Hydrolytic dehalogenation of the chlorothalonil isomer *o*-tetrachlorophthalonitrile by *Pseudochrobactrum* sp. BSQ-1

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Abstract

Microbial dehalogenation plays key roles in the biodegradation and detoxification of halogenated aromatics. Although the hydrolytic dehalogenation of halogenated aliphatic hydrocarbons and carboxylic acids has been extensively studied, there are few reports on the hydrolytic dehalogenation of halogenated aromatics. In this study, the aerobic strain BSQ-1, isolated from soils contaminated with halogenated aromatics in Jiangsu, China, was able to completely degrade 0.12 mM *o*-tetrachlorophthalonitrile in 84 hours in the presence of 2 mM sodium acetate trihydrate and released one equivalent of chlorine ions. MS-MS and NMR analysis revealed that *o*-tetrachlorophthalonitrile was dehalogenated to 4-hydroxyl-*o*-trichlorophthalonitrile. The dehalogenation of *o*-tetrachlorophthalonitrile could occur under both aerobic and anaerobic conditions, showing that the observed dehalogenation was a hydrolytic process. The optimal temperature and pH for *o*-tetrachlorophthalonitrile dehalogenation by strain BSQ-1 were 30°C and 8, respectively. It was found that 0.2 mM Zn²⁺, Mg²⁺ or Co²⁺ enhanced dehalogenation activity, whereas 0.2 mM Fe³⁺, Ni²⁺, Cu²⁺, Ca²⁺, or Pb²⁺ inhibited dehalogenation activity. Strain BSQ-1 was able to dehalogenate not only

o-tetrachlorophthalonitrile but also its two isomers, chlorothalonil and *p*-tetrachlorophthalonitrile. This study presents a new example of the hydrolytic dehalogenation of halogenated aromatics by microorganisms and provides a good candidate strain for the clean-up of tetrachlorophthalonitrile-contaminated sites.

Keywords: hydrolytic dehalogenation; *Pseudochrobactrum*; *o*-tetrachlorophthalonitrile; chlorothalonil isomer

1. Introduction

Halogenated aromatic compounds are widely used in agriculture and industry, for example, as defatting agents, herbicides and fungicides. Due to the substitution of halogens, these compounds are toxic and environmentally persistent, and they have therefore caused great problems for ecosystems and human health. Microbial removal of the halogens from halogenated aromatics can increase their biodegradation and reduce the risk of toxic intermediate formation, in addition to promoting the biogeochemical halogen cycle (Janssen et al., 2001; Adriaens et al., 2004). Thus, the key reaction in the microbial degradation and detoxification of halogenated aromatics is dehalogenation (Slater et al., 1995). Four microbial dehalogenation mechanisms of halogenated aromatics have been revealed to date: reductive, thiolytic, oxidative, and hydrolytic (Van Pee et al., 2003). In the oxidative dehalogenation reaction, the transformation of halogenated aromatics is catalyzed by monooxygenase (Orser et al., 1993), dioxygenases (Schweizer et al., 1987; Stefan et al., 1998; Tamara et al., 1999; Xun et al., 1999) and peroxidase (Gert et al., 1997). Reductive dehalogenation of halogenated aromatics occurs extensively in strict anaerobes in a process termed organohalide respiration, using halogenated compounds as the terminal electron acceptors (Gert et al., 1997; Adrian et al., 2000; Bunge et al., 2003). Reductive dehalogenation can also occur in aerobes, catalyzed by either glutathione S-transferase (Wilce et al., 1994) (termed thiolytic dehalogenation) or aerobic BhbA homologs, which share some features with the anaerobic “respiration-linked reductive dehalogenase” (non-organohalide respiration) (Chen et al., 2013).

