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Eco-friendly biotransformation of penicillin G by free and immobilized marine halophilic *Bacillus pseudomycoides* AH1

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Abstract

Background Several antibiotics are partially metabolized by patients after administration and end up in municipal sewage systems. The fate of biodegradation in aquatic environments and the role of biodegradation in the development of bacterial resistance are poorly understood. Thus, as a crucial step in an environmental risk assessment, the biodegradability of many therapeutically significant antibiotics was investigated.

Results A marine halophilic bacteria that degrades penicillin G (PEN-G) was isolated and identified based on morphology, physio-biochemical characteristics, and 16S rDNA sequences as *Bacillus pseudomycoides* AH1 (accession no. MF037698). The effects of various concentrations of PEN-G and carbon and nitrogen sources on the biotransformation ability at 30°C and pH 7.0 were evaluated. Cells grown in medium supplemented with glucose as an additional carbon source and yeast extract as a nitrogen source exhibited maximal PEN-G biotransformation efficiency and rate (71.678% ±1.28 and 2.99 mg/h, respectively). The culture conditions for *B. pseudomycoides* AH1 cells were optimized using a Plackett–Burman design (PBD). Six key determinants ($p < 0.05$) significantly affected the process outcome, as deduced by regression analysis of the PBD data, and modified MSM broth achieved PEN-G biotransformation efficiency (100%) under aerobic shaking conditions at 35°C, irrespective of HPLC analysis. Additionally, the present investigation could strongly support the application of immobilization approaches for the removal of PEN-G-contaminated environmental sites.

Conclusion To the best of the authors' knowledge, this is the first detailed study on the efficient biotransformation of PEN-G by an alginate-bacteria system as a simple, green, and inexpensive process, as well as a promising method.

Keywords *Bacillus*, Penicillin biotransformation, Immobilization

Introduction

In recent years, many different kinds of antibiotics have been developed and used in various fields, including for the treatment of diverse microbial diseases and for stimulating the growth of poultry, livestock, and fish (Van et al. 2020). These antibiotic residues have been detected in numerous environmental samples, notably wastewater from cities (McArdell et al. 2003), sediments and sludge (Lindberg et al. 2005), and ground and surface water (Batt and Aga 2005). Antibiotic residues may disrupt the physiological processes of microbial populations or may lead important environmental groups of microorganisms to disappear from the environment (Kulik et al. 2023).

Penicillins are β-lactam antibiotics that have a broad spectrum of activity against both gram-positive and gram-negative organisms. Penicillin G (PEN-G) is

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especially produced on a large scale, with between 30 and 90% of it being dumped into waste streams (Wu et al. 2019). The danger of exposure to antibiotics has become a significant issue that has been repeatedly addressed in the clinical treatment of numerous illnesses. Consequently, methods for the efficient decomposition of PEN-G residues in the environment are required to lower the danger of exposure (Wang et al. 2015).

Several methods have been employed to remove β-lactam antibiotics, including photocatalysis, UV and solar-based processes, and chemical oxidation, which includes ozonation (Michael-Kordatou et al. 2018). Unfortunately, there are several drawbacks to these methods, including their high cost, the development of hazardous secondary byproducts, ecological risks, and nonsustainability (Panigrahy et al. 2020). Hence, microbial bioremediation has been considered an effective method that offers a more reliable, economical, and sustainable choice for cleaning different contaminated locations (Kamaz et al. 2019; Nas et al. 2020). Microbes that can mineralize, biodegrade, or biotransform antibiotics can also be identified (Reis et al. 2020). Additionally, bioremediation has been applied to a variety of methods, including the use of single-strain microorganisms (Shao et al. 2018), microbial consortia and genetically engineered microorganisms (Liu et al. 2017).

Physicochemical and nutritional factors could have an impact on the efficacy of bioremediation for eliminating contaminants. Therefore, to identify the optimum biodegradation conditions for pollutant removal, it is crucial to ascertain their effects and optimize the most significant conditions. Multivariable optimization investigations of different biotechnological, environmental, and chemical processes have effectively employed statistical approaches such as the use of Plackett–Burman design (PBD) (Plackett and Burman 1946; El-Borai et al. 2016; Farag et al. 2021). They are also considered to be reliable and effective methods for rapid screening of significant factors from a multivariable system with a few experiments (Favier et al. 2021).

A number of bacterial strains can biodegrade several antibiotics (Wang et al. 2015; Anan et al. 2018; Wang et al. 2020). *Bacillus* species are aerobic, endospore-forming, gram-positive bacteria with a low level of pathogenicity. Numerous researchers have examined the ability of *Bacillus* species to break down various antibiotic types (Liyanage and Manage 2015; Fiedler et al. 2019; Zhang and Wang 2021).

Cell immobilization has several advantages that could be crucial for the biodegradation of xenobiotic compounds (Chen et al. 2002; Farag et al. 2022), including increased tolerance to a range of toxic and resistant compounds in an appropriate matrix, ease of separation and reuse, high rates of volumetric reaction and high local cell concentrations (Bergero and Lucchesi 2015).

As a result, the aim of the current work was to develop a cost-effective and efficient method for the biotransformation of PEN-G through the use of a local marine bacterial isolate that can utilize PEN-G as its only source of energy and carbon. Additionally, the best parameters for maximum PEN-G biotransformation were investigated. Furthermore, to achieve efficient PEN-G biotransformation, several matrices were employed during the immobilization procedure.

Materials and methods

Chemicals

All of the analytical-grade compounds were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

Sampling and culture media

Ten sediment samples were collected in sterile screw-capped bottles from multiple locations, such as the Suez Government, Burllus Lake (Kafr-Elsheikh), and two samples from pharmaceutical effluents (Alexandria), Egypt. The samples were then transported to the laboratory in an ice box and kept at 4°C until further analysis.

Minimal salt medium (MSM) containing (g/l) NH_4NO_3 (1.0), K_2HPO_4 (0.5), KH_2PO_4 (0.5), MgSO4·7H2O (0.2), and 10 ml of the trace element solution was used. The trace element solution consisted of the following (g/l): $FeSO_4$, 0.1; $MnSO_4$, 0.1; $ZnSO_4$, 0.1; $CaCl_4$, 0.1; $CuSO_4$, 0.1; and Na_2MoO_4, 0.1. After the pH of the media was adjusted to 7.0, the media were sterilized for 20 minutes at 121°C. Following sterilization, sterile PEN-G (50.0 mg/l) was added to the medium after filtration under aseptic conditions using a sterile filtration system with a hydrophobic membrane filter measuring 0.45 μm. Agar (2%) is utilized in the solid medium production process.

Enrichment and acclimatization of PEN-G-degrading *bacteria*

Five grams of each sample was mixed in 50 ml of sterile seawater before the proper aseptic glass beads were placed in 250-ml Erlenmeyer flasks. To disperse the samples, the flasks were shaken for 30 min at 30°C in a rotary incubator shaker. Next, one ml of each sample was added separately to flasks containing 99.0 ml of sterile MSM (pH 7.0) supplemented with 50 mg/l of PEN-G. Then, the flasks were incubated for 5 days at 30°C and 160 rpm in an orbital incubator. The bacterial cultures were then subjected to gradually increasing PEN-G concentrations to acclimatize the bacterial strains to the antibiotic. After incubation, a 250-ml Erlenmeyer flask containing 99.0 ml of sterilized MSM supplemented with 100 mg/l PEN-G was loaded with one ml of fermentation liquid, which was then incubated

at 30°C and 160 rpm. Similarly, for another hebdomadal acclimation, one ml of suspension from each medium cultivated for five days was added to flasks containing 99.0 ml of MSM and 150 mg/l of PEN-G. Next, 99.0 ml of fresh MSM and 200 mg/l of PEN-G were combined with 1.0 ml of each of the previous cultured media. The flasks were cultivated for 5 days at 30°C and 160 rpm. Thus, isolates with higher tolerance and remediation abilities toward the antibiotic were obtained. Next, solid MSM plates containing 100 mg/l were streaked with the enrichment suspension, and the plates were incubated for 72 h at 30°C. The isolates were also tested for their pathogenicity by subculturing on blood agar plates. For further identification, bacterial colonies of various morphological types were chosen, purified, subcultured, and kept at -20°C (Liyanage and Manage 2015).

nm). Distilled water was used as the blank control. The absorbance values and the PEN-G concentrations had a strong linear relationship (Yang et al. 2019).

To test the biodegradability of the isolates, one ml (1.6 $\times 10^6$ CFU) of each bacterial suspension was inoculated in a 250-ml Erlenmeyer flask containing 50 ml of fresh fermentation medium (MSM) and 100 mg/l PEN-G and then cultivated at 30°C for 72 h on an orbital shaker (160 rpm). Following incubation, a cooling centrifuge was used to centrifuge the culture broth at 6000 rpm and 4°C. Uninoculated medium was used as the control treatment.

The biotransformation efficiency of the bacterial isolates toward the antibiotic was calculated as described by Al-Gheethi et al. (2017) as follows:

$$\text{Biotransformation efficiency (\%)} = (\text{initial PEN}-G\text{ concentration}) - (\text{final PEN}-\text{G concentration})/(\text{initial PEN}-G\text{ concentration}) \times 100$$

$$\text{Biotransformation rate (mg/h)} = (\text{degraded PEN}-\text{G (mg)})/(\text{fermentation period (h)})$$

Bacterial inoculum preparation

According to Mo et al. (2019), the standardized bacterial inoculum was prepared by transferring a full loop of bacterial isolate from a 24 h old slant into 10 ml of sterilized MSM and incubating at 30°C for 72 h on a rotary shaker (160 rpm).

Analytical determinations

Bacterial biomass determination

To assess bacterial growth, the turbidity at 600 nm was determined using a spectrophotometer to record the optical density of the bacterial isolate (Farag et al. 2021).

