Removal of dichloromethane from waste gases in one- and two-liquid-phase stirred tank bioreactors and biotrickling filters

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Abstract

The removal of dichloromethane (DCM) from polluted air was studied both in biotrickling filters and in continuous stirred tank bioreactors, using either a single-liquid aqueous phase or a combination of an aqueous–organic liquid phase. The presence of the organic phase, *i.e.* silicone oil, at a volume ratio of 10% of the liquid phase, increased the maximum EC by about 25% in the BTF, reaching 200 g m^3/h, and by as much as 300% in the CSTB, reaching 350 g m^3/h. Based on data of chloride release in the aqueous phase and carbon dioxide production in the gas phase, complete dechlorination and mineralization of the pollutant could be confirmed. When applying shock loads, a more stable behaviour was observed in the presence of the organic phase. Generally, the completely mixed reactors were also more stable than the plug-flow biotrickling filters, irrespective of the presence of the organic phase. The use of molecular techniques allowed showing that the originally inoculated DCM-degrading *Hyphomicrobium* strains remained present, although not dominant, after long-term bioreactor operation. Different new bacterial populations did also appear in the systems, some of which were unable to degrade DCM.

Keywords

Activated sludge reactor; Air pollution; Completely mixed reactor; Dichloromethane; *Hyphomicrobium*; Plug flow; Shock load

1. Introduction

Dichloromethane (DCM) or methylene chloride is a toxic organic compound and a common air pollutant. It is an irritating chemical with fragrant odour, which is harmful to the respiratory system and the central nervous system. Its MAK value (maximal concentration at work) should not exceed 360 mg/m^3. The MAK value is the maximum concentration of a single pollutant to which workers may be exposed without health risk for an average continuous exposure of 8 h/day and 40 h/week, according to German regulations (DFG, 2008). DCM is produced in large amounts by the chemical industry and is widely used as solvent in paint removers, acetate film production, pharmaceutical industries, metal degreasing and as an aerosol propellant, among others. Different technologies, such as incineration, adsorption and wet scrubbing have been tested for the elimination of DCM from waste gases. However, such methods are costly above all when dealing with relatively low pollutant concentrations and large flow rates. Some factors that significantly affect costs are fuel consumption in case of incineration, catalysts in case of catalytic oxidation processes, activated carbon replacement or regeneration in case of adsorption, and chemicals required for scrubbing. Bioprocesses represent an attractive alternative technology for the removal of DCM from waste gases, based on the activity of cheap biocatalysts operating at room temperature (Kennes and Veiga, 2001).

The biodegradability of DCM was shown for the first time in the early 1980's (Kennes et al., 2006). Different types of bioreactors are available for the removal of VOC as dichloromethane from waste gases. The best choice in terms of reactor configuration depends on factors such as the nature and characteristics of the pollutant as well as the nature of the end products. In case of chlorinated compounds, as DCM, their biodegradation will lead to the formation and release of acid end products. Free liquid-phase bioreactors, as biotrickling filters or bioscrubbers, perform usually better than conventional biofilters, in the removal of acid producing pollutants (Kennes and Thalasso, 1998). Some recent studies have also shown that the addition of a second liquid, organic phase, in suspended growth bioreactors may significantly increase the performance of such reactors (Daugulis, 2001). This has mainly been tested with very poorly soluble pollutants, which is the case of some high molecular weight aromatic hydrocarbons and most VOC-compounds. The presence of such an organic phase is particularly interesting when the microbial activity may be inhibited by the presence of high concentrations of a specific pollutant, as it lowers the concentration in the aqueous phase where biodegradation takes place.

In the present study, the removal of dichloromethane was compared in a biotrickling filter (BTF) and a completely mixed stirred tank bioreactor (CSTB), operated under non-aseptic conditions. The reactors were seeded with pure *Hyphomicrobium* strains and the stability of the inoculated organisms was evaluated after long-term operation. Complete pollutant removal and optimal reactor performance were determined. In a second stage, 10% (vol:vol) silicone oil was added as organic phase to both reactors and performance was compared to the one-liquid-phase systems. The influence of the organic phase when applying shock loads was studied as well. The fate of the inoculated microorganisms was evaluated after long-term operation. Amplified ribosomal DNA restriction analysis (ARDRA) method (Massol-Deyá et al., 1997) was used as a fast molecular fingerprinting technique to track the patterns characteristic for the inoculated

strains. In addition, DCM dehalogenase gene sequences were also targeted as an alternative functional marker.

2. Materials and methods

2.1. Bacteria and medium

The biotrickling filter (BTF) and the completely stirred tank bioreactor (CSTB) were inoculated with a biomass suspension of *Hyphomicrobium* KDM2 and KDM4 (Nikolausz et al., 2006) grown in shake-flasks. Before undertaking the studies reported in this paper, the reactors were operated for about 250 days (eight months) to perform several assays (data not shown). At the end of that period, observations under the microscope showed the presence of a mixed microbial community. As the reactors were operated under non-sterile conditions, after a total of one-year continuous operation samples of both bioreactors were taken in order to characterise the microbial community and to check the presence of the originally inoculated organisms.

The mineral medium used contained per litre distilled water: 1.5 g KH_2PO_4, 4.69 g $Na_2HPO_4 \cdot 12H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 ml of a trace mineral solution and 1 ml of a vitamins solution. The trace mineral solution contained per litre distilled water: 5.3 mg $CaCl_2$, 2 mg $FeSO_4 \cdot 7H_2O$, 0.2 mg $MnSO_4 \cdot 5H_2O$, 0.2 mg $CuSO_4 \cdot 5H_2O$, 0.2 mg $ZnSO_4 \cdot 7H_2O$, 0.03 mg H_3BO_3, 0.4 mg $CoCl_2$ and 4 mg $Na_2MoO_4 \cdot 2H_2O$. The vitamin solution contained per litre distilled water: 0.2 mg biotin, 0.2 mg folic acid, 0.5 mg riboflavin, 0.5 mg thiamine, 0.5 mg nicotinic acid, 0.001 mg vitamin B12, 0.5 mg *p*-aminobenzoic acid, 1 mg pyridoxamine and 0.5 mg lipoic acid. The pH of the medium was adjusted at 7.

