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Optimization of TCE Degradation in Counter-Diffusional, Membrane-Attached, Methanotrophic Biofilms for Remediation of Contaminated Groundwater

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Abstrak

Penelitian ini dimaksudkan untuk mengembangkan, mengevaluasi dan sekaligus mengoptimasi potensi dari suatu reaktor inovatif "counter-diffusional membrane biofilm" untuk pengolahan tanah dan air tanah yang tercemar oleh bahan kimia pelarut industri TCE. Tujuan dari penelitian ini adalah untuk mengkaji dan mengevaluasi faktor-faktor disain dan operasional yang mempengaruhi kecepatan dan keberlanjutan proses penguraian dan transformasi TCE.

Sebagai langkah awal untuk mencapai tujuan penelitian, koefisien transfer material dari reaktor diukur melalui serangkaian percobaan, disain 2^3 percobaan laboratorium dilakukan, model matematika dan simulasi komputer untuk mendeskripsikan profil konsentrasi dari substrat dan TCE dalam lapisan biofilm dikenalkan.

Nilai maksimum yang konsisten dari pemisahan TCE sebesar 205 □mol/m²/day dicapai dengan sukses pada tingkat penggunaan gas metan (CH_4) sebesar 11,67 mmoles/m²/hr dengan tingkat pembebanan TCE mendekati 400 □mol/m²/day. Kurva Normal Probabilitas dan Pareto menunjukan bahwa Tekanan Pasial Gas Methane (P) dan Bilangan Reynolds (Re) mempunyai efek yang signifikan positif terhadap kecepatan penguraian TCE. Rata-rata efisiensi penguraian (pemisahan) TCE berada pada kisaran 78,6 – 94,7%.

***Kata kunci**: Bioremediasi, biofilm,model komputer & matematik, air tanah tercemar, aliran difusi berlawanan, membran, gas metan, bakteri methanof, pelarut kimia dan TCE.*

Abstract

This study develops, evaluates, and optimizes the potential of a novel "counter-diffusional" membrane biofilm reactor to biologically treat and remove trichloroethylene (TCE) from contaminated soil and groundwater caused by industrial activities (industrial solvent). The objectives of the research are to investigate and evaluate design and operational factors affecting the sustainability and degradation rates of TCE transformation in a counter-diffusional membrane-attached methanotrophic biofilm reactor system.

As a first step attaining this objective, an overall mass transfer coefficient of the bioreactor was developed, a 2^3 laboratory experimental design have already conducted, and the development of a mathematical model and computer simulation describing the concentration profile of substrates and TCE within the biofilm has been introduced.

A maximum sustainable TCE removal flux of 205 □mol/m²/day was successfully attained when the CH_4 utilization rate was 11.67 mmoles/m²/hr, the TCE loading rate was approximately 400 □mol/m²/day. Normal probability plot and pareto chart indicated that methane partial pressure (P) and hydraulic Reynolds's numbers (Re) have important and significant positive effects on the TCE degradation rates. The average percentage of TCE removal efficiency falls between 78.6 and 94.7%.

***Keywords**: Bioremediation, biofilm, computer & mathematical model, contaminated groundwater, counter-diffusional, membrane, methane, methanotrophic, solvent and TCE.*

PERPUSTAKAAN PUSAT
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1. Introduction

Trichloroethylene (TCE; C_2HCl_3), a common industrial solvent, is a chlorinated aliphatic hydro-carbon (CAH) that has been widely used for degreasing aircraft engines, automobile parts, and electronic components [7]. TCE also has been used as a household and industrial dry-cleaning solvent as well as an extractive solvent in foods industry [11]. Its relative chemical stability, non-flammability, volati-lity, and poor water solubility make TCE a very useful solvent.

Because of widespread industrial usage, inadequate disposal techniques, and accidental spillages, CAHs and their transformation products such as TCE, dichloroethylene (DCE), and vinyl chloride (VC) have become crucial contaminants in soil and groundwater. CAHs are important environmental pollutants because they are commonly detected in groundwaters and industrial wastestreams, are carcinogenic and/or toxic, are resistant to biodegradation, and can contribute to photochemical smog formation and stratospheric ozone depletion.

Although no known bacteria use TCE as a growth substrate, numerous studies have demonstrated that rapid co-metabolic degradation rates can be attained in methanotrophic cultures because of the broad substrate specificity of soluble methane monooxygenase (MMO). However, the following problems must be overcome to effectively treat TCE in methanotrophic bioreactor system:

- Methane (CH_4), a sparingly soluble gas, must be transferred to the micro-organisms as efficiently as possible.
- If methanotrophic cells are simultaneously exposed to CH_4 and TCE, the two compounds will compete for soluble MMO active sites, resulting in decreased TCE degradation rates.

