Microbiological treatment of iridium-containing organic wastewater

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Abstract. Biodegradation of iridium-containing organic wastewater and simulated wastewater by different strains was studied in this paper. By qualitative and quantitative analysis of each organic component in the wastewater and determination of the content of total organic carbon in the organic wastewater, the decomposition effect of microorganisms on organic materials in the iridium containing organic wastewater was discussed. The results showed that the decomposition effect of the combined bacteria was better. In the simulated wastewater system, the degradation rate of the three strains of LA-2, LB-1 and LZ-1 reached about 95%, and in the actual wastewater system, the degradation rate of the combination of LA-2 and LZ-1 reached about 49%. The results show that microorganisms can effectively degrade organic pollutants and are environmentally friendly, which proves the feasibility of biodegradation of organic matter in iridium-containing organic wastewater.

1. Introduction

1.1. Refractory organic pollutants

1.1.1. Volatile Organic Compounds

Industrial production produces a large number of intractable organic pollutants and toxic compounds [1]. Volatile organic compounds (VOCs) are one of them. At present VOCs are the primary pollutants controlled and treated in most countries in the world, including China. Volatile Organic Compounds with boiling point between 50°C and 260°C and saturated vapor pressure over 133.322kPa at room temperature are generally referred to as Volatile Organic Compounds (VOCs). VOCs are widely distributed and their sources can be mainly divided into three aspects. The first, mobile sources (tail gas), the second, fixed sources (oil refining [2], industry using volatile organic solvents, etc.), and some of them are pollution from microplastics (MP) [3] and lampblack emitted by families. Industrial production is the most important source of volatile organic compounds in water. Many volatile organic compounds, such as halogenated organic compounds, benzene series and phenols, are important solvents and intermediate products of chemical synthesis in the process of industrial production. Many
industrial industries are involved in the production, manufacture and transportation of volatile organic compounds.

With the rapid development of industry in China, the storage and transportation of volatile organic compounds are increasing, which poses a serious threat to the living environment of human beings. VOCs have carcinogenic, teratogenic, mutagenic and other risks, and long-term exposure to VOCs increases the prevalence of asthma, bronchitis and other respiratory diseases [4]. VOCs are effective precursors of ozone and secondary organic aerosols (SOA) [5, 6]. This problem exists in the treatment of environmental pollution in many global cities [7]. At the same time, with the rapid development of the global national economy, people have higher and higher requirements on the quality of living environment. Some western developed countries have issued relevant laws and regulations to strictly control the emission of volatile organic compounds. According to the Clean Water Act in the United States, 129 pollutants (mostly volatile organic compounds) from industrial production have been classified as toxic substance. At the same time, China has also promulgated relevant laws, namely the Law of the People's Republic of China on the Prevention and Control of Air Pollution, which requires the treatment or recovery of volatile organic compounds produced in various industrial production processes. Therefore, it is very important to treat volatile organic compounds in industrial wastewater. Those with simple structure are easier to degrade than those with complex ones, while those with small molecular weight are easier to degrade than those with large molecular weight. Polymer and complex are resistant to biodegradation. In general, the chain hydrocarbon is easier to degrade than the cyclic hydrocarbon; the unsaturated hydrocarbon is easier to degrade than the saturated hydrocarbon; the straight chain hydrocarbon is easier to degrade than the branched chain hydrocarbon; and the more branched alkyl the more difficult to degrade. When all the hydrogen on the carbon atom is replaced by an alkyl or an aryl group, a bioimpedance substance is formed.

1.1.2. PAHs and their heterocyclic analogues
Natural oil seeps, oil spills, bilge effluents and land-based inputs have released large quantities of PAHs and their heterocyclic analogues (N, S, O) into the Marine environment. Many of these compounds are toxic and have a deleterious effect on Marine life. Nitrogen-containing compounds in crude oil usually exist in the form of cyclic compounds, such as polycyclic aromatic nitrogen heterocycles (PANHs), which are usually classified as B-PANHs (basic compounds) and N-PANHs (non-basic compounds) [8]. The coking chemical industry with high energy consumption and high pollution has discharged a large number of volatile organic compounds into the atmosphere [9]. (CWW) due to the existence problems of coking wastewater treatment of organic matter, coking wastewater of high toxicity, bad biodegradability [10], because of its high concentrations of phenolic compounds and nitrogen heterocyclic compounds (NHC) [11], it has long been a serious challenge of an anaerobic treatment, a series of organic waste water produced in coal chemical production process, coal gasification wastewater (CGW), a variety of organic pollutants in wastewater is toxic, mutagenic and carcinogenic, including phenolic compounds, nitrogen, sulfur, heterocyclic compounds and polycyclic aromatic hydrocarbons (10, 12). Both Fenton and ozone chemicals can remove many polycyclic aromatic compounds and polyphenols, but ozone produces a large number of aliphatic compounds with high bioavailability [13]. The combination of Fenton technology and biodegradation can achieve a good degradation effect.

