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Degradation of Tetrachloroethene by Several Co-metabolism Substrates in Groundwater

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Abstract: Tetrachloroethene (PCE) is biodegraded by reductive dechlorination with co-metabolism substrates under anaerobic conditions. By inoculating sludge from an anaerobic pool, a biodegradation test of PCE is conducted in the anaerobic condition. In the test, several substrates including methanol, ethanol, formate, acetate, lactate and glucose, are conducive to the conversion from PCE to TCE and 1,1–DCE. The results show the microbe can be cultivated well under the anaerobic circumstances of mixture of sewage (sludge) and soil with the index of COD after eleven days. Degradation of PCE accords with one order reaction kinetics equation. The sequence of the reaction rate constant is $K_{acetate} > K_{glucose} > K_{lactate} > K_{ethanol} > K_{formate} > K_{methanol}$, and acetate is an outstanding co-metabolism substratum whose reaction rate constant is $0.6632d^{-1}$.

Key words: tetrachloroethene, biodegradation, reductive dechlorination, Co-metabolism substrate

1 Introduction

Since 1940s, chlorohydrocarbon, as an organic solvent, has been widely employed as a degreasing solvent in modern industry, including electron, car parts, plane engine, machinery, printing, dry-clean, etc. Because of inappropriate disposal of sewage and the leaking, chlorohydrocarbon has become the commonest contaminants in groundwater, and PCE is one of the main contaminants arousing environmental problem (Beneteau et al., 1999; He et al., 2004; Kao et al., 2003). As a persistent organic compound, PCE is listed as one of the priority contaminants under control by environmental protection administration USA (USEPA), which is believed to be "carcinogenic, teratogenic and mutagenesis"(Shen, 2002; USEPA, 2002). Because PCE is a dense non-aqueous phase liquid (DNAPL) and chemically stable, it may exist as non-dissolve residual liquid phase, dissolve phase, gas phase, or adsorption phase as long as it can once it enters unsaturated zone and groundwater system, it is difficult to investigate and remediate the contamination (Villarante et al., 2001). Because PCE is widely detected in groundwater as drinking water resouce, it has become a world-wide major issue and also a field which has attracted great interest of scientists.

Since the natural attenuating rate of PCE is slow, microbe uses co-metabolism substrates to biodegrade PCE under anaerobic conditions. At present, main study focused on this field is to choose substrates as electron donors and carbon source, which are conducive to microbe growth, metabolism, and cultivation. So it is necessary to study the effects of different substrates as co-metabolism to degrade PCE (Gao et al., 1997; Ndon et al., 2000; Skeen et al., 1995).

In this paper, it is investigated that microbe is cultivated and acclimated under anaerobic conditions with co-metabolism substrates including methanol, ethanol, formate, acetate, lactate and glucose.

2 Methods and Materials

2.1 Anaerobic microbe cultivation

Anaerobic microbe cultivation means that some microbe is inoculated in a sealed and filled with liquid culture medium container, and microbe grows and reproduces under certain temperature, pH and anaerobic conditions (Zhou and Gao, 2000).

2.1.1 Source of anaerobe microbe

The mixture of sludge and sewage from an anaerobic pool in a sewage plant.

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Table 1 The component concentration of groundwater

Common ion ($mg \cdot L^{-1}$)									
Na^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_3^-	SO_4^{2-}	HCO_3^-	pH
8.38	3.49	15.94	34.75	0.28	17.99	5.52	31.99	148.63	7.38
Trace element ($\mu g \cdot L^{-1}$)									
Ti	V	Cr	Mn	Co	Ni	Cu	Zn	Rb	Sr
210.00	4.36	8.24	52.16	1.76	40.88	16.52	64.48	36.56	852.00
Ba	Pb	Bi	As						
206.96	2.56	0.24	3.72						

2.1.2 Experimental apparatus

Anaerobic glove box, jar, COD quick determination apparatus.

2.1.3 Cultural condition

Groundwater from a well (Table 1) as inorganic salt cultural liquid. Carbon source uses glucose and yeast as nitrogen source. Temperature is controlled at 20°C in the process of cultivation. pH is about 7.5.