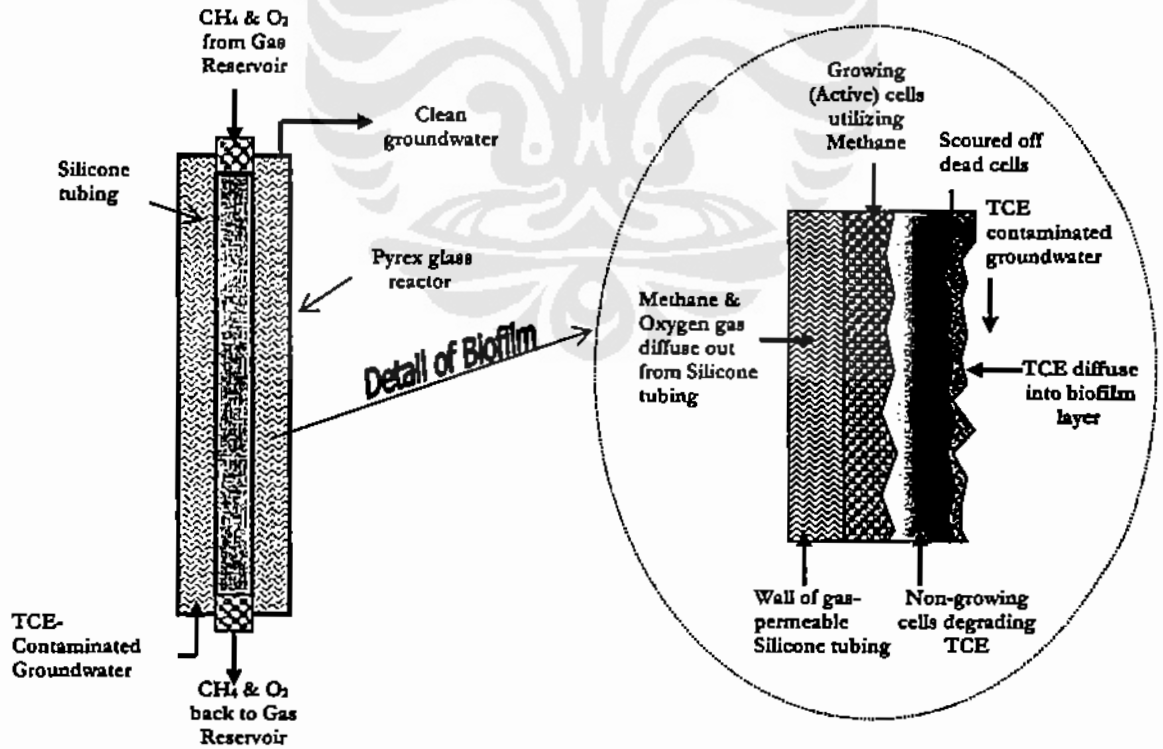

Figure 1.
Detail of The Counter-Diffusional Membrane-Attached Methanotrophic Biofilm and Biofilm Cell Stratification

Cultivating a methanotrophic biofilm on a gas-permeable silicone membrane could potentially solve the difficulties described above. The problem of low CH_4 solubility would be overcome because diffusion through the membrane would result in 100% transfer efficiencies. The problem of CH_4 competing with TCE for sMMO active sites would be avoided with this technique because the exterior of the biofilm would have high TCE concentrations and low CH_4 concentrations, while the interior of the biofilm would have low TCE concentrations and high CH_4 concentrations; therefore TCE and CH_4 competition could be minimized

In this research a novel and potentially important methanotrophic biofilm design and operational factors for overcoming some key problems inherent in co-metabolic bio-degradation of CAHs were studied.

The objectives of the research are to investigate and evaluate design and operational factors affecting the sustainability and degradation rates of TCE transformation in a novel counter-diffusional membrane attached methanotrophic biofilm (CDMAMB). This configuration is applied to optimize the degradation process of TCE to treat contaminated groundwater.

2. Gaps in Knowledge

It was investigated and demonstrated that the CDMAMB reactor system configuration could overcome substrate mass transfer limitation, inactive biomass accumulation, and competition between primary and secondary substrates for the operative co-metabolizing enzyme problems [2], [8]. Compared with conventional methanotrophic bioreactor, the system that incorporating methanotrophic biofilm could be advantageous in treating CAH-contaminated groundwater; however, there is very little information about design and operational factors that available for a full scale application of the system in bioremediation fields [15].

Biofilm reactors are advantageous because they tend to have higher biomass densities than dispersed and suspended growth systems and thus allow for shorter wastestream retention times and become more effective for accumulating specific slow-growing bacteria [12].

Previous researches demonstrate that the maximum TCE degradation rate in a methanotrophic bioreactor system was controlled by methanotrophic biomass production rate and the TCE transformation capacity (mass of TCE degraded per mass of cell inactivated during process). Reported biomass production rates in methanotrophic biofilm reactors have been low due to CH_4 and O_2 mass transfer limitations [6], [8]. Therefore, increasing gas mass transfer for substrates (primary and secondary) within the system will potentially increase the biomass production that will also increase TCE degradation rates.