Quantitative evaluation of PEN-G-degrading bacteria

The analysis was carried out according to the methodology outlined by Feng (2009). After a total of 72 h of incubation, the residual concentration of PEN-G in the MSM medium, which contained 100 mg/ml of the purified strains, was assessed using UV spectrophotometry. A total of 25 ml of PEN-G solution (100 µg/ml) was added to a 50 ml volumetric bottle, and the absorbance was measured from 310 to 340 nm to determine the maximum detection wavelength. The results indicated that the maximum detection wavelength was approximately 325 nm, which is in the range detected by other studies (Feng 2009, Yang et al. 2019).

To establish the standard curve, an accurately weighed quantity of sodium benzyl penicillin was dissolved and diluted to 100 ml to obtain standard solutions with the following concentrations of antibiotics: 20, 40, 20, 40, 60, 80, and 100 µg/ml. The antibiotic absorbance was assessed at the maximum absorption wavelength (325

Determination of residues of PEN-G biotransformation by HPLC

PEN-G and its bioremediation intermediates were detected by high-performance liquid chromatography (Agilent HC-C18 (2)) and an Ultimate XB C18 column (4.6 × 150 mm, 5 µm). All the analytical grade reagents used in the HPLC analyses were of this grade. The samples were tested at 30°C with a flow rate of 1.0 ml/min and using a methanol/phosphate mobile phase (1 mM KH_2PO_4, pH, flow rate: 1.0 ml/min; the injected volume of the sample was 20 µl; the wavelength of the detector was 225 nm) (Wang et al. 2020).

Identification of the most potent bacterial isolate

Morphological, microscopic, biochemical, and 16S rDNA gene sequence studies were used to characterize and identify the pure target isolate that was isolated from the PEN-G-enriched cultures.

DNA isolation and strain identification

The identification method mainly consisted of DNA extraction, PCR amplification, and sequencing according to Sambrook and Russell (2001). The partial 16S rDNA gene was amplified using the universal primers forward (5-AGAGTTTGATCATGGCTCAG-3) and reverse (5-TACGGTTACCTTGTTACGAC-3). PCR amplification was performed using IU of Taq DNA polymerase. A Perkin ABI PRISM 377 DNA sequencer (Perkin Elmer, Foster City, CA, U.S.A.) was used to detect 16S rDNA sequencing fragments. The acquired nucleotide sequences were compared to those found in the NCBI database using a BLAST search. The

sequence was determined using the BioEdit sequence alignment editor tool (version). The neighbor-joining method was used to conduct phylogenetic analysis.

Factors influencing PEN-G biotransformation

Effect of initial PEN-G concentration, carbon, and nitrogen source

To determine the effect of the initial concentrations of PEN-G, several concentrations of the antibiotic ranging from 50 to 500 mg/l were combined with sterilized MSM and incubated at 30°C for 72 h in an orbital shaker (160 rpm). Following incubation, microbial growth was assessed; the residual PEN-G concentration in the cell-free culture was then assayed, and the PEN-G biotransformation efficiency and rate were calculated. The effects of 0.1% additional carbon sources (glucose, fructose, sucrose, maltose, lactose and starch) and 0.1% nitrogen sources (yeast extract, casein, tryptone, peptone, NH_4NO_3, KNO_3, $(NH_4)_2SO_4$, NH_4Cl and $NaNO_3$) on PEN-G biotransformation were also tested in MSM supplemented with 300 mg/l PEN-G. All experiments were conducted at least in triplicate, and the mean values were calculated.

Plackett–Burman experimental design

An effective approach for identifying factors that have a major impact on the biotransformation of PEN-G by the most potent bacterial strain is the Plackett–Burman experimental design (Plackett and Burman 1946). Eleven variables were tested in 13 trials for the current investigation. High (+) and low (-) levels were tested for each variable (Table 1). Every experiment was carried out in duplicate, and the response was determined by averaging the PEN-G biotransformation.

Table 1 Variables and their levels utilized in the optimization experiment

Variables	Code	Levels		
		Low (-1)	Basal (0)	High (+1)
Yeast extract (g/l)	X_1	**1.0**	2.0	3.0
PEN-G (g/l)	X_2	0.15	0.30	0.45
Glucose (g/l)	X_3	0.5	1.0	1.5
K_2HPO_4 (g/l)	X_4	0.5	1.0	1.5
KH_2PO_4 (g/l)	X_5	0.15	0.3	0.45
$MgSO_4.7H_2O$ (g/l)	X_6	0.25	0.5	0.75
Trace element (%)	X_7	5	10	15
Inoculum size (%)	X_{10}	1	2.0	3
pH	X_8	6.0	7.0	8.0
Temperature (°C)	X_9	**25**	30	35
Time of incubation	X_{11}	48	72	96

The main effect for each variable was calculated by the following equation:

$$\sum xi = \left(\sum pi + - \sum pi\right)/N$$

In the given context, ∑xi represents the variable main effect, Σpi+ and Σpi–are the PEN-G biotransformation responses in trials with high and low concentrations of the independent variable (Xi), respectively, and N represents the number of trials divided by two. The factors that were significant at the 95% level ($P<0.05$) were chosen because they were considered to have significant effects on the biotransformation of PEN-G and thus were used for further optimization. Using regression analysis, the biotransformation coefficients were calculated. The effects of different factors on biotransformation were examined using p values and F tests.

Cell immobilization procedures

By Entrapment

According to Farag et al. (2015), entrapment was carried out in 3% sodium alginate or K-carrageenan solution. A volume of approximately 3 ml (OD 600, 1.0) of bacterial suspension was introduced into sterile alginate or k-carrageenan solution. The gel-mixing cells were drawn out using a sterile syringe and put into a cross-linking solution (2% KCl for k-carrageenan and 2% $CaCl_2$ solution for alginate) to create circular beads with a diameter of 3 to 4 mm. After two hours of hardening, the beads were rinsed with sterile distilled water. After that, the beads were added to previously sterilized MSM medium containing PEN-G, and the mixture was shaken (160 rpm) at 35°C.

By adsorption

For cell adsorption, several solid support matrices (luffa pulp, polyurethane foam, and charcoal cubes) were utilized after sterilization at 121°C for 30 min. Before being immobilized, 2 ml of bacterial cells (OD_{600}, 1.0) was added to sterile MSM containing PEN-G. The mixture was then incubated at 35°C in the dark in an orbital incubator. Subsequently, each support matrix was introduced, and the mixture was incubated at the optimal temperature on a rotating shaker (160 rpm) (El-Borai et al. 2022; Farag et al. 2022).

Scanning *electron* microscopy (SEM)

The biocatalysts were rinsed with sterilized distilled water and vacuum dried to remove moisture. After being sliced and coated with gold, the samples were

investigated using a scanning electron microscope (JEOL JEM-2100 F; JEOL Ltd., Japan).

Biotransformation of different initial PEN-G concentrations by immobilized cells

To determine the optimal initial concentration of PEN-G (0.2 to 3.5 g/l), the immobilized cells were introduced to the optimum medium supplemented with various concentrations of PEN-G and incubated in an orbital shaker incubator (160 rpm) at 35°C. Following incubation, a predefined volume was aseptically collected at regular intervals, and the concentration of residual PEN-G was measured. Every treatment was carried out in triplicate.

Reuse and storage of immobilized cells

Cells immobilized within the most suitable material support were incubated at 35°C. Following incubation, the culture medium was removed from the flask for determination of residual PEN-G, while the immobilized cells were kept inside for another cycle. The previously described procedure was carried out many times; fresh sterile MSM supplemented with PEN-G was added, and the cells were incubated. At the end of each run, the residual concentration of PEN-G was calculated.

Results

Isolation of high-efficiency PEN-G-degrading *bacteria*

After acclimatization and purification, a total of four isolates that showed the ability to grow on PEN-G as the sole carbon source were chosen for the next investigation. BL-1, NSC-1, PW-1, and NSC-2 were isolated from Burullus drains, the New Suez canal, pharmaceutical wastes, and the New Suez canal, respectively. The plants were cultured on sterile MSM supplemented with 100 mg/l PEN-G as the only carbon and energy source for 72 h at 30°C and 160 rpm to compare their remediation capabilities. Following incubation, microbial growth (absorbance at 600 nm) and the residual PEN-G concentration were assessed. The data (Fig. 1) revealed that all bacterial isolates recorded optical density for growth in the range 0.8-1. Also they were capable of utilizing more than 60% PEN-G. Furthermore, among all the isolates that were investigated, the bacterial strain BL-1 showed the greatest ability to remediate PEN-G, with biotransformation efficiencies and rates of 98.0% and 1.361 mg/h, respectively. Consequently, the BL-1 strain was selected as the target isolate, despite its slightly least growth (0.8) among the tested isolates, as it had the highest biotransformation capability. Moreover, the isolate showed no hemolysis when subcultured on blood agar, thus confirming no pathogenicity, while the others showed slight hemolysis. The purity of the isolate was confirmed by microscopic examination of the morphological

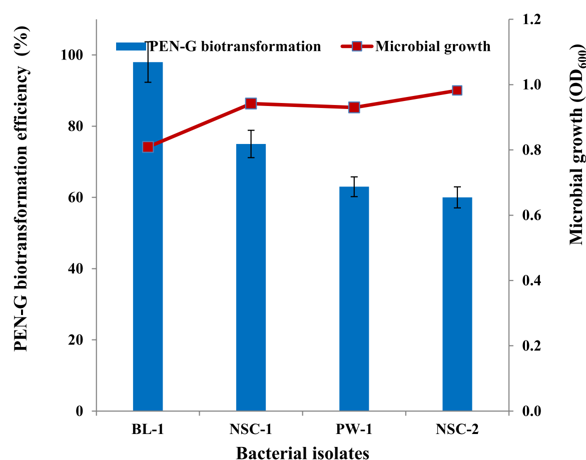

Fig. 1 PEN-G degradation by marine bacterial isolates using PEN-G as the sole carbon and energy source. Bacterial cultures were cultivated at 30°C under shaking conditions (160 rpm) for 72 h. Data are expressed as the mean ± SD

uniformity of cells from a single colony subcultured on solid media plates.

