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ABSTRACT

Title of Thesis:

**Experiments
in Aerobic and Anaerobic Biodegradation
of Trichloroethylene**

Qiong Liao, Master of Science in Chemistry, 1991

Thesis Directed by: Dr. Sam S. Sofer

The removal of trichloroethylene (TCE) from industrial waste has been intensively studied in recent years. Biodegradation has been determined to be an effective treatment for TCE in the environment. Many researchers have studied the biodegradation of TCE and obtained successful results based on the use of specific isolated bacteria. At the NJIT Biotechnology Laboratory, a new method is being developed in which immobilized activated sludge is used to degrade several toxic chemicals in various reactor configurations. The objective of this research is to determine if these methods can be extended to treat TCE.

Activated sludge from a wastewater treatment plant was acclimated with TCE and then used in aerobic experiments. Some commonly and effectively used methods from our laboratory, such as membrane, shaker flask, and microassay reactors were studied with activated sludge.

In the membrane reactor experiment, microorganisms from activated sludge were immobilized on microporous plastic sheet (MPS) by attachment. TCE solution was pumped through the reactor containing microorganisms attached on MPS sheet and TCE variation was analyzed with GC. Experiments were conducted in batch recirculation flow mode.

A series of shaker flask experiments were constructed to check the result of preinduction for activated sludge with some inducers.

The activity of activated sludge was studied by examining the oxygen uptake in a microassay reactor. This reactor has been a valuable tool in the enzyme kinetic studies due to its reproducibility and accuracy of the data acquired.

Anaerobic sludge was also used in this study, and was taken from a secondary treatment plant. It was digested in an anaerobic digester with a specific nutrient medium and then incubated in an incubator at $37^{o}C$ for anaerobic treatment.

In shaker flask experiments, TCE was degraded by NJIT activated and preinduced sludge with inducers. Biodegradation rates were found to be 0.36, 0.49, 0.44, 0.32 nmol/min per mg of dry biomass with sodium acetate, phenol, toluene, and sodium benzoate, respectively. They are greater than the rates for bacterial strains 46-1, G-4, and Alcaligenes eutrophus JMP134 reported in the literature.

However, the results of the membrane reactor, the microassay reactor, and the anaerobic experiments indicate that TCE biodegradation rates are far lower than typical biodegradation rate, which is 2.4 nmol/min per mg of dry biomass, for phenol, chlorophenol, and other substrates in NJIT Biodegradation System.

EXPERIMENTS IN AEROBIC AND ANAEROBIC BIODEGRADATION OF TRICHLOROETHYLENE

by
Qiong Liao

A Thesis
Submitted to the Faculty of the Graduate Division of the
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in Partial Fulfillment of the Requirements for the
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Department of Chemical Engineering, Chemistry,
and Environmental Science
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APPROVAL SHEET

Experiments
in Aerobic and Anaerobic Biodegradation
of Trichloroethylene

by
Qiong Liao

12/18/90

Dr. Sam S. Sofer, Thesis Adviser
Chair Professor of Biotechnology and Professor of Chemical Engineering, Chemistry, and Environmental Science, NJIT

12/18/90

Dr. Barbara Kebbekus, Committee Member
Chairperson for Chemistry and Professor of Chemical Engineering, Chemistry, and Environmental Science, NJIT

12/18/90

Dr. Reginald Tomkins, Committee Member
Associate Chairperson for Chemistry and Professor of Chemical Engineering, Chemistry, and Environmental Science, NJIT

BIOGRAPHICAL SKETCH

Author: Qiong Liao

Degree: Master of Science in Chemistry

Date: January, 1991

Date of Birth:

Place of Birth:

Undergraduate and Graduate Education:

* Master of Science in Chemistry, New Jersey Institute of Technology, Newark, NJ, 1991
* Bachelor of Science in Chemistry, Peking University, Beijing, P. R. of China, 1987

Major: Chemistry

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TABLE OF CONTENTS

Page

Chapter 1 Introduction 1

Chapter 2 Materials and Experimental Methods

2.1 Aerobic Experiments

2.1.1 Microorganism 5

2.1.2 Nutrient Medium 5

2.1.3 Measurement of Biomass 6

2.1.4 Experiment in Membrane Reactor 6

2.1.5 Shaker Flask Experiments 11

2.1.6 Microassay Reactor Experiment 119

2.1.7 Experiment with Phenol Oxygenase using Surfactant 13

2.2 Anaerobic Experiments

2.2.1 Microorganism 15

2.2.2 Nutrient Medium 15

2.2.3 Anaerobic Experiments 18

Chapter 3 Results and Data

3.1 Aerobic Experiments

3.1.1 Acclimation of Activated Sludge 19

3.1.2 Experiment with Membrane Reactor ... 22

3.1.3 Shaker Flask Experiments 25

3.1.4 Microassay Reactor Experiments 30
3.1.5 Experiments with Phenol using N-57 32
3.2 Anaerobic Experiments
3.2.1 Anaerobic Experiment without N-57 .. 35
3.2.2 Anaerobic Experiment with N-57 37

Chapter 4 Discussion and Recommendation 40

Chapter 5 Conclusions 44

Reference 45

LIST OF TABLES

Table Page
1 TCE Variation in Experiment with N-57 (ppm) 14
2 TCE Variation in Shaker Flasks (ppm) 27
3 Percentage of TCE Removed in Shaker Flasks 27
4 Effect of TCE on Biomass Activity 31
5 TCE Variation in Anaerobic Experiment without N-57 (ppm) 36
6 Protein Analysis in Anaerobic Experiment without N-57 36

7 TCE Variation in Anaerobic Experiment
with N-57 (ppm) 38
8 Comparison of Biodegradation Rates 41

LIST OF FIGURES

Figure Page
1 Experimental Setup of Membrane Reactor 7
2 Biomass Loading Variation in Absorbance
and Pressure 9
3 Effect of Biomass Loading on Operating Pressure .. 10
4 Experimental Setup of Micromassay Reactor 12
5 TCE Variation during Acclimation 20
6 D. O. Variation during Acclimation 21
7 TCE Variation in Membrane Reactor 23
8 D. O. Variation in Membrane Reactor 24
9 TCE Variation in Shaker Flasks 28
10 Percentage of TCE Removed in Shaker Flasks 29
11 TCE Variation in Experiment with Phenol Oxygenase 33
12 D. O. Variation in Experiment with
Phenol Oxygenase 34
13 TCE Variation in Anaerobic Experiment with N-57 .. 39

CHAPTER 1 INTRODUCTION

Activated sludge has been recognized as one of the most versatile methods known for wastewater treatment. Sawyer (1965) has given an excellent account of the developments in the activated sludge process in the fifty years of its use.

Trichloroethylene (TCE) is the most frequently reported contaminant at hazardous waste sites on the National Priority List of the U.S. Environmental Protection Agency (U.S. Environmental Protection Agency, 1985). This compound and other chlorinated alkenes present a serious groundwater contamination problem to industrial societies; they are suspected carcinogens and generally resist biodegradation in the environment.

The biodegradation of TCE, both aerobic and anaerobic, has been a field of intensive study in the last decade. Nelson *et al.* (1986) studied bacterial strain G4. Biodegradation of TCE by G4 resulted in complete dechlorination of the compound, as indicated by the production of inorganic chloride. Strain G4 degraded TCE in the presence of chloramphenicol only when preinduced with phenol. The inducer of TCE metabolism apparently induced the same aromatic degradative pathway that cleaved the aromatic ring by *meta* fission.

A type I methanotrophic bacterium, strain 46-1, was demonstrated to degrade TCE by Little et al. (1987). TCE biodegradation by strain 46-1 appeared to be a cometabolic process that occurred when the organism was actively metabolizing a suitable growth substrate, such as methane or methanol. They proposed that TCE biodegradation by methanotrophs occurs by formation of TCE epoxide, which breaks down spontaneously in water to form dichloroacetic and glyoxylic acids and one-carbon products.

Wackett and Gibson (1988) have studied the degradation of TCE by the bacterium Pseudomonas Putida F1. They present evidence that toluene dioxygenase is the enzyme responsible for TCE degradation by P. putida F1. Because the current investigation of NJIT biodegradation system oxidizes toluene, we decided to check if it is responsible for TCE degradation as well.

Arciero et. al.(1989) studied the degradation of TCE by an ammonia-oxidizing bacterium Nitrosomonas europaea. They found that fresh cells catalyzed the reaction in the absence of added ammonium but in older cells, however, TCE degradation depended on the addition of ammonia. Thus degradation of TCE was dependent on and possibly catalyzed by the ammonia oxidizing enzyme.

Tsien et al. (1989) studied another bacterium, Methylosinus trichosporium OB3b, a type II methanotroph

(1989). This bacterium, which was obtained from a pure culture grown in a fermenter, degraded TCE following the appearance of soluble methane monooxygenase in continuous and batch cultures. M. trichosporium OB3b cells that contained soluble methane monooxygenase also degraded vinyl chloride, 1,1-dichloroethylene, cis-1,2-dichloroethylene, and trans-1,2-dichloroethylene.

