PENTACHLOROPHENOL DEGRADATION IN MEMBRANE BIOREACTORS

by

Le Ngoc Thu

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Examination Committee:	Prof. C. Visvanathan (Chairman) Prof. Nguyen Cong Thanh Dr. Preeda Parkpian
Nationality:	Vietnamese
Previous Degree:	Bachelor of Engineering in Environmental Technology Ho Chi Minh City University of Technology Ho Chi Minh City, Vietnam
Scholarship Donor:	The Royal Netherlands Government

Asian Institute of Technology
School of Environment, Resources and Development
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Abstract

Pentachlorophenol (PCP) and sodium pentachlorophenol has been used as a pesticide, herbicide, antifungal agent, bactericide and wood preservative, which can cause environmental pollution. The potential of using membrane bioreactor for treating PCP contaminated wastewater has been accessed. Synthetic wastewater includes PCP and glucose has COD around 600 mg/L was using in this study. During different loading stages in the range of 12 to 40 mg/m^3.day of the MBR operation, a removal rate of 99% of PCP and 95% of COD could be achieved at hydraulic retention time of 12 hours and mixed liquor suspended solid of 10,000 mg/L. With NaPCP loading from 20-200 mg/m^3.day (10-100 mg/L in the influent), the capacity of removal in terms of NaPCP was still higher than 99% as well as more than 96% of COD.

In this study with high concentration of biomass, biosorption played an important role besides the biodegradation process. The equilibrium time of 60 min was achieved and biosorption was found 0.6289mg/gVSS. This phenomenon could enhance the PCP degradation though increase the contacting between microorganism and contaminant.

Mycobacterium chlorophenolicum had lower capacity in mineralization PCP compared with activated sludge from reactors. It also did not grow on NaPCP environment. Predominant microorganism R1-C1, R1-C2, R2-C1, and R2-C2, R2-C4 that could degrade PCP were isolated from the membrane bioreactor systems depending on their colony morphology. Their log phase was in the range of 18-72 hours and applied for bioaugmentation technique to enhance the performance of system in inhibition case.

The inhibition level of NaPCP for different kind of predominant organism was different. 150mg/L (300 mg/m^3.day) in the influent was found causing inhibition.

Membrane resistance was low (14 KPa) after more than 100 days of operation. The toxic level of the influent make an increase in viscosity of the mixed liquor though producing more bound EPS to protect microorganism to the adverse environmental condition. The result was faster increased in transmembrane pressure.

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List of Abbreviations

ATSDR: Agency for Toxic Substances and Disease Registry
ATU: Allylthiourea
COD: chemical oxygen demand
CFU: colony forming unit
CST: Capillary suction time
DO: Dissolved oxygen
DSVI: Diluted Sludge Volume Inde**x**
EC: Effective concentration
EPS: Extracellular Polymeric substances
F/M: food to microorganism ration (mg/mg.day)
HRT: hydraulic retention time
LC: lethal concentration
MBR: membrane bioreactor
MLSS: mixed liquor suspended solid
MLVSS: mixed liquor volatile suspended solid (mg/L)
NaPCP: Sodium Pentachlorophenol
OSHA: The Occupational Safety and Health Administration
OUR: oxygen uptake rate
PCP: Pentachlorophenol
SMP: soluble microbial products
SRT: suspended solids retention time
SVI: Sludge Volume Inde**x**
s: second
TMP: transmembrane pressure
TOC: total organic carbon (mg/l)
TSS: total suspended solid
TU: toxicity units

Chapter 1

Introduction

1.1 Background

Pentachlorophenol (PCP) is a synthetic chlorinated organic compound released into the environment as a result of its manufacture, storage and transportation, along with its use as fungicide, bactericide, algaecide, and herbicide for crops, leathers and textiles. A major source of PCP is the wood preserving industry. Approximately 40 million kilograms of this chemical was manufactured and used in 1977. This widespread use has resulted in the uncontrolled release of PCP into the soil and aquatic environments. The contaminants present in these soils are often transported into freshwater streams and lakes or ground water by soil runoff. The use of PCP was discontinued because of its toxicity and persistence in the environment. PCP is significantly toxic to mammals, plants, and many microorganisms and has a relatively high potential for aqueous phase migration in soils. It is listed as priority pollutant by the U.S. Environmental Protection Agency.

PCP is toxic to plants, animals and humans beings (Packham et al., 1982 cited in Ho and Bolton, 1998). It causes not only adverse long-term effects in the aquatic environment but also to human being with damage to the central nervous system when people are exposed to levels over 0.001 mg/L for relatively short periods of time; affects reproduction and causes damage to liver and kidneys and cancer due to a lifetime exposure at levels above the 1ppm (US Environmental Protection Agency). The Occupational Safety and Health Administration (OSHA) has set a limit of 0.5 milligrams of pentachlorophenol per cubic meter of workplace air (0.5 mg/m^3) for 8 hour shifts and 40 hour work weeks (ATSDR-Agency for Toxic Substances and Disease Registry, Atlanta).

1.2 Problem statement

PCP is highly toxic and therefore, there is a need to decontaminate PCP. Several physical, biological and chemical treatment methods have been used to treat the wastewater containing PCP. Several treatment procedures such as: activated carbon adsorption, ion exchange, incineration, advanced oxidation processes, and biological degradation have been proposed for treating or recovering chlorophenolic compounds. The reduction of PCP occurs via reductive dechlorination, in which chlorine removal and substitution with hydrogen results in a reduced organic compound with fewer chlorine.

PCP has been shown to degrade both aerobically and anaerobically under the appropriate conditions by several bacterial and fungal species (Wilson et al, 1997; Moos, et al. 1983,). However, PCP also acts as an inhibited compound to certain microorganism, thereby limiting the application of biological processes or increasing the requirements such as long retention time to acclimate to obtain the stable condition of the treatment system.

Bioaugmentation has been proved to be beneficial in enhancing the removal efficiency of toxic compounds such as phenol, chlorinated aromatics and aromatic hydrocarbons (Foster and Whiteman, 2003). By adding selected organisms, higher efficiency in removing pollutants can be achieved. Different strains of microorganism for PCP degradation have been developed such as *Pseudomonas* sp. (Watanabe, 1973), *Flavobacterium* (Saber and Crawford, 1985), *Rhodococcus* (Haggblom et al. 1988), etc. Besides these microorganisms, bio-treatment process facilities also play an important role in the performance of the

biological system. Membrane bioreactor (MBR) is one of the most promising newer technologies that can be applied.

Membrane bioreactor is a system that combines membrane filtration with biological process. In MBR, solid, liquid separation occurs through filtration. Solid materials, biomass, pathogenic bacteria are retained in the system while allowing water and smaller solution species to pass through the membrane (Zhang and Versatraete, 2002) so that a very good quality effluent is obtained. MBR is also better than conventional activated sludge process in terms of reducing sludge production, separation of solid retention time and hydraulic retention time, low sludge loading rate, etc. Microbial populations in an MBR are capable of degrading a wider range of substrates than a culture from the conventional activated sludge process (Chiemchaisri et al., 1992). However, membrane fouling is one of the main barriers in MBR application. It leads to increased financial requirement for operation because of the need for extra energy in maintaining a high cross flow velocity. Extracellular polymers or soluble microbial products and concentration polarization are the prime causes for biofouling in MBR (Visvanathan et al., 1997).

The efficient operation of biological treatment system is largely dependent on the kinetic properties of the microbial population. Thus, determination of these parameters is important of designing and application of biological process.

The present study focuses on application of membrane bioreactor with mixed cultured of microorganism and mixed culture of microorganism blended with specific microorganism to treat pentachlorophenol wastewater to meet the effluent discharge standards.

1.3 Objectives

The objectives of this study are the following:

1. Investigate the performance of membrane bioreactor to treat PCP containing wastewater in terms of removal efficiency, stability of process and membrane fouling.

2. Enhancement the performance of system by bioaugmentation technique with microorganism isolated to degrade PCP.

1.4 Scope of the work

All the experimental runs were carried out with the synthetic wastewater.

Batch studies on PCP degradation using *Mycobacterium chlorophenolicum* and isolated microorganisms were carried out in laboratory scale.

The efficiency of membrane bioreactor with mixed culture of bacteria and mixed culture of bacteria augmented with microorganism isolated in PCP biodegradation was studied in a pilot scale reactor.

Biokinetic studies on the sludge taken from the reactor were conducted in a lab scale respirometer.

Chapter 2

Literature Review

This chapter gives the literature about the PCP, its characteristics and toxicity, biodegradation of PCP; membrane bioreactor and sludge characteristics relating to fouling problem; and finally about the biokinetic parameters.

2.1 Toxic chlorinated compounds

Chlorine compounds are used widely in our life. They vary in their application and can be used as pesticide, refrigerants, propellants, solvents, bleaching agents, etc. These substances cause many environmental problems such as global warming, ozone depletion, environmental degradation and toxicity to human and animals.

The use of pesticides such as Aldrin, Chlordane, DDT, PCP,etc has been proved to cause deterioration of ecological systems. The major problem is that, these compounds are stable in the environment. They accumulate in the food chain and increase in concentration in an individual, eventually poisoning animals to death. In the 1950's, large numbers of dead birds were found around cereal- growing farms. Analysis of the corpses showed high levels of DDT in the bodies. Even though many toxic compounds such as PCP, DDT have been banned for a long time in certain countries, they still exist in the environment and are of environmental concern due to their application in the past.

2.2 Pentachlorophenol

Pentachlorophenol is a wide spectrum biocide, disinfectant, and ingredient in antifouling paints. Since 1987, wood preservatives, and other pesticides, containing pentachlorophenol were no longer available for home and garden use (Fact Sheet, Washington State Environmental Health Programs Office of Environmental Health & Safety). Currently, the principal use for pentachlorophenol is as a commercial wood preservative for power line poles, cross arm, and fence posts.

Pentachlorophenol is categorized as cancer causing in humans and toxic compound in environment. PCP is toxic to algae at 0.001 mg/L, with an LC50 for large size goldfish of 0.25 ppm at 24 hours and 0.19 ppm at 96 hours (Verschueren, 1983). Pentachlorophenol residues have been found worldwide in soil, water, and air samples; in food products; and in human and animal tissues and body fluids, and are likely to get bioaccumulated in the environment. (PCP is persistent in soil, having a half-life of up to 5 years).

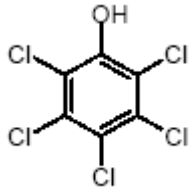
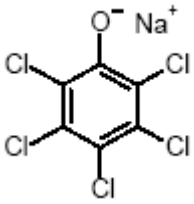
Thus, there is a need to convert PCP present in the water, soil into harmless forms in order to protect the environment.

2.2.1 Characteristics of PCP

Pentachlorophenol has a pK_a of 4.75 and at low pH is relatively hydrophobic. Thus, in subsurface environments, it will partition from the aqueous phase to the solid phase. Assessing chemical reactivity is an important component of contaminated site characterization. Characterizing redox and pH conditions in aqueous systems assists in the understanding of how different environments influence the fate and mobility of PCP and oxidation byproducts (Petrie et al., 2002).

Pentachlorophenol has two forms of chemical structures. The first form is pentachlorophenol and another form is sodium pentachlorophenate. Characteristics of PCP are presented in Table 2.1.

Table 2.1 Physical and chemical properties of PCP (ATSDR, 2003)

Property	PCP	NaPCP
Synonym(s)	PCP; penchlororol; penta; pentachrophenate; 2,3,4,5,6-pentachorophenol.	Pentachlorophenol sodium; pentachlorophenol sodium salt
Chemical formula	C_6HCl_5O	C_6Cl_5ONa
Chemical structure		
Molecular weight	266.35	288.34
Color	Colorless or white (pure); dark gray to brown (crude product)	White or tan
Physical state	Crystalline solid (pure); pellets or powder (crude product)	Flakes or powder
Melting point	190^0C	No data
Boiling point	$309\text{-}310^0$C	No data
Density	1.978 g/mL at 22^0C/4^0C	No data
Odor	Phenolic; very pungent (only when hot)	No data
Odor threshold Water	0.875 mg/L at 30^0C 12.0mg/L at 60^0C	330,000 mg/L at 25 ^{o}C
Solubility in organic solvent(s)	Very soluble in alcohol and ether; soluble in benzene; slightly soluble in cold petroleum ether	Soluble in acetone and Ethanol
Vapor pressure at 25^0C	0.00011 mmHg	No data
Photolysis	$t_{1/2}$ = 48h	No data
Henry's law constant at 25^0C	$3.4*10^{-6}$ atm-m^3/mol	No data

2.2.2 PCP solubility

Dean (1973,); Weast (1987), Mackay and Shiu, (1981) (cited in Arcand et al., 1994) pointed out that PCP has aqueous solubility between 10-20 mg per kg of water at room temperature.

PCP is a weak acid, so there is a partially dissociation in water.

$$C_6Cl_5OH + H_2O \Leftrightarrow C_6Cl_5O^- + H_3O^+$$

The ratio between PCP and pentachlorophenoxide ion (PCP^-) depend on pH. Solubility of PCP increases drastically with increasing pH.

There is a model to calculate the relationship between total PCP aqueous solubility (PCP_t) and pH. This model can be applied in the pH range 3-9.

$[PCPt]_{maq}$ (mol/L) = 10^{-5}*{1+$10^{(pH-4.35)}$} (Arcand et al., 1995) Eq. 2.1

Based on this model, the PCP solubility at different pH obtained is presented in the Figure 2.1.

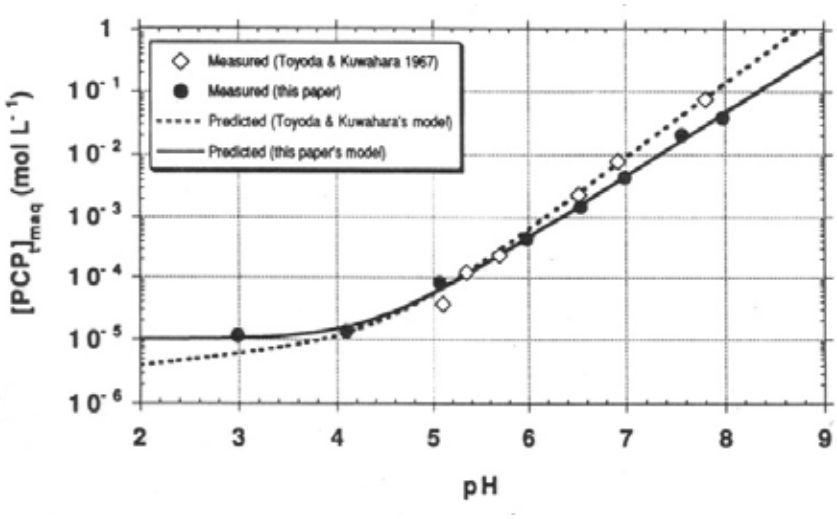


Figure 2.1 PCP solubility in water at 20-25^{0}C (Arcand et al. 1995)

The solubility of PCP is compared with other chlorophenols in table 2.2

Table 2.2Comparison of the solubility of chlorophenol compounds (Antizar-Ladislao and Galil, 2004)

Chemical name	Formula and molecular weight (g/mol)	Solubility (mg/L)
Phenol	C_6H_6O(94.1)	82000 (25°C)
2-MCP	C_6H_5ClO(128.6)	28000(25°C)
2,4,6-TCP	$C_6H_3Cl_3O$(197.5)	5170 (pH =7.15,25°C)
PCP	C_6HCl_5O(266.5)	3670 (pH =7.13,25°C)

Thus, it could be said that PCP is more difficult to dissolve in water than other chlorophenol compounds. This characteristic is affected by the pH and temperature.

2.2.3 Environmental concerns of pentachlorophenol

2.2.3.1 Toxicological effects

Acute toxicity

Pentachlorophenol is moderately toxic via the oral route. It is toxic via inhalation as well as causes irritation to the mucous membranes, skin, and eyes of test animals. Skin penetration may be the most dangerous route of exposure, being responsible for about 50 known cases of PCP poisoning, 30 of which have resulted in death (ATSDR, 2003). Immersion of a human hand in a 0.4 percent PCP solution for 10 minutes caused pain and inflammation. High acute exposure to PCP can cause elevated temperature, profuse sweating, dehydration, loss of appetite, decrease body weight, nausea, and neurological effects such as tremors, uncoordinated movement, leg pain, muscle twitching, and coma. Some of the symptoms maybe due to the impurities in the formulation, rather than the pentachlorophenol itself.

Chronic toxicity

Pure PCP, and also technical PCP without dioxin contamination, produced only slight enlargement of livers and kidneys. Purified PCP also did not produce toxic effects such as liver damage and immune system alterations. In humans, the most common exposure to PCP is inhalation in the workplace. Abdominal pain, nausea, fever, and respiratory irritation, as well as eye, skin, and throat irritation, may result from such exposure. Very high levels may cause obstruction of the circulatory system in the lungs and cause heart failure. Survivors of toxic exposures may suffer permanent visual and central nervous system damage. Persons regularly exposed to PCP tend to tolerate higher levels of PCP vapors than persons having little contact with these vapors.

Carcinogenic effects

There have been reports of the possible association between occupational exposures to technical PCP and Hodgkin’s disease, acute leukemia, and soft tissue sarcoma. No convincing evidence of PCP’s carcinogenic effects in humans is available.

2.2.3.2 Humans and ecological effects

Fate in humans and animals

PCP is rapidly absorbed through the gastrointestinal tract following ingestion. Accumulation is not common, but if it does occur, the major sites are the liver, kidneys, plasma protein, brain, spleen, and fat. Unless kidney and liver functions are impaired, PCP is rapidly eliminated from blood and tissues, and is excreted mainly unchanged or in conjugated form, via the urine.

Effect on aquatic organisms

PCP may be highly to very highly toxic to many species of fish. Several species of fish, invertebrates, and algae have had levels of PCP that were significantly higher (up to 10,000 times) than the concentration in the surrounding waters. Once absorbed by fish, pure PCP is rapidly excreted, as is its metabolite, with a biological half-life of only 10 hours. Biomagnifications, that is the progressively higher concentration of a compounds as it

passes up to food chain, is not thought to be significant because of PCP rapidly breaks down in living organisms.

2.2.3.3 Environmental fate

Break down in soils and groundwater

PCP is moderately persistent in the soil environment. PCP degrades most rapidly in flooded or anaerobic (airless) soils, at higher temperatures and in the presence of organic matter in the soil. Breakdown is mainly by anaerobic biodegradation while break down by sunlight and hydrolysis does not appear to be significant processes. It is poorly sorbed at neutral and alkaline conditions, and may be mobile in many soils. Sorption will be slightly greater (and mobility slightly lesser) in soils with higher proportions of soil organic matter.

Breakdown in water

In the water environment, PCP is mainly bound to sediments and suspended particles in water. PCP will dissociate by releasing a hydrogen ion and may then be more readily degraded by sunlight or microorganisms. In water, biodegradation occurs, mainly at the surface, with a half-life ranging from hours to days. It does not evaporate to a significant degree. PCP has been detected at very low levels in rivers and stream, surface water system, and seawater.

2.2.3.4 Toxicity of PCP

PCP has been investigated and proved toxic by many researchers. Kahru et al. (1996) have carried out study to analyze the toxic effect of pesticides by using Luminescent bacteria *Photobacterium phosphoreum*. The toxicity of priority pesticides is presented in Table 2.3. Among 13 pesticides analysis by Biotox™ and Microtox, PCP was found to be the most toxic substrate with 5-min EC50 equal to 0.55 mg/L.

Table 2.3 Comparison the toxicity of different pesticides (Kahru et al., 1996)

Chemical	Biotox™ 5-min EC_{50},mg/L	Biotox™ 5-min EC_{20},mg/L	Microtox™ 5-min EC_{50},mg/L	Rodent LD_{50} mg/kg
Paraquat	14800	6000	603	100
Ferrous sulfate	2340	620	197	1520
Nicotine	434	210	36.3	230
Lindane	60.8	12	6360	88
Glyphosate	2.19	0.75	7.7	1570
Pentachlorophenol	0.55	0.28	1.1	146
Arsenic trioxide	7.6	1.9	7350	15
$CuSO_4*5H_2O$	138	12.5	2.5	960

(EC: Effective concentration)

The toxicity of pentachlorophenol and its intermediate from the UV treatment was studied by Ho et al.(1998). The results obtained are presented in table 2.4.

Table 2.4 The toxicity of PCP and other chlorinated aromatics (Ho and Bolton, 1998)

Compounds (pK_a)	% Chlorine (w/w)	EC_{50}(µM)		LC_{50}(µM)	
		Toxich-chromo test	Microtox® Test	Fathead Minnow	24h Goldfish
Pentachlorophenol (4.7)	66.6	14	4-10	0.6	1.0
2,3,4,6-Tetrachlorophenol (5.2)	61.2	23	6.0-9.1	2.3	
2,3,5,6-Tetrachlorophenol	61.2		0.22-9.5		3.2
Tetrachloro-p-benzoquione	57.7	20	1-3		
Tetrachlorocatechol	57.2	16.7			
2,4,5-Trichlorophenol(6.9)	53.9	20	6.6-9.1		8.6
			8		
2,6-Dichlorophenol(6.6)	43.5	110	61-81		
					124
3-Chlorop)	27.6				
4-Chlorophenol(9.2)	27.6				70
Phe	0		19	361	638

It was suggested from the study that the toxicity of the compounds increase with the increasing chloride content. Decreasing values of EC50 or LC50 corresponds to high levels of toxicity. PCP was found to be more toxic than phenol and most of other chlorinated aromatics. Table 2.5 gives the PCP toxicity in different organisms.

Table 2.5 Toxicity of PCP to different kinds of organisms (Extoxnet PIP, 2003)

Species	Pentachlorophenol	
	LC50	LD50
Birds		
Japanese quail	5,139 ppm	
Mallards duck		380 mg/kg
Pheasants		504mg/kg
Aquatic organisms		
Chinook salmon	68 µg/L	
Rainbow trout	52 µg/L	
Fathead minnow	205 µg/L	
Channel catfish	68 µg/L	
Bluegill sunfish	32 µg/L	

2.2.4 PCP aerobic degradation pathways

In aerobic process, with dissolved oxygen concentration at least 2 mg/L, phenol ring could be broken down during the early stage of the process and can be completely mineralized to carbon dioxide, water and chloride faster than in anaerobic conditions. According to Mahaffey (1997, cited in MacEwen et al. 2001), initial intermediate products that form prior to breaking the phenol ring may include tetrachloroatechol, tetrachlorohydroqinone (TeCHQ), trichlorohydroxylbenzoquione (TCBHQ) and tetrachlorobenzoquinone (TeCBQ).

The intermediate products of PCP have been found to be relatively innocuous. None of these intermediate products are listed in EPA Region III RBC tables (MacEwen et al., 2001). Also none are listed in EPA's Integrated Risk Information System (IRIS) and Health effects assessment Summary table (HEAST) databases.

The intermediate breakdown products have fewer chloride atoms than PCP, so they break down quickly by cleavage of the phenol ring. They are labeled as short lived, as they have not been found to accumulate in the environment at similar sites. So, it is assumed that the risk posed by these intermediate products is significantly less than PCP. (MacEwen et al., 2001)

2.3 Technologies for treating pentachlorophenol

Many studies have been carried out to investigate the method for treating PCP. Technologies such as carbon adsorption, enzyme catalyzed polymerization and precipitation process, oxidation by ozone, biodegradation, etc have been applied. Carbon adsorption has an advantage of low retention time and simple operation. However, it is very expensive because of high cost required for activated carbon regeneration and waste residue production. Among these methods, biological treatment system is an effective and economical technique in PCP treatment. The objective of the treatment is to reduce the PCP concentration contained in wastewater and thereby, reduces its toxicity.

Studies have been done on biological degradation of PCP by anaerobic and aerobic organisms in different environment. PCP is believed to be resistant to biodegradation due to its high chlorine content and acute toxicity. However, some organisms have been found to degrade the PCP molecule completely to carbon dioxide and chloride.

Woods et al (1989) worked on anaerobic degradation of PCP using a continuous anaerobic sludge blanket reactor in the presence of high concentrations of readily biodegradable organic compounds. The original inoculums used were from a municipal sludge digester. The organisms were acclimated to a dilute caustic extraction effluent for two years and then grown in the column for three months prior to the addition of the chlorinated substrate to the column. A PCP concentration of 100 ppb was completely degraded into 2,3,4,5-tetrachlorophenol and 3,4,5-trichlorophenol within 80 hours. 2,3,4,5-tetrachlorophenol was removed at 95-97% during the last six days of the experiment. It was concluded that dechlorination of chlorides occurs at *ortho* position than *meta* position.

Another experiment to treat PCP under anaerobic condition using a fluidized-bed granular carbon has also been done (Khodadoust et al. 1996). The efficiency of removing PCP was found to be greater than 99.9% with the PCP loading as high as 4 kg/kg of GAC per day and the influent concentration was 1,333mg/L with the retention time of 9.3h. The results

show that under this condition, the concentration of PCP reduced but it is not completely mineralized. It was converted into intermediates such as 3-and 4- chlorophenol, which inhabited the methanogenic culture. It was suggested that in order to prevent the failure of the system, the influent PCP concentration must be controlled.

Wilson et al (1997) carried an experiment to treat PCP in GAC fluidized bed reactor. PCP and ethanol as input entered the anaerobic phase first and followed by aerobic stage. The effluent from the first stage showed that PCP was converted to mainly monochlorophenol (MCP). The second stage aerobic reactor mineralized the remaining aromatic chlorinated phenols. With the concentration of PCP and ethanol kept constantly, the mass and hydraulic loading rates was simultaneously doubled to evaluate the effect of 50% reduction in empty bed contacting time (EBCT). They found out that the optimum EBCT was 2.3 hours in the first stage for converting PCP into MCPs and stable reactor operation. More than 99% PCP was found to be converted to MCP in the first stage. And the second stage completely removed MCP and phenol.

PCP is biodegraded by only a limited number of bacteria and this bacterial mineralization occurs mainly aerobically. According to Stanlake and Finn (1982), PCP degrader can be isolated and characterized from the habitats as soil, aquatic environment that have a history of PCP contamination. Kinetic parameters such as: growth rate; length of lag phase; toxicity were determined in this study. *Arthrobacter* is one of the organisms used for degradation of PCP. Before PCP mineralization, a lag phase about 10 h or more was required. Because of the long time of the lag phase, the research also found that the continuous culture methods were more suitable than batch culture method. For the batch study, the length of lag period was directly proportional to the PCP concentration. They also found that bacteria that have capacity to mineralize PCP could also be susceptible to PCP toxicity. Efficiency of the degradation was low when PCP was utilized as sole carbon source. pH also affected PCP toxicity.

Another approach involves the use of a fluidized-bed reactor (FBR) with aerobic enriched microbial culture to degrade PCP (Melin et al., 1997). Oligotrophic bacteria grown at low substrate concentrations had characteristic of high substrate affinity even at low minimum substrate concentration with PCP as the sole source of carbon and energy. The chlorophenol-degrading culture in the FBR was enriched from the activated sludge by using a mixture of 2,3,4,6-tetrachlorophenol and PCP as sole sources of carbon and energy with feed concentration of 22 mg/L and 3.1 mg/L respectively. The effluent concentration below 2 µg/L was achieved at the retention time of 268 min and 2.5 mg/L PCP feed concentration. Table 2.6 gives the advantages and disadvantages of various methods for treating PCP.

Many kinds of microorganism are characterized and isolated for the purpose of treating PCP. Arthrobacter sp. was introduced by Stanlake and Finn (1982). *Flavorbacterium* sp. was determined by Saber and Crawford (1985). Radehause and Schmidt (1992) isolated *Pseudomonas* sp. strain RA2 from polluted soil. It has ability to minimize a higher concentration of PCP than any previous reported PCP degrading pseudomonad. Table 2.7 gives the different strains used in PCP degradation.

Table 2.6 Comparison of different available technologies for treating PCP

Treatment process	Advantages	Disadvantages
Carbon absorption	• Low retention time. • Simple in operation.	• Expensive because of high cost for carbon regeneration. • Waste residue must be deposed in a suitable way to protect environment.
Biological degradation	• Cost effectiveness	• Long retention time • Capacity to face adverse effects of phenols on microbial ecosystems.
Oxidation combined with catalytic process such as horseradish peroxidase.	• Can be applied to wide variety of phenolic compounds including those that are bio-refractory and toxic to microorganisms • Wide ranges of contaminant concentration, pH, temperature. • Insensitive to shock loading. • Low volume of sludge and residues production. • High reaction rate than bioprocesses.	• High cost of catalysis. • Residual toxicity of treated solution sometimes is higher than original toxicity of untreated solutions.

Table 2.7 PCP degrading strains

PCP-degrading strains	Sources	PCP tolerance	References
Pseudomanas luteola	Soil	400 mg/L as PCP salt	Otte et al.(1994)
Arthrobacter (ATCC 33790)	Soil	300 mg/L PCP salt	Edgehill and Finn (1982)
Flavobacterium	Soil	160-200 mg/L	Radehause and Schimdt (1992)
Mixed culture *KC-3*	Continuous-flow enrichment culture	20 mg/L pure PCP	Chu and Kirsch (1972)
Pseudomanas RA2	Soil	200 mg/L PCP salt	Radehause and Schmidt (1992)
Mycobacterium chlorophenolicum PCP1 and *Sphingomonas chlorophenolica RA2*	Soil	30 and 100 mg/kg soil	Miethling and Karlson (1996)

2.4 Membrane bioreactor

Combining membrane technology with biological reactors for the treatment of wastewaters has led to the development of three generic membrane bioreactors: for solid separation, bubble-less aeration within the bioreactor, and for extraction of priority organic pollutant from wastewater. The coupling of a membrane to a bioreactor has increased interest both

academically and commercially because of the inherent advantages of the process offered over conventional biological wastewater treatment systems.

2.4.1 MBR process description

A membrane bioreactor process for separation and retention of biological sludge is generally regarded as one alternative to the conventional activated sludge process.

The combination of activated sludge biodegradation and membrane separation is known as membrane bioreactor process (MBR). A membrane is manufactured in order to achieve the reasonable mechanical strength and can maintain a high throughput of a desired permeate with a high degree of selectivity.

In a MBR, the membrane will separate the biosolids from water. The separation will not depend on the hydrodynamic conditions of the bioreactor and the settling characteristics of the sludge.

There are two types of membranes, which can be applied for MBR. They are microfiltration (pore size from 0.1-0.4μm) and ultrafiltration (pore size from 2-50nm).

MBR systems can be categorized by two different configurations based on the location of membranes modules. Figure 2.2 and 2.3 show the two different configurations.

- The first one is *externally pressured cross flow MBR*. It is the use of cross-flow membrane modules in conjunction with mixed liquor in the bioreactor being circulated through the membrane. Although relatively easy to operate, but they require high-speed pumping devices. It leads to the high operation cost and impose a high level of shear stress on the biological suspension. Shear stress usually involves the breakage of microbial floc and subsequent damage to microbial activities (Cho and Lee, 1996).

- The second one is *submerged membrane bioreactor system*, in which a membrane module is directly immersed in an aeration tank. It is becoming more widespread because it mitigates the mentioned problems in the first type of MBR. This process can also be easily used to retrofit the conventional activated sludge process without significant modifications to the existing plant (Kim and Lee, 2003).

The advantage and disadvantage of MBR configurations are listed in the Table 2.8.

Table 2.8 Advantages and disadvantages of MBR configurations

Submerged MBR	Externally pressured cross MBR
Aeration costs high (nearly 90%)	Aeration costs low (nearly 20%)
Very low liquid pumping costs (higher if suction pump is used nearly 28%)	High pumping cost (60-80%)
Lower flux (larger footprint)	Higher flux (smaller footprint)
Less frequent cleaning required	More frequent cleaning required
Lower operating costs	Higher operating costs
Higher capital costs	Lower capital costs

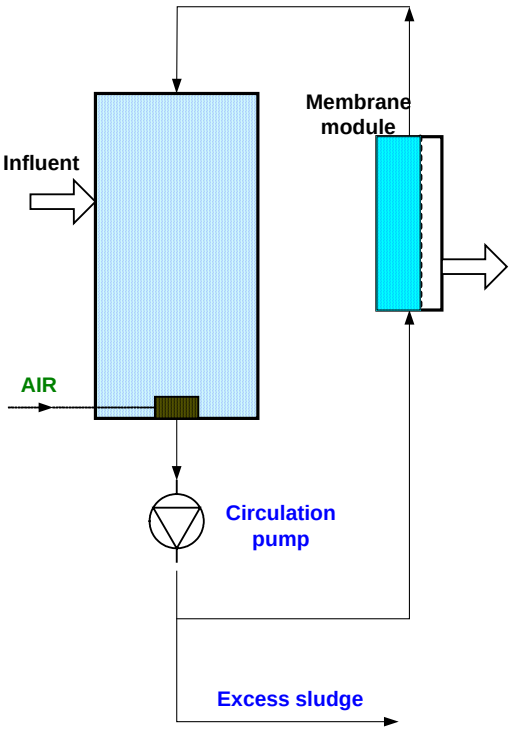


Figure 2.2 Externally pressured cross flow

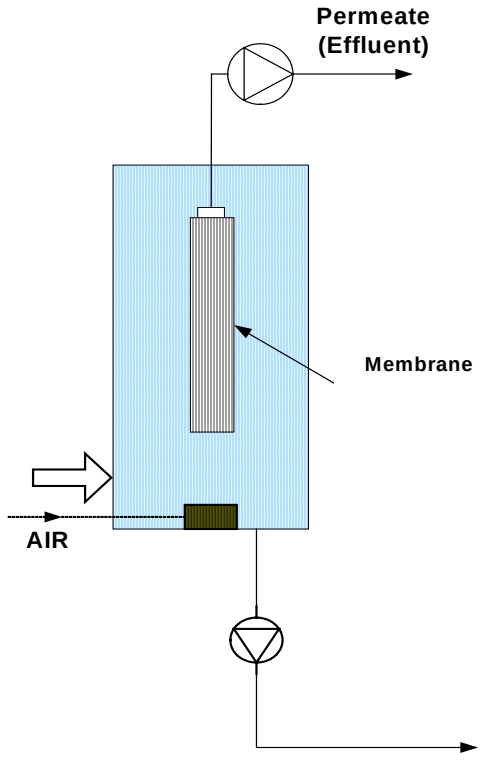


Figure 2.3 Submerged MBR

2.4.2 Advantage and disadvantage of MBR

By using membrane bioreactor in PCP treatment, there are advantages and disadvantages of than conventional biological treatment. Domestic and industrial wastewater can be treated by MBR (Mouthon et al., 2002, Yoo et al., 2004).

2.4.2.1 Advantage

MBR has advantage of a space saving, and the module design can be expanded to increase the capacity when needed. Membrane will continue to decrease in cost in the coming years.

MBR has been proved to be more efficiency than conventional biological treatment process in the following ways: MBR

- MBR makes it possible to completely separate the hydraulic retention time (HRT) and suspended solids retention time (SRT), which gives an opportunity to control the biological reactions, such as the system can run at very long SRT providing favorable conditions for the growth of slow-growing microorganism to degrade biorefractory compounds.

- Biomass concentration can be greater than conventional systems. It can be up to 30g/L in MBR. Therefore, the system can tolerate high volumetric loading rate. The reactor volume can also be reduced.

- The membrane can retain soluble material with a high molecular weight, improving its biodegradation in the bioreactor.

- Very good effluent quality, reuse of wasted effluents comes into view, which makes it a sustainable technology. They can be used for cooling, toilet flushing, lawn watering, or with further polishing as process water.

- Low sludge loads resulting in low sludge production. Low F/M and low wastage of biomass are the result of this. The sludge production of different processes is given in Table 2.9

- The settling tank is unnecessary because of the membrane separation.

Table 2.9 Sludge production for various wastewater treatment processes (Mallia and Till, 2001)

Treatment process	Sludge production (kg/kgBOD)
Submerged MBR	0-0.3
Structured media biological aerated filter (BAF)	0.15-0.25
Trickling filter	0.3-0.5
Conventional activated sludge	0.6
Granular media BAF	0.63-1.06

2.4.2.2 Limitations

The high biomass concentration in MBR has a high fouling tendency on the membrane, which increases energy consumption. Another problem is the accumulation of soluble microbial products (SMP) inside the bioreactor resulting due to the existence of the membrane.

2.4.3 Membrane fouling

In membrane filtration of wastewater, fouling is a major problem. Principally, because it reduces the flux applied, therefore increasing the membrane cost. Figures 2.4 shows the membrane fouling.

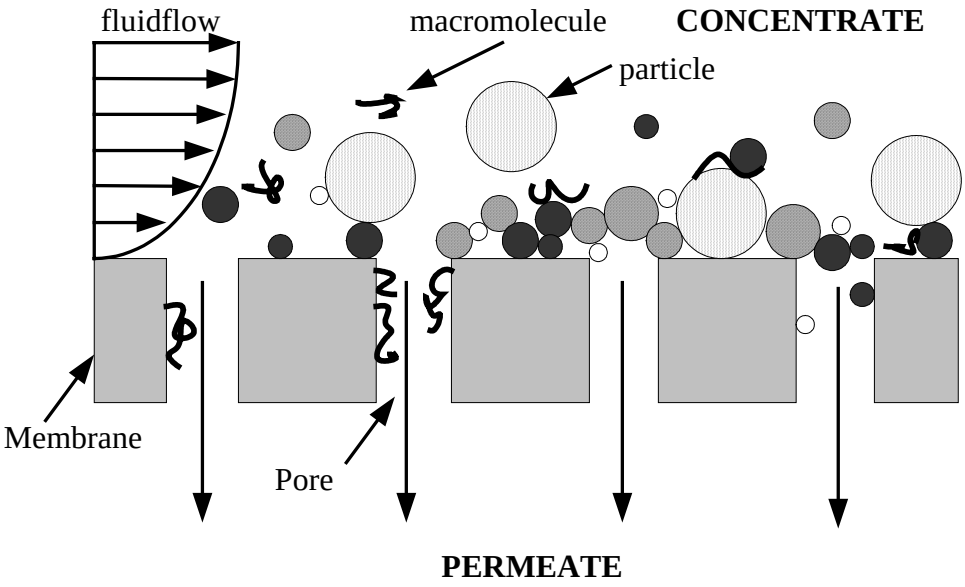


Figure 2.4 Membrane fouling

Foulant or fouling layer, are general terms for deposits on or into the membrane that adversely affect filtration. The term 'Fouling' is often used indiscriminately in reference to any phenomena that result in reducing product rate. These phenomena can be desirable or undesirable, reversible or irreversible.

Fouling can be characterized by mechanism and location. Membranes can be fouled in two ways: on or into membranes. Cake gel layer built on the membrane surface extends outward into the feed channel. If the constituents of foulant layer are smaller than the pore of the membrane, internal pore fouling occurs. This is usually difficult to remove.

Fouling can also be understood in terms of physical and chemical forces involved. Attractions between solutes and/or attractions between solutes and membrane result in foulants. Solvent and ionic environments are also key factors that mediate solute-solute and membrane-solute interactions.

Control of fouling is of utmost importance. Several techniques such as pretreatment of feed, chemical cleaning and optimization of operational conditions such as temperature, transmembrane pressure, cross flow velocity, etc can be considered to reduce fouling.

For a constant pressure membrane process, the permeation flux can be expressed by the resistance as:

$$J = \frac{\Delta P}{\mu R_t}$$

Eq. 2.2

J: permeate flux ($m^3/m^2.s$)

ΔP : Transmembrane pressure (Pa)

μ : viscosity of feed solution (Pa.s)

Rt: total resistance

Total resistance can be expressed as follows:

$$\mathbf{R_t = R_m + R_{ef} + R_{if}}$$

Eq. 2.3

R_m: the resistance of clean membrane, which can be obtained from the pure water flux data.

R_{ef}: the external fouling resistant which includes concentration polarization and deposition of colloids/macromolecules on the membrane surface.

R_{if}: the internal fouling resistance, due to internal fouling into the membrane pores.

These two resistances can be calculated from the solution flux data and from clean water flux before and after membrane cleaning.

