erythropolis Cells: Comparison of Conventional Aqueous Phase Dehalogenation and Nonconventional Gas Phase Dehalogenation

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Abstract: Biofiltration of air polluted by volatile organic compounds is now recognized by the industrial and research communities as an effective and viable alternative to standard environmental technologies. Whereas many studies have focused on solid/liquid/gas biofilters, there have been fewer reports on waste air treatment using other biological processes, especially in a solid/gas biofilter. In this study, a comparison was made of the hydrolysis of halogenated compounds (such as 1-chlorobutane) by lyophilized *Rhodococcus erythropolis* cells in a novel solid/gas biofilter and in the aqueous phase. We first determined the culture conditions for the production of *R. erythropolis* cells with a strong dehalogenase activity. Four different media were studied and the amount of 1-chlorobutane was optimized. Next, we report the possibility to use *R. erythropolis* cells in a solid/gas biofilter in order to transform halogenated compounds in corresponding alcohols. The effect of experimental parameters (total flow into the biofilter, thermodynamic activity of the substrates, temperature, carbon chain length of halogenated substrates) on the activity and stability of lyophilized cells in the gas phase was determined. A critical water thermodynamic activity (a_w) of 0.4 is necessary for the enzyme to become active and optimal dehalogenase activity for the lyophilized cells is obtained for an a_w of 0.9. A temperature of reaction of 40°C represents the best compromise between stability and activity. Activation energy of the reaction was determined and found equal to 59.5 KJ/mol. The pH effect on the dehalogenase activity of *R. erythropolis* cells was also studied in the gas phase and in the aqueous phase. It was observed that pH 9.0 provided the best activity in both systems. We observed that in the aqueous phase *R. erythropolis* cells were less sensitive to the variation in pH than *R. erythropolis* cells in the gas phase. Finally, the addition of volatile Lewis base (triethylamine) in the gaseous phase and the action of the lysozyme in order to permeabilize the cells was found to be highly beneficial to the effectiveness of the biofilter.

Keywords: waste air treatment; bioremediation; solid/gas biofilter; haloalkane dehalogenase; *Rhodococcus erythropolis*

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INTRODUCTION

Biological waste air treatment is an emerging technology which is becoming more popular with industries facing increasingly stringent environmental regulation (US EPA, 1990). This technique offers a cost-effective and environmentally friendly alternative to conventional air pollutant control technologies such as catalytic oxidation or adsorption onto activated carbon (Menig and Krill, 1997; Cox and Deshusses, 1998). Gaseous effluent treatment processes were reviewed and compared by Jorio and Heitz (1999), who showed that the cost of biological treatment was among the lowest. Indeed, biological waste air treatment is achieved at ambient temperatures and does not involve high running costs. Moreover, it does not generate secondary pollutants, such as nitrogen oxides (Jorio and Heitz, 1999). Generally, pollutants are converted to carbon dioxide, water, mineral salts, and biomass.

Although there have been many articles dealing with biofilters (solid/liquid/gas bioreactor), there have been fewer reports on waste air treatment by other biological processes, especially on the direct treatment of gaseous effluents in a solid/gas biofilter. However, this kind of process could be of interest in the transformation of volatile organic compounds (VOCs) (Maugard et al., 2001; Goubet et al., 2002). Indeed, solid/gas catalysis does not require the preliminary solubilization of compounds since substrates are directly treated in the gas phase. This could be of great interest in the treatment of VOCs, which often have a low solubility in water and are relatively volatile. With solid/gas catalysis, the viability of

the biofilter is not limited by biomass development since the dehydrated catalyst does not grow. In addition, it does not need to be fed with nutrients and the bioreactor's efficiency does not depend on cell viability. Moreover, mass transfers in such systems are more efficient than in a conventional solid/liquid/gas system (Lamare and Legoy, 1993; Lamare et al., 2001).

Among the VOCs, halogenated compounds form one of the most important groups of industrially produced chemicals. Many of them have been designated as priority pollutants by the US EPA because of their recalcitrance, toxicity, carcinogenicity, and potential teratogenicity (Keith and Teillard, 1979; Belkin, 1992). Biodegradation of short chain chloroalkanes in a solid/gas biofilter by the haloalkane dehalogenase of *Rhodococcus erythropolis* has recently been reported (Dravis et al., 2000). This work showed that haloalkane dehalogenase can hydrolyse chlorinated substrates into the corresponding alcohols and HCl in the gas phase. Since enzyme purification would increase the cost of a gaseous effluent treatment process, it was decided to use dehydrated cells as catalyst. Moreover, if no cofactor is needed for successive reactions, the use of whole-dehydrated cells could favor the catalysis of several steps of the conversion, which is particularly valuable for bioremediation. Recently, it has been shown that lyophilized *Saccharomyces cerevisiae* cells (baker's yeast) can be used as a catalyst for the transformation of alcohols and aldehydes (Maugard et al., 2001; Goubet et al., 2002).

