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Research Article

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Degradation of tritchloroethene and chloroform using methanotrophs isolated from landfills biocovers soil

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ABSTRACT

Bioremediation techniques were considered to be the most effective way to treat and dispose of chlorinated hydrocarbons and methanotrophs are potentially useful to do so. Municipal solid waste (MSW) landfill biocovers, where methanotrophs exist widely, played an important role in reducing emissions of methane and bioremediation of chlorinated hydrocarbon. Methylocystis strain JTA1, a new kind of facultative methanotroph isolated from age-fused, showed high tolerance for trichloroethene (TCE) and chloroform (CF). Then, effect of chlorinated hydrocarbon on methane oxidation and the degradation kinetic of chlorinated hydrocarbon were carried out. Strain JTA1 showed high-activity of methane oxidation at the chloroform concentration of 20-50 mg L^{-1}, which was much higher than the highest rate of reported bio-covers. In addition, the kinetic equation of TCE degradation by methanotrophs community was also fitted, the maximum degradation rate ($q_{s,max}$= $1.51*10^{-4}$ min^{-1}) and half-saturation constants (K_S=2.58 mg L^{-1}, R^2=0.961) were calculated according to kinetic equation of trichloroethylene degradation which fitted to Monad model well. In conclusion, bioremediation of chlorinated hydrocarbon using biocovers Soil opens up a new possibility for environmental biotechnology, such as soil or landfills bioremediation and wastewater decontamination.

Keywords: methanotrophs; landfill biocovers; chlorinated hydrocarbons; bioremediation

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INTRODUCTION

Volatile chlorinated hydrocarbons (VCH), such as trichloroethylene (TCE) and chloroform (CF), seriously polluted ecological environment and threat to human health because of unreasonable application in industry and human life[1-2]. Bioremediation of chlorinated hydrocarbons by microorganism has attracted more and more attentions with its high efficiency, non-toxicity, non secondary pollution and other benefits[3-4]. Among all kinds of microbes involved in biodegradation, facultative methanotrophs have revealed spacious prospect on engineering application, since they can utilize multi-carbon compounds as source of carbon and energy and were easy to be cultured compared with obligate methanotrophs. Facultative methanotrops can utilize acetate, pyroracemic acid, hydroxysuccinic acid and ethanol etc. Moreover, some compounds can be prior to use and some chlorinated hydrocarbons can be degraded by these cells[5-6].

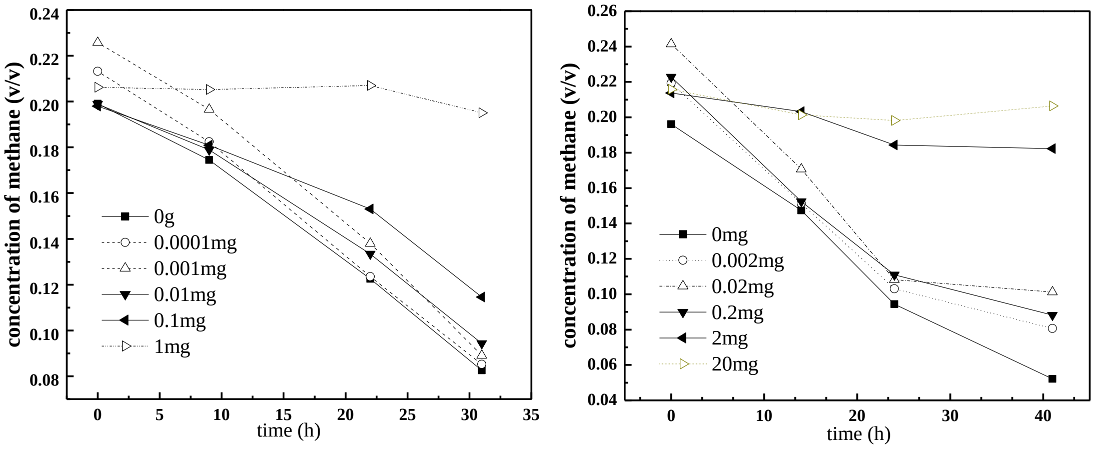
The degradation of TCE by the facultative methanotrophs isolated from aged refuse was studied and the kinetic equation of degradation was also fitted. These studies would provide theoretical guidance and foundations in microbiology for bioremediation of chlorinated hydrocarbons.

1.1 The tolerance of landfill biocover soil for tce and dichloromethane (DCE)

Adding different concentrations of TCE and DCE into biocover soil, the methane consumption was studied. From **Fig.1**, after 32 hours, the maximum methane consumption of 12 ml occurred in TCE control experiment and the maximum methane consumption was 15ml in DCM control experiment. but the methane oxidation activity of biocover soil was inhibited when amount of TCE exceeded 1 mg and amount of DCE exceeded 2 mg, respectively.

The result was consistant with Oldenhuis's study on toxic effect of TCE on methanotrophs[7].

Two possible reasons could about these phenomenons. The activity of methane monooxygenase was inhibited by the toxic effect of chlorinated hydrocarbons, which resulted in slow consumption rates of methane. In addition, the competitive inhibition which generated in process of degradation of chlorinated hydrocarbons by facultative methanotrophs existed in soil. Whatever the reason was, the desired result was that greenhouse gas reduction targets was realised by bio-medium and the volatile organic compounds were also eliminated at the same time, then isolation, domestication and utilization of facultative methanotrophs was significant to solve these problems.



