Bioresource Technology 231 (2017) 19–25

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Kinetic analysis and degradation pathway for *m*-dichlorobenzene removal by *Brevibacillus agri* DH-1 and its performance in a biotrickling filter

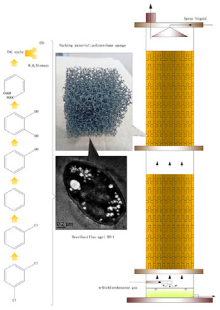
Bai-ren Yang [a,b], Zhu-qiu Sun [b], Li-ping Wang [a,*], Zhao-xia Li [b], Cheng Ding [b]

[a] School of Environment Science and Spatial Informatics, China University of Mining and Technology, Xuzhou, Jiangsu 221116, China
[b] School of Environmental Science and Engineering, Yancheng Institute of Technology, Yancheng, Jiangsu 224051, China

HIGHLIGHTS

- Growth and degradation kinetics for *m*-dichlorobenzene degradation by a strain DH-1 were studied.
- A putative pathway for *m*-dichlorobenzene degradation was presented.
- *m*-Dichlorobenzene removal and pressure drop in a biotrickling filter inoculated with strain DH-1 was analyzed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 18 January 2017
Accepted 20 January 2017
Available online 25 January 2017

Keywords:
m-Dichlorobenzene
Kinetic analysis
Degradation pathway
Biotrickling filter

ABSTRACT

A strain, *Brevibacillus agri* DH-1, isolated from dry lands was used to remove *m*-dichlorobenzene. After 48 h culturing, the concentrations of *m*-dichlorobenzene decreased from 26–130 to 7.87–28.87 mg/L and dry cell weight for bacterial growth reached 52.43–75.05 mg/L. The growth and degradation kinetics were analyzed by the fitting of Haldane-Andrews model and pseudo first-order model. A degradation pathway was proposed according to major intermediates (phenol), chloride ion variation, ring-opening enzyme activity, and high mineralization (0.47 $g_{Cl^-}/g_{m\text{-dichlorobenzene}}$, 0.65 $g_{co2}/g_{m\text{-dichlorobenzene}}$, 0.15 $g_{DCW}/g_{m\text{-dichlorobenzene}}$). In addition, the performance in a biotrickling filter (BTF) was evaluated through removal efficiency and pressure drop values with increasing inlet loading rate from 4.10 to 122.57 g/m^3/h at three empty bed residence time points (30 s, 60 s, and 90 s). The results demonstrated that strain DH-1 possessed high removal efficiency and stable operation in a BTF.

1. Introduction

Chemicals spread throughout the environment due to human activities, causing great harm to human health and ecosystems (Geelen et al., 2009). The emission of *m*-dichlorobenzene frequently leads to endocrine disorders, immune dysfunction, and cancer. A majority of the volatile organic compounds (VOCs) are toxic, generating severe photochemical pollution and health issues. The removal of VOCs has become a common concern of human health (Zhang et al., 2016). *m*-Dichlorobenzene was widely utilized as a raw material in the production of synthetic resin and pesticides (Mato et al., 2014), thereby releasing abundant quantities into the environment due to its refractory characteristic and volatility. Therefore, the study on waste-gas treatment technology for *m*-dichlorobenzene is significant and urgent.

Comparing to other treatment methods (physical and chemical), biological treatment is not only economical and effective for

* Corresponding author.
E-mail address: wlpkd123@163.com (L.-p. Wang).

http://dx.doi.org/10.1016/j.biortech.2017.01.038
0960-8524/

VOCs at low concentrations but also environmentally friendly (Rene et al., 2015). Biofilter (BF) is an optimal choice for VOCs treatment in various bioreactors (Rene et al., 2012). Conventional BFs and biotrickling filters (BTFs) are the two forms of BFs. The concentration of nutrients and pH in BTFs can be easily regulated by adding fresh medium, acids, and bases (Zamir et al., 2015; Kennes et al., 2009; Kennes and Veiga, 2013; Zhang et al., 2011). The high-efficiency degrading strains are vital for the stability of BTFs and removal performance. Several studies showed the ability of microorganisms for VOCs removal, for example, a chlorobenzene (CB)-degrading organism, *Ralstonia pickettii* L2, was isolated by Zhang et al. (2011) from a BTF. Biodegradation efficiency, degradation extent, and metabolic pathway of CB have been reported in their study. Wang et al. (2015) recently investigated a strain *Pandoraeas sp.* WL1 that was isolated from a pharmaceutical wastewater treatment plant. In addition, *a Brevibacillus borstelensis* GIGAN1 was under exploration for the potential as hydrophobic methanotroph on methane degradation (Li et al., 2015). However, kinetic analysis and degradation pathway for *m*-dichlorobenzene degradation by a *Brevibacillus agri* strain and its performance on *m*-dichlorobenzene removal in BTFs are still lacking.

In the present study, a strain *Brevibacillus agri* DH-1 that was isolated from soil was tested for its ability for *m*-dichlorobenzene removal. The degradation pathway and kinetic analysis of *m*-dichlorobenzene degradation by *Brevibacillus agri* DH-1 were studied. In addition, its performance in a BTF for *m*-dichlorobenzene removal was evaluated.

