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Perspective

# Biodegradation of 3,5-dimethyl-2,4-dichlorophenol in saline wastewater by newly isolated *Penicillium* sp. yz11-22N2

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ABSTRACT

In this study, the performance of 3,5-dimethyl-2,4-dichlorophenol (DCMX) degradation by a screened strain was investigated. 18S rDNA and the neighbor-joining method were used for identification of the isolated strain. The results of phylogenetic analysis and scanning electron micrographs showed that the most probable identity of the screened strain should be *Penicillium* sp. Growth characteristics of *Penicillium* sp. and degradation processes of DCMX were examined. Fourier transform infrared spectroscopy of the inoculated DCMX solution was recorded, which supported the capacity of DCMX degradation by the screened *Penicillium* sp. Under different salinity conditions, the highest growth rate and removal efficiency for DCMX were obtained at pH 6.0. The removal efficiency decreased from 100% to 66% when the DCMX concentration increased from 5 to 60 mg/L, respectively. Using a Box–Behnken design, the maximum DCMX removal efficiency was determined to be 98.4%. With acclimation to salinity, higher removal efficiency could be achieved. The results demonstrate that the screened *Penicillium* sp. has the capability for degradation of DCMX.


## Introduction

Chlorophenols are introduced into the environment through disinfection by chlorination or as components of personal care products (Thomas et al., 2015), and have recently gained public attention as a pervasive problem. Chlorophenols are characterized by high persistence and bioaccumulation (Van Aken et al., 2015), and can result in severe ecotoxicological problems (Furukawa, 2006). Chlorophenols consist of a benzene ring, –OH group and chlorine atom(s) and other possible substituents, such as methyl and ethyl groups (Ivanciuc et al., 2006). Chlorophenols are considered to be harmful to human health due to their potential carcinogenic and mutagenic activity (Kumar and Min, 2011) and toxicity. These compounds are listed by the USA EPA as priority environmental pollutants, and the amount of these compounds in drinking water is stipulated

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to less than 1 mg/L (ATSDR, 2007). Van Aken et al. (2015) proved that the increasing toxicity of higher chlorinated phenolic compounds could be ascribed to their higher lipophilicity. Chlorophenols can be transported through the cell membrane and bio-accumulated in aquatic organisms due to their lipophilicity (González et al., 2010). The toxicity of chlorophenols depends on the number of chlorine atoms and the position of the substitution (Annachhatre and Gheewala, 1996), which can dramatically influence the lipophilicity of chlorophenols. Recently, the interest in these chlorophenols and the search for approaches leading to their complete removal has resulted in a significant increase in related studies (Jiang et al., 2005; Galíndez-Mayer et al., 2008).

Numbers of physical, chemical and biological methods have been used to remove chlorophenols from industrial effluents (Olaniran and Igbinosa, 2011). Oxidation methods are the most widely used techniques for chlorophenol removal (Fukushima and Tatsumi, 2001; Denizli et al., 2004). However, oxidation processes have several disadvantages, including considerably high temperatures and pressures, large amounts of chemical reagents and complex equipment, *etc.* As a consequence, some more cost-effective processes, including anaerobic or aerobic biological treatment, have been considered as the most promising methods for treating chlorophenols (Denizli et al., 2004).

Biodegradation is an important removal process during wastewater treatment and for the purification of water through soil passage and groundwater recharge. Biodegradation of chlorophenolic compounds has been carried out with microorganisms (Bergauer et al., 2005; Galíndez-Mayer et al., 2008). Many bacteria and fungi are capable of using chlorophenols as a carbon and energy source (Murialdo et al., 2004; Reddy and Gold, 2000; Cortés et al., 2002; Crawford et al., 2007), such as white and brown rot (Fahr et al., 1999); *Phanerochaete chrysosporium* (Denizli et al., 2004), and *Bacillus subtilis* (Matafonova et al., 2006). These processes are attributed to the participation of their enzymatic systems. The ability of laccase and other related enzymes to eliminate organic pollutants is a conventional concept which has been reported in many studies (Pérez et al., 1997; Olaniran and Igbinosa, 2011; Phan and Sabaratnam, 2012). However, the chlorophenols with chlorine atoms at ortho position on the aromatic ring were preferentially attacked. As we all know, chlorine atoms at meta- and para-positions are more resistant to degradation than those at the ortho position (Liu et al., 1982; Saito et al., 1991; Reddy and Gold, 2000). Due to the large amounts of chlorine atoms and substitution positions, some chlorophenols, such as 3,5-dimethyl-2,4-dichlorophenol (DCMX), which is a kind of fungicide and widely exists in water environments, have been hard to remove. Hence, with growing environmental concerns worldwide, efforts are now focused on studies about the degradation of difficultly degradable chlorophenols.

