Use of membrane bioreactors for the bioremediation of chlorinated compounds polluted groundwater

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Abstract Chlorinated compounds are widely used in agricultural applications where they are employed as components of pesticides; this leads often to pollution of groundwater near to agricultural sites, with serious effects for human health. The aim of the present study was the development of a membrane bioreactor, a new and effective water treatment technology, for the bioremediation of water polluted by 1,2-dichloroethane, 1,2-dichlorobenzene and 2-chlorophenol. Before starting-up the MBR system, a biomass was acclimated, to simultaneously degrade the three chlorinated compounds; then the acclimated biomass was inoculated into the MBR. The results showed a higher removal rate for 1,2-dichloroethane than for 1,2-dichlorobenzene; besides, the presence of 1,2-dichlorobenzene together with 1,2-dichloroethane decreased 1,2-dichloroethane specific removal rate. 2-chlorophenol was degraded only in presence of phenol as co-substrate, and the presence of phenol and 2-chlorophenol decreased 1,2-dichloroethane specific removal rate of approximately eight times, while 1,2-dichlorobenzene specific removal rate was not affected.

Keywords Chlorophenol; cometabolism; dichlorobenzene; dichloroethane; membrane bioreactor; phenol

Introduction

The widespread use of chlorinated compounds for pesticides production and use in agricultural applications, leads to water pollution and ecosystems alterations, due to the toxic and bioaccumulating nature of chlorinated compounds and to their poor biodegradability by non-acclimated bacterial populations. Contaminating agents can permeate the soil and pollute groundwater, or flow into surface waters, with serious effects for human health. 1,2-dichloroethane (1,2-DCA), 1,2-dichlorobenzene (1,2-DCB), used as production intermediates for agricultural chemicals, such as herbicides and insecticide for termites and borers, and 2-chlorophenol (2-CP), used as pesticides component, are often detectable in waters near to agricultural and industrial sites. For chlorinated compounds polluted waters remediation, biological treatments are coming into increasing consideration; 1,2-DCA, 1,2-DCB and 2-CP can be aerobically degraded (Janssen et al., 1985; Haigler et al., 1988; Freitas Dos Santos and Livingston, 1995; Herbst and Wiesmann, 1996; Armenante et al., 1999; Seignez et al., 2001; Farrel and Quilty, 2002; Hirschorn et al., 2004). Due to their xenobiotic nature, chlorinated compounds biodegradation requires long biomass acclimation time and high retention time in the bioreactor.

Membrane bioreactor (MBR) technology is an emerging wastewater treatment which couples an ultrafiltration membrane module to a suspended biomass biological reactor. It allows a decrease in the foot-print of the plant (since it substitutes the mixed liquor sedimentation) and improves the effluent quality (while achieving the very low maximum concentration limits). Besides, MBRs allow the decoupling of sludge and hydraulic retention times, thus creating appropriate conditions for the development of a xenobiotic acclimated biomass, since toxic by-products are washed out of the reactor, while biomass is retained inside it.

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In the present study, an MBR was operated in SBR modality (MSBR, membrane sequencing batch reactor); SBRs have been used for a few years, for their ascertained efficiency in treating streams with high contaminant concentrations and highly variable influent composition, due to the flexibility given by the possibility of alternating different metabolic and kinetic conditions in the reactor, with different phases length. The aim of the present study was to investigate the capability of a 1,2-DCA acclimated biomass to degrade 1,2-DCA, 1,2-DCB and 2-CP, together or in different combinations, in a MSBR.

Methods

Batch tests
An activated sludge obtained from the aerobic reactor of the municipal wastewater plant “Is Arenas in Cagliari” (Italy) was investigated for its capability of degrading the three compounds (singularly and simultaneously) in different metabolic conditions (i.e. aerobic and anaerobic conditions) by means of batch tests. Aerobic batch reactors consisted of glass bottles of 1L volume to allow sufficient headspace, while anaerobic batch tests were carried out in serum bottles of 120mL volume. Biological sludge, mineral medium and chlorinated compounds, with a total working volume of 100mL, were inoculated in the bottles which were sealed with grey butyl rubber PTFE faced caps, and aluminium seals, in order to avoid chlorinated compounds volatilisation.