The purpose of this study was to investigate if lyophilized *R. erythropolis* cells could be used for the dehalogenation of gaseous substrates. We focused on the effect of culture and dehydration conditions of the microorganism, as well as on the effect of operating conditions on the conversion efficiency of a solid/gas biofilter. We compared conventional aqueous phase dehalogenation with nonconventional gas phase dehalogenation.

MATERIALS AND METHODS

Microorganisms, Culture Conditions, and Chemicals

Rhodococcus erythropolis NCIMB 13064, was obtained from the National Collection of Industrial and Marine Bacteria (Aberdeen, Scotland). The organism was grown in 1-L Erlenmeyer flasks sealed with Teflon-lined screw caps containing 200 mL of medium at pH 7.0. All substrates were purchased from Sigma Chemical (St. Louis, MO) except tryptone and yeast extract, which were obtained from Fluka (Ronkonkoma, NY). Deionized water was obtained via a Milli-Q system (Millipore, France).

Culture Conditions

Four different media were tested: 2YT, M9, and two mineral media described by Curragh et al. (1994) and Sorkhoh et al. (1991), respectively. The 2YT medium had the following composition (in g/L): 16.0 tryptone, 10.0 yeast extract, and 5.0 NaCl. The M9 medium had the following composition (g/L): 6.0 $Na_2HPO_4{\cdot}2H_2O$ 3.0 KH_2PO_4, 1.0 NH_4Cl, 0.5 NaCl, 0.05 $MgSO_4{\cdot}H_2O$, 0.0002 thiamine/HCl, and 0.004 $CaCl_2{\cdot}H_2O$. The mineral medium described by Curragh et al. (1994) had the following composition (g/L): 1.6 $NaH_2PO_4{\cdot}2H_2O$, 1.4 Na_2HPO_4, 0.5 $(NH_4)_2SO_4$, 0.2 $MgSO_4{\cdot}7H_2O$, and 0.5 K_2SO_4; the medium was supplemented with a trace element solution (10 mL/L) containing: 12.0 NaEDTA$\cdot 2H_2O$, 2.0 $FeSO_4{\cdot}7H_2O$, 1.0 $CaCl_2$, 0.4 $ZnSO_4{\cdot}7H_2O$, 0.4 $MnSO_4{\cdot}4H_2O$, and 0.1 $CuSO_4{\cdot}5H_2O$. The pH of the medium was adjusted to 7.0 with HCl. The mineral medium described by Sorkhoh et al. (1991) had the following composition (g/L): 0.85 $NaNO_3$, 0.56 KH_2PO_4, 0.86 Na_2HPO_4, 0.17 K_2SO_4, 0.37 $MgSO_4{\cdot}7H_2O$, 0.007 $CaCl_2{\cdot}2H_2O$, and 2.5 ml of a trace element solution consisting of (g/L): 2.32 $ZnSO_4{\cdot}7H_2O$, 1.78 $MnSO_4{\cdot}4H_2O$, 0.56 H_3BO_3, 1.0 $CuSO_4{\cdot}5H_2O$, 0.39 $NaMoO_4{\cdot}2H_2O$, 0.42 $CoCl_2{\cdot}6H_2O$, 0.66 KI, 0.1 EDTA, 0.4 $FeSO_4{\cdot}7H_2O$, and 0.0004 $NiCl_2{\cdot}6H_2O$. The pH of each medium was adjusted to pH 7.0 with HCl. The media were sterilized by autoclaving for 15 min at 121°C. After cooling, 100–200 μL (0.96–1.92 mmol) of filter-sterilized 1-chlorobutane was added once or twice to each screw cap-sealed flask. Cultures were incubated at 28°C on an orbital shaker (160 rpm). Growth of the organism was monitored by measuring the Absorbance at 690 nm (A_{690nm}).

Preparation of Cells

Cells grown for 24–48 h were harvested by centrifugation at 7,000 rpm for 10 min. The cell paste was washed with 50 mM Tris/HCl buffer at pH 6.2, 7.0, 7.5, 8.0, 8.5, or 9.0 and resuspended in 50 mM Tris/HCl buffer at the same pH. To perform degradation tests the volume of buffer used for suspension of cells was half of the volume used for culture. The volume of buffer used to suspend cells before freeze-drying was one-tenth of the volume used for culture. One part was used for substrate conversion tests in the aqueous phase and the rest was lyophilized prior to use in the solid/gas biofilter.