2.1.4 Cultivation manner

The cultivation manner is standing and intermittent batch cultivation. The sludge and sewage from an anaerobic pool in a sewage plant are inoculated to the soil (collected at intervals of 20 cm and 30 cm under ground in a park), groundwater is used to produce a ratio of water to soil 3:2; at the same time, glucose (carbon source) and yeast (nitrogen source) are added. Then the mixture is placed in an anaerobic glove box at 20°C.

2.2 Anaerobic microbe acclimation

Acclimation means that microbe adapts to some specific conditions step by step by manual control so as to obtain some resistant strains and promote metabolizing (Shen, 2002). In this experiment, some anaerobic microbes are acclimated to the change of PCE condition and then are used to degrade PCE at a quick rate.

2.2.1 Experimental apparatus and chemicals

Biochemistry incubator, glass bottle with rubber plug, gas-tight glass syringe, and constant temperature water tank, Teflon film. As co-metabolism substrate of the experiment, a kind of chemical reagent is required, including PCE (99.0% analytically pure), methanol chromatogram pure (≥ 99.5%), ethanol (≥ 99.7% analytically pure), sodium formate (analytically pure), sodium acetate (analytically pure), sodium lactate (50%–60%) (analytically pure), and glucose (analytically pure). All of those reagents are purchased from one chemical company of Beijing. As nitrogen source, yeast gets from one biotechnological company.

2.2.2 Testing apparatus and conditions

Testing apparatus: HP6890 gas chromatograph (GC), HP7694 trap auto-sampler. Testing conditions: HP–5 capillary column (30m×0.25mm×0.25μm film thickness, inlet temperature 160° C, electron capture detector temperature (ECD) 300°C, over from 50°C with the rate of 5° C /min to 70° C and hold 6min, chromatographic column flow rate 1.0mL/min, split ratio 5:1, carrier gas N_2, and flow 30mL/min, vial 50° C, Loop 60° C, translate line 70° C, injecting time 1.00min, staking time 5min, method detector limit 0.05μg/L.

2.2.3 Acclimation mode

The cultured anaerobic microbes are acclimated with six co-metabolism substrates, including methanol, ethanol, formate, acetate, lactate, glucose and PCE. In the anaerobic glove box, the cultured mixture of water and soil (as inoculum) is put into six glass bottles (500mL) respectively, and then the solution volume is amount to 500mL through adding groundwater. At the same time 0.02% yeast is added in them. After methanol, ethanol, formate, acetate, lactate, glucose (co-metabolism substrates) and PCE solution added respectively, the bottles are sealed with rubber stopper and teflon film. Finally, these bottles are put into biochemistry incubator to be acclimated at 20°C.

2.3 Degradation experiment of PCE

The glass bottles filled different substrates and PCE are put into constant temperature bain-marie and shaken 2h to mix homogeneously. After 2h standing, the solution is taken and the content of chlorohydrocarbon is measured. The concentration of PCE and degrading product are measured every time. After sampling with injector, the caps of glass bottles must be sealed with teflons immediately to prevent from volatilization of PCE.

3 Results and discussion

3.1 Culture of anaerobic microbe

Glucose, as the carbon source, is added to cultivate anaerobic microbes in above-mentioned mode. The COD concentration of water sample is measured to assess activity of anaerobic microbes. The cultivation carrys three cycles, and the measure results are showed in Table 2.

In the first two culture cycles, the same phenomena can be observed that the removal rate of COD is maximal at the 11^{th} day, the first cycle is 89.06% and the second 93.14%, and the change of rate is little if cultured further. Correspondingly, in the continual 3^{rd} cycle, the removal rate of COD is 95.81% at the 11^{th} day. The activity of microbe in mix culture solution is strong enough now that it is the time to acclimate.

3.2 Acclimation of microbe

With six different co-metabolism substrates and the condition of increasing PCE concentration, the cultured

Table 2 The COD change in the process of anaerobic microbe culture

First cycle (COD)			Second cycle (COD)			Third cycle (COD)		
Initial concentration ($mg \cdot L^{-1}$)	Concentration of 11^{th} ($mg \cdot L^{-1}$)	Removal rate (%)	Initial concentration ($mg \cdot L^{-1}$)	Concentration of 11^{th} ($mg \cdot L^{-1}$)	Removal rate(%)	Initial concentration ($mg \cdot L^{-1}$)	Concentration of 11^{th} ($mg \cdot L^{-1}$)	Removal rate (%)
4473.5	489.2	89.06	5649.2	387.5	93.14	5147.6	215.6	95.81

Table 3 The concentration of PCE in the process of anaerobic microbe acclimation (theoretical value $\mu g \cdot L^{-1}$)

Times of acclimation							
The 1st time	The 2nd time	The 3rd time	The 4th time	The 5th time	The 6th time	The 7th time	The 8th time
32.4	32.4	32.4	64.8	64.8	129.6	64.8	324

anaerobic microbes are acclimated. The concentration of pure PCE every acclimation is showed in Table 3.