2.2. Fixed bed biotrickling filter (BTF)

A scheme of the biotrickling filter is shown in Fig. 1. The glass reactor had an internal diameter of 0.09 m and a total packed volume of 2.1 L lava rock. Both the waste gas and the trickling liquid were fed co-currently in a downflow mode. The polluted gas stream was generated by mixing two different air streams. A small stream of air was bubbled through a vaporization chamber containing pure dichloromethane and was mixed afterwards with a large pure air stream in a mixing chamber. Gas-phase concentrations ranging from 0.1 to 15.7 g/m^3 were obtained by changing the flow rate of the dichloromethane stream. The total gas flow rate was kept at 0.084 m^3/h. The gas velocity was 13.20 m/h and the empty bed retention time 90 s.

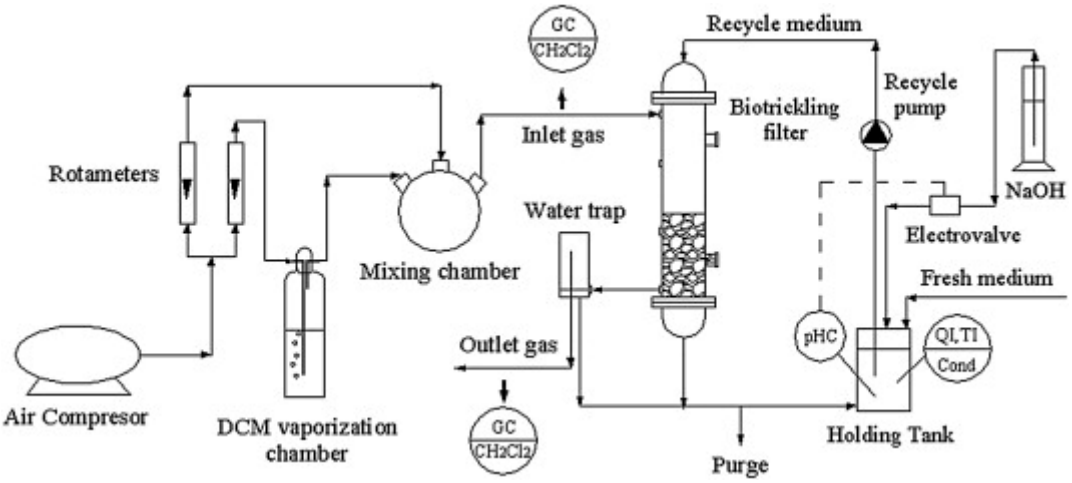

Fig. 1.

Schematic of the biotrickling filter (BTF).

The gas flow rates were adjusted by means of flowmeters (Mohammad et al., 2007). The liquid velocity was kept at 6.9 m/h with a Watson Marlow peristaltic pump. The reactor was maintained at room temperature, around 21 ± 2 °C. The liquid in the holding tank was gently mixed with a magnetic stirrer. The pH of the recirculated liquid medium was kept between 6.90 and 7.05, by means of a pH controller coupled to an electrovalve, by dosing a 2 N NaOH solution to neutralize the HCl formed during DCM biodegradation. This resulted in NaCl accumulation. High concentrations of this salt are known to inhibit the microbial activity (Gälli and Leisinger, 1985, Hartmans and Tramper, 1991, Diks et al., 1994 and Okkerse et al., 1999). Therefore, the conductivity, which is directly related to the NaCl concentration, was measured in the holding tank and was kept below 28 mS/cm^2 by replacing part of the liquid solution when needed. This allowed also to maintain a high enough nutrient supply. The limit of 28 mS/cm^2 was obtained from a salt tolerance test showing that up to such a conductivity microbial inhibition was not significant (Bailón et al., 2007). Air samples were regularly taken from the inlet and outlet gas streams.

2.3. Continuous stirred tank bioreactor (CSTB)

The experimental set-up is shown in Fig. 2. The 2 L BioFlo 110 reactor (New Brunswick Scientific) was equipped, among others, with a PCU, a level control module, dissolved O_2 and pH control modules. A water jacket allowed to maintain a constant temperature.

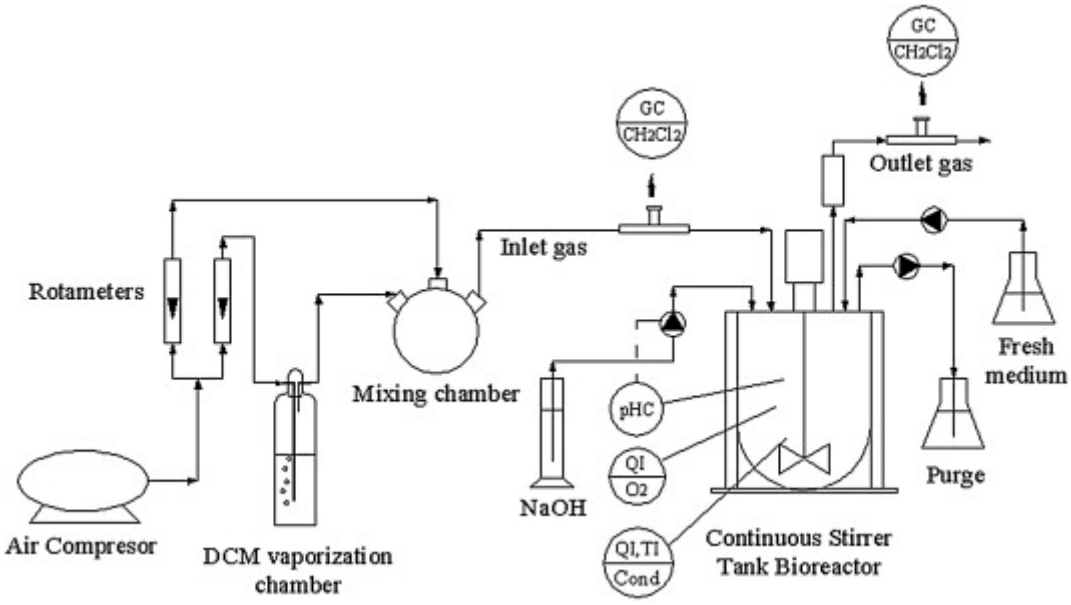

Fig. 2.

