In-situ bioremediation of soil pollution with electric heating temperature regulation Bio-Ventilation

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Abstract. The relation between activity of soil indigenous microorganisms and temperature is an important research topic. The method of low rate flow nutrition and oxygen injection to a soil by an electrokinetic technique is studied to be used in aerobic polluted soil bioremediation treatments. The proper temperature nutrient solution and oxygen could be heated and poured into the polluted soil to promote the growth of indigenous bacteria in the soil and improve the efficiency of degrading inorganic pollutants. The present study was conducted to clarify oxygen levels and soil temperature influence on Bio-Ventilation bioremediation technology.

1. Introduction

Study on the seriousness and universality of remediation of heavy metal pollution in soil has become an important research content in the fields of agriculture, ecology and environment. Bioremediation can adsorb and accumulate heavy metals, improve rhizosphere soil microenvironment. Thereby, the absorption, volatilization or fixation efficiency of heavy metals in plants can be improved. The effects of microorganisms on the activity of heavy metal pollutants in soils mainly include dissolution, biosorption, oxidation-reduction, mycorrhizal fungi and bioavailability coefficient of heavy metal in soils [1]. There are two basic approaches of bioremediation include degradation and adsorption. Degradation is the advantage of microbial bioremediation. In-situ bioremediation can promote the degradation of organic pollutants by adding special microbial reagents, nutrients (N, P, etc.) and soil amendments to the soil without changing the location of contaminated soil [2]. Figure 1 shows that in-situ bioremediation has two functions: aerobic respiration and anaerobic respiration. There are three phases for anaerobic decomposition: hydrolysis fermentation phase, acidification phase, acetic acid production and methanogenic phase, which can degrade organic matter into small molecular organic acid. In general, contaminated soils are treated by aerobic respiration at the following reasons:

- The degradation rate of aerobic system is much faster than that of anaerobic system.
- It is difficult for anaerobic systems to treat soil in isolation from air, but there is no special requirement for aerobic systems.
- The final products of aerobic treatment system are CO\(_2\) and H\(_2\)O, which are harmless to human beings. While the products of anaerobic system are CH\(_4\) and H\(_2\)S will cause the new environmental pollution.

Under aerobic conditions, when organic matter enters the cells of degrading microorganisms, assimilation is used to carry out a very complex degradation process. The bioremediation technology...
can completely oxidize organic pollutants and decompose them into inorganic substances such as \(\text{H}_2\text{O}, \text{CO}_2, \text{NO}_2^-, \text{SO}_4^{2-}, \text{PO}_4^{3-}\) and so on.

![Figure 1. Fundamental principles of bioremediation technology.](image)

The success of in-situ remediation relies highly on the existence of suitable microbial species to stimulate pollutant degradation and the improvement or effective management of environmental conditions in contaminated sites[1]. Microorganisms used for in-situ remediation may not compete with indigenous microorganisms and difficult to adapt to the environment. This might lead to unsatisfactory remedial effect. Therefore, the method of temperature regulation and infusion of nutrient solution with high oxygen content can be used to enhance the activity of indigenous microorganism.

2. Materials and methods
Rehabilitation technology can make full use of in-situ microbial community, supplemented by plant, physical and chemical methods and combined with the geological environment to change the macro-environment with micro-effect. Table 1 listed the Control factors primarily include soil moisture, redox potential, oxygen levels, nutrients, soil temperature, improvement of geological environment, etc.

| Factor            | Requirements for microbial activity                      | Optimum degradation |
|-------------------|----------------------------------------------------------|---------------------|
| Soil moisture     | 0.25-0.85 of soil maximum moisture capacity             | 0.30-0.90           |
| Soil pH           | 5.5-8.5                                                  | 7.5-7.8             |
| Redox Potential   | Aerobes and facultative anaerobes>50mV                  |                     |
| Oxygen levels     | Aerobes<50mV with low air-filled porosity of 10%         | 10%-40% \(\text{O}_2\) |
| Nutrition         | Anaerobes<1.0%, \(\text{N}\) and \(\text{P}\) of microbial growth |                     |
| Soil temperature  | C:N:P=120:10:1                                           | 20-30               |

Enhancing Bio-remediation can stimulate the activities of microbes involved the modification of waters and soils by the addition of oxygen or nutrients[4].
2.1. Regulation of soil temperature

Temperature is one of the important factors affecting the growth and survival of microorganisms. The activity intensity and biochemical action of microorganisms are related to the regulation of soil temperature [5]. Increasing soil temperature can increase microbial degradation activity and volatility of pollutants, and accelerate the degradation of organic pollutants. Degradation during the process of microbial remediation is mostly carried out within a certain temperature range (20-40) ℃, generally no more than 40℃. Microorganisms grow slowly and have poor metabolic activity at low temperature. Higher temperature within the range of 5-30℃ accelerated the degradation due to the activation of microbial enzyme at high temperature. On the contrary, the degradation rate decreases with the increase of temperature if the temperature exceeds the physiological allowable temperature. Regulation methods of soil temperature include hot air injection, vapour injection, electrical heating and microwave heating.