These positive and negative aspects of membrane bioreactor can be summarized according to the types of membrane bioreactor (Stephenson et al, 2001). The summary is presented in table 2.10

2.4.4 Sludge characteristic

Sludge characteristics are measured to find out the relationship between parameters concerned with membrane operation and fouling and to assess the characteristics for the sludge treatment.

2.4.4.1 Sludge settleability

Conventionally, the sludge settling properties have been described by the Sludge Volume Index (SVI).

But this parameter gives a poor description of settleability because it strongly depends on the concentration of activated sludge (MLSS). It has been proposed that the diluted SVI (DSVI) can be used instead.

2.4.4.1.1 Sludge Volume Index (SVI).

The SVI is the volume of 1 g of sludge after 30 minutes of settling.

Measurement of SVI: Place a mixed-liquor sample in a one to two Liter cylinder and measure the settled volume after 30 minutes and the corresponding sample MLSS concentration.

$$SVI = \frac{(\text{settled volume of sludge, mL/L})(10^3 \text{mg/g})}{(\text{suspended solids, mg/L})} = \frac{\text{mL}}{\text{g}} \qquad \textbf{Eq. 2.4}$$

- SVI = 100 mL/g is considered a good settling sludge
- SVI >150 mL/g are typically associated with filamentous growth (Metcalf and Eddy, 2003)

Table 2.10 Advantages and Disadvantages of MBR

Advantages	Disadvantages
Membrane Separation Bioreactors	
Small footprint	Aeration limitations
Complete solids removal from effluent	Membrane fouling
Effluent disinfection	Membrane costs
High loading rate capability	
Combined COD, solids and nutrient removal in a single unit	
Low/zero sludge production	
Rapid start up	
Sludge bulking not a problem	
Modular/retrofit	
Membrane Aeration Bioreactors	
High oxygen utilisation	Susceptible to membrane fouling
Highly efficient energy utilisation	High capital cost
Small footprint	Unproven at full-scale
Feed-forward control of O demand	Process complexicity
Modular/retrofit	
Extractive Membrane Bioreactors	
Treatment of toxic industrial effluents	High capital cost
Small effluents	Unproven at full-scale
Modular/retrofit	Process complexity
Isolation of bacteria from wastewater	

2.4.4.1.2 Diluted SVI (DSVI)

Metcalf and Eddy (2003) introduced DSVI test as follow: The sludge sample is diluted n times with process effluent until the settled volume after 30 minutes is 250mL/L or less. And after that, we apply SVI standard method for this sample.

$$DSVI = \frac{(\text{settled volume of sludge, mL/L})(10^3 \text{mg/g})}{(\text{suspended solids, mg/L})} = \frac{\text{mL}}{\text{g}} \qquad \textbf{Eq. 2.5}$$

$$SVI = DSVI^* n \quad (\text{mL/g}) \qquad \textbf{Eq. 2.6}$$

2.4.4.2 Sludge dewatering

The dewatering of sludge is very important in terms of membrane fouling. It helps assessing the easiness in filterability of the sludge. Capillary suction time test is used to characterize the sludge dewaterability. The time the filtrate requires to travel a fixed distance in the filter paper is referred to a CST. A large CST usually implies poor sludge filterability.

The capillary suction pressure generated by standard filter paper is used to "suck" water from the sludge. The rate at which water permeates through the filter paper varies depending on the condition of the sludge and the filterability of the cake formed on the filter paper. The CST is obtained from two electrodes placed at a standard interval from the funnel. The time taken for the waterfront to pass between these two electrodes constitutes the CST.

The force generated by capillary suction is much greater than the hydrostatic head within the funnel, so the test is independent of the amount of sludge tested, as long as there is sufficient to generate the CST. Each test can be completed in a few minutes. Table 2.11 presents CSTs of different sludge types.

Table 2.11 Variation of filterability (CST) of sludge in 18mm reservoir (Carberry and Englane 1983).

Type of Sludge	Total solids concentration (%)	Filterability CST (sec.)
Mixed primary sludge	3.3	283
Humus (low rate)	4.9	1023
Humus (high rate)	3.3	580
Activated (very low rate extended-aeration) sludge	1.1	7
Activated (slow rate) sludge	2.0	14
Activated (high rate) sludge	2.0	223
Anaerobically digested sludge	2.5	278

2.4.4.3 Extracellular Polymeric substances (EPS)

EPS containing protein and polysaccharide have been thought to be a source of membrane fouling in MBR process. Therefore, it is important to control the amount of EPS to reduce membrane fouling during the operation and maintenance of in MBR.

Many researchers reported that the amounts of protein and carbohydrate in sludge have significance in sludge dewaterability. Some found that the effect of protein component in EPS was positive, and while some found it negative. The effect of the carbohydrate in EPS was mostly negative (Liu and Fang, 2003).

EPS has a relationship with dewaterability of sludge. It has been widely reported that the increase of EPS in sludge would lower the sludge dewaterability measured as CST, floc strength, etc.

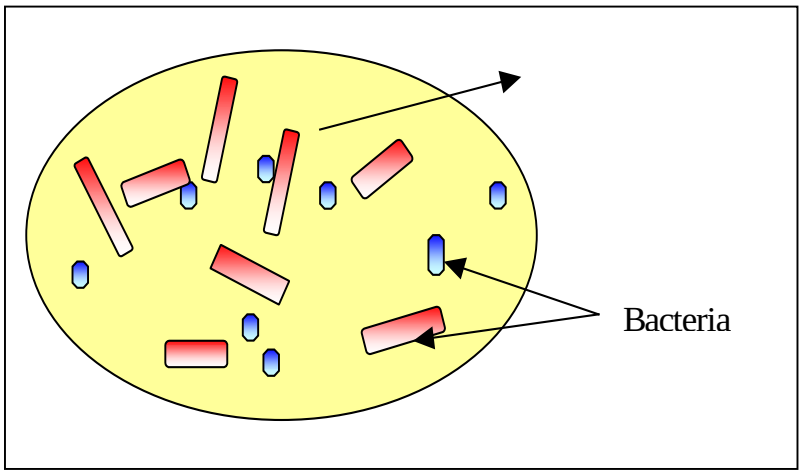


Figure 2.5 EPS

Characklis and Wilderer (1989, as cited in Wingender 1999) define EPS as organic polymers of microbial origin that in biofilm systems which are frequently responsible for binding cells and other particulate materials together (cohesion) and to the substratum (adhesion). Although EPS enhances the settleability of sludge, excess EPS causes high viscosity, thus increasing the MBR fouling.

EPS is considered in two types:

- Bound EPS (sheaths, capsular polymers, condensed gel, loosely bound polymers, attached organic material).
- Soluble EPS (soluble macromolecules, colloids, slimes).

Figure 2.6 illustrates the two types.

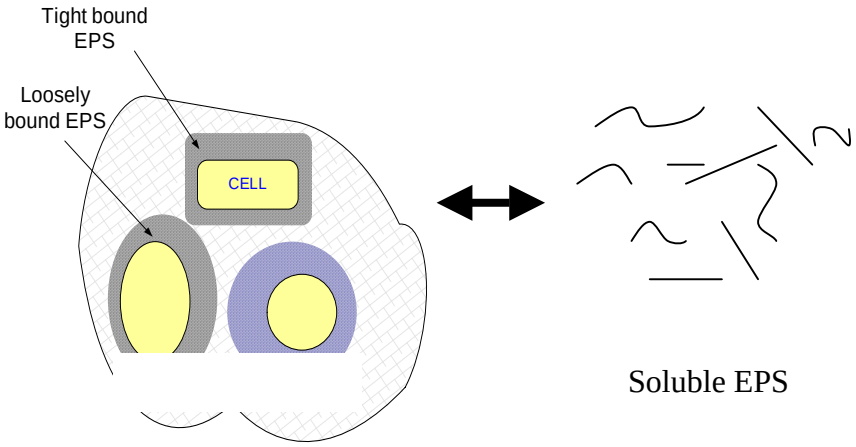


Figure 2.6 Types of EPS
(Nielsen and Jahn, 1999 as mentioned in Wingender, 1999)

EPS not only contains high-molecular-weight mucous secretions from the microorganisms, but also products of cellular lysis and hydrolysis of macromolecules. EPS from aggregates is a heterogeneous material. The compositions of the EPS in activated sludge floc are reported in table 2.12.

Table 2.12 Compositions of EPS of activated sludge floc and range of component concentration (Wingender et al., 1999)

Component	Content in EPS(%)
Polysaccharides	40-95
Protein	<1-60
Nucleic acids	<1-10
Lipids	<1-40

Main organic fractions of EPS are protein and carbohydrates (Wingender et al., 1999). EPS accumulation both in the mixed liquor and on the membrane may cause an increase the viscosity of the mixed liquor and an increase in the filtration resistance of the membrane (Nagaoka, et al. 1996).

The relationship between EPS and the resistance of the membrane R is displayed by Nagaoka et al. (1996) by equation 2.7.

$$\frac{dR}{dt} = \alpha.J.C_p - k.R \qquad \textbf{Eq. 2.7}$$

Where R is resistance (m^{-1}), t is the time (d), α is specific resistance of EPS (m/kg), J is the flux (m/d), C_p is concentration of EPS in the mixed liquor and k is specific decrease rate of the resistance (day^{-1}).

R is increased by the accumulation of EPS on the membrane, which is caused by the movement of bulk water into the membrane, and is decreased by the detachment and/or biological self-decay of the attached EPS.

2.5 Biokinetic theorical model

Knowledge of the biodegradation kinetics is necessary for the application of reactor engineering principles to predict effluent concentrations under various operating conditions. It also is important to know how changes in the operation of the plant, which might affect the biodegradation rates. With this information, the design engineer can make decisions regarding process alternatives with the goal of achieving a cost-effective design that will have high probability of meeting permit requirements.

The performance of biological processes used for wastewater treatment depends on the dynamics of the substrate utilization and microbial growth. Understanding clearly about the microbial growth will help engineer to have an effective design and operation of these systems.

The overall growth of microorganism is expressed by the following equation:

$$\text{Substrate (A)} \xrightarrow{\text{cell(C)}} \text{New cells (C) + product (R)}$$

2.5.1 Specific growth rate

2.5.1.1 Monod Kinetics (growth without inhibition)

In this model, Monod assumes that a single essential substrate is the growth limiting factor.

$$\mu = \mu_m \times \frac{S}{K_s + S} \qquad \textbf{Eq. 2.8}$$

Where

μ : specific growth rate, time^{-1}

μ_m : maximum specific growth rate, time^{-1}

S: concentration of substrate in solution, mass/unit volume

Ks: half-velocity constant, substrate concentration at one-half maximum growth rate, mass/unit volume

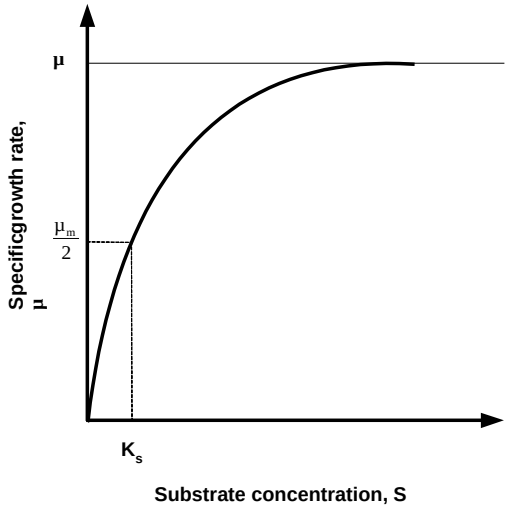


Figure 2.7 Relationship between substrate concentration and specific growth rate

2.5.1.2 Extended Monod kinetics for inhibition

In the practice, there are many inhibition effects on the growth of microorganism such as substrate, cells, and product. For instance,

- n-butanol inhibits the growth of Arthrobacter AK 19;
- 3-and 4- chlorophenol inhibit the methanogenic bacteria (Khodadoust, et al. 1996).

The general Monod equation for inhibition:

$$\mu = \mu_m \left(1 - \frac{C_I}{C_I^*}\right)^n \frac{S}{S + K_s (1 - \frac{C_I}{C_I^*})^m} \qquad \textbf{Eq. 2.9}$$

Where:

C_I: inhibitor concentration

C_I^* : the critical inhibitor concentration above which reaction stops m,n are constant.

Many researchers invest many kinetic models for inhibition. They are displayed in Table 2.13.

Table 2.13 Kinetic models for inhibition (Han and Levenspiel, 1987)

Equation	Reference
For substrate inhibition	
$\mu = \mu_m \frac{1}{1 + K_s/S + S/K_I}$	Adrews and Noack
$\mu = \mu_m \frac{S(1 + K_1 S/K_2)}{K_s + S + S^2/K_2}$	Webb
$\mu = \mu_m \frac{S}{K_s + S} \exp(-S/K_s)$	Aliba and co-workers
$\mu = \mu_m \frac{S}{K_s + S}$, when S<S' $\mu = \mu_m \frac{S}{K_s + S} - K_I (S - S')$, when S>S'	Wayman and Tseng
(K_I, K_1. K_2 : constant)	
For product inhibition	
$\mu = \mu_m \left(1 - \frac{C_I}{C_I^*}\right) \frac{S}{S + K_s}$	Ghose and Tyagi
$\mu = \mu_m \left(1 - \frac{C_I}{C_I^*}\right)^{0.5} \frac{S}{S + K_s}$	Bazua and Wilke
$\mu = \mu_m \left(1 - \frac{C_I}{C_I^*}\right)^{n} \frac{S}{S + K_s}$	Levenspiel
$\mu = \mu_m \frac{S}{K_s + S} \exp(-K_1 C_I)$	Aliba and co-workers

2.5.2 Growth yield coefficient (Y)

Y is defined as the ratio of the mass of cells formed to the mass of substrate consumed expressed as, mass/mass.

The relationship between substrate removal and bacterial growth rate is expressed as following

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \qquad \textbf{Eq. 2.10}$$

Y: true growth yield coefficient (mg SS/g substrate removed.day)

X: biomass concentration (mass/volume)

S: substrate concentration (mass/volume)

2.5.3 Substrate utilization

Substrate utilization can be expressed as

$$\frac{dX}{dt} = \mu X \qquad \textbf{Eq. 2.11}$$

From [2.8], [2.10], [2.11, the rate of substrate utilization (r_{su}) is:

$$r_{su} = -\frac{\mu_m XS}{Y(K_s + S)} \qquad \textbf{Eq. 2.12}$$

Also,

$$-\frac{dS}{dt}\frac{1}{X} = \frac{\mu_m}{Y}\frac{S}{k_s + S} \qquad \textbf{Eq. 2.13}$$

Substitution of r_x for $-(dS/dt)/X$ and $r_{x,m}$ for μ_m/Y in equation [2.13] would result in

$$r_x = r_{x,m}\frac{S}{k_s} + S \qquad \textbf{Eq. 2.14}$$

Where r_x and $r_{x,m}$ are actual and maximum substrate removal rates, respectively.

2.5.4 Maximum rate of substrate utilization per unit mass of microorganism

We defined maximum rate of substrate utilization per unit mass of microorganism by following equation as

$$k_S = -\frac{\mu_m}{Y} \qquad \textbf{Eq. 2.15}$$

Methods for measuring k_s and $r_{x,m}$ can be divided as follows (Drtil et al. 1993):

(a) Batch feed method

(b) Chemostat method

(c) Infinite-dilution method

(d) Respirometric method

The simplest is the respirometric method.

2.5.5 Respirometric technique

Many authors have used respirometric techniques for the determination of microbial kinetic parameter (CokGor et al., 1998; Spanjers et al., 1995; Kappeler and Gujer, 1992).

Respirometric is the measurement and interpretation of the respiration rate of the activated sludge, and is defined as the amount of the oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge.

The advantages of this method for measuring kinetic parameter are:

- It can be used for those substrates, which cannot be easily determined analytically.
- It is much more sensitive than other methods.
- It determines kinetic constants at given conditions, without changing the qualitative or quantitative composition of biomass.

Although this method constitutes a rapid and easy way, there are many complicating factors associated with the measurement of kinetic and stoichiometric parameters such as feed pattern (Chudoba et al., 1992), substrate storage, etc (Chudoba et al. 1992, Dircks et al, 1993) which restrict or limit its applicability.

2.5.5.1 Measurement of oxygen uptake rate

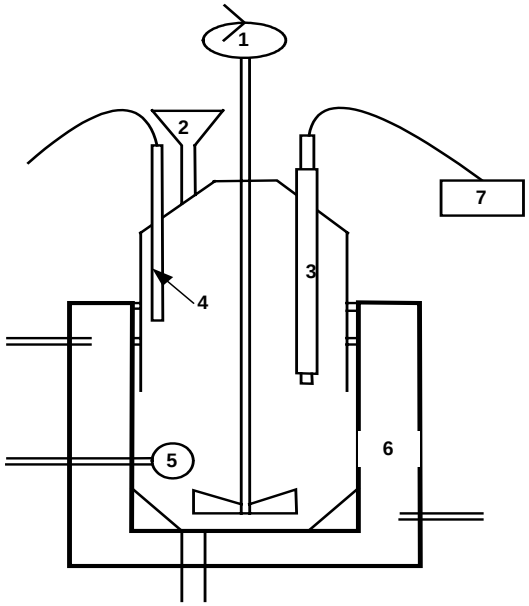
The measurement system consists of an oxygen sensor, temperature sensor, continuous recorder, an airtight, circular measuring chamber, with variable height and volume, a temperature-controlling water bath surrounding the bottom and the wall to maintain a constant temperature of 20^0C, a stirrer. Figure 2.7 illustrates a measuring system. An air stone is used to increase the amount of dissolved oxygen if needed. The stirrer will create the necessary turbulence; the content of the cell can be thoroughly stirred. The concentration of dissolved oxygen is measured with an oxygen electrode of high stability. Its signals are registered with the course of temperature within the measuring chamber. A ceiling of the system is oblique in order that air bubbles can easily escape from the cell. The funnel is served for dosing of the substrate solution and for escaping air bubbles during period of aeration.

2.5.5.2 Experimental procedure and evaluation of respirogram

A working concentration of biomass, X_0, is prepared by either thickening or diluting the original suspension. In the case of diluting, washing medium or effluent or supernatant can be used according to the necessity. The working concentration of biomass will generally depend on the level of heterotrophic endogenous respiration rate. In order to suppress the process of nitrification, allylthiourea is used in suitable concentrations.

The biomass is transferred into the respirometric system and aerated to increase the dissolved oxygen concentration to 6-8mg/L. When DO is reached to this level, the aeration is stopped.

The DO change is recorded as respirogram. A typical respirogram is interpreted as in Figure 2.8.



1: Stirrer
2: Funnel
3: Oxygen sensor
4: Temperature sensor
5: Aeration stone
6: Water bath
7: Recorder

Figure 2.8 Air tight chamber (Kappeler and Gujer, 1992) for respirometric measurement

When the aeration is stopped, a slow decrease in oxygen concentration is due to heterotrophic endogenous respiration, as demonstrated by the line A-B-C. At time B, a small known volume of concentrate substrate solution is injected into the system to get the initial S/X required. All necessary nutrients are also added with the substrate. At this time, there is a temporary increase in respiration rate, is shown by line BD. This line is maximum-value tangent to the curve BE and means the total respiration rate at the substrate concentration S. When substrate concentration decreases with time, the respiration rate also decreases. At point E, substrate is removed, the respiration rate returns to the value (line D-E) which equal to or slightly different from the original endogenous rate.

When the measurement with one concentration is finished, a new dose of substrate is injected into the cell and the next respirogram is recorded. This step can be repeated several times when DO concentration drops below 2.

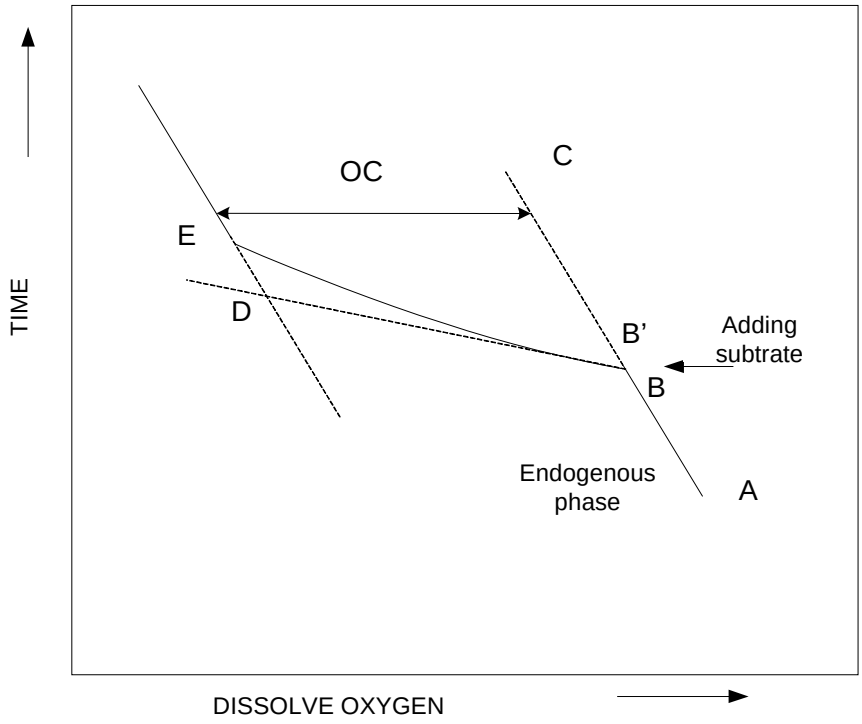


Figure 2.9 Recorder chart with a typical respirogram

2.5.5.3 Determination of kinetics parameter

Evaluating the respirogram, we can get these data:

Endogenous respiration rate : $r_{X,e}$

Total respiration rate : $r_{X,t}$

Net oxygen concentration : OC

Using these data, the following rates and coefficients can be computed.

Specific substrate oxidation rate at concentration S:

$$\mathbf{r_{X,ox} = r_{X,t} - r_{X,e}} \qquad \textbf{Eq. 2.16}$$

Specific substrate removal rate at concentration S:

$$r_X = \frac{r_{X,OX}}{OC/S} \qquad \textbf{Eq. 2.17}$$

Coefficient of substrate oxidation

$$1 - Y = \frac{OC}{S} \qquad \textbf{Eq. 2.18}$$

Coefficient of biomass yield

$$Y = 1 - \frac{OC}{S}$$ **Eq. 2.19**

All quantities are expressed in oxygen units.

This method is so sensitive that even substrate concentrations below 1 mg/L bring about recordable changes in respiration rates.

Specific growth rate

$$\mu = Yr_X$$ **Eq. 2.20**

2.5.6 Biokinetics of PCP degrading microorganisms

Many studies have determined the biokinetic parameters of PCP degrader micororganisms. Table 2.14 and 2.15 summaries the results.

Table 2.14 Kinetic constants for PCP degrader (Melin et al., 1997)

Culture	**Enrichment method**	**Ks (mg/L)**	**K_I (mg/L)**	**n**	**Reference**
Flavobacterium sp. [a,b]	Batch	25	1.5	1	Hu et al. (1994)
Flavobacterium sp. [a,b]	Batch	38	81	1	Gu and Korus (1995)
Flavobacterium sp. [a,b]	Batch	13	70	4	O'Reilly and Kovach (1992)
Pseudomonas aerubinosa and P. putida	NS	164	6	1	
Mixed	NS	1097	1	1	Carberry and Kovach (1992)
Mixed	Continuous	0.06	1.38	1	Klecka and Maier (1985)
Mixed [c]	Continuous	1.1	1.7	3	Melin et al. (1995)
Mixed	Continuous	0.016	5.3	3.5	Melin et al. (1997)

[a] In mixture with glumamate

[b] reclassified and identified as Sphingomonas chlorophenilica

[c] In the mixture with 2,3,4,6-tetrachlorophenol

NS: not specified.

K_I: the inhibition constant

K_S: the half-saturation constant

n: the inhibition response coefficient.

Table 2.15 Comparison of the kinetic and stoichiometric parameters of different kind of wastewater

	Yield (gCOD/ gCOD)	**Maximum specific growth rate μ_{max} (d^{-1})**	**Half-saturation constant K_s (d^{-1})**	**Decay rate b_H (d^{-1})**	**Reference**
PCP wastewater in CSTR		$\mu_{max}/K_s = 0.0017$ l $\mu g^{-1} day^{-1}$		0.046	Moos et al., 1983
Nitrogen removal of municipal wastewater in MBR	0.72	5.7	4.3	0.51	Mouthon et al., 2002
Domestic Wastewater in MBR	0.5	3	100	0.028	Yoo et al., 2004

2.6 Toxicity test

Toxicity tests indicate whether a sample of chemical, waste, effluent, sediment or soil causes a toxic effect on biota. They can provide information about unknown substances. The advantage of biological toxicity test s over chemical analysis is the direct assessment of potential biotic impact without the need for extrapolation from the individual impacts of the different chemical constituents. Observed toxicity can be an early warning or even prediction of potential environmental damage. It also takes into account the effects of toxicant mixtures.

Over the last twenty years, test systems using lower organisms (e.g. Microtox TM) have so far mainly been used in wastewater treatment, for screening the toxicity of the effluent. (Kahru et al., 1996). Microbiological toxicity tests include test designs using defined bacteria and to some extent unicellular algae. Most of the tests are based on measurements of growth inhibition, respiration, mineralization or metabolism, enzyme activity and viability of bacterial cells

Presently, the most common microbiological test in effluent toxicity assessment is the luminescent bacteria test. The inhibition of light production by *Vibrio fischeri* bioluminescent bacteria indicates disturbance in the energy metabolism. The luminescent measurement assesses the metabolic status of this bacterium and the change in bacterial bioluminescence after exposure to wastewater samples can be used as an indicator for potential toxicity.

This kind of test kits is also commercially available for example Microtox^{TM}-USA, Lumistox^{TM}-Germany, Biotox^{TM}-Finland. Micotox^{TM} test is carried out by using the Microtox Model 500 Analyzer. This instrument is a laboratory-based temperature controlled photometer (15 or 27^0C) that maintains the luminescent reagent and test samples at the appropriate test temperature. This instrument measures the light production from the luminescent bacteria reagent and is designed to interface with a PC for collecting, analyzing and storing the test data.

The results from this test can be interpreted in terms of:

- EC_{50} (%) = Effective concentrations of sample that causes 50% inactivation of the test bacteria.

- EC_{20} (%) = Effective concentrations of sample that causes 20% inactivation of the test bacteria.

- TU_{50} = toxicity units where $TU_i = C_i/ED_{50}$ and C_i is the concentration of component i (normally used TOC concentration, mg/L).

- The increase of EC value means the reduction of toxicity.

For solutions containing a single toxicant, EC50 is expressed as a ratio that can be converted to a mass or molar concentration. For treated compounds, which may consist of one or more toxicants, EC50 can only be expressed as a multiple of the solution concentration, because of unknown actual concentration of toxicants.

According the research of Kahru et al., (1996), Microtox™ bacteria (free-dried *P. Phosphoreum*) are 8.7 times more sensitive toward pesticides than Biotox™.

There are some researchers using Microtox test for measuring the toxicity of aromatic compounds including PCP during many degradation processes such as ozonation, enzyme catalyzed polymerization and precipitation.

According to Shang N.C., et al. (2003), the ozonation of aromatic compounds such as phenol, benzoic acid, 2-CP, 3-CP and 4-CP induced new toxicity during the early period of the reaction. After the subsequent biological treatment, the toxicity is decreased. Acute toxicity of pure compounds and their intermediates are evaluated by Microtox toxicity assay.

From the toxicity test conducted by Zhang and Nicell (2000), the EC50 of PCP in water was found to be 0.97mg/L compare to other results, which were 1.5 mg/L or 1.3 mg/L. PCP concentration and the toxicity dropped sharply during the first 10 min of reaction time and then continued to decrease lowly. However, the soluble products from PCP transformation process contributed to the overall toxicity leading to real toxicity higher than theoretical toxicity (only for PCP).

2.7 Bioaugmentation

Bioaugmentation is defined as the application of selected microorganisms to enhance the microbial populations of an operating waste treatment facility to improve water quality or lower operating costs.

A prevailing belief has been that, over the time, the proper microbes will grow in the system and become acclimated to the influent. Indigenous microbes are those that occur naturally in a given environment. The indigenous population may act but not quickly enough to prevent the spread of the contaminant. In reality, even the natural population may develop into an acceptable one though there may be performance limitations that only can be overcome through the introduction of superior strains of microorganisms.

Without bioaugmentation, the indigenous population consists of numerous types of organisms. Some of these organisms are more efficient and effective than others at degrading various organic compounds and producing a settleable biomass.

As shown in figure 2.10, biomass can be categorized as Population A (desired indigenous organisms), Population B (other indigenous organisms), and Population C (selected bioaugmentation organisms). The goal of the bioaugmentation program is to enhance the growth of Population A, establish the selected organism of Population C, and minimize Population B. The net result is to improve both the quality and quantity of the bacterial population.

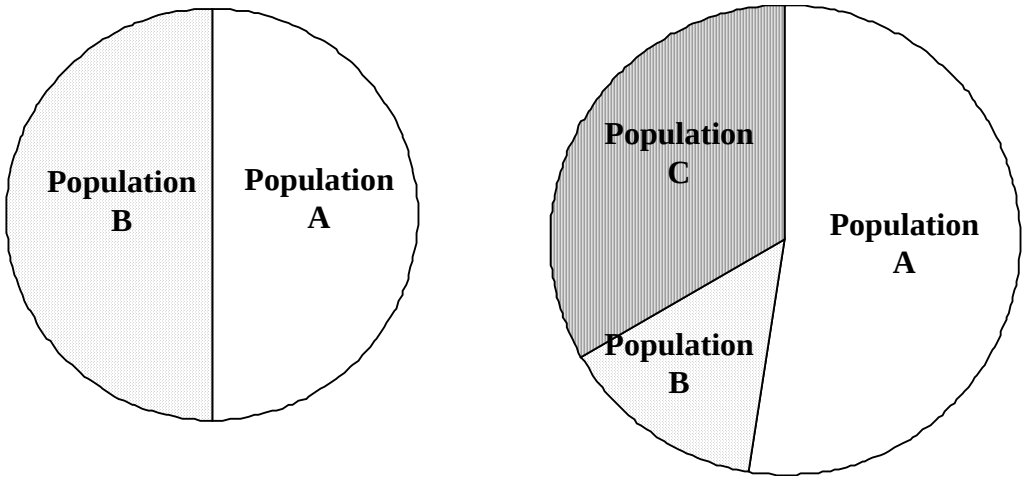


Figure 2.10 Impact of bioaugmentation (Foster and Whiteman, 2003)

Controlled addition of specific microbial cultures to treatment systems for stimulation of the degradation of specific compounds is a discipline in environmental engineering. Pure cultures studies are most often conducted under favorable laboratory conditions, and therefore the results only indicated the potential for the treatment process. The success for implementation of bioaugmentation as an integrated treatment process will depend on the competitiveness and activity of such specific cultures when exposed to environmental conditions common for wastewater treatment process (Jacobsen and Arvin, 1996). However, when successfully applied though, bioaugmentation has show to assist with plant start up, organic or hydraulic overloading, removal of toxic compounds or other problematic wastewater components and sludge settleability (Belia and Smith, 1997)

For Edgehill and Finn (1983), when adding Arthrobacter to soil, the reduction of the half life for PCP degradation from 2 weeks to less than one day. Bioaugmentation in wastewater was studied by Stanlake and Finn (1983), when adding Arthrobacter to an activated sludge reactor spiked with PCP, PCP degradation happen after 1 day whereas a parallel system without addition need 6 days for acclimation before degradation began. And continuous adding improves the stability of plant performance after exposed to a shock loading of 120 mgPCP/L.

Not only PCP as an organic compounds, can be applied with bioaugmented technique, there are target substrates have been used in study of this technique. According to the research of Wilderer et al. (1991), *Pseudomonas putida* was added to activated sludge from

a municipal wastewater treatment plant to treat 3-Chlorobenzoate. On the long run, there were no significant differences between the supplemented and non-supplemented system. However, the start-up time for the supplemented as significantly shorter compared to non-supplemented one. After support of 3-CB for some time, the non-supplemented system failed and required several days to recover the 3-CB removal capacity when this compound appeared again in the influent, while the supplemented system could keep its degradation capabilities.

2.8 Biosorption

Besides the degradation of chlorophenols by microorganism, the biosorption is also one of aspects to be considered. Although the contribution of biosorption for toxic organics removal is negligible in the long run, compared with degradation, it significant affects the fate of organics and performance of the treatment system (Ning et al, 1996).

Previous studies at the laboratory scale (Jacobsen et al., 1991) showed that sorption of PCP accounts for up to 50% removal of influent load at low (< 3days) solid retention time (SRT) in the activated sludge process. No biodegradation took place at the low SRT, on the other hand at SRT > 14 days, the sorption accounted for < 10% removal of PCP.

Several researchers have investigated biosorption of chlorophenols by selected microorganisms. According to Benoit et al (1998), there was 40% of the overall uptake of 4-MCP after 7 days due to physicochemical sorption and the rest due to biodegradation and bioaccumulation. Ning et al. (1996) studied the sorption of 2,4-dichlorophenol on inactivated anaerobic granules and recognized that there was not much different between live and chemically inactivated sludge. They also suggested that the aerobic biosorption was mainly a physico-chemical process. The results from many research showed that sorption capacity was influenced by pH, biomass concentration, and hydrophobicity of the target pollutants and biomass sources (Antizar-Ladislao and Galil, 2004).

Antizar-Ladislao and Galil (2004) had conducted biosorption experiments with biomass collected from the site contaminated with phenol and chlorophenols. Results, indicated that the equilibrium time for the pollutants is rapid. pH increase from 6-9 lead to decreasing of equilibrium biosorption capacity. They also applied Freundlich mathematical models to simulate the fate of these pollutants in the aquifer.

Biosorption process is carried out in two stages: (1) Kinetic sorption test; (2) sorption isotherm tests.

This is the Freundlich model with the assumption that adsorbent has heterogeneous surface composed of different classes of adsorption sites.

$$\left(\frac{x}{m}\right) = KC^{1/n} \Rightarrow \log(\frac{x}{m}) = \log(K) + \frac{1}{n}\log C \qquad \textbf{Eq. 2.21}$$

where

x is amount of material adsorbed (mg)

m is weight of absorbent (mg)

C: concentration of material remaining in solution after adsorption is completed (mg/L).

K and n are constants.

Biosorption is affected by many factors such as pH, octanol/water distribution coefficient K_{OW}, nutrient condition, influent of co-solutes,etc. According to Jacobsen et al. (1995), pH had the most important impact on the linear sorption coefficient K_p, but the dissolve organics matter and added P-buffer also had some impact on the sorption of PCP on biomass.

2.9 Summary

The characteristics of PCP, which include both the physical-chemical and toxicity aspect, have been mentioned in this part. Though PCP is toxic substance but biodegradable. An advanced technology such as membrane combining with degradation process of microorganism has been applied to remove PCP in wastewater. The biokinetic of the microorganism cultures must be determined as useful information for engineering work in design and operation. The toxicity test has been applied to check the level of mineralization of PCP with the time.

Chapter 3

Methodology

3.1 Introduction

Membrane bioreactor is an advanced wastewater treatment process, and has been successful in treating organic pollutants with high removal efficiency. With toxic compounds like PCP, there is the need to use a large amount of biomass, for which MBR process would be beneficial.

The objective of the study was to verify the applicability of membrane bioreactor system in degrading wastewater-containing PCP; a toxic compound used widely as wood preservative material. The efficiency of this process with acclimatized activated sludge and activated sludge bioaugmented with specific microorganism was compared with shock loading to check the inhibitory effect of toxic compound.

Two reactors were operated in parallel. One MBR was operated with mixed cultured of microorganism while the other with mixed culture bioaugmented with *Mycobacterium chlorophenolicum.* The comparison was carried out in terms of the removal efficiency, system stability and membrane fouling.

The study consist of three parts: (1) Part 1: Preliminary study, (2) Part 2: Batch studies, (3) Part 3: MBR studies with mixed bacterial culture and MBR with mixed bacterial culture blended with *Mycobacterium chlorophenolicum.* These stages of the study are displayed in Figure 3.1.

3.2 Materials and methods

3.2.1 Feed wastewater characteristics

Commonly, PCP concentration in municipal sewage is in the range of a few μg/L or lower. In extreme conditions, influent PCP concentration can reach 12 mgPCP/L (Melcer and Bedford, 1988). Thus, a PCP concentration of 12 mg/L was used in the study during optimization of operational parameters.

In both MBR, synthetic wastewater having PCP concentration of 12 mg/L along with glucose was used to maintain a total COD of 600 mg/L. Nutrient salts were added to provide a suitable condition for the growth of bacteria. Mineral salt composition used in the feed is displayed in Table 3.1.

Table 3.1 Mineral salt composition (Stanlake and Finn, 1982)

Composition	Concentration (mg/L)
K_2HPO_4	1730
KH_2PO_4	680
$(NH_4)_2SO_4$	1000
$MgSO_4.7H_2O$	100
$FeSO_4.7H_2O$	20
$CaCl_2.2H_2O$	30
$MnSO_4.H_2O$	30

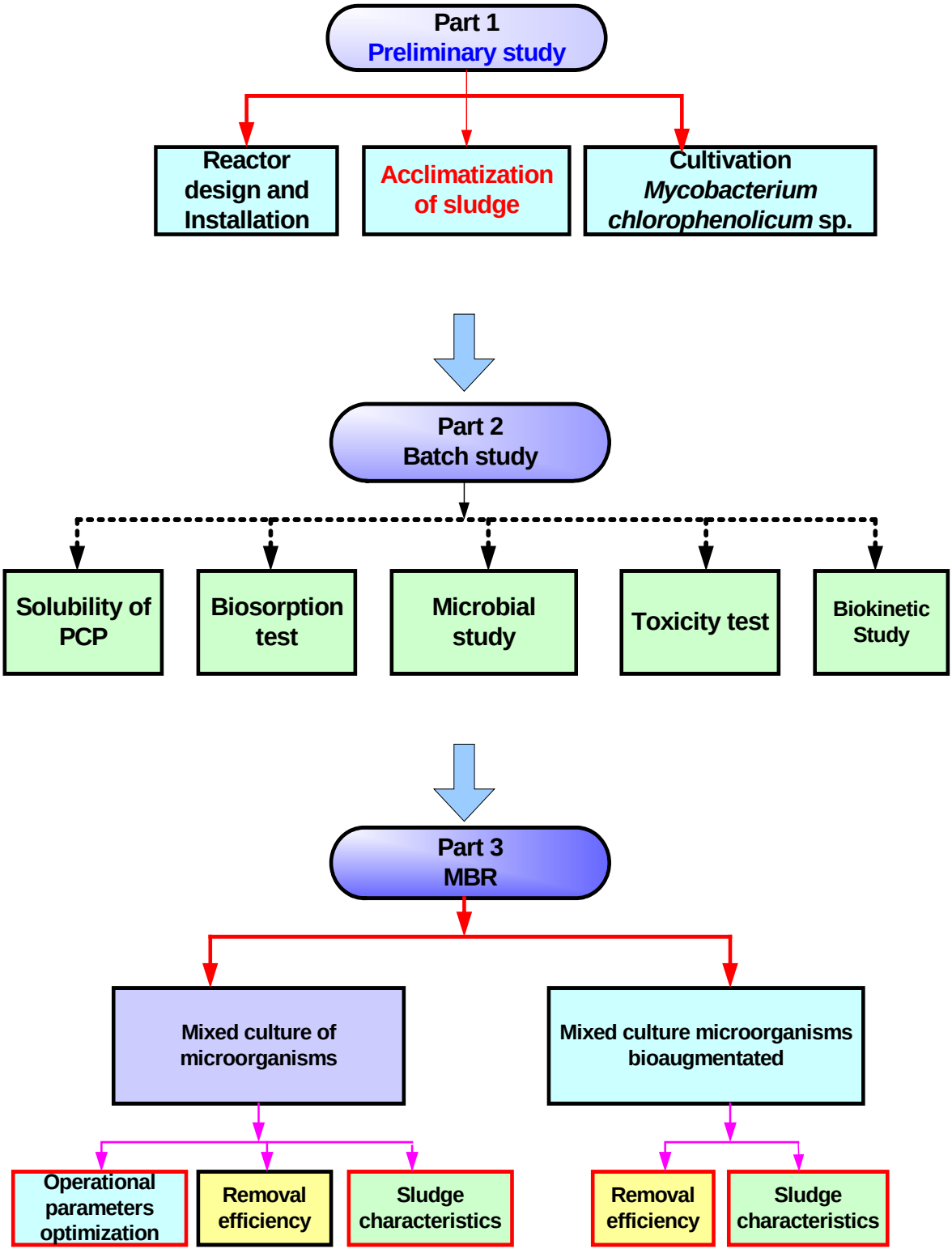


Figure 3.1 Stages of the study

PCP was added from the stock solution (400 mg/L) that was prepared in 1N NaOH.