Schematic of the completely stirred tank bioreactor (CSTB).

The vessel was filled with 1.5 L liquid containing the suspended biomass solution. The temperature was kept constant at 30 °C, the turbine stirrer at 400 rpm and the oxygen saturation at ~80%. As in the BTF, the pH of the culture was kept constant, at 7.0, by utilizing a 2 N NaOH solution as neutralizing agent, automatically added by means of a peristaltic pump. The conductivity was here also kept below 28 mS/cm^2. Mineral medium was either added or removed when needed to reach this aim. The total reactor volume was maintained constant. The removed biomass solution was allowed to settle down and the supernatant or the settled biomass recycled to the reactor when needed to maintain a constant biomass concentration. The waste gas stream was created in the same way as for the BTF. The flow rate was also 0.084 m^3/h while the gas residence time was 64 s. The DCM concentration was determined in the inlet and outlet gas streams of the bioreactor.

2.4. Partition coefficient

The air/oil partition coefficient of DCM (P_{DCM}, oil) was determined at room temperature (~22 °C) and at 30 °C. 12 ml Teflon coated closed vials were filled with 5 ml each of the silicone oil (viscosity: 10 mPa s, 25 °C). A known mass of DCM was injected into the vials (by triplicate), vortexed for 1 min and left at room temperature or at 30 °C overnight. The concentration of DCM in the oil phase was between 0.22 and 2.29 kg/m^3. After equilibrium was established between the phases, the headspace DCM concentration was measured by GC analysis. The air/oil partition coefficient was then obtained from the slope of the plot of the DCM concentration in the gas phase *vs* DCM concentration in the liquid phase.

2.5. Analytical methods

The DCM concentration of the gas samples was determined with a gas chromatograph, Hewlett Packard HP 6890, equipped with a flame ionisation detector. An HP PLOT-Q column of 30 m length and 0.53 mm I.D. was used. The oven temperature was held at

180 °C during 5 min. Carbon dioxide was detected and quantified on an HP 5890 series II gas chromatograph equipped with a TCD (Mohammad et al., 2007). The injection and oven temperatures were 90 and 25 °C, respectively, with the TCD set at 100 °C.

2.6. Molecular techniques

DNA was isolated from the bioreactor samples and from the pure and mixed cultures by using DNeasy kit (Qiagen, Hilden, Germany) according to the manufactureŕs protocol. 16S rRNA were amplified from purified DNA by PCR using HotStar *Taq* polymerase with the buffer supplied by the manufacturer (Qiagen) and universal 16S rRNA gene primers 27f (Lane, 1991) and 1387r (Heuer et al., 1997). The annealing temperature was decreased to 50 °C according to the suggestion of Sipos et al. (2007) in order to reduce PCR bias. PCR amplification of the DCM halogenase gene (*dcmA*) using primers DM for DMrev was performed as described by Vuilleumier et al. (2001). PCR products were separated on a 1.2% agarose gel stained with ethidium bromide and were visualized with UV excitation.

The 16S rRNA gene PCR amplicons were digested in separate reactions with 1 U of the tetrameric site restriction endonucleases Hin6I and BsuRI (Fermentas, Vilnius, Lithuania) in 1× buffer supplied by the manufacturer in a final volume of 25 μl. The digestion mixture was incubated for 3 h at 37 °C to ensure full digestion of the PCR products. The fragments were separated by electrophoresis for 35 min at 100 V in a 1.6% agarose gel in 1× TAE buffer.

Partial 16S rRNA gene sequence of two strains was determined using the Big Dye Terminator Cycle Sequencing Kit V3.1 (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. Primers used for 16S rDNA sequencing reactions were 27F and 907R (Lane, 1991). Sequencing reaction mixtures were separated with an ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Analysis of sequences and homology searches were completed using the BLAST algorithm with BLAST server of the National Centre for Biotechnology Information (Altschul et al., 1997). The partial sequences determined in this study have been deposited in the NCBI database under accession numbers FM177766 and FM177767.

3. Results and discussion

3.1. Partition coefficients

The DCM air/water and air/oil partition coefficients appearing in Table 1 show that DCM is 10 times more soluble in silicone oil than in water.

Table 1.

DCM partition coefficients air/oil, air/water.

Temperature (°C)	**22**	**30**
$P_{DCM,\ water}$ $[(mg/L)_{air}/(mg/L)_{water}]$ (Diks, 1992)	0.07	0.11
$P_{DCM,\ oil}$ $[(mg/L)_{air}/(mg/L)_{oil}]$ (this study)	6×10^{-3}	7.7×10^{-3}

