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Assistance of aminated straw on enhanced biodegradation of phenol through bacteria and potential prediction of machine learning for bioremediation

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ABSTRACT

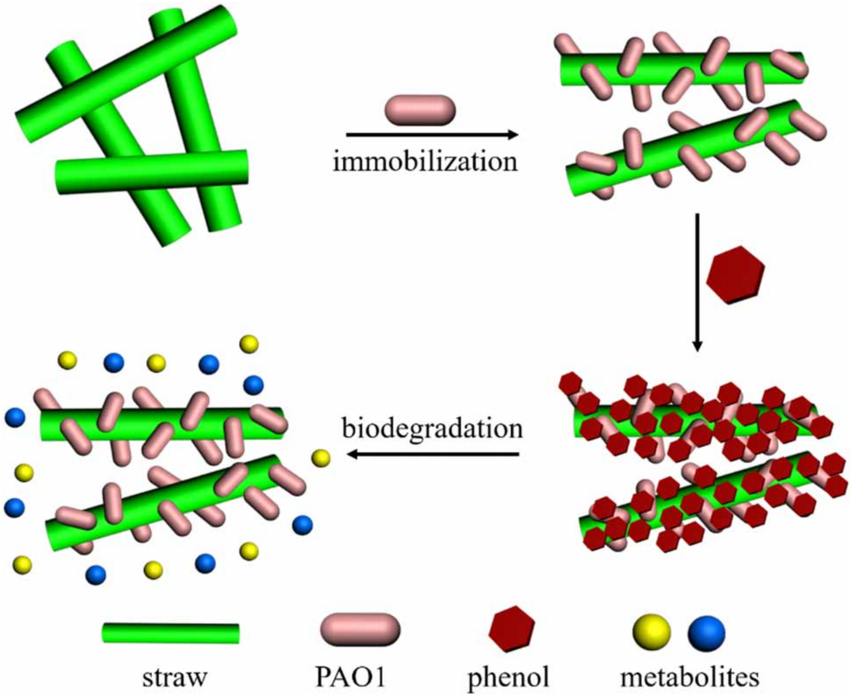
How to treat phenol-containing wastewater harmlessly is an urgent problem to be solved. In this study, a kind of biomaterial was prepared through strain PAO1 immobilized on aminated straw to enhance the phenol removal rate and efficiency. The aminated straw assisted PAO1 to increase the phenol degraded concentration from 1,900 to 1,500 mg/L, and shorten the degraded time by 44 h at 1,500 mg/L phenol. The immobilized PAO1 could remove phenol at pH 10 and 11, which was 2.7 and 3.8 times higher than free bacteria, respectively. In addition, the immobilized PAO1 could totally remove phenol, which was twice as high as that of free bacteria, at 4% NaCl stress. Furthermore, the removal efficiency of immobilized PAO1 was higher than that of free bacteria under the stress of various metal ions, especially for Co^{2+} and Pb^{2+}. The determination coefficients R^2 and root mean square error showed that the back propagation artificial neural network model could predict the degradation of phenol under various conditions, saving time and economic cost. The present study envisions that this biomaterial has great potential in the bioremediation of organic pollutions.

Key words: biodegradation, machine learning, phenol, straw

HIGHLIGHTS

- Straw-assisted PAO1 to increase phenol concentration and shorten time for degradation.
- Aminated straw helped strains degrade phenol in harmful environments.
- BP-ANN predicted the degradation of phenol under different conditions.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The pollution of phenol is urgent to effectively be solved, due to its high biological toxicity and wide pollution area which seriously threatens human health and the diversity of biological species (Barik 2021). The microbial method is considered as a promising treatment because of its economical and thoroughness of degradation features (Iqbal *et al.* 2018). There are many environmental microorganisms which are capable of degrading for phenol (Shahryari *et al.* 2018; Panigrahy *et al.* 2020; Zhao *et al.* 2021). Generally, those microorganisms face problems such as low tolerance concentration, long degradation period, degradation and survival ability decrease with the increase in external pollutant concentration (Long *et al.* 2019; Duraisamy *et al.* 2020; Nouri *et al.* 2020; Wen *et al.* 2020; Gong *et al.* 2021). In addition, the existence of complicated pH, high salt concentration and numerous metal ions in the wastewater inhibit the survival of microorganisms (Mei *et al.* 2019; Xu *et al.* 2021). Therefore, it is very necessary to develop strategies that can not only remove high concentrations of phenol but also maintain or improve microbial viability and degradation efficiency in harsh wastewater.

Recently, the immobilization of microorganisms was used to fix strains on the carriers to protect microorganisms from the toxicity of high concentration pollutants, so as to improve their ability to withstand harsh conditions. However, most studies focused on the concentration of phenol up to 1,500 mg/L, high concentrations have been rarely reported (Bera & Mohanty 2020; Dong *et al* 2020; Zhao *et al* 2020). Biomass and its derived materials have been applied as carriers of microorganisms, due to their economical features, excellent mechanical strength and structural stability. Cashew apple bagasse was applied to immobilize *Candida tropicalis* ATCC 750 to biodegrade phenol, showing that the adapted and immobilized strain was more resistant to higher phenol concentration than the free cell (Silva *et al.* 2019). Areca nut husks and luffa sponge fibers immobilizing bacterial consortium could degrade 1,000 mg/L phenol in 28 and 30 h, respectively (Bera & Mohanty 2020). Currently, most studies focus on the concentration of phenol at 250–1,500 mg/L, while high concentrations have rarely been reported. Due to the complexity of the actual wastewater, associated with various pH, heavy metals and salts, numerous verifications are required during the actual treatment process to determine the optimal conditions, which results in a waste of time and economic cost. Back propagation artificial neural network (BP-ANN), as a kind of machine learning method, could recognize complex non-linear relationships between input and output data sets, which further estimate output values referring to training and learning processes (Bao *et al.* 2019; Zhang *et al.* 2024). BP-ANN can not only solve the complicated biodegraded problems but can also be applied to predict the optimization of degraded conditions. BP-ANN has been developed for the prediction of soil heavy metals and persistent organic pollutants. However, few studies on immobilized microorganisms for biodegradation of phenol by the usage of BP-ANN have been reported, which is in great demand for savings of time and cost.

