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Microbial Ecology and Kinetic Study of a Biofilter Treating Hydrogen Sulfide and Methanol
(Under the Direction of KESHAV DAS)

Biofiltration is a preferred way of removing H_2S and CH_3OH, two important industrial air emissions. The microbial community of a H_2S, CH_3OH dual-biofilter system was studied using 16S rRNA gene libraries. The kinetic study was conducted using traditional reaction kinetics methodology. Biofilter-H received H_2S only and Biofilter-HM received both H_2S and CH_3OH. *Thiobacillus* and *Sulfobacillus* dominated the microbial community after 20 days of H_2S treatment. After CH_3OH introduction, the microbial community shifted from *Thiobacillus* to a community with greater diversity which included CH_3OH oxidizers. A one-tail t-test verified that the first order rate constant of Biofilter-HM (0.031±0.011/sec) after methanol introduction was higher than that for Biofilter-H (0.021±0.009/sec). With a higher first order rate constant, Biofilter-HM removed more H_2S in the first order stage than Biofilter-H.

INDEX WORDS: Biofiltration, Hydrogen sulfide, Methanol, 16S rRNA gene, Diversity indices, Phylogenetic tree, Operational taxonomic unit, Kinetics, Rate constants, Reaction rate

MICROBIAL ECOLOGY AND KINETIC STUDY OF A BIOFILTER TREATING HYDROGEN SULFIDE AND METHANOL

by

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Finally, I thank my parents who taught me to become a person who is useful to others, hard working, and persistent.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

BACKGROUND

Air pollution is a worldwide environmental issue today. In the USA, approximately 200 million tons of waste gases are released into the air annually [15]. Many industrial processes are sources of air pollutants and therefore are regulated by environmental protection agencies. In the U.S., the enactment of the federal Clean Air Act Amendments of 1990 has brought about stricter regulation of air emissions and calls for techniques that can effectively control air emissions from industrial processes. Commonly, both process control to reduce emissions and end of pipe treatments, like scrubbers, incinerators, thermal oxidizers and biofilters are used to achieve this objective[4].

Biofiltration is the preferred way of treating large volume emissions that contain low concentrations of contaminants. It has low operating and capital costs and produces minimal secondary waste streams [6, 9]. It uses a biologically active, solid medium bed to absorb compounds from the air stream and retain them for subsequent biological oxidation [2]. The performance of a biofilter depends on the microbial community of the medium. Heterotrophic microorganisms are commonly found in biofilters, which treat VOCs (Volatile Organic Compounds) [13]. When influent gases contain only inorganic constituents, chemoautotrophic microorganisms are dominant [21].

Hydrogen sulfide is commonly found in sewage gas streams and methanol is an important VOC in the forest products industry. The combination of those two gases exists in air emissions of several industrial processes, especially, the pulp and paper industry. They have different properties affecting their behavior in a biofilter. Hydrogen sulfide is an inorganic sulfur compound with a Henry's Constant of 0.373 $H_{cc,\ 20°C}$ [20], where $H_{cc} = C_G/C_L$ at 20 °C, and possesses a unique odor with an odor threshold of only 0.00047 ppmv. Methanol is a highly water-soluble organic compound with a Henry's Constant of 1.91E-4 $H_{cc,\ 20°C}$ [20]. Biofiltration of hydrogen sulfide and methanol requires distinct groups of microorganisms.

Microorganisms commonly use hydrogen sulfide as a source of energy and electrons by oxidizing to sulfate [23]. Traditionally, colorless sulfur bacteria such as genera *Thiobacillus, Thiomicrospira, Thiosphaera, Sulfolobus, Acidianus, Thermothrix, Thiovulum, Beggiatoa, Thiothrix, Thioploca, Thiodendron, Thiobacterium, Macromonas, Achromatium,* and *Thiospira* are recognized as being able to grow on reduced sulfur compounds. In addition, *Paracoccus, Hyphomicrobium, Alcaligenes, Pseudomonas,* and *Hydrogenobacter* are also able to use reduced sulfur compounds [11, 12]. Different physiological types of sulfur-metabolizing microbes exist in the environment based on the ratio of inorganic to organic sulfur substrates available [7]. In this study, because the available sulfur substrate is hydrogen sulfide, the dominant sulfur-metabolizing microbes are expected to be obligate chemolithotrophs, which require an inorganic source of energy and obtain their cell carbon from carbon dioxide fixation [14]. Many *Thiobacillus* species, at least one species each from *Sulfolobus* and *Hydrogenobacter*, and all of the known species of *Thiomicrospira* fall into this group [17]. Methanol is commonly oxidized by methylotrophs to formaldehyde. The resulting formaldehyde can be oxidized to carbon dioxide or incorporated into cell material. *Methylomonas* and *Methylococcus* are known genera that can utilize methanol [3].

In this research, a biofiltration system treating hydrogen sulfide and methanol was constructed to study the effect of methanol introduction on hydrogen sulfide biofiltration. The microbial community and system performance were evaluated to achieve this goal.

LITERATURE REVIEW

In the last decade, 16S rRNA gene sequencing technique has been used extensively in the molecular ecology field to describe many environmental habitats of microorganisms. This approach is based on the use of 16S rRNA gene sequence comparisons to define relationships. It is dependent on the fact that all prokaryotes contain 16S rRNA gene sequences that are conserved enough to be compared in a pair-

wise manner, yet have diverged enough to reflect evolutionary change. With an average length of 1,500 nucleotides, when fully or almost fully analyzed, the 16S rRNA gene provides sufficient information for reliable phylogenetic analysis [1]. This technique avoids the traditional laboratory incubation or cultivation of samples, which imposes a significant bias on the analysis, because cultivable microorganisms may represent less than 1% of the microorganisms present in a natural microbial community [1].

Rheims and Stackebrandt used this method to identify the bacterial diversity inside a peat sample from northern Germany [16]. Of the total clones they obtained, 42% belonged to the alpha subclass of Proteobacteria, 15% was remotely related to the Actinomycetales, and 10% was remotely related to the Acidobacterium phylum. Voordouw et al. used this technique to characterize bacteria in an oil field [22]. A variety of gram-negative, sulfate-reducing bacteria were detected (16 members of the family *Desulfovibrionaceae* and 8 members of the family *Desulfobacteriacea*). In contrast, a much more limited number of anaerobic, fermentative, or acetogenic bacteria were found (one *Clostridium sp*., one *Eubacterium sp*., and one *Synergistes sp*.). Potential sulfide oxidizers and microaerophiles (*Thiomicrospira, Acrobacter, Campylobacter,* and *Oceanospirillum sp*.) were also detected.

Recently the 16S rRNA gene sequencing method has been used in the bioremediation field. So and Young identified a sulfate-reducing bacterium that anaerobically degraded alkanes [19]. It was found to be closely related to the genera *Desulfosarcina, Desulfonema,* and *Desulfococcus* in the delta subdivision of Proteobacteria. In another study, both 16S rRNA and ammonia monooxygenase (amoA) genes were used to characterize the heterotrophic and ammonia-oxidizing bacteria in an ammonia biofilter during a 102-day experiment [18]. The overall diversity of the heterotrophic microbial population appeared to decrease by 38% by the end of the experiment. The community structure of the heterotrophic population shifted from predominantly members of two subdivisions of the Proteobacteria (beta and gamma

subdivision) to members of one subdivision (the gamma subdivision). Juteau et al. used the 16S rRNA gene sequencing method to study the relative abundance of bacteria capable of toluene degradation in a compost biofilter [8]. The population was divided into 11 genotypic groups based on DNA fingerprints. Identification of a member of each group using 16S rRNA gene sequencing comparison showed that they belonged to seven genera: *Acinetobacter, Azoarcus, Mycobacterium, Nevskia, Pseudomonas, Pseudonocardia* and *Rhodococcus*.

Performance analyses on hydrogen sulfide biofiltration have been done extensively. Yang and Allen determined the optimal design and operating parameters of a laboratory scale biofilter system for treatment of hydrogen sulfide [24]. Composts from various sources were used for hydrogen sulfide removal. They reported that the maximum hydrogen sulfide loading capacity was compost specific and suggested the following operating conditions for treating hydrogen sulfide in a compost biofilter system: operating temperature: 25 to 50 °C, pH: >3.0, compost water content: 50±15 %, compost sulfate content: <25 mg-S/g medium, and retention time: >15 seconds. The kinetics of hydrogen sulfide oxidation in the biofilter was evaluated and the reaction rates were determined to be first-order at low inlet hydrogen sulfide concentrations (<200 ppmv), zero-order at high inlet concentrations (>400 ppmv), and fractional-order in the intermediate concentration range [25]. In 200 days of operation, the compost biofilter showed good buffering capacities to variations in gas flow rate and pollutant loading. System acidification and sulfate accumulation were identified as inhibitors to required biological activity.

Methanol biofiltration was studied by Krailas et al who reported the effect of inlet mass loading, water content and total bacteria count on methanol elimination in upward and downward flow biofilters [10]. They found that both the upward and downward flow biofilters had similar performance in terms of elimination capacities at different inlet mass loadings. The maximum elimination capacity was approximately 101 $g/m^3 \cdot h$ with

an optimum methanol loading rate of 169 $g/m^3 \cdot h$ (7.5 g/m^3 of methanol with superficial velocity of 7.6 m/h). In addition, it was found that when the water content in the compost was below 35% by weight, microbial activity was impaired. Similar trends were shown by both the elimination capacity and total bacteria count which initially increased, went through a plateau, and then decreased with increased methanol loading.

Dhamwichukorn et al. reported studies on the thermophilic biofiltration of methanol and α-pinene [5]. Two bench-scale thermophilic biofiltration systems were used to examine methanol removal at different residence times, with influent concentrations of 110 ppmv methanol and 15 ppmv α-pinene. At a residence time of 10.85 minutes, the smaller of the two systems had removal efficiencies of >98% for methanol, but only 23% for α-pinene. The larger system was operated with the same parameters to evaluate residence time and surfactant effects on compound removal. At a residence time of 18.24 minutes, the removal rates of both methanol and α-pinene were >95%. However, α-pinene removal dropped to 26% at a residence time of 6.08 minutes while methanol removal remained unaffected.

OBJECTIVES

Although many studies of the microbial communities in bioremediation sites have been successfully conducted using 16S rRNA gene sequencing technique, fewer studies have been reported for biofilter systems. Most of those studies on biofilter systems only address single compound situations. The use of 16S rRNA gene sequencing technique to study interaction between microorganisms caused by simultaneously treating different gases in a biofilter system has not been reported before. Although biofiltration of hydrogen sulfide and methanol has been reported earlier, simultaneous hydrogen sulfide and methanol biofiltration has not been reported. This information would prove to be extremely helpful for obtaining a deeper understanding of how the biofilter reacts to a multi-substrate situation.

Thus, the objective of this research is to characterize the effect of methanol introduction on hydrogen sulfide biofiltration through the study of both the microbial community and the biofilter system performance.

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CHAPTER 2

CHANGES IN THE MICROBIAL ECOLOGY OF A BIOFILTER TREATING HYDROGEN SULFIDE AND METHANOL[1]

[1]Ding, Y., K. C. Das, W. B. Whitman, J. R. Kastner. To be submitted to Applied and Environmental Microbiology

ABSTRACT

Microbial community of a hydrogen sulfide biofilter in the presence and absence of methanol was studied to identify the effect of adaptation to hydrogen sulfide oxidation on the initial microbial community and the effect of methanol introduction on the adapted hydrogen sulfide biofilter microbial community. The biofilter's initial microbial community possessed a high diversity, species richness and species evenness. There was low possibility that any dominant microorganism would exist in the start medium. After 20 days of hydrogen sulfide addition, *Thiobacillus* and *Sulfobacillus* genera dominated the biofilter's microbial community. With time, the population of sulfur oxidizers shifted from mainly *Thiobacillus* to *Sulfobacillus* genus. After introduction of methanol, the microbial community experienced a significant change. Although many species detected in the medium of the biofilter when it reached a steady state of hydrogen sulfide treatment remained, many more species were detected in the medium at the end of experiment. Those species included potential methanol-oxidizers such as methylotrophic proteobacterium and microorganisms from genera *Methylosinus and Methylocella*.

KEYWORDS: Hydrogen sulfide, Methanol, Biofiltration, Microbial community, 16S rRNA gene, Diversity, Coverage, Evenness

INTRODUCTION

Air pollution is an important aspect of the worldwide environmental issue. In the USA only, approximately 200 million tons of waste gases are released into the air annually [12]. Many industrial processes are sources of air pollutants and therefore are regulated by environmental protection agencies. In the USA, the enactment of the federal Clean Air Act Amendments of 1990 has brought about stricter regulation of air emissions and calls for techniques that can effectively control air emissions from industrial processes. Commonly both process control to reduce emissions and end of pipe treatments, like scrubbers, incinerators, thermal oxidizers and biofilters are used to achieve this objective[5].

Biofiltration is a preferred way of treating large volume emissions that contain low concentrations of contaminants. It has low operating and capital costs and produces minimal secondary waste streams [6, 9]. It uses a biologically active, solid medium bed to absorb compounds from the air stream and retain them for subsequent biological oxidation. A properly designed and operated biofilter converts target waste gas chemicals to end products such as carbon dioxide, water, inorganic salts and biomass. The performance of a biofilter depends on the microbial community of the medium. Heterotrophic microorganisms are commonly found in biofilters treating VOCs (Volatile Organic Compounds) [10]. When influent gases contain only inorganic constituents, chemoautotrophic microorganisms are dominant [19].

Hydrogen sulfide is commonly found in sewage gas streams and methanol is an important VOC in the forest products industry. The combination of those two gases exists in air emissions of several industrial processes, especially, the pulp and paper industry. They have different properties affecting their behavior in a biofilter. Hydrogen sulfide is an inorganic sulfur compound with a Henry's Constant of 0.373 $H_{cc,\ 20°C}$ [18] and possesses a unique odor with odor threshold of only 0.00047 ppmv, where $H_{cc} = C_G/C_L$ at 20 °C. Methanol is a highly water-soluble organic compound with a Henry's Constant of

1.91E-4 $H_{cc,\ 20°C}$ [18]. And biofiltration of hydrogen sulfide and methanol requires distinct groups of microorganisms.

Hydrogen sulfide is commonly used by microorganisms as sources of energy and electrons by oxidizing to sulfate (Figure 1) [21]. Typically, colorless sulfur bacteria such as genera *Thiobacillus, Thiomicrospira, Thiosphaera, Sulfolobus, Acidianus, Thermothrix, Thiovulum, Beggiatoa, Thiothrix, Thioploca, Thiodendron, Thiobacterium, Macromonas, Achromatium* and *Thiospira* are traditionally recognized as being able to grow on reduced sulfur compounds. While *Paracoccus, Hyphomicrobium, Alcaligenes, Pseudomonas and Hydrogenobacter* are also found to be able to use reduced sulfur compounds. Different physiological types of sulfur metabolizing microbes exist in the environment based on the ratio of inorganic to organic sulfur substrates available (Figure 2) [7]. When the available sulfur substrate is hydrogen sulfide, the dominant sulfur metabolizing microbes are likely to be obligate chemolithotrophs, which require an inorganic source of energy and obtain their cell carbon from the fixation of carbon dioxide. Many *Thiobacillus* species, at least one species each from *Sulfolobus* and *Hydrogenobacter* and all of the known species of *Thiomicrospira* fall into this group [14]. Methanol is commonly oxidized by *Methylotrophs* to formaldehyde. The resulting formaldehyde can be oxidized to carbon dioxide or incorporated into cell materials. The methanol oxidation pathway is shown in Figure 3. *Methylomonas* and *Methylococcus* are known genera that can utilize methanol [4].

In a biofilter that has an inlet gas stream composed of both hydrogen sulfide and methanol, it is possible that consortiums of microorganisms reside and the interactions between them are inevitable. Little is known about how and to what extent those microorganisms interact. One of the possible reasons for limited work in this field is that it is generally assumed that changes in the microbial population and its activity within a biofilter are slow compared to other phenomena [1]. Difficulty in sampling the biofilter may also be a possible reason. Reactions of microbial oxidation may be rapid and interaction between microorganisms may be extremely sensitive to ambient conditions,

therefore happening too quickly to be measured. Clear understanding of the microbial community and the interactions between microorganisms inside such a biofilter is of importance in maintaining optimal performance. Determination of the microbial community inside a hydrogen sulfide and methanol biofilter provides the basis for deciding nutrient requirements and the optimal operating condition of the biofilter system. Better understanding of the interaction between microorganisms can help us decide whether hydrogen sulfide and methanol can be treated together favoring to each other or not.

Previous studies on microorganisms used in biofilters commonly derived their conclusions on overall biofilter response, without detailed explanation of the microbial ecology. Those studies reported pH, temperature and removal efficiency changes with time without direct description of the microorganisms residing in the biofilter. Many practical problems exist in the process of studying microorganisms in a solid fermentation system. For example, traditional methods for characterization of the structure and activity of natural microbial communities require laboratory incubation or cultivation of samples. Laboratory incubation of samples imposes a significant bias on the analysis and cultivable microorganisms may represent less than 1% of the microorganisms present in a natural microbial community. Viable plate count or most-probable-number techniques have been used for quantification of active cells in environmental samples. However, because they select for certain organisms, these methods are inadequate for describing microbial consortia [2].

In the last decade, 16S rRNA gene sequencing technique has been used extensively in the molecular ecology field to describe many environmental habitats of microorganisms. This approach is based on the use of 16S rRNA gene sequence comparisons to define relationship. It is dependent on the fact that all prokaryotes contain 16S rRNA gene sequences that are conserved enough to be compared in a pair-wise manner, yet have diverged enough to reflect evolutionary change. With an average

length of 1,500 nucleotides, when fully or almost fully analyzed, the 16S rRNA gene contains sufficient information for reliable phylogenetic analysis [11]. This technique does not need the traditional laboratory incubation or cultivation of samples, thus avoiding the significant bias involved in incubation or cultivation as discussed before. Principal steps used to identify microbial communities inside an environmental sample using 16S rRNA gene sequencing technique are listed in Figure 4 [2]. The first step is to obtain a representative collection of pure nucleic acids. Then the nucleic acids are amplified using a number of universal and bacterial primers. The primer pair 27F and 1392R have been frequently used. The PCR amplified rRNA gene is then cloned and sequenced. The sequences obtained are phylogenetically analyzed to identify most similar microorganism of the sequence and to infer any inner structure of the microbial community where the sample comes from [11]. Rheims and Stackebrandt used this method to identify the bacterial diversity inside a peat sample from northern Germany [13]. Of the total clones they obtained 42% belonged to the alpha subclass of Proteobacteria, 15% were remotely related to the *Actinomycetales* and 10% were remotely related to the *Acidobacterium capsulatum* phylum. Voordouw et al. used this technique to characterize bacteria in an oil field [20]. A variety of gram-negative, sulfate-reducing bacteria were detected (16 members of the family *Desulfovibrionaceae* and 8 members of the family *Desulfobacteriacea*). In contrast, a much more limited number of anaerobic, fermentative, or acetogenic bacteria were found (one *Clostridium sp*., one *Eubacterium sp*. and one *Synergistes sp*.). Potential sulfide oxidizers and microaerophiles (*Thiomicrospira, Acrobacter, Campylobacter* and *Oceanospirillum sp*.) were also detected.

Recently this technique has been used in the bioremediation field. So and Young identified a sulfate-reducing bacterium that anaerobically degraded alkanes [17]. It was found to be closely related to the genera *Desulfosarcina, Desulfonema* and *Desulfococcus* in the delta subdivision of Proteobacteria. In another study, both 16S rRNA and

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Although the microbial communities of some biofiltration systems have been studied, microbial community analysis of a combined hydrogen sulfide and methanol biofilter has not been reported. Information on the population dynamics is likely to be extremely useful in understanding the combined hydrogen sulfide, methanol biofiltration and in deciding the operating environment for such a system based on the microbial community of the biofilter medium. Hence, the aims of this work were to characterize the changes in the microbial community and the interaction between microorganisms in a biofilter system during biofiltration of hydrogen sulfide and methanol using 16S rRNA gene sequencing technique.

