3921

AEROBIC AND ANAEROBIC PROCESS FOR PCBs REMOVAL FROM AQUEOUS AND OIL PHASES

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ABSTRACT

Although the manufacture and use of polychlorinated biphenyls (PCBs) were banned, they are still widespread in the environment. Technologies available for PCBs remediation are limited and often impractical. In this study, aerobic and anaerobic treatment processes were investigated for PCBs removal from both aqueous and oil phases, respectively. The cell immobilization method, Entrapped Mixed Microbial Cells (EMMC) technology, and Intermittent Up-Flow Anaerobic Bioreactor (IUFAB), were introduced for PCBs removal from aqueous and oil phases, respectively.

The investigation for using EMMC technology to remove PCBs from the aqueous phase indicates high efficiency that PCBs removal efficiency of more than 90% can be achieved with HRT of 1 day by using aerobic EMMC up-flow reactor with EMMC carrier packing ratio of 40%. Also, EMMC technology shows high potential to treat the PCBs contaminated oil directly by using EMMC carriers with suspended culture aerobic batch reactor.

For PCB contaminated oil, a glass column reactor to avoid the possible adsorption of PCBs, having effective volume of 2 liters with 7000mg/L MLSS of initial activated anaerobic sludge, was installed as an IUFAB. Methanol was used

as the sole carbon source. After 45 days of operation with HRT of 1 day and COD loading rate of 1g/L/day, COD removal efficiency of more than 95% was achieved. Then, PCBs contaminated oil was then introduced into IUFAB with COD loading rate of 0.25 g/L/day and oil loading rate of 3.2 g/L/day. Based on the HRT of 4 days, and the ambient temperature of 23°C ± 2°C, PCBs removal efficiency of 50%-65% is able to achieve for a period of 6-month investigation. A mass balance of calculating the PCB removal efficiency in the oil phase was conducted. The value of the calculated removal efficiency is closed to the actual measurement of PCB removal efficiency from the steady state condition. This removal is due to the biodegradation based on the measurement of biogas production and chloride concentration in the effluent. Both biogas production and effluent chloride concentration can indicate the effective PCB removal from IUFAB. The IUFAB has the advantages of high biomass content; easy to separate the treated oil and liquid parts; PCBs removal efficiency meeting the requirement of PCBs contaminated oil to be discharged; stable process performance; easy operation; shorter HRT applied. It is highly possible that it can be applied for the biological removal of PCBs contaminated oil.

Keywords: polychlorinated biphenyls (PCBs); Entrapped Mixed Microbial Cells (EMMC); EMMC carrier packing ratio; Intermittent Up-Flow

Anaerobic Bioreactor (IUFAB); Chemical Oxygen Demand (COD); Hydraulic Retention Time (HRT)

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LIST OF ABBREVIATIONS

CAW	**Cellulose Acetate Waste Material**
COD	**Chemical Oxygen Demand**
CTA	**Cellulose Triacetate**
EMMC	**Entrapped-Mixed-Microbial-Cells**
GAC	**Granular Activated Carbon**
GC	**Gas Chromatograph**
HRT	**Hydraulic Retention Time**
IUFAB	**Intermittent Up-Flow Anaerobic Bioreactor**
MLSS	**Mixed Liquid Suspended Solids**
NH_3-N	**Ammonia-nitrogen**
NO_2-N	**Nitrite-nitrogen**
NO_3-N	**Nitrate-nitrogen**
PAHs	**Polynuclear Aromatic Hydrocarbon**
PCBs	**Polychlorinated Biphenyls**
PCP	**Pentachloro Phenol**
SCOD	**Soluble Chemical Oxygen Demand**
SRT	**Solid Retention Time**
TSS	**Total Suspended Solid**
EPA	**Environmental Protection Agency, U.S.A.**

UASB	**Up-flow Anaerobic Sludge Blanket**
VSS	**Volatile Suspended Solids**
WHO	**World Health Organization**

CHAPTER 1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are a family of compounds synthesized by the catalytic chlorination of biphenyl. These compounds are produced as complex mixtures containing specific levels of chlorine. A PCB is a chlorinated biphenyl compound with the general formula $C_{12}H_{(10-n)}Cl_n$. PCBs generally occur as mixtures, where n can vary from 1 to 10. The 10 sites available for possible chlorine substitution result in 209 possible PCB compounds or congeners (Kim et al. 2000).

PCBs were widely used in industrial application from 1929. This was mainly due to their advantageous characteristics: thermal stability, resistance to oxidation, reduction, chemical agents, and their excellent dielectric properties. While the production of PCBs was banned in 1977 due to their carcinogenicity, recalcitrance and accumulation for the environment and human health, those released to the environment still persist in water, oil, soils and sediments. At the present time, PCBs, owing to their demonstrated toxicity for human and for the environment, are considered as one of the most dangerous pollutants.

In the early days, the PCBs were considered as non-biodegradable. However, in the past 10~15 years, PCBs have been shown to be biodegradable via two distinct microbially mediated mechanisms, aerobic biodegradation and anaerobic dechlorination. Based on the pervious experiments, PCBs indicate very low removal efficiency compared with other hazardous wastes. How to develop innovative bioreactor and improve the PCBs removal efficiency have come to be an urgent task for the environmental engineer. In this study, all experiments will focus on the PCBs removal from the aqueous and oil phases.

Three general bioremediation methods have been applied to hazardous wastes such as chlorinated benzenes, pentachlorophenol (PCP) and some polynuclear aromatic hydrocarbons (PAHs) and PCBs: land treatment or composting, in-*situ* bioremediation, and bioreactor treatment. Although land treatment is least expensive, it requires significant land area and extended treatment duration. The in-*situ* bioremediation approach avoids problems associated with transportation of hazardous wastes; however, controlling in-*situ* environmental conditions are difficult and it is equally difficult to predict treatment performance. The bioreactor treatment approach can avoid the weaknesses of the other two treatment approaches. The cost of using the

bioreactor treatment approach may be higher, but it is more effective in treating most hazardous wastes.

Many studies on biological treatment of PCBs have been carried out in recent years. In these studies, various configurations of reactors were used to treat PCBs from aqueous and sediments (Tang et al., 2000; Tartakovsky et al., 2000; Tartakovsky et al., 2001; Saponaro et al., 2003). Based on the results, the configuration of upflow anaerobic sludge blanket (UASB) indicates high potential to remove the PCBs from the aqueous phase. Also these studies indicate, in order to increase the PCBs removal, that the key problem is how to increase and maintain high-activated biomass content in the reactor if biological treatment process is to be practiced. However, for PCBs removal from the oil phase, the application of the design and operation of upflow anaerobic reactor needs to be modified and investigated further, since the physical properties of oil, such as lighter density compared with water, only 0.719g/ml, and very sticky. So the study also will focus on development and improvement of the innovative bioreactor for PCBs removal from the aqueous and oil phases.

The objectives for this study are to identify, demonstrate, and evaluate innovative treatment technologies for PCBs removal from the aqueous and oil

phases with the most reasonable bioreactor configuration and optimal operational conditions with potential development of design/operation criteria and scale-up.

CHAPTER 2. LITERATURE REVIEW

2.1 Definition of PCBs

Polychlorinated biphenyls (PCBs) are a family of compounds synthesized by the catalytic chlorination of biphenyl. These compounds are produced as complex mixtures containing specific levels of chlorine and sold under the trade names such as Aroclor, Clophen, Fenclor, Phenoclor, Pyralene, and Kanechlor. Structurally, PCB is a chlorinated biphenyl compound with the general formula of $C_{12}H_{(10-n)}Cl_n$. PCBs generally occur as mixtures, where n can vary from 1 to 10. The 10 sites available for possible chlorine substitution result in 209 possible PCB compounds or congeners (Kim et al. 2000). The congeners differ in the number (from 1 to 10) and position of chlorines on a biphenyl molecule, for example, 2, 3, 4, 3', 4'- pentachlorobiphenyl (abbreviated as 234-34-CB) as shown in Figure 2.1 (Abramowitz and Olson, 1995). This figure shows the process how the biphenyl is converted to the polychlorinated biphenyl (PCB) by chlorination.

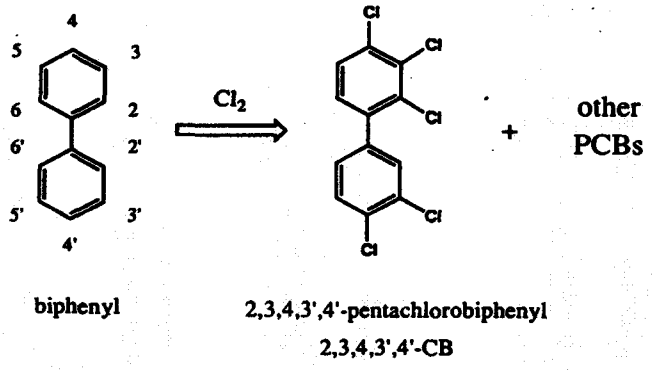

Figure 2.1. The synthesis of PCBs via the direct chlorination of biphenyl and 2, 3, 4, 3', 4'- CB as an example

Aroclor 1260 (CAS registry number 11096-82-5) is a colorless liquid with an average molecular weight of 376 (USAF 1989). It is a polychlorinated biphenyl (PCB) mixture containing approximately 38% $C_{12}H_4Cl_6$, 41% $C_{12}H_3Cl_7$, 8% $C_{12}H_2Cl_8$, and 12% $C_{12}H_5Cl_5$ with an average chlorine content of 60% (USAF 1989).

2.2 Properties of PCBs

Most of PCB mixtures are extremely thermo stabile (up to 350°C), resistant to oxidation, acids, bases, and have excellent electrical insulating as well as dielectric characteristics. While the PCB may not exhibit the acute toxicity

originally ascribed to them, they and their byproducts remain as significant factors for adverse effects in the ecological food chain. The physical and chemical properties of PCB mixtures made them industrially useful. The most important physical properties of the mixtures are that they are liquids, have low vapor pressures, low water solubility, and excellent dielectric properties. Chemical properties include stability to oxidation, flame resistance, and relative inertness (Hutzinger et al. 1974).

2.3 History of PCB

Because of excellent flammability, electrical, and stability properties, PCBs were widely used for a variety of industrial purposes, such as heat transfer fluids, hydraulic fluids, lubricants, plasticizers, and dielectric fluids in capacitors and transformers. These PCB mixtures have been synthesized since 1929 in a number of different countries, such as Japan, France, Great Britain, German, USA, etc. Most of PCB's congeners have low volatility and high lipophilicity, hence more than 99% of PCBs are found in soil and sediment.

PCBs were manufactured starting in North American from 1929 to 1977 (Brown et al. 1984). They were produced by Monsanto and marketed under the trade name Aroclor. Polychlorinated biphenyls (PCBs) have attained considerable

infamy as everywhere environmental contaminants (WHO, 1993). Despite where they were used, many industrialized countries have been restricted since 1970s. The subsequent disposal or dumping in landfills of PCB has resulted in universal contamination of air, water, soils, etc. They have been included in the priority pollutants listing implemented by the U.S. Environmental Protection Agency (EPA) and by the European Commission. Approximately, 640 million kilograms were manufactured, and several hundred million kilograms were released into the environment as sediments and waste streams, and as components of the lipoidal compartments of plant and animal wildlife in this meantime (Tanabe et al. 1983). One study reported that the Canadian Arctic presently shows little evidence of reduced PCB loading and concluded that the lifetimes of PCB in Arctic measure in decades (MacDonald et al. 2000).

Federal and state legislation regulating the disposal, management, and cleanup of hazardous waste has led to a search for new treatment technologies in the past few years. Scientists have been working on dealing with PCBs for some years. There are different kinds of methods in dealing with PCB, including long-term storage, high-temperature incineration, photolytic or radiochemical destruction, solvent extraction, thermal desorption, chemical treatment, stabilization, and biodegradation (Brunelle et al. 1985). In the following, the use

of microorganisms for bioremediation of PCBs, an innovative method for the treatment of PCBs, are reviewed and discussed, as well as the other methods through traditional physical or chemical treatment.

2.4 Risks of PCBs to environment and health

Contamination of the environment by PCBs was firstly reported in 1966 (Jensen, 1966), and the presence and persistence of PCBs has been a matter of concern since then. The regulatory history concerning PCBs dates to the 1970s, at which time several industrial nations instituted PCB phaseouts, although some countries continued to permit PCB production into the 1980s (U. S. Public Law 94-469,1976).

PCBs are found in many different geographic locations in air, water, oil and soil, and are environmentally persistent. A significant reason why PCBs pose a risk to people is that they are bio-accumulated in the food chain. The hydrophobic nature of the compounds causes the PCBs to be preferentially attracted to lipids, or fats, resulting in the accumulation of the chemicals in living cells. This causes the concentration of PCBs to increase as they move from simple aquatic life forms, to fish, to humans through ingestion. If humans are exposed to the PCBs or ingest a food source that has been exposed to PCBs, the chemical

remains in their system. Repeated exposure leads to the accumulation of the compound and can result in a toxic effect.

PCBs are known to be carcinogenic and have the potential to pose a significant threat to humans and the environment. Data are suggestive but not conclusive concerning the carcinogenicity of PCBs in humans. The EPA has not determined a weight-of-evidence classification or slope factor for Aroclor 1260 specifically. However, hepatocellular carcinomas in three strains of rats and two strains of mice have led the EPA (1996b) to classify PCBs as group B2, probable human carcinogen. PCBs also have significant ecological and human health effects other than cancer, including neurotoxicity, reproductive and developmental toxicity, immune system suppression, live damage, skin irritation, and endocrine disruption (Faroon et al. 2000). Additionally, the specific binding of PCB to rat liver cytosol proteins has been documented (Buff et al. 1992). More reviews of human health effects (Kimbrough, 1987) and of mutagenicity and carcinogenicity of PCB (Safe, 1989) are also available. Toxic effects have been observed from acute and chronic exposures to PCB mixtures with varying chlorine content. These materials have been implicated in a number of well-publicized catastrophic events (Kimbrough, 1987). The high lipophilicity and stability of PCB resulted in their widespread distribution in the global ecosystem

and led to their bans by EPA in 1977 and EEC in 1976. And PCBs are now regulated under the Toxic Substances Control Act (40 CFR761).

2.5 Principles of Biodegradation of PCBs

In the early days, the PCBs were considered as non-biodegradable. However, at the present time, PCBs have been shown to be biodegradable via two distinct microbially mediated mechanisms: aerobic biodegradation, which involves the oxidative destruction of PCB molecules through a series of degradation intermediates; and anaerobic dechlorination, which involves the removal of chlorine atoms and replacement by hydrogen atoms in the absence of oxygen. These biodegradations are shown in the Figure 2.2 and 2.3. Studies on the microbial breakdown of PCBs have been reported as early as 1973 (Ahmed et al. 1973). Rhee and co-workers have provided some initial evidence for the anaerobic degradation of PCBs (Rhee et al. 1989).

2.5.1 Aerobic Biodegradation

The aerobic bacterial biodegradation of PCBs is widely known and has been studied extensively (Bedard and Kamely et al. 1990). Numerous microorganisms have been isolated that can aerobically degrade a wide variety of

PCBs, although the more lightly chlorinated congeners are preferentially degraded. These organisms attack PCBs via the 2, 3 – dioxygenase pathway, converting PCB congeners to their corresponding chlorobenzoic acids as shown in Figure 2.2. Then, these chlorobenzoic acids can be readily degraded by indigenous bacteria, producing carbon dioxide, water, chloride, and biomass (Harkness et al, 1993).

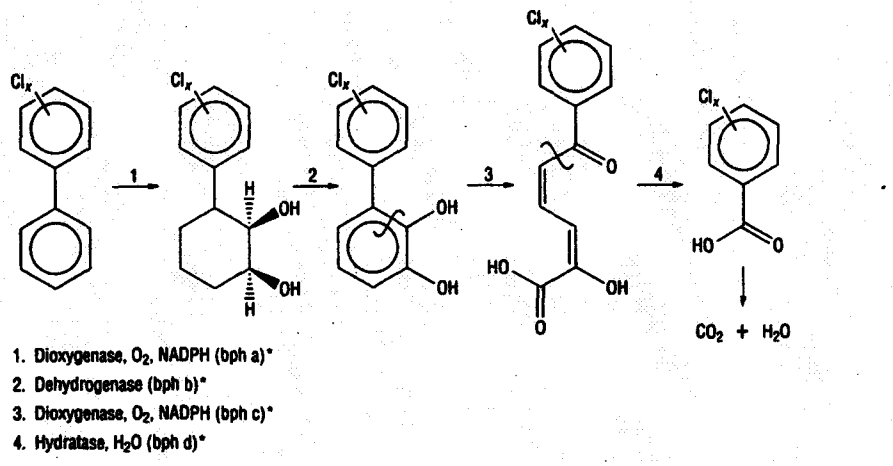

Figure 2.2. Aerobic polychlorinated biphenyl (PCB) biodegradation by 2, 3 – dioxygenase pathway (Abramowicz and Olson, 1995)

2.5.2 Anaerobic Dechlorination

Anaerobic bacteria attack more highly chlorinated PCB congeners through reductive dechlorination as shown in Figure 2.3. In the proposed scheme, the organisms utilize PCBs as an electron acceptor, with addition of the electron to

the carbon-chlorine bond, chloride loss, and hydrogen abstraction from an unknown species. In general, this microbial process affects the preferential removal of *meta* and *para* chlorines, thus converting highly chlorinated PCB congeners to lower chlorinated, *ortho* – substituted congeners. The altered congener distribution of residual PCB contamination observed in several aquatic sediments was the earliest evidence of the anaerobic dechlorination of PCBs (Brown et al, 1987). This same activity has occurred in the laboratory; the selective removal of *meta* and *para* chlorines was observed (Quensen et al, 1990).

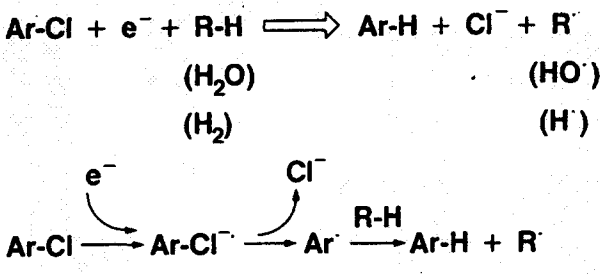

Figure 2.3. Possible mechanism for reductive dechlorination catalyzed by anaerobic microorganisms. (Abramowitz, 1990)

2.5.3 Two – step combined anaerobic / aerobic process to biodegrade PCBs

Two – step combined anaerobic / aerobic process was used to biodegrade PCBs. In this scheme (as shown in Figure 2.4), initial anaerobic treatment

converts highly chlorinated PCBs to lightly chlorinated derivatives. Subsequent aerobic treatment destroys the remaining material (Abramowitz, 1990).

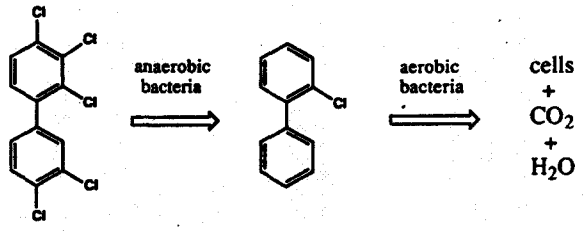

Figure 2.4. Two – step combined anaerobic / aerobic process to biodegrade PCBs (Abramowitz, 1990)

2.6 Innovative Bioreactor Technology for the Treatment of PCB contaminated Oil

Physical methods such as adsorption, filtration, or extraction are effective for many wastes, but additional treatment often is required since the waste is separated out but not destroyed. Chemical treatment may be applicable to a wide variety of materials but can leave hazardous byproducts or residual sludge. Isolating or altering waste through stabilization, solidification, or encapsulation is a rapid means to control some wastes. Incineration and other thermal methods are expensive yet, but very effective in reducing volumes of waste and completely destroying them. Gaseous emissions and ash residues still require further treatment.

With support from EPA Administrator, treatment firms and customers are looking for more cost-effective technologies that can meet cleanup standards and provide permanent disposal of hazardous waste, such as PCBs. Bioremediation, in which microorganisms are used to destroy or detoxify wastes, is classified as one such innovative technology. Somewhat surprising is, many microbes and enrichment cultures have been shown to metabolize and utilize PCBs as carbon and energy sources under anaerobic or aerobic conditions. Rates for the metabolism observed in the laboratory can be high. This suggests that bioremediation may ultimately be a solution for the treatment of PCBs contaminated materials.

Three general bioremediation methods have been applied to hazardous wastes: land treatment or composting, bioreactor treatment, and *in-situ* bioremediation. In land treatment, solids, sludge, or liquid wastes are mixed into surface soils or composted. Composting is similar to land treatment except that bulking agents generally are added. Land treatment usually requires adequate amounts of land area and long treatment times, but it is one of the least expensive methods. Bioreactor is a more rapid and efficient mean of degrading hazardous wastes. *Ex-situ* reactors may allow for effective mixing, aeration, and bacterial growth with greater control over residence time, nutrient addition, temperature

control, pH control and concentration control. Bioreactor can provide on-site treatment and avoid some of the problems related with the transportation of hazardous wastes. The *in-situ,* unlike bioreactor, may be more difficult to control, which makes it sometimes a slower process and for which the outcome is harder to predict. In favor of *in-situ* treatment is the chance to reach, and have undisturbed sites of contamination that are inaccessible by other methods. Generally, land or *in-situ* treatment may require months or years to complete, but bioreactor may need only weeks to be effective. The bioreactor approach can avoid the weaknesses of the other two treatment approaches. The cost of using the bioreactor approach may be higher, but it is more effective in treating many hazardous wastes.

Due to either physical or economic reasons, an interest in bioremediation-based alternatives has increased. Just as mentioned above, bioremediation is now considered an innovative technology for the cleanup of hazardous wastes, such as PCBs.

2.6.1 Recent Researches for biological treatment of PCBs

Bioremediation of lighter halogenated aromatics such as chlorinated benzenes, pentachloro phenol (PCP) and some polynuclear aromatic hydrocarbon

(PAHs) has been applied successfully for years. However, until 1990, biodegradation of higher molecular weight and more structurally complex compounds such as DDT (pesticide), some PAHs, dioxins, and polychlorinated biphenyls (PCBs) was considered to be impractical. Considerable R&D progress in biodegradation of the higher and more structurally complex compounds was made in the 1990's, which has opened the door for using bioremediation instead of incineration, fixation, landfilling, or chemical treatment. Also researchers have reported some successes in biodegrading PCBs.