Bacterium Acaligenes eutrophus JMP134(pJP4) was studied by Harker and Kim (1989). This bacterium degraded TCE by a chromosomal phenol-dependent pathway and by the plasmid-encoded 2,4-dichlorophenoxyacetic acid pathway. The two pathways were independent and exhibited different rates of removal and capacities for quantity of TCE removed. The phenol-dependent pathway was found more rapid and consumed all detectable TCE.

The most recent research was about bacterium Pseudomonas cepacia G4 (Folsom). The intact cells of Pseudomonas cepacia G4 completely degraded TCE following growth with phenol. Degradation kinetics were determined for both phenol, used to induce requisite enzymes, and TCE, the target substrate. Since the NJIT biodegradation system degrades phenol very well, it is reasonable to think that this method may also work on TCE.

Due to significant differences among methods reported in the literature,a direct comparison of TCE

degradation rates among different organisms is difficult. However, a rough comparison of TCE biodegradation rates between the reported methods and NJIT biodegradation methods will be presented in the Discussion.

CHAPTER 2

MATERIALS AND EXPERIMENTAL METHODS

2.1 Aerobic Experiments

2.1.1 Microorganism

Activated sludge from the Parsippany Wastewater Plant (NJ) was used in this study. It was sieved through a 900 mesh screen first and then acclimated with TCE at room temperature. H_2O_2 was used as the oxygen source. Usually, the activated sludge was fed with 40 ppm TCE and 17 ppm oxygen. The TCE and oxygen concentrations were analyzed daily. At the end of acclimation the culture was centrifuged at 3000 rpm and 5^oC to obtain concentrated pellets.

2.1.2 Nutrient Medium

The nutrient medium used in this study consisted of the following chemicals:

Magnesium chloride ....100 mg

Manganese sulfate ....10 mg

Ferric chloride ....0.5 mg

Potassium phosphate ....10 mg

Ammonium sulfate ....10 mg

Water ....100 ml

The above solution was then diluted to 1000 ml by adding distilled water and aerating for 15 minutes. The

composition of this nutrient medium was developed in an earlier study (Lakhwala _et. al._).

2.1.3 Measurement of Biomass

A known weight of pellets obtained after centrifugation, was dried at 120°C for 24 hours. The dry weight so obtained was expressed as dry biomass per unit weight of pellets. For the present set of experiments, biomass concentration was 52.2 mg biomass/g pellet.

2.1.4 Experiments in Membrane Reactor

a. Microporous membrane module

The polymeric membrane was a 1 inch wide and 36 ft long strip, wound in a spiral configuration with a spacing of 0.013 inches between adjacent layers. The inner surface of the membrane had projecting ribs throughout the length, which provided this spacing. The spirally wound membrane was supported between two plates, which gave it mechanical strength and rigidity.

The membrane had a pore size of 1.5 u and was slightly hydrophilic with good wetting characteristics.

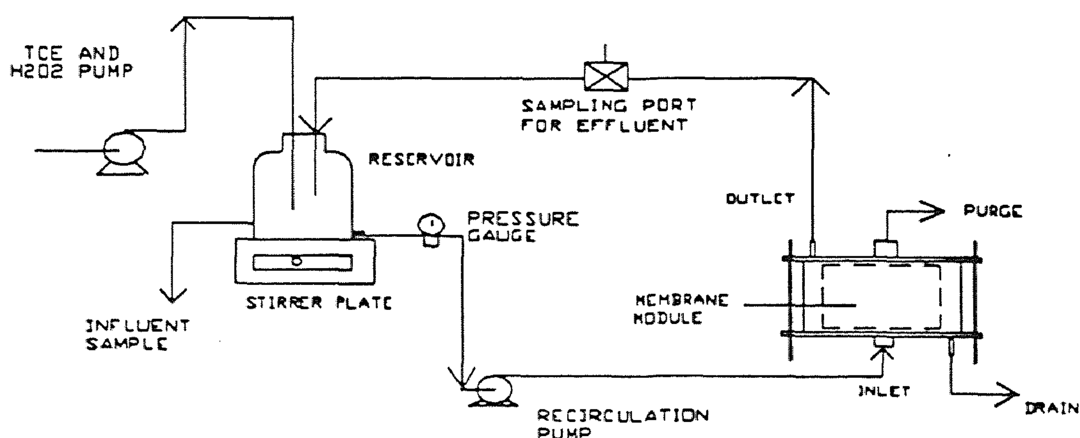
b. Experimental setup of the reactor

Fig. 1 shows the experimental setup of the reactor. The membrane module is fixed in between two steel plates

a plastic housing which forms the complete reactor. The steel plates have openings for inlet and outlet streams. The inlet and outlet connections may be reversed depending on the direction of flow, i.e. inside-out (going in at the center and out at the periphery) or outside-in (going in at the periphery and out at the center of the loop). A high pressure pump is used to pump the feed from the reservoir in inside-out direction.

Since TCE is highly volatile, the reservoir was tightly sealed to avoid physical losses. An additional pump was used to pump TCE and H_2O_2 solution to the reservoir. For batch recirculation experiments, the total working volume was about 600 ml, 200 ml in the reactor and 400 ml in the reservoir.

Fig. 1 Experimental Setup of Membrane Reactor



c. Inoculating the bioreactor

The reactor was flushed with nutrient medium (pH 7.2) to assure that the pH inside the reactor was around neutral. The reservoir was filled with 600 ml nutrient medium. After adequate recirculation and removal of any air bubbles inside the reactor, the feed pump was stopped. One gram of bacterial pellets was added to the reservoir and vigorously mixed. A 10 ml reservoir sample was removed and analyzed on a Spectronic 20D for absorbance. The medium, along with cells, was then recycled through the reactor at a flow rate of 25 ml/min and a pressure of 15 psig. Every 25 minutes, samples were taken from the reservoir and analyzed for absorbance. The absorbance decreased over a period of time due to attachment of cells over the membrane (Fig. 2). This was also evident from a gradual increase in pressure.

A total of 10 grams of pellets was added to the reactor with a subsequent increase in pressure from 15 to 20 psig (Fig. 3) at a flow rate of 25 ml/min. The reactor was then injected with 100 ppm TCE and 85 mg ammonium sulfate, and the initially attached biomass was allowed to grow for two days. This was done in order to have an actively attached biofilm in addition to the initial passive biofilm.