Fermentor
Acclimation of biomass to the chlorinated compounds was carried out in a 3L fermentor (B. Braun Biostat MD), equipped with dissolved oxygen online recording; it was maintained at 30°C and at a pH value of 7, by automatic addition of 1N NaOH. The acclimation was carried out in SBR modality; the working volume was 2.1L, and at the beginning of any cycle 1,200mL of mineral medium (in 15 min) and 400mL of aqueous solutions of the different chlorinated compounds (in 5 min) were fed; after the reaction phase, sedimentation (45 min) and discharge phase (15 min) took place. An initial concentration of 1,2-DCA in the reactor was about 50mg/L; first, the reaction phase of any cycle lasted until the 1,2-DCA was completely degraded, so that any cycle lasted about 4 days. Then, after about 50 days, cycles became daily (organic load of 105mg 1,2-DCA/d); after about 120 days, two cycles per day were performed (organic load of 210mg 1,2-DCA/d). Also the acclimation of 1,2-DCA acclimated biomass to 1,2-DCB took place in the fermentor, where 1,2-DCA and 1,2-DCB were fed together; 1,2-DCA and 1,2-DCB concentrations at the beginning of any cycles in the first phase were about 50 and 5mg/L, respectively, while in a second phase, 1,2-DCB initial concentration was increased up to the value of 15mg/L.

Membrane bioreactor
Membrane bioreactor consisted of a 3L volume glass vessel in which a hollow fibers ultrafiltration polymeric membrane module (ZW 1, Zenon Inc.) was submerged. Its filtering surface was 0.047m² and the average and maximum pore sizes were 0.04 and 0.1μm, respectively. Before use, membrane module was conditioned by dipping it for 24h in a NaOCl solution (200mg/L) at 35°C. Transmembrane pressure was always monitored, either with a manometer or a pressure transducer, connected to a datalogger for pressure data automatic recording. The working volume of the MBR was 2.1L and it was operated in SBR modality with the same flow rates and volumes of mineral medium and chlorinated compounds solutions that were fed to the fermentor. Differing from the latter, at the end of the reaction phase, sedimentation and discharge phases were replaced by the filtration phase. It consisted of six filtration cycles, each of them constituted by
10 min of real filtration and 1 min backwashing. Moreover, in order to minimise membrane fouling, at the end of any cycle 30 min coarse bubble aeration took place by air blowing inside the membrane module.

**Medium**

In any reactors configuration, the same mineral medium was fed with the following composition (g/L): K₂HPO₄ 5.54, KH₂PO₄ 2.66, CaCl₂ 0.07, MgSO₄ 7H₂O 0.35, (NH₄)₂SO₄ 0.09, solution A 100 mL; the composition of the solution A was the following (in g/L): FeSO₄ 7H₂O 0.2, EDTA 0.5, solution B 100 mL; the composition of the solution B was the following (in g/L): ZnSO₄ 7H₂O 2, MnSO₄ 6H₂O 0.51, Na₂B₄O₇ 0.24, CoCl₂ 6H₂O 0.2, CuCl₂ 2H₂O 0.01, NiCl₂ 6H₂O 0.02, (NH₄)₆MoO₂₄ 4H₂O 0.153. Mineral medium was diluted 1.75 times when fed to the reactors. Since the chlorinated compounds used in this experimentation are very volatile (vapour pressure: 87 hPa for 1,2-DCA, 1.33 hPa for 1,2-DCB, 13.3 hPa for 2-CP), aerobic metabolism could not be carried on by direct air sparging in the reactor, which could cause compounds volatilisation; therefore, oxygen supply was performed by mineral medium aeration before its inoculation into the reactors.

**Analytical methods**

1,2-DCA and 1,2-DCB were analysed with a GC Agilent 6890N, equipped with a HP-5 column, 30 m long, with an inner diameter of 0.25 μm; carrier gas was helium (10 mL/min). The compounds detection was performed by FID and ECD detectors operating in parallel. The sample preparation was performed through the headspace technique: each sample was put in a vial, then sealed and heated for 10 min at 70 °C in a water bath; after that, 100 μL of the vial gas phase were taken with a gas-tight syringe and injected in the GC. The temperature ramp was the following: 35 °C for 2 min; 35 °C/min until 70 °C; 45 °C/min until 190 °C. 1,2-DCA and 1,2-DCB standards were injected at any analysis session. 2-CP and phenol were analysed with a HPLC-UV Dionex, equipped with a C8 Acclaim120 column and a D2 lamp for UVD 160/320, set to 245 nm. Mobile phase consisted of 66% acetonitrile and 34% aqueous solution of 0.4% H₃PO₄; the total flux was 1.5 mL/min. Chlorides and nitrates were analysed with a Dionex IC, with a IonPac AS14 column and a AG14 precolumn; the flux was set at 0.5 mL/min and the injection volume of the sample was 0.2 mL.