With regard to hydrolytic dehalogenation, although the hydrolytic dehalogenation of halogenated aliphatic hydrocarbons and carboxylic acids has been extensively studied, there are only two reports on the hydrolytic dehalogenation of halogenated aromatics, involving the hydrolytic dehalogenation of 4-chlorobenzoyl-CoA and chlorothalonil (Scholten et al., 1991; Schmitz et al., 1992). Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile), a broad-spectrum chlorinated aromatic fungicide, is

highly toxic to fish, birds and aquatic invertebrates and is commonly detected in ecosystems (Zhang et al., 2007). Bacteria that are capable of the hydrolytic dechlorination of chlorothalonil to form 2,4,5-trichloro-6-hydroxybenzene-1,3-dicarbonitrile (4-TPN-OH) were previously isolated (Liang et al., 2010; Wang et al., 2010; Liang et al., 2011; Liang et al., 2012). Due to the common use of chlorothalonil in agriculture, the microbial degradation of chlorothalonil has attracted much attention. However, there are no reports on the microbial degradation of the isomers of chlorothalonil, the *o*-tetrachlorophthalonitrile (3,4,5,6-tetrachloro-1,2-benzenedicarbonitrile) and *p*-tetrachlorophthalonitrile (2,3,5,6-tetrachloro-1,4-benzenedicarbonitrile).

o-Tetrachlorophthalonitrile, which is an important organic intermediate, plays vital roles in the pharmaceutical and chemical industry. For example, *o*-tetrachlorophthalonitrile is used as a key intermediate in the synthesis of phthalocyanine, clinafloxacin, sitafloxacin, and other fluoroquinolones (Volkova et al., 2007; Galanin et al., 2012). *o*-Tetrachlorophthalonitrile exhibits lower solubility and longer permanent resistance than chlorothalonil in alkaline environments, and it is more difficult to degrade using microorganisms because of its high stability. The identification of potential *o*-tetrachlorophthalonitrile degraders and investigation of the microbial degradation mechanism involved holds great interest for us. In this study, a bacterial strain of *Pseudochrobactrum* sp. BSQ-1, that was capable of degrading *o*-tetrachlorophthalonitrile was isolated and characterized. The metabolite of *o*-tetrachlorophthalonitrile during the degradation process was identified, and the dehalogenation process of *o*-tetrachlorophthalonitrile was elucidated. This study presents a new example of the hydrolytic dehalogenation of halogenated aromatics by microorganisms and provides a good candidate strain for the clean-up of tetrachlorophthalonitrile-contaminated sites.

2. Materials and methods

2.1 Chemical and medium

o-tetrachlorophthalonitrile (3,4,5,6-tetrachloro-1,2-benzenedicarbonitrile) (purity > 98%) was purchased from the Nanjing Chemlin Chemical Industry Co., Ltd (Nanjing, China). Chlorothalonil (purity > 98%) and *p*-tetrachlorophthalonitrile (purity > 98%) were provided by the Jiangsu Weunite Fine Chemical Co., Ltd (Xinyi, China).

Lysogeny broth (LB) medium, mineral salt medium (MM; 1.0 g NH₄NO₃, 1.5 g K₂HPO₄, 0.5 g KH₂PO₄, 0.2 g MgSO₄ and 1.0 g NaCl per liter of water, pH 7.0) and non-halide MM (MNMM; 1.0 g NH₄NO₃, 1.5 g K₂HPO₄, 0.5 g KH₂PO₄, and 0.2 g MgSO₄ per liter of water, pH 7.0) supplemented with

0.12 mM *o*-tetrachlorophthalonitrile were used for strain culture and dehalogenation tests.

2.2 Strain isolation and characterization

The isolation of *o*-tetrachlorophthalonitrile-degrading bacteria was carried out by the conventional enrichment culture technique (Li et al., 2010; Yang et al., 2013). Soil samples (5 g) collected from a halogenated-aromatic manufacturing factory in Liyang, Jiangsu, were added to 100 ml MM supplemented with 0.12 mM *o*-tetrachlorophthalonitrile, followed by incubation in a rotary shaker at 160 rpm at 30°C. The enrichment was finally spread on MM agar supplemented with 0.12 mM *o*-tetrachlorophthalonitrile and 1/10 LB and then incubated at 30°C. Colonies with transparent hydrolytic halos were picked and purified using the streaking method. One pure isolate that could efficiently degrade *o*-tetrachlorophthalonitrile, designated strain BSQ-1, was selected. Strain BSQ-1 was identified based on its morphological, physiological, and biochemical properties, referring to *Bergey's Manual of Determinative Bacteriology* and 16S rRNA gene sequence analysis (Bergey et al., 1994; Sarioglu et al., 2012; Benkiara et al., 2013).