All these studies provide valuable insight into TCE degradation in methanotrophic biofilm reactors such as biomass dynamic within biofilm system. A mathematical model along with computer simulation will help us understand the dynamic of biofilm process within CDMAMB system

3. Literature Review

In general, bacteria cannot directly metabolize most CAHs, they can be fortuitously co-metabolized by methane utilizing bacteria (methanotrophs) due to their possessing of a methane mono-oxygenase (MMO) enzyme with broad substrate specificity.

Methanotrophic microorganisms, using CH_4 as a primary substrate, offer several advantages over other aerobic oxygenase-producing bacteria in degrading common groundwater contaminants, such as certain CAHs. Due to their high conversion-rates as well as their capability of using CH_4, as a nontoxic oxygenase inducers in TCE co-metabolic degradation process, methano-trophic microorganisms such as *Methylosinus trichosporium (Mt) OB3b* recently have been intensively investigated [4], [7], [15].

Methanotrophic microorganisms have been shown to produce a specific enzyme of

MMO. Although CH_4 is the only substrate that will support rapid methanotrophic growth, MMO has broad substrate specificity and will fortuitously oxidize a range of generally recalcitrant hydrocarbons, including chlorinated methanes, ethanes, and ethenes [10].

Studies have shown that membrane biofilm provide a number of advantages in biological treatment as well as a support surface for microbial growth [1], [9]. These include operational flexibility, reduced energy requirements, and less stripping of volatile compounds to atmosphere. However, methanotrophic TCE co-metabolism has to overcome several problems in order to optimize the TCE degradation process in a biofilm system, including mass transfer limitation [5], [7], product toxicity [10], and competitive inhibition [15].

A counter-flux gas-permeable membrane pressurized with CH_4 and O_2 was used to resolve common problem found in conventional methanotrophic biofilm systems. Using this innovative configuration, methanotrophic biofilm was cultivated to attain greater sustainable TCE degradation rates than that in a conventional methanotrophic biofilm reactor [2], [8].

3.1. Methanotrophic Biofilm

Many types of microorganism enzymes involved in dehalogenation reaction are known, and their properties have been reviewed recently [4], [14], [15].

One of the promising applications of methanotrophic microorganisms is the cleanup of polluted groundwater that is contaminated by low molecular weight halogenated hydrocarbons, such as chloroform, TCE, DCE, chloropropanes, and 1,1,1-trichloroethane.

Further work was stimulated by the observation of Wilson and Wilson (1985), who demonstrated that methane could stimulate biological TCE degradation in soil. Since then, various pure cultures of methanotrophic microorganism were found capable of co-metabolic transformation of halogenated hydrocarbon [15], [16].

It will be necessary for successful application to develop ways to specifically stimulate methanotrophs that show high conversion rates. Reducing the availability of copper could be a way to stimulate the conversion rates, but will be difficult to manipulate if copper is present in the material to be treated.

3.2. Aerobic Treatment of CAHs

CAHs containing oxygen, such as alkanoic acids, are known to serve as primary substrate (carbon and energy sources) for aerobic microbes. However, only very few microbes are able to use monooxygenated CAHs as a growth substrate [8]. Aerobic microbes are not believed to have the ability to use the more electrophilic **tri-** and **tetra-**chlorinated CAHs as a growing substrate. However, a variety of bacteria have been shown to be capable of cometabolizing CAHs, such as methanotrophs propane oxidizers, toluene, cresol and phenol oxidizers, methane-oxidizing cultures, and an ammonia oxidizer [10].

MMO is one of the four enzymes required for the conversion of methane to carbon dioxide (CO_2). There are two types of MMO enzymes; soluble-MMO and particulate-MMO. There are two categories of methanotrophs, which have been identified (type I and type II) based on several characteristics. These characteristics include the pattern of internal membranes, carbon assimilation pathway, and predominant fatty acid chain length. One strain, *Methylococcus capsulatus*, which is classified as type X, has characteristics of both MMOs and the second one, *Mt*-OB3b, produces solely the sMMO enzyme [8], [14].

MMO is a large enzyme that consists of three proteins. Certain methanotrophic bacteria produce MMO that capable of catalyzing the oxidation of methane into methanol and thus provide a source of carbon and energy for the bacteria [14].

Two other types of aerobic bacteria that are capable to degrade trichloroethylene are

Burkholderia cepacia G4 (formerly known as *Pseudomonas cepacia*) and *Pseudomonas putida* F1. In addition, a cloning to different strains of *P. putida* F1 was conducted. The result was a specific growth rate that affected sMMO activity; hence, TCE degradation was enhanced at higher growth rates.

The first step of the biological pathway of methane oxidation is the mono-oxygenase-catalyzed oxidation of methane to methanol, which requires O_2 and reducing equivalents in the form of $NADH+H^+$, such as the epoxidation of TCE does. Unlike methane catabolism, subsequent oxidation steps do not regenerate the NADH down the pathway. When adding formate, an intermediate in the catabolic pathway, as a reductant to pure and mixed methanotrophic cultures, the rate and total amount of TCE degraded increased; however, this high level of activity declined quickly. An advantage of using formate as a source of reducing equivalents is that, unlike methane, it does not compete with TCE for the catalytic site of MMO. Formate acts to stimulate the activity of existing MMO, which results in an increasing TCE degradation [8].