Fig. 2 Biomass Loading Variation in Absorbance and Pressure

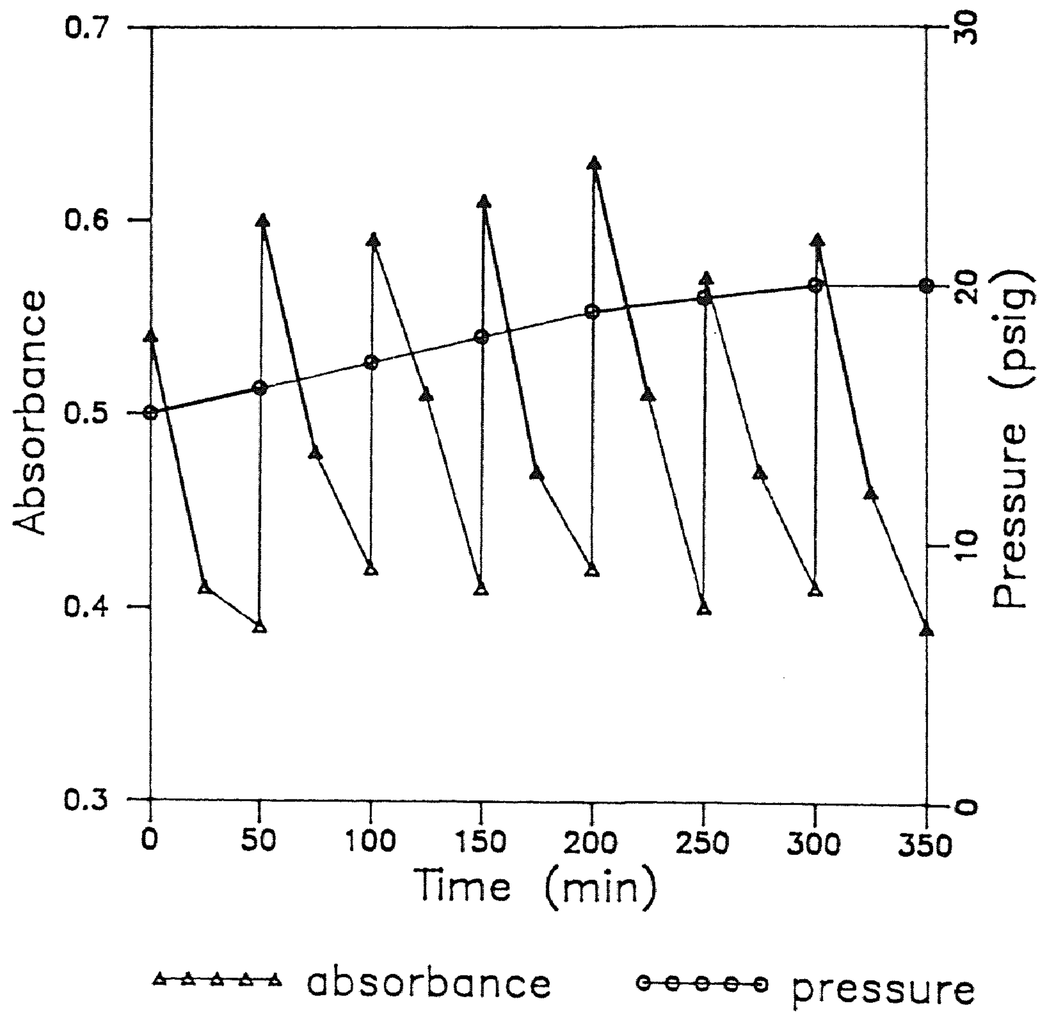
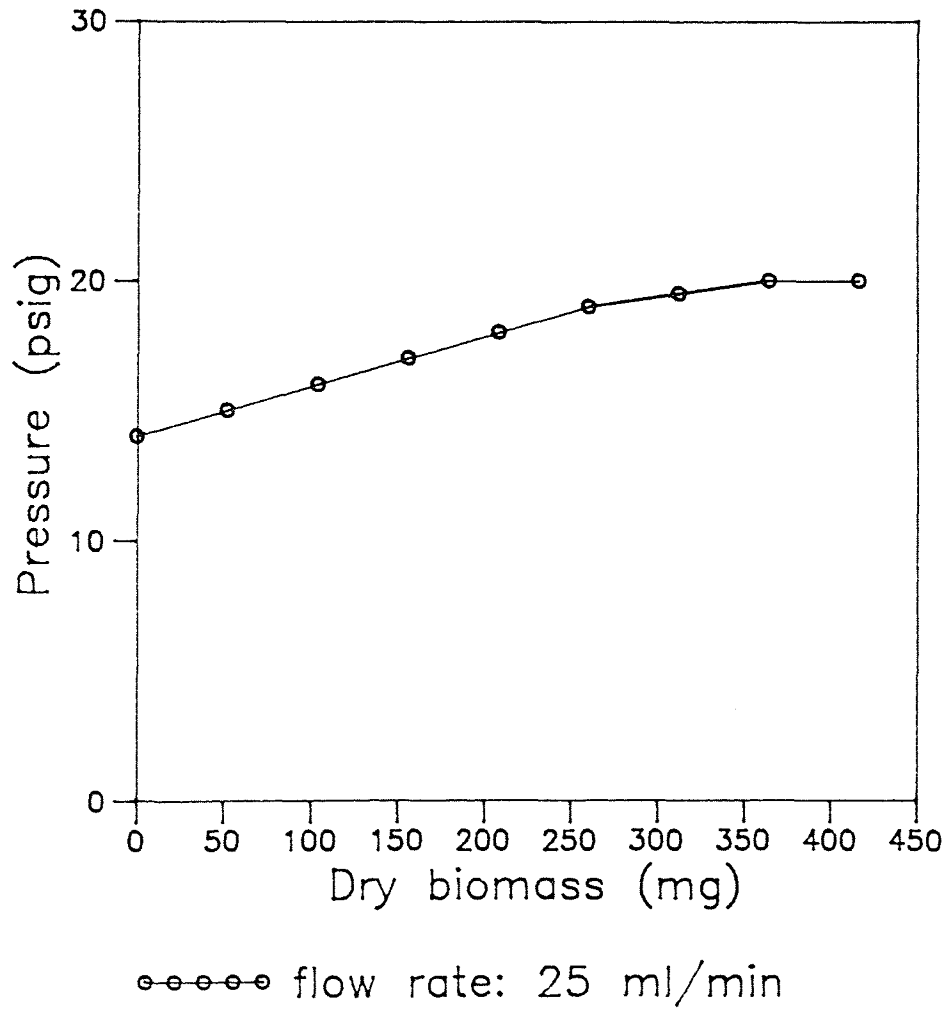


Fig. 3 Effect of Biomass Loading on Operating Pressure



d. Analytical methods

Throughout the experiments, liquid samples were taken and analyzed for TCE, and dissolved oxygen (D.O.). TCE concentration was measured on a Perkin-Elmer 8500 gas chromatograph equipped with ECD (Electron Capture Detector). An 8' x 1/8" OD stainless steel column packed with 1% SP-1000, 60/80 Carbopack was used at an oven temperature of 175°C.

D.O. was monitored using an Orion (97-08 model) oxygen probe.

2.1.5 Shaker Flask Experiments

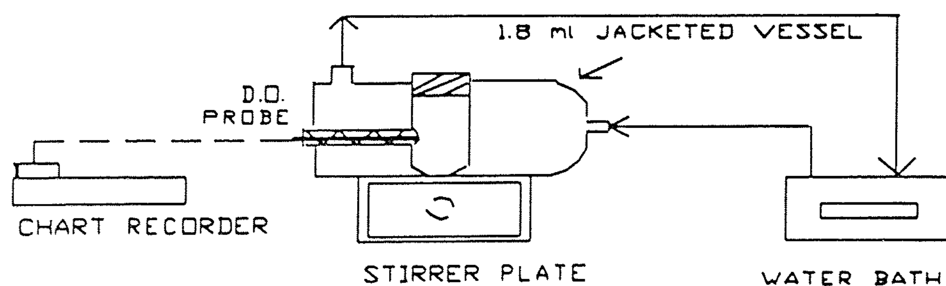
According to the literature (Nelson <u>et al</u>), TCE degradation requires bacteria preinduced with phenol, toluene or other inducers. Some shaker flask experiments were conducted to check the effect of preinduction. Flasks containing samples were kept in an Orbit Environ-Shaker at 33°C. Liquid samples were taken and analyzed for TCE and chloride ion. Chloride concentration was measured with an Orion Specific Ion Electrode.

2.1.6 Microassay Reactor Experiments

The experimental setup of the microassay reactor is shown in Fig. 4. The microassay reactor is a small jacketed vessel of 1.8 ml capacity, with provision for a

Clark-type dissolved oxygen probe. This reactor is a valuable tool in the enzyme kinetic studies due to its reproducibility and accuracy of the data. It is also economical as it requires very small amounts of reagents. In the present experiment, the reactor proved suitable for easy yet explicit measurements of dissolved oxygen, the parameter to be monitored. Water at the required temperature was circulated through the jacket, and the reactor was mounted on a magnetic stirrer plate.

Fig. 4 Experimental Setup of Microassay Reactor



Before the start of each run, the reactor was sterilized in an autoclave at 120°C, and then washed successively with methanol and sterile distilled water. It was then rinsed several times with a sterile nutrient medium. 1.5 ml of nutrient medium with biomass was added to

the reactor and saturated with oxygen by bubbling water saturated air through it. The saturation concentration of oxygen from air in water at 37°C is about 6.25 ppm (200 nmoles/ml). After saturation with oxygen, the air was stopped and the reactor was run for about 20 minutes. Then a TCE solution of various concentrations was added to the reactor and the concentration of oxygen was monitored by the oxygen probe and recorded on a chart recorder.

2.1.7 Experiment with Phenol Oxygenase using surfactant N-57

Trichloroethylene is a highly volatile compound, which causes a serious problem in this study. Triton N-57, nonylphenoxypolyethoxyethanol nonionic surfactant, and Triton X-405, Octylphenoxypolyethoxyethanol nonionic surfactant were determined to be effective in keeping volatile compounds in reaction solution and thus increasing the reaction efficiency.

A experiment was conducted to check the ability of the N-57 and X-405 surfactants to hold TCE in the liquid phase. A series of samples with the same TCE concentration and different surfactant concentration were prepared and analyzed for TCE daily. The result is shown in Table 1. From the data, it was determined that the N-57 solution with concentration of 3% was most effective for TCE.

Table 1 TCE Variation in Experiment with Surfactant (ppm)

Time (hr)	0	20	68	88
X-405 (0%)	900	180	110	52
X-405 (1%)	900	200	136	100
X-405 (3%)	900	230	200	165
X-405 (5%)	900	240	240	195
N-57 (0%)	900	190	130	40
N-57 (1%)	900	360	340	240
N-57 (3%)	900	380	330	320
N-57 (5%)	900	250	340*	160

*: Might be error of analysis.

Phenol oxygenase, extracted from the reservoirs of activated sludge in recirculation bioreactors, has been used in this experiment. It serves in the phenol biodegradation pathway by activated sludge.

2.2 Anaerobic Experiments

2.2.1 Microorganisms

The anaerobic sludge used in this study was taken from the Secondary Treatment Plant (STP, Livingston, NJ.). 1.5 kg of sludge was mixed with 2 liters of nutrient medium #1 and then digested into the anaerobic reactor. Nitrogen was bubbled through it for 20 minutes. The reactor was sealed and covered with aluminum foil to protect it from light. Typically, during the process of digestion, a certain amount of biomass was harvested for use in the lab, and then the same amount nutrient medium #2 was immediately digested into the reactor to keep the bacteria active. The nutrient medium #2 contained higher concentrations of glucose and other components to provide enough nutrients for the bacteria. The temperature was kept at 35°C in the reactor by using a water bath. This method of anaerobic digestion was introduced by Gourdon et. al. (1989).

