



# 厭氧廢水處理氯化脂肪族化合物臨界負載率之估計

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## 摘要

在生物轉化工業廢水之優先管制污染物過程中，厭氧反應器具備有相當的應用潛力。經過適當的馴化後，氯化脂肪族化合物可與乙酸或丙酸以顯著的速率同時被分解轉化。依據宿命模式推估顯示，在乙酸或丙酸的反應器中，生物轉化在移除氯化脂肪族化合物上為主要機制，佔了移除率的67%至99%；而揮發則為次要機制，可貢獻0.2%至33%的移除率。特定氯化脂肪族化合物之臨界負載率，定義為主要基質利用率因受氯化脂肪族化合物影響而降為原值50%時之負載率。代謝丙酸時，氯化脂肪族化合物之臨界負載率介於0.4與24 mg/g cell-day；而代謝乙酸時，此臨界負載率介於0.1與21mg/g cell-day。而氯化脂肪族烯烴類比氯化鏈烷烴類可以較快的速率被厭氧微生物轉化。若不超過此臨界負載率，厭氧處理程序可以穩定地同時將主要基質伴同氯化脂肪族化合物一起生物轉化。

**關鍵詞：**氯化脂肪族化合物、甲烷菌、揮發有機性化合物、可處理性

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# Estimation of Chlorinated Aliphatics Critical Loading Rates for Anaerobic Wastewater Treatments

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## ABSTRACT

Anaerobic reactors have considerable potential of biotransforming priority pollutants from industrial wastewaters. With proper acclimation, selected chlorinated aliphatics were transformed simultaneously with acetic acid or propionic acid at significant rates. Based on fate model estimation. Biotransformation, accounted for from 67% to 99% of the total removal, was the major mechanism of chlorinated aliphatics removal; while volatilization, ranged from 0.2% to 33% of removal, was the secondary one for reactors supplied with either HPr or HAc. The critical loading rate was defined as the loading rate of a specific chlorinated aliphatic which resulted in reduction of the primary substrate utilization to 50%. The chlorinated aliphatics critical loading rates for the microbes metabolizing HPr were from 0.4 to 24 mg/g cell-day, while those rates for the microbes metabolizing HAc ranged from 0.1 to 21 mg/g cell-day. On the other hand, anaerobic microorganisms biotransformed chlorinated aliphatic alkenes at a higher rate than those of alkanes. Under the critical loading, an anaerobic wastewater treatment process could stably and simultaneously biotransform primary substrate along with chlorinated aliphatics.

**Keywords:** Chlorinated aliphatics, Methanogen, VOCs, Treatability.

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## 1. INTRODUCTION

Halogenated aliphatic compounds are used in industrial processes and are prevalent groundwater contaminants and significant components of hazardous wastes and landfill leachates (Chaudhry and Chapalamadugu, 1991; Zhang and Bennett, 2005). They are also the high-risk chemicals found in drinking water in the United States (Crouch et al., 1983). Many halogenated compounds are highly toxic, and because they are often recalcitrant or insoluble, they escape degradation. However, microbes exposed to these synthetic chemicals have developed the ability to utilize some of the halogenated compounds (Chaudhry and Chapalamadugu, 1991). Field and Sierra-Alvarez (2004) also reported chlorinated compounds are also degraded under anaerobic conditions in which they are utilized as an electron donor and carbon source. Cometabolism occurs when a compound, a co-metabolite, is not metabolized as a source of carbon or energy but is incidentally transformed by organisms using another primary substrate (Kobayashi and Rittmann, 1982; Liu, 1986). Acclimation plays a key role in such biodegradation of inhibitory compounds.

The objective of this study was to evaluate the treatability of an anaerobic treatment process, such as used in industrial wastewater treatment, to biotransform seven chlorinated aliphatics, while simultaneously converting the primary substrate to methane. Acetic acid (HAc) and propionic acid (HPr) were used as the primary substrates because they represented key intermediates in anaerobic digestion of organic pollutants. The critical loading rate of chlorinated aliphatic which reduced the utilization rate of the primary substrate to 50% of a control was also evaluated.

