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BACTERIAL DEGRADATION OF VINYL CHLORIDE.

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SUMMARY

Mycobacterium L1 can grow on vinyl chloride as sole carbon and energy source. Application of this bacterium to remove vinyl chloride from waste gases is proposed. From air containing 1% vinyl chloride 93% of the vinyl chloride was removed by passing the air through a fermentor containing a growing population of *Mycobacterium* L1.

INTRODUCTION

Vinyl chloride is one of the largest commodity chemicals, with a world-wide production estimated at 17 million tons in 1982 (Kirk-Othmer, 1983). Approximately 200 thousand tons of this gaseous compound were released into the environment that year, mainly during the production of PVC. Vinyl chloride does not accumulate in the environment due to its relatively short half-life in the troposphere (20 hours). The average vinyl chloride concentration in The Netherlands was 0.2 $\mu g/m^3$ air in 1983 ($8 * 10^{-5}$ ppm), but in the vicinity of factories producing or processing vinyl chloride, the average concentrations were 50 to 100 times higher (Criteriadocument over vinylchloride, 1984).

Dutch government policy aims at a drastic reduction of vinyl chloride emission because of its carcinogenic action in animals and human beings (Maltoni and Lefenine, 1974; Creech and Johnson, 1974; Infante, 1981). Until now, vinyl chloride emissions have been reduced significantly from 4000 tons in 1975 to 360 tons in 1981. Further reduction of vinyl chloride emission is desirable. This necessarily will involve the

removal of relatively low concentrations of vinyl chloride from large volumes of waste gases. Unfortunately physical processes (Scamehorn, 1979) for the removal of vinyl chloride at low concentrations are relatively expensive. For example the use of activated carbon involves steam regeneration and drying, which are energy intensive operations. Therefore it would be of great interest to develop a (bio)catalyst which would oxidize vinyl chloride completely, also at very low concentrations.

The isolation of a *Mycobacterium* capable of growing on vinyl chloride (de Bont and van der Linden, 1985) thus prompted us to investigate the possibility of using this organism to remove vinyl chloride at low concentrations from industrial waste gases.

MATERIALS AND METHODS

CHEMICALS: Vinyl chloride and ethene were obtained from Hoekloos, Schiedam (NL) and all other chemicals were from Merck, Darmstadt (BRD). CULTIVATION OF *Mycobacterium* L1:The growth medium contained per litre deionized water: 5.0 g K_2HPO_4, 2.0 g $NaH_2PO_4.2H_2O$, 2.0 g $(NH_4)_2SO_4$, 0.075 g $MgCl_2.6H_2O$ and 0,2 ml of a trace element solution as described by Visniac and Santer (1957). Batch grown cells were cultivated in 5 litre Erlenmeyer flasks containing 1 litre growth medium. The flasks were sealed with rubber stoppers fitted with Suba-seals through which 150 ml vinyl chloride or ethene was injected. The cultures were incubated at 28°C and shaken at 1 Hz. Growth on ethene in a 3 litre fermentor (2 litre working volume) was at dilution rate of 0.02 hr^{-1} with ethene supplied as a 50 ml/min 4% ethene in air mixture. The flow of ethene, vinyl chloride and air was controlled by Brooks mass flow controllers (5850 TR series; Brooks Veenendaal (NL)). Cells were harvested by centrifugation at 16,000 g, washed with 50 mM potassium-phosphate buffer pH 7.0 and stored at -20°C. ANALYTICAL METHODS: Vinyl chloride and ethene were determined with a Packard 430 gas chromatograph fitted with a Porapak R column. The column temperature was 180°C and the carrier gas was N_2. Samples of 100 µl of gas-phases were analyzed. CO_2 was measured with a Packard 427 gas chromatograph fitted with a Porapak Q column. The column temperature was 100°C and the carrier gas He. Chloride ion concentrations were determined with a Marius micro-chlor-o-counter (Marius, Utrecht (NL)). Total organic carbon (TOC) was analyzed with a Xertex, Dohrmann Envirotech Organic Analyzer. DETERMINATION OF DOUBLING TIME: The doubling time on vinyl chloride

