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Comparison Analysis of Dust-Depressor Using Microbial Induced Carbonate Precipitation with Urease Reagent

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Abstract

The dust-depressors have been developed utilizing a method of microbial induced carbonate precipitation. This microbial dust-depressor has the characteristics of high efficiency for dust suppression and environmental protection. Optimal compositions of urease dust-depressor and microbial dust-depressor have been studied. In addition, pure water, CaCl2 and super absorbent polymer are chosen to compare with new dust-depressors on the performances. The results showed that the microbial dust-depressor had 3.13 mm/min of seepage velocity and 79.1% of dust suppression efficiency, which were superior to other dust-depressors on the performance of seepage velocity and dust suppression efficiency.

Keywords

microbial induced carbonate precipitation, Bacillus pasteurii, Urease, dust-depressor, composition proportion, application performance

1. Introduction

Dust refers to small particles that are susceptible to external forces. Bare ground, industrial processes, dust buildup at construction sites, and motor vehicle emissions are the main sources of dust pollution. Dust could affect air quality, and have the adverse effects on the human health, such as bronchitis, lung cancer. Dust depressor could effectively control the dust of roads, factories, construction sites, mines, coal and other places, and has good environmental and economic benefits. Most condensation type depressors could damage the growth of plants because of the corrosivity (Goodrich et al., 2010). Under the economic background of energy saving and emission reduction and human consensus on environmental protection, the environment-friendly dust suppressant with excellent performance is the current research direction.

Microbial-Induced Carbonate Precipitation (MICP) is a biochemical induced mineralization in environment (Boquet et al., 1973). Urea hydrolysis is the most efficient and easy-to-control route to

calcium carbonate precipitation (Muynck et al., 2010a; Muynck et al., 2010b). The commonly used bacterium for urea hydrolysis of MICP is Bacillus pasteurii, which continuously secretes high activity of urease to the outside of the cell, increasing the rates of urea decomposition to 104-fold (Benini et al., 1999; Jabri et al., 1995). Generated NH3 is rapidly hydrolyzed to NH4 +. And then, the Ca2 + with HCO3-hydrolyzed from CO2 in solution precipitate CaCO3.

The new dust suppressant contains urease reagent (bacterium suspension of Bacillus pasteurii), urea, calcium chloride and polymer water-absorbing resin. CaCO3 generated by the reaction has a certain bonding property, bonding dust particles together to form large particles. On the other hand, calcium carbonate precipitation covered above the dust, effectively preventing the rising dust. Bacillus pasteurii in the particles continually produce urease, which ensure the effects of dust suppression.

The new type of microbial dust suppressants is environmentally friendly, and urea is decomposed to gas by urease secreted from bacteria. The objectives of the present study were to (1) investigate the optimum methods of making microbial dust suppressants; (2) compare the effects of microbial dust suppressants with the urease agentia; (3) provide the value references to the dust pollution control.

2. Materials and Methods

2.1 Experimental Materials

The freeze-dried powder of Bacillus pasteurii (ATCC11859) was purchased from the Ruichu Biotech Co., Ltd., Shanghai, China. According to the manufacturer instructions, the optimum temperature was 30 °C and the bacteria was aerobic cultivation. The urease (CA: 9002-13-5) was purchased from the Harvey Bio, Beijing, China, which contained protein, fat, sugar and nucleic acid. Super Absorbent Polymer (SAP), produced by FNA (Shanghai) Co., Ltd., has a particle size of 120-150 um and is a low degree of crosslinking or partial crystallization with many hydrophilic groups of the polymer. The polymer is sodium polyacrylate bridge into a network structure, and the molecular formula is [-CH2-CH (COONa)] n. Superfine coal, purchased in Xinxin Mining, China, and the particle size of pulverized coal was 200-325 mesh.

