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Removal of dichloromethane from waste gases by a biotrickling filter

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Abstract: Dichloromethane is harmful to human health and hazardous to atmospheric environment. In this study, two strains were isolated which were identified as *Pseudomonas* sp. and *Mycobacterium* sp., and utilized dichloromethane (DCM) as sole carbon and energy sources. The optimal culture conditions were temperature of 28℃ and pH of 6.5 for obtaining the two mixed bacterial strains. The investigation on the purification of DCM-contaminated gas was carried out in a bench-scale biotrickling filter which was inoculated with the two strains and operated under these optimal conditions. The DCM removal efficiencies varied between 72% and 99% in the biotrickling filter when empty-bed residence time was 9.6 s with the inlet concentrations ranged from 0.7 to 3.12 g/m^3 under the conditions of pH of 6.5±0.5 and temperature of 28℃. It was also found that NaCl accumulation in the broth would inhibit the DCM biodegradation dramatically when the accumulated NaCl concentration was over 35.1 g/L.
Keywords: dichloromethane; biodegradation; biotrickling filter; air pollution control

Introduction

Dichloromethane (DCM) has been widely used as an intermediate and solvent in chemical industries and many other analytical fields. Due to its low boiling point (40.1℃) and high vapour pressure (47 kPa at 20℃), large amounts of dichloromethane enter the environment via gaseous emissions (Adachi and Komiyama, 2000, 2001; Adachi and Hamamoto, 2005). DCM is harmful to human health and hazardous to atmospheric environment. For instance, it is known that DCM is toxic to the central nerves of human beings. Its fifteen-minute EC_{50} value was as low as 0.25 mg/ml which strongly correlated with hydrocarbon concentrations. It is essential to develop effective technologies to purify DCM-contaminated gas streams. Previous study has shown that DCM could be biodegraded and mineralized by activated sludge (Kirchner and Schlachter, 1989). Methanol and hydrochloricacid are the main products of DCM biodegradation that mediated by glutathione-DCM dehalogenase (Staub and Leisinger, 1985; Gälli and Leisinger, 1985; Okkerse *et al.*, 1999; Diks and Ottengraf, 1991). Methanol could be further oxidized into formic acid and finally transformed into CO_2 and cellular substances. Several studies on DCM removal in biotrickling filters have been reported (Oh and Bartha, 1994; Ergas *et al.*, 1994, 1996). The study result of Hartmans and Tramper (1991) showed that the removal efficiency of DCM reached 57.0% and 85.0% when empty-bed residence time (EBRT) was 9.6 and 20.8 s, respectively. Oh and Bartha (1994) found that high liquid-phase circulating flow-rate and accurate automatic control of pH were the key factors to keep stable operation of a biotrickling filter.

The objectives of this study were to isolate DCM-degrading bacteria, to optimize bacterial culture conditions and to evaluate the DCM removal performance of a biotrickling filter inoculated with the bacteria.

1 Materials and methods

1.1 Medium

A special inorganic salt medium was selected for the culture, separation and purification of DCM-degrading bacteria (Hartmans and Tramper, 1991; Oh and Bartha, 1994). The medium was prepared by diluting the mixture of 10 ml liquid A and 25 ml liquid B to 1000 ml solution with deionized water. Liquids A and B were prepared with salts and de-ionized water, whose compositions were liquid A: $(NH_4)_2SO_4$ (200 g/L), EDTA (1.0 g/L), $CaCl_2 \cdot H_2O$ (0.1 g/L), $ZnSO_4 \cdot 7H_2O$ (0.2 g/L), $FeSO_4 \cdot 7H_2O$ (0.5 g/L), $Na_2MnO_4 \cdot 2H_2O$ (0.02 g/L), $CuSO_4 \cdot 5H_2O$ (0.02 g/L), $CoCl_2 \cdot 6H_2O$ (0.04 g/L), $MnCl_2 \cdot 2H_2O$ (0.1 g/L), $MgCl_2 \cdot 2H_2O$ (10 g/L); liquid B: K_2HPO_4 (155 g/L), $NaH_2PO_4 \cdot 2H_2O$ (85 g/L).