MATERIALS AND METHODS

Medium Sampling

The biofiltration experiment was conducted using a dual-tower system as shown in Figure 5. One biofilter was denoted as Biofilter-H, where H stand for hydrogen sulfide. The other biofilter was denoted as Biofilter-HM, where H and M stand for

hydrogen sulfide and methanol respectively. Both biofilters treated hydrogen sulfide until a steady state was reached (i.e. outlet hydrogen sulfide concentration changed less than 15% from one day to the next). This steady state was referred to as middle stage. Then methanol was combined with hydrogen sulfide as inlet gases to Biofilter-HM. Biofilter-H continued to treat hydrogen sulfide only. The experiment was ended at day 49 which was referred to as final stage. Five samples were taken from biofilters at different stages and their microbial communities were analyzed. For each sample, one gram of medium was taken from each sampling port of the individual biofilter and mixed. One gram of that mixture was used. An original biofilter medium sample was taken at day zero (denoted as S, which stand for start). One sample was taken from each of the biofilters when they reached middle stage at day 20 (denoted as M1 and M2 respectively, M stand for middle stage), the last samples from each of the biofilters at final stage at day 49 (denoted as F1 and F2 respectively, where F stand for final stage). Comparing S with M1 or M2, hydrogen sulfide-metabolizing microbes could be identified. By comparing F1 with F2, the shift of the microbial community caused by methanol introduction could be identified. The inlet hydrogen sulfide concentration for both biofilters ranged from 55 ppmv to 160 ppmv. The inlet methanol concentration for Biofilter-HM ranged from 100 ppmv to 1000 ppmv.

One gram of each sample was then mixed with 0.2 gram of PVPP (Polyvinylpolypyrrolidone) and placed in a 10 ml centrifuge tube.

DNA Extraction and Purification

Lysis buffer (3 ml; 0.15 M NaCl, 0.1 M Na_2EDTA, pH 8.0) was added to each sample and the sample was vortexed extensively for 1.5 minutes. Lysozyme (3 mg/ml) was then added and the sample was vortexed briefly for few seconds. The sample was incubated at 37 °C in a water-bath for one hour, with brief vortexing every 15 minutes. While vortexing, 500 µl of the sample was transferred to a 1.5 ml centrifuge tube and 400

µl of bead beating solution (0.1 M NaCl, 0.5 M Tris.Cl, pH 8.0, 10% SDS) and 0.1 gram of 0.1 mm diameter glass bead were added. Turbomixing at maximum speed was maintained for 3 minutes and the sample was centrifuged for 3 minutes, 14,000 rpm at room temperature. The supernatant was transferred to a new 1.5 ml centrifuge tube and equal volumes of phenol:chloroform:IAA (isoamyl-alcohol) solution were added. The resultant sample was vortexed briefly and centrifuged for 10 min at maximum speed. The supernatant from this step was transferred to a new 1.5 ml centrifuge tube and equal volumes of chloroform:IAA (24:1) were added. The sample was vortexed briefly and centrifuged for 10 minutes at 14,000 rpm.

The supernatant was removed and 2 volumes of ice cold 100% ethanol were added and mixed gently and the nucleic acids were allowed to precipitate at -20 °C for at least one hour. The sample was centrifuged for 15 minutes and the pellet was dried in vacuum. The pellet was dissolved in 500 µl of water and treated with 1 µl of DNase free RNase (10 mg/ml) for one hour at 37 °C. A commercially available Wizard® DNA Clean-Up Kit from Promega was used to purify the extracted DNA. The sample was then desalted using Millipore Type VS 0.025 µm filter paper for at least two hours.

PCR Amplification

Hot start PCR amplification was performed using a DNA-thermal cycler (Thermolyne Company) using 1 µl eubacterial primer Eub27f (20 mM) with sequence 5'-AGA GTT TGA TCM TGG CTC AG-3', 1 µl eubacterial primer Eub1392r (20 mM) with sequence 5'-ACG GGC GGT GTG TRC-3', both from Integrated DNA Technologies, 1 µl DNA template, 22 µl sterile water and a commercially available Ready-To-Go™ PCR bead from Pharmacia Biotech Company. Less than 10 ng of DNA template was used and the following amplification program was implemented: dwell at 94 °C for 5 minutes, followed by 29 cycles consisting of 94 °C for 1 minute, 61 °C for 1

minute and 72 °C for 2 minutes. Extension was at 72 °C for 8 minutes at the end of the cycles.

Ligation and Transformation

The PCR product was immediately ligated into the pCR® 2.1 phagemid using the original TA Cloning® Kit from Invitrogen®. The ligation reaction included 17.5 ng PCR product, 1 µl 10 × ligation buffer, 2 µl pCR® 2.1 vector (25 ng/µl), 1 µl T4 DNA ligase (4.0 Weiss units) and sterile water was added to bring the total volume to 10 µl. The reaction was incubated at 14 °C for overnight.

The ligation product was transformed into One Shot™ INVαF' competent cells and was plated on LB (Luria-Bertani) plates (1.0% Tryptone, 0.5% Yeast Extract, 1.0% NaCl) containing 50µg/ml of kanamycin and 40µl of 40mg/ml X-Gal.

High-throughput Sequencing

Aliquots of 1.5 ml of TB medium (1.2% Tryptone, 2.4% Yeast Extract, 0.4% glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4) with 50 µg/ml kanamycin were placed in each well of Beckman 96 deepwell using the Eppendorf repeat pipettor. Clones were picked from the LB plates and were incubated in the deepwell at 37 °C, 250 rpm for 20 hours. Two Dynex V-bottom plates were inoculated with 30 µl of 50% glycerol and 100 µl overnight culture cells for permanent storage at -80 °C.

Bacteria were harvested by centrifugation for 5 minutes at 3000 rpm. Cell lysis was carried out as following: 200 µl TE-RNase (10 mg/ml) solution was added, the plate was shaken on a Titertek shaker at speed 7 for 30 minutes. SDS/NaOH solution, 200 µl, was added, and the plate was shaken for 45~60 minutes. KOAc (pH 5), 200 µl 3 M, was then added and the plate was vortexed completely for 3 minutes and shake at speed 4 until all blocks had been vortexed, placed in an incubator for 20 minutes (37 °C, 250 rpm) and then put in the –80 °C freezer for a minimum of 8 hours.

The deepwell block was then thawed for 2 hours and centrifuged for 45 minutes at 3850 rpm and 200 µl of the supernatant was transferred into a clean Costar deep well block. DNA precipitation was carried out as follows: add 500 µl of 95% EtOH, immediately centrifuge for 30 minutes at 3850 rpm, gently decant supernatant into sink, let block drain for 3 minutes and add 250 µl of 70% EtOH. Immediately centrifuge for 15 minutes at 4000 rpm, gently decant supernatant into sink and kept blocks inverted on super absorbent paper towel for 5 minutes, dry blocks upside up at 37°C for at least 20 minutes and add 150 µl of ddH_2O to each well and put on the Titertek shaker at speed 7 for 10 minutes. Transfer 150 µl to Dynex V-bottom plate, cover with foil and store at -20 °C.

Ten or more purified plasmids from previous precipitation step were picked for DNA concentration measurement using DynaQuant fluorometer after EcoR I digestion. The digestion reaction included 3 µl of plasmid, 14 µl of ddH_2O, 2 µl of 10×buffer, 1 µl of EcoR I, 1 µl of BSA, incubated at 37°C for 1 hour. The reaction was then run on the agrose gel to identify the correct insertion.

Suitable amounts of the purified plasmids were then prepared for sequencing. The cycle sequencing MasterMix reaction for 96 well was: ddH_2O 53.5 µl, DMSO 32.5 µl, 5×buffer 133 µl, 300 pmol/µl primer 14 µl, BigDye 67 µl, to a total of 300 µl. Eubacterial primer Eub27f (20 mM) with sequence 5'-AGA GTT TGA TCM TGG CTC AG-3' was used. The thermal cycling procedure was as follows: 96 °C for 10 minutes, 50 °C at 5 minutes, 60 °C for 4 hours and hold at 4 °C. Thermal cycling products were purified using Sephadex G-50 plates, and sequences were determined by an ABI PRISM™ 3700 DNA Sequencer (Perkin Elmer Co.).

Sequencing Data Analysis

Chromas program was used to open the sequence files generated by PRISM ABI™ 3700 Sequencer. Generally, around 400 bases of each sequence were used in

further analysis. A similarity matrix of all the sequences from an individual sample was constructed using the SEQBOOT and DNADIST program of the Phylip 3.5 software available at the www.evolution.genetics.washington.edu/Phylip.html Phylip home website. All sequences were compared to each other and duplicate sequences (>98% sequence similarity) were grouped into an operational taxonomic unit (OTU), and only one sequence from each OTU was used for further analysis. A BLASTN search was conducted on all unique sequences to determine the closest relative in GenBank. Those sequences were used later as reference sequences in building taxon-specific phylogenetic tree. An alignment of unique sequences and reference sequences were created using the PILEUP program available at the RCR (Research Computing Resource) of the University of Georgia. The Jukes-Cantor algorithm was used to calculate sequence distance and the Neighbor-joining algorithm was used to construct the phylogenetic tree using the Phylip 3.5 program package. Phylip program SEQBOOT was used to generate multiple trees for calculating bootstrap values and distances between sequences for the trees were generated by DNADIST program of Phylip. Phylip program NEIGHBOR returned data for plotting the trees and a consensus tree was built by the CONSENSE program of Phylip. Branch lengths were generated by using Phylip program DNADIST. Taxon-specific phylogenetic tree was plotted by using the DRAWGRAM program in the Phylip package and was further edited using Adobe Illustrator software.

Species diversity indices related the number of species and the relative importance of individual species [3]. A widely used measure of species diversity, Shannon-Weaver index of diversity (H), was calculated for all the samples using the following formula:

$$H=C/N(N\log N - \sum n_i \log n_i)$$

Where C = 2.3, N =number of individuals and n_i=number of individuals in the i^{th} OTU.

Two major components of species diversity, the species richness, which measured the number of species in the community and evenness, which indicated whether there were dominant populations, were calculated using following formulas:

Species Richness (d): $d = (S-1)/\log N$, where S = number of species, N = number of individuals.

Species Evenness (e): $e = H/\log S$, where H = Shannon-Weaver diversity index, S = number of species.

Comparison of the prokaryotic community between samples was performed by using the LIBSHUFF program written by David Singleton et al. of University of Georgia and available at the www.arches.uga.edu/~whitman/libshuff.html site [16]. This program compared two clone libraries of sequence information and determined if they were significantly different. Homologous coverages ($1-N_x/n$) of each library and heterologous coverages ($1-N_{xy}/n$) between libraries were also generated by this program, where N_x = number of unique sequences, N_{xy} = number of sequences in X that are not found in Y, n = total number of sequences. A Δ-C value (square sum of the difference between homologous and heterologous coverages at different revolutionary distance) and a p-value (r/(N+1)) were also generated, where r = rank of the empirical value of Δ-C, N = time of shuffling of the sequences. Two libraries were considered significantly different when $p < 0.05$.

RESULTS AND DISCUSSION

The Microbial Community of the Start Medium (S)

The start medium generated around 200 clones, 96 of them were randomly selected and sequenced. Among them, 83 clones yielded good sequencing result. From the similarity matrix, 67 OTUs were identified. Of those, 9 were encountered more than once. The closest relative of the most abundant OTU was a clone MK12, which belonged to the Cytophaga/Flexibacter/Bacteroides group. Other OTUs were quite

uniform in number of sequences within. Further phylogenetic analysis of those 67 unique sequences were conducted and their most similar microorganisms in the GenBank database were used to assign each sequence to a major phylogenetic group, those groups and the proportion of sequences within that group of the S sample were listed in Table 1 under the S column. From Table 1, it was found out that sequences belonging to the CFB group and Proteobacteria group were most abundant, representing around 33.7% and 43.4% of total sequences in the sample S. Among the Proteobacteria group, γ-proteobacteria was most abundant, representing around 16.9% of the total, and followed by β-Proteobacteria, which accounted for 15.7% of the total. Other sequences were more evenly distributed among Firmicutes (7.2% of the total), Actinobacteria (3.6%), Candidate division TM7 (3.6%), Planctomycetales (1.2%), Cyanobacteria (1.2%), Green non-sulfur bacteria (1.2%) and Green sulfur bacteria (1.2%) groups.

Percentage coverage, diversity, richness and evenness values were calculated for the S sample and were listed in Table 2. The coverage for the S sample was relatively low, only around 28%, which suggested a majority of the S sample microbial community had not been sequenced. As the diversity, richness and evenness values were all close to maximum value, it suggested that the sample S microbial community was highly diverse and relatively uniformly distributed population. It was of low possibility that any singly dominant microorganism was present in the sample S.

The Microbial Community of the Sample from Biofilter-H at Middle Stage (M1)

Hundreds of clones were obtained from M1 sample, 96 of them were selected randomly for sequencing. Among them, 62 sequences of sufficient quality were obtained. Twelve OTUs were identified. However, 4 were most abundant and contained 54 of the total sequences. The most abundant OTU of M1 sample, which consisted of 36 clones and represented 58.1% of the total sequences, grouped with *Alicyclobacillus hesperidensis* of the Firmicutes phylum. The second largest OTU consisted of 13 clones

and grouped with *Thiobacillus thermosulfatus* of the β-Proteobacteria group. The majority of the sequences found in M1 belonged to the Firmicutes group (69.4%) (Table 1). Within the Firmicutes phylum, sequences weakly related to *Alicyclobacillus hesperidensis* were most abundant, representing 83.7% of the sequences grouped in the Firmicutes phylum. Within the Firmicutes phylum, 1.6% of M1 sequences were grouped with *Sulfobacillus*. *Sulfobacillus* is a Gram-positive, strictly aerobic and facultative chemolithoautotroph. It is a moderate thermophile and can oxidize sulfides or elemental sulfur. Hence, there appear to be the hydrogen sulfide oxidizer in the biofiltration system. The next most abundant sequences in M1 sample grouped with *Thiobacillus thermosulfatus* of the β-Proteobacteria group. *Thiobacillus* genus is Gram-negative, obtains energy from oxidation of reduced sulfur compounds. Hence, they may also be a hydrogen sulfide oxidizer in the biofiltration system. So, the total percentage of potential sulfur oxidizers identified in M1 sample was 22.6%.

Percentage coverage, diversity, richness and evenness values were calculated for the library from M1 (Table 2). The coverage for the M1 sample was relatively high, with a value of 0.87. Thus, the majority of the abundant species in the microbial community had been sequenced. Hence the information obtained from the M1 sample would be a reliable representation of the microbial community inside the Biofilter-H when the hydrogen sulfide treatment reached middle stage. The diversity, richness and evenness values were much lower than the maximum values. It suggested that the microbial community of M1 sample was of low diversity and dominated by a few microorganisms. As compared to sample S, which was composed of a community of high diversity and relatively uniform distribution, the introduction of hydrogen sulfide into the biofiltration system appears to have acclimated the medium. In response, the microbial community may have adapted for the oxidation of hydrogen sulfide to obtain energy and survive at the low pH environment by enriching reduced sulfur oxidizers *Sulfobacillus* and *Thiobacillus* genera.

Microbial Community of the Sample from Biofilter-HM at Middle Stage (M2)

Hundreds of clones were obtained from M2 sample, 96 of them were selected randomly for sequencing. Among them, 74 sequences were obtained. Only 7 unique sequences were found in the M2 library. The most abundant OTU consisted of 33 clones, which represented 44.6% of the total sequences. It grouped with *Alicyclobacillus hesperidensis* of the Firmicutes phylum. The second largest OTU consisted of 19 clones and it grouped with *Thiobacillus thermosulfatus* of the β-Proteobacteria group. The third largest OTU consisted of 13 clones and it grouped with *Bacillus cycloheptanicus*. The majority of the sequences belonged to the Firmicutes group (64.9%) (Table 1). Within the Firmicutes phylum, sequences grouped with *Alicyclobacillus hesperidensis* were most abundant, representing 68.8% of the sequences grouped in the Firmicutes phylum. Unlike M1 sample, *Sulfobacillus* genus was not detected. The next most abundant sequences grouped with *Thiobacillus thermosulfatus* of the β-Proteobacteria group. Hence, the major sulfur oxidizer in biofilter-HM would be *Thiobacillus thermosulfatus.* So, the total percentage of potential sulfur oxidizers identified in M2 was 25.7%.

Percentage coverage, diversity, richness and evenness values were calculated (Table 2). The coverage for M2 was rather high, with a value of 0.97. Thus, almost all of the microbial community species in the M2 library had been sequenced. Hence the information obtained from the M2 sample would be a reliable indicator of the microbial community inside the Biofilter-HM when the hydrogen sulfide treatments reached middle stage. The diversity, richness and evenness values were much lower than the maximum values. Those indices suggested that the microbial community of M2 sample possessed a low diversity and only a few types of microorganisms dominated. As compared to sample S, which exhibited high diversity and a relatively uniformly distributed microbial community, the introduction of hydrogen sulfide to the biofiltration system had shifted the microbial community of the medium to obtain energy from the oxidation of hydrogen sulfide and adapt to the low pH.

The Microbial Community of the Sample from Biofilter-H at Final Stage (F1)

Hundreds of clones were obtained from F1 sample, 96 of them were selected randomly for sequencing. Among them, 46 sequences were obtained. However, 3 OTUs accounted for 41 of those. The number of unique sequences in F1 was 8. The most abundant OTU of F1 consisted of 20 clones, which represented 43.5% of the total sequences. It grouped with *Sulfobacillus yellowstonensis* of the Firmicutes phylum. The second largest OTU consisted of 19 clones and was also grouped with *Sulfobacillus yellowstonensis*. The third largest OTU consisted of only 2 clones and was grouped with *Alicyclobacillus hesperidensis* of Firmicutes phylum. Further phylogenetic analysis of those 8 unique sequences was conducted and their most similar microorganisms in the GenBank database were used to assign each sequence to a major phylogenetic group (Table 1). Almost all of the sequences belonged to the Firmicutes group (95.7%). Within the Firmicutes phylum, sequences grouping with *Sulfobacillus yellowstonensis* were the most abundant, representing 88.6% of the sequences grouped in the Firmicutes phylum and 84.8% of all the sequences of F1 sample. No sequences were identified to group with *Thiobacillus thermosulfatus* of the β-Proteobacteria group. Hence the major sulfur oxidizer in Biofilter-H at the final stage would be within the *Sulfobacillus* genus and the total percentage of sulfur oxidizers was 84.8%.

The coverage for the F1 sample was relatively high, with a value of 0.73 (Table 2). Thus, majority of the microbial community species in the F1 sample had been sequenced and the information obtained from the F1 sample would be a reliable representation of the microbial community inside the Biofilter-H at the final stage. The diversity, richness and evenness values were much lower than the maximum values. Thus, the microbial community of F1 presented a low diversity and was dominated by a few microorganisms.

The Microbial Community of the Sample from Biofilter-HM at Final Stage (F2)

Hundreds of clones were obtained from F2 sample, 96 of them were selected randomly for sequencing. Among them, 74 sequences were obtained. The number of unique sequences in F2 was 34, but only 14 OTUs represented 54 of the total number of sequences. The most abundant OTU consisted of 10 clones, which represented 13.5% of the total sequences. It grouped with *Frateuria aurantia* of the γ-Proteobacteria group. The second largest OTU consisted of 7 clones and was grouped with *Frateuria sp. NO-16*. Another OTU that had 7 clones was grouped with uncultured eubacterium WD247. The third and forth most abundant OTUs consisted of 5 and 4 clones each and they both grouped with *Sulfobacillus yellowstonensis* of Firmicutes phylum. The sizes of all other OTUs were relatively uniformly distributed. The most abundant sequences were grouped in the Proteobacteria, Firmicutes and Actinobacteria phylum (Table 1). Within the Proteobacteria phylum, sequences grouped with γ-Proteobacteria were most abundant, representing 58.7% of the sequences grouped in the Proteobacteria phylum. Of less abundance, sequences grouped with α-Proteobacteria, which represented 32.4% of the sequences grouped in the Proteobacteria phylum. Sequences grouped with *Thiobacillus thermosulfatus* of β-Proteobacteria consisted of 2.7% of total sequences. Within the Firmicutes phylum, 16.2% of the total sequences were grouped with the genus *Sulfobacillus*. As discussed previously, *Sulfobacillus* and *Thiobacillus* are hydrogen sulfide oxidizers. Hence, the major potential sulfur oxidizers in Biofilter-HM at the final stage would be from the genera *Sulfobacillus* and *Thiobacillus.* The total percentage of potential sulfur oxidizers in F2 was 18.9%. In the α-Proteobacteria group, sequences grouped with methanol-oxidizers were also identified. The potential methanol-oxidizers grouped were *Methylosinus sporium* and *Methylocella palustris*. They composed of 5.41% of the total sequences of F2.

