Trichloroethylene elimination assay by naural consortia of heterotrophic and methanotrophic bacteria

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Abstract Xenobiotic compounds generated from the various industrial activities are toxic and could affect the natural ecosystem. So far biological processes have been used for treatment of those compounds. It has been reported that xenobiotic compounds can be degraded in pure cultures of methanotrophic bacteria. Therefore, the aim of this study is to demonstrate the capacity of several natural consortia of heterotrophic and methanotrophic bacteria in degradation of trichloroethylene (TCE). Treatability of TCE was studied using 3 different consortia of heterotrophic and methanotrophic bacteria. After a first culture with methane and contact with TCE, all the consortia tested showed a biological TCE degradation efficiency between 29 and 43%. Using acetylene as MMO inhibitor, the implication of this enzyme on the three inocula was demonstrated very well. It was found that the toxicity threshold of TCE to the tested bacteria fell into a range of 30 to 40 mg/l. At not toxic TCE concentration of 5 and 10 mg/l, the maximal TCE specific activity was observed after an incubation of 15 minutes. This initial degradation rate could be used as indicator of the efficiency of a natural inoculum for TCE degradation. The impact of the initial TCE and biomass concentration on the TCE degradation kinetics was also evaluated. In the experiments, the TCE degradation was subject to first order kinetics. The maximum specific degradation rate of TCE was estimated at 48.9 mg TCE/mg SST.h.

These experiments clearly demonstrate that methanotrophic bacteria are ubiquitous in the environment, and a lot of them can degrade TCE. This shows good perspectives for in situ treatment of TCE-contaminated sites by enrichment of the methanotrophic natural populations.

Keywords Methanotrophic bacteria; mixed culture; trichloroethylene

Introduction

Xenobiotic compounds, generated by recent industrial activity, are toxic, and a high concentration could affect the natural ecosystem. Trichloroethylene (TCE) is currently used as solvent and degrease in metallurgic, electronic, painting, paper and textile industries. This compound is toxic and carcinogenic, and cannot be efficiently degraded under aerobic conditions (Wilson and Wilson, 1984). However, it has been reported that pure cultures of methanotrophic bacteria are able to degrade these compounds (Oldenhuis *et al.*, 1989, Tsien *et al.*, 1989). Methanotrophic bacteria under aerobic condition are able to oxidise methane to methanol, using a complex enzymatic system, the Methane Mono Oxygenase (MMO) (Krebs and Strater, 1994). This enzyme is not specific, and confers on the methanotrophic bacteria the ability to oxidise a lot of xenobiotic compounds by co-metabolism (Whittenbury *et al.*, 1970, Tonge *et al.*, 1977). However, TCE degradation requires the presence of methane to induce the MMO synthesis. In this case, acclimatisation of the methanotrophic bacteria to the TCE is not necessary. The biochemical pathway for methane and TCE oxidation is presented in Figure 1.

Figure 1 shows that the MMO plays a major role in the first stage of the TCE oxidation by introducing an oxygen atom. Complete transformation of the TCE to CO_2 requires the action of other kinds of heterotrophic bacteria, which can use the degradation product as their growth substrate. Therefore, it appears that mixed culture could have advantages in

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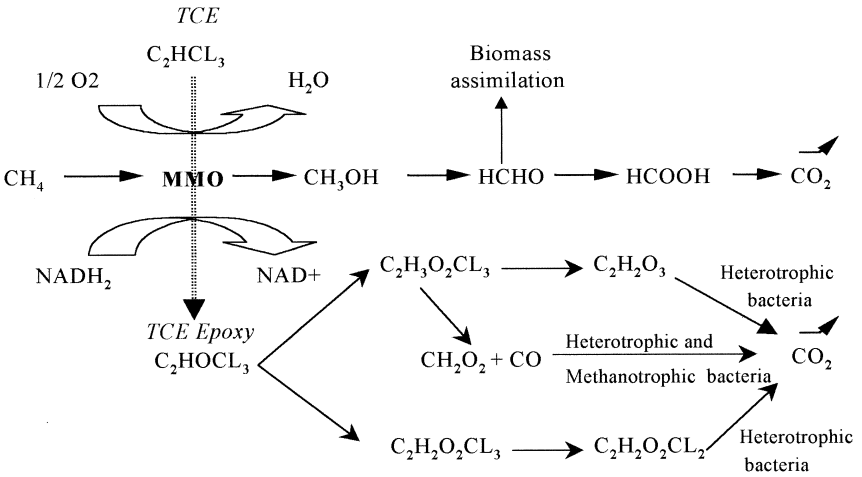

