Optimum Condition for 2，6-DNT –Microbe Degrading Using Uniform Design

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Abstract: The GC-ECD was used in this study. The optimization of preparation was performed by U9(94)uniform design method, With the rate of 2, 6-DNT -degrading result as investigative indexes, the influences of of ratio the concentration of substrate and PH,The speed of shaker and Temperature .the result were screened by Uniform Design to found the optimum condition of degrading . Result the optimum condition for 2, 6-DNT -degrading as fllowing: The concentration of substrate is 135mg/L, pH = 7.3, Temperature is 29℃, speed of shaker is 129 rpm/min. The result show: It is scientific and feasible to screen the experimental condition of for 2, 6-DNT - degrading by using the uniform design and regression.

Keywords: uniform design;COD; degradation; domestication

1 Introduction

Dinitrotoluene (DNT) is a nitro-aromatic organic pollutant, which has a certain chemical activity. it is broken down by heated,potential application of DNT and its derivatives have been of interest in the past few years in a broad range of weapon industry and the production of military and civilian goods. it is mutagenic and suspected carcinogenic, having toxic effects on both animals and human beings, on the human central nervous system,especially[1]. It is a potential hazard to human health. the 2,4-DNT and 2,6-DNT are common in the municipal sludge and groundwater. It is hard to degradable [2-3], they can cause the pollution of soil and crops and the impact on the growth, development and reproduction of the aquatic life [4]. When the concentration is up to 10mg/L, they can cause the death of fish and aquatic organisms [5]. And the synergy of binary mixtures makes toxicity much stronger, which brings a serious damage to the aquaticecosystem. it is classified as a member of the "excellent control of pollutants" by the United States National Environmental Protection Agency (USEPA) [6] .Therefore, it is necessary to find a degradation method that is economical and does not cause secondary pollution.

Biodegradation is a phenomenon that biological organisms turn to simpler compounds through a series of biochemical reactions .by the help of enzymes; sometimes they can completely turn to inorganic. In most aerobic and anaerobic environments, a lot of recalcitrant compounds can be degraded effectively through the metabolic activities of the environmental microbe. Microbial degradation becomes a principal degradation method of environmental pollutants, because of the advantages such as the low price, easy to control, the simple condition, not any secondary pollution and so on. In this study 2,6-DNT-degrading activity sludge was gained from the domestication of the strain; multi-factor multi-level Uniform Design was also invited to gain the.

2 Materials and methods

2.1 Experimental strain

Bacteria come from the activated sludge in the aeration tank of Harbin Sewage Treatment Plant.

2.2 Instruments and reagents

Inorganic salt medium: MnSO4.7H2O 0.28mg; FeSO4.7H2O 0.3mg; MgSO4.7H2O 0.06mg; CaCl2 1mg; CuSO4 0.05mg; ZnSO4 0.05mg; H3BO3 0.05mg; distilled water 1000mL. Test reagent: 2,6-Dinitrotoluene standard (AccuStandard); 2,6-Dinitrotoluene analytically pure (MERCK); chromatography pure benzene (Tianjin Chemical Reagent Research Institute), analytically pure ethanol (Tianjin Tianxin Fine Chemicals Development Center), distilled water.

Instrument: HDL turbulence incubator HZQ-F16 (vibration mode: maneuver); Aikele Analytical Balance (ALC-110.4); Angilent6890 gas chromatography-electron capture detector instrument (United States); DPS statistical software (Beijing Zhongnongbosi Science and Technology Development Corporation)

2.3 Methods

2.3.1 Domestication and Ex-cultivation of Degradation Bacteria

Add 50 mL inorganic salt medium into a 250mL-conical beaker, and then add some 2,6-DNT(the concentration is up to the following experimental conditions). Shake it then it becomes the domestication medium. Take 1mL of the supernatant of activated sludge into it and cultivated in the incubator shaker at 30℃, 130r/min. Take 50mg/L as the initial concentration, 5d as a cycle to domesticate it.

And during this period increase the concentration of 2,6-Dinitrotoluene gradually as 50,70,90,110,130,150,170 mg/L. After the domestication, add 100 mL inorganic salt medium into a 500mL-conical beaker and then add a small amount of beef extract. Take it for high-pressure sterilization. After the sterilization, take 10mL domesticated bacilli into the 500 mL-conical beakers to expand the training and achieve the logarithmic growth phase.

- Analytical Methods Sample Treatment: Extract the 2mL-degradated fluid twice through 2mL chromatography with Benzene and combine the extractions. Dry it over through the anhydrous sodium sulfate column and filter under the use of microporous membrane.

  GC-ECD Determination Conditions: capillary column HP-5MS (30m×0.25mm×0.25μm); 63Ni electron capture detector; carrier gas flow rate 0.8mL/min, inlet temperature: 230℃; temperature 300℃; column temperature 165℃; split injection, split ratio 50:1; injection volume 0.2μL.
- *Factors Affecting Degradation*

  Conditions affect the degradation are mainly: PH value, temperature, oxygen demand (shaker speed), the substrate concentration. Selected ranges for the major factors are as shown in Table 1[7].

  Carry out the uniform experimental design to the data by the use of DPS software. Add 100mL domesticated medium into the conical flask, and then add 4mL domesticated bacilli. The degradation time is 5d. Sample respectively at 24,48,72,96,120h, and each for 1mL.Calculate the degradation rate, half-life, degradation rate constant, and verify the optimal conditions through screening.