Efficient microbial degradation of PCBs requires diverse metabolic activities due to the high number of congeners. In addition, degradation of PCBs has been shown to occur primarily via co-metabolism in that the microorganisms responsible for PCBs transformation are unable to grow on PCBs as a sole carbon source (Abramowitz et al., 1990; Boyle et al., 1992) and require a co-substrate (a additional carbon source) for microbial growth and degradation activity. Composting is one way to provide both a highly diverse microbial community with a range of metabolic capabilities and co-substrate(s) for PCB metabolism. This method also offers an inexpensive and effective way for bioremediation of contaminated soils (Funk et al, 1993).

The elevated temperatures generated during composting, and the air convection through compost piles, could potentially increase the extent of PCB volatilization from contaminated soils. Numbers of research groups have studied the atmospheric volatilization as one of the fates of environmental PCBs (Chiarenzelli et al. 1997). They reported that 16% of a low chlorinated Aroclor mixture was volatilized after 35 days of composting around 50°C; however, the volatilization of higher chlorinated mixtures such as 1248, 1254, and 1260 from composts has not previously been determined. The measurement for PCB volatilization in the process of composting is still a technical problem. Since compost biodegradation of PCBs is mostly an aerobic process, this method might be improved by combining with additional remediation technologies capable of reducing PCB congeners with greater than 4 chlorines. Currently, composting is primely used for PCBs contaminated soil. But this method can be adjusted to treat PCBs contaminated sediments or oil.

Tang et al. (2000) has done a feasibility study to evaluate some natural processes for cleaning PCB-contaminated dredged material using actual sediment. The overall objective of their study was to identify those variables that could be manipulated to enhance natural cleaning-up of PCBs. And PCBs were monitored

over time in five experimental aquariums. The experimental matrix is given in Table 2.1.

Aquarium	Description	Simulation
1	Sediment under simulated UV radiation and no tilling (Ts)	Conventional
2	Sediment under simulated UV radiation (UV) with 5Ts/week	Tilling effect
3	Sediment with 5Ts/week but no UV	Photolysis effect
4	Sediment with 5Ts/week plus water addition (WA) but no UV	Volatilization
effect		
5	Sediment with 5Ts/week and WA plus mercuric chloride but no UV	Biodegradation
effect		

Table 2.1. Experimental Matrix for simulation of PCB clean up

According to their description, "Aquarium 1 simulated disposal of dredged material in a CDF with conventional management, that is, evaporative dewatering with no tilling." Thus, aquarium 1 served as the control. "Aquarium 2 simulated intervention by tilling and included sunlight (specifically UV radiation)." "Tilling, 5 times per week," so that "it renewed the surface exposed to sunlight". "Aquarium 3 was a companion of aquarium 2, but in aquarium 3, these sediment was tilled without simulated sunlight." "Aquarium 4 was identical to aquarium 3 except that aquarium 4 had periodic addition of water in order to maintain the sediment in a damp condition and encourage biodegradation." Damp conditions also enhance volatilization (Valsaraj et al. 1999). "Aquarium 5 was identical to aquarium 4 except that aquarium 5 was poisoned with mercuric chloride to inhibit

the growth of microorganisms." All aquariums started at about the same PCB concentration and the same PCB congeners.

Their research results showed that, the total concentration dropped in nearly all aquariums at the second week sampling time, but increased above the setup day value at the fourth and sixth week sampling time. However, all value for the 3 and 5 months sampling times were lower than that of the setup day. Furthermore, the values of the fifth month sampling were significantly lower than that of setup day. Similar evidence of PCB dechlorination in sediment from the Saginaw River, Michigan has been reported (Tang et al. 2000). The mass of PCB with two chlorines also declined during the 5-month experiments.

According to Tang et al. (2000), a 40% reduction of PCBs in contaminated sediments from the New York / New Jersey Harbor was achieved through the use of periodic tilling over a period of about 5 months. Analysis of the variance showed that there was a greater likelihood that PCB disappearance was caused by a combination of photolysis, volatilization, and biodegradation mechanisms. It is not a single process. Periodic tilling exposed new sediment surface to oxygen and light and reduced mass transfer limitations on oxygen penetration and contaminant volatilization therefore, enhancing PCBs disappearance.

Tartakovsky et al. (2000) used a single – stage coupled Anaerobic/Aerobic bioreactor to treat the highly chlorinated PCB, Aroclor 1242 (as shown in Figure 2.5). The coupled anaerobic/aerobic bioreactor consisted of 1 L up-flow anaerobic sludge bed (UASB) – type reactors connected to 0.5 L aeration columns. To minimize the sorption of biphenyl and Aroclor 1242, the reactors were made of glass and recirculation and feeding lines were made of lindone (Vinton). The reactors were operated with a hydraulic retention time (HRT) of 2.1 days and at the temperature of 30°C. But the result was not impressive, only Aroclor 1242 removal efficiency of 16~19% could be achieved.

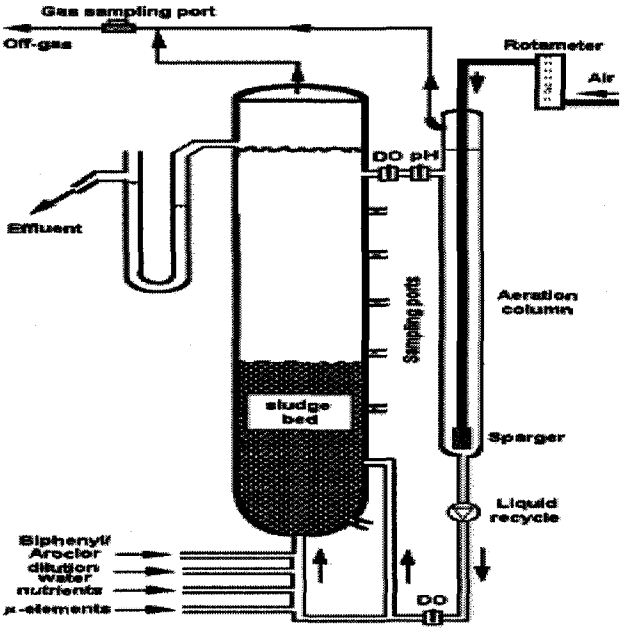

Figure 2.5. Set up the single – stage coupled Anaerobic/Aerobic bioreactor

Tartakovsky et al. (2001) used a continuously operated up-flow anaerobic sludge bed (UASB) reactor to degrade the Aroclor 1242. Experiments were performed in a 5 liters up-flow anaerobic sludge bed (UASB) reactor. To minimize adsorption of Aroclor 1242, the reactor was made of glass and recirculation and feeding lines were made of viton. The reactor was inoculated with 1 liter (39.2 g volatile suspended solids, VSS) of anaerobic sludge obtained from a UASB reactor treating wastewater from a food industry. This reactor was operated at a temperature of 30°C, a residence time of 5 days, and a pH in the range of 7.0 ~ 7.5. In this study, an addition of Tween 80 was used to improve solubility of Aroclor 1242, and it led to self-inhibition of the dechlorination process at a load of 1.3 mg Aroclor 1242/ (g VSS day). The maximal dechlorination rate observed in this study was 0.6 mg Aroclor/ (g VSS day). That means, at most, Aroclor removal efficiency of about 45% could be achieved.

Saponaro et al., (2003) used the batch systems to test the removal efficiency of saturated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in slurry phase biological treatment of lagoon sediments. Sediments were contaminated by saturated hydrocarbons (958 mg/kg d.w.), polyaromatic hydrocarbons (PAHs) (29 mg/kg d.w.) and polychlorinated biphenyls (PCBs) (236 μg/kg d.w.). Biodegradation studies were

carried out at 21 ± 1°C, in completely mixed slurry phase aerobic, anaerobic and sequential anaerobic/aerobic batch systems (3.5 l), with a solid to liquid ratio of 10% w/w with reaction time of 10 and 22 days. Abatements of PAHs were between 43% and 69% in the aerated tests, and between 17% and 51% in the non-aerated treatments. Concerning PCBs, tests evidenced that reductive dehalogenation mechanisms have occurred in the anaerobic reactors with the most stable pH values, resulting in an increase of 2,4,4"-CB and 2,2', 5,5'-CB concentrations; the aerobic treatments did not modify the PCB mixture. In both types of systems, no variation of the total PCB concentration could be observed.

2.3.2 Potential of EMMC technology for PCBs removal from aqueous and oil phases

Cell immobilization is defined as any technique that limits the free movement of cells, and consists of two broad types: attachment and entrapment. Cell immobilization is relatively new technology in the field of water and wastewater treatment. In the past 10 years, removal of hazardous wastes, heavy metals, nitrification and denitrification, and methane and hydrogen production has become the focus of application for this technology.

For entrapped cell system, it offers the potential to provide longer SRT's for slow growing bacteria and solve liquid-solid separation problems. This is especially suitable for treating the toxic/hazardous organic material contained in the water/oil mixture, i.e., washout of effective microorganisms is impossible which is beneficial for the degradation of toxic organics. Over the past 15 years, University of Hawaii has investigated the application of entrapped microbial cell systems, called Entrapped Mixed Microbial Cell (EMMC), at laboratory and pilot scale for various wastewater treatments (Yang et al., 1995; Yang et al., 1997; Yang and Chou 1990; Yang et al., 2002; Yang et al., 2003; Yang et al., 2004). Based on those studies, the application of EMMC has its own advantages: long SRT (minimum production of sludge); higher density of activated microorganisms contained (about 10 times higher than the existing conventional activated sludge process); low effluent-suspended solid content, etc.

Since most of the xenobiotic compounds can be removed through the so-called coupled oxidative and reductive reactions, the EMMC technology can be used to carry the synchronous oxidative and reductive reaction for achieving complete biodegradation of xenobiotic compound. This is because of its characteristics relating to the diffusion resistance and protects the ability of the gel materials against toxic effect. This ultimately will lead the EMMC technology to

improve the existing bioremediation of contaminated soil and water. Therefore, the removal of toxic or xenobiotic compounds, such as PCBs in the aqueous phase, is highly possible. In this study, it was proposed to use EMMC technology to treat PCBs in aqueous phase.

2.6.3 Summary

A few years ago, polychlorinated biphenyls (PCBs) were considered that they could not be biodegraded. Physical and chemical methods were used to treat PCBs directly, but these methods could be costly, might also result in residual pollution. According to the above review, bioremediation might open a novel way for the treatment of PCBs and other hazardous wastes. In the current years, based on some studies (Chiarenzelli et al., Tang et al., 2000; 1997; Tartakovsky et al., 2000; Tartakovsky et al., 2000; Saponaro et al., 2003), PCBs already showed high biodegradable potential via aerobic and anaerobic treatment processes.

Based on previous studies (Abramowitz et al., 1990; Boyle et al., 1992), PCBs cannot be directly used to provide the necessary energy for microorganisms to complete the metabolism and synthesis. An additional carbon source needs to be introduced as energy source and electron donor. Also, based on their studies,

different types of bacteria were required for effective microbial degradation of PCBs, because of high number of PCB congeners. So composting activated sludge is considered to be used as the materials to fill the reactor.

EMMC, as an innovative technology, has its own advantages, such as long SRT; higher density of activated microorganisms contained, etc. And, EMMC technology can carry the synchronous oxidative and reductive reaction for achieving complete biodegradation of hazardous waste. Based on the precious studies (Yang et al., 1995; Yang et al., 1997; Yang and Chou 1990; Yang et al., 2002; Yang et al., 2003; Yang et al., 2004), EMMC technology had used to treat the different types of waste, like glucose, phenol, and its derivatives, carbaryl (1-naphtyl-N-methylcarbamae), diluted pig wastewater, sugar mill wastewater, food processing wastewater, and nitrate. Therefore, the removal of PCBs from the aqueous phase by using EMMC technology is highly possible. In this study, EMMC technology will be applied and investigated for the PCBs removal from the aqueous and oil phases.

For anaerobic process, there are many reactor configurations used for the treatment of municipal and industrial wastewater or sludge. For example, completely mixed process, anaerobic contact process, up-flow and down-flow

packed bed process, fluidized and expended bed process, up-flow anaerobic sludge blanket (UASB), etc. These various types of reactor configurations have their own advantages and disadvantages for the treatment of different kind of wastewater. Based on previous studies (Tartakovsky et al., 2000; Tartakovsky et al., 2000; Saponaro et al., 2003), the configuration of up-flow anaerobic sludge blanket (UASB) indicates high potential to remove the PCBs from the aqueous phase. However, for PCBs removal from contaminated oil, since the physical properties of waste oil (such as density lighter than water, only 0.719g/ml, and very sticky), the application of the design and operation of up-flow anaerobic reactor needs to be modified and investigated further in this study.

CHAPTER 3. METHODOLOGY

Part I. PCB Removal from Aqueous Phase

3.1 (Experiment 1) Selection of polymeric materials for EMMC carriers

In the previous studies, various polymeric materials, such as calcium alginate, polyacrylamide, K-carrageenan, cellulose triacetate and a combination of cellulose triacetate and calcium alginate were evaluated for potential application of EMMC technology. Cellulose triacetate (CTA) was found as the best material for entrapping the microbial cells because of its simple preparation, easy gel formation and better mechanical strength.

Because of the economic reasons, cellulose acetate waste material (CAW) was also used to prepare the carriers. In this study, both carriers, CAT carrier and CAW carrier, were investigated under the same operational conditions (i.e., HRT of 24 hours and influent of 200mg COD /L) in order to compare the difference of effectiveness of these two different polymeric materials.

3.1.1 Immobilization of EMMC

3.1.1.1 Cell immobilization

The EMMC carriers for EMMC processes were prepared by following the procedures developed by Ma (1994) and modified by Zhang (1995) as shown in Figure 3.1. The cellulose triacetate (CTA) and cellulose acetate waste materials (CAW) were chosen as the gel-polymers for carriers because of their highly stable process performance and strong mechanical strength (Yang et al., 1988; Yang and See, 1991). The mixed cell suspension was taken from the exiting suspended culture tank operated in our Bioenvironmental Engineering laboratory, and it was harvested by a tubular bowl centrifuge at 15,000rpm for 10 minutes. The mixtures of 60(g) of 10%(w/v) wet cell, and 60(g) distilled water were added into 10%(w/v) cellulose triacetate (CTA) and cellulose acetate waste material (CAW) dissolved in methylene chloride, respectively. It was mixed thoroughly until it became uniform. Then, the mixture was poured into the toluene solution from which methylene chloride and water had been extracted. Once the mixture was hardened, it was cut into small cubes (10mm x 10mm x 10mm) and flushed with tap water to remove residual toluene and methylene chloride.

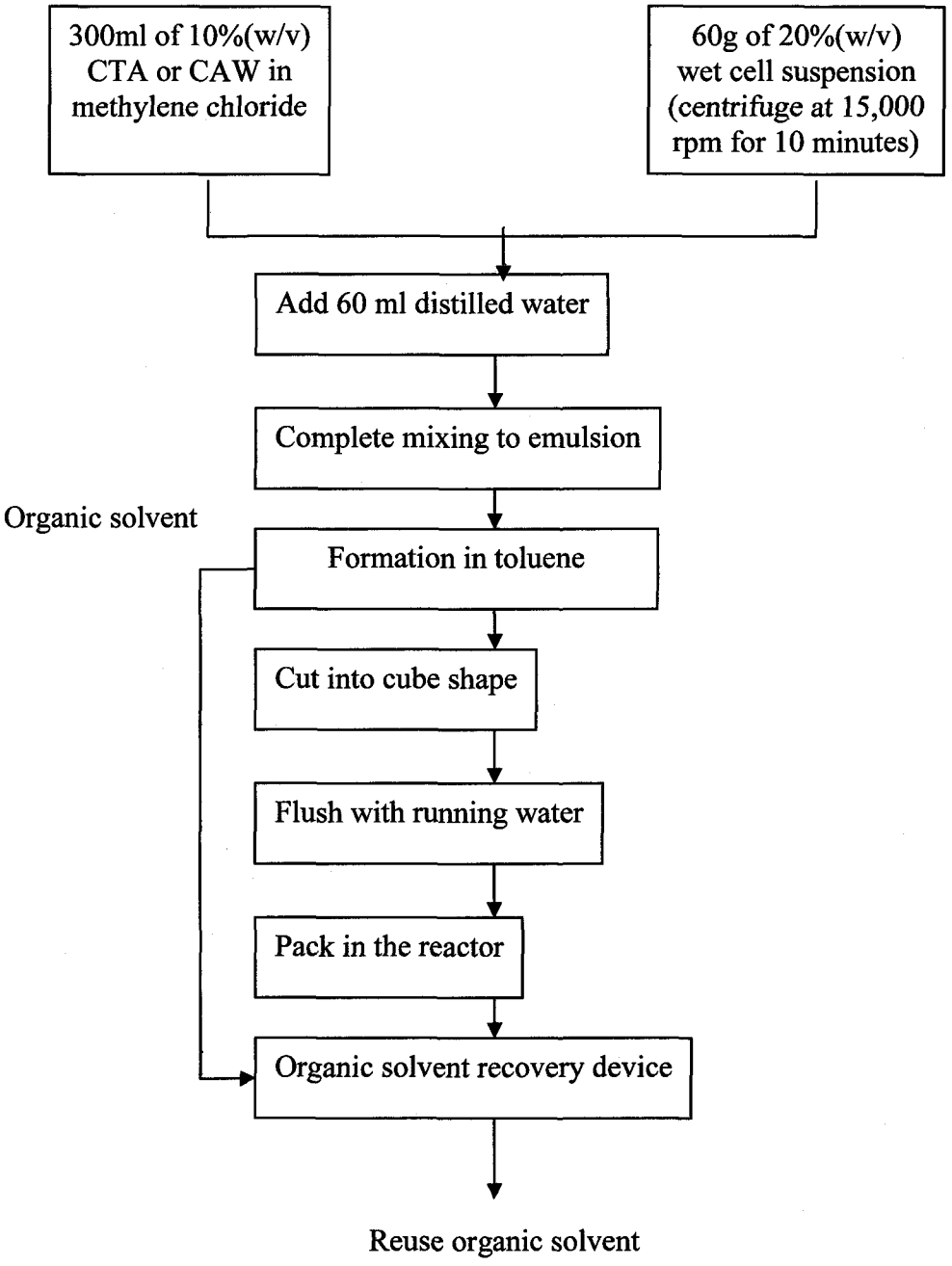

Figure 3.1. Preparation of Entrapping Mixing Microbial Cells

3.1.1.2 Substrate and experimental conditions

The synthetic wastewater was used in this study. The composition of the synthetic wastewater is presented in the Table 3.1 and the experimental conditions for the experiment is presented in Table 3.2.

Table 3.1. Composition of the synthetic wastewater for EMMC aerobic process

Content	Concentration (mg/L)	
MeOH (CH_3OH)	200	as COD
$(NH_4)_2SO_4$	30	as N
$MgSO_4.7H_2O$	1.98	as Mg
$FeCl_3.H_2O$	0.03	as Fe
KH_2PO_4	95	as PO_4
K_2HPO_4	144	as PO_4
$MnSO_4.H_2O$	0.81	as Mn
$CaCl_2$	0.68	as Ca
pH	7.5~8.6	

Table 3.2. Experimental Conditions of EMMC aerobic process

HRT (hour)	24
Liquid temperature (°C)	23 ± 2
Influent SCOD concentration (mg/L)	200
Influent NH_3-N concentration (mg/L)	30
Total effective volume of reactor (ml)	4700
Total carrier volume (ml)	1085
Packing ratio (%)	23%
pH of influent	6.5~8.3

3.1.2 EMMC System set-up and operation

Two Plexi-glass reactors having effective volume of 4.7 Liters for both reactors installed for the use of aerobic EMMC reactors are shown in Figure 3.2. For these two reactors, the packing ratio (total volume of EMMC carriers / total volume of the EMMC aerobic reactor) of 23% is installed. The packed carriers are supported and fixed in these reactors by using aluminum frames. Two pumps were used to continually pump the influent, synthetic wastewater, into these two up-flow EMMC aerobic reactors. Air was pumped into these two aerobic EMMC

reactors continuously. Effluent come out from the top of the reactors were monitored for COD and NH_3-N concentrations.

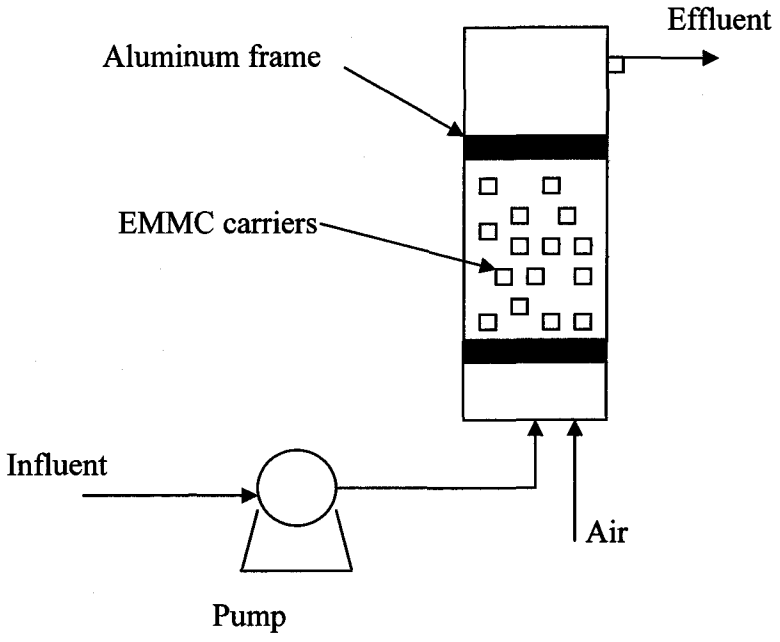

Figure 3.2. The Schematic diagram of the EMMC Aerobic Process

3.2 (Experiment 2) Comparison of Aerobic and Anoxic PCBs Degradation using Synthetic Wastewater

3.2.1 EMMC systems set-up and operation

After the necessary polymeric material for the EMMC carriers was decided, three aerobic and anoxic EMMC reactors were started to feed the synthetic wastewater with methanol as a carbon source. The same packing ratio (total volume of EMMC carriers / total volume of the EMMC aerobic reactor) of EMMC carriers, 40%, was used to these three EMMC reactors. These two aerobic EMMC reactors were operated with a constant air supply (as shown in Figure 3.3(b)), and the anoxic EMMC reactor was operated without air supply (as shown in Figure 3.4(b)). The aeration schedule of the two aerobic EMMC reactors was operated with continuous aeration and intermittent aeration with 1-hour air-on and 1-hour air-off (as shown in Table 3.3). These bioreactors were made of glass to avoid the possible adsorption of PCBs by the reactor wall. The synthetic wastewater with PCBs of 2 mg/L and 600 mg COD/L was prepared by spiking the PCBs (Aroclor 1260) in the synthetic wastewater and fed with continuous flow modules with HRT of 24 hours. The effluent gas discharged from the aerobic EMMC reactor was passed through a column packed with granular activated

carbon (GAC) to absorb any volatile compounds exhausted with the air. Biogas from anoxic EMMC reactor is collected in a gasbag for further analysis. In this study, COD concentration, NH_3-N, NO_3-N, NO_2-N and PCBs concentration were monitored and analyzed.