Herein, a kind of biomaterial was prepared by the electrostatic interaction between the negative *Pseudomonas aeruginosa* PAO1 and positive aminated straw, to apply on phenol degradation (Figure 1). This study aimed to (1) investigate the degraded rate and efficiency at high concentrations of phenol by immobilized PAO1; (2) verify the degraded ability of immobilized PAO1 in harsh environments; (3) determine the predictive ability of the BP-ANN model in predicting phenol biodegradation. This study provides an alternative for biodegradation of phenol wastewater, which offers predictions of phenol remediation in complicated surroundings.

2. MATERIAL AND METHODS

2.1. Chemicals

The corn straw was from Jilin Agricultural University. Triethylamine (99%, v/v) and ethylenediamine (99%, v/v) were from Beijing Chemical Works. *N*, *N*-dimethylformamide (DMF) (99.5%, v/v), $Cr_2(SO_4)_3$ and $K_2Cr_2O_7$, were the products of Sinopharm Chemical Reagent Co. Ltd. Potassium ferricyanide (99.5%, w/w), $CoCl_2$ and $HgCl_2$ were the products of Tianjin Kermel Chemical Reagents Development Centre. $CdCl_2$, $NiCl_2$, Ag_2SO_4 and $ZnCl_2$ were from Shanghai Macklin Biochemical Co. Ltd. Phenol (99%, w/v), epichlorohydrin (99%, v/v), 4-aminoantipyrine (98.5%, w/w), $MnCl_2$, $CuCl_2$, and $Pb(NO_3)_2$ were bought from Tianjin Hengxing Chemical Preparation Co. Ltd, Xi'an Regent, Shanghai Yuanye Biotechnology Co. Ltd, Beijing Chemical Works and Xilong Chemical Co. Ltd, respectively. *Pseudomonas aeruginosa* PAO1 was provided by the Pathogenic Microbiology Team, College of Life Sciences, Northwest University, China.

2.2. The preparation of aminated straw

After drying and crushing, the straw was screened with a particle size of about 75 μm, washed with distilled water until the filtrate was clarified, and dried at 65 °C in an oven for 24 h. Then, the straw was immersed into an excess of NaOH (1 mol/L), stirred for 60 min, followed by filtering and washing until the filtrate was neutral, and dried to constant weight to obtain alkali pretreatment straw. Straw (4 g), epiclorohydrin (20 mL, 99% wt) and DMF (20 mL, 99% wt) were placed together and heated

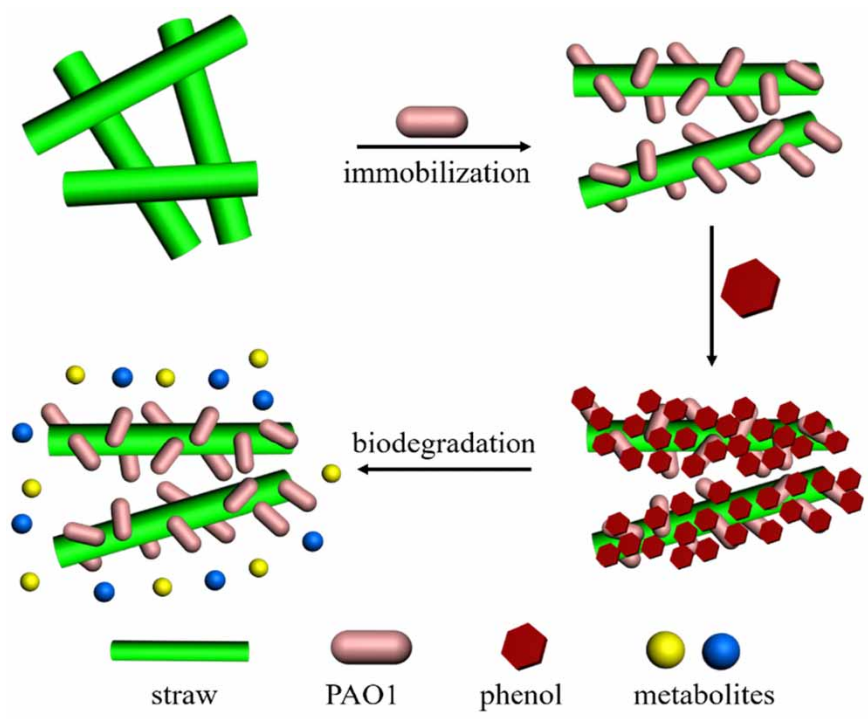


Figure 1 | The schematic drawings of PAO1 immobilized on straw for phenol degradation.

to 85 °C with stirring for 60 min, followed with the addition of triethylamine (20 mL, 99% wt) for another 60 min. After cooling to room temperature, the straw was cleaned with anhydrous ethanol three times, and then washed with distilled water until the filtrate was clarified and neutral, and then dried at 80 °C to constant weight to obtain the amine-modified straw.