**Results and discussion**

**Acclimation to 1,2-DCA**

The activated sludge was checked in batch tests for its capability of aerobically degrade 1,2-DCA. After a short lag-phase (24 h), 1,2-DCA was degraded with a specific removal rate of 0.04 g 1,2-DCA/(d gVSS). In the whole acclimation phase, initial concentration of 1,2-DCA was around 50 mg/L. During the phase in which one cycle per day was performed, specific removal rate increased up to the value of 0.19 g 1,2-DCA/(d gVSS). During the phase in which two cycles per day were performed, a specific removal rate of 1.38 g 1,2-DCA/(d gVSS) was achieved.

VSS concentration was about 3 gVSS/L at the beginning of acclimation, and stabilised at a minimum of 200 mg VSS/L when one cycle per day was performed.

**Acclimation to 1,2-DCB**

The activated sludge was checked in batch tests for its capability of both aerobically and anaerobically degrading 1,2-DCB at an initial concentration of 5 mg/L. No degradation of 1,2-DCB was observed in 15 days, even with the addition of methanol as co-substrate,
neither in aerobic, nor in anaerobic conditions. 1,2-DCA acclimated biomass was then checked in a batch test for its capability of aerobically degrading 1,2-DCB; the test showed a 1,2-DCB specific oxidation rate of 0.004 g 1,2-DCB/(d gVSS) when 1,2-DCB was fed at 5 mg/L. The same specific removal rate was observed when 1,2-DCB was treated in the fermentor with an initial concentration of 5 mg/L.

**Acclimation to 2-CP**

The batch tests with the activated sludge showed no capability of both aerobically and anaerobically degrade 2-CP at 50 mg/L when it was the only organic substrate. Even the 1,2-DCA acclimated sludge did not show degradation of chlorophenol with only 2-CP as organic substrate. Three aerobic batch tests were performed in which 2-CP was fed together with 1,2-DCA as possible co-substrate, but while 1,2-DCA fed at 50 mg/L, was degraded with a specific removal rate of 0.175 g 1,2-DCA/(d gVSS), 2-CP at the same concentration was not degraded. A batch test was performed in which 2-CP and methanol were fed together as organic substrates, and another batch test was performed in which 2-CP, 1,2-DCA and methanol were fed together, but in both cases no degradation was observed in 5 days. Figure 1 shows the results of 2-CP degradation with phenol as co-substrate. While phenol was degraded without lag-phase, 2-CP was oxidised after a lag phase of 3 days, and when phenol decreased at a low concentration (less than 4 mg/L), 2-CP degradation was interrupted. After a new addition of phenol in the batch, degradation of 2-CP took place again, thus indicating that phenol was a necessary co-metabolite for 2-CP oxidation. The mean value of 2-CP degradation in presence of phenol was 0.17 g 2-CP/(d gVSS), when 2-CP initial concentration was around 70 mg/L. The same test was performed in anaerobic conditions, but while phenol was degraded, no 2-CP degradation was observed.

**Simultaneous degradation of different chlorinated compounds**

MBR was inoculated with the biomass coming from the fermentor, and both reactors were operated simultaneously; in each of them different chlorinated compounds were fed together. In parallel, batch tests were performed with the biomass taken from the fermentor and fed with different combinations of chlorinated compounds.

**Feeding of 1,2-DCA and 1,2-DCB in the fermentor and in the MBR**. 1,2-DCA and 1,2-DCB were fed together in the fermentor; in Figure 2, 1,2-DCA, 1,2-DCB, Cl\(^-\) and NO\(_3^+\) concentrations during one cycle are shown. The highest production of chlorides is observed within the first 2 days, when 1,2-DCA is oxidised; when only 1,2-DCB remains in the reactor, chlorides production rate decreases, thus showing that the main biological activity in the reactor is 1,2-DCA oxidation. Due to this low 1,2-DCB removal rate, cycles in which 1,2-DCA and 1,2-DCB were fed together lasted even 6 days, much longer than cycles with only 1,2-DCA fed.

**Figure 1** Degradation of 2-CP in presence of phenol as co-substrate
During the first 60 days of MBR operation, 1,2-DCA and 1,2-DCB were fed separately or together. For about 15 days, the MBR was fed with only 1,2-DCB at a concentration of about 15 mg/L; its specific removal rate stabilised at a value of 0.02 gDCB/(d gVSS), which was higher than the value observed when 1,2-DCB was fed at initial concentration of 5 mg/L. When only 1,2-DCA was fed in the MBR at 50 mg/L, its specific removal rate stabilised at a value of 0.16 gDCA/(d gVSS).