1.2. Introduction to Iridium Containing Organic Wastewater
Paraffins, substituted benzenes, polycyclic aromatic hydrocarbons and phthalates were the main types of organic pollutants in water area. The main organic compounds in iridium-containing organic wastewater are methanol, ethanol, dichloromethane, ethyl acetate, water, acetone, ethyl ether and ethylene glycol monoethyl ether, etc. The main iridium complexes are nitrogen-containing heterocyclic compounds and diketones, such as phenylpyridine, phenylquinoline and their derivatives.

At present, the treatment of iridium containing organic wastewater mainly focuses on the recovery of iridium, ignoring the treatment of organic pollutants in wastewater. Iridium exists in wastewater in
the form of an organic iridium compound, which is volatile. Iridium loss can be caused by direct heating or calcination, so the removal of organic matter is a key step in the recovery of iridium from iridium-containing organic wastewater.

2. Materials and Methods

2.1. Test wastewater and microorganisms

2.1.1. Test wastewater
The iridium-containing organic waste liquid used in this experiment was purchased from Yunnan Platinum Industry Co., Ltd. Before the experiment, the main chemical components of the wastewater and the properties and characteristics of each organic matter were analyzed.

2.1.2. Test strain
Strains LA-2, LB-1, LZ-1, LZ-3 and LZ-4 were screened from wastewater.

2.2. experimental method

2.2.1. Strain culture
In this experiment, Potato glucose medium was used for the culture of bacteria, and the ingredients were: NH₄Cl 0.53 g/L, KH₂PO₄ 1 g/L, FeCl₂·6H₂O 0.5 g/L, CaCl₂·7H₂O 0.2 g/L, MgSO₄·7H₂O 1 g/L, {CH₃OH 11.8 mL(9.3 g)/L or C₂H₅OH 8.5 mL(6.7 g)/L or C₅H₈O₂ 6.0 ml(5.8 g)/L, adding deionized water, pH natural.

2.2.2. Determination of organic components in waste liquid
The types and contents of organic compounds in organic wastewater were determined by gas chromatography-mass spectrometry. The molecular formula, molecular weight, relative density, boiling point and physical and chemical properties of each organic component are also defined.

2.2.3. Isolation and purification of highly degradable bacteria
The organic wastewater was coated on a plate with improved solid medium (replacing carbon source), and cultured at 28 °C for 2-3 days. Then, single bacterial colonies were selected, separated and purified by lines, and the above operations were repeated until pure bacterial colonies were obtained. After separation and purification, the strain was obtained and cultured in an inclined plane at 30 °C for 3-5 days, then stored at 4 °C for later use. The purified strains were inoculated and added to iridium containing culture medium. Strains with good resistance to Ir were selected and cultured in an inclined plane at 30 °C for 3-5 days, then stored at 4 °C for later use.

2.2.4. Degradation experiment
Set the liquid-solid ratio to 1%, the microbial inoculation amount to 5% (v/v), and a prepared 100mL solution is added to a 250 mL Erlenmeyer flask. and the degradation test of Degradation bacteria is performed under the conditions of 30 degrees Celsius, the pH is 3.0 and the speed is 160r/min.

2.2.5. Analysis of degradation characteristics
The prepared bacterial suspension was inoculated into the organic wastewater to be degraded, and the culture was carried out in the constant temperature oscillation culture at 30 °C, 170 r/min, until the growth of the bacteria reached the stable growth stage. The bacterial liquid was centrifugated for 4 min, 12 000 r/min, and the supernatant was taken to measure the degradation amount of organic matter with TOC meter, and the degradation rate was calculated.

(FUT-SOP (Y) -3.1) is used to detect organic components such as methanol, ethanol, acetone and ethyl acetate, (HJ 810-2016) is used to detect toluene and dichloromethane, etc., (HJ 601-2011) is used to detect formaldehyde, and (HJ 503-2009) is used to detect the content of volatile phenol.
2.2.6. Identification of single bacteria
Molecular identification of strain LZ series, LA-2, LB-1. ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used as primers, and the PCR reaction system was set up according to the routine. The amplification procedure was as follows: pre-denaturation at 94 °C for 3 min; 94 °C denaturation, 30 s, 57 °C annealing, 30 s, 72 °C extension, 60 s, a total of 30 cycles; Extended at 72 °C for 10min and stored at 4°C. The quality of 5 μL PCR product was tested by 1.2% agarose gel electrophoresis, and the electrophoresis result was confirmed to be a single band. The remaining PCR products are sent to the company for sequencing. The obtained sequences were compared by BLAST on NCBI, and sequences of strains with high homology and similar morphology were selected. The phylogenetic tree was constructed by Neighbor-joining method in Mega V7.0.6, and the phylogenetic tree was verified by Bootstrap with 1 000 replicates.