The coverage for F2 sample was relatively high, with a coverage value of 0.87 (Table 2). Hence, the majority of the microbial community species in the F2 library had

been sequenced. Thus the information obtained from the F2 sample would be a reliable representative of the microbial community inside the Biofilter-HM at the final stage. The diversity, richness and evenness values were much closer to the maximum values when compared with F1 sample. It suggested that the microbial community of F2 sample was of higher diversity and the microorganisms were more evenly distributed than in F1.

Microbial Community Comparison of the Samples from Biofilter-H and Biofilter-HM at Middle Stage (M1, M2)

The LIBSHUFF program was used to compare the microbial community of M1 and M2 based on 62 sequences of 16S rRNA gene of M1 and 74 sequences of 16S rRNA gene of M2. The comparison between M1 and M2 sequences had a Δ-C value of 0.013 and a P-value of 0.805 (Figure 6). The comparison between M2 and M1 had a Δ-C value of 0.002 and a P-value of 1.000. Thus, M1 and M2 were not significantly different. They were composed of almost the same species and possessed similar diversity and evenness. Also based on coverage values shown on Figure 6, both of the clone libraries (M1, M2) were reliable representatives of the microbial community of Biofilter-H and Biofilter-HM when they reached the middle stage. So, the microbial community of Biofilter-H and Biofilter-HM were almost identical when they reached the middle stage at day 20.

Microbial Community Comparison of the Start Sample (S) and the Sample from Biofilter-HM at Middle Stage (M2)

As previously discussed, M1 and M2 were actually identical in phylogenetic structure. As M2 had a bigger sample size, the comparison between sample S and the sample from middle stage was conducted by using S and M2 samples. LIBSHUFF program was used to compare microbial community of S and M2 based on 83 sequences of S and 74 sequences of M2 (Figure 7). The comparison between S and M2 had a Δ-C

value of 17.108 and a P-value of 0.001. The comparison between M2 and S had a Δ-C value of 10.070 and a P-value of 0.001. Thus, S and M2 samples were significantly different. This result confirmed the discussion based on the comparison between the diversity indices calculated for S and M1, M2. The S sample contained high diversity and evenness, with a low possibility of dominant microorganisms. In contrast, M1 and M2 samples contained low diversity and low evenness and were dominated by only a few microorganisms. With a high coverage value, M1 and M2 samples were reliable representatives of the microbial community of Biofilter-H and Biofilter-HM at the middle stage. *Alicyclobacillus* and *Thiobacillus* genera had a high possibility of dominating the microbial community of Biofilter-H and Biofilter-HM at the middle stage. Those differences were caused by the continued presense of hydrogen sulfide, which forced the microbial community of the medium to adapt to a high sulfur and low pH environment.

Microbial Community Comparison of the Samples from Biofilter-H and Biofilter-HM at the Final Stage (F1, F2)

The LIBSHUFF program was used to compare the microbial community of F1 and F2 based on 46 sequences of 16S rRNA gene of F1 and 74 sequences of F2 (Figure 8). The comparison between F1 and F2 sequences had a Δ-C value of 0.126 and a P-value of 0.456. The comparison between F2 and F1 had a Δ-C value of 3.905 and a P-value of 0.001. From Figure 8, it was found out that the microbial community of F2 included the microbial community of F1. This meant that the microbial community of F2 included a majority of the species in the F1 sample. However, some of the species in F2 sample could not be found in F1 sample. Based on coverage values shown on Figure 8, both of the clone libraries (F1, F2) were reliable representatives of the microbial community of Biofilter-H and Biofilter-HM at the final stage. So, the microbial community of Biofilter-HM included a majority of the species in Biofilter-H, while some of the species in Biofilter-HM could not be found in Biofilter-H at the final stage. This

was supported by diversity indices calculation which showed F2 having higher diversity and species richness than sample F1. This result was also supported by examination of the OTUs. Some of the methanol-oxidizers were only identified in F2 sample and were not identified in F1 sample.

Microbial Community Comparison of the Samples from Biofilter-H at Middle Stage (M1) and the Sample from Biofilter-H at Final Stage (F1)

The LIBSHUFF program was used to compare microbial community of M1 and F1 based on 62 sequences of M1 and 46 sequences of F1 (Figure 9). The comparison between M1 and F1 had a Δ-C value of 1.471 and a P-value of 0.001. The comparison between F1 and M1 had a Δ-C value of 1.547 and a P-value of 0.001. From Figure 9, it was found out that M1 and F1 samples were significantly different. Even though this result was not supported by diversity indices calculation, as diversity indices of M1 and F1 showed the same pattern, this result was confirmed by OTUs and unique sequences phylogenetic identification which indicated a shift in sulfur oxidizers from mainly *Thiobacillus thermosulfatus* (21% of total M1 sequences) to *Sulfobacillus* genus (84.8% of total sequences of F1).

Microbial Community Comparison of the Sample from Biofilter-HM at Middle Stage (M2) and Sample from Biofilter-HM at Final Stage (F2)

The LIBSHUFF program was used to compare prokaryotes community of M2 and F2 based on 74 sequences of M2 and 74 sequences of F2. The result was shown in Figure 10. The comparison between M2 and F2 had a Δ-C value of 0.189 and a P-value of 0.105. The comparison between F2 and M2 had a Δ-C value of 9.537 and a P-value of 0.001. From Figure 10, it was found out that the microbial community of F2 included most of the species in the M2 sample. However, many of the species in F2 sample could not be found in M2 sample. This result was supported by OTUs and unique sequences

phylogenetic identification. As the methanol oxidizers in F2 sample were not found in the M2 sample, which was prepared before methanol had been introduced into Biofilter-HM.

Taxon-specific Phylogenetic Trees of Start Sample (S), Samples from Biofilter-H and Biofilter-HM at Middle Stage (M1, M2) and Samples from Biofilte-H and Biofilter-HM at Final Stage (F1, F2)

Six taxon-specific phylogenetic tree (Figure. 11-16) of the samples S, M1, M2, F1 and F2 combined together were constructed containing all the OTUs grouped in that taxon and most of the sequences for those five samples (92.0%).

The CFB group phylogenetic tree contained 30 sequences in the total of 339 sequences from samples S, M1, M2, F1 and F2 (Figure 11). Most of them (93.3%) came from sample S. One OTU of sample F2 also fell into this group, consisting of two sequences. None of the M1, M2 and F1 sequences belonged to the CFB group. Except for one OTU grouped with a clone MK12, all other OTUs belonged to the CFB group from sample S were relatively evenly distributed. Cultured genera included in the CFB phylogenetic tree were *Flavobacterium, Cytophagles* and *Sporocytophaga*. Sequences grouped with *Flavobacterium* were most abundant, representing around 40.0% of the sequences grouped in the CFB group.

The Firmicutes phylum phylogenetic tree contained 163 sequences, representing 48.1% of the sequences from samples S, M1, M2, F1 and F2 (Figure 12). Among them, 6 sequences were from sample S. Sequences from M1 and M2 totaling 91 represented 55.8% of all the sequences grouped in Fimicutes phylum. Sequences from F1 and F2 totaling 66 represented 40.5% of the sequences in this phylum. Majority of the M1, M2 sequences belonged in this phylum were grouped with *Alicyclobacillus*. Representing around 75.8% of all the M1, M2 sequences in this phylum. Majority of the F1, F2 sequences belonged in this phylum were grouped with *Sulfobacillus,* representing around

77.3% of all the F1, F2 sequences in this phylum. Results indicated that Firmicutes microbial community shifted from mainly *Alicyclobacillus* genus to *Sulfobacillus* genus between middle and final stages. The genera included in Firmicutes phylogenetic tree were *Bacillus, Paenibacillus, Brevibacillus, Saccharococcus, Alicyclobacillus, Sulfobacillus, Actinomycetales, Clostridium, Propionibacterium* and *Oscillospira*.

The Actinobacteria phylogenetic tree contained 13 sequences, representing 3.8% of the total sequences from samples S, M1, M2, F1 and F2 (Figure 13). Majority of them came from sample F2, representing 76.9% of the 13 sequences. Two sequences from sample S were also grouped in this phylum. Relatively, all the sequences grouped in this phylum were evenly distributed. The genera included were *Nocardioides, Kitasatospora, Amycolatopsis, Dactylosporangium, Micromonospora, Actinoplanes, Thermomonosporaceae, Microbispora, Sarraceniospora* and *Microbacteriaceae*.

The α-Proteobacteria phylogenetic tree contained 23 sequences, representing 6.8% of the total sequences from samples S, M1, M2, F1 and F2 (Figure 14). None of the F1 sequences were grouped in this class. Number of sequences came from sample S totaling 8 represented 34.8% of the sequences in this class. Number of sequences from sample F2 totaling 11 represented 47.8% of the sequences in this class. It is worth mentioning that 3 of the F2 sequences belonged in α-Proteobacteria class were grouped with potential methanol oxidizer (Methylotrophic proteobacterium, *Methylocella palustris* and *Methylosinus sporium*), representing 27.3% of the F2 sequences grouped in this class. Cultured genera included in this phylogenetic tree were *Phaeospirillum, Rhizobium, Pedomicrobium, Methylocella, Methylosinus, Sphingomonas, Caulobacter, Rhodobacter* and *Acidiphilium*.

The β-Proteobacteria phylogenetic tree contained 48 sequences, representing 14.2% of the sequences from samples S, M1, M2, F1 and F2 (Figure 15). None of the F1 sequences were grouped in this class. Among the 48 sequences, 13 came from sample S, representing 27.1% of all the sequences grouped in this class. Number of F2 sequences

in this class were 3, representing 6.3% of all the sequences grouped in this class. Sequences from samples M1 and M2 represented a majority of the sequences grouped in this class (66.7%), and were exclusively grouped with *Thiobacillus thermosulfatus* which was a potential hydrogen sulfide oxidizer. This phylogenetic tree analysis indicated that M1, M2 sequences grouped with *Thiobacillus thermosulfatus* were decreasing in number between the middle and final stages. Cultured genera included in the β-Proteobacteria phylogenetic tree were *Ralstonia, Azoarcus, Polaromonas, Zoogloea, Roseateles, Burkholderia* and *Thiobacillus*.

The γ-Proteobacteria phylogenetic tree contained 35 sequences, representing 10.3% of the sequences from samples S, M1, M2, F1 and F2 (Figure 16). None of the M1, M2 sequences were grouped in this class. Number of sequences from sample S grouped in this class was 14, representing 40.0% of the 35 sequences. Sequences from sample F2 consisted of 57.1% of all the sequences grouped in this class and 95.0% of them were grouped with genus *Frateuria*. One of the F1 sample sequences was also included in this class. Cultured genera included in the γ-Proteobacteria phylogenetic tree were *Pseudomonas, Cellvibrio, Thiorhodospira, Frateuria, Thermomonas* and *Stenotrophomonas*.

Sequences of sample S were found in all six phylogenetic trees constructed. And in each phylogenetic tree they were relatively evenly distributed. Table 1 showed sample S sequences that were not grouped in those six phylogenetic trees were also relatively evenly distributed among phylogenetic groups: Candidate division TM7, Planctomycetales, Cyanobacteria, Fibrobacteria, Green non-surfur and Green sulfur bacteria. While for the M1 and M2 samples, none of their sequences were found in the CFB group, Actinobacteria phylum and γ-Proteobacteria class phylogenetic trees. For the F1 sample, none of its sequences were grouped in the CFB group, α- and β-Proteobacteria class phylogenetic trees. For the F2 sample, like sample S, its sequences were found in all six phylogenetic trees. Except for 7 sequences that were from unknown

taxon, none of the sequences were found outside those grouped in the six phylogenetic trees (Figure 11-16). The above discussion on the six taxon-specific phylogenetic trees supported the results gained from the diversity indices study that microbial community of sample S was of high diversity and evenness, composed of diversely and evenly distributed microorganisms. While microbial community of samples M1, M2 and F1, due to the effect of hydrogen sulfide oxidation, were of low diversity and evenness, possibly dominated by few sulfur oxidation microorganisms (e.g. microbes from *Thiobacillus* and *Sulfobacillus* genera). For sample F2, due to the introduction of methanol, its microbial community was of higher diversity and evenness comparing to sample F1. But its microbial community was not as diversely and evenly distributed as sample S, possibly due to the effect of adaptation to both hydrogen sulfide and methanol oxidation.

CONCLUSIONS

The microbial community of the initial compost medium for biofiltration of hydrogen sulfide and methanol possessed high diversity, species richness and high species evenness. There was low possibility that any dominant microorganism would exist in this start medium. After the introduction of hydrogen sulfide, the microbial community in the medium adapted to the high load of reduced sulfur and low pH by an increase in the number of potential sulfur oxidizers. *Thiobacillus* and *Sulfobacillus* genera dominated the microbial community after only 20 days of hydrogen sulfide treatment.

The media microbial communities of Biofilter-H and Biofilter-HM at the stage when hydrogen sulfide treatment reached stable were comparable, with a P-value from LIBSHUFF program of >0.05. Between the time when Biofilter-H and Biofilter-HM reached a steady state of hydrogen sulfide treatment (day 20^{th}) and end of the experiment

(day 49th), the sulfur-oxidizers in the media shifted from mainly *Thiobacillus* to *Sulfobacillus* genus.

After introduction of methanol to Biofilter-HM, its microbial community experienced a significant change. Although many species detected in the medium from Biofilter-HM at the 20th day remained, many more species were detected in the medium at the end of experiment (day 49). These species included potential methanol-oxidizers, such as methylotrophic proteobacterium, and microorganisms from genera *Methylosinus and Methylocella*.

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Table 1. Proportion of S, M1, M2 and F1, F2 16S rRNA gene sequences in different phylogenetic groups.

Phylogenetic Groups Represented	S (n=83)[1]	M1 (n=62)	M2 (n=74)	F1 (n=46)	F2 (n=74)
CFB (Cytophaga/Flexibacter/Bacteroides) Group	0.337	0	0	0	0.027
Firmicutes (*Sulfobacillus*)[2]	0.072 (0)	0.694 (0.016)	0.649 (0)	0.957 (0.848)	0.297 (0.162)
Actinobacteria	0.036	0	0	0.022	0.122
Proteobacteria					
α-Proteobacteria (Methanol oxidizers)[4]	0.096 (0)	0.032 (0)	0.027 (0)	0	0.149 (0.012)
β-Proteobacteria (*Thiobacillus thermosulfatus*)[3]	0.157 (0)	0.210 (0.210)	0.257 (0.257)	0	0.041 (0.027)
γ-Proteobacteria	0.169	0	0	0.022	0.270
δ-Proteobacteria	0.012	0	0	0	0
Candidate division TM7	0.036	0	0	0	0
Candidate division OP12	0	0.016	0	0	0
Planctomycetales	0.012	0	0	0	0
Cyanobacteria	0.012	0	0	0	0
Fibrobacteria	0.036	0	0	0	0
Green non-sulfur bacteria	0.012	0	0	0	0
Green sulfur bacteria	0.012	0	0	0	0
Unknown Taxon	0	0.048	0.068	0	0.095

[1]n here represents number of sequences in each clone library.

[2]Numbers in parentheses after each number at the Firmicutes line represent the proportion of sequences grouped with *Sulfobacillus* in the Firmicutes group of the total sequences.

[3]Numbers in parentheses after each number at the β-Proteobacteria line represent the proportion of sequences grouped with *Thiobacillus thermosulfatus* in the β-Proteobacteria group of the total sequences.

[4]Numbers in parentheses after each number at the α-Proteobacteria line represent the proportion of sequences grouped with potential methanol oxidizers in the α-Proteobacteria group of the total sequences.

Table 2. Comparison of diversity indices for S, M1, M2, F1 and F2 samples.

Diversity Index[1]	S	M1	M2	F1	F2	Index Minimum[2]	Index Maximum[2]
%Coverage	0.28	0.87	0.97	0.73	0.87	0.00	1.00
Diversity	3.65	1.34	1.33	1.28	3.02	0.00	3.82
Richness	23.94	5.23	2.77	4.21	15.10	0.00	27.06
Evenness	2.26	1.36	1.79	1.42	2.13	0.00	2.30

[1]For calculation of the diversity indices, the sample sizes were reduced to the size of the smallest clone library (n=46) by randomly removing sequences from the larger libraries. This process was repeated 10 times, and the values shown were the averages.

[2]Minimum and maximum values were obtained for 46 sequences at minimum and maximum diversity.

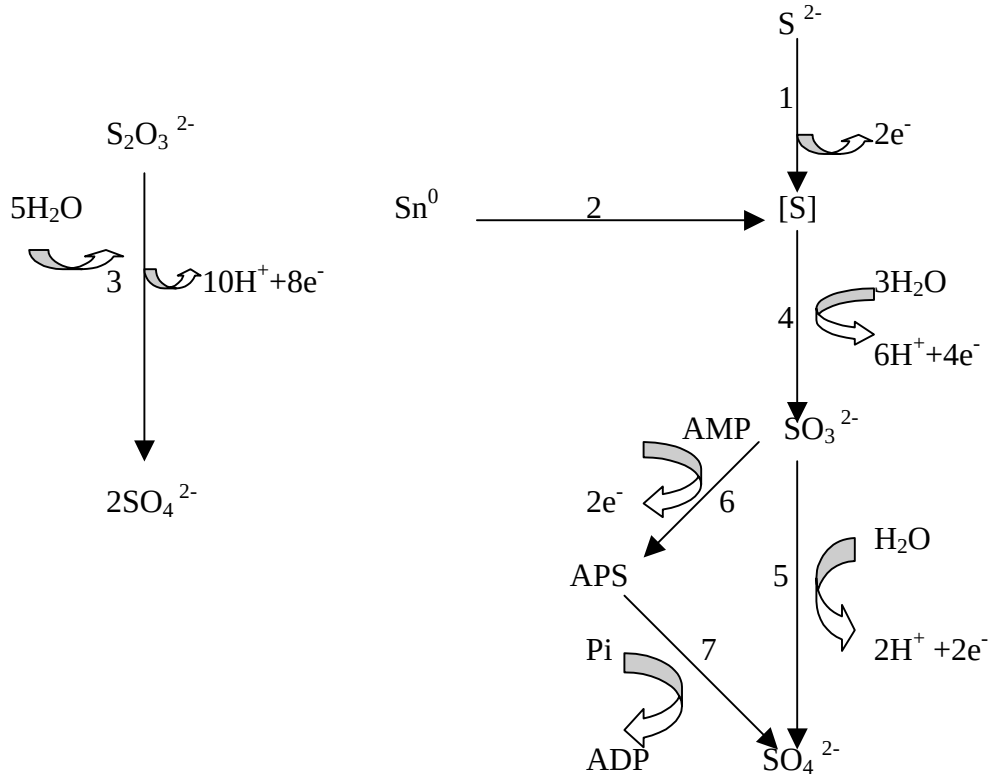

Figure 1. Summary of sulfur oxidation pathways [21].

(1) Oxidation of sulfide to linear polysulfide; (2) conversion of elemental sulfur to linear polysulfide; (3) thiosulfate multienzyme complex; (4) sulfur oxidase; (5) sulfite oxidase; (6) APS reductase; (7) ADP sulfurylase.

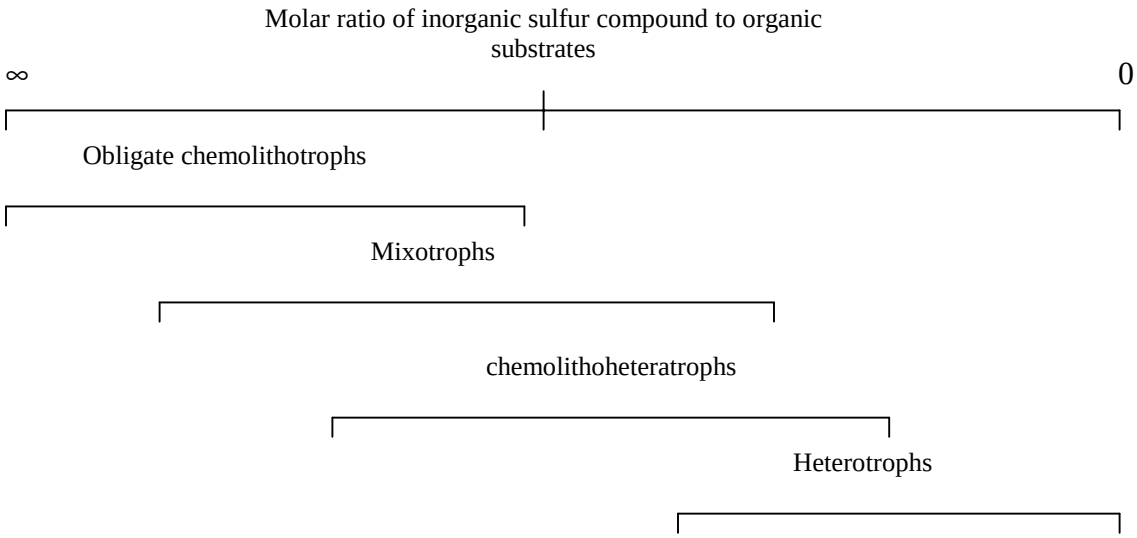

Figure 2. A model to describe selection of different physiological types of sulfur metabolizing microbes based on the ratio of inorganic to organic sulfur substrates [7].