The acclimation is carried out 8 times and the results are showed in Fig.1. Because PCE is difficult to dissolve in water, there is deference between the actual measure value of PCE and the theoretical value of PCE. In view of the toxicity of PCE, the added PCE is increased from 32.4μg/L of theoretical value of PCE at the first time of acclimation. With the low concentration of PCE at the beginning, the concentration of trichloroethene (TCE) is also low and other products are not found. The reason of this is that the weak activity of microbes resulted from the elimination of microbes unadapted to PCE. The microbes begin to adapt to the circumstance and the degradation is enhanced as the acclimation continues. As the result, the concentration of TCE is increasing and other products, 1,1–Dichloroethene (1,1–DCE), can be detected. Besides, the experiment shows that the concentration of TCE is higher when acetate, lactate, and glucose are co-metabolism substrates. It may be concluded that the

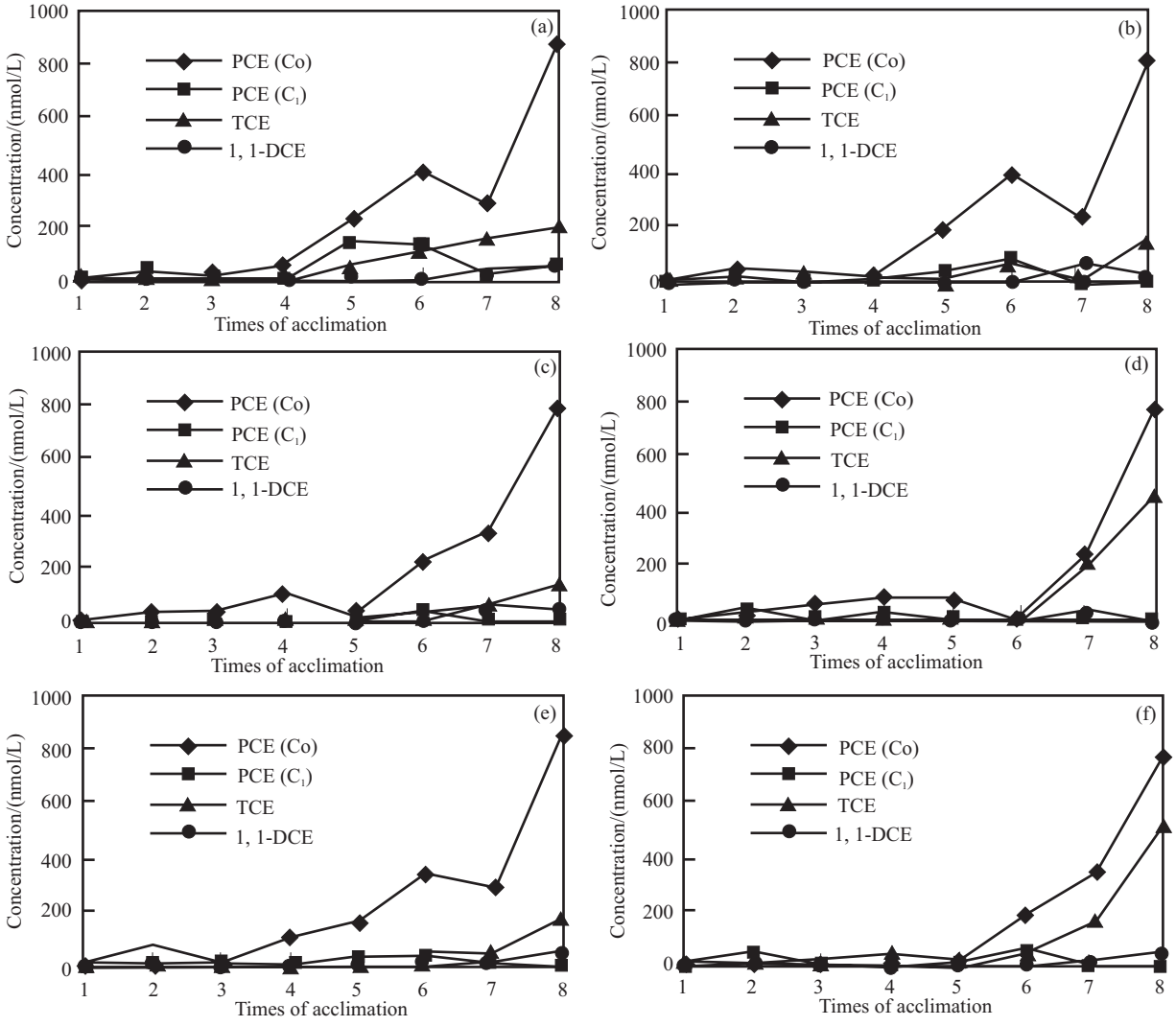

Fig. 1. The acclimation process of six substractes.

a – methanol; b – ethanol; c– formate; d – acetate; e – lactate; f – glucose; C_0 – initial concentration; C_1 – final concentration

