Microbial degradation of organic pollutants in groundwater related to underground coal gasification

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Abstract
Groundwater pollution is regarded as one of the most serious environmental risks related to underground coal gasification (UCG). In this paper, two kinds of high efficient phenol degrading strains were isolated from activated sludges, which were obtained from coking wastewater and domestic sewage, and were named as JC and WX correspondingly. The isolated bacteria were identified by a combination method of physiological and biochemical analyses and 16SrDNA sequencing. The total organic carbon (TOC) and organic pollutants in gas washing water produced from the UCG model test of lignite were measured by TOC analyzer and gas chromatography-mass spectrometer (GC-MS), and the microbial degradation effect of gas washing water was finally studied by the isolated bacteria. The results reveal that JC and WX bacteria were pseudomonas aeruginosa and achromobacter xylosoxidans, respectively, and that phenolic compounds were the main organic pollutants in gas washing water, taking 95.01 percent of the total organics. The removal of TOC exceeded 72.9%, the degradation efficiency of total organic pollutants (TOM) achieved 79.03% and the degradation efficiency of phenolic compounds could be 98.76% by JC and 99.04% by WX, respectively. Most benzene series (BTEX) could not be degraded by the screened two kinds of bacteria, whereas the concentrations of BETX were increased. Some short-chain hydrocarbon compounds with low concentrations were also detected in wastewater after microbial degradation.

KEYWORDS
activated sludges, groundwater pollution, microbial degradation, phenolic compounds, underground coal gasification

1  INTRODUCTION

“Coal is not about to go away,”¹ as it is one of the major primary energies and constitutes approximately 65% of the fossil fuel reserves in the world,² and the coal consumption will cover 45% of the world energy needs by 2030.³ However, the traditional mining and utilization of coal resource have caused serious environmental problems such as generation of coal refuse, fly ash, particulate matters, SO2, CO2, NOx, etc, which result in the pollution of air, soil, surface water and groundwater.⁴,⁸ UCG is a clean coal technology which converts coal into combustible gas in situ. The produced
combustible gas can be centrally purified and subsequently used for electricity generation, hydrogen generation, chemical synthesis, etc eliminating most of the above mentioned environmental problems.\textsuperscript{9,10} UCG can be applicable for the exploitation of high ash and high sulfur coal seam, deep coal deposits, steeply dipping coal seams and the residual coal in abandoned coal mine.\textsuperscript{10-12} In addition, UCG has good economic benefit. Compared with surface gasifier, the cost of syngas produced by UCG is 1/2 to 1/4 of that produced by surface gasifier,\textsuperscript{13} the cost of natural gas substitutes produced by UCG is lowered by 10\%-18\% and the cost of power generation reduces by 27\%.\textsuperscript{14} Post-UCG sites hold high potential for carbon capture and storage (CCS).\textsuperscript{15} Based on the above mentioned benefits, UCG is regarded as one of the most innovative technologies associated with the exploitation of coal deposits.\textsuperscript{16}

Since the UCG process is conducted in underground coal seams, the produced coal gas together with organic pollutants and heavy metal vapours may migrate to aquifer through the pores and cracks in surrounding rocks and pollute the groundwater in aquifer. Meanwhile, the harmful substances in residues in combustion cavity will leach out with the invasion of groundwater into the cavity after UCG, thus also resulting in the contamination of groundwater.\textsuperscript{17} The groundwater pollution is viewed as one of the most serious environmental risks related to UCG. The main organic contaminants in the effluents and groundwater caused by UCG were phenolic compounds and aromatic hydrocarbons.\textsuperscript{18} Seventy kinds of inorganic pollutants, phenolic compounds, volatile and semi-volatile organics were detected in groundwater sampled from one UCG site.\textsuperscript{19} The high concentrations of inorganic and organic components, such as phenolic compounds, polycyclic aromatic hydrocarbons (PAHs) and BTEX, were detected in the ex situ UCG wastewater.\textsuperscript{20} In our earlier studies, phenolic compounds, PAHs and aliphatic hydrocarbons were also detected in gas washing water obtained from the gasification of three types of Chinese coal under laboratorial simulation conditions.\textsuperscript{21,22} Therefore, the removal of organic pollutants in polluted groundwater is very important for the protection of groundwater resource as well as the application and promotion of UCG technology.

The treatment methods for the organic contamination of groundwater include physical, chemical, physicochemical and biological treatments, of which the biological treatment is considered as one of the most promising methods due to the cost effectiveness and little impact on the environment. Bioremediation has been widely reported for the treatment of PAHs,\textsuperscript{23,24} other hydrocarbons,\textsuperscript{25,26} synthetic dyes\textsuperscript{27} and phenol in industrial effluents.\textsuperscript{28} But relevant information or study on the bioremediation of polluted groundwater associated with UCG was scarce. The aim of this study is to seek suitable bacteria which can be used for the remediation of organic contaminants in groundwater caused by UCG and to examine the degradation effect.

\section*{2 | MATERIALS AND METHODS}

\subsection*{2.1 | Isolation and screening and identification of phenol degrading bacteria}

Cultivation and domestication of activated sludge is a vital step for the microbial degradation of organic pollutants. Since phenolic compounds are the main organic contaminants in groundwater caused by UCG,\textsuperscript{17,20,29} bacteria capable of degrading phenolic compounds should be cultivated and domesticated in this study.