Table 3.3. Aeration schedule of those three EMMC aerobic and anoxic reactors

Type	**Aerobic Reactor 1**	**Aerobic Reactor 2**	**Anoxic**
Aeration	**Continuous Aeration**	**Intermittent Aeration (1-hour off, 1-hour on)**	**No Aeration**

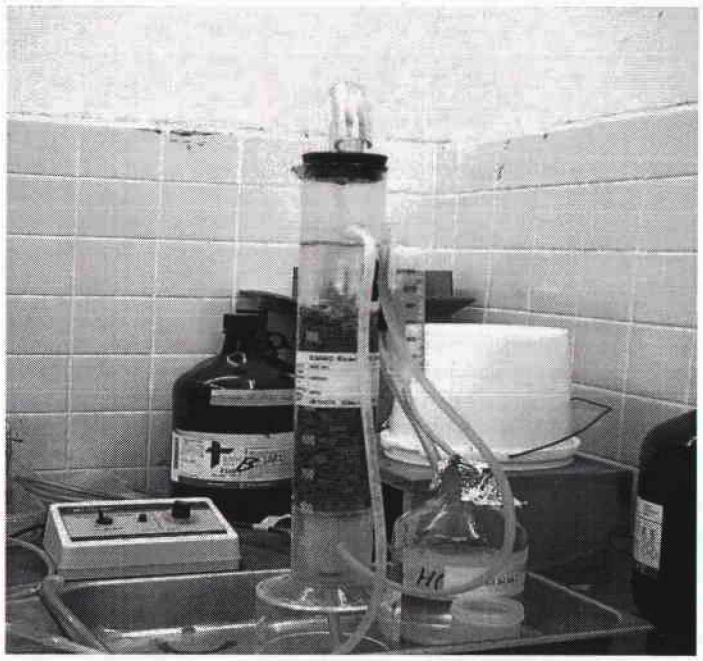

Figure 3.3(a). Photo of the Down-Flow EMMC Aerobic Reactor

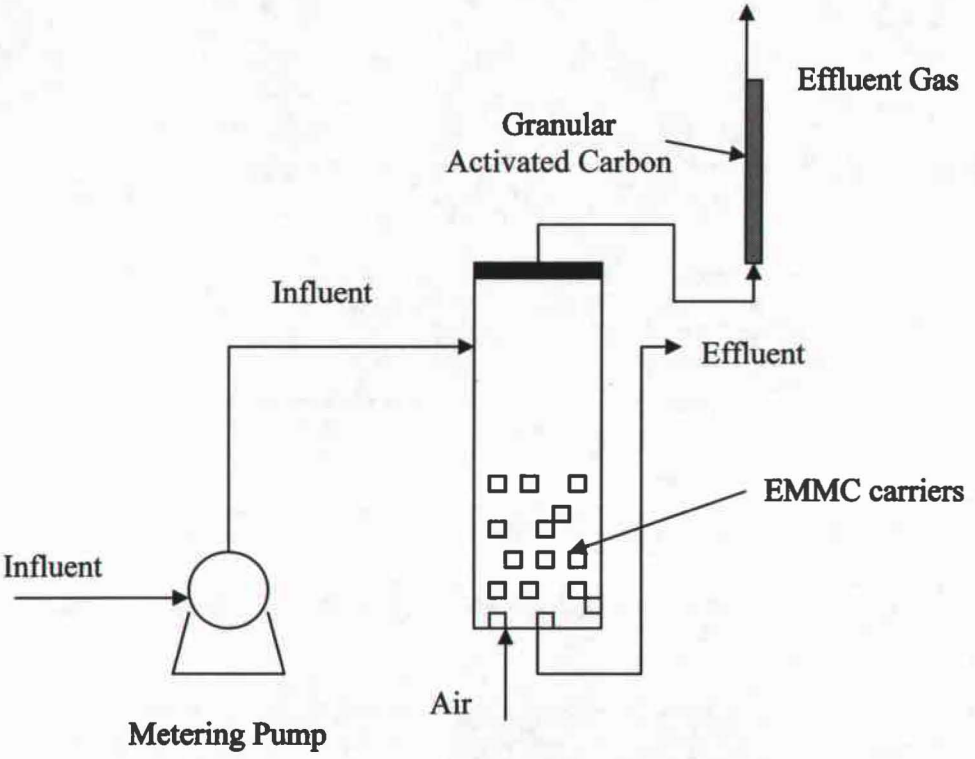

Figure 3.3(b). Schematic diagram of the Down-Flow EMMC Aerobic Reactor

Figure 3.4(a). Photo of the Down-Flow Anoxic EMMC Reactor

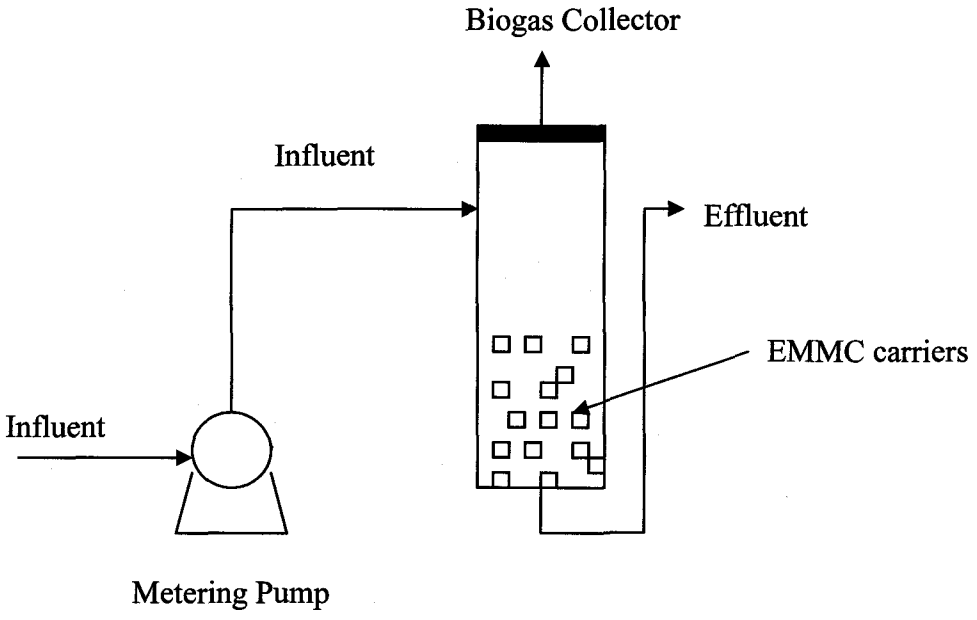

Figure 3.4(b). Schematic diagram of Down-Flow Anoxic EMMC Reactor

3.2.2 Synthetic wastewaters for both aerobic and anoxic EMMC reactors

Table 3.4. Composition of the Synthetic Wastewater for PCBs Removal from the aqueous phase by EMMC Aerobic Bioreactors

Content	Concentration (mg/L)
PCB	2
MeOH (CH_3OH)	600
$(NH_4)_2SO_4$	40
$MgSO_4.7H_2O$	5.87
$FeCl_3.H_2O$	0.08
KH_2PO_4	272.4
K_2HPO_4	425.9
$MnSO_4.H_2O$	2.41
$CaCl_2$	2.02
$NaCO_3.H_2O$	121

Table 3.5. Composition of the Synthetic Wastewater for PCBs Removal from the aqueous phase by EMMC Anoxic Bioreactor

Content	Concentration (mg/L)
PCB	2
MeOH (CH_3OH)	600
$NaNO_3$	50
$MgSO_4.7H_2O$	1.19
$FeCl_3.H_2O$	0.02
KH_2PO_4	54.5
K_2HPO_4	85.2
$MnSO_4.H_2O$	0.48
$CaCl_2$	0.40

3.2.3 Experimental Conditions

Table 3.6. Experimental conditions of EMMC Aerobic Process for PCBs Removal

HRT (hour)	24
Liquid temperature (°C)	23 ± 2
Influent SCOD concentration (mg/L)	600
Influent NH_3-N concentration (mg/L)	40
Total effective volume of reactor (ml)	980
Total carrier volume (ml)	392
Packing ratio (%)	40%
pH of influent	8.6~9.0
pH of effluent	7.6~8.0

Table 3.7. Experimental conditions of EMMC Anoxic Process for PCBs Removal

HRT (hour)	24
Liquid temperature (°C)	23 ± 2
Influent SCOD concentration (mg/L)	600
Influent NO_3-N concentration (mg/L)	40
Total effective volume of reactor (ml)	980
Total carrier volume (ml)	392
Packing ratio (%)	40%
pH of influent	6.8~7.0
pH of effluent	7.0~7.4

3.3 (Experiment 3) Using EMMC Aerobic Reactor To Treat Anaerobically Treated Effluent

In this experiment, the oil contained in the effluent of the anaerobic bioreactor fed with PCBs contaminated oil was removed for the further disposal / treatment (if necessary), and the liquid part was introduced to aerobic EMMC reactor to sweep away the remained PCBs and remove residual COD.

3.3.1 Pretreatment for Anaerobiclly Treated Effluent

Anaerobically treated effluent in the amount of 500ml was collected from Anaerobic Batch Reactor and 4ml of 6N NaOH was added to adjust pH from 6.4 to 8.0. Alum ($Al_2(SO_4)_3$) was also added to maintain the Alum concentration in the treating anaerobically effluent at 450mg/L. Then Jar test was used to separate the oil, solid and liquid parts in the anaerobically effluent. Mixing occurred in two stages: rapid mixing was first done at 100 rpm for five minutes then slow mixing was done at 30 rpm for 12 to 15 minutes. The mixtures of anaerobically treated effluent with NaOH and Alum were settled for about 12 hours. The separation was occurred and is shown in Figure 3.5. In order to achieve complete removal of oil, air was introduced to allow oil floatation. Both SCOD and PCBs

concentrations were monitored. The oil portion contained PCBs (shown in Figure 3.5) was proposed to be recycled and be retreated in the anaerobic treatment process.

3.3.2 EMMC system set-up and operation

After most of oil and solid were removed from the pretreatment process, the suspension (liquid part) was maintained at a pH of 8.0, SCOD of 17600mg/L, and MLSS of 4500mg/L. This suspension was then used as the feed for a one-liter EMMC aerobic reactor (as shown in Figure 3.6). The packing ratio (i.e. total volume of EMMC carriers/total volume of this unit) of the EMMC system was 30%. This EMMC unit was operated with a HRT of 20 days. The experimental conditions are shown in the Table 3.8.

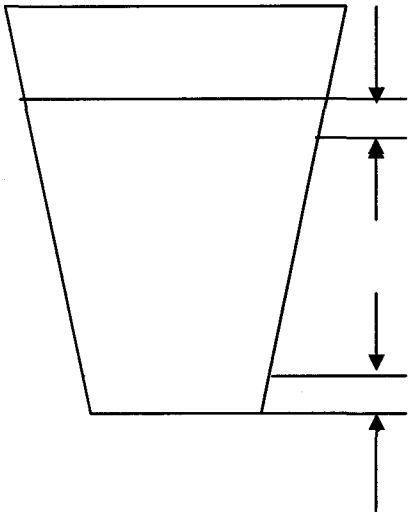

Figure 3.5. Separation after chemical coagulation of anaerobically treated effluent

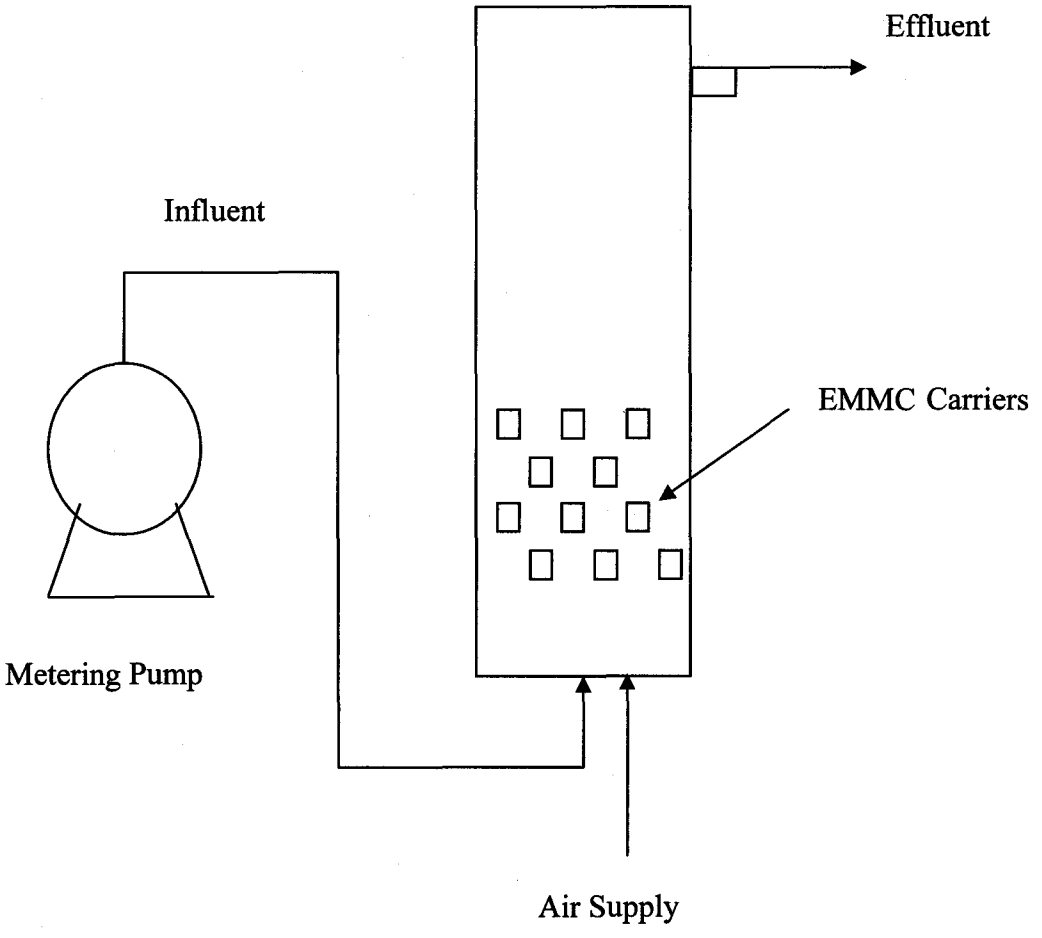

Figure 3.6. Schematic diagram of Up-Flow EMMC Aerobic Reactor

3.3.3 Experimental Conditions

Table 3.8. Experimental conditions of the Up-Flow EMMC Aerobic Reactor

HRT (day)	20
Liquid temperature (°C)	23 ± 2
Influent PCBs concentration (mg/L)	0.12
Influent SCOD concentration (mg/L)	176000
Effluent SCOD concentration (mg/L)	100000
Total effective volume of reactor (ml)	1000
Total carrier volume (ml)	300
Packing ratio (%)	30%
pH of influent	8.0
pH of effluent	7.6~7.8

3.4 (Experiment 4) Using EMMC Carriers with Suspended Culture Aerobic Batch Reactor to treat PCBs contaminated oil directly

Although the EMMC technology shows a high potential of biodegradation of PCBs, using EMMC Up-flow Aerobic Bioreactor, the clogging problem existed. In order to correct this clogging problem, EMMC carriers with suspended culture operated with aerobic batch reactor was investigated.

3.4.1 System set-up and operation

A reactor having effective volume of 4 liters with 5% of EMMC carriers (total volume of 200 ml) and MLSS of 2000mg/L was installed and operated with reaction time of 10 days (as shown in Figure 3.7).

For operation of EMMC carriers with suspended culture aerobic batch reactor, the following parameters was maintained (as shown in Table 3.9): initial COD (Methanol) concentration of 1000mg/L, a MLSS 2000mg/L for suspended culture, oil and surfactant concentration of 12800mg/L, and PCBs concentration of 0.09mg/L. Methanol was added intermittently into the reactor as the feed for microorganisms every two days.

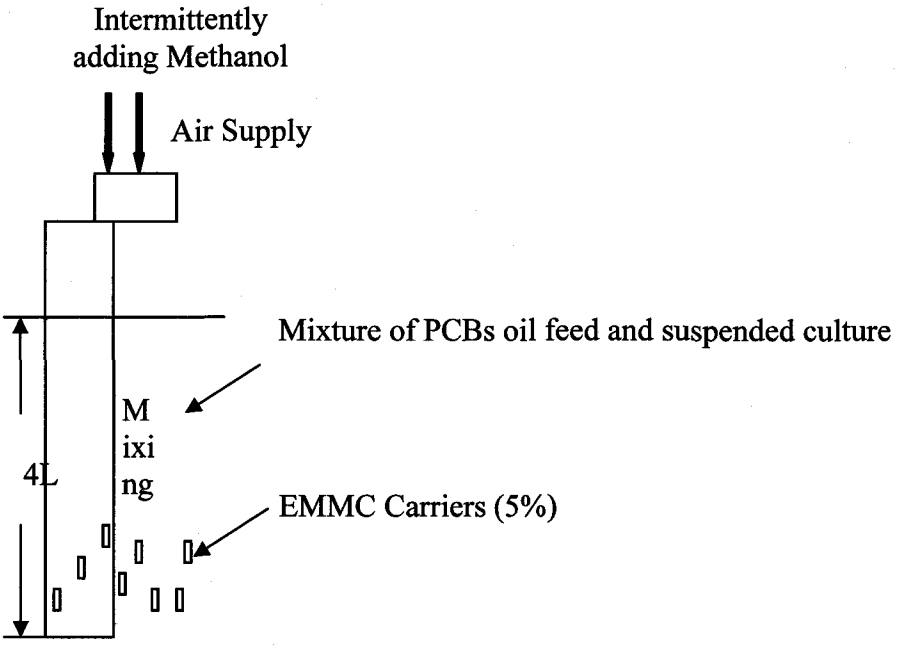

Figure 3.7. Schematic diagram of EMMC carriers with Suspended Culture Aerobic Batch Reactor

Table 3.9. Experimental conditions of the EMMC carriers with Suspended Culture Aerobic Batch Reactor

Reaction Time (day)	10
Liquid temperature (°C)	23 ± 2
Initial PCB concentration (mg/L)	0.09
Initial SCOD (MeOH) concentration (mg/L)	1000
Initial MLSS concentration (mg/L)	2000
Initial oil concentration (mg/L)	12800
Total effective volume of reactor (ml)	4000
Total carrier volume (ml)	200
Carbon Source added (ml / every 2 days)	4.3
pH of influent	7.6

Part II. PCB Removal from Oil Phase

3.5 (Experiment 5) Direct PCBs Removal from the Oil phase via Intermittent Up-Flow Anaerobic Bioreactor (IUFAB)

The objective of this experiment is to use the Intermittent Up-flow Anaerobic Bioreactor (IUFAB) to directly treat the PCBs contaminated oil. In this experiment, both PCBs enhanced (spiked) oil, which was spiked to PCBs concentration of 7.85 mg/L by using Aroclor 1260, and original PCBs contaminated oil with PCBs concentration of about 3 mg/L were introduced into the Intermittent Up-flow Anaerobic Bioreactor (IUFAB) to test potential of PCB removal from oil phase.

The PCBs concentration of the treated oil is monitored. For the operation of the Intermittent Up-flow Anaerobic Reactor (IUFAB), optimal HRT (Hydraulic Retention Time), COD loading rate and pH were investigated in this study.

3.5.1 Preliminary studies for determination of the bioreactor configuration

3.5.1.1 Anaerobic Reactor with Three-layer of media with high biomass content

Three medias made of steel were inserted into a glass column reactor as a three-layer up-flow anaerobic reactor, which has effective volume of 2 liters (as shown in Figure 3.8), with 7000mg/L MLSS of initial activated anaerobic sludge. The PCB feeding solution (as shown in Table 3.10) with PCBs concentration of 0.09 mg/L and COD concentration of 1000 mg/L was pumped to pass through the reactor intermittently (with the pumping schedule of 1-hour on and 7-hour off) from the bottom to the top. This bioreactor was operated with COD loading rate of 0.25g /L/day based on methanol and HRT of 4 days (as shown in Table 3.11).

Table 3.10. Compounds of the PCB feeding solution for three-layer up-flow anaerobic reactor

Content	Concentration (mg/L)
PCB based on PCB enhanced oil (7.85 mg/L)	0.09
MeOH (CH_3OH) as carbon source	1000
Initial oil concentration	128000
Initial surfactant concentration (Tween 80)	128000
NH_4Cl	26.7
$CaCl_2$	5.7
$MgCl_2.6H_2O$	10.1
$FeCl_2.4H_2O$	1
KH_2PO_4	25.2
$MnCl_2.4H_2O$	0.25
H_3BO_3	0.125
$ZnCl_2$	0.125
$CuCl_2.2H_20$	0.11
$CoCl_2.6H_20$	1.25
$NiCl_2.6H_20$	0.125
$NaMoO_4.2H_20$	0.025
$CaHCO_3$	1200
pH	6.6

COD loading rate (g/L/day)	0.25
PCB loading rate (mg/L/day)	0.0125
Oil loading rate (g/L/day)	3.2
pH	6.4

Table 3.11. Operational Conditions of three-layer up-flow anaerobic reactor

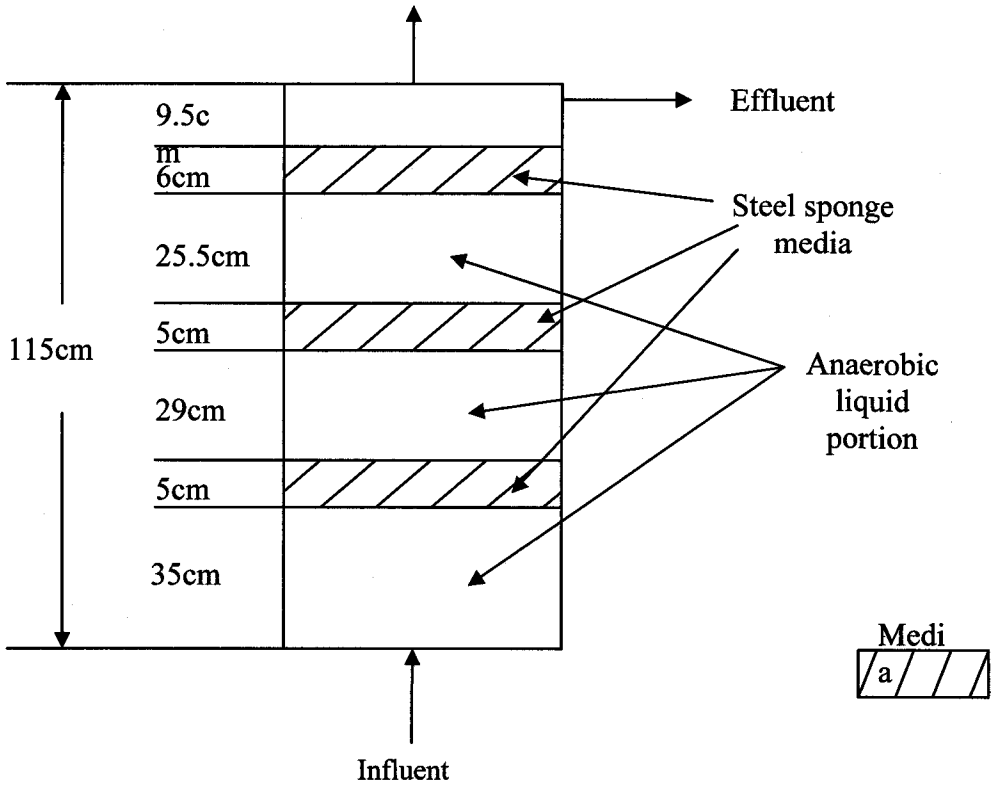

Figure 3.8. Schematic diagram of three-layer up-flow anaerobic reactor

3.5.1.2 Anaerobic Batch Reactor integrated with Three-layer Up-flow Anaerobic Reactor System

In this experiment, three-layer up-flow anaerobic reactor and anaerobic batch reactor were integrated together for improving PCBs removal from oil. A batch reactor with effective volume of 4 liters (as shown in Figure 3.9) with initial activated anaerobic sludge of MLSS of 7000mg/L was installed and operated with HRT of 10 days as the first stage, and the three-layer up-flow anaerobic reactor was used as the second stage (as shown in Figure 3.9). The PCB feeding solution is similar with the three-layer up-flow anaerobic reactor (as shown in Table 3.10) and the HRT of this system is operated with 14 days.