Figure 3 shows 1,2-DCA and 1,2-DCB concentrations during successive cycles in which they were fed together.

The contemporary feeding of 1,2-DCB decreased 1,2-DCA specific removal rate down to the value of 0.06 gDCA/(d gVSS), while 1,2-DCB removal was not affected by the presence of 1,2-DCA (specific removal rate of 0.02 gDCB/(d gVSS)); initial concentrations of 1,2-DCA and 1,2-DCB compounds were respectively around 50 and 15 mg/L.

**Batch tests with 1,2-DCA, 1,2-DCB, 2-CP and phenol.** Three batch tests with the biomass coming from the fermentor and fed with 1,2-DCA, 1,2-DCB, 2-CP and phenol in different combinations were performed; in Batch test A, 1,2-DCA, 2-CP and phenol were fed; in Batch test B, 1,2-DCB, 2-CP and phenol were fed; in Batch test C, 1,2-DCA, 1,2-DCB, 2-CP and phenol were fed all together. The initial concentrations of the compounds were the following: 50 mgDCA/L, 5 mgDCB/L, 50 mgCP/L and 50 mgphenol/L. The specific removal rates of the chlorinated compounds in the different combinations are shown in Table 1. The presence of 2-CP and phenol negatively affects 1,2-DCA specific removal rate, which decreases about eight times (mean value of 0.019 g 1,2-DCA/(d gVSS) when 1,2-DCA, phenol and 2-CP are fed, and 0.16 g 1,2-DCA/(d gVSS) when only 1,2-DCA is fed). Also 1,2-DCB seems to be negatively affected by the presence of 2-CP and phenol in batch tests (1,2-DCB average specific removal rate of 0.0005 g 1,2-DCB/(d gVSS) versus 0.004 g 1,2-DCB/(d gVSS) when 1,2-DCB was fed at 5 mg/L without 2-CP and phenol). Also 2-CP specific removal rate is lower than the one
Feeding of chlorinated compounds in different combinations in the fermentor. Two cycles were then performed in the fermentor: in cycle A, which lasted 4 days, 1,2-DCA, 2-CP and phenol were fed; in cycle B, which lasted 8 days, 1,2-DCB, 2-CP and phenol were fed. The initial concentration of the compounds were the following: $50 \text{ mg}_{1,2\text{-DCA}}/\text{L}$, $5 \text{ mg}_{1,2\text{-DCB}}/\text{L}$, $50 \text{ mg}_{2\text{-CP}}/\text{L}$ and $50 \text{ mg}_{\text{phenol}}/\text{L}$. The specific removal rates of the chlorinated compounds are shown in Table 2. Similar effects of 2-CP on 1,2-DCA are observed in the fermentor, but not on 1,2-DCB, whose specific removal rate is about the value observed when it was fed alone or together with 1,2-DCA ($0.004 \text{ g}_{1,2\text{-DCB}}/(\text{d g}_{\text{VSS}})$) at the same concentration.

Feeding of chlorinated compounds in different combinations in the MBR. In parallel with the fermentor, two identical cycles were carried out in the MBR: in cycle A, which lasted 4 days, 1,2-DCA, 2-CP and phenol were fed; in cycle B, which lasted 8 days, 1,2-DCB, 2-CP and phenol were fed. The initial concentration of the compounds were the following: $50 \text{ mg}_{1,2\text{-DCA}}/\text{L}$, $5 \text{ mg}_{1,2\text{-DCB}}/\text{L}$, $50 \text{ mg}_{2\text{-CP}}/\text{L}$ and $50 \text{ mg}_{\text{phenol}}/\text{L}$. The specific removal rates of the chlorinated compounds in the MBR are shown in Table 3; they are similar to those observed in the fermentor.

2-CP removal efficiencies
The 2-CP specific removal rates obtained in this experimentation are about twenty times higher than those observed in several past studies, where 2-CP was anaerobically degraded with rates of $0.00025 \text{ g}_{2\text{-CP}}/(\text{d g}_{\text{VSS}})$ (Basu et al., 1996) and of $0.0006 \text{ g}_{2\text{-CP}}/(\text{d g}_{\text{VSS}})$ (Bae et al., 2002). In terms of removal rates, the mean values obtained in the present study are of $8.1 \text{ mg}_{2\text{-CP}}/(\text{L d})$ and of $81.4 \text{ mg}_{\text{phenol}}/(\text{L d})$. These rates are much higher than those obtained in past studies where anaerobic degradation of 2-CP was performed: 0.5 mg 2-CP/(L d) was obtained by Becker et al. (1999), who used a biomass taken from the sediments of tidal muds, then incubated with 2-CP and mineral medium. A similar value (2.58 mg 2-CP/(L d)) was obtained by Cole et al. (1994), who used an inoculate coming from a small stream, enriched with 2-CP, fumarate, acetate and mineral medium. Lower values were achieved in aerobic conditions by Lu et al. (1996) (0.67 mg 2-CP/(L d) obtained with a bacterial population coming from a petroleum WWTP, fed with phenol, 2-CP, 3-CP, 4-CP, 2,3-DCP and 2,4,6-TCP), and by Farrel and Quilty (2002) (0.27 mg 2-CP/(L d), obtained with a pure and a mixed culture of $P.\ putida$). Only one experimentation showed higher removal rates than those observed in the present