In this paper, two strains named W and J were isolated and screened from two kinds of activated sludges, which were obtained from the oxidation ditch of a sewage treatment plant and the aeration tank of a coking wastewater treatment plant correspondingly. Using phenol as the sole carbon source, the cultivation and domestication of activated sludge were performed in marine mineral cultures, which were composed of 0.5 g/L of K$_2$HPO$_4$, 0.5 g/L of KH$_2$PO$_4$, 0.2 g/L of NaCl, 0.5 g/L of MgSO$_4$·7H$_2$O, 0.1 g/L of CaCl$_2$, 0.01 g/L of FeSO$_4$·7H$_2$O, 0.01 g/L of MnSO$_4$ and 1 g/L of NH$_4$NO$_3$.

The cultivation and domestication process was as follows: (a) 10 millilitre (mL) of activated sludge were separately pipetted and added into test tubes containing the above marine mineral culture with phenol concentration of 500 mg/L, and domesticated in an incubator shaker with shaking speed of 120 rpm at 40°C; (b) then 10 mL of the above culture solution were pipetted and added into another test tube containing marine mineral culture with phenol concentration of 800 mg/L and domesticated under the same conditions as step (a); (c) by the above method, the cultivation and domestication of activated sludge were sequentially conducted in a series of marine mineral cultures containing phenol solution with higher concentrations of 1000, 1200, 1500, 2000 mg/L. An ultraviolet and visible spectrophotometer (Tu1810: Beijing Persee Universal Apparatus LLC) was applied to measure the absorbance of phenol in culture solution at wavelength of 270 nm,\textsuperscript{30} the concentration of phenol could be calculated from the known calibration curve according to the measured absorbance, and then the corresponding phenol degradation efficiency could be also calculated. Finally, the phenol degrading strains were obtained from the two kinds of activated sludges and designated as W and J bacteria, respectively.
2.1.2 | Isolation and purification of mixed bacteria

The W and J bacteria are both mixed strains. In order to screen the high efficient phenol degrading bacteria, the mixed bacteria must be further isolated and purified.

Firstly, 1 mL of bacteria solution taken from the marine mineral culture with initial phenol concentration of 2000 mg/L was introduced into a test tube and diluted 10 times with sterile water. Secondly, 1 mL of the above diluted bacteria solution was transferred into another test tube and well mixed with 9 mL of sterile water, thus the bacteria solution was diluted by a factor of 10^2. The bacteria solution was repeatedly diluted by this method until it was diluted by a factor of 10^8. Thirdly, the bacteria solutions diluted by a factor of 10^6, 10^7 and 10^8 were separately coated on glass plates, which were covered by isolated culture medium comprising 3 g/L of beef extract, 10 g/L of peptone, 5 g/L of NaCl, 20 g/L of agar and 300 mg/L of phenol, and then the glass plates with bacteria solutions were put in an incubator at constant temperature of 37°C and cultured for 48 hours. Fourthly, single colony was selected by streak plate method for further isolation. In order to obtain the purified strain, the selected bacteria were continuously transferred and cultured for three times. All the above operations were carried out under aseptic conditions. Finally, the species of selected bacteria were identified according to the colony shape features and gram staining observation.

2.1.3 | Screening of bacterium with high phenol degradation efficiency

The cultivation and domestication of activated sludge as well as the isolation and purification of mixed bacteria just indicated that the bacteria could survive in culture solution with high phenol concentration of 2000 mg/L, but bacterium which can efficiently degrade phenol needs to be examined. Therefore, the screening of bacterium with high phenol degradation efficiency was performed.

Firstly, the isolated and purified bacteria were continuously cultivated for 30 hours in separate marine mineral culture with phenol concentration of 300 mg/L. Afterwards, 10 mL of the bacterial solutions were separately pipetted into a set of marine mineral culture with phenol concentrations of 500, 1000, 1500 and 2000 mg/L and were cultivated at 40°C and 120 rpm. The phenol concentrations at different incubation times were measured and the corresponding phenol degradation efficiencies were calculated according to the method described in Section 2.1.1. Based on the phenol degradation efficiency, two kinds of bacterium with high phenol degradation efficiency were separately screened from J bacteria and W bacteria.

2.1.4 | Identification of bacterium with high phenol degradation efficiency

Because of different enzyme systems, different species of bacteria digest different nutriments, thus producing various metabolites. In order to identify the species of the screened bacteria, the physiological and biochemical tests and 16SrDNA sequencing were conducted.

1. Physiological and biochemical tests

The physiological and biochemical tests performed in this research included gelatin liquefaction test, starch hydrolysis test, methyl red test, Voges-Proskauer test, catalase test, indole test, nitrate reduction test and oxidase test. The gelatin culture medium was composed of 100 mL of beef extract peptone fluid and 12 g of gelatin.

The reagents for starch hydrolysis test included starch medium consisting of 10 g/L of peptone, 5 g/L of NaCl, 20 g/L of agar, 5 g/L of beef extract and 2 g/L of soluble starch and lugol iodic fluid comprising 1 g of iodine crystals, 2 g of potassium iodide and 300 mL of distilled water.

The methyl red test utilized glucose peptone water medium consisting of 5 g/L of peptone, 5 g/L of glucose and 5 g/L of K_2HPO_4 and methyl red reagent comprising 0.04 g of methyl red, 60 mL of 95% ethanol and 40 mL of distilled water.

The Voges-Proskauer test utilized the same glucose peptone water medium as that used for methyl red test and KOH (40%) and 5% a-naphthol solution (5 g of a-naphthol dissolved in 100 mL of absolute ethyl alcohol).

The catalase test used 3% hydrogen peroxide solution.