Table 1. Factors in the table

NO	Substrate concentration (mg/L)	PH Valve	Temperature (℃)	Shaker Speed (r/min)
1	15	5	5	100
2	30	5.5	10	110
3	45	6	15	120
4	60	6.5	20	130
5	75	7	25	140
6	90	7.5	30	150
7	105	8	35	160
8	120	8.5	40	170
9	135	9	45	180

3 Results and Analysis

3.1 Drawing of the Standard Curve and the Linear Range

Weigh 10 mg of 2,6-Dinitrotoluene standard accurately into 100mL-flask, adding benzene to the scale in order to gain the 100mg/L standard solution. Dilute it to a series of concentrations as 30,60,90,120,150,180,210,240,270,300 μg/L 2,6-Dinitrotoluene standard solution. Detect with GC-ECD. Take peak area (AUS) as the vertical axis, the concentration (C) as the abscissa, and then the standard curve is Y = 275353X + 600000, R = 0.9997. It shows that 2,6-DNT has a good linear relationship within the concentration scope of 30 ~ 300μg/L.

3.2 Result Analysis on the Degradation Conditions by Uniform Design Analysis

Gain the 9 units testing conditions by using U9 (94) uniform design method for optimal degradation conditions [8]. And after the 5d-degradation, degradation rates under different conditions can be know. The equation is fitted to a kinetic equation, and the degradation rate constants and half-life are shown in Table 2.

The optimization equation is obtained through the quadratic polynomial regression of the four factors and the degradation rate in Table 2 by the use of DPS software: Y = 197.92947122-22.92992881 3X1-0.7058404862X3+1.4521173438X1X1+0.0028964509323X3X3-0.0008412500401X4X4-0.0005513381186X1X2+0.04325027929X1X4, significance level p=0.0492, statistical value F=244.1291, correlation coefficient R=0.9997, adjusted correlation coefficient Ra=0.9977. Goodness of fit shows a good equation, and factors are significant. The above data indicate that the optimal degradation conditions based on the degradation rate, half-life and degradation rate constant were: PH value 7.2749, temperature 28.5388℃, speed 128.9894r/min, substrate concentration 135mg/L.

Table 2. U9 (94) UNIFORM DESIGN, DEGRADATION RATE AND HALF-LIFE（N=3）

NO	Initial concentration (mg/L)	PH	Temperature (℃)	Shaker Speed (r/min)	Degradation rate (%)	Degradation rate Constant	half-life (d)
1	30	5.5	40	120	61.05	0.943	0.73
2	90	7	45	160	60.13	0.920	0.75
3	105	6.5	25	100	75.42	1.403	0.494
4	75	9	15	110	59.5	0.904	0.77
5	45	8.5	30	180	67.45	1.122	0.62
6	60	5	20	150	55.37	0.807	0.86
7	120	6	10	170	60.68	0.933	0.74
8	135	8	35	130	85.21	1.794	0.39
9	15	7.5	5	140	45.49	0.607	1.14

3.3 Compliance Test

In the optimizing conditions (PH value 7.3, temperature 29℃, speed 129r/min, substrate concentration 135mg/L), the verification experiment is carried out. Take 4mL domesticated activated sludge into 100mL medium. Set three parallel sample groups. Placed them in the constant temperature shaker incubator cultured for 120h. Sample for 1mL respectively at 2,4,10,24,36,48,72,96,120h, accompanying supplement. After the sample extraction, dry

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it through anhydrous sodium sulfate and then filter it through the membrane, dilute it to the linear range of standard curve, detect it with GC-ECD and calculate the concentration of 2,6-DNT with the one point external standard method. Under these conditions, the degradation rate of 2,6-DNT was 96.5%, the degradation half-life was 0.38d, and the degradation rate constant was 1.821. The degradation curve is shown in Figure 1. The verification results were similar to the predicted one through the uniform design experiment, which indicated that the degradation conditions of this experiment was accurate and feasible.

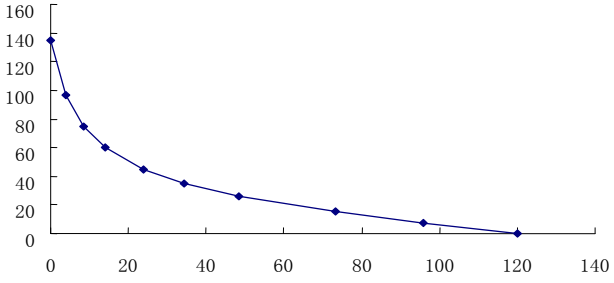

Figure 1. 2,6-DNT degradation curve

4 Conclusion

Domesticate the activated sludge comes from the aeration tank of Harbin Sewage Treatment Plant, taking 2,6-DNT as the sole carbon and nitrogen source, and gradually increasing its concentration. Therefore, the activated sludge has a much stronger degradation ability to 2, 6-DNT the degradation rate of which can reaches 96.5% in 5d.

The uniform design is a multi-factor experimental design method which can reduce the reduce the number of experiments; make a quantitative prediction of the optimum conditions and results. In this study, with the uniform design, the ultimate optimizing conditions for the degradation of 2,6-DNT were: PH value 7.5, temperature 29℃, speed 129r/min, substrate concentration 135mg/L. In this degradation condition, the degradation rate of 2,6-DNT was 96.5%, the degradation half-life was 0.38d, and the degradation rate constant was 1.821.

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