Strain identification

Based on morphological, microscopic, and biochemical evaluations, the most potent bacterial isolate (BL-1) that degrades PEN-G was identified (Additional File; Table 1). In addition, this identification has been verified by a molecular method based on the study of the 16S rDNA gene sequence. The sequence's nucleotide sequence analysis was carried out at the NCBI server's Blast N site. The sequence of isolate BL-1 was 652 bp (Fig. 2), 100% similar to that of members of the genus *Bacillus*, and it was identified as *Bacillus pseudomycoides* AH1 and deposited in GenBank with accession number MW052694.

Determination of PEN-G biotransformation by HPLC

PEN-G biotransformation was analyzed by HPLC 90 min after injection with a standard (noninoculated) sample of 100 mg/l PEN-G and another sample of culture medium containing the same concentration of PEN-G and previously inoculated with *Bacillus pseudomycoides* AH1 (Fig. 3). This method could be used to determine the concentration of PEN-G in aqueous media. Fig. 3A shows the chromatogram of a standard PEN-G solution (in medium without inoculation), whereas Fig. 3B shows the PEN-G residues (in medium inoculated with *Bacillus pseudomycoides* AH1). Based on a comparison of the two chromatograms, PEN-G had a 4.15 min retention time (Fig. 3A), which decreased, and the appearance of two additional peaks with varying retention times (2.221 and 3.284 min, respectively) is shown in Fig. 2B. These analytical results imply that the *Bacillus pseudomycoides* AH1 strain

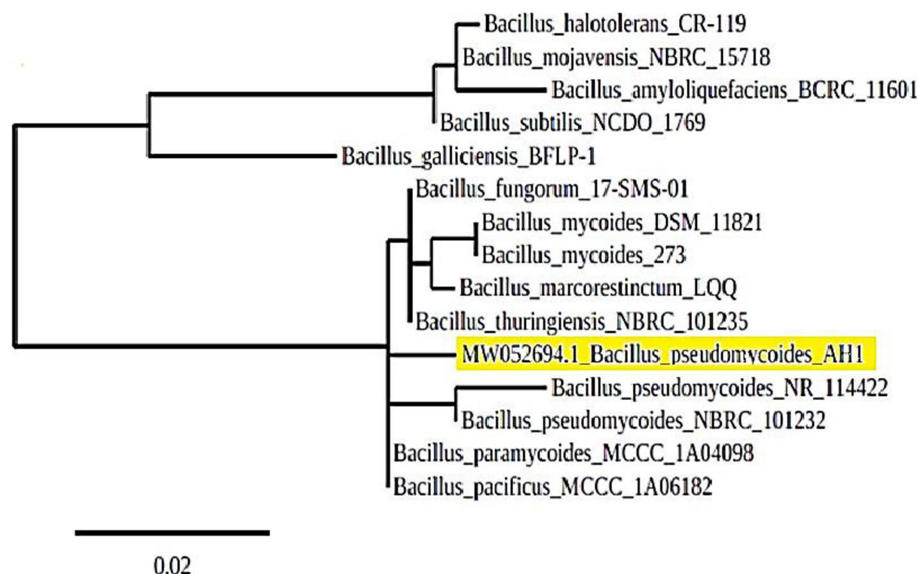

Fig. 2 Phylogenetic tree showing the relationships between *Bacillus pseudomycoides* AH1 and closely related strains

degrades PEN-G and that two biotransformation products are produced in solution throughout the process.

Optimization of PEN-G biotransformation

Effect of initial PEN-G concentration and additional carbon and nitrogen sources

The effect of different initial PEN-G concentrations on PEN-G biotransformation was assessed. The highest biotransformation rate (2.26±0.04 mg/h) was observed at 300 mg/l, and the lowest (0.46±0.01 mg/h) was observed at 500 mg/l (data not shown; Additional file 2). In contrast, as the initial PEN-G concentration increased, microbial proliferation decreased.

The present findings generally showed that the inclusion of carbon substrates increased the cell density and decreased the efficiency of PEN-G biotransformation (Fig. 4A). However, glucose was an exception; its addition slightly increased the cell density (0.754) and biotransformation efficiency and rate (61.450% ± 0.45 and 2.56 mg/h, respectively) compared with those of the control.

The results of the addition of an additional nitrogen source revealed that the addition of yeast extract resulted in a maximum PEN-G biotransformation efficiency and rate (71.678% ±1.28 and 2.99 mg/h, respectively) (Fig. 4B). Repressed PEN-G biotransformation efficiency was observed in a medium lacking a nitrogen source, in which the efficiency was approximately 36.40% of the maximum efficiency detected in the presence of yeast extract. Therefore, yeast extract was chosen as the nitrogen source for the experimental design and was used to assess a variety of physiochemical factors impacting the ability of *B. pseudomycoides* AH1 cells to biotransform PEN-G.

Optimization of PEN-G biotransformation by Plackett–Burman design

The presence of many variables needs to be taken into consideration because optimizing the fermentation conditions of the culture medium is considered a laborious and complex process. Therefore, the Plackett–Burman design was employed to estimate the most significant factors affecting PEN-G biotransformation. The PEN-G biotransformation efficiency (%) was assessed in the present study (Table 2). Trial number 11 had the highest PEN-G biotransformation efficiency (99.989%), whereas trial number 10 had the lowest PEN-G biotransformation efficiency (38.558%). This in turn demonstrated that performing the optimization step was needed.

The PEN-G biotransformation efficiency (%) was negatively impacted by high levels of glucose and incubation time within the test ranges but was positively impacted by all other factors (Fig. 5). ANOVA was used to evaluate the adequacy of the model and the impact of the medium components on the percentage of PEN-G biotransformation by *B. pseudomycoides* AH1 cells, as listed in Table 3. Fisher's statistical test (F test) was utilized to determine the model's statistical significance. Consequently, a component was regarded as significant if it had a statistical confidence above 98% ($P = 0.02$). Based on multiple linear regressions of the Plackett–Burman design data (Table 3), six investigated independent factors were found to have a significant impact ($p < 0.05$) on process performance.

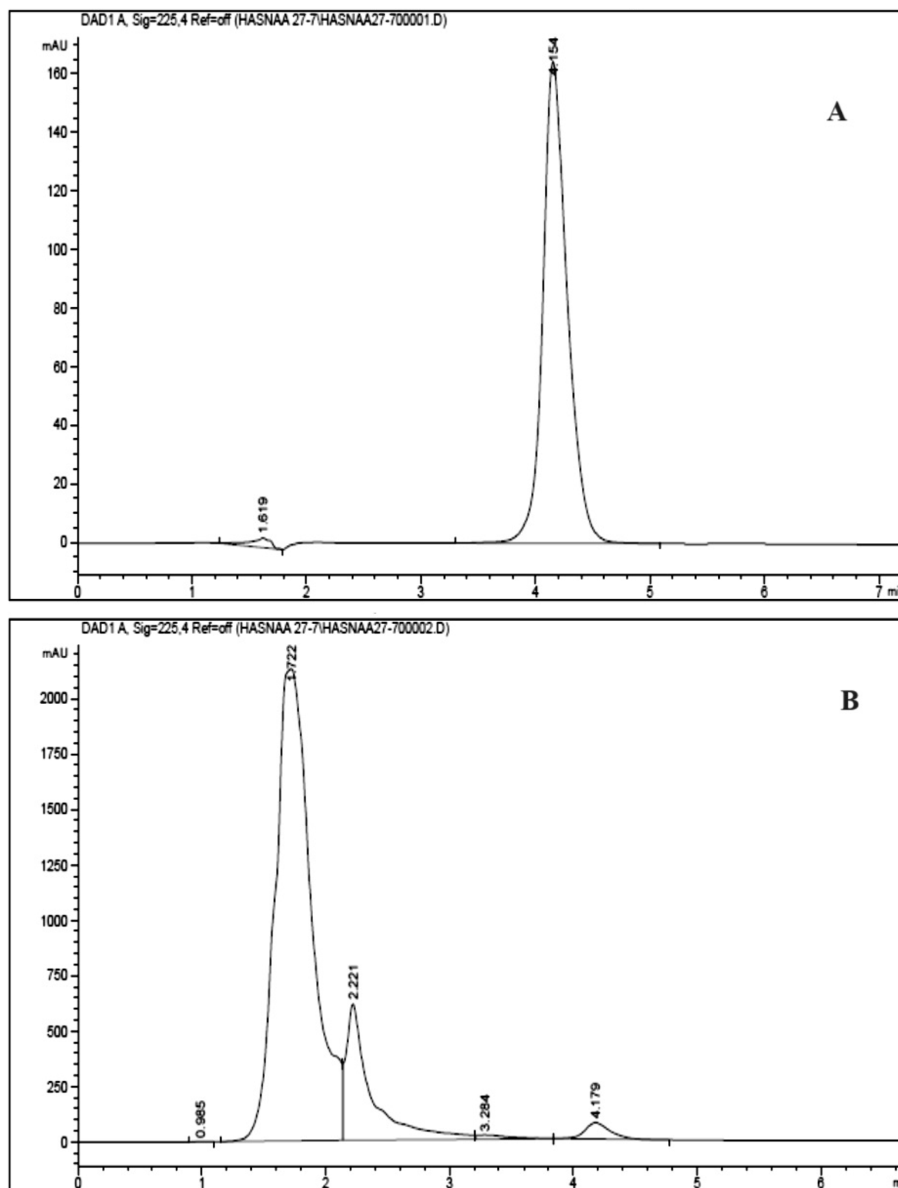

Fig. 3 HPLC results of (**A**) standard sample and (**B**) culture medium of *Bacillus pseudomycoides* AH1

All of these variables were predicted to have the greatest impact on PEN-G biotransformation efficiency (%).