Figure 1 Biochemical pathway for methane and TCE degradation by the MMO

TCE degradation, such as high TCE degradation capacity, best toxicity resistance, and no accumulation of intermediate products (Anderson *et al.*, 1996, Sutfin and Ramey, 1997).

The aim of this paper is to show that several natural consortia of heterotrophic and methanotrophic bacteria are able to degrade TCE. Biological degradation of TCE by three natural inocula was tested, as well as the real implication of the MMO, and the TCE toxic concentration. The influence of the initial TCE and biomass concentration was also discussed.

Materials and method

In this study, three different types of consortia of heterotrophic and methanotrophic bacteria from lagoon (A), activated sludge (B), and lake sediments(C) were enriched by successive cultures (Houbron, 1995). Bacteria were cultivated under atmosphere air-methane (70/30) in a Nitrate Mineral Salt (NMS) as proposed by Whittenbury *et al.* (1970) with KNO_3 as nitrogen source. The initial pH was 6.8 and the agitation speed was kept at 300 rpm, while the reactor temperature was controlled at 30ºC. The medium was free of copper in order to favour synthesis of the MMO soluble form (Oldenhuis *et al.*, 1989). EDTA was used to increase the growth culture, methane and TCE oxidation. A technical assay was developed. In a first time, the consortia were cultivated in 160 ml of medium in a sealed 1 litre flask under air-methane (70/30) atmosphere to obtain biomass and active MMO (Houbron *et al.*, 1999). Bacterial growth was measured by total suspended solids (TSS). Later, at a determined biomass concentration and after elimination of methane by air stripping, an aliquot of 4 ml was introduced into a sealed glass vial of 4.5 ml and the co-substrate TCE was then added. Each experiment was reproduced in triplicate, and three samples were taken using a control sample containing bacteria killed by thermal shock.

In the experiments, the TCE concentration was determined by gas chromatography after pentane extraction. A 1 ml centrifuged sample was analysed by chromatograph HP5890 serie II using a capillary column CP-Sil 13CB with a FID detector (250ºC) (gas vector: He, oven: 100ºC, injector: 150ºC). The adsorbed TCE on sludge and abiotic loss by evaporation or adsorption on the vessel walls were considered in determination of TCE degradation efficiency. Thus, the efficiency of TCE degradation was calculated as follows:

$$\text{Efficiency } (\%) = 1 + (A - B)/B \times 100$$

with A = final TCE concentration and B = TCE concentration in the control sample with

Table 1 Degradation of 10 and 20 mg/l of TCE by the consortia A, B, C during two hours

Consortia	TSS (mg/l)	Initial TCE (mg/l)	Residual TCE (mg/l)	Efficiency %
A	378	9.8	5.6	43
A	378	19.8	13.2	33
B	359	10.3	6.7	35
B	359	19.8	16.0	19
C	228	10.1	5.6	44
C	228	20.1	14.8	26

Table 2 Comparative culture of the three consortia with killed bacteria and with acetylene

Consortia	Initial TCE (mg/l)	Residual TCE (mg/l) Killed bacteria	Residual TCE (mg/l) Culture with acetylene
A	10	9.8	9.2
B	10	9.9	9.5
C	10	9.6	9.7

bacteria killed. Inhibition of the MMO activity was realised by using acetylene (Fogel *et al.*, 1986).

Results and discussion

Biological degradation of TCE

The 3 consortia were cultivated in Erlenmeyer flasks under air methane (70/30) atmosphere until a biomass from 228 to 378 mg TSS/l. After methane stripping by air, 10 and 20 mg/l of TCE were added to the sample of 4 ml of each consortia. The result obtained after 2 hours of incubation without aeration are presented in Table 1. The residual TCE concentration represents an average of six analyses.