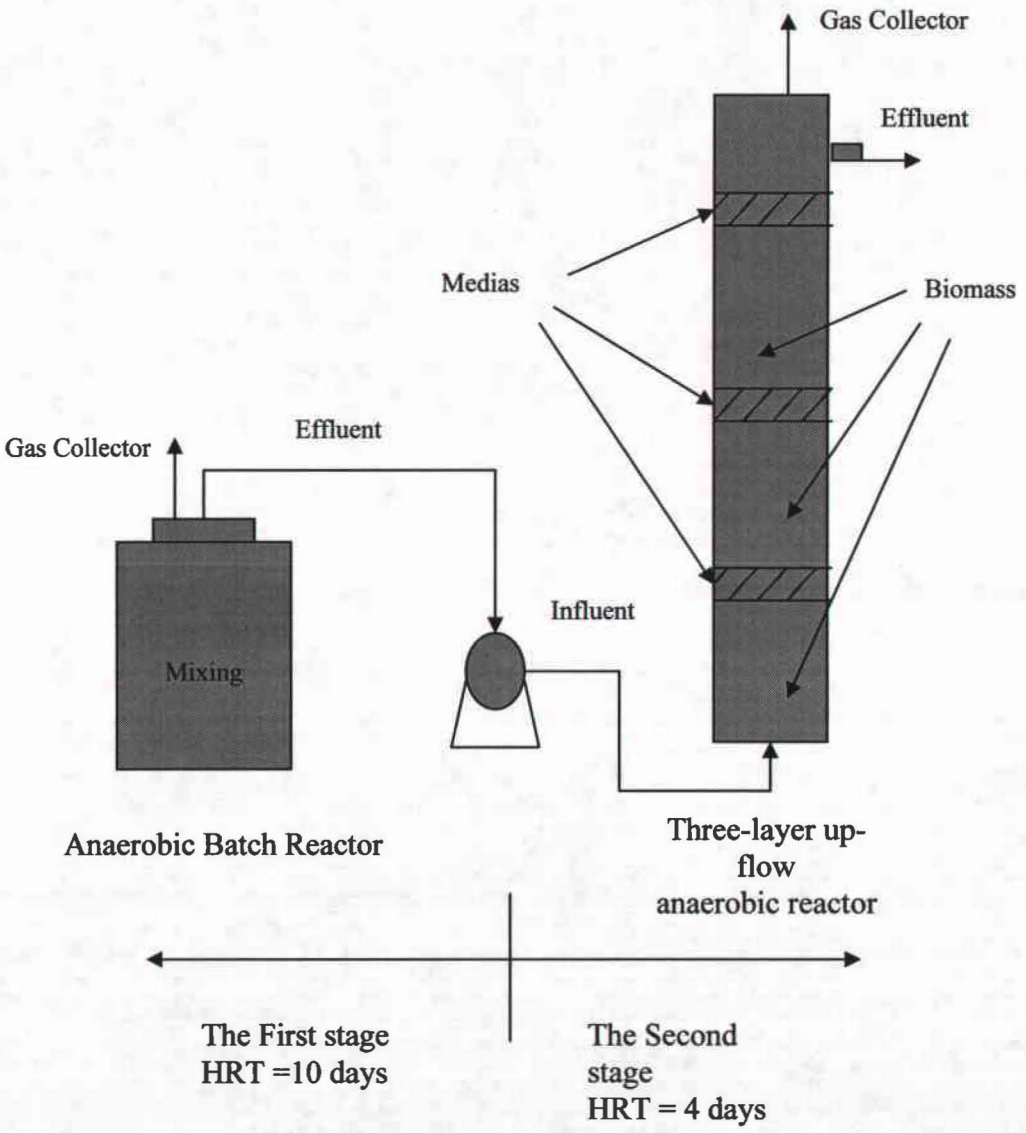

Figure 3.9. Schematic diagram of two-stage anaerobic system

3.5.2 Intermittent Up-Flow Anaerobic Bioreactor system set-up and operation

A glass column reactor having effective volume of 2 liters (as shown in Figure 3.10(a) & (b)) with 7000mg/L MLSS of initial activated anaerobic sludge was installed as an Intermittent Up-flow Anaerobic Bioreactor (IUFAB). This bioreactor is operated with HRT of 4 days in the ambient temperature of 23°C ± 2°C. The PCBs feeding solution (as shown in Table 3.12.), as influent, was pumped to pass through the reactor intermittently from the bottom to the top. The intermittent pumping schedule for pump is 1-hour on and 7-hour off. The oil layer was formed by the treated oil on the top of Intermittent Up-Flow Anaerobic Bioreactor (IUFAB), where effluent was separated to two parts, oil part and liquid part, and most of the treated oil was accumulated and formed oil layer, also the liquid part was discharged. The oil part of the effluent, the treated oil, was collected and measured for PCBs content by using Gas Chromatograph (GC) machine. The liquid part of the effluent come out from the top was collected and measured for PCBs content. Also, gas production collected by gas collector was analyzed for the biogas composition. In this experiment, PCBs concentration of the treated oil taken from the top of the reactor was monitored as the most important parameter.

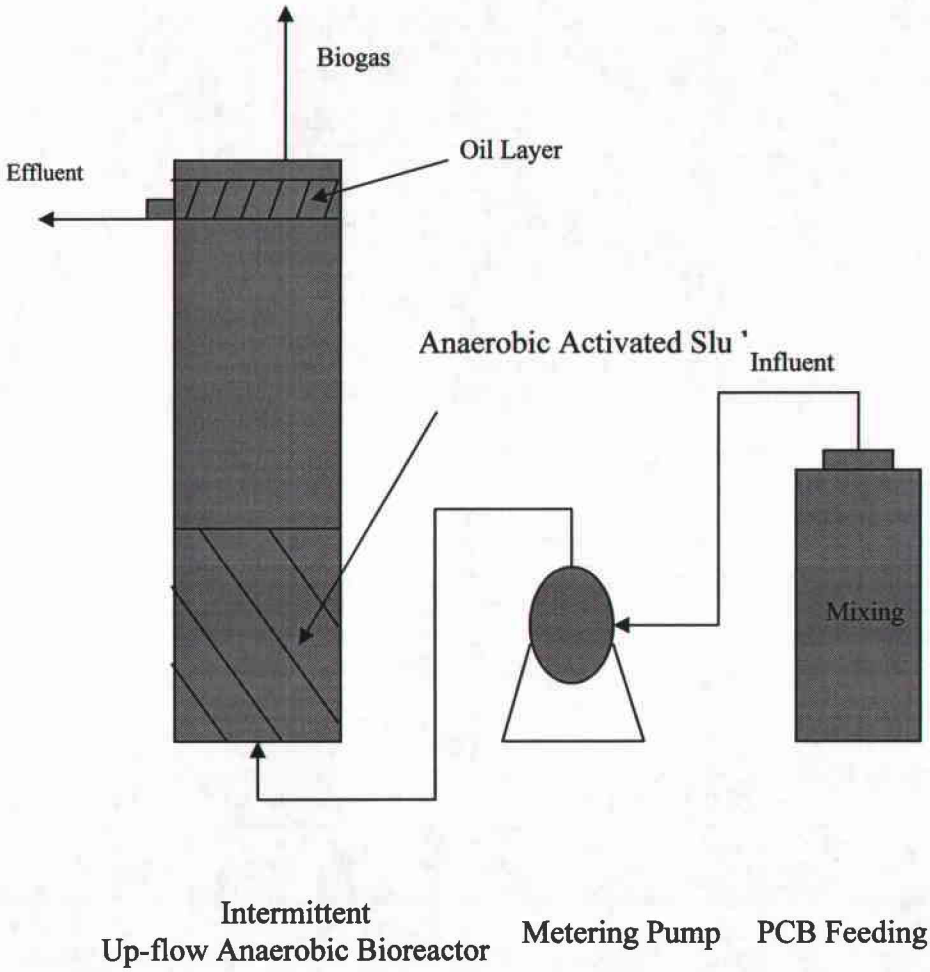

Figure 3.10(a). Schematic Diagram of Intermittent Up-Flow Anaerobic Bioreactor

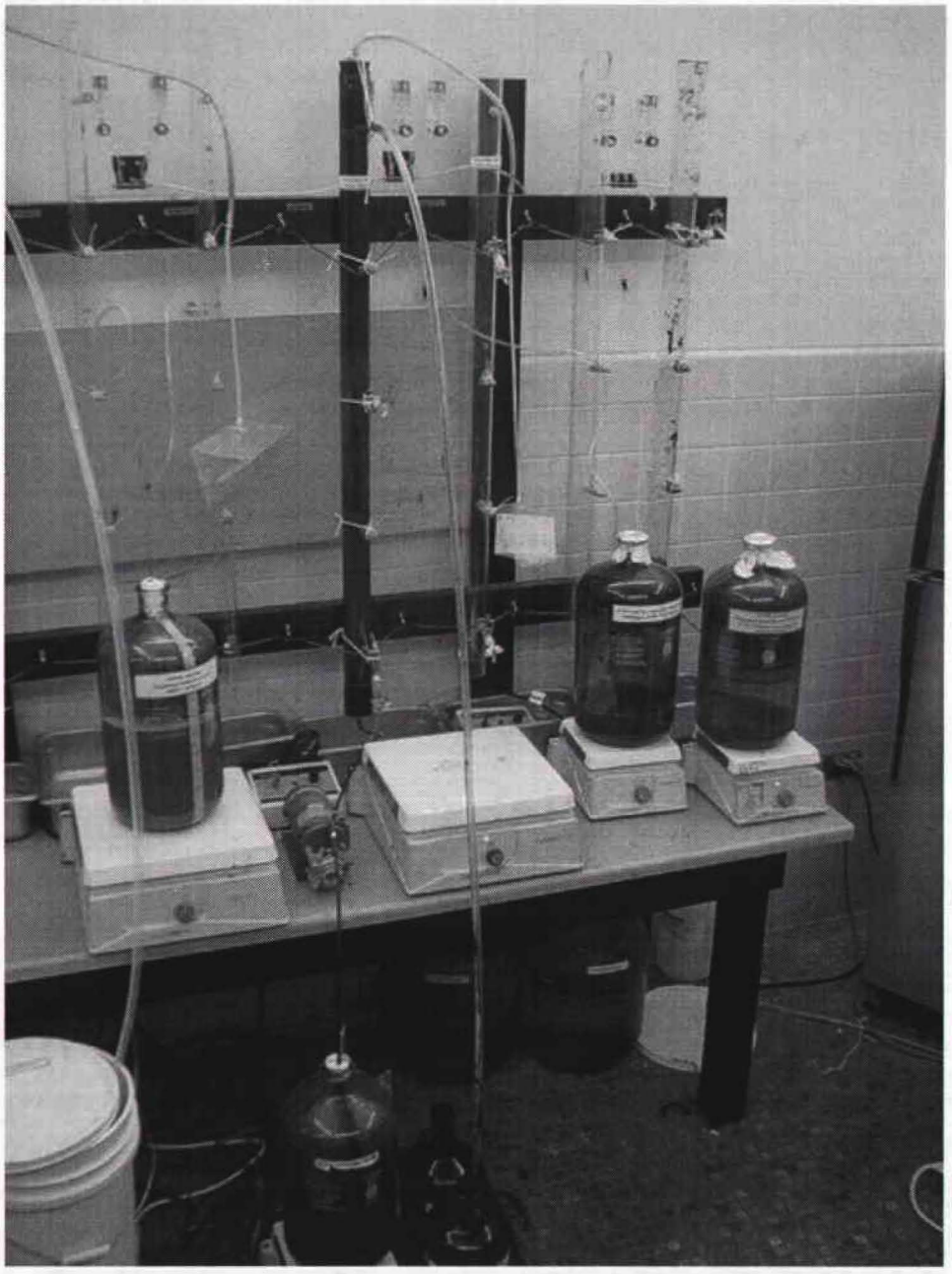

Figure 3.10(b). Photo of the Intermittent Up-flow Anaerobic Bioreactor (IUFAB)

3.5.3 Preparation of the PCB feeding solution and experimental conditions for IUFAB

Based on our preliminary experiments, the PCBs feed was prepared (as shown in Table 3.12) with the ratio of volume, 1: 40 (Oil: Carbon source and Nutrients solution). The surfactant was added with the same amount of oil. Oil, surfactant and carbon source and nutrients solution were mixed continuously in the feeding tank. To prepare the PCB feeding solution, both the oil with enhanced PCB concentration up to 7.85 mg/L and the original PCB contaminated oil with PCB concentration of 3 mg/L were used to make the PCB feed, and methanol was used as the carbon source. COD loading rate of 0.25g /L/day based on methanol and HRT of 4 days were applied for the operation of Intermittent Up-flow Anaerobic Bioreactor (IUFAB) (as shown in Table 3.13).

Table 3.12. Compounds of the PCB feeding solution for Intermittent Up-flow Anaerobic Bioreactor (IUFAB)

Content	Concentration (mg/L)
PCB concentration (mg/L) – PCB Enhanced oil	0.09
PCB concentration (mg/L) – Original oil	0.04
MeOH (CH_3OH)	1000
Initial oil concentration	128000
Initial surfactant concentration (Tween 80)	128000
NH_4Cl	26.7
$CaCl_2$	5.7
$MgCl_2.6H_2O$	10.1
$FeCl_2.4H_2O$	1
KH_2PO_4	25.2
$MnCl_2.4H_2O$	0.25
H_3BO_3	0.125
$ZnCl_2$	0.125
$CuCl_2.2H_20$	0.11
$CoCl_2.6H_20$	1.25
$NiCl_2.6H_20$	0.125
$NaMoO_4.2H_20$	0.025
$CaHCO_3$	1200
pH	6.6

Table 3.13. Experimental conditions of Intermittent Up-flow Anaerobic Bioreactor (IUFAB)

HRT (day)	4
Intermittent Feeding	1-hour on 7-hour off
Ambient temperature (^{o}C)	23 ± 2
Influent PCB concentration (mg/L) – PCB Enhanced oil	0.09
Influent PCB concentration (mg/L) – Original oil	0.04
Influent SCOD (MeOH) concentration (mg/L)	1000
Influent oil concentration (mg/L)	12800
Initial MLSS concentration in IUFAB (mg/L)	7000
Total effective volume of reactor (ml)	2000
pH of Influent	6.4
pH of Effluent	6.2

Part III. Analytic Methods

3.6 Sample Analytic Methods

Influent and effluent samples were analyzed for TCOD, SCOD, NH_3-N, NO_3-N, TS, TSS, pH and PCB concentration. Also, the biogas production and composition were monitored. PCBs contaminated oil and treated oil samples were analyzed for PCBs concentrations by using Gas Chromatograph (GC) machine.

3.6.1 Analyses of Fundamental Parameters

* COD (Chemical Oxygen Demand):

  COD values were determined by using Reactor Digestion Method with HACH spectrophotometer DR/3000 (HACH Co., 1992).

* NO_3-N (Nitrate nitrogen):

  The concentration of NO_3-N values in the samples was analyzed by the Cadmium Reduction Method with HACH spectrophotometer DR/3000 (HACH Co., 1992). Use of HACH methods are applied by U.S. EPA (U.S. Environmental Protection Agency).

* NH_3-N (Ammonia):

The Nessler Method was used in the HACH spectrophotometer, Model DR/3000 to determine ammonia nitrogen concentration.

* TSS (Total Suspended Solids):

TSS value was measured by drying the filter attached with biomass at 103oC – 105oC. According to procedure in ***Standard Methods*** for the Examination of Water and Wastewater (American Public Health Association, 1995), 19th Edition.

* pH

pH value of raw synthetic wastewater and treated water were measured by using ORION 250 pH meter.

* Gas composition

Gas composition of the biogas production from the anaerobic bioreactor was measured by using Gas chromatography (GC) machine, SHIMADZU GC-3BT.

* GC analysis

Analysis of Total PCBs and PCB Congeners and Trans-nonachlor in Fish by Gas Chromatography / Negative Chemical Ionization Single Ion Mass Spectrometry

By using Standard Operating Procedure SOP No. HC 519.D

3.6.2 Analyses of PCB Concentration in the Aqueous and Oil Phrase

Based on our pervious study, a variety of chemicals including Methanol, Acetonitrile, Acetone, Dimethyl Sulfoxide (DMSO) and Hexane were used to extract PCBs from PCB contaminated oil. Among these chemicals, hexane was the best chemical for extracting PCBs from the PCB contaminated oil because it has the best recovery efficiency and it is more cost-effective (as shown in Figures 3.11 & 3.12) (Yang and Kim, 2002).

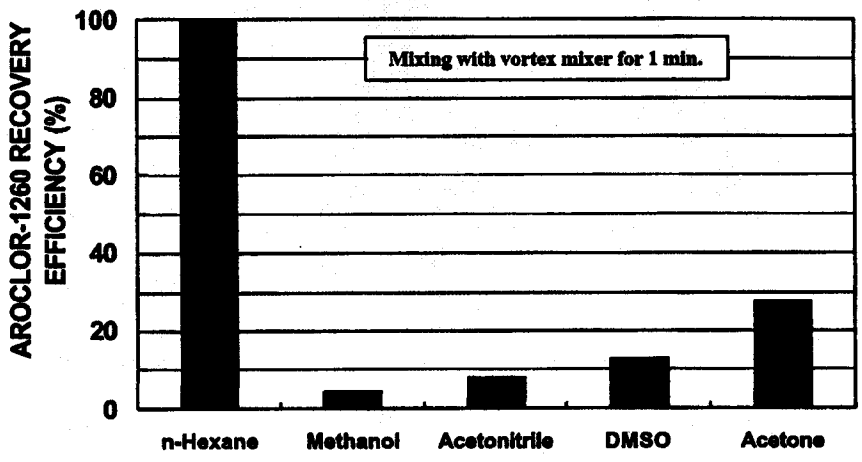

Figure 3.11. Comparison of Aroclor–1260 recovery efficiency extracted with various solvents

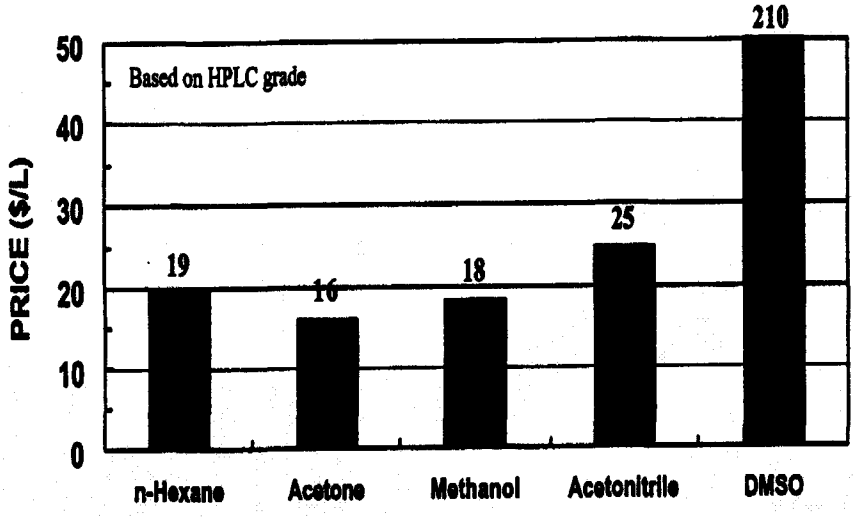

Figure 3.12. Comparison of solvent price

3.6.2.1 Analysis of PCB Concentration in the Aqueous Phrase

Method:

1. An influent or effluent liquid sample of 0.5 ml was taken and put into the vial, and Hexane of 5 ml was also added. A Vortex Mixer was used to completely mix sample and Hexane for at least 30 seconds. In the meantime, the PCBs in the sample were extracted by Hexane.
2. Sulfuric acid (H_2SO_4) of 2.5 ml was added into vial and mixed by Vortex Mixer for another 30 seconds for removal of the residual organic matters.

3. Sample was centrifuged at 300 rpm for 20 minutes.
4. Part of solvent layer was transferred to 1 ml vial for GC analysis.

3.6.2.2 Analysis of PCB Concentration in the Oil Phrase

The procedure is same as analysis of PCB concentration in the aqueous phrase. The only difference is that the treated oil of 0.05 gram was weighed as the sample instead of 0.5 ml of aqueous sample. If PCB concentration is very low, treated oil of 0.1 gram can be taken as the sample.

CHAPTER 4. RESULTS AND DISCUSSION

Part I. PCBs Removal from the Aqueous Phase via EMMC Technology

4.1 (Experiment 1) Selection of EMMC carriers for PCBs removal from aqueous phase

In order to investigate the potential application of EMMC technology for the PCB removal, the appropriate carrier preparation requires further investigation. The objective of this experiment is to determine the polymeric materials for the preparation of the EMMC carriers, which can be used to investigate COD and nitrogen removal. Both CTA carriers (made by cellulose triacetate) and CAW carriers (made by cellulose acetate waste material) were used to observe the process performance for potential PCBs removal from aqueous phase.