3.2. Performance of one-liquid-phase bioreactors

Dichloromethane is relatively water soluble despite being a typical volatile air pollutant. It is sometimes also found in wastewaters (Moura et al., 2007) and its solubility in aqueous phase is around 19.4 g/L at room temperature (Kennes et al., 2006). Although its Henry coefficient is somewhat higher than 10^{-2}, DCM removal should certainly be feasible in either biotrickling filters or bioscrubbers, *i.e.* stirred tank bioreactors. Conversely, conventional biofilters would not be the best choice, as confirmed by the poor performance reported for such reactors for DCM removal (Ergas et al., 1994). Although DCM is a common industrial air pollutant, only few research papers have been published on its removal from waste gases in bioreactors. The main objective of this experiment was to compare the removal of DCM in a biotrickling filter and in a stirred tank bioreactor. Although biotrickling filters have most often been used so far, industries already having a biological wastewater treatment plant as, for example, an activated sludge bioreactor, could advantageously feed the waste gas to such bioreactor with limited additional cost. In order to compare the efficiency of a biotrickling filter and a stirred tank bioreactor, both systems were started up simultaneously, and the inlet DCM concentration was gradually increased. Fig. 3 shows elimination capacity data *vs* inlet load for the BTF, while Fig. 4 shows the same data for the CSTB, as well as the corresponding removal efficiencies. Comparison of both figures allows to make some interesting observations. With respect to the maximum elimination capacity obtained in each case, a 45% higher value was reached in the BTF compared to the CSTB, namely 110 g/m^3 h in case of the suspended growth bioreactor *vs* 160 g/m^3 h in the attached growth system. Considering that the BTF was operated at room temperature, while the CSTB was operated at 30 °C, the higher performance of the BTF would most probably even have been somewhat more significant if both systems had been operated at the same temperature. When focusing on the removal efficiencies, more than 90% removal was always maintained in the CSTB even when reaching the maximal elimination capacity of 110 g/m^3 h. Conversely, in the packed bed bioreactor, removal efficiencies exceeding 80% could only be maintained at loads below 40 g/m^3 h. The performance curve gradually deviated from the 100% removal line when further increasing the load. The removal efficiency did hardly reach 50% at the maximum elimination capacity of 160 g/m^3 h in the BTF, corresponding to a load of almost 350 g/m^3 h. This does also confirm data previously published on DCM removal in BTF (Hartmans and Tramper, 1991 and Okkerse et al., 1999). For example, the maximum elimination capacity reached by Hartmans and Tramper (1991) in a BTF was 103.5 g/m^3 h with a removal efficiency of only 59.3%. In that study, RE above 80% were only possible at loads below 20–30 g/m^3 h. The present data suggest that, although the one-liquid-phase CSTB does not allow reaching maximum elimination capacities as high as the one-liquid-phase BTF, it allows maintaining much higher removal efficiencies at high loads.

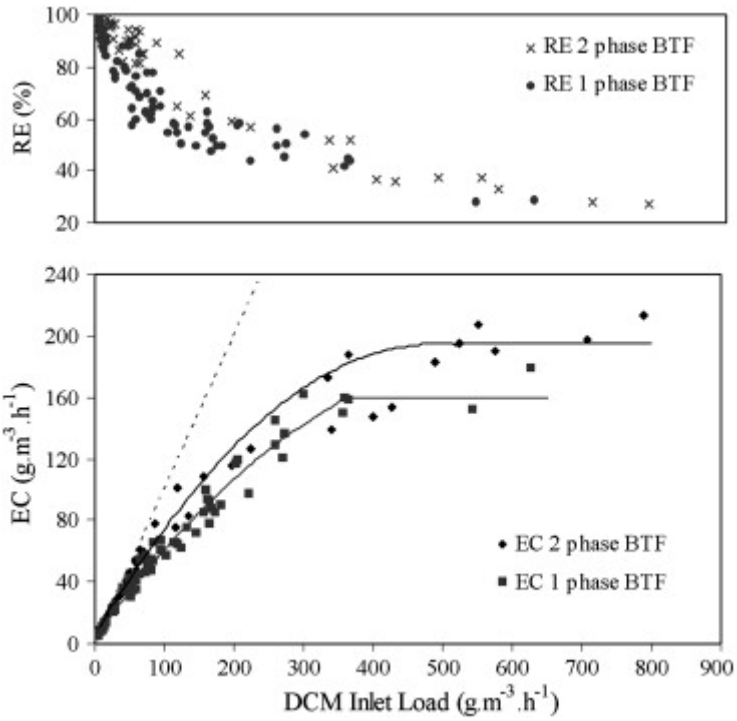

Fig. 3.

EC and RE *vs* DCM-inlet load in one- and two-liquid-phase biotrickling filters.

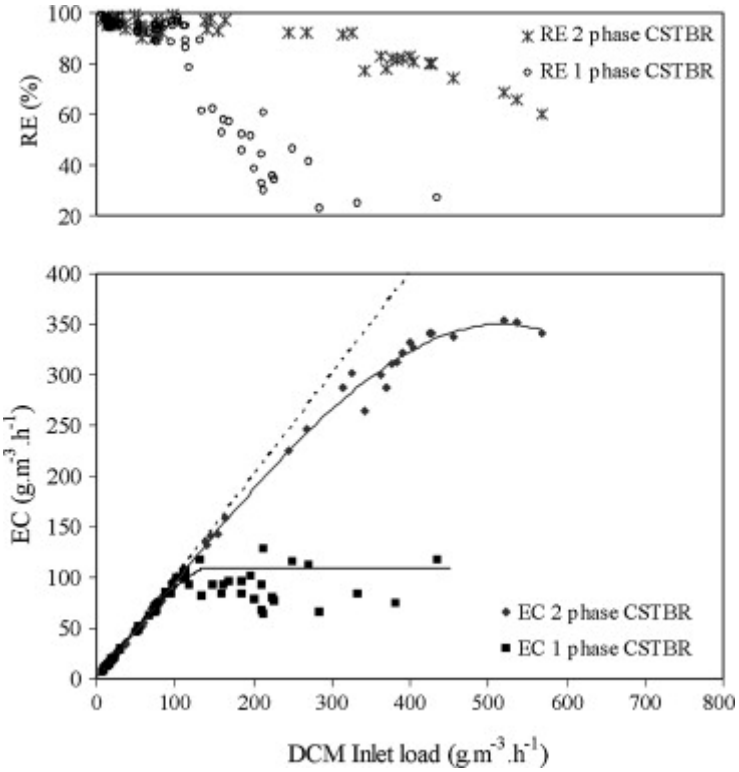

Fig. 4.

EC and RE *vs* DCM-inlet load in one- and two-liquid-phase Completely Stirred Tank Bioreactors.

3.3. DCM mineralization and mass balance calculations

Carbon dioxide and hydrogen chloride are both end products of DCM biodegradation, according to the following stoichiometric equation, when neglecting biomass growth (Kennes et al., 2006):

$CH_2–Cl_2 + O_2 \rightarrow CO_2 + 2HCl$

In order to confirm, respectively, complete dechlorination and complete mineralization, both the amounts of HCl and CO_2 formed were checked.