2.3 Degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1

Cells of strain BSQ-1 were pre-cultured in LB at 30°C for 24 h, washed and re-suspended in MNMM. The OD_{600 nm} (cell density) was adjusted to 5.0. To measure the degradation of *o*-tetrachlorophthalonitrile, cells of strain BSQ-1 were inoculated into 100 ml MNMM (containing 0.12 mM *o*-tetrachlorophthalonitrile) to obtain a final density of OD_{600nm} = 0.2. The inoculum was incubated at 30°C on a shaker at 160 rpm. The chloride ions released during degradation were detected and quantified according to the method of Bergmann (Bergmann et al., 1957).

The effect of other carbon sources on *o*-tetrachlorophthalonitrile degradation by strain BSQ-1 was investigated by adding 2 mM sodium acetate trihydrate to the MNMM. The effect of temperature on *o*-tetrachlorophthalonitrile degradation by strain BSQ-1 was evaluated at 15, 20, 25, 30 and 37°C in MM in the presence of 2 mM sodium acetate trihydrate. The effect of pH on *o*-tetrachlorophthalonitrile degradation was determined by incubation of the MM reaction system (containing 2 mM sodium acetate trihydrate) at 30°C with initial pH values ranging from 4 to 10. To investigate the effect of different metal ions on *o*-tetrachlorophthalonitrile degradation, various metal ions (FeCl₃, ZnCl₂, CoCl₂, NiSO₄, CuSO₄, CaCl₂, MgCl₂, and PbAc) at 0.2 mM, together with 2 mM sodium acetate trihydrate, were added to MM in the presence of 2 mM sodium acetate trihydrate, followed by incubation at 30°C. The degradation rate of *o*-tetrachlorophthalonitrile was determined after 48 hours. All experiments

were carried out in triplicate.

2.4 Dechlorination assay under anaerobic conditions

Cells of strain BSQ-1 were washed twice with phosphate-buffered saline (PBS; 8.0 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, 0.24 g KH₂PO₄ per liter of water, pH 7.4) and disrupted using French pressure (Thermo Spectronic, USA). Anaerobic dechlorination experiments with the crude enzyme were performed in GasPak Anaerobic Systems according to the manufacturer's instructions. Trace amount of oxygen in the solution were removed by the addition of a reduced form of glutathione to a concentration of 10 mM. Resazurin (0.002 mM) was used as an indicator of anoxic conditions in the assay. The residual *o*-tetrachlorophthalonitrile, which had previously been added to the reaction system (0.12 mM), was measured as described below.

2.5 *o*-Tetrachlorophthalonitrile detection assay

o-Tetrachlorophthalonitrile in the liquid medium was extracted with an equal volume of dichloromethane. The extract was dried over anhydrous Na₂SO₄ and then evaporated at room temperature. The *o*-tetrachlorophthalonitrile residue was subsequently dissolved in 1 ml of acetonitrile and filtered through a 0.22- μ m-pore-size Millipore membrane, and then analyzed by high-performance liquid chromatography (HPLC, Shimadzu LC-20AD, Waters 2487 Dual λ Absorbance Detector). The injection volume was 20 μ l and separation was carried out in a Waters Nova-Pak C18 column (internal diameter, 4.6 mm; length, 250 mm) with acetonitrile/water (80:20, v/v) as the mobile phase. The flow rate was 1.0 ml min⁻¹, and the column temperature was 40°C. The detection wavelength was 240 nm..

2.6 Detection and identification of the metabolite during *o*-tetrachlorophthalonitrile degradation

Resting cells were used for the collection of the metabolite during *o*-tetrachlorophthalonitrile degradation. Briefly, the cells were pre-cultured in LB until mid-log phase, then harvested and washed twice with ice-cold MM and re-suspended in 20 ml MM. The resting cells were inoculated at a final density of OD_{600 nm} = 0.2 into MM with 0.12 mM *o*-tetrachlorophthalonitrile and 2 mM sodium acetate trihydrate and then incubated at 30°C. Samples (100 ml) were collected after incubation for 96 hours and acidulated by adding 5 M HNO₃ to obtain a final pH value of 3.0, followed by extraction with an equal volume of ethyl acetate. The extract was dried over anhydrous Na₂SO₄ and then evaporated via rotary evaporation at room temperature. The residue was dissolved in 1 ml of acetonitrile and then separated via thin-layer chromatography (TLC) on silica gel G using a hexane-isopropanol-methanol (5:3:2, vol/vol/vol) solvent system. The metabolite was visualized under UV light at 240 nm. The spots

corresponding to the metabolite were all scratched from the plates, then extracted in acetonitrile and dried as described above. The metabolite was first identified through tandem mass spectrometry (MS/MS; Finnigan TSQ Quantum Ultra AM). Characteristic fragment ions were detected via second-order MS. To determine the position of the dehalogenated chlorine atom, the purified metabolite in solid form was tested through nuclear magnetic resonance (NMR) (Bruker, Switzerland), and the crystal structure was identified with an x-ray diffractometer (Apex/CCD, German). In the NMR analysis, the assignment of signals was carried out by considering their relative heights and through comparison with the calculated chemical shift values using the substituent shift.