2.2.2 Nutrient Mediums

a. Nutrient medium #1 and #2

The two mediums consisted of the same components but with different concentrations. They were prepared by mixing the following compounds and then diluting with tap water:

Compound	Concentration (g/l)	
	#1	#2
Glucose	1.5	35
Yeast extract	0.5	15
Peptone	0.25	
Low viscosity sodium alginate	0.5	10
NH_4Cl	0.15	3
$K_2HPO_4 \cdot 3H_2O$	0.05	1.5
$CaCl_2 \cdot 2H_2O$	0.025	0.5
$MnSO_4 \cdot H_2O$	0.005	0.1
$MgSO_4 \cdot 7H_2O$	0.01	0.2
$FeCl_3 \cdot 6H_2O$	0.005	0.1

The pH value of the solutions were adjusted to 7.5----7.0 by adding 0.1 N NaOH solution. Nitrogen gas was bubbled through the medium for 20 minutes.

b. Nutrient Medium #3

This medium was used in the anaerobic experiments. It contained nutrients and vitamins for mixed anaerobic cultures as adapted from studies by Owen and McCarty (1979). It was prepared by the following procedure:

First, four kinds of solutions were made as follows:

Solution	Compound	Concentration (g/l)
S1	$(NH_4)_2HPO_4$	26.7
S2	$CaCl_2 \cdot 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120
	KCl	86.7
	$MnCl_2 \cdot 4H_2O$	1.33
	$CoCl_2 \cdot 6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2 \cdot 2H_2O$	0.18
	$Na_2MoO_4 \cdot 2H_2O$	o.17
	$ZnCl_2$	0.14
S3	$FeCl_3 \cdot 4H_2O$	370
S4	$Na_2S \cdot 9H_2O$	500

5.4 ml of S1 and 27 ml of S2 solution were diluted to 1.8 liters and then boiled for 15 minutes. When it was cooled to room temperature, 1.8 ml of S3 and 1.8 ml of S4 solution were added and mixed well. Nitrogen gas was flushed throughout the whole procedure. The flask was sealed to minimize introduction of air into the container.

2.2.3 Anaerobic Experiments

After digestion for a few weeks, biomass was taken from the reactor. 40 ml of biomass solution and various amounts of TCE were added to 70 ml serum bottles and the bottles were sealed properly. N_2 gas was flushed during the process. Then the bottles were stored in an incubator at 37°C. Samples were taken out to analyze TCE concentration both in gas and liquid phase. At the end of the experiment, samples were also analyzed for chloride ion and protein.

Protein analysis was undertaken by using the Lowry Method, which has been found to be a reliable and satisfactory procedure for quantification of soluble proteins. First, a 1.0 ml sample was added to a labelled test tube. Then 1.0 ml Lowry Reagent Solution was added to each tube and mixed well. The solution was allowed to stand at room temperature for 20 minutes. With rapid and immediate mixing, 0.5 ml of Folin & Ciocalteu's Phenol Reagent Working Solution was added to each tube. Color was allowed to develop for 30 minutes. The absorbance of the sample vs. blank at 600 nm was measured. Protein concentration was determined by absorbance from the standard curve.

CHAPTER 3

RESULTS AND DATA

3.1 Aerobic Experiments

3.1.1 Acclimation of Activated Sludge

In this study, activated sludge was acclimated for 25 days. The variation of TCE concentration is shown in Fig. 5. In both acclimation and control experiments, TCE concentration decreased quickly on the first day. Over the next few days, TCE kept decreasing but at a slower rate. This could be because the gas phase had been saturated with TCE so that the rate of evaporation decreased and finally the system approached equilibrium.

A strange phenomenon can be seen in Fig. 5. TCE concentration during acclimation decreased less than it did in the control experiment. It seemed that the activated sludge held TCE in the matrix but did not react with it as expected.

The oxygen variation is shown in Fig. 6. Over three days, the oxygen concentration remained constant in both acclimation and control experiments. At the end of the run, oxygen concentration decreased to about 13 ppm. It is possible that the decrease of oxygen was because of physical losses rather than of consumption by bacteria. This result matches with the TCE variation and thus provides further evidence against biodegradation.

Fig. 5 TCE Variation during Acclimation

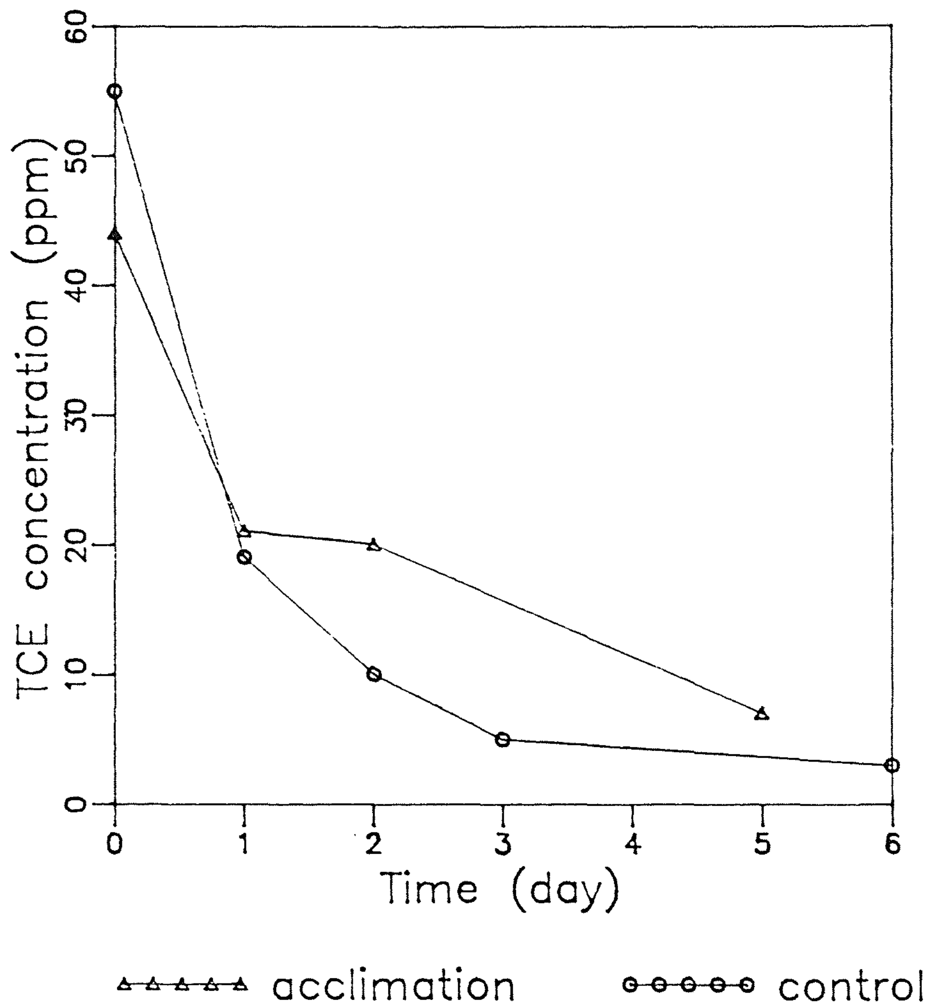
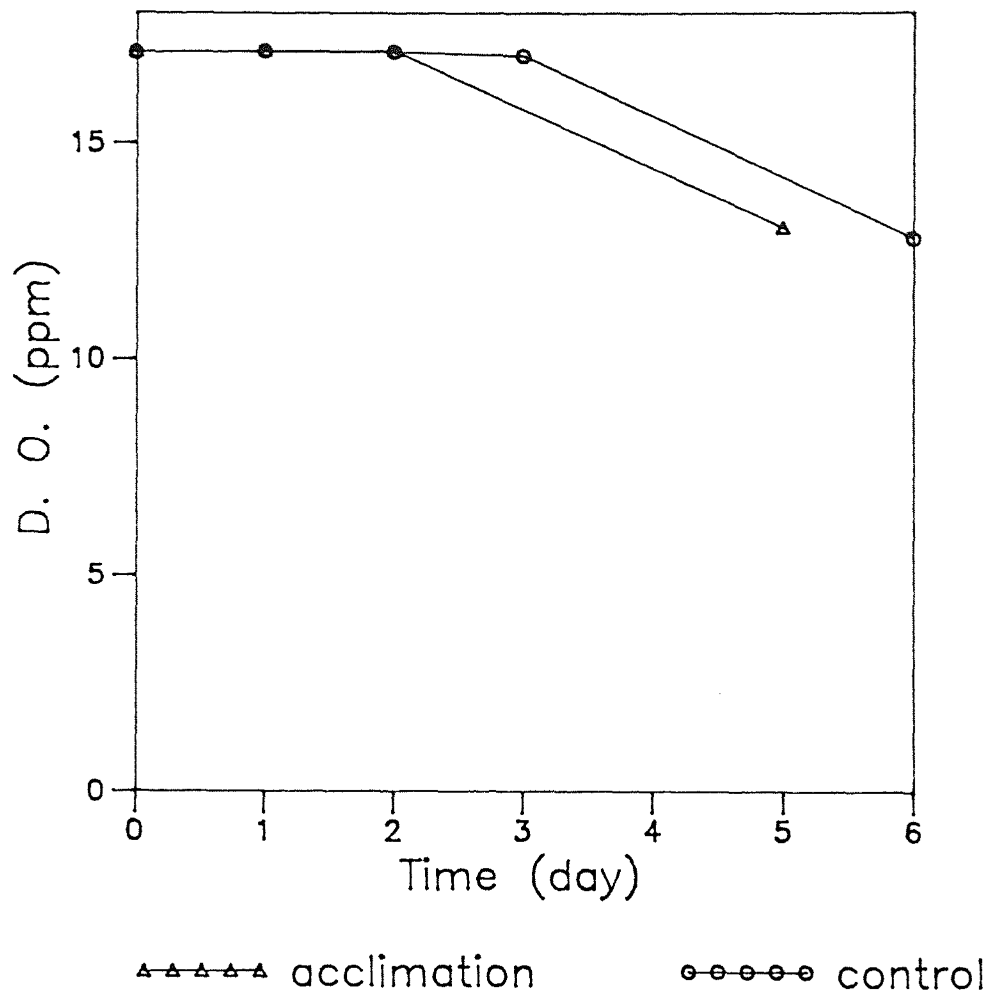


Fig. 6 D. O. Variation during acclimation

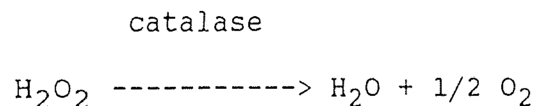


3.1.2 Experiments with Membrane Reactor

All these experiments were conducted under the same conditions, initial TCE concentration of 25 ppm and flow rate of 25 ml/min.