(a) The tolerance of landfill cover for trichloroethene **(b) The tolerance of landfill cover for dichloromethane**

Fig.1 The TCE and DCM tolerance of landfill biocover soil at different concentrations of chlorinated hydrocarbons

Methylocystis strain JTA1, a new kind of facultative methanotroph isolated from landfills, can effectively enhance methane oxidation capacity of biocovers, and have high tolerance to chlorinated hydrocarbons[8]. As shown in **Fig. 2,** the activity of strain JTA1, compared with control experiment, was strengthened when CF concentration was less than 80 mg L^{-1}. In particular, the removal rate of CH_4 reached 100% after 22 days when the concentration of CF was 50 mg L^{-1}. At the CF concentration of 50 mg L^{-1}, the methane-oxidation rate of biocovers reached 0.114 ml d^{-1} g^{-1}, which is much higher than that of reported aged refuse (0.0068 ~ 0.0135 ml d^{-1} g^{-1}). In other words, high CF tolerance of *M.* strain JTA1 could be not only conductive to the methane oxidation, but also suitable for the integrated technology to reduce the emission of methane from the landfills.

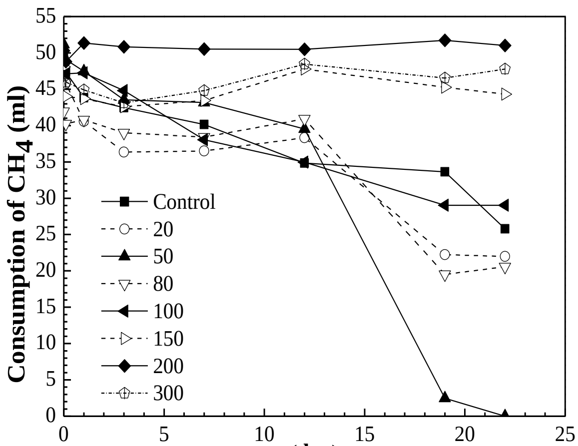


Fig. 2 The chloroform tolerance of *Methylocystis* strain JTA1 at different concentrations of chloroform

1.2 Degradation kinetics of TCE by methanotrophs community

Methanotrophs community was isolated from cover soil of landfill biocover soil of Changsheng qiao in Chongqing, and enriched as methane as carbon and energy sources for 7 d. The degradation of TCE by methanotrophs community and kinetics of degradation were studied, the result was shown in **Fig. 3**. When the initial concentration of TCE and methanotrophic cells were 45.5 mg L^{-1} and 1.728 g L^{-1} respectivly, the degradation rate of TCE was 79% degradation after 300 min. The methanotrophs community had high tolerance and affinity to TCE, degradation kinetics of TCE corresponded to Monad equation ($r_s = \frac{q_{s,max} C_s}{K_s + C_s} C_x$). The maximum rate of consumption $q_{s,max}$ was $1.51*10^{-4}$ min^{-1}, and the half-saturation constant K_S was 2.58 mg L^{-1} (R^2 = 0.961) which was much lower than that of reported methanotroph *Methylosinus trichosporium* OB3b (19.1 mg L^{-1}).

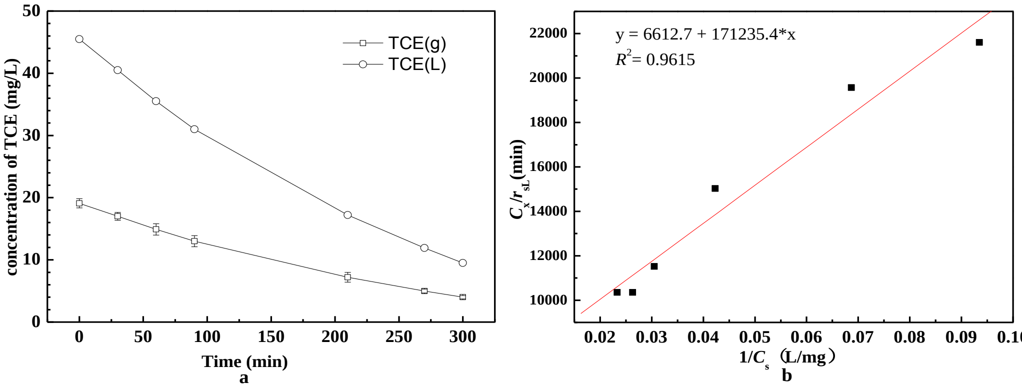


Fig. 3 Degradation curve of TCE by methanotrophs and degradation kinetics of TCE based on monad equation
Where C_x, r_{sL} and C_S are dry weight of the cell, average degradation rate of TCE and concentrations of TCE respectively

CONCLUSION

The results indicated that the methanotrophs existed in landfill biocover soil have high tolerance to some VCH. In particular, the methane-oxidation rate of strain JTA1 reached 100% after 22 days when the concentration of CF was 50 mg L^{-1}. The kinetic equation of TCE degradation by methanotrophs community fitted to Monad model well. According to the study, facultative methanotrophs utilizing mutil-carbon compounds overcame the disadvantage that methanotrophs was difficult to culture resulting from insolubility of methane in aqueous solution. The research on greenhouse gas emission reduction and biodegradation of contaminants in landfill biocovers strengthened by facultative methanotrophs was both challenging and significative work, these studies had theoretical and practical instruction value on improving ecological environment.

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