Indole test utilized pancreatic protein water medium consisting of 10 g of tryptone, 5 g of NaCl, one litre of distilled water and kovacs comprising 2 g of p-dimethylamino-benzaldehyde, 190 mL of 95% ethanol and 40 mL of concentrated hydrochloric acid.

Nitrate reduction test utilized the nitrate medium consisting of 10 g of peptone, 0.2 g of potassium nitrate and one litre of distilled water and two kinds of nitrate reducing reagents (A and B). Reagent A was composed of 0.8 g of p-aminobenzene sulfonic acid and 100 mL of 5 mol/L acetic acid, and reagent B was composed of 0.5 g of a-naphthylamine and 100 mL of 5 mol/L acetic acid.

The reagents for oxidase test included 1% dimethyl-p-phenylenediamine hydrochloride (0.1 g of dimethyl-p-phenylenediamine hydrochloride dissolved in 10 mL of distilled water) and 1% a-naphthol-ethanol solution (0.1 g of a-naphthol dissolved in 10 mL of 95% ethanol).
2. 16SrDNA sequencing

16SrDNA sequencing of the screened bacteria was conducted and with reference to GenBank, the species were finally identified according to the sequencing results.

2.2 | Model experiment of UCG

2.2.1 | Experimental system of UCG

The schematic view of UCG model test system applied in this experiment is presented in Figure 1. It is composed of four parts: (a) gasification agent supplying unit including air compressor, oxygen cylinder, steam generator and cistern, (b) gasifier unit, (c) coal gas processing and analysis unit including scrubber, dehumidifier and gas analyzer, and (d) temperature and pressure control unit including thermocouples denoted as No. 1-10, temperature sensor denoted as T, pressure sensor denoted as P and T&P control cabinet. The simulated coal seam of 800 × 400×400 mm dimensions is piled up with coal blocks in size of approximately 200 × 200 × 200 mm.

The vertical injection well and production well are inter- connected by a horizontal channel drilled in the coal seam at the base of the wells. By this experimental system, the simulations of UCG process can be performed by using gasification agents like air, oxygen and steam as well as their mixtures in different volumetric ratios.

2.2.2 | UCG model test

Coal blocks used in this experiment were obtained from Inner Mongolia, China. The results of the proximate and ultimate analyses of coal samples are presented in Table 1.

After completing the construction of simulated coal seam and the assembly of the experimental setup, coal blocks around the bottom of the injection well were ignited by char-coal in contact with oxygen which was injected through the injection well. After the successful ignition, oxygen was replaced by oxygen-enriched air with oxygen volume fraction of 24.7%, coal seams in oxidation zone kept burning until the temperature of oxidation zone exceeded 1000°C and maintained for some time in order to accumulate sufficient heat for the subsequent steam gasification process. Oxygen-enriched air was then replaced by steam, and the coal in high temperature zone was gasified by steam. The produced gas was discharged through the production well and purified by the coal gas processing and analysis unit. Due to the endothermic reactions during the steam gasification process, the temperature in gasification zone dropped. When the temperature dropped to about 700°C, the oxygen-enriched air would be injected to burn the coal seam again. Meanwhile, the UCG process went to the next cycle. By this oxygen-enriched air/steam two stage gasification method, the simulated gasification of lignite was completed.

In the UCG process, gas washing water produced at the steam gasification stage was collected for further microbial degradation experiment.

| No. | Composition (analytical) | Value (wt, %) |
|-----|-------------------------|--------------|
| 1   | Moisture, M_{ad}        | 13.69        |
| 2   | Ash, A_d               | 13.94        |
| 3   | Volatiles, V_{daf}     | 49.73        |
| 4   | Total Sulfur, S_{td}   | 1.03         |
| 5   | Carbon, C_{daf}        | 76.06        |
| 6   | Hydrogen, H_{daf}      | 5.25         |
| 7   | Nitrogen, N_{daf}      | 1.37         |
| 8   | Oxygen, O_{daf}        | 16.12        |

*aad-air dried basis; d-dry basis; daf-dry ash-free basis; O_{daf}-by subtraction method.*
2.3 | Microbial degradation of organic pollutants in gas washing water

2.3.1 | Determination of TOC and organic pollutants in gas washing water

Total organic carbon and the organic components in gas washing water were measured by TOC analyzer (Apollo 9000; Teledyne Tekmar) and GC-MS (HP7890/5975; Agilent), respectively. As for the determination of the concentration of organic pollutants by GC-MS, external standard method was applied.

Before the determination of pollutants in gas washing water, a filter membrane with mean pore size of 0.45 μm was used in order to remove the solid particles.

2.3.2 | Optimum conditions for the growth of bacteria with high phenol degradation efficiency

Before studies of the microbial degradation of UCG gas washing water, experiments were carried out to investigate the optimum conditions favourable for the growth of high efficient phenol-degrading bacteria. The experimental parameters including temperature, pH, shaking speed and inoculation amount are listed in Table 2. With phenol as the sole carbon source, the high efficient phenol-degrading bacteria were firstly cultured in separate marine mineral culture with phenol concentration of 300 mg/L at 37°C for 24 hours with shaking speed of 120 rpm, and then different amounts of culture solution (inoculation amount) were transferred separately into 100 mL marine mineral culture with phenol concentration of 800 mg/L and continued to be cultured for 48 hours at a specified temperature, pH value and shaking speed. Based on the phenol degradation efficiency, the optimal conditions favourable for the growth of bacteria were obtained.