To demonstrate the significance of each investigated independent variable on the process output in ascending order, a Pareto chart (Fig. 6) was developed.

The first-order models for power generation were determined to be appropriate after ANOVA was performed. A linear model equation was then presented to determine the optimal levels of these variables for PEN-G biotransformation efficiency (%) and can be written as:

$$Y = 72.432+8.23X_1+6{,}479\ X_2-1.185X_3+1.315X_4+7.103X_5+7.403X_6+4.017X_7+2.593X_8-2.710X_9+1.25310_{10}-5.974X_9$$

where Y is the response of the PEN-G biotransformation efficiency (%), and the concentrations of the independent variables are represented as X_1:$X_{11,}$ as reported in Table 1 in the Materials and Methods section.

Within the limits of the experimental conditions, the PEN-G biotranformation efficiency (%) may be predicted via the Plackett–Burman design. In addition, there is considerable agreement among the predicted and actual response levels (Fig. 7). For the PEN-G

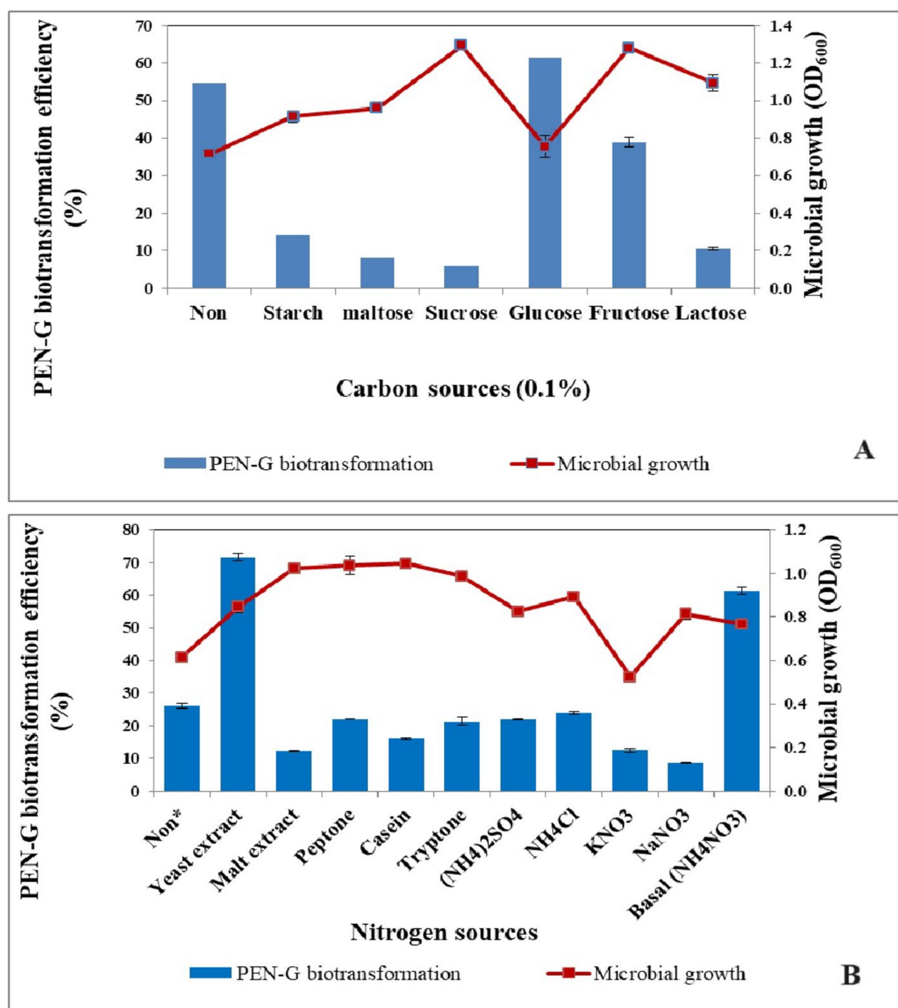

Fig. 4 Influence of additional carbon sources (A) and nitrogen sources (B) on PEN-G-biotransformation and growth. Bars indicate the standard deviation of the mean.

biotransformation efficiency (%), the highest experimental value was 99.653, whereas the predicted response was 99.159.

The Plackett–Burman experimental data suggest that the following medium composition (g/l) is likely to be close to optimal: yeast extract, 1.0; PEN-G, 0.45; glucose, 0.5; K_2HPO_4, 1.5; KH_2PO_4, 0.45; $MgSO_4.7H_2O$, 0.75; trace element solution, 15 ml; inoculum size, 3%; and volume of medium, 50 ml. The medium was adjusted to pH 8 and incubated at 35°C on a rotary shaker at 160 rpm for 48 h.

By applying the Plackett–Burman experimental design, a validation experiment was carried out in triplicate to verify the performance of *B. pseudomycoides* AH1 cells. The PEN-G biotransformation efficiency was 99.653 ± 1.5%, showing a 3.12-fold increase. There was a 0.59% difference between the predicted and actual results.

PEN-G biotransformation by immobilized *B. pseudomycoides* AH1 cells

To evaluate the effect of the immobilization approach on PEN-G biotransformation by *B. pseudomycoides* AH1, cells were batchwise cultured at 35°C in 250 ml flasks containing 50 ml of optimized MSM supplemented with 450 mg/l PEN-G. Samples were taken every 8 h for up to 96 h. According to the results (Fig. 8), PEN-G was degraded to differing degrees by the cells immobilized on various supports or in the gel matrix. The average biotransformation rates of cells immobilized in alginate beads, polyurethane foam, and luffa pulp were greater than those of free cells in comparison to those of other materials (12.5, 11.50, and 11.45 mg/h, respectively). However, free cells exhibited greater PEN-G biotransformation than immobilized cells in K-carrageenan and on charcoal cubes and nut particles. Based on these findings, the immobilization method employing Ca-alginate-entrapped cells

Table 2 Plackett–Burman experimental design for the evaluation of variables affecting PEN-G biotransformation efficiency (%)

Trial No.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	PEN-G biotransformation efficiency (%)	
												Actual	Predicted
1	-1	-1	1	1	1	-1	1	1	-1	1	-1	68.749	68.679
2	1	1	1	-1	1	1	-1	1	-1	-1	-1	99.804	99.734
3	-1	1	-1	-1	-1	1	1	1	-1	1	1	70.098	70.028
4	1	1	-1	1	-1	-1	-1	1	1	1	-1	83.717	83.648
5	-1	1	1	-1	1	-1	-1	-1	1	1	1	59.330	59.261
6	1	1	-1	1	1	-1	1	-1	-1	-1	1	80.896	80.827
7	-1	-1	-1	1	1	1	-1	1	1	-1	1	68.856	68.786
8	1	-1	1	1	-1	1	-1	-1	-1	1	1	60.641	60.572
9	1	-1	1	-1	-1	-1	1	1	1	-1	1	59.340	59.271
10	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	38.558	38.489
11	1	-1	-1	-1	1	1	1	-1	1	1	-1	99.989	99.919
12	-1	1	1	1	-1	1	1	-1	1	-1	-1	80.034	79.965
13	0	0	0	0	0	0	0	0	0	0	0	71.597	72.432

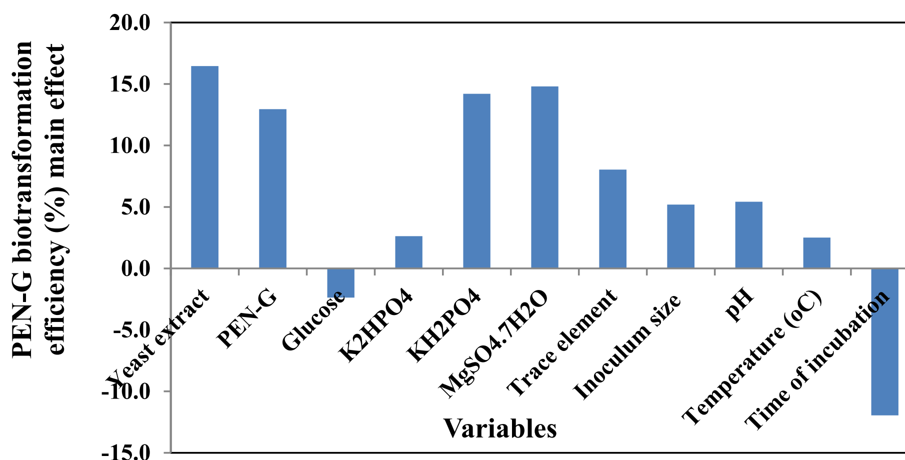

Fig. 5 The main effect of different variables on PEN-G biotransformation efficiency (%) by *B. pseudomycoides* AH1

was chosen for achieving the greatest level of PEN-G biotransformation.

Cells of *B. pseudomycoides* AH1 immobilized in alginate beads and polyurethane foam and cultured on optimized media supplemented with PEN-G (450 mg/l) were collected and investigated by SEM (Fig. 9). SEM revealed unevenly distributed, tightly packed cells.

Effect of different initial PEN-G concentrations on PEN-G biotransformation using immobilized *B. pseudomycoides* AH1 cells

Different PEN-G concentrations (200–3500 mg/l) were applied to the immobilized cells to determine their tolerance limits (Fig. 10). The findings indicated that immobilized *B. pseudomycoides* AH1 cells could degrade PEN-G in 36 h at a biotransformation rate of 41.67 mg/h when the initial concentration was 1500 mg/l. However, when the PEN-G concentration was increased to 3500 mg/l, the biotransformation efficiency of PEN-G decreased and eventually decreased to its lowest value (17.20%).

