbicide concentrations in Midwestern streams in relation to changes in use. *Sci Total Environ* 248 : 255—263.
  31. Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2003) Atrazine induced hermaphroditism at 0.1 PPB in American leopard frogs (*Rana pipiens*): Laboratory and field evidence. *Environ Heal Perspect* 111 : 568—575.
  32. Muller K, Bach M, Hartmann H, Spiteller M, Frede HG (2002) Point and non-point source pesticide contamination in the Zwester Ohm Catchment (Germany). *J Environ Qual* 31 : 309—318.
  33. Kaushik G, Satya S, Naik SN (2009) Food processing a tool to pesticide residue dissipation - A review. *Food Res Int* 42 : 26—40.
  34. Struthers JK, Javachandran K, Moorman TB (1998) Biodegradation of atrazine by *Agrobacterium* radiobacter J14 and use of this strain in bioremediation of contaminated soil. *Appl Environ Microbiol* 64 : 3368—3375.
  35. Ghosh PK, Philip L (2004) Atrazine degradation in anaerobic environment by a mixed microbial consortium. *Water Res* 38 : 2277—2284.
  36. Sene L, Converti A, Secchi GA, Cassia RD, Simao G (2007) New aspects on atrazine biodegradation. *Brazilian Archiv Biol Technol* 50 : 487—496.
  37. Kumar A, Singh N (2016) Atrazine and its metabolites degradation in mineral salts medium and soil using an enrichment culture. *Environ Monit Assess* 188 : 1—12.
  38. Mateo C, Grazu V, Palomo JM, Lopez-Gallego F, Fernandez-Lafuente R, Guisan JM (2007) Immobilization of enzymes on heterofunctional epoxy supports. *Nature Protocols* 2 : 1022—1033.
  39. Chan KH, Chu W (2005) Atrazine removed by catalytic oxidation processes with or without UV irradiation Part II : An analysis of the reaction mechanisms using LC/ESI-tandem mass spectrometry. *Appl Catalysis B: Environ* 58 : 165—174.
  40. Cai B, Han Y, Liu B, Ren Y, Jiang S (2003) Isolation and characterization of an atrazine - degrading bacterium from industrial wastewater in China. *Letters in Appl Microbiol* 36 : 272—276.
  41. Ghosh PK, Philip L (2006) Environmental significance of atrazine in aqueous systems and its removal by biological processes: An overview. *Global Nest* 8 : 71—90.
  42. Lima DLD, Schneider RJ, Scherer HW, Duarte AC, Santos EBH, Esteves V (2010) Sorption-desorption behavior of atrazine on soils subjected to different organic long-term amendments. *J Agric Food Chem* 58 : 3101—3106.
  43. Wong F, Bidleman TF (2011) Aging of organochlorine pesticides and polychlorinated biphenyls in muck soil: Volatilization, bioaccessibility and degradation. *Environ Sci and Technol* 45 : 958—963.
  44. Chen S, Dong YH, Chang C, Deng Y, Zhang XF, Zhong G, Song H, Hu M, Zhangm LH (2013) Characterization of a novel cyfluthrin-degrading bacterial strain *Brevibacterium aureum* and its biochemical degradation pathway. *Bioresour Technol* 132 : 16—23.
  45. Rousseaux S, Hartmann A, Lagacherie B, Piutti S, Andreux F, Soulas G (2003) Inoculation of an atrazine-degrading strain, *Chelatobacter heintzii* Citi, in four different soils: Effects of different inoculum densities. *Chemosphere* 51 : 569—576.
  46. Chegini S, Sani B, Darvishi HH (2015) Atrazine biodegradation by stimulating the activity of soil bacterial population in maize field. *Int J Biosci* 6 : 293—297.
  47. Ricart M, Barcelo D, Guasch H, de Ald ML, Romani AM, Vidal G, Villagrana M, Sabater S (2009) Effects of low concentrations of the phenylurea herbicide diuron biofilm algae and bacteria. *Chemosphere* 76 : 1392—1401.
  48. Popat A, Hartono SB, Stahr F, Liu J, Qiao SZ, Lu GQ (2011) *Mesoporous silica* nanoparticles for bioadsorption, enzyme immobilization and delivery carriers. *Nanoscale* 3 : 2801—2818.