2.2 Experimental Design

2.2.1 Activated Culture of Bacillus Pasteurii

According to the manufacturer instructions, after dissolving with 0.3-0.5 mL sterile water, the freeze-dried powder of Bacillus pasteurii was cultured in the CASO AGAR + Urea medium. And then the culture mediums were cultivated at 30 °C for 72 h, activated successfully after appearing significant milky colony.

Bevel solid medium with colonies was inoculated into a flask containing liquid medium (CASO + urea medium). The flasks were placed in a shaker at 30 °C for 48 h. If the culture medium was turbid, after centrifuged at 4000 r/min for 20 min, removed the supernatant in the liquid medium, and then added the new liquid medium with additional culture for 48 h. The bacterium solution was stored at 4 °C for subsequent experiments.

Turbidimetry method was used to measure the number of Bacillus pasteurii in the liquid medium. The number of cells was determined using an ultraviolet spectrophotometer at an absorbance (OD600) of a wavelength of 600 nm. The calculation method of actual cell number referenced Santosh et al. (2001).

2.2.2 Effects of Environmental Factors on Bacterial Urease Activity

According to formula (1-4), with the progress of the urea hydrolysis reaction, conductive ammonium ions and carbonate ions in the solution increase. With the increasing urease activity, conductive ions increase in the solution, enhancing solution conductivity. Whiffin (2004) demonstrated that the amount of urea hydrolysis is proportional to the variation of solution conductivity, and the conductivity change of 1 mS/min corresponds to the urea hydrolysis amount of 11 mM urea hydrolysed/min. In this study, 1.5 mL of 10 g L-1 urease solution (or Bacillus pasteurii solution with OD600 = 0.9) mixed with 13.5 mL of 0.8 M urea solution. After the reaction for 30 min, the conductivity change within 5 min was measured with a conductivity meter (Mettler Toled Electrodes LE438) to obtain the average conductivity change value per minute (ms / min), and then the amount of hydrolyzed urea per minute of the bacterium solution (mM urea hydrolysed / min) was converted.

2.2.3 Study on the Optimal Composition of New Dust Suppressants

The effect of the microbial dust suppressant mainly depends on the yield of calcium carbonate precipitation. The increasing calcium carbonate generation could bond more dust particles, and had a large cover area on dust, showing better the dust suppression effects. Therefore, based on the generation amount of calcium carbonate, the study researched the optimal composition of the new dust suppressant.

1.5 mL of urease solution (or bacterial solution) was added to 13.5 mL of urea and CaCl2 mixed solution, the reaction was cultivated in 50 mL tube for 3 days. Most of generated CaCO3 adhered on the centrifuge tube wall, and a small part of the precipitate was in the bottom of the centrifuge tube. After filtration and removing supernatant from the centrifuge tubes using the filter papers and then the filter papers and tubes were washed with deionized water. After drying, the filter papers and centrifuge tubes together weighed, marked as W1 + 2. Then the filter papers and centrifuge tubes together were immersed in 0.1M nitric acid solution was washed until no bubbles, washing with deionized water and dried, weighed as W2. The difference between the two weights, W1 = W1 + 2 - W2, is the amount of CaCO3 produced by microbial-induced calcium carbonate precipitation.

In order to explore the optimal composition of the dust suppressant, this study researched the effects of concentration of urease solution (bacterial liquid), the concentrations of urea and CaCl2 mixed solution on the production of CaCO3.

2.2.4 Comparison Test on the Performance of Dust Suppressant

(1) Infiltration rate

First, a certain amount of pulverized coal was placed into test tubes (φ × h as 20 × 200 mm), compacted by a glass rod, so that the height of pulverized coal in the test tube was 15 cm, and then the test tubes were fixed on the test tube rack. 2 mL dust suppressant solution was slowly added into the test tubes

containing pulverized coal, measuring the penetration depth for 20 minutes. Under the same conditions, the suppressant solution had high infiltration rate, suggesting the better permeability of the suppressant solution.