4. Material and Methods

4.1. Experimental System Set-Up

The previous preliminary studies have provided valuable insight for the design of the experimental systems required to successfully meet the research objectives. Hence, the set-up of the experimental system should allow for: (1) independent control of CH_4, O_2, and N_2 partial pressures in the gas reservoirs, (2) prevention of CO_2 accumulation in the gas reservoirs, (3) prevention of N_2 back-diffusion through membranes; (4) independent control of nutrient and TCE concentrations in the liquid phase and (5) independent control of hydraulic residence times and Reynolds numbers (Re).

A CDMAMB reactor configuration was used in these experiments as shown in Figure 3. The reactor is constructed from Pyrex glass tubing with an internal diameter (ID) of 39 mm and total volume of 358-mL. The total length of the glass reactor is 30 cm. The silicone permeable tubing was placed in the center inside of the glass reactor unit. The silicone tubing ID is 16 mm, with a 3.2-mm wall thickness, and an effective length of 26.2 cm.

The outside of the silicone tubing served as the substratum for the biofilm development. Methane and oxygen gasses are pressurized and diffused through the silicone-tubing wall to the interior of the biofilm.

A nutrient solution (NMS) was pumped from an influent reservoir through the tubular biofilm reactor. The flow distribution is designed to yield uniform hydraulics. The effective hydraulic volume of the reactor is approximately 170 mL (under pressure).

By using a recycle pump to attain nearly completely mixed hydraulic condition within membrane module as well as to attain target Reynolds numbers, part of the effluent was pumped back to the influent line at different recycle ratios. The final effluents were collected in a 16-L effluent reservoir.

4.2. Bioreactor Start-up

After some abiotic experiments, the bioreactor was seeded with *MT*-OB3b obtained from the American Type Culture Collection (ATCC). *Mt*-OB3b is a strict methanotrophs that use methane as both a carbon and energy source. The suspended growth bacteria were then continuously recycled past the silicone tubing until visible biofilm is formed on the outside surface.

4.3. Primary Substrate and Nutrient Supply

For co-metabolism to occur, an active population of microorganisms having the co-metabolizing enzymes must be present. This means that the appropriate primary substrates for growth and maintenance of these organisms must also be present. Since *Mt*-OB3b is a strict aerobe-methanotrophs, CH_4 and O_2 were used as primary substrates in this study.

The methane-oxidizing culture used in these studies was grown in a NMS medium [4]. The NMS solution is made up of 996.60 mg/L $NaNO_3$, 179.81 mg/L K_2SO_4, 36.96

