CHAPTER IV

RESULTS AND DISCUSSIONS

This chapter describes in details of characteristic and morphology dominant species from biofilter and biofiltration performance of three selected dominant species in term of removal efficiency and elimination capacity.

4.1 Biofilter Media Moisture Content

The biofilter media was determined initial moisture content at 105 °C 24 hr. The result for initial moisture contents of component in packing materials were 14.5 % for coconut husk, 18.1 % for manure and 70.5 % for waste water sludge. Water content of components in packing materials is shown in Table 4-1.

Table 4-1 Biofilter Media Moisture Content

Biofilter Media	% Moisture Content
Coconut Husk *	14.5 %
Manure*	18.1 %
Waste Water Sludge*	70.5 %
Mixed Sterilized Packing Materials **(coconut husk : manure ; 70:30)	15.5 %

Remark : * = packing materials for acclimatization step

** = packing materials for performance study

4.2 Operation Control Condition

The operation condition of biofiltration of acclimatization step and performance study were 50 % moisture content with room temperature 28-33 °C and low pressure drop 0.25-1 cm of water. pH was changing 5.5-8.5 for all operation period (Table 4-2).

Table 4-2 Operation Condition

Parameters	**M 1**	**M 2**	**M 3**
Ph	6-8	5.5-8	5.5-8.5
Temperature	28-33 °C	28-33 °C	28-33 °C
Pressure Drop	0.25-1cm of water	0.25-1 cm of water	0.25-1 cm of water
Moisture content	50 %	50 %	50 %

4.3 Isolation and Identification of Xylene Degrading Microorganisms

The sample of packing materials were determined after acclimatized with xylene vapor 8 hrs a day at flow rate of 1 L/m for 30 and 60 days. All degrading xylene microorganisms were selected with enrich media. Two strains of bacteria and 4 strains of fungi were found from the first acclimatization in 30 days. (Figure 4-1, 4-2, 4-3, 4-4 and 4-5). Only 4 types of fungi were found in 60 days after acclimatization. There was no bacteria or other microbial growth in enrich media.

In general, microbial ecosystems are able to response in a very dynamic manner to the environmental change, particularly to the changes that can stress to community. Total microorganisms after acclimatization 1 month was 9.0×10^7 to 8.2×10^8 CFU/g with M1, M2, M3, M4, B1, B2 in 22, 23, 23, 20, 5, and 6 % respectively. Total microorganisms after acclimatization 2 month were 8.5×10^6 to 8.0×10^7 CFU/g with M1, M2, M3, M4 in 22, 33, 33, and 11 % respectively (Table 4-3 and 4-4).

Table 4-3 Microorganisms from Biofilter

	1 Month	**2 Months**
Total plate Count (CFU/g)	9.0×10^7 to 8.2×10^8	8.5×10^6 to 8.0×10^7
Amount of Microorganisms (%)		
M1	22	22
M2	23	33
M3	23	33
M4	20	11
B1	5	Not found
B2	6	Not found

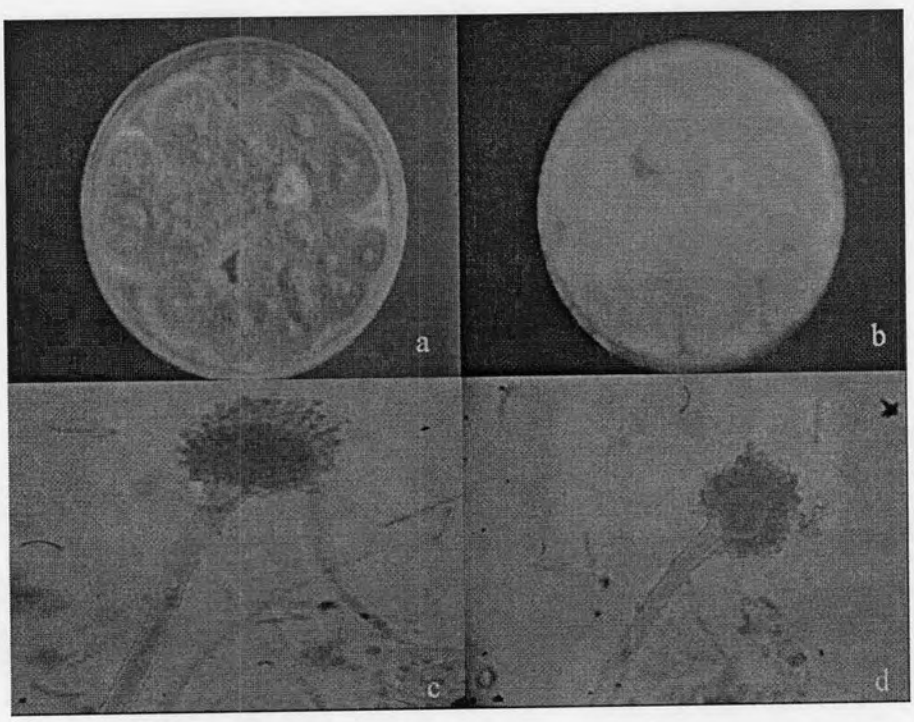

Figure 4-1 *Aspergillus flavus* (M1)

a,b : Colonies on SDA c,d : Columnar Vescicle, Hyphae and Conidia

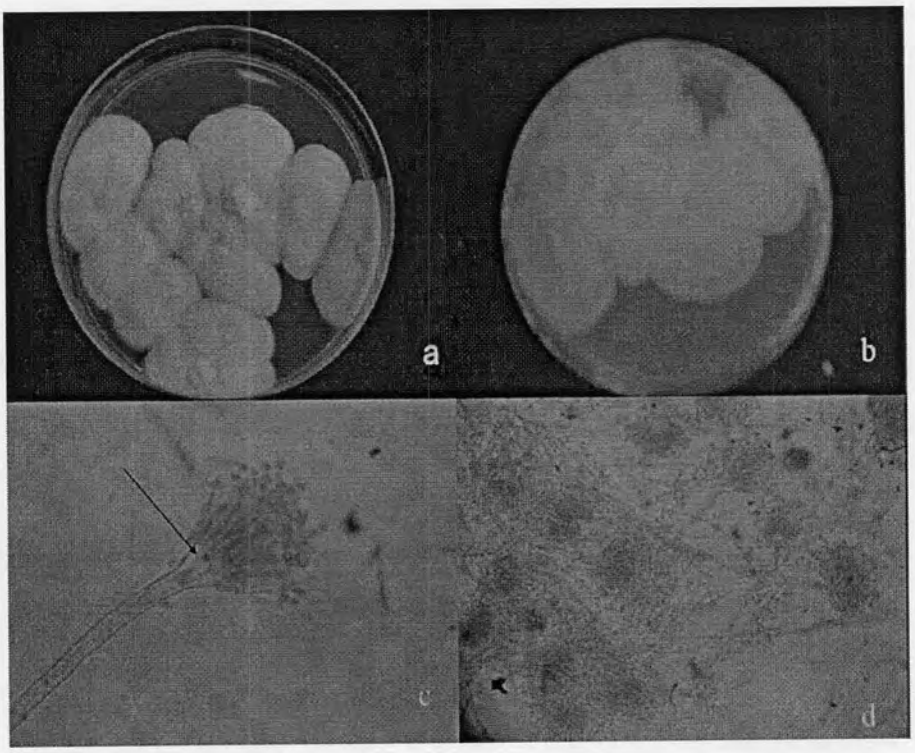

Figure 4-2 *Aspergillus terreus* (M2)