High salinity has great influence on microbial internal molecules, including microbial products, extracellular polymeric substances, and microbial enzymes. However, studies on organic pollutant treatments have focused on their removal from fresh wastewater, and few studies have focused on chlorophenol removal from saline wastewater (Wang et al., 2014). Changes in microbial internal molecules have very dramatic influences on the removal of organic pollutants (He et al., 2016). Faced with tightening regulations, the interest in treatment of organic matter in saline wastewater has been increasing rapidly. Saline wastewaters are usually treated through physico-chemical methods, as conventional biological treatment is strongly inhibited by salt (mainly NaCl). Biological processes perform poorly when treating wastewater with high salinity, because microbial degradation of organic matter mixtures could be inhibited by high salinity in water, which would affect the growth of microorganisms due to an inhibition of microbial activity (Lefebvre and Moletta, 2006). In spite of the detrimental effect of salt on microbial activity, moderate acclimation to high salinity is possible.

The aim of the current study is to obtain a strain capable of DCMX degradation in the presence of high salinity. To our knowledge, this is the first report of DCMX biodegradation, especially in high salinity wastewater. The effects of important variables, such as pH, initial concentration of DCMX, and culture salinity on the growth characteristics and removal capacity for DCMX of the screened strains were investigated in batch experiments. A Box–Behnken design was applied to determine the optimum conditions, and also to explain the relationships between DCMX removal and three operating parameters, namely, temperature, culture pH, and the salinity of wastewater.

## 1. Materials and methods

### 1.1. Wastewater collection and pretreatment

Raw wastewater was obtained from the sterilizing agent production process (SAPP) in Chemical Synthesis Corporation, Hunan, China. The components of the wastewater were 3,5-dimethylphenol (MX), 4-chloro-3,5-dimethylphenol (PCMX), DCMX and a certain amount of salt (mainly NaCl). The main organic pollutant was DCMX, surmised according to the processing technique and verified by High Performance Liquid Chromatography (HPLC) (1100, Agilent Tech Co., Ltd) and Gas Chromatograph-Mass Spectrometer (GC–MS) (QP2010, SHIMADZU Co., Ltd).

Minimal medium (MM) was used for isolation of degrading microorganisms. Diluted raw wastewater was added into the MM as the sole carbon source. The screened strains were purified by the plate streaking method. Finally, purified strains were inoculated into MM with the diluted raw wastewater as the sole carbon source. The strains with higher growth rate were chosen.

### 1.2. Strain screening, isolation and cultivation conditions

#### 1.2.1. Cultivation conditions

The composition of MM included (in g/L): NaCl 30.0, $KH_2PO_4$ 3.0, $(NH_4)_2SO_4$ 1.0, Na-Citrate 1.0, $MnCl_2{\cdot}4H_2O$ 0.04, $CoCl_2{\cdot}6H_2O$ 0.07, $ZnSO_4{\cdot}7H_2O$ 0.05, $CaCl_2$ 0.05, $NiCl_2{\cdot}6H_2O$ 0.05, $FeSO_4{\cdot}7H_2O$ 0.075, $MgSO_4{\cdot}7H_2O$ 0.2, $CuSO_4{\cdot}5H_2O$ 0.05; $C_6H_6O_6$ 10.0 g/L as the carbon source. The solid medium used for the isolation process consisted of (in g/L): NaCl 30.0, $KH_2PO_4$ 3.0, $(NH_4)_2SO_4$ 1.0, Na-Citrate 1.0, Peptone 15.0, Beef extract 15.0, Agar 16.0 (Liang et al., 2015). The pH value of the screening culture medium was 7.0, which was adjusted by adding NaOH or HCl.



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