## 2. MATERIALS AND METHODS

Methylene chloride (MC;  $\text{CH}_2\text{Cl}_2$ ), chloroform (CF;  $\text{CHCl}_3$ ), carbon tetrachloride (CT;  $\text{CCl}_4$ ), 1,1-dichloroethylene (1,1-DCE;  $\text{CCl}_2\text{CH}_2$ ), trichloroethylene (TCE;  $\text{CCl}_2\text{CHCl}$ ), tetrachloroethylene (PCE;  $\text{CCl}_2\text{CCl}_2$ ), and 1,1,1-trichloroethane (1,1,1-TCA;  $\text{CCl}_3\text{CH}_3$ ), as common industrial solvents, were assayed.

A continuous flow stirred-tank reactor (CSTR) with high concentrations of suspended-growth biomass was used. Fourteen reactors were used for testing the 7 chlorinated aliphatic compounds, with each of the 2 primary substrates, HPr or HAc. A 2 L, wide-mouth Pyrex glass bottle was used as the reactor for each compound. The chlorinated organics were

delivered through Teflon tubing. A pH electrode entered the reactor through the top mouth of the bottle. To evaluate the removal of chlorinated compounds by volatilization, gas productions and contents were daily recorded and analyzed. The selected substrate was added into each reactor through a glass tubing. All materials that contacted with chlorinated compounds were glass, Teflon, or stainless steel to eliminate the possibility of adsorption. All reactors were incubated at a temperature of  $35 \pm 2$  °C. To eliminate the effects of photodegradation (Glaze et al., 1993), the incubation room was always kept dark, except when sampling and recording. A syringe pump driven by a stepping motor was used to add chlorinated compounds into the reactor.

A computer controlled pH-Stat system was used to carry out this study. A computer read the pH signal once per 45 seconds, then compared it with a default value to set the on/off of the primary substrate injection pump. The pH probes were calibrated by an off-line pH meter daily. In this way, the pH value in the reactor was automatically kept at  $6.8 \pm 0.1$ . Since the primary substrate (HPr or HAc) was acids, maintenance of a nearly constant pH, coupled with a controlled alkalinity, resulted in maintenance of nearly constant primary substrate concentrations. In this system, with an alkalinity of  $1,200 \pm 200$  mg  $\text{CaCO}_3/\text{L}$ , the HAc in reactors were from 1,500 to 2,500 mg/L, and the HPrs in reactors ranged between 500 and 1,500 mg/L. For single reactor, the HPr or HAc concentration variation was less than 100 mg/L.

Based on half-velocity constant ( $K_s$ )= 40 mg/L (Costello et al., 1991), the HPr concentrations of 500~1,500 mg/L resulted in 93% to 97% of the maximum substrate utilization rate during operation. While for  $K_s = 400$  mg/L (Takashima and Speece, 1989), the HAc concentrations of 1,500~2,500 mg/L resulted in 79% to 86% of the maximum substrate utilization rate during operation. Hence, a primary substrate-unlimited and pH-Stat environment were automatically maintained by computerized control. Therefore, these variations in substrate utilization were interpreted as being principally related to inhibition by the injected specific chlorinated aliphatic and not to primary substrate variation.

The solid retention time (SRT) of 20 days was controlled by wasting 1/20 of the mixed contents daily and replacement with an equal volume inorganic basal media shown in Table 1. The pH of basal media was about 8 with 6,000 mg/l  $\text{NaHCO}_3$  as a buffer. To avoid sudden drops in pH during the feeding of primary substrates, the concentrated organic acids were diluted to 10% and 40% concentration for HPr and HAc, respectively. A trace metal solution was added daily into each reactor ( 5 mg Fe/L, 1 mg Ni/L, and 1 mg Co/L of reactor-day ) to promote predomination of a high-rate *Methanosarcina* enrichment vs. the low rate

*Methanothrix (Methanosaeta)*. This procedure was described in detail by Takashima and Speece (1989).

### *Fate Model*

Biotransformation, biomass adsorption, abiotic transformation, and volatilization are the major mechanisms for the removal of chlorinated aliphatics (inhibitors) during wastewater treatment. A model was developed to clarify the contributions of these mechanisms in the system.