a,b : Colonies on SDA c,d : Columnar Vescicle, Hyphae and Conidia

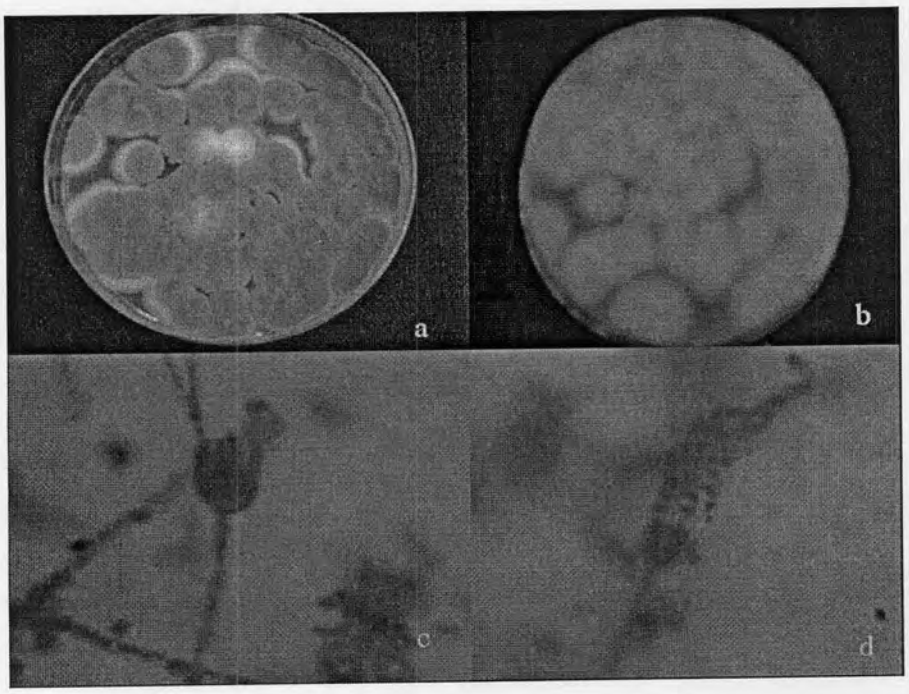

Figure 4-3 *Penicillium glabrum* (M 3)

a,b : Colonies on SDA c,d : Septate Hyphae, Sterigma and Conidia

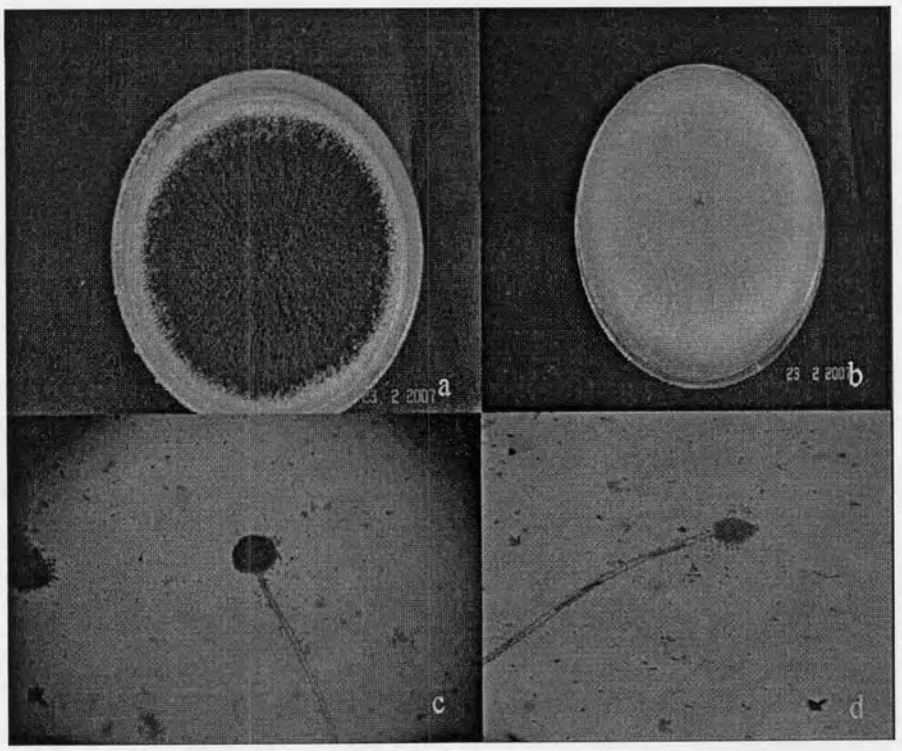

Figure 4-4 *Aspergillus niger* (M 4)

a,b : Colonies on SDA c,d : Conidiospores

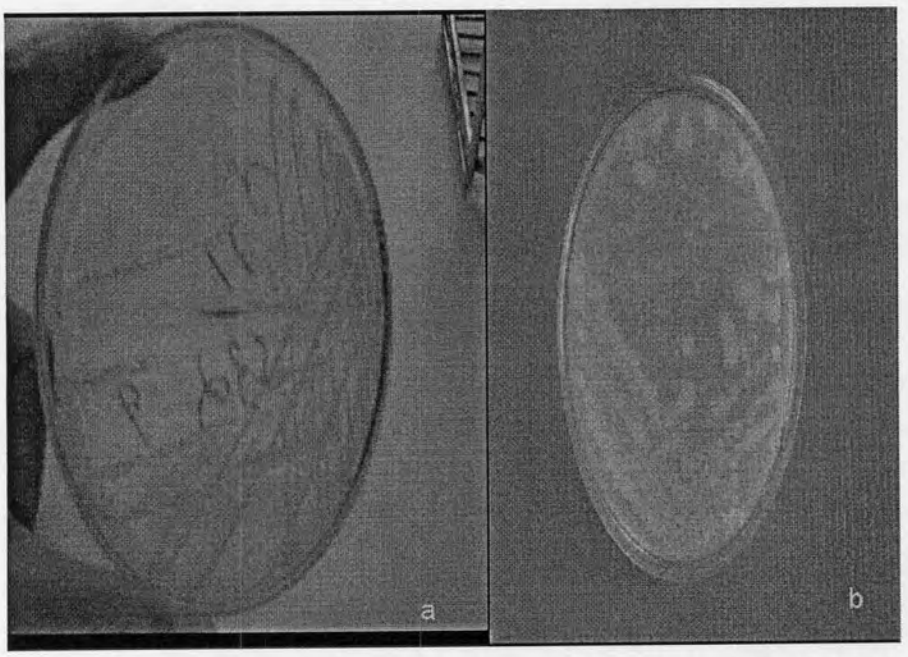

Figure 4-5 Colonies of Glucose Non Fermentative Bacteria on Nutrient Agar

a: Bacteria type 1 ; b : Bacteria type 2

4.3.1 Selected Degrading Xylene Microorganisms

All microorganisms from filter media were tested in selective media with xylene adding as a carbon source. The result shows 4 fungi that can grow up with xylene at 0.3 ml, 0.5 ml per plate but when adding too much xylene at 1 and 2 ml it was toxic to microorganisms. For both types of bacteria they could not survive in a selective media even a small amount of xylene (0.2 ml). It could be implied that bacteria could not degrade xylene in this experiment.

Table 4-4 Type of Microorganisms in Biofilter

1 month	**Morphology**	**Type of Microorganisms**
Bacteria	White to yellow colonies, size 1-2 mm. Gram negative rod	B1:Glucose non_fermentative bacteria (GNB)
	White Colonies size 2-3mm. Gram negative rod	B2:Glucose non_fermentative bacteria (GNB)
Fungi	Dark green colonies	M1: *Aspergillus flavus*
	Yellow to brown colonies	M2: *Aspergillus terreus*
	Gray colonies	M3: *Penicillium glabrum*
	Black colonies	M4: *Aspergillus niger*.
2 months		
Bacteria	Not found	
Fungi	Dark green colonies	M1: *Aspergillus flavus*
	Yellow to brown colonies	M2: *Aspergillus terreus*
	Gray colonies	M3: *Penicillium glabrum*
	Black colonies	M4: *Aspergillus niger*.