2.7 The range of dehalogenation substrates of strain BSQ-1

The range of dehalogenation substrates of strain BSQ-1 was determined using the same conditions described for *o*-tetrachlorophthalonitrile in the presence of 2 mM sodium acetate trihydrate in MNMM, but with different halogenated substrates (including chlorothalonil, *p*-tetrachlorophthalonitrile and other halogenated aromatics). These halogenated aromatics were detected by the same method described for the *o*-tetrachlorophthalonitrile assay.

3. Results

3.1 Isolation and characterization of the *o*-tetrachlorophthalonitrile-degrading strain BSQ-1

A bacterial strain, designated BSQ-1, that was capable of efficiently degrading *o*-tetrachlorophthalonitrile was isolated from soils collected from a halogenated-aromatic manufacturing factory in Liyang, Jiangsu, China. Strain BSQ-1 is a Gram-negative, aerobic, rod-shaped (0.5-0.7 μm $\times$ 1.2-1.5 μm), non-motile bacterium. Strain BSQ-1 was positive for arginine dihydrolase, lysine decarboxylase, urease, and catalase, but negative for β -galactosidase, tryptophan deaminase, and gelatinase. It could not hydrolyze starch or gelatin. Strain BSQ-1 could assimilate citrate but could not use sucrose or D-mannitol for growth. The phylogenetic tree of 16S rRNA gene sequences showed that strain BSQ-1 was related to the *Pseudochrobactrum* species lineage and clustered closely with *Pseudochrobactrum saccharolyticum* CCUG 33852, with a sequence similarity score of 99.87%. Based on these phenotypic characteristics and phylogenetic analyses, strain BSQ-1 was identified as *Pseudochrobactrum* species.

3.2 Degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1

In MNMM, strain BSQ-1 degraded nearly 22% of 0.12 mM *o*-tetrachlorophthalonitrile in 84 hours and was unable to utilize *o*-tetrachlorophthalonitrile as the sole carbon source for growth. However, in

with the presence of 2 mM sodium acetate trihydrate, 0.12 mM *o*-tetrachlorophthalonitrile was completely degraded by strain BSQ-1 in 84 hours, and released one equivalent of chloride ions, and the cell number increased from 1.94×10^7 CFU ml⁻¹ to 1.06×10^8 CFU ml⁻¹ (Fig. 1). These results showed that the degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1 was greatly affected by exogenous carbon sources.

Fig. 1

3.3 Identification of the metabolite during *o*-tetrachlorophthalonitrile degradation

The metabolite produced from *o*-tetrachlorophthalonitrile degradation was identified via MS/MS. In standard MS, a prominent deprotonated molecular ion [M-H]⁻ at $m/z = 244, 246, 248$ and 250 enabled the assignment of molecular ion [M] at $m/z = 245, 247, 249$ and 251 , which was same as the compound hydroxyl-trichlorophthalonitrile (Fig. 2). The position of the hydroxyl group on the aromatic ring was first determined through proton-NMR. Proton-NMR analysis of the purified metabolite showed that the characteristic displacement of the hydrogen atom was 7.25, which was less than 10. Therefore, we speculated that the position of the hydroxyl group was on the fourth carbon atom of the aromatic ring (Fig. 3).

Fig. 2

Fig. 3

To further confirm the position of the hydroxyl group, a crystal of the metabolite was obtained, and X-ray crystallographic diffraction analysis was performed using a single-crystal diffractometer (Apex/CCD, Germany). The results showed that the position of the hydroxyl group was, in fact, on the fourth carbon atom of the aromatic ring (Table 1 and Fig. 3). Thus, the metabolite was identified as 4-hydroxyl-*o*-trichlorophthalonitrile.