If complete dechlorination takes place, 2 mol NaOH would be needed to neutralize the acid formed from the biodegradation of 1 mol DCM, according to Eqs. (1) and (2).

$2HCl + 2NaOH \rightarrow 2NaCl + 2H_2O$

Since the amount of DCM fed to the reactors is known, by recording the amount of sodium hydroxide consumed and used to neutralize the HCl formed, it was possible to confirm that complete dechlorination took place in both reactors, since that experimental value matched with the calculated value based on Eqs. (1) and (2).

Regarding CO_2 data, generally higher carbon dioxide concentrations were generated in the reactors than the ones expected from the DCM load and stoichiometric equation. This has sometimes already been observed and reported by others and can be attributed to phenomena as endogenous respiration. Based on the above results, complete dechlorination was clearly proven and complete mineralization could reasonably be assumed.

3.4. Stability of inoculated strains

The presence of the inoculated strains after one-year reactor operation was investigated by amplified ribosomal DNA restriction analysis (ARDRA) and by the detection of the DCM dehalogenase gene. Fig. 5 shows the comparison of the restriction pattern obtained from the inoculated strains and from both reactors. Re-isolation attempts of the DCM-degrading *Hyphomicrobium* strains from the BTF resulted in two mixed cultures designated as DCM A and DCM B. While the ARDRA pattern specific for the *Hyphomicrobium* strains is clearly highly present in the CSTR, careful inspection of the Hin6I pattern (Fig. 5B) of the BTF suggests that *Hyphomicrobium* KDM strains were not the predominant microorganisms in the sample. Mineral medium plates under DCM atmosphere (Nikolausz et al., 2005) were used for re-isolation of the potential degraders in the system. The first isolation attempt resulted in mixed cultures based on direct sequencing of the PCR products. The major bands of the ARDRA patterns of these two mixed cultures corresponded to the patterns of the BTF. Further purification steps resulted in pure cultures designated as DCM F1 and DCM F2. The ARDRA patterns of the DCM F1 corresponded to the major band of the BTF samples (data not shown). However, these strains grew very slowly on the mineral medium plate without any detectable DCM degradation. While the activity of the original *Hyphomicrobium* strains resulted in a strong colour change of the pH indicator added to the plate, such colour change was not observed with the DCM F1 and F2. After the second transfer on mineral medium plate the strains did not grow at all. DCM F1 was identified as

Stenotrophomonas maltophilia (100% identity, 840/840 bp) and DCM F2 was closely related to a *Mesorhizobium* sp. (98%, 636/643 bp).

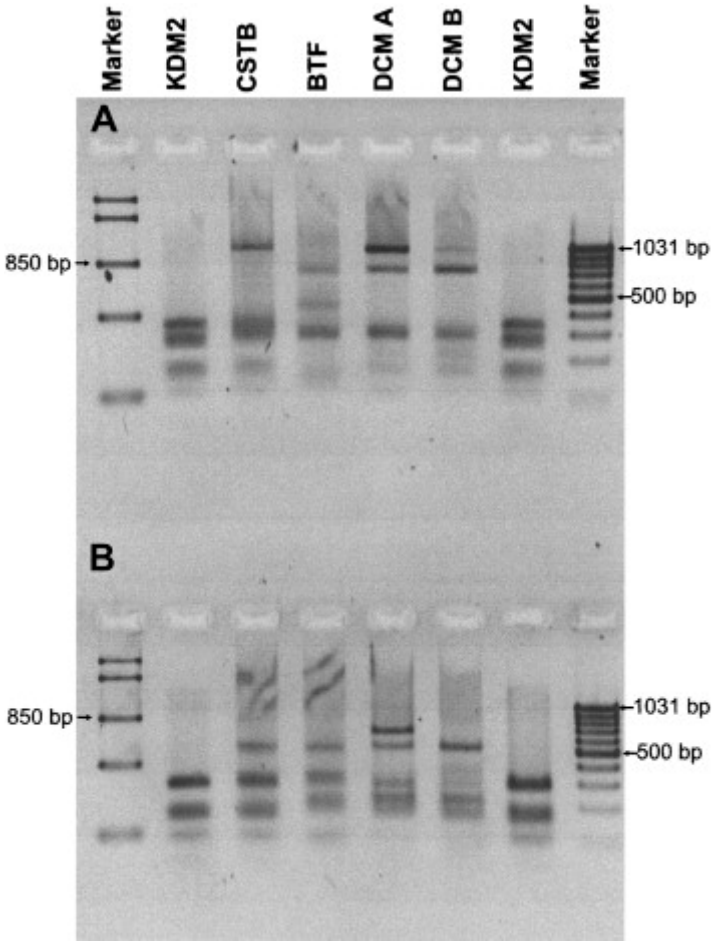

Fig. 5.

ARDRA patterns of the microbial communities and cultures retrieved from the different reactors by using Hin6I (A) and BsuRI (B) restriction enzymes. Samples were run parallel with molecular weight markers (Marker).

Figure options

The DCM dehalogenase gene was not detected in these pure cultures (Fig. 6) but it was detected in the parent mixed culture. The functional gene was also present in the two reactor samples (Fig 6) which indicates that the functionality is linked to the presence of the DCM dehalogenase gene. Our data suggest that the horizontal transfer of the functional gene has not occurred.

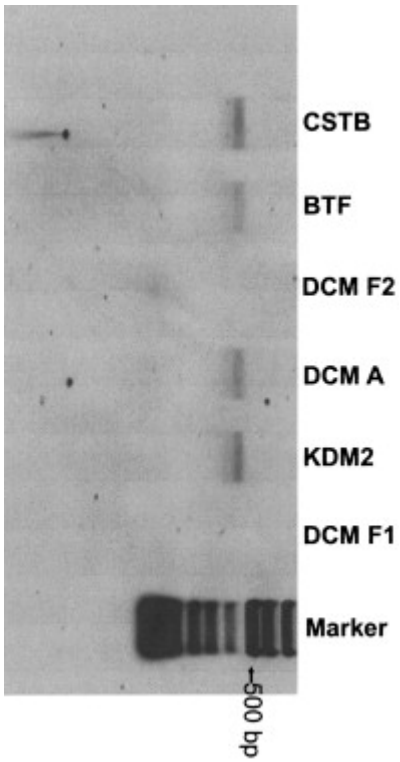

Fig. 6.