4.3.2 Identification

Four dominants species were identified by text, picture, index description and DNA sequencing method. There were identified as *Aspergillus flavus, Aspergillus terreus*, *Penicillium glabrum*, and *Aspergillus niger*. The morphologies of them are as the following:

***Aspergillus flavus* (M1)**

Macroscopic Morphology

Colony diameter after seven days on malt extract agar (MEA) and on potato dextrose agar (PDA) was 50-70 mm in diameter. Colonies were yellowish-green, consisting of a dense felt of conidiophores at room temperature (28-33 °C). Colonies on sabouraud dextrose agar (SDA) was olive to lime green with texture woolly to cottony and light brown exudate present. The growth rate was rapid.

Microscopic Morphology

Hyphae were septate with conidial heads typically radiated, later splitting into several loose columns, yellow-green becoming dark yellow-green. The Conidiophores was hyaline, coarsely roughened up to 1.0 mm in length. The shape of vesicle was globosely with 25-45 µm in diameter. Phialides of 6-10 x 4.0-5.5 µm in size was directly on the vesicle.

***Aspergillus terreus* (M2)**

Macroscopic Morphology

Colonies on malt extract agar (MEA) and on potato dextrose agar (PDA) at 28-33°C were cinnamon, reverse site was yellow and yellow soluble pigments were frequently present. The growth rate was moderate to rapid. Colonies became finely granular with conidial production. Colonies on malt extract agar were 29-39 mm in diameter with cinnamon conidia. Colonies on sabouraud dextrose agar

(SDA) were yellowish-brown to cinnamon-brown with a yellow reverse, texture cottony.

Microscopic Morphology

Hyphae were septate and hyaline and conidial heads were in the form of compact columnar. Conidiophores were smooth walled, with length ranging from 70 - 300 μm long. Conidia were globose, smooth, and small with size of 2 – 2.5 μm in diameter. Accessory conidia were hyaline with size of 2 – 6 μm, and were frequently produced on submerged hyphae.

Macroscopic Morphology

Colonies on malt extract agar (MEA) and on potato dextrose agar (PDA) at 28-33°C were cinnamon, reverse side was yellow and yellow soluble pigments were frequently present. The growth rate was moderate to rapid. Colonies become finely granular with conidial production. Colonies on malt extract agar was 29-39 mm in diameter with cinnamon conidia. Colonies on sabouraud dextrose agar (SDA) was yellowish-brown to cinnamon-brown with a yellow reverse, texture cottony.

***Penicillium glabrum* (M3)**

Macroscopic Morphology

Colonies on malt extract agar (MEA) and on potato dextrose agar (PDA) at 28-33°C were flat, filamentous, and velvety, woolly, or cottony in texture. The colonies were initially white and become gray green and olive gray. Colonies on sabouraud dextrose agar (SDA) were velvety, gray-green and reverse yellow to yellow-orange. The growth rate was rapid.

Microscopic Morphology

There was no foot-cell, the stalk arising from an undifferentiated cell of the mycelium. The conidiophore was *septate* and ends in a whorl of short branches, each of which beared a whorl of little branches or *sterigmata*, which formed the conidia. They were simple or branched and were terminated by clusters of flask-

shaped phialides. The spores (conidia) were produced in dry chains from the tips of the phialides, with the youngest spore at the base of the chain, and were nearly always green.

Aspergillus niger **(M4)**

Macroscopic Morphology

Colonies on malt extract agar (MEA), on potato dextrose agar (PDA) and on sabouraud dextrose agar (SDA) at 28-33°C were initially white, quickly becoming black with conidial production. Reverse site was pale yellow and growth may produce radial fissures in the agar.

Microscopic Morphology:

Hyphae were septate and hyaline. Conidial heads were radiate initially splitting into columns at maturity. The species was biseriate (vesicles produces sterile cells known as metulae that support the conidiogenous phialides). Conidiophores were long (400-3000 μm) smooth and hyaline becoming slightly brown near the vesicle. terminating in a globose vesicle (30-75 μm in diameter). Metulae and phialides coverd the entire vesicle. Conidia were brown to black, very rough, globose, and measured 4-5 μm in diameter. Vesicle was globose. Metulae and phialides covered entire vesicle.

4.4 DNA Sequencing

Dominant species were confirmed and identified by DNA sequencing method. DNA was extracted using the method of Makimura *et al.* (1994). The internal transcribed spacer (ITS) was amplified using the primer pairs ITS-1 (5' TCC GTA GGT GAA CCT GCG G) and ITS-4 (5' TCC TCC GCT TAT TGA TAT GC). Four sequence of them were indicated as *Aspergillus flavus, Aspergillus terreus, Penicillium glabrum, and Aspergillus nige* by following.

Aspergillus flavus

TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGTAGGGTTCC
TAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCG
GGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCG
CCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGT
ATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGA
TGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTG
AATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGC
ATGCCTGTCGAGCGTCATGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTC
GTCGTCCCCCATCATCCCAGGGG

genomic DNA 422 bp *Aspergillus flavus*

Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;

Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.

REFERENCE 1 (bases 1 to 422)

BASE COUNT 84 a 122 c 116 g 100 t

ORIGIN

1 tccgtaggtg aacctgcgga aggatcatta ccgagtgtag ggttcctagc gagcccaacc
61 tcccacccgt gtttactgta ccttagttgc ttcggcgggc ccgccattca tggccgccgg
121 gggctctcag ccccgggccc gcgcccgccg gagacaccac gaactctgtc tgatctagtg
181 aagtctgagt tgattgtatc gcaatcagtt aaaactttca acaatggatc tcttggttcc
241 ggcatcgatg aagaacgcag cgaaatgcga taactagtgt gaattgcaga attccgtgaa
301 tcatcgagtc tttgaacgca cattgcgccc cctggtattc cgggggggcat gcctgtcgag
361 cgtcatgctg cccatcaagc acggcttgtg tgttgggtcg tcgtccccca tcatcccagg
421 gg //

Aspergillus terreus

TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTC
GTGGCCCAACCTCCCACCCGTGACTATTGTACCTTGTTGCTTCGGCGGGCC
CGCCAGCCCTGCTGGCCGCCGGGGGGCGTCTCGCCCCCGGGCCCGTGCCC
GCCGGAGACCCCAACATGAACCCTGTTCTGAAAGCTTGCAGTCTGAGTGT

GATTCTTTGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGC
ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATT
CAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG
GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTT
GGGTCCTCGTCCCCCGGCTCCGGGGGACGGGCCCGAAAGGCAGCGGCGG
CACCGCGTCCGTCTTTTTTTATTGAAAATGGGGTTTGTCTTCCGCTCCGCCT
CCCCCCCCCCTTTTTTTTTCC

genomic DNA 522 bp *Aspergillus terreus*

Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.

REFERENCE 1 (bases 1 to 522)

BASE COUNT 87 a 164 c 143 g 128 t

ORIGIN

1 tccgtaggtg aacctgcgga aggatcatta ccgagtgcgg gtcctcgtgg cccaacctcc
61 cacccgtgac tattgtacct tgttgcttcg gcgggcccgc cagccctgct ggccgccggg
121 gggcgtctcg ccccgggccc cgtgcccgcc ggagacccca acatgaaccc tgttctgaaa
181 gcttgcagtc tgagtgtgat tctttgcaat cagttaaaac tttcaacaat ggatctcttg
241 gttccggcat cgatgaagaa cgcagcgaaa tgcgataact aatgtgaatt gcagaattca
301 gtgaatcatc gagtctttga acgcacattg cgcccctgg tattccgggg ggcatgcctg
361 tccgagcgtc attgctgccc tcaagcccgg cttgtgtgtt gggtcctcgt ccccggctc
421 cgggggacgg gcccgaaagg cagcggcggc accgcgtccg tcttttttta ttgaaaatgg
481 ggtttgtctt ccgctccgcc tccccccccc ctttttttt cc //

Penicillium glabrum

TCCGTAGGTGAACCTGCGGAAGGATCATTACTGAGTGAGGGCCCTC
TGGGTCCAACCTCCCACCCGTGTTTATTGTACCTTGTTGCTTCGGTGCGCC
CGCCTCACGGCCGCCGGGGGGCTTCTGCCCCCGGGTCCGCGCGCACCGGA
GACACTATTGAACTCTGTCTGAAGATTGCAGTCTGAGCATAAACTAAATA
AGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAAC
GCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCG
AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTC

CGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTCCGTCCCC
CCGGGGACGGGTCCGAAAGGCAGCGGCGGCACCGAGTCCGGTCCTCGAG
CGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCCAGCCGAC
AACCAATCATCCTTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACTTAAGC

genomic DNA 560 bp DNA *Penicillium glabrum.*

Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Penicillium.