In order to better interpret the result in Table 1, it is necessary to consider an error percentage (dosage and manipulation error due to the high volatility of the TCE) above which the variation is not significant. As proposed by Wackett *et al.* (1989), a significant percentage was fixed to 10%. All variation higher than 10% was considered as bacterial oxidation.

Table 1 showed that the degradation efficiency of TCE by 3 consortia varied between 19 to 44%. The high efficiency displays a microbial biodegradation of the TCE. Since the microbial composition of each consortia was unknown and the initial biomass was different, it seems difficult to conclude which consortia is more efficient to TCE degradation.

This experiment shows that consortia from different places, enriched in methanotrophic bacteria, can efficiently degrade TCE.

Implication of the MMO of the methanotrophic bacteria

The aim of this experiment is to verify that TCE degradation results from the oxidative action of the MMO of the methanotrophic bacteria and not from other enzyme or oxidative bacteria of the consortia. Cultures of the 3 consortia with 0.2 ml of acetylene in 50 ml of culture medium (Fogel *et al.*, 1986), were compared with culture killed by thermal shock. The initial concentration of TCE was fixed at 10 mg/l with an incubation time of 2 hours. Results are showed in Table 2.

As shown in Table 2, when MMO of the methanotrophic bacteria was inactivated by acetylene, only 4 to 7% of initial TCE disappear. Similar results were obtained in the bacteria-killed cultures. Obviously, such low efficiency can not be attributed to biodegradation of

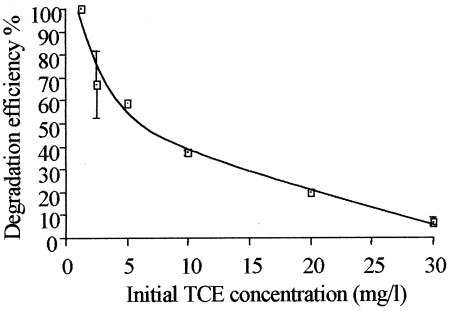

Figure 2 Degradation efficiency versus initial TCE concentration on consortium B

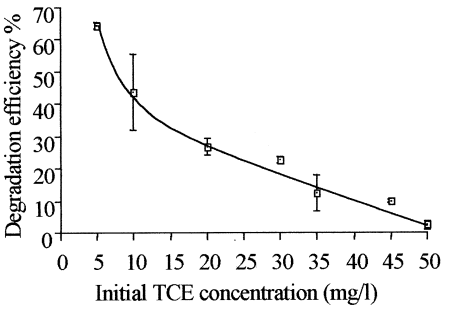

Figure 3 Degradation efficiency versus initial TCE concentration on consortium C

TCE. Compared with the results given in Table 1,Table 2 confirms that the MMO of the 3 consortia enriched in methanotrophic bacteria were responsible of the TCE degradation.

Toxicity level

As a solvent, TCE is probably toxic to bacteria, even at low concentrations. To evaluate TCE biodegradability, experiments were conducted in a range of non toxic concentrations. The objective of this experiment is to determinate the toxicity level of TCE on the consortia B (TSS=228 mg/l) and C (TSS=359 mg/l). Bacteria were incubated for one hour at initial TCE concentration ranging from 0 to 50 mg/l, and the TCE degradation was then determined. Figures 2 and 3 present respective degradation efficiency versus initial TCE concentration on consortia B and C.

Considering that efficiency values above 10% were not significant, Figures 2 and 3 show that toxicity levels were around 30 mg/l for consortium B and 40 mg/l for C. These values were similar to the toxic level of 46 mg/l reported by Oldenhuis *et al.* (1989) with Methylosinus trichosporium OB3b in pure culture. Complete degradation was not observed, even for non-toxic TCE concentration. This is probably due to presence of a limiting element. Incubation vials were aerated during methane stripping, and no more oxygen was added during the incubation. Oxygen could be limiting factor for complete TCE oxidation.