The experiment was repeated five times in the reactor. The TCE variation is shown in Fig. 7. The results did not indicate that TCE could be biodegraded. In the first hour, TCE concentration decreased quickly because of the dilution of the reaction solution. Then it remained constant during the next few hours. In about twenty hours, it decreased to zero. A control experiment without biomass was also done under the same conditions. TCE concentration in the control experiment changed at almost the same rate as that in the membrane reactor experiment. It is possible that TCE was lost due to its high volatility rather than by biodegradation.

Also from the variation of D.O. (Fig.8), the same conclusion could be drawn. The initial D.O. was about 17 ppm. During the run, both influent and effluent D.O. decreased and the effluent D.O. was always higher than influent D.O. This result might be because of the decomposition of hydrogen peroxide by some catalases. The process can be expressed by the following equation:



$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2$$

Fig. 7 TCE Variation in Membrane Reactor

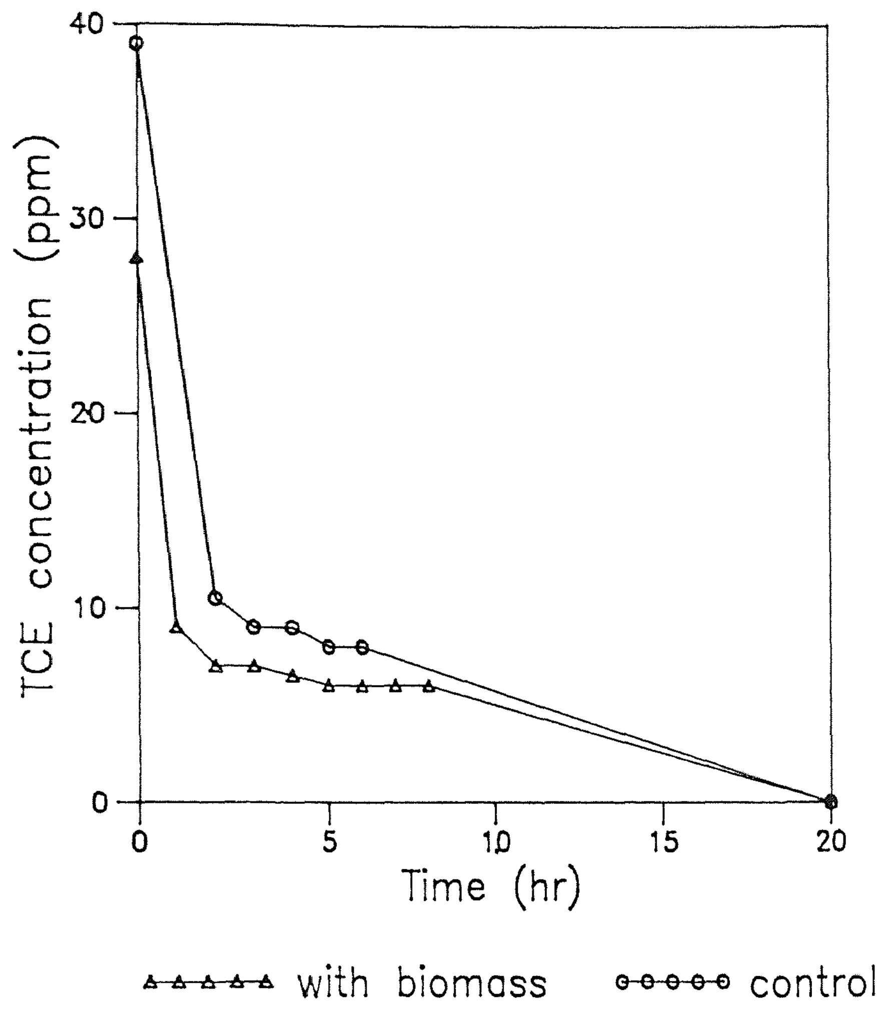
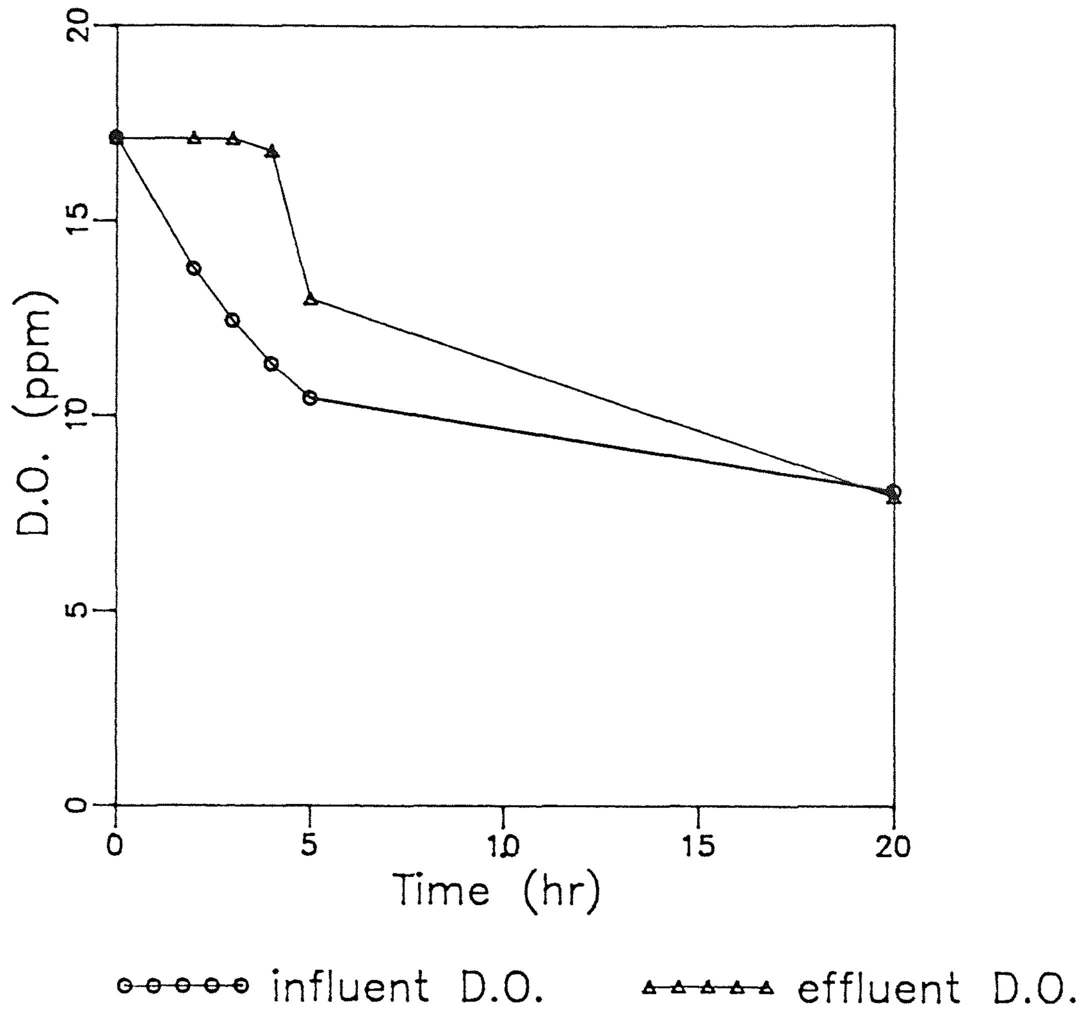


Fig. 8 D.O. Variation in Membrane Reactor



3.1.3 Shaker Flask Experiments

These shaker flask experiments were conducted following procedures in the literature (Nelson et. al.) about the preinduction of bacteria with phenol and other inducers. Six shaker flasks were prepared as follows:

Flask	Biomass	TCE (400 ppm)	H_2O_2 (3%)	Inducer @
No. 1	none	10 ml	5 ml	none
No. 2	1 g	10 ml	5 ml	2 ml phenol (10000 ppm)
No. 3	1 g	10 ml	5 ml	100 mg sodium acetate
No. 4	1 g *	10 ml	5 ml	2 ml phenol (10000 ppm)
No. 5	1 g	10 ml	5 ml	0.5 ml pure toluene
No. 6	1 g	10 ml	5 ml	10 mg sodium benzoate

*: This biomass was preacclimated with phenol.