As presented in Figures 4.2 and 4.5, both EMMC systems with CTA and CAW carrier are able to achieve the steady state after 10 days and 20 days of starting up, respectively. Figures 4.2 and 4.5 also show that the CTA carrier

system has the faster starting-up period than the CAW carrier system based on the COD and nitrogen removal.

As shown in Figure 4.2, the COD removal efficiencies of CTA carrier system and CAW carrier system are in the average values of 95% and 93%, respectively. As shown in Figure 4.3, the concentrations of effluent ammonia nitrogen in CTA and CAW carrier systems are 3.5 mg/L and 5 mg/L, respectively. Also, the concentrations of effluent nitrate nitrogen (NO_3-N) are about 15 mg/L for both CTA and CAW carrier systems (as shown in Figure 4.4). The total nitrogen removal (including NO_3-N and NH_3-N) efficiencies are 35% and 32% for the CTA and CAW carrier systems, respectively (as shown in Figure 4.5). Based on all the results, the EMMC reactor with CTA carriers indicates faster starting-up, higher COD and nitrogen removal compared to CAW carriers.

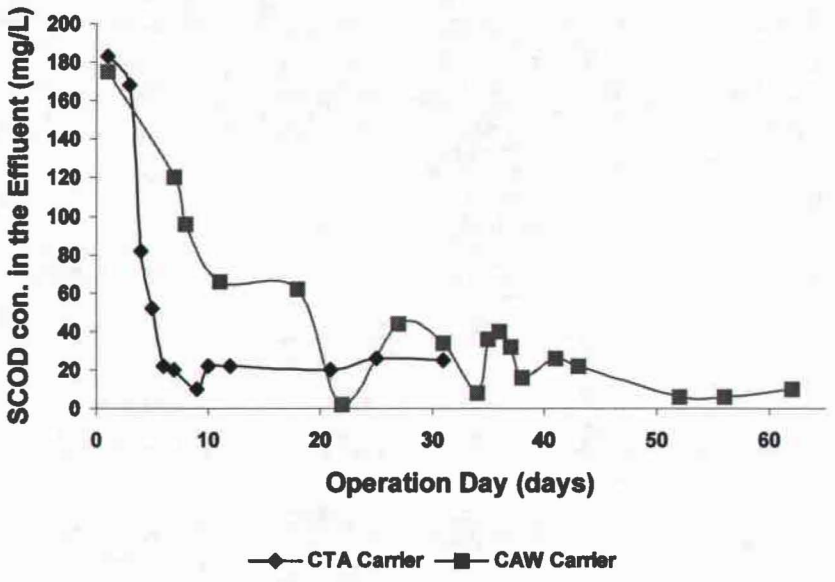

Figure 4.1. Comparison of effluent SCOD conc. of CTA and CAW carrier systems

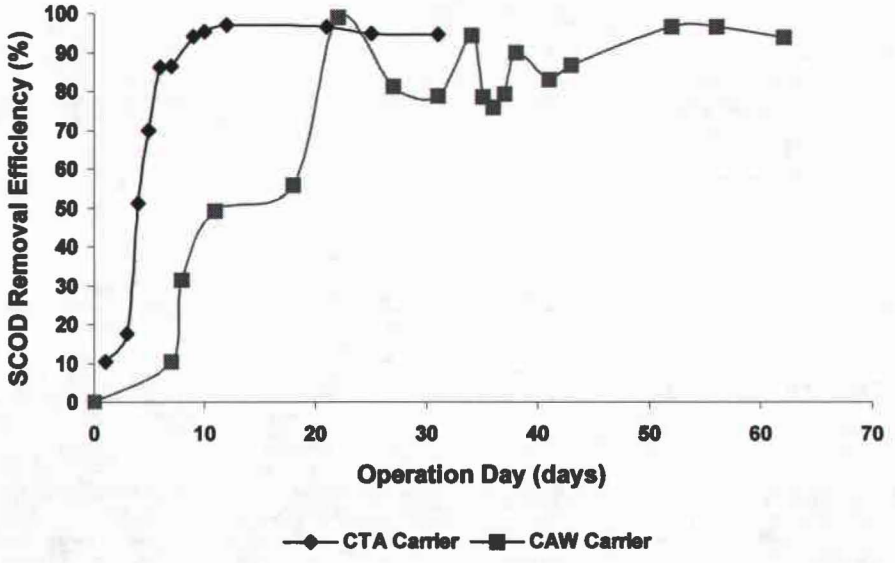

Figure 4.2. Comparison of SCOD Removal Efficiencies of CTA and CAW carrier systems

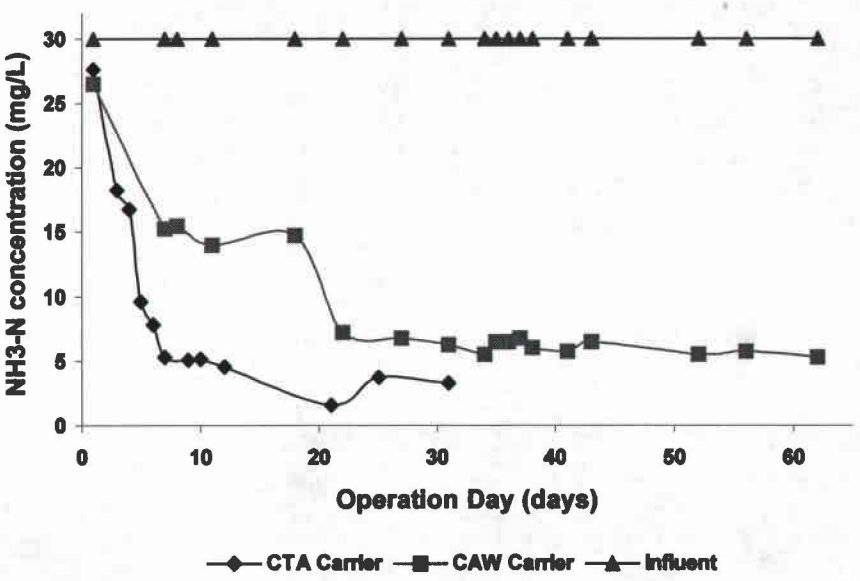

Figure 4.3. Comparison of effluent NH_3-N conc. of CTA and CAW carrier systems

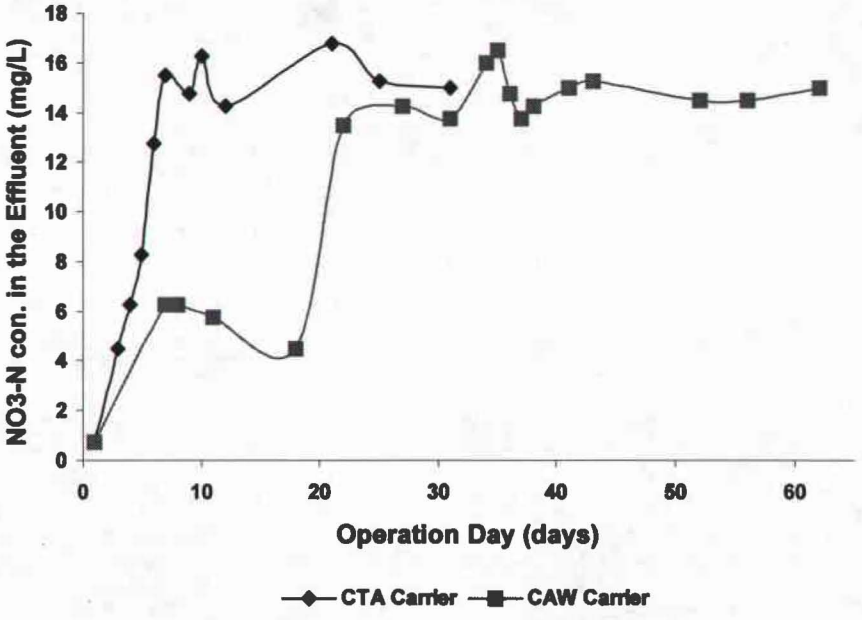

Figure 4.4. Comparison of effluent NO_3-N conc. of CTA and CAW carrier systems

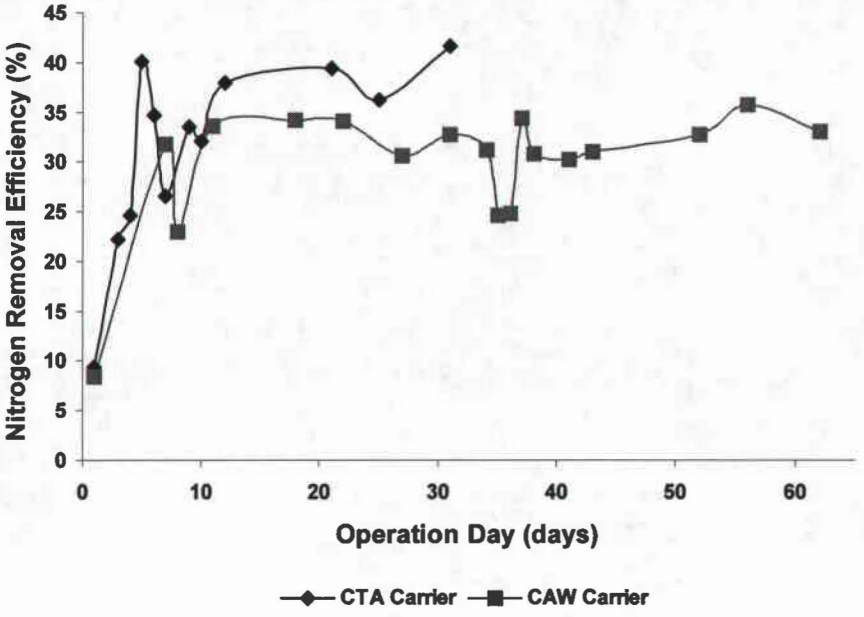

Figure 4.5. Comparison of Nitrogen Removal Efficiencies of CTA and CAW carrier systems

4.2 (Experiment 2) Comparison of EMMC Aerobic and Anoxic processes for PCBs Degradation using Synthetic Wastewater

Under the HRT of 24 hours, influent COD concentration of 600mg/L and influent PCBs concentration of 2 mg/L, those aerobic and anoxic EMMC reactors were investigated for the process performance. As presented in Table 4.1, the COD removal efficiency and Total-N (including NH_3-N, NO_3-N, and NO_2-N) removal efficiency of EMMC aerobic reactor operated with continuous aeration are 94.7-97.4% (average 96.9% ± 0.4%) and 40.1-47.1% (average 44.6% ± 2.1%),

respectively. The COD and Total-N removal efficiencies of EMMC aerobic reactor operated with intermittent aeration (1-hour on and 1-hour off) are 92.3-96.3% (average 94.9% ± 1.1%) and 38.9-42.1% (average 41.6% ± 0.5%) for COD and Total-N removal efficiencies, respectively. However, the COD removal efficiency and NO_3-N removal efficiency of EMMC anoxic reactor are 33.1-46.7% (average 38.7% ± 1.4%) and 87.4-99.4% (average 96.8% ± 1.7%), respectively. Compared to the EMMC aerobic reactors, COD removal efficiency of EMMC anoxic reactor is low (as shown in Table 4.1).

Table 4.1. COD and total nitrogen removal efficiencies of aerobic and anoxic reactors

	Aerobic reactor (Continuous aeration)	Aerobic reactor (Intermittent aeration)	Anoxic reactor
COD removal efficiency (%)	94.7-97.4	92.3-96.3	33.1-46.7
Total Nitrogen removal efficiency (%)	40.1-47.1	38.9-42.1	87.4-99.4

As shown in Figures 4.6, 4.7, 4.8, and Table 4.2, PCBs removals for various types of operations of EMMC bioreactor are presented. The Aroclor-1260 and Aroclor-total degradation efficiencies of EMMC aerobic reactor operated

with continuous aeration are 93.4-99.8% (average 98.4% ± 1.1%) and 94.7-99.8% (average 98.3% ± 0.9%), respectively. The efficiencies of 82.7-97.2 % (average 95.3% ± 1.5%) for Aroclor-1260 and 85.7-97.7% (average 95.7% ± 1.8%) for Aroclor-total are also high in the EMMC aerobic reactor operated with intermittent aeration. However, the degradation efficiency is gradually decreased from 80% to 40% in anoxic condition.

In this experiment, methanol was added as additional carbon source into both aerobic and anoxic EMMC reactors, and it was also used as the electron donor and energy source to attain necessary energy and electrons for the microorganisms to complete their metabolism and synthesis. For the anoxic reactor, sodium nitrate was also introduced for denitrification. Denitrification proceeds in a stepwise manner in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and N_2 gas. Each half-reaction and enzyme catalysing it are shown below (Rittmann and McCarty, 2000):

$NO_3^- + 2\ e^- + 2\ H^+ = NO_2^- + H_2O$	Nitrate Reductase
$NO_2^- + e^- + 2\ H^+ = NO + H_2O$	Nitrite Reductase
$2NO + 2\ e^- + 2\ H^+ = N_2O + H_2O$	Nitric Oxide Reductase
$N_2O + 2\ e^- + 2\ H^+ = N_2(g) + H_2O$	Nitrous Oxide Reductase

In the anoxic reactor, it was assumed that a part of energy had to be used by denitrification to produce the nitrogen gas (N_2), and rest was used to biodegrade the PCBs by completing the dechlorination, and convert the PCBs to their corresponding acids. However, in the aerobic reactors, almost all energy was utilized to metabolism and synthesis of microorganisms. That causes the difference on the PCBs removal efficiency between these aerobic and anoxic reactors, i.e., the aerobic EMMC reactor provides higher PCBs removal than the anoxic EMMC reactor.

Based on the results, it is apparent that it is more effective for the EMMC bioreactor operated with continuous or intermittent aeration to biodegrade PCBs than the anoxic EMMC reactor. Therefore, in the next phase, the anaerobically effluent from the anaerobic reactors fed with PCBs contaminated oil is investigated for further treatment by using EMMC aerobic reactor.

Table 4.2. Aroclor-1260 and total PCBs removal efficiencies of aerobic and anoxic reactors

	Aerobic reactor (Continuous aeration)	Aerobic reactor (Intermittent aeration)	Anoxic reactor
Aroclor-1260 removal efficiency (%)	93.4-99.8	82.7-97.2	27.3-89.7
Total PCBs removal efficiency (%)	94.7-99.8	85.7-97.7	32.6-91.4

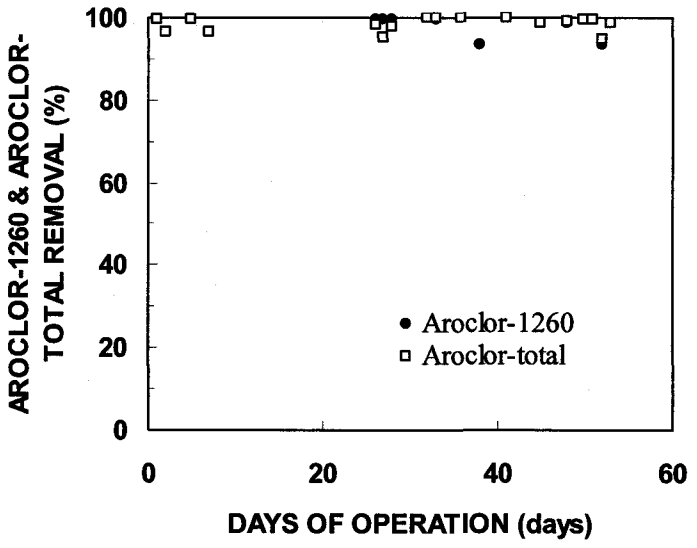

Figure 4.6. Aerobic degradation of Aroclor-1260 and Aroclor-total in aerobic EMMC reactor operated with continuous aeration

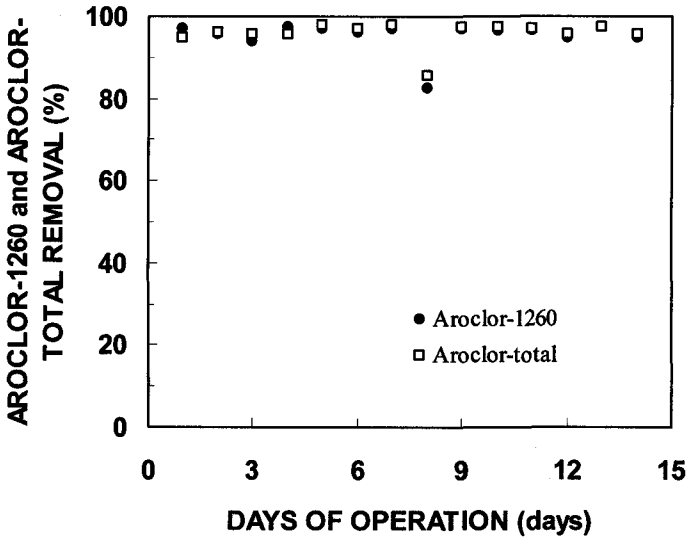

Figure 4.7. Aerobic degradation of Aroclor-1260 and Aroclor-total in aerobic EMMC reactor operated with intermittent aeration

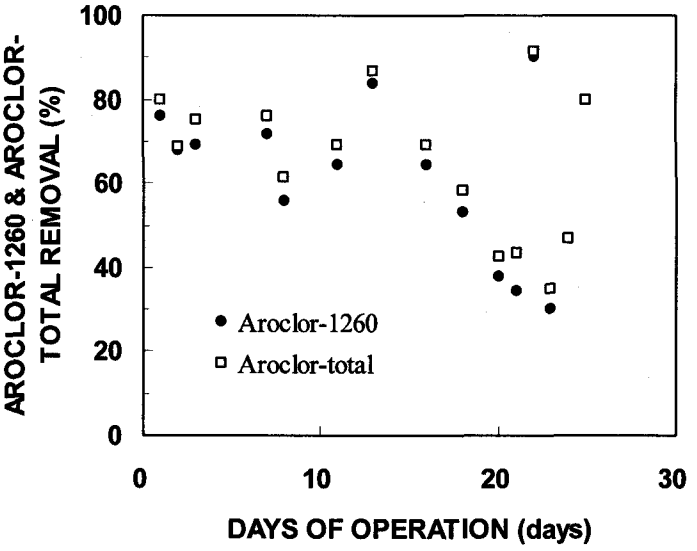

Figure 4.8. Anoxic degradation of Aroclor-1260 and Aroclor-total in anoxic EMMC reactor

4.3 (Experiment 3) Using EMMC Aerobic Reactor To Treat Anaerobically Treated Effluent

As shown in Figures 4.9 and 4.10, based on the mass balance (considered total COD and PCBs loaded, and total COD and PCBs remained), COD and PCB removal efficiency of 63.5% and 40% are achieved at the 20th day, respectively at the HRT of 20 days and COD loading rate of 4.4 g/L/day.

Based on this preliminary study, both COD and PCBs contained in the anaerobically treated effluent can be further removed by using the aerobic reactor with EMMC technology. However, it was observed that the problem of clogging in the EMMC reactor, which could slow down the treatment efficiency (as shown in Figure 4.10). Further study of this application is required.

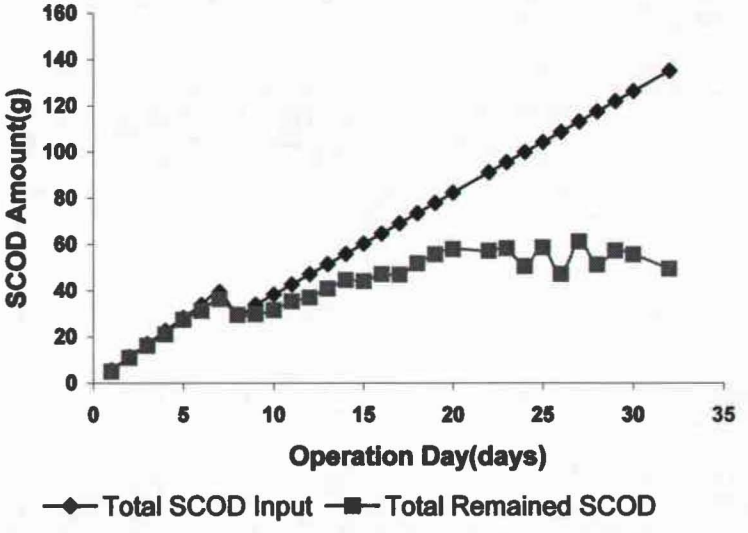

Figure 4.9. Comparison Total SCOD Amount between input and remained (HRT=20d)

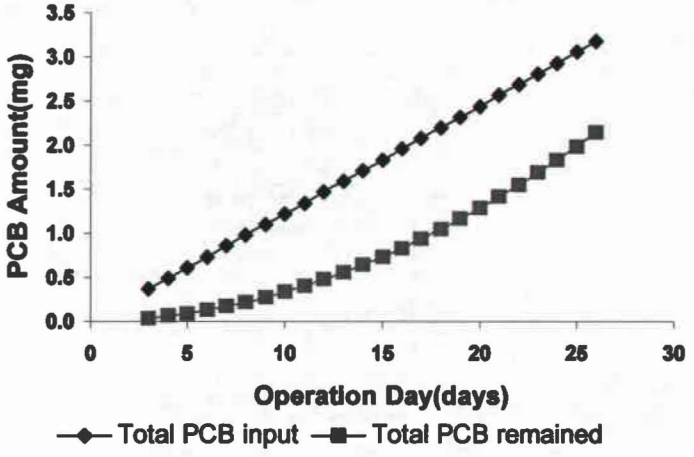

Figure 4.10. Comparison of Total PCB Amount between input and remained (HRT= 20d)

4.4 (Experiment 4) Using EMMC Carriers with Suspended Culture of Aerobic Batch Reactor to treat PCBs contaminated oil directly

In order to correct the problem of clogging occurred in the fixed bed of EMMC bioreactor, an EMMC carriers (5%) with aerobic suspended culture batch reactor with reaction time of 10 days was investigated. As shown in Figure 4.12, the PCBs concentration in the mixed liquor is decreased to a more stable level after about 10 days of reaction. The removal efficiency of PCBs in the mixed liquor is about 77% (as shown in Figure 4.11). During the period of treatment, methanol was intermittently added as the feed for microorganisms in every two days (as shown in Figure 4.12). This result indicates a certain degree of promise for direct removal of PCBs from oil, but because there are a lot of impurities contained in the sample, those impurities eventually caused the analytical problem with the sample for PCBs analysis by using GC machine. Therefore, unless an improvement of GC analysis is developed, the direct biological treatment of PCBs contaminated oil using EMMC carriers (5%) in the aerobic suspended culture is difficult to be monitored. This requires further study if this direct biological removal process is desired to implement.