2.3.3 | Microbial degradation of gas washing water

Phenolic compounds were the major organic contaminants in gas washing water produced from UCG. The determination results of gas condensate water, which was collected from a field-scale UCG trial during the production process, indicated that the TOC value and phenolic compounds were 616 and 484 mg/L, respectively. Generally, the concentrations of organic contaminants in condensate water are higher than that of polluted groundwater around the gasification zones. It was also found that the total organics in groundwater sample taken from the combustion cavity was 103 mg/L and that the phenolic compounds were 100 mg/L.

Total organic carbon in gas washing water produced in the steam gasification stage of this UCG model test was measured to be 1089.19 mg/L, and based on the above relevant information, the gas washing water was diluted before the study of microbial degradation. TOC in diluted gas washing water was 175.07 mg/L, and the total organic pollutants (TOM) as well as the concentration of each organic component could be calculated according to the dilution ratio of 175.07/1089.19. With 250 mL volumetric flasks used, the microbial degradation of gas washing water with inoculation amount of 16 mL was studied under conditions of 120 rpm and 40°C.

3 | RESULTS AND DISCUSSION

3.1 | Cultivation and domestication results of activated sludge

Figure 2 presents the cultivation and domestication results of the two kinds of activated sludges, wherein the W-curve and J-curve represent the degradation effects of phenol by W bacteria and J bacteria, respectively.

The results indicated that both strains exhibited better degradation effects at lower concentrations. The phenol degradation trends by W and J bacteria were alike at phenol concentrations of 500 mg/L (Figure 2A) and 1000 mg/L (Figure 2B), but became different at higher concentrations. The phenol degradation effect by J bacteria became better than that by W bacteria for long time degradation (Figure 2C), and the better degradation effect by J bacteria was significantly observed at phenol concentration of 2000 mg/L (Figure 2D). This can be explained by the stronger restraining effect of phenol on W bacteria than J bacteria at higher phenol concentrations.

3.2 | Isolation and purification results of mixed bacteria

According to the different colony morphology features presented in Table 3, the J bacteria are classified into three categories, designated as JA, JB and JC, and the W bacteria are classified into two categories, designated as WX and WD, respectively. The results of gram staining experiments of the above five kinds of bacteria are listed in Table 4. Based on the results of gram staining observation and the colony morphology, the isolated five kinds of bacteria are identified as five different strains.
FIGURE 2 The cultivation and domestication results of activated sludge, wherein W represents the phenol degradation effect by bacteria isolated from activated sludge obtained from the oxidation ditch of a sewage treatment plant and J represents the phenol degradation effect by bacteria isolated from activated sludge obtained from an aeration tank of the coking wastewater treatment plant: (A) 500 mg/L, (B) 1000 mg/L, (C) 1500 mg/L, and (D) 2000 mg/L.

3.3 Screening and identification of bacterium with high phenol degradation efficiency

Phenol degradation results of the above five kinds of strains are shown in Figure 3. As depicted in Figure 3(A), the degradation efficiencies of phenol by J bacteria were apparently superior to those by W bacteria at phenol concentration of 500 mg/L, the maximum degradation efficiencies reached about 85% for the five kinds of strains and it could easily find that JC and WX were the dominant strain in J and W bacteria, respectively. With phenol concentration being increased up to 1000 mg/L, the degradation efficiencies were approximately the same, indicating that 1000 mg/L was a suitable phenol concentration for the growth of all the isolated and purified strains (Figure 3B), wherein the degradation efficiencies by JC and WX were still higher than those by the other three strains. At phenol concentration of 1500 mg/L, the degradation trends became almost the same for the above five strains, but different strains also exhibited different degradation features (Figure 3C). At phenol concentration of 2000 mg/L, the degradation efficiency of phenol by JC bacterium was apparently higher than those by other bacteria (Figure 3D); Based on the relatively lower degradation efficiencies, it also indicated that the phenol solution at concentration of 2000 mg/L was unfavourable for the growth of bacteria. According to the experimental results, JC and WX were selected and identified as strains with high phenol degradation efficiency, respectively.

TABLE 3 The colony morphology characteristics

| Strain | Colony morphology characteristics |
|--------|---------------------------------|
| JA     | Turquoise, irregular border, convex, large thin colony, wet surface. |
| JB     | Round, milky white, regular border, convex, relatively larger and thicker colony, wet surface. |
| JC     | Round, yellow, regular border, convex, small thicker colony, wet surface. |
| WX     | Round, primrose yellow, regular border, convex, relative smaller colony of a certain thickness, wet surface. |
| WD     | Turquoise, irregular border, convex, relatively larger and thinner colony, wet surface. |

TABLE 4 The results of gram staining experiments

| Bacterium | Results of gram staining (G+/G−) | Individual bacteria morphology |
|-----------|---------------------------------|-------------------------------|
| JA        | G+                              | Short-bar                     |
| JB        | G−                              | Short-bar                     |
| JC        | G−                              | Long stem                     |
| WX        | G−                              | Rod forms                     |
| WD        | G+                              | Long stem                     |
In order to identify the species of the screened bacteria, the physiological and biochemical tests and 16SrDNA sequencing were conducted. The results of physiological and biochemical tests are presented in Table 5. According to the results of physiological and biochemical tests and gram staining observations, JC belongs to pseudomonas and WX belongs to achromobacter. Combined with the results of 16SrDNA sequencing, JC and WX are finally identified as pseudomonas aeruginosa (NCBI number: Z76651) and achromobacter xylosoxidans (NCBI number: Y14908), respectively.

### 3.4 Optimum conditions for the growth of bacterium with high phenol degradation efficiency

Based on the results of phenol degradation by JC and WX bacterium under different conditions, which were shown in Figure 4, the optimum conditions favourable for the growth of bacteria with high phenol degradation efficiency were discussed.