Effect of reuse and storage of immobilized cells

B. pseudomycoides AH1-immobilized cultures were reused for 10 cycles. The gel beads containing the cells could entirely degrade PEN-G by the fifth cycle, with a biotransformation rate equal to 41.64 mg/h. However, the biotransformation efficiency and rate titer decreased in subsequent cycles (Fig. 11). The results showed that for five batches in succession, the amount of PEN-G substantially decreased in the immobilized cells. After five cycles of repetition, cell leakage occurred, and the efficiency of PEN-G biotransformation decreased.

Table 3 Statistical analysis of the effects of Plackett–Burman design on PEN-G biotransformation efficiency (%)

Variables	Coefficients	*t* value	Main Effect	*p* value	Confidence %))
	72.432	300.710		0.002	99.8
Yeast extract (g/l)	8.230	32.828	16.460	0.019	98.1
PEN-G (g/l)	6.479	25.843	12.958	0.025	97.5
Glucose (g/l)	-1.185	-4.725	-2.369	0.133	86.7
K_2HPO_4 (g/l)	1.315	5.243	2.629	0.120	88.0
KH_2PO_4 (g/l)	7.103	28.332	14.206	0.022	97.8
$MgSO_4.7H_2O$ (g/l)	7.403	29.527	14.805	0.022	97.8
Trace elements (%)	4.017	16.021	8.033	0.040	96.0
Inoculum size (%)	2.593	10.342	5.186	0.061	93.9
pH	2.710	10.810	5.420	0.059	94.1
Temperature (°C)	1.253	4.997	2.506	0.126	87.4
Time of incubation (h)	-5.974	-23.829	-11.948	0.027	97.3

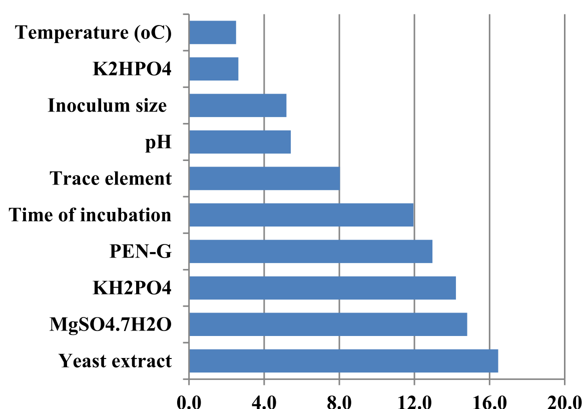

Fig. 6 Pareto diagram showing the effect of variables on PEN-G biotransformation efficiency (%) by *B. pseudomycoides* AH1

The stability and activity of microorganisms are also significantly affected by prolonged storage. The PEN-G biotransformation efficacy of immobilized *B. pseudomycoides* AH1 after storage at 30°C for ten weeks was tested (Table 4). The data indicated that with increasing storage time, the removal of PEN-G decreased gradually from 99.87% to 73.24%, which indicated that the immobilized biocatalyst had good storage stability.

Discussion

Penicillin is considered an inexpensive β-lactam antibiotic that exhibits a broad spectrum of antimicrobial action. Ecosystems and human health may be negatively impacted by penicillin residues, which have the potential to be hazardous, leading to the development of penicillin-resistant strains. Currently, the removal of penicillin from water is an important issue. Therefore, it is crucial to isolate a potential local microbial strain that can withstand, grow, and degrade PEN-G even at extremely high initial concentrations.

The present study has several advantages compared to other anaerobic/aerobic biodegradation and abiotic techniques. In addition, the use of a single bacterium rather than a microbial consortia, a high rate of PEN-G biotransformation, efficient biotransformation, and aerobic process execution are highly important. There are few papers

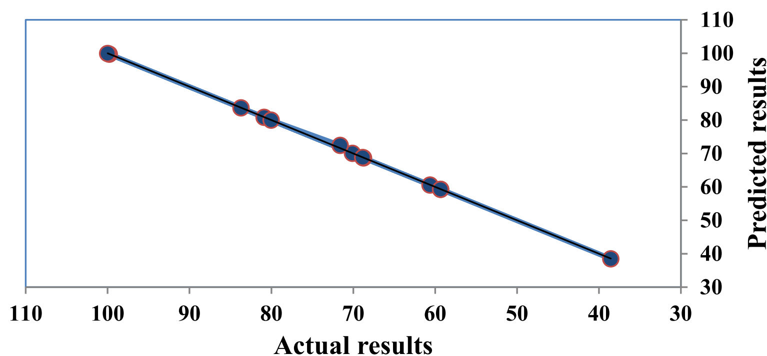

Fig. 7 Predicted versus actual plot obtained by the Plackett–Burman design

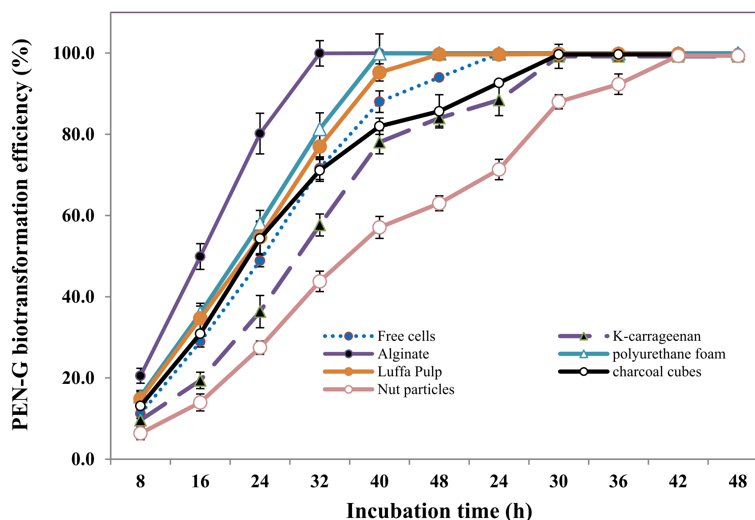

Fig. 8 PEN-G biotransformation by immobilized *B. pseudomycoides* AH1 cells at different incubation times. The values represent the means ± SDs of 3 replicates

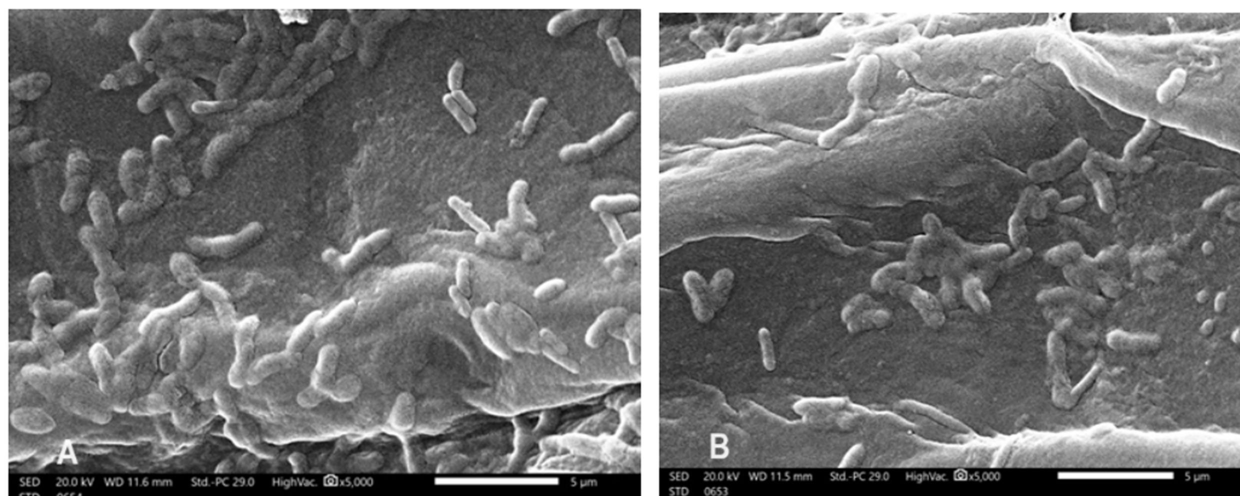

Fig. 9 Scanning electron micrographs showing (**A**) cells entrapped in alginate beads and (**B**) cells adsorbed on PF

discussing the biotransformation of antibiotics in general and PEN-G in particular in the most recent reviews of the literature. The LB-1 strain succeeded in nearly completely eliminating PEN-G, with the biotransformation efficiency reaching 99.65% after 48 h of incubation. However, the strain showed the least growth (0.8) among the tested isolates which may be related to accumulation of some metabolites from the degradation process which can act as suppressors for cell growth (Wang et al. 2015). Moreover, the use of microorganisms in biodegradation processes is safe, energy saving and eco-friendly.

The present study also showed the ability of four bacterial isolates from different locations to grow well on media supplemented with penicillin G as the sole carbon source. Most microorganisms cannot use antibiotics as a sole carbon source and depend on cometabolism by adding additional carbon sources for reproduction (Wang et al. 2021).