Media was sterilized by autoclaving at 121^0C for 20 min. The calcium, manganese and iron salts were added separately from other nutrients to avoid the formation of precipitate. The culture medium was strongly buffered by KH_2PO_4, K_2HPO_4 at pH around 7 to avoid inhibition of microbial activity due to hydrochloric acid production during PCP degradation.

The COD: N: P ratio of this synthetic wastewater was 2.2:1:1.7

3.2.2 Microbial cultures

3.2.2.1 Acclimatized activated sludge

There were two sources of microbial population, which had been acclimatized. The first one was activated sludge from the aeration tank of wastewater treatment plant and the second one was activated sludge, which was used to treat phenol.

The procedure for acclimation of these populations to PCP was as follows:

During the acclimatization process, a pH of 7 and DO of 3 mg/L were maintained. The acclimatization was conducted by fill and draw process. A HRT of 24 hours was remained with 15-min filling, 20-h aeration, 3.5-h settling and 15-min draw. COD of 600 mg/ was maintained throughout the acclimatization process.

- **Fresh Activated Sludge**

The activated sludge collected from aeration tank from a wastewater treatment plant was run with Glucose as carbon source with 600 mg COD /L for the first two days. After monitoring the consistency of the biomass, 1 mg/L PCP was added with Glucose content. MLSS, MLVSS concentrations were monitored to see the change in biomass with the toxicity of PCP. Once in three days, PCP and COD of the supernatant were measured.

Once a sufficient (greater than 70%) removal was obtained, a higher concentration of PCP was added and sludge was allowed to acclimatize. The process was continued until a sufficient COD and PCP removal and an increased biomass was obtained (MLSS around 8,000 mg/L). The PCP concentration used was 1, 5 and 10 mg/L.

- **Phenol adapted Activated Sludge**

Initially, feed concentration of 200 mg/L phenol and 112.5 mg/L glucose had been added as the carbon source. The MLSS, MLVSS concentration along with the phenol and COD/TOC removal were verified after 3 days.

When the phenol reduction was greater than 80%, 1mg/L PCP concentration was added. The phenol, COD and PCP removal were monitored. Once the removal was high, phenol was reduced while increasing PCP. Later, the acclimatization was continued with only PCP and glucose as the carbon source.

Once a sufficient (greater than 70%) removal was obtained, a higher concentration of PCP was added and sludge was allowed to acclimatize. The removal was monitored along with MLSS and MLVSS concentration. The process was continued until a sufficient COD and

PCP removal with an increased biomass was obtained. The PCP concentrations used were 1, 5, 10 mg/L.

The acclimatization process of two kinds of sludge was monitored and compared. After that, they were mixed together and added to the MBR.

3.3 Experimental set up

A pilot scale tank membrane bioreactor was built for the present study. The reactor was made of transparent acrylic sheet having the dimensions as shown in Figure 3.2 and 3.3. The working volume was 6 liters. The hollow fiber polyethylene membrane manufactured by Misubishi Rayon Company, Japan (Sterapore) was submerged in the reactors. Characteristics of the membrane are given in Table 3.2

Table 3.2 Characteristics of the membrane module.

Membrane type	Hollow fiber
Membrane material	Polyethylene (PE)
Pore size	0.4μm
Surface area	0.2 m^2

Two MBR was operated in parallel to compare removal efficiency. The schematic diagram of the experiment is presented in Figure 3.4.

The feed wastewater was mixed in the mixing tank, which was pumped into the feed tank. From feed tank, water enters the level control tank by gravity force. The influent part of reactor present in the bottom was connected to the level control tank. This tank operated with an automatic valve that controls the influent flow with the help of floating buoy. This set-up helped to maintain the rate of feed wastewater flow into the reactor equal to the rate being withdrawn. The reactor was aerated with compressed air using two stone diffusers with airflow rate of 5L/min to maintain DO in the reactor in the range of 2-3 mg/L. Permeate was pumped out by suction with a circulation pump and speed controller to adjust the flow rate.

The system was operated with a 5 minutes on/off filtration cycle. Timer and solenoid valves controlled the operations of the alternative membrane filtration and nonfiltration period. The transmembrane pressure (TMP) was measured using a U shaped Hg manometer.

The system was cleaned every week to prevent the development and accumulation of microorganisms in the storage system, which could decrease the concentration of substrate before entering the system.

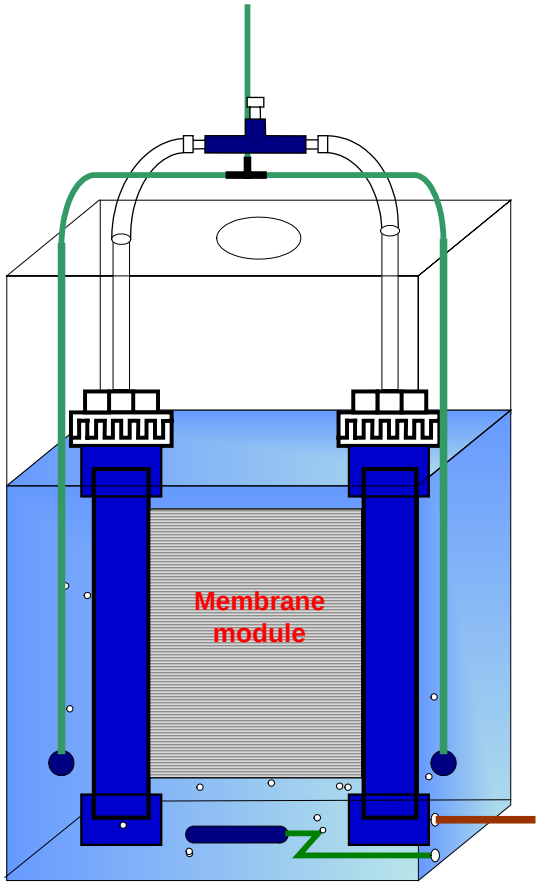


Figure 3.2 Membrane reactor

3.4 Batch studies

Biosorption of PCP on sludge; biokinetic of sludge, toxicity of influent and effluent and the degradation PCP of *Mycobacterium chlorophenolicum sp.* and organisms isolated from the MBR was carried out in the batch condition.

3.4.1 Biosorption study

3.4.1.1 Preparation of chemical and biomass

The stock PCP solution (86% purity) was prepared in 0.1N NaOH solution. The working solution with different PCP concentrations was prepared by diluting the stock solution with 0.1 M $NaNO_3$, 0.01% NaN_3 solution. NaN_3 is a respiratory inhibitor, which was used to prevent the active uptake and metabolism of PCP. The solution was adjusted to pH 7.2 using H_2SO_4.

Activated sludge from the domestic wastewater treatment plant was used as the biomass for the biosorption studies. The microbial biomass was washed thrice with 0.1M $NaNO_3$ (8.5g/L) (Antizar-Ladislao and Galil, 2004) and centrifuged at 4000 rpm for 10 minutes, in order to inhibit the microbial metabolism, while maintaining the viability of the microorganisms.

MEMBRANE BIOREACTOR

Working volume 6 liters

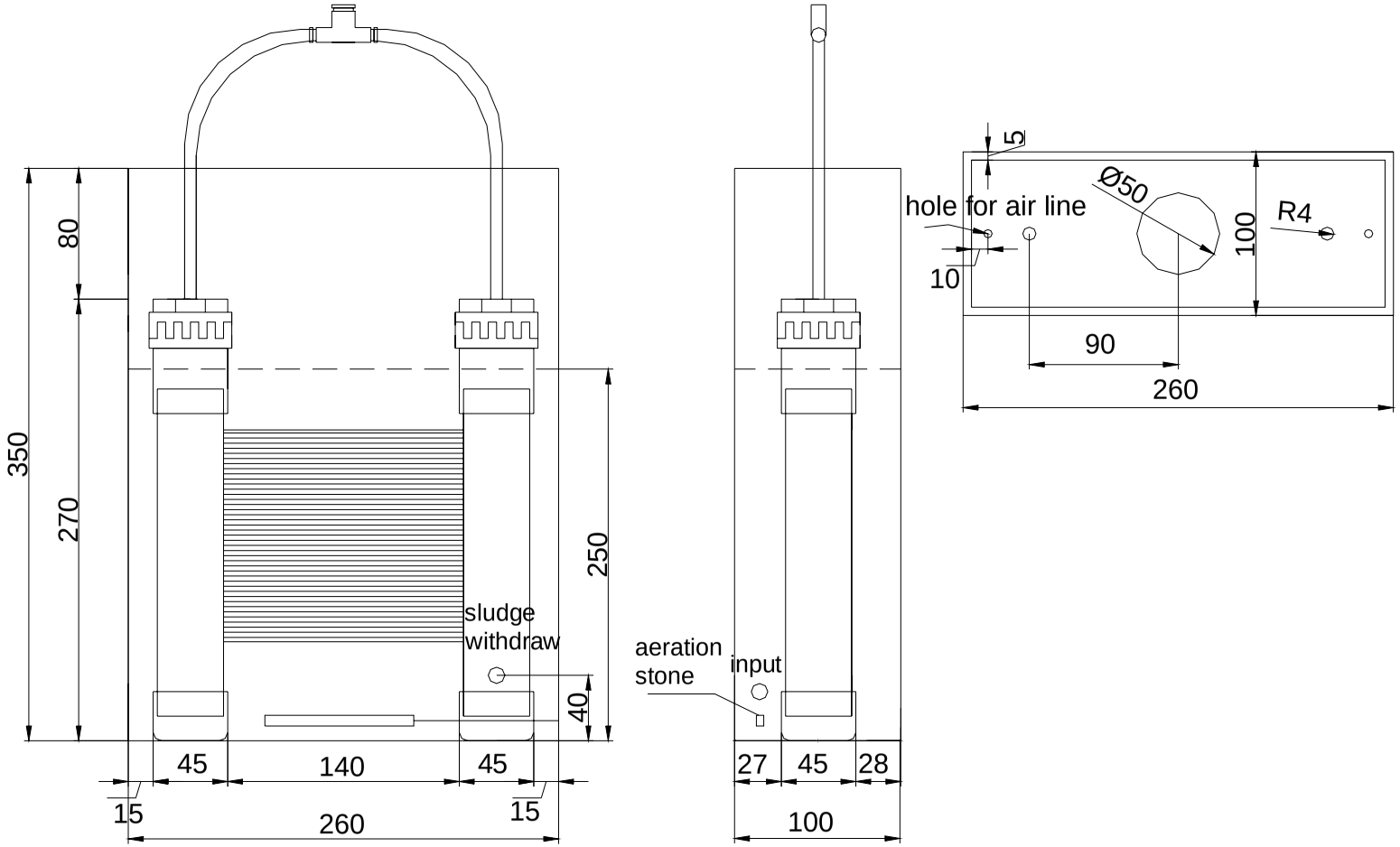


Figure 3.3 Dimensions of the MBR (unit:mm)

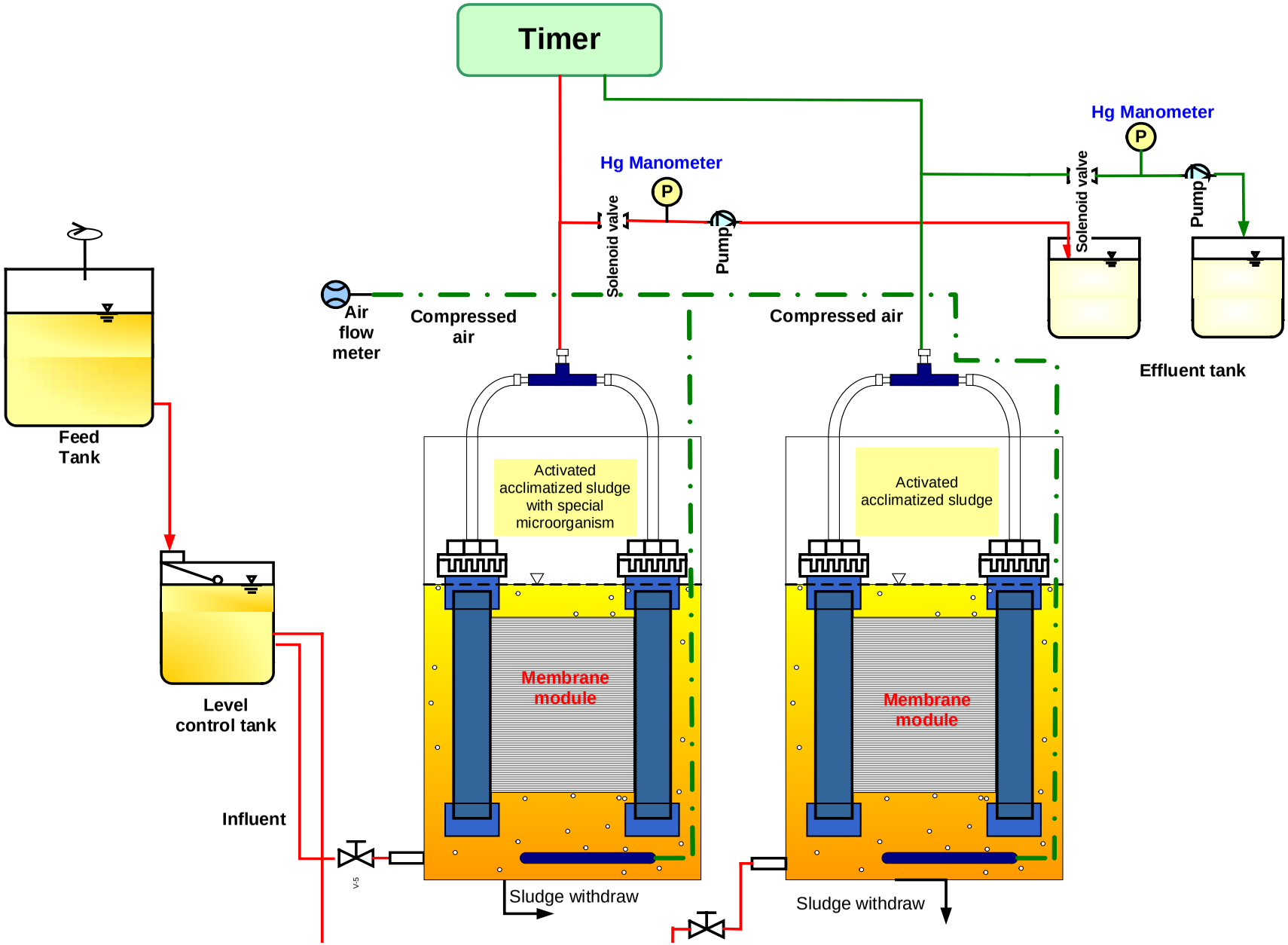


Figure 3.4 Schematic diagram of the experiment

3.4.1.2 Biosorption equilibrium

Uniform volume (50mL) with a known biomass concentration (MLVSS) was added to 100 mL of PCP solution in a 500 mL flask. These flasks are rotated in a shaker with rotational speed of 120 rpm at 25ºC ± 1. Samples were taken at contact time of 0, 10, 20, 40, 60, 120 and 180 minutes. Sampling was done by allowing the sludge to settle and removing 20 mL of the supernatant. The samples were filtered using 0.2μm-cellulose acetate filter and the filtrate was analyzed for PCP using GC. The PCP concentration in the experiment was varied from 7 – 15 mg/L. The equilibrium time was the time beyond which there was no significant decrease in PCP concentration.

3.4.1.3 Biosorption isotherm

After obtaining the equilibrium time from experiment described in 3.4.1.2, the optimum equilibrium time was used to obtain biosorption isotherm. PCP concentration of 15 mg/L with varying biomass concentration was used for the experiment. Sampling and analysis was carried out as mentioned in 3.4.1.2.

3.4.2 Biokinetic study

3.4.2.1 Materials required

(a) Respirometer vessels

The shape of the respirometer has been described as in the Figure 3.5. It should be water-jacketed in order to maintain stable temperature during the tests. If the temperature is not controlled, rises during the experiments due to the heat generated by the stir plate and friction from the stir bar.

(b) **Dissolved Oxygen monitor**

Any dissolved oxygen meter with recorder output can be used to continuously monitor the DO.

3.4.2.2 Preliminary preparation prior to experimental analysis

(a) Sludge preparation

Sludge was collected from the reactor and let to settle. The supernatant is replaced by buffered distilled water (so that the organism does not die because of pH change) in order to reduce the initial concentration of substrate in the sludge. The nutrient was added to the sludge to avoid nutrient limitations. Table 3.3 displays the concentration of nutrients.

Table 3.3 Composition of the mineral (Dircks et al.,1998)

Compounds	Concentration (mg/L)
$MgSO_4$	166
$FeCl_3$	2.3
$CaCl_2$	412
NH_4Cl	6.8
And phosphate buffer (maintain pH = 7.24)	
KH_2PO_4	90
K_2HPO_4	410

To inhibit nitrification, N-allylthiorea of concentration 12 mg/L was added (Dircks et al., 1998).

(b) Preparation the DO probes**.**

When high sensitivity membranes are used, it is recommended to change them before performing respirometric tests and to leave it overnight on aerated water. Then, the DO probe was calibrated at the same condition of mixing and temperature as the kinetic test.

3.4.2.3 Procedure of analysis

The experiment procedure is as follows:

(a) DO probes prepared.

(b) Buffer was added to the biomass sample to maintain the desired pH

(c) Biomass was aerated for 30 minutes prior to testing to oxidize residual substrate.

(d) VSS and the pH of the biomass sample were measured.

(e) Sludge was aerated at least two hours until endogenous respiration is reached. OUR of endogenous phase was recorded ($r_{X,e}$).

(f) An accurate amount of concentrated substrate is added to obtain the desired S_0/X_0 (0.01-0.2 mgCOD/mgVSS) (Chudoba et al., 1992; Ekama et al, 1986, Dircks, et al., 1998). Total OUR was recorded ($r_{X,t}$). DO must be maintained higher than 2 mg/l.

(g) DO decrease due to microbial respiration was monitored every 10 seconds using DO meter. DO change and OUR data were calculated. When the measurement with one concentration is finished, a new dose of substrate is injected into the cell and the next respirogram is recorded.

(h) Applying equations [2.16]-[2.20], we can determine kinetic parameters.

The study could be divided into 3 parts:

(1) Low concentration of PCP for Monod kinetics

(2) High concentration of PCP for inhibited kinetic

(3) Glucose and PCP plus glucose were used as different substrates in order to evaluate whether effect of glucose on PCP degradation.

All the experiments were conducted at temperature of 25 ± 1^0C in 20^0C temperature room.

The respirometer used in this study is presented in Figure 3.5.

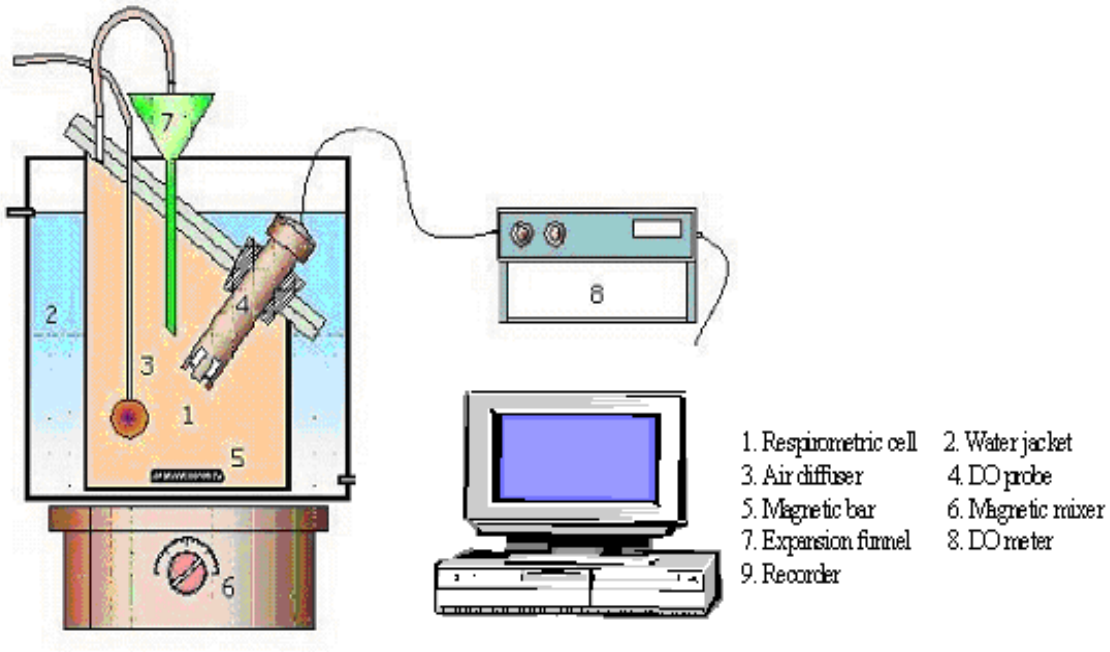


Figure 3.5 Respirometer

3.4.3 Toxicity test

Microtox test is a quick bioassay using marine bioluminescent bacterium (Photobacterium *phosphoreum*) as the test organism. The light emitted by the bacteria after they have been exposed to a sample of unknown toxicity is compared with the light emitted by the bacteria in the controls containing no sample. The decrease in the light emission due to the exposure, which is a function of metabolic inhibition in the bacteria, indicates the degree of toxicity of the sample.

The Microtox reagent contains living bioluminescent bacterium (*Vibrio fisheri*) that has been grown under optimal conditions, harvested and then lyophilized (freeze-dried under vacuum). The lyophilize bacteria are rehydrated with the Microtox Reconstitution (is specially prepared distilled water, free from toxic material) to provide a ready-to-use cell suspension.

The Microtox system is normally employed for the determination of a dose-response curve, from which the effective concentration (EC) of the sample causing a specified effect is found. Dose-response curve is a graph on the log-log scale plotting the concentration of the sample (dose) against its effect on the test organisms; the response is represented by Gamma (Γ). Gamma is the ratio of light lost by the test organism after exposure to a sample to the light remaining after that exposure. On the log-log scale, the plot of the concentration against Gamma is approximately a straight line, in most cases, following easy interpolation and extrapolation.

Toxicity of a sample is expressed as EC50 which is the effective concentration of a toxicant causing a 50% decrease in the test organism light output under defined conditions of exposure time (5, 15 minutes) and test temperature of 15 $\pm$ 0.5^0C. This value is obtained by calculating the point at which Gamma equals 1.0 on the dose response curve.

3.5 Studies on MBR

The study was carried out with the objective of standardizing the suitable condition for operating MBR for PCP removal as well as maintaining the stability of the system. Two reactors were operated in parallel to compare the efficiency with two kinds of microorganism seed in case of poor performance of MBR because of shock loading or inhibited effect of toxic compound. One was operated with acclimatized activated sludge and another with acclimatized activated sludge with *Mycobacterium chlorophenolicum*

3.5.1 Optimum HRT and sludge characteristics

To obtain an appropriate MBR operation, different HRTs were investigated with the influent concentration of 600 mg/l with PCP 12 mg/L and glucose. Mixed culture microorganisms after acclimatization (indigenous) were added to both MBR with the initial concentration around 7,000 mgSS/L. Three different HRT used in this study were 12, 18 and 24 hours. The effluent, influent, mixed liquor were monitored for COD, TOC, PCP, Cl^- to assess the removal efficiency. In terms of system stability, sludge characteristics such as MLSS/MLVSS, pH, DO, TMP, F/M, viscosity, DSVI, Protein and polysaccharide were monitored.

Biomass concentration in both reactors was maintained at 10,000 mg/L.

The permeate flux was controlled by volumetric measurement of the effluent to maintain the desired HRT. Table 3.4 shows the expected permeate flux at different HRT.

Table 3.4 Permeate Flux at different HRTs

HRT	Flowrate (L/d)	Permeate Flux (m^3/m^2.day)
24	6	0.06
18	8	0.08
12	12	0.12

3.5.2 Inhibition experiment

For the inhibition studies, NaPCP was used in place of PCP because at pH around 7, PCP solubility was maximum 20mg/L. Thus, further increasing of PCP concentration, may lead to precipitation of PCP in the solution.

Initial NaPCP concentration used was 10 mg/L and was gradually increased to 20, 30, 50 and 100 mg/L with HRT of 12hours.

The performance of both reactors was monitored. Bioaugmentation was used when the performance of MBR started deteriorating.

3.5.3 Bioaugmentation technique

With decrease in MBR performance, bioaugemetnation technique was applied. After cultivating a stock solution of each culture, a small volume (0.1mL) of the culture will be transferred to sterile synthetic wastewater under sterile condition.

For each organism, the cultivated culture would be added to the reactor at their log phase at HRT 12 hours by withdrawing indigenous population and maintain the MLSS of 10,000 mg/L. Performance of the reactor was monitored to know the affect of bioaugmentation in enhancing the degradation of PCP.

3.6 Analytical methods

Most of the analytical techniques used in this study were as mentioned in the standard methods (APHA et al., 1998). Table 3.5 lists parameters and their analytical methods.

Table 3.5 Analytical methods

Parameters	Analytical methods	Analytical Equipment	Interference	Range	Source
pH		pH meter			
DO		DO meter			
TOC	Oxidative combustion infrared analysis	TOC analyzer (Shimadzu TOC-V_{CSN})	HCO_3^-, suspended solid	>1 mg/L	APHA et al., 1998
COD	Dichromate reflux	Titration	Halides	20 – 900 mg/l	APHA et al., 1998
Cl-		Mercuric nitrate	Titration	All range	APHA et al., 1998
DSVI		1L graduated cylinder and timer			Jenkins 1993
MLSS	Dry at 103-105^{0}C	Oven			APHA et al., 1998
MLVSS	Dry at 550^{0}C	Oven			APHA et al., 1998
Carbohydrate	UV absorbance (490)	Spectrophotometer (Hitachi U-2001)			Dubois et al.,1956
Protein	UV absorbance (750)	Spectrophotometer (Hitachi U-2001)			Lowry et al.,1951.
CST		CST apparatus (Triton electronic limited)			APHA et al., 1998
PCP and NaPCP	Gas chromatography	GCECD	Any contaminants	µg	This study

PCP. Pentachlorophenol was measured by using gas chromatography Electron capture detector HP 5890Series I with nitrogen carrier gas. Operating conditions were as follows: make up gas of 42.5 cm/s, split flow of 1.25 mL/min, split ratio 40:1 and split- less mode. Column, injector, and detector temperature were 70, 250 and 250°C. The initial column temperature was 70°C, increasing 10°C/min to 250°C. Injection volume was 2µL. Ethyl acetate was used as solvent solution. The standard curves are produced with triplicate measurement.

PCP was extracted using ethyl acetate; 10 ml of sample was added to 10 ml of ethyl acetate. Later, solutions were mixed for 5 minutes using vortex mixer. After which, the

sample was allowed to stabilize for 10 minutes. The top layer having solvent containing PCP was collected in a GC vial of volume 2mL.

The PCP extraction method was modified from the procedure of Liao et al.(2001)

EPS. The compositions of EPS such as protein and carbohydrate were measured as mentioned in figure 3.6. The centrifugation rate for separate soluble EPS from the bound was 3200 rpm for 20 minutes. Thermal extraction at temperature 80^0C for 1hour was carried out. (Chang and Lee, 1998).

(a) Protein. Protein in the mixed liquor and bound EPS was measured by Lowry Assay method as Lowry et al. (1951) mentioned. The range of the sensitivity is 5- 100 µg/mL. The absorbance of the color after 30 min was read against a blank at 750 nm. The standard curve was constructed by using Bovine Serum Albumen (BSA).

(b) Carbohydrate. Carbohydrate in mixed liquor and bound EPS was measured by phenol/sulphuric acid method of Dubois et al. (1956). The color obtained was allowed to develope 30 min, after which the sample was read against a blank at 490 nm. The standard curve was constructed by using glucose.

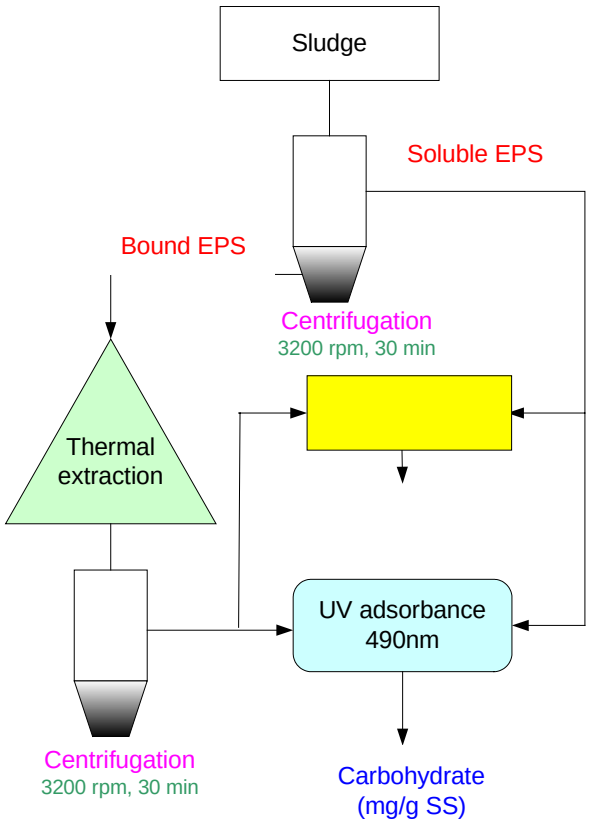


Figure 3.6 EPS analysis

3.7 Membrane cleaning

After a long period of MBR operation, the TMP increases significantly. It is the sign of membrane fouling. Chemical cleaning is required to reduce the increases trans-membrane pressure back down to a level close to the initial level and this would enable stable

operation over an extended period of time. The procedure for cleaning membrane is as follows. First, membrane module was removed from the reactor. Using the tap water to remove activated sludge and other material adhering to the interior of the membrane unit. Immerse the unit completely into a chemical cleaning tank with chemical solution containing a mixed of sodium hypochloride (effective chloride about 3000mg/L) and 4% (wt/vol) of aqueous sodium hydroxide solutions. It is allowed to stand for 6-24 hours. After which, the membrane was rinsed with water to remove chemicals prior to its installation back to the reactor. The resistance of the membrane was checked to ensure a high percentage recovering of the membrane.

Chapter 4

Results and Discussion

4.1 Acclimatization process

Acclimatization of activated sludge is done in order to make the activated sludge resistant to toxic chemical compound and increase the ability of the microorganisms to degrade PCP. In the present study, batch acclimatization was carried out with two kinds of sludge: one with 9 L of fresh activated sludge and another with 2 L of phenol-adapted sludge to increase their ability to degrade PCP. Glucose was used as the supplementary carbon source during the acclimatization process. One liter of synthetic wastewater with COD 600 mg/L consisting of glucose, PCP and mineral salt was added to each system everyday. In fresh activated sludge, PCP concentration was gradually increased from 1 mg/L to 10 mg/L, through 5 mg/L. The phenol-adapted sludge was initially fed with 200 mg/L of phenol and 112.5 mg/L glucose to maintain COD of 600 mg/L. After providing phenol for initial activation, the feed wastewater as used for fresh activated sludge was used. The acclimatization was done by fill and draw process. pH in the influent was maintained at about 7 to avoid pH variation from the feeding. The supernatant was removed everyday and the performance was checked in terms of COD and PCP.

The experimental results of the acclimatization process are given in Appendix A-1. The performance of changes occurring in acclimatization process is illustrated is figure 4.1, 4.2, and 4.3.

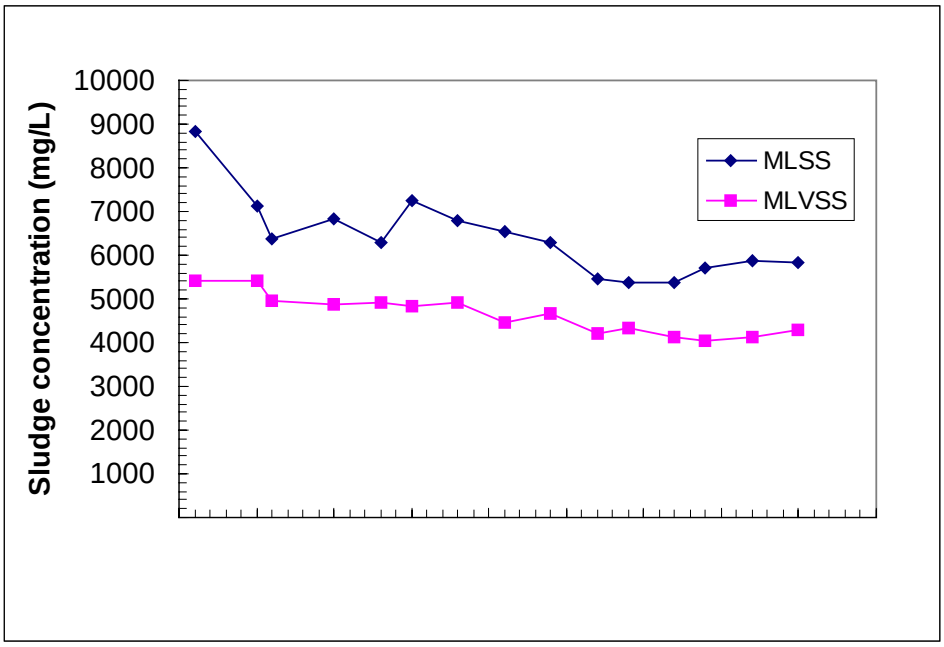


Figure 4.1 Biomass Variation of Fresh Activated Sludge

From the result obtained, it could be concluded that phenol adapted sludge was well adapted for PCP more than fresh activated sludge as COD removal capacity of phenol-adapted sludge was higher than fresh activated sludge.

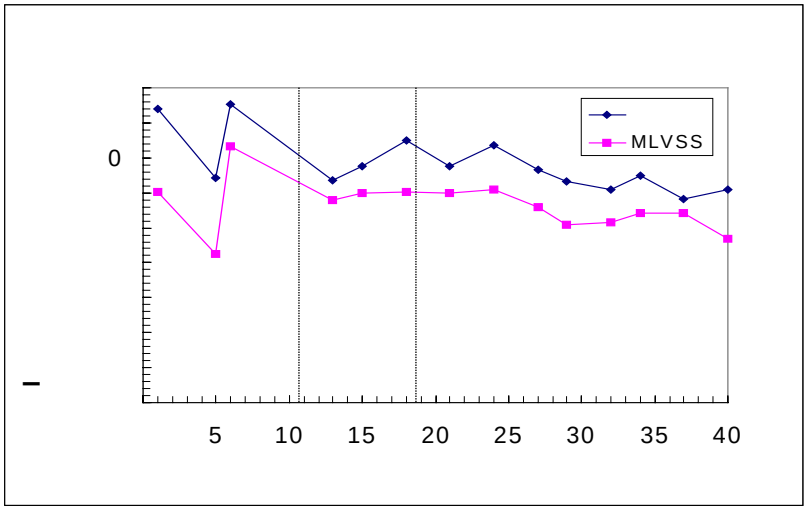


Figure 4.2 Biomass Variation of Phenol-Adapted Sludge

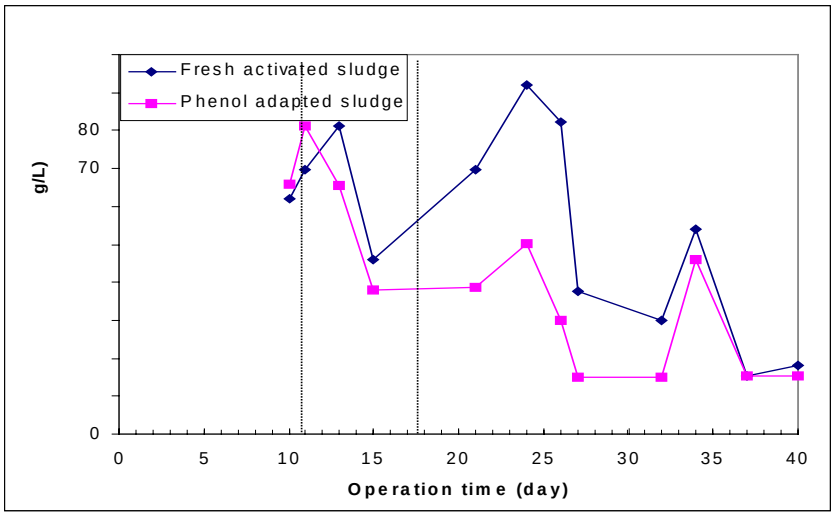


Figure 4.3 COD variation in the effluent

The reason for this would be that the microorganisms present in the fresh activated sludge were not resistant to toxic compound, so that they required a longer period for adaptation, whereas phenol adapted sludge was not only resistant but also adapted to degradation of phenol, thus giving better removal efficiency in degradation of chlorinated compound. Meanwhile, the COD removal efficiency of fresh activated sludge reached the same as phenol-adapted sludge after one month of operation. COD removal in fresh activated sludge was 85%- 97%. The capacity of COD removal of phenol-adapted sludge was from 86.4% to 97%. It confirmed that the microorganism in the phenol-adapted sludge were more resistant to PCP toxicity. PCP of the effluent from both reactors was found to be lower than 0.1 mg/L.

pH in both reactors dropped down after every cycle. pH inside the reactors was adjusted by NaOH so that it did not decrease below 6. Chloride in the effluent was higher than the influent (refer to appendix B-1) and pH drop were noted. These two factors are indicator of PCP degradation.

While adding PCP, microorganism concentration decreased from around 7000mg/L to 6000 mg/L MLSS in the fresh activated sludge, and later gradually increased and got the stability of each PCP concentration. After 40 days operation, the performance of two kinds of sludge was not different. Both of sludge types were mixed together and used as biomass source in MBR.

Even though PCP with 10 mg/L was showed to significantly inhibit the growth of PCP degrading microorganism (Watanabe, 1977 cited in Moos et al., 1983), acclimatization process has been done relatively easy in one month and a half following the critical procedure of starting acclimatization at low concentration and then increasing the concentration gradually to the required input level.

4.2 Biosorption study

Sorption of lipophilic compounds like pentachlorophenol may be an important removal process in bioreactors. Study done by Jacobsen et al. (1991) showed that sorption of PCP may be accounted for up to 50% removal of influent load at low SRT (<3days) in activated sludge. No biodegradation took place at the low SRT, on the other hand at SRT higher than 14 days, the sorption accounted for less than 10% removal of PCP. Another result of Melcer and Berford (1988, cited in Jacobsen et al. 1996) confirmed the high distribution of sorption PCP on the overall percentage removal of 30% to 5 days of SRT (no biodegradation)

4.2.1 Biosorption equilibrium

The experiment was done with PCP concentrations to obtain biosorption equilibrium. Three sets of experiments were conducted before standardizing the optimum time. The data for the three sets of experiments are presented in Appendix A-2. The biomass and PCP concentration used in the first experiment was 5,200 mgVSS/L and 10 mg/L, respectively. Figure 4.3 gives the PCP concentration and biosorption equilibrium with time for the first experiment.