(2) Dust suppression efficiency

The weight of glass dishes was measured as m1 and then the pulverized coal of 20 g was placed in these glass dishes. 15 mL of each dust suppressant were added evenly on the surface of coal powder, determining the weight as n2. After formed the solidified layer on the surface of pulverized coal, the wind resistance experiment was conducted. Mechanical fan (M403B3, Midea, China) was used to fan the glass dish (up to 5 wind speed, wind speed 8.0 ~ 10.7 m·s-1). Blade shaft faced to the glass dish, with the 1 m distance for 8 h. After the end of the test, the pulverized coal and the glass dish were weighted together as n2. Dust suppression efficiency Ƞ reflects the ability of dust suppressants to suppress dust under light wind conditions, and the higher of the wind resistance efficiency, indicating that the less dust is blown up, and dust suppression has the better effects. Dust suppression efficiency of the formula as follows:

$$\eta = \frac{n_2 - m_1}{n_1 - m_1} \times 100\%$$

Where Ƞ is the dust suppression efficiency (%); m1 is the weight of glass dish (g). n1 is the weight of pulverized coal and glass dish (g); n2 is the weight of pulverized coal and glass dish after wind-resistance experiment (g).

3. Results and Discussion

3.1 Urease Activity

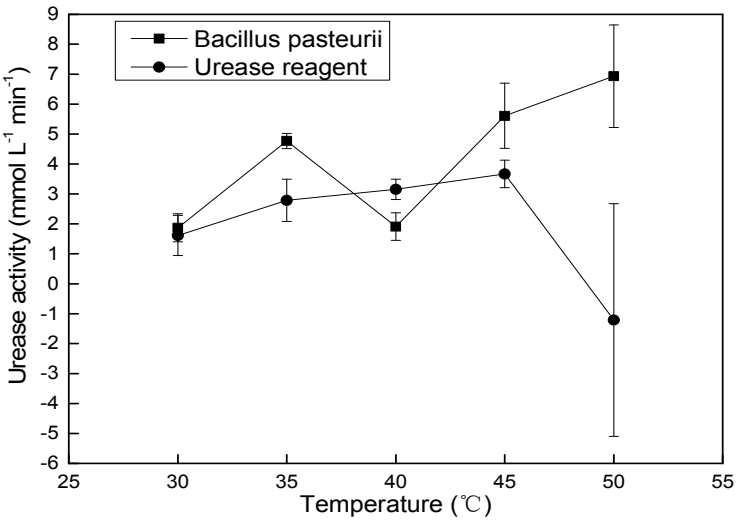

Figure 1. The Effect of Temperature on Urease Activity

It can be seen from Figure 1 that the urease activity of urease reagent increased gradually with the increase of temperature in the range of 30~45 °C, and the urease activity increased to the maximum value (3.67 mmol urea hydrolysed·min-1) at 45 °C; When the temperature reached 50 °C, the activity of urease reagent began to decline rapidly and became negative, which indicated that higher temperature led to the denaturation of urease protein, and then affected urease activity of urease reagent.

Urease activity of Bacillus pasteurii increased with the increase of temperature in the mass in the range of 30~50 °C and increased rapidly, which fluctuated slightly at 40 °C and reached the maximum (6.93 mmol urea hydrolysed·min-1) at 50 °C, within the temperature range studied.