was calculated from chloride formation or from vinyl chloride consumption by growing cells. DETERMINATION OF K_M: The K_M of non-growing cells in potassium-phosphate buffer (50 mM, pH 7.0) at 30°C was calculated by fitting the integrated Michaëlis-Menten equation to vinyl chloride depletion curves (300-10 ppm). Substrate transport limitation was eliminated by employing low biomass concentrations and by shaking vigorously. STABILITY EXPERIMENTS: Stability experiments were performed using washed cells in 25 ml potassium-phosphate buffer (50 mM, pH 7.0) in 130 ml serum bottles. Vinyl chloride (3 ml) was added and incubation was at 30°C by shaking at 130 rpm in a Gallenkamp orbital incubator. Remaining vinyl chloride in the bottles was periodically assayed for. The relative activities were calculated from the vinyl chloride depletion curves.

RESULTS AND DISCUSSION

Vinyl chloride degrading bacterium

The vinyl chloride utilizing bacterium L1 - that was isolated from soil which had been contaminated with vinyl chloride containing water for several years (de Bont and van der Linden, 1985) - strongly resembled previously isolated alkene-utilizing Mycobacteria (de Bont *et al.* 1980), and was accordingly classified as a species of the genus *Mycobacterium*. Strain L1 was also capable of growth on ethene. Propene did not support growth. The growth rate of the isolate increased substantially as a result of continuous subculturing with vinyl chloride as growth substrate. In the course of a year the doubling time dereased from 40 to 12 hours, indicating further adaptation to growth on vinyl chloride. Suspensions of washed resting cells of *Mycobacterium* L1 grown on either vinyl chloride or ethene, were capable of degrading vinyl chloride. Acetate grown cells exhibited no vinyl chloride degrading activity. The vinyl chloride was degraded to concentrations below the detection limit (less than 1 ppm) and both vinyl chloride and ethene grown cells had a K_M of approximately 100 ppm vinyl chloride (1.75 µM). This high affinity of *Mycobacterium* L1 towards vinyl chloride is very promising with respect to a possible application of this bacterium to remove vinyl chloride at low concentrations. Subsequently we have examined two possibilities to degrade vinyl chloride using *Mycobacterium* L1: A) degradation by non-growing cells and B) degradation by growing cells.

A) Vinyl chloride degradation by non-growing *Mycobacterium* L1.

The most efficient way to quantitively remove vinyl chloride from waste gas can be achieved by using a plug-flow bioreactor *e.g.* a column packed with immobilized vinyl chloride degrading bacteria. To assess the possibility of using *Mycobacterium* L1 in such a reactor, the operational stability of free cells was tested. Using batch-grown cells (either vinyl chloride or ethene as growth substrate) the vinyl chloride degrading activity decreased very rapidly. After 24 hours 70-75% of the original activity was lost. Batch-grown cells are harvested in the late exponential growth phase, which might influence the stability of the vinyl chloride degrading activity. The use of vinyl chloride to grow the biocatalyst can be avoided because ethene grown cells of *Mycobacterium* L1 are also capable of degrading vinyl chloride. The stability experiment was therefore repeated with washed cells from a chemostat with ethene as growth substrate. In such cells the initial activity was lower than in batch-grown cells (respectively 0.9 and 2.4 µmol/ mg carbon hr), which could be explained by the low growth rate ($D = 0.02\ hr^{-1}$) at which the chemostat cells had been grown. The operational stability of the chemostat cells depended on the biomass concentration (Fig. 1).