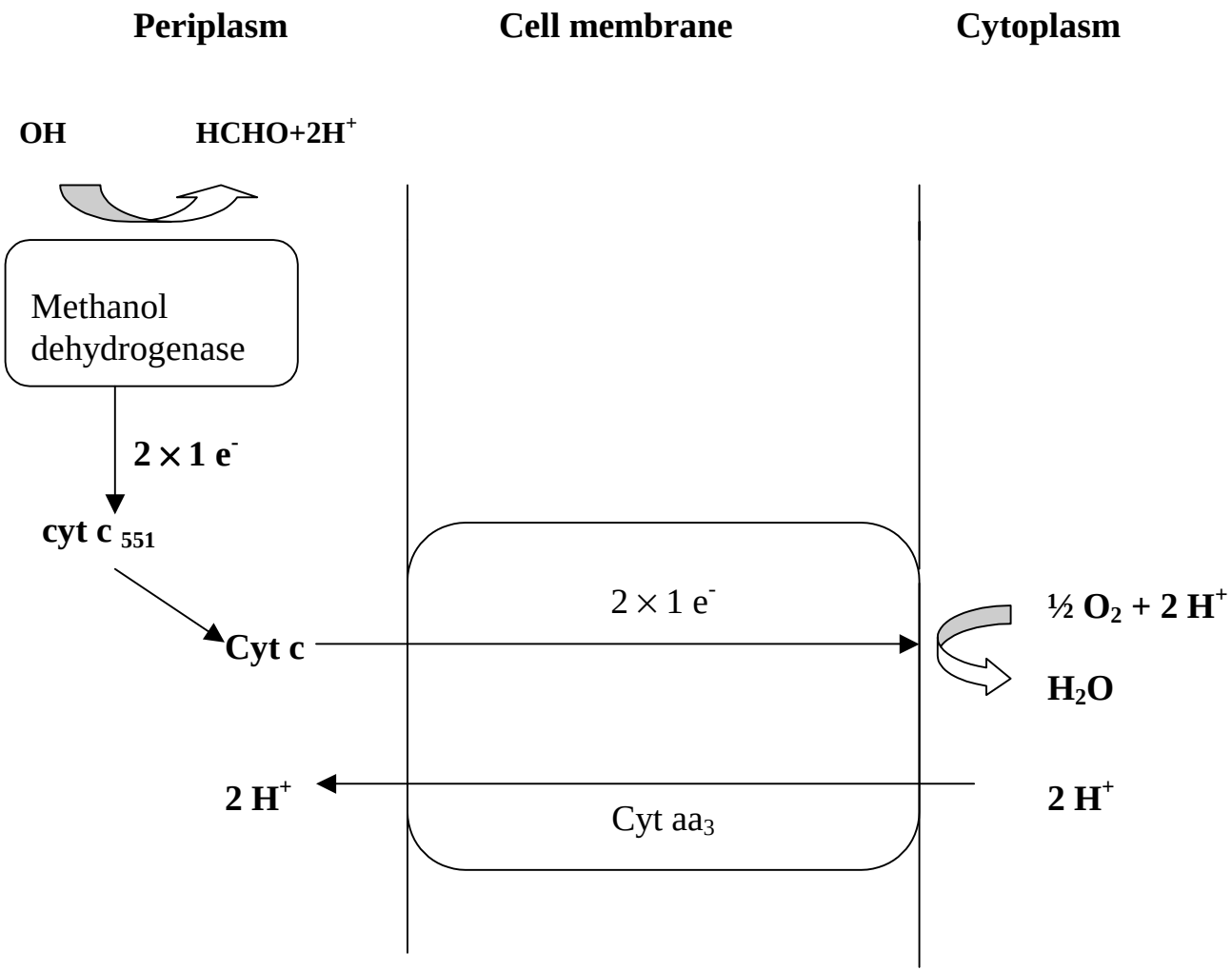

Figure 3. CH_3OH oxidation pathway [21].

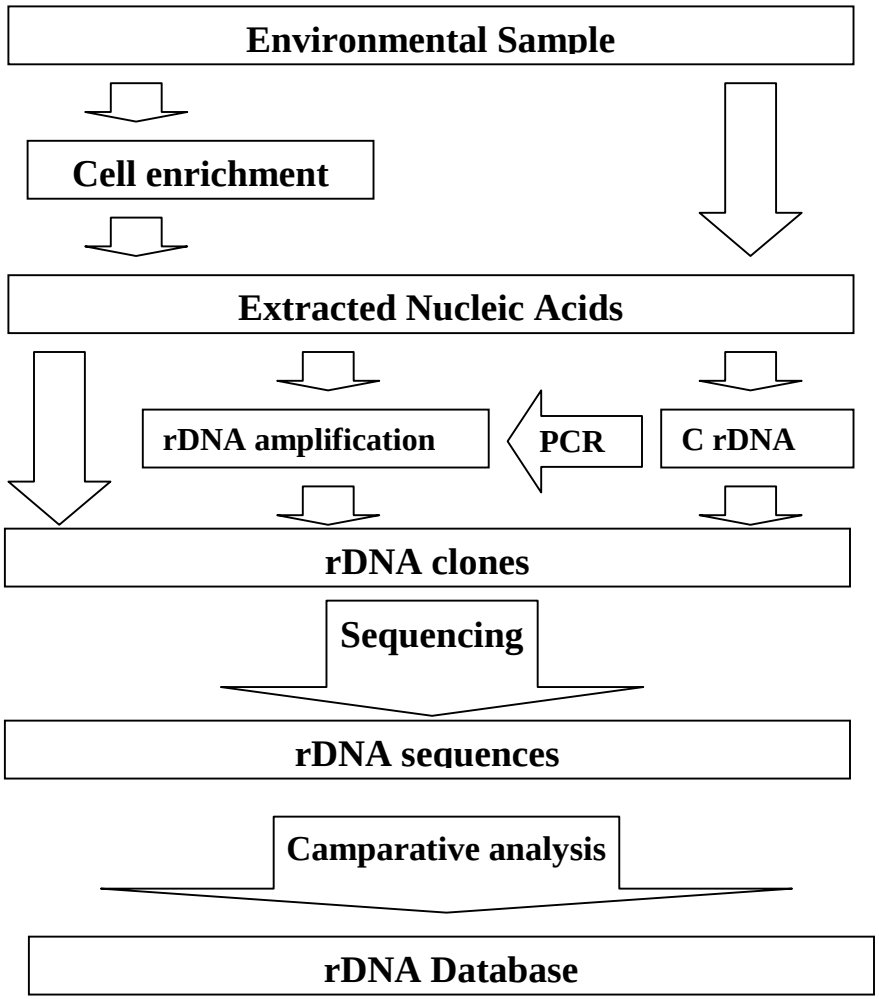

Figure 4. Flow chart showing different possibilities to characterize an environmental sample by comparative rDNA sequence analysis [2].

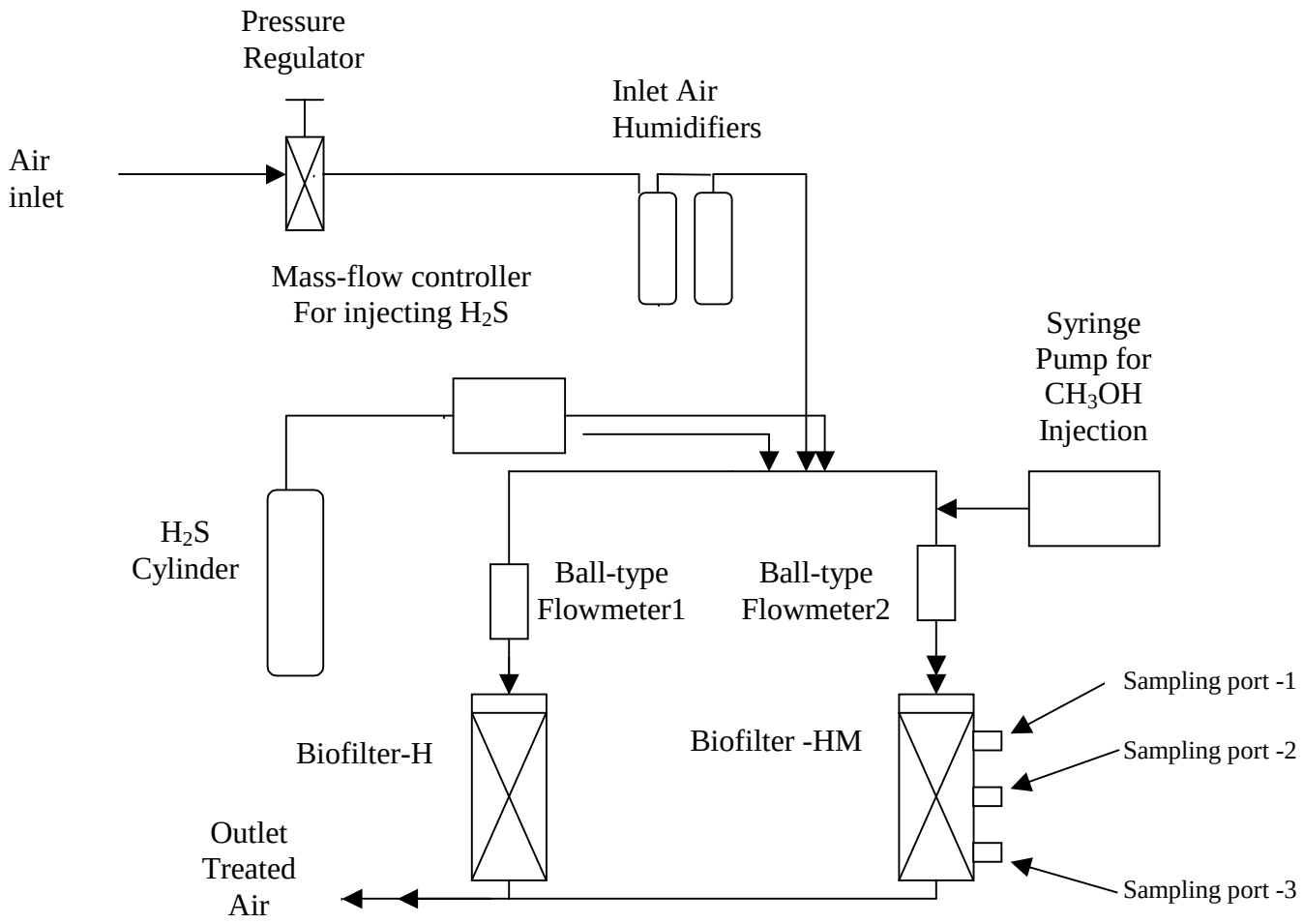

Figure 5. Schematic of experimental biofilter system for treating H_2S and CH_3OH simultaneously or sequentially.

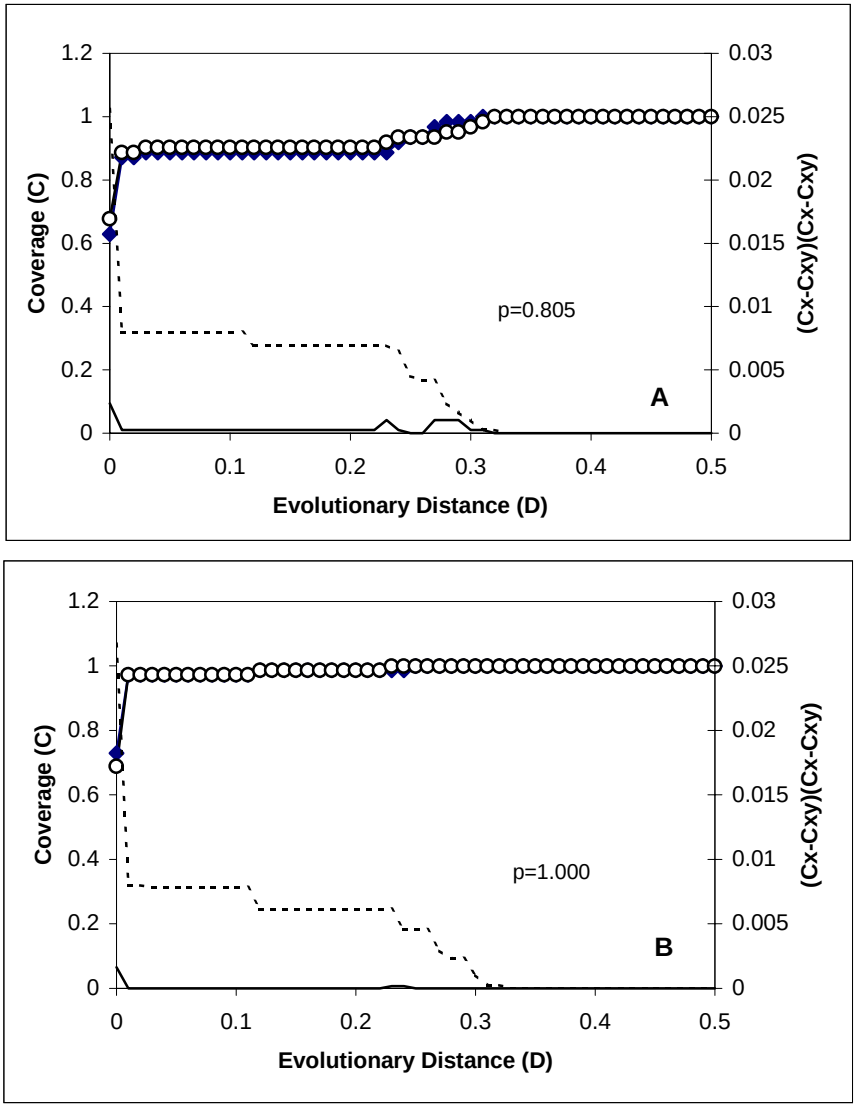

Figure 6. Clone library comparison of M1 (X) to M2 (Y), not significantly different (A). Clone library comparison of M2 (X) to M1 (Y), not significantly different (B). Diamonds indicate homologous coverage of X (Cx), circles are heterologous coverage of XY (Cxy). Solid lines are the difference between the Cx and Cxy as determined by the Cramer von-Mises test statistics and broken lines indicate the 95% statistical difference as determined by LIBSHUFF program. Solid lines greater than broken lines would indicates levels of D where significant difference occur between the two samples.

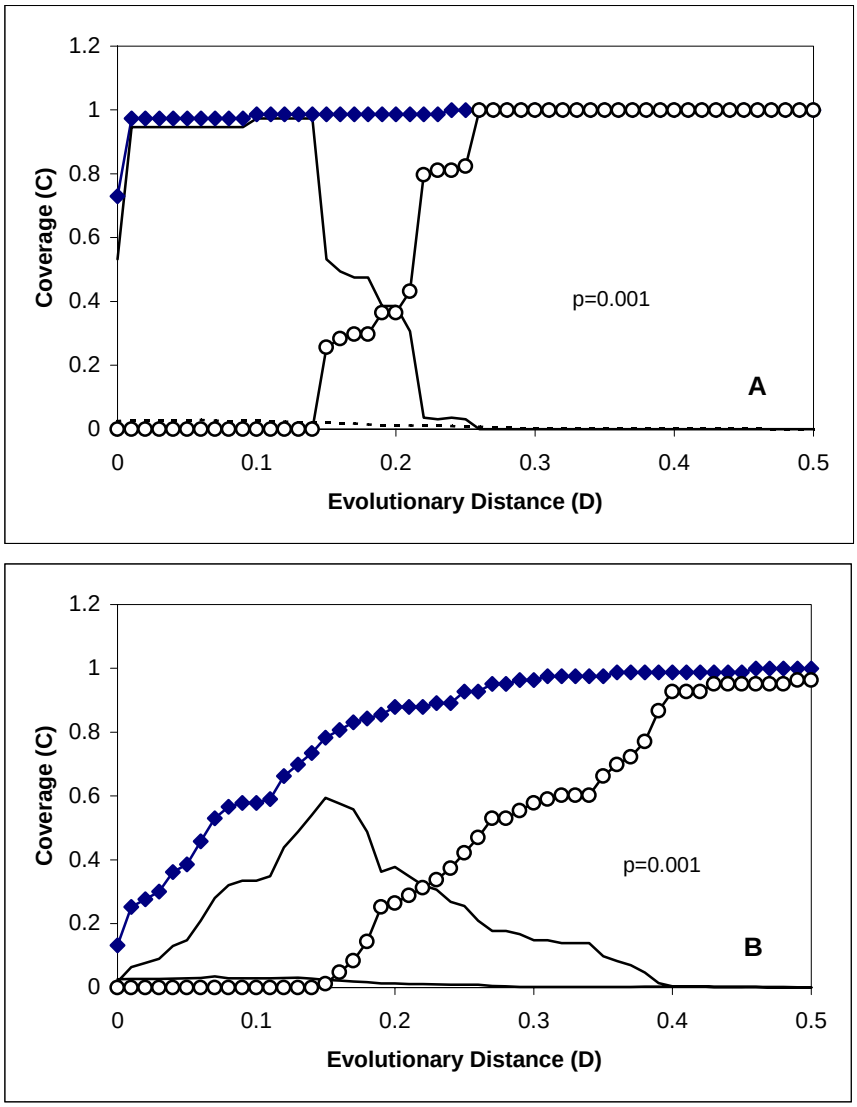

Figure 7. Clone library comparison of M2 (X) to S (Y), significantly different (A). Clone library comparison of S (X) to M2 (Y), significantly different (B). Diamonds indicate homologous coverage of X (Cx), circles are heterologous coverage of XY (Cxy). Solid lines are the difference between the Cx and Cxy as determined by the Cramer von-Mises test statistics and broken lines indicate the 95% statistical difference as determined by LIBSHUFF program. Solid lines greater than broken lines would indicates levels of D where significant difference occur between the two samples.