PCR amplification and confirmation of the presence of the DCM dehalogenase by agarose gel electrophoresis. Samples were run parallel with a molecular weight marker (Marker).

Although not so many data are available in the literature, some researchers observed that in gas-phase bioreactors inoculated microorganisms may remain dominant after several months operation, above all under rather selective or extreme conditions, as observed in some fungal biofilters (Estévez et al., 2005 and Jin et al., 2007). Conversely, other researchers observed that sometimes bacteria originally inoculated in such bioreactors may still remain present but not necessarily as dominant population. Sercu et al. (2005) used *Hyphomicrobium* VS strain as inoculum in a two-stage biotrickling filter for the removal of hydrogen sulphide and dimethyl sulphide. After a few weeks operation, the strain was not anymore the dominant microorganism in the reactor. A similar result was observed by Moller et al. (1996) where the introduced *Pseudomonas putida* strain represented only a minor part of the community, i.e. around 4%, but corresponded to about 65% of the toluene degraders in a BTF. These results are in good agreement with our findings that the inoculated microorganisms can become a minor part of the community after short-term operation but may still be responsible for a considerable part of the biodegradation. Although PCR bias caused by preferential amplification may skew the relative abundance of different populations obtained by molecular techniques, several recent studies showed that non-stringent PCR conditions as used in this study can reduce this effect (Ishii and Fukui, 2001 and Sipos et al., 2007).

3.5. Performance of two-liquid-phase bioreactors

In waste gas treatment, the use of a second, organic, liquid phase is known to be able to significantly increase the bioreactors efficiency mainly in case of hardly water soluble pollutants and for toxic compounds, which will be released slowly from the organic to the aqueous phase as biodegradation proceeds. Although DCM is not very poorly soluble in water, the effect of adding an organic phase was checked.

As with the one-liquid-phase systems, the inlet DCM concentration was gradually increased in order to evaluate both reactors' performance. Data of EC *vs* load as well as the corresponding removal efficiencies are shown in Fig. 3 and Fig. 4, respectively, for the BTF and the CSTB and are compared with the results of the one-liquid-phase reactors. It can be seen that the presence of a second, organic, phase improved the overall performance. However, such improvement was much less significant in the BTF than in the CSTB. Indeed, when adding 10% silicone oil to the BTF, the maximum EC increased by 25%, reaching 200 g/m^3 h to be compared to 160 g/m^3 h for the one-liquid-phase BTF. High RE, exceeding 80%, could only be maintained up to a load of about 80 g/m^3 h, after which the performance curve started deviating from the 100% removal line (Fig. 3). Conversely, in the two-liquid-phase CSTB, more than 80% RE could be maintained up to a load of about 330 g/m^3 h, corresponding to an inlet DCM concentration of about 6 g/m^3. The maximum EC in this system was 350 g/m^3 h, which is more than three times higher than in the one-liquid-phase CSTB. To the best of our knowledge, this is also almost three times higher than the best results reported so far for DCM removal in the literature (Table 2). Information on two-liquid-phase BTF is very scarce in the literature. Although a few researchers have studied the removal of VOC from polluted air in two-liquid-phase stirred tank bioreactors (Boudreau and Daugulis, 2006, Césario et al., 1995, Déziel et al., 1999 and Davidson and Daugulis, 2003), there are only very few reports systematically comparing the removal of such pollutants in one- and two-liquid-phase systems. One study reported on the removal of hexane in a two-liquid-phase BTF, in which EC close to 100 g/m^3 h was reached with 90% RE (Van Groenestijn and Lake, 1999). Although the data were not compared to any control system with one single-liquid-phase in that study, such EC is slightly higher than those commonly reported in the literature for one-liquid-phase BTF (Kennes and Veiga, 2001). A much more recent study compared the removal of that same pollutant, namely hexane, in fungal BTF and CSTB, with either one- or two-liquid-phases (Arriaga et al., 2006). The authors observed an increase in the maximum EC from 110 to 180 g/m^3 h in their BTF and from 50 to 120 g/m^3 h in the stirred tank reactor, respectively, with one- or two-liquid-phases. This means a 60% improvement in case of the BTF and a 2.4 times increase of the maximum EC in the suspended growth bioreactor, confirming that the improvement is significantly more important in CSTB than in BTF, when adding an organic phase. The better results obtained with the two-liquid-phase CSTB compared to the two-liquid-phase BTF could be explained by the formation of many small oil droplets in suspension in the aqueous phase as a result of the high agitation speed used in the CSTB which is highly favourable for an optimal and fast mass transfer of the pollutant from the organic phase to the aqueous phase where biodegradation takes place. Such behaviour is not found in packed bed bioreactors.

Table 2.

DCM removal from waste gases in bioreactors.