2.3. Culture, acclimation and degradation of phenol of PAO1

Luria-Bertani medium (LB: tryptone 10 g/L, NaCl 10 g/L and yeast extract 5 g/L) and minimal salt medium (MSM: $MgSO_4 \cdot 7H_2O$ 0.2 g/L, $CaCl_2 \cdot 2H_2O$ 0.02 g/L, KH_2PO_4 1.0 g/L, K_2HPO_4 1 g/L, NH_4NO_3 1 g/L and $FeCl_3$ 0.05 g/L) were used for activation and acclimation, respectively. The above medium was adjusted by 6 mol/L NaOH or HCl to pH 7.0–7.2, then sterilized at 115 °C under high temperature and pressure for 20 min, and cooled to room temperature before use. When using solid medium, 1.5–2% agar can be added.

The strain was cultured in MSM with phenol as the sole carbon source, and the phenol concentration was continuously increased each time (250 mg/L) to achieve maximum biodegradation (about 1,500 mg/L). Then, the strains were inoculated (10%, v/v) into MSM (phenol, 1,500 mg/L), the above operations were repeated at 37 °C and 180 rpm to shorten the degradation time, until degradation was stable. The concentration of phenol was detected by the 4-aminoantipyrine method (Baird *et al.* 2017).

2.4. The biodegradation of phenol by immobilized strains

The fermentation broth of PAO1 was centrifuged at 5,000 rpm at 4 °C for 20 min. Then, the supernatant was discarded, washed with sterile water and centrifuged again, and repeated three times. After that, the strains were suspended with sterile water, with an optical density value of 1.0 ± 0.11. The linear relationship between the number of bacteria and the optical density (OD) value can be obtained by the plate dilution coating culture method, as shown in Supplementary material, Figure S1. PAO1 bacterial suspension and aminated straw were immobilized at the ratio of solid to liquid 1:100 at 30 °C and 120 rpm for 6 h and repeated three times. After fixation, 300-mesh nylon cloth was used for filtration, and the OD600 value of filtrate was determined. The fixed number of bacteria could be obtained according to Supplementary material, Figure S1 and the OD600 difference of bacterial suspension before and after fixation. After fixation, nylon cloth with 300-mesh was used for filtration. According to the solid–liquid ratio of 1:100, the immobilized material was added into the MSM containing phenol to perform the phenol degradation experiment at 37 °C and 180 rpm.

2.5. The effect of pH

The sterilized MSM was adjusted to pH 5, 6, 7, 8, 9, 10 and 11, with 6 mol/L NaOH or HCl, followed by the addition of 1,000 mg/L phenol. The immobilized material was treated with the solid–liquid ratio 1:100, and cultured at 37 °C and 180 rpm.

2.6. The effect of NaCl concentration

The MSM with 1,000 mg/L phenol was added to 0, 1, 2, 3, 4, 5, 6 and 7% NaCl, respectively, and autoclaved at 121 °C for 20 min. The pH was adjusted to 7.0–7.2. The cultural conditions were the same as before.

2.7. The effect of metal ions

Heavy metal ions Cr^{3+}, Cr^{6+}, Ag^{+}, Co^{2+}, Mn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+} and Ni^{2+} were added to MSM, respectively, where the concentration of heavy metal salt ions was 5 mmol/L. The cultural conditions were the same as before.

2.8. Back propagation artificial neural network

According to the factors affecting the biodegradation of phenol, including phenol concentration (1,000, 1,500, 1,750, 1,800, 1,900 and 2,000 mg/L), pH (5, 6, 7, 8, 9, 10 and 11), NaCl concentration (0, 1, 2, 3, 4, 5, 6 and 7%) and time, with three repetitions per group (total of 295 groups), a BP-ANN model was established to test and predict whether the expected value and empirical value was consistent. Every set of data contained the effective values of all variables in each ML model data set, which were excluded in the case of missing data. Here, the BP-ANN model used 286 sets of data and was randomly divided into a training group and a test group, according to a ratio of 9:1. In order to ensure the accuracy of the predicted data, the training group had 256 sets of data and the test group had 30 sets of data.

2.9. Measurements

Fourier transform infrared (FTIR) data were performed on a Bruker IFS66 V FTIR spectrometer (32 scans), using KBr pellets, and the spectra were recorded with a resolution of 4 cm^{-1}. Zeta potential data were recorded on a Malvern Instruments Zetasizer Nano ZS. Element analysis (EA) was carried out on a Flash EA1112 analyzer from ThermoQuest Italia S.P.A