Effect of Cell Permeabilization on Dehalogenase Activity

Cells grown for 48 h on Sorkhoh's mineral medium supplemented twice with 100 μL of 1-chlorobutane were permeabilized by treatment with lysozyme. Then 200 mg of lysozyme were added to 600 mg of harvested cells washed and resuspended in 30 mL of Tris/HCl buffer (50 mM, pH 9.0). After a 15-min incubation at 22°C, the preparation was frozen at −20°C and lyophilized. Lysozyme was obtained from Sigma Chemical Co.

Enzyme Assay for Dehalogenation in the Aqueous Phase

Tests were performed with 10 mL of cell suspension (in Tris/HCl buffer) in Teflon-lined screw cap-sealed tubes to

which 7 μL (67 μmol) of substrate was added. The reaction was stopped by addition of 2 mL of dichloromethane. Concentrations of the remaining substrate and corresponding alcohol in the extract were monitored by gas chromatography analysis using 1-hexanol (or 1-butanol) as an internal standard and the same analytical conditions as for the analysis of the gas phase leaving the reactor. Controls containing buffer and substrate alone were used to measure any abiotic dehalogenation and additional controls containing cells grown on 2YT medium without 1-chlorobutane (also lacking the dehalogenase) were used to measure the amount of 1-chlorobutane adsorbed on cells.

Enzyme Assay for Dehalogenation in the Solid/Gas Biofilter

The solid/gas biofilter used in this study has been described previously by Lamare and Legoy (1995). It consists of a 9 cm long glass tube in which the lyophilized *R. erythropolis* cells are packed between two layers of glass wool. Nitrogen was used as a carrier gas. It first passed through the substrate saturation flasks and a continuous flow through the biofilter ensured a reaction with the lyophilized *R. erythropolis* cells. The gas leaving the biofilter was injected into a gas chromatograph for analysis. Acquisition and control of the operating parameters (thermodynamic activity of substrates and of water, temperature, and pressure) were monitored online using a compatible IBM computer.

The key parameters considered for the solid/gas biocatalysis are the ''availability'' of the different chemical species in the gas phase for the cell. The thermodynamic parameter corresponding to each ''availability'' is the activity of each compound. For each compound present in the gas phase, determination of its thermodynamic activity requires knowledge of its partial pressure in the gaseous phase to be transformed and knowledge of the vapor pressure curve as a function of temperature.

The thermodynamic activity of each compound (X) in the reactor was calculated as follows:

$$a_x = \frac{Pp_x}{Pp_x^{Sat}}$$

with Pp_x: partial pressure of compound X (atm) in the gas entering the biofilter Pp_x^{Sat}: saturation vapor pressure of pure compound X (atm) at the operating temperature.

A typical experiment was run at 40°C with 100 mg of lyophilized cells. The total flow through the biofilter was 500 μmol/min. The 1-chlorobutane thermodynamic activity (a_{ClBut}) was set at 0.06 (corresponding to 8.1 μmol/min of 1-chlorobutane at 40°C) and the water thermodynamic activity (a_w) at 0.8 (corresponding to 30.0 μmol/min of water at 40°C).

With these experimental conditions, less than 5% of the substrates were converted; it could thus be assumed that the initial rates (expressed in μmol of 1-butanol produced per minute and per g of cell) were measured. The vapor phase leaving the biofilter was sampled using a 250 μL loop on a six-way valve (Valco, Houston, TX) maintained at 190°C. Samples were automatically injected into the split injector of a gas chromatograph.

Chromatographic Analysis

Analysis were performed on a gas chromatograph (Hewlett Packard, Corvallis, OR, model 5890 A), equipped with a flame ionization detector (FID). The column used was an OV 1701 fused silica capillary column (25 m × 0.25 mm i.d. × 0.25 μm film thickness; Chrompack, France). The split ratio was 43.2/2.7. The injector was kept at 200°C, and the detector was kept at 250°C. The column temperature was held at 40°C for 2.5 min, then programmed to increase at 15°C/min to 110°C and kept 1 min at this temperature. Nitrogen was used as carrier gas and the flow rate in the column was 2.7 mL/min. Hydrogen and air were supplied to the FID at 38 and 398 mL/min, respectively. Quantitative data were obtained after integration on an HP 3396A integrator. An internal standard was used for analysis of the liquid phases and an external standard method was used for analysis of the gaseous phase leaving the reactor.