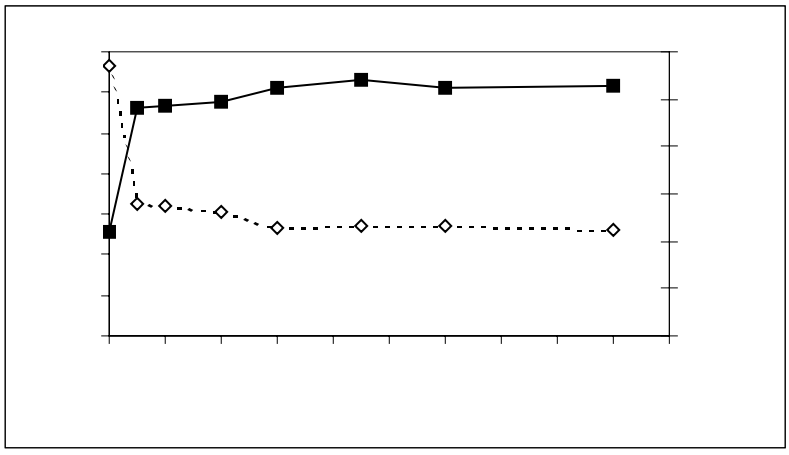


Figure 4.3 Biosorption Equilibrium at PCP 10 mg/L and MLVSS 5,200 mg/L

The second experiment was conducted with a higher biomass (10,300 mgVSS/L) and with a PCP concentration of 7.8 mg/L. It was found that the PCP concentration decreased up to 60 min and was stable thereafter. Third experiment was done in order to confirm the equilibrium time. Figure 4.4 presents the trend of second experiment.

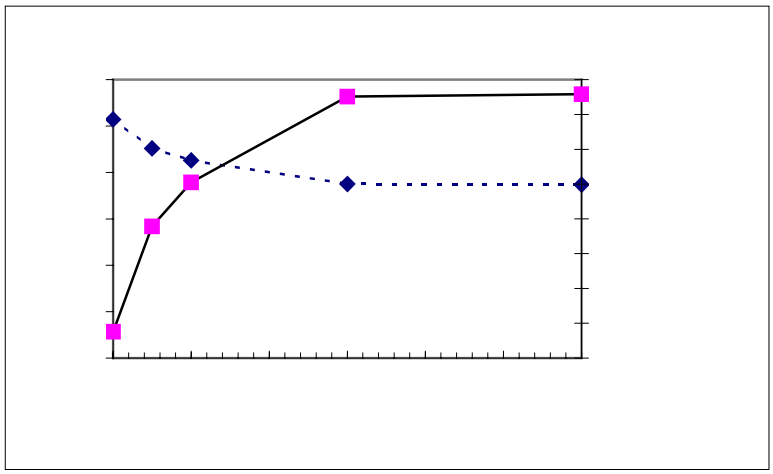


Figure 4.4 Biosorption Equilibrium at PCP 7.8 mg/L and MLVSS 10,300 mg/L

The third experiment was conducted with PCP 14.8mg/L and MLVSS is 10,300 mg/L. Figure 4.5 represents the results of the experiment.

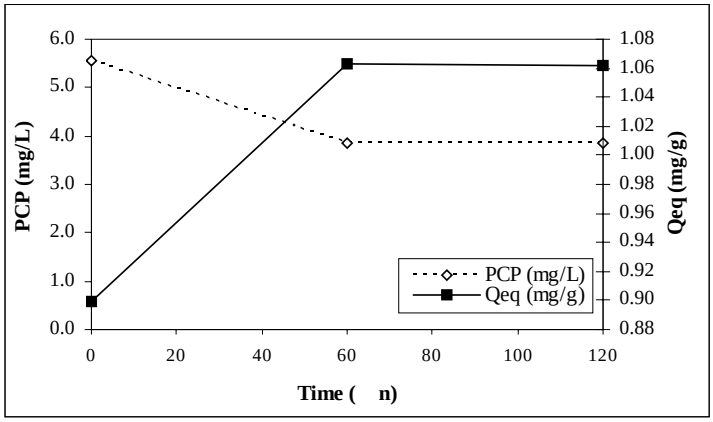


Figure 4.5 Biosorption Equilibrium at PCP 14.8 mg/L and MLVSS 10,300 mg/L

The result showed that the biosorption capacity of the sludge increases with contacting time. The PCP concentration decreased rapidly in the first 10 min and then gradually decreased up to 60 min, after which biosorption stabilized. Thus, it could be said that sorption equilibrium could be achieved at 60 min. The equilibrium time of 60 min was used for isotherm studies. The results were in accordance with other researchers (cited on Antiza-Ladislao and Galil, 2004). It was reported that PCP stabilized before 2h while using the activated sludge and at 2h for the anaerobic granular sludge.

4.2.2 Biosorption isotherm

Biosorption isotherm experiments were carried out at PCP concentration of 14.8 mg/L with varying biomass concentrations from 4750 to 10,300 mg/L with equilibrium time of 60 min. The data for the experiment is presented in Appendix A-3. The amount of PCP absorbed by the biomass was determined as the difference between the initial and final concentration. The specific capacity at varied biomass concentrations was calculated by dividing the loss of PCP by the biomass concentration.

Figure 4.6 depicts the specific removal rate with varying equilibrium PCP concentration. The figure shows that the percentage removal of PCP increased with increasing in biomass concentration, which is due to a better contact between the pollutant and the biomass. The sorption capacity was found to be 0.6289 mgPCP/g biomass and occurred rapidly within 60 minutes. As PCP is a hydrophobic organic compound, its sorption into the sludge was found to be high; moreover its solubility also depends on pH. Looking into MBR system, it could be confirmed that with high concentration of biomass, biosorption will play an important role besides the biodegradation process. As the SRT in MBR was high, more than 40 days, sorption capacity also enhanced the degradation process, as the biomass would be in contact with the degrading compound. In the MBR treatment process, the PCP would reach the equilibrium, as the HRT (12-24h) is high. The sorption phenomena and degradation occurred continuing in the sludge particles. As the SRT in MBR was high, more than 40 days, PCP biosorption also to great extent is dependent on COD and PCP of the influent. Higher organic carbon source would lead to higher biomass, which would indeed increase the sorption capacity.

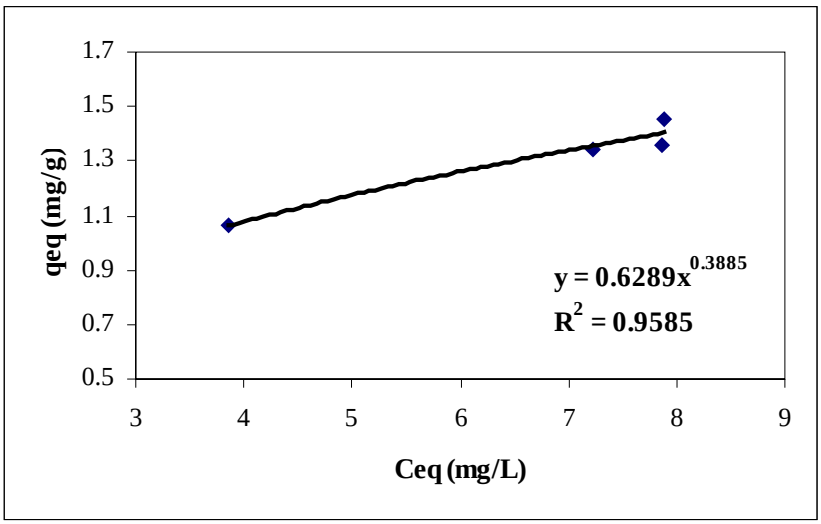


Figure 4.6 Biosorption Isotherm of PCP

As each study has varying conditions such as biomass viability, biomass cell wall structure, lipid composition and content, etc (Antizar-Ladislao and Galil, 2004); it is difficult to generalize the sorption capacity of the biomass with regard to the toxic organic chemical compound.

In the present study, Freundlich Model was used for sorption study. The equation describing the model is as follows:

$$q_{ep} = K_F C_{eq}^n \quad \textbf{Eq. 4.1}$$

$$\ln(q_{ep}) = \ln(K_F) + n\ln C_{eq} \quad \textbf{Eq. 4.2}$$

Figure 4.7 presents the trend of PCP sorption, when Freundlich Model was applied. The R^2 obtained was 0.959. Thus, it could be mentioned that the PCP sorption into the activated sludge follows Freundlich Model. In addition, the Freundlich isotherm constants, namely- the sorption capacity and sorption intensity were measured. 'K_F', which indicates the sorption capacity of the biomass, was found to be 0.63 and 'n', which is an indicator of sorption intensity was equal to 0.3881. Table 4.1 gives the comparison K_F and n values obtained from other research studies with that of the present study.

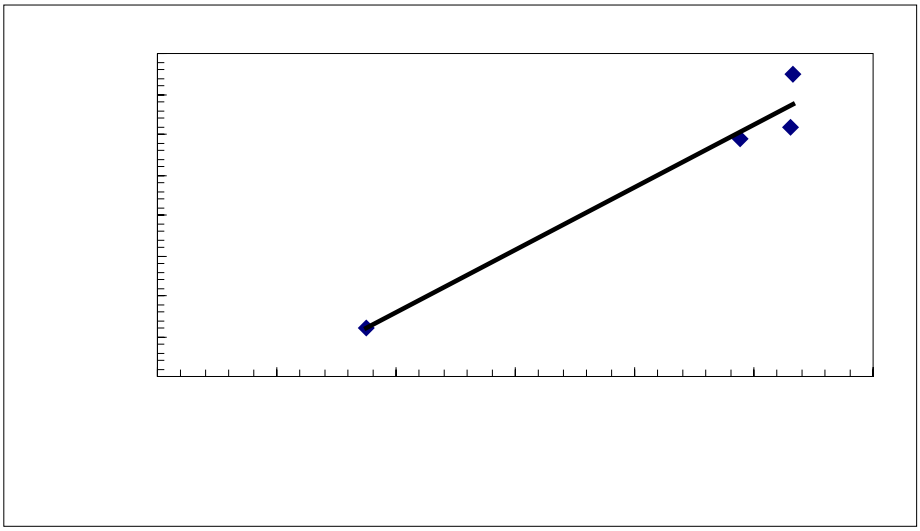


Figure 4.7 Freundlich model for biosorption of PCP

Table 4.1 Comparison of Freundlich isotherm constants for PCP sorption
(Antizar-Ladisdao and Galil, 2004)

K_F	n	R^2	Comments
32.1	0.56		Rhizopus arrhizus
85.1	0.60		Activated sludge
0.76	1.00		Microbial biomass, pH=6.99-7.02
34.1	0.74	0.985	Activated sludge, pH=7.0; 0.5g/L biomass
26.6	0.80	0.968	Activated sludge, pH=7.0; 1 g/L biomass
0.14	0.42	0.977	Acclimatized sludge, pH = 7, 0.9g/L biomass
0.63	***0.388***	***0.959***	***Present Study***

The report also suggested that the pH of the working solution to the great extent affected sorption capacity than the sorption intensity. The results are presented in Appendix A-3.

As the sludge source, degree of acclimatization, pH etc would play an important role in determining the sorption capacity; each case study would be situation specific.

4.3 Activation of Mycobacterium chlorophenolicum

The bacteria were obtained from Microbial Genomics and Bioprocessing, National Center for Agricultural Utilization Research, Agricultural Research Service, USDA, Peoria, Illinois. The bacterium has been preserved in a dormant state by drying a heavy suspension of cells in sterile bovine serum. The cells can be brought to an active state of growth by transfer to a suitable liquid medium.

The usual procedure follows: Make a file scratch in the centre of the tube and wipe the tube with cotton moistened with alcohol. Break the tube and lightly flame open end and transfer the pellet into the broth. The resulting culture may be used immediately as a source of inoculum, but usually it is preferable first to incubate until growth occurs (Microbial Genomics and Bioprocessing, National Center for Agricultural Utilization Research). This mentioned procedure above was followed to activate the microorganism *Mycobacterium chlorophenolicum.* The pellet was initially transferred into sterilized nutrient broth (A). In addition, some amount of nutrient broth was added to the tube containing lyophilized bacteria and transferred back into the media (B). The media was incubated at 37 ºC.

The growth did not occur in the media A immediately, while it occurred in the media B. It was allowed further to be activated in the incubator for around two weeks. The growth occurred after a period of two weeks. There could be two possible reasons for this. One could be absence of specific conditions required by the microorganisms and the other probable reason could be as the microorganisms were in inactive form for a long period of time, it would require a longer time period for resuming its activity. Wilderer *et al*, 1991 suggested that selective advantages must be provided in order to favor the survival and growth of any specialist. The cultures were later confirmed for purity by streaking them in the agar plate and checking for single form of colony formation. The cultures were pure culture.

After activation of microorganism in the nutrient media, the resistance of the culture to the PCP was tested. The culture was transferred into nutrient broth with 1.25 mg/L PCP and nutrient broth without PCP. The growth was similar in both the broth with and without PCP, with around 2×10^7 CFU/mL. As the microorganism could grow in 1.25 mg/L PCP, this culture was transferred into 5 and 10 mg/L to verify the growth as well as degradation.

The wastewater composition to be used as a feed in the membrane bioreactor was also used for experiments and inoculated with culture B. Four flasks with the following media was used for the experiments: Nutrient Broth with 5 mg/L PCP (Culture B), Nutrient Broth with 10 mg/L PCP (Culture B), Feed wastewater with 5 mg/L PCP (Culture B) and Feed wastewater with 5 mg/L PCP (Activated sludge).

After 24h, it could be observed through turbidity that the growth in 10 mg/L was much lower than that in 5 mg/L, which was resumed after another 24 h. A similar observation could be seen while acclimatizing the activated sludge with PCP concentration.

4.4 Microbial studies

4.4.1 Degradation of PCP with *Mycobacterium chlorophenolicum*

The experiment was conducted to test the efficiency of the microorganism to degrade PCP with varied concentrations. Two media were used for the experiment: Synthetic wastewater used in the MBR and Nutrient Broth (common media for bacterial growth). The experiments were conducted in the 500-mL shake flask. The volume of the media taken in the flask was 200 mL. The conditions used in the experiment are given in Table 4.2. In the experiment, the pH, microbial population with plate count method and PCP were monitored.

Table 4.2 Conditions used in the experiment to verify activity

Conical Flask	Media	PCP (mg/L)	Microbial Characteristics	
			Culture	Count (CFU/ mL)
A	Synthetic wastewater (with Glucose)	2.0	Specific Organism	1.33E+04
B	Synthetic wastewater (with Glucose)	8.2	Specific Organism	1.33E+04
C	Synthetic wastewater (with Glucose)	12.6	Specific Organism	1.33E+04
D	Synthetic wastewater (with Glucose)	16.9	Specific Organism	1.33E+04
E	Nutrient Broth	2.0	Specific Organism	1.33E+04
F	Nutrient Broth	8.2	Specific Organism	1.33E+04
G	Nutrient Broth	16.9	Specific Organism	1.33E+04
H	Nutrient Broth	16.9	Activated Sludge	9.30E+03

The PCP was added to the media from a stock solution of 200 mg/L prepared in NaOH solution. The experimental condition for experiment conducted on the degradation of PCP with *M. chlorophenolicum* is presented in Table 4.3.

Table 4.3 Degradation of PCP at different concentration

Conical Flask	Initial		After 24h			After 48h		
	PCP (mg/L)	Microbial Count (CFU/mL)	PCP (mg/L)	PCP Removal (%)	Microbial Count (CFU/mL)	PCP (mg/L)	PCP Removal (%)	pH
A	2.0	1.33E+04	1.8	9	2.00E+05	1.4	29	6.8
B	8.2	1.33E+04	8.5	-	4.00E+03	8.0	-	6.8
C	12.6	1.33E+04	11.3	10	1.00E+03	9.5	24	6.8
D	16.9	1.33E+04	14.3	15	3.30E+04	13.6	20	6.8
E	2.0	1.33E+04	1.1	43	2.00E+03	1.1	43	8.6
F	8.2	1.33E+04	6.6	20	2.00E+03	5.9	29	8.5
G	16.9	1.33E+04	14.8	12	NA	13.1	23	8.5
H	16.9	9.30E+03	13.1	23	NA	9.6	43	8.8

*NA- Not Available

The results from the experiment indicated that the initial microbial concentration in all the flasks was almost the same. After 24h, when the microbial concentration was observed, it

was found that the bacterial concentration decreased in all the reactors. The toxicity of PCP and its intermediate products could pose toxicity to the microorganisms. Comparing flasks A, B, C and D, it was found that as the PCP concentration increased there was a decrease in biomass in the synthetic wastewater though at 16mg/L, the microbial concentration was relatively stable. Looking into the PCP degradability, the overall reduction in PCP was found to be very low. In synthetic wastewater and nutrient broth, the PCP degradability decreased with increasing concentration. Figure 4.8 shows the PCP degradation after 48h in synthetic wastewater and nutrient broth with increasing initial PCP concentration. Though, the PCP degradation increased from 24 to 48h, the reduction in effluent PCP was not significant. Figure 4.9 shows the increase in PCP removal from 24h to 48h. Nutrient Broth with 1 mg/L PCP and specific organism culture and Nutrient broth with 10mg/L PCP and activated sludge showed better degradability compared to other samples.

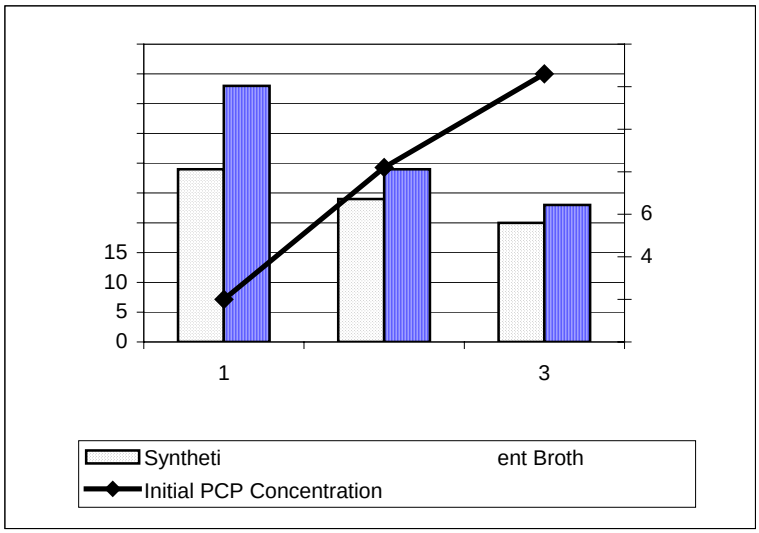


Figure 4.8 PCP degradation at 48h in synthetic wastewater and nutrient broth

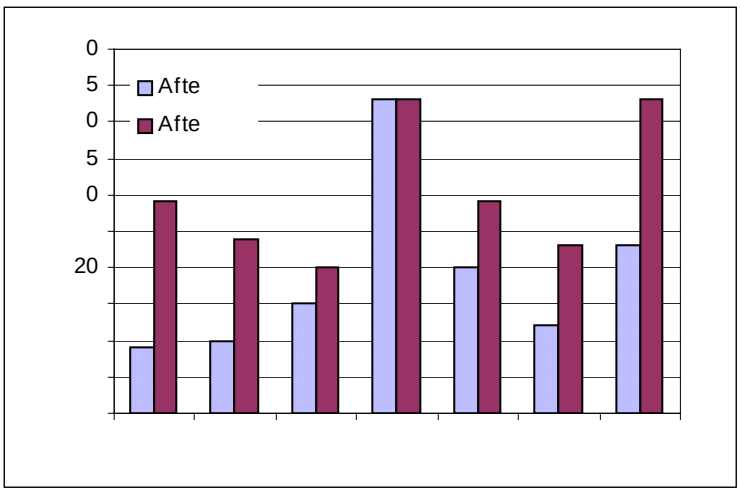


Figure 4.9 PCP Degradation after 24 and 48h

In the experiment, it is interesting to note that the pH in the synthetic wastewater decreases with time whereas the pH of the nutrient broth increases with time. The probable reason for this observation could be the different degradation pathways taking place in the different media.

However, the degradability of PCP was found to be much lower than expected. The probable reason could be external carbon source other than PCP in all the working solutions. The microorganisms would prefer to degrade easily degradable compound compared to that of PCP. For this reason, the next set of experiments was conducted with PCP as the sole carbon source. Moreover as nutrient broth could not be practically used, the study was done with different synthetic wastewater composition to arrive at a suitable media for the growth of the microorganism.

4.4.2 Selection of Media and comparative study with activated sludge

The experiment was conducted with three sets of media: Media I (Kao *et al*, 2004), Media II (Kao *et al*, 2004) and Synthetic wastewater (Stanlake and Finn, 1982). Different media was tested in order to obtain the most suitable media for bacterial growth. Instead of ammonium nitrate, sodium nitrate was used as the nitrate source. Calcium chloride was added separately after sterilization to avoid precipitation. The difference in the composition of the media is given in Table 4.4.

Table 4.4 Compositions of different media

Media I (mg/L)		Media II (mg/L)		Synthetic Wastewater (mg/L)	
Na_2HPO_4	2400	Na_2HPO_4	800	K_2HPO_4	1700
KH_2PO_4	2000	KH_2PO_4	800	KH_2PO_4	680
$NaNO_3$	100	$NaNO_3$	500	$(NH_4)_2SO_4$	1000
$MgSO_4.7H_2O$	10	$MgSO_4.7H_2O$	200	$MgSO_4.7H_2O$	100
$CaCl_2$	10	$CaCl_2$	10	$CaCl_2.2H2O$	30
1 mL of Micronutrient		1 mL of Micronutrient		$FeSO_4.7H_2O$	
$FeSO_4.7H_2O$	5000	$FeSO_4.7H_2O$	5000	$MnSO_4.H_2O$	30
$ZnSO_4.7H_2O$	4000	$ZnSO_4.7H_2O$	4000		
$MnSO_{4.4}H_2O$	200	$MnSO_{4.4}H_2O$	200		
$NiCl.6H_2O$	100	$NiCl.6H_2O$	100		
H_3BO_3	150	H_3BO_3	150		
$CoCl_2.6H_2O$	500	$CoCl_2.6H_2O$	500		
$ZnCl_2$	250	$ZnCl_2$	250		
EDTA	2500	EDTA	2500		

Six working solutions were taken for the experiment. Two sets of all the three media were used. One set was inoculated with specific microorganism, while the other set was inoculated with mixed activated sludge taken from the two reactors. The initial PCP concentration and the microbial count are given in the following Table 4.5.

Table 4.5 Conditions for the experiment with different media

Flask ID	Media	Culture	Initial PCP Conc. (mg/L)	Initial Microbial Concentration (CFU/mL)
A	Media I	Mixed Activated Sludge	0.88	1.20E+05
B	Media I	Specific Organism	1.00	3.80E+03
C	Media II	Mixed Activated Sludge	0.83	1.20E+05
D	Media II	Specific Organism	1.00	3.80E+03
E	Synthetic Wastewater	Mixed Activated Sludge	0.61	1.20E+05
F	Synthetic Wastewater	Specific Organism	1.00	3.80E+03

The biomass growth and the PCP were measured after 24h, 48h and 96h. The Table 4.6 indicates the change in biomass and the PCP.

Table 4.6 Variation in biomass and PCP with time

Conical Flask ID	Initial PCP Conc. (mg/L)	After 24h			After 48h			After 96h		
		PCP (mg/L)	Removal (%)	Microbial Conc. (CFU/mL)	PCP (mg/L)	Removal (%)	Microbial Conc. (CFU/mL)	PCP (mg/L)	Removal (%)	Microbial Conc. (CFU/mL)
A	0.88	0.31	65	6.30E+04	0.04	96	3.60E+06	0.05	94	1.00E+05
B	1.00	0.86	14	1.14E+06	0.80	20	1.50E+07	0.72	28	1.00E+07
C	0.83	0.19	77	7.20E+04	0.11	87	4.00E+05	0.05	94	1.00E+06
D	1.00	0.82	NA	2.38E+06	0.89*	NA	1.40E+08	0.81	NA	3.00E+08
E	0.61	0.04	93	1.10E+04	0.11	82	4.00E+05	0.05	92	5.00E+06
F	1.00	0.53	47	1.64E+06	1.42	NA	1.00E+08	1.06	NA	3.00E+06

* Increase in PCP concentration was noted; which could be linked to analytical measurement error in GC

The number of microorganism in the media inoculated with activated sludge decreased after 24h, whereas it increased after 24h in the media having specific microorganism. The reason for this would be that the activated sludge microorganisms might have contained consortia of microorganism degrading both PCP and glucose as the activated sludge was fed with glucose as additional source. When PCP was used as the sole carbon source, this would have removed the microorganisms surviving on the glucose as carbon source and the organism degrading the PCP would have survived. As the specific organism used in the study is specialized for PCP degradation, they were able to survive well and grow rapidly compared to that of the activated sludge. Though the activated sludge organisms decreased after 24 h, the growth increased after that time period. The growths in the working solutions are presented in Figure 4.10.

The difference in the number of organisms with different media was not different. Media-I did not have any precipitation. While comparing the three media in terms of PCP degradation, synthetic wastewater used in the membrane bioreactor was found to be best suitable for PCP degradation, as it would provide the similar condition as that of the membrane bioreactor.

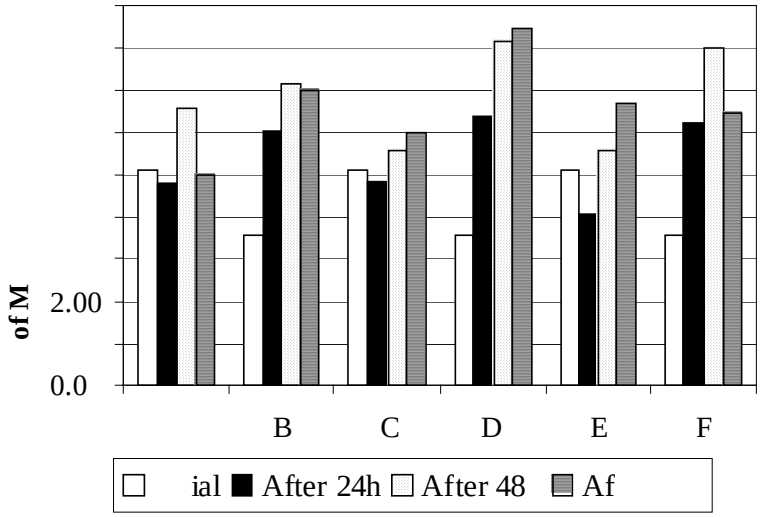


Figure 4.10 Microbial growth with time

While comparing the degradation in the different media and cultures, the PCP degradation was found to be much better with activated sludge than the specific microorganism as shown in Table 4.6. The probable reason for this could be the activated sludge taken from the membrane bioreactor was well adapted and acclimatized to degrade a PCP concentration of 10 mg/L. Moreover, the activated sludge would contain consortium of microorganisms capable of degrading PCP, thus giving better removal efficiency. Figure 4.12 represents the degradation after 24h.

The results suggest that isolation of microorganism from the activated sludge is a better option than using specific organism. Further experiments were done to further confirm this. Another reason for this could be that the activated sludge has 4-5 specific organisms for degrading PCP (Wilderer, *et al,* 1990), and additional organism could increase the activity of PCP further.

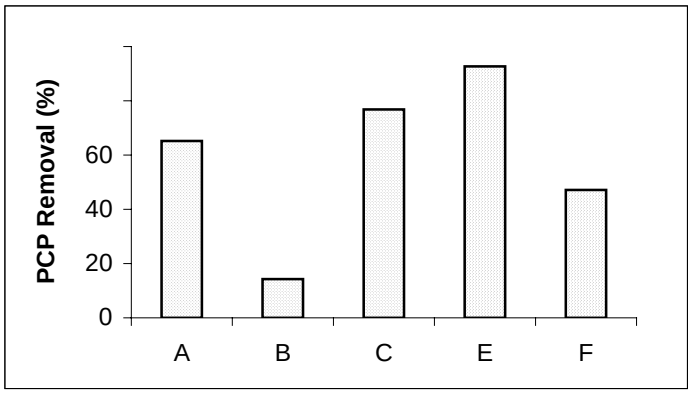


Figure 4.11 PCP degradation after 24h

4.4.3 Cultivation of Microorganisms

Cultivation of the microorganisms was as follows. A similar procedure was also followed for specific microorganism to acclimatize it. The activated sludge from the MBR (treating 10 mg/L PCP) was be taken and added (1ml) to the defined medium as described in Kao *et al*, (2004) having a PCP concentration of 1 mg/L as the sole carbon source. Prior to addition, the activated sludge was centrifuged to separate the sludge from the working solution. After few days of cultivation of the microorganisms, the microbial count was done. Once it exceeds 10^5 CFU/mL, the culture was shifted to defined media containing 5mg/L PCP. The procedure was continued till the PCP concentration of 15 mg/L is achieved.

After cultivating, the microorganisms at a PCP concentration of 15 mg/L were developed. The microbial culture needs to be cultured in an agar plate at different dilutions in buffer solutions. The colonies appearing in the agar plate was to be isolated and cultivated in the fresh defined media. Among the culture appearing in the plate, the 5-6 commonly occurring cultures was isolated and cultivated. A comparison of their efficiency in degrading PCP was tested by measuring their removal efficiency. The cultures with very high removal efficiency were the microorganism to be cultivated in order to be augmented into the MBR reactor.

The data of the experiment is presented in Table 4.7. Though the above mentioned procedure was followed, the results obtained did not signify PCP degradation and biomass growth. The PCP degradation was slow as well as the biomass did not significantly increase. After certain period, the complete destruction of the biomass occurred. The probable reason for this could be the by-products formed by the degradation of PCP or the change in pH, which could have lead to toxicity of the media to the microorganisms. A similar trend was obtained in both the activated sludge and specific microorganism media.

Table 4.7 Results obtained while cultivating the specific microorganism and the activated sludge

Activated Sludge				Specific Microorganism			
Days	**PCP**	**Microbial Count (CFU/mL)**	**Removal**	**Days**	**PCP**	**Microbial Count (CFU/mL)**	**Removal**
1	0.86	2.16E+05		1	0.86	6.00E+05	
3	0.37	1.00E+07	57.36	3	1.06	4.00E+05	Error
5	0.06	3.00E+09	93.02	5	0.88	5.00E+03	Error
7	0.06	1.00E+08	93.02	7	0.95	2.80E+04	Error
10	<0.005	1.00E+07	Greater than 99.4%	10	1.20	1.00E+03	Error
16	<0.005	NA	Greater than 99.4%	16	1.04	NA	Error
10	3.50	1.00E+07		10	3.94	1.00E+03	
16	3.46		1.20	16	3.47		11.97

4.4.4 Isolation of Microorganisms

The microorganisms present in the activated sludge from the Reactor 1 and 2 were isolated by both spread plate and streak plate method. The streak plate results are shown in Figure 4.12.

The spread plate was done at different dilutions in order to find out the predominant microorganisms present in the activated sludge. It was found that only few type of microorganisms persisted in the activated sludge. The microbial population of Reactor 2 was found to be higher than the Reactor 1.

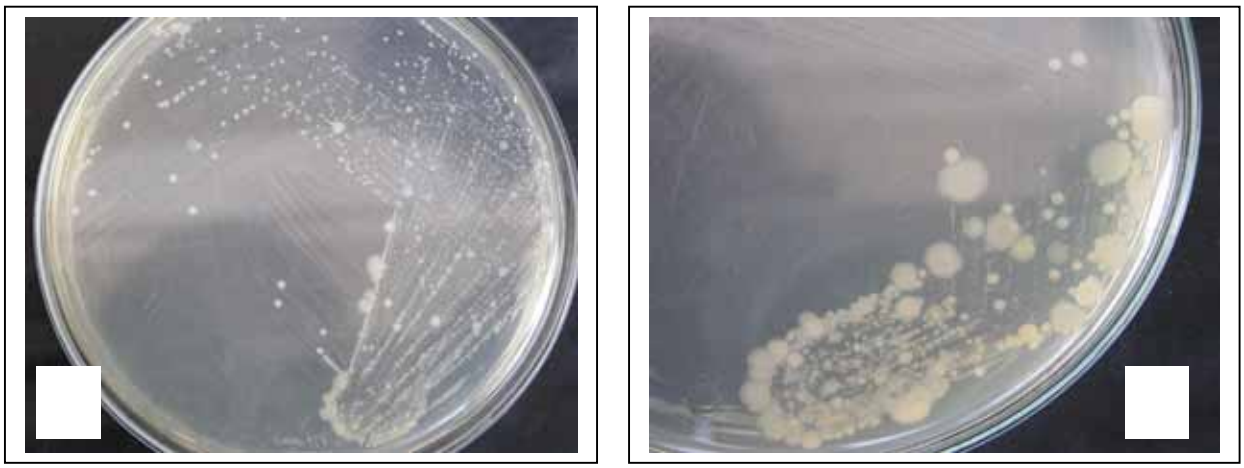


Figure 4.12 Isolation of bacteria by streak plate method (A- Reactor 1, B-Reactor 2)

Once the microorganisms were isolated by the streak plate method, the organisms were separated based on the colony morphology. Each type of colony was assumed to be a single type of the organism as each bacterium has a characteristic colony formation. In the Reactor 1, three different types of microorganisms were isolated. The cultures were labeled as R_1-C_1, R_1-C_2 and R_1-C_3.

When tested with PCP, only the first two cultures (R_1-C_1 and R_1-C_2) could grow in PCP containing nutrient broth media. Four cultures were obtained in Reactor 2 labeled as R_2-C_1, R_2-C_2, R_2-C_3 and R_2-C_4. All the four cultures could growth in a nutrient broth with PCP. The cultures in Reactor 1 and 2 are shown in Figure 4.13.

4.4.5 Degradation of PCP by Isolated culture

The degradation of PCP with isolated cultures was tested in a flask experiment. The initial PCP concentration used in the experiment was around 2mg/L. The PCP was added to the synthetic wastewater used in the membrane bioreactor with peptone and yeast extract as a supplementary nutrient source. Peptone and yeast extract were used in order to cultivate sufficient amount of microorganisms.

The degradability of PCP by the isolated microorganisms was low. When compared to the specific microorganism, it was found that at the end of 96h, the special microorganism performed better than each isolated species. As mentioned earlier, the better performance of the activated sludge from the reactor could be due to the cumulative effect of the various PCP degrading microorganisms. The results obtained are expressed in Table 4.8. Though the degradability was not high, this experiment confirmed the ability of the

microorganisms to degrade PCP. The difference in the performance of the different organism could be not only their difference in capacity to degrade PCP, but also difference in the bacterial number.

M.Chlorophenolicum showed a higher degradation than that of the previous experiments as additional carbon source other than PCP was used and moreover, the initial microbial culture inoculated could be higher than the microbial count inoculated earlier.

Table 4.8 PCP degradability of the Isolated Species

Culture	Initial PCP (mg/L)	After 24h		After 96h	
		PCP (mg/L)	Removal (%)	PCP (mg/L)	Removal (%)
Specific Organism	2.5	2.3	8	1.0	61
R_1-C_1	2.5	2.2	12	1.9	24
R_1-C_2	2.6	2.1	18	1.5	42
R_2-C_1	2.5	1.9	24	1.7	30
R_2-C_2	2.3	2.2	5	2.0	13
R_2-C_3	2.3	2.1	6	2.3	Error
R_2-C_4	2.0	2.1	Error	1.9	4

4.4.6 Growth Pattern of the isolated species

The growth of the isolated species was monitored with time in order to find out the logarithmic phase of the individual organism and culture them into sufficient amount to enhance PCP degradation. As the feed solution was changed from PCP to Na-PCP, the organisms were first cultivated in Na-PCP solution.

The special organism did not growth with NaPCP. R_2-C_3 showed no growth, while the organisms R_1-C_2 and R_2-C_1 showed slow growth. These two cultures took a period of 48h before colonies started developing on the agar plate. Figure 4.12 gives the growth pattern of the isolated microorganisms.

The growth pattern was determined in synthetic wastewater with glucose concentration similar to that of the MBR system so that the organism got adapted to similar conditions. The Na-PCP concentration was around 10-12 mg/L. The results of the experiment are presented in Table 4.9. The logarithmic phase of the isolated microorganisms is presented in Table 4.10. Log phase could be defined as the time taken by the organism for rapid growth. Though the log phase may differ at different concentration, it could be said that it will not fluctuate to a significant level.

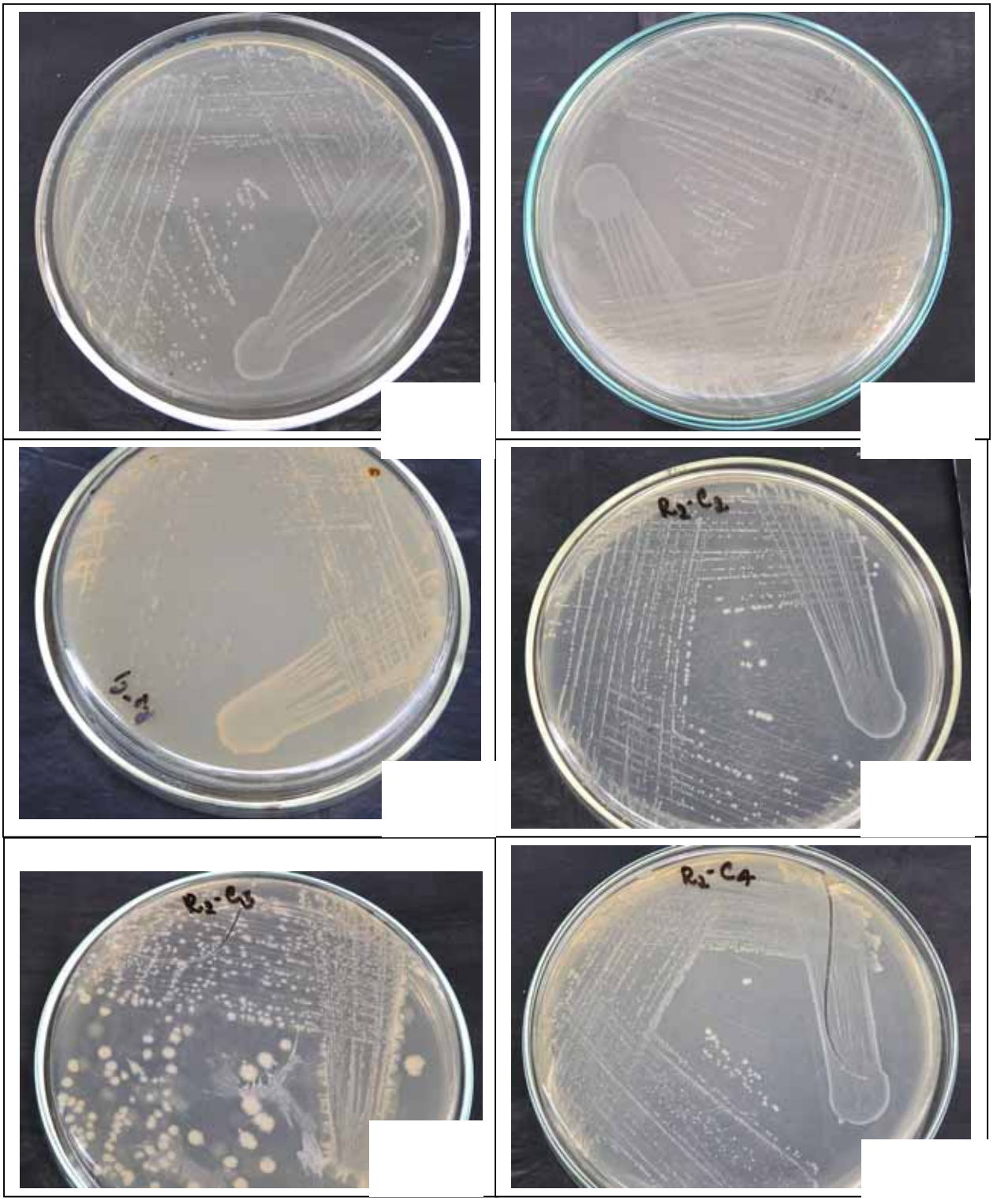


Figure 4.13 Isolated Microorganisms in Reactor 1 and 2

Table 4.9 Microbial Count with time of the Isolated Cultures

Time (h)	Log of Microbial Count (CFU/mL)				
	R_1-C_1	R_1-C_2	R_2-C_1	R_2-C_2	R_2-C_4
0	2.90	5.81	3.68	3.48	4.60
2	4.01	5.30	3.76	3.84	4.59
5	NA	5.56	NA	NA	NA
8	NA	NA	4.67	NA	NA
11	8.60	6.72	NA	8.36	6.28
18	9.09	6.85	NA	8.93	8.93
25	9.76	6.85	4.90	8.26	8.73
31	7.75	NA	5.20	7.58	NA
47	7.39	6.83	5.30	7.34	8.78
71	6.92	6.60	5.70	7.25	6.75
83	6.78	5.68	5.43	6.90	6.36
95	6.05	5.58	5.17	6.26	5.86

Table 4.10 Log phase of the Isolated Cultures

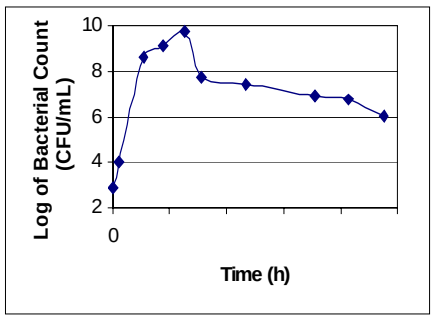
Cultures	Time (h)
R_1-C_1	25
R_1-C_2	25
R_2-C_1	71
R_2-C_2	18
R_2-C_4	18

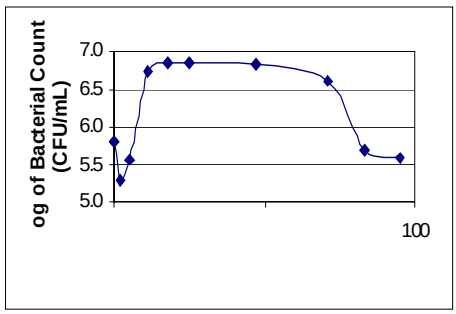
The pattern of growth of R_1-C_1, R_2-C_2 and R_2-C_4 were similar, which showed a gradual increase in the bacterial number after a short lag phase. In culture R_1-C_2, there was decrease in bacterial number, which could be due to loss of certain organisms prior to its acclimatization. Culture R_2-C_1 showed a sloe growth rate compared to that of the other cultures. From the results obtained, it could be said that in a long run, R_1-C_2 and R_2-C_1 could be ignored for bioaugmentation though initially they may be used.