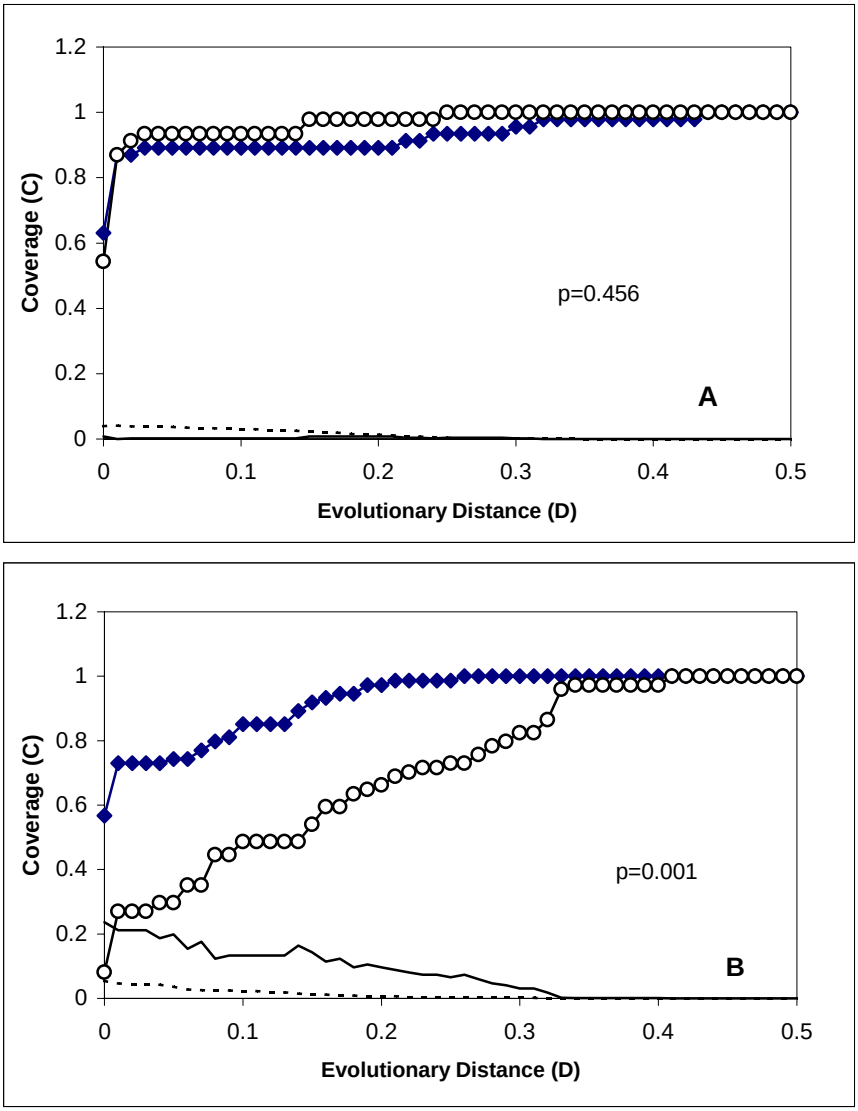

Figure 8. Clone library comparison of F1 (X) to F2 (Y), not significantly different (A). Clone library comparison of F2 (X) to F1 (Y), significantly different (B). Diamonds indicate homologous coverage of X (Cx), circles are heterologous coverage of XY (Cxy). Solid lines are the difference between the Cx and Cxy as determined by the Cramer von-Mises test statistics and broken lines indicate the 95% statistical difference as determined by LIBSHUFF program. Solid lines greater than broken lines would indicates levels of D where significant difference occur between the two samples.

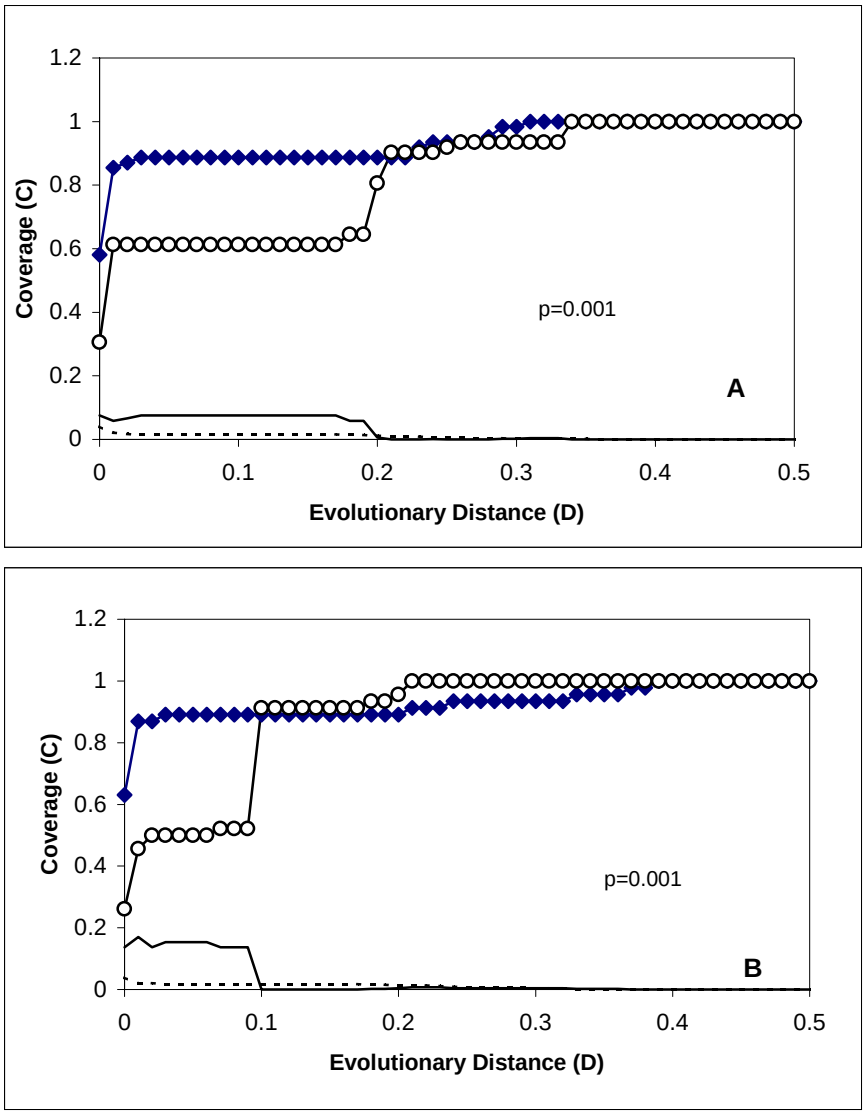

Figure 9. Clone library comparison of M1 (X) to F1 (Y), significantly different (A). Clone library comparison of F1 (X) to M1 (Y), significantly different (B). Diamonds indicate homologous coverage of X (Cx), circles are heterologous coverage of XY (Cxy). Solid lines are the difference between the Cx and Cxy as determined by the Cramer von-Mises test statistics and broken lines indicate the 95% statistical differences as determined by LIBSHUFF program. Solid lines greater than broken lines would indicates levels of D where significant difference occur between the two samples.

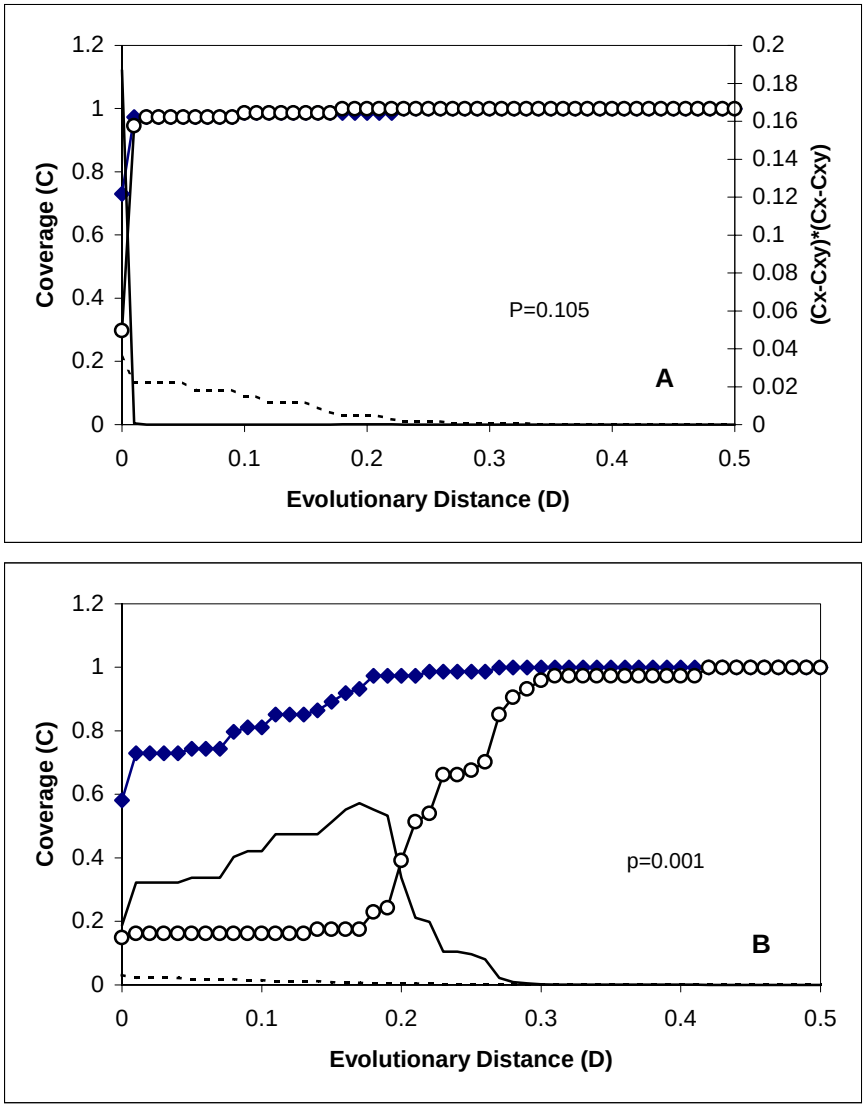

Figure 10 Clone library comparison of M2 (X) to F2 (Y), not significantly different (A). Clone library comparison of F2 (X) to M2 (Y), significantly different (B). Diamonds indicate homologous coverage of X (Cx), circles are heterologous coverage of XY (Cxy). Solid lines are the difference between the Cx and Cxy as determined by the Cramer von-Mises test statistics and broken lines indicate the 95% statistical difference as determined by LIBSHUFF program. Solid lines greater than broken lines would indicates levels of D where significant difference occur between the two samples.

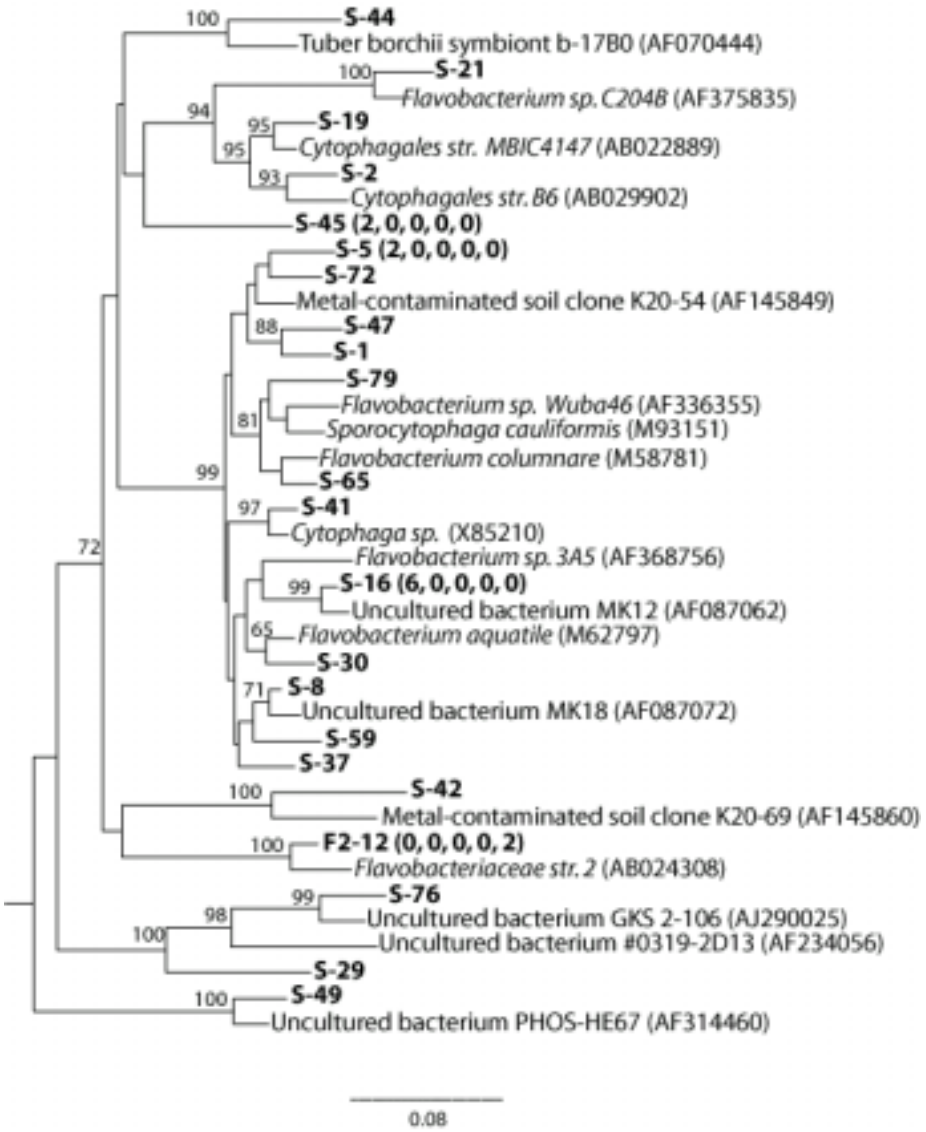

Figure 11. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the CFB group organisms based on analysis of 351 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which come from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Planctomyces staleyi*.

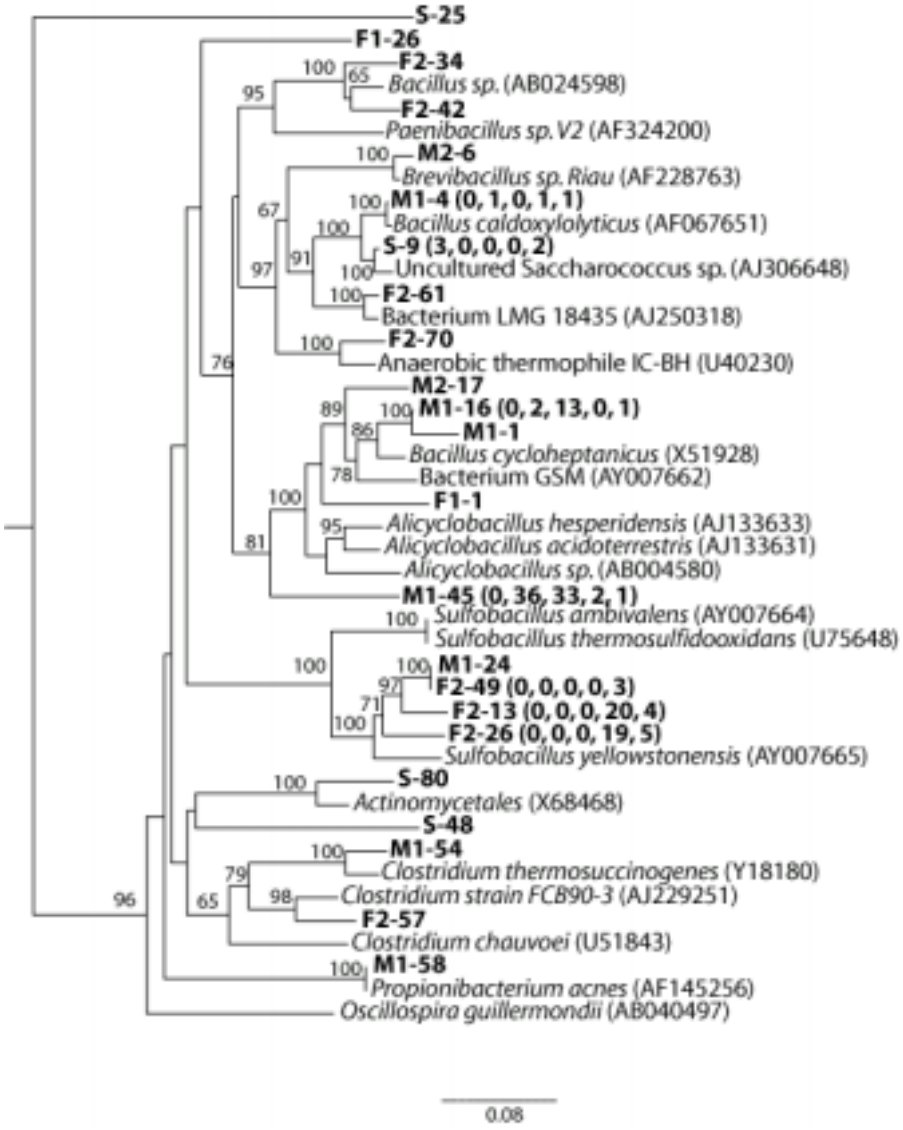

Figure 12. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the Firmicutes group organisms based on analysis of 460 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which come from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Flavobacterium aquatile*.

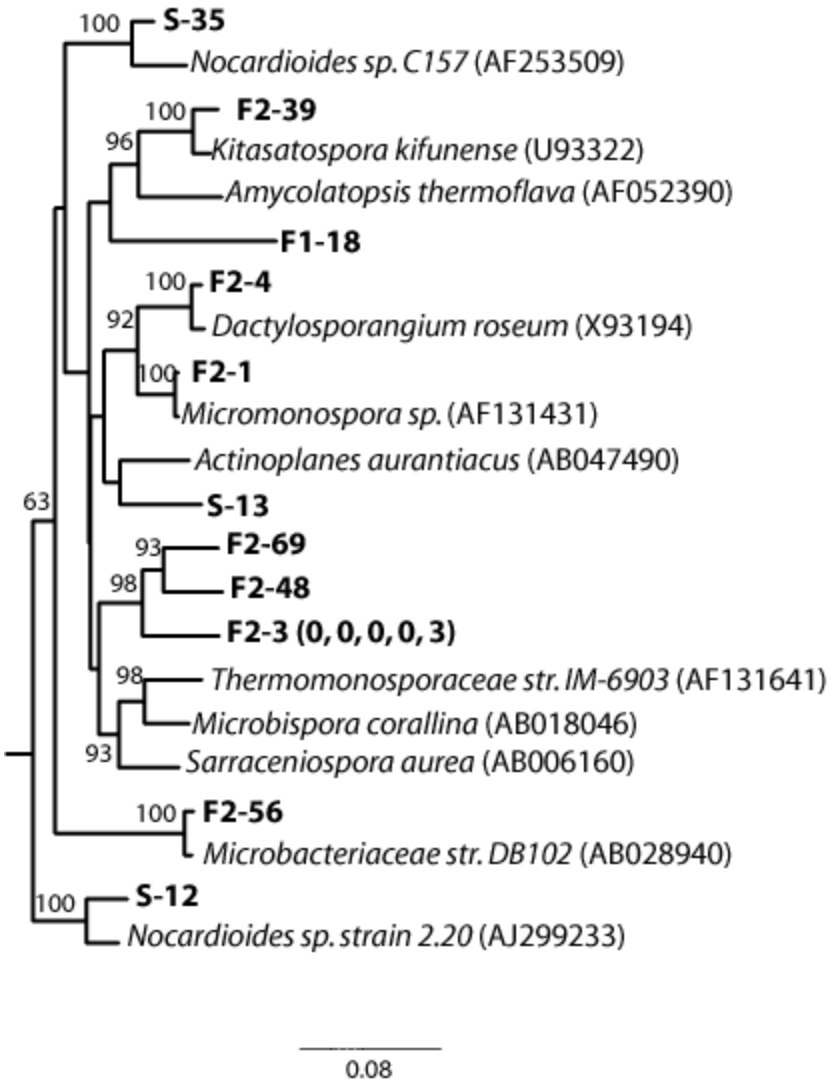

Figure 13. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the Actinobacteria organisms based on 559 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which come from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Flavobacterium aquatile*.