Reactor type	Elimination capacity (g/m^3 h)	Corresponding RE (%)	References
BTF (one-liquid-phase)	157	–	Diks and Ottengraf, 1991
BTF (one-liquid-phase)	103.5	59.3	Hartmans and Tramper, 1991
BTF (one-liquid-phase)	102	70	Okkerse et al., 1999
Compost biofilter	10.3	98	Ergas et al., 1994
BTF (one-liquid-phase)	160	44	This study
BTF (two-liquid-phase)	195	42	This study
CSTB (one-liquid-phase)	117	92	This study
CSTB (two-liquid-phase)	351	68	This study

3.6. Shock loads

In case of shock loads, the presence of an organic phase is expected to buffer the inhibitory effect of the sudden higher pollutant concentrations in the feed. However, only few experimental data are available in the literature to evaluate this potentially favourable effect of using mixed aqueous–organic phases in CSTB operated under transient or high load conditions (Nielsen et al., 2005, Boudreau and Daugulis, 2006 and Aldric and Thonart, 2008) and no data are available for BTF. Some results have also been published on the effect of placing an oil-absorber prior to a one-liquid-phase suspended growth bioreactor when applying shock loads of either monochlorobenzene (Oliveira and Livingston, 2003) or dichloroethane (DCE) (Koutinas et al., 2006). Such configuration showed that a previous oil absorption stage allows to buffer shock loads and avoid biomass inhibition and/or wash-out. In the present research both liquid phases, aqueous and organic, were combined in one single stage, namely the bioreactor. Three different experiments were performed with the four systems, *i.e.* one- and two-liquid-phase BTF and CSTB: (1) switching from a “low” load (15 g/m^3 h) to a “low” overload (200 g/m^3 h), (2) switching from a “medium” load (50 g/m^3 h) to a “medium” overload (250–300 g/m^3 h), and (3) switching from a “high” load (70 g/m^3 h) to a “high” overload (500 g/m^3 h). The results showing the loads, EC and corresponding RE during the overloads are shown in Fig. 7, Fig. 8 and Fig. 9 for experiments (1), (2), and (3), respectively. The data of Fig. 7 show that the low overload inhibits all the systems except the two-liquid-phase CSTB, in which the RE remained close to 100% throughout the entire duration of the shock load. Actually, this is not surprising as a shock load of 200 g/m^3 h is still below the maximum EC reachable in

that system (Fig. 4). Regarding the other three systems, although the one-liquid-phase CSTB was previously shown to exhibit the lowest maximum EC under normal conditions (110 g/m^3 h) (Fig. 4) compared to both BTF (160 and 200 g/m^3 h) (Fig. 3), that suspended growth bioreactor was less inhibited during the shock load than the BTF, as can easily be seen from the RE data (Fig. 7). In the one-liquid-phase CSTB, the RE dropped later and less than in the packed bed bioreactors. This can easily be explained by the flow regime in each one of these systems. Indeed, the CSTB is basically a completely mixed reactor, while plug-flow regime is typically found in packed bed BTF (Mendoza et al., 2004). In the mixed tank, the high inlet concentration reaching the reactor is immediately diluted in the whole reactor volume. Thus, the biocatalyst is actually not exposed to very high pollutant concentrations during the shock load, resulting in minimal inhibition. Therefore, the RE does only start dropping around the end of the 6-h shock load. In case of both BTF, the RE dropped almost immediately once applying the shock load because the attached biomass in both plug-flow bioreactors is directly exposed to the high, inhibitory, inlet concentration. A quite similar behaviour is observed in Fig. 8, when increasing the shock load to a somewhat higher value: almost no inhibition in the two-liquid-phase CSTB, slow and little inhibition in the one-liquid-phase CSTB, and immediate and more significant inhibition in both BTF. In the third experiment, the shock load (500 g/m^3 h) was well above the critical load of all four bioreactors. In such case, inhibition appears in all four systems. Nevertheless, the two-liquid-phase CSTB is the least affected, followed by the one-liquid-phase CSTB, where the RE dropped to minimum values of 60% and 50%, respectively, at the end of the 6-h shock load. In the BTF, inhibition seemed to be slightly less in the two-liquid-phase bioreactor although the difference was not really significant compared to the one-liquid-phase configuration (Fig. 9). Both BTFs quickly reached a minimum value close to 40% which remained almost constant throughout the overload period. This does again show the positive effect of the oil phase in the CSTB, combined to a better behaviour of completely mixed systems compared to packed bed bioreactors.

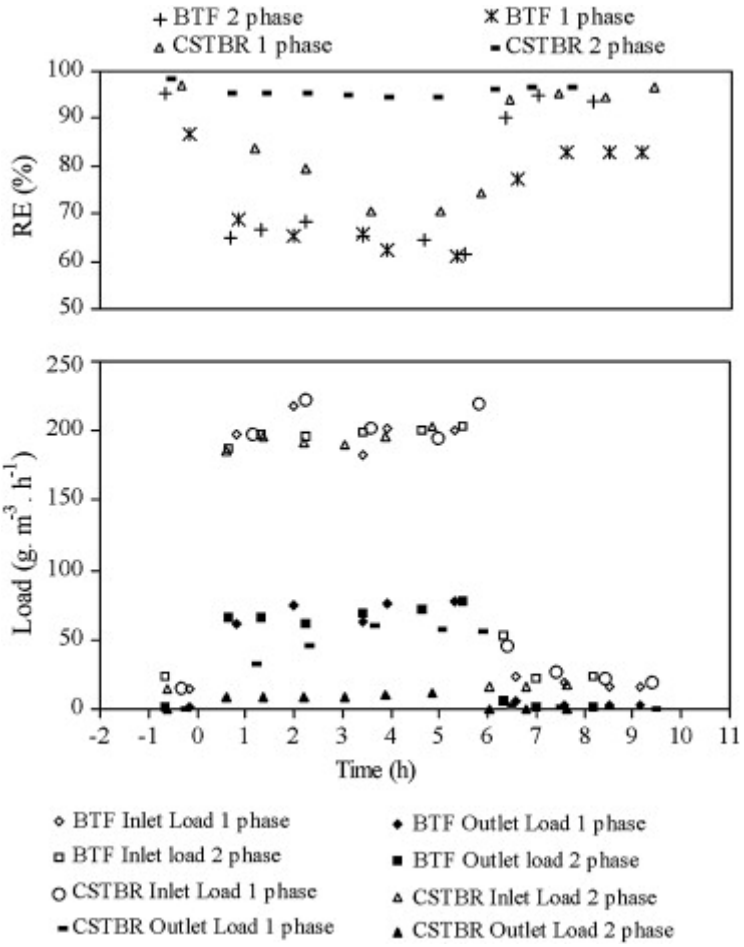

Fig. 7.

