Biofiltration of dichloromethane vapours: isolation of microorganisms

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ABSTRACT. This paper presents preliminary results about realization of a DCM degrading vapour-phase biofilter. Specific microorganisms were isolated from chloorganics contaminated soils and selected after screening growth tests in the presence of DCM at different concentrations. A strain of *Penicillium* and a microbial consortium were isolated and separately inoculated into two identical lab-scale bioreactors (0.5L volume) packed with expanded clay in granular form. Results revealed better performance for the consortium than Penicillium, but for both the biomasses elimination capacity was very low (< 50 $gm^{-3}h^{-1}$) and removal efficiency decreased below 20% after about 2 months operation. A new isolation process was then carried out from a municipal waste sludge and a new microbial consortium was isolated and batch growth kinetics in the presence of dichloromethane at different pHs was investigated.

1 INTRODUCTION

Dichloromethane (DCM) is a widely used chemical solvent with a diverse number of applications. It was introduced as a replacement for more flammable solvents over 60 years ago. It is commonly used in paint removers and industrial adhesive formulations. It is also employed in the production of flexible urethane foams, pharmaceutical products, and plastics, as a cleaning agent for fabricated metal parts, and as an extraction solvent. It has been shown to cause lung and liver cancer in rodends, and a number of harmful effects on humans have been reported (Sakai *et al.,* 2002). DCM shows high persistence in water and atmosphere (estimated life time 130 days), thus there is a growing interest in studying biodegradative processes of DCM in natural environment. Dichloromethane and chloromethane are the only chlorinated hydrocarbons of which it is known that they can serve as growth substrates for aerobic as well as anaerobic microorganisms (De Best *et al.,* 2000; Herbst and Wiesmann, 1996). Biodegradation of DCM is carried out in four steps with GSH (glutathione) as co-factor necessary for the separation of chlorine atoms. Free oxygen is only used for the degradation of formaldehyde. Following dehalogenation, HCl forms and accumulates bringing toxicity for microorganisms. That's why most of the researches

about application of biological techniques for treatment of chlorinated compounds used biotrickling filters which allow for an easy pH control of the trickling phase (Diks and Ottengraf, 1991; Hartmans and Tramper, 1991; Lee *et al.,* 2003; Okkerse *et al.,* 1999a,b). However, some studies (only one for dichloromethane) reported promising results about employment of conventional biofilters for removal of chlorinated compounds (Den and Pirzbazari, 2002; Devinny *et al.,* 1995, Ergas *et al.,* 1994; Kim *et al.,* 1998). In particular, at low inlet gas concentrations, biofilters are more favorable to mass transfer than biotrickling filters due to higher specific surface areas and smaller liquid film thickness (Ergas *et al.,* 1995).

Biofiltration is used on commercially scale for odor control in waste treatment, in food, and flavors manufacture and, generally, to treat large volume of air with low pollutants concentrations. Biofilters consist of packed beds containing packing material (e.g. peat, compost) which support the growth of microorganisms in the form of biofilms. Waste gases are conveyed through this bed. Pollutants are transferred from the gas phase to the biofilms where they diffuse and undergo aerobic biological degradation. For specific compounds, specialized pure or mixed cultures are needed in order to get high efficiency and reduce the lag-phase of adaptation to the pollutant compounds (Veiga and Kennes, 2001).

In the present work some specific DCM degrading microorganisms (bacteria and fungi) were isolated from chloroorganics contaminated soils and tested in a vapor-phase lab-scale bioreactor. A second isolation procedure was carried out starting from a municipal waste sludge. The obtained culture was tried to be characterized for its growth kinetics in the presence of DCM at different pHs.

2 MATERIALS AND METHODS

2.1 Isolation of microorganisms

The first isolation was carried out from soils stored in an abandoned steel plant in the northern Italy and containing chloroorganic compounds. Soil samples were extracted with sterilised water, and dilutions of the filtered solution were spread onto Czapek Dox Agar (Oxoid Ltd, England), a selective medium for fungi and bacteria added with lactate and yeast extract. An enriched mixed culture was obtained and growth tests in Yeast Nitrogen Base (YNB, a medium lacking carbon sources, Difco) at different DCM concentrations (05-0.1-0.01 g/L) and in the presence or absence of a primary substrate (glucose or lactate, 5 g/L) were carried out.