RESULTS AND DISCUSSION

Effect of the Medium on the Growth and Dehalogenase Activity of *Rhodococcus erythropolis* Cells

Four media were first compared for the development of *R. erythropolis* and the expression of dehalogenase activity. Media assessed included one nutrient media (2YT) and three mineral media (the M9 and two media previously used for *R. erythropolis* culture [Sorkhoh et al., 1991; Curragh et al., 1994]). In all media, 0.5 μL of 1-chlorobutane per mL of medium was added. In the mineral media it was the sole source of carbon and energy. As shown in Table I, growth in 2YT was approximately twice that obtained with the other media, but the cells obtained had a weak dehalogenase

Table I. Growth and dehalogenase activity of *R. erytropolis* cells in different culture media (supplemented with 1-chlorobutane).

Culture medium	Growth (A_{690nm})	Dehalogenase activity (μmol/min. A_{690nm})
2YT medium	1.96	1.27
M9 medium	1.03	1.32
Minimal salt medium[a]	0.95	1.52
Minimal salt medium[b]	0.86	3.15

Cells were grown for 24 h on 2YT medium and for 48 h on the other media, at 28°C in 1-L sealed flasks containing 200 mL of medium to which 100 μL (0.96 mmol) of 1-chlorobutane was added. Cells were then harvested, washed twice with Tris/HCl buffer (50 mM, pH 9.0), and suspended in Tris/HCl buffer (50 mM, pH 9.0); 1-chlorobutane was added at a 700 μL/L concentration; dehalogenation was measured by GC analysis.

[a]Minimal salt medium described by Curragh et al. (1994).

[b]Minimal salt medium described by Sorkhoh et al. (1991).

activity. Conversely, cell production in Sorkhoh's medium was low but a significant dehalogenase activity was obtained. This medium was selected for further tests and for the production of a biocatalyst.

Effect of the Amount of 1-Chlorobutane Added to the Culture

In order to determine the optimal concentration of 1-chlorobutane for the growth of *R. erythropolis* cells, different concentrations of 1-chlorobutane in the medium were studied. Cells were grown for 48 h at 28°C in 1-L Teflon-sealed flasks containing 200 mL of Sorkhoh's medium. The addition of 100 μL of substrate once at the beginning of culture resulted in the production of cells with the highest specific activity (Table II). The best conditions were obtained with twice the addition of 100 μL of substrate, which provided both strong growth (A_{690nm}: 1.21) and a high specific activity, thus a high amount of catalytic activity. A very high concentration of 1-chlorobutane in the culture medium inhibited cell growth (A_{690nm}: 0.26) and resulted in a low dehalogenase activity.

Effect of the Total Flow of the Gas Phase Through the Biofilter

After checking that the lyophilized preparations were still active when resuspended in the aqueous phase, we used lyophilized cells in a solid/gas biofilter. The biofilter was packed with 100 mg of lyophilized cells and fed with 500 μmol/min of a gaseous phase at 40°C with an a_w fixed at 0.8 (corresponding to 30.0 μmol/min at 40°C) and an a_{ClBut} fixed at 0.06 (corresponding to 8.1 μmol/min at 40°C). The course of the reaction was followed by GC analysis. The decrease of 1-chlorobutane concentration was seen to be concomitant with the synthesis of 1-butanol. No other compound could be detected in the gas phase. In the absence of lyophilized cells in a solid/gas biofilter, product did not appear.

Table II. Effect of the amount of 1-chlorobutane added to the medium on growth and dehalogenase activity of *R. erythropolis* cells grown on 200 mL of Sorkhoh's minimal salt medium.

Amount of 1-chlorobutane added	Growth A_{690nm}	Dehalogenase activity (μmol/min.A)
100 μL	0.51	5.38
200 μL	1.41	3.02
400 μL	0.26	0.59
2 × 10 μL	1.21	4.69
2 × 200 μL	1.10	2.18

Cells were grown for 48 h at 28°C in 1-L sealed flasks containing 200 mL of medium to which 100, 200, or 400 μL of 1-chlorobutane was added. When substrate was added twice, the second addition was made 24 h after the first. Cells were then harvested, washed twice with Tris/HCl buffer (50 mM, pH 9.0), and suspended in Tris/HCl buffer (50 mM, pH 9.0); 1-chlorobutane was added at a 700 μL/L concentration; dehalogenation was measured by GC analysis.