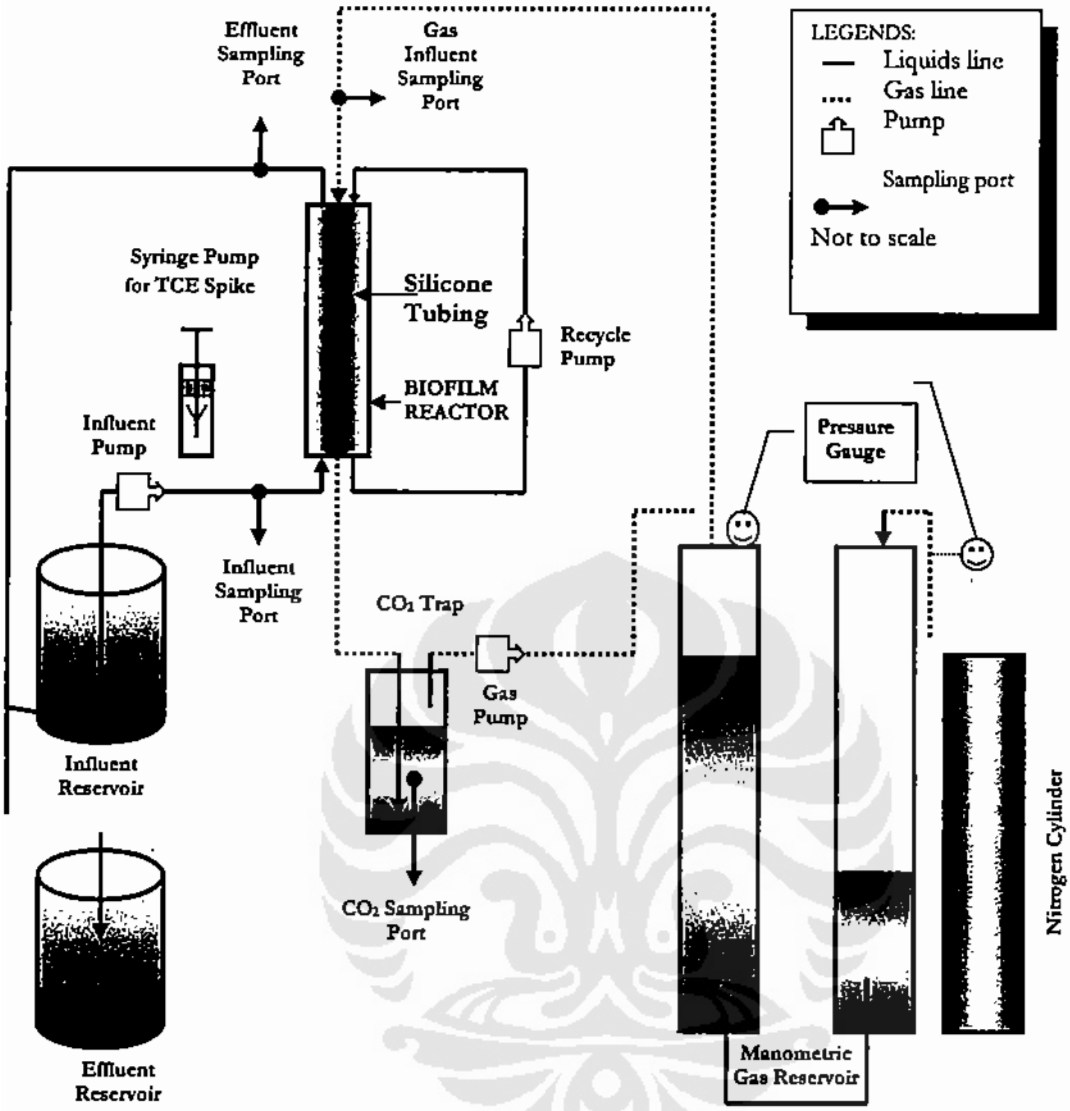

Figure 3
Counter-Diffusional Membrane-Attached Methanotrophic Biofilm Reactor Set-Up

mg/L $MgSO_4 \cdot H_2O$, 12.05 mg/L $CaSO_4 \cdot 2H_2O$, 22.24 mg/L $Fe_2SO_4 \cdot 7H_2O$, 886.20 mg/L $Na_2HPO_4 \cdot 2H_2O$, 530.78 mg/L KH_2PO_4, and some trace compound solution. The trace compound solution contained 0.166 mg/L KI, 0.575 mg/L $ZnSO_4 \cdot 7H_2O$, 0.338 mg/L $MnSO_4 \cdot H_2O$, 0.124 mg/L H_3BO_3, and 0.947 mg/L $CoMoO_4 \cdot H_2O$

4.4. Experimental Method

Several experiments were conducted to quantify CH_4 & O_2 utilization and CO_2 production rates; TCE degradation rates; and biofilm detachment. The studies investigated the relationship between three basic operational variables (liquid Reynolds numbers Re, CH_4 partial pressures P, and TCE organic loading rates L_{TCE}) toward TCE removal rates.

1. Quantifying CH_4 and O_2 Utilization and CO_2 Production Rates

These studies were conducted to test the hypothesis that the counter-diffusional biofilm configuration could be exploited to maximize rates of CH_4 and O_2 transfer, and sustainable TCE degradation.

2. Quantifying TCE Degradation Rates

TCE degradation rates were quantified by routinely measuring the influent and effluent TCE concentrations. TCE and non-polar metabolites will be extracted into the hexane and then injected onto a gas chromatograph (GC) equipped with an electron capture detector (ECD). Additional influent and effluent samples were collected and analyzed for chloride ion concentrations using an ion chromatograph (IC).

3. Quantifying and Characterizing Biofilm Detachment

The rates of biofilm detachment (the combined effects of biofilm erosion, sloughing, and abrasion) were quantified by routinely measuring the effluent total suspended solids (TSS) concentrations.

4. Fitting the Experimental Data into a Mathematical Biofilm Model

The structured model for co-metabolic TCE degradation in methanotrophic biofilm developed by Anderson and McCarty (1994) as well as the mathematical model for the counter-diffusional membrane attached methanotrophic biofilm reactor developed by Clapp & Noguera (2000) were adapted to fit the experimental data and simulate the TCE degradation process. These models were used to mathematically evaluate the effects of varying CH_4/O_2 partial pressure, hydraulic Reynolds numbers (Re), and TCE loads on active biofilm thickness. Matlab® computer software were used for the simulation and interpretation of the experimental data.

5. Optimizing TCE Degradation Process

Designed experiments can be used to systematically investigate some operational parameters that influence the sustainability and optimum performance of TCE degradation process in the biofilm reactor.

Optimization of a sustained TCE degradation process within biofilm system was performed using Design of Experiment (DOE) method. Statistical data analysis for DOE was focused on every operational and design parameters that being used in this study. Minitab® statistical software was used in designing of experiments, data analysis, and data and result plots.

6. Quality Assurance

A quality assurance research plan was prepared at the start of the research. Negative and positive controls were used whenever possible. Abiotic CH_4 and TCE mass balance tests were performed with the membrane systems to verify that no significant leaks exist. Standards were analyzed concurrently with all experimental samples. Data were reported as means and sample standard deviations. Accuracy during analysis was obtained by appropriate calibration of the instruments.