2.3. Detection method
The test time was 1, 3, 5, 7, 9, 11 and 15 days, respectively. The concentration of organic matter in the solution was measured by total organic carbon analyzer, and pH meter was used to detect pH and ORP changes during the leaching process.

3. Results & Discussion

3.1. Organic constituents and their contents in iridium-containing organic wastewater
The organic waste liquid was produced in the process of producing the organic iridium compound in the Chemistry and Medicine Laboratory of Kunming Institute of Precious Metals. The iridium-containing organic waste liquid consists of an organic phase, an aqueous phase and a small amount of solid precipitation. The organic phase is at the top, the aqueous phase is at the top of the container, the aqueous phase is at the lower levels of the container, and a small amount of precipitation is at the bottom of the container. The main organic compounds in wastewater and their physical and chemical properties are shown in Table 1.

| Name          | Content/mg•L⁻¹ | Boiling point/°C | Vapor pressure/kPa |
|---------------|----------------|------------------|--------------------|
| ethanol       | 1.62×10⁻³      | 78.4             | 5.8(20°C)          |
| methanol      | 50.3           | 64.7             | 11.83(20°C)        |
| acetone       | 54.0           | 56.53 (329.4 K)  | 53.32(39.5°C)      |
| formaldehyde  | 0.23           | -19.5            | 13.33(-57.3°C)     |
| Ethyl acetate | 3.1            | 77 °C(350.25 K)  | 13.33 (27°C)       |
| Volatile phenol | 1.48         | /                | /                  |
| Methylene chloride | 2.09×10⁴   | 39.75            | 30.55(10°C)        |

3.2. Morphological characteristics during microbial action
The degradation of each strain was determined by the determination of total organic carbon (TOC). LA-2, LB-1, LZ-1, LZ-3 and LZ-4 are the names of the strains I screened. Fig. 1 shows the morphological change characteristics of the wastewater treatment process by LA-2, LB-1 and LZ-1 from left to right (A), (B) and (C).
3.3. Degradation of five strains in organic wastewater

Fig. 2(a) and Fig. 2(b) shows changes in pH and potential of microorganisms inoculated in iridium-containing organic wastewater. The results showed that microorganisms began to adapt to growth after inoculation for 24 h. It shows that the organic wastewater has complex composition and contains toxic and harmful organic matter, some of which inhibit its growth. Fig. 2(c) and Fig. 2(d) respectively simulated the TOC degradation rate change trend of wastewater and wastewater containing organic iridium, the results clearly show that the TOC degradation of organic wastewater rate is significantly higher than the TOC degradation rate of the wastewater containing iridium. The possible reason is that the bacterial strain was selected by replacing the carbon source of the culture medium with specific organic matter, so the bacterial strain had targeted degradation effect on the simulated organic wastewater.

Fig. 2 Changes in pH, potential and degradation effect
3.4. Molecular identification of microorganisms
The single genomic DNA of the five isolated degradation strains was amplified by PCR and sent to Sangon Biotech for sequencing. After bidirectional sequencing, double strands were spliced and the sequences were uploaded to NCBI. BLAST was used to compare the gene sequences with the known sequences in GenBank to find the strains with the highest similarity, determine the classification status of the screened single bacteria, and obtain the relatives and similar species of the bacteria strains. The comparative analysis results showed that LA-2, LB-1, LZ-1, LZ-2 and LZ-3 all belonged to *Aspergillus*. Figure 3 shows the phylogenetic tree of microorganism LZ1. The strain with the highest sequence similarity to LZ-1 was *Aspergillus sp*. Strain H4307 (100.00%)

![Phylogenetic tree of isolated microorganisms LZ-1](image)

**Fig. 3** The phylogenetic trees of isolated microorganisms LZ-1.

4. Conclusions
a) The iridium-containing organic wastewater contains a variety of toxic and harmful pollutants with high boiling point besides the complex organic composition. It cannot be separated and recovered by simple physical vacuum distillation, nor can it be directly discharged into the environment. The application of chemical methods to remove the consuming reagents will increase the environmental burden. Therefore, it is feasible to selectively breed microorganisms and then use them for targeted degradation.

b) The microorganisms screened out under specific conditions can degrade organic matter in a targeted way, and it is also feasible for the degradation of complex organic matter by the combination of a variety of microorganisms.

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