For the second isolation procedure, a sludge was kindly provided by a plant (in Piacenza-Italy) treating different liquid wastes. Another enriched mixed culture was selected after successive transfers in liquid Czapek Dox Agar added with diphenil to avoid moulds growth and select bacteria. Growth tests were carried out at different pH (7 or 9) in a DCM saturated environment to investigate the best development conditions. Saturation was achieved holding inside the growth flasks an open tube filled with DCM and mass development was monitored by reading absorbance at 660 nm. The growth kinetics of the selected culture was investigated at constant pH (the value was periodically corrected by addition of 0.1N NaOH as neutralizing agent) and variable pH (in this case the value was regulated only at the beginning of the trial). All the selected cultures were observed with a Scanning Electron Microscope (SEM Hitachi S-2300) and stored on slants at 4°C.

2.2 Lab-scale bioreactor

The selected biomasses from the first isolation were inoculated into two identical bioreactors assembled in parallel as schematised in Figure 1. The plant is the same as that optimised in a previous work for degradation of hexane (Spigno and De Faveri, 2005). Each biofilter consisted in a jacketed glass column (to work at a constant temperature of 30°C), 0.4 m overall height and 0.04 m i.d, packed with expanded clay in granular forms. The polluted gas stream was artificially created by mixing two air streams (one stripping DCM, and the other one passing into a humidifying system) and introduced at the bottom of the reactor. Connecting tubes were Viton based. DCM concentrations in the inlet and outlet gas streams were monitored by a Perkin Elmer Autosystem KL GC equipped with a SPB-1 column (15m x 0.53mm x 3 μm) and FID detector.

For the inoculum the *Penicillium* was grown on Malt Extract Agar (Oxoid, Ltd) at 30°C for 4-5 days, 0.5g of mycelium was suspended in MEB and the solution introduced into the column packed with pre-humidified clay. YNB liquid cultures were made develop at 30°C, centrifuged and re-inoculated into MEB with DCM 0.1 g/L. The developed cultures were collected and introduced into the column as done with the *Penicillium*. In both the cases the columns were held filled and horizontal for about a week, with the polluted gas flowing in, in order to adapt the biomass and enhance a more as possible uniform support colonization. After the reactors were drained off, nutritive medium (MEB) was fed and recirculated down-flow, each day for the first 5 days and then whenever an important decrease in the removal efficiency (RE) was observed. RE was calculated as $[(C_{in}-C_{out})/C_{in}]$, with C_{in} C_{out} the inlet and outlet pollutant concentrations respectively.

Operating conditions were the following:

- ✓ *Penicillium*: flow rate 5 - $6.3 \cdot 10^{-3}$ m^3/h, C_{in} 2-30 g/m^3
- ✓ Consortium: flow rate $1.3 \cdot 10^{-3}$ m^3/h, C_{out} 2-40 g/m^3

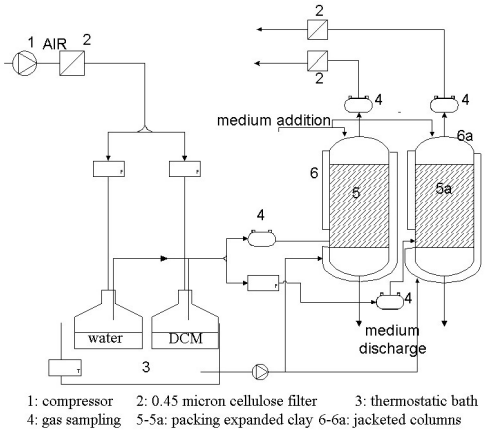

Figure 1. Scheme of the lab-scale biofilter.

3 RESULTS AND DISCUSSION

3.1 Microorganisms from contaminated soils

After the first isolation procedure we selected a mould and a mixed culture. The mould was identified (by SEM observation) as a strain of *Penicillium* and chosen because always present and characterized by an aerial and thin development: these latter properties should allow in a vapour-gas bioreactor for a reduced clogging effect and an enhanced mass transfer of poor-water soluble compounds between the polluted air and the biomass (Kennes and Veiga, 2004; Spigno and De Faveri, 2005). The mixed culture, composed of bacteria and yeasts, was chosen to investigate a possible synergic effect on DCM degradation by yeasts and bacteria, since, even though in our isolates fungi were always predominant, most of the literature works on chlorinated compounds use bacteria.

Growth tests in the presence of different DCM concentrations revealed a very poor development with 0.5 g/L and a similar development at 0.01 and 0.1 g/L. As suggested by Gao and Skeen (1999), we examined whether or not the addition of a water-soluble, non-toxic substrate could stimulate aerobic biodegradation of DCM. In fact, generally cometabolism of chlorinated compounds is achieved by adding specific substrates, such as ammonia, methane, propane, propylene or phenol, which can be in their turn environmentally hazardous materials. Addition of lactate enhanced biomass development only slightly more than glucose.