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of aminated straw

Straw is a common agricultural waste, showing lots of functional groups on the microstructure, such as carboxyl and hydroxyl, which could be further modified. Herein, an aminated straw was prepared through the reaction of epichlorohydrin, ethylenediamine and triethylamine, which was pre-treated with NaOH for the exposure of reactive groups. The FTIR spectra showed that the strong absorption peak at 1,363 cm^{-1} was the characteristic peak of the C–N bond originating from aminated straw. The strong absorption peak at 1,463 cm^{-1} was assigned to the N–H bond, which was another covalent bond in the amine groups (Figure 2(a)). In addition, the peak at 863 cm^{-1} was attributed to the characteristic absorption peak of the C–Cl bond. This indicated that a large number of amine groups were introduced into the straw, which was beneficial to improve the adsorption ability (Xing *et al.* 2011; Ma *et al.* 2013). After modification, the charges of the straw would be changed, as shown in Figure 2(b). The zeta potential of the unmodified straw was negative even at pH 2, while the aminated straw was positive with the potential of 90.4 mV. The positive charges would be decreased with the increase in pH. The isoelectric point of the aminated straw was 10.1, much higher than that of the original straw, indicating that the surface of the modified straw contained more amine groups. EA results showed that the content of the N element was increased significantly after modification, indicating that more amine functional groups were introduced and consistent with the results of FTIR (Table 1).

3.2. The biodegradation of PAO1

Microbial treatment of phenol has become a hotspot in recent years, with the advantages of low cost, easy operation and relatively thorough removal. However, the problems for long range processing and low degradation concentration need in-depth study and solution. As shown in Figure 3(a), with the increase in phenol concentration, the time for phenol biodegradation was prolonged. The maximum degradation concentration of phenol by PAO1 was 1,500 mg/L, using 288 h. Microbial acclimation is when substrates are gradually added to the culture, which makes the microorganisms gradually adapt to the targeting environment, so as to improve their degraded ability. In order to shorten the degradation time of phenol by PAO1, the strain

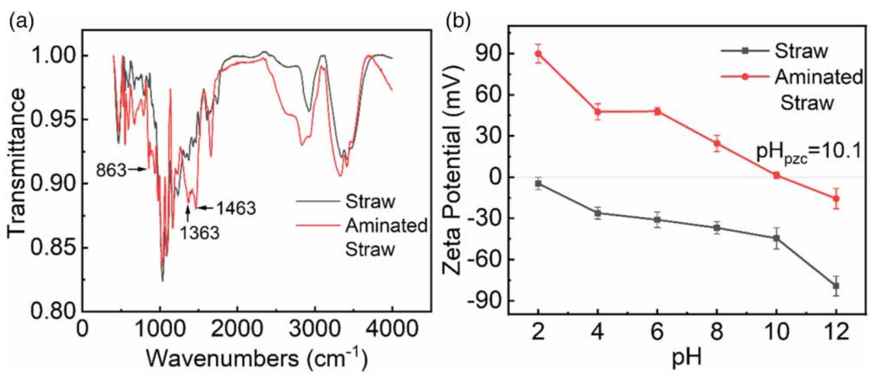


Figure 2 | (a) FTIR spectra and (b) zeta potential of original and aminated straw.

Table 1 | EA data of straw

Type of materials	Type of elements N (%)	C (%)	H (%)
Original straw	0.58	36.43	5.47
Aminated straw	3.69	35.82	7.49

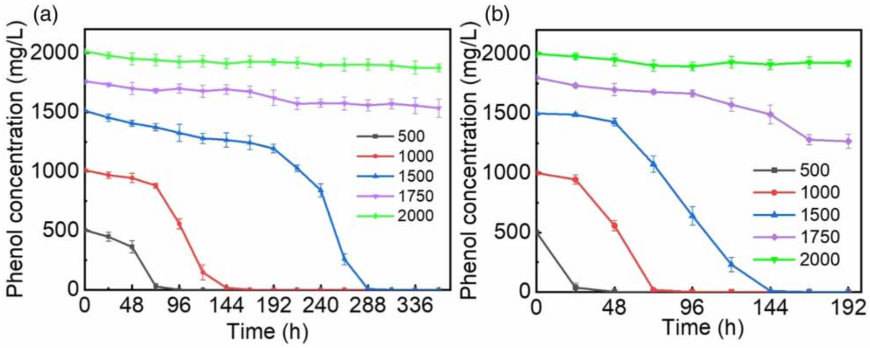


Figure 3 | Degradation of phenol by strain PAO1 (a) before and (b) after acclimation.

was acclimated at 1,500 mg/L phenol. When the concentration of phenol was degraded to 100–200 mg/L, it was used as the seed broth to degrade phenol for the next batch, which was repeated until the degraded time was stable. In this process, the external continuous stimulation of PAO1 with a high concentration of phenol improved the continuous expression of related degrading enzymes. After acclimation, the highest degradation concentration of PAO1 was still 1,500 mg/L, as shown in Figure 3(b), due to the limitations of structure of PAO1. The time for complete phenol degradation was decreased to 144 h at 1,500 mg/L, much faster than the original 288 h. Correspondingly, the degradation time of phenol at 500 and 1,000 mg/L was also significantly shortened. In addition, the lag phase was significantly shortened, indicating that the expression and synthesis of intracellular protective solutes were also improved, which effectively helped the bacteria to resist the external adverse environment.