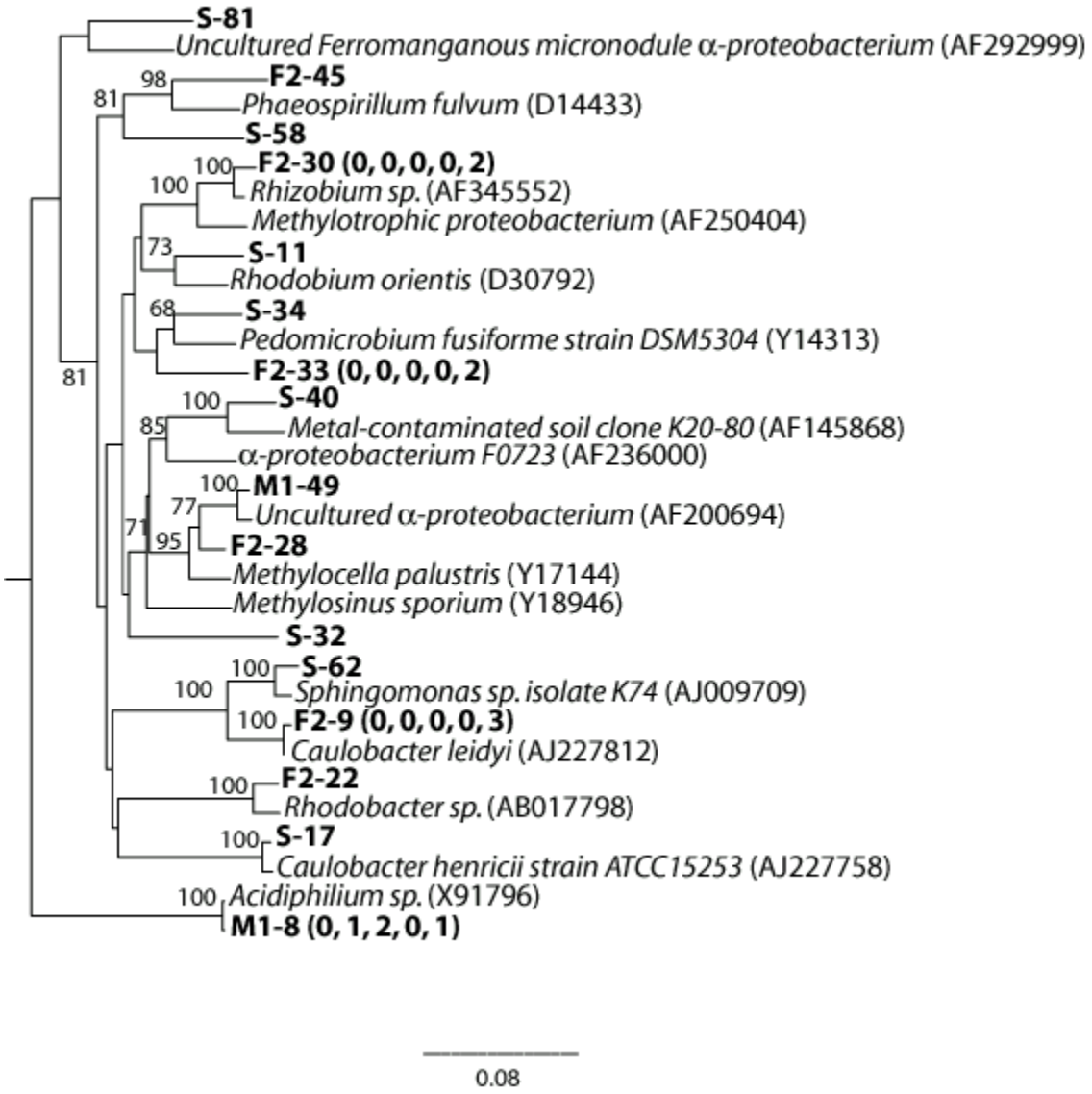

Figure 14. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the α-Proteobacteria organisms based on analysis of 568 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which come from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Planctomyces staleyi*.

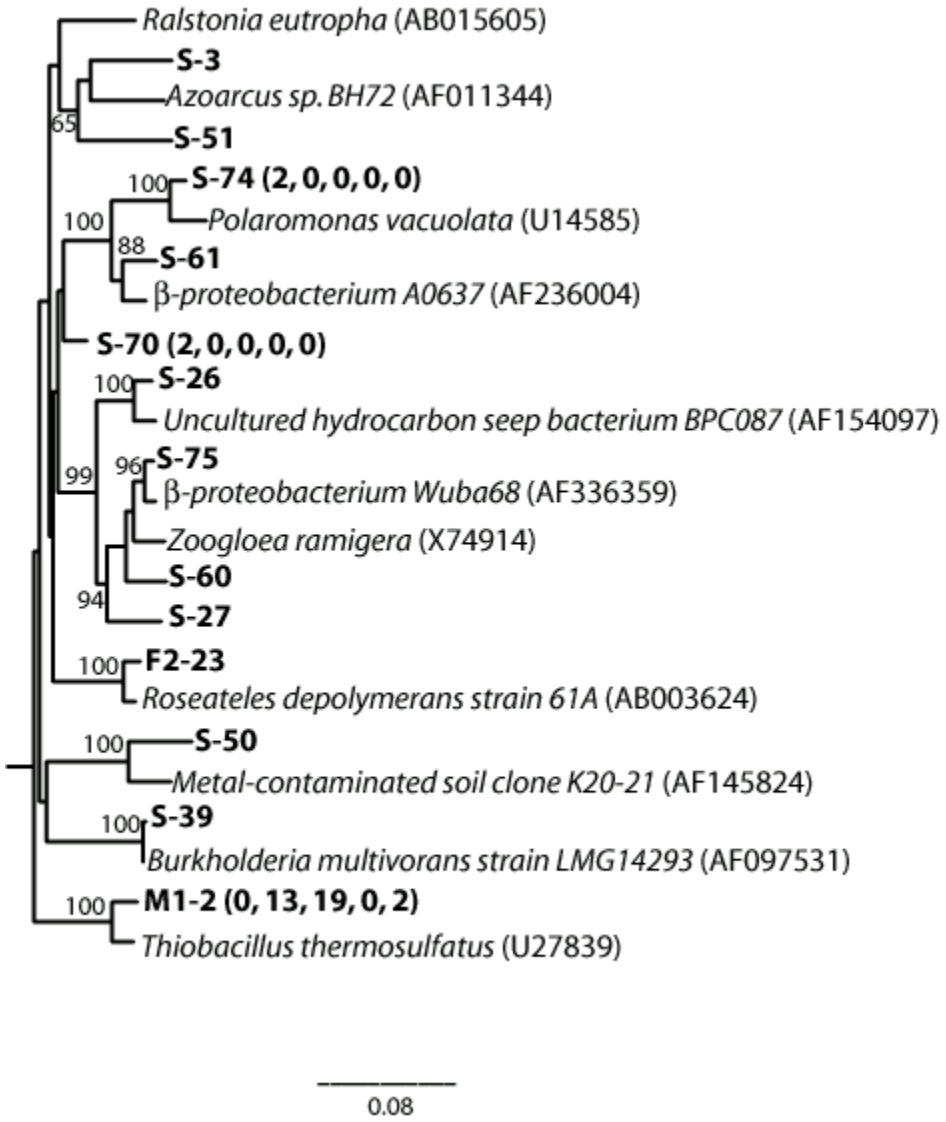

Figure 15. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the β-Proteobacteria organisms based on analysis of 588 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which comes from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Planctomyces staleyi*.

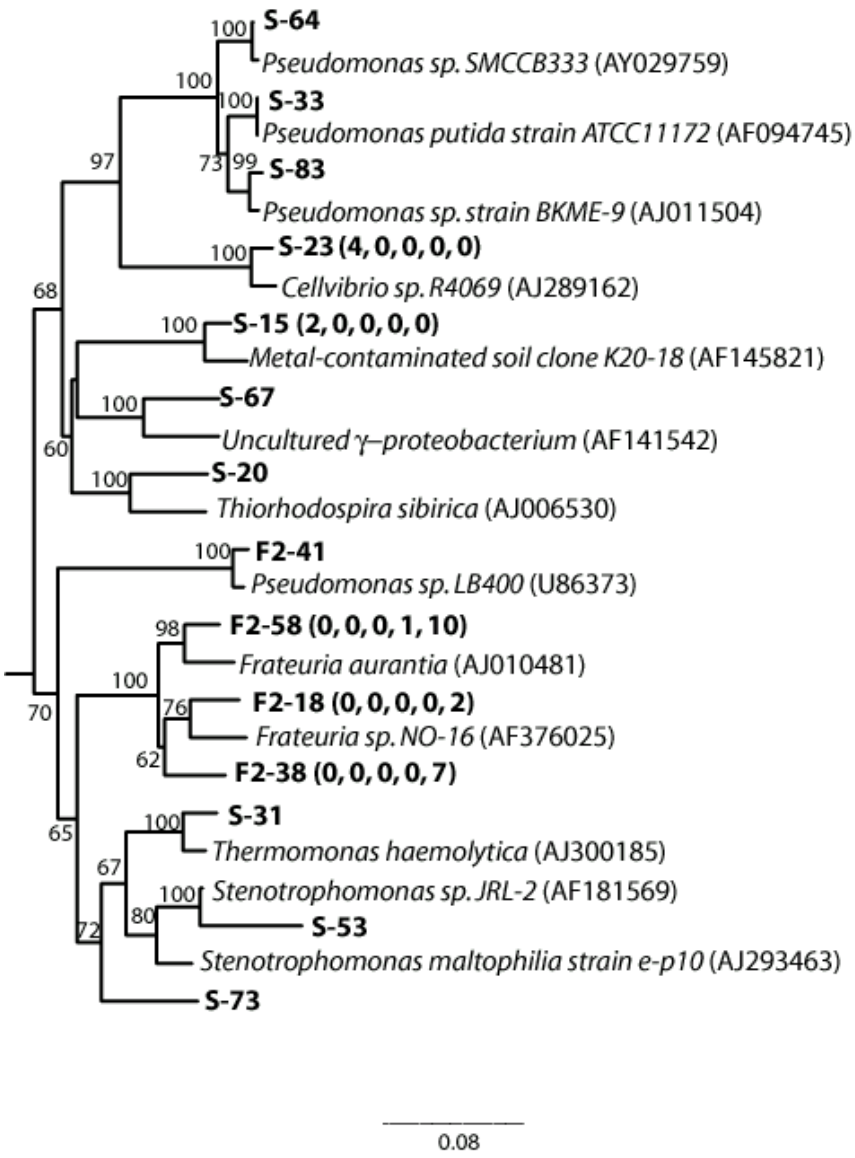

Figure 16. Neighbor-joining tree showing the relationship of sequences from samples S, M1, M2, F1 and F2 to reference members of the γ-Proteobacteria organisms based on analysis of 472 bases of aligned 16S rDNA sequences. OTUs are all in bold and begin with the sample name followed by the sequence number. The numbers in parentheses after each OTU designation refer the number of sequences within that OTU which come from samples S, M1, M2, F1 and F2 respectively. The number in parentheses after each reference sequences refers to the accession number. Bootstrap values greater than 60% are indicated at the nodes. The scale bar represents Jukes-Cantor distance. This tree was rooted with *Planctomyces staleyi*.

CHAPTER 3

KINETIC STUDY OF A BIOFILTER SYSTEM TREATING HYDROGEN SULFIDE AND METHANOL[1]

[1]Ding, Y., K. C. Das, J. R. Kastner, W. B. Whitman. To be submitted to the Journal of the Air & Waste Management Association

ABSTRACT

Degradation kinetics of hydrogen sulfide removal in the presence and absence of methanol was studied using a dual-column biofiltration system. The first biofilter (Biofilter-H) was set up to study the degradation kinetics of hydrogen sulfide only, while the second biofilter (Biofilter-HM) was used to study degradation kinetics of hydrogen sulfide in the presence of methanol. The removal efficiencies for hydrogen sulfide in both biofilters remained at around 50% before the introduction of methanol to Biofilter-HM. The Biofilter-H showed a clear shift from first order to fractional order kinetics. At inlet hydrogen sulfide concentration levels of 0.116 g/m^3 to 0.275 g/m^3, Biofilter-H followed the first order kinetic model. The reaction rate could be represented by the following equation:

$$r = 0.021/sec \times C$$

where r is the reaction rate, 0.021/sec is the average first order rate constant, and C is the inlet hydrogen sulfide concentration in g/m^3. For inlet hydrogen sulfide concentrations between 0.275 g/m^3 and 0.337 g/m^3, Biofilter-H followed a fractional order kinetic model, with reaction rate as follows:

$$r = 0.011\ g^{1/2}m^{-3/2}sec^{-1} \times C^{1/2}$$

Biofilter-HM followed first order kinetics for all the inlet hydrogen sulfide concentrations (0.113 g/m^3 to 0.249 g/m^3) after methanol introduction. The reaction rate was:

$$r = 0.031/sec \times C$$

A one-tail Student's t-test verified that the mean of the first order kinetics rate constant was higher for Biofilter-HM after methanol introduction than that for Biofilter-H. Therefore Biofilter-HM can be expected to remove a greater amount of hydrogen sulfide during first order stage than Biofilter-H. The difference between the two biofilters could be attributed to the presence of methanol in Biofilter-HM.

KEYWORDS: Hydrogen sulfide, Methanol, Kinetic model, Zero order, First order, Fractional order, Rate constant, Reaction rate.

INTRODUCTION

Air pollution is a worldwide environmental issue today. In the USA, approximately 200 million tons of waste gases are released into the air annually [9]. Many industrial processes are sources of air pollutants and therefore are regulated by environmental protection agencies. In the U.S., the enactment of the federal Clean Air Act Amendments of 1990 has brought about stricter regulation of air emissions and calls for techniques that can effectively control air emissions from industrial processes. Commonly both process control to reduce emissions and end of pipe treatments, like scrubbers, incinerators, thermal oxidizers and biofilters are used to achieve this objective [2, 3].

Biofiltration is a preferred way of treating low concentrations of contaminants in large volumes of air. It has low operating and capital costs and produces minimal secondary waste streams [5, 6]. It uses a biologically active, solid medium bed to absorb compounds from the air stream and retain them for their subsequent biological oxidation (Figure 17) [8, 11].

Hydrogen sulfide and methanol are two common air pollutants. Hydrogen sulfide is commonly found in sewage gas streams, and methanol is an important VOC in the forest products industry. The combination of those two gases exists in air emissions of several industrial processes, e.g. the pulp and paper industry. The two gases have different properties that affect their behavior in a biofilter. Hydrogen sulfide is an inorganic sulfur compound. It has a Henry's Constant of 0.373 $H_{cc,\ 20\ °C}$ [10], where $H_{cc} = C_G/C_L$ at 20 °C, and possesses a unique odor with odor threshold of only 0.00047 ppmvv. Methanol is a highly water-soluble organic compound with a Henry's Constant of 1.91E-4 $H_{cc,\ 20\ °C}$ [10]. Biofiltration of hydrogen sulfide and methanol requires distinct groups of microorganisms.

Study on hydrogen sulfide biofiltration has been done extensively. The most representative work has been conducted by Yang and Allen [12]. They determined the

optimal design and operating parameters of a laboratory scale biofilter system for treatment of hydrogen sulfide using compost medium from various sources. They found that the maximum hydrogen sulfide loading capacity was compost specific. The following optimum operating conditions are suggested for control of hydrogen sulfide emissions by compost biofilter systems: temperature: 25 to 50 °C, pH: >3.0, compost water content: 50±15 %, compost sulfate content: < 25 mg-S/g medium, retention time: >15 seconds. The kinetics of hydrogen sulfide oxidation in the biofilter was evaluated and the reaction rates were determined to be first-order at low concentrations (<200 ppmv), zero-order at high concentrations (>400 ppmv), and fractional-order in the intermediate concentration range for hydrogen sulfide [13]. In 200 days of operation, the compost biofilter showed good buffering capacities to variations in gas flow rate and pollutant loading. System acidification and sulfate accumulation were identified as inhibitors to required biological activity.

Methanol biofiltration was studied by Krailas et al who reported the effect of inlet mass loading, water content and total bacteria count on methanol elimination in upward and downward flow biofilters [7]. They found out that both the upward and downward flow biofilters had similar performance in terms of elimination capacity at different inlet mass loadings. The maximum elimination capacity was approximately 101 $g/m^3 \cdot h$ with an optimum methanol loading rate of 169 $g/m^3 \cdot h$ (7.5 g/m^3 of methanol with superficial velocity of 7.6 m/h). In addition, it was found that when the water content in the compost was below 35% by weight, microbial activity was impaired. Similar trends were shown by both the elimination capacity and total bacteria count which initially increased, went through a plateau and then decreased with increased methanol loading.

Dhamwichukorn et al. reported studies on the thermophilic biofiltration of methanol and α-pinene [4]. Two bench-scale thermophilic biofiltration systems were used to examine compound removal at different residence times, with influent concentrations of 110 ppmv methanol and 15 ppmv α-pinene. At a residence time of 10.85 minutes, the

smaller of the two systems had removal efficiencies of >98% for methanol, but only 23% for α-pinene. The larger system was operated with the same parameters to evaluate residence time and surfactant effects on compound removals. At a residence time of 18.24 minutes, the removal rates of both methanol and α-pinene were >95%. However, α-pinene removal dropped to 26% at a residence time of 6.08 minutes while methanol removal remained unaffected.

Although the biofiltration of hydrogen sulfide and methanol have been studied before, the study on combined biofiltration of hydrogen sulfide and methanol has not been reported. As both hydrogen sulfide and methanol are common industrial air pollutants, and their combination occurs in many industrial settings, an understanding of the combined biofiltration of hydrogen sulfide and methanol would be useful. It is also useful to identify any interaction between hydrogen sulfide and methanol during their biofiltration. Therefore, the objective of this section of the research was to identify the effect of methanol introduction on hydrogen sulfide biofiltration through biofilter performance and kinetics study.

MATERIALS AND METHODS

Biofiltration was conducted using a dual-column laboratory-scale biofilter system as shown in Figure 5. The two biofilters were denoted as Biofilter-H and Biofilter-HM. Biofilter-H was supplied with hydrogen sulfide as the influent gas throughout the 49 days of the experiment, thereby developing a microbial community adapted to hydrogen sulfide metabolism. Biofilter-HM was supplied with hydrogen sulfide as the influent gas until a steady-state of hydrogen sulfide treatment was obtained (i.e. change in the outlet hydrogen sulfide concentration between two consecutive days was less than 15%), followed by an influent gas composed of a mixture of hydrogen sulfide and methanol. A microbial community comprised mainly of hydrogen sulfide and methanol metabolizing microbes was expected to develop in Biofilter-HM. A steady-state of hydrogen sulfide

treatment was reached by both biofilters at day 20th. Methanol was then introduced to Biofilter-HM only at day 20th.

Yard trimming compost in a ratio of 3:1 (v/v) of 0.11-0.25 inch and 0.25-0.50 inch particle sizes was used as the biofilter medium. A 100 g sample of the medium was dried in an oven at 105 °C for 18 hours for moisture content measurement. Bulk density was measured by weighing a 500 ml sample of the medium in a graduated cylinder. Particle density was measured using an air pycnometer [1]. Porosity of the medium was calculated from bulk density and particle density. The ash content of the medium was measured by incinerating 25 g of sample in a muffle furnace at 550 °C for 6 hr. Carbon, sulfur, nitrogen contents of the medium were measured using a Leco CNS analyzer. Trace elements were analyzed using an ICP mass spectrometer. Inlet concentrations of hydrogen sulfide and methanol were selected based on maximum degradable concentrations and loading rates reported by Devinny et al [3]. The hydrogen sulfide concentrations ranged from 0.116 g/m^3 to 0.337 g/m^3 for Biofilter-H, and 0.113 g/m^3 to 0.249 g/m^3 for Biofilter-HM.

The biofilters were glass columns with a total height of 69 cm, an active medium height of 52.5 cm, a 10-cm internal diameter, and an 11.5-cm external diameter (Figure 5). Each biofilter had three sampling ports evenly distributed along the column. The packing heights were 6 cm, 11.5 cm, 11.5 cm, 23.5 cm between inlet and port 1, port 1 and port 2, port 2 and port 3, port 3 and outlet, respectively. Air was introduced into the biofilter system from the top of the column after stabilizing pressure in an impinger and humidification by bubbling through a water column containing deionized water. Airflow was maintained using rotameters (1 to 10 lpm). Gaseous hydrogen sulfide was provided from a standard cylinder of 5% hydrogen sulfide in nitrogen. Flow rate of hydrogen sulfide was controlled using an AALBORG Mass Flow Controller (0 to 0.2 lpm). Hydrogen sulfide was continuously mixed with humidified air in the inlet line to each column. Liquid methanol was introduced only into Biofilter-HM using a syringe pump

(Cole–Parmer ® Instrument Company 74900 Series). Before the start of the experiment, the biofilter system was tested for any leaks by tracing with an UltroFlow ™ Primary Gas Flow Calibrator (0.001-6 lpm) produced by A. P. Buck Inc.

The system was tested for plug flow behavior by determining the residence time distribution using methane as a tracer gas. A 1 ml tracer of 10,000 ppmv methane was injected into the inlet of the biofilter and the outlet methane concentration was monitored for 6 minutes using a Model 680 Hydrocarbon Vapor Meter. The outlet methane concentrations for Biofilter-H and Biofilter-HM as a function of time was plotted to evaluate the shape of the distribution.

Fractional conversions for hydrogen sulfide or methanol of the biofilters were calculated as the difference between inlet and outlet concentration divided by the inlet concentration for the particular gas. Concentration of hydrogen sulfide gas was measured using a Jerome 631-X Hydrogen Sulfide Analyzer (Arizona Instrument Company). The calibration curve of Jerome 631-X is shown in Appendix 1. Concentration of methanol was measured using a Model 680 Hydrocarbon Vapor Meter (Thermo Environmental Instruments Inc.). The pH of the medium was measured on a 5:1 (v/v) deionized water to solids extract using an Accumet-125 pH/mV meter.