1.2 Biotrickling filter

The bench-scale biotrickling filter consisted of a vertical plexiglas column with an inner diameter of 50 mm and a total height of 750 mm. Polypropylene rings with a specific surface area of 118 m^2/m^3 were packed in the column to a height of 400 mm as a biotrickling filter. The simulated DCM-contaminated gas was generated by pumping air through a buffer tank and a portion of the flow was bubbled through a reservoir containing DCM (analytical grade) in liquid phase. The air streams were controlled as shown in Fig.1 to achieve the desired inlet gas concentration. The gas was then fed to the bottom of the filter.

1.3 Culture, purification and identification of bacteria

Two activated sludge samples were taken from aerated wastewater treatment ponds located in Huadong Pharmaceutical Factory (Y) and Hangzhou Pesticides Factory (N) respectively. The suspension of

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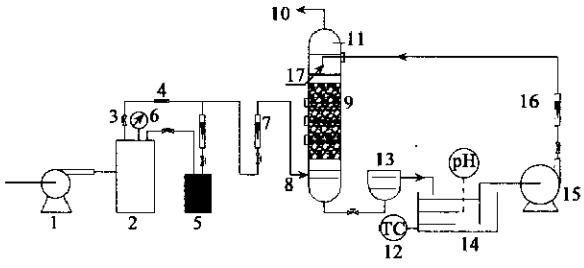

Fig.1 Schematic of biotrickling filter
1. pump; 2. buffer storage; 3. valve; 4. non-return valve; 5. waste gas generator; 6. pressure meter; 7. gas rotameter; 8. inlet port; 9. biotrickling filter; 10. outlet port; 11. sample port; 12. temperature controller; 13. liquid capper; 14. tank; 15. pump; 16. liquid rotameter; 17. liquid distributor

the samples was filtered by sieve and then its pH value was adjusted. A 10% (v/v) pretreated suspension was inoculated into each 250 ml flask. The flasks were covered by 4 layers of gauze, and cultured on the shaking table at 200 r/min and 28℃. A small amount of DCM was added into the medium three times every day and the medium was refreshed once a week. The H^+ accumulation in the broth due to DCM degradation resulted in the decrease of pH, which could indirectly determine the DCM degradation. The final broth was spread onto an agar plate without additional carbon source and then the plate was placed upside down with a large piece of aseptic cotton on the bottom cover. About 200 μl of DCM was dropped onto the cotton every 3 h. The colonies on the plate were spread again to obtain an apparently single colony. The culture was transferred onto the slant of medium in a big test tube (Φ20 mm×200 mm) with one stack of cotton containing DCM on the bottom side of the horizontally placed tube when the slant was placed upside down for strain incubation. The morphology of the isolated strain was observed under a microscope.

1.4 Optimization of bacterial culture conditions

The synergistic action of many microorganisms in the same environment can often be utilized to form a degradation reaction system (Burgess *et al.*, 2001). In this study, two bacterial strains isolated from sludge were mixed in equal proportions to form a biological system, in which each strain was expected to grow well and cooperate to improve the DCM degradation. The pH and temperature were designed as two factors in an orthogonal test to obtain the optimal culture conditions of mixed bacteria.

1.5 Analytical methods

The DCM concentration in the liquid phase was determined by a gas chromatography (GC-14C, Shimadzu, Japan) equipped with a flame ionization detector and a capillary column (AU-1, 10 m long, 0.25 mm ID, 0.25 μm film thickness). The conditions were as follows: carrier gas, hydrogen; column temperature, 180℃ ; detector temperature, 230℃ ; carrier gas flow rate, 30 ml/min. The Cl^- concentration in the circulating liquid was determined by a chloride ion selective electrode and a double-liquid junction reference electrode. An automatic pH controller (Shanghai Fine Instrument Co. Ltd., China) in the effluent liquid stream was used to control the pH in the filter, by adding a buffer solution into the circulating liquid. The buffer solution contained 4 g/L NaOH and 1 g/L K_2HPO_4.