3.3. The biodegradation of immobilized PAO1

Microorganism immobilization is a kind of biological technology, which can load microorganisms on the carrier stably, so that the microorganisms are highly dense and maintain high biological activity, and can proliferate rapidly under suitable conditions. Aminated straw can be used as an excellent immobilized carrier because of the graft of amino groups, showing positive charges on the surface, improving the adsorption capacity of bacteria. In this study, the bacteria were fixed on the aminated straw carrier through electrostatic interaction, with a number of 1.83×10^9 CFU/g, as shown in Supplementary material, Figure S1. The degradation of phenol by free and immobilized bacteria was studied at different initial concentrations of phenol. When the initial concentration of phenol was 1,500 mg/L, the degradation time of phenol by immobilized bacteria was 108 h, shortened by 36 h, compared with free bacteria (144 h), as shown in Figure 4. Aminated straw could adsorb some phenol, reducing its content and accelerating the degradation rate by immobilized bacteria. Besides, the decrease in phenol content also reduced its toxicity on the bacteria and increased the number of bacteria. The free bacteria could not degrade phenol, whose concentration was higher than 1,500 mg/L. The immobilized bacteria could degrade 1,900 mg/L phenol, improving 400 mg/L, in comparison with free bacteria. When the concentration of phenol reached 2,000 mg/L, only the aminated straw could adsorb phenol, PAO1 could not further remove phenol at this concentration. Overall, immobilized PAO1 can improve the degradation concentration and shorten the degradation time, which is largely related to the ability of aminated straw to adsorb phenol.

3.4. The effect of pH

In the actual application, pH, salt concentration and heavy metal ions of wastewater have great effects on the removal of phenol through biodegradation. Thus, the influences of pH were investigated. As shown in Figure 5, the immobilized and free bacteria are suitable for survival at pH 7–9. Herein, the degradation rate at pH 8 was the highest. Between pH 5–6, the degradation efficiency of phenol was almost unchanged over time, indicating that the free and immobilized bacteria could only remove part of the phenol in the interval, so it was not detected at 240 h. In the case of too much acid or base, the degradation rate was decreased, especially for the free bacteria, indicating that PAO1 was sensitive to pH. The degradation rate of free bacteria was low, at pH lower than 7, indicating that PAO1 was not suitable for survival under acidic conditions. In addition to the aminated straw, the immobilized PAO1 can adsorb and degrade phenol. The bacteria tend to survive in neutral or weakly alkaline conditions. Under the conditions of pH 7 and 8, phenol was completely removed

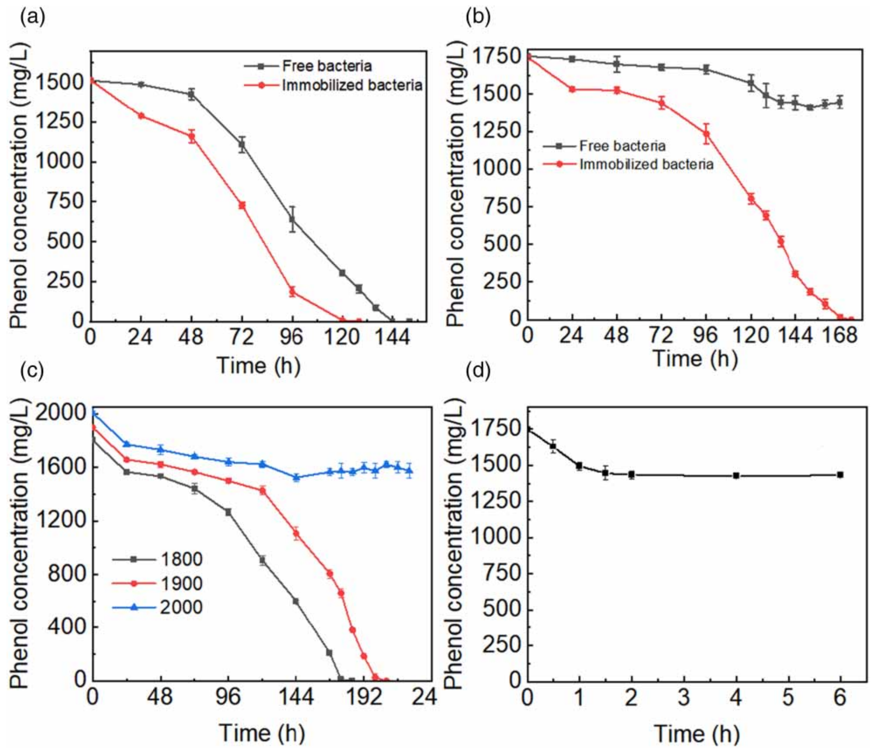


Figure 4 | Immobilized and free PAO1 for phenol degradation at concentration of (a) 1,500, (b) 1,750, and (c) 1,800, 1,900 and 2,000 mg/L, and (d) aminated straw for phenol adsorption.

