Study on the Screening for Chloronitrobenzene: Degrading Bacteria and Degradation of Chloronitrobenzene

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Abstract. With sewage plant aeration tank activated sludge as mixed bacteria source, Nitrobenzene concentration gradient of the chlorine used to increase the aerobic bacterial degradation of oscillating bottle method of domestication and enrichment culture. Separation and purification out of one can right-chloronitrobenzene as the sole carbon, nitrogen and energy of bacteria. Through physiological and biochemical reactions characteristic morphology and 16S rDNA sequence analysis, to determine the strain is Bacillus subtilis. The degradation kinetics analysis showed that the degradation of single strain 4CNB kinetic characteristics consistent with features.

Keywords: Chloro-nitrobenzene, Uniform design, Bacillus subtilis.

1 Introduction

Chloronitrobenzene are a class of chlorine-containing nitro-aromatic compounds and widely used as dyes, pesticides, pharmaceutical intermediates production, According to incomplete statistics, the country's right-chloronitrobenzene (4CNB, 4 - chloro-nitrophenyl) total production is about 120,000 tons in 2000th(1). wastewater in the process of chloro-nitrobenzene production, for containing chlorinated nitrobenzene, nitro-phenol, chlorobenzene, sulfuric acid, nitric acid and other pollutants, especially for the chlorinated nitrobenzene toxic and refractory, result in serious water discharge exceeded. Once poured into the soil and groundwater will be difficult to repair environmental damage(2). Due to the toxicity of chlorinated nitrobenzene toxicity including blood, spleen toxicity, liver toxicity, immunotoxicity, and also result in kidney damage, damage to the nervous system, etc.(3, 4) even the mutations and cancer. Therefore, the EC had list chloronitrobenzene as a pernicious and difficult to degrade in the environment of organic pollutants.(5) In this study, 4CNB pollution of the environment for the purpose of repair, the multi-source mixing domesticated bacteria, enrichment, separation and purification of a strain able to 4CNB as the sole carbon and nitrogen source of the bacterial strain, to be identified as Bacillus subtilis, and also the degradation characteristics of bacteria have been studied. The 4CNB predominant strains of the environment to provide bioremediation for the degradation of 4CNB provide the scientific theoretical approach.
2 Materials and Methods

2.1 Experimental Strain

Activated sludge, provide from Harbin Wenchang Wastewater Treatment Plant.

2.2 Instruments and Reagents

Experimental Instruments: HDL turbulence incubator model is HZQ-F19(Harbin East Union Electronics Co., Ltd.), Angilent6890 Gas chromatography - electron capture detector(Agilent Co., Ltd.) DPS statistical software (Beijing Boshi Technology Development Corporation)

Experimental reagents: Chloro-nitrobenzene(J & K Chemical Technology Co., Ltd., Standard sample, purity 100%); Benzene(Tianjin Chemical Reagent Research Institute, Chromatography pure)

Inorganic salt medium: Na2HPO4 14.3g/L, KH2PO4 3g/L, MgSO4.7H2O 0.06mg/L, FeSO4.7H2O 0.3 mg/L, MnSO4.7H2O 0.28mg/L, CaCl2 1mg/L, CuSO4 0.05mg/L, ZnSO4 0.05mg/L, H3BO3 0.01mg/L, NaOH(PH adjusted to 7.0), Medium (MSB) composed of Na2HPO4 1g/L, KH2PO4 0.5g/L, MgSO4·7H2O 0.03g, trace element solution, 5mL, (6) pH 7.0. Solid medium by adding 15g / L agar.

2.3 Analysis Method

Sample treatment: 4CNB the solution obtained after degradation of the sample bottle by adding 10mL in 10mL of benzene chromatographic eluent oscillation extraction 3 times, combined extract, using GC-ECD was determined 4CNB content.

GC-ECD determination conditions(7): HP-5 (30m×0.25mm×0.25μm) capillary, Column temperature 135℃; injection volume: 0.2μL; Split ratio : 50:1; Injection temperature; 230℃; Carrier gas flow rate: 0.8mL/min; 63Ni electron capture detector; Detector temperature 300℃.

2.4 Strain Enrichment, Separation and Purification

Sludge sample volume of 10% of the access to vaccination in the inorganic medium at 30℃, 130rpm shaking the bed shaking culture for about one week, Sampling measurement of the chlorine content of nitrobenzene, pending on the chlorine concentration of nitrobenzene is reduced, then 1% of the amount transferred to the new access medium to cultivate a week or so, so repeated 3 times. and then crossed on agar plates separated until they have a pure, single colony (8, 9).

2.5 Strain Identification

1) Degradation of physiological and biochemical characteristics of bacteria analyzed in accordance with conventional methods of identification (10, 11)

2) DNA extracted using CTAB / NaCl method (12)

3) 16S rDNA sequence analysis