2 Results and discussion

2.1 DCM-degrading strains

After the active sludge samples Y and N were cultured in flasks for 25 d, the pH of the medium decreased from 6.8—7.9 to 3.4—4.0, and numerous rod-shape bacteria could be observed under a microscope, while there were almost no changes in the pH of the control medium. Single colonies were observed on the surface of the agar plate medium after the broth was spread onto a plate and then the plate was incubated for 2 d. The observation suggested that the bacteria isolated from sludge samples could use DCM as sole carbon and energy sources for growth. Based on general identification methods for common strains and Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974), the bacteria were tentatively identified as *Pseudomonas* sp. and *Mycobacterium* sp.

2.2 Optimization of bacterial culture conditions

A series test of the culture condition was carried out in flasks. The results indicated that temperature and pH had significant influences on the culture of mixed strains. It was found that the proper culture temperature ranged from 28 to 32℃ and the proper pH of medium ranged from 5.0 to 7.5. Therefore, the temperature and pH were selected as the main factors for designing optimization test. The cell concentration was determined by the $OD_{600\,nm}$ of broth. Table 1 shows

Table 1 Optimization test results for the mixed strains

Sample	pH	Temperature,℃	$OD_{600\,nm}$	$C_{bacteria}$, $\times 10^8$
1	5	28	1.08	5.834
2	5	30	0.65	1.390
3	5	32	0.48	0.950
4	6.5	28	1.10	7.101
5	6.5	30	0.91	3.908
6	6.5	32	0.58	2.703
7	7.5	28	1.08	6.512
8	7.5	30	0.45	1.104
9	7.5	32	0.70	5.110
$K_1=K_1/3$	2.725	6.482		
$K_2=K_2/3$	4.571	2.134		
$K_3=K_3/3$	4.242	2.921		
R_j	1.846	4.348		

that temperature was the major factor to the bacteria growth. So the optimum conditions for mixed strains were 28℃ and pH 6.5.

2.3 Stability of biotrickling filter

The bacteria suspension was inoculated onto the surface of packing materials after batch culture of mixed strains in inorganic salt medium with 0.2% (v/v) DCM. To ensure the continuous growth of bacteria on the surface of packing materials, liquid A, NaOH and K_2HPO_4 were added once a week. Under the conditions of pH of 6.5 ± 0.5 and temperature of 28℃, DCM removal efficiency became relatively stable after 28 d, as shown in Fig.2, and the DCM removal efficiency fluctuated between 72% and 99% in the biotrickling filter when EBRT was 9.6 s and DCM concentration 0.7—3.12 g/m³.

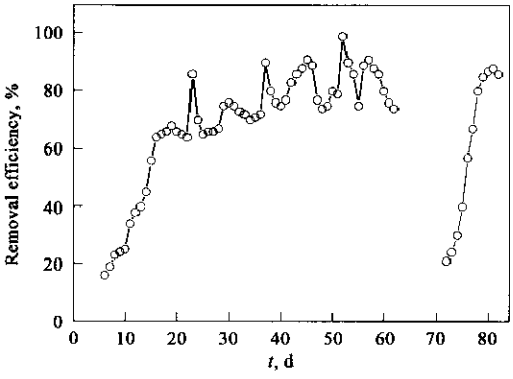

Fig.2 Variations of DCM removal efficiency during the operation period at EBRT of 9.6 s and inlet concentration of 0.7—3.12 g/m³

After 2 months' operation, the biotrickling filter system was halted for 10 d. After that the system was restarted. It was found that the filter could quickly reach the original removal efficiency within one week when DCM was supplied, and that if no NaOH was added during the operating period, the pH of the circulating liquid would decrease to 3.5, and the DCM removal efficiency greatly decreased. The DCM elimination capacity recovered again in a few days, once the pH was adjusted to 6.5 ± 0.5. The obtained high performance and stability of the biotrickling filter suggested its favorable application in purification of DCM-contaminated gas.

2.4 Relationship between elimination capacity and inlet load

Fig.3 shows the relationship between elimination capacity and inlet load at various EBRTs under the conditions of pH of 6.5 ± 0.5 and temperature of 28℃.