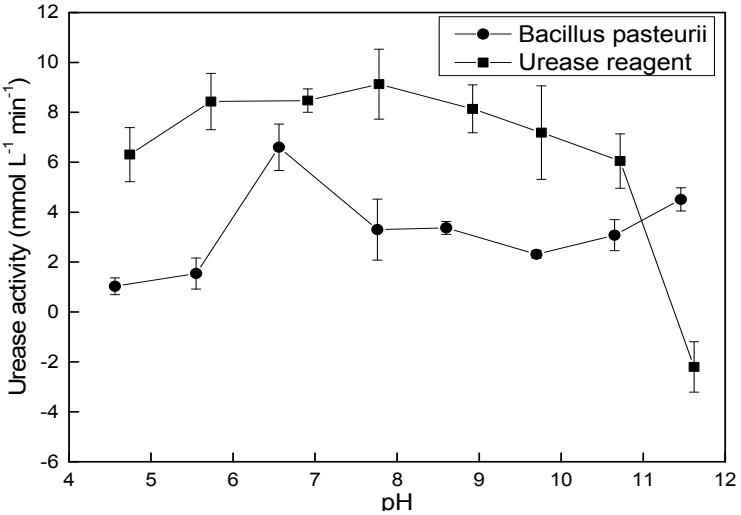

Figure 2. The Effect of pH on Urease Activity

In general, Bacillus pasteurii and urease dust suppressants are more conducive to play the role in the hot summer or high temperature environment.

Urease is a biologically active enzyme, indicating that it also has the most suitable pH value for growth. Within a certain range of the optimum pH value, urease reagent has higher enzyme activity. As shown in Figure 2, the urease activity of urease reagent increased with the increase of pH in the range of 4.74~7.78, and decreased with the increase of pH in the range of 7.78~11.62, which reached maximum (9.13 mmol urea hydrolysed·min-1) at pH=7.78.

Urease activity of Pasteurella multocida increased with the increase of pH in the range of 4.56~6.56, which reached maximum (6.6 mmol urea hydrolysed·min-1) at pH=6.56. Bacterial urease activity decreased with the increase of pH value in the mass in the range of 6.56~9.70, and slightly increased at pH=8.60; Urease activity of Bacillus pasteurii increased with the increase of pH in the range of 9.70~11.46.

In general, urease reagents and Bacillus subtilis dust suppressants have a wide range of applicable pH, but they are more suitable for use in neutral and weakly alkaline environments.

3.2 Optimum Composition Ratio of Microbial Dust Suppressant

3.2.1 Optimum Urease (Bacterial) Concentration

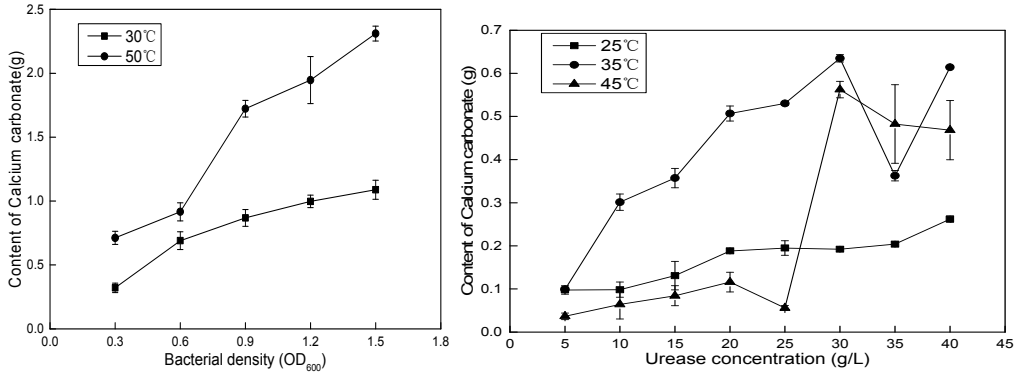

Figure 3. The Effect of Bacterial Density and Urease Concentration on Content of CaCO3

As shown in Figure 3, the optimum temperature of urease reagent was 25 °C, but the yield of calcium carbonate was not ideal. The yield of calcium carbonate increased slowly with the increase of urease concentration. When the urease concentration was 30 g/L, the yield of calcium carbonate decreased slightly, and the maximum yield of calcium carbonate reached 0.262 g when the urease concentration was 40 g/L. The formation of calcium carbonate was the best at 35 °C; When the concentration of urease was in the range of 5~30 g/L, the yield of calcium carbonate increased with the increase of urease concentration, and the calcium carbonate yield reached the maximum value of 0.635 g at 30 g/L. In the high temperature environment of 45 °C, when urease concentration in the range of 5~25 g/L, calcium carbonate production had been at a very low level, but in the range of 30~40 g/L, calcium carbonate production was higher; when urease concentration was 30 g/L, calcium carbonate production rapidly increased to 0.562 g, which reached the maximum value.