Table 1. Composition of basal inorganic nutrients used in the reactor

Constituent	Concentration in Reactor (mg/L)
NH <sub>4</sub> Cl	1,200
MgSO <sub>4</sub> . 7H <sub>2</sub> O	400
KCl	400
Na <sub>2</sub> S . 9H <sub>2</sub> O	300
CaCl <sub>2</sub> . 2H <sub>2</sub> O	50
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	80
FeCl <sub>2</sub> . 4H <sub>2</sub> O	40
CoCl <sub>2</sub> . 6H <sub>2</sub> O	10
KI	10
(NaPO <sub>3</sub> ) <sub>6</sub>	10
MnCl <sub>2</sub> . 4H <sub>2</sub> O	0.5
NH <sub>4</sub> VO <sub>3</sub>	0.5
CuCl <sub>2</sub> . 2H <sub>2</sub> O	0.5
ZnCl <sub>2</sub>	0.5
AlCl <sub>3</sub> . 6H <sub>2</sub> O	0.5
NaMoO <sub>4</sub> . 2H <sub>2</sub> O	0.5
H <sub>3</sub> BO <sub>3</sub>	0.5
NiCl <sub>2</sub> . 6H <sub>2</sub> O	0.5
NaWO <sub>4</sub> . 2H <sub>2</sub> O	0.5
Na <sub>2</sub> SeO <sub>3</sub>	0.5
Cysteine	10
NaHCO <sub>3</sub>	6,000

**Biotransformation.** Biotransformation of a chlorinated aliphatic can be expressed by Monod kinetics. Corapcioglu and Hossain (1991) concluded that a first-order rate expression could be satisfactorily quantified for biotransformation processes of chlorinated aliphatic

hydrocarbons under methanogenic conditions. When individual compounds were biodegraded through a secondary utilization mechanism, the concentration of active microorganisms could be approximated as the total active biomass in the reactor (Namkung and Rittmann, 1987). Therefore, the Monod equation can be re-arranged as:

$$R_{\text{bio}} = -k_1 X_a S_I V \quad (1)$$

Where

$R_{\text{bio}}$  = Rate of chlorinated compound removed by biotransformation, mg/day.

$k_1$  = Pseudo first-order biological reaction constant, L/mg cell-day.

$X_a$  = Concentration of active microorganisms, mg/L.

$S_I$  = Concentration of chlorinated compound in the reactor, mg/L.

$V$  = Volume of reactor, L.

**Volatilization.** In continuous stirred-tank reactors (CSTRs), the volatile compounds can be stripped out by the off-gas. For a quasi-equilibrium process, the transfer of a volatile compound between a liquid phase and a gas phase could be modeled as follows (Harrington et al., 1993; Namkung and Rittmann, 1987; Rittmann et al., 1988).

$$R_{\text{vol}} = -\frac{GH_M S_I}{RT} = -GH_c S_I \quad (2)$$

where

$R_{\text{vol}}$  = Rate of compound removed by volatilization, mg/day.

$G$  = Gas volumetric flow rate, L/day.

$R$  = Universal gas constant ( $= 8.206 \times 10^{-2}$  L-atm/ $^{\circ}$ K-mole).

$T$  = Absolute temperature,  $^{\circ}$ K.

$H_M$  = Henry's Law constant, atm-m<sup>3</sup>/mole.

$H_c$  = Henry's Law constant, dimensionless.

However, the assumption that off gas was saturated with the chlorinated aliphatics as it left the liquid phase was doubtful. Therefore, a factor of the fractional saturation of chlorinated aliphatic in the off gas was added as Equation 3 (Matter-Muller et al., 1981; Parker et al., 1993).

$$f = 1 - \exp\left(-\frac{\alpha K_{La} V}{H_c G}\right) \quad (3)$$

Where

$f$  = Fractional saturation, dimensionless.

$\alpha$  = Process water to clean water correction factor, dimensionless.

$K_{La}$  = Overall mass-transfer coefficient, day<sup>-1</sup>.

The factor for process water ranges from 0.4 to 0.8 (Metcalf and Eddy, 1991), therefore, the middle value of 0.6 was used. The values of overall mass-transfer coefficient ( $K_{La}$ ) was found to be 17.28 day<sup>-1</sup> in the laboratory. For compounds with higher Henry's Law constant, such as PCE, CT, 1,1,1-TCA, the  $f$ -values were low at high gas production. For MC and CF, with lower Henry's Law constant, the  $f$ -values were greater than 0.7 when gas productions were less than 70 L/day.