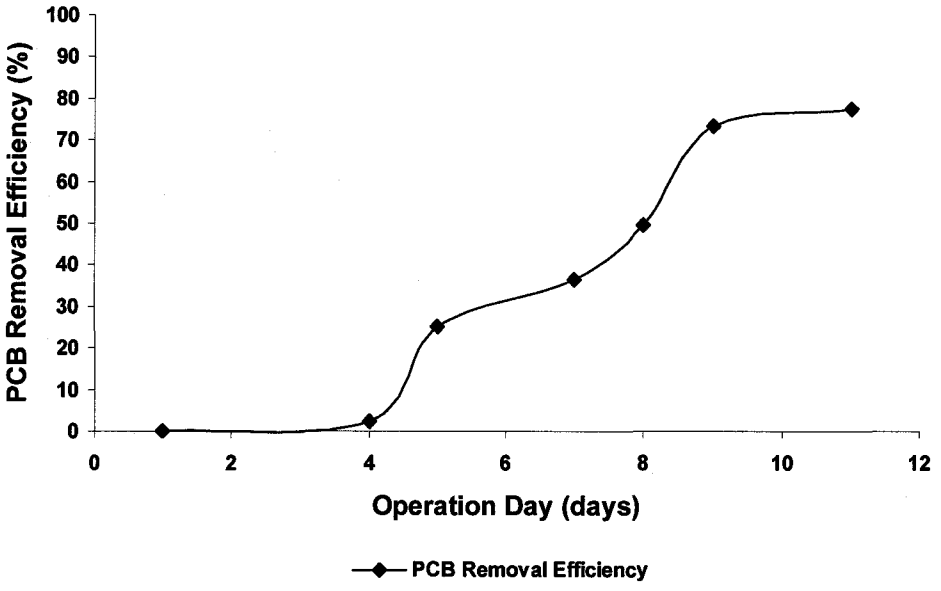

Figure 4.11. Removal Efficiency of PCBs in the mixture liquor in the EMMC with Suspended Culture Aerobic Batch Reactor

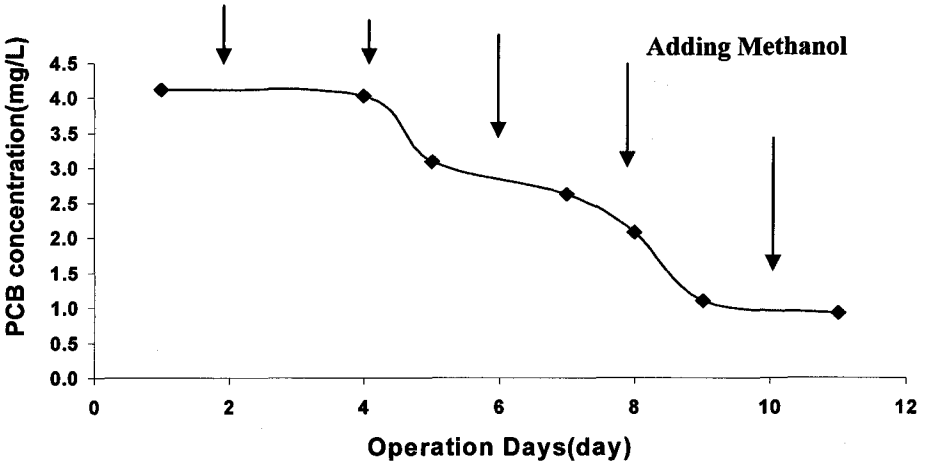

Figure 4.12. PCBs concentration of the mixture liquor in the EMMC with Suspended Culture Aerobic Batch Reactor

Part II. PCBs Removal from Oil Phase

4.5 (Experiment 5) Direct PCBs Removal from the Oil phase via Intermittent Up-Flow Anaerobic Bioreactor (IUFAB)

Based on the previous studies (Tang et al., 2000; Tartakovsky et al., 2000; Tartakovsky et al., 2001; Saponaro et al., 2003) of the unsuccessful cases of cleanup PCBs in both aqueous and slurry phases, the key problem is how to increase and maintain high-activated biomass content in the reactor if biological treatment process is to be practiced. Therefore, various types of the bioreactors were designed and investigated in this experiment, such as, three-layer up-flow anaerobic reactor, two-stage anaerobic system and intermittent up-flow anaerobic bioreactor.

4.5.1. Preliminary evaluation of various anaerobic bioreactors with high biomass content

4.5.1.1. Anaerobic Reactor with Three-layer of media

In order to maintain high biomass content and increase the SRT, three media made of steel were inserted into a glass column reactor, which had an

effective volume of 2-liter (as shown in Figure 3.8), with 7000mg/L MLSS of initial activated anaerobic sludge as a three-layer up-flow anaerobic reactor. This reactor was investigated in the ambient temperature of 23°C ± 2°C. The PCB feeding solution (as shown in Table 3.10) with PCBs concentration of 0.09 mg/L and COD concentration of 1000 mg/L was pumped to pass through the reactor intermittently (with the pumping schedule of 1-hour on and 7-hour off) from the bottom to the top. COD loading rate of 0.25g /L/day based on methanol and HRT of 4 days were applied for the operation of this reactor (as shown in Table 3.11).

After 13 days of operation, based on the mass balance of fed and remained PCBs amount, the three-layer up-flow anaerobic reactor with HRT of 4 days could achieve PCBs removal of 25% from oil (as shown in Figure 4.13).

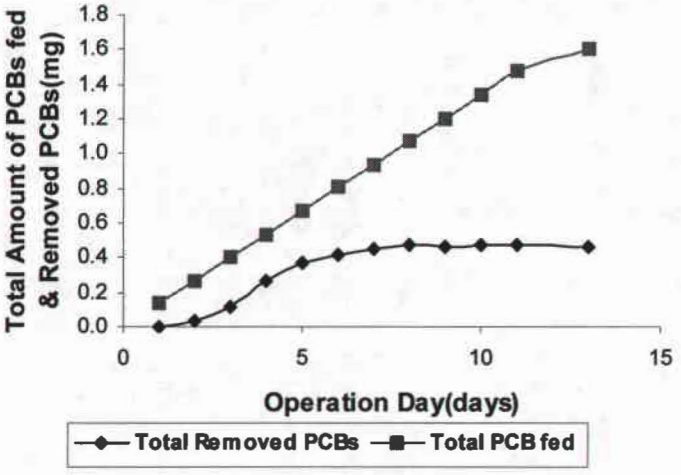

Figure 4.13. Total PCB loaded amount VS total PCB removed from the three-layer up-flow anaerobic reactor

4.5.1.2. Anaerobic Batch Reactor integrated with Three-layer Up-flow Anaerobic Reactor System

To increase the PCB removal efficiency, three-layer up-flow anaerobic reactor and anaerobic batch reactor were integrated together for improving PCBs removal from oil. A batch reactor with effective volume of 4 liters (as shown in Figure 3.9) with initial activated anaerobic sludge of MLSS of 7000mg/L was installed and operated with HRT of 10 days as the first stage. Initial COD (Methanol) concentration of 1000mg/L, oil and surfactant concentration of

12800mg/L, and PCB concentration of 0.09mg/L were maintained at the starting of the reaction. The three-layer up-flow anaerobic reactor was operated as the second stage (as shown in Figure 3.9). The PCB feeding solution is similar with the three-layer up-flow anaerobic reactor (as shown in Table 3.10) and the HRT of this system is operated with 14 days.

This two-stage system is more effective than the single stage of three-layer up-flow anaerobic reactor. Based on the calculation of the PCB mass balance, PCB removal of 40% can be reached easily after 14 days of operation. However, for the long-term operation (after 45 days of operation), the problem of clogging was found in the second stage, i.e., the three-layer reactor.

4.5.1.3. Intermittent Up-Flow Anaerobic Bioreactor

In order to solve the clogging problem and simplify the design and operation of the three-layer anaerobic reactor integrating with anaerobic batch reactor, the intermittent up-flow anaerobic bioreactor (IUFAB) was designed and operated to treat the PCB contaminated oil directly. A glass column reactor having effective volume of 2-liter with 7000mg/L MLSS of initial activated anaerobic sludge was installed as an Intermittent Up-flow Anaerobic Bioreactor (IUFAB). This reactor was operated in the ambient temperature of 23°C ± 2°C

with HRT of 4. The PCB feeding solution was pumped directly to pass through the reactor intermittently from the bottom to the top.

This IUFAB system has been operated for 212 days with a HRT of 4 days, COD loading rate of 0.25 g/L/day, oil loading rate of 3.2 g/L/day, PCB loading rate of 0.024 mg/L/day (from 1st to 92nd day based on PCB enhanced oil) and 0.011 mg/L/day (from 93rd to 212th day based on original oil), and PCB feed of pH 6.4, and the PCB removal efficiency of about 50% can be achieved for both enhanced PCB oil and original PCBs contaminated oil. Based on the 6-month data, the IUFAB demonstrated a very good stability to maintain the high PCB removal efficiency. Further detailed analysis of this process is presented as follows:

4.5.2. Development of IUFAB for treatment of PCBs contaminated oil

4.5.2.1. PCBs removal from PCBs enhanced oil and original oil

For the first experiment of Intermittent Up-flow Anaerobic Bioreactor (IUFAB), PCBs concentration in the oil was enhanced from 2.85 mg/L to 7.85 mg/L by adding pure high concentration PCBs (Aroclor 1260) in order to investigate the performance of this reactor. As shown in Figures 4.14 & 4.15, PCBs concentration in the treated oil is decreased from 7.85 mg/L to 3.5 mg/L,

and PCBs removal efficiency of about 51.2% ± 4.3% can be achieved. As shown in Figure 4.19, the PCBs concentration of liquid part of effluent is in the range of 0.01 mg/L - 0.1 mg/L (Average 0.02 mg/L).

Based on Figure 4.16, Aroclor 1260 concentration in the original oil is about 2.2 mg/L, and after enhanced by adding high concentration of PCBs, the Aroclor 1260, in the oil, the concentration of the Aroclor 1260 in the oil is about 7.2 mg/L. After the treatment from IUFAB, Aroclor 1260 removal efficiency of about 50% is achieved at HRT of 4 days. The Aroclor 1260 remained in the treated oil is about 3.3 mg/L. Because Aroclor 1260 concentration in the treated oil was always higher than 2.2 mg/L (Aroclor 1260 concentration in original oil), it was not sure that the removed Aroclor 1260 was from the initial Aroclor 1260 contained in the original oil or from the additional Aroclor 1260. It is, therefore, the direct treatment study of PCBs in the original oil is required for further study.

The original oil with PCBs concentration of 3.55 mg/L is introduced into the feed solution for the first IUFAB. On the 92nd day, as shown in Figures 4.17 & 4.18, the PCBs concentration in the original oil is decreased from an initial value of 3.55 mg/L to a range of from 1.4 to 1.6 (Average 1.5 mg/L) at HRT of 4 days. This can be translated to a PCBs removal efficiency of more than 50%. Also, the

PCBs concentration in the liquid part of effluent has an average value of 0.0034 mg/L, which has been removed from PCBs concentration in the feeding solution of 0.046 mg/L (as shown in Figure 4.19).

For the first IUFAB, based on the calculation of the mass balance for a period of 6-month operation (212 days), which combines total PCBs amount of loaded, remained in the treated oil and biomass in the IUFAB and discharged in the liquid part of the effluent, the result indicates the IUFAB with the operation of HRT of 4 days can achieve PCBs removal of 42.6%. The calculation of PCB mass balance based on the analysis conducted by May 2004 is as follows:

In the period of 6-month operation (212 days), and oil loading rate of 3.2g/L/day, 6.4-gram oil (having effective volume of 8.9ml) were introduced into the IUFAB every day. As a matter of fact, from the first day to 92nd day, PCB enhanced oil was applied; from 93rd day to 212th day, original oil was introduced into the IUFAB, and their averaged PCB concentrations are 7.85 mg/L and 3.68 mg/L, respectively (as shown in Table 4.3).

Table 4.3. Various PCB concentrations in first IUFAB

PCB concentration (mg/L)	Operation day (0 ~ 92nd day)	Operation day (93rd ~ 212th day)
In feed oil	7.85 (Average)	3.68 (Average)
In treated oil	3.42(Average)	1.54(Average)
In aqueous effluent	0.02(Average)	0.0034(Average)

In the last day of experiment, the biomass was taken from bottom of IUFAB. PCB concentration of 1.288 mg/L was detected in the biomass (total volume of 0.3 liter).

1) Total input PCBs amount is:

$$7.85 \text{ mg/L} * 0.0089 \text{ L/day} * 92 \text{ day} + 3.68 * 0.0089 * 120 = 10.36 \text{ mg}$$

2) Total remained PCBs amount in treated oil is (sum of all PCB in the treated oil):

$$3.42 \text{ mg/L} * 0.0089 \text{ L/day} * 92 \text{ day} + 1.54 * 0.0089 * 120 = 4.44 \text{ mg}$$

3) Total PCBs remained in biomass is (Total biomass volume is 0.3 Liter in IUFAB):

$$1.288 \text{ mg/L} * 0.3 \text{ L} \cong 0.39 \text{ mg}$$

4) PCBs in the aqueous effluent are:

$$0.02 \text{ mg/L} * 0.5 \text{ L/day} * 92 \text{ day} + 0.0034 * 0.5 * 120 = 1.12 \text{ mg}$$

5) PCBs removal efficiency = $(10.36 - 4.44 - 0.39 - 1.12) / 10.36 = 42.6\%$

Based on the mass balance, the result of PCBs removal efficiency of 42.6% is closed to the previous experimental result, PCBs removal efficiency of 50%, based on the PCBs concentrations in the feed oil and treated oil.

A second IUFAB was investigated for the repeatability of IUFAB. This IUFAB was started up by using methanol as the sole carbon source. On day 45, COD removal efficiency of more than 95% was achieved. The same preparation method of the PCB feeding solution as used in the first experiment of IUFAB was used to prepare the PCBs feed by using original oil with PCBs concentration of about 3 mg/L as influent. As shown in Figures 4.20 & 4.21, averaged PCB removal efficiency of 52.4% ± 2.6% is achieved, and PCBs concentration in the treated oil can be maintained at 1.4 mg/L to ~1.8 mg/L at HRT of 4 days. This result is able to achieve the regulated cleanup level of 2.0 mg/L.

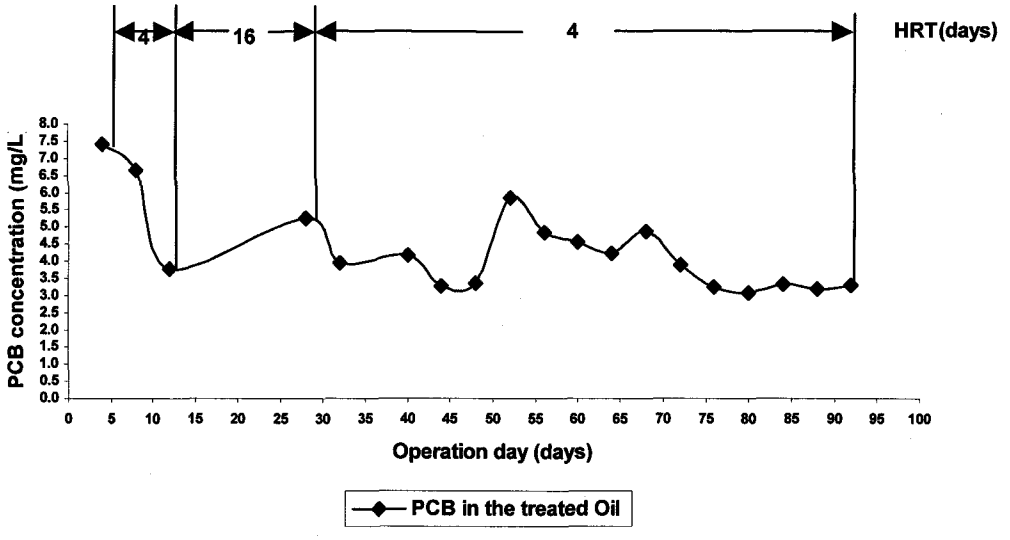

Figure 4.14. PCBs concentration of the treated oil part of the effluent with PCB enhanced oil

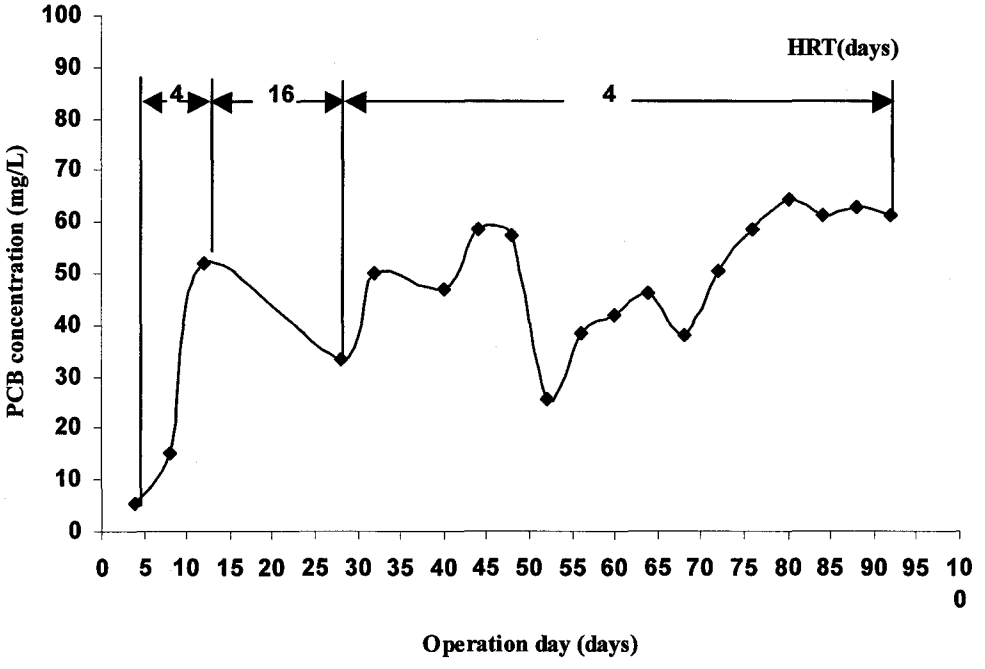

Figure 4.15. PCBs removal efficiency of the treatment of PCBs enhanced oil

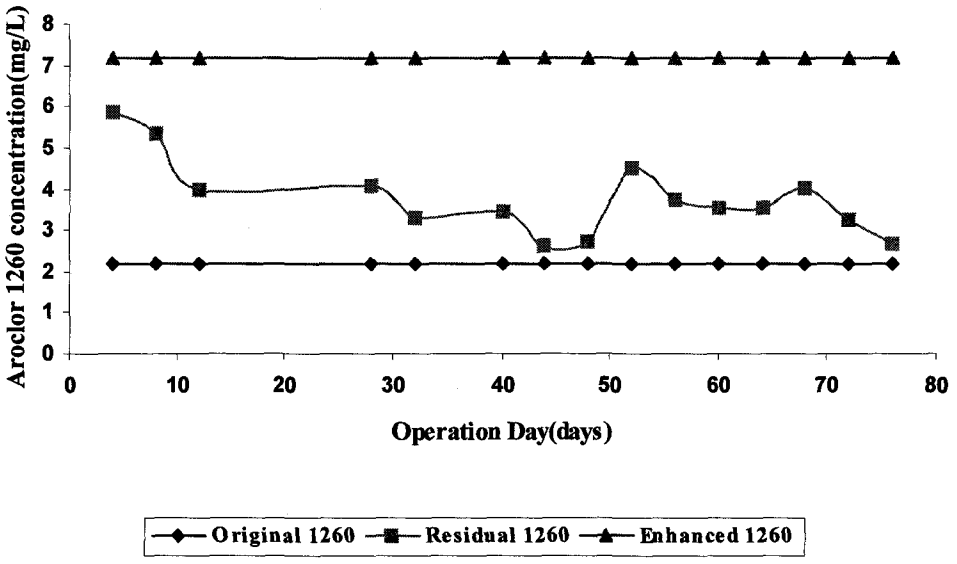

Figure 4.16. Aroclor 1260 concentrations in the various forms of oil

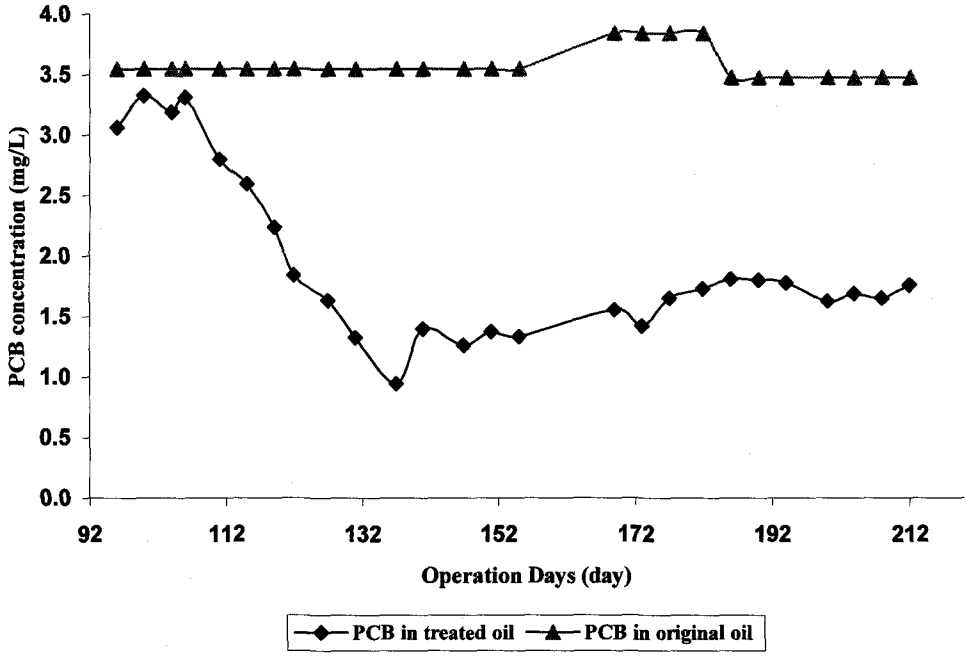

Figure 4.17. PCBs concentration of the oil part of the effluent using original oil