In order to determine the kinetic model for each biofilter, different inlet concentrations of hydrogen sulfide were chosen. For each inlet concentration, hydrogen sulfide concentrations at five sampling sites: inlet, port 1, port 2, port 3 and outlet were measured and recorded everyday until the hydrogen sulfide treatment reached a steady-state. The hydrogen sulfide concentrations of the last three days of sampling at steady-state were averaged for each sampling site and used in calculating the rate constant for each assumed kinetic model. Three kinetic models were assumed for each biofilter, namely, [1] zero order, when reaction rate equals the rate constant; [2] first order, when reaction rate equals the rate constant times inlet compound concentration in g/m^3; and [3] fractional order, when reaction rate equals the rate constant times the inlet compound

concentration raised to ½ order. The mean and standard deviation of the rate constant at any one hydrogen sulfide inlet concentration was calculated for each kinetic model. The ratio between standard deviation and mean was used to verify the order of kinetics, where the smallest ratio indicated the correct kinetic model for that concentration level.

RESULTS AND DISCUSSION

Plug Flow Behavior of the System

Both Biofilters showed similar plug flow pattern (Figure 18). Both biofilters reached peak outlet concentration of methane at around 140 seconds after methane introduction into the biofilter, suggesting relatively close residence time at the given flow rate. The two biofilters were comparable in initial medium structure and no distinct channeling existed in either biofilter at the beginning of the biofiltration experiment.

Initial and Final Medium Properties of Biofilter-H and Biofilter-HM

The physical and chemical properties of the initial medium are listed in Table 3. The pH of initial medium was slightly acidic (5.8), probably because of humic acids in the medium. Moisture content was found to be 54.9%, a value considered acceptable for biofiltration and bulk density was 447.2 kg/m^3.

By the end of the experiment, the moisture content of both biofilters had increased around 10% compared to the initial medium due to the introduction of water throughout the biofiltration process (Table 3). The moisture contents of the final medium in both biofilters were similar within experimental error. Bulk densities of both biofilters had increased compared to the initial medium probably because the media packed tighter during the biofiltration. The bulk densities of both biofilters were comparable within experimental error. The pH values of both biofilters had decreased compared to the initial medium because of the accumulation of sulfate during hydrogen sulfide oxidation. The pH of Biofilter-HM was slightly higher than Biofilter-H. Carbon contents for both

biofilters had increased comparing to initial medium and carbon content for Biofilter-HM was higher than Biofilter-H. Sulfur contents of both biofilters also increased due to the accumulation of sulfur - an intermediate in hydrogen sulfide microbial oxidation. Biofilter-HM had lower sulfur content at the end of the experiment comparing to Biofilter-H. The sulfate content of both biofilters had increased compared to the initial medium and sulfate content was higher for Biofilter-HM than Biofilter-H. With lower concentration of sulfur (an intermediate in hydrogen sulfide microbial oxidation) and higher concentration of sulfate (end product of hydrogen sulfide microbial oxidation), the hydrogen sulfide microbial oxidation might be more complete for Biofilter-HM than for Biofilter-H. The chloride contents for both biofilters decreased which suggested leaching or that the biofiltration of hydrogen sulfide and methanol utilized chloride. The nitrate contents did not change much between initial and final samples. The phosphate concentrations increased for final samples compared to initial medium due to the input of phosphate buffer which was used to maintain pH. Other trace elements showed similar decrease or increase pattern between Biofilter-H and Biofilter-HM final media.

System Performance of Biofilter-H for Hydrogen Sulfide Treatment

The inlet, outlet concentrations and fractional conversion of Biofilter-H are shown in Figure 19. The fractional conversion of Biofilter-H was high (~90%) at the beginning and then dropped and stayed around 50% when the inlet hydrogen sulfide concentration changed from 0.116 g/m^3 to 0.295 g/m^3. The higher initial fractional conversion was probably due to medium absorption of hydrogen sulfide. When the medium was saturated with hydrogen sulfide the fractional conversion began to drop. As the microbial community gradually adapted to the hydrogen sulfide introduction, the drop of fractional conversion slowed and remained stable at around 50% throughout the rest of the experiment.

Hydrogen Sulfide Biofiltration Kinetics in Biofilter-H

The kinetic models selected for each inlet hydrogen sulfide concentration for Biofilter-H are listed in Table 4 (sample calculation for selecting a kinetic model was shown in Appendix 2). For inlet hydrogen sulfide concentrations of 0.116 g/m^3 to 0.275 g/m^3, the hydrogen sulfide biofiltration followed first order kinetics. At inlet hydrogen sulfide concentrations around 0.337 g/m^3, the Biofilter-H began to follow fractional order kinetics. The average of all those calculated first order rate constants after methanol introduction was found to be 0.021±0.009/sec and the rate constant for fractional order kinetics was 0.011 $g^{1/2}m^{-3/2}sec^{-1}$.

System Performance of Biofilter-HM for Hydrogen Sulfide Treatment

The inlet and outlet hydrogen sulfide concentration and fractional conversion of Biofilter-HM are show in Figure 19. Similar to Biofilter-H, in the initial 20 days of the experiment, the fractional conversion of Biofilter-HM was high (~90%) and then dropped to around 60%. The fractional conversion of Biofilter-HM after methanol introduction was initially lower than Biofilter-H and from day 27^{th} began to increase to a value (average fractional conversion between day 27^{th} and day 49^{th} was 0.600±0.088) higher than that of Biofilter-H (average fractional conversion between day 27^{th} and day 49^{th} was 0.468±0.091). One-tail t-test verified that the average fractional conversion of Biofilter-HM from day 27^{th} to day 49^{th} was higher than that of Biofilter-H with a p-value of < 0.005.

Determination of Hydrogen Sulfide Biofiltration Kinetic Model for Biofilter-HM

The kinetic models selected at each inlet hydrogen sulfide concentration for Biofilter-HM are listed in Table 5. For all the inlet hydrogen sulfide concentration of 0.113 g/m^3 to 0.249 g/m^3, the hydrogen sulfide biofiltration followed first order kinetics. The average of all the calculated first order rate constants was 0.031±0.011/sec.

Hydrogen Sulfide Biofiltration Kinetics Comparison between Biofilter-H and Biofilter-HM

Both biofilters would show a similar pattern in kinetics with a shift from first to fractional order. This shift was identified in Biofilter-H. For Biofilter-HM, more data need to be collected to demonstrate the shift from first to fractional order in kinetics. The difference in kinetics pattern between Biofilter-H and Biofilter-HM was that the rate constants for first order kinetic model of Biofilter-HM (0.031±0.011/sec) after methanol introduction was higher than that of Biofilter-H (0.021±0.009/sec). A one-tail t-test was conducted to test the hypothesis that the mean first order rate constant for Biofilter-HM after methanol introduction on day 20 was higher compared to that for Biofilter-H. The calculated p-value was 0.042, indicating that at confidence level of 0.05, the hypothesis should be accepted. With a larger first order rate constant, Biofilter-HM would remove more hydrogen sulfide during the first order kinetics stage than Biofilter-H. This difference was possibly caused by the introduction of methanol to Biofilter-HM. This behavior is graphically illustrated as a plot of reaction rate at different inlet concentrations for the two biofilters (Figure 20).

System Performance of Biofilter-HM for Methanol Removal

Methanol was introduced into Biofilter-HM at three concentration levels. They were 1.33g/m^3, 0.40g/m^3, and 0.13g/m^3. The biofiltration performance of biofilter-HM for methanol is shown in Table 6. For all three situations, the methanol removal rate was around 65%.

CONCLUSIONS

The final medium for Biofilter-HM was of lower sulfur (an intermediate of hydrogen sulfide microbial oxidation) and higher sulfate (end product of hydrogen sulfide microbial oxidation) compared to that of Biofilter-H. This suggests that the

microbial oxidation in Biofilter-HM may be more complete than that in Biofilter-H. Therefore, more energy per gram of substrate can be gained in Biofilter-HM microbial oxidation than in Biofilter-H.

Both biofilters removal efficiencies for hydrogen sulfide were maintained at around 50% before the introduction of methanol to the Biofilter-HM. After introduction of methanol to Biofilter-HM at day 20th, its fractional conversion declined until day 27th, and then became higher than that of Biofilter-H. Biofilter-H showed a clear shift from first order kinetics to fractional order kinetics. At the inlet hydrogen sulfide concentration between 0.116 g/m^3 to 0.275 g/m^3, hydrogen sulfide removal in Biofilter-H followed the first order kinetics. The reaction rate can be determined using the following formula:

$$r = 0.021/sec \times C$$

where r is the reaction rate, 0.021/sec is the first order rate constant, and C is the inlet hydrogen sulfide concentration in g/m^3. Between the inlet hydrogen sulfide concentration of 0.275 g/m^3 and 0.337 g/m^3, Biofilter-H followed the fractional order kinetics where the reaction rate was:

$$r = 0.011\ g^{1/2}m^{-3/2}sec^{-1} \times C^{1/2}$$

Biofilter-HM hydrogen sulfide removal was of first order for all the inlet hydrogen sulfide concentrations used (0.113g/m^3 to 0.249 g/m^3) with a reaction rate as follows:

$$r = 0.031/sec \times C$$

One-tail t-test between first order rate constants of Biofilter-HM after methanol introduction at day 20th and that of Biofilter-H verified that the means of the first order kinetics rate constants were significantly higher for Biofilter-HM than Biofilter-HM (at 0.05 confidence level). Therefore Biofilter-HM would remove more hydrogen sulfide during first order stage than Biofilter-H. This difference might have been caused by introduction of methanol to Biofilter-HM.

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Table 3. Physical and chemical properties of initial and final media for Biofilter-H and Biofilter-HM.

Properties	Initial medium	Final medium for Biofilter-H	Final medium for Biofilter-HM
Moisture content (%)	54.92±1.50	63.77±1.21	62.64±2.66
Bulk density (kg/m3)	447.2±4.5	696	693.8
pH	5.8	2.0-3.0	3.0-4.0
Chemical properties			
Total Nitrogen (ppm)	3.278	1.089	1.031
Medium Leco CNS Analysis (%)			
Carbon	31.71	34.69	37.63
Sulfur	0.381	1.758	1.307
Nitrogen	1.196	0.849	0.741
Extracted Nutrients (ppm):			
Chloride	2.31±0.061	0.53±0.10	0.68±0.022
Nitrate	0.20±0.028	0.19±0.21	0.11±0.069
Phosphate	0.32±0.16	56.04±2.50	74.79±4.10
Sulfate	11.26±0.092	178.77±18.93	256.83±8.82
B	382±3	354±140	248±17
Na	2008±113	84601±6428	142784±1950
Mg	3475±20	2069±1505	2934±449
Al	6196±433	1232±30	1812±296
P	892±37	50012±5031	61194±7767
K	69275±4964	31395±2402	50116±317
Ca	13918±711	4215±876	3994±166
Cr	5.03±0.21	1.48±1.19	2.06±0.87
Mn	247.30±3.83	65.67±24.08	41.10±6.26
Fe	3015.4±10.3	437.5±29.7	846.2±93.2
Ni	4.49±0.28	2.94±0.042	3.82±3.35
Co	3.68±0.12	2.28±0.53	1.56±0.37
Cu	27.90±6.36	20.70±9.87	37.77±0.037
Zn	38.73±5.45	87.61±3.12	48.06±12.94
As	3.08±0.13	1.56±0.16	1.50±0.21

Table 3 continued

Se	0.77±0.30	0.16±0.041	1.99±0.00
Mo	3.76±1.38	0.32±0.091	0.15±0.031
Ag	0.08±0.025	0.07±0.024	0.78±1.05
Cd	0.15±0.0052	0.24±0.075	0.45±0.22
Cs	0.18±0.011	0.26±0.032	0.18±0.0049
Ba	47.64±0.86	149.73±0.97	115.58±5.16
Hg	0.43±0.0031	0.11±0.040	0.28±0.050
Pb	10.85±1.25	7.03±1.53	4.84±0.94
Solid Medium ICP Analysis (ppm):			
Na	61.179	2288.127	3517.028
Mg	1941.707	1025.372	995.251
Al	6593.179	8424.313	7649.629
P	671.514	2703.521	2951.514
K	3932.035	2549.963	2948.606
Ca	8084.124	4014.336	22.067
Cr	37.312	13.978	12.1
Mn	355.598	56.604	54.237
Fe	9316.107	8589.672	7984.852
Ni	7.691	7.668	10.32
Co	4.985	2.461	2.204
Cu	17.164	25.635	19.912
Zn	113.412	11.737	82.5
As	1.242	1.31	1.176
Se	/	12.353	0.087
Mo	0.772	0.76	0.573
Ag	0.386	11.33	6.321
Cd	326.615	46.03	55.484
Cs	649.756	775.074	794.689
Ba	88.776	126.78	90.798
Hg	6.466	3.037	0.205
Pb	20.139	24.892	23.544

Table 4. Selected kinetic models for each inlet hydrogen sulfide concentration for Biofilter-H.

Inlet concentration (g/m^3)	Kinetic model selected	Rate constant value
0.337	Fractional order	0.011 $g^{1/2}m^{-3/2}sec^{-1}$
0.275	First order	0.014/sec*
0.217	First order	0.013/sec*
0.202	First order	0.015/sec*
0.178	First order	0.015/sec*
0.147	First order[1]	0.019/sec*
0.144	First order	0.036/sec*
0.139	First order	0.025/sec*
0.131	First order	0.041/sec
0.116	First order	0.032/sec*

[1]The kinetic model at this inlet concentration was zero order according to calculation. But first order should be the reasonable kinetic model at this concentration level based on other results.

*Those first order kinetics rate constants were obtained after methanol introduction to Biofilter-HM at day 20^{th}.

Table 5. Selected kinetic models for each inlet hydrogen sulfide concentration for Biofilter-HM when methanol was present.

Inlet concentration (g/m^3)	Kinetic model selected	Rate constant value (sec^{-1})
0.249	First order	0.021
0.218	First order	0.034
0.217	First order	0.018
0.194	First order	0.055
0.174	First order[1]	0.029
0.137	First order[1]	0.034
0.128	First order[1]	0.032
0.113	First order	0.024

[1]The kinetic model at this inlet concentration was zero order according to calculation. But first order should be the reasonable kinetic model at this concentration level based on other results.

Table 6. Methanol biofiltration performance of Biofilter-HM.

	Methanol Concentration (g/m^3)		
Inlet	1.33	0.58	0.120
Port one	0.57	0.48	0.054
Port two	1.20	0.13	0.036
Port three	0.84	0.11	0.035
Outlet	0.51	0.14	0.041
Removal efficiency	62.0%	75.9%	65.8%

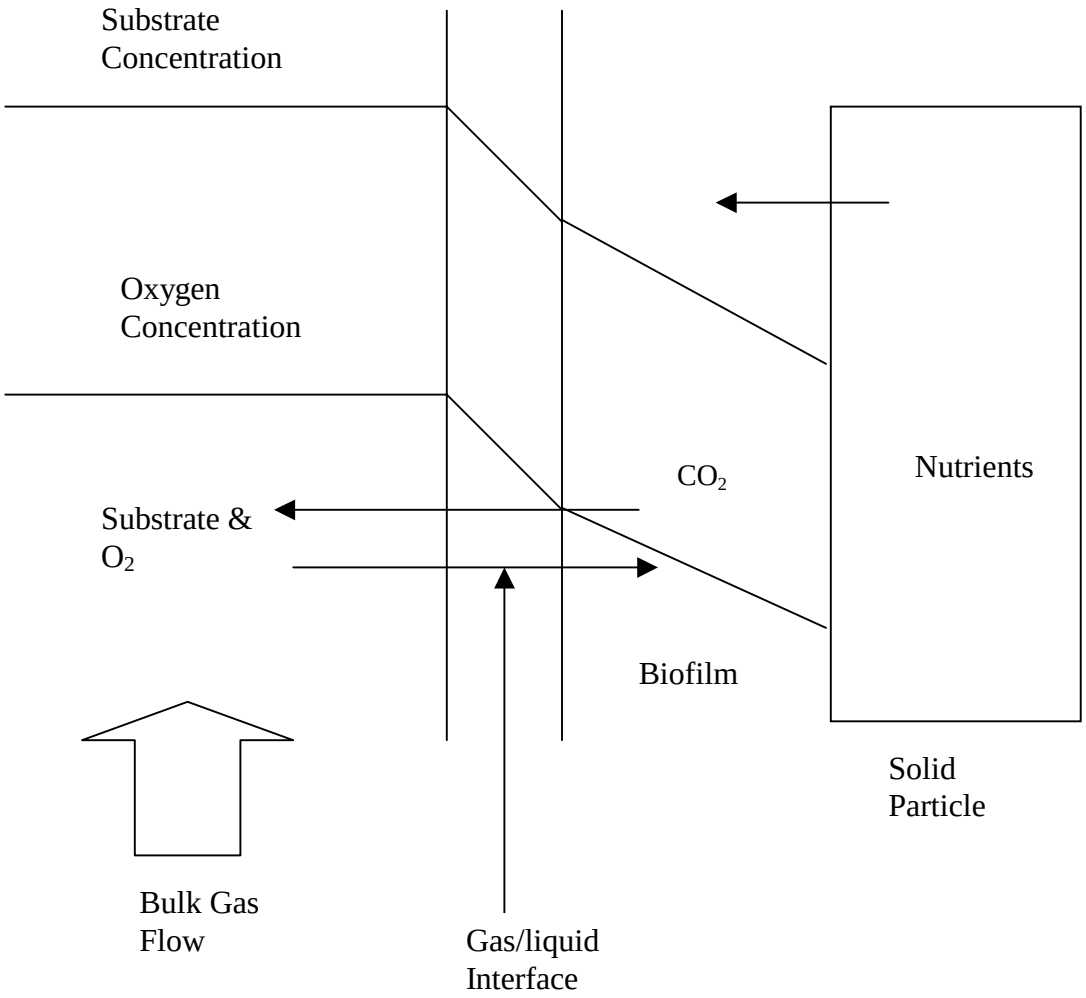


Figure 17. Principle of Biofiltration [8].

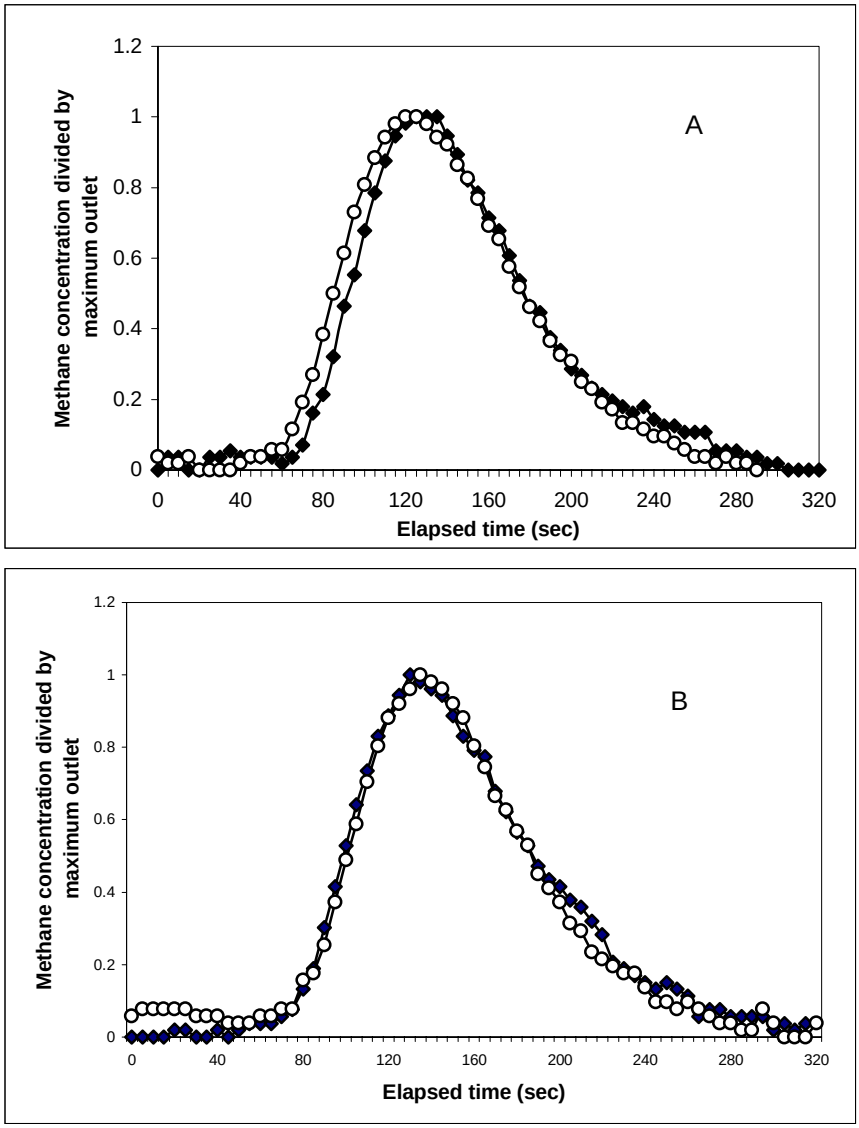


Figure 18. Residence time distribution of (A) Biofilter-H and (B) Biofilter-HM. Shapes indicate near plug flow behavior with average residence time of 130 seconds. The two curves (°, ♦) indicate two independent replications.