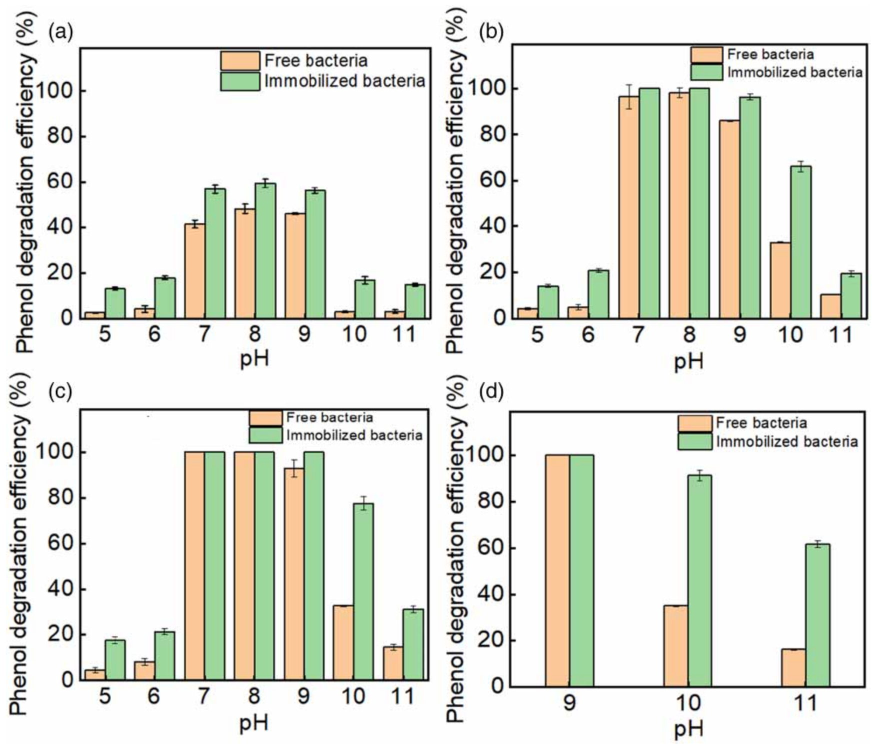


Figure 5 | The effect of pH on the degraded efficiency of phenol by free and immobilized PAO1 at (a) 48 h, (b) 72 h (c) 144 h and (d) 240 h, respectively.

after 144 h, so the subsequent detection was not carried out. In addition, the removal rate of phenol by immobilized bacteria was higher than that of free bacteria, proving that immobilized bacteria could effectively improve the degradation rate. The advantage of immobilized bacteria was exhibited, at pH of 10 and 11, which was much better than free bacteria. The removal efficiency of immobilized bacteria was 2.7 and 3.8 times higher than free bacteria at pH 10 and 11, respectively. The excellent physical and chemical properties of aminated straw with lots of carboxyl, amine and hydroxyl groups, reduced acid and base content in the wastewater, and promoted bacteria to survive in a neutral environment. In addition, a neutral environment was also conducive to maintaining high enzyme activity, which directly affected the biodegradation of phenol.

3.5. The effect of NaCl

Phenol-containing wastewater usually include a high content of salt. So far, most of the phenol degrading bacteria were not very good at salt tolerance. Herein, phenol degradation by free and immobilized bacteria under high NaCl concentration was studied. As shown in Figure 6, both immobilized and free bacteria can grow at a NaCl concentration of 0–3%, indicating that strain PAO1 has an excellent salt-tolerance ability. Therefore, this strain can be applied to several kinds of high-salt environments to deal with organic pollutants, especially in marine wastewater. Under the NaCl concentration of 4%, the phenol removal efficiency of immobilized PAO1 was higher than that of free bacteria at 144 and 240 h. The immobilized PAO1 could completely remove phenol, which was twice as high as that of free bacteria. The immobilized PAO1 can alleviate the stress by reducing NaCl concentration through adsorption so that the degradation of PAO1 can be exerted. However, under the double stress of NaCl and phenol, the phenol degradation efficiency was inhibited. When the NaCl concentration continued to increase, the phenol degradation by PAO1 was significantly weakened. It has been reported that the phenol biodegradation efficiency of free strains (33.6%) was lower compared with immobilized cells (99.9%) by NaO-Fe_3O_4 carrier at a salinity of 9%, because the carriers could prevent strains from being poisoned (Jiang *et al.* 2017). The increase in salt concentration resulted in the change of osmotic pressure of PAO1, which destroyed the cell membrane and inhibited the intracellular phenol degradation enzyme activity so that the strain could not degrade phenol and even could not survive. In addition, the anions in the high salt solution competed with immobilized PAO1 for the sites on the cationic straw taking positive charges on its surface. Thus, the straw loses the protective effect on the strain and inhibits the phenol degradation.