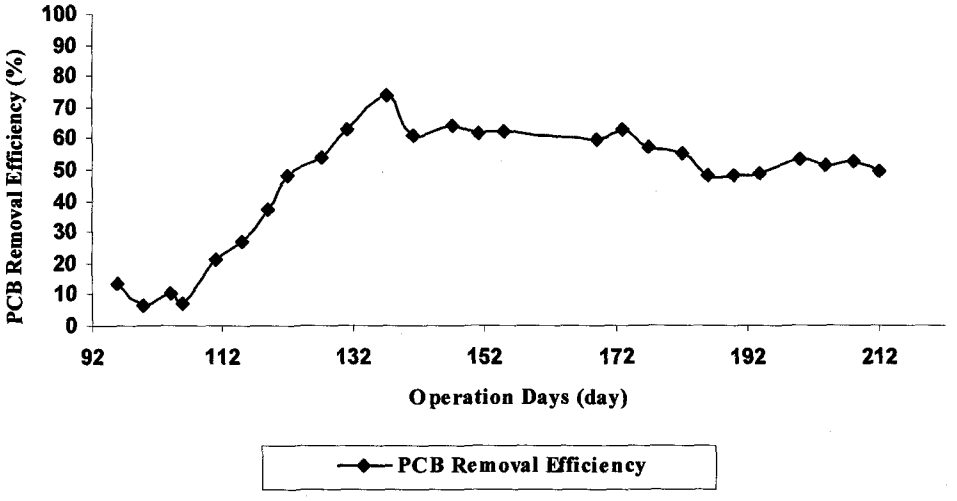

Figure 4.18. PCBs removal efficiency using original oil

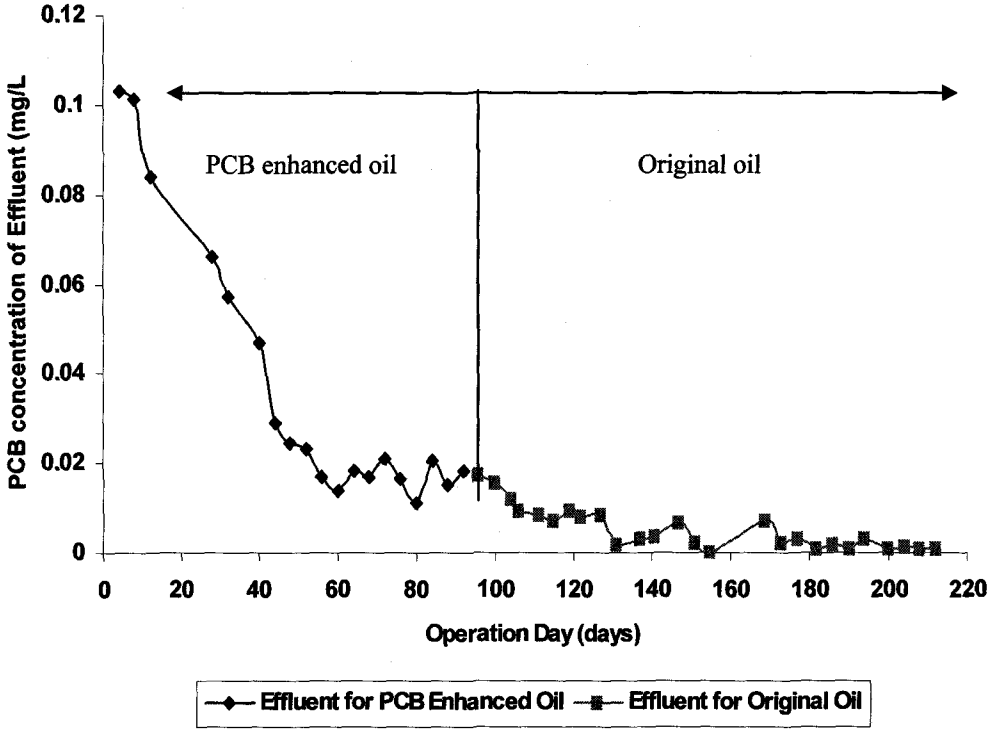

Figure 4.19. PCBs concentrations of liquid part of the effluent from the IUFAB (First experiment)

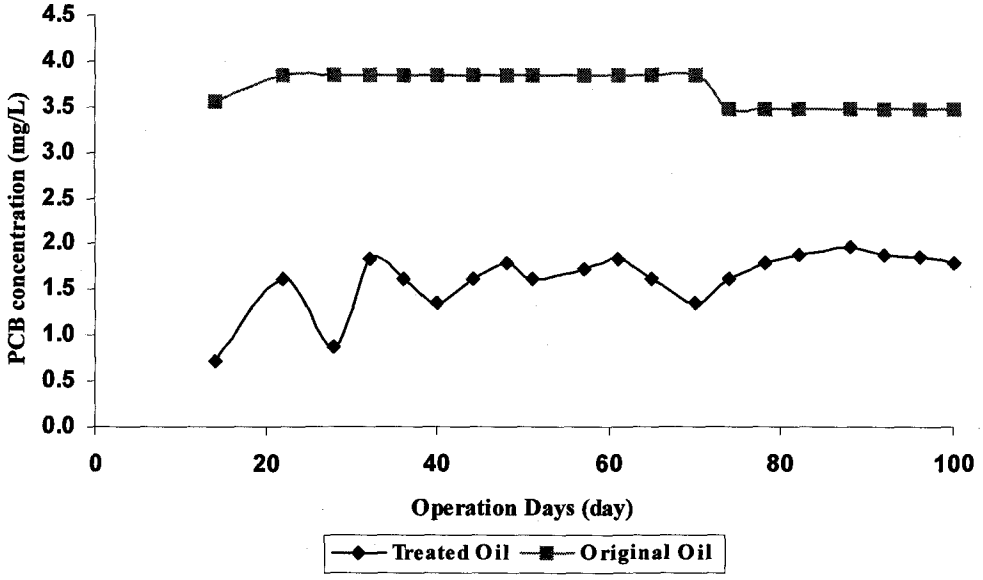

Figure 4.20. PCBs Concentration of treated Oil in the IUFAB with original oil (Second experiment)

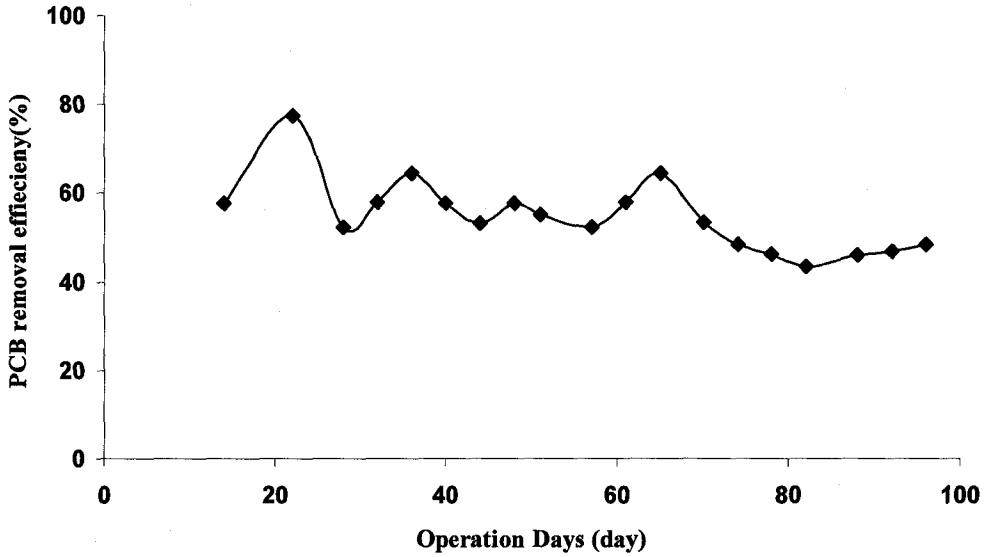

Figure 4.21. PCBs removal efficiency in IUFAB using original oil (Second experiment)

4.5.2.2. Nutrient and environmental factors for the treatment of the PCBs contaminated oil

4.5.2.2.1. Application of cometabolism for PCBs removal from the oil phase

Metabolism is the sum total of the all the biochemical processes (a series of biochemical oxidation-reduction reactions) performed by living organisms to

yield energy for synthesis, motility, and respiration to remain viable. In the metabolism, organic matter is the substrate used as an energy source for the heterotrophic microorganisms. However, the majority of organic matter in wastewater is in the form of large molecules that cannot penetrate the bacteria cell membrane. Therefore, the first biochemical reaction is the hydrolysis of complex organics into diffusible fractions.

In this study, the main objective is to use biological treatment process to remove PCBs from the oil phase. PCB as a synthesized high-molecular-weight substrate has its own chemical properties, for example, stability to oxidation, etc. Based on the PCBs properties, PCBs cannot be hydrolyzed easily, and in the meantime, because chlorinated organic materials frequently resist microbial degradation, necessary enzyme is required to break down the carbon-chlorine bond. This means that PCBs cannot provide the electrons and enough energy for the microorganisms to grow and enzymes to act. Also, the major limitation of anaerobic growth is energy production. The anaerobic decomposition is a low energy yield per unit of substrate, which results from an incomplete reaction. In other words, the limiting factor in anaerobic metabolism is a lack of hydrogen acceptors. When the supply of biologically available energy is exhausted, the processes of metabolism and synthesis will stop.

Organic matter that can be used by microorganisms to obtain energy for growth is the one, which is the easiest to be biodegraded in the natural environment and in engineered systems. Methanol, toluene, propane and butane are used to support the cometabolism. In this experiment, methanol, because of its simplest formula and the property of easy biodegradation, was selected as additional substrate to provide the necessary energy and promote the PCBs removal from the oil phase. In this study, PCBs cannot be used as a source of energy for microorganisms, primarily because no microorganism has the enzymes, which are necessary for their complete biodegradation. They have to be transformed through cometabolism.

In the cometabolism, the additional substrate, methanol, was readily degraded to acquire the energy and electrons by microorganisms. These energy and electrons were combined with the coenzyme (co-E), and transferred to the enzyme (E), which may have the function to break down the carbon-chlorine bond of PCBs after it was activated. To activate the enzyme (E), the coenzyme (co-E) with electrons and enough energy has to combine or attach with the enzyme (E), and change its shape. Activated enzyme (E) may be able to biodegrade the PCBs by completing the dechlorination, and convert the PCBs to

their corresponding acids. The Schematic diagram is shown in followed Figure 4.22:

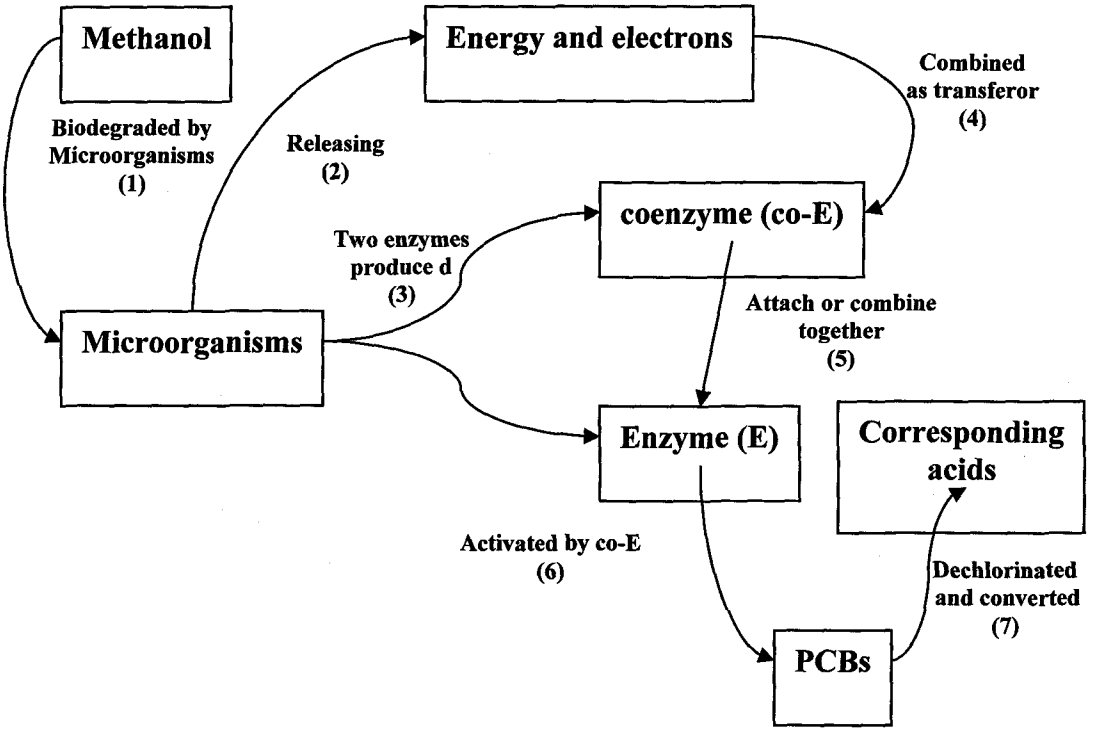

Figure 4.22. Schematic diagram of possible cometabolism for PCBs removal

Because of its own properties, PCBs cannot be used as the energy source and electron donor to provide necessary energy and electron for microorganisms to complete the metabolism. Therefore, cometabolism was introduced into this experiment. Methanol as the energy source was added to obtain the energy, and it makes dehalogenation of PCBs possible. In this experiment, the PCBs feed used

for IUFAB contains 1000 mg COD (methanol) / L. Based on the principle of cometabolism, additional organic matter is very necessary for PCBs removal from PCBs contaminated oil.

4.5.2.2.2. Impact of HRT

For the first IUFAB, two different HRTs, 4 days and 16 days, were used to investigate the difference on PCBs removal efficiency. The results (shown in Figures 4.23 & 4.24) indicate that when HRT is changed from 4 days to 16 days, and COD loading rate and PCBs loading rate decrease from 0.25g/L/day and 0.024mg/L/day to 0.06g/L/day and 0.006mg/L/day, respectively, PCBs concentration in the treated oil increases from 3.5 mg/L to 5.2 mg/L. Thus, PCBs removal efficiency decreases from 52% to 33%. When HRT is changed back to 4 days, the PCBs removal efficiency increases back to about 50%. Apparently, HRT of 4 days with COD loading rate of 0.25g/L/day and PCBs loading rate of 0.024mg/L/day is much better than HRT of 16 days for the PCBs removal. It is apparent that at the lower HRT of 4 days, the necessary enzymes may be produced for meeting the requirement of the cometabolism of PCBs. For the operation of HRT of 16 days, it may be short of producing necessary enzymes for the need of cometabolism of PCBs. Therefore, a critical HRT with a certain COD

concentration of 1000 mg/L is required to operate in order to be able to produce the necessary enzymes for the cometabolism of PCBs.

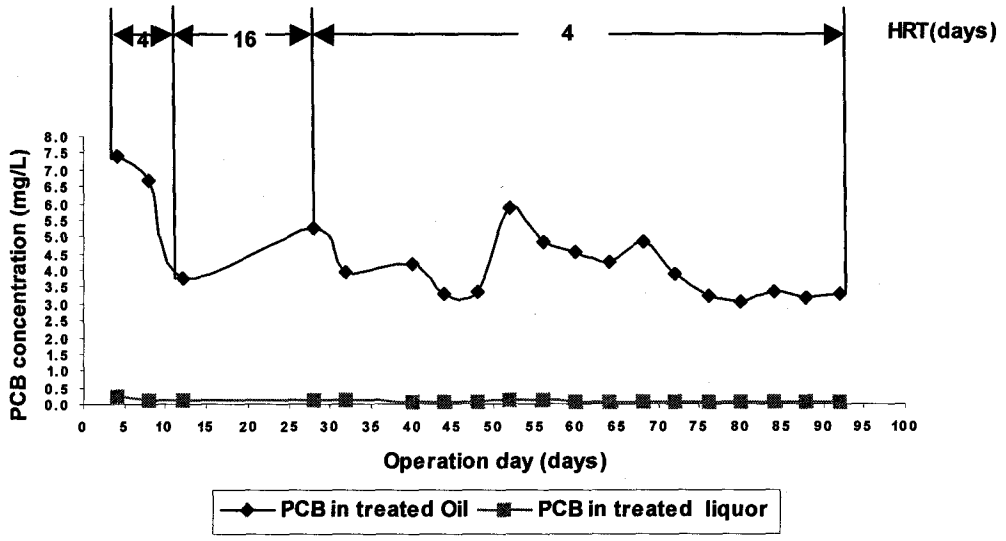

Figure 4.23. Effect of HRT on PCBs remaining in the treated oil and liquid

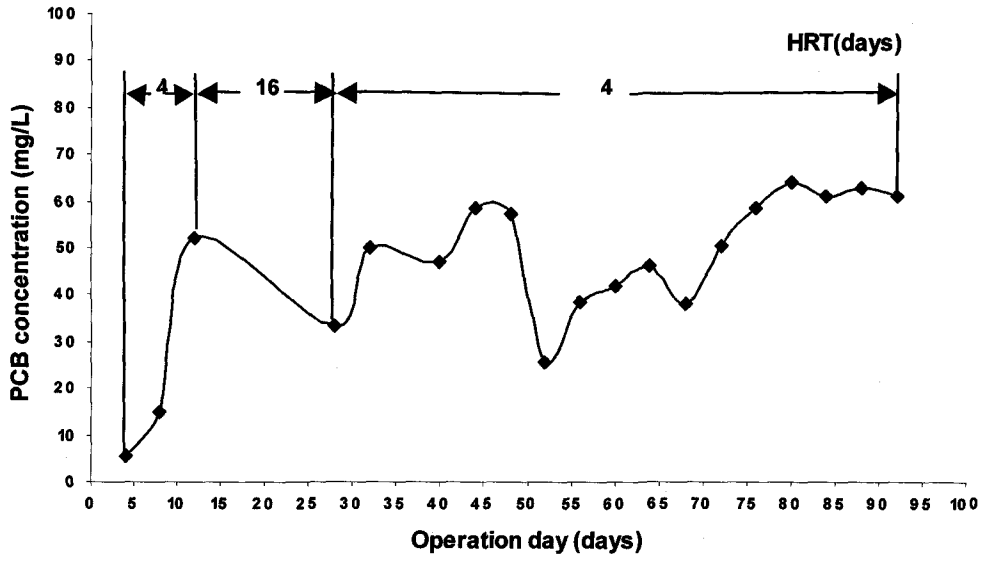

Figure 4.24. Effect of HRT on removal efficiency of PCBs

4.5.2.2.3. Impact of pH

In order to observe the impact of pH on the PCB removal, the original oil with PCBs concentration of 3.55 mg/L was introduced into the feed solution on the 92nd day (as shown in Figures 4.17, 4.18 & 4.19) with the pH value in the feed of 7.2 instead of 6.4. This is to maintain the neutral pH as suggested for the optimal pH for the growth and metabolism of microorganisms in the anaerobic wastewater treatment process. However, it is found that the operation of pH of 7.2 in the feed has created the problem of biomass washing out from the bioreactor. The PCBs concentration in the treated oil is increased to 3 mg/L and PCBs

removal efficiency is decreased to about 20% as shown in Figure 4.18. After adjusting the pH value in the feed back to 6.4, the PCBs concentration in the treated oil is decreased to about 1.6 mg/L as shown in Figure 4.17, and the PCBs removal efficiency for the original oil is increased back to 50% as shown in Figure 4.18. Again, the initial pH of 6.4 needs to be maintained in the feed, and this may require to induce the production of necessary enzymes produced for the cometabolism of PCBs and prevention of the biomass washing out.

4.5.2.3. Relationship between PCBs removal rate and biogas production rate and residual chlorine concentration

4.5.2.3.1. PCBs removal efficiency or rate and biogas production

For the first IUFAB fed by PCBs enhanced oil was investigated for the relationship between gas production rate and PCB removal rate. This relationship is presented in Figures 4.25 & 4.26. It is shown that high biogas production rate provides high removal of PCBs from the oil. For the second IUFAB fed by the original oil, this relationship is further confirmed (as shown in Figures 4.27 & 4.28). Therefore, biogas production rate can be used as an indicator parameter for assessing PCBs removal rate in IUFAB.

Anaerobic process consists of two distinct stages that occur simultaneously in the digestion of sludge (as shown in Figure 4.29). The first stage consists of hydrolysis of the high-molecular-weight organic compounds and conversion to organic acids by acid-forming bacteria. The equation is following as:

Organics ------ intermediates + CO_2 + H_2O + energy

The second stage is the gasification of the organic acids to methane gas and carbon dioxide by the acid-splitting methane-forming bacteria. The equation is following as:

Organic acid intermediates ------ CH_4 + CO_2 + energy

Biogas produced in anaerobic treatment process usually consists of methane (65%-69%), carbon dioxide (31%-35%), and trace levels of other gases such as hydrogen, carbon monoxide, nitrogen, oxygen, and hydrogen sulfide. The relative percentage of these gases in biogas depends on the feed material and management of the process. Generally, pending failure of the anaerobic treatment process is evidenced by a decrease in gas production, a lowering in the percentage of methane gas produced, etc, because they all indicate reduced activity of the acid-splitting methane-forming bacteria. As shown in Figure 4.30, in this study, the composition of the biogas production from IUFAB contains 65% of methane gas (CH_4), 28% of carbon dioxide (CO_2) and 7% of other gases. The results,

higher percentage of methane gas and lower percentage of carbon dioxide, show a good methane fermentation process in the present IUFAB system. Furthermore, the good methane fermentation process may also be used as the key for the biological PCBs removal from the oil phase. The necessary enzymes produced from the methane fermentation of methanol used as carbon source in this study can be successfully used for the cometabolism of PCBs. Detailed metabolic mechanism requires further studied.