**Biomass Adsorption.** Hydrophobic organic compounds were known to adsorb to organic solids, of which biological solids were a prime example. Therefore, the adsorbed chlorinated aliphatics were removed from the system when biomass was removed with the effluent (Namkung and Rittmann, 1987). The partitioning of each hydrophobic chlorinated compounds between the water phase and biomass was estimated by Equations 4 and 5 (Harrington et al., 1993; McCarty et al., 1980).

$$K_p = 3.06 \times 10^{-6} K_{ow}^{0.67} \quad (4)$$

$$R_{abs} = -Q X K_p S_I \quad (5)$$

Where

$K_p$  = Partition coefficient, L/mg cell.

$K_{ow}$  = Octanol/water partition coefficient, m<sup>3</sup> H<sub>2</sub>O/m<sup>3</sup> octanol

$R_{ads}$  = Rate of chlorinated compound removed by adsorption onto biomass, mg/day.

$Q$  = Continuous flow rate, L/day.

$X$  = Concentration of wasted cells, mg cell/L.

**Abiotic Transformation.** Bouwer and McCarty (1983) reported that the transformation of the chlorinated aliphatics was the result of biological action, whereas a combination of biological and chemical processes appeared responsible for the transformations of bromoaliphatic compounds under reducing conditions. For surface waters, photolysis often controls the fate of chlorinated compounds. But in anaerobic wastewater treatment, the

digesters were always closed systems and kept dark. Therefore, photolysis should not contribute to the breakdown of chlorinated aliphatics. At constant pH, the reactions of abiotic hydrolysis or dehydrohalogenation follow first-order kinetics (Vogel, 1988). Therefore, the pseudo first-order rate constant ( $K_{abi}$ ) could be derived from the half-life ( $t_{1/2}$ ) as expressed by Equation 6.

$$K_{abi} = 0.69/t_{1/2} \quad (6)$$

**Model Developed.** For a CSTR system, a mass balance equation (Equation 7) was derived by combining the mechanisms discussed above:

$$\frac{dI}{dt} V = Q I_i - Q I + R_{bio} + R_{vol} + R_{ads} + R_{abi} \quad (7)$$

Where

$I_i$  = Chlorinated compound concentration in influent, mg/L.

$I$  = Chlorinated compound concentration in reactor, mg/L.

Due to dynamic change in the system, the finite difference method was used for analysis. The data at time  $t+\Delta t$  were derived from data at time  $t$  based on the change during the time interval  $\Delta t$ . Based on analytical data, the  $\Delta t$  of 1 day was used in this model. Table 2 listed the values of the parameters used in the model to estimate the contributions.

#### *Analytical Method and Procedures*

A gas chromatography (Shimadzu GC-6AM) equipped with FID was found suitable for analyzing higher concentrations of chlorinated compounds. Nitrogen gas (40 mL/min) served as the carrier of the injected samples. An 1.7 m glass column, packed with 0.3% Carbowax 20M/0.1%  $H_3PO_4$ , 60/80 Carbopack-C (Supelco, Inc., Bellefonte, PA) was used. After receiving a 10.0 mL sample from the liquid phase of the reactor, the 15 mL vial was capped with a Teflon septum, then was shaken for 3 minutes and followed by standing still for 1 minute to reach gas-liquid equilibrium. Next, a 50.0  $\mu$ L aliquot from the head space was injected into the gas chromatography column for analysis. The practical quantification range was found to be from 0.25 to 50 mg/L, when temperatures of detector and oven were 200 °C and 120 °C, respectively. Acetic acid and propionic acid were also measured by the Shimadzu GC-6AM. The sample from reactor was prepared by centrifugation at 4,000 rpm for 10



minutes. A supernatant of 1.0 mL was acidified (pH<3) by 10% formic acid, then a 1.0  $\mu$ L aliquot from the liquid mixture was injected into the column for analysis. The practical quantification range was found to be from 50 to 1,000 mg/L. The procedures in Standard Methods (APHA,1998) were followed for determining the mixed liquor suspended solids(Method 2540D) and the mixed liquor volatile suspended solid (MLVSS)(Method 2540E).