Inlet load, outlet load (*i.e.*, EC) and RE *vs* time during shock load experiment #1.

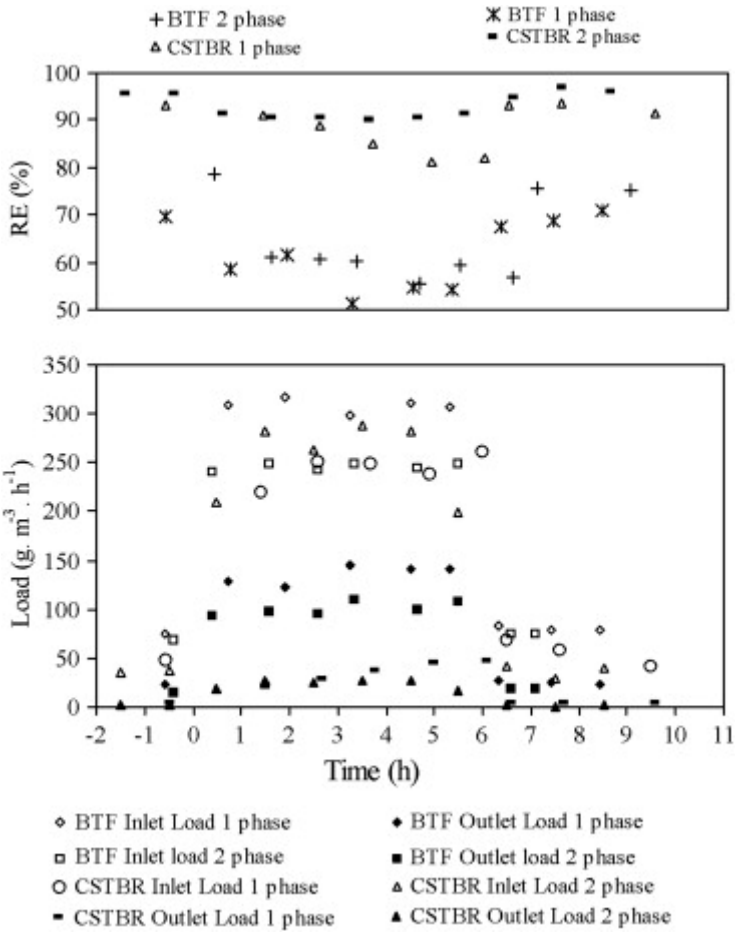

Fig. 8.

Inlet load, outlet load (*i.e.*, EC) and RE *vs* time during shock load experiment #2.

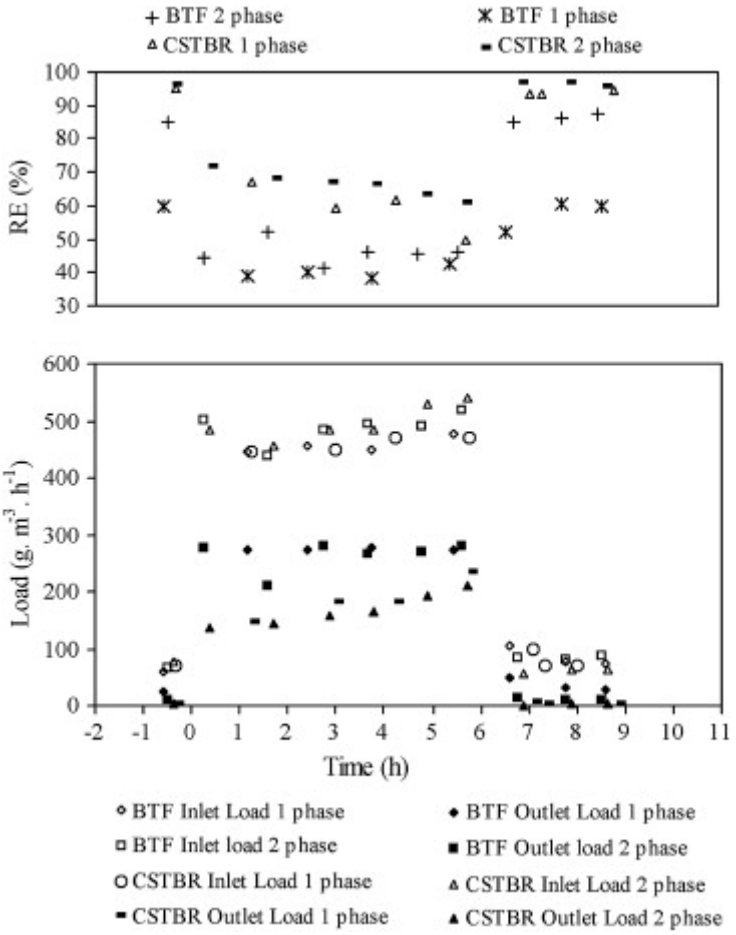

Fig. 9.

Inlet load, outlet load (*i.e.*, EC) and RE *vs* time during shock load experiment #3.

4. Conclusions

- The two-liquid-phase CSTB performs always better than the other three bioreactors, both in terms of maximal performance (highest maximum EC and highest RE) and in terms of its resistance against shock loads.

- The BTF (either with one- or two-liquid-phases) reaches higher maximum EC than the one-liquid-phase CSTB. Nevertheless, up to its critical load the CSTB performs better as its RE remains close to 100% up to that critical load, i.e. about 110 g/m^3 h. The RE of the BTF quickly deviates from the 100% removal line when reaching loads of about 40 g/m^3 h.

- The CSTB can better withstand shock loads, above all in the case of the two-liquid-phase system which benefits not only from its completely mixed nature

but also from the buffering effect of the organic phase. When increasing the shock load to higher values, the difference between the one-liquid-phase CSTB and the BTFs becomes somewhat less pronounced.

- The inoculated *Hyphomicrobium* strains became only a minor part of the total bacterial community in both reactors, after long-term operation. Unambiguous detection in the BTF was only possible with the functional gene marker. However, this minor community may play a considerable role in biodegradation despite its relative low abundance.

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