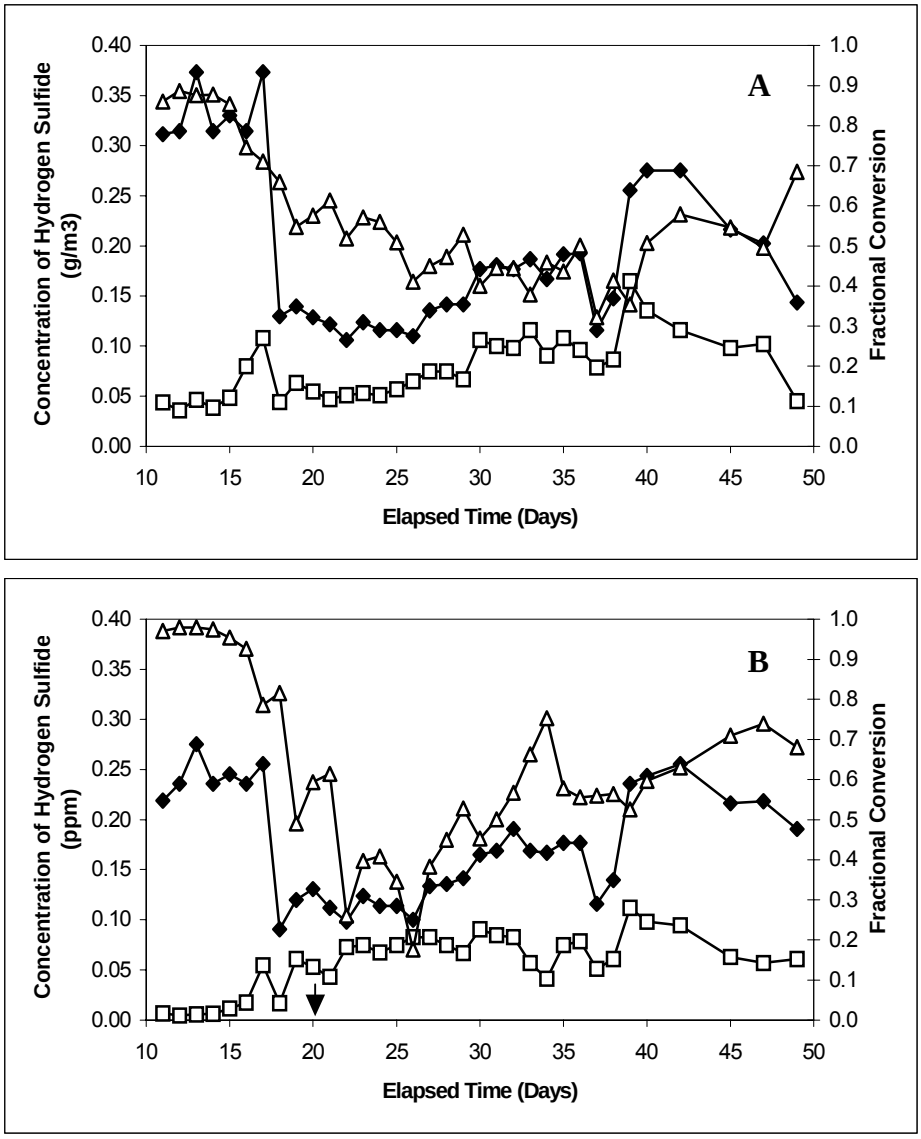


Figure 19. Inlet, outlet and fractional conversion of Biofilter-H (A) and Biofilter-HM (B). Curves (♦, □, Δ) represent inlet concentrations of hydrogen sulfide, outlet concentrations and fractional conversion. Array points to the day when methanol began to be introduced to Biofilter-HM.

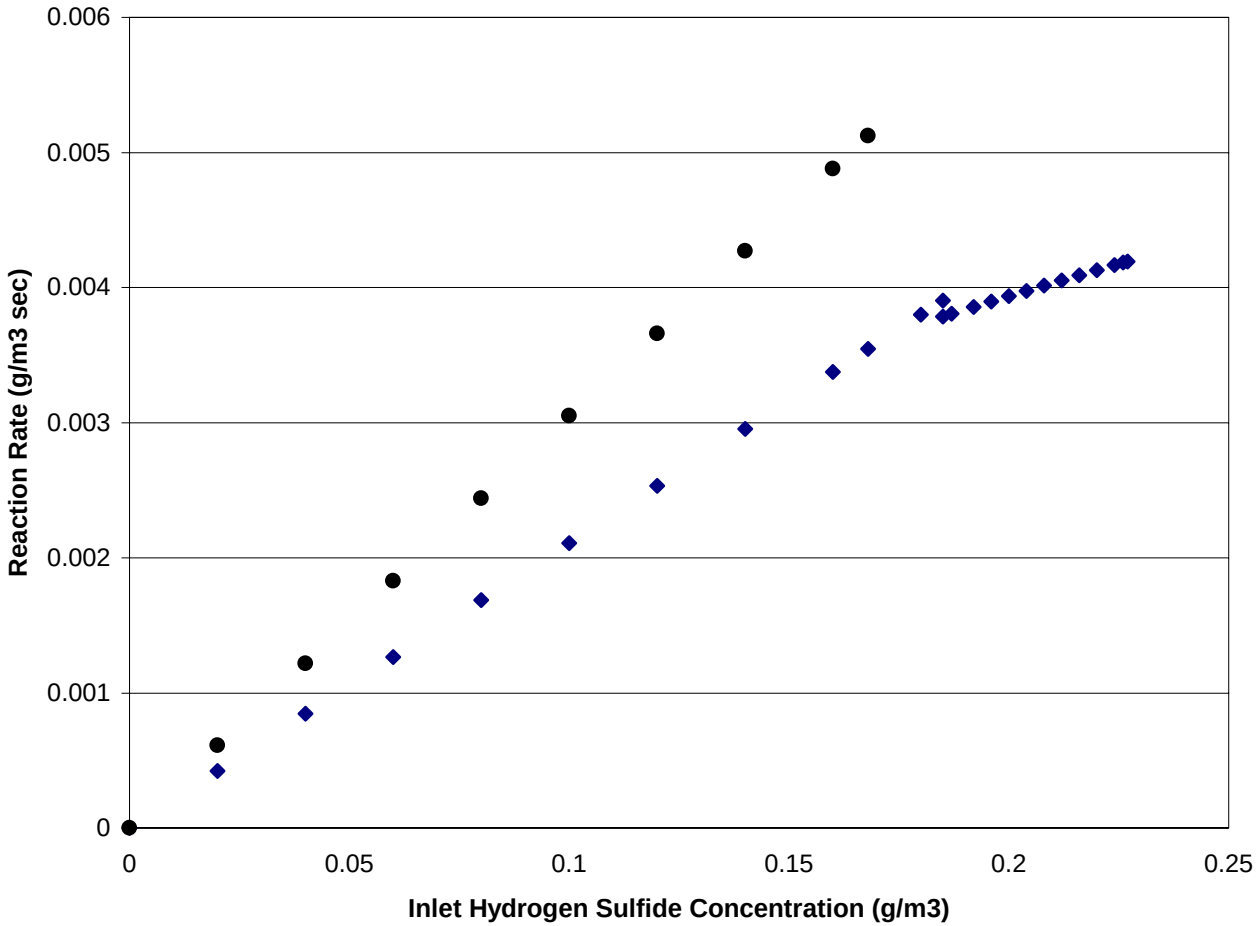


Figure 20. Reaction rate verses inlet hydrogen sulfide concentrations for Biofilter-H and Biofilter-HM. Diamonds represent Biofilter-H, solid circles represent Biofilter-HM.

CHAPTER 4

CONCLUSIONS

SUMMARY OF FINDINGS

Microbial Community Study of the Biofiltration System

The microbial community of the original compost medium for biofiltration of hydrogen sulfide exhibited high diversity, species richness and high species evenness. After the introduction of hydrogen sulfide, the medium adapted to the high sulfur, and low pH environment by enriching reduced sulfur oxidizers to gain energy. *Thiobacillus* and *Sulfobacillus* genera dominated the medium microbial community after only 20 days of hydrogen sulfide introduction. With time, the population of reduced sulfur oxidizers shifted from mainly *Thiobacillus* to *Sulfobacillus* genus.

After the introduction of methanol, the microbial community experienced a significant change. Although the final medium at the end of experiment still included a majority of the species in the sample taken at the 20^{th} day (hydrogen sulfide treatment steady-state), many new species were identified in final medium. These species included methanol oxidizers, such as methylotrophic proteobacterium, *Methylosinus and Methylocella*.

Kinetic Study of the Biofiltration System

Removal efficiencies for hydrogen sulfide were maintained at around 50% for both biofilters before the introduction of methanol to the Biofilter-HM. Biofilter-H showed a clear shift from first to fractional order kinetics. At the concentration level of inlet hydrogen sulfide between 0.116 g/m^3 to 0.275 g/m^3, the Biofilter-H followed the first order kinetic model. The reaction rate was:

$$r = 0.021/sec \times C$$

where r is the reaction rate, 0.021/sec is the first order rate constant, and C is the inlet hydrogen sulfide concentration. Between inlet hydrogen sulfide concentration of 0.275 g/m^3 and 0.337 g/m^3, Biofilter-H hydrogen sulfide removal followed the fractional order kinetics with reaction rate expressed as:

$$r = 0.011\ g^{1/2}m^{-3/2}sec^{-1} \times C^{1/2}$$

Between inlet hydrogen sulfide concentrations of 0.113 to 0.249 g/m^3 in Biofilter-HM the reaction rate was found to be:

$$r = 0.031/sec \times C$$

As the first order rate constant for Biofilter-HM was higher than Biofilter-H, Biofilter-HM would remove more hydrogen sulfide during first order stage than Biofilter-H. This difference between Biofilter-H and Biofilter-HM might have been caused by introduction of methanol to Biofilter-HM.

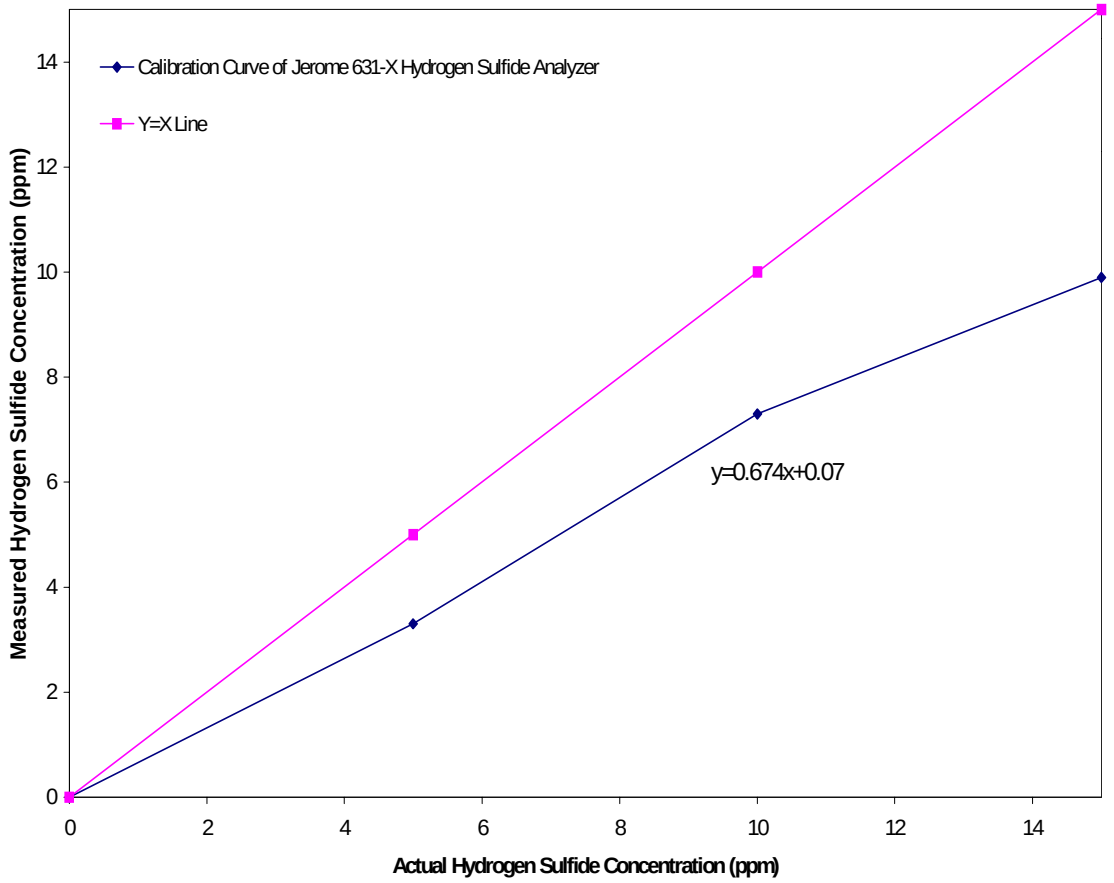
CONCLUSIONS

1. Original compost medium used in the biofiltration system exhibited high diversity and species richness.
2. *Thiobacillus* and *sulfobacillus* genera dominated the microbial community of the medium after only 20 days of hydrogen sulfide treatment.
3. The microbial community shifted from *Thiobacillus* domination to *Sulfobacillus* domination with time.
4. After the introduction of methanol the microbial community of Biofilter-HM shifted from *Thiobacillus* domination to a more diversity and relatively higher species richness and methanol oxidizers were identified in the medium of Biofilter-HM after around 30 days of combined hydrogen sulfide and methanol biofiltration.
5. Removal efficiency of both biofilter one and two were around 50% before the introduction of methanol to Biofilter-HM.
6. The rate constants of first order kinetics were 0.21/sec, and 0.31/sec for Biofilter-H and Biofilter-HM respectively. The rate constants of fractional order kinetics for Biofilter-H was 0.011 g$^{1/2}$m$^{-3/2}$ sec^{-1}.

7. As the rate constant of first order for Biofilter-HM was higher than Biofilter-H, Biofilter-HM could remove more hydrogen sulfide than Biofilter-H in the first order stage.

APPENDICES

Appendix 1. Jerome 631-X Hydrogen Sulfide Analyzer Calibration Curve.



The corresponding calibration equation is:

Actual hydrogen sulfide concentration (ppm) = 1.484 × Measured value - 0.104

Appendix 2. Sample calculation for selecting a kinetic model at a given inlet hydrogen sulfide concentration

For example, at given inlet hydrogen sulfide concentration of 0.337 g/m^3, the measured hydrogen sulfide concentrations for port 1, port 2, port 3 and outlet were 0.283 g/m^3, 0.224 g/m^3, 0.175 g/m^3, 0.059 g/m^3, respectively. The corresponding residence times were 0, 7.068, 20.615, 34.162 and 61.845 seconds. Three kinetic models were assumed, the zero, first and fractional order. General formula for calculating reaction rate for each kinetic model is:

$$r = K \times C^n$$

where r is the reaction rate, K is the rate constant, C is the concentration of hydrogen sulfide in g/m^3 and n = 0, 1, ½ based on order of reaction.

Rate constants at each sampling site for each kinetic model were calculated as follows:

Rate constants for zero order kinetics (K0):

$$K0 = (C_{in} - C_{out})/\tau$$

where C_{in} = hydrogen sulfide concentration of previous sampling site, C_{out} = hydrogen sulfide concentration of current sampling site and τ is the residence time difference between current and previous sampling sites.

Inlet: K0 = 0

Port 1: K0 = (0.337-0.283)/(7.068-0) = 0.00764 $g/m^3 \cdot sec$

Port 2: K0 = (0.283-0.224)/(20.615-7.068) = 0.00436 $g/m^3 \cdot sec$

Port 3: K0 =(0.224-0.175)/(34.162-20.615) = 0.00362 $g/m^3 \cdot sec$

Outlet: K0 = (0.175-0.059)/(61.845-34.162) = 0.00418 $g/m^3 \cdot sec$

The average of K0 for port 1, port 2, port 3 and outlet was:

Average K0 = (0.00764+0.00436+0.00362+0.00418)/4 = 0.00495 $g/m^3 \cdot sec$

And the standard deviation of K0 for port 1, port 2, port 3 and outlet were calculated:

Standard deviation of K0 = 0.00182 $g/m^3 \cdot sec$

The ratio between standard deviation and average was:

0.00182/0.00495 = 0.368.

Rate constants for first order kinetics (K1):

$$K1 = \ln(C_{in}/C_{out})/\tau$$

where C_{in} = hydrogen sulfide concentration of previous sampling site, C_{out} = hydrogen sulfide concentration of current sampling site and τ is the residence time difference between current and previous sampling sites.

Inlet: K1 = 0

Port 1: K1 = ln(0.337/0.283)/(7.068-0) = 0.0247/sec

Port 2: K1 = ln(0.283/0.224)/(20.615-7.068) = 0.0173/sec

Port 3: K1 =ln(0.224/0.175)/(34.162-20.615) = 0.0182/sec

Outlet: K1 = ln(0.175/0.059)/(61.845-34.162) = 0.0390/sec

The average of K1 for port 1, port 2, port 3 and outlet was:

Average K1 = (0.0247+0.0173+0.0182+0.0390)/4 = 0.0248/sec

And the standard deviation of K1 for port 1, port 2, port 3 and outlet were calculated:

Standard deviation of K1 = 0.0100/sec

The ratio between standard deviation and average was:

0.0100/0.0248 = 0.405.

Rate constants for fractional order kinetics (K1/2):

$$K1/2 = 2 \times (\text{sqrt}(C_{in}) - \text{sqrt}(C_{out}))/\tau$$

where C_{in} = hydrogen sulfide concentration of previous sampling site, C_{out} = hydrogen sulfide concentration of current sampling site and τ is the residence time difference between current and previous sampling sites.

Inlet: K1/2 = 0

Port 1: K1/2 = $2 \times$ (sqrt(0.337)-sqrt(0.283))/(7.068-0) = 0.0137 $g^{1/2}m^{-3/2}sec^{-1}$

Port 2: K1/2 = $2 \times$ (sqrt(0.283)-sqrt(0.224))/(20.615-7.068) = 0.0087 $g^{1/2}m^{-3/2}sec^{-1}$

Port 3: K1/2 =$2 \times$ (sqrt(0.224)-sqrt(0.175))/(34.162-20.615) = 0.0081 $g^{1/2}m^{-3/2}sec^{-1}$

Outlet: $K1/2 = 2 \times (\text{sqrt}(0.175)-\text{sqrt}(0.059))/((61.845-34.162) = 0.0126\ g^{1/2}m^{-3/2}sec^{-1}$

The average of K1/2 for port 1, port 2, port 3 and outlet was:

Average $K1/2 = (0.0137+0.0087+0.0081+0.0126)/4 = 0.0108\ g^{1/2}m^{-3/2}sec^{-1}$

And the standard deviation of K1/2 for port 1, port 2, port 3 and outlet were calculated:

Standard deviation of $K1/2 = 0.0028\ g^{1/2}m^{-3/2}sec^{-1}$

The ratio between standard deviation and average was:

$0.0028/0.0108 = 0.261$.

As the ratio between standard deviation and average of the rate constants was smallest for fractional order, hence fractional order kinetic model was selected as the most suitable kinetic model for inlet hydrogen sulfide concentration at 0.337 g/m^3.