Few bacterial strains have been found to degrade PEN-G, such as *Klebsiella pneumoniae* Z-1 (Wang et al. 2015), *Phanerochaete chrysosporium* (Mullai and Vishali 2007), *Actinobacillus pleuropneumoniae* (Hathroubi et al. 2015), and *Paracoccus* sp. KDSPL-02 (Wang et al. 2020). The most potent strain reported in this study was *Bacillus pseudomycoides* AH1. This strain is one of nine novel 454 species of the *Bacillus cereus* group, as reported

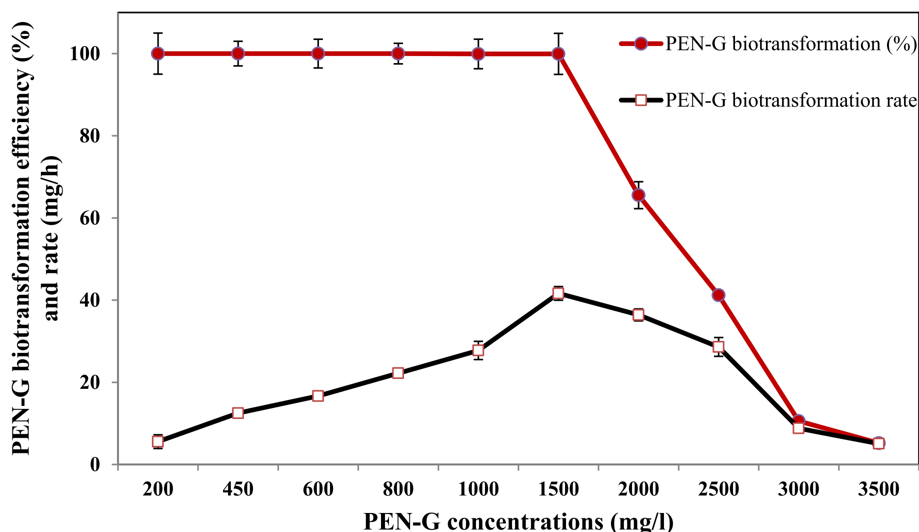

Fig. 10 PEN-G biotransformation efficiency and rate at different PEN-G concentrations. The experiments were performed in triplicate, and the data are expressed as the mean ± SD

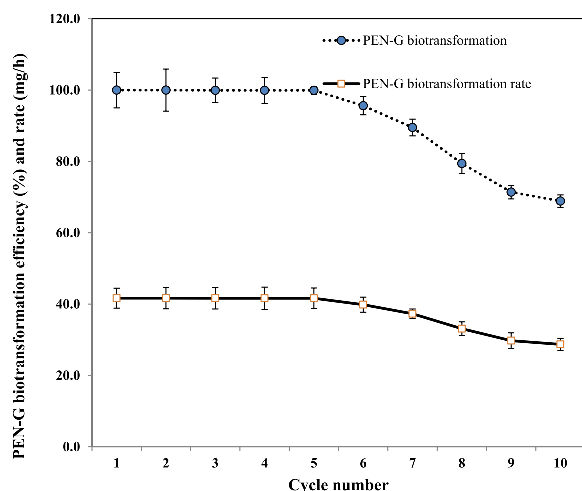

Fig. 11 Reuse of immobilized *B. pseudomycoides* AH1 cells. The experiments were performed in triplicate, and the data are expressed as the mean ± SD

Table 4 Effect of long-term storage of immobilized *B. pseudomycoides* AH1 cells on PEN-G biotransformation. The experiments were performed in triplicate, and the data are expressed as the mean ± SD

Storage time (week)	Residual PEN-G (mg/l)	PEN-G biotransformation efficiency(%)	PEN-G biotransformation rate (mg/h)
1	1.9	99.87±0.06	41.61±0.16
2	3.56	99.76±0.12	41.57±0.22
3	5.32	99.65±0.08	41.52±0.32
4	15.91	98.94±1.04	41.22±0.30
5	58.10	96.13±1.97	40.05±0.34
6	87.84	94.14±2.60	39.23±0.41
7	127.28	91.51±3.85	38.13±0.55
8	205.00	86.33±2.67	35.97±1.05
9	315.34	78.98±2.05	32.91±1.02
10	401.34	73.24±1.19	30.52±0.87

by Liu et al. (2017). Numerous researchers have documented *Bacillus cereus* resistance to β-lactam antibiotics (Kim et al. 2015; Fiedler et al. 2019; Nguyen et al. 2022). Bacterial strains comparable to *B. pseudomycoides* MT32 have been isolated from soil samples in the Tenth Rhamadan district of Sharkia Governorate, Egypt, which is an industrialized area contaminated with heavy metals (El-Saadony et al. 2019). Liyanage and Manage (2015) reported that *B. cereus, Enterobacter ludwigii* and *Enterobacter* sp. were used for sulfanomide and amoxicillin degradation. Additionally, Al-Gheethi and Ismail (2014) demonstrated that four different β-lactam antibiotics (ampicillin, cefalexin, cefuroxime, and amoxicillin) can potentially be degraded by *Bacillus subtilis* strains. To date, a review of the literature has not revealed any information concerning the ability of *Bacillus pseudomycoides* to degrade penicillin G.

The bacterial strains, along with the fermentation conditions, have a significant impact on the rate of biotransformation. The PEN-G biotransformation efficiency decreased with increasing initial concentration of PEN-G, possibly due to substrate inhibition, as demonstrated by Mullai and Vishali (2007). Another potential explanation for the reduced PEN-G biotransformation

might be the diminished activity of the β-lactamase enzyme at high levels of substrate (PEN-G) (Nguyen et al. 2022). The PEN-G biotransformation efficiency slightly increased with the addition of glucose (0.1%). The cell growth of *B. pseudomycoides* AH1 in our study is also slightly increased when using additional glucose from that reported by using PEN-G alone as a carbon source, indicating that the strain can use also PEN-G alone as a carbon source for growth. Anan et al. (2018), used glucose as an additional carbon source for *Achromobacter xylosoxidans* strain Cef6 to achieve maximum sodium ceftriaxone biotransformation. Additionally, Wang et al. (2015) suggested that the decrease in the biodegradation rate of PEN-G with the addition of glucose might be due to the preference of *Klebsiella pneumoniae* Z1 for utilizing external organic glucose rather than antibiotics. In contrast to our results, Leng et al. (2016) reported that the addition of glucose increased the cell density and decreased the rate of tetracycline biotransformation in *Stenotrophomonas maltophilia* DT1 cells. In cell cultures, sucrose was shown to support high rates of growth, although low PEN-G biotransformation efficiency (%) was also observed. It is commonly known that sucrose is a disaccharide that is formed when the monosaccharides glucose and fructose are combined. This combination of molecules can offer high-sufficient carbon sources to support the growth of bacteria. The current findings are in contrast with earlier research by Sudha et al. (2022), who found that the addition of sucrose as an additional carbon source increased the oxytetracycline degradation efficiency of *Lysinibacillus* sp. strain 3+I, up to a maximum of 14%. Tan et al. (2022) additionally found that, among the six carbon sources, potassium acetate exhibited the greatest rates of tetracycline biodegradation by the yeast strain *Cutaneotrichosporon dermatis* M503, whereas glucose, sucrose, and maltose gave lower rates of biodegradation. Therefore, the species determines the best carbon source, and our isolate could represent a novel *B. pseudomycoides* variant.

The majority of bacterial cells are able to employ multiple compounds as their sole supply of nitrogen. The addition of yeast extract to the MSM increased the biotransformation efficiency. The maximum rate of PEN-G biodegradation by *Paracoccus* sp. KDSPL-02 was achieved using a combination of NH_4NO_3 and yeast extract (Wang et al. 2020). Similarly, the addition of yeast extract to cultures of *Klebsiella* strain TR5 enhanced tetracycline degradation (Yin et al. 2020).

The traditional approaches for medium optimization, known as OFATs, require a great deal of time and are not appropriate for assessing a large number of independent parameters. Consequently, a crucial statistical method known as the Plackett–Burman experimental design is usually employed to determine the key variables in a multivariable system and to provide the basis for additional optimization (Anan et al. 2018). The results of the experimental design revealed that a higher glucose concentration has a negative impact on the biotransformation efficiency, which may be a result of reduced glucose adsorption by the strain or detrimental osmotic pressure (Dike et al. 2014). The effect of temperature and pH on the biotransformation process was also revealed by the design in which both parameters positively affected the biotransformation process. The optimum temperature and pH were 35°C and 8, respectively, similar to the results reported by some authors who also reported that the stability of penicillin is usually lower in alkaline media than in neutral and acidic media (Wang et al. 2021). The results obtained from the optimization methods revealed that free cultures of *Bacillus pseudomycoides* AH1 had a good ability to degrade PEN-G in a medium containing an initial concentration of 450 mg/l, which was superior to the results of other investigations (Wang et al. 2015; Yang et al. 2019). The determination coefficient was used to evaluate the model's goodness of fit (R2). In this instance, the calculated R2 value was 0.9998, whereas the adjusted R2 value was 0.9974. The two values are close to one another, confirming good model fitting. As stated by Guilford and Fruchter (1973), a regression model with an R2 value near 1.0 is considered to have a remarkably strong correlation. The model in the study is appropriate, as demonstrated by the strong correlation established between the predicted and experimental results. These results are in good agreement with other authors' reports of the use of statistical optimization techniques and mathematical modeling for enhancing the biodegradation of antibiotics and phenolic compounds (Anan et al. 2018; Chris Felshia et al. 2018; Yang et al. 2020; Farag et al. 2021; Favier et al. 2021).