REFERENCE 1 (bases 1 to 560)

BASE COUNT 111 a 164 c 158 g 127 t

ORIGIN

1 tccgtaggtg aacctgcgga aggatcatta ctgagtgagg gccctctggg tccaacctcc
61 cacccgtgtt tattgtacct tgttgcttcg gtgcgcccgc ctcacggccg ccgggggggct
121 tctgcccccg ggtccgcgcg caccggagac actattgaac tctgtctgaa gattgcagtc
181 tgagcataaa ctaaataagt taaaactttc aacaacggat ctcttggttc cggcatcgat
241 gaagaacgca gcgaaatgcg ataactaatg tgaattgcag aattcagtga atcatcgagt
301 ctttgaacgc acattgcgcc ccctggtatt ccgggggggca tgcctgtccg agcgtcattg
361 ctgccctcaa gcacggcttg tgtgttgggc tccgtcccct cggggacggg tccgaaaggc
421 agcggcggca ccgagtccgg tcctcgagcg tatggggctt tgtcacccgc tctgtaggcc
481 cggccggcgc cagccgacaa ccaatcatcc ttttttcagg ttgacctcgg atcaggtagg
541 gatacccgct gaacttaagc//

Aspergillus niger

TCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTT
TGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCC
CGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCC
CGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTG
ATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCA
TCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTC
AGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
GGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTG

GGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGC
ACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGAT
TGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATAT

genomic DNA 585 bp *Aspergillus niger*

Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes;
Eurotiales; Trichocomaceae; mitosporic Trichocomaceae; Aspergillus.

REFERENCE 1 (bases 1 to 585)

BASE COUNT 107 a 175 c 170 g 133 t

ORIGIN

1 tccgtaggtg aacctgcgga aggatcatta ccgagtgcgg gtcctttggg cccaacctcc
61 catccgtgtc tattgtaccc tgttgcttcg gcgggcccgc cgcttgtcgg ccgccggggg
121 ggcgcctctg ccccccgggc ccgtgcccgc cggagacccc aacacgaaca ctgtctgaaa
181 gcgtgcagtc tgagttgatt gaatgcaatc agttaaaact ttcaacaatg gatctcttgg
241 ttccggcatc gatgaagaac gcagcgaaat gcgataacta atgtgaattg cagaattcag
301 tgaatcatcg agtctttgaa cgcacattgc gccccctggt attccggggg gcatgcctgt
361 ccgagcgtca ttgctgccct caagcccggc ttgtgtgttg ggtcgccgtc cccctctccg
421 gggggacggg cccgaaaggc agcggcggca ccgcgtccga tcctcgagcg tatggggctt
481 tgtcacatgc tctgtaggat tggccggcgc ctgccgacgt tttccaacca ttctttccag
541 gttgacctcg gatcaggtag ggatacccgc tgaacttaag catat //

4.5 Biofiltration Performance

The performance of a biofilter was evaluated in term of removal efficiency, or pollutant elimination capacity as a function of pollutant loading of gases. Biofiltration of xylene vapor was carried out over a periods of 95 days in a various operation conditions, the type of microbial, flow rate, inlet concentration, carbon dioxide production , moisture content , pH and temperature. The biofilter was initially acclimatized by operating the biofilter at low concentration and low gas flow rate for 7 days. This was necessary to obtain sufficient biomass concentration in the filter bed. During the 95 days the operation was under the conditions of 50 % moisture content

pressure drop 0.25-1 cm of water, pH ranging from 5.5-8.5 and at room temperature of 28-33° C.

There dominants species selected from 4 strains are 2 strains of Aspergillus and one of Penicilium. Penicillium was select to study performance as a different genus fungi. Among of 3 strains of Aspergillus, *A. Flavus* and *A. terreus* were selected instead of *A. niger* because both of them were slightly in higher amount in plate than *A. niger*. In this study, fungi were at room temperature and pH range 5.5-8.5 with initial moisture content 50 %. Only fungi were found as dominant species from biofilter because fungi could grow in more general conditions than bacteria. Fungi are able to grow under both neutral as well as acidic conditions and to be metabolically active over a wide pH range of approximately 2 -7, depending on the species. Bacteria are usually considered to be less tolerant to pH fluctuations and require a near neutral environment for their activities (Kennes and Veiga, 2004). From the study of Mohammad *et al*, 2007 for the long term operation reactor, dominant species were also fungi. In the mesophilic reactor, fungi became dominant after long-term operation, while bacteria dominated in the thermophilic unit. Microbial acclimation was achieved by exposing the biofilters to initial BTEX loads of 2 to 15 g m^{-3} h^{-1}, at an empty bed residence time of 96 s. After adaptation, the elimination capacities ranged from 3 to 188 g m^{-3} h^{-1}, depending on the inlet load, for the mesophilic biofilter with removal efficiencies reaching 96%.

In this experiment, fungi were found to be more effective to degrade xylene than bacteria; similar results have been observed from other studies. Form the study of biofilters of toluene (Aizpuru *et al.,* 2005), elimination rates was higher than those of bacterial systems. However, strong mycelia growth can cause clogging ((Aizpuru *et al.,* 2005). From the study on the treatment of mixed VOCs (Qi *et al.,* 2005), biofilter packed with a cubed polyurethane foam media initially inoculated with a pure culture of the fungus *Cladosporium sphaerospermum*, was maintained under acidic conditions throughout the duration of the experiments to treat a mixture of *n*-butyl acetate, methyl ethyl ketone, methyl propyl ketone, and toluene. The results

demonstrate that fungal biofilters can consistently maintain high removal efficiency for paint VOC mixtures over extended periods of operation.

4.5.1 Influence of Gas Flow Rate

An important hydrodynamic parameter in the biofilter is the input gas flow rate because it quantifies the amount of pollutant, which is to be treated per unit time. Table 4-5 shows the biofilter operation condition of the experiment. Three dominants species were inoculated in 2, 3, and 4 for xylene treatment in 95 days. Control column (column 1) was operated without inoculate microorganism as same condition of three treatment column (column2, 3 and 4). The results are shown in Table 4-6, Table 4-7 and Table 4-8 for M1, M and M3, respectively. The effects of gas flow rate on removal efficiency and elimination capacity were investigated at three flow rates, i.e. 0.2, 0.5 and 1 L/m. The increase in gas flow rate led to decrease in the time of contact between xylene and filter material and so a decrease in the residence time of xylene gas which caused a reduction in the removal efficiency. Figure 4-6 illustrates the influence of gas flow rate on the removal efficiency for M1, M2 and M3 in 95 days. For higher flow rate of 1 L/m (EBRT of 140 s), the removal efficiency was lower than at flow rate of 0.5 L/m (EBRT of 240 s) and 0.2 L/m (EBRT of 705 s).