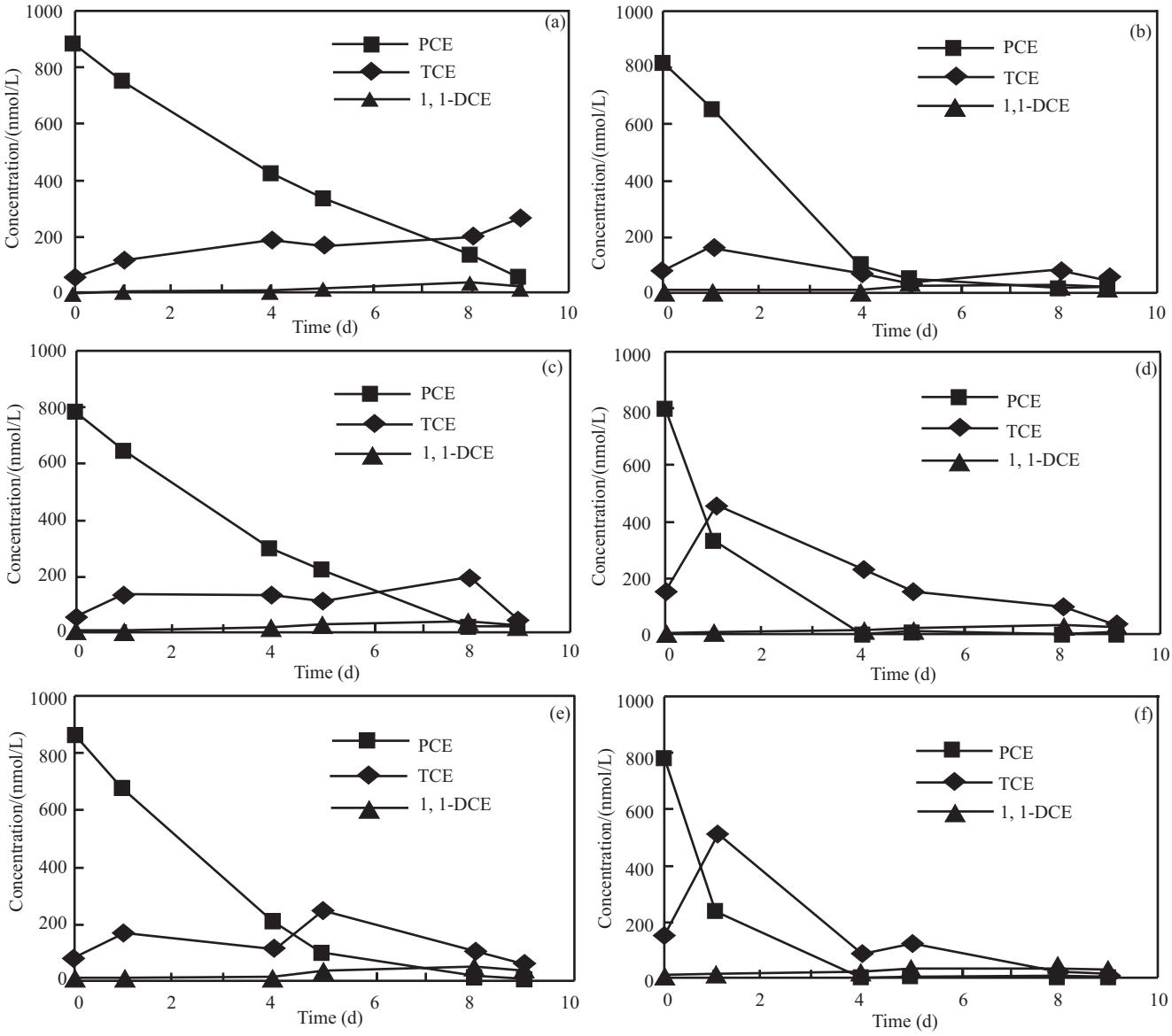

Fig. 2. The concentration change of PCE and the degradation product.
a – methanol; b – ethanol; c – formate; d – acetate; e – lactate; f – glucose

activity and reduction dechlorination of microbes is stronger and PCE is easier to be reduced and dechlorinated to be TCE with the three kinds of substrates.

3.3 Degradation experiment of PCE

Now, the acclimated anaerobic microbe can be used to degrade PCE. Six substrates and PCE are added to six glass bottles respectively. The theoretical value of added PCE is 324 μg/L (1951.8nmol/L). The experiment lasts 9 days, the concentration of PCE and its degradation products are showed in Fig. 2.

The degradation rate of PCE is high and the degradation ratio of PCE is about 90% at the 4th day when acetate, lactate, and glucose are co-metabolism substrates. By contrast, the degradation rate of PCE is only 60% when the other three substrates are co-metabolisms. As the concentration of PCE decreases, TCE and 1,1–DCE, two daughter products of PCE, are produced. The concentration of TCE increases gradually in the early of the experiment and decreases with time. Unlikely, the concentration of 1,1–DCE begins to increase until the end of the experiment at a small scale, then decreases. The possible explanation of that phenomenon is other byproducts, such as chloroethene, ethane and so on, are produced. The detection of these products will be made in next step.

3.4 Degradation kinetics of PCE

The fitting curves of PCE degradation at the six substrates are listed in Fig. 3. All of curves are according with one order kinetics equation. The fitting results of six substrates as follows: methanol, $y = 1061e^{-0.2825x}$, $R^2 =$