A few drawbacks of biodegradation/biotransformation technology include microbial cell death, substrate inhibition, and a lack of ability to withstand extreme conditions (Lou et al. 2019). Numerous authors have presented data on the viability and efficacy of antibiotic biological degradation in immobilized microbial cells (Wang et al. 2020; Al-Dhabi and Arasu 2022; Xia et al. 2023). According to Wang et al. (2020), immobilized *Paracoccus* sp. KDSPL-02 cells in alginate beads had greater effectiveness than free cells in the breakdown of PEN-G. Furthermore, Liu et al. (2023) enhanced the capability of *Alcaligenes* sp. R3 cells to remove tetracycline by immobilizing tetracycline in both polydopamine and carboxymethylcellulose. Consequently, immobilized cells are utilized more frequently and have greater biotransformation efficiency than free cells (Zur et al. 2016). Among all the tested supports, immobilized cultures of *Bacillus pseudomycoides*

AH1 in alginate beads had the highest PEN-G biotransformation efficiency, which was even greater than that obtained from free cultures. This might be because of the structure of the alginate beads, which provide an internal living environment for microorganisms to shield them from outside contaminants and improve the probability of surviving in an antibiotic-contaminated environment (Zhao et al. 2019). Furthermore, alginate is an anionic polysaccharide that has outstanding chemical and physical stability, stable properties, and a low toxicity profile. It additionally enhances the possibility of reuse and decreases the risk of cell leakage or contamination in the fermentation medium (Ramakrishnan and Prakasham 2011; Rivas-Sanchez et al. 2022). The investigated bacterium can completely degrade PEN-G by immobilized cultures in alginate beads when the initial antibiotic concentration is raised to 1500 mg/l, which is a higher concentration than that reported by Wang et al. (2020), who reported that entrapped *Paracoccus* sp. KDSPL-02 cells in alginate beads entirely degraded PEN-G at an initial concentration of 1200 mg/l, and the cells maintained their degradation efficiency up to 2000 mg/ml. In accordance with our findings, *Phanerochaete chrysosporium* has the ability to tolerate PEN-G concentrations of up to 2000 mg/l; however, its degradation capability is limited to 61.4% (Mullai and Vishali 2007). A key aspect that influences the efficacy of biotransformation over time in industrial treatment is the reuse of immobilized cultures (Lin and Cheng 2020). Immobilized cultures of *Bacillus pseudomycoides* AH1 in alginate beads were reused for several cycles since reusing immobilized microbial cells could save money and could be considered together with storage stability as a key parameter of their utilization in practice (Wang et al. 2015). *Bacillus pseudomycoides* AH1-immobilized cultures maintained their biotransformation efficiency until the fifth cycle, after which the efficiency decreased. Cell inactivation, cell leakage, the buildup of hazardous substances inside the carrier, and mass transfer restrictions might all be the reasons for this decline (Partovinia and Rasekh 2018). Immobilized cultures of *Bacillus pseudomycoides* were also stable under storage conditions and could conserve 73.23% of its biotransformation efficiency after 10 weeks. Similarly, immobilized *Bacillus subtilis* cultures are extremely stable during storage and can be efficiently and continually utilized for three cycles to remove chlortetracycline (Zhang and Wang 2021). Zhang and Wang (2021) showed, in line with our findings, that immobilized *Bacillus subtilis* exhibited excellent storage stability and that as the storage time increased, the percentage of chlortetracycline removed decreased from 78.83% to 38.52%. These findings therefore emphasize the significance of the use of immobilized *B. pseudomycoides* AH1 to improve PEN-G biotransformation.

Conclusion

Organic pollutants, especially antibiotics, are substances of high environmental concern globally due to the severe issues they generate. Consequently, the present study reported a new source for PEN-G removal from a marine environment. Several bacterial strains were isolated and screened for their ability to degrade PEN-G, and the most potent strain was then phylogenetically identified as *Bacillus pseudomycoides* AH1 (accession no. MF037698). The goal of using free and immobilized cells was to enhance the biotransformation efficiency of PEN-G. Immobilizing bacterial cells in alginate beads is an easy, affordable, and environmentally friendly way to degrade the majority of contaminants found in wastewater. To enhance efficiency and outcomes, this study could offer more ecologically friendly materials that may be combined and used in wastewater treatment systems.

Abbreviations

PEN-G Penicillin G
h hour
MMS minimal mineral synthetic
mg/h milligram per hour
ml milliliter
OD optical density
rpm Rounds per minute

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-024-01774-7.

Additional file 1: Table 1. Morphology, culture characteristics and biochemical tests of the BL-1 isolate.

Additional file 2: Fig. 1. Growth, PEN-G biotransformation efficiency and rate of *Bacillus pseudomycoides* AH1 at different PEN-G concentrations. Bacterial cultures were cultivated at 30°C under shaking conditions (160 rpm) for 72 h. Data are expressed as the mean± SD.

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Authors' contributions

Prof. Dr. AMF participated in the research idea, plan of the work, strain identification, statistical experiments and analysis, and writing of the manuscript. Dr. AME participated in the optimization experiments, statistical experiments, immobilization methods, and revision of the manuscript. Dr. HEBG participated in the optimization experiments, statistical experiments, immobilization methods, and revision of the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interest
The authors declare that they have no competing interests. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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References
Al-Dhabi NA, Arasu MV (2022) Effective degradation of Chlortetracycline using dual bio catalyst. Environ Res 204(Pt C):112339. https://doi.org/10.1016/j.envres.2021.112339
Al-Gheethi AA, Ismail N (2014) Biodegradation of pharmaceutical wastes in treated sewage effluents by *Bacillus subtilis* 1556 WTNC. Environ Processes 1:459–481. https://doi.org/10.1007/s40710-014-0034-6
Al-Gheethi AA, Efaq AN, Mohamed RM, Norli I, Kadir MO (2017) Potential of bacterial consortium for removal of cephalexin from aqueous solution. J Assoc Arab Univ Basic Appl Sci 24(1):141–148. https://doi.org/10.1016/j.jaubas.2016.09.002
Anan A, Ghanem KM, Embaby AM, Hussein A, El-Naggar MY (2018) Statistically optimized ceftriaxone sodium biotransformation through *Achromobacter xylosoxidans* strain Cef6: an unusual insight for bioremediation. J Basic Microbiol 58:120–130. https://doi.org/10.1002/jobm.201700497
Batt AL, Aga DS (2005) Simultaneous analysis of multiple classes of antibiotics by ion trap LC/MS/MS for assessing surface water and groundwater contamination. Anal Chem 77:2940–2947. https://doi.org/10.1021/ac048512+
Bergero MF, Lucchesi GI (2015) Immobilization of *Pseudomonas putida* a (ATCC 12633) cells: a promising tool for effective degradation of quaternary ammonium compounds in industrial effluents. Int Biodeterior Biodegr 100:38–43. https://doi.org/10.1016/j.ibiod.2015.02.004
Chen KC, Chen CY, Peng JW, Houng JY (2002) Real-time control of an immobilized-cell reactor for wastewater treatment using ORP. Water Res 36(1):230–8. https://doi.org/10.1016/s0043-1354(01)00201-9
Chris Felshia S, AshwinKarthick N, Thilagam R, Gnanamani A (2018) Elucidation of 2,4-dichlorophenol degradation by *Bacillus licheniformis* strain SL10. Environ Techno 20:1–12. https://doi.org/10.1080/09593330.2018.1498923
Dike KS, Obiukwu CE, Ekwealor IA (2014) Optimization of nutritional parameters for the production of L-methionine from newly isolated *Bacillus cereus* strain. Microbiol Res J Int 14(11):1248–1261. https://doi.org/10.9734/BMRJ/2014/2444
El-Borai AM, Eltayeb KM, Mostafa AR, El-Aassar SA (2016) Optimization and Statistical Evaluation of Medium Components Affecting Crude Oil Biodegradation by Some Locally Isolated Bacteria. Egy Bot J 56 (3): 753-767. https://doi.org/10.21608/ejbo.2016.2733
El-Borai AM, Gadallah EE, El-Aassar SA, Beltagy EA (2022) Enhancement of β-1,3-1,4-Glucanase Production by Marine Halomonas meridiana ES021 via Statistical Optimization and Cell Immobilization. Egy J Aquat Biol Fish 26(6):781–802. https://doi.org/10.21608/EJABF.2022.276391
El-Saadony MT, El-Wafai NA, El-Fattah HA, Mahgoub SA (2019) Biosynthesis, optimization and characterization of silver nanoparticles using a soil isolate of *Bacillus pseudomycoides* MT32 and their antifungal activity against some pathogenic fungi. Adv Anim Vet Sci 7(4):238–249. https://doi.org/10.17582/journal.aavs/2019/7.4.238.249
Farag AM, Hassan SW, Beltagy EA, El-Shenawy MA (2015) Optimization of production of anti-tumor L-asparaginase by free and immobilized marine *Aspergillus terreus*. Egypt J Aquat Res 41(4):259–302. https://doi.org/10.1016/j.ejar.2015.10.002
Farag AM, Fawzy A, El-Naggar MY, Ghanem KM (2021) Biodegradation and enhancement of 2,4-dichlorophenol by marine halophilic *Bacillus subtilis* AAK. Egypt J Aquat Res 47(2):117–123. https://doi.org/10.1016/j.ejar.2021.04.005
Farag AM, El-Naggar MY, Ghanem KM (2022) 2, 4-Dichlorophenol biotransformation using immobilized marine halophilic *Bacillus subtilis* culture and laccase enzyme: application in wastewater treatment. J Genet Eng Biotechnol 20:134. https://doi.org/10.1186/s43141-022-00417-1
Favier L, Ungureanu CV, Simion AI, Bahrim G, Vial C (2021) Enhancing the biodegradation efficiency of a emergent refractory water pollutant by a bacterial isolate through a statistical process optimization approach. Process Saf Environ Prot 148:1133–1145. https://doi.org/10.1016/j.psep.2021.02.012
Feng LZ (2009) Research on the Biochemical Treatment of Penicillin Wastewater. Northeastern University, Shenyang, China
Fiedler G, Schneider C, Igbinosa EO, Kabisch J, Brinks E, Becker B, Stoll DA, Cho GS, Huch M, Franz CMAP (2019) Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. BMC Microbiol 19(1):250. https://doi.org/10.1186/s12866-019-1632-2
Guilford JP, Fruchter B (1973) Fundamental statistics in psychology and education. McGraw-Hill, New York, pp 154–155. https://api.semanticscholar.org/CorpusID:145224068
Hathroubi S, Fontaine-Gosselin SÈ, Tremblay YDN, Labrie J, Jacques M (2015) Subinhibitory concentrations of penicillin G induce biofilm formation by field isolates of *Actinobacillus pleuropneumoniae*. Vet Microbiol 179(3–4):277–286. https://doi.org/10.1016/j.vetmic.2015.06.011
Kamaz M, Wickramasinghe SR, Eswaranandam S, Zhang W, Jones SM, Watts MJ, Qian X (2019) Investigation into micropollutant removal from wastewaters by a membrane bioreactor. Int J Environ es PuRblic Health 16(8):1363. https://doi.org/10.3390/ijerph16081363
Kim C, Cho S, Kang S, Park Y, Yoon M, Lee J, No W, Kim J (2015) Prevalence, genetic diversity, and antibiotic resistance of *Bacillus cereus* isolated from Korean fermented soybean products. J Food Sci 80(1):M123–M128. https://doi.org/10.1111/1750-3841.12720
Kulik K, Lenart-Boroń A, Wyrzykowska K (2023) Impact of Antibiotic Pollution on the Bacterial Population within Surface Water with Special Focus on Mountain Rivers. Water 15(5):975. https://doi.org/10.3390/w15050975
Leng Y, Bao J, Chang G, Zheng H, Li X, Du J, Snow D (2016) Biotransformation of tetracycline by a novel bacterial strain *Stenotrophomonas maltophilia* DT1. J Hazard Mater 318:125–133. https://doi.org/10.1016/j.jhazmat.2016.06.053
Lin YH, Cheng YS (2020) Phenol degradation kinetics by free and immobilized *Pseudomonas putida* BCRC 14365 in batch and continuous-flow bioreactors. Processes 8:721. https://doi.org/10.3390/pr8060721
Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson BAV (2005) Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environ Sci Technol 15;39(10):3421–9. https://doi.org/10.1021/es048143z
Liu Y, Du J, Lai Q, Zeng R, Ye D, Xu J, Shao Z (2017) Proposal of nine novel species of the *Bacillus cereus* group. Int J Syst Evol Microbiol 67(8):2499–2508. https://doi.org/10.1099/ijsem.0.001821
Liu Z, Yang Y, Liu G, Fang J (2023) Study on a novel immobilized microbe pellets constructed with *Alcaligenes* sp. R3 and its ability to remove tetracycline. J Environ Chem Eng 11(2):109378. https://doi.org/10.1016/j.jece.2023.109378
Liyanage Y, Manage PM (2015) Evaluation of Amoxicillin and Sulfanomide removal by *B.cereus, E. ludwigii* and *Enterobacter* sp. Environ Nat Resour J 14: 4339-4343. https://ph02.tcithaijo.org/index.php/ennrj/article/view/68430
Lou L, Huang Q, Lou Y, Lu J, Hu B, Lin Q (2019) Adsorption and degradation in the removal of nonylphenol from water by cells immobilized on biochar. Chemosphere 228:676–684. https://doi.org/10.1016/j.chemosphere.2019.04.151