This clearly shows that 1-chlorobutane can be directly converted to 1-butanol and HCl in the gas phase using lyophilized *R. erythropolis* cells, but it would seem that this alcohol is not metabolized further. This could be due either to the fact that the only enzyme active in the gas phase is the dehalogenase or to the fact that only small amounts of catalyst were used and that we were not able to detect traces of secondary products. A third hypothesis is that the enzymes involved in the conversion of 1-butanol require cofactors and that since only 1-chlorobutane was supplied as a substrate, these enzymes were unable to regenerate their cofactors and remain active for a sufficiently long period. Poelarends et al. (2000) proposed that 1-butanol is further converted in butanal by a dehydrogenase requiring a cofactor. If such an enzyme is involved in the conversion of 1-butanol by *R. erythropolis* cells, it is logical that no secondary products were detected in our experiment.

The maximal initial reaction rate of 1-butanol formation observed in the gas phase with a 500 μmol/min total flow was 2.9 μmol/min.g of lyophilized cells. Dravis et al. (2000) observed 7 times lower rates of conversion in the gas phase with pure dehalogenase (0.4 μmol/min.g of lyophilized dehalogenase). This difference could be due to the fact that in our experiment a_{ClBut} was maintained at 0.06, whereas Dravis et al. (2000) used 1-chlorobutane saturated vapor (activity close to 1), which could inhibit the enzyme. Moreover, in our experiment the products were continuously flushed by nitrogen, whereas Dravis et al. used a static system.

The reaction rate was measured with three gaseous flows while maintaining the same water and substrate activities (Table III). When the total flow decreased, the initial rate of 1-butanol formation increased. When the total flow in the reactor was 750 μmol/min (11.7 μmol/min of 1-chlorobutane) 1-chlorobutane was transformed into 1-butanol at a rate of 1.9 μmol/min.g of cells, when the total flow was 500 μmol/min (8.1 μmol/min of 1-chlorobutane) the substrate was transformed with a rate of 2.9 μmol/min.g, and when the total flow was 250 μmol/min (43 μmol/min of 1-chlorobutane) the substrate was transformed at rate of 3.7 μmol/min.g. Decreasing the flow in the reactor from 750 to 250 μmol/min increased the conversion rate from ~1.6% to 8.6% (with 100 mg of dehydrated cell), which could be explained by the longer residence time in the bioreactor.

Effect of pH on the Dehalogenase Activity of *Rhodococcus erythropolis* Cells

In nonconventional media, as in aqueous media, enzyme activity is dependent on the enzyme ionization state. Enzyme activity is dependent on the last aqueous pH to which the enzyme is exposed prior to drying (''pH memory'') (Zaks and Klibanov, 1985; Halling, 2000). It might also be expected that the pH of the buffer used for the suspension of cells prior to lyophilization would influence the solid catalyst activity. Consequently, we studied the effect of pH

Table III. Effect of the total flow of gas phase through the reactor on the rate of 1-butanol formation by lyophilized cells of *R. erythropolis*.

Total flow (μmol/min)	Flow of 1-chlorobutane (μmol/min.g)	Residence time (Sec)	Rate of production 1-butanol (μmol/min.g)	Conversion yield with 100 mg of cell (%)
250	4.3	10.9	3.7	8.6
500	8.1	5.5	2.9	3.5
750	11.7	3.6	1.9	1.6

on the dehalogenase activity of lyophilized *R. erythropolis* cells. Cells grown for 48 h on Sorkhoh's mineral medium supplemented twice with 100 μL of 1-chlorobutane were harvested, washed with 50 mM Tris/HCl buffer (pH 6.2, 7.0, 8.0, or 9.0), and resuspended in Tris/HCl buffer at the same pH.

In both the aqueous and the gas phases, and in the range of pH studied (pH 6.2–9.0), an increase of cellular dehalogenase activity was observed. In both cases, for the range of pH studied, the maximal dehalogenase activity was observed at pH 9.0 (Fig. 1). Moreover, we observed that in the aqueous phase *R. erythropolis* cells were less sensitive to the variation in pH than *R. erythropolis* cells in the gas phase. Indeed, in the gas phase a reduction in pH of 0.5 from pH 9.0 to 8.5 induced a drop of 50% in the dehalogenase activity, whereas in the aqueous phase this decrease was obtained with a reduction in pH of 1.5. In the absence of cells in gas phase or in aqueous phase, 1-chlorobutane was not transformed.

Effect of Water Thermodynamic Activity on the Half-Life and Dehalogenase Activity

The biofilter was packed with 100 mg of lyophilized cells, maintained at 40°C, and fed with 500 μmol/min of a gaseous phase with a a_{ClBut} fixed at 0.06 (8.1 μmol/min). A critical a_w of 0.4 is necessary for the *R. erythropolis* cells to become active (Fig. 2), whereas the pure dehalogenase of *R. erythropolis* remains active even at lower a_w (Dravis et al., 2000). Similar results have been observed with yeast alcohol dehydrogenase (YADH) and whole baker's yeast cells (Yang and Russel, 1995; Maugard et al., 2001; Goubet et al., 2002). This is probably because of a different partitioning of water due to the presence of cellular material around the enzyme, thus leading to a greater need of water to reach sufficient ''enzymatic flexibility.''