@: The amounts of inducers added were calculated from published information.

All the six mixtures were diluted to 150 ml with nutrient medium and agitated in an Orbit Environ-Shaker at 33°C. Samples were taken and analyzed for TCE concentrations. The result is shown in Table 2 and Fig. 9. All the data are normalized and the percentage of TCE removed is shown in Table 3 and Fig. 10.

Table 3 and Fig. 10 make it easier to discuss the results in the shaker flask experiments. During 25 hours, the rates of TCE decreasing in the flasks with biomass and inducers are greater than the rate in the control. The degradation rates of the NO. 3, No. 4, No. 5, No. 6 flasks are 0.36, 0.49, 0.44, 0.32 nmol/min per mg of dry biomass. These TCE degradation rates are apparently greater than previously published rates for *Alcaligenes eutrophus* JMP134 (Harker *et. al.*) and rates calculated from data reported for bacteria Strain 46-1 (Little *et. al.*) and G4 (Shields *et. al.*).

The interesting point is the difference between the No. 2 and No. 4 flasks. The biomass used in No. 2 flask was acclimated by TCE and that used in No. 4 flask was acclimated by phenol. They both had phenol as inducer. The TCE in the No. 2 flask was not degraded but the No. 4 experiment had the greatest degradation rate among the shaker flask experiments. It seemed that the acclimation with TCE did not increase the activity of the bacteria as hoped for but made the bacteria less active.

Table 2 TCE Variation in Shaker Flasks (ppm)

Time (hr)	0	5	10	23
No.1 (control)	29	27	22	12
No.2 (phenol)	32	28	20	13
No.3 (sodium acetate)	29	28	20	6
No.4 (phenol & biomass*)	36	16	12	5
No.5 (toluene)	36	14	10	8
No.6 (sodium benzoate)	30	16	14	10

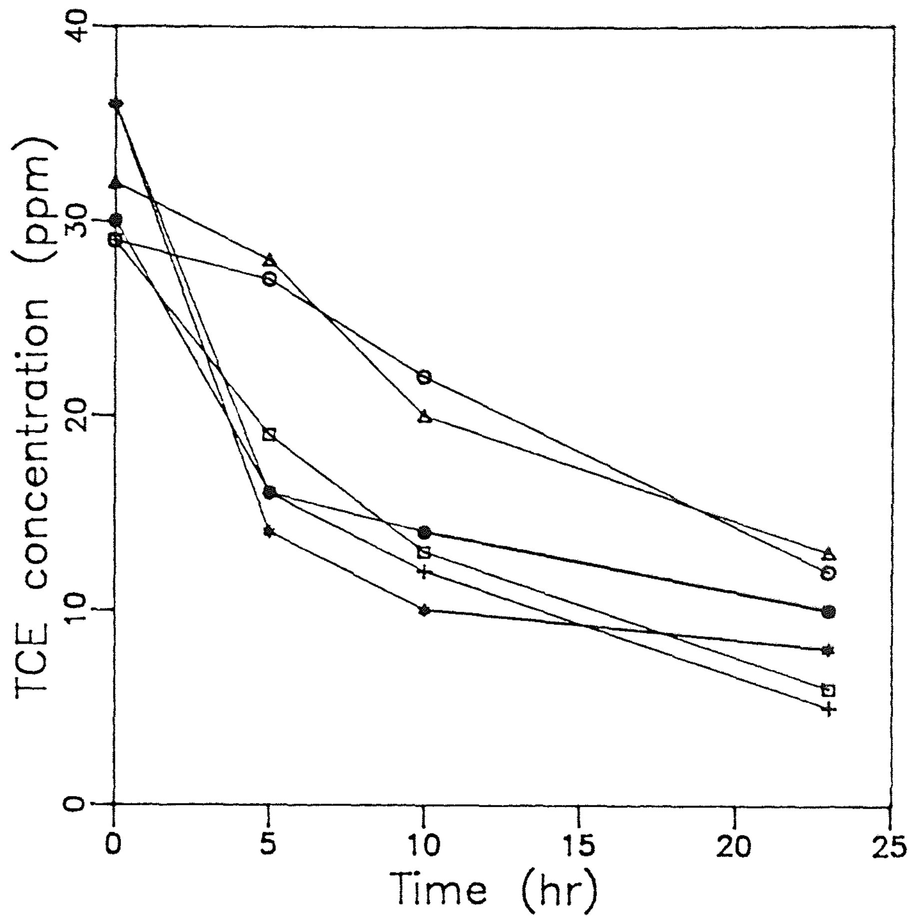
*: Preacclimated with phenol.

Table 3 Percentage of TCE removed in Shaker Flasks (%)

Time (hr)	0	5	10	23
No.1 (control)	0	7	24	59
No.2 (phenol)	0	13	38	59
No.3 (sodium acetate)	0	34	55	79
No.4 (phenol & biomass*)	0	56	67	86
No.5 (toluene)	0	61	72	78
No.6 (sodium benzoate)	0	47	53	67

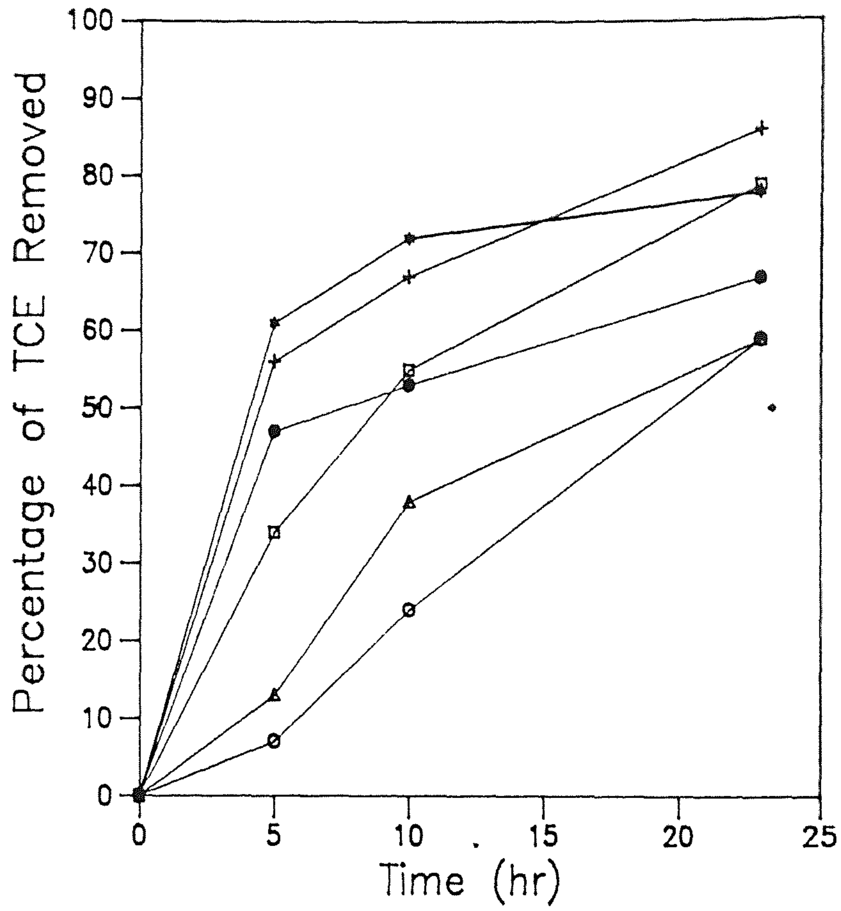
*: Preacclimated with phenol.

Fig. 9 TCE Variation in Shaker Flasks



- No.1 (control)
- No.2 (phenol)
- No.3 (sodium acetate)
- No.4 (phenol & biomass*)
- No.5 (toluene)
- No.6 (sodium benzoate)

Fig 10 Percentage of TCE Removed in Shaker Flasks



No.1 (control)

No.2 (phenol)

No.3 (sodium acetate)

No.4 (phenol & biomass*)

No.5 (toluene)

No.6 (sodium benzoate)

3.1.4 Microassay Reactor Experiments

a. Effect of TCE on bacterial activity

Activated sludge which was acclimated with TCE was used in this series of microassay reactor experiments to determine the effect of TCE on the activity of bacteria. First, 1.5 ml 5% biomass solution was added to the reactor and an activity curve was determined. Then 0.3 ml additional solution of 100 ppm phenol and various TCE concentrations (0-600 ppm) were added and a small change of the activity could be seen from the curve. The results of these experiments are shown in Table 4. The first column of this table indicates the concentrations of TCE solution which were added to the reactor after the reactor had run a certain time with biomass. The second and third column show the activities of before and after these various TCE solution were added to the reactor.

In all the eight TCE concentrations, the activities without TCE (A) are higher than those with TCE (A'). The ratios of A and A' are shown in the last column. It is obvious that with TCE the activity of bacteria did not increase as observed for phenol, toluene, and other substrates, but decreased. The values of A/A' are not exactly the same but very close. The small differences might be caused by random errors.