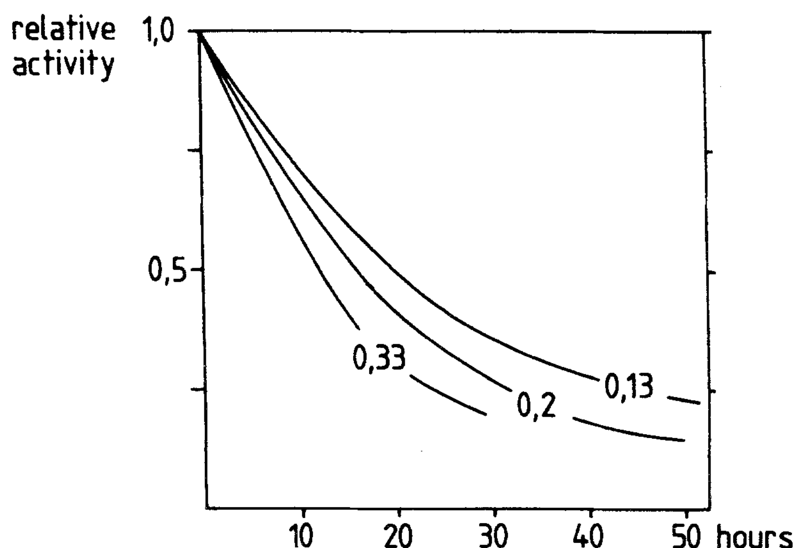

Figure 1: Relative vinyl chloride degrading activity by non-growing *Mycobacterium* L1 at different biomass concentrations (mg C/ml)

At a concentration of 0.33 mg carbon/ml the activity loss after 24 hours (75%) was comparable with batch grown cells. At lower biomass

concentrations the operational stability was somewhat higher (Fig. 1). The decrease in stability at higher biomass concentrations can perhaps be ascribed to a (temporary) accumulation of a toxic intermediate of vinyl chloride degradation.

The low operational stability of vinyl chloride degradation by non-growing cells, especially at higher biomass concentrations, probably prevents commercial application of non-growing cells to remove vinyl chloride from waste gases. It can however not be excluded that the stability of the vinyl chloride degradation at very low concentrations (less than 100 ppm) may be much higher, making certain applications possible.

B) Vinyl chloride degradation by growing *Mycobacterium* L1.

Removal of vinyl chloride from a waste gas by growing cells rather than by immobilized cells might be an attractive alternative. To investigate this possibility, strain L1 was grown in a fermentor with a 2 litre working volume with vinyl chloride as the sole carbon and energy source. Vinyl chloride was supplied as a 40 ml/min mixture of 1% (v/v) vinyl chloride in air. After reaching steady state at a dilution rate of 0.012 hr^{-1} the outgoing air contained 0.07% vinyl chloride. Thus 93% of the vinyl chloride from the ingoing air was removed. A carbon and chloride balance over the fermentor is shown in Table 1.

Table 1. Carbon and chloride balance over fermentor in steady state at D = 0.012 hr^{-1} with a 2 litre working volume.

	carbon (mg/hr)	chloride (mg/hr)
ingoing vinyl chloride	23.31	34.48
outgoing vinyl chloride	1.63	2.41
outgoing CO_2	15.15	-
effluent (TOC and chloride)	7.07	31.68

With the results in table 1 recovery percentages for carbon and chloride of respectively 102.2 and 98.8 can be calculated, implying that the removed vinyl chloride was completely degraded. Recovery of 70% of carbon from the degraded vinyl chloride in CO_2 is a reflection of the low growth rate at which this experiment was executed. The volumetric vinyl chloride degradation capacity that can be calculated from table 1 is 0.68 kg vinyl chloride /m^3 day.

Vinyl chloride degradation is accompanied by hydrochloric acid formation, which must be neutralized by the addition of base. The resulting salt formation could become growth limiting. In batch growth experiments it was shown that *Mycobacterium* L1 could only tolerate up to 100 mM NaCl added to the growth medium during growth on vinyl chloride, whereas growth on ethene and acetate was still possible at higher NaCl concentrations.

Using a conventional fermentor with *Mycobacterium* L1 at a dilution rate of 0.03 hr^{-1} (approximately half μ_{max}) and limiting the chloride concentration to 100 mM, a maximum volumetric vinyl chloride degrading capacity of 4.5 kg/m^3 day could be achieved. Preliminary calculations indicate that this volumetric capacity might be commercially competitive with activated carbon treatment of vinyl chloride containing waste gas.

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