Overall, the effect of calcium carbonate precipitation induced by urease was best at the temperature of 35 °C, worse under the condition of higher urease concentration at 45 °C, worst at 25 °C. Optimum urease concentration was 40 g·L-1 at 25 °C, and optimum urease concentration was 30 g·L-1 at 35 and 45 °C.

For Bacillus pasteurii, the yield of calcium carbonate increases with the increase of the concentration of Bacillus cereus at 30 or 50 °C. Under the same concentration of Pasteurella Bacillus, when the reaction temperature was 50 °C, the yield of calcium carbonate was always greater than 30 °C. This was because, in the temperature range studied, the urease activity of Bacillus pasteurii increased with the increase of temperature, and the precipitated calcium carbonate would become more and more. The yield of calcium carbonate reached the maximum value of 2.311 g at 50 °C, when the OD600 of Bacillus pasteurii was 1.5; The yield of calcium carbonate reached the maximum value of 1.089 g at 30 °C, when the OD 600 of Bacillus pasteurii was 1.5.

Regardless of the reaction temperature of 30 or 50 °C, the optimum OD600 of Bacillus pasteurii was 1.5.

3.2.2 The Optimum Concentration of Urea and Calcium Chloride

It can be seen from Figure 4, for urease reagent, at 25 °C, the yield of calcium carbonate increased with the increase of the concentration of the mixed solution when the mixed solution concentration of urea and calcium chloride was in the range of 0.2~0.6 mol·L-1. When urea and calcium chloride concentration increased to 0.6 mol·L-1, calcium carbonate production reached the maximum value of 0.576 g. With the increase of urea and calcium chloride concentration, the yield of calcium carbonate decreased rapidly. At 35 °C, the yield of calcium carbonate increased with the increase of the concentration of the mixed solution when the mixed solution concentration of urea and calcium chloride was in the range of 0.2~0.8 mol·L-1. When urea and calcium chloride concentration increased to 0.8 mol·L-1, calcium carbonate production reached the maximum value of 0.635 g. Under the condition of high temperature (45 °C), when the mixed solution concentration of urea and calcium chloride was in the range of 0.4~1.0 mol·L-1, calcium carbonate production increased rapidly and then gradually stabilized, and reached the maximum value of 0.568 g when the mixed solution concentration of urea and calcium chloride was 0.6 mol· L-1.

To sum up, the optimum composition ratio of urease inhibitor was urease solution of 40 g·L-1, mixed solution of urea and calcium chloride of 0.6 mol·L-1 under the optimum temperature (25 °C); Urease solution of 30 g·L-1, mixed solution of urea and calcium chloride of 0.8 mol·L-1 under normal temperature in summer (35 °C); Urease solution of 30 g·L-1, mixed solution of urea and calcium chloride of 0.6 mol·L-1 under high temperature (45 °C).