Table 4 Effect of TCE on Biomass Activity

TCE concentration (ppm)	activity (A) (without TCE)	activity (A') (with TCE)	A/A'
0	3.09 x E-3	2.59 x E-3	1.20
10	2.13 x E-3	1.53 x E-3	1.39
50	2.70 x E-3	2.33 x E-3	1.16
100	3.36 x E-3	2.27 x E-3	1.48
200	2.98 x E-3	1.77 x E-3	1.68
400	2.72 x E-3	1.54 x E-3	1.77
500	2.58 x E-3	1.50 x E-3	1.72
600	1.69 x E-3	1.39 x E-3	1.54

b. Comparison of unacclimated and acclimated sludge

This experiment was designed to check the result of the acclimation of activated sludge with TCE. Samples taken from unacclimated and acclimated sludge were centrifuged and their weights were determined and then diluted. Each sample of 1.5 ml was added to the reactor, and its activity was determined from the activity curves. The activity of unacclimated activated sludge was about eighty times stronger than that of the acclimated activated sludge. This result is counter to what was ex-

pected. TCE acclimation decreased oxidative actiiety of bacteria.

3.1.5 Experiment with Phenol Oxygenase by using N-57

10 ml enzyme solution, 10 ml TCE solution, 3 ml 3% H_2O_2, 3 ml N-57 surfactant and 100 ml nutrient medium were added to a container. The solution was stirred throughout the experiment and samples were taken out for TCE and D.O. analysis daily. A control experiment without enzyme was also tested later. Fig. 11 and Fig. 12 show the variation in TCE and D.O. during six days of acclimation. There is no obvious sign that TCE could be degraded by this enzyme. TCE concentrations in acclimation and control experiments decreased at almost the same rate.

From Fig. 12, D.O. concentration decreased a little faster in the presence of enzyme as compared to the control. It indicated that the enzyme was active and consumed oxygen in the presence of TCE. This was a good sign for TCE degradation. Since this experiment lasted only 5 days, it was not clear if the TCE could be biodegraded by this enzyme. In further research, further experiments could be performed to confirm if the enzyme works on TCE degradation.

Fig. 11 TCE Variation in Experiment with Phenol Oxygenase

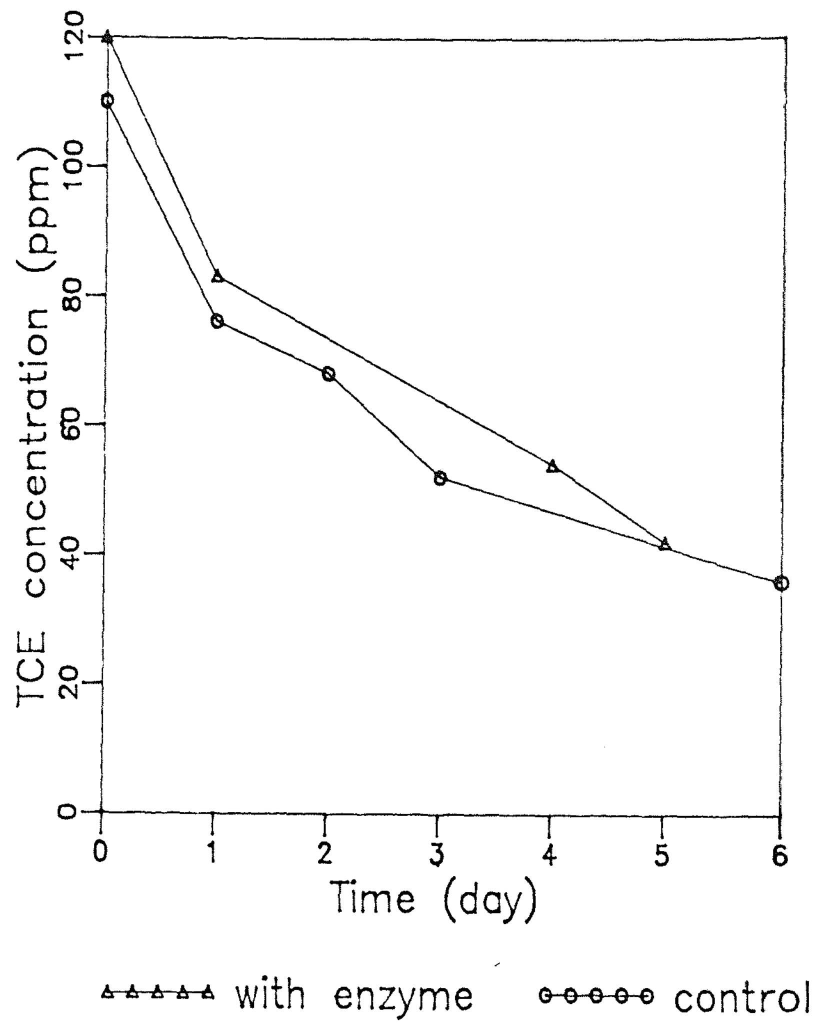
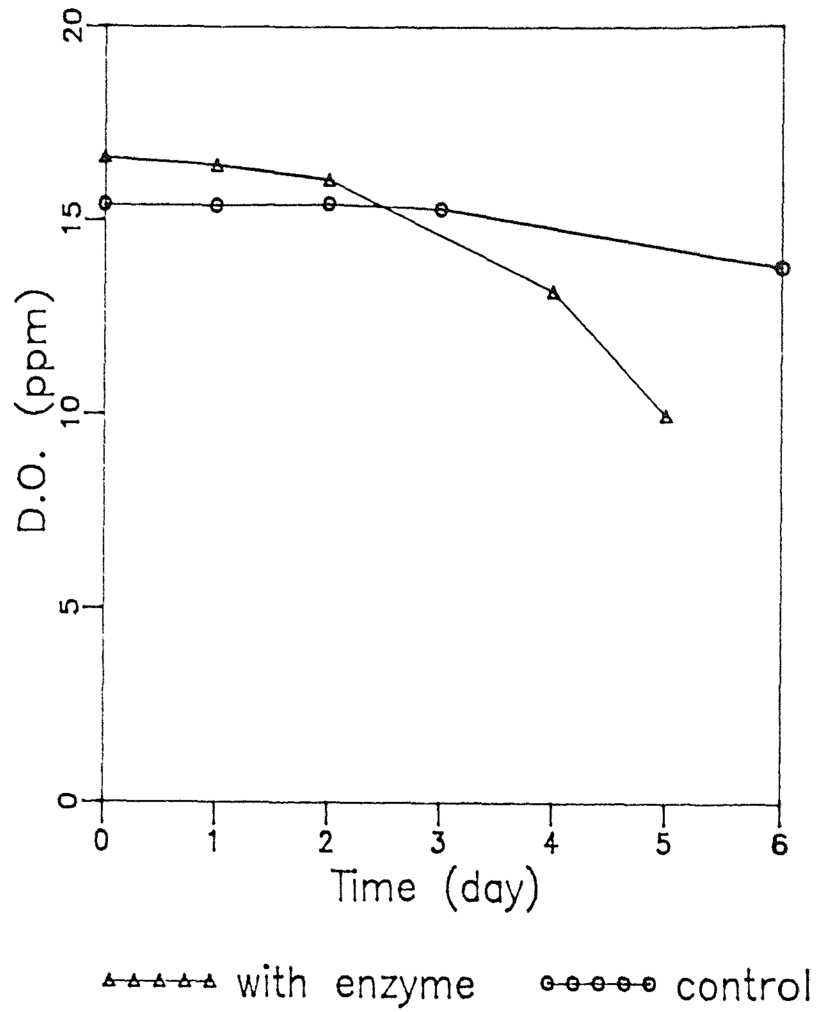


Fig. 12 D.O. Variation in Experiment with Phenol Oxygenase



3.2 Anaerobic Experiments

3.2.1 Anaerobic Experiment without N-57

Five sample groups were prepared with different TCE concentrations in this experiment. In each group, the first and second were duplicates and the third one was a control without biomass inside. The results are shown in Table 5. There is no obvious difference between the control and reaction samples. In each identical pair of sample, TCE did not vary at exactly the same rate. This might be caused by errors in preparation and analysis of the sample. It seemed that the high volatility of TCE acted as a prominent role in the experiment similiar to that in the previous aerobic experiments.

The chloride ion was analyzed with the Specific Ion Chloride Electrode. The results show that no chloride ions were produced in the experiment.

Also protein analysis was done, and the results are shown in Table 6. These data indicate that there were large amounts of protein in both the reaction solutions and control solutions. The protein in the control samples was from the nutrient medium #1 and #2, which contained yeast extract and were used in the digestion of anaerobic sludge. But the protein concentrations in reaction samples were all higher than that in control samples. It meant that the sludge had active enzymes.

Table 5 TCE Variation in Anaerobic Experiment

without N-57 (ppm)

time (day)	0	3	10
A 1	7	1	0
A 2	5	1	0
A 3 (control)	7	1	0
B 1	28	8	1
B 2	25	8	1
B 3 (control)	19	5	0
C 1	72	26	4
C 2	59	26	1
C 3 (control)	83	19	0
D 1	150	90	35
D 2	140	76	36
D 3 (control)	110	55	35
E 1	280	182	72
E 2	230	190	105
E 3 (control)	170	215*	60

*: Possible error of analysis.