When a_w increased the dehalogenase activity of dehydrated cells increased, in agreement with what has previously been reported for pure enzymes (Dravis et al., 2000). This behavior can partly be ascribed to the increase of mobility in the system and also to an increase of diffusion of substrates and products into cells. The increase in flexibility of the enzyme could also partly explain it. Graber et al. (2003) have shown that in a solid/gas system, increasing a_w has a positive effect on the flexibility of the catalyst. Moreover, water is one of the substrates involved in the reaction and, logically, increasing its activity also increases the rate of conversion and the amount of 1-butanol produced.

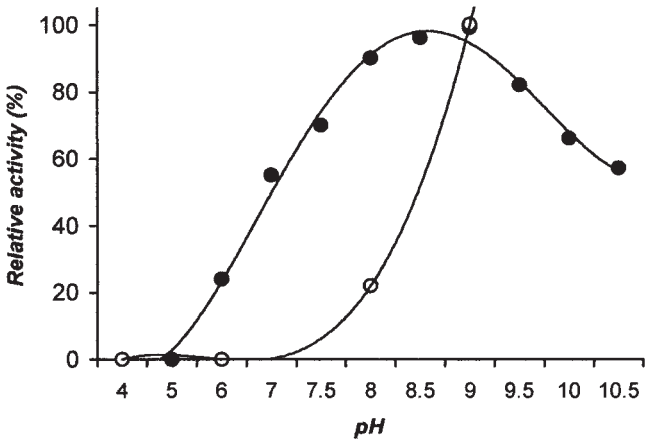

Figure 1. Effect of pH on relative dehalogenase activity of *R. erythropoli* cells in the gas phase (○) and in the aqueous phase (●). Cells grown for 48 h on Sorkhoh's minimal medium supplemented by 2 × 100 μl of 1-chlorobutane were harvested and washed twice with Tris/HCl buffer (50 mM, pH 6.2, 7.0, 7.5, 8.0, 8.5, or 9.0) and then incubated in Tris/HCl buffer (50 mM, same pH as used for washing). Dehalogenation was measured as described in Materials and Methods and expressed relative to that observed at pH 9.0.

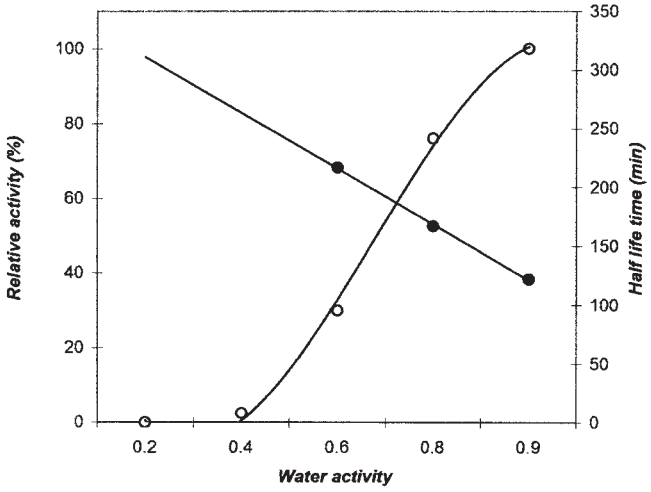

Figure 2. Effect of water activity on the half-life (●) and relative initial reaction rate (○) of 1-butanol formation by lyophilized cells of *R. erythropolis*. The reaction was carried out at 40°C with 100 mg of cells. The total flow into the biofilter was 500 μmol/min. The 1-chlorobutane activity was fixed at 0.06.

The maximal a_w for the conversion of 1-chlorobutane by lyophilized cells was 0.9 and in the range studied no decrease in activity was noticed when the water activity increased.

Conversely to the reaction rate, which increased with a_w, the stability decreases. This decrease in stability is probably not due to thermal denaturation of the enzyme, since 40°C is its optimal temperature in the aqueous phase (Stafford, 1993) and since it has already been observed that enzymes used as catalysts in the gas phase are more stable than in the aqueous phase (Lamare et al., 2001; Cameron et al., 2002). However, it could be explained by an accumulation of HCl produced during dehalogenation. Indeed, HCl is a very hydrophilic molecule and probably diffuses very slowly through the membrane of cells in nonconventional media. The accumulation of HCl in the cells during the process decreases the pH of the microenvironment of the dehalogenase and consequently decreases the dehalogenase activity, as we previously showed (Fig. 1). This hypothesis is confirmed by the fact that the activity of the catalyst was restored by the addition of a volatile base such as triethylamine in the gaseous phase, or by the permeabilization of the cells by lysozyme (Fig. 3).