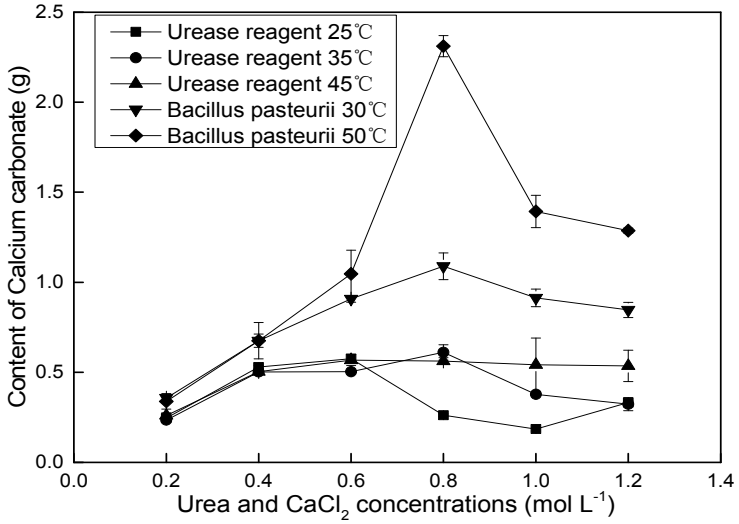

Figure 4. The Effect of Urea and CaCl2 Concentration on Production of CaCO3

For Bacillus pasteurii, increasing the concentration of urea and CaCl2 in the concentration range of

0.2~0.8 mol·L-1 under the reaction temperature of 30 and 50 °C could both effectively increase the production of CaCO3. When the urea and CaCl2 concentration was more than 0.8 mol·L-1, calcium carbonate production began to decline with the increase of urea and CaCl2 concentration, which was because the obvious inhibitory effect of high concentration CaCl2 on urease activity of Bacillus pasteurii. When the urea and CaCl2 concentration of 0.8 mol·L-1, calcium carbonate production reached the maximum value of 2.311 g (50 °C) and 1.089 g (30 °C).

So, whether the reaction temperature was 30 or 50 °C, the optimum OD600 of Bacillus pasteurii dust suppressant was 1.5, and the optimum concentrations of urea and calcium chloride were both 0.8 mol·L-1.

3.3 Contrast Test Results of Application Performance

Microbial dust suppressant was prepared according to the best composition ratio: the OD600 of Bacillus pasteurii was 1.5, the concentration of urea and CaCl2 mixed solution was 0.8 mol·L-1; Because the experiment was carried out under the summer high temperature, urease inhibitor was prepared according to the optimum composition ratio under normal temperature in summer (35 °C): the concentration of urease solution was 30 g·L-1; the concentration of urea and calcium chloride mixed solution was 0.8 mol·L-1.

3.3.1 Seepage Velocity

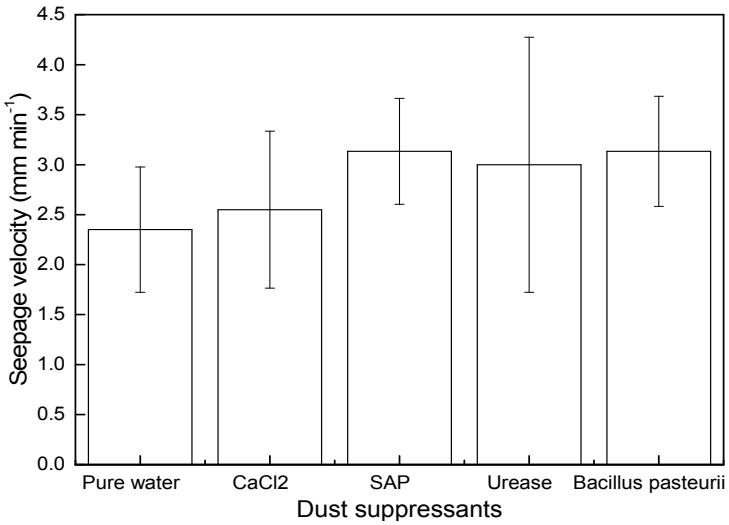

Figure 5. The Seepage Velocity of Different Dust-Depressor

As shown in Figure 5, the seepage velocity of the five dust suppressants from high to low in turn were as follows: microorganism = SAP＞urease＞CaCl2＞pure water. The faster the penetration rate of dust inhibitor was, the faster the velocity in the wetting depth dust after wetting surface dust, which made the rise of surface dust and deep dust under control. The seepage velocity of pure water and CaCl2 dust suppressant was poor, the velocity of wetting deep dust slow after spraying, and the control of deep raise dust could not be guaranteed. The seepage velocities of microbial dust suppressant and SAP were

the highest of 3.13 mm min-1, but microbial dust suppressant could generate sticky calcium carbonate deep inside the dust, the firm bond from outside to inside more conducive to raise dust control. The seepage velocity of urease depressor was the second, which was just beneficial to the control of deep raise dust.