Removal efficiency of biofilter varies with the media characteristics including dimensions and type of media, air flow rate, inlet loading and gases to be removed. In this experiment, flow rate was varied at a time, while keeping other parameters constant. Figure 4-6 shows the removal efficiency of xylene at different flow rates. The removal efficiency of M1 at 0.2 L/m was 63.2-96.6 % with highest at day 26 when inlet loading was 0.91 g $m^{-3}h^{-1}$. The highest removal efficiency of 0.5 L/m was 74.0 % at day 74 when inlet loading rate was 32.8 g $m^{-3}h^{-1}$. For the flow rate of 1L/m, the highest removal efficiency of 61.8 % was observed at day 86 with inlet loading rate of 138 g $m^{-3}h^{-1}$.

The removal efficiency of M 2 at 0.2 L/m was similar to M1 at 62.2-96.4 % with highest at day 41 after operating with inlet loading 2.2 g $m^{-3}h^{-1}$. For flow rate of 0.5 L/m, the highest removal efficiency was 74.5 % at day 74 for inlet loading rate of 32.8 g $m^{-3}h^{-1}$. The highest removal efficiency of 1 L/m was 61.2 % at day 83 with inlet loading rate of 135 g $m^{-3}h^{-1}$.

The removal efficiency of M3 was higher than those of M1 and M2 at all flow rates. For low flow rate of 0.2 L/m, the removal efficiency was 70.6-99.2 % which was higher than 63.2-96.6 % of M1 and 62.2-96.4 % of M 2. The highest removal rate was found at day 32 with inlet loading of 1.30 g $m^{-3}h^{-1}$. The highest removal efficiency of 0.5 L/m was 81.0 % at day 74 with inlet loading rate of 31.3 g $m^{-3}h^{-1}$. For low rate of 1 L/m, the highest removal efficiency of 77.3 % was observed at day 83 for inlet loading rate of 149 g $m^{-3}h^{-1}$.

Table 4-5 Operational Sequence and Conditions for Biofilter

Period (d)	Inlet Conc.($g.m^{-3}$)	EBRT(s)	Purpose
0-7	0.01-1.3	705	Biofilter start-up
8-65	0.01-1.3	705	Steady-state performance test
66-79	1.9-3.3	280	Steady-state performance test
80-95	5.2-6.3	140	Steady-state performance test

Table 4-6 Performance of Microorganisms 1 (M1)

Flow Rate (L/m)	Operation time (d)	Inlet Conc. ($g\ m^{-3}$)	%RE	EC ($gm^{-3}h^{-1}$)	IL ($gm^{-3}h^{-1}$)	EBRT (s)
0.2	1-65	0.01-1.1	63.2-96.6	0.05-4.6	0.06-6.3	705
0.5	66-79	1.9-3.3	68.8-74.0	17.7-28.8	24.8-41.9	280
1	80-95	5.3-6.4	51.9-61.8	70.7-88.4	136-162	140

Table 4-7 Performance of Microorganisms 2 (M2)

Flow Rate (L/m)	Operation time (d)	Inlet Conc. (gm^{-3})	%RE	EC ($gm^{-3}h^{-1}$)	IL ($gm^{-3}h^{-1}$)	EBRT(s)
0.2	1-65	0.01-1.11	62.2-96.3	0.06-4.00	0.07-5.65	705
0.5	66-79	1.9-3.3	65.2-74.5	17.7-27.5	24.9-42.3	280
1	80-95	5.3-6.3	52.7-61.2	74.0-90.8	135-162	140

Table 4-8 Performance of Microorganism 3 (M3)

Flow Rate (L/m)	Operation time (d)	Inlet Conc. (gm^{-3})	%RE	EC ($gm^{-3}h^{-1}$)	IL ($gm^{-3}h^{-1}$)	EBRT(s)
0.2	1-65	0.01-1.2	70.6-99.2	0.07-4.7	0.07-6.3	705
0.5	66-79	2.0-3.2	77.1-81.0	19.6-32.5	25.4-41.0	280
1	80-95	5.3-6.3	57.4-77.3	91-115	134-161	140

Table 4-9 Performance of Control Column

Flow Rate (L/m)	Operation time (d)	Inlet Conc. (gm^{-3})	%RE	EC ($gm^{-3}h^{-1}$)	IL ($gm^{-3}h^{-1}$)	EBRT(s)
0.2	1-65	0.01-1.3	7.4-28.8	0.01-1.5	0.08-6.8	705
0.5	66-79	2.3-3.0	14.3-25.4	4.1-12.6	38.2-42.6	280
1	80-95	5.2-6.3	9.4-19.1	16.3-24.2	158-160	140

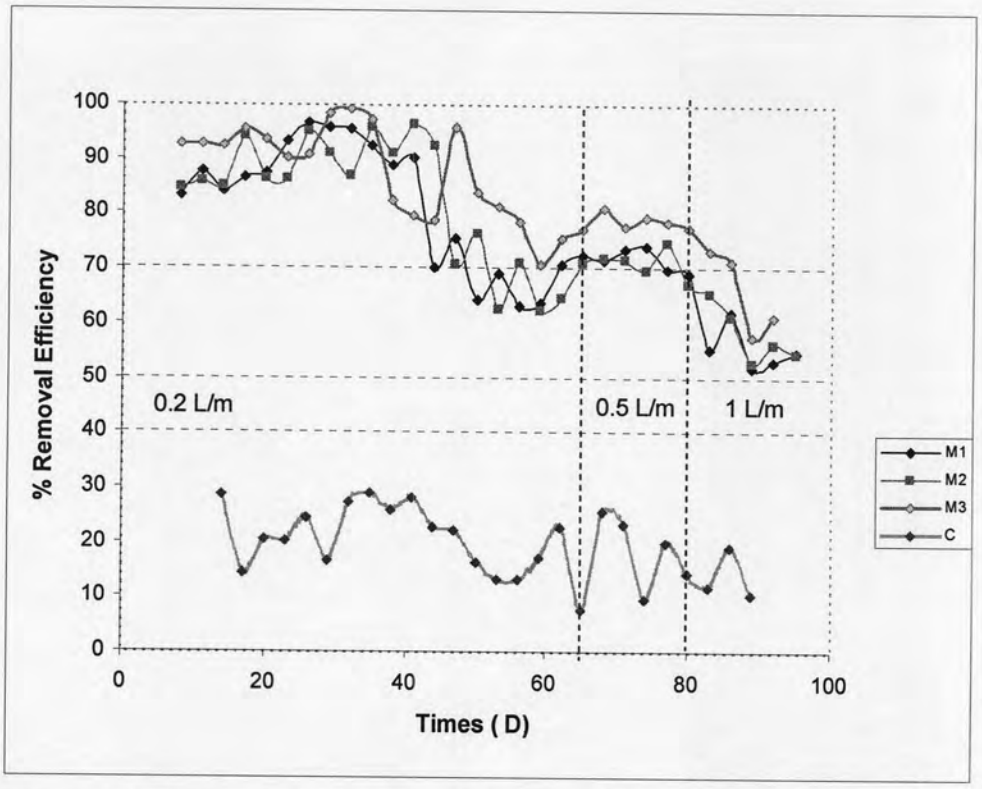

Figure 4-6 Removal Efficiency and Times at Different Flow Rate

In this study, the acclimation period was 7 days and it was the time required to reach higher removal efficiency. During this period, the microorganisms presumably produce enzymes capable of degrading xylene vapor. After the acclimation period, the removal efficiency for lowest flow rate increase rapidly to reach approximately 96.6, 96.4, and 99.2 % on the 26th, 44th and 32nd day operating for M1, M2 and M3, respectively.

The variations in percentage of xylene removal with time trend to gradually decrease from the first week as shown in Figure 4-7. The performance of M1 and M2 was almost the same but M 3 had a slightly higher performance.