The permeabilization of cells means that HCl can diffuse more freely and accumulate more slowly in the cells. The decrease in pH and the inhibition of the dehalogenase also occurs later. The combined action of triethylamine and lysozyme confirms our assumptions. We added (at steady state) a nonreactive hydrophobic base (triethylamine), which is not a substrate of the dehalogenase. When the triethylamine is added (thermodynamic activity fixed at 0.05, corresponding to 3.9 μmol/min.g at 40°C), the conversion of 1-chlorobutane is stimulated and the initial rate of 1-butanol formation increases. This stimulation is not only the result of the increase of the pH of the microenvironment of the enzyme, but also the result of the increase of the rate of release of the Cl^- from the active site, thanks to the triethylamine action.

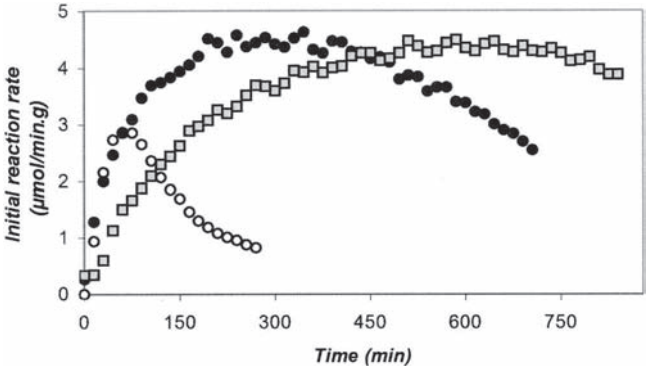

Figure 3. Continuous initial rate of the hydrolysis of 1-chlorobutane catalyzed by *R. erythropolis* as a function of reaction time. The reaction was carried out at 40°C with 100 mg of dehydrated cells (○), cells treated with Lysozyme prior dehydration (●), or cells treated with lysozyme and with triethylamine in the gaseous phase ($a_{TEA} = 0.12$ (■)). The total flow into the biofilter was 500 μmol/min. The water activity and 1-chlorobutane activities were fixed at 0.8 and 0.06, respectively.

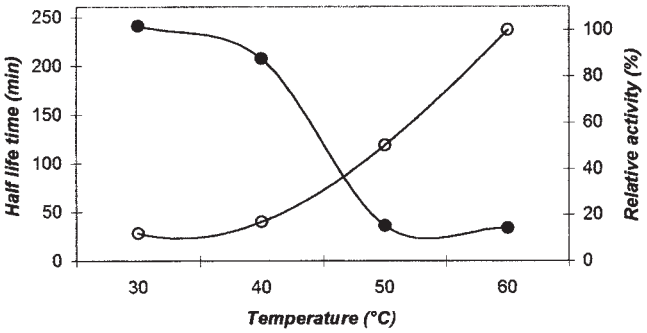

Figure 4. Effect of temperature on half-life (●) and relative initial reaction rate (○) of conversion of 1-chlorobutane by lyophilized *R. erythropolis* cells. The reaction was carried out with 100 mg of cells. The total flow passing into the biofilter was 500 μmol/min. The water activity and 1-chlorobutane activities were fixed at 0.8 and 0.06, respectively. Rate obtained with a 30°C temperature was taken as reference.

Triethylamine plays the role of a volatile buffer that controls the local pH and the ionization state of the dehalogenase and prevents the inhibition of the dehalogenase. This phenomenon has been described for the free haloalkane dehalogenase in gas phase (Dravis et al., 2000). These two experiments confirmed that the dehalogenase was not denatured, but only inhibited by HCl.