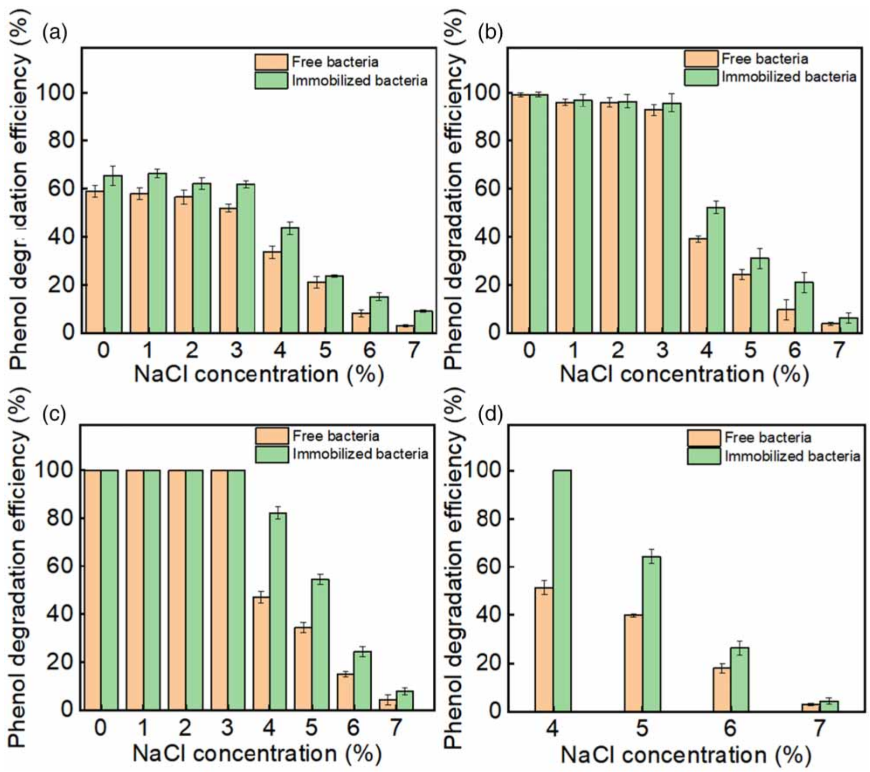


Figure 6 | The effect of NaCl on phenol biodegradation at (a) 48 h, (b) 72 h, (c) 144 h and (d) 240 h, respectively.

3.6. The effect of metal ions

Generally, the composition of phenol-containing wastewater is quite diversified, especially in the polluted seawater near the coastline. In addition to the presence of organic pollutants such as phenol, there are several kinds of heavy metal ions, which makes phenol-containing wastewater become difficult to degrade. Heavy metal ions such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Cr^{6+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+} and Co^{2+} were added to MSM to realize phenol removal. As shown in Figure 7, the removal efficiency of immobilized PAO1 was higher than that of free bacteria under the stress of metal ions. Among them, Mn^{2+} showed no toxicity to PAO1, whose removal efficiency was not different from that of control. The addition of Zn^{2+} has little effect on the biodegradation of phenol. Zn^{2+} is conducive to the formation of proteins with special spatial structures by bending and folding. The degradation of phenol is closely related to the synthesis of enzymes, which contributes to the rapid degradation of phenol. At 240 h, in the presence of Cu^{2+}, Co^{2+} and Pb^{2+}, the phenol removal efficiency of immobilized PAO1 was significantly higher than that of free bacteria. In the presence of Cu^{2+}, the immobilized PAO1 could completely remove phenol at 240 h, while the free bacteria could only remove 68.5% under the presence of Cu^{2+}. The main reason might be that Cu^{2+} could coordinate with the amine groups on the modified straw, resulting in a part of Cu^{2+} being absorbed on the straw (Zhang *et al.* 2024). Thus, the toxic effect of Cu^{2+} on the strain was weakened, which could be completely degraded phenol in a sufficient time. However, for Cr^{3+} and Cr^{6+}, the phenol degradation capacity of both free and immobilized PAO1 was significantly inhibited. It might be that the Cr^{3+} and Cr^{6+} were not conducive to the growth of the strain and inhibited the enzyme activity, so the complete degradation of phenol could not be achieved (Bing *et al.* 2024). Although the presence of cationic straw could adsorb part of the Cr^{3+} and Cr^{6+} and slow down its toxicity to the strain, the inhibition on the strain was particularly strong, so it significantly delayed the degradation of phenol. In addition, the phenol removal efficiency of immobilized PAO1 was nearly twice that of free bacteria, under the stress of Co^{2+} and Pb^{2+}. The aminated straw containing lots of amine groups could combine with heavy metal ions to remove them, thus weakening their toxicity on PAO1 and maintaining the degrading efficiency of bacteria.

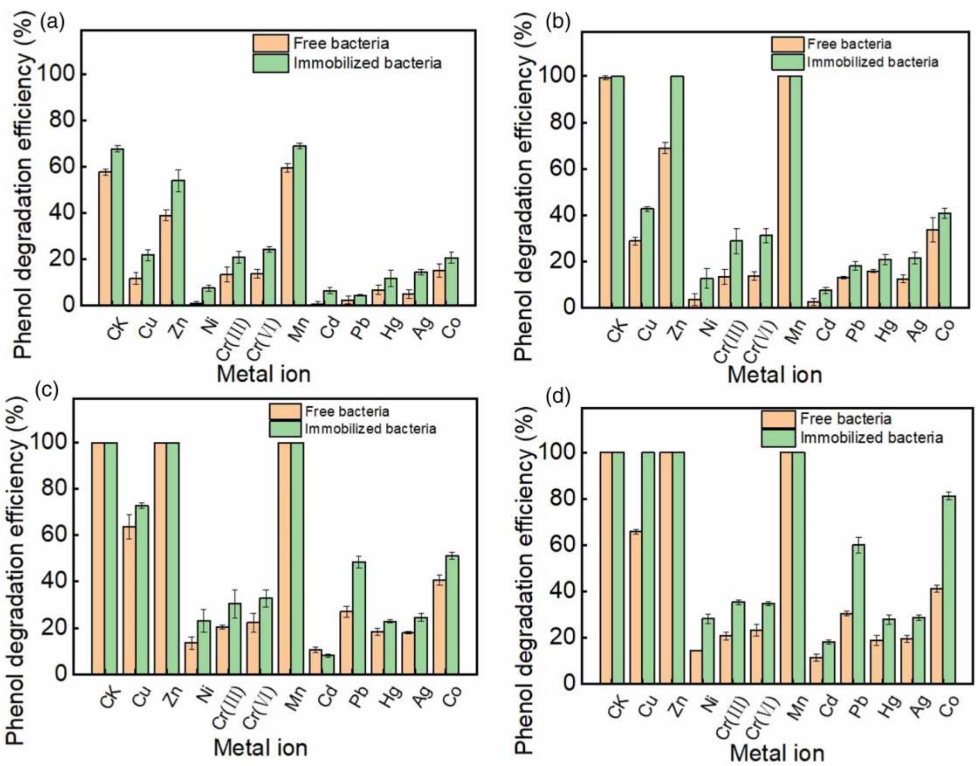


Figure 7 | The effect of metal ions on the phenol degradation at (a) 48 h, (b)72 h, (c)144 h and (d)240 h, respectively.