3.3.2 Dust Suppression Efficiency

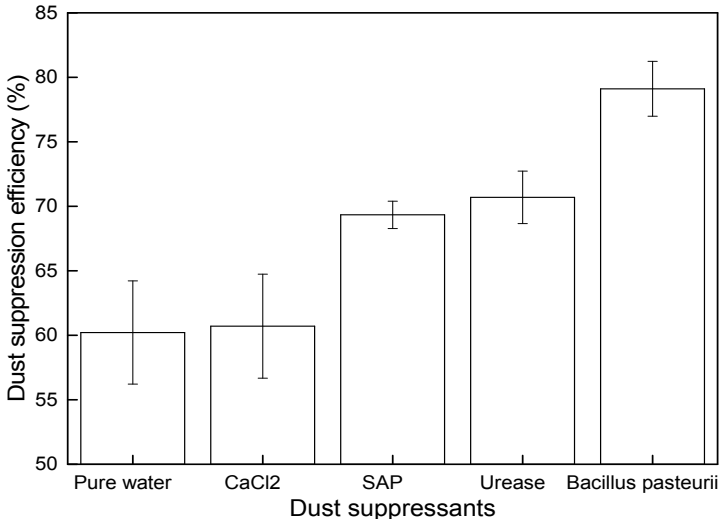

Figure 6. The Dust Suppression Efficiency of Different Dust-Depressor

The dust suppression efficiency intuitively reflects the raising volume of dust under blowing and dust suppressant condition. The higher the dust suppression efficiency is, the less dust is raised and the better dust suppression effect is. As shown in Figure 7, the dust suppression efficiency of the 5 dust suppressants from high to low in turn were as follows: microorganism > urease > SAP > CaCl2 > pure water. Among them, the microbial dust suppressant had the highest dust suppression efficiency of 79.1%. Under the blowing condition, the dust raising amount was the least, and the dust suppression effect was the best. The dust suppression efficiency of urease inhibitor was the second. Under long time wind resistance test, the dust suppression efficiency of urease inhibitor could reach 70.7%; The dust suppression efficiency of pure water and CaCl2 dust suppressant was the lowest, both in the range of 60% ~ 61%, which indicated more dust raised and poor dust suppression effect.

4. Conclusion

The new dust-depressor can effectively improve the dust suppression effect by using the theories of biological degradation, biological calcification and bonding. It has the advantages of strong wind resistance, high dust depression efficiency and green environmental protection.

The new dust suppressants include urease (Bacillus pasteurii), urea and calcium chloride. The optimal proportion of urease suppressant at 25 °C were urease solution of 40 g·L-1, urea and calcium chloride mixed solution of 0.6 mol·L-1; urease solution of 30 g·L-1, urea and calcium chloride mixed solution of

0.8 mol·L-1 at 35 °C; urease solution of 30 g·L-1, urea and calcium chloride mixed solution of 0.6 mol·L-1 at 45 °C. The optimal proportion of Bacillus pasteurii dust suppressant were bacterial solution of OD600=1.5 and urea-calcium chloride mixed solution of 0.8 mol·L-1.

Compare with the common dust-depressor available in the market such as pure water, calcium chloride and SAP dust suppressants, urease and microbial dust suppressant have faster seepage velocity as well as better suppression efficiency than the three kinds dust-depressor mentioned before. Among them, microbial dust suppressant has the highest penetration rate of 3.13 mm min-1 and dust suppression efficiency of 79.1% respectively.

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