4.5. Analytical Methods

1. Gas Chromatograph

A Varian 3600CX gas chromatograph (GC) was used to measure TCE, trichloro-ethanol (TCOH), and trichloroacetaldehyde (TCAA) concentrations in hexane-extracted liquid influent and effluent samples, as well as TCE concentration in the extract gas-phase samples. As shown in Figure 4, the GC was equipped with a Varian 8200 carousel-type autosampler a Varian split/splitless injector and a Varian pulsed electron capture detector (ECD). Glass vials (2 mL) with Teflon-coated septa were used in the autosampler.

Figure 4
Gas Chromatograph Varian 3600CX

2. Ion Chromatograph

Cl influent and effluent concentrations were determined using a Dionex DX 500 Ion Chromatograph (IC) equipped with a conductivity detector, a self-regenerating eluent suppressor, a IonPac AS11 analytical column, and a IonPac guard column.

Figure 5
Dionex DX 500 Ion Chromatograph

6. Result and Discussions

6.1. Modeling and Computer Simulation

As a first step attaining the objective, an overall mass transfer coefficient of the bioreactor was developed, abiotic and biotic laboratory experiments have conducted, and the development of a mathematical model and computer simulation describing the concentration profile of substrates and TCE within the biofilm has been introduced.

In recent years mathematical and computer modeling of biofilm has been developed in order to better understand biofilm system behavior in mass transfer, diffusion and utilization. Clapp's model (1999) is based on McCarty's model (1984) that simulated concentration profiles in a system with a traditional biofilm. Clapp simulated TCE and CH_4 concentration profiles by using substrate utilization and competitive inhibition equations and neglected the influence of O_2 by assuming that it is not the limiting factor of the process.

Clapp added the non-steady-state biomass accumulation terms to the model and defined a steady-state biomass thickness corresponding to CH_4, O_2, and TCE concentrations. In addition, they calculated TCE degradation rates with TCE influent concentrations ranging between 0 – 500 µmol/L. The model described in this study is based on Clap's model. The model primarily focuses on concentration profiles of CH_4, O_2, and TCE to find potential for process optimizations.

The kinetics of the degradation of TCE and CH_4 and O_2 utilization was modeled for the CDMAMB reactor. The flux and concentration profile of CH_4 and TCE were calculated and simulated using a model of biofilm diffusion and co-metabolic degradation kinetic. The model simulated the effects of competition inhibition between methane and TCE on the enzymatic oxidation by MMO enzyme.

The substrate utilization rate in a biofilm can be written by applying the Monod kinetics as follows:

$$r_s = -\frac{q_s \cdot C_S \cdot X_a}{K_s + C_S} \qquad (1)$$

where r_s is the reaction rate (mmol/l·d), K_s the half-velocity constant for the substrate (mmol/l), C_S is the carbon substrate concentration in the biofilm (mmol/l), and q_s is the substrate maximum utilization rate (mmol/mg d).

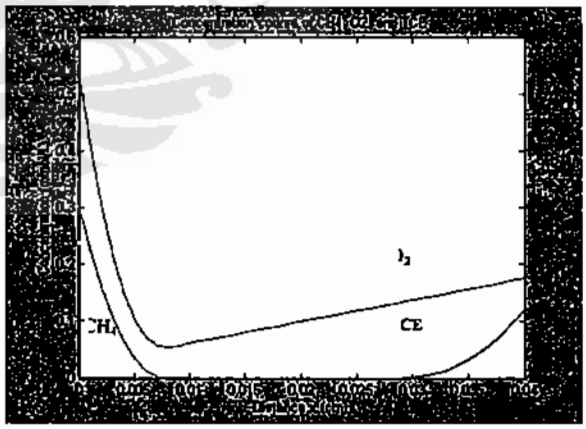

Figure 6
Concentration Profile of CH_4, O_2, and TCE In a Biofilm System (Biofilm Thickness of 0.03 cm)

The simulation (Figure 6) shows that low O_2 concentrations in the gas phase can support TCE diffusion through the biofilm. The TCE diffusion also depended on the dissolved O_2 concentration in the water and

the biofilm thickness. A low O_2/CH_4 ratio can lead to O_2 becoming the limiting factor for the process and support TCE diffusion through the biofilm into the membrane.

6.2. TCE Degradation Rates

A series of laboratory experiments was performed to investigate the relationships among CH_4 partial pressure, TCE loading rate, and hydraulic shearing forces (Re) at exterior of biofilm toward methanotrophic cell production, TCE degradation rates, and biofilm detachment rates to obtain the optimum values of those parameters for the sustainability of the TCE degradation rates. These studies were conducted by cultivating methanotrophic biofilm on gas-permeable membranes pressurized with CH_4 and O_2 and then subjecting them to a range of CH_4 partial pressures (P_{CH4} =0.4 and 0.6 atm), hydraulic Reynolds numbers (Re = 115 and 225), and TCE loading rates (L = 400 and 800 μmol/m^2/day). The CH_4/O_2 utilization, CO_2 production, biofilm detachments rates, and TCE degradation rates were investigated and quantified.