Both the biomasses were tested on the lab-scale biofilters, evaluating the RE and the elimination capacity ($EC=(C_{in}-C_{out})/V_{reactor}$, where V is the reactor volume). The resulting EC showed the typical EC trend of a biofilter for pollutants degradation as a function of inlet pollutants load (Figure 2a): generally, for a given compound, the bulk EC increases with increasing concentration in the air stream (mass transfer limitation) until it reaches an asymptote value (reaction limitation). The maximum is determined by the biodegradability of the compound and/or, for aerobic process, the availability of oxygen to the microorganisms (Berger and Peters, 1999).

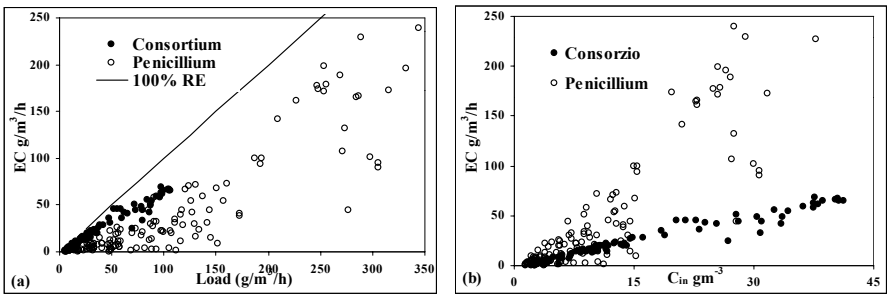

Figure 2. EC of the two tested biomasses as a function of inlet load (a) and concentration (b).

In our systems it seemed reaction-limitation regime was never reached (Figure 2b), anyway it must be noted that for inlet DCM concentration higher than 7g/(m^3h) it is reported oxygen transfer would become rate limiting (Okkerse *et al.,* 1999a).

Maximum EC was over 150 $gm^{-3}h^{-1}$ for the *Penicillium*, while it was about 50 $gm^{-3}h^{-1}$ for the consortium since inlet pollutant load was lower.

Even considering the removal efficiency (Figure 3a), consortium expressed better performances than *Penicillium*: RE was higher and more stable. The better removal

could also be due to the higher EBRT (empty bed residence time) of DCM in the bioreactor inoculated by the consortium: 23 min against 5 min for *Penicillium*. 23 minutes is a very high EBRT if compared with other biofilters normally used for VOCs treatment, where contact times even lower than 1 min are reached. However, many authors report the positive effect of quite long EBRT (up to some minutes) for treatment of chlorinated compounds (Diks and Ottengraf, 1991; Ergas *et al.,* 1995; Kim *et al.,* 1998; Speitel and McLay, 1993).

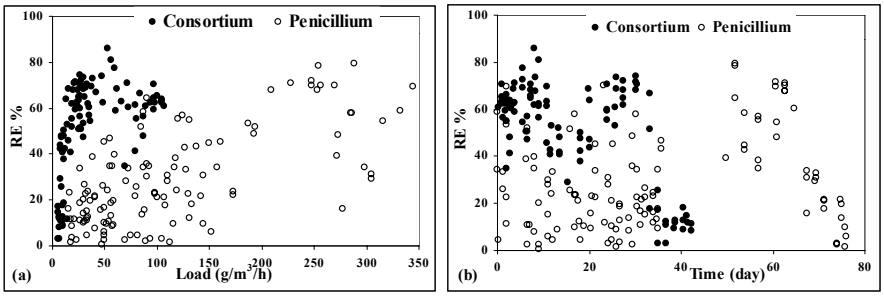

Figure 3. RE of DCM by the two tested biomasses as a function of inlet load (a) and time (b).

Even though from Figure 3a it seems the consortium was less efficient for very low concentrations, it must be noted that these data corresponded to the last period of operation, when RE drastically reduced (Figure 3b) after 42 days for the consortium and 75 days for *Penicillium*.

It must be underlined that these preliminary tests were carried out with inlet DCM concentrations higher than 1 g/m^3, the upper limit for typical VOCs containing effluents to be treated by biofiltration, even though other authors tested concentrations of chloroorganics similar as the present work and up to 50 ppm_v (Ergas *et al.,* 1995; Lee *et al.,* 2003). Moreover, pH of the medium was neither checked nor regulated, such as the composition of the nutrient supply as concerns the presence of co-metabolites and substrates acting as electron-acceptor. Then, the loss in degradation ability could be due to a toxic effect of the pollutant amount or to acidification of the medium. Lower inlet loads, and employment of a medium with buffering properties could easily reduce this problems as already described in literature. Anyway, the high concentrations tested allowed selecting biomasses able to survive in highly polluted environment for short periods and, then, to tolerate sudden and unwanted fluctuations in off-gas composition which can occur in real situations.