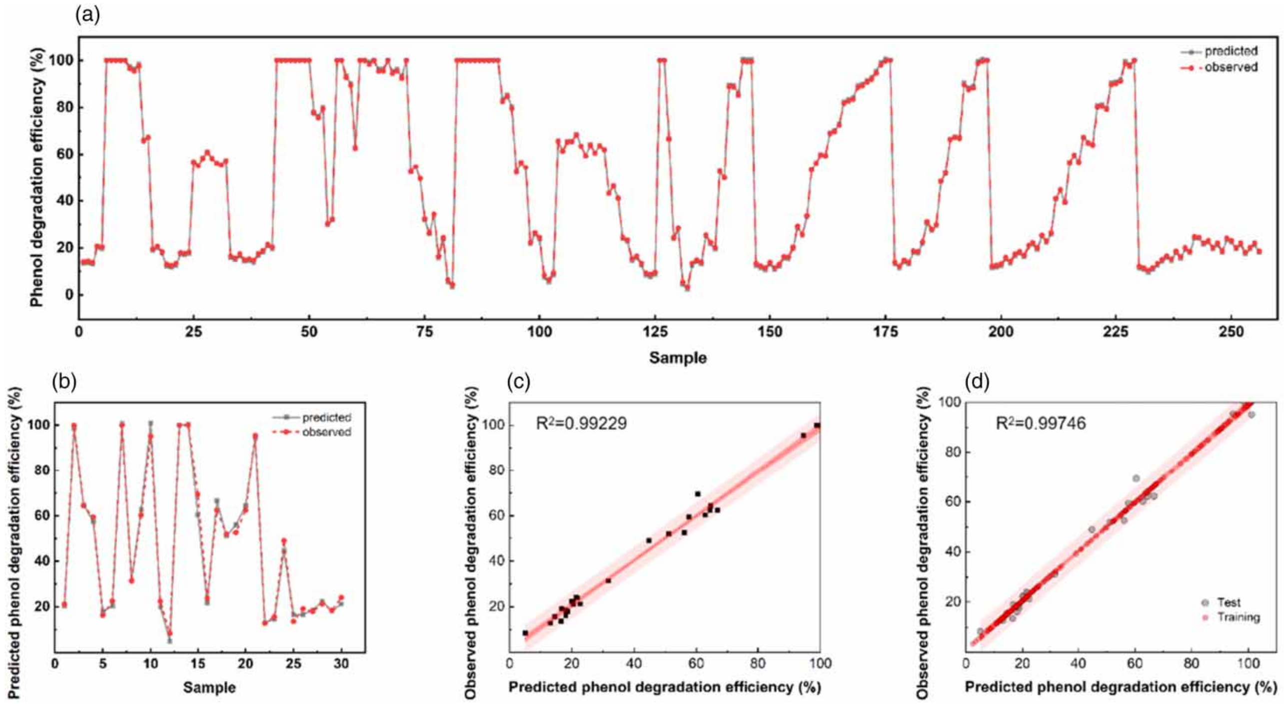


Figure 8 | Observed and predicted values obtained through the BP-ANN model in the (a) training, and (b) testing data set, and (c) the correlation between the observed and predicted values. The line represents the regression, and the shadow area represents the 95% confidence interval.

3.7. The prediction of phenol removal by PAO1-aminated straw

BP-ANN was used to predict the removal efficiency of phenol by aminated straw-PAO1. The phenol concentration, pH, NaCl concentration and time were used as input parameters, and the removal efficiency of phenol was used as output parameters. Herein, a total of 286 valid data sets were selected and randomly divided into training and test sets in a ratio of 9:1, to evaluate the ability of the BP-ANN model. Based on the regression coefficient (R^2) and root mean square error (RMSE) of the test set, when the number of hidden layer nodes of the model is 15, RMSE is the smallest (2.8066) and R^2 is 0.997, indicating that the model has better prediction ability under this hidden layer, and can be used as a fast tool for predicting phenol removal efficiency of aminated straw-PAO1. As shown in Figure 8, the predicted and experimental data are identical during the training and testing period. The model effectively avoids a large number of experimental attempts on complex wastewater in practical applications, which reduces time and economic costs. In order to better apply the BP-ANN model to the broader phenol removal process, a large data collection database needs to be established in future work.

4. CONCLUSION

In conclusion, a kind of biomaterial was prepared through strain PAO1 immobilized on aminated straw to enhance the phenol removal rate and efficiency. The aminated straw assisted the strain in increasing the phenol degraded concentration and shortening the degraded time. Compared with free bacteria, aminated straw helped the strain to degrade phenol in the external harmful environment. Furthermore, the degradation efficiency of phenol by the immobilized strains under different conditions was also predicted by BP-ANN, which could be applied to complex wastewater in practical applications, reducing time and economic costs. The present study envisioned that this biomaterial has great potential for the bioremediation of organic pollution.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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