2. Material and methods

2.1. Chemicals and cultivation medium

m-Dichlorobenzene (>99.5%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pyrocatechol (AR) was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). KH_2PO_4 (for molecular biology, ⩾99%), Na_2HPO_4 (for molecular biology, ⩾99.50% (T)), $(NH_4)_2SO_4$ (for molecular biology,⩾99%), $CaCl_2$ (99.99% metals basis), $MnSO_4{\cdot}H_2O$ (for cell culture, for insect cell culture,⩾99%), $ZnCl_2$ (for molecular biology,⩾98% (AT)), $CuSO_4{\cdot}H_2O$ (99.999% metals basis), $CoCl_2{\cdot}6H_2O$ (99.998% metals basis), $Na_2MoO_4{\cdot}2H_2O$ (99.95% metal basis), $AlCl_3{\cdot}6H_2O$ (99.99% metals basis) all were obtained from Shanghai Aladdin Biochemical Technology Co.,Ltd.

The formula of mineral salts medium (MSM): KH_2PO_4 0.50 g, Na_2HPO_4 0.50 g, $(NH_4)_2SO_4$ 2 g, $CaCl_2$ 0.01 g, $MnSO_4{\cdot}H_2O$ 0.13 mg, $ZnCl_2$ 0.23 mg, $CuSO_4{\cdot}H_2O$ 0.03 mg, $CoCl_2{\cdot}6H_2O$ 0.42 mg, $Na_2MoO_4{\cdot}2H_2O$ 0.15 mg, $AlCl_3{\cdot}6H_2O$ 0.05 mg were mixed in one liter of water (Zhou et al., 2016). *m*-Dichlorobenzene was added as the sole carbon and energy source.

2.2. Strain Brevibacillus agri DH-1

In the current study, the strain *Brevibacillus agri* DH-1 (GenBank accession JX170207) is an aerobic, Gram-positive, encapsulated, sporiferous, motile, and rod-shaped bacterium (Fig. S1 in the Supplementary Materials). The cell morphology was observed using a JEM-2100F transmission electron microscope (TEM) (JEOL, Japan). Genotypic identification was carried out by 16S rRNA gene sequencing (Su et al., 2013; Zhang et al., 2010).

2.3. Experimental equipment

A BTF was used in the present experiment as presented in Fig. 1. The main body of BTF comprised of two sections of plexiglass with diameter 20 cm and height 50 cm. A gas pressure meter was placed at the top of the column and the packing material was constituted of polyurethane sponge. The gas streams of *m*-dichlorobenzene and spray liquid were regulated by rotameters. Gas-liquid two-phase flow in this BTF was designed for gas flowing upward and sprays liquid flowing downward. The gas streams of *m*-dichlorobenzene were introduced through the bottom of the column of the BTF. The spray liquid was raised to the top of BTF, and a sprinkler was placed at the top of the column for liquid redistribution.

2.4. Growth of strain DH-1 and m-dichlorobenzene degradation

50 mL of MSM was added to a 250 mL culture flask sealed with silicone rubber septa. The culture flask was then disinfected by high-pressure steam sterilization pot. *m*-Dichlorobenzene was added to the sterilized culture flask using a 1 μL gas-tight microsyringe. The concentration of *m*-dichlorobenzene increased to 26, 52, 78, 104, and 130 mg/L, respectively (Nkulu et al., 2015; Wang et al., 2013). Consecutively, 5 mL of the microbial suspension with an initial cell concentration of 20–40 mg DCW/L (dry cell weight) was administered to the culture flask, respectively. All the experiments were performed in duplicate.

2.5. Degradation pathway

The variation of Cl^- was monitored in the *m*-dichlorobenzene degradation for investigating dechlorination of *m*-dichlorobenzene. In the 2 d culture process, a sample was withdrawn every 6 h for testing Cl^-. All the extraction procedure was completed in a sterile environment. The samples were extracted using disposable sterile syringes with needles. Then, they were injected into the sample bottle of ion chromatography. In addition, the pin hole on the rubber stoppers of culture flask needs to be sealed using waterproof sealant at the end of the extraction.

In order to verify *m*-dichlorobenzene open-loop way, the enzyme activity of catechol-1,2-dioxygenase ($C_{12}O$) and catechol-1,2-dioxygenase ($C_{23}O$) that catalyzes the oxidation of aromatic ring to different products was assessed. A crude enzyme fluid was placed for 20 min from the logarithmic phase in 4 mL enzymatic reaction system with added catechol. Subsequently, the UV–visible spectrophotometric estimation was determined at 260 nm and 275 nm. The enzyme activity was computed as follows (Sala-Trepat and Evans, 1971; Leng, 2011):

$$\mathrm{U/mg\ Protein} = (\Delta A \times \Delta A \times V)/(\varepsilon \times M) \quad (1)$$

where, ΔA is the change of light absorption value per minute, V is the volume of enzymatic reaction system (L), ε is the molar extinction coefficient (1/mmol/cm), M is the quality of protein in enzymatic reaction system (mg).

Brevibacillus agri DH-1 was cultured in a 250-mL culture flask containing 50 mL MSM with 6.5 mg of initial *m*-dichlorobenzene, and the culture flask was agitated for 36 h in a water-bath (37 °C, 130 rpm) in order to detect the intermediates of *m*-dichlorobenzene degradation.

m-Dichlorobenzene mineralization was confirmed by determining the CO_2 production, Cl^- production, and bacterial yield (Li et al., 2015). *m*-Dichlorobenzene added to the sterilized culture flask (as described above) was calculated to be 1.30, 2.60, 3.90, 5.20, and 6.50 mg, respectively. The CO_2 and Cl^- production and bacterial yield were determined until the added *m*-dichlorobenzene was completely consumed. The coefficients of CO_2 and Cl^- production and bacterial yield were calculated by linear fitting of the test results.