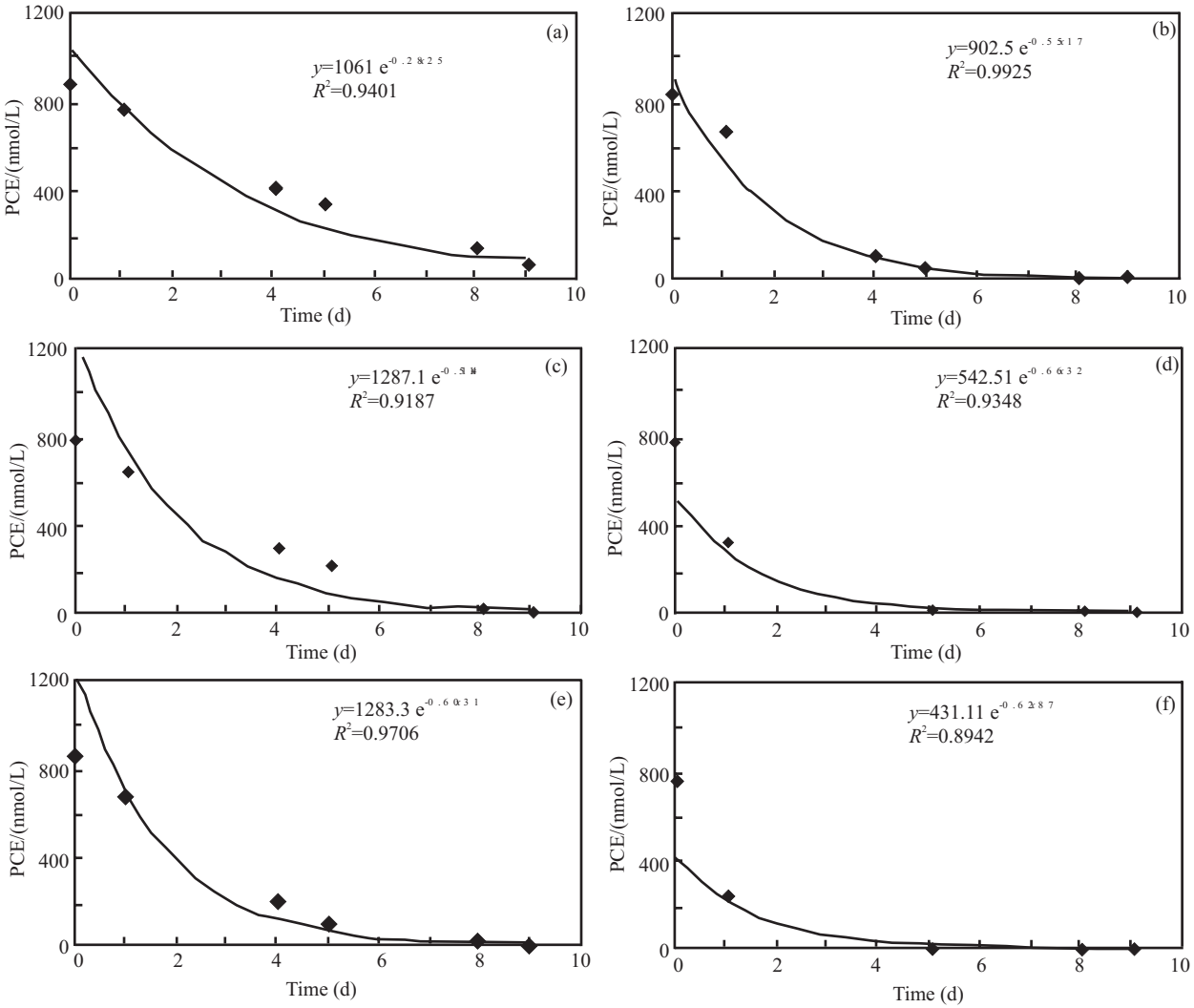

Fig. 3. The fitting curve of PCE degradation.
a – methanol; b – ethanol; c – formate; d – acetate; e – lactate; f – glucose

0.9401 (n=6), the reaction rate constant is 0.2825d^{-1}; ethanol, $y = 902.5e^{-0.5517x}$, $R^2 = 0.9925$ (n=6), the reaction rate constant is 0.5517d^{-1}; formate, $y = 1287.1e^{-0.5114x}$, $R^2 = 0.9187$ (n=6), the reaction rate constant is 0.5114d^{-1}; acetate, $y = 542.51e^{-0.6632x}$, $R^2 = 0.9348$ (n=5), the reaction rate constant is 0.6632d^{-1}; lactate, $y = 1283.3e^{-0.6031x}$, $R^2 = 0.9706$ (n=6), the reaction rate constant is 0.6031d^{-1}; glucose, $y = 431.11e^{-0.6287x}$, $R^2 = 0.8942$ (n=5), the reaction rate constant is 0.6287d^{-1}. The sequence of the reaction rate constant is $K_{acetate} > K_{glucose} > K_{lactate} > K_{ethanol} > K_{formate} > K_{methanol}$, which shows that the degradation rate of PCE is the fastest one when acetate is seemed as co-metabolism substrate under this experiment condition. So acetate is a best co-metabolism substrate.

4 Suggestion and Conclusion

(1) With the index of COD, the microbe can be cultured well at the anaerobic circumstance of mixture of sewage (sludge) and soil after eleven days.

(2) For six substrates, i.e. methanol, ethanol, formate, acetate, lactate and glucose, the activity of microbe is stronger when the last three substrates are co-metabolism substrates of microbe acclimation.

(3) With the six substrates, PCE is reduced and dechlorinated to TCE and 1,1–DCE. The fitting curves of degradation of PCE at the six substrates accord with one order kinetics equation. The sequence of the reaction rate constant is $K_{acetate} > K_{glucose} > K_{lactate} > K_{ethanol} > K_{formate} > K_{methanol}$, which shows that the degradation rate of PCE is the fastest one when acetate is seemed as co-metabolism substrate under this experiment condition, so acetate is a best co-metabolism substrate for the degradation of PCE.

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