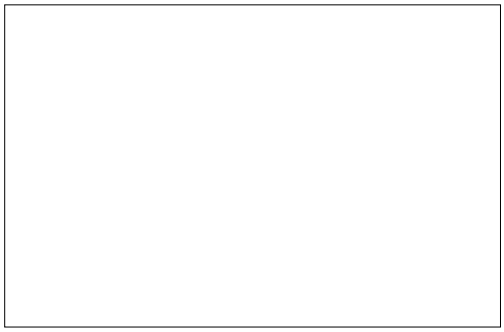
4.5 Membrane bioreactor operation

4.5.1 Optimization the operation condition

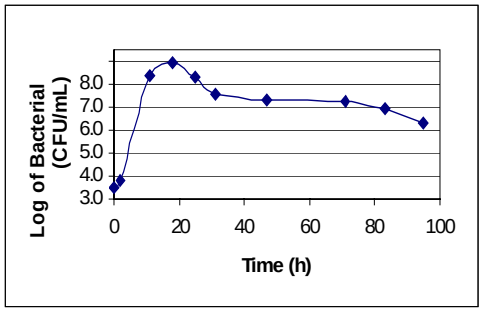
With the objective is standardizing the suitable condition of MBR for treating wastewater containing PCP as well as maintaining the stability of the system, different HRT of 24, 18, 12 hours were carried out. The COD of the influent was maintained at 600mg/L through out this run. The experiment data were presented in Appendix B.



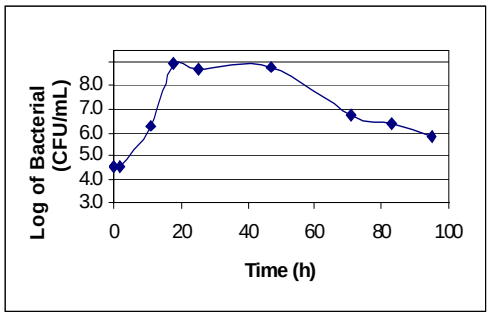




(c) R_2-C_1



(d) R_2-C_2



(e) R_2-C_4

Figure 4.14 Growth patterns of Isolated Species

The detecting limit of GC ECD was 0.005mg/L. From the result (figure 4.16) it could be seen that the percentage of PCP removal was very high, greater than 97%. PCP in the effluent was high during certain period and reached 0.45 mg/L because of fluctuating pH inside the reactors and instability condition of the system in the first time of setting. In general, the PCP concentration in the effluent was 0.045-0.3 mg/L. In certain period, the PCP was below detection limit. It is evident that microorganisms in both reactors were well adapted to PCP and able to degrade PCP completely. Even though the PCP in the effluent was still higher than the WHO standard (10μg/L), microorganism activities in MBR showed a very efficiency in removing high concentration of PCP to very low concentration. Though it is said that PCP of more than 10 mg/L showed substrate inhibition (Moos et al. 1983), this performance of MBR with 12 mg/L PCP was stable and interesting. Figure 4.19 shows the biomass concentration in MBR systems.

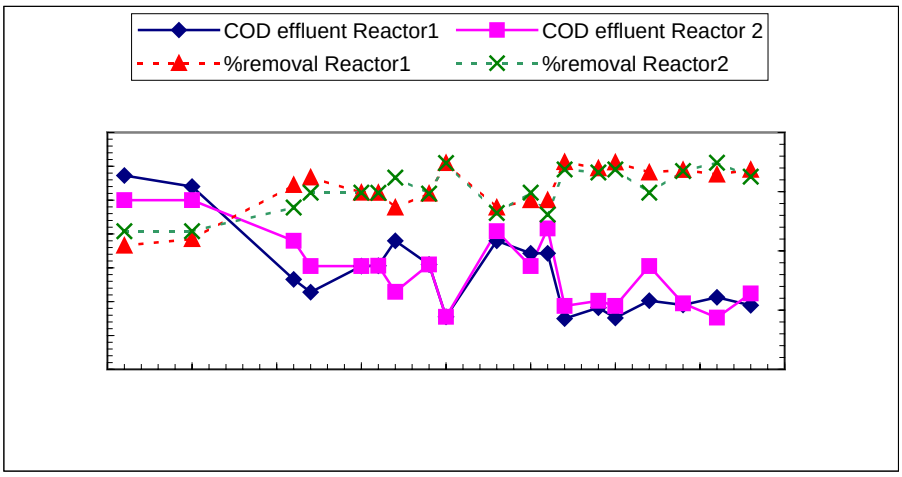


Figure 4.15 Performance of MBR in term of COD

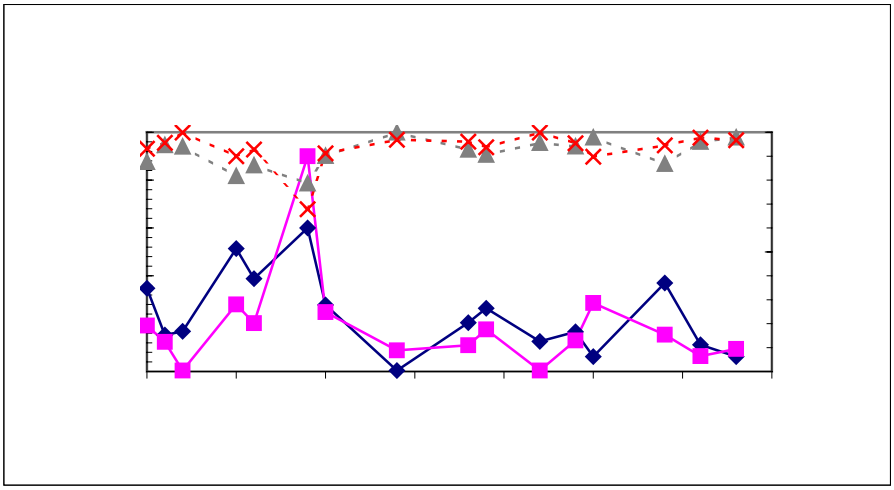


Figure 4.16 Performance of MBR in terms of PCP

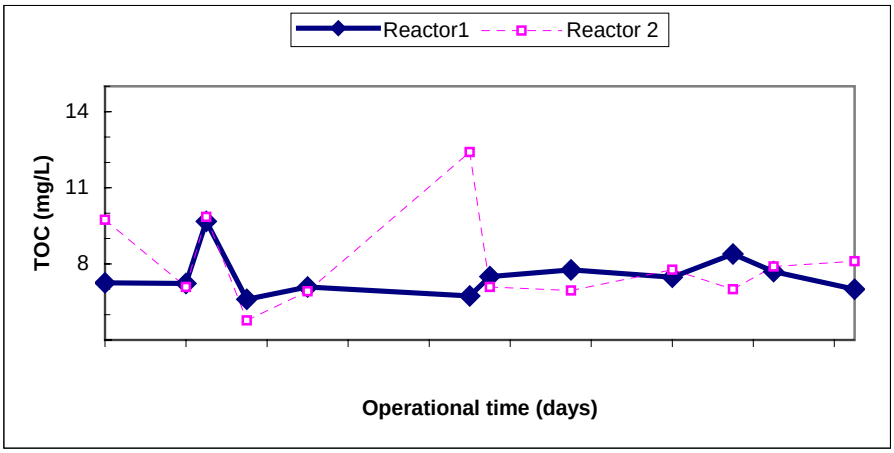


Figure 4.17 TOC in permeate

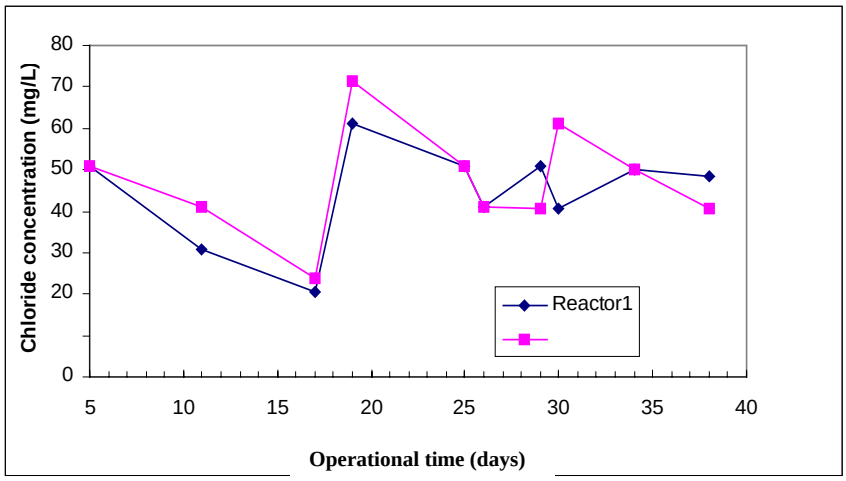


Figure 4.18 Chloride concentration in the effluent

There was a decrease in biomass concentration in both reactors after 10 days due to pH fluctuating within reactor and changing condition from the batch to continuous. At this stage, in order to verify the pH change, pH was monitored within reactors. The results confirmed that the degradation of PCP produced HCl, which over the buffer capacity of the influent, cause a pH drop down and continued decrease (Figure 4.20). So the adjustment of pH in range 7.5- 7 must be done to prevent the effect of pH on the activities of microorganisms. The decrease pH within the MBR was a symbol of PCP degradation and was observed by many studies. For example, in the experiment of Barbeau et al. 1997, after 30 days of treatment with the loading rate of 250mg/kg to 300mg/kg of soil, pH decreased from the initial value of 6.6 to 5.7. The pH decrease caused a decrease in biomass, lowering the performance of reactors. When pH was maintained about 7-7.5, biomass increased gradually.

At the end of this stage, biomass concentration was around 6000 mg/L and 5000 mg/L in reactor 1 and 2, respectively with MLSS/MLVSS ration in range 87% to 96%.

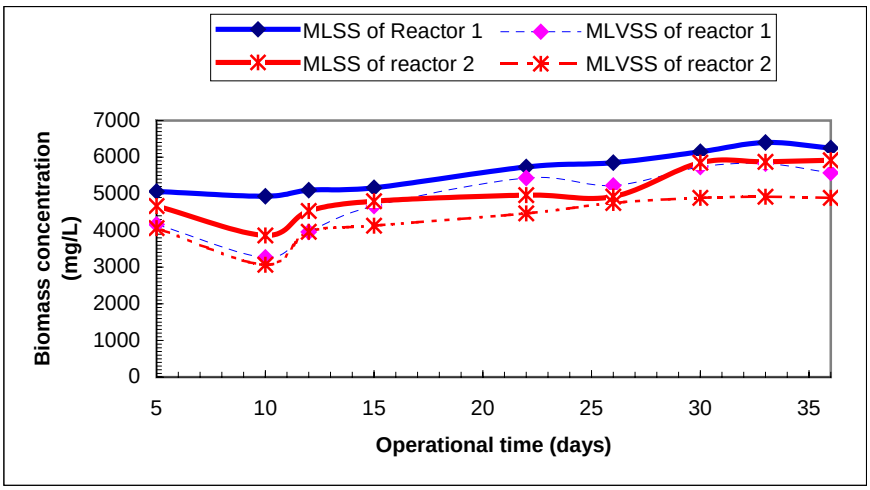


Figure 4.19 Biomass variation in MBR

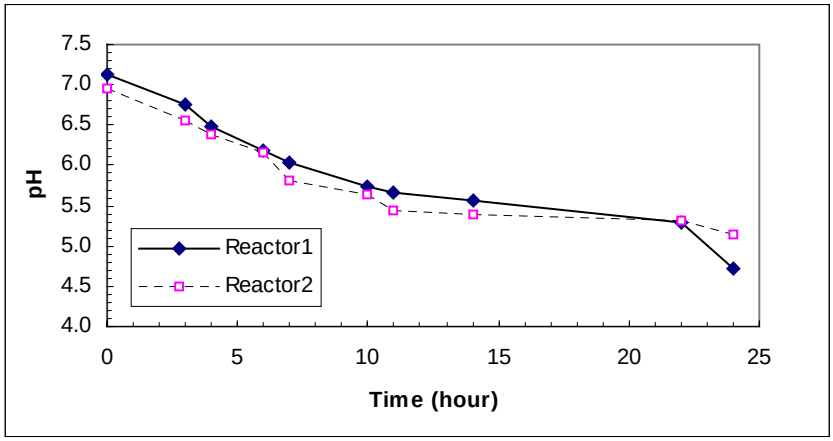


Figure 4.20 pH in mixed liquor with time

4.5.1.1 HRT 18hours

As the performance of the MBR was stabilized at 24hours, HRT was further reduced to 18 hours. At hydraulic retention time (HRT) of 18 hours with average flowrate 11.1 mL/min, PCP loading rate was 96mg/day. The performance of MBR is displayed in figure 4.21 and 4.22. Change in biomass is displayed in figures 4.23.

Biomass concentration increased in both reactors and was in between 8,000 to 10,000 mg/L with MLVSS/MLSS of 70-73%. The performance of MBR with HRT 18 hours was better than HRT 24 hours period in terms of PCP removal and COD removal even at higher loading rate. The reason for the trend must be the higher concentration of microorganism and the adaptation of the bacteria to PCP.

During the first day of HRT 18 hours operation, PCP concentration was high with 0.08mg/L in reactor 1 and 0.03 mg/L in reactor 2, because of increase in PCP loading rate from 72 mg/L to 96mg/L. From second day onwards, the PCP concentration in the effluent of both reactors was lower than detecting limit of GC-ECD (0.005 mg/L). Generally, the treatment efficiency of MBR for PCP was higher than 99.96% in this period. In terms of COD removal, the performance of MBR was also more stable than 24 h HRT with percentage removal of 93-97%. COD in the effluent was lower than 50 mg/L.

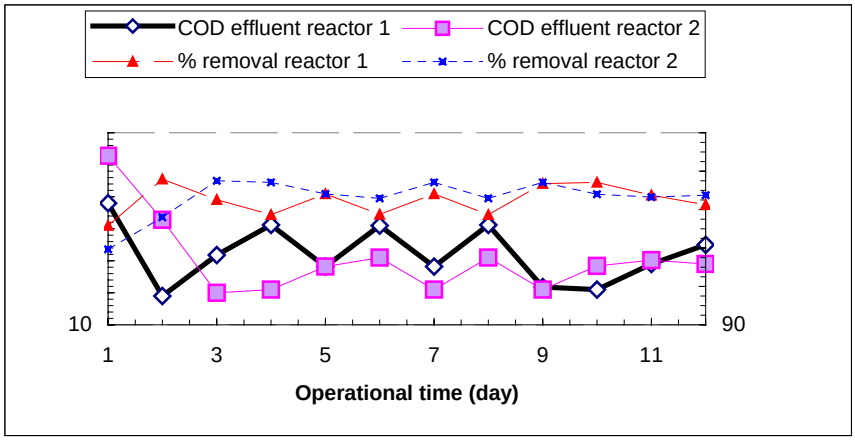


Figure 4.21 Performance of MBR in terms of COD removal

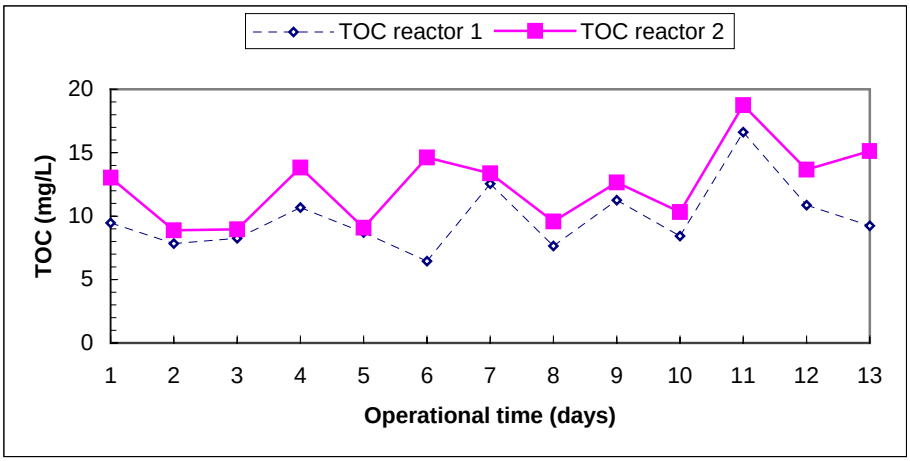


Figure 4.22 Performance of MBR in terms of TOC removal

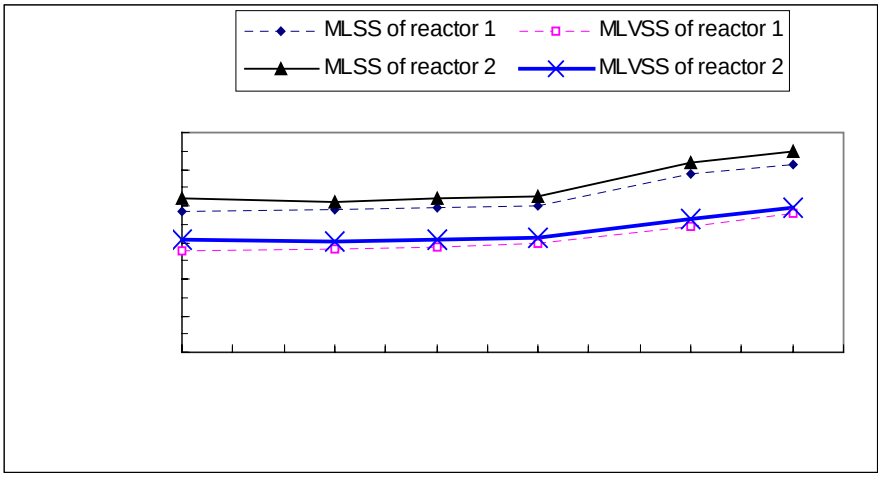


Figure 4.23 Biomass variations in MBRs with HRT 18 hours

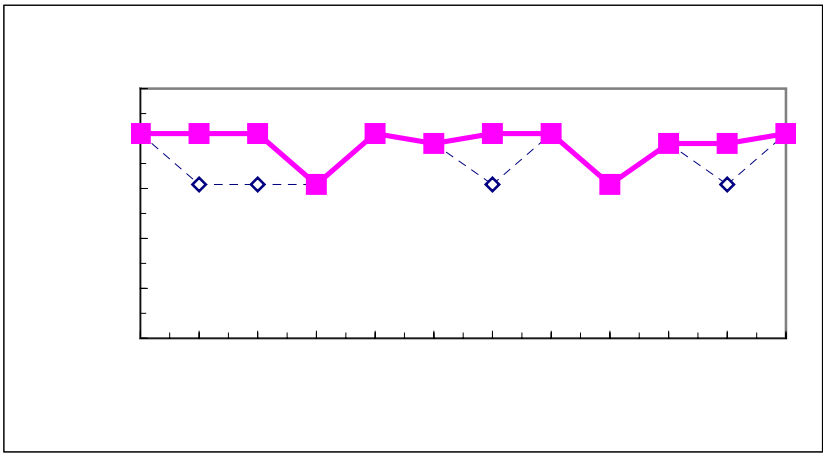


Figure 4.24 Chloride concentration in the effluent

4.5.1.2 HRT 12hours

Further to increase organic loading, HRT was reduced to 12hours and PCP loading of 144mg/day. With SRT of 25 days, MLSS in both reactors was maintained around 10,000mg/L. The performance of reactors is displayed in figure 4.25 and 4.26.

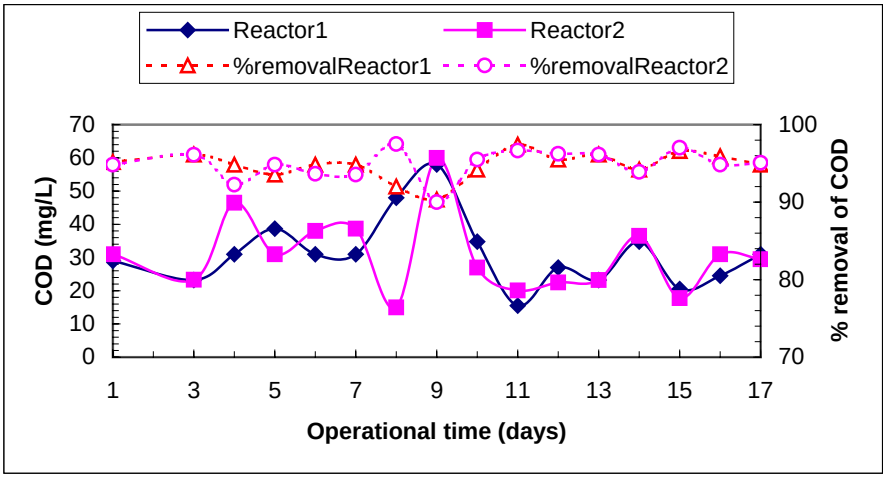
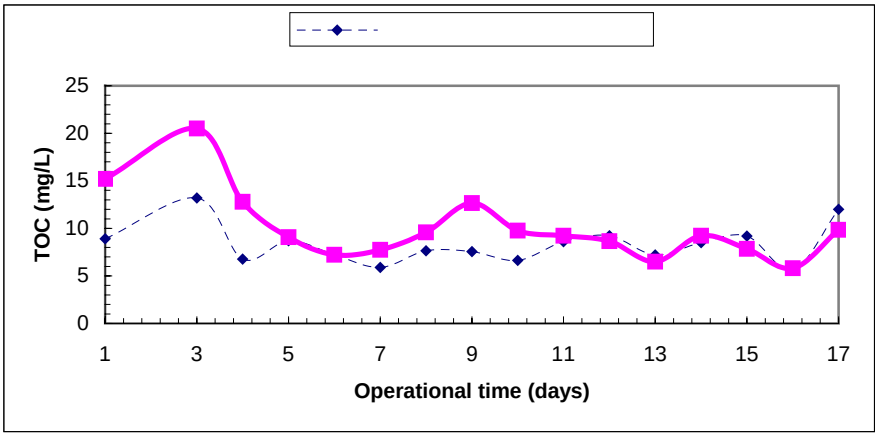


Figure 4.25 Performance of MBR in terms of COD removal



The results showed that MBR performance was stable in terms of COD, TOC and Cl- concentration in permeate. With PCP loading of 144mg/day, PCP in the effluent was below detection (detecting limit was 0.005mg/L). It is suggested that microorganism in both are resistant and adapted for PCP degradation. The PCP loading could also be increased further.

4.5.1.3 HRT 12, 20mg/L PCP

As the performance of system was stable, to further increase the loading rate and to test the performance of system with higher concentration of PCP, PCP was increased form 12 mg/L to 20 mg/L in the influent (240mg/L). With SRT of 12 hours and biomass concentration 10,000mg/L, and flowrate of 16.67 mL/min, similar conditions were maintained. PCP was degraded well in both reactors. PCP could not be detected in permeate. It can be concluded that MBR system is well adapted with high PCP loading.

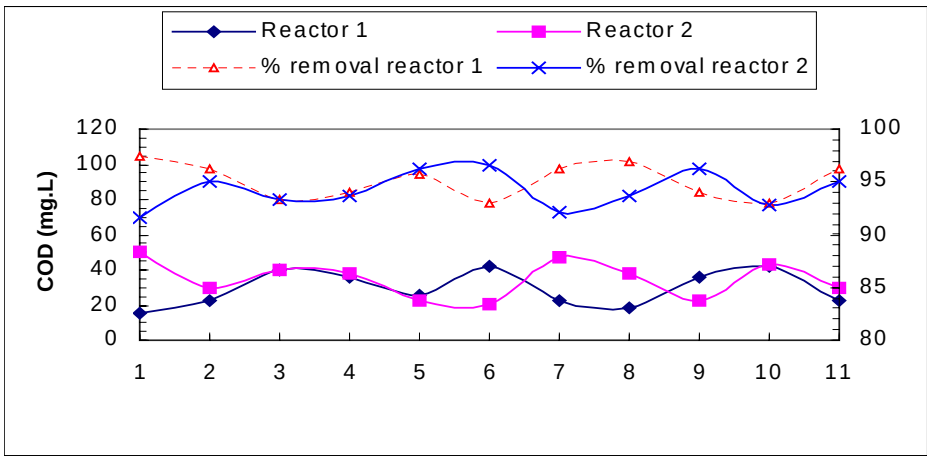


Figure 4.27 Performance of MBRs in terms of COD

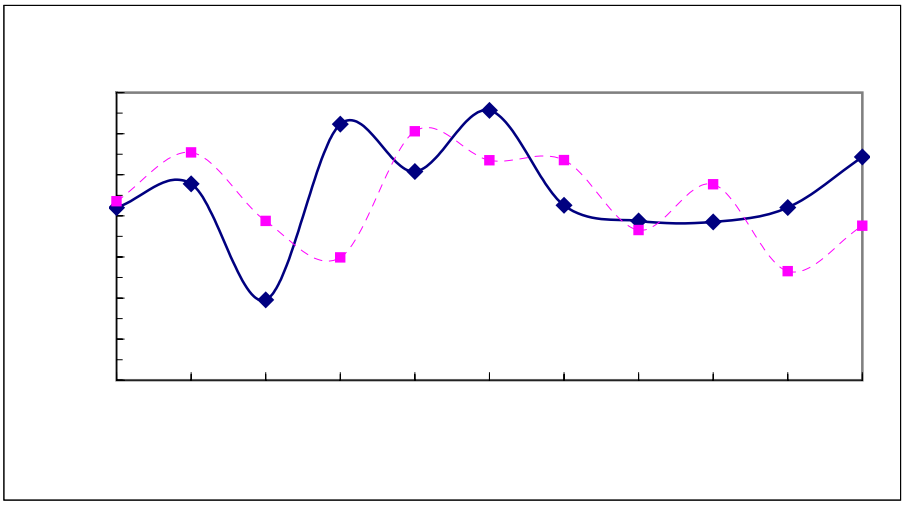


Figure 4.28 Performance of MBR in terms of TOC

Except with HRT 24 hours, the effluent still remain PCP with low concentration, MBRs system was so efficient for treating the PCP contaminating wastewater. With the MLSS of mixed liquor around 8,000 to 10,000 mg/L and PCP loading from 72-240mg/day, the MBR system produced excellent quality of effluent. At HRT 18, 12 hours, even with PCP loading rate of 240mg/day, PCP removal (more than 99%) and COD removal was higher than 95%. The chloride concentration in permeates were also stable in the range of 35-60mg/L. There was not much difference in the performance of MBR with different HRTs in terms of COD removal. Most of the time, the MBR system had COD value in permeate lower than discharge standard of 50mg/l (figure 4.30).

The result showed the advantages of the MBR system. Even with the sludge, which has been acclimatized, the biomass concentration played an important role in the removal capacity of the toxic compounds. It should maintain the high MLSS around 8,000 to 10,000 mg/l for removal of high concentration of PCP. Compared to the normal MLSS of the activated sludge process is around 3,000mg/L, MBR can satisfy the requirement of

high MLSS in reactor. This founding confirm the conclusion which have been done in the experiment of Moos et al. (1983), chosen a high MLSS concentration will result in a more stable process.

Table 4.11 Permeate quality of MBR with different HRT

HRT	PCP loading (mg/day)	Permeate (mg/L)				% Removal		
		COD (mg/L)	TOC (mg/L)	Cl- (mg/L)	PCP (mg/L)	COD (mg/L)	TOC (mg/L)	PCP (mg/L)
24	72	15-50	6-10	20.4-61	ND-0.3	90-97.5	94-96	96.8-99.9
18	96	14.5-36.4	6.5-18.8	41-51	ND	94-97.5	89.6-96.3	99.9
12	144	15-60	5.6-20.5	30.5-62	ND	90-97.5	89-95	99.9
12	240	15-50.25	5.8-12.6	45.5-61.5	ND	92-98		99.9

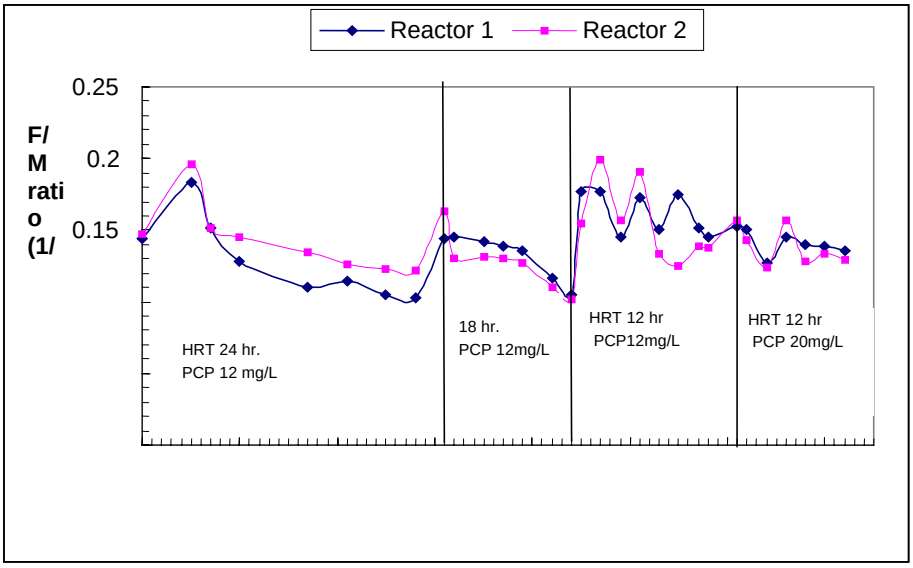


Figure 4.29 F/M ratio in the reactors with difference operation conditions.

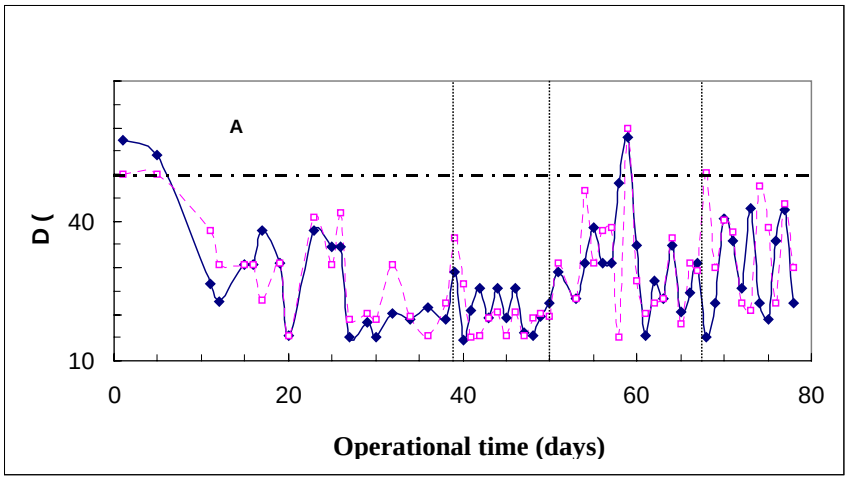


Figure 4.30 COD in permeate with different operation conditions.

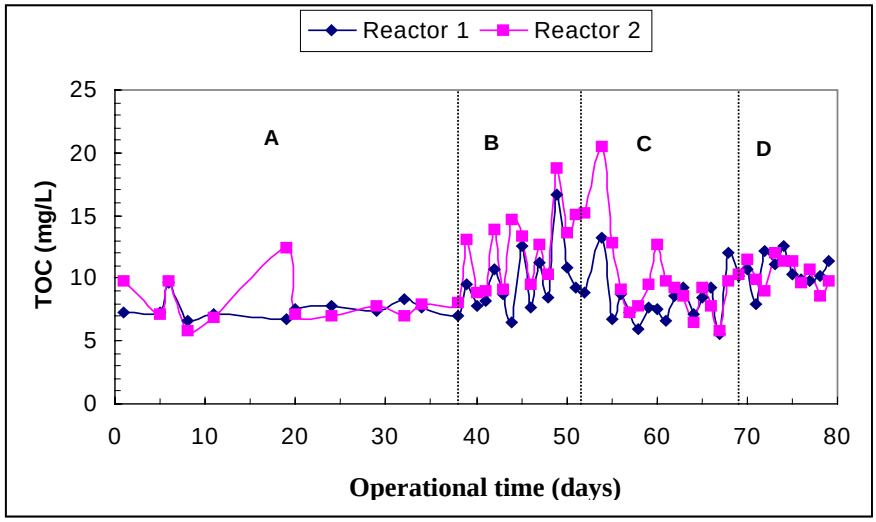


Figure 4.31 TOC in permeates with different operation conditions.

Besides, membrane also has an advantage role in keeping all the useful bacterial in reactor, while in normal activated sludge, they can wash out in the effluent. Besides that the quality of permeates also increase in term of COD and TOC.

Because of separating the HRT and SRT, MBR also provided more convenience. The SRT in this experiment was around 25 days, compared to 5-15 days in the conventional activated sludge leads to low F/M ratios. This ratio in the MBRs system was 0.1-0.19 (kgCOD/kgMLVSS.day) compare to 1.5-2 of high rate aeration or 0.2-0.4 of conventional plug flow (Metcalf and Eddy, 2003). The energy substrate could be used for other purpose than cellular growth thus minimizing biomass production (Scholz and Fuchs, 2000). This phenomenon leads to a slow wastage of biomass and reduced the cost for treating the sludge. In case toxic compound was absorbed in the sludge, the minimize sludge production was expected. And longer SRT reduced not only the average pollutant concentration but also the degree of inhibition exhibited by PCP (Moos, et al., 1983)

As the result from the biosorption capacity and the experiment of acclimatized activated sludge with PCP as the sole carbon, PCP has a trend to be highly absorbed to sludge. But with the HRT 12 and 18 hours, the absorption capacity in the sludge reached to the equilibrium level. The sorption phenomenon and degradation occurred continuing in the sludge particles, so the degradation capacity was enhanced though increasing contacting time between the pollutant and the biomass.

4.5.1.4 Chloride balance

The chloride material balance was a measure of the biotransformation of the PCP. The difference between the chloride concentration and the total chloride potential of the influent was indicative of the degree of PCP dechlorination.

Chloride is equal to 66.7% of molecule weight of PCP. With PCP concentration in the influent of 12mg/L (vary in range 10.6-14.5 mg/L), the chloride concentration in effluent must be higher from 8 to 10 mg/l compare to the influent when PCP is completely degraded. Actually, chloride concentration in the influent was around 20 mg/L and the chloride in effluent was higher 40 - 61 mg/L. Chloride in permeate higher than the influent

was an evidence for PCP degradation. Dechlorination production due to the PCP degradation was the main cause for pH decrease and chloride production. Figure 4.32 shows the variation in chloride in effluent at different operation condition.

$$C_6HCl_5O+O_2 \Rightarrow CO_2+H_2O+5Cl^-$$

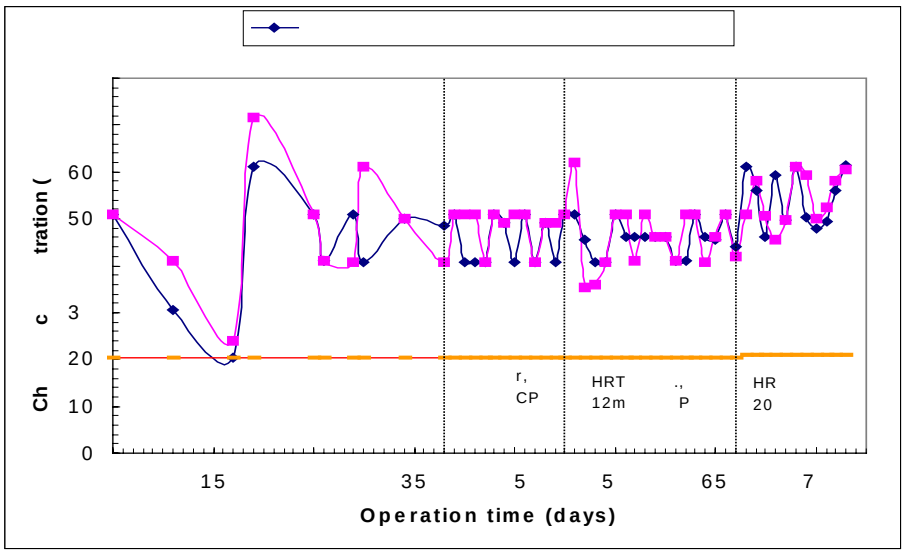


Figure 4.32 Chloride concentration in permeates with different operation conditions.

4.5.1.5 NaPCP

Apparently, from the performance of MBR, the system was able to adapt to increasing PCP concentration. So, higher PCP in the influent was applied to check the performance of the MBR. As PCP has very low solubility, with PCP concentration higher than 20 mg/L, PCP would precipitate at pH 7. Therefore, NaPCP was used instead of PCP for running MBR at higher PCP concentration. In practice, NaPCP is more easily found in the wastewater. The same operation condition was applied for this study with MLSS of 10,000 mg/L, SRT 25 days and HRT of 12 hours. NaPCP loading was gradually increased.

First, NaPCP with 10mg/L was used for a few days to get microorganisms acclimatized to Na-PCP. Then after, it was gradually increased to 20, 30, 40, 60 and 100 mg/L. The experimental data for these stages are summarized in appendix B.

With NaPCP concentration of 10 and 20 mg/L, MBR systems produced very good permeate in terms of PCP removal. There was no NaPCP was detected in the effluent.

During the time of running MBR with NaPCP, with different toxic loading, the performance of systems was stable and high organic removal efficiency in terms of COD. COD in permeate was also stable, lower than 40 mg/L with percent removal more than 95%.