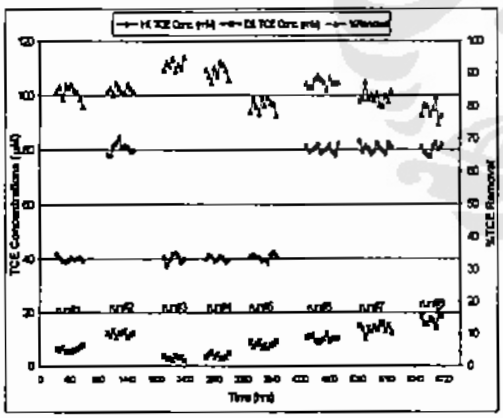

Figure 7.
Influent and Effluent TCE Concentrations and TCE Removal Rates During Experiments

A maximum sustainable TCE removal flux of approximately 205 μmol/m^2/day was successfully attained with the membrane-attached methanotrophic biofilm when the CH_4 utilization rate was 11.67 mmoles/m^2/hr, the TCE loading rate was 400 μmol/m^2/day, CH_4 partial pressure was 0.6 atm, and the hydraulic Reynolds number was 225. This TCE removal flux was 1.5 times greater than attained in previous studies by Clapp (1999).

This result supported the hypothesis that the attainment of greater CH_4 utilization rates per biofilm surface area would translate into higher sustainable TCE degradation rates per biofilm surface area.

As expected, the experimental results also demonstrated that higher biofilm detachment rate of 32.4 mg/L in measured of effluent TSS occurred when the CH_4 utilization rate was 10.52 mmoles/m^2/hr, the TCE removal efficiency was 92.7%, the TCE loading rate was 800 μmol/m^2/day, CH_4 partial pressure was 0.6 atm, and the hydraulic Reynolds number was 225. This results support the hypothesis that applying high hydraulic shearing forces (Re) on the surface of biofilms with maximum TCE loading rate (800 μmol/m^2/day) would cause outer layer of biofilms that contain most inactive cell removed much easy and faster from the surface of biofilms

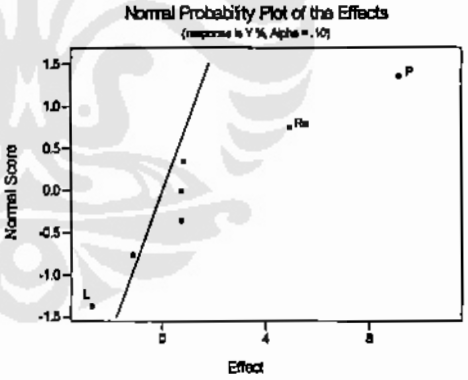

Figure 8.
Normal Plot of The Main and Interaction Effects of P, Re, and L on TCE Removal Efficiencies

Normal probability plot and pareto chart indicated that methane partial pressure (P) and hydraulic Reynolds's numbers (Re) have important and significant positive effects on the TCE degradation rates. The estimated main effects for the methane partial pressure (P), hydraulic Reynolds's numbers (Re), and TCE loading rates (L) were 4.90, 9.15, and –2.75, respectively. There is no interaction effect occurring between the parameter observations. In this case, every parameter

acts individually on TCE degradation rates. The average percentage of TCE removal efficiency falls between 78.6 and 94.7%.

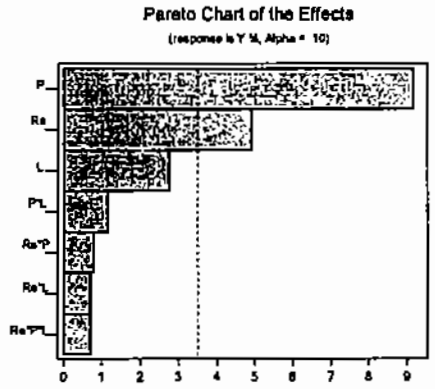

Figure 9.
Pareto Chart of The Main and Interaction Effects of P, Re, and L on TCE Removal Efficiencies

Experimental results also demonstrated that TCE back-diffusion thorough the membrane occurred when TCE loading rates were increased from 400 to 800 $\mu mol/m^2/day$. The TCE back-diffusion significantly affected the methane utilization rates as well as TCE degradation rates. These results support the hypothesis that not only TCE byproduct toxicity would be the limiting factor in attaining higher sustainable TCE degradation, but also the TCE back-diffusion even though higher methane partial and total gas pressure was used during the experiments.

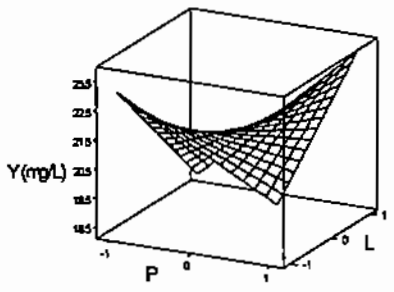

Figure 10.
Cube Plots of The Main and Interaction Effects of P and L on Biofilm Detachment Rates

Normal probability plot indicated that methane partial pressure (P), hydraulic Reynolds's numbers (Re), and TCE loading rates (L) seem to be important and significant effects on the CH_4 utilization rates. The estimated main effects for the methane partial pressure (P), hydraulic Reynolds's numbers (Re), and TCE loading rates (L) were 1.35, 1.26, and - 1.14, respectively.