### 3. RESULTS AND DISCUSSION

Acclimation and start-up were carried out simultaneously. During this period, an amount of 0.1 mg/L-day of a specific chlorinated compound was injected daily into each reactor to allow contact with the microbes for acclimation. Due to interacting with different substrates and chlorinated compounds, each reactor reached a specific stable substrate utilization rate and biomass concentration after 6 months, under unlimited primary substrate supplement. For reactors fed with HPr, the propionate utilization rates (PURs) ranged from 2.2 to 4 g HPr/g cell-day, with biomass concentrations of 2,000 to 5,000 mg/L. While reactors fed HAc, the acetate utilization rates (AURs) were from 3 to 6 g HAc/g cell-day, with biomass concentrations of 4,500 to 11,000 mg/L. The reactors using HAc as primary substrates were injected daily with additional 200 mg- $\text{NaHCO}_3$ / L of reactor to maintain stable alkalinities ( $1,200 \pm 200$  mg as  $\text{CaCO}_3$ /L). The reactors using HPr as primary substrates always maintained stable alkalinities with the basal media.

Biotransformation, biomass adsorption, abiotic transformation, and volatilization were the major mechanisms for the removal of chlorinated aliphatics. The contributions of the various mechanisms were the results of competition. The developed model (Equation 7) was used to clarify the contributions of these mechanisms in the system. The biotransformation constants ( $k_1$ ) of the pseudo-first-order reaction were retrieved from the previous experiment used the same system (see Table 2). The estimated contribution of each mechanism was summarized in Table 3.

Gas productions from reactors supplied with HAc (5 to 15 L gas/L of reactor-day) were generally higher than in those reactors supplied with HPr (less than 5 L gas/L of reactor-day). Higher gas production could enhance the chlorinated compounds removals by volatilization. However, those reactors supplied with HAc always supported more biomass, which encouraged biotransformation. Based on the model result, biotransformation was the major

mechanism of chlorinated aliphatics removal for reactors supplied with either HPr or HAc. For these compounds, biotransformation accounted for from 67% to 99% of the total removal, while removal by volatilization ranged from 0.2% to 33%. Since CT had the highest Henry's Law constant, CT with HAc had the highest volatilization contribution. On the other hand, biomass adsorption did not significantly affect the transformation of chlorinated aliphatics (less than 0.12%). The effect of abiotic transformation on removal of chlorinated aliphatics was almost negligible.

During the experiment period, a new loading of a specific chlorinated compound could immediately reduce the substrate utilization rate (PUR or AUR). However, with a continuous loading at the same level, the substrate utilization rate might partially recover and stay stable. Sometimes, the loading was too torrential to recover the substrate utilization rate, and then the loading was terminated. A lighter loading was applied after the system retrieving the normal substrate utilization rate. All the responses of substrate utilizations resulted from different loadings of chlorinated aliphatics were recorded to establish dose-response curves. The carbon tetrachloride loading and PUR response was used as an example, and illustrated in Figure 1. The critical loading rate was estimated from the 50% PUR of the regressed line. Exponential regression was found to give better correlation than linear regression. Table 4 summarized the estimated critical loading rates of the chlorinated aliphatics for cultures fed with HPr or HAc.

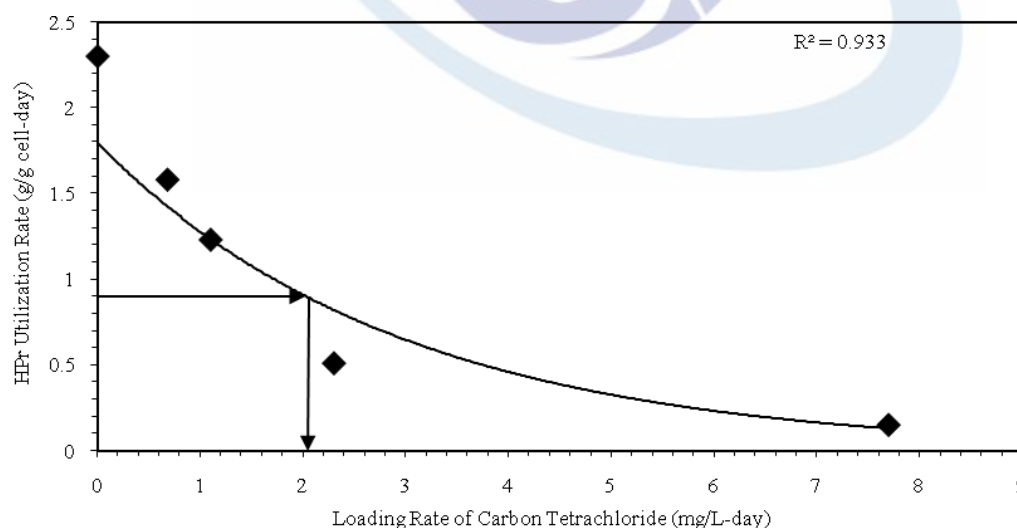


Figure 1. Carbon tetrachloride loading and toxicity response when propionic acid was the Primary Substrate.