The elimination capacity gradually increased with inlet load increasing. When the inlet DCM load was at a low level, almost 100% removal of DCM was achieved. However, with further increasing of inlet loads, the elimination capacity increased slowly at various EBRTs but the removal efficiency decreased. Although there was enough DCM dissolved in the

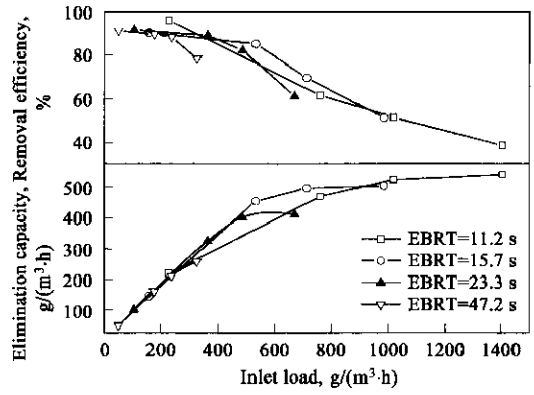

Fig.3 Relationship between elimination capacity and inlet load at various EBRTs

biofilm at a high inlet load, the bacteria could not degrade all the DCM. The elimination capacity of 455 g/(m³·h) (removal efficiency more than 85%) could be obtained at an inlet load of 534.3 g/(m³·h) (inlet concentration around 2.33 g/m³) with an EBRT as low as 15.7 s. Under that condition, both removal efficiency and elimination capacity reached their optimal levels. Although high removal efficiency (more than 98%) was achieved at a longer EBRT, the elimination capacity decreased quickly. Similar biodegradation behavior was reported using a compost-based biofilter by Ergas *et al.* (1994, 1996).

2.5 Inhibition of NaCl concentration to DCM degradation

During the DCM removal process, NaCl was found to accumulate in the medium. To determine whether NaCl could cause any possible effect on DCM degradation, a test was conducted at five NaCl concentrations: 0, 11.7, 23.4, 35.1 and 46.8 g/L.

Fig.4 shows that DCM degraded by the mixed strains would be inhibited when NaCl concentration was more than 35.1 g/L and almost no DCM degradation could be detected when NaCl concentration reached 46.8 g/L. Therefore, a proper NaCl concentration should be controlled for DCM biodegradation. When DCM was biodegraded by the mixed bacteria, H^+ and Cl^- were the by-products.

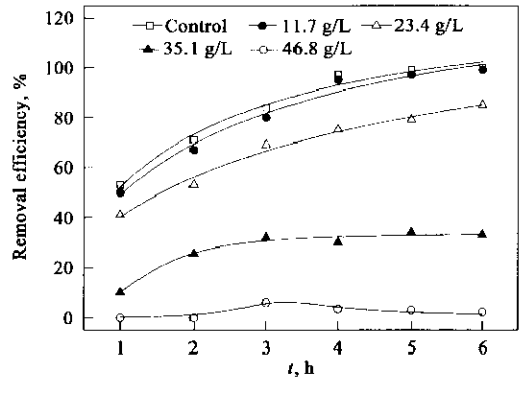

Fig.4 Inhibition of NaCl to DCM degradation

During the operation, NaOH was used to neutralize H^+, thus NaCl would be accumulated. The excessive NaCl would lead to the rise of bacterial permeate pressure, which would cause the poisoning of protein enzyme. At the same time, Cl^- itself was known to be toxic to the cell of bacteria. So NaCl could inhibit DCM degradation.

3 Conclusions

Two strains, identified as *Pseudomonas* sp. and *Mycobacterium* sp., could degrade DCM and utilize DCM as sole carbon and energy sources. The mixed culture of the two strains had relatively high removal efficiencies in flasks and the biotrickling filter under conditions of temperature of 28℃ and pH of 6.5. The DCM removal in the biotrickling filter was stabilized in 28 d after startup. An elimination capacity of 455 g/(m^3·h) and a removal efficiency of about 85% were achieved under conditions of an inlet load equal to 534.3 g/(m^3·h), inlet concentration around 2.33 g/m^3, and an EBRT as low as 15.7 s. Under these conditions, both high removal efficiency and elimination capacity could be obtained. The accumulation of NaCl in the circulating liquid could inhibit the growth of the mixed strains and had a negative effect on the DCM removal efficiency. Results indicated that NaCl concentration should be maintained below about 35.1 g/L.

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