Initial degradation rate

The aim of this experiment was to evaluate the ability of the consortium to degrade TCE. Consortium B was used at a TSS concentration of 250 mg/l, at initial TCE concentration of 5 and 10 mg/l respectively. Vials were incubated, and sacrificed at different incubation times (15, 30, 60, 120, 180, 300 minutes). The residual TCE concentration was measured.

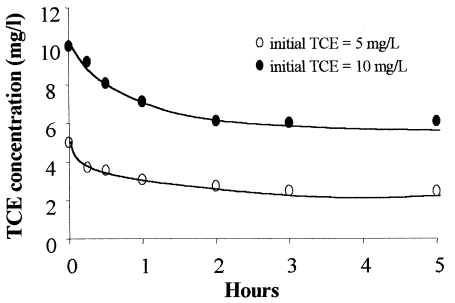

Figure 4 TCE concentration versus time for initial TCE concentration of 5 and 10 mg/l, with Consortium B (TSS=250 mg/l)

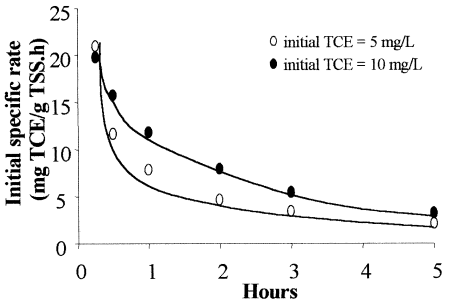

Figure 5 Initial specific TCE degradation rate versus time for initial TCE concentration of 5 and 10 mg/l, with Consortium B (TSS = 250 mg/l)

Evolution of TCE concentration and initial degradation rates are presented in Figure 4 and 5, respectively.

Similar evolution profiles were observed for the two initial concentrations. As shown in Figure 5, the specific rate was higher at the beginning of each experiment. Oxygen limitation could explain the kinetic reduction observed. The maximal specific degradation rates of TCE was observed after 15 minutes of incubation (21 and 19.8 mg TCE/g TSS.h for the cultures fed with 5 and 10 mg/l of TCE respectively). In fact, the initial rate reflects the ability of a consortium to remove TCE, and could be used as consortium efficiency indicator.

However, we observed for the two initial TCE concentration tested (with a biomass of 250 mg TSS/l), a similar initial rate. This observation was not in agreement with the literature according to a first order kinetics for TCE removal (Arvin,. 1991; Oldenhuis *et al.*, 1990). This observation could be elucidated by the following experiments.

Influence of initial TCE concentration on initial TCE degradation rate

The objective of this experiment was to evaluate the influence of the initial TCE concentration on the initial rate. Consortium B was used with a bacterial concentration of 92 mg/l. Vials were incubated with initial TCE concentrations of 2.5, 5, 8 and 10 mg/l, and sacrificed for residual TCE analyse after 15 and 30 minutes. Initials specific TCE degradation rates are presented in Figure 6.

As observed in the previous experiments, kinetic values were higher after 15 minutes than after 30 minutes. Also, this figure shows a linear relation between initial rate and initial TCE concentration. Enzymatic oxidation of TCE follows first-order kinetics as described in the literature. This proportionality was only observed when the consortium has a TSS concentration of 92 mg/l, but not with a TSS of 250 mg/l. This in turn suggests that bacterial

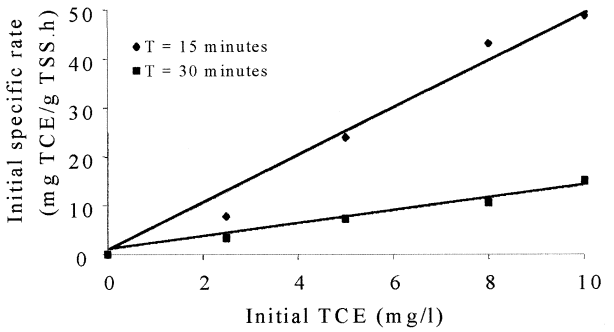

Figure 6 Initial specific rate versus initial TCE concentration for consortium B at TSS of 92 mg/l