Effect of Temperature on the Half-Life and Dehalogenase Activity

The effect of temperature on the behavior of the catalyst was then studied. The biofilter was packed with 100 mg of lyophilized *R. erythropolis* cells fed with 500 μmol/min of gaseous phase at an a_w fixed at 0.8 and an a_{ClBut} fixed at 0.06. Reactions were carried out at temperatures ranging from 40–60°C. As shown on Figure 4, the maximum initial reaction rate of 1-butanol formation increased with temperature but the half-life decreased. It was observed that the best compromise between the dehalogenase activity and the half-life of *R. erythropolis* cells was 40°C.The exponential effect of temperature on activity of the catalyst is shown in

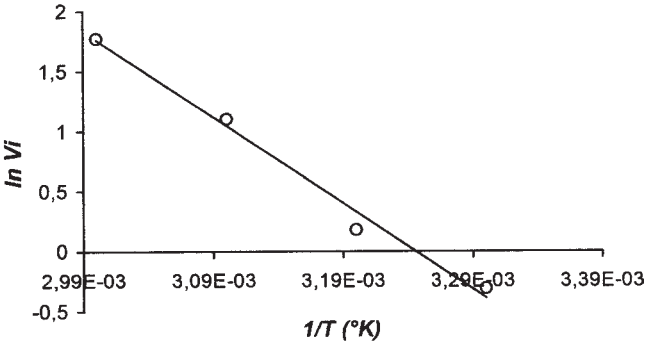

Figure 5. Effect of temperature on rate of formation of 1-butanol by lyophilized cells of *R. erythropolis*. The reaction was carried out with 100 mg of cells. The total flow passing into the biofilter was 500 μmol/min. The water activity and 1-chlorobutane activities were fixed at 0.8 and 0.06, respectively.

Figure 5. Since a linear relationship between the logarithm of the rate and the inverse of temperature was noticed, it can be deduced that there is no diffusional limitation and that the system is kinetically controlled. The activation energy of the reaction as determined by an Arrhenius plot was found to be 59.5 KJ/mol, which is similar to values reported for haloalkane dehalogenases (Stafford, 1993).

Dehalogenase Activity of *Rhodococcus erythropolis* Cells With Various Substrates

The ability of *R. erythropolis* cells to dehalogenate a range of halogenated substrates in the aqueous phase and in the solid/gas phase is shown in Table IV. When harvested cells washed and resuspended in Tris/HCl buffer (pH 9.0), were exposed to 1-chlorobutane, 1-chloropentane, 1-chlorohexane, or 1-bromobutane, the same rate of degradation in a liquid system was observed ($4.9\ 10^{-3}$ μmol/min.g). We observed a slightly lower deha logenase activity using 1-bromohexane as substrate, in agreement with the lower dehalogenase activity observed with the pure enzyme and this substrate (Stafford, 1993). No dehalogenase activity was observed with 1-chlorodecane and 2-chlorobutane. These results demonstrate that the dehalogenase of *R. erythropolis* has a preference, in the aqueous phase, for short chain compounds and for terminally substituted substrates. These results are in agreement with data published on resting cells or pure enzymes (Stafford, 1993; Curragh et al., 1994; Armfield et al., 1995).

In the solid/gas biofilter, the longer the carbon chain of the compound, the higher the initial rate of dehalogenation. This preference for C_5 and C_6 halogenated substrates was not observed in the aqueous phase. On the other hand, no activity was found with 1-chlorodecane and 2-chlorobutane, as was the case in the aqueous phase.

Table IV. Relative dehalogenase activity of *R. erythropolis* cells with various substrates.

	A	B
Substrate	Relative dehalogenase activity of harvested cells in aqueous phase (%)	Relative dehalogenase activity of lypholized cells in gaseous phase (%)
1-chlorobutane	100	100
1-chloropentane	101	186
1-chlorohexane	100	194
1-chlorodecane	0	0
1-bromobutane	98	98
1-bromohexane	87	301
2-chlorobutane	0	0

Cells grown on minimal medium described by Sorkhoh et al. (1991) supplemented by 2 × 100 μL of 1-chlorobutane were harvested and washed twice with Tris/HCl buffer (50 mM, pH 9.0) and then either incubated in Tris/HCl buffer (50 mM, pH 9.0) with halogenated substrates (A) or lyophilized. In the solid-gas biofilter (B), the reaction was carried out with 100 mg of lyophilized cells. The total flow in the biofilter was 500 μmol/min. The water activity and halogenated compound activities were fixed at 8.0 and 0.06, respectively.

CONCLUSION

For the first time, this study has shown the ability of whole *R. erythropolis* cells to dehalogenate a range of chlorinated and brominated substrates in the gas phase at significant rates compared to those observed in the aqueous phase. The influence of parameters such as water thermodynamic activity, temperature, and total flow into the solid/gas biofilter were studied. A decrease in the stability of the solid/gas biofilter versus time was observed. This was related to the accumulation of HCl in the cells, lowering local pH, and could be limited by the addition of a volatile base or by the permeabilization of the cells.

Studies are currently in progress to determine if further degradation of the alcohols and also a multistep conversion can be obtained with the same microorganism in a solid/gas biofilter.

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