2.2. Bio-Ventilation

Bio-Ventilation is an effective and inexpensive in-situ bioremediation technology for polluted soils [6]. The method generally involves at least two wells with blowers and vacuum pumps installed in contaminated soil [7].

In Figure 2, low-power electric heater, which is controlled by temperature sensors are used to collect the temperature of contaminated soil, suspended above the ground heats the low rate flow nutrition and fresh air beneath. The proper temperature nutrient solution and oxygen are heated and poured into the polluted soil to promote the growth of indigenous bacteria and improve the efficiency of degrading inorganic pollutants. Fresh air containing sufficient oxygen is forcibly discharged into unsaturated soil to enhance air flow between soil and atmosphere.

3. Results and discussion

3.1. Relationship between indigenous microorganisms and temperature
The effect of temperature on the growth rate of indigenous microorganisms could be satisfactorily described by Arrhenius Law, which is presented in this paper quantifying a chemical reaction:

\[ k = A e^{\frac{-\Delta E}{RT_k}} \]  \\
\[ \ln k = -\frac{\Delta E}{RT_k} + \ln A \]

This equation (1) describes the linear relationship between the logarithm of the inactivation rate \( k \) and the reciprocal of the temperature \( T_k \), where \( A \) is the frequency factor, \( \Delta E \) is the activation energy, and \( R \) is the ideal gas constant [8]. The mathematical models showed from the natural logarithmic function were established on the relationship between activation energy \( \Delta E \) and \( \ln k \). This model is commonly used to quantify the impact of temperature on the growth rate.

The sterilization of culture medium should not only eliminate the spores of hybrid bacteria, but maintain the growth of indigenous bacteria. The temperature of \( T_k \) is increased to a certain extent to accelerate the rate of spore death of hybrid fungi. Suitable temperature in a short time can not only sterilize quickly, but preserve the active ingredients of indigenous bacteria in the culture medium, while prolonged high temperatures can also lead to the destruction of the active ingredients in the medium.

3.2. Discussion

The aerobic culture degradation and temperature increase of indigenous microorganisms were studied by flask-shaking tests. The fresh culture medium with 5 mL of the domesticated indigenous microorganism suspension was aerobic cultured in for 2-3 days in shaking flask. Then it was coated on a plate of selected medium. Based on the optimal flask culture medium composition and fermentation conditions, four 1 mL suspensions were prepared and transferred into four triangular flasks containing 50 mL medium by pipette. The measurement of the indigenous bacterial growth curve shows that 4 strains of bacteria at different temperature can reach a higher concentration of incubating within 30 hours. They are fit for industrial production. OD (Optical Density) value, which indicates the optical density absorbed by the detected substance, is an index to the growth state of microorganisms.

![Figure 3. The growth curve of indigenous microorganisms, 20-35°C.](image-url)

When the temperature is 20, 25, 30 and 35°C, the OD value of bacteria is measured every 2 hours, and the time-OD curve is drawn in Figure 3. The growth of indigenous bacteria will go through four phases: 0-5h is lag phase, 5-22h is exponential phase, 22-27h is stationary phase, and 27h later is senescence phase. In Bio-Ventilation, inoculation of logarithmic microorganisms in the growth of
indigenous microorganisms can shorten the delayed period of pollutant degradation. Considering Bio-Ventilation can provide the microbes with a growing environment, the experiment confirm that 30°C is optimal temperature. On the contrary, the growth state of microorganisms decreases with the increase of temperature when the temperature reaches 35°C.

4. Conclusions
Bioremediation is a feasible method for the remediation of containing soils highly contaminated with organic pollutants. This study focuses on the effect of temperature on the growth of soil indigenous microorganisms using different temperatures at 20, 25, 30 and 35°C, by Bio-Ventilation with nutrient solution and hot fresh air injection. The Arrhenius Law could be used successfully to quantify the impact of temperature on the rate of inactivation of indigenous microorganisms. The results of this study demonstrated the future application of Bio-Ventilation bioremediation under soil temperature regulation in bioremediation sites.

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