Table 6 Protein Analysis in Anaerobic Experiment

without N-57

sample number	protein concentration (ug/ml)
B 1	720
B 3 (control)	240
C 1	400
C 3 (control)	220
D 1	400
D 3 (control)	160
E 1	360
E 3 (control)	140

3.2.2 Anaerobic Experiment with Surfactant N-57

One more anaerobic experiment was studied with the surfactant N-57. A series of samples were prepared and sealed in serum bottles as follows:

Sample	Biomass	TCE	Surfactant N-57
No.1	40 ml medium	5 ml	0 ml
No.2	40 ml medium	5 ml	1.2 ml
No.3	40 ml	1 ml	1.2 ml
No.4	40 ml	3 ml	1.2 ml
No.5	40 ml	5 ml	1.2 ml
No.6	40 ml	10 ml	1.2 ml

All these serum bottles were stored in an incubator at 37°C for twenty days, and samples were taken out for TCE analysis. The results are shown in Table 7 and Fig. 13. In the No. 1 bottle, TCE concentration decreased rapidly because it lacked N-57. The TCE concentration in the No. 2 bottle, which was same as the No. 1 except that it contained N-57, decreased much slower than that in the No. 1 bottle.

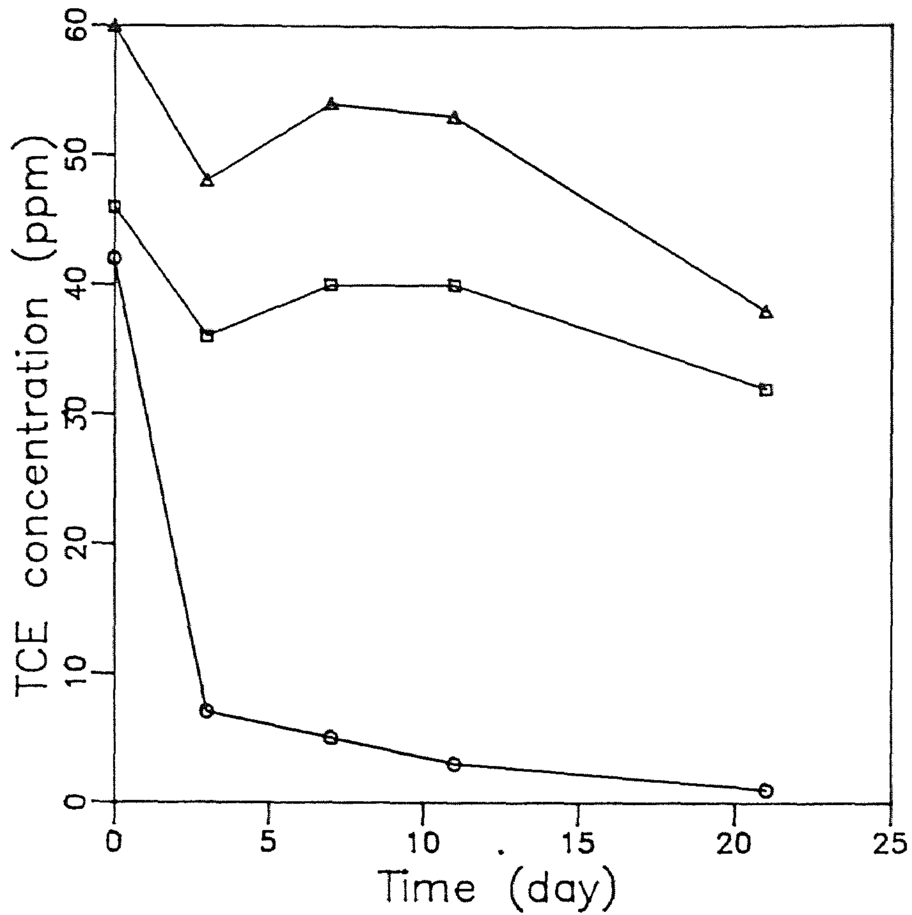
This result means that the surfactant works very well to hold volatile TCE in the liquid phase and therefore effectively solves the problem of high volatility,

which causes experimental difficulties in the whole research field. Nevertheless, it does not produce a positive change in TCE degradation. All the TCE concentrations in the last four bottles, which contained biomass and different initial TCE concentrations, decreased slowly just as that in the No. 2 bottle. According to Fig. 13, the TCE concentration in the No. 2 and No. 4 bottles decreased at almost the same rate.

Table 7 TCE Variation in Anaerobic Experiment with N-57 (ppm)

Time (day)	0	3	7	11	13
No.1 (no biomass, no N-57)	42	7	5	3	1
No. 2 (no biomass)	60	48	54	53	38
No. 3	20	17	20	20	14
No. 4	46	36	40	40	32
No. 5	52	50	50	56	40
No. 6	134	126	122	128	83

Fig. 13 TCE Variation in Anaerobic Experiment with N–57



No.1 (no biomass, no N–57)

No.2 (no biomass & N–57)

No.4 (with biomass & N–57)

CHAPTER 4

DISCUSSION AND RECOMMENDATIONS

The results of these experiments show that the NJIT treatment systems do not treat TCE under the conditions similar to those developed for other compounds at the high rates experienced for those compounds.

However, the shaker flask experiments and the experiment with phenol oxygenase show more positive indications.

Due to significant differences between methods reported in the literature, direct comparison of TCE degradation rates between different organisms is difficult. But a rough comparison between the rates observed in the shaker flask experiments and the previously published rates and the rates calculated from data reported is given in Table 8.

The TCE degradation rates for activated sludge are 0.36, 0.49, 0.44, 0.32 nmol/min per mg of dry biomass with sodium acetate, phenol, toluene, and sodium benzoate as inducer, respectively. They are greater than the rates for bacterial strains 46-1, G 4, and *Alcaligenes eutrophus* JMP134. This means the induction of these inducers works positively on TCE biodegradation.

Table 8 Comparison of Biodegradation Rates

Substrate	Biodegradation rate (nmol/min per mg of dry biomass)
Pseudomonas putida F1	1.8 [(a)]
Methylosinus trichosporium OB3b	20 [(b)]
Strain 46-1	0.011 [(c)]
G4	0.024 [(d)]
Alcaligenes eutrophus JMP134	
phenol-dependent pathway	0.2 [(e)]
plasmid-encoded 2,4-dichlorophenoxyacetic pathway	0.06
Nitrosomonas europaea	1.1 [(f)]
Pseudomonas cepacia G4	8 [(g)]
NJIT activated sludge	
with sodium acetate	0.36
with phenol & biomass*	0.49
with toluene	0.44
with sodium benzoate	0.32

(a): Published (Wackett and Gibson).

(b): Published (Tsien et. al.).

(c): Calculated from data reported (Little et. al.).

(d): Calculated from data reported (Nelson <u>et. al.</u>).

(e): Published (Harker and Kim).

(f): Published (Arciero <u>et. al.</u>).

(g): Published (Folsom <u>et. al.</u>).

The activated sludge has been determined, by coworkers, to degrade phenol, BTX, and several chlorinated phenol compounds (Lakhwala <u>et. al.</u>). They concluded that phenol oxygenase in the activated sludge was involved in the biodegradation of these compounds. This enzyme was used in the experiment with TCE and the results indicated that although the TCE was not degraded significantly, the enzyme was still active and consumed oxygen in the presence of TCE.

Besides, there are two problems found in TCE research, the low solubility in water and high volatility of TCE. From the data-base, TCE's solubility in water is around 1100 ppm at 25°C. In this study, a TCE solution of maximum concentration about 900 ppm was obtained by keeping the solution stirred.

The high volatility of TCE caused problems in this work. One way to solve this problem is to seal the culture vessel as tightly as possible. This is not very effective in aerobic experiments because the system has to be open for sample analysis. Another method, using sur-

factant N-57, is determined to be very effective in both aerobic and anaerobic experiments. It does not even cause any trouble in TCE analysis with GC as feared. It is suggested that in the further research of TCE, surfactant N-57 is a good way to overcome the trouble caused by the high volatility of TCE.

Three recommendations are presented:

(1). Repeat the shaker flask experiments shown in Fig.9, keeping TCE volatility at a minimum by using surfactant N-57.

(2). Repeat the experiment with phenol oxygenase for a longer time.

(3). Repeat the anaerobic experiments shown in Fig.13, with lower TCE concentrations of 10 ppm, 5ppm, and 1 ppm.

CHAPTER 5

CONCLUSIONS

From the results of these experiments, a few conclusions can be drawn.

(1). In the acclimation with TCE, the membrane reactor experiments, the microassay reactor experiments, and the anaerobic experiments, TCE biodegradation rates were far lower than the typical biodegradation rates for phenol and other substrates in NJIT Biodegradation System.

(2). In the shaker flask experiments, TCE was degraded with the use of inducers and the degradation rates were greater than the rates reported in literature for some other bacteria. The rates are 0.36, 0.49, 0.44, 0.32 nmol/min per mg of dry biomass with sodium acetate, phenol, toluene, and sodium benzoate, respectively.

(3). In the experiment with enzyme phenol oxygenase, TCE was not degraded significantly, but the enzyme was active and consumed oxygen in the presence of TCE.

(4). Surfactant N-57 was determined to be effective to keep TCE in the liquid phase.

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