The results indicated that the suitable temperature was in range of 30-40°C, and 35°C was the optimum temperature (seen in Figure 4A). When temperature was above 40°C, the degradation efficiencies of phenol dropped rapidly due to the possible denaturation of enzyme protein.31

The pH favourable for the growth of JC and WX bacteria was in range of 6-8, and the degradation efficiency of phenol reached the maximum at pH = 7 as shown in Figure 4B. It was also observed that the degradation efficiency of phenol by WX bacteria was higher than that by JC bacteria in weak acid or weak alkali solutions, but significantly decreased and became far lower than that by JC bacteria at pH = 9. This can be explained by the different growth conditions for JC and WX bacterium and can be attributed to the stronger alkali-resistance ability of JC than that of WX.

With the increasing of shaking speed, the degradation efficiency of phenol first significantly increased as a result of the increasing oxygen content in solution, and then the increasing trend became slow when the shaking speed exceeded 120 rpm (shown in Figure 4C). Therefore, 120 rpm can be regarded as the optimum shaking speed.

Inoculation amount is another critical factor for the degradation of organic contaminants. Predictably, the phenol degradation efficiency increases with the augment of inoculation amount within a certain range. For WX bacterium, the phenol degradation efficiency increased exponentially with the augment of inoculation amount in range of 1-16 mL. However, the increasing trends of phenol degradation

### Table 5 The results of physiological and biochemical tests

| Test items               | JC  | WX  |
|-------------------------|-----|-----|
| Gelatin liquidized test | +   | +   |
| Starch hydrolysis test  | −   | −   |
| Methyl red test         | −   | −   |
| Voges-Proskauer test    | −   | −   |
| Catalase test           | +   | +   |
| Indole test             | −   | −   |
| Nitrate reduction test  | −   | −   |
efficiency slowed for both strains when the inoculation amount exceeded 16 mL (as illustrated in Figure 4D). Besides, in consideration of the cost, 16 mL was viewed as a reasonable inoculation amount.

According to the above experimental results, the optimum conditions favourable for the growth of JC and WX bacteria, under which the bacteria can effectively degrade phenol, can be concluded as: the optimum temperature is 35°C, pH is 7, shaking speed is 120 rpm and inoculation amount is 16 mL. However, it should be noted that the above optimum conditions are determined by variable-controlling approach. For further studies, orthogonal experiment should be conducted to investigate the interaction effects of the above discussed factors on the microbial degradation of phenol.

3.5 | Organic pollutants in gas washing water

Total organic carbon in gas washing water produced in the steam gasification stage of UCG model test was measured to be 1089.19 mg/L by TOC analyser, and the organic components in gas washing water were measured by GC-MS, the results of which were presented in Table 6. The results showed that the TOM in gas washing water was about 901 mg/L and that the total amount of phenols (TPC) was 856.06 mg/L, taking 95.01% of the organic pollutants. It proved that phenols were the main organic pollutants in polluted groundwater caused by UCG.

Total organic carbon in diluted gas washing water, which was used for microbial degradation, was detected at a level of 175.07 mg/L, and according to this dilution ratio of 175.07/1089.19, the concentrations of different organic components in diluted gas washing water could be calculated.

3.6 | Microbial degradation of organic contaminants in gas washing water

Based on the optimum conditions favourable for the growth of the screened bacteria and that the temperature of groundwater at depth of 700 m in China is about 40°C, the experiments of the microbial degradation of organic contaminants in gas washing water were performed at pH = 7, 40°C, 120 rpm.

3.6.1 | Degradation efficiency of TOC

Total organic carbon is an index for evaluating the degrees of organic pollution, and is also one of the important parameters in water monitoring. The degradation results of TOC are shown in Figure 5. The results indicated that TOC could be efficiently degraded by JC and WX. After degradation for 36 hours, TOC decreased from 175.07 to 52.63 mg/L by JC and to 64.61 mg/L by WX, and the removal of TOC finally achieved 76% and 72.9% correspondingly. It was also observed that the bacterium with high TOC degradation efficiency for the treatment of UCG gas washing water could be cultivated and domesticated from activated sludge obtained from an aeration tank of the coking wastewater treatment plant.
3.6.2 Degradation of organic components

After being degraded by JC and WX bacterium, the organic components in diluted gas washing water were also detected by GC-MS. Table 7 presents the organic pollutants as well as the corresponding concentrations in diluted gas washing water before and after the microbial degradation. Some organics such as phenol, o-cresol, m-cresol and 2, 4-dimethylphenol disappeared and some organics such as 3, 4, 5-trimethylphenol and 2,3-dihydro-1H-indene-5-ol decreased significantly. It was also found that the TOM decreased from 144.82 to 29.30 mg/L after the degradation by JC bacterium and to 30.37 mg/L by WX bacterium, and the corresponding degradation efficiencies were obtained as 79.77% and 79.03%, respectively. The TPC decreased from 137.60 to 1.70 mg/L degraded by JC and to 1.32 mg/L by WX, and the