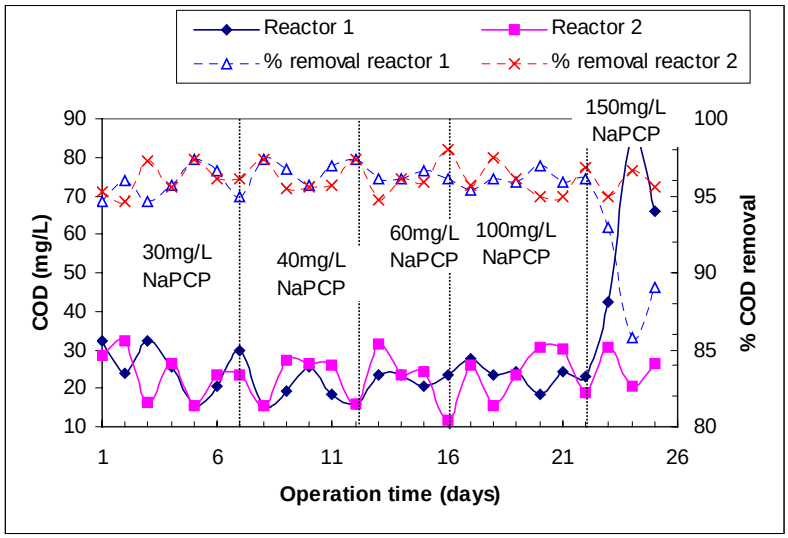


Figure 4.33 COD in permeate with different NaPCP concentrations in the influent.

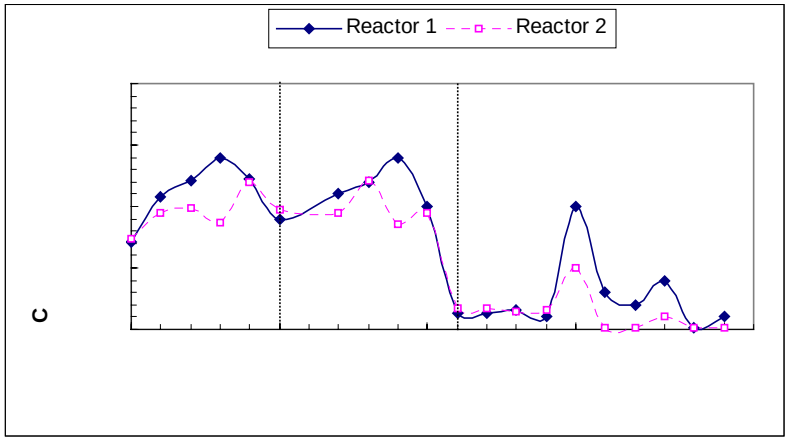


Figure 4.34 NaPCP in permeate with different NaPCP concentrations in the influent

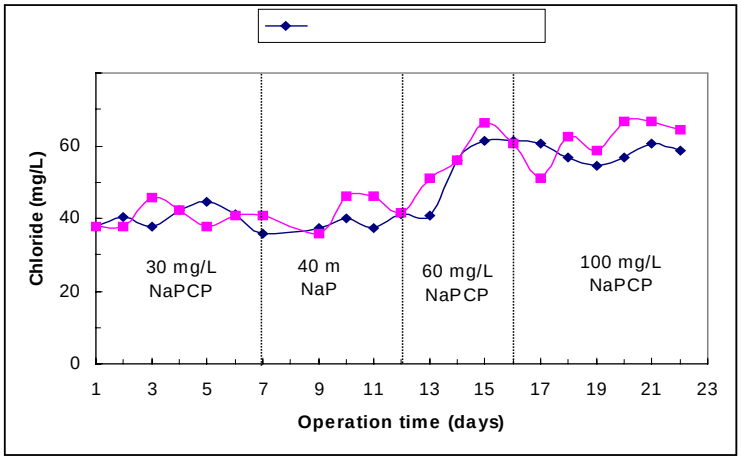


Figure 4.35 Chloride in permeate with different NaPCP concentrations in the influent

When PCP concentration increase to 30 mg/L and 40 mg/L, permeate contain NaPCP with low concentration vary from 0.01-0.014 mg/L in reactor 1 and 0.0075-0.012 mg/l in reactor 2. This result confirmed the results of batch studies. When using NaPCP instead of

pure PCP, some kinds of organisms which have been isolated from the MBR do not growth or take longer time to growing in the new kind of toxic compound, especially with higher concentration. However, when increasing the concentration of PCP salt to 60 mg/L, the PCP concentration was lower than those with 30 or 40 mg/L of influent. NaPCP in permeate in this stage was around 0.001 mg/L. The result could be the adaptation of organism in reactor with new toxic substrates. Higher NaPCP of 100 mg/L was applied, the system was still stable with NaPCP in permeate in range of 0.001 to 0.004 mg/L. Two reactors had different color, and the reactor 2 seemed working better than reactor 1 though the NaPCP in the effluent was lower than reactor 1.

However, when 150mg/L of NaPCP was applied, the reactor 1 turned to poor performance while reactor 2 still could degrade NaPCP. Reactor 1 did not have the phenomenon of pH drop down. The poor performance also occurred in terms of COD removal. Table 4.12 shows the performance of the systems in terms of permeate quality with 150 mg/L of NaPCP in the influent.

Table 4.12 Effluent of system with NaPCP 150mg/L in the influent

Operation date (days)	NaPCP (mg/L)		COD (mg/L)		Chloride (mg/L)	
	Reactor1	Reactor 2	Reactor1	Reactor 2	Reactor1	Reactor 2
1	12.18	0.32	42.5	31	30.6	91.88
2	79.2	0.54	85.2	50	20.4	71.47
3	81.9	0.2	65.8	45	26.5	89.5

The reason for the difference in behavior of two reactors could be the different kinds of predominant in different reactors as the result of isolated organisms, which could have different tolerant level with NaPCP.

Depend on the poor performance of the Reactor 1; bioaugmentation technique was applied to enhance the NaPCP degradation capacity.

4.5.2 Membrane fouling

By controlling the flowrate, we can maintain the desired HRT. The resistance of membrane was described through transmembrance pressure. Figure 4.36 presents the variation of the TMP during the operation stages.

There was not much different between the TMP of two reactors through different stages. The TMP gradually increased with the time. When decreasing the HRT from 24 to 18 and 12 hours, the TMP gradually increase from 5.9kPa to 6.4kPa by the end of HRT 18 hours stage and 7.1 kPa by the end of 12 hours in reactor 1 and from 5.9 to 6.4 and 7.2 kPa in reactor 2 respectively. After 100 days of operation, through visual observation it was noted that membrane surface was coated with a gel layer, but not with a cake layer formation due to microbial flocs. Under this condition, the membranes were removed from the reactors and washed with the tap water. However, even after this simple surface washing, when the filtration process was restarted no notable TMP reduction was observed. This demonstrates that this gel layer formation could be linked to EPS production in reactors.

One reason also affected on the fouling problem in the system was the design of the air diffuser system. Even with this experiment, air backwashing was not applied; with the

position and the shape of air diffusers in the middle of the membrane contributed the removal of cake layer in the membrane.

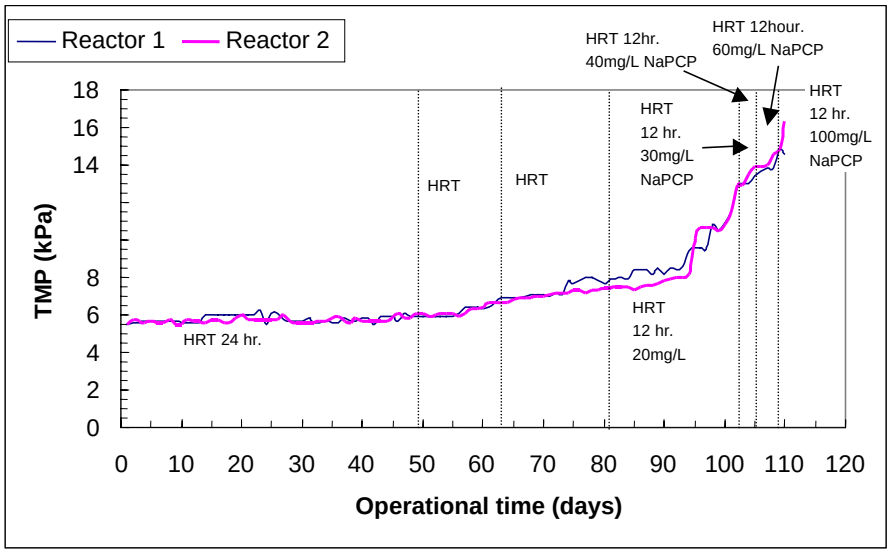


Figure 4.36 Membrane resistance with different operation condition

4.5.3 EPS and sludge characteristics.

EPS has a function of forming a protective layer for the cells against the harmful external environment, such as biocides, sudden changes of pH, etc, or aggregating bacterial cells in flocs (Wingerder et al., 1999). Consequently, EPS play a crucial role in the flocculation, settling, and dewatering of activated sludge (Morgan et al. 1990; Liao et al., 2001). Not only EPS, the amounts of protein and carbohydrate have significance to the sludge characteristic such as dewaterability (Houghton et al, 2000,2001, Wu et al. 1998). So EPS components mainly in terms of protein and carbohydrate were also checked.

EPS level has been identified as being primarily responsible for fouling in MBR. Each component of EPS has a different effect on characteristic of sludge. There were two kinds EPS level has been identified as being primarily responsible for fouling in MBR. Each component of EPS has different effect on characteristic of sludge. There are two kinds of There were two kinds of founlant in the membrane, the first one is irreversible, and the second one is reversible. The bound EPS link with the reversible fouling due to more higher bound EPS, more stronger the cake layer attach to the membrane, while the soluble EPS was relative to irreversible fouling.

Table 4.13 Operation stage condition

Stage of operation	COD* concentration (mg/L)	Toxic compounds concentration influent (mg/L)	Flowrate (L/day)	HRT (hours)
A	600	12 as PCP	6	24
B	600	12 as PCP	8	18
C	600	12 as PCP	12	12
D	600	20 as PCP	12	12
E	600	20 as NaPCP	12	12
F	600	40 as NaPCP	12	12
G	600	60 as NaPCP	12	12

*Actually, the COD value varied in range 310-650 mg/L

Table 4.14 EPS and its components at different operation condition

Condition		Soluble (mgC/L)				Bound (mgC/gVSS)			
		EPS	Protein	Carbo-hydrate	P/C	EPS	Protein	Carbo-hydrate	P/C
A	R1	66.94	16.44	50.5	0.32	34.88	18.98	15.9	1.19
	R2	74.25	19.65	54.6	0.36	36.90	19.9	17.00	1.17
B	R1	68.23	41.64	26.59	1.56	21.69	9.88	11.81	0.84
	R2	74.94	40.06	34.88	1.15	16.22	8.92	7.30	1.22
C	R1	71.60	46.28	45.32	1.02	25.15	11.82	13.33	0.88
				50.76	0.13	24.56	11.34	13.23	0.86
	R2	42.96	8.02	34.94	0.23	22.06	10.6	11.46	0.92

The condition of different stages of MBR was summarized in table 4.13. Sludge characteristic at different operation stage of the MBRs system was presented in table 4.14.

From figure 4.38 and 4.39, it concluded that there was not much change in terms of soluble EPS with different HRT with PCP in the feed wastewater for both reactors. However, with NaPCP, the same condition of operation, the soluble EPS has a trend increase slightly when concentration increased. The increase of soluble EPS could lead to the rapid fouling rate of the membrane. Many research confirmed that EPS carry negative charges due to the ionization of the anionic functions groups, such as carboxylic and phosphate (Liao et al. 2001, Pere et al., 2001). So mineral cations in wastewater tend to complex with soluble EPS inside the membrane to increase the flogging. With the same condition of operation, there was not much difference between the EPS of two reactors.

In terms of bound EPS, figure 4.38 shows that, in the stable condition of reactors, the bound EPS produced was lower than unstable condition. As discussion in MBR performance, in condition of A and F, the performance of reactors was worse than other conditions; the result was higher bound EPS value. The result for this trend was higher EPS was produce to protect the microorganism to the harmful condition of the environment.

The EPS value in this study was the same with the result of Houghton et al. (2000) (Table 4.15). However it was lower than the result from Thuy (2003). Each condition of operation and each chemical characteristic of the wastewater leaded to the different behavior of the microbial cultures.

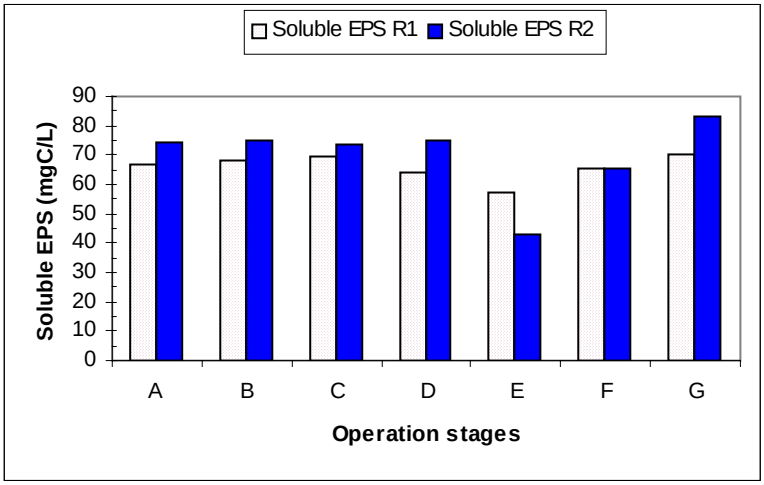


Figure 4.37 Changing of soluble EPS at different operation stages

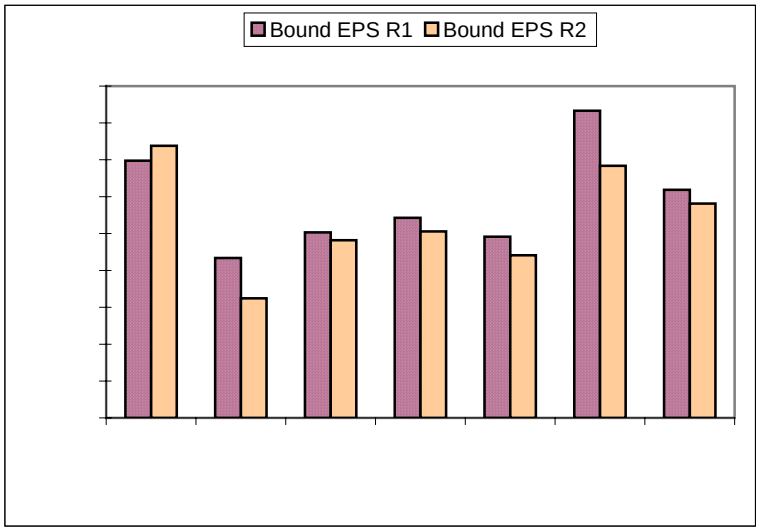


Figure 4.38 Changing of bound EPS at different operation stages

Table 4.15 Comparison of EPS and its components

Type of treatment	Type of bacterial growth	Type of wastewater	Bound EPS		Reference
			EPS (mgC/gVSS)	P/C	
MBR- aeration	Suspended	PCP wastewater	16.22- 41.66	0.75-1.61	**This study**
MBR- aeration	Suspended	Phenol wastewater	37.6-83.06	0.86-2.72	Thuy, 2003
Convention activated sludge	Suspended	Municipal wastewater	14.6-43.7	1.25-1.7	Houghton, et al., 2000
Convention activated sludge	Suspended	Municipal wastewater	128.9	0.58	Morgan et al, 1990

Table 4.16 showed the sludge characteristic with different operation conditions.

Table 4.16 Sludge characteristic with different operation condition

Condition	Reactor	SVI (mL/gVSS)	CST (s)	Viscosity (cP)
A	R1	150	15.5	7.8
	R2	159	12.4	7.3
B	R1	169	45.4	16.6
	R2	162	60.9	16.1
C	R1	144	53.0	17.5
	R2	126	39.0	22.5
D	R1	154	52.3	15.6
	R2	129	57.1	17.6
E	R1	142	69.1	46.5
	R2	148	33.0	62.6
F	R1	99.5	31.0	173
	R2	116	70.1	92
G	R1	82	32.4	123
	R2	114	67.2	87

Sludge Volume index (SVI): SVI is a quick parameter in determining the effectiveness of settling of activated sludge. This characteristic of MBRs considered satisfactory with stages running with NaPCP due to lower than 150. Figures 4.40 showed the SVI value of system with different stages.

The reason was the chemical nature of wastewater leaded to changing EPS constituents as well as SVI. It is recognized that the higher NaPCP concentration in the influent the lower SVI parameter. Actually, SVI was not an important parameter for MBR system because we do not use the sedimentation tank. According to Liu and Fang (2003), sludge settled better with lower EPS. However, in this study, the relation between these parameters was not clear. The changing in the SVI was caused not only by the effect of EPS but also by the adding alkali to maintain pH in the MBRs (Liu and Fang, 2003) and other effects. Even both reactors running in the same conditions, there was difference in the sludge characteristic due to the different of microbial cultures, density, DO, pH, substrate, bacteria growth phase, etc.

Viscosity: Viscosity has a relation to membrane fouling, the higher viscosity the higher TMP**.** The higher adverse level of the environment (more toxic), the higher bound EPS was produced lead to higher viscosity of the mixed liquor. The viscosity of the membrane increase with the time was one of the factors contributed to the increase of TMP. Figures 4.43 showed the relationship between viscosity and TMP of the MBR system..

EPS, its components and the relationship with different sludge parameters was still a lot of things have to be done before we could get the accurate trend. As above discussion, there was many factors could affect on the changing of these parameters. Further research should be done to know more about the contacting effects of EPS and sludge characteristics in MBR.

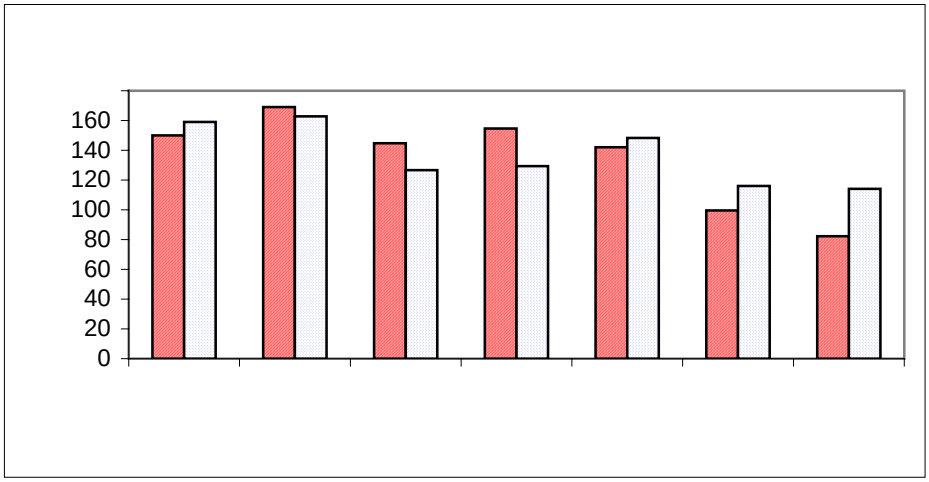


Figure 4.39 SVI with different operation condition

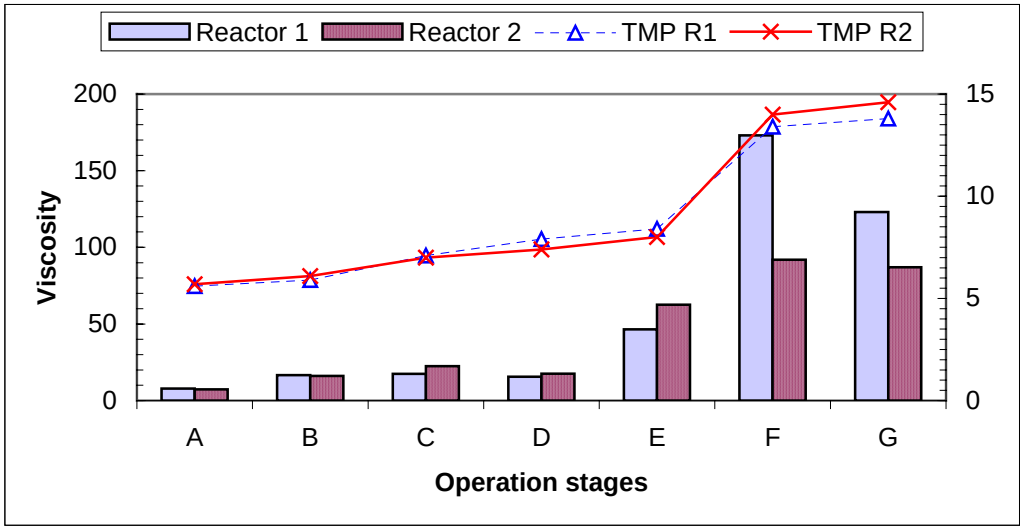


Figure 4.40 Relation between viscosity and TMP

4.6 Biokinetic study

Specific growth rates (μ) of the sludge collected from the MBR system was measured using respirometric method. Biokinetic studies were conducted twice (Study-I and II). Results are presented in Table 4.17 and 4.18; Figure 4.44 and 4.45. Calculations were based on the equations mentioned before. Thus, μ_{max} of the study I and II were equal to 2.392 (day^{-1}) and 2.72 (day-1); while K_s was 38 mgCOD/L and 27.55 mgCOD/L, respectively.

The MBR system from which the sludge was collected contained two substrates, PCP and glucose. The organic carbon contributed by PCP constituted less than 5% of the feed COD. According to Timothy *et al.* (1998), the effects of simultaneous substrate biodegradation were small; single-substrate extant kinetic tests should be adequate for describing the capacities of the biomass in degrading a particular substrate in multi-substrate environments. Moos *et al.* (1983) suggested PCP had little impact on the removal of the other organic matter based on their study conducted with influent 600mgCOD/L glucose and 20mg/L PCP. Thus, glucose was used as the substrate, which contributed to the COD

for the feed wastewater for this study. The result from this test has been fixed by the Monod's model without inhibition. Table 4.19 shows the values of the parameters in an activated sludge process.

Table 4.17.Results of Biokinetic Study of the Sludge collected from MBR (Study– I)

Parameters	Values					
S (mgCOD/L)	5.16	8.60	12.05	14.63	17.21	20.65
Re,x(mgO_2/L.h)	5.51	5.62	5.80	5.58	4.90	5.80
Rx,t(mg/L.h)	18.97	23.83	27.72	28.44	28.22	30.85
Rx,ox(mgO_2/gVSS.h)	3.88	5.26	6.33	6.60	6.73	7.23
OC/S(mgO_2/mgCOD)	0.19	0.16	0.16	0.16	0.13	0.12
Rx(mgCOD/gVSS.h)	20.79	32.41	38.51	42.50	53.69	60.54
Y(mgVSS/mgCOD)	0.57	0.59	0.59	0.59	0.62	0.62
μ (day^{-1})	0.29	0.46	0.54	0.61	0.79	0.90

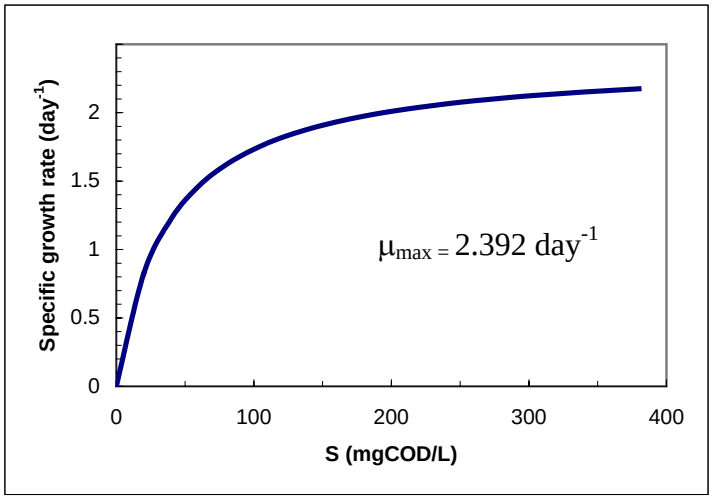


Figure 4.41 Relationship between substrate and specific growth rate (Study-I)

Table 4.18. Results of Biokinetic Study of the Sludge collected from MBR (Study–II)

Parameters	Values						
S(mgCOD/L)	5.96	9.93	15.89	19.87	28.60	32.17	42.89
Re,x(mgO_2/L.h)	6.83	5.61	5.72	5.90	6.18	6.46	7.09
Rx,t(mg/L.h)	24.45	32.76	36.96	39.96	42.01	38.24	40.34
Rx,ox(mgO_2/gVSS.h)	4.99	7.68	8.84	9.64	10.14	8.99	9.41
S(mgCOD/L)	5.96	9.93	15.89	19.87	28.60	32.17	42.89
OC/S(mgO_2/mgCOD)	0.16	0.14	0.12	0.11	0.11	0.11	0.10
Rx(mgCOD/gVSS.h)	32.09	55.03	75.55	84.25	91.12	82.65	93.85
Y(mgVSS/mgCOD)	0.59	0.61	0.62	0.62	0.63	0.63	0.63
Specific growth rate,u,(day^{-1})	0.46	0.80	1.13	1.26	1.37	1.24	1.43

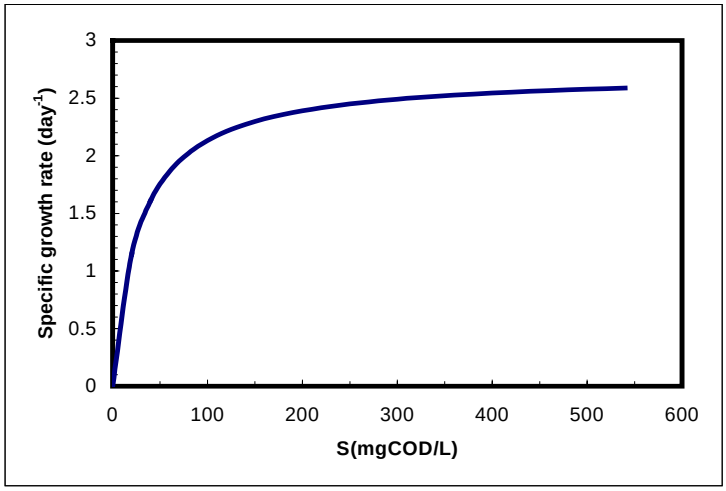


Figure 4.42 Relationship between substrate and specific growth rate (Study-II)

Table 4.19 Biokinetic parameters of the Activated Sludge

Coefficients	Unit	Range	Typical	Reference
K_s	mg/L BOD	25-100	60	Metalf and Eddy (2003)
	mg/LCOD	27.55-38		This study
Y	mgVSS/mgBOD	0.4-0.8	0.6	Metalf and Eddy (2003)
	mgVSS/mgCOD	0.57-0.62		This study

There was a small difference between the values of biokinetic parameters of study I and II, which may be due to change in sludge as Na-PCP was increased from 30mg/l to 40mg/L.

Chapter 5

Conclusions and Recommendations

The biodegradation of pentachlorophenol (PCP) and its salt (NaPCP) was carried out by the membrane bioreactor system. There were four main parts of this study. The first part involved setting the pilot scale of the MBR, acclimatization on the organism and accessing the effect of biosorption. The second part was investigating the performance of special organism: *Mycobacterium chlorophenolicum*, isolating the organisms from the MBR system, comparing PCP degradation capacity of these organisms and finding out the growth pattern of each type of microorganism. The third part allowed determination the performance of MBR system with different HRT 24, 18 and 12 hours with different PCP as well as NaPCP loading. Kinetics of the microorganism for removing glucose and bioaugmentation technique to enhance the performance of system with inhibition level of NaPCP were conducted in the last part. From the results of the experiments the following conclusions can be drawn.

5.1 Conclusions

1. PCP was biodegradable and acclimation could be achieved by using fill and draw process with HRT 24 hours.

2. The biosorption of PCP on biomass was high 0.6289 mg/g biomass and occurred rapidly within 60 minutes. It could be confirmed that with high concentration of biomass, biosorption played an important role besides biodegradation and might enhance the degradation process by increasing the contact between biomass and PCP.

3. The PCP degradation was found to be much better with activated sludge than the specific microorganism *(Mycobacterium chlorophenolicum)*. There were three predominant kinds of microorganism (R1-C1, R1-C2 and R1-C3) which were isolated from reactor 1 while 4 kinds of organism were detected in reactor 2 (R2-C1, R2-C2, R2-C3, R2-C4) in the period of MBR running with PCP. However, when NaPCP was used, special organism, R1-C3, R2-C3 did not grow. The log phase of isolated organisms was found in the range of 18-71 hours.

4. With PCP in the influent, except with HRT 24 hours, the effluent still containing PCP with low concentration, the MBR system produced excellent quality of effluent. PCP removal (more than 99%) with PCP loading rate from 12 to 40 mg/m^3.day, COD removal was higher than 95%. Most of the time, the MBR system had COD value in permeate lower than discharge standard of 50mg/L.

5. Biomass concentration played an important role in removal capacity of the system. For removing PCP, high biomass concentration around 8,000 to 10,000 mg/L was applied.

6. pH adjustment has applied to maintain suitable condition for the development of biomass due to the product HCl produced as a result of the mineralization process of PCP and its salt lead to the decrease in pH in the system.

7. When NaPCP was used instead of PCP, with low concentration, NaPCP was completely removed. However, after certain period of operation, the microorganisms became highly adapted, NaPCP remained being very lower even at higher concentration (60, 100 mg/L) of influent.

8. With 150mg/L of NaPCP in the influent, due to different predominant organisms in different reactors, the behavior was different. Reactor 1 turned to poor performance, while reactor 2 still could degrade NaPCP. Bioaugmentation technique was applied to enhance the performance of reactor 1.

9. After more than 120 days operation, the transmembrane pressure in both reactors was low: 13.9 kPa for reactor 1 and 14.7 kPa for reactor 2. The main reason for increasing TMP was the irreversible foulant. The design of reactor was suitable to prevent fouling during operation of the system. Chemical cleaning is required if fouling occurs.

10. The kinetic parameters of system for removing glucose was in range 27.55-38 mgCOD/L for K_S and 0.57-0.62 for Y. These values were similar as those of activated sludge.

11. The higher viscosity of the mixed liquor could be linked to the increase in TMP. The dewaterability of the system was good though SVI was lower with time.

5.2 Recommendations for future study

To ensure PCP and its salt are mineralized completely; toxicity test and intermediate text should be done.

As PCP contributes to very low carbon source to the microbial culture, while high biomass concentration is applied with MBR system for removal PCP and its salt, lead to lack of food for the growth of biomass. In practice, in order to treat the PCP containing wastewater in wood processing or pesticide manufacturing industry, domestic wastewater or food wastewater should be combined to enhance the removal of both substrates as well as in terms of economic aspects.

Wastewater from pesticide industry or wood processing not only contain PCP but also many phenolic compounds such as 2,3,4,6-tetraclorophenol, 2,4,5-trichlorophenol, 1,2,3,4- trichlorophenol, phenol, etc. The study in MBR for mixture of these substrates is recommended.

Instead of using acclimatized technique, the special kinds of microorganism which specialized for removing PCP and phenolic compounds should be added to activated sludge of the wastewater system to shorten the time of acclimatization as well as supply the sources of organism useful for removing toxic compounds.

Further studies about the biokinetic shoud be done in case of inhibition as well as the effect of PCP with other carbon sources such as glucose. It would help in finding out the suitable substrates, which can be combined with PCP wastewater.

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Appendix A

Experimental result of preliminary study

Table A- 1 Experimental results of batch acclimatization

Operation time (day)	Influent PCP (mg/L)	Fresh activated sludge					Phenol adapted sludge				
		MLSS (mg/L)	*MLVSS (mg/L)*	*COD (mg/L)*	*Cl- (mg/L)*	*PCP (mg/L)*	*MLSS (mg/L)*	*MLVSS (mg/L)*	*COD (mg/L)*	*Cl- (mg/L)*	*PCP (mg/L)*
	0	7067	4960				5267	4360			
1	1	8833	5400				8400	6033			
5	1	7133	5400				6433	4233			
6	1	6367	4967				8533	7333			
10	1	6825	4875	61.9					65.8		
11	1			69.7					81.26		
12	5										
13	5	6275	4900	81.3		< 0.1	6350	5775	65.6		< 0.1
15	5	7250	4825	46			6750	6000	38		
18	5	6800	4925			< 0.1	7500	6025			< 0.1
21	5	6525	4475	69.7			6750	5975	38.7		
24	10	6300	4650	92		< 0.1	7350	6075	50.3		< 0.1
26	10			82.4					30		
27	10	5450	4200	37.5		< 0.1	6650	5575	15		< 0.1
29	10	5375	4350			< 0.1	6325	5100			< 0.1
32	10	5375	4125	30	61.2		6100	5150	15	71.5	
33	10				61.2	< 0.1				56	< 0.1
34	10	5700	4050	54	56.5		6475	5425	46	51	
37	10	5875	4125	15.5	61.3	< 0.1	5824	5424	15.5	66.4	< 0.1
40	10	5850	4275	18	66.7	< 0.1	6075	4675	15.5	56.2	< 0.1

Note: COD in influent: 600 mg/L; Chloride in influent 20.8 mg/L,

Table A- 2 Biosorption equilibrium at different PCP concentration and biomass

(a) PCP 10mg/L; 5,200 mgVSS/L (b) PCP 7.8 mg/L; 10,300 mgVSS/L (c) PCP 14.8 mg/L; 10,300 mgVSS/L

a)

Contacting time (min)	Equilibrium PCP (mg/L)	Qeq (mg/g)
0	5.528	0.86
10	4.848	0.990
20	4.837	0.992
40	4.816	0.996
60	4.736	1.012
90	4.742	1.019
120	4.743	1.010
180	4.725	1.014

(b)

Contacting time (min)	Equilibrium PCP (mg/L)	Q (mg/g)
0	2.57	0.507
10	2.26	0.537
20	2.13	0.550
60	1.87	0.575
120	1.87	0.575

(c)

Time (min)	PCP concentration (mg/L)	Q (mg/g)
0	5.54	0.899
60	3.85	1.063
120	3.86	1.062

Table A- 3 Biosorption Isotherm Studies with 14.8 mg/L PCP

MLVSS (mg/L)	Equilibrium PCP concentration C_{eq} (mg/L)	% Removal	Specific removal rate q_{eq} (mgPCP/gVSS)
10300	3.85	73.98	1.063
5633	7.23	51.15	1.344
5100	7.86	46.87	1.360
4750	7.88	46.72	1.455

Appendix B

MBR performance

Table B- 1 Performance of MBR with HRT 24 hours, COD input 600 mg/L and PCP input concentration 12 mg/L

Operating date	Reactor 1						Reactor 2					
	MLSS (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)	**MLSS** (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)
1				57.3		7.26				50.0		9.74
5	5066	4166	0.174	54.0	51.0	7.25	4666	4066	0.096	50.0	51.0	7.10
6			0.076			9.68			0.062			9.85
7			0.084						ND			
8						6.60						5.77
10	4933	3266	0.257				3866	3066	0.140			
11			0.194	26.6	30.6	7.10			0.101	38.0	41.0	6.92
12	5100	3960		22.8			4533	3966		30.5		
14			0.300						0.450			
15	5166	4666	0.139	30.5			4800	4133	0.124	30.5		
16				30.6						30.6		
17			0.23	38.0	20.4					23.0	24.0	
19			ND	31.0	61.2	6.74			0.044	31.0	71.5	12.41
20				15.5		7.50				15.5		7.10
21												
22	5733	5433					4966	4466				
23			0.102	38.0					0.055	40.80		
24			0.132			7.76			0.088			6.95
25				34.3	51.0					30.50	51.0	
26	5850	5225		34.3	41.0		4925	4750		41.60	41.0	
27			0.063	15.0					< Limit	18.75		
29			0.083	18.2	51.0	7.50			0.065	20.25	40.8	7.77
30			0.031	15.2	40.8				0.143	18.75	61.2	

32				20.3		8.40				30.50		7.00
33	6400	5824					5875	4920				
34			0.185	19.0	50.0	7.70			0.077	19.50	50.0	7.90
36	6250	5570	0.056	21.3			5915	4893	0.032	15.30		
38			0.031	18.9	48.5	7.00			0.047	22.40	40.8	8.10

Table B- 2 Performance of MBR with HRT 18hours, COD input 600 mg/L and PCP input concentration 12mg/L

Operating date	**Reactor 1**						**Reactor 2**					
	MLSS (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)	**MLSS** (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)
1	7650	5525	0.08	29	51	9.46	8425	6150	0.031	36.4	51	13.03
2			ND	14.5	40.8	7.83			ND	26.4	51	8.88
3			ND	20.9	40.8	8.25			ND	15.0	51	8.96
4	7825	5650	ND	25.6	40.8	10.68	8200	6100	ND	15.5	40.8	13.83
5			ND	19.1	51	8.71			ND	19.1	51	9.08
6	7900	5750	ND	25.5	49	6.45	8450	6150	ND	20.5	49	14.62
7			ND	19.1	40.8	12.55			ND	15.5	51	13.38
8	8020	5900	ND	25.6	51	7.65	8500	6300	ND	20.5	51	9.58
9			ND	15.9	40.8	11.25			ND	15.5	40.8	12.66
10			ND	15.5	49	8.43			ND	19.2	49	10.32
11	9766	6866	ND	19.5	40.8	16.62	10400	7280	ND	20.1	49	18.75
12			ND	22.5		10.87			ND	19.5		13.66

Table B- 3 Performance of MBR with HRT 12hours, COD input 600 mg/L and PCP input concentration 20mg/L

Operating date	Reactor 1						Reactor 2					
	MLSS (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)	**MLSS** (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)
1	11366	7956	ND	15.00	61.25	10.2	10733	8366	ND	50.25	51	10.356
2			ND	22.50	56.15	10.78			ND	30.00	58.26	11.544
3	11866	9433	ND	40.50	46	7.956	12700	9700	ND	40.25	50.5	9.875
4			ND	35.60	59.45	12.233			ND	37.65	45.5	8.988
5	11533	8266	ND	25.50	49.65	11.078	10300	7666	ND	22.50	49.65	12.058
6			ND	42.56	61.25	12.566			ND	20.80	61.25	11.354
7	11100	8560	ND	22.50	50.25	10.256	12766	9387	ND	47.55	59.35	11.355
8			ND	18.80	47.8	9.874			ND	38.45	50.15	9.655
9	10566	8633	ND	35.60	49.55	9.853	12000	8966	ND	22.25	52.45	10.766
10			ND	42.50	56.15	10.205			ND	43.56	58.26	8.654
11	10233	8866	ND	22.50	61.5	11.436	12566	9266	ND	30.00	60.5	9.758

Table B- 4 Performance of MBR with HRT 12hours, COD input 600 mg/L and NaPCP input concentration 10, 20mg/L

NaPCP (mg/L)	Operating date	Reactor 1						Reactor 2					
		MLSS (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)	**MLSS** (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)
10	1			ND	24.32	32.5	12.35			ND	24.35	38.5	11.235
	2	10950	8250	ND	27.30	40.6	10.785	10655	8456	ND	28.65	40.6	9.786
	1			ND	32.23	42	8.652			ND	32.55	30.6	7.956
	2	11255	8656	ND	32.30	44.55	12.536	12563	9256	ND	26.55	48.55	12.534
	3			ND	26.80	50.6	11.285			ND	32.45	52.55	10.754
	4	10986	8264	ND	30.65	60.55	7.596	10655	8424	ND	25.88	58.95	8.423
	5			ND	26.56	44.25	10.586			ND	23.35	46.56	12.325
20	6	11250	8956	ND	25.25	50.25	8.973	10365	8265	ND	25.68	55.35	10.985

Table B- 5 Performance of MBR with HRT 12hours, COD input 600 mg/L and NaPCP input concentration 30, 40, 60, 100, 150 mg/L

NaPCP concentration	Operating date	Reactor 1						Reactor 2					
		MLSS (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)	**MLSS** (mg/L)	**MLVSS** (mg/L)	**PCP** (mg/L)	**COD** (mg/L)	**Cl-** (mg/L)	**TOC** (mg/L)
	1	11566	8655	0.0071	32.23	37.8	10.256	10896	8256	0.0074	28.65	37.8	8.233
	2			0.0108	23.75	40.55	11.563			0.0095	32.23	37.8	7.956
	3	10950	8466	0.0121	32.32	37.8	9.745	11250	8620	0.0099	16.25	45.6	11.258
	4			0.0139	25.68	42.25	9.562			0.0086	26.25	42.25	12.15
	5	11560	8652	0.0123	15.64	44.5	8.15	10950	8554	0.012	15.64	37.8	9.785
	6			0.009	20.50	41.25	9.66			0.0098	23.36	40.8	13.25
30mg/L	7				30.00	35.7	8.549				23.50	40.8	