McArdell CS, Molnar E, Suter MJF, Giger W (2003) Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environ Sci Technol 37:5479–5486. https://doi.org/10.1021/es034368i

Michael-Kordatou I, Karaolia P, Fatta-Kassinos D (2018) The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. Water Res 129:208–230. https://doi.org/10.1016/j.watres.2017.10.007

Mo J, Zhang Z, Wang Z, Yu Z, Li S, Jiang S, Liu H, Ao J (2019) Isolation and identification of a psychrotolerant dimethyl phthalate-degrading bacterium from selected frozen soil of high-latitude areas in China and optimization of its fermentation conditions using response surface methodology. Biotechnol Biotechnolog Equip 33(1):1706–1720. https://doi.org/10.1080/13102818.2019.1696703

Mullai P, Vishali S (2007) Biodegradation of penicillin-G wastewater using *Phanerochate chrysosporium*–an equilibrium and kinetic modeling. Afr J Biotechnol 6(12):1450–4

Nas B, Argun ME, Dolu T, Ates H, Yel E, Koyuncu S, Dinç S, Kara M (2020) Occurrence, loadings and removal of EU-priority polycyclic aromatic hydrocarbons (PAHs) in wastewater and sludge by advanced biological treatment, stabilization pond and constructed wetland. J Environ Manag 268:110580. https://doi.org/10.1016/j.jenvman.2020.110580

Nguyen TD, Pham MH, Lam NH, Pham CQ, Le TD, Tran BM, Tra TV (2022) Amoxicillin degradation ability of *Bacillus cereus* C1 isolated from catfish pond sludge in Vietnam. Heliyon 8(11):e11688. https://doi.org/10.1016/j.heliyon.2022.e11688

Panigrahy N, Barik M, Sahoo RK, Sahoo NK (2020) Metabolic profile analysis and kinetics of *p*-cresol biodegradation by an indigenous *Pseudomonas citronellolis* NS1 isolated from coke oven wastewater. Int Biodeterior Biodegrad 147:104837. https://doi.org/10.1016/j.ibiod.2019.104837

Partovinia A, Rasekh B (2018) Review of immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environment. Crit Rev Env Sci Tec 48(1):1–38. https://doi.org/10.1080/10643389.2018.1439652

Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Biometrika 33(4):305–25

Ramakrishnan SV, Prakasham RS (2011) Microbial fermentation with immobilized cells. Curr Sci 77:87–100

Reis AC, Kolvenbach BA, Nunes OC, Corvini PFX (2020) Biodegradation of Antibiotics: The New Resistance Determinants—Part I. New Biotechnol 54:34–51. https://doi.org/10.1016/j.nbt.2019.08.002

Rivas-Sanchez A, Cruz-Cruz A, Gallareta-Olivares G, González-González RB, Parra-Saldívar R, Iqbal HM (2022) Carbon-based nanocomposite materials with multifunctional attributes for environmental remediation of emerging pollutants. Chemosphere 303(Pt 1):135054. https://doi.org/10.1016/j.chemosphere.2022.135054

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York

Shao S, Hu Y, Chen C, Cheng J, Chen Y (2018) Simultaneous degradation of tetracycline and denitrification by a novel bacterium, *Klebsiella* sp. SQY5. Chemosphere 209:35–43. https://doi.org/10.1016/j.chemosphere.2018.06.093

Sudha S, Parthasarathi N, Prabha D, Velmurugan P, Sivakumar S, Anitha V, Shrestha A, Chinnathambi A, Alharb SA (2022) Lakshmanaperumalsamy P (2022) Oxytetracycline Degrading Potential of *Lysinibacillus* sp. Strain 3+I Isolated from Poultry Manure. Evid Based Complement Alternat Med 24:2750009. https://doi.org/10.1155/2022/2750009

Tan H, Kong D, Ma Q, Li Q, Zhou Y, Jiang X, Wang Z, Parales RE, Ruan Z (2022) Biodegradation of Tetracycline Antibiotics by the Yeast Strain *Cutaneotrichosporon dermatis* M503. Microorganisms 10(3):565. https://doi.org/10.3390/microorganisms10030565

Van TTH, Yidana Z, Smooker PM, Coloe PJ (2020) Antibiotic Use in Food Animals Worldwide, with a Focus on Africa: Pluses and Minuses. J Glob Antimicrob Resist 20:170–177. https://doi.org/10.1016/j.jgar.2019.07.031

Wang P, Liu H, Fu H, Cheng X, Wang B, Cheng Q, Zhang J, Zou P (2015) Characterization and mechanism analysis of penicillin G biodegradation with *Klebsiella pneumoniae* Z1 isolated from waste penicillin bacterial residue. J Ind Eng Chem 27:50–8. https://doi.org/10.1016/j.jiec.2014.12.018

Wang P, Liu S, Wang X et al (2021) Assessment of the efficiency of synergistic photocatalysis on penicillin G biodegradation by whole cell *Paracoccus* sp. J Biol Eng 15:25. https://doi.org/10.1186/s13036-021-00275-4

Wang P, Shen C, Wang X, Liu S, Li L, Guo J (2020) Biodegradation of penicillin G from industrial bacteria residue by immobilized cells of *Paracoccus* sp. KDSPL-02 through continuous expanded bed adsorption bioreactor. J Biol Eng 14:5. https://doi.org/10.1186/s13036-020-0229-5

Wu X, Xiong J, Huang Z, Cao S, Zong M, Lou W (2019) Improving biocatalysis of cefaclor with penicillin acylase immobilized on magnetic nanocrystalline cellulose in deep eutectic solvent based co-solvent. Bioresour Technol 288:121548. https://doi.org/10.1016/j.biortech.2019.121548

Xia M, Niu Q, Qu X, Zhang C, Qu X, Li H, Yang C (2023) Simultaneous adsorption and biodegradation of oxytetracycline in wastewater by *Mycolicibacterium* sp. immobilized on magnetic biochar. Environ Pollution 339:122728. https://doi.org/10.1016/j.envpol.2023.122728

Yang X, Li M, Guo P, Li H, Hu Z, Liu X, Zhang Q (2019) Isolation, Screening, and Characterization of Antibiotic-Degrading Bacteria for Penicillin V Potassium (PVK) from Soil on a Pig Farm. Int J Environ Res Public Health 16(12):2166. https://doi.org/10.3390/ijerph16122166

Yang X, Guo P, Li M, Li H, Hu Z, Liu X, Zhang Q (2020) Optimization of Culture Conditions for Amoxicillin Degrading Bacteria Screened from Pig Manure. Int J Environ Res Public Health 17(6):1973. https://doi.org/10.3390/ijerph17061973

Yin Z, Xia D, Shen M, Zhu D, Cai H, Wu M, Zhu Q, Kang Y (2020) Tetracycline degradation by *Klebsiella* sp. strain TR5: Proposed degradation pathway and possible genes involved. Chemosphere 253:126729. https://doi.org/10.1016/j.chemosphere.2020.126729

Zhang S, Wang J (2021) Removal of chlortetracycline from water by immobilized *Bacillus subtilis* on honeysuckle residue derived biochar. Res Square. https://doi.org/10.21203/rs.3.rs-400808/v1

Zhao F, Yang L, Chen L, Xiang Q, Li S, Sun L, Yu X, Fang L (2019) Soil contamination with antibiotics in a typical peri-urban area in eastern China: seasonal variation, risk assessment, and microbial responses. J Environ Sci 79:200–212. https://doi.org/10.1016/j.jes.2018.11.024

Zur J, Wojcjeszynska D, Guzik U (2016) Metabolic responses of bacterial cells to immobilization. Molecules 21(7):958. https://doi.org/10.3390/molecules21070958

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