Table 1

3.4 Dechlorination of *o*-tetrachlorophthalonitrile under anaerobic conditions

The microbial substitution of chlorine by a hydroxyl group may be carried out through either oxidative or hydrolytic dechlorination. To confirm the dechlorination process, the efficiency of dechlorination by the crude enzymes of strain BSQ-1 in the absence or presence of oxygen was compared. A similar dechlorination efficiency was observed in the presence and absence of oxygen. These data showed that the oxygen atom in the hydroxyl group was derived from water instead of molecular oxygen. Therefore, we conclude that the dehalogenation of *o*-tetrachlorophthalonitrile by

strain BSQ-1 is a hydrolytic process (Fig. 4). The reaction time was 24 hours.

Fig. 4

3.5 Effect of environmental factors on the biodegradation of *o*-tetrachlorophthalonitrile

The degradation rates of *o*-tetrachlorophthalonitrile were studied through inoculation with strain BSQ-1 in liquid culture medium under different conditions. The optimal temperature for the degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1 was determined to be 30°C (Fig. 5A). The optimal pH for degradation was determined to be 8.0, with an over 80% relative degradation rate being observed from pH 7.0-8.0 (Fig. 5B). The degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1 was strongly inhibited by 0.2 mM Zn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Ca^{2+} , Pb^{2+} or Mg^{2+} but was slightly enhanced by 0.2 mM Zn^{2+} , Mg^{2+} , or Co^{2+} (Fig. 6).

Fig. 5A and 5B

Fig. 6

3.6 The range of the dehalogenation substrates of strain BSQ-1

Interestingly, strain BSQ-1 could dehalogenate not only *o*-tetrachlorophthalonitrile but also its two isomers, chlorothalonil and *p*-tetrachlorophthalonitrile (Fig. 7). However, other tested halogenated aromatics including chlorobenzene, *p*-dichlorobenzene, 4-chloronitrobenzene, pentachloronitrobenzene, 4-chlorobenzoate, 4-chlorophenylacetic acid, *m*-chloroaniline, *p*-chloroaniline, and pentachlorophenol were not dehalogenated by strain BSQ-1 (data not shown). Among the three chlorothalonil isomers, strain BSQ-1 showed a faster degradation rate for chlorothalonil than *o*-tetrachlorophthalonitrile and *p*-tetrachlorophthalonitrile (Fig. 7).

Fig. 7

4. Discussion

o-Tetrachlorophthalonitrile is generally used in the fields of industry and pharmacy. As an isomer of chlorothalonil, *o*-tetrachlorophthalonitrile is a good candidate halogenated aromatic for the study of hydrolytic dehalogenation. Although the microbial degradation of chlorothalonil, including hydrolytic dechlorination and reductive dechlorination, has been investigated in detail (Liang et al., 2010; Wang et al., 2010; Liang et al., 2011; Liang et al., 2012), there are no available reports on the microbial degradation of *o*-tetrachlorophthalonitrile. Strain BSQ-1 is the first reported bacterium with the ability to dehalogenate *o*-tetrachlorophthalonitrile. Previously reported chlorothalonil-degrading bacteria can efficiently dehalogenate chlorothalonil in the absence of other carbon sources (Liang et al., 2010; Wang

et al., 2010). Although these chlorothalonil-degrading bacteria cannot use chlorothalonil as a carbon source for growth, it is presumed that the dehalogenase gene is continuously expressed and that a special transport protein responsible for the transport of chlorothalonil into cells exists. However, strain BSQ-1 showed a lower dehalogenation rate for *o*-tetrachlorophthalonitrile in the absence of other carbon sources, and the dehalogenation rate increased when another carbon source was added. The results indicate that the dehalogenation of *o*-tetrachlorophthalonitrile is dependent on the biomass of strain BSQ-1, which can be increased in the presence of other carbon sources.

Based on the results of NMR and X-ray crystallographic diffraction analyses, the chlorine atom at the fourth site of *o*-tetrachlorophthalonitrile was substituted by a hydroxyl group. During the microbial degradation of chlorothalonil, dehalogenation also occurs at the fourth site (Wang et al., 2010). The 4-chlorine atom of chlorothalonil is presumed to be the most susceptible chlorine atom to being replaced (Binkley et al., 1977). It appears that the 4-chlorine atom of *o*-tetrachlorophthalonitrile is also the most susceptible chlorine atom to being substituted. Dehalogenation at the fourth site is hypothesized to be affected by the electron-withdrawing properties of nitrile and other chlorine atoms at the *ortho* or *para* positions.