3.2 Microorganisms from sludge

The second isolation procedure started from a sludge collected at a waste water treatment plant. The employment of this kind of source for isolation of biomass, even though polluted with different classes of compounds besides that under analysis, is a common practice in many works related to bioremediation techniques, because sludges are characterized by the presence of a mixed population able to degrade a wide range of substances. The isolation process brought, after a 4 months acclimatisation periods, to an enriched culture of rod bacteria and yeasts. With aging, this culture tended to get darker and produce abundant extra-cellular gelatinous material, which could be an unfavourable aspect for employment in a biofilter plant giving clogging problems.

Some works (Brunner *et al*., 1980; Diks and Ottengraf, 1991; Okkerse *et al.,* 1999; Olaniran *et al.*, 2001) reported an optimal basic pH for the dehalogenase activity, that's

why some screening tests were performed to evaluate biomass growth at different pHs. Having observed that with a starting pH = 9, microbial development was higher, other trials were carried out in batch culture both at constant pH = 9 and variable pH. The results showed no apparent influence of pH on the growth that was, in both the cases, very law. Anyway, the influence of pH reduction (in our tests final pH values were around 6.0) on biomass survival and DCM effective degradation is to be further investigated. Batch cultures, in fact, present the problem of accumulation of either HCl or NaCl (in case of neutralisation) which provide both an inhibitory effect (NaCl should be kept under 150 mM). Anyway, experimental data were elaborated according to the Gompertz equation modified by Zwietering *et al*. (1990).

$$y = B\exp\left\{-\exp\left[\frac{\mu * e}{B}(\lambda - t)+1\right]\right\} \qquad \text{(Eq. 1)}$$

Microbial growth often shows a lag-phase (□. *time*) in which the specific growth rate begins at 0 and then increases up to a maximum value μ^* *(time*$^{-1}$*)*. In a final phase, growth decreases and becomes 0 reaching an asymptote value (*B*). When the growth curve is defined as the logarithm of number of microorganisms vs. time, a sigmoid curve is obtained that can be described by eq. (1). In the present case *y* is the biomass measured as optical density (values above 1 were obtained through linear correlation on the basis of readings of different dilutions of the culture). Data were interpolated by a non-linear regression procedure (SPSS v.11.5) (Figure 4 reports two examples), obtaining an average μ^* of 0.008 h^{-1} at both constant and variable pH.

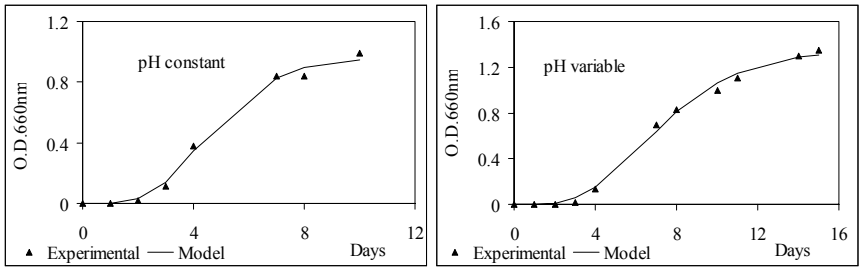

Figure 4. Interpretation of experimental growth data according to the Zwietering model (O.D. is optical density).

The calculated μ^* are lower than other reported in literature for aerobic degradation of DCM by pure cultures of *Pseudomonas* (0.11-0.22 h^{-1}) or *Hyphomicrobium* (0.11 h^{-1}) and not identified mixed culture (0.037 h^{-1}) (Herbst and Wiesmann, 1996). The low value and the apparent absence of a pH effect, could be due to the effect of NaCl (as already said) together with a marked darkening of the medium over the time which gave high O.D. reading so that O.D. could not be used anymore as a measure of cell growth.
These results confirm the difficulty of enrichment and adaptation of cultures for degradation of chloroorganic compounds. Many works, in fact, report of very long adaptation period, even up to one year (Duhamel *et al.*, 2002). This is due to the complex metabolism of halogenated compounds, which, in general, requires the presence of co-metabolites that could stimulate the production of enzymes which could fortuitously start the dehalogenation process, together with opportune electrons acceptors/donors.

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