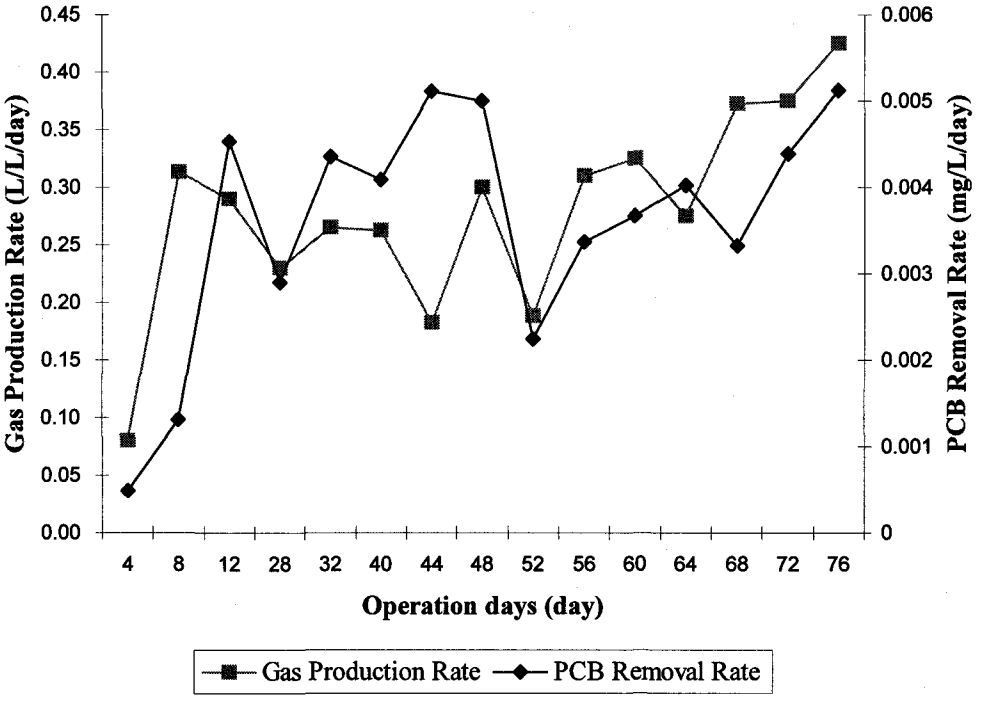

Figure 4.25. Comparison of Biogas production rate and PCBs Removal Efficiency in the first IUFAB

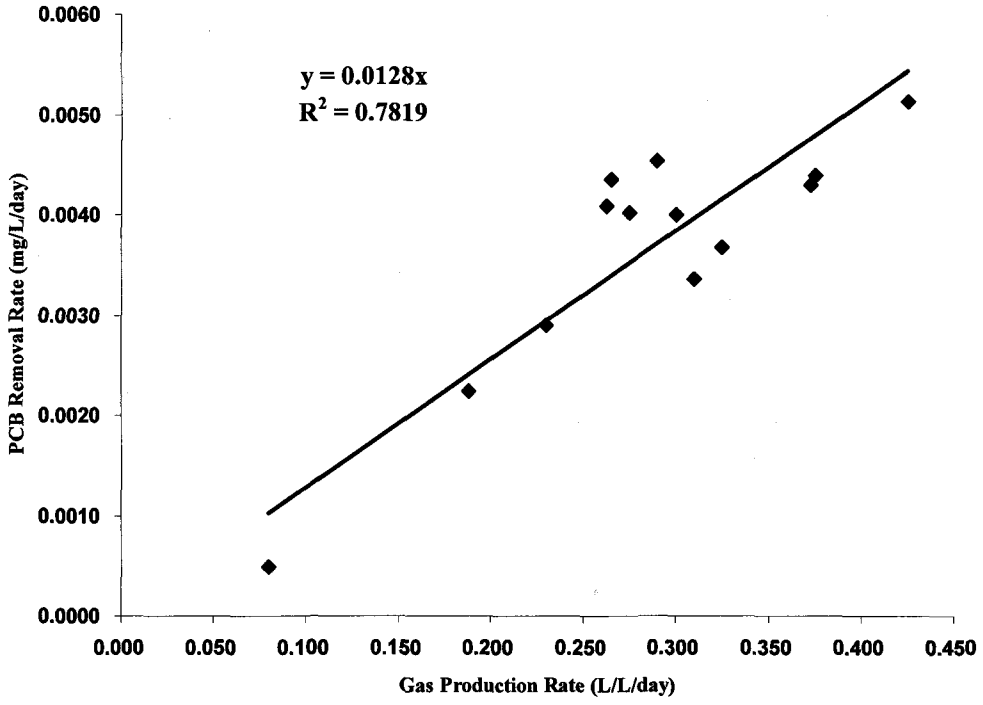

Figure 4.26. Relationship between gas production rate and PCB removal rate in the first IUFAB

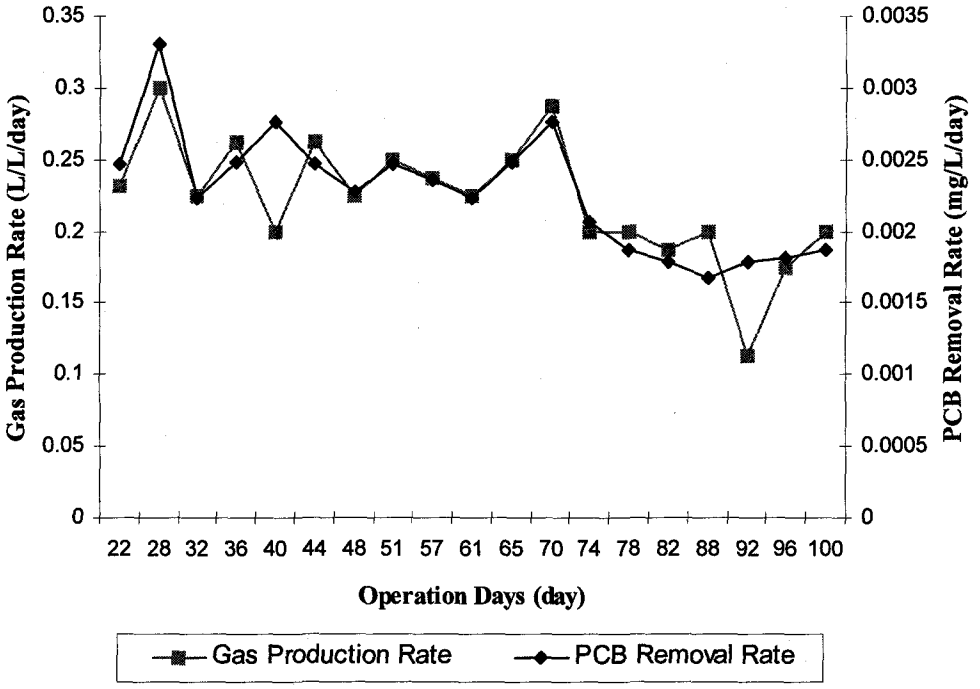

Figure 4.27. Comparison of Biogas production and PCBs Removal Efficiency in the second IUFAB

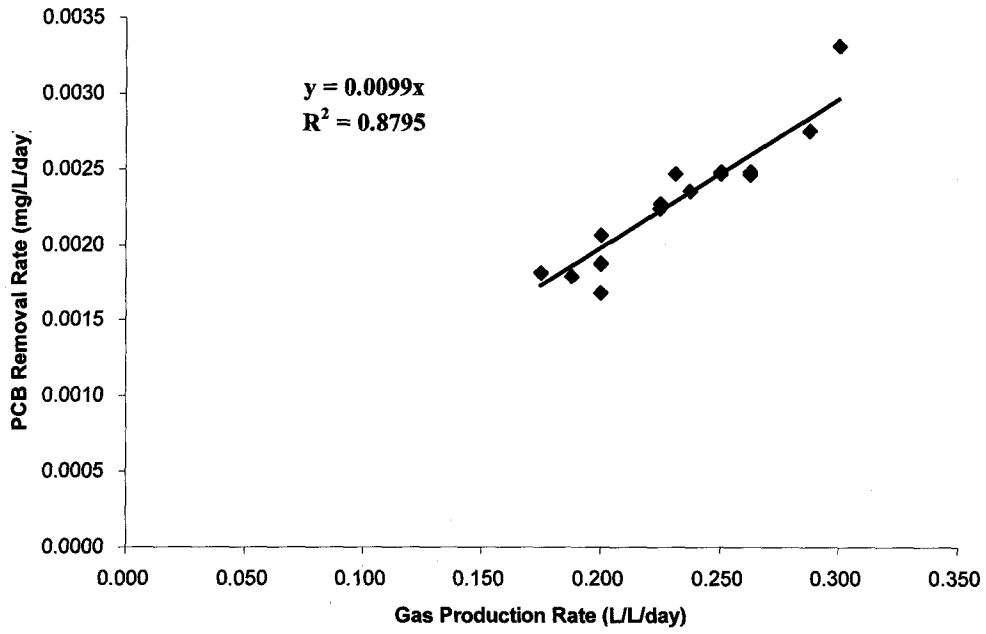

Figure 4.28. Relationship between gas production rate and PCB removal rate in the second IUFAB

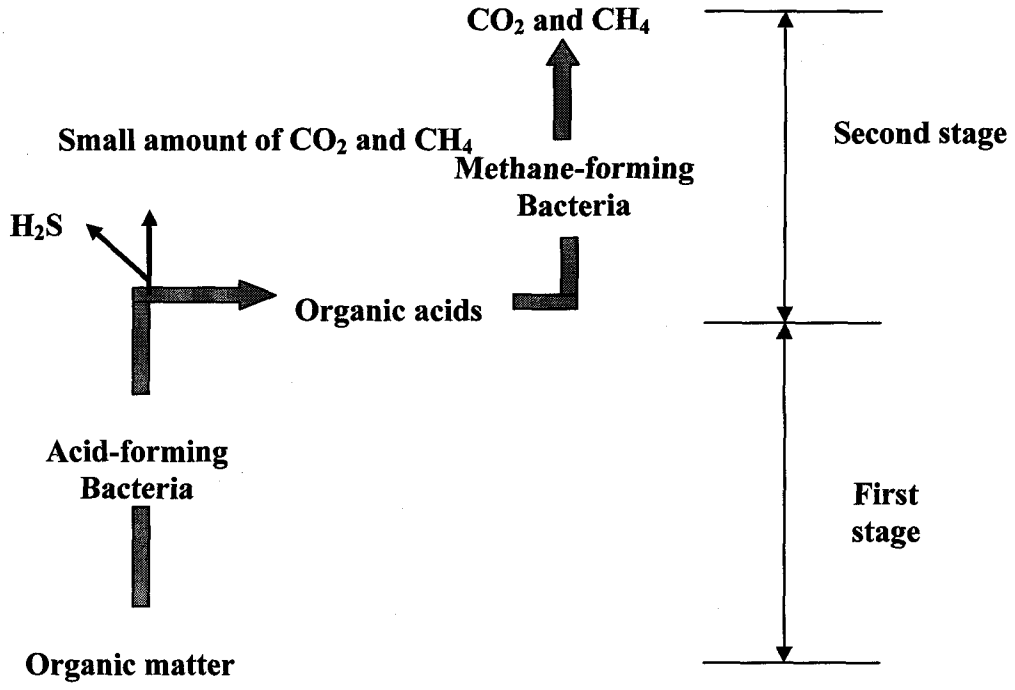

Figure 4.29. Simplified diagram of two stages in anaerobic digestion (Warren, etc., 1998)

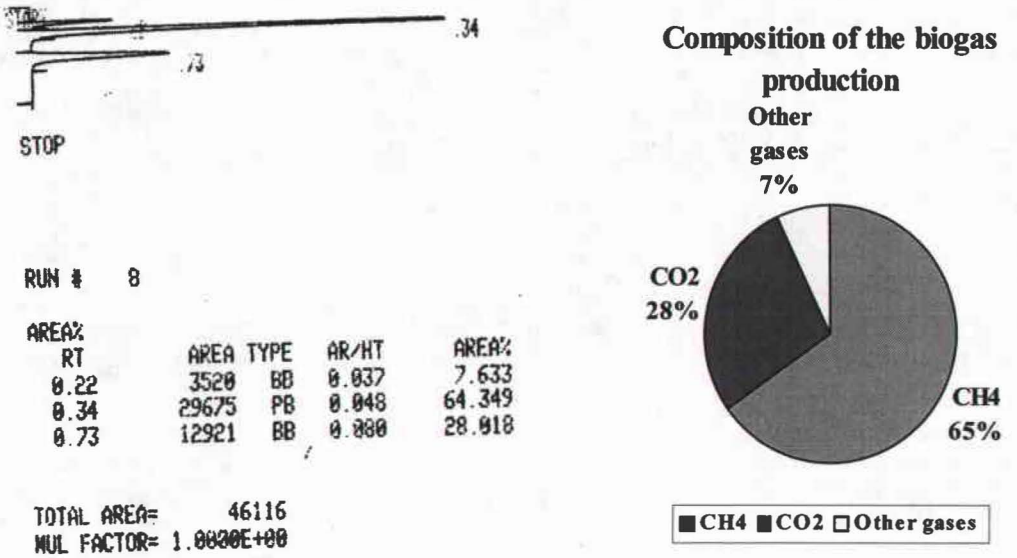

Figure 4.30. Composition of the biogas produced in the IUFAB

4.5.2.3.2. PCBs removal and chloride concentration

Based on the principle of dechlorination of PCBs in the anaerobic treatment process, PCBs may be metabolized by the microorganisms via cometabolism, and be further biodegraded to corresponding acids. The possible mechanism for reductive dechlorination catalysed by anaerobic microorganisms is that the microorganisms utilize PCBs as an electron acceptor, with the addition of the electron to the carbon-chlorine bond, chlorine loss, and hydrogen abstraction

from other carbon sources, respectively (as shown in Figure 4.31). In other words, in the meantime, some chlorides will be produced by dechlorination.

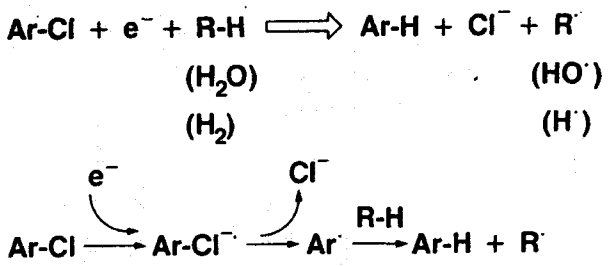

Figure 4.31. Possible mechanism for reductive dechlorination catalyzed by anaerobic microorganisms (Abramowitz, 1990).

In the PCBs feeding solution, we assume that all chlorides are from the feed nutrition (added inorganic materials), such as $CaCl_2$, $MgCl_2$, etc. By calculating those inorganic materials, it is able to find that the initial chloride concentration in the feeding solution is about 25.9 mg/L. However, in the aqueous effluent from IUFAB, the chloride concentration of 34 – 93 mg/L was detected based on data from Tetra Tech EM Inc. in May 2004 (as shown in Table 4.4). The feeding solution with low chloride concentration was introduced into the IUFAB, and the aqueous effluent with high chloride concentration was discharged.

Apparently, the difference of the chlorides concentrations was caused by the dechlorination of PCBs. In other words, certain amounts of PCBs were biodegraded in IUFAB under the anaerobic condition, which caused the difference of chloride concentrations between influent and effluent. During this period, the PCBs concentrations in both treated oil and original oil were measured, and the PCBs removal efficiency of 50% was achieved. It is apparent that the net increase of chloride concentration in the effluent from IUFAB can be used as an effective indicator for progress of PCBs removal or dechlorination of PCBs.

Table 4.4. Comparison of chloride concentrations between influent and effluent

Operation days (day)	Influent chloride (mg/L)	Effluent chloride (mg/L)	Difference (mg/L)
0	25.9		
4 (Sample 1)	25.9	92.1	+ 66.2
8 (Sample 2)	25.9	34	+ 8.1
12 (Sample 3)	25.9	93	+ 67.1
16 (Sample 4)	25.9	81.7	+ 55.8

4.5.3. Design and operational criteria developed for IUFAB for the treatment of PCBs contaminated oil regarding PCBs removal efficiency, implementation and cost evaluation

For IUFAB, the general criteria for the evaluation and design of the bioreactor configuration include removal efficiency, implementability and cost. In this study, anaerobic up-flow reactor with three-layer media, anaerobic batch reactor integrated with three-layer up-flow reactor, and intermittent up-flow anaerobic bioreactor (IUFAB) were designed and investigated. PCBs removal efficiencies of 25%, 40% and 50% can be achieved by these various type reactors, respectively. Among those three reactors, IUFAB indicates consistent stability and high removal efficiency, also for the end product, the treated oil can meet the target cleanup level. Compared to other reactors, IUFAB shows its own advantages in implementation, for example, easy to separate the treated oil and liquid parts; stable process performance; easy operation, etc. All the information about IUFAB including cost estimation of pilot-scale study were summarized and shown in the followed Tables 4.5 & 4.6.

Table 4.5. Summary of IUFAB regarding PCBs removal efficiency, implementation and cost estimation

Basis for Selection	IUFAB
General/PCB removal	Good PCB removal efficiency, 50-70% in oil phase, can be achieved with 6-month stable performance. Also, based on the PCBs mass balance including oil / liquid / biomass phases, PCBs removal efficiency of about 50% can be achieved.
Implementability	Advantages: ♦ Higher biomass content ♦ Easy to separate the treated oil part and liquid part ♦ Higher PCB removal efficiency ♦ Stable process performance ♦ Easy operation ♦ Shorter HRT applied Disadvantages: ♦ Need to use surfactant, which may cause high COD content in the treated effluent
Cost	Based on IUFAB of 10 m3 system with 4-day HRT, the total cost is $390,000 (Tax not included) (Details as shown in following Table 4.6)

For the operational criteria, they will be presented in following:

A. For 2-Liter bench-scale IUFAB:

1) HRT = 4 days (Flow rate = 0.5 L / day)
   COD (Methanol as carbon source) Loading rate = 0.25 g/L/day
   PCB Loading rate = 0.0125mg/L/day
   (Based on PCB concentration of 3.6 mg/L in original oil)

2) pH = 6.2~6.6
To adjust pH value to 6.2 ~ 6.6, $NaHCO_3$ of 6 gram should be added to 10-liter PCB feed.

3) Ambient temperature = 23°C ± 2°C

4) Intermittent Feeding schedule: one hour on, and seven hours off

5) Initial MLSS of anaerobic activated sludge concentration in IUFAB = 7000 mg/L

6) Original Oil concentration in PCB feed = 12.8 g/L
Original Oil Loading rate = 3.2 g/L/day
Original Oil treated in ONE HRT = 25.6 g / HRT

7) To prepare the PCB feed:

COD Loading rate / Oil Loading rate ratio = 1: 12.8
Oil / surfactant ratio = 1: 1
Carbon source and Nutrient solution / Oil = 56: 1

B. For 10 m^3 Pilot-scale IUFAB:

1) HRT = 4 days (Flow rate = 2.5 m^3 / day)
COD (Methanol as carbon source) Loading rate = 0.25 g/L/day
PCB Loading rate = 0.0125mg/L/day
(Based on PCB concentration of 3.6 mg/L in original oil)

2) pH = 6.2~6.6

3) Ambient temperature = 23°C ± 2°C

4) Intermittent Feeding: one hour on, and seven hours off

5) Initial MLSS of anaerobic activated sludge concentration in IUFAB = 7000 mg/L

6) Original Oil concentration in PCB feed = 12.8 g/L
Original Oil Loading rate = 3.2 g/L/day
Original Oil treated in ONE HRT = 128000 g / HRT = 128 Kg / HRT
(178-Liter PCB contaminated Oil / HRT)

In every 4 days (1 HRT), 178-liter PCB contaminated Oil, which is equal to 1335 liter per month or 352 gal per month, will be treated by $10m^3$ IUFAB system.

7) To prepare the PCB feed:

COD Loading rate / Oil Loading rate ratio = 1: 12.8
Oil / surfactant ratio = 1: 1
Carbon source and Nutrient solution / Oil = 56: 1

C. Materials Required:

1) For nutrient preparation:

Methanol is used as carbon source and some trace elements (listed as followed) will be added. (Trace: NH_4Cl, $CaCl_2$, $MgCl_2.6H_2O$, $FeCl_2.4H_2O$, KH_2PO_4, $MnCl_2.4H_2O$, H_3BO_3, $ZnCl_2$, $CuCl_2.2H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $NaMoO_4.2H_2O$.)

2) For adjusting pH:

$NaHCO_3$ will be used as buffer.

3) For preparing the PCB feed:

Surfactant (Tween 80) will be used.

Table 4.6. Cost Estimation of IUFAB For PCBs Biological Treatment Pilot Scale Study (One Year)

	Item	Capacity (m^3)	Quantity	Material	Unit Price ($)	Cost ($)	Freight
1	Feed Tank	5	2	Stainless steel	5160	10320	90%
2	Bioreactor	10	1	Stainless steel	13900	13900	90%
3	Storage Tank	10	2	Stainless steel	6720	13440	90%
4	Mixer for feeding tank	10	2	Stainless steel	2040	4080	90%
5	Seed Tank	10	1	Fiberglass	6840	6840	90%
6	Monitor (PCBs&Biogas Production)				10000	10000	90%
7	Pipe/fitting			Brass or Stainless	10%	5858	90%
8	Construction and installation				25%	16110	
9	Engineering Design				45%	36246	
10	O/M cost				30%	35038	
11	Labor and other service	2 full time workers				150000/Year	
12	Miscellaneous Cost				10%	30183	
13	Total					332015	57994
Total Cost						390, 009 (Tax not included)	
NOTE:	Based on IUFAB of 10 m^3 system with 4-day HRT						

CHAPTER 5. CONCLUSIONS

The EMMC process and Intermittent Up-Flow Anaerobic Bioreactor (IUFAB) were investigated for the PCBs removal for both aqueous and oil phases, respectively. It is apparent that EMMC process is technically feasible for the PCBs removal under the different operational conditions, such as aerobic and anoxic conditions, for the aqueous phase. The bioreactor of IUFAB is able to provide a stable PCBs removal efficiency from both PCBs enhanced and original oil based on the results of the investigation of the 6-month period. The specific conclusions resulting from this study are presented as follows:

For PCB removal from the aqueous phase, the EMMC technology was introduced in this study. It was found that both aerobic EMMC reactors, which were operated under continues aeration and intermittent aeration, can achieve more than of 95% PCBs removal efficiency with initial of COD concentration of 600mg/L and PCB concentration of 2mg/L at HRT of one day. For the anoxic reactor, PCBs removal efficiency is low, only 40% ~ 80%. Because EMMC technology can provide high PCBs removal efficiency from the aqueous phase, they also were applied for further removal of the remained PCBs and residual COD from the effluent of the anaerobic bioreactor, and directly treat the PCBs

contaminated oil under a reaction time of 10 days using a suspended culture with EMMC carrier (5%). Because of the problems of clogging and GC analysis, the result is not clear.

For PCBs removal from the oil phase, three types of anaerobic bioreactors were evaluated. They are three-lay up-flow anaerobic reactor, two-stage bioreactor system, and Intermittent Up-Flow Anaerobic Bioreactor (IUFAB). It was found that only IUFAB could avoid the clogging problem. The IUFAB is able to achieve PCBs removal efficiency of 45%-65% for a period of 6-month operation with COD loading rate of 0.25 g/L/day, oil loading rate of 3.2 g/L/day, influent pH of 6.4, and HRT of 4 days in the ambient temperature of 23°C ± 2°C. It was also found that methanol is required to be added for the production of the co-metabolism of PCB. This was also revealed by the calculation of mass balance of chloride content and production of biogas production for the confirmation of the anaerobic bioconversion process.

Necessary design and operational criteria for treating PCB contaminated oil by using IUFAB were developed. Furthermore, a proposed pilot plant set-up (10 m^3 bioreactor) and cost evaluation were estimated.

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