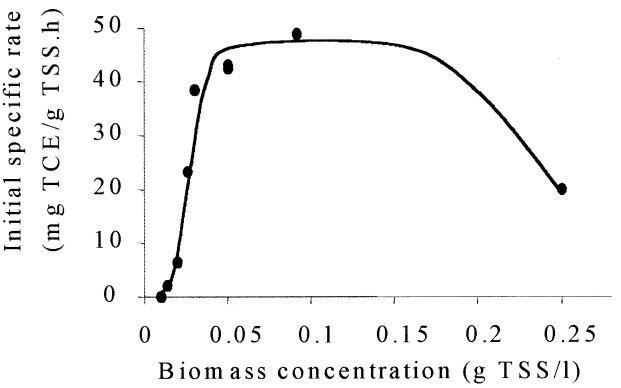

Figure 7 Initial specific TCE degradation rate versus biomass concentration for consortium B

re-partition between methanotrophic and heterotrophic bacteria changed during growth under air methane atmosphere.

Influence of initial biomass concentration on initial TCE degradation rate

The objective of this experiment was to show the effect of biomass concentration on degradation rate in a batch culture. Consortium B was cultivated in a 3 litres of sealed Erlenmeyer under air methane atmosphere. At different time and biomass concentration, aliquots of 4 ml were inoculated with a TCE concentration of 10 mg/l. Vials were incubated for 15 minutes, and initial rate was then determined. Figure 7 shows the evolution of the initial specific TCE degradation rate versus biomass concentration.

This figure shows that in the range of 0 to 100 mg/l of biomass, a linear relation appears between initial rate and biomass concentration. A maximum rate of 48.9 mg TCE/g TSS.h was observed at a biomass concentration of 92 mg/l: this rate is comparable with those obtained in pure culture by Tsien *et al.* (1989) and Oldenhuis *et al.* (1989). Beyond 100 mg/l of TSS, the initial rate began to decline. Further experiment is thus needed to determinate the microbiological composition of the consortium during the growth culture. Certainly, at low biomass concentration, methanotrophic bacteria are predominant. In this case, the ratio of active MMO to total bacteria should be high. As pointed earlier, high ratio of active MMO to total biomass would result in a high specific rate. This in turn provides a plausible explanation for the observed phenomenon.

For a higher biomass concentration, the methanotrophic bacteria activity favours the growth of heterotrophic bacteria that can utilise methane oxidation product as substrate.

Under such a circumstance, the consortium should have a low ratio of active MMO to biomass, thus the initial specific rate is low.

Considering that a mixed culture could be more efficient for a complete degradation of TCE, a particular attention should be given to the growth phase or MMO production phase. Although methanotrophic bacteria do not need acclimatisation for TCE degradation, heterotrophic bacteria, to be more efficient for assimilation of TCE oxidation products, need an acclimatisation period.

Design of processes using consortia enriched in methanotrophic bacteria for TCE biodegradation, has to consider various stages, such as a period of production or activation of the MMO, a period of TCE degradation and a period of acclimatisation of the heterotrophic population or assimilation of the TCE oxidation product.

Conclusions

This research clearly showed that methanotrophic bacteria are ubiquitous in the environment, and demonstrated that the consortia enriched in methanotrophic bacteria can degrade TCE, with a similar efficiency to that obtained in pure culture. For the three consortia tested, the toxicity threshold of TCE is in a range of 30 to 40 mg/l. At non-toxic concentrations, the maximal specific degradation rate was obtained after an incubation of 15 minutes. This initial degradation rate could be used as an indicator of the efficiency of natural inoculum for TCE degradation. The maximum specific rate of 48.9 mg TCE/g TSS.h obtained in this research is comparable with those reported in the literature. Results showed that TCE degradation follows a first-order kinetics, and both biomass concentration and composition have a significant influence on the initial degradation rate of TCE. These results suggest good perspectives for in or ex situ treatment of TCE contamined sites by enrichment of natural methanotrophic populations.

Acknowledgements

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