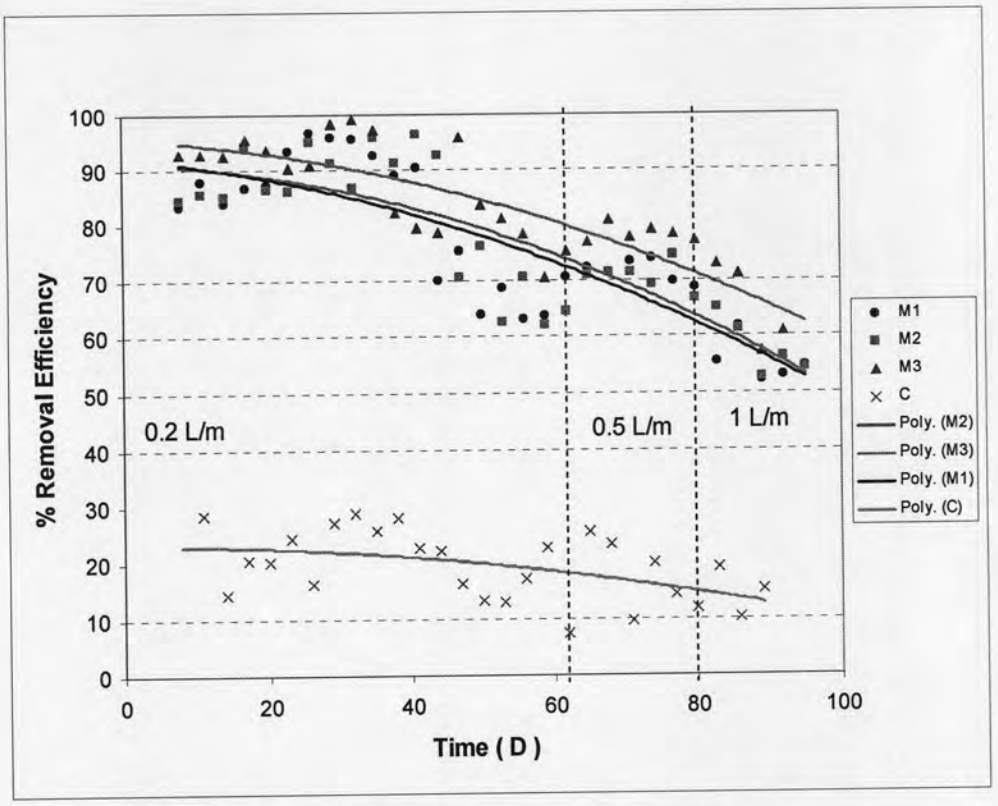

Figure 4-7 Trend Line of Removal Efficiency and Times

4.5.2 Influence of Inlet Concentration

The inlet concentration is an important factor for biofiltration process, with too high inlet concentration will be toxic to the microorganisms. At the start up period of about 7 days, xylene, as sole carbon source for microorganisms, was fed to biofilter with low concentration of about 0.01-1.3 g m^{-3}. During this period, the microorganisms acclimatize to packing materials. After 7 days, the removal efficiencies are increased to the highest efficiency at almost 100 % for M1, M2, and M 3 as shown in Figure 4-8.

In this study, with longer empty bed retention time and lower flow rate condition, it was found that single culture of microorganisms can degrade highly concentration (0.01-6.3 g $m.^{-3}$) than mixed culture of microorganisms in biofilter of other studies at 0.2–4 g m^{-3} (Jorio *et al.*, 2000) and 1.39 g m^{-3} (Elmrini *et al.*, 2004).

From the research result as shown in Figure 4-8, the removal efficiency of control column was in average of 18.9 % ,therefore the adsorption process can reduce the amount of xylene when it take more time in bed filter.

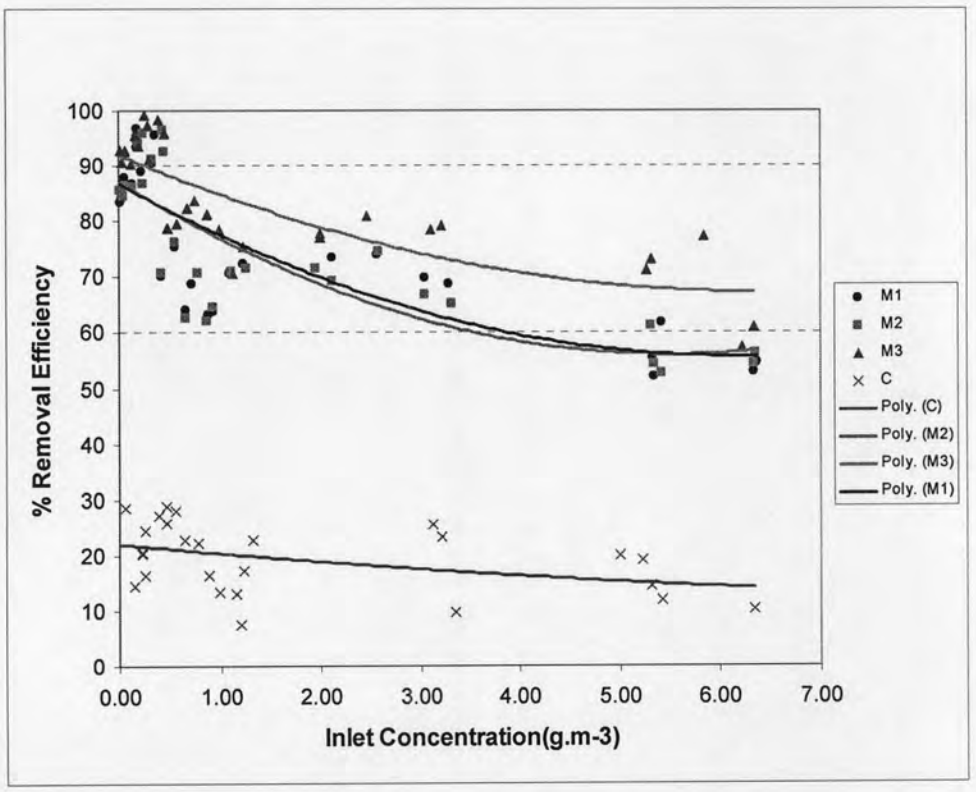

Figure 4-8 % Removal Efficiency and Inlet Concentration

4.5.3 Elimination Capacity

The influence of the inlet load on the elimination capacity in all microorganisms of M1, M2 and M3 are shown in Figure 4-9. As can be observed, the maximum removal capacity was higher for the M3. The maximum elimination capacity for the M 3 was 115 $gm^{-3}h^{-1}$ at a loading rate of 149 $gm^{-3}h^{-1}$ with removal efficiency of almost 77.3 %. The maximum elimination capacity of xylene by M1 and M2 were nearly equal at 88.3 and 90.8 $gm^{-3}h^{-1}$ for the same loading rate of 162 $gm^{-3}h^{-1}$ with removal efficiency of about 54.5 and 56.1 %, respectively. The average ratios of elimination capacity /loading rate were 0.76, 0.76 and 0.83 for M 1, M 2, and M 3, respectively.

The average elimination capacity of M1, M2, and M3 were 18.5, 16.1 and 22.2 $g.m^{-3}$ h^{-1}, respectively. The elimination capacity of control on average was 5.1 $gm^{-3}h^{-1}$. The maximum elimination capacity of M1 and M2 are nearly the same at the value of 88.4 and 90.8 gm^{-3} h^{-1}. However, M3 have higher maximum value of elimination capacity at 115 gm^{-3} h^{-1}. It could be implied that M3 had a better activity than M1 and M2. The elimination capacities with different loading rates are shown in Figure 4-9. It also showed the linear relationship between biofilter loading rate and elimination capacity for xylene by M1 M2 and M3. The slopes of all lines were similar at 0.9913, 0.9936 and 0.9816, respectively.