The results regarding the effect of oxygen on dehalogenation rates confirmed the observed dehalogenation was a hydrolytic process. This is the third example of microbial dehalogenation of halogenated aromatics in a hydrolytic manner, although there are many reports of hydrolytic dehalogenation of halogenated aliphatic hydrocarbons and carboxylic acids. Despite the fact that only one chlorine was dehalogenated, the metabolite 4-hydroxyl-*o*-trichlorophthalonitrile exhibited higher solubility than its parent compound, and it can therefore be easily cleaned from solid particles in a water system and might provide opportunities for other organisms to utilize and even, ultimately, mineralize this metabolite. In general, this study presents a new example of the hydrolytic dehalogenation of halogenated aromatics by microorganisms and provides a good candidate strain for the clean-up of tetrachlorophthalonitrile-contaminated sites.

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Figure legends

Fig. 1 Degradation of *o*-tetrachlorophthalonitrile (●, with carbon source; ○, without carbon source) and cell growth of strain BSQ-1 (▲, with carbon source; △, without carbon source), with the release of chloride ions (■) in MNMM with carbon source (2 mM sodium acetate trihydrate). The data are represented as the means ± standard deviation for triplicate incubations. When the error bar is not visible, it is within the data point.

Fig. 2 Identification of the metabolites associated with *o*-tetrachlorophthalonitrile degradation via MS-MS. *o*-tetrachlorophthalonitrile shows negatively charged ions (with m/z 244.81, 246.82, 248.81 and 250.79, $[M-H]^-$) in standard MS (A). The characteristic second-order MS fragment ions peak are observed at m/z =111.98 and m/z =146.82 (B).

Fig. 3 Identification of the purified metabolite via NMR. The peaks at 2.485, 3.657 and 7.302 correspond to the solvent DMSO, H₂O and the hydroxyl group of the metabolite, respectively.

Fig. 4 Effect of oxygen on the degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1; ck (a) is the blank control (without the strain BSQ-1) under aerobic conditions; ck (an) is the blank control (without strain BSQ-1) under anaerobic conditions.

Fig. 5 Effects of temperature (A) and pH values (B) on the degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1.

Fig. 6 Effects of various metal ions on the degradation of *o*-tetrachlorophthalonitrile by strain BSQ-1.

Fig. 7 Degradation of *o*-tetrachlorophthalonitrile (■), chlorothalonil (▲) and *p*-tetrachlorophthalonitrile (●) by strain BSQ-1 in MM with 2 mM sodium acetate trihydrate. The data are presented as the means ± standard deviation for triplicate incubations. When the error bar is not visible, it is within the data point.

T: - c QIMS [100.00-350.10]

Fig 2.

T: - c Full ms2 245.00@55.00 [30.00-275.00]

(B)

Hubincheng-1H
2014-06-25 1H-4 DMSO HBC-216 (ZC-1)

Fig 3.

Fig 4.

(A)

(B)

Fig 5.

Fig 6.

1 Table 1 Crystal data of X-ray diffraction analysis

Items	
Temperature / K	296
Empirical formula	C ₈ HCl ₃ N ₂ O
Formula weight / g mol ⁻¹	247.46
Crystal system	Monoclinic
Space group	<i>C2/m</i>
<i>a</i> /Å	7.3852(12)
<i>b</i> /Å	11.0858(18)
<i>c</i> /Å	11.913(2)
/°	90.00
/°	105.86(3)
/°	90.00
Volume/Å ³	1358.26(6)
<i>Z</i>	4
ρ_{calc} /mg/mm ³	1.21
μ /mm ⁻¹	0.8507
<i>F</i> (000)	488
Reflections collected	6665
Independent reflections	4556
	<i>R</i> _{int} = 0.0242
Goodness-of-fit on <i>F</i> ²	1.057
Final <i>R</i> indexes	<i>R</i> ₁ = 0.0403
[<i>I</i> ≥ 2σ(<i>I</i>)]	<i>wR</i> ₂ = 0.0647
Final <i>R</i> indexes	<i>R</i> ₁ = 0.0424
[all data]	<i>wR</i> ₂ = 1.0239
Largest diff. peak/hole / e Å ⁻³	0.485/-0.713