	8			0.011	15.64		11.563			0.0095	15.64		7.956
	9	11550	9466	0.012	19.35	37.5	8.544	11200	9066	0.0121	27.10	35.7	14.258
	10			0.014	25.68	40.1	12.07			0.0086	26.40	46	16.89
	11			0.01	18.56	37.5	7.264			0.0095	25.80	46	8.353
40mg/L	12	11733	10333	0.0013	15.70	41.25	7.564	11566	95423	0.0017	15.70	41.5	10.24
	13	12966	11366	0.00129	23.60	40.8		12433	10466	0.00168	31.47	51.1	
	14			0.00156	23.60	56.15				0.00142	23.60	56.15	
	15			0.0011	20.50	61.25				0.0016	24.50	66.36	
60mg/L	16	11563	9750	ND	23.60	61.4		12560	10600	ND	11.80	60.5	
	17			0.01	27.50	60.5				0.005	25.90	51	
	18	12560	9876	0.003	23.60	56.6		11600	10354	ND	15.40	62.5	
	19			0.002	24.50	54.5				ND	23.40	58.5	
	20	11330	9533	0.004	18.60	56.6		10900	9850	0.0001	30.50	66.8	
	21			ND	24.50	60.5				ND	30.40	66.8	
100 mg/L	22	11800	9480	0.001	23.20	58.5		12534	10890	ND	18.90	64.2	
	23			12.18						0.32			
	24	11000		79.2				12500	10055	0.54			
150mg/L	25			81.9						0.2			

Appendix C

Standard curve

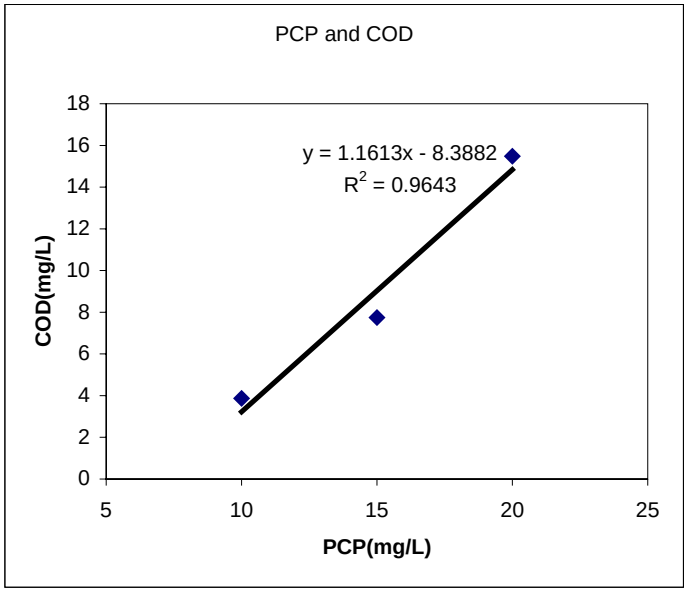


Figure C- 1 Relationship between PCP and COD

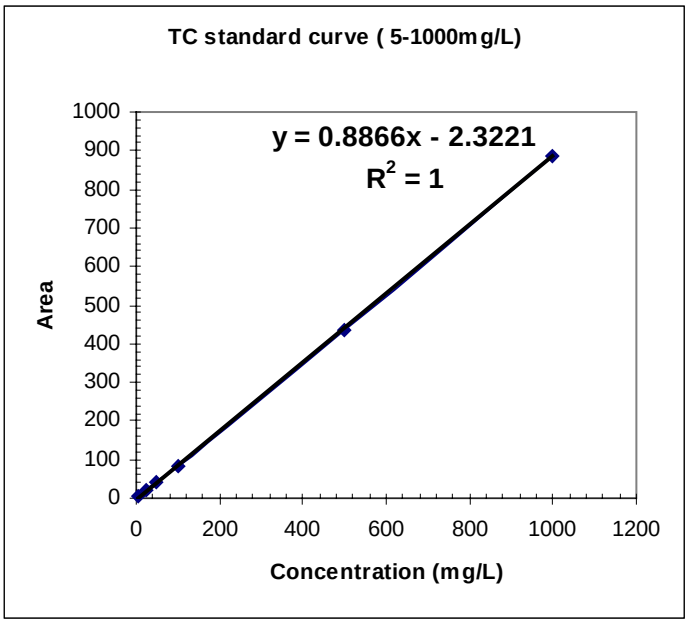


Figure C- 2 TOC standard curve (5-1000 mg/L)

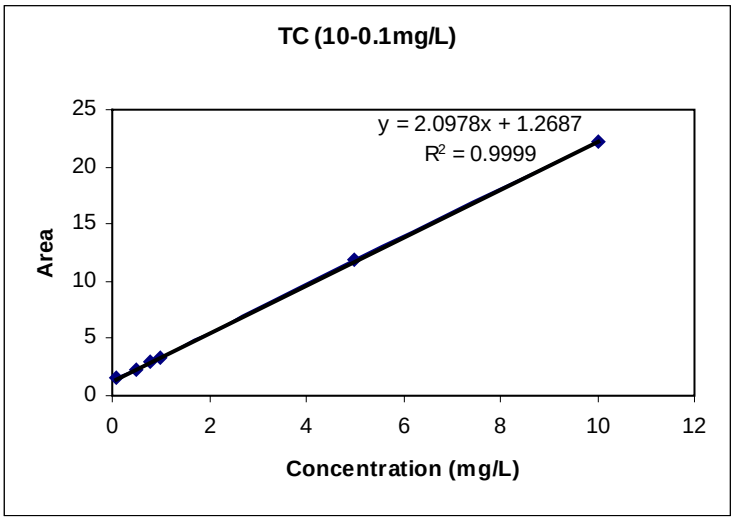


Figure C- 3 TOC standard curve (0.1-10 mg/L)

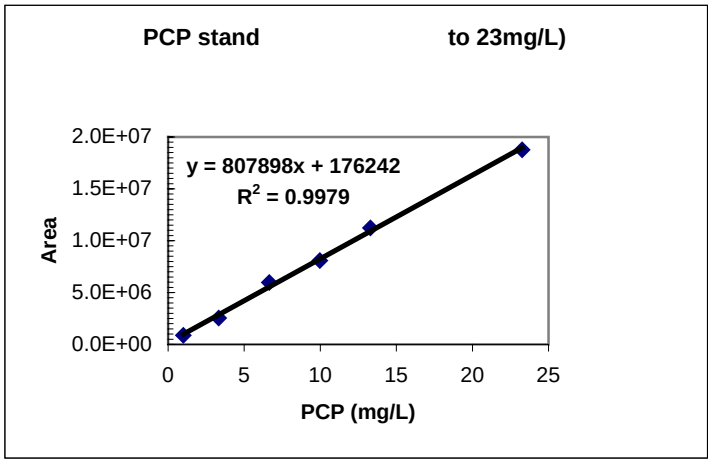


Figure C- 4 PCP standard curve (1-23 mg/L)

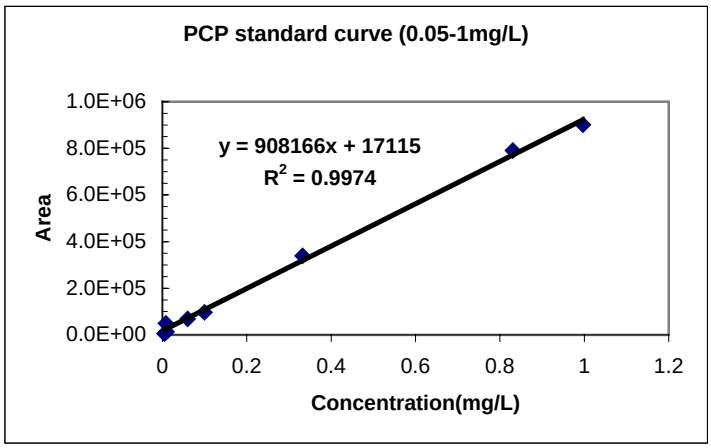


Figure C- 5 PCP standard curve (0.05-1 mg/L)

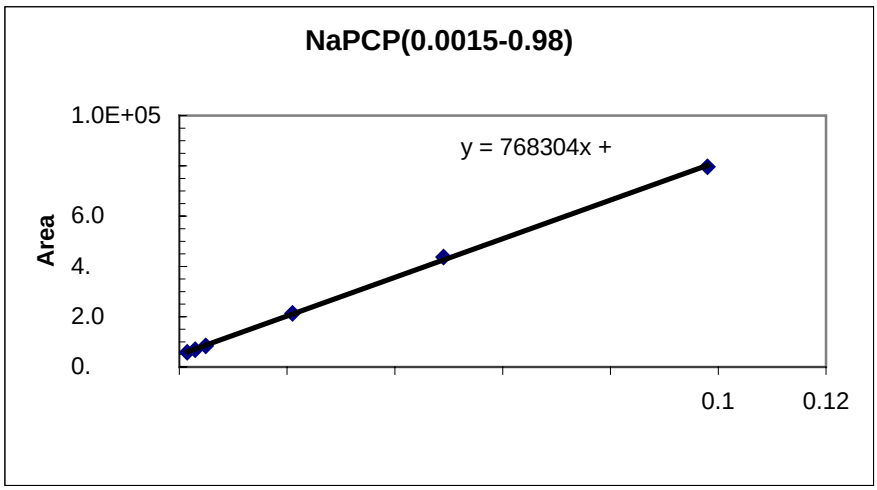


Figure C- 6 NaPCP standard curve (0.0015-0.98 mg/L)

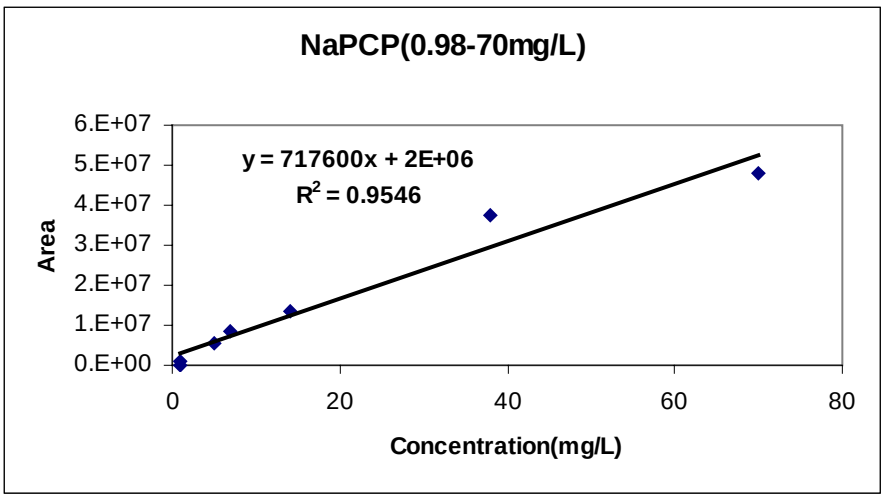


Figure C- 7 NaPCP standard curve (0.98-70 mg/L)

Appendix D

Membrane resistance

Table D- 1 Membrane resistance of new membrane reactor 1

Flux (ml/min)	Different TMP (mm Hg)	Filtration flux ($L/m^2.h$)	TMP (kPa)
56.4	35	16.9	4.7
109.3	40	32.8	5.3
189	49	56.7	6.5
238.5	60	71.6	8.0

Membrane resistance Rm = **2.67068E+11**

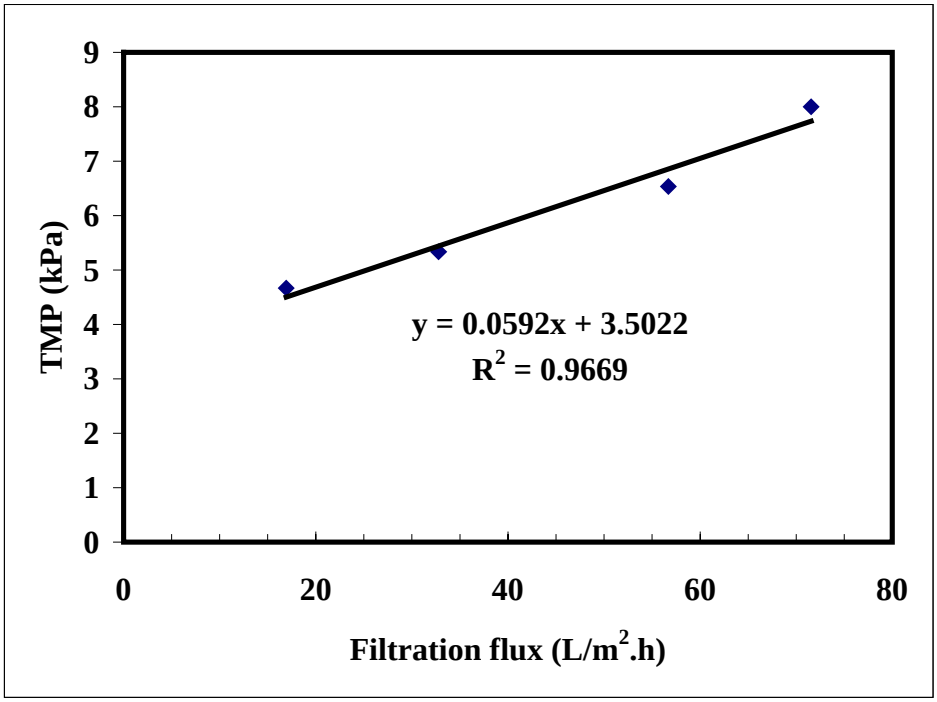
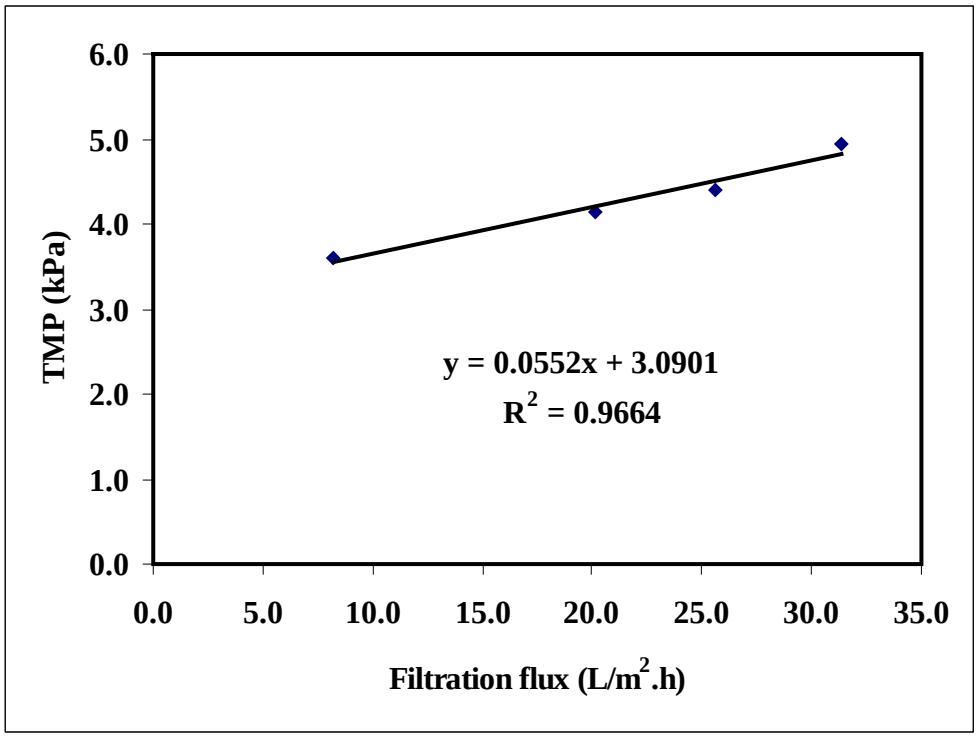


Table D- 2 Membrane resistance of new membrane reactor 2

Flux (ml/min)	Different TMP (mm Hg)	Filtration flux ($L/m^2.h$)	TMP (kPa)
27.30	27	8.2	3.6
67.00	31	20.1	4.1
85.33	33	25.6	4.4
104.50	37	31.4	4.9

Membrane resistance Rm = **2.49023E+11**



Appendix E

Photographs on Experiments

Figure E- 1 Membrane bioreactor system

Figure E- 2 New membrane and reactor

Figure E- 3 Biokinetic experiment

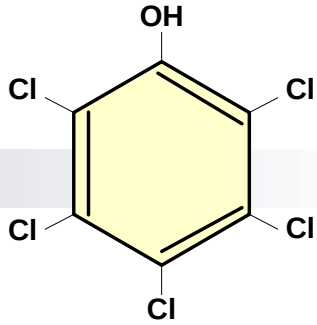
PENTACHLOROPHENOL DEGRADATION IN MEMBRANE BIOREACTOR

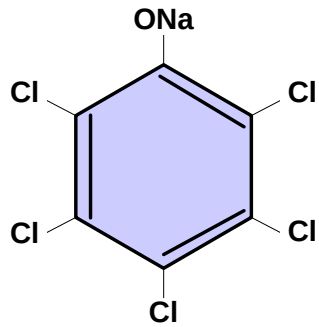
Committee: Prof. C. VISVANATHAN (Chairman)
Prof. NGUYEN CONG THANH
Dr. PREEDA PARKPIAN

Student: LE NGOC THU
ID No.: EVA 037066

AIT-July 2004

INTRODUCTION

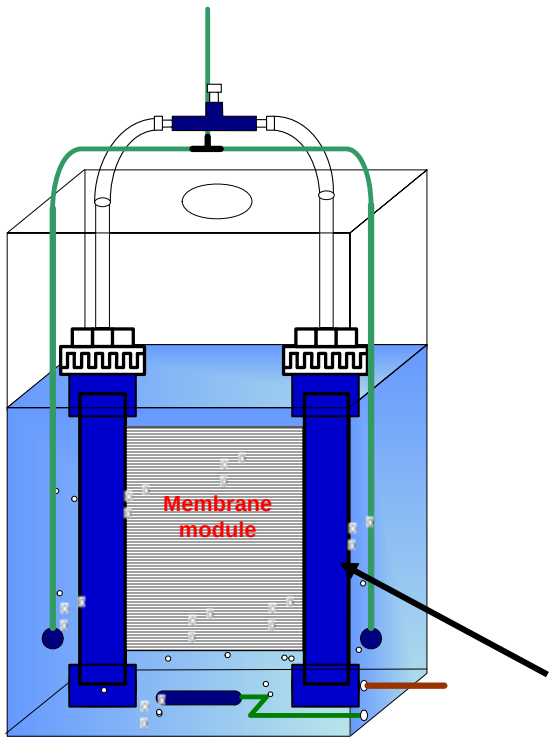




Substrate: Pentachlorophenol (PCP) and its salt (NaPCP)

Source: wood preservation, herbicide, fungicide, pesticide

Environmental risks: toxic, bioaccumulation, persistent in soil



Membrane bioreactor:

combination of activated sludge biodegradation and membrane separation

Advantage:

better effluent quality and lower sludge production.

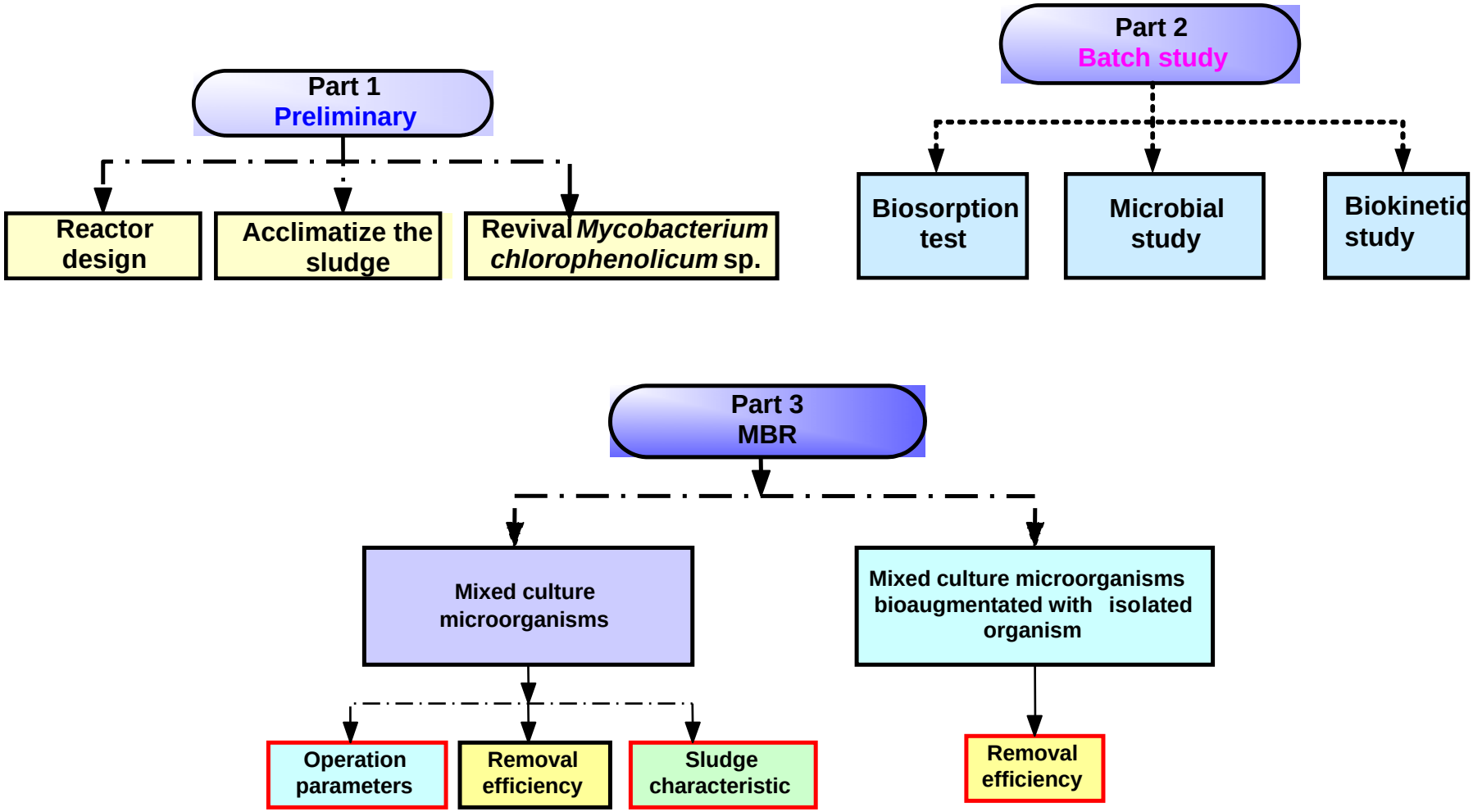
OBJECTIVES

Objectives

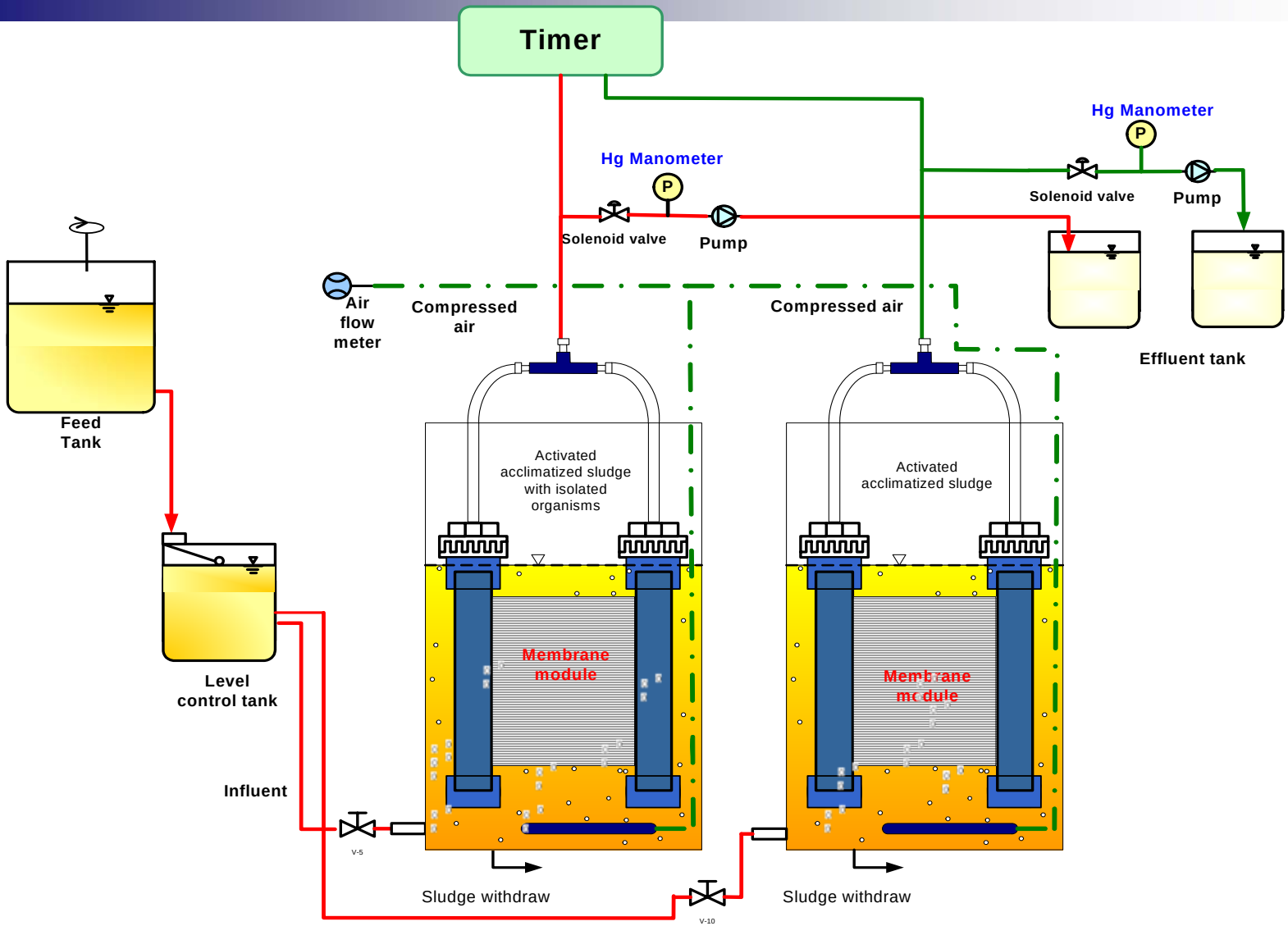
- Investigate the performance of membrane bioreactor to treat wastewater containing PCP in terms of removal efficiency, stability of process and membrane fouling.

- Enhancing the efficiency of removing PCP by bioaugmentation technique in inhibition case

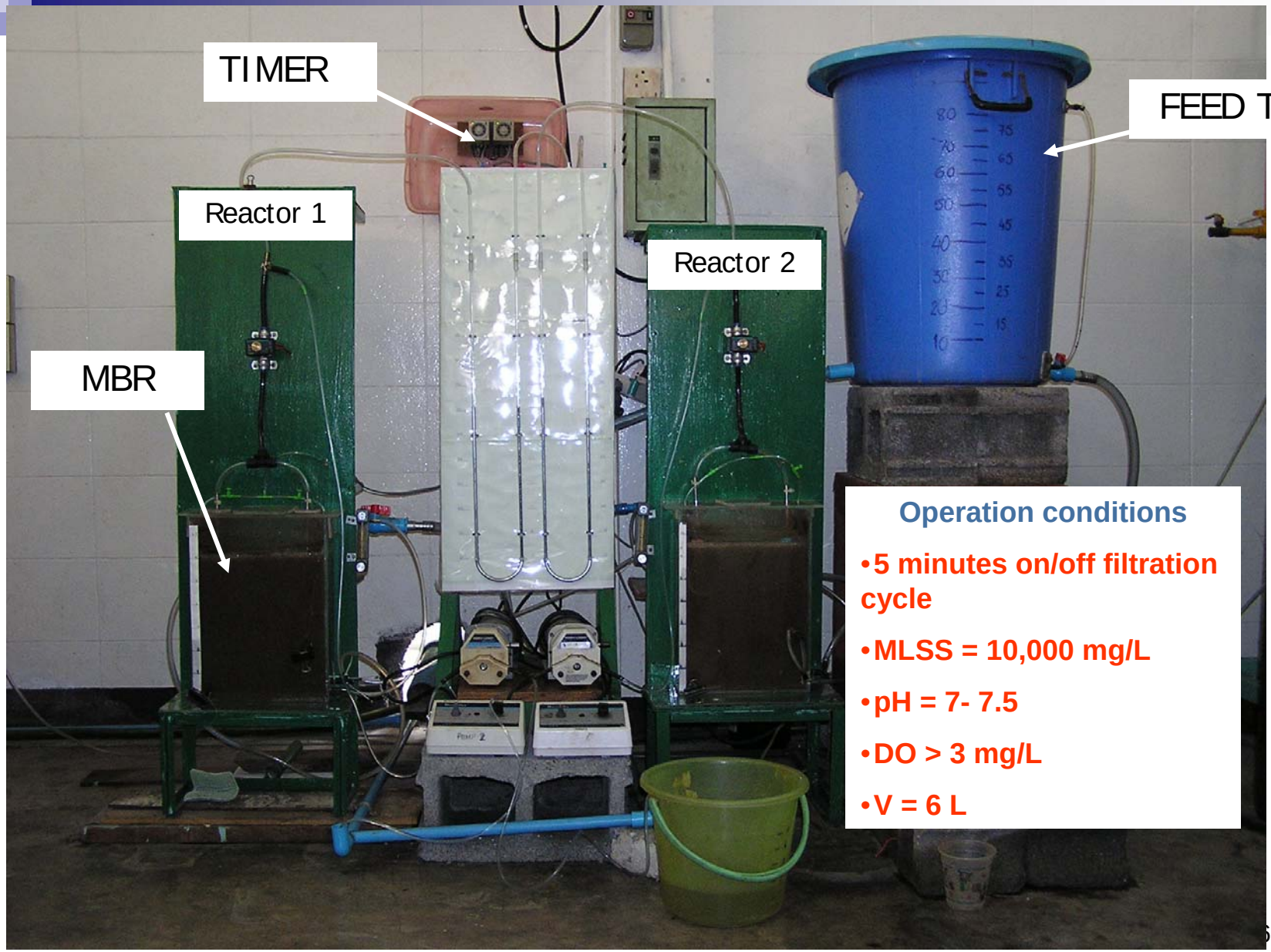
Research Methodology



Reactor Design



Reactor Design



Operation conditions

- **5 minutes on/off filtration cycle**
- **MLSS = 10,000 mg/L**
- **pH = 7- 7.5**
- **DO > 3 mg/L**
- **V = 6 L**

Wastewater Characteristics

COD value: 600 mg/L (actually 310-650mg/L)

Composition: PCP (NaPCP) and glucose, mineral salts

Composition of mineral salt for MBR system

Composition	Concentration (mg/L)
K_2HPO_4	1730
KH_2PO_4	680
$(NH_4)_2SO_4$	1000
$MgSO_4.7H_2O$	100
$FeSO_4.7H_2O$	30
$CaCl_2.2H_2O$	20
$MnSO_4.H_2O$	30

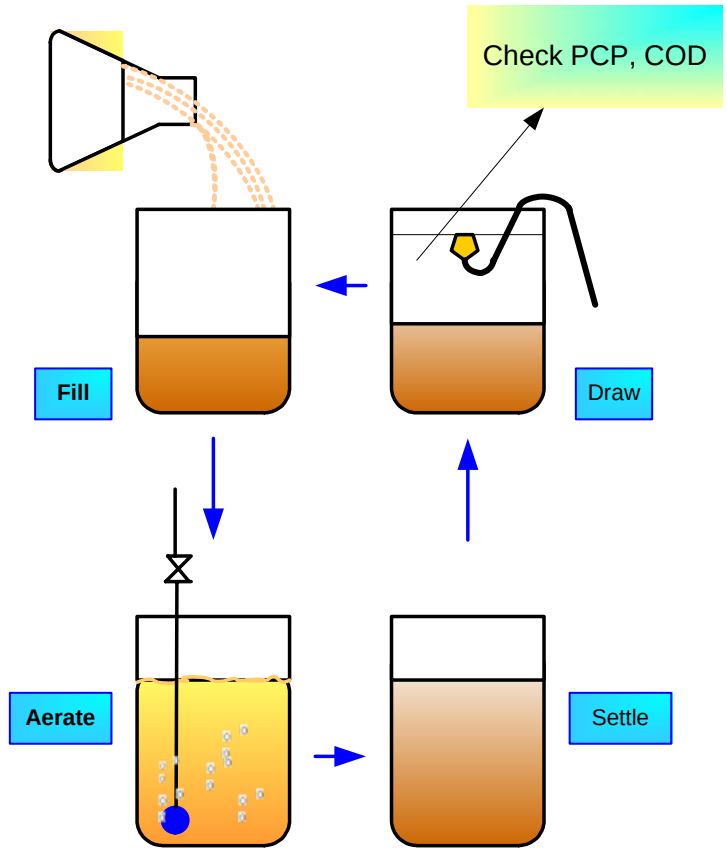
Acclimatization

Target: activated sludge resistant to PCP, increase the ability to degrade PCP

COD removal: 85-97%

PCP in the effluent lower than 0.1 mg/L

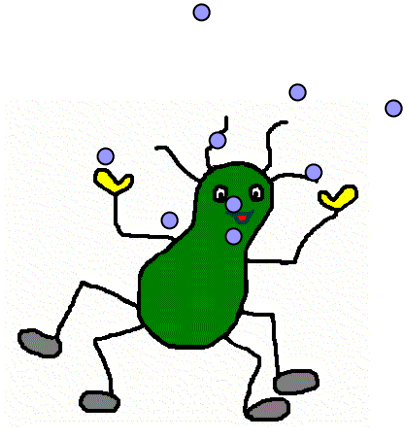
Operation conditions	Activated sludge
HRT (h)	24
MLSS (mg/L)	6500
COD (mg/L)	600
PCP (mg/L)	1, 5, 10
pH	7



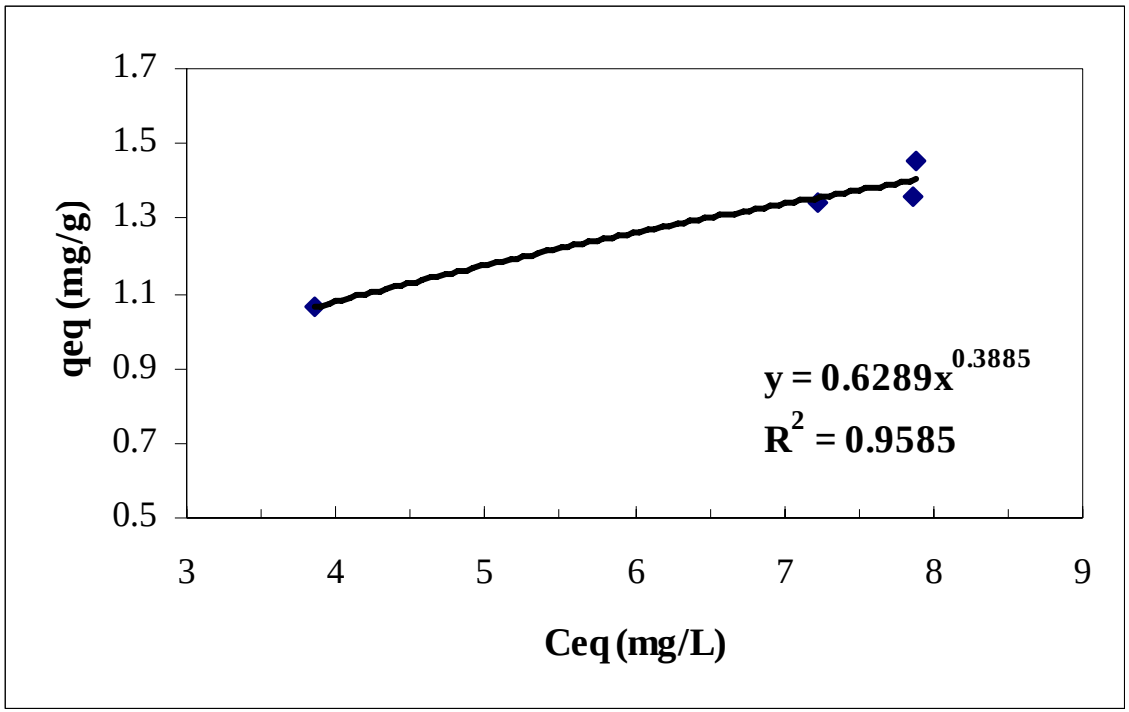
Biosorption Study

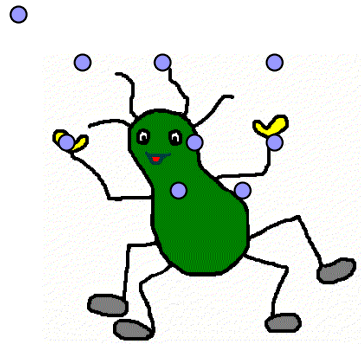
A. Biosorption equilibrium: **Sorption equilibrium could be achieved at 60 min**

B. **Biosorption isotherm:**



Biosorption Isotherm of PCP



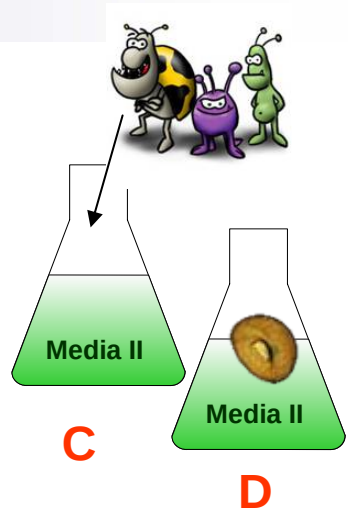


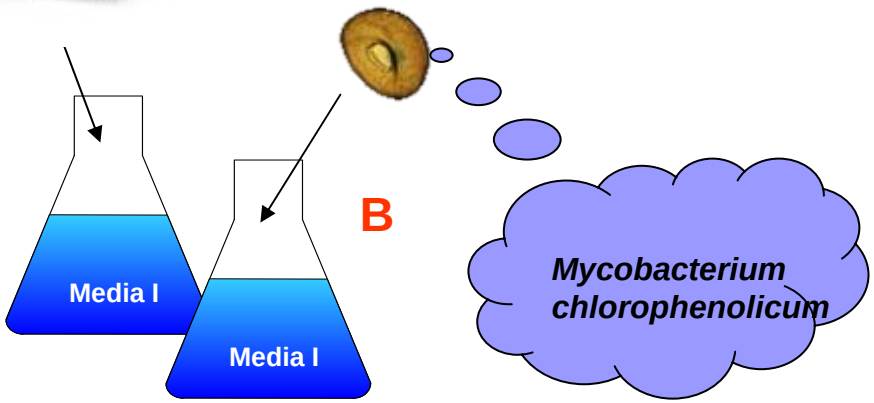
- **The sorption capacity 0.6289 mg/g VSS**
- **Enhance the degradation process**

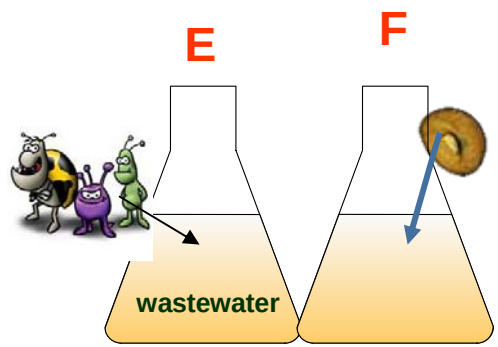
Compare the degradation of PCP with *Mycobacterium chlorophenolicum* and activated sludge

Conditions for the experiment with different media

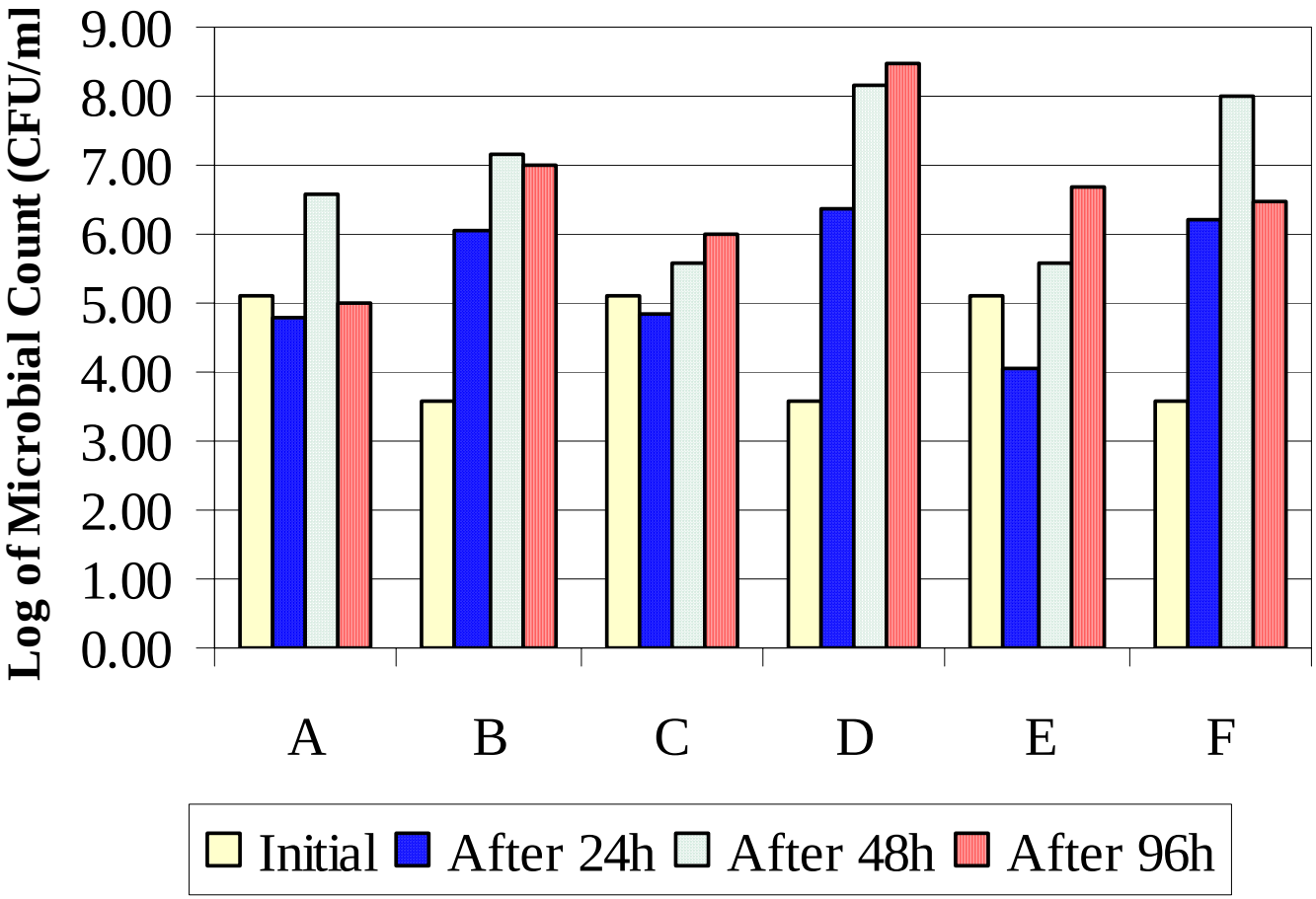
Flask ID	Media	Culture	Initial PCP Conc. (mg/L)	Initial Microbial Concentration (CFU/mL)
	Media I	Mixed Activated Sludge	0.88	1.20E+05
	Media I	*Specific Organism*	1.00	3.80E+03
	Media II	Mixed Activated Sludge	0.83	1.20E+05
	Media II	*Specific Organism*	1.00	3.80E+03
	Synthetic Wastewater	Mixed Activated Sludge	0.61	1.20E+05
	Synthetic Wastewater	*Specific Organism*	1.00	3.80E+03







Compare the degradation of PCP with *Mycobacterium chlorophenolicum* and activated sludge (cont.)