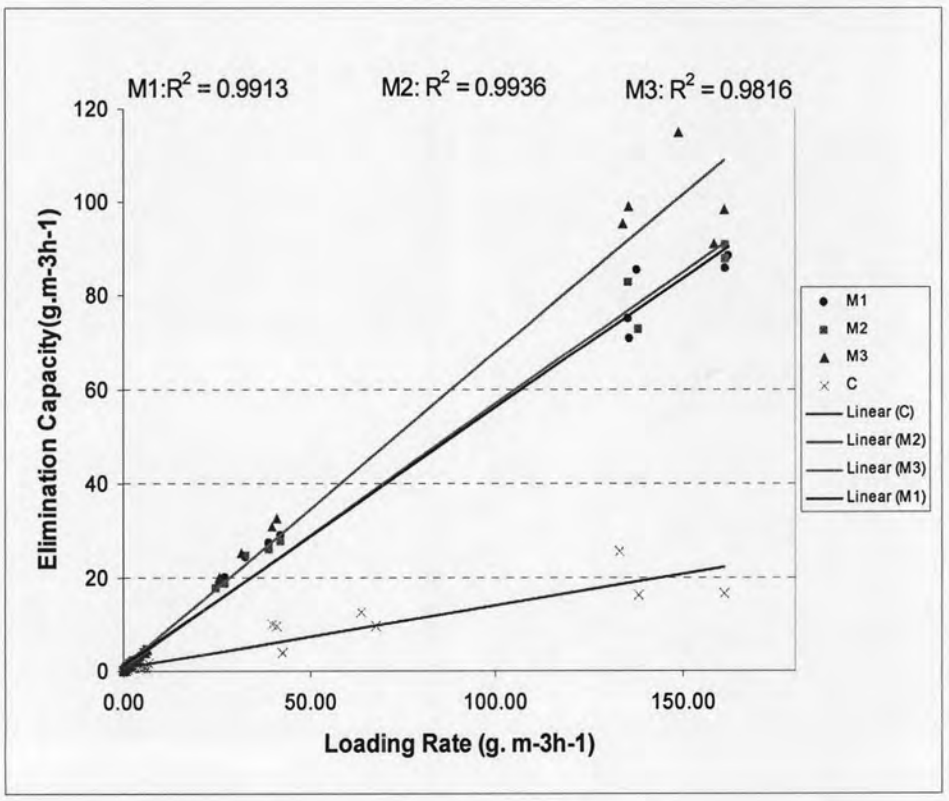

Figure 4-9 Elimination Capacity and Loading Rate

4.5.4 Carbon dioxide Production

Production of carbon dioxide is an important indicator for degree of biodegradation of organic pollutant by microorganisms. The organic contaminants are

degraded by aerobic heterotrophic microbial species in biofiltration to water and carbon dioxide and utilized as carbon source to form biomass for microbial growth. Hence, the profile of carbon dioxide concentration in gas phase at inlet and outlet of biofilter provides valuable information on biofiltration performance. The ratio between CO_2 production and EC, the mass of CO_2 produced per mass of xylene removed should be 3.3 in the case of complete oxidation of xylene to water and carbon dioxide according to the following stoichiometric reaction:

$$\underset{106\ +336}{C_8H_{10}+10.5O_2} \longrightarrow \underset{352+\ 90}{8\ CO_2+5H_2O}$$

Mass balance can evaluate from the xylene degradation to carbon dioxide and water. 1 mole of xylene will degrade to be 8 moles of carbon dioxide and 5 moles of water, then 106 g of xylene should degrade and generate 352 g of carbon dioxide in completed degradation. The completed reaction ratio was 3.3 (352/106).

In case of biodegradation of organic pollutants, a fraction of consumed organic carbon was used for the microbial growth according to the following metabolism:

Organic pollutant+Oxygen→Carbondioxide+Water+Cellular material

In this study, the relationship of CO_2 production and loading rate is shown in Figure 4-10. The mass ratio of the practical PCO_2 and EC for M1 was on average of 1.82 and in range of 0.76 – 3.0 The average ratio of PCO_2 and EC for M2 was 1.96 with the range of 0.62 – 2.9 In case of M3, the ratio was a slightly higher than M1 and M2, in the range of 0.67-3.1 with average ratio of 1.96. The average mass ratio of the practical PCO_2 was approximately 1.89 which was lower than theoretical theory value of 3.3. The possible reasons are that some biodegrade xylene was converted into biomass for microbrial growth and the CO_2 produced may be accumulated in aqueous phase in some forms, such as HCO_3^-, H_2CO_3, CO_3^{-2}.

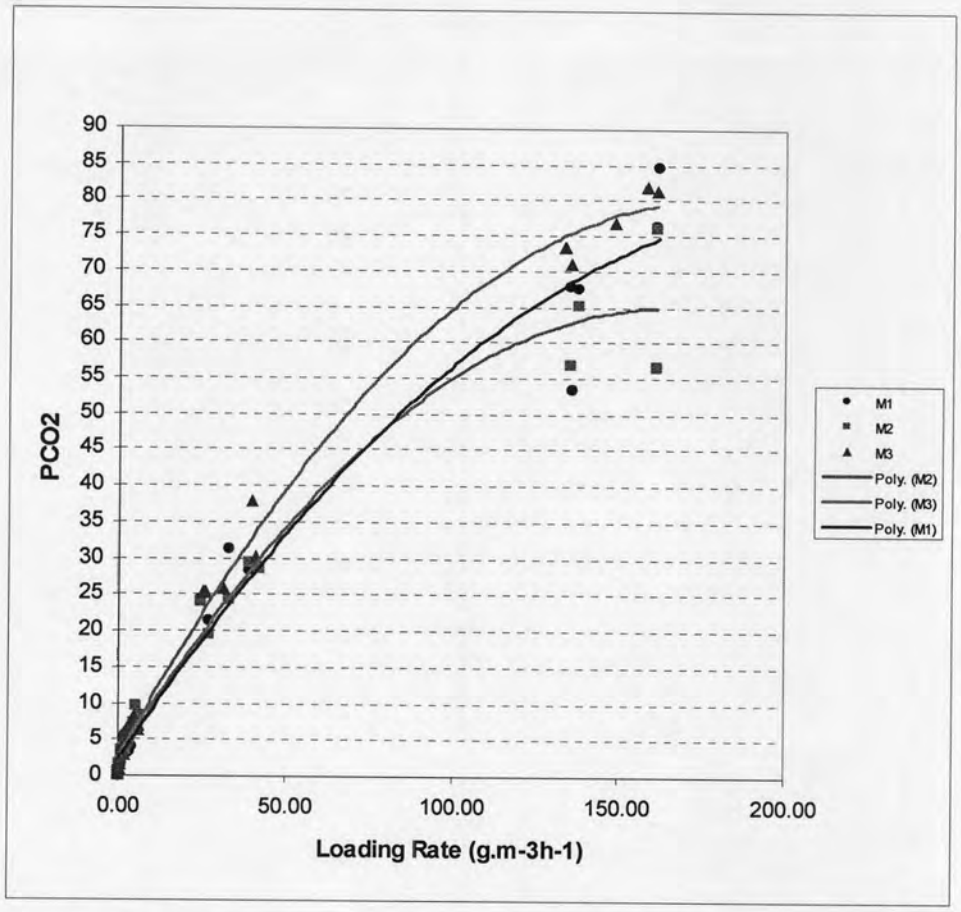

Figure 4-10 Carbon Dioxide Production and Loading Rate

The relationship between elimination capacity and carbon dioxide production are shown in Figure 4-11. At higher elimination capacity, the carbon dioxide production is also increased. The amount of carbon dioxide production was increased when higher xylene was fed in system (Figure 4-10) with higher elimination capacity too. At lower inlet loading the biofiltration system produces less carbon dioxide with high removal efficiency. From this research result shows the limitation of system, as at higher loading rate more amount of carbon dioxide also generate too. Although one product from the system is carbon dioxide (greenhouse gas), the small amount emission of it is less toxic and less harmful than xylene or any VOCs generate to atmosphere.