Based on reactor volume, the critical loading rates of chlorinated alkenes (1,1-DCE, TCE, and PCE) ranged from 8 to 130 mg/L of reactor-day (0.01 to 0.1 mmole/g cells-day).

Chlorinated alkanes (MC, CF, CT, and 1,1,1-TCA) lowered those values to the levels between 1 to 4.5 mg/L of reactor-day (0.001 to 0.007 mmole/g cells-day). The response of TCE could be loaded at higher dose for cultures fed with HPr and HAc than both CF and TCA. Therefore, compounds with 3 chlorines but containing a double bond could get faster biotransformation.

For the cultures fed with HAc as the primary substrate, the more chlorinated the alkene, the higher the critical loading rate. However, the chlorinated alkanes fed with HAc did not present the same result. Blum and Speece (1991) reported the inhibitory concentration of 50% (IC<sub>50</sub>) of chlorinated aliphatics for unacclimated methanogens, fed with acetate, was in the order of PCE > TCE > DCE > MC > CT. This order was identical with the critical loading results of this study. Therefore, for the acclimated cultures fed HAc, the critical loading rate loading rate was still correlated to the toxicity of chlorinated aliphatic to the unacclimated methanogens. In the experiment period, due to a pH probe failure, the microbes in reactor receiving CF were lifeless. Therefore, the culture was replaced by biomass that had been acclimated to MC for 1 year. The inter-acclimated culture resulted in prompt biotransformations of CF and the intermediate MC. The mechanism needs further study to explore. For the cultures fed HPr, the critical loading rates of chlorinated aliphatics were in the same order of PCE > TCE > MC > CT > CF as fed HAc, when based on biomass.

Comparing the ratios of primary substrates utilized to chlorinated aliphatic biotransformed, the reactors supplied with HPr always had a higher values than the reactors supplied with HAc. During anaerobic fermentation, HPr can be converted to hydrogen and acetate, then to methane and carbon dioxide, while HAc is directly converted into methane and carbon dioxide. Hydrogen might be the electron donor used directly for dechlorination (Maymo-Gatell et al., 1997). Therefore, the dechlorination of chlorinated aliphatics might be enhanced by the presence of hydrogen. These results were consistent with those of DiStefano et al. (1992). Since all chlorinated aliphatics could be biotransformed using HAc as the primary substrate, which produced no hydrogen intermediate. Therefore, dechlorination of chlorinated aliphatics by hydrogen might not be the only path.

#### 4. CONCLUSIONS

Anaerobic reactors for the removal of organic pollutants from industrial wastewaters had significant potential for biotransformation of some priority pollutants. With proper acclimation, the anaerobic treatment process had a considerable potential for simultaneous biodegradation

of toxic chlorinated aliphatics during conversion of the primary substrate to methane. These critical loading rates gave ranges for stable anaerobic wastewater treatment while biodegrading chlorinated aliphatics. Based on biomass, the chlorinated aliphatics critical loading rates for the microbes metabolizing HPr were from 0.4 to 24 mg/g cell-day, while those rates for the microbes metabolizing HAc ranged from 0.1 to 21 mg/g cell-day. Anaerobic microorganisms biotransformed chlorinated aliphatic alkenes at a higher rate than those of alkanes.