Compare th… CP with *Mycobacterium* …ivated sludge

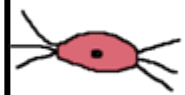
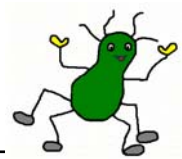
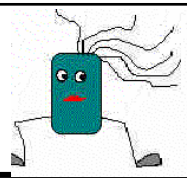
Isolation of microorganism from the activated sludge is a better option than using specific organism for bioaugmentation

					After 96h	
				Removal (%)	PCP (mg/L)	Removal (%)
				96	0.05	94
			0.80		0.72	28
C	0.83		77	0.11	0.05	94
D	1.00	0.82	NA	0.89*	NA	NA
E	0.61	0.04	93	0.11	82	92
F	1.00	0.53	47	1.42	NA	1.0

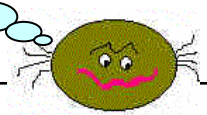
NaPCP is not suitable for me

- **Synt… …d in the membrane bioreactor was suitab… …ion**
- **Activat… sludge was much better than the specific r… …ng PCP**

Isolation of Microorganisms

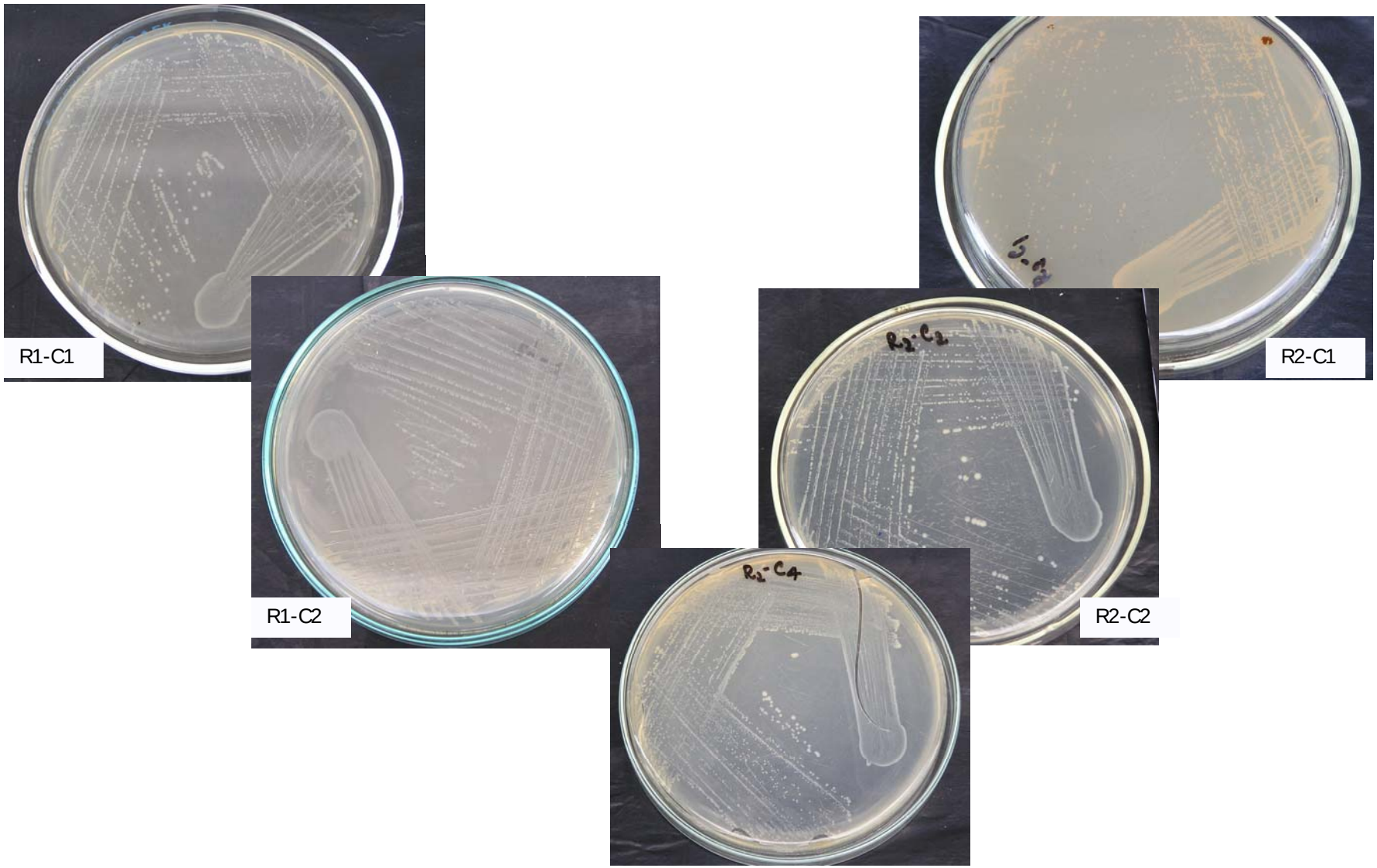
Reactor 1	Reactor 2
 R1-C1	R2-C1 
 R1-C2	R2-C2 
R1-C3	R2-C3 
	R2-C4 

I do not like PCP alone



I can not service with NaPCP

Isolation of microorganisms



Degradation of PCP by Isolated culture

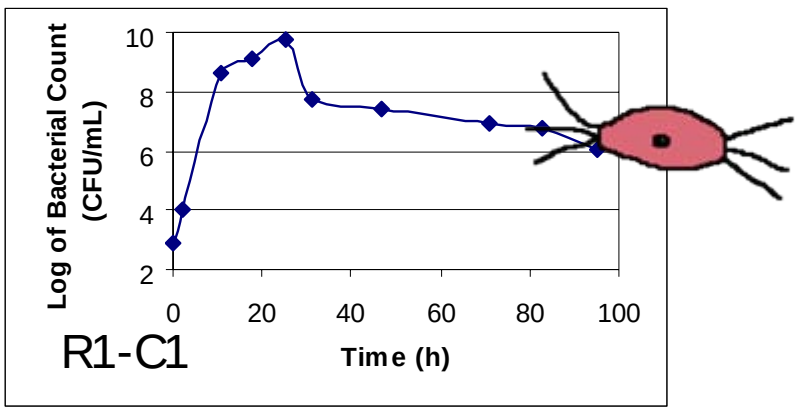
Culture	Initial PCP (mg/L)	After 24h		After 96h	
		PCP (mg/L)	Removal (%)	PCP (mg/L)	Removal (%)
Specific Organism	2.5	2.3	8	1.0	61
R_1-C_1	2.5	2.2	12	1.9	24
R_1-C_2	2.6	2.1	18	1.5	42
R_2-C_1	2.5	1.9	24	1.7	30
R_2-C_2	2.3	2.2	5	2.0	13
R_2-C_3	2.3	2.1	6	2.3	Error
R_2-C_4	2.0	2.1	Error	1.9	4

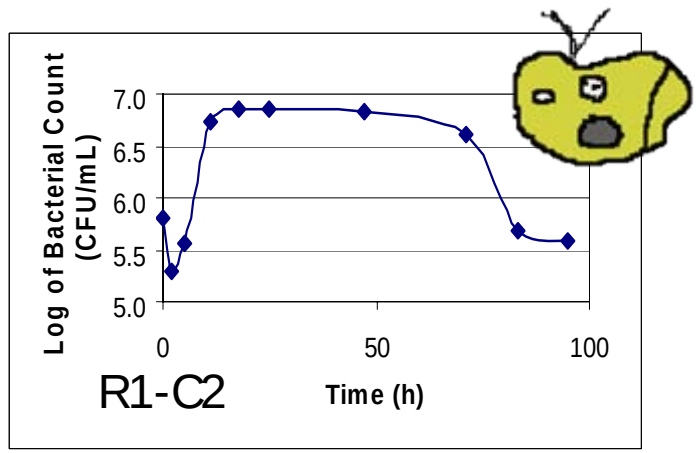
•The degradability of PCP by the isolated microorganisms was low. It was found that at the end of 96h, the special microorganism performed better than the isolated species.

•The better performance of the activated sludge from the reactor could be due to the cumulative effect of the various PCP degrading microorganisms

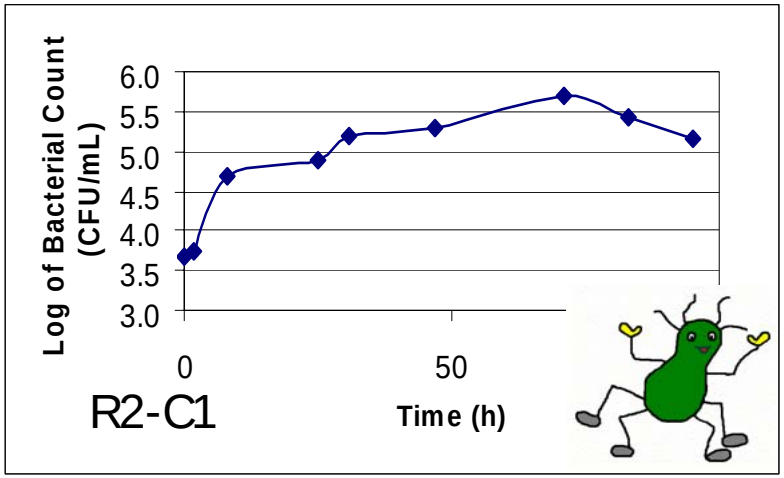
Log phase of the Isolated Cultures

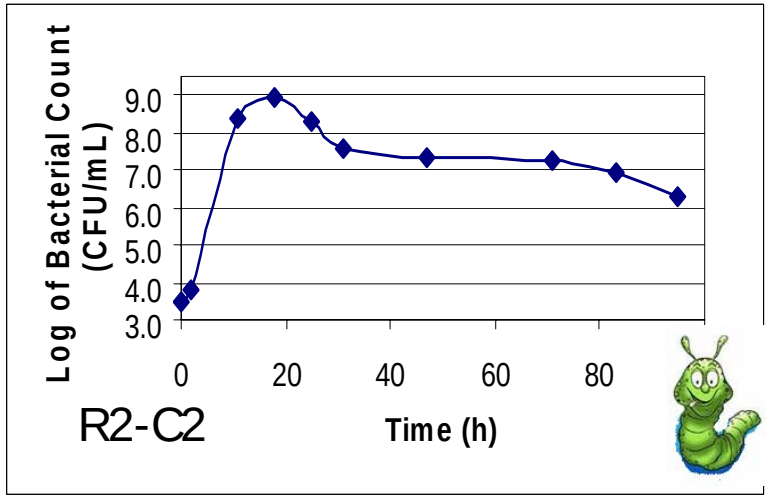
Cultures	Time (h)
R_1-C_1	25
R_1-C_2	25
R_2-C_1	71
R_2-C_2	18
R_2-C_4	18

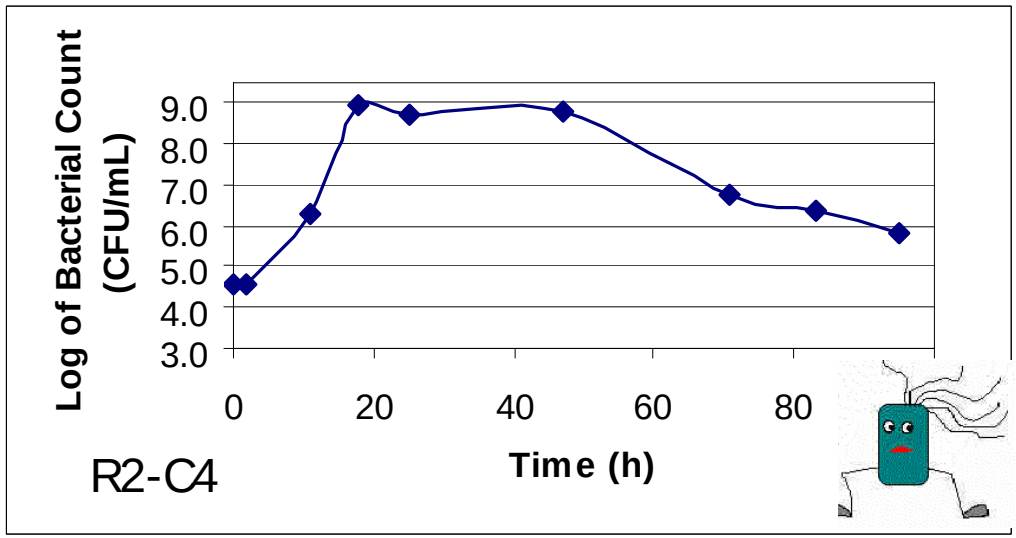




Log phase of the Isolated Cultures (cont.)



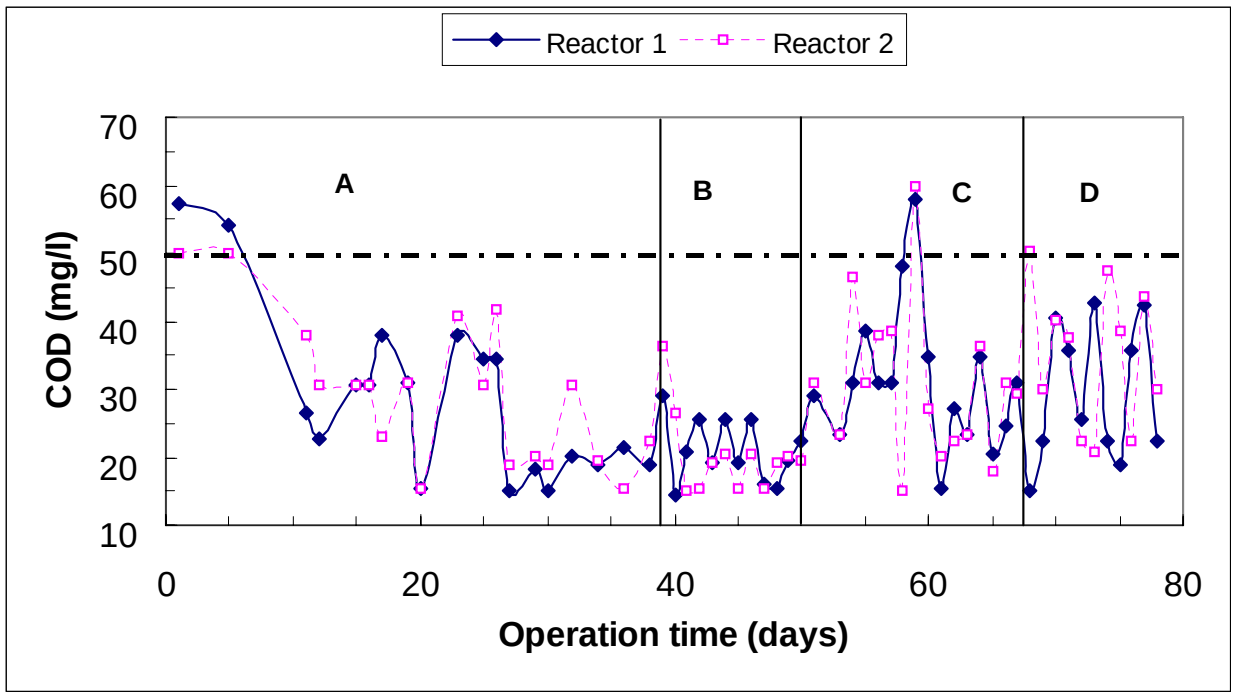




Performance of MBR

With PCP

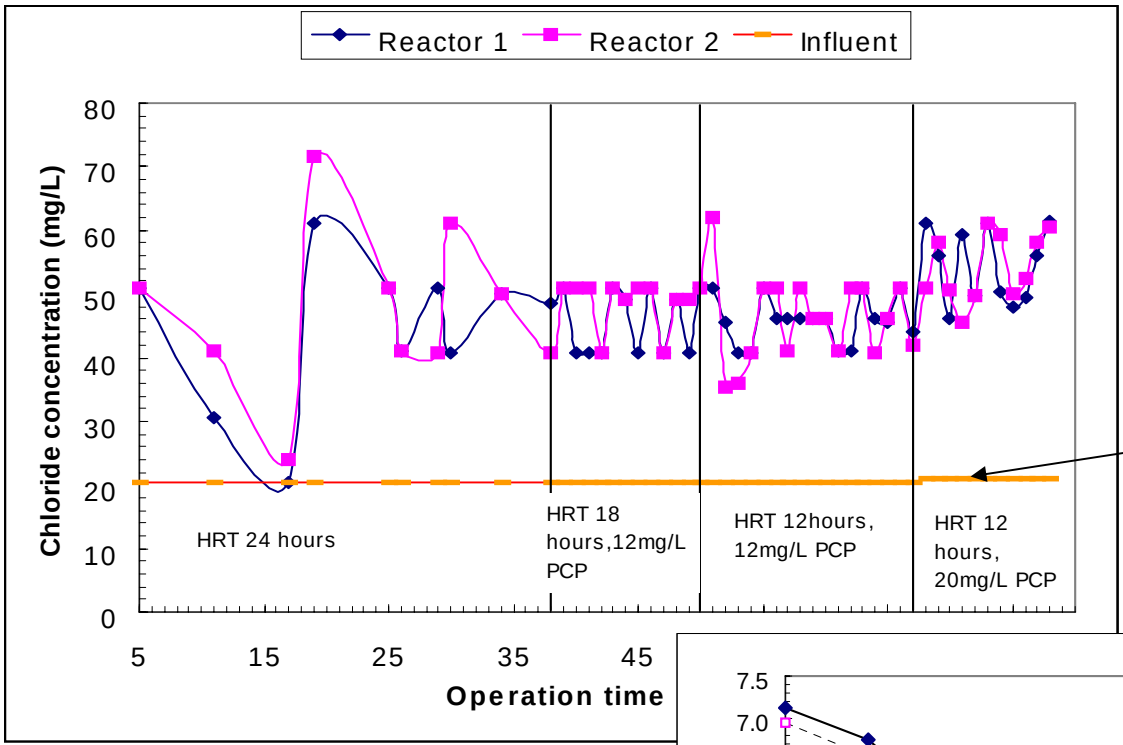
Stage of operation	MLSS	Toxic compounds concentration influent (mg/L)	Flowrate (L/day)	HRT (hours)
A	5,000-6,000	12 as PCP	6	24
B	8,000-10,000	12 as PCP	8	18
C	10,000±1,000	12 as PCP	12	12
D	10,000±1,000	20 as PCP	12	12



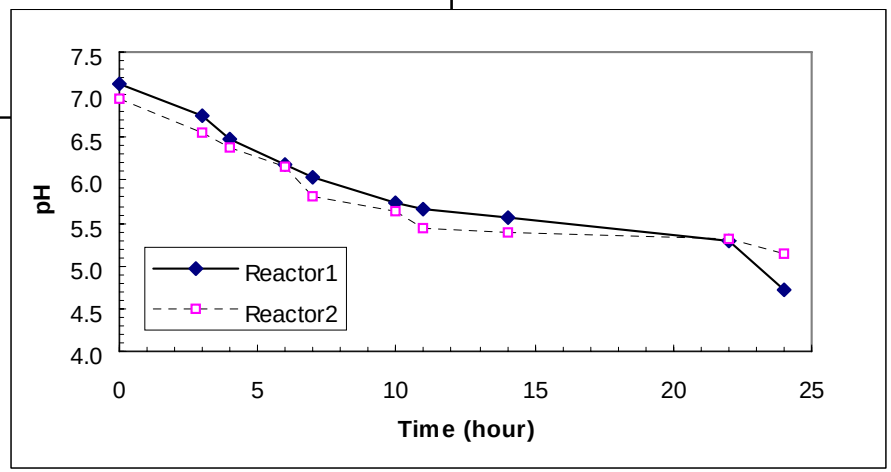
- PCP removal: 99%
- PCP lower than detected limit 0.005 mg/L
- COD removal 95%
- COD lower than 50mg/L

Performance of MBR

$$C_6HCl_5O + O_2 \rightarrow CO_2 + H_2O + 5Cl^-$$

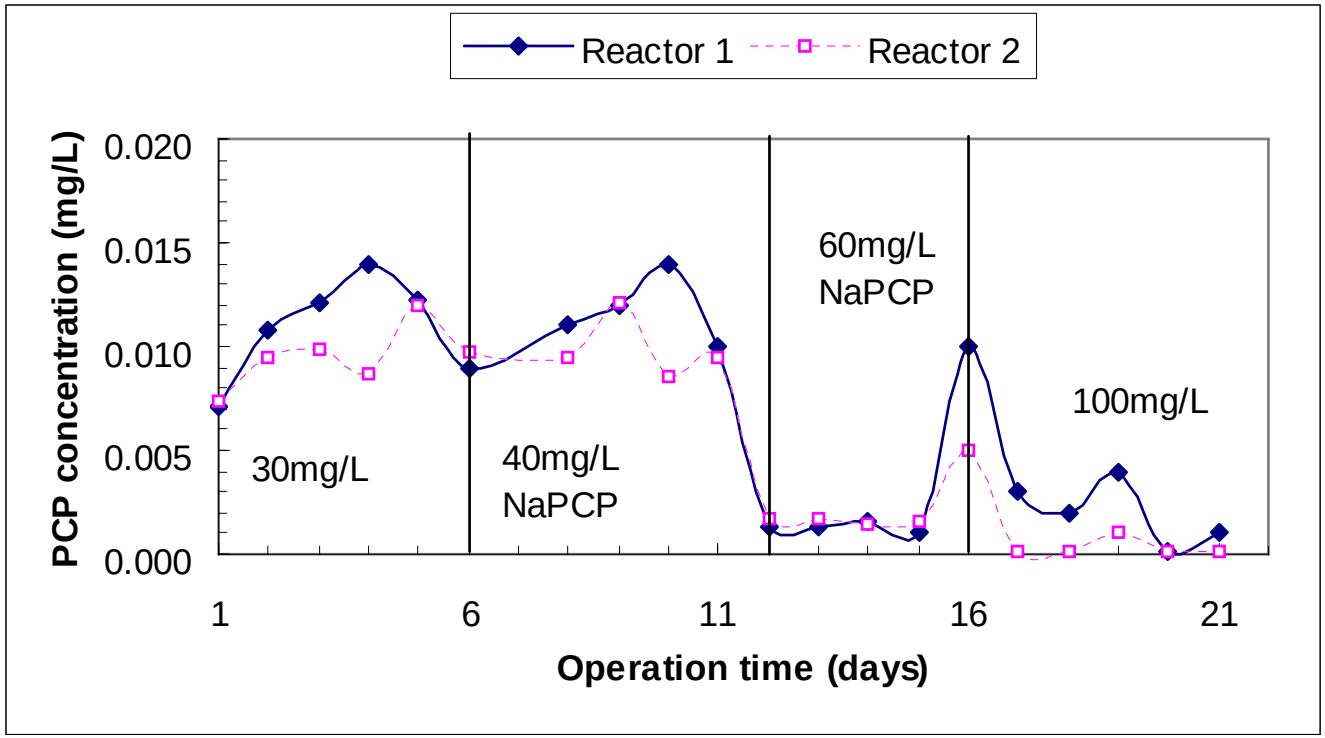


- Chloride in permeate is higher than in the influent
- pH drop down



Performance of MBR (cont.)

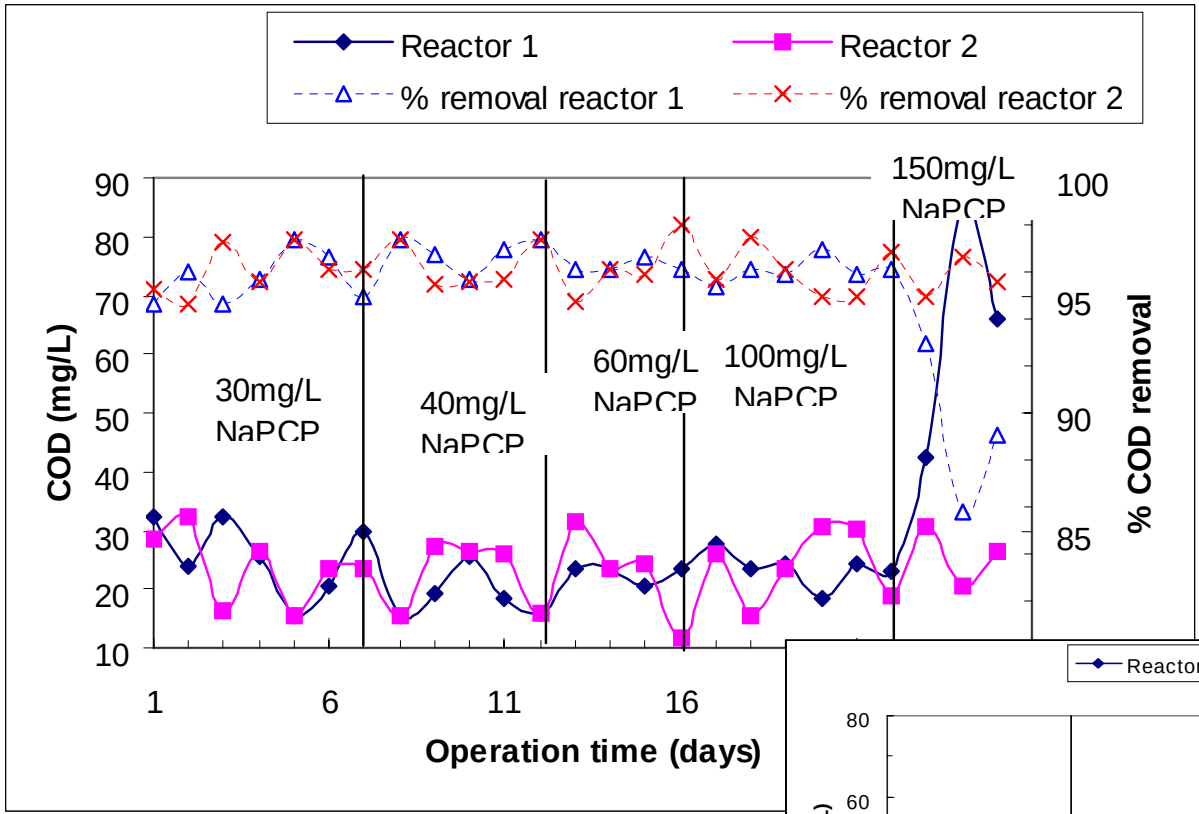
With NaPCP

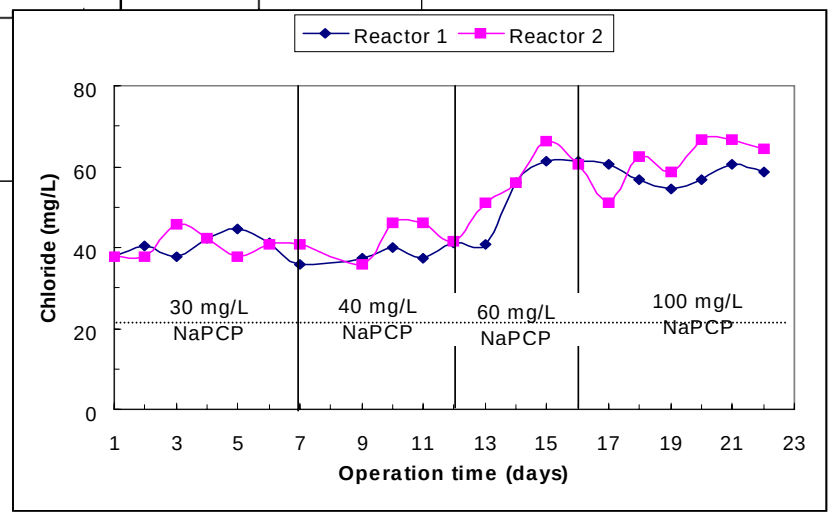


PCP removal 99%

COD removal 95%

Performance of MBR (cont.)





Inhibition

With 150 mg/L of NaPCP

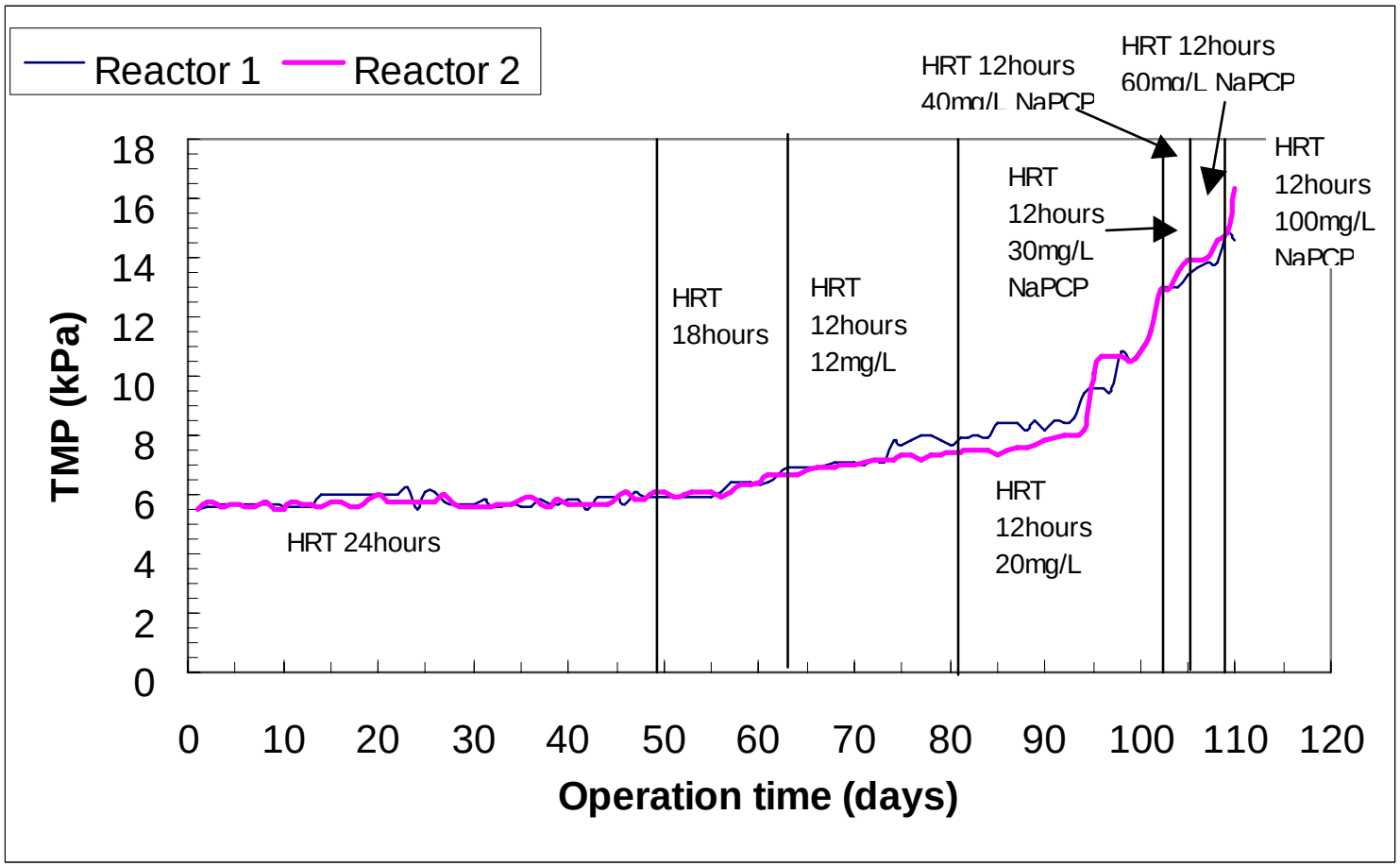
PCP concentration in permeate

Operation days	Reactor 1 (mg/L)	Reactor 2 (mg/L)
1	12.2	0.32
2	79.2	0.54
3	81.9	0.2

Different predominant organisms, different inhibition level of NaPCP

Bioaugmentation technique has applied to enhance performance

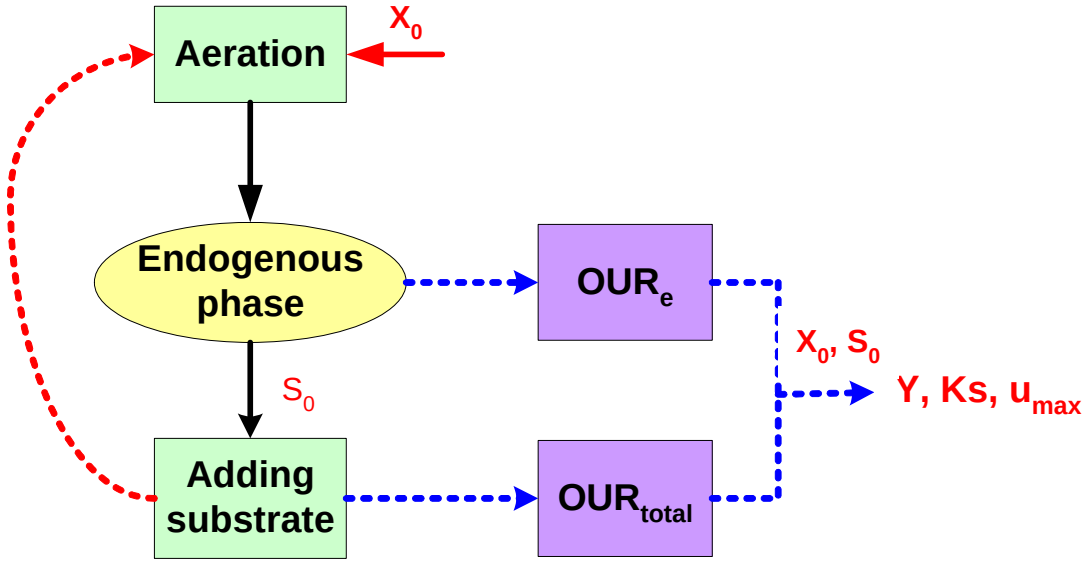
Membrane Resistance



Membrane fouling was low (15 kPa)

Irreversible fouling is mainly

Biokinetics Study



Coefficients	*Unit*	*Range*	*Typical*	*Reference*
K_s	mg/L BOD	25-100	60	Metalf and Eddy (2003)
	mg/LCOD	*27.55-38*		*This study*
Y	mgVSS/mgBOD	0.4-0.8	0.6	Metalf and Eddy (2003)
	mgVSS/mgCOD	*0.57-0.62*		*This study*
μ_{max}	day^{-1}	*27.55-38*		*This sudy*

Conclusions

1. **Biosorption of PCP on biomass was high 0.6289 mg/g biomass and occurred rapidly within 60 minutes.**

2. **Predominant organisms was isolated depending on their colony morphology. Their log phase was found in range of 18-71 hours.**

3. **For removing PCP, high biomass concentration around 8,000 to 10,000 mg/L was applied.**

4. **With PCP loading rate from 12 to 40 mg/m^3.day, PCP removal was higher than 99% and COD removal more than 95%.**

Conclusions (cont.)

5. **With NaPCP loading from 20- 200 mg/m^3.day, the NaPCP removal higher than 99%, COD removal higher than 96%.**

6. **150mg/L of NaPCP in the influent (300mg/m^3.day) was found causing inhibition.**

7. **After more than 120 days operation, the transmembrane pressure in both reactors was low 15 kPa.**

8. **The kinetic parameters of system for removing glucose was in range 27.55-38 mgCOD/L for K_S and 0.57-0.62 for Y. These values were similar as those of activated sludge.**

Recommendations

- **Toxicity test and intermediate test should be done to ensure completely mineralization of PCP.**
- **In practice, domestic wastewater or food wastewater should be combined with PCP containing wastewater in wood processing or pesticide manufacturing industry, to enhance the removal of both substrates as well as in terms of cost aspects.**
- **Wastewater from pesticide industry or wood processing not only contain PCP but also many phenolic compounds such as 2,3,4,6-tetraclorophenol, 2,4,5-trichlorophenol, 1,2,3,4- trichlorophenol, phenol, etc. The study in MBR for mixture of these substrates is recommended.**
- **Further studies about the biokinetic should be done in case of inhibition as well as the effect of PCP with other carbon sources such as glucose.**
- **Bioaugmentation technique should be continue in case of inhibition.**


Thank You