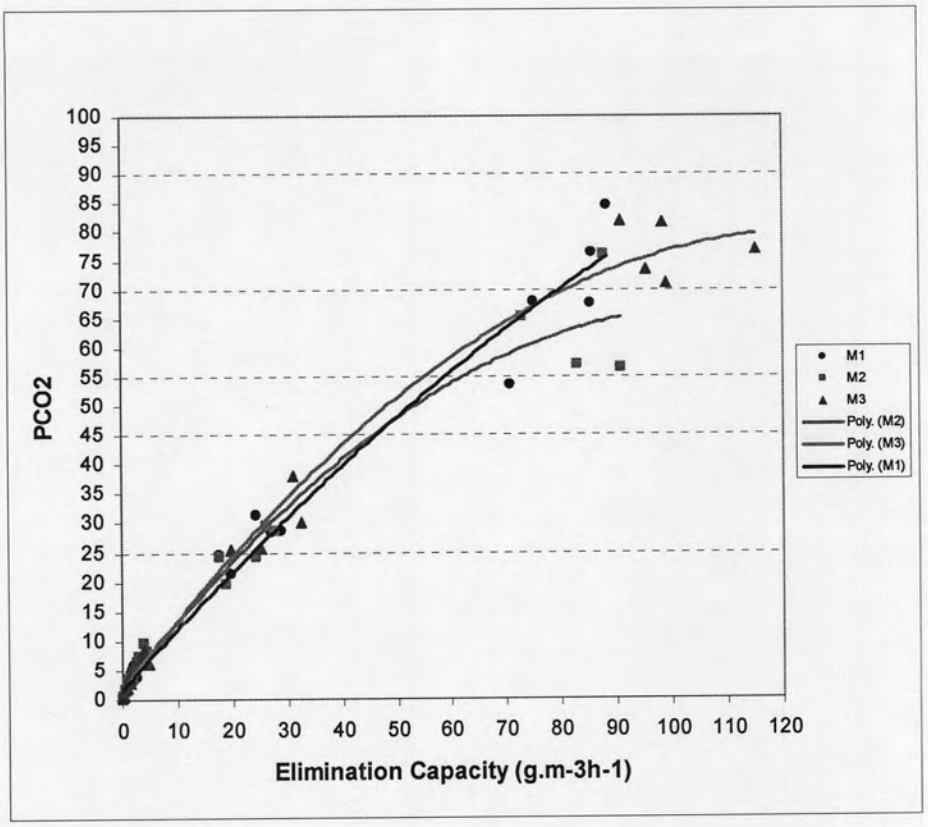

Figure 4-11 Carbon Dioxide Production and Elimination Capacity

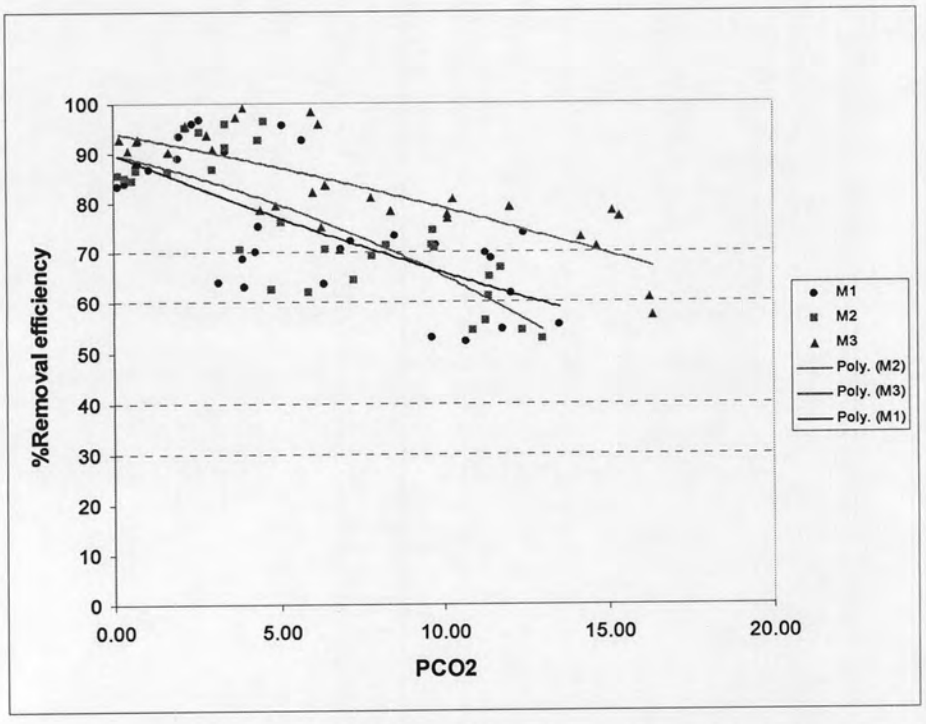

Figure 4-12 % Removal Efficiency and Carbon Dioxide Production

The relationship between % removal efficiency and carbon dioxide in Figure 4-12 was shown that at higher removal efficiency, the biofiltration system produces less carbon dioxide. The explanation is that at the high removal efficiency region, xylene was fed to biofiltration system with lower inlet concentration therefore there is less production of carbon dioxide. In this experiment, carbon dioxide production of M3 was in average of 20.4 g $m^{-3}h^{-1}$ which is higher than M1 (18.3 $gm^{-3}h^{-1}$) and M2 (17.1 g $m^{-3}h^{-1}$).

4.6 Monitor on Intermediate Species of Xylene

Intermediate species of xylene were monitored in the experiment. The spectra of inlet and outlet species of xylene from column 2 (inoculated with M1), column3 (inoculated with M2), and column 4 (inoculated with M3) are shown in Appendix C. The result shows that the inlet and outlet spectra and chromatogram were similar for all column and only ethybenzene and p,o,m xylene were found. (Table 4-10) The intermediate species were not observed in this experiments because of biofiltration process was not long enough time for degradation and generation of intermediate species as in liquid degradation.

Table 4-10 Monitor on Intermediate Species from Biofilters

	Column 2 (for M1)	**Column 3 (for M2)**	**Column 4 (for M3)**
Inlet Spectra	Ethylbenzene, o,m,p-xylene	Ethylbenzene, o,m,p-xylene	Ethylbenzene, o,m,p-xylene
Outlet Spectra	Ethylbenzene, o,m,p-xylene	Ethylbenzene, o,m,p-xylene	Ethylbenzene, o,m,p-xylene

4.7 Monitor on Microorganisms

At the end of the experiment, the type of microorganisms from each column was monitored to compare with the start up microorganisms. The

microorganisms were found as same types of inoculated microorganisms at the end of process.

The microorganisms were defined as *Aspergillus flavus* (M1) from column 2, *Aspergillus terreus* (M2)from column3 and *Penicillium glabrum* (M3) from column 4 with 2.5×10^{9},1.3×10^{10} and 4.1×10^{9}CFU /g, respectively The result of total plate count of microorganisms is shown in Table 4-11.

Table 4-11 Total Plate Count of Microorganisms

	M1 in Column 2	**M2 in Column 3**	**M3 in Column 4**
Total Plate Count (CFU/g)	2.5×10^{9}	1.3×10^{10}	4.1×10^{9}
Type of Microorganisms	*Aspergillus flavus*	*Aspergillus terreus*	*Penicillium glabrum*

Three types of dominant species could degrade xylene vapor in biofilter at a flow rate of 0.2 L/m at the highest removal efficiency of 96.6 %, 96.4 % and 99.2 % for M1, M2 and M3, respectively. It was found that there was no significant difference in removal efficiency and elimination capacity of M1 and M2 for genus Aspergillus. However, the higher removal efficiency was observed for M3 of genus Penicillium. At the end of operation, amount of microorganisms was very high at 10^{9} - 10^{10} CFU/g. For overall biofiltration performance, there was no significant in quantity of microorganisms in each column but the differentiation of microbial type was more important parameter in this study.