Table 2. The values of the parameters used in the fate model

Compound	Primary Substrate	$k_1^a$ (L/mg cell-day)	$H_c^b$ (Dimensionless, 35 °C)	$\log K_{ow}^c$ (L/mg cell-day)	$t_{1/2}^d$ (year)
Methylene Chloride (MC)	HPr	$2 \times 10^{-2}$	0.129	1.30	1.5
	HAc	$0.8 \times 10^{-2}$			
Chloroform (CF)	HPr	$4 \times 10^{-2}$	0.223	1.90	1.3
	HAc	$1 \times 10^{-2}$			
Carbon Tetrachloride (CT)	HPr	$2 \times 10^{-2}$	1.823	2.73	7000
	HAc	$1 \times 10^{-2}$			
1,1,1-Trichloroethane (1,1,1-TCA)	HPr	$4 \times 10^{-2}$	0.987	2.18	0.5
	HAc	$2 \times 10^{-2}$			
1,1-Dichloroethylene (1,1-DCE)	HPr	$1 \times 10^{-2}$	0.83 <sup>c</sup>	2.13	1.5 <sup>e</sup>
	HAc	$0.9 \times 10^{-2}$			
Trichloroethylene (TCE)	HPr	$3 \times 10^{-2}$	0.591	2.53	0.9
	HAc	$2 \times 10^{-2}$			
Tetrachloroethylene (PCE)	HPr	$1 \times 10^{-2}$	1.116	2.88	0.7
	HAc	$0.8 \times 10^{-2}$			

a: Rhee, 1990. b: Gossett, 1987. c: Montgomery and Welkom, 1990. d: Vogel et al., 1987. e: Dilling et al., 1975. .

Table 3. Processes responsible for observed removal of chlorinated aliphatic compounds

Compound	Primary Substrate	Estimated Contribution of Removal							
		Biotransformation		Biomass Adsorption		Abiotic		Volatilization	
		(%)	(mg/L-day)	(%)	(mg/L-day)	(%)	(mg/L-day)	(%)	(mg/L-day)
Methylene Chloride	Propionic Acid	99	1	0.01	0	0.01	0	0.2	0.003
	Acetic Acid	98	7	0.01	0	0	0	2	0.1
Chloroform	Propionic Acid	99	1	0.03	0	0	0	0.3	0
	Acetic Acid	95	4	0	0	0	0	5	0.2
Carbon Tetrachloride	Propionic Acid	95	2	0.1	0.002	0	0	5	0.1
	Acetic Acid	67	2	0.07	0	0	0	33	0.9
1,1,1-Trichloroethane	Propionic Acid	93	2	0.04	0	0.02	0	7	0.1
	Acetic Acid	86	2	0.04	0	0	0	14	0.4
1,1-Dichloroethylene	Propionic Acid	87	37	0.04	0.01	0	0	13	5
	Acetic Acid	82	2	0.03	0	0	0	18	0.5
Trichloroethylene	Propionic Acid	95	28	0.07	0.02	0.01	0	5	2
	Acetic Acid	86	12	0.06	0.01	0	0	14	2
Tetrachloroethylene	Propionic Acid	90	45	0.12	0.06	0	0	10	8
	Acetic Acid	94	16	0.1	0.02	0	0	6	0.8

\* HRT=20 days.

Table 4. Estimated critical loading rate of chlorinated aliphatic compounds in pH-stat system (HRT=20 days)

Compound	Primary Substrate	Average Biomass (mg/L)	Substrate Utilization Rate * (g/g cells-day)	Critical Loading Rate		
				Based on Reactor Volume (mg/L of reactor-day)	Based on Biomass (mg/g cells-day)	Based on Primary Substrate Utilization (mg/g substrate)
Methylene Chloride	HPr	2,600	3.2	2.3	0.8	0.7
	HAc	10,000	5.6	4	0.4	0.2
Chloroform	HPr	5,000	2.0	2	0.4	0.4
	HAc <sup>†</sup>	6,000	3.0	> 4.5	> 0.8	> 0.5
Carbon Tetrachloride	HPr	2,700	1.8	1.8	0.7	0.6
	HAc	4,500	7.8	1.7	0.3	0.1
1,1,1-Trichloroethane	HPr	2,000	1.6	1.2	0.9	0.3
	HAc	11,000	3.2	1	0.1	0.04
1,1-Dichloroethylene	HPr	2,300	1.8	-	-	-
	HAc	9,000	4.0	8	1.1	0.4
Trichloroethylene	HPr	3,500	2.4	56	24	8
	HAc	8,000	3.2	90	14	5
Tetrachloroethylene	HPr	2,300	2.4	40	19	11
	HAc	10,000	2.6	130	21	6

\* Based on the regressed equations.

<sup>†</sup>Conducted in a mix-liquor of MC and CF acclimated cultures.

- R<sup>2</sup> is too low to estimate.

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