| Table 1 | Specific removal rates of the chlorinated compounds in different combinations in batch tests |
|-----------------|-----------------|-----------------|-----------------|
|                 | 1,2-DCA (g/d g_{VSS}) | 1,2-DCB (g/d g_{VSS}) | 2-CP (g/d g_{VSS}) | Phenol (g/d g_{VSS}) |
| Batch test A    | 0.019            | –                | 0.0148           | 0.212                |
| Batch test B    | –                | 0.0003           | 0.0126           | 0.202                |
| Batch test C    | 0.012            | 0.0007           | 0.0277           | 0.195                |

| Table 2 | Specific removal rates of the chlorinated compounds in different combinations in the fermentor |
|-----------------|-----------------|-----------------|-----------------|
|                 | 1,2-DCA (g/d g_{VSS}) | 1,2-DCB (g/d g_{VSS}) | 2-CP (g/d g_{VSS}) | Phenol (g/d g_{VSS}) |
| Cycle A         | 0.0187          | –                | 0.0133           | 0.207                |
| Cycle B         | –                | 0.011            | 0.0182           | 0.206                |
study: Gallego et al. (2003) employed a biomass coming from a polluted river, then cultivated in a synthetic mineral medium of 2-CP, phenol and m-cresol; when 2-CP was fed alone, the rate of 88 mg 2-CP/(l d) was observed, while when 2-CP was fed together with phenol and m-cresol, the rates of 67 mg 2-CP/(l d) and 33 mg phenol/(l d) were observed; however no indication is given on the biomass concentration.

Further considerations
At the beginning and at the end of any cycle, samples for chlorides analysis were taken, in order to verify the complete mineralisation of chlorinated compounds. The moles of degraded chlorinated compounds were always about the half of those of produced chlorides, thus indicating that complete mineralisation of chlorinated compounds took place. An ammonia nitrifying activity was also observed, with a production of 0.4 mmol of nitrates per cycle; this shows that the chlorinated compounds under study in this experimentation do not affect the ammonia-oxidiser bacteria, which are usually sensitive to xenobiotic compounds, thus suggesting the possibility of coupling a nitrification activity with the oxidation of these chlorinated compounds.

Conclusions
The experimentation showed a higher removal rate for 1,2-DCA than for 1,2-DCB; moreover, feeding 1,2-DCB together with 1,2-DCA decreased 1,2-DCA specific removal rate of about twice.

1,2-DCA and 1,2-DCB acclimated biomass showed the ability of degrading 2-CP only in aerobic conditions and only in the presence of phenol as co-substrate at a concentration of more than 4 mg/L.

The presence of 2-CP and phenol negatively affected 1,2-DCA specific removal rate, which decreased by about eight times.

While 1,2-DCB seems to be negatively affected by the presence of 2-CP and phenol in batch tests, this trend has not been observed in the fermentor and in the MBR.

The 2-CP specific removal rates obtained in this experimentation are about twenty times higher than those observed in several past studies.

The biological treatment of the chlorinated compounds studied in this experimentation is suitable to the activity of nitrifying bacteria, usually sensitive to xenobiotic compounds.

If compared, chlorinated compounds specific removal rates observed in the MBR and in the fermentor are similar; the advantages of the MBR system rely in achieving a much better supernatant in terms of clearness and absence of solids and in shorter treatment times than those necessary in a traditional SBR system, where a perfect separation between solids and supernatant is strongly linked to a long settling phase.

In the future, daily cycles will be performed for 2-CP and phenol, in order to achieve higher specific removal rates. The possibility of degrading 2-CP without phenol as co-substrate will be checked in batch tests, by feeding decreasing amounts of phenol with the same amounts of 2-CP; this is in order to reduce the management costs of the system and to avoid the employment of a pollutant agent itself as phenol is.
References