The cube plot that shows that the biofilm detachment rates falls between 12.1 and 32.7 mg/L of measured Total Suspended Solids (TSS) in the bioreactor's effluents.

7. Conclusions

Intensively study combine with model development and computer simulation would give a better understanding in degrading TCE biologically with cost effective and timely manner. Several conclusions were drawn from the study.

- The chloride results supported the TCE data to demonstrate that the biofilm reactor was capable of degrading TCE and also indicated that most of the TCE degraded was mineralized to chloride.
- The model and computer simulation shows that low O_2 concentrations in the gas phase can support TCE diffusion through the biofilm. The TCE diffusion also depended on the dissolved O_2 concentration in the water and the biofilm thickness.
- The model can be used as a tool optimizes the TCE degradation process in this counter-diffusional membrane-attached methanotrophic biofilm.

References

[1]. Ahmed, T., and Semmens, M. J. "Use of transverse flow hollow fibers for bubbleless membrane aeration." *Water Research*, 1996, 40, 440-446.

[2]. Ali, F., Clapp, L., Regan, J. M., Newman, J., Noguera, D. R., and Park, J. K. "Activity, Structure, and Stratification of Membrane-attached Methanotrophic Biofilms Cometabolically Degrading TCE."

Water Science & Technology, 1998, 39 (7), 153-161.

[3]. Ali, Firdaus, "Mass transfer in a methanotrophic gas permeable membrane counter-Flux biofilm reactor for the treatment of trichloroethylene (TCE) in groundwater" Master Thesis, University of Wisconsin-Madison (USA), 1998

[4]. Alvarez-Cohen, L. "Application of methanotrophic oxidations for the bioremediation of chlorinated organics. in: microbial growth on C1 compounds." J. C. Murrell, ed., Intercept, Ltd., Andover, 1994, UK, 337-350.

[5]. Anderson, J. E., and McCarty, P. L. "Model for treatment of trichloroethylene by methanotrophic biofilms." *Environmental Engineering*, 1994, 120, 379-399.

[6]. Arcangeli, J. P. "Biodegradation of *cis*-1,2-dichloroethylene at low concentrations with methane-oxidizing bacteria in a biofilm reactor. Water Res., 1996, No. 30, pp-1885-1893, 1996" *Water Research*, 30, 1885-1893.

[7]. Clapp, L., Hartono, R., Newman, J., and Park, J. K. "Trichloroethylene Degradation in a Novel Membrane Bioreactor." *The 69th Annual WEF Conference and Exposition*, Dallas, Texas, 1996, 344-357.

[8]. Clapp, L. W. "Sustained TCE degradation in counter-diffusional membrane-attached methanotrophic biofilms," Ph.D. Disertation, UW-Madison, Madison, 1999

[9]. Cote, P., Bersillon, J.-L., and Huyard, A. "Bubble-free aeration using membranes: Mass transfer analysis." *Membrane Science*, 1989, 47, 91-106.

[10]. Fox, B. G., Borneman, J. G., Wackett, L. P., and Lipscomb, J. D. "Haloalkene oxidation by the soluble methane mono-oxygenase from methylosinus trichosporium OB3b: Mechanistic and environmental implications." *Biochemistry*, 1990, 29, 6419-6427.

[11]. Huff, J. E. "New evidence on the old problems of trichloroethylene." *Industry Medical.*, 1971, 40(25).

[12]. Korber, D. R., and Lawrence, J. R. "Growth of microorganisms on surfaces. In: Microbial biofilms." H. M. Lappin-Scott and J. W. Costerton, eds., Cambridge University Press, Cambridge, 1995. U.K., 15-35.

[13]. McCarty, P. L., Kissel, J. C., and Street, R. L. "Numerical simulation of mixed-culture biofilm." *Journal of Environmental Engineering*, 1984. 110, 393.

[14]. Moran,R. A. "Methane monooxygenase." Personal Website, University of Wisconsin-Eau Claire, 1997

[15]. Speitel, G. E., and Segar, R. L. "Cometabolism in biofilm reactors." *Water Science & Technology*, 1995.31, 215-225.

[16]. Tsien, H.-C., Brusseau, G. A., Hanson, R. S., and Wackett, L. P. " Biodegradation of TCE by methylosinus trichosporium OB3b." *Applied & Environmental Microbiology*, 1989 No. 55, 3155-3161.

[17]. Urbain, V., Trouve, E., and Manem, J. "Membrane bioreactors for municipal wastewater treatment and recycling." *Proceedings of the 67th Annual WEF Conference and Exposition*. New Orleans, LA, 317-323.

[18]. Wilson, J. T., and Wilson, B. H. "Biotransformation of trichloroethylene in soil." *Applied & Environmental Micro-biology*, 1985 No. 49, 242-243.