### TABLE 6 Organic components in gas washing water collected from the steam gasification stage of lignite (mg/L)

| Organic component                  | Content  | Organic component                  | Content  |
|------------------------------------|----------|------------------------------------|----------|
| phenol                             | 320.8912 | 5-isopropyl-2-methylphenol         | 0.7838   |
| o-cresol                           | 302.5198 | 2-methoxymethylphenol              | 0.7748   |
| m-cresol                           | 63.9620  | 2-isopropyl-5-methylphenol         | 0.7568   |
| 2,4-dimethylphenol                 | 53.7536  | 1-ethyl-4-isopropylbenzene         | 0.7208   |
| 4-ethylphenol                      | 24.8046  | hexatoluene                        | 0.6668   |
| 2-ethylphenol                      | 14.2448  | naphthalene                        | 0.6308   |
| 3,4-dimethylphenol                 | 13.5330  | 2-methylnaphthalene                | 0.6126   |
| 3,4,5-trimethylphenol              | 12.8122  | 6-methyl-2,3-dihydro-1H-indene-4-ol| 0.5586   |
| 3-ethylphenol                      | 9.3254   | 2-ethyl-6-methylphenol             | 0.5226   |
| m-xylene                           | 8.8838   | (1E,2E)-1,2-bis(1-phenylethylidene)hydrazine| 0.4956 |
| 2-ethyl-5-methylphenol             | 7.5954   | 2-propylphenol                     | 0.4776   |
| 2,3-dihydro-1H-indene-5-ol         | 6.8836   | 1-(3,4-dimethylphenyl)ethan-1-one  | 0.4506   |
| toluene                            | 6.2078   | 2-(sec-butyl)phenol                | 0.4234   |
| 2,3-dimethylphenol                 | 5.2438   | 1-methylnaphthalene                | 0.4144   |
| p-xylene                           | 4.6582   | 4,4-dimethylcyclopent-2-en-1-one   | 0.4054   |
| 2-isopropylphenol                  | 4.3968   | 2,3-dimethylcyclopent-2-en-1-one   | 0.3784   |
| 2,3-dihydro-1H-indene-1-ol         | 3.7032   | 3,4-dimethylcyclopent-2-en-1-one   | 0.3604   |
| 3-ethyl-5-methylphenol             | 3.4598   | 2,3,6-trimethylphenol              | 0.2884   |
| 2,6-dimethylphenol                 | 3.2076   | phenanthrene                       | 0.2704   |
| 2-ethyl-4-methylphenol             | 2.8742   | 2,6-dimethylpyridine               | 0.2522   |
| 2-methylcyclopent-2-en-1-one       | 2.6580   | 3-(tert-butyl)phenol               | 0.2252   |
| p-cresol                           | 2.4146   | 2,3,5-trimethylphenol              | 0.2252   |
| 4-isopropyl-1,2-dimethylbenzene    | 2.1534   | azolamide                          | 0.2252   |
| 2,4,5-trimethylphenol              | 1.6488   | fluorene                           | 0.1532   |
| ethylbenzene                       | 1.6038   | 2-methylpyridine                   | 0.1262   |
| 2,4,6-trimethylphenol              | 1.4596   | 2-methylcyclopentanone             | 0.1262   |
| 2-allyl-4-methylphenol              | 1.1894   | 2-ethyl-4,5-dimethylphenol         | 0.0992   |
| cyclopentanone                     | 1.1714   | 2,5-dimethylpyridine               | 0.0992   |
| 2,3,5,6-tetramethylphenol          | 1.0902   | dibenzo[b,d]furan                  | 0.0720   |
| 3-isopropylphenol                  | 1.0542   |                                   |          |

**FIGURE 5** The relationship between TOC in gas washing water with time degraded by WX and JC at pH = 7, 40°C, and 120 rpm
| Organic component                  | Contents of organic components (mg/L) | Before degradation | Degraded by WX | Degraded by JC |
|------------------------------------|---------------------------------------|--------------------|----------------|----------------|
| phenol                             |                                       | 51.5782            | —              | —              |
| o-cresol                           |                                       | 48.6253            | —              | —              |
| m-cresol                           |                                       | 10.2809            | —              | —              |
| 2,4-dimethylphenol                 |                                       | 8.6400             | —              | —              |
| 4-ethylphenol                      |                                       | 3.9869             | —              | —              |
| 2-ethylphenol                      |                                       | 2.2896             | —              | —              |
| 3,4-dimethylphenol                 |                                       | 2.1752             | —              | —              |
| 3,4,5-trimethylphenol              |                                       | 2.0594             | 0.0195         | 0.1811         |
| 3-ethylphenol                      |                                       | 1.4989             | —              | —              |
| m-xylene                           |                                       | 1.4279             | 12.8829        | 9.5653         |
| 2-ethyl-5-methylphenol             |                                       | 1.2208             | —              | —              |
| 2,3-dihydro-1H-indene-5-ol         |                                       | 1.1064             | 0.0538         | 0.0349         |
| toluene                            |                                       | 0.9978             | 0.2244         | 0.4618         |
| 2,3-dimethylphenol                 |                                       | 0.8429             | 0.1727         | 0.1866         |
| p-xylene                           |                                       | 0.7487             | 2.6524         | 2.0387         |
| 2-isopropylphenol                  |                                       | 0.7067             | —              | —              |
| 2,3-dihydro-1H-indene-1-ol         |                                       | 0.5952             | —              | —              |
| 3-ethyl-5-methylphenol             |                                       | 0.5561             | —              | —              |
| 2,6-dimethylphenol                 |                                       | 0.5156             | —              | —              |
| 2-ethyl-4-methylphenol             |                                       | 0.4620             | 0.0991         | —              |
| 2-methylcyclopent-2-en-1-one       |                                       | 0.4272             | 4.0733         | 0.5919         |
| p-cresol                           |                                       | 0.3881             | —              | —              |
| 4-isopropyl-1,2-dimethylbenzene    |                                       | 0.3461             | 0.0286         | 0.0272         |
| 2,4,5-trimethylphenol              |                                       | 0.265              | 0.0617         | 0.0352         |
| ethylbenzene                       |                                       | 0.2578             | 2.3639         | 1.7609         |
| 2,4,6-trimethylphenol              |                                       | 0.2346             | —              | —              |
| 2-allyl-4-methylphenol             |                                       | 0.1912             | —              | —              |
| cyclopentanone                     |                                       | 0.1883             | 0.0085         | 0.0662         |
| 2,3,5,6-tetramethylphenol          |                                       | 0.1752             | —              | 0.0308         |
| 3-isopropylphenol                  |                                       | 0.1694             | 0.1128         | 0.2054         |
| 5-isopropyl-2-methylphenol         |                                       | 0.1260             | 0.0559         | 0.0082         |
| 2-methoxyphenol                    |                                       | 0.1245             | —              | —              |
| 2-isopropyl-5-methylphenol         |                                       | 0.1216             | —              | —              |
| 1-ethyl-4-isopropylbenzene         |                                       | 0.1159             | 0.0362         | —              |
| hexatoluene                        |                                       | 0.1072             | —              | —              |
| naphthalene                        |                                       | 0.1014             | —              | —              |
| 2-methylnaphthalene                |                                       | 0.0985             | —              | —              |
| 6-methyl-2,3-dihydro-1H-indene-4-ol|                                       | 0.0898             | 0.0289         | 0.0671         |
| 2-ethyl-6-methylphenol             |                                       | 0.084              | —              | —              |
| (1E,2E)-1,2-bis(1-phenylethylidene)hydrazine |               | 0.0797             | 0.5229         | 0.4981         |
| 2-propylphenol                     |                                       | 0.0768             | —              | —              |
| 1-(3,4-dimethylphenyl)ethan-1-one  |                                       | 0.0724             | —              | —              |
| 2-sec-butylphenol                  |                                       | 0.0681             | —              | —              |

(Continues)
| Organic component                                      | Before degradation | Degraded by WX | Degraded by JC |
|--------------------------------------------------------|--------------------|----------------|----------------|
| 1-methylnaphthalene                                    | 0.0666             |                |                |
| 4,4-dimethylcyclopent-2-en-1-one                       | 0.0652             | 0.2158         | 0.4301         |
| 2,3-dimethylcyclopent-2-en-1-one                       | 0.0608             | 0.0030         | 0.5760         |
| 3,4-dimethylcyclopent-2-en-1-one                       | 0.0579             | 1.1230         | 0.9391         |
| 2,3,6-trimethylphenol                                  | 0.0464             |                |                |
| phenanthrene                                           | 0.0435             |                |                |
| 2,6-dimethylpyridine                                   | 0.0405             |                |                |
| 3-(tert-butyl)phenol                                   | 0.0362             | 0.0030         | 0.5760         |
| 2,3,5-trimethylphenol                                  | 0.0362             | 0.0280         | 0.0258         |
| azolamide                                              | 0.0362             | 0.0030         | 0.5760         |
| fluorene                                               | 0.0246             |                |                |
| 2-methylpyridine                                       | 0.0203             | 0.0046         | 0.0261         |
| 2-methylcyclopentanone                                 | 0.0203             |                |                |
| 2-ethyl-4,5-dimethylphenol                            | 0.0159             | 0.0030         | 0.5760         |
| 2,5-dimethylpyridine                                   | 0.0159             |                |                |
| dibenzo[b,d]furan                                      | 0.0116             |                |                |
| 3-ethyl-2-methylpentane                                | —                  | 0.0511         | 0.0349         |
| 3-ethylhexane                                           | —                  | 0.0793         | 0.0407         |
| styrene                                                | —                  | 0.1502         | 0.3449         |
| 2,5-dimethylhexa-2,4-diene                             | —                  | 0.3110         | 0.1902         |
| 1,2-dimethylcyclohexene                                | —                  | 0.2067         | 0.1459         |
| 3-methylcyclopent-2-en-1-one                           | —                  | 0.5943         | 5.8222         |
| 2,4-dimethylheptane                                    | —                  | 0.1599         | 0.1216         |
| 2,4-dimethylcyclopent-2-en-1-one                       | —                  | 0.1225         | 0.0275         |
| 1,6-dimethylcyclohexene                                | —                  | 0.0976         | 0.0431         |
| 5-ethyl-2-methylheptane                                | —                  | 0.0462         | 0.0372         |
| 3-ethyl-5-methylheptane                                | —                  | 0.1137         | 0.0943         |
| 4-ethyl-3-methylphenol                                 | —                  | 0.0368         | 0.0100         |
| 3-ethyl-5-methylphenol                                 | —                  | 0.0268         | 0.0114         |
| 2,5-diethylphenol                                      | —                  | 0.0784         | 0.0311         |
| 5-methyltridecane                                      | —                  | 0.2113         | 0.2030         |
| 3,5-diethylphenol                                      | —                  | 0.0240         | 0.1743         |
| 2-ethyl-4,5-dimethylphenol                             | —                  | 0.0155         |                |
| 4-allylphenol                                          | —                  | 0.0164         | 0.1497         |
| 2-allylphenol                                          | —                  | 0.0079         |                |
| 2-ethyl-4,6-dimethylphenol                             | —                  | 0.0340         | 0.0229         |
| 2-isopropyl-5-methylphenol                             | —                  | 0.0413         | 0.0621         |
| 4-methylnonane                                         | —                  | 0.0468         | 0.0337         |
| 4-methyltridecane                                      | —                  |                | 0.0381         |
| 2-methyltridecane                                      | —                  |                | 0.0193         |
| 3,3-dimethyloctane                                     | —                  | 0.2909         | 0.1617         |
| 2,3,4-trimethylcyclopent-2-en-1-one                    | —                  | 0.1192         | 0.1128         |
| 1-(cyclohex-1-en-1-yl)ethan-1-one                      | —                  | 0.0544         | 0.0082         |

(Continues)
The degradation efficiency of TPC achieved 98.76% and 99.04% correspondingly.

It should be noted that some organic pollutants such as m-xylene, p-xylene and ethylbenzene, increased after microbial degradation and benzene series are typical compounds among these pollutants. Meanwhile, the contents of benzene series in the gas washing water degraded by JC were lower than those degraded by WX. The reason for the increasing concentrations of benzene series in gas washing water after microbial degradation needs to be investigated in future research. In addition, fifty-six kinds of organic compounds appeared after microbial degradation, which were not detected in the initial gas washing water. Most of these newly produced organic compounds were lower concentrations of short-chain hydrocarbons, such as alkanes, olefine, etc. It can be deduced that these small molecular hydrocarbon compounds may be the decomposition products of organics like phenols and polycyclic aromatic hydrocarbons (PAHs) during the microbial degradation, but the exact production mechanism needs further research.

Most organic pollutants in gas washing water can be degraded by JC and WX, but BTEX (except for toluene) cannot be degraded in the process. Because of the carcinogenicity of BTEX, subsequent questions of how to clean up the BTEX in groundwater caused by UCG and how to control the secondary benzene pollution of groundwater during the microbial degradation process are proposed.

| Organic component                  | Contents of organic components (mg/L) |
|------------------------------------|----------------------------------------|
|                                    | Before degradation | Degraded by WX | Degraded by JC |
| 2,3,3-trimethyloctane               | —                      | 0.0511         | 0.0469         |
| 4,5-dimethylnonane                 | —                      | 0.0727         | 0.0158         |
| 2,6-dimethylnonane                 | —                      | 0.0277         | 0.0202         |
| 5-methyldecane                     | —                      | 0.0344         | 0.0334         |
| 4-methyldecane                     | —                      | 0.0280         | 0.0094         |
| 5-methylundecane                   | —                      | 0.0219         | 0.0305         |
| 4-methylundecane                   | —                      | 0.0325         | 0.0328         |
| 2-methylundecane                   | —                      | 0.0489         | 0.0481         |
| 3-methylundecane                   | —                      | 0.0836         | 0.0557         |
| 2,5-dimethyl phenol                | —                      | 0.0505         | 0.0296         |
| 2,4,6-trimethyl phenol             | —                      | 0.1198         | 0.0451         |
| 3-methyldecane                     | —                      | 0.0313         | 0.0264         |
| 2-ethyl-5-methylphenol             | —                      | 0.2423         | 0.4442         |
| 4-methyltridecane                  | —                      | 0.0638         | —              |
| 2-methyltridecane                  | —                      | 0.0292         | —              |
| 1,4-diethyl-2-methylbenzene        | —                      | 0.0106         | 0.0214         |
| 2,4-diethyl-1-methylbenzene        | —                      | 0.0340         | 0.0103         |
| nonylcyclopentane                  | —                      | 0.0578         | 0.0586         |
| 5-methyl-2,3-dihydro-1H-indene-4-ol| —                      | —              | 0.1254         |
| 1-isopropyl-2,4-dimethylbenzene    | —                      | 0.0219         | —              |
| 2-methylquinoline-8-ol             | —                      | 0.0733         | 0.0481         |
| 2,7,10-trimethyldodecane           | —                      | 0.0766         | 0.0703         |
| 1-(4-hydroxy-3-methylphenyl)ethan-1-one| —                   | 0.0672       | —              |
| 1-(2-hydroxy-5-methylphenyl)ethan-1-one| —                   | 0.0496       | 0.0445         |
| 5-methylheptadecane                | —                      | 0.0462         | 0.0378         |
| octadecane                         | —                      | 0.0155         | 0.0202         |
| octadec-1-ene                      | —                      | 0.0654         | 0.0665         |
| 5-methyloctadecane                 | —                      | 0.0377         | 0.0401         |
| 2-methyloctadecane                 | —                      | 0.0222         | 0.0182         |
| (E)-2-methylnonadec-7-ene          | —                      | 0.0787         | 0.0583         |

Note: “—” indicates that the corresponding organic compound was not detected in solution.
4 | CONCLUSIONS

1. Two kinds of bacteria with high phenol degradation efficiency, named JC and WX, are isolated and screened from two types of activated sludges, and correspondingly identified as pseudomonas aeruginosa (NCBI number: Z76651) and achromobacter xylosidoxans (NCBI number: Y14908).

2. Phenolic compounds are the main organic pollutants in UCG gas washing water, taking 95.01 percent of the total organics.

3. Most organic pollutants in gas washing water, such as phenolic compounds, can be effectively degraded by JC and WX bacteria. The removal of TOC reached 76.0% by JC and 72.9% by WX, respectively. The degradation efficiency of total organic pollutants exceeds 79%, and the degradation efficiency of total phenolic compounds achieves 98.76% by JC and 99.04% by WX.

4. BTEX except for toluene in gas washing water cannot be degraded by JC and WX bacteria, but additional BTEX are produced in the process of microbial degradation.

5. Low concentrations of short-chain hydrocarbon compounds, having little effect on